IN-SILICO IDENTIFICATION OF POTENTIAL DRUG TARGETS FOR BOVINE ONCHOCERCIASIS USING PROTEINS FROM METABOLIC PATHWAYS OF *Wolbachia* ENDOSYMBIONT OF *Onchocercaochengi*

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DEPARTMENT OF BIOCHEMISTRY,

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ZARIA, NIGERIA

MAY, 2021

DECLARATION

I declare that the work in this dissertation entitled "*In-silico* identification of potential drug targets for bovine onchocerciasis using proteins from metabolic pathways of *Wolbachia* endosymbiont of *onchocerca ochengi''*, which was presented in the Department of Biochemistry, represents my own work. The information derived from literature has been acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other institution.

……	……	……
Name of student	Signature	Date

CERTIFICATION

This dissertation entitled "*IN-SILICO* IDENTIFICATION OF POTENTIAL DRUG TARGETS FOR BOVINE ONCHOCERCIASIS USING PROTEINS FROM METABOLIC PATHWAYS OF *WOLBACHIA* ENDOSYMBIONT OF *ONCHOCERCA OCHENGI"* by Cheluchi Solumtochukwu Okpoko , meets the regulations governing the award of the degree of Masters in Biotechnology of the Ahmadu Bello University, and is approved for its' contribution to knowledge and literary presentation.

……..……	**……**	**……**
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Member, Supervisory Committee	**Signature**	**Date**
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Dean, School of Postgraduate Studies	**Signature**	**Date**

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ABSTRACT

Chemotherapy with antibiotics against the *Wolbachia* endosymbiont is a method of eliminating adult filarial parasites. Unfortunately, rapidly evolving drug resistance to antibiotics is limiting the use of these drugs against Gram-negative bacteria, thereby demanding novel approaches in drug target identification and chemotherapy.

In-silico approaches offer a more rapid and cost effective strategy in drug discovery and design. In this study, computational techniques which involved a systematic protein subtractive approach, were employed to identify putative drug targets for bovine onchocerciasis, as a model of filariasis, using the biological pathways of *Wolbachia* endosymbiont of *Onchocerca ochengi* (*wOo*) available in KEGG (Kyoto Encyclopedia of Genes and Genomes). This study identified a total of 70 biological pathways of *wOo* from KEGG, with 13 of these pathways being unique to *wOo* and 57 being common to both cattle (*Bos taurus*) and *wOo*. Out of 349 proteins associated with the 70 pathways of *Wolbachia* of *Onchocerca ochengi,* 204 proteins were identified as non-homologous to cattle proteins by NCBI BLAST search tool. From the 204 proteins, 180 proteins were further identified as essential to the survival of *Wolbachia* of *Onchocerca ochengi* using DEG (Database of Essential genes). Prioritization of the resultant 180 proteins revealed 58 proteins as druggable targets for bovine onchocerciasis. Molecular docking studies of the three dimensional structures of these 58 proteins with 36 drug structures from drug bank database resulted in only 32 proteins of *wOo* completing the molecular docking process. This study therefore revealed 32 drug targets for bovine onchocerciasis. Molecular docking results of malic enzyme with NADH, N5-carboxyaminoimidazole ribonucleotide synthase with carglumic acid and replicative DNA helicase with zinc showed the best, moderate and least binding energies repectively. This study has identified potential drug targets of *wOo* for bovine onchocerciasis, towards control of human onchocerciasis

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LIST OF ABBREVIATIONS

wOo- Wolbachia endosymbiont of *Onchocerca ochengi*

KEGG- Kyoto Encyclopedia of Genes and Genomes

DEG- Database of Essential Genes

BLAST- Basic Local Alignment Search Tool

NCBI- National Centre for Biotechnology Information

NADH- Nicotinamide adenine dinucleotide

DNA-Deoxyribonucleic acid

MDA – Mass Drug Administration

APOC- African Program for Onchocerciasis Control

Kd- Dissociation constant

Fig.- Figure

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Filariasis is one of the neglected tropical diseases (NTDs) that occurs frequently in developing countries (Barton *et al.,* 2014). This important parasitic disease is caused by filariae, and has a worldwide distribution, with effects on both man and animals (Hashem and Badawy, 2007). Nigeria is believed to have the greatest burden of Lymphatic filariasis, with an estimated 80 to 120 million people at risk (Okorie *et al.,* 2013). Filariae are thread-like parasitic nematodes which are spread by arthropod vectors (Cross, 1996) such as mosquitoes and black flies.

During the life cycle of filariae, microfilariae (stage 1 or L1) are ingested by the vector (mosquito and blackfly) during a blood meal. The microfilariae then moult and develop till the larvae reaches the infective stage (stage 3 or L3) in the vector. In every process of this development, the larvae moves from the ingested blood to other tissues, depending on the vector species. While the vector takes another blood meal, infective larvae are transferred from the intermediate host (vector) to the dermis of the vertebrate's skin. For *Onchocerca* and *Dirofilaria* genera ,the L3 larvae moults into stage 4 larvae within two days, while moulting for the other genera is about ten days (Bain and Babayan, 2003). Most filarial species migrate through the host's body from the skin to their final niches, which include the lymphatic system, the coelomic cavities, the cardiopulmonary system, and connective tissues. The fouth moult, which is also the final moult, gives rise to adult male or female worms (Bouchery *et al*., 2013).

Chronic filarial disease pose serious social and economic effects. Individuals with elephantiasis and hydrocele (caused by filarial infections) are often socially marginalized and poor. Acute attacks and chronic disability lead to low economic output and poverty (W.H.O, 2007). However, avoidance of mosquito bites through personal or community-level vector control is the best method to prevent lymphatic filariasis (W.H.O, 2019).

Onchocerciasis, also known as river blindness, is a parasitic disease caused by one of the filarial worms- *Onchocerca volvulus*. Onchocerciaisis is the world's second-leading infectious cause of blindness (Ngorok and Bush, 2011). Recently, W. H. O recorded that more than 99% of people infected with *O. volvulus* live in thirty-one African countries and that the disease also exists in some foci in Yemen and Latin America (W.H.O, 2020). Human onchocerciasis occurs due to repeated bites of infected blackfly (Diptera: Simuliidae) vectors of the parasitic nematode *Onchocerca volvulus* (Hendy *et al.,* 2018). These black flies breed in fast-flowing streams and rivers, increasing the risk of blindness to individuals living near these water bodies (W. H. O, 2019). The disease is regarded as an inhibitor of social and economic progress and causes considerable suffering (Mopecha and Thieme, 2003).

Filarial nematodes affect cattle. Generally, the filarioid nematodes of economic importance in cattle are *Setaria digitata, Setaria labiatopapillosa, Setaria marshalli, Onchocerca gibsoni, Onchocerca gutturosa, Onchocerca armillata, Onchocerca lienalis, Onchocerca ochengi, Parafilaria bovicola* and *Stephanofilaria* spp. (Solismaa *et al.,* 2008)**.** However, there are four *Onchocerca* species which are common parasites of cattle in Africa. These include: *Ochocerca gutturosa, O. armillata, O. ochengi* and *O. dukei* (Wahl *et al*., 1994). *Onchocerca*

ochengi is the closest relative of *O. volvulus*, the causative agent of river blindness. The parasites also share the same vector (*Simulium damnosum*) (Mbah *et al*., 2016).

Wolbachia bacterial endosymbionts are present in filarial nematode species. *Wolbachia* is a target for antibiotic therapy with tetracyclines (Bouchery *et al.,* 2013), which have serious effects on the development, viability and fertility of filarial worms. The endosymbiont has a mutualistic relationship with the *Onchocercidae,* and also provides essential metabolites to the filariae (Bouchery *et al.,* 2013). *Wolbachia* is also found in arthropods. *Wolbachia* has an influence on the reproductive functions of its host (male killing, cytoplasmic incompatibility, parthenogenesis), which may lead to host evolution and species formation (Gordon *et al.,* 2012). The genus '*Wolbachia*' belongs to the class of parasitic alphaproteobacteria and are very common and widespread (Gordon *et al.,* 2012). Antibiotics have been seen to produce an effect on filarial nematodes due to their symbiotic relationship with *Wolbachia*. The tetracycline class of antibiotics depletes *Wolbachia* and results in the sterilization of the female filarial nematode. (https://microbewiki.kenyon.edu/index.php/The_use_of_antibiotics_on_Wolbachia_as_treatment_for_filarial_diseases).

Ivermectin, another drug for the treatment of onchocerciasis, was introduced in 1987 for mass treatment of the disease (African Programme for Onchocerciasis Control, 2008). OEPA (Ohio Environmental Protection Agency) initiated the use of twice per year treatments of ivermectin, at high coverage rates, and in so doing has eliminated onchocerciasis from some countries affected in the region. In December 2015, APOC published a report describing strategic options and alternative treatment strategies for hastening the elimination of onchocerciasis in Africa, and proposed the use of twice per year MDA, a core strategy developed and implemented by OEPA (Cupp *et al.,* 2019).

This drug- ivermectin, is soley microfilaricidal (Mbah *et al*., 2016) and it has also been reported that cattle protected from onchocerciasis with ivermectin are highly vulnerable to infection, after drug withdrawal (Njongmeta *et al*., 2004).

Drug target discovery is one of the first steps to drug discovery. It is easy to identify drug targets at the genomic level for any given pathogen when the pathogen and host genome sequences are available (Mondal *et al*., 2015). Bioinformatics and cheminformatics are fasinating alternative methods employed in the identification of drug targets. Darby *et al*., (2012) sequenced the complete genome of *Wolbachia* strain *wOo*. This information can help in identifying the most suitable drug targets for *wOo*.

This study aimed to propose potential drug targets in *Wolbachia* of *Onchocerca ochengi* (*wOo*) for bovine onchocerciasis using proteome subtractive technique and molecular docking.

1.2 Statement of research problem

One of the major challenges of the use of doxycycline, a tetracycline antibiotic and the current "standard" anti-*Wolbachia* macrofilaricide in the treatment of filariasis, is its prolonged treatment period in order to deliver a cure. (Hong *et al.*, 2019). Drug resistance can occur due to prolonged treatment with this antibiotic as gram-negative bacteria are becoming resistant to nearly all the antibiotic drugs available (Ventola, 2015).

Ivermectin, which is used for the treatment of onchocerciasis, is only lethal to the microfilarial stage of the parasite, and not the adult worm (Mbah *et al*., 2016, Cho-Ngwa *et al*; 2019).

These problems make it necessary for potential drug targets for bovine onchocerciasis to be investigated in order to develop new filaricidal strategies.

1.3 Justification

In the past and even in recent times, *Onchocerca ochengi* has been reported to infect cattle in Africa (Akusu *et al.*,1983; Hansen *et al.,* 2010; Eisenbarth *et al.*,2013; Hildebrandt *et al.*,2014; Kalmobé *et al*., 2017).

Many parasitic nematodes of livestock have become resistant to ivermectin (Lustigman and McCarter 2007) and a case of *Onchocera spp*. resistance to the drug has been recorded (Osei-Atweneboana *et al.,* 2007). The occurrence of *O. ochengi* infections in cattle; and ivermectin resistance by *Onchocera spp*. makes it important for potential drug targets for bovine onchocerciasis to be investigated in order to develop new drugs for bovine onchocerciasis; hence, the need to undertake this study.

1.4 Aim

To identify potential drug targets for bovine onchocerciasis with bioinformatics tools, using proteins associated with biological pathways of *Wolbachia* endosymbiont of *O. ochengi* (*wOo*).

1.5 Specific objectives

1. Prioritize essential non-homologous proteins of *wOo* by identifying subcellular localization of proteins, investigating their molecular weights and analyzing the druggability of essential non-homologous proteins of *wOo*.

2. Molecular docking of 3D structures of drug targets with drugs from drug bank

3. Identify drugs which could be repurposed for the treatment of bovine onchocerciasis.

CHAPTER TWO

2.0 LITERATURE REVIEW

2. 1 Onchocerciasis in cattle

Onchocerciasis refers to microfilariosis where only members of the genus *Onchocerca* are involved and clearly identified (Soulsby, 1982; Georgi and Georgi, 1990). Many *Onchocerca* species are parasites of ungulates (Neary *et al*; 2010). One of such ungulates infected by *Onchocerca* species is cattle.

Four *Onchocerca* species are common parasites of cattle in Africa: *O. gutturosa, O. armillata, O. ochengi and O. dukei. O. gutturosa is known in many parts of th*e world such as some African countries neighbouring the Sudan (Fain, 1955); Chad (Grarer, 1969); and Uganda (Elbihari and Hussein 1978). Reports have revealed that *Onchocerca gutturosa* is transmitted by different vectors. In the Sudan and Sierra Leone, it is transmitted by *Culicoides* species (Davies *et al*., 1989), while it was reported to be transmitted by *Simulium vorax* in Tanazania (Mwaiko, 1981). *O. armillata* (Raillet and Henri, 1909) is a parasite which lives in the intima of the aorta of cattle (Wahl *et al*., 1994). The vector of *O. armillata* is not yet known. (Wahl, 1991). The aortic lesions caused by the parasite are commonly regarded as being unharmful, however, Chodnik (Chodnik, 1958) and Patnaik (Patnaik, 1962) indicated that *O. armillata* might cause vascular aneurysms in the affected aortae. Aortic onchocerciasis is common in countries of the West African savannah (Schillhorn, 1974). *O. ochengi* and *O. dukei* are parasites which form nodules in the ventral skin and on the fasciae of the thoracic muscles (Wahl *et al*., 1994). The vector which transmits *Onchocerca ochengi* is *Simulium damnosum* s.1. (Denke and Bain, 1978)

and *O. dukei* is transmitted by *S. bovis* (Wahl and Renz, 1991). In Europe, the presence of *Onchocerca lienalis* was reported (Trees *et al*., 1987).

Different kinds of *Onchocerca spps* cause bovine onchocerciasis. However, *Onchocerca ochengi* is peculiar and of great importance because of its similarities and close relationship with the *O. volvulus,* the causative agent of human onchocerciasis. *O. ochengi* and *O. volvulus* have so many things in common (Trees *et al.,* 2000). Molecular and biochemical comparisons of both have revealed almost identical antigenic and genomic profiles (Xie *et al.,* 1994). Both parasites are also equally vulnerable to the same filaricides- tetracyclines (Langworthy *et al.,*2000), ivermectin, suramin (McCall *et al.,* 1992). Again, *O. ochengi* forms nodules that closely resemble those of *O. volvulus* (Wildenburg *et al*., 1997).

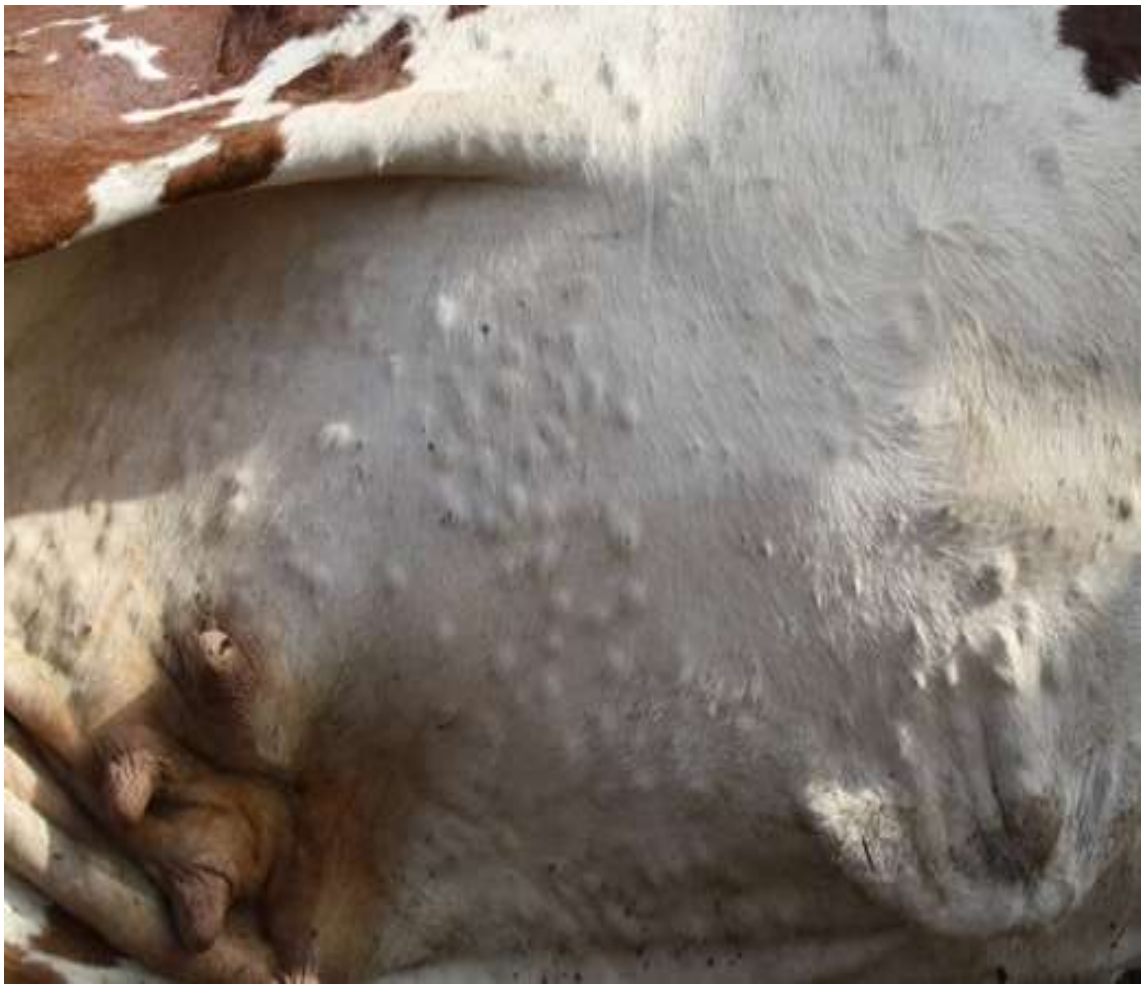

Fig. I: Ventral view of the abdominal area of a zebu cattle with many *O. ochengi* nodules (https://www.riverblindness.eu/epidemiology/bovine-onchocercoss/; 2020, March 08)

2.2 Onchocerciasis in humans

Onchocerciasis, also known as river blindness, is a parasitic disease caused by *Onchocerca volvulus*. In West Africa, there are at least two extant strains of the parasite. They include the savannah strain and the forest strain. The "savanna" strain is associated with higher degree of blindness than the "forest" strain which causes a less severe form of onchocerciasis (Prost *et al.,* 1980). Onchocerciaisis is the world's second-leading infectious cause of blindness (Ngorok and Bush, 2011). Recently, W. H. O recorded that more than 99% of people infected with *O. volvulus* live in thirty-one African countries and that the disease also exists in some foci in Yemen and Latin America (W.H.O, 2020).

Onchocerciasis is regarded as an inhibitor of social and economic progress and causes considerable suffering (Mopecha and Thieme, 2003). In 2018, W.H.O reported that a total of 18 million people were infected with the disease and have dermal microfilariae (W.H.O, 2018).

Human onchocerciasis occurs due to repeated bites of infected blackfly vectors of the parasitic nematode *Onchocerca volvulus* (Hendy *et al.,* 2018). These black flies place their eggs into fast flowing rivers where highly oxygenated water is present, after which young adult flies spring up. There are two main species of the black fly vector in Africa: *Simulium damnosum* and *Simulium naevi*. *S. damnosum* can carry infection to distant locations because of the long distances it can travels and is found predominantly in west and central Africa (Ngorok and Bush, 2011).

The life cycle of *O. volvulus* involves a black fly injecting the infective larvae stage of the parasite into their human host. The larvae then migrates to the subcutaneous tissue where they form nodules on the skin as they develop into adult worms. The adult worms mate and produce a very large number of microfilaria. The microfilariae migrate to the whole body and activate intense inflammatory reaction. The black flies, which feed during the day, then take up the microfilaria which further mature into infective larvae within the black flies. These infective larvae can further be transmitted to another individual (Ngorok and Bush, 2011).

Understanding onchocerciasis in animals could be vital in understanding some aspects of the disease in humans (Mellorp, 1973). It has been revealed that cattle protect from some serious parasitic diseases, either by diverting the vectors coming to take a bloodmeal or by transmitting bovine parasites to humans which do not further develop within humans but activate their immune system (Renz *et al.,* 1994).

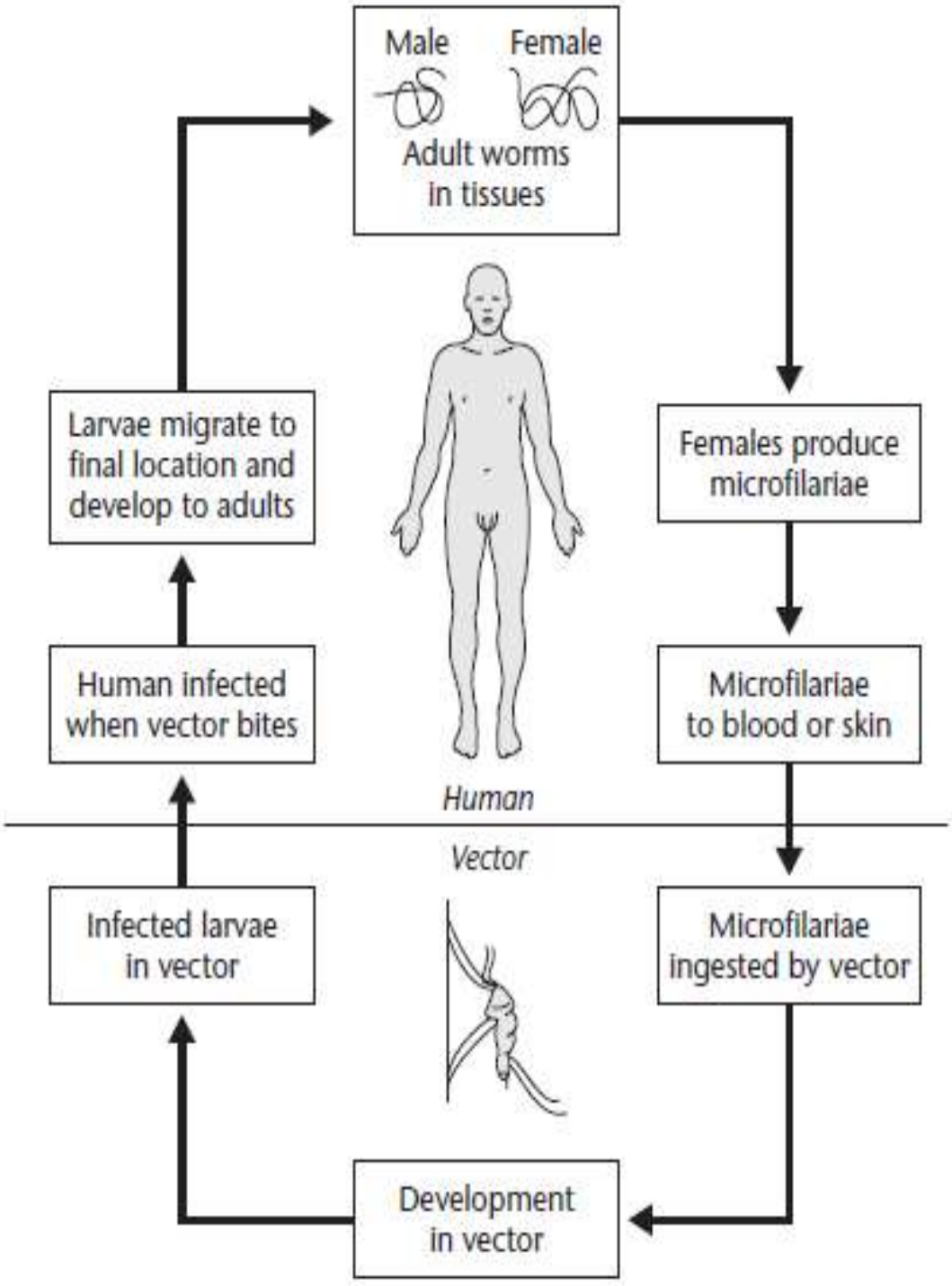

Fig. II: General life cycle of filiariae (Simonsen, 2008)

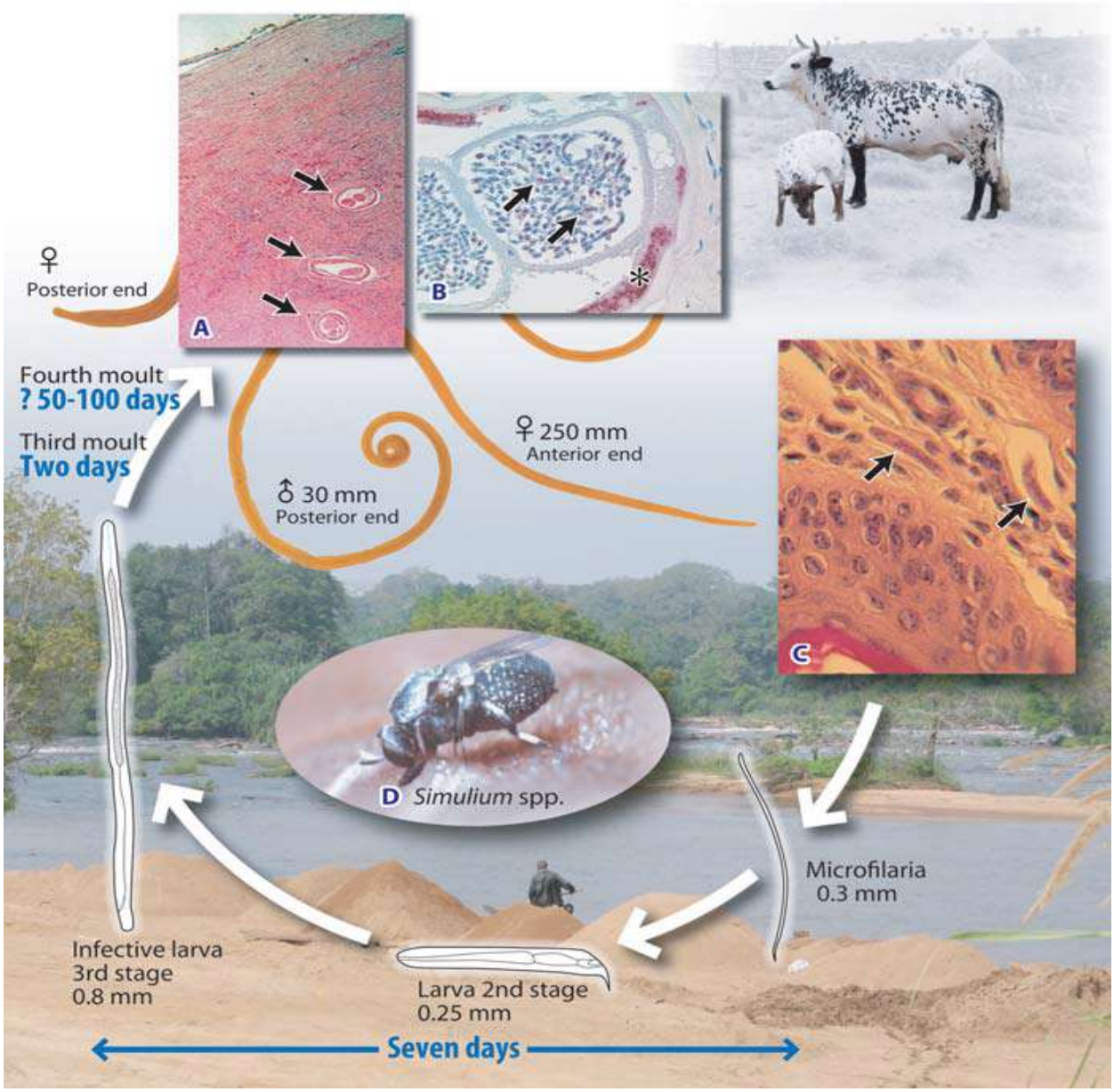

Fig. III: Life cycle of *Onchocerca volvulus* and *Onchocerca ochengi* (Allen *et al.*, 2008).
Stage A shows the bites on the skin of a cattle/human by *S. damnosum* which implies that *S. damnosum* has transferred the infective larvae of the parasite to the human/cattle. The infective larvae (L3) then develops into adult worms in the body of the human/cattle.
Stages B and C show the microfilariae produced by adult worms of the parasite in the body of human/cattle.
Stage D shows *S. damnosum* taking a blood meal through the skin of human/cattle. During this blood meal, microfilariae are ingested by *S. damnosum*.

2.2.1 Diagnosis of human onchocerciasis

Microscopy is the bedrock for laboratory diagnosis with blood and tissue parasite (Garcia, 2007). In the laboratory diagnosis of onchocerciasis, microscopy of multiple Giemsa-stained "skin snips," which demonstrate microfilaria can be employed (Udall, 2007). Skin snips are small bloodless biopsies taken down to the dermal papillae with a razor blade or corneoscleral biopsy instrument. Skin snips can be gotten from tissue around a nodule which is likely to contain adult worms or from random body sites overlying the scapula or iliac crest. Fresh unstained wet preparations of skin snips should also be examined after incubation in saline at 37^{o}C for 2–24 hrs. For correct microscopy results, the skin snips must be properly obtained and must be transported to the laboratory as quickly as possible. The skin snips must be processed in an appropriate time to preserve organism's (*Onchocerca spps*) viability and/or morphology (Rosenblatt, 2009).

Apart from microscopy, there are several other ways to diagnose human onchocerciasis, such as, diethylcarbamazine (DEC) patch test which achieves the indirect detection of microfilaria (Udall, 2007; Winthrop *et al*., 2011), detection of antibodies to onchocercal antigens (Udall, 2007; Winthrop *et al.*, 2011), PCR detection of *O.volvulus* DNA in skin snips (Udall, 2007; Winthrop *et al*., 2011), detection of microfilaria in histopathologic sections of skin biopsies stained with hematoxylin and eosin (Rosenblatt, 2009), Ultrasonic detection (Rosenblatt, 2009), observation of microfilaria in the eye by slit-lamp exam (Rosenblatt, 2009) and the surgical removal of adult worms from a subcutaneous nodule (Rosenblatt, 2009).

2.2.2 Control of human onchocerciasis

Treatment of water sources with insecticides to kill the larvae (larviciding) of the blackfly (*Simulium spp.*) was the previous attempt of onchocerciasis control. The WHO/ UNDP Onchocerciasis Control Programme (OCP) reduced the burden of onchocerciasis in savannah regions of West Africa after using this approach for over 25 years (Thylefors and Alleman, 2006). Another factor that has contributed to the success of onchocerciasis control is the extensive mass treatment of individuals with the ivermectin, governed by institutions of the World Health Organization, such as the African Programme for Onchocerciasis Control (WHO, 2013). Ivermectin (Mectizan, Merck & Co.) was introduced in 1987 for mass treatment of onchocerciasis (African Programme for Onchocerciasis Control, 2008).

Interruption of the transmission of *Onchocerca volvulus*, has been verified for a growing number of endemic foci on the American continent (Cruz-Ortiz *et al.*, 2012), in West (Diawara *et al.*, 2009) and East Africa (Katabarwa *et al.*, 2014).

2.3 *Wolbachia*

Wolbachia are bacteria in the genus *Rickettsiae*, which are inherited via the cytoplasm and found in reproductive tissues (ovaries and testes) of arthropods (O'Neill *et al.*, 1992, Rousset *et al.*, 1992) and also in nematodes. *Wolbachia* are also present in isopods (Rousset *et al.*, 1992) and mites (Johanowicz and Hoy, 1995). The first detection of *Wolbachia* was in the mosquito, *Culex pipiens,* in 1924 (Hertig and Wolbach, 1924). *Wolbachia* has been a subject of intense interest due to its unusual diversity (Gordon *et al.*, 2012)

Wolbachia comprises ten supergroups namely A, B, C, D, E, F, H, I, J and K– with most found either in arthropods or nematodes (Ferri *et al.,* 2011). *Wolbachia* within the supergroups C and D found in nematodes while *Wolbachia* within supergroups A, B, E, H, I, K are found in arthropods (Ros *et al.,* 2009). Both arthropod and nematode hosts harbor supergroup F *Wolbachia*, implying horizontal transfer between the two host phyla (Casiraghi *et al*., 2005). The J supergroup has been recognized to be associated with filarial nematodes (Haegeman *et al*., 2009).

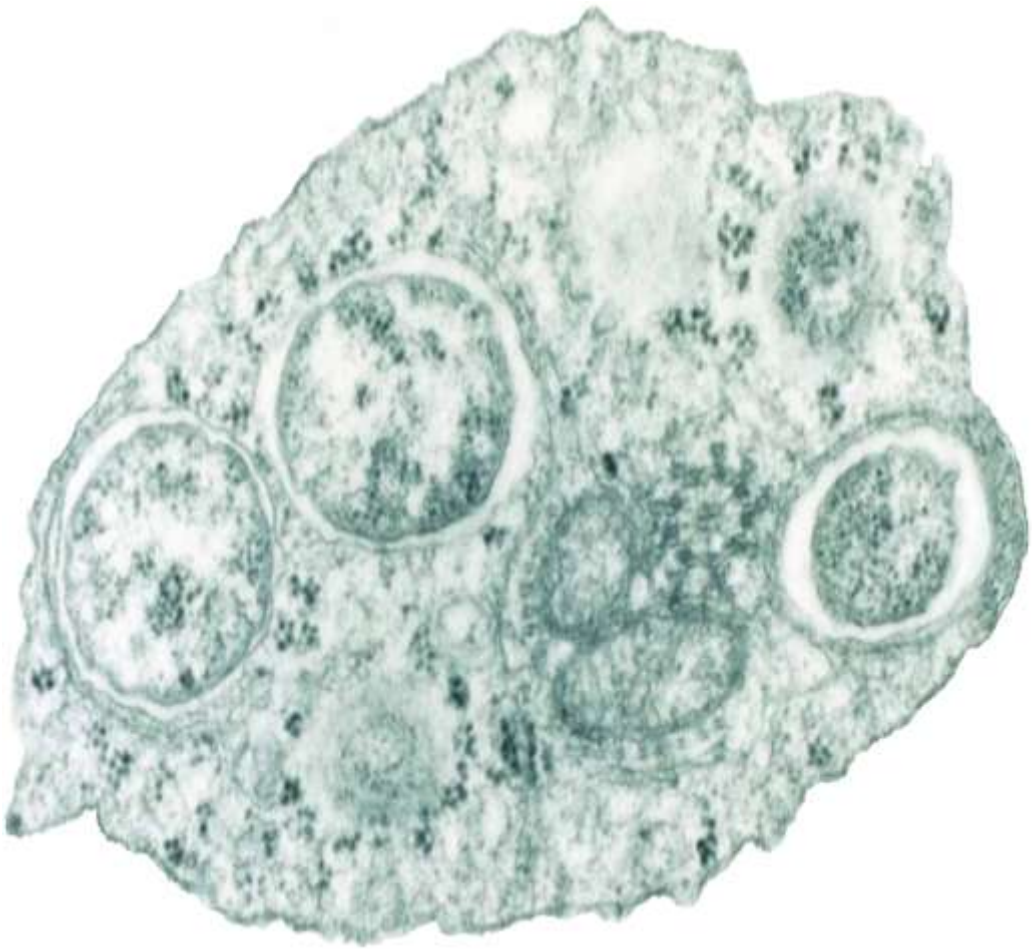

Fig. IV: *Wolbachia* (O'Neil, 2004)

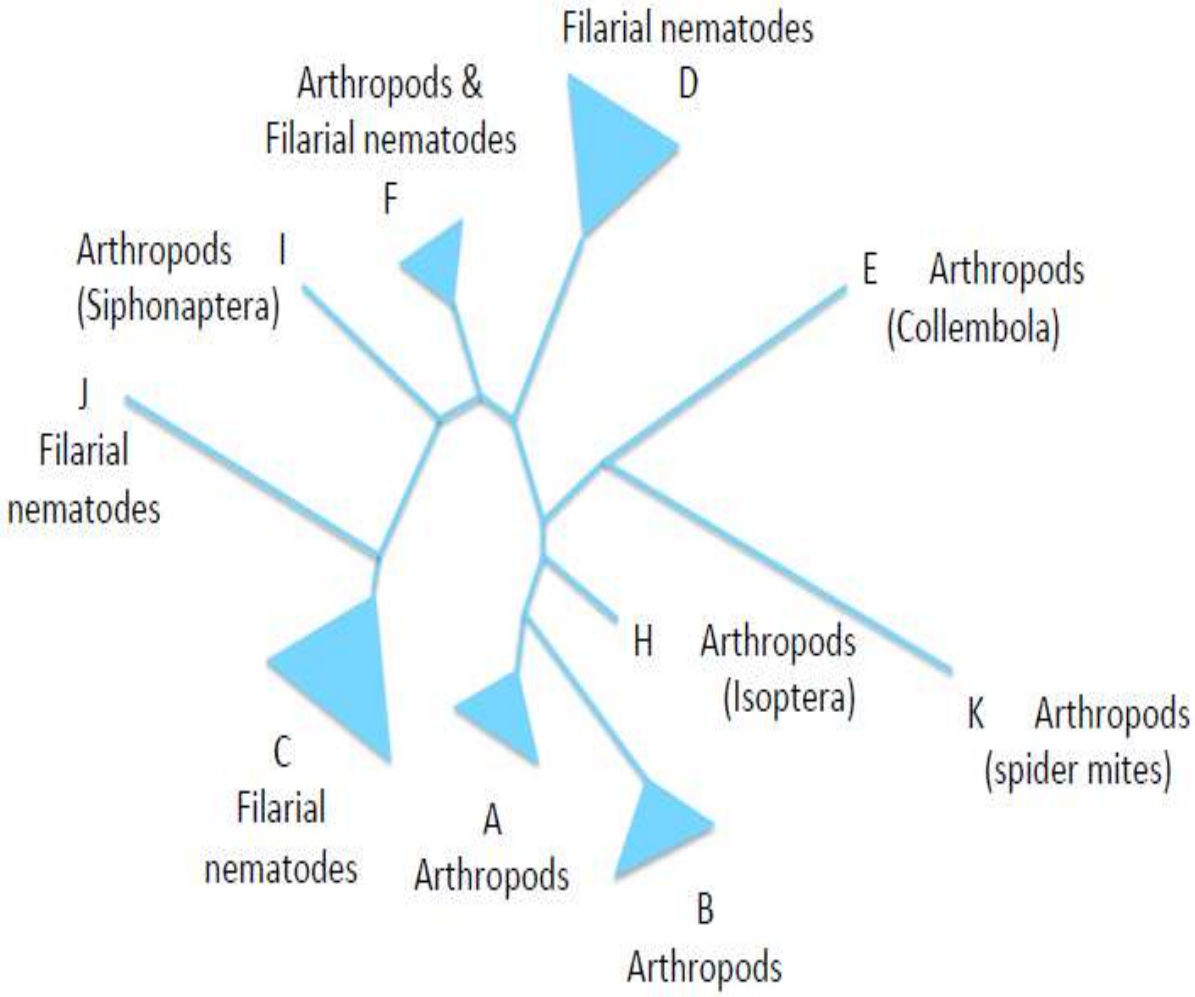

Fig. V: A phylogenic representation of the 10 supergroups of *Wolbachia* (Ros *et al.*, 2009).

2.3.1 *Wolbachia* in arthropods

Wolbachia associated with arthropods are generally regarded as reproductive parasites (http://www.sas.rochester.edu/bio/labs/WerrenLab/WerrenLab-WolbachiaBiology.html; 2020 March, 21). Mutualistic features of the symbiotic relationship between *Wolbachia* and certain arthropod hosts have also been reported (Slatko *et al.,* 2010). *Wolbachia* is found in the reproductive tissues of arthropods and are transmitted horizontally between arthropod species (Werren, 1997). PCR results signify that *Wolbachia* may infect up to 70% of all insect species (Jeyaprakash and Hoy 2000; Hilgenboecker *et al.,* 2008).

In arthropods, *Wolbachia* infections alter their hosts' reproduction leading to cytoplasmic incompatibility (Sinkins *et al.,* 1995; Stouthamer *et al*., 1999), parthenogenesis induction (PI) (Stouthamer *et al.,* 1993), male killing (Simoes, 2012) and feminization of genetic males (Rousset *et al.,* 1992). These reproductive modifications impart a selective advantage for the bacteria (Turelli 1994).

1. Cytoplasmic incompatibility (CI): There are two types of CI that can occur namely; unidirectional and bidirectional CI. In unidirectional CI, uninfected females mate with *Wolbachia* infected males to produce inviable offsprings and *Wolbachia*-infected females also mate with both infected and uninfected males to produce viable offsprings. In bidirectional CI, *Wolbachia*-infected females are not compatible with males infected with a different *Wolbachia* strain (Sutton *et al.,* 2014). Cytoplasmic incompatibility related strategies have been proposed for both the inhibition and replacement of host populations (Plichart and Legrand, 2005). *Wolbachia* can serve as biocontrol against insect vectors that help to spread pathogens to humans

and livestock (Iturbe-Ormaetxe *et al.*, 2011), through the utilization of Cytoplasmic Incompartibility (Metcalf, 2014).

2. Male-killing: Male killing increases the fitness of infected females by either helping the infectious transmission of the symbiont or the survival of their infected female relatives (Simoes, 2012).

3. **Feminisation**: *Wolbachia* causes genetic males to become females (Werren *et al.*, 2008). Genetic conflict as regards to sex determination is likely to occur when the feminizing bacterium is present, which can further result in rapid evolution in the sex-determining system (Rigaud and Juchault, 1993; Juchault *et al*., 1994).

4. **Parthenogenesis**: Parthenogenesis occurs in haplodiploid species where the parasites' presence in unfertilised eggs (which normally give origin to males) brings about the duplication of chromosomes and, hence, the asexual production of infected females. (Simoes, 2012)

2.3.2 *Wolbachia* in filaria nematodes

Nearly all filarial nematodes contain *Wolbachia* within their tissues, needed for fertility, development and survival of the nematodes, consequently, providing targets for filariasis control (Barton *et al.*, 2014). These bacteria were first detected in tissues of several filarial species by electron microscopy (Kozek and Marroquin, 1977; McCall *et al.*, 1999) and later identified as *Wolbachia* by molecular analyses (Sironi *et al.*,1995). However, *Loa loa* (a filarial nematode) does not harbour *Wolbachia* (Desjardins *et al.*, 2013). *Wolbachia* in filarial nematodes are

obligate mutualists. The phylogenetic relationships between *Wolbachia* strains are nearly identical to the phylogenetic relationships between their filarial hosts (Casiraghi *et al.*,2004). The extended and stable co-evolution that led to these matching phylogenies implies interdependence. On the contrary, insects and parasitic *Wolbachia* do not have these concordant phylogenetic patterns. (Werren *et al.,* 2008).

At all developmental stages of filarial worms, *Wolbachia* is present but as the nematode is introduced from the vector to the mammalian host, *Wolbachia* rapidly increases. The titer further enlarges as the larvae develops to the adult stages and within the oocytes and embryos (Fenn and Blaxter, 2004; McGarry *et al.*,2004). The ovaries, oocytes, developing embryos within females and the hypodermal cells of the lateral cords of both sexes house *Wolbachia* in filarial nematodes. The presence of *Wolbachia* in ovaries, oocytes and developing embryos within females supports the notion of vertical transmission of *Wolbachia* through the egg cytoplasm (Landmann *et al.,* 2010). There is high level of interest in using *Wolbachia* to indirectly suppress the incidence of vector borne human diseases such as malaria, dengue fever or filariasis (Sinkins 2013). Administration of effective antibiotics against *Rickettsia* for 4-6 weeks, on a daily basis, has a strong impact on *Wolbachia*-dependent filarial worms (Hoerauf *et al*. 1999).

Among the four *Onchocerca spps* which affect African cattle, three have been reported to be *Wolbachia* positive (*O. gutturosa, O. ochengi and O. armillata*) while *O. dukei* is of unknown *Wolbachia* status (Neary *et al.,* 2010). *Ex vivo* studies of *O. ochengi* nodules during antibiotic treatment has brought about the hypothesis that *Wolbachia* may aid long term worm survival by creating a neutrophil-dominated cellular environment around the worms to prevent eosinophil attack (Nfon *et al*., 2006).

One method of combating adult filarial parasites is chemotherapy by antibiotics against the *Wolbachia* endosmbiont (Kumar *et al.,* 2013) and antibiotic treatment of cattle infected with the *Wolbachia*-positive *O. ochengi* kills *O. ochengi* adult worms as a result of the depletion of *Wolbachia*, implying that *Wolbachia* contributes to worm survival (Gilbert *et al*., 2005). Unfortunately, drug resistance can occur after prolonged mass treatment with antibiotics as gram-negative bacteria (*Wolbachia* inclusive) are becoming resistant to nearly all the antibiotic drugs (Ventola, 2015). This necessitates the need to investigate new potential drug targets for bovine onchocerciasis in order to develop new macrofilaricidal strategies.

2.4 Drugs for treatment of bovine onchocerciasis related to *o. ochengi* infections

Previous reports have shown some drugs *O.ochengi* is susceptible to, which include the following: sumarin**,** ivermectin (McCall *et al.,* 1992), tetracyclines (Langworthy *et al.,*2000), melarsomine (Tchakoute *et al.,* 2006) and UMF-078 (a modified flubendazole) (Bronsvoort *et al*., 2008).

a. **Suramin**: Suramin is a complex cabamide and is regarded as the premier nonmetallic drug used in chemotherapy (Olenick, 1975). The mechanism of action for this drug is unknown, although its action in the treatment of onchocerciasis is macrofilaricidal and partially microfilaricidal (Wishart *et al.,* 2006). The adverse reactions associated with this drug include: kidney damage, exfoliating dermatitis and adrenal cortex damage. Suramin is also plays a role in hepatic and bone marrow toxicity and death (Babalola, 2011). These adverse effects are limitations to the use of sumarin for treatment of onchocerciasis.

b. **Ivermectin** (brand name Mectizan®): Ivermectin is used to treat onchocerciasis. Ivermectin is safe and can be used on a wide scale, unlike previous treatments, which had serious and sometimes fatal side effects. Ivermectin was introduced in 1987 (W.H.O, 2018). Ivermectin binds to glutamate-gated chloride ion channels in invertebrate muscle and nerve cells of the microfilaria. This binding brings about an increase in the penetrability of the cell membrane to chloride ions and results in hyperpolarization of the cell, leading to paralysis and death of the parasite (Wishart *et al.,* 2006). This drug is solely microfilaricidal (Mbah *et al*., 2016) and previous report has shown that cattle protected from onchocerciasis with ivermectin are highly vulnerable to infection, after drug withdrawal (Njongmeta *et al*., 2004). Thus, novel drugs need to be designed due to these limitations.

c. **Tetracyclines** : Tetracyclines are antibiotics which target *Wolbachia,* and has serious effects on the development, viability and fertility of filarial worms (Bouchery *et al.,* 2013). Tetracycline interferes with protein synthesis by passively diffusing through porin channels in the bacterial membrane and reversibly binding to the 30s ribosomal subunit, preventing binding of tRNA to the mRNA-ribosome complex (Wishart *et al.,* 2006). Unfortunately, gram-negative bacteria (*Wolbachia* inclusive) are becoming resistant to nearly all the antibiotic drug options available (Ventola, 2015), implying that novel strategies should be employed in combating with filarial nematodes.

d. **UMF-078 (a modified flubendazole)**: Flubendazole is an anthelmintic that is used to treat worm infection in humans (Wishart *et al.,* 2006). UMF-078 (a modified

flubendazole) has been reported to have potent macrofilaricidal activity against *Onchocerca ochengi* in African cattle. However, the specific target for the drug was not investigated. Also, neurological side effects in the mammalian host was observed after repeated dosing with UMF-078, suggesting that it may exhibit an adverse effect against the nervous system of cattle (Bronsvoort *et al*., 2008), thus, the need for novel drugs to be designed.

2.5 *In-silico* methods for identification of drug targets

Animals are used for drug screening in scientific research to find suitable drugs to treat diseases that affect human beings. Using animals for drug development in laboratories involves cost and is time consuming. Traditional drug discovery methods are time-consuming, expensive, yield few drug targets most of the times (Mondal *et al*., 2015) and the animals involved tend to suffer. In fact, animal right activists antagonize the use of animals. Bioinformatics plays a significant role in drug discovery, drug assessment and drug development. It provides a wide range of drug-related databases and softwares which can be used for various purposes related to drug design and development (Kumar and Chordia, 2017).

Vaccines and drugs can be designed based on genetic engineering principles through the employment of bioinformatics tools (Tambunan and Parikesit, 2012). However, bioinformatics does not completely rule out the use of animals in research because a better model of the complex interaction of the physiological processes in the body is provided by the intact animal (Arora *et al*., 2011).

In order to develop a drug for a particular disease, drug targets for that disease need to be known. Identification of drug targets is the first process in drug discovery (Chan *et al*., 2010). A drug target is a native protein in the body whose activity is modified by a drug, thereby resulting in a desirable therapeutic effect.

Targets of approved drugs are majorly proteins (Bull and Doig 2015). In recent times, computational techniques have been employed for the identification of potential drug and vaccine targets in different pathogenic microorganisms (Mondal *et al*., 2015). Subtractive and comparative genomics combined with metabolic pathway analysis is an effective approach to single out the proteins essential for the pathogen's survival but absent in the host (Rahman *et al.*, 2014).

2.5.1 Properties of drug targets and bioinformatics tools for identification of drug targets

For a protein to be a potential drug target, it should play a significant role in the metabolic pathway of the pathogen (Sharma and Kumar, 2016). Kyoto Encyclopedia of genes and genomes (KEGG) is a database for methodical analysis of gene functions linking genomic data with higher order functional data by computerizing current knowledge on cellular operations and by standardizing gene annotations. KEGG possesses three databases namely: GENES database, PATHWAY database and LIGAND database (Kanehisa and Goto, 2000). GENES database is a collection of gene catalogs for all the completely sequenced genomes and some partial genomes. PATHWAY database is a knowlegebase for representation of higher order functions in terms of the network of interacting molecules. LIGAND (Goto *et al*., 2000) database is a knowledgebase for the collection of chemical compounds in the cell, enzyme molecules and enzymatic reactions. The following tools are available in KEGG: Computational

tools for sequence comparison, graph comparison and path computation as well as JAVA graphics tools for browsing genome maps, comparing two genome maps and manipulating expression maps. The KEGG database is updated on diurnal bases and its website is freely assessible to all (Kanehisa and Goto, 2000).

Severe side effects can occur in the host species if a drug interferes with any requisite protein of the host. Also, the similarity in the coding region of a particular gene or functional domain of any protein of prokaryotic and eukaryotic organisms may result in cross-reactivity of a therapeutic agent against the host (Parvege *et al.,* 2014). Therefore, a protein sequence of a pathogen that is a potential drug target must be non homologous to the hosts protein sequences. This can be determined using BLAST (Basic Local Alignment Search Tool) and sequence similarity search. NCBI BLAST is a suitable BLAST search tool. NCBI BLAST shows areas of similarity between biological sequences. NCBI BLAST compares nucleotide or protein sequences to the catalog of sequences stored in NCBI databases and calculates the statistical significance.

A protein is a potential drug target if it is extremely important for the survival of the pathogen. DEG (Database of Essential Genes) contains essential genes of organisms. DEG contains genes that are requisite for support of cellular life. Individuals using DEG website can BLAST query sequences against DEG to find homologous sequences/ essential genes and these essential genes can be searched for by their function or name. Individuals can also browse and extract all the records in DEG.

The location of the protein in the cell determines whether it is a suitable drug target. Proteins located in the cytoplasm and inner membrane are regarded as potential drug targets (Sharma and Kumar, 2016). Various bioinformatics tools can be used to predict the subcellular

localization of proteins. Some of these tools include CELLO v.2.5 server (Prediction of subcellular localization of proteins of prokaryotes and eukaryotes), GnegPLoc (for determination of subcellular localization of proteins of gram negative bacteria), BaCelLo (for predicton of subcellular localization of proteins in eukaryotes) amongst others.

Finally, a potential drug target must be druggable. A protein that is druggable is one that has endogenous or extraneous folds which permit and support interactions with small drug-like molecules. It is one that contains a binding site (Bull and Doig 2015). The drug bank database and the TTD (Therapeutic Target Database) can be used to identify potential targets that are druggable by running a stand alone BLAST of query proteins against the drug targets in the drug bank and TTD. The Drug Bank database is a comprehensive bioinformatics and a cheminformatics resource which contains information on drugs and drug targets. The Drug Bank database is a freely accesible, online database and it combines detailed drug (i.e. chemical, pharmacological and pharmaceutical) data with comprehensive drug target (i.e. sequence, structure, and pathway) information. (Wishart *et al.*, 2017). TTD is a collection of the known and explored therapeutic protein and nucleic acid targets, the targeted disease, pathway information and the analogous drugs for each of these targets (Li *et al.*, 2018).

2.6 Molecular docking

Molecular docking is an in-silico tool which gives a prediction of ligand-receptor complex structure. Molecular docking is key in structural molecular biology and in-silico drug design (Meng *et al.*, 2011). This tool can demonstrate the possibility of any biochemical reaction because molecular docking is carried out before the experimental part of any investigation (Dar and Mir, 2017). The aim of carrying out ligand-protein docking is to

predict the key binding mode(s) of a ligand with a protein whose three-dimensional structure is already known (Morris and Lim-Wilby, 2008). Molecular docking can be used to model the interaction between a small molecule and a protein at the atomic level, which aids in depicting the behavior of small molecules in the binding site of target proteins as well as to expound on essential biochemical processes (McConkey *et al.,* 2002). The docking process involves two fundamental steps: prediction of the ligand configuration, position and orientation within these sites, which is regarded as *pose,* and the binding affinity assessment (Meng *et al.,* 2011).

Tools employed in molecular docking use search algorithms such as genetic algorithm, fragment-based algorithms, Monte Carlo algorithms and molecular dynamics algorithms. Asides from this, there are some tools such as DOCK, GOLD, FlexX and ICM which are mainly used for high throughput docking simulations (Dar and Mir, 2017).

Additionally, there are varieties of molecular docking approaches involving either ligand/target being flexible or rigid based upon the objectives of docking simulations. These include:

Flexible ligand docking: This is a situation where the target is the rigid molecule (Dar and Mir, 2017). For complexes whose actions are that of the induced fit pattern (Hammes, 2002), putting to mind the flexibilities of both the ligand and receptor is key since both the ligand and receptor modify their conformations to form a minimum energy perfect-fit complex. Nonetheless, the presence of a flexible receptor signifies higher cost implications. Hence, the common approach is handling the ligand as flexible whereas, the receptor is treated as rigid during docking. AutoDock (Morris *et al.*,1998) and FlexX (Rarey *et al.*,1996) and other docking programs have adopted this methodology. (Meng *et al.,* 2011).

Rigid body docking: In this case, both the target and ligand are rigid molecules. . This method keeps the ligand and receptor rigid during the process of the docking and was used in the former versions of DOCK (Kuntz *et al.*,1982; Ewing *et al.,* 2001), FLOG (Miller *et al.,* 1994) and some protein-protein docking programs, such as FTDOCK (Gabb *et al.*,1997).

Flexible docking: In this case, both interacting molecules are flexible. Implementing the receptor flexibility is a problem in the field of docking. However, various methods are available to overcome this challenge. The simplest one known as soft-docking (Totrov and Abagyan, 2001), decreases the van der Waals repulsion energy term in the scoring function to permit a degree of atom-atom overlap between the receptor and ligand (Meng *et al.,* 2011).

2.6.1 Softwares used for molecular docking processes

PyRx: PyRx is a graphical user interface for AutoDock 4.2 and AutoDock Vina to perform virtual screening. It reveals binding affinities and RMSD scores for each ligand and presents nine different poses. (Zaveri *et al.,* 2015). It is written in Python programming language and it can be operated on almost all modern computer (Dallakyan and Olson, 2015).

Discovery Studio: Discovery studio is a software suite for computational chemists and computational biologists. Discovery studio aids in examining the properties of large and small molecules (Sahu, 2013).

Chimera: UCSF Chimera is a program for the interactive visualization and analysis of molecular structures and related data, designed for use by structural biologists and biomedical researchers. Visualization capabilities of UCSF Chimera has been enhanced, its set of features has been increased to include more computationally intensive tasks due to the improvement in

desktop performance and the use of web services (Huang *et al.*,2014).

Other docking tools and programs developed for both academic and commercial use include AutoDock (Österberg *et al*., 2002), DOCK (Venkatachalam *et al.*, 2003), MOE-Dock (Corbeil *et al.*, 2012), FlexX (Rarey *et al.*, 1996), Surflex (Jain, 2003), GOLD (Jones *et al.*, 1997) and many more.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Flow chart

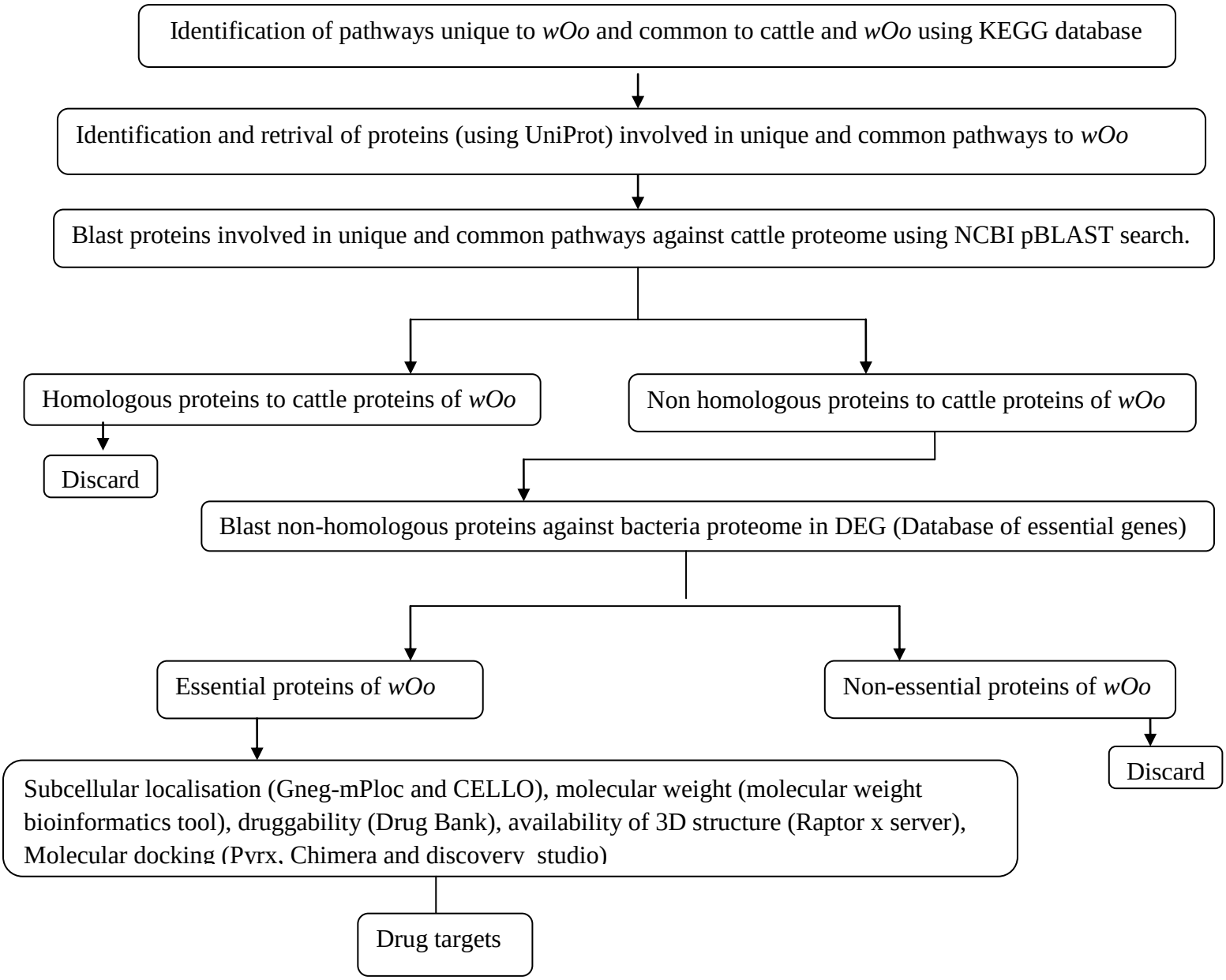

Fig. V: Steps involved in identification of drug targets in *wOo*. (Modified from Sharma and Kumar, 2016)

3.2 Materials

The materials used in this study were majorly computational tools comprising of databases, servers, software tools, internet access and a computer system.

The **databases** used in this study include: KEGG, Database of essential genes, DrugBank, NCBI and Uniprot database.

The **servers** used in the study include: Gneg-mPLoc server, CELLO server, Raptor X server.

The **software tools** used in this work include: Protein molecular weight bioinformatics tool, RAMPAGE, Modrefiner, PyRx, Chimera and Discovery studio.

3.3 Methods

The methodology used in this work is a modified version of the study done by Sharma and Kumar, 2016.

3.3.1 Identification of all metabolic pathways of *Wolbachia* of *Onchocerca ochengi (wOo)* and retrival of protein sequences associated with *wOo* pathways.

Metabolic pathways of *wOo* were identified using KEGG (Kyoto Encyclopedia of Genes and Genomes). Identification numbers of all the metabolic pathways of *wOo* were retrieved (rest.kegg.jp/link/woo/pathway; 2020, March 08; Kanehisa and Goto; 2000, Kanehisa *et al;* 2019, Kanehisa, 2019. These IDs were used to get the respective names of each pathway in *wOo* (Kanehisa and Goto; 2000, Kanehisa *et al;* 2019, Kanehisa, 2019). Also, KEGG identification number (KEGG ID) of all the proteins associated with each metabolic pathway of *wOo* were also retrived from the database (Kanehisa and Goto; 2000, Kanehisa *et al;* 2019, Kanehisa, 2019). Identification numbers of all the metabolic pathways of *Bos taurus* (cattle)

were also retrieved (rest.kegg.jp/link/bta/pathway; 2020, March 08; Kanehisa and Goto; 2000, Kanehisa *et al;* 2019, Kanehisa, 2019). These *Bos Taurus* metabolic pathway IDs helped in the identification of the respective names of each pathway in *Bos taurus* (Kanehisa and Goto; 2000, Kanehisa *et al;* 2019, Kanehisa, 2019). The pathways in *Bos taurus* and *wOo* were compared. The pathways that are present in *wOo* but not in *Bos taurus* were regarded as unique pathways while those present in both were regarded as common pathways. Protein sequences associated with each metabolic pathway in *wOo* were retrieved from UniProt (www.uniprot.org, 2020, March 08) using the KEGG id of each protein. These proteins were saved and used for further analysis.

3.3.2` Identification of non homologous proteins to cattle (*Bos taurus*) proteins of *wOo*.

Protein sequences of *wOo* retrieved from UniProt were subjected to NCBI Basic Local Alignment Search Tool (BLAST) sequence similarity search (https://www.ncbi.nlm.nih.gov/,2020, March 07) against *Bos taurus* proteome database. This sequence similarity search was done to find sequences of *wOo* that are non homologous to cattle protein sequences because similarity in the coding region of a particular gene or functional domain of a protein may bring about cross-reactivity of a therapeutic agent against the host (Parvege *et al.*,2014). E- value < 0.0001 and a minimum bitscore >100 (Hassan *et al.*, 2014) were used as cutoffs to determine the homologous and non homologous protein sequences. Only the non-homologous sequences of all the proteins were used for further study, the homologous proteins were not considered.

3.3.3 Essentiality assessment of resultant non homologous proteins to cattle *wOo* proteins

After the NCBI blast search, the resultant non homologous proteins to cattle proteins were assessed for essentiality in the Database of Essential genes (DEG). Essential proteins of *wOo* are proteins that are considered indispensible for its survival. DEG is a collection of information on the essential genomic elements present in bacteria, archaea and eukaryotes. The non-homologous protein sequences were subjected to protein BLAST tool and similarity search against the essential protein sequences of bacteria from DEG using E-value < 0.0001 and a minimum bit score > 100 as cutoffs (Hassan *et al.*, 2014). Protein sequences similar to the bacterial protein sequences in DEG were regarded as essential proteins.

3.3.4 Prioritization of essential non homologous proteins of *wOo*

Aside from the parameters listed above, other paramaters also play important roles in determination of suitable drug targets. Such parameters include determination of subcellular localization of proteins, prediction of number of transmembrane helices of proteins and determination of molecular weight of potential drug targets.

Determination of subcellular localization of proteins was done using the Gneg-mPloc (Shen and Chou, 2010) and CELLO v.2.5 server (Yu *et al.*, 2004, Yu *et al.*, 2006). Gneg-mPLoc is a tool for identification of subcellular location of gram negative bacteria proteins. It identifies these proteins in the following locations: cytoplasm, extracellular, fimbrium, flagellum, inner membrane, nucleoid, outer membrane and periplasm (Shen and Chou, 2010).

CELLO v.2.5 server makes use of multi-class support vector machine classification system to predict the subcellular localization of the proteins of gram negative bacteria, gram positive bacteria and eurkaryotes. Proteins located at the cytoplasm and inner membrane were regarded as potential drug targets (Sharma and Kumar, 2016). Extracellular proteins and outer membrane proteins were also considered as drug targets (Bakheet and Doig, 2009). All bacterial proteins are synthesized in the cytoplasm, and most remain there to carry out their peculiar functions. Other proteins, however, contain export signals that direct them to other locations in the cell. In Gram-positive bacteria, these include the cytoplasmic membrane, cell wall and extracellular space. In Gram-negative bacteria, they include the cytoplasmic membrane, the periplasm, the outer membrane and the extracellular space. (Butt *et al.*, 2012). These explains why proteins in the cytoplasm, cytoplasmic membrane, the periplasm, outer membrane and extacellular space should be used as drug targets in *Wolbachia*. Also, membrane proteins are prime drug targets because they perform essential processes in the cell including controlling the flow of information and materials between cells and mediating activities like hormone action and nerve impulses (https://www.elsevier.com/books/membrane-proteins-as-drug-targets/lunn/978-0-12-381288-9 2020, March 13). This also explains why inner membrane and outer membrane proteins are to be chosen as suitable drug targets in *insilico* studies.

Molecular weight (MW) of each of the potential drug targets was determined using protein molecular weight bioinformatics tool (https://www.bioinformatics.org/sms/prot_mw.html ,2020 March 08). Previous literature suggests that smaller proteins are suitable targets for drug development because they are more

soluble and easier to purify in comparison with large proteins (Duffield *et al*., 2010). Protein solubility is a vital parameter in protein purification. (https://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Sigma-Aldrich/General_Information/1/ge-strategies-for-protein-purification.pdf 2020, march 14). Protein purification is essential for the characterization of the function, structure and interactions of the protein of interest. (https://en.wikipedia.org/wiki/Protein_purification#Free-flow-electrophoresis 2020, march 13). One of the most widely applied methods to characterize proteins that bind specifically to candidate compounds is based on affinity chromatography combined with massspectrometry-based quantitative proteomics. Small molecule-based affinity chromatography was first used for the purification of protein targets as early as the 1960s. In affinity chromatography, a small bioactive molecule is immobilized onto a solid phase support and then incubated with a protein extract. After incubation, the affinity resin is washed extensively with an aqueous buffer to elute any non-binding proteins from the resin. Bound proteins are then eluted from the affinity matrix under denaturing conditions or by incubation with the free ligand and resolved by SDS-PAGE. Finally,the proteins are identified by mass spectrometric analysis (Hu *et al.*, 2012).

ın SDS-Polyacrylamide Gel Electrophoresis- a method of separating molecules based on the difference of their molecular weight-,when a mixture of proteins is applied to a gel and electric current is applied, smaller proteins migrate faster than larger proteins through the gel. The rate of movement is influenced by the gel's pore size and the strength of the electric field. The pores in a highly cross-linked polyacrylamide gel are quite small. Such a gel could resolve small proteins and peptides, but large proteins would not be able to move through it. (Lodish *et al.*, 2000).

These explanations above show how important it is to use proteins with smaller molecular weights as drug targets. In this study, proteins which had MW larger than 110 kDa were excluded and not considered as drug targets..

3.3.5 Druggability analysis of essential non homologous proteins of *wOo*.

The druggability of each drug target was analysed. The druggability of a protein is its capability to be modulated by drug-like molecules (Liu and Altman, 2014). Drug targets of FDA approved drugs were retrieved from DrugBank (Wishart *et al.*,2006) for druggability analysis. DrugBank is a colossal collection of drugs with target information. Drug targets identified from this study underwent a similarity search with the drug targets downloaded from the DrugBank database using stand alone BLASTp tool. The targets which had hits with approved drug targets from drug bank were considered as druggable targets.

3.3.6 Tertiary structure identification

The presence of druggable proteins in Protein data bank (PDB) was detected by running a PSI-BLAST (Poition-Specific Iterative Basic Local Alignment Search Tool) search against *wOo* proteins present in PDB database. PDB serves as the single global repository for atomic-level, 3D structure data, making greater than 144,000 experimentally-determined structures of proteins, DNA, and RNA, and their complexes with metal ions, drugs, and other small molecules freely available without restrictions on use. The PDB is universally regarded as a core data resource essential for understanding the functional roles that macromolecules play in biology and medicine. (wwPDB consortium, 2019). Computationally solved 3D structures were also detected using Raptor X :http://raptorx.uchicago.edu/StructurePrediction/predict/, 2020, March 7 (Källberg *et al.*, 2012).

RaptorX is a protein structure prediction server, which predicts 3D structures for protein sequences that do not have close homologs in the PDB. Raptor X predicts the secondary and tertiary structures, contacts, solvent accessibility, disordered regions and binding sites of sequences submitted to the server. RaptorX also provides the following: P-

value for the relative global quality, GDT (global distance test) and uGDT (un-normalized GDT) for the absolute global quality, and modeling error at each residue; which are confidence scores to signify the quality of a predicted 3D model: http://raptorx.uchicago.edu/about/, 2020, March 7 (Källberg *et al.*, 2012).

3.3.7 Structure refinement

3D structures obtained from raptor x server were refined to reduce local structural distortion by subjecting the predicted 3D structure to ModRefiner (https://zhanglab.ccmb.med.umich.edu/ModRefiner/, 2020, March 7; Xu and Yang, 2011). Also, the accuracy of the refined 3D structures were analysed by RAMPAGE (http://mordred.bioc.cam.ac.uk/~rapper/rampage.php, 2020, March 7) which showed the Ramachandran plot for each predicted model. RAMPAGE produces a clear graphical output of the Ramachandran plot which shows the proportion of residues in favoured, allowed and outlier regions, which in turn indicates the stereochemical quality of the model. (Monie *et al*., 2016).

3.3.8 Molecular docking and calculation of dissociation constant (Kd)

Molecular docking of protein drug targets with drugs from drug bank was done. The drugs used for molecular docking were drugs that could bind to drug targets (in drug bank) which had significant sequence similarity with potential drug targets. The 3D structures of each of the drugs were downloaded from drug bank in pdb format. Docking studies were also done to compare the dockimg result of tetracycline (which was used as a standard) with other antibiotics that were also known to bind to 30S ribosomal subunit of bacterial cells but were

not used in anti-*Wolbachia* therapy. PyRx (Trott and Olson 2010) and Chimera (Pettersen *et al.*, 2004) were used to accomplish the molecular docking process of refined 3D structure of drug targets with drugs. The visualization and analysis of interaction between docked complexes were made using Discovery studio 2016 client (Dassault Systèmes BIOVIA).

The binding energies gotten from this study were used to calculate the dissociation constants of each drug-protein binding, using the formular:

Kd= Ki= Exp (ΔG/RT) (http://mgldev.scripps.edu/pipermail/autodock/2009-April/005619.html ; 2020, March 19)

Where Kd= Dissociation constant

Ki= Inibition constant

ΔG= Gibbs free energy

R= Gas constant= 1.986cal/mol-k

T= Temperature of the reaction in Kelvin= 298k

(https://www.chemguide.co.uk/physical/entropy/deltagandk.html, 2020, March 19)

CHAPTER FOUR

4.0 RESULTS

4.1 Identification of non homologous proteins to cattle (*bos taurus*) proteins of *wOo*

Presently, there are 70 metabolic pathways of *wolbachia* of *Onchocerca ochengi* in KEGG database. Identification numbers (IDs) of the metabolic pathways of *wOo* (rest.kegg.jp/link/woo/pathway ,2020 March 08; Kanehisa and Goto; 2000, Kanehisa *et al;* 2019, Kanehisa, 2019), the respective names of each pathway in *wOo* (Kanehisa and Goto;

2000, Kanehisa *et al;* 2019, Kanehisa, 2019) and KEGG IDs of all the proteins associated with each metabolic pathway of *wOo* (rest.kegg.jp/link/woo/pathway 2020, March 08; Kanehisa and Goto; 2000, Kanehisa *et al;* 2019, Kanehisa, 2019) are reported in this study (Appendix I). Also, there are 319 metabolic pathways of *Bos taurus* in KEGG database. Identification numbers of all the metabolic pathways of *Bos taurus* (cattle) (rest.kegg.jp/link/bta/pathway; 2020, March 08; Kanehisa and Goto; 2000, Kanehisa *et al;* 2019, Kanehisa, 2019) and the respective names of each pathway in *Bos taurus* (Kanehisa and Goto; 2000, Kanehisa *et al;* 2019, Kanehisa, 2019) are reported in this study (Appendix II).

The comparison between the biological pathways of *Bos taurus* and *wOo* revealed 13 pathways unique to *wOo* and 57 pathways common to both *Bos taurus* and *wOo*. List of common and unique pathways are documented in appendix iii.

The retrieved protein sequences associated with each *wOo* metabolic pathway (www.uniprot.org; 2020, March 08) were all in FASTA format. Additionally, some proteins were involved in more than one pathway (Appendix I). This could be because some bacterial proteins synthesized in the cytoplasm, contain export signals that direct them to other cellular locations. In Gram-negative bacteria, such proteins could be exported to the cytoplasmic membrane, the periplasm, the outer membrane and the extracellular space. In most cases the whole protein is located in a single compartment; however, proteins can also span multiple localization sites (Butt *et al.*, 2012). Again, the patterns displayed by proteins inside bacterial cells can be complex. Some proteins are known to change location over time, such as during the course of cell cycle (Laloux and Jacobs-Wagner, 2014).

About 349 proteins were associated with the metabolic pathways of *wOo* (Appendix IV). NCBI protein BLAST search of the 349 protein sequences of *wOo* against *Bos taurus* (cattle) proteome, revealed the following results: 204 protein sequences which were non homologous to cattle proteins and 145 protein sequences which were homologous to cattle proteins. The 145 homologous proteins were excluded while the 204 non-homologous proteins were used for further study (Appendix V).

4.2 Identification of essential proteins of *wOo* using DEG database.

The potential of the identified non homologous proteins to be a therapeutic target of a given pathogen gene product is dependent on the role of the gene in the growth and survival of the pathogen (Damte *et al.,* 2013). After the NCBI blast search, the BLASTp search of the 204 non homologous proteins to cattle proteins against the proteins of bacteria in DEG database revealed the following results: 180 protein sequences which were homologous to proteins of bacteria in DEG database and 24 protein sequences which were non homologous to proteins of bacteria in DEG database. DEG is a collection of information on the essential genomic elements present in bacteria, archaea and eukaryotes. The 180 protein sequences were used for further study since they are essential to *wOo*.

4.3 Prioritization of essential proteins of *wOo*

After using Gneg-mPloc and CELLO bioinformatic tools for the determination of the subcellular localization of proteins, there were discrepancies in their results. However, results from CELLO server were chosen.

Out of the 180 proteins, CELLO server identified the following proteins: 24 proteins located in the outermembrane, 2 extracellular proteins, 148 proteins located in the cytoplasm, 33 proteins located in the inner membrane and 11 periplasmic proteins. Results also showed that 31 proteins had more than one subcellular localization. There were more proteins in the cytoplasm in this study because in reality, all bacterial proteins are synthesized in the cytoplasm and most remain there to carry out their peculiar functions (Butt *et al.*, 2012).

Results from CELLO server showed that all the 180 proteins were suitable drug targets because previous studies reported that proteins located in the cytoplasm (Sharma and Kumar, 2016), extracellular and membrane regions (Bakheet and Doig, 2009) could be considered as drug targets. Proteins in these locations are regarded as drug targets because bacterial proteins are synthesized in the cytoplasm and most of them remain in the cytoplasm, while other proteins are directed to other cellular locations with the help of export signals. *Wolbachia* is a Gram-negative bacteria. In Gram-negative bacteria, such proteins could be exported to the cytoplasmic membrane, the periplasm, the outer membrane and the extracellular space (Butt *et al.*, 2012).

Out of 180 protein sequences predicted as potential drug targets, molecular weight bioinformatics tool (https://www.bioinformatics.org/sms/prot_mw.html; 2020, March 08) showed 5 proteins to have molecular weights more than 110 KD (Parvege *et al.*, 2014), which were then excluded from the list of suitable drug targets.

4.4 Results of assessment of druggability of proteins.

The resultant drug targets identified after prior steps of the proteome subtractive technique were subjected to a local blast search against the downloaded drug targets from DrugBank to ascertain the druggability of the proteins. FDA approved drugs were downloaded from DrugBank for druggability analysis. An e-value of 0.005 was used as cut off mark. Out of 175 proteins, 58 proteins were identified as druggable targets (Appendix VII). These 58 proteins showed similarity with targets available in DrugBank database.

4.5 Tertiary structure identification and refinement.

After the blast search of the 58 druggable proteins against the protein databank database, results did not show any single hit with the protein data bank database. This indicated that none of the experimentally solved 3D structures of the 58 proteins were present in the protein data bank. All the 58 proteins had computationally solved 3D models of proteins from Raptor X protein structure prediction server. The 58 protein structures were identified by Raptor X server and downloaded in pdb format. Raptor X structures were refined after being subjected to ModRefiner.

4.6 Molecular docking of computationally solved 3D structures of drug targets with drugs from drug bank.

All the structures could not complete the molecular docking process. Out of the 58 drug targets only 32 were completed (Appendix X). Results of molecular docking showed binding affinity

ranging from -9.1 kcal/mol to -1.3 kcal/mol (Appendix X). Also different types of interactions were shown for the various molecular docking results. The docking results are documented in appendix viii. The presence of hydrogen bonds implies that the bond between protein ligand complexes is stable. Similarly, presence of hydrophobic amino acid residues within the binding site highly contributed to the stability during molecular interaction between the protein and small compounds. The resultant hydrophobic interaction between the docked complexes also contributed to strong binding affinity of different drugs towards the drug targets. Molecular docking of malic enzyme and NADH gave a binding energy of -9.1kcal/mol (Fig VI). Also, tetracycline, a known drug for anti-*Wolbachia* therapy, docked with 30S ribosomal protein S3, to give a binding energy of -7.4kcal/mol (Fig. VII).

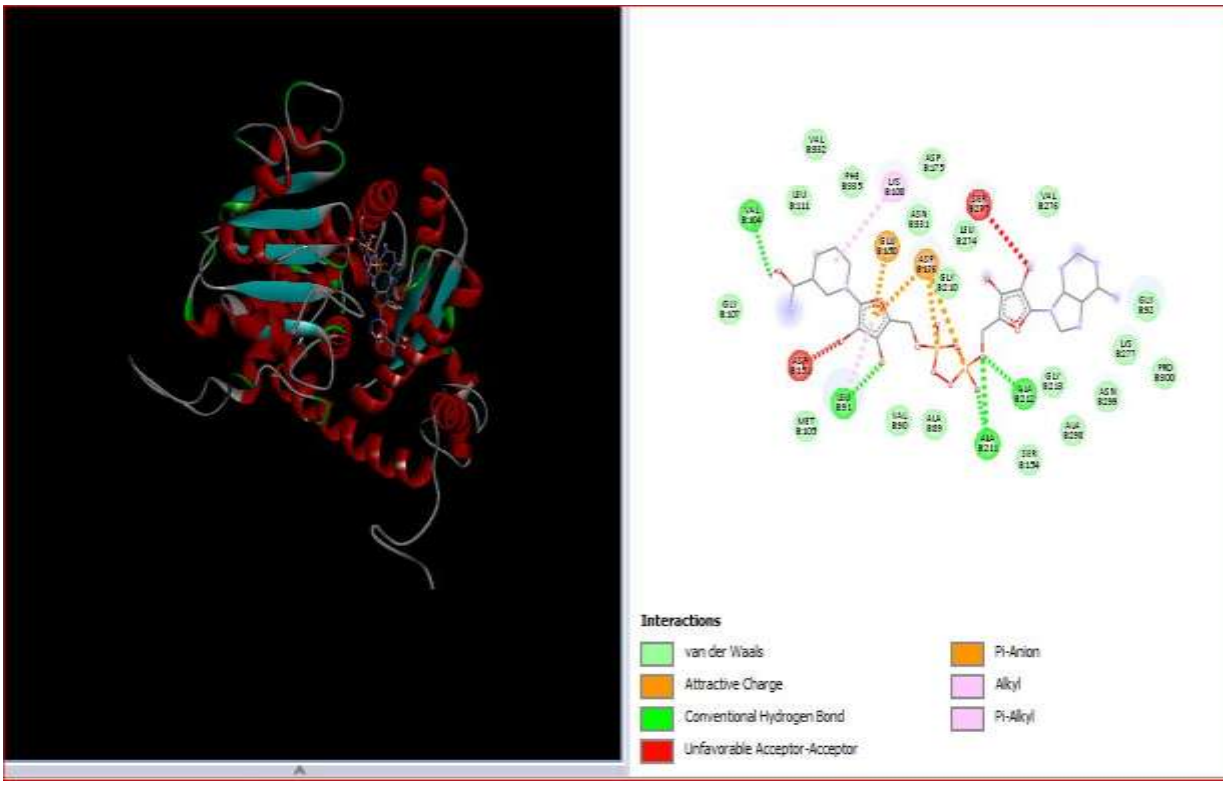

Fig. VI: Molecular docking result for malic enzyme and NADH (binding energy=

-9.1kcal/mol).

Fig. VI shows the same interaction, but in different formats. The figure by the left is the 3D structure of the result. The one by the right side is a 2D diagram of the result showing the interactions involved. The interactions involved include: Vander waals, attractive charge, conventional hydrogen bond, unfavourable acceptor acceptor, pi-anion (electrostatic), alkyl (hydrophobic) and pi-alkyl (hydrophobic) interactions. This interaction has a low binding energy and when compared with tetracycline-a known drug for anti-*Wolbachia* therapy- it has a better binding energy. However, NADH is a co-enzyme. This implies that it promotes the activity of malic enzyme and does not inhibit it. Therefore malic enzyme cannot be regarded as a good drug target.

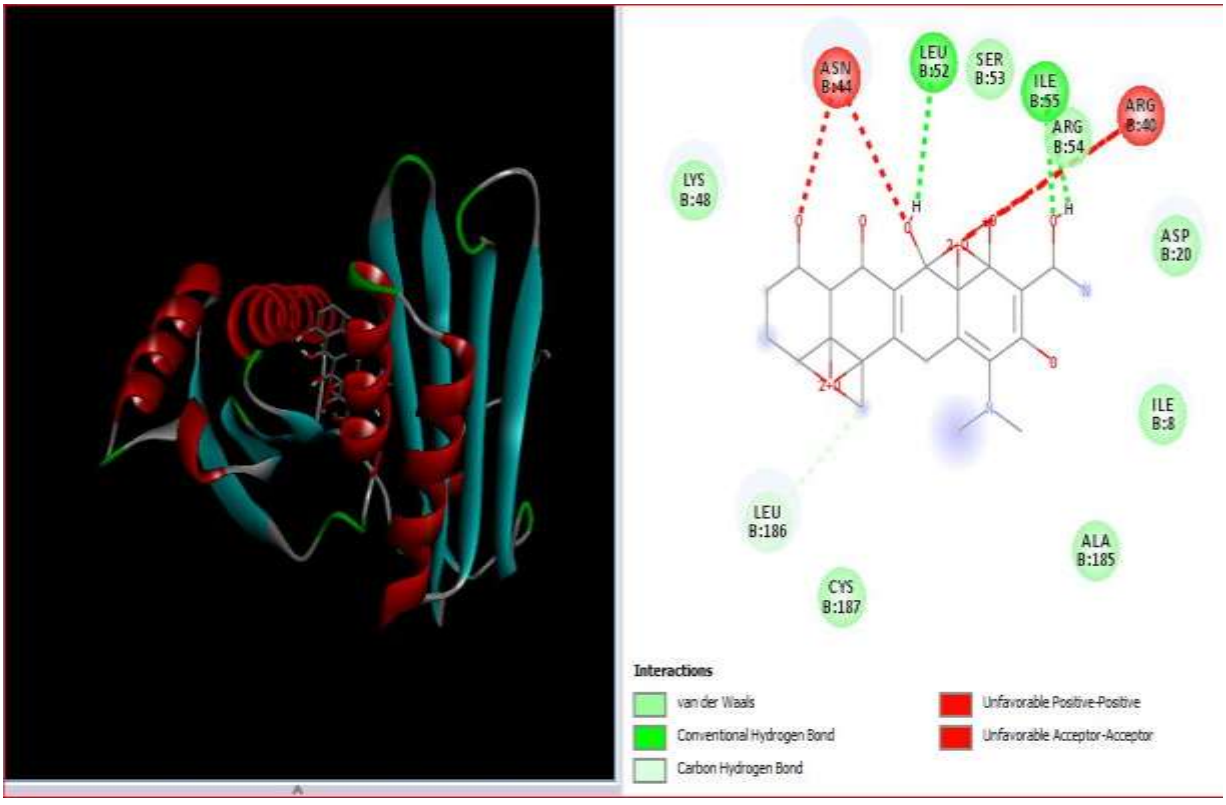

Fig. VII: Molecular docking result for 30S ribosomal protein S3 and Tetracycline (binding energy= -7.4kcal/mol)

Fig. VII shows the same interaction, but in different formats. The figure by the left is the 3D structure of the result. The one by the right side is a 2D diagram of the result showing the interactions involved. The interactions involved include: Vander waals, conventional hydrogen bond, carbon hydrogen bond, unfavourable positive-positive and unfavourable acceptor-acceptor interactions. This result authenticates previous reports about tetracycline being a known drug for anti-*Wolbachia* therapy.

Another molecular docking study was done to find out whether some antibiotics used in this study could be repurposed for the treatment of bovine onchocerciasis. Since *Wolbachia* bacteria are targets of antibiotic therapy with tetracyclines (Bouchery *et al.,* 2013), tetracycline was used as a standard. The dissociation constants (Kd) of tetracyclines interaction with four *wOo* proteins (30S ribosomal protein S4, 30S ribosomal protein S10 and 30S ribosomal protein S3, 30S ribosomal protein S8) and the binding energies of tetracycline with the same proteins were compared with other antibiotics to identify potential drugs for bovine onchocerciaisis. The antibiotics that were compared with tetracycline (Clomocycline and Paromomycin) were drugs which were also known to bind to 30S ribosomal subunit but have not been used in anti-*Wolbachia* therapy. These drugs were compared with tetracycline based on their binding energies and dissociation constants (Table 4.1).

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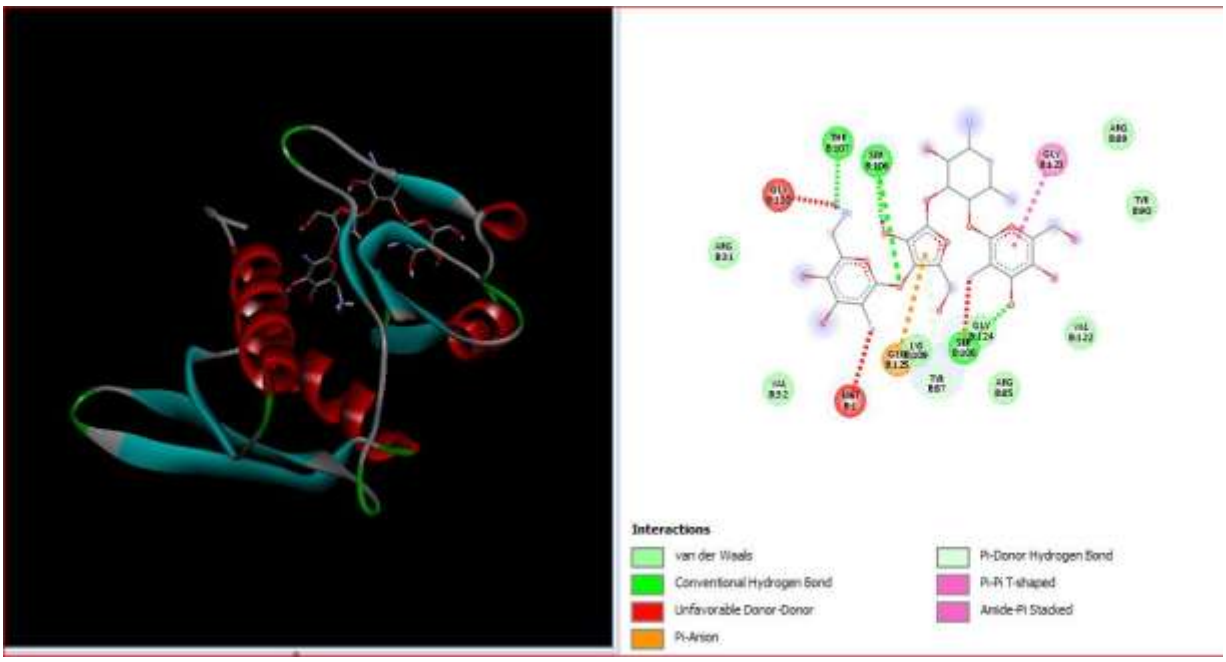

Fig. VIII: Molecular docking result for paromomycin and 30S ribosomal protein S8 (binding energy = -6.4kcal/mol; kd= 2.01 x 10^{-5}).

Fig. VIII shows the same interaction, but in different formats. The figure by the left is the 3D structure of the result. The one by the right side is a 2D diagram of the result showing the interactions involved. The interactions involved can be identified by their different colours as

shown in the figure. This result had the same binding energy and kd as the result for the standard (Table 4.1), which suggests that paromomycin could be used for drug repurposing in anti-*Wolbachia* therapy.

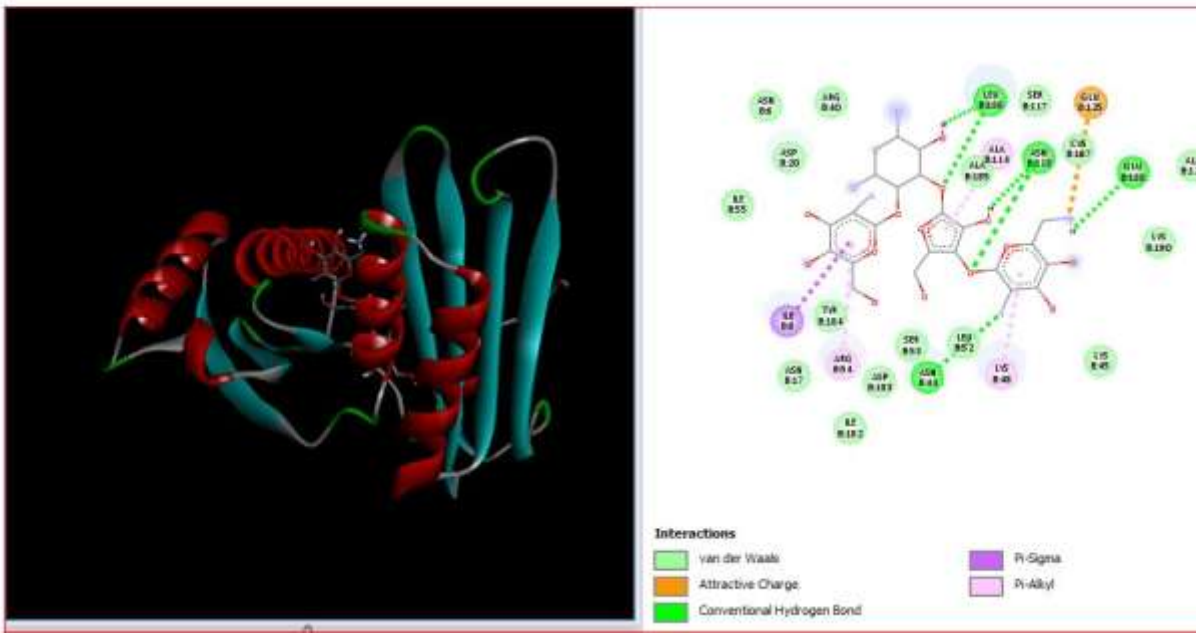

Fig. IX

Molecular docking result for Paromomycin and 30S ribosomal protein S3(binding energy = -8.2, kd= 9.61 x 10^{-7})

Fig. IX shows the same interaction, but in different formats. The figure by the left is the 3D structure of the result. The one by the right side is a 2D diagram of the result showing the interactions involved. The interactions involved can be identified by their different colours as shown in the figure. This result had the a lower binding energy and kd than the result for the standard (Table 4.1), which suggests that paromomycin could be used for drug repurposing in anti-*Wolbachia* therapy.

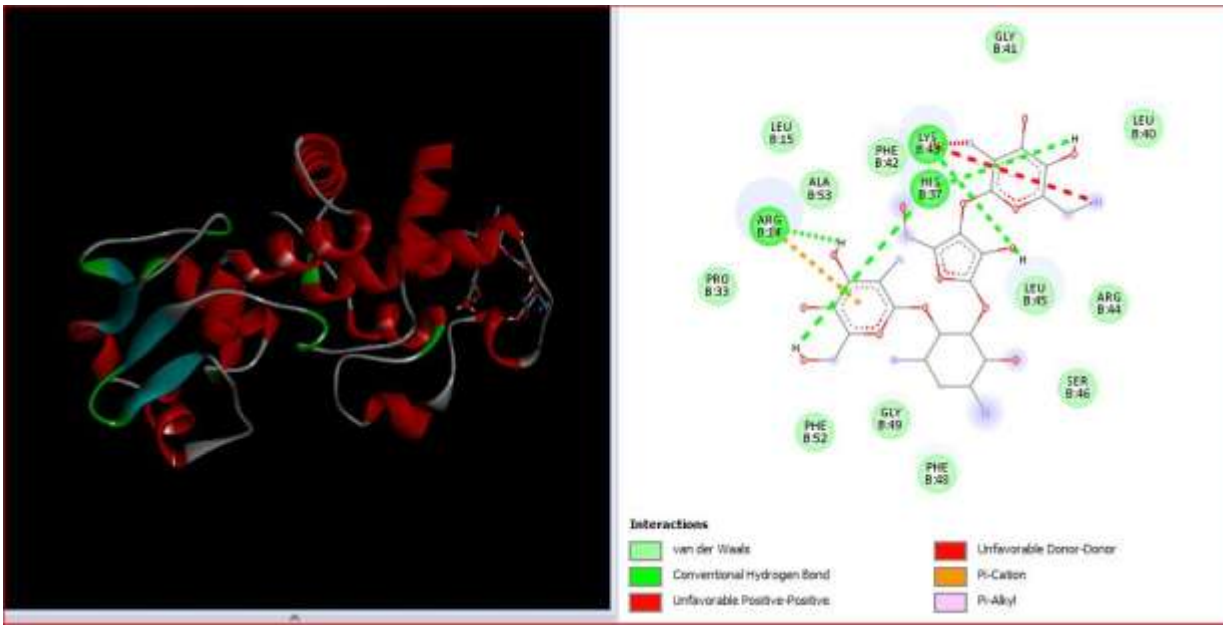

Fig. X: Molecular docking result for paromomycin and 30S ribosomal protein S4

Fig. X shows the same interaction, but in different formats. The figure by the left is the 3D structure of the result. The one by the right side is a 2D diagram of the result showing the interactions involved. The interactions involved can be identified by their different colours as shown in the figure. This result had a higher binding energy and kd than the result for the standard (Table 4.1), though its binding energy and kd were very close to that of the standard. This suggests that paromomycin could be used for drug repurposing in anti-*Wolbachia* therapy.

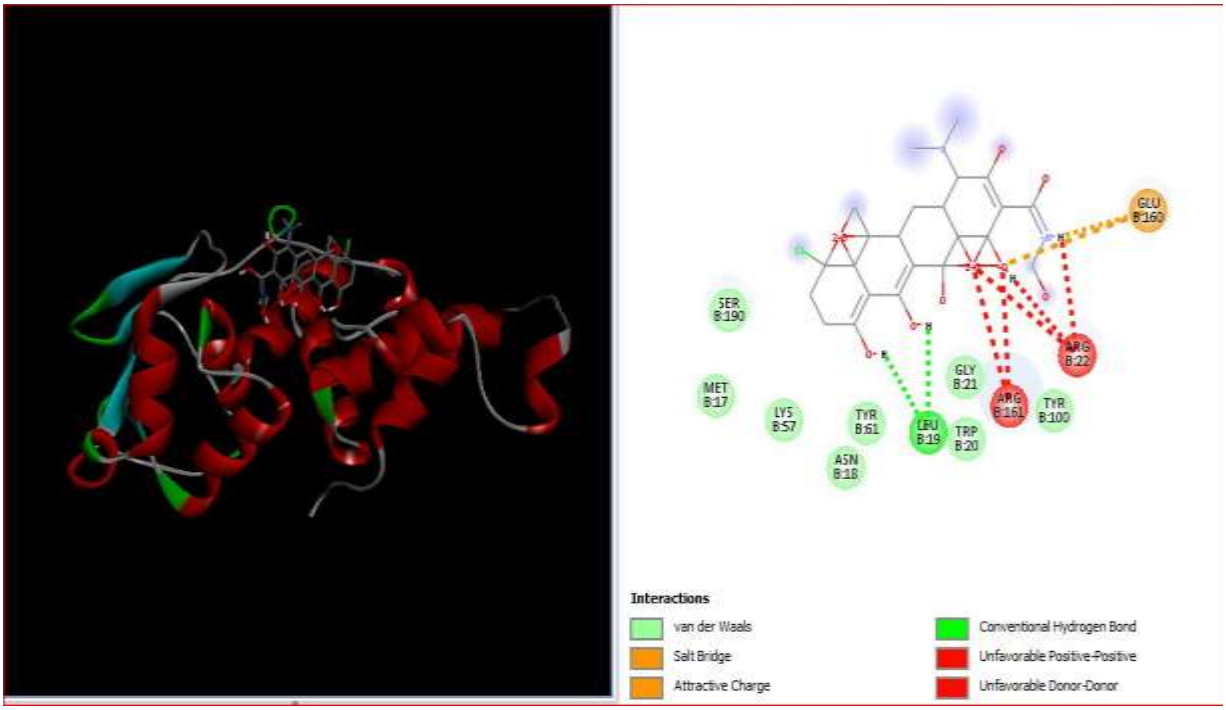

Fig. XI: Molecular docking result for 30S ribosomal protein S4 and clomocycline

Fig. XI shows the same interaction, but in different formats. The figure by the left is the 3D structure of the result. The one by the right side is a 2D diagram of the result showing the interactions involved. The interactions involved can be identified by their different colours as shown in the figure. This result had the same binding energy and kd as the result for the standard (Table 4.1). This suggests that clomocycline could be used for drug repurposing in anti-*Wolbachia* therapy.

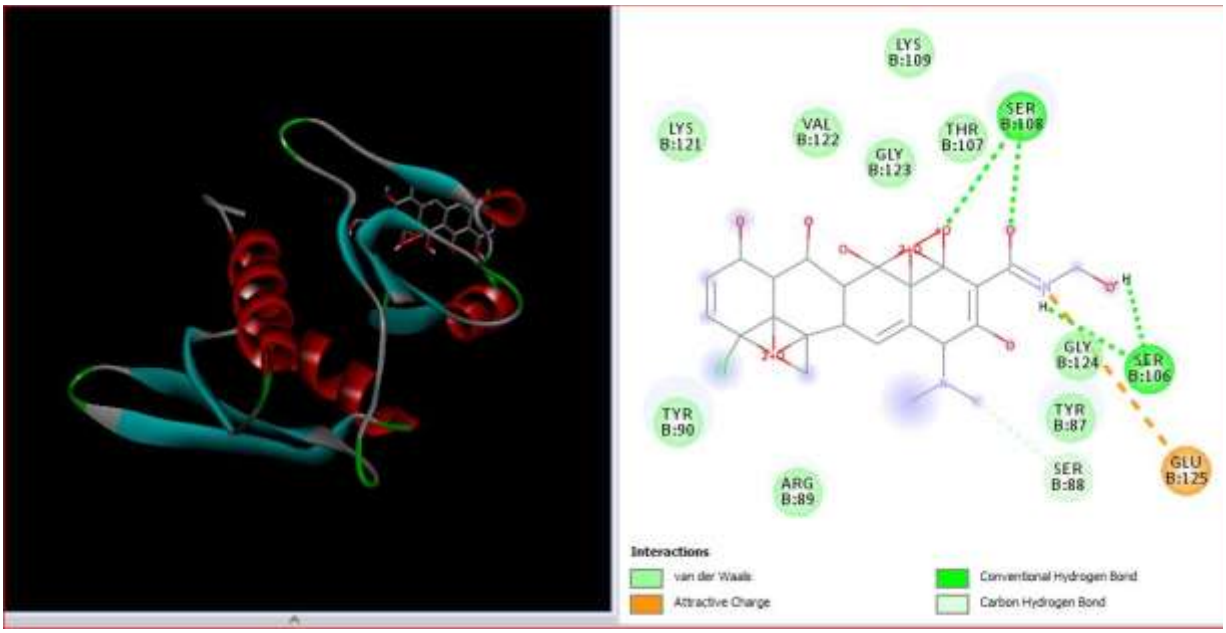

Fig. XII: Molecular docking result for Clomocycline and 30S ribosomal protein S8

Fig. XII shows the same interaction, but in different formats. The figure by the left is the 3D structure of the result. The one by the right side is a 2D diagram of the result showing the interactions involved. The interactions involved can be identified by their different colours as shown in the figure. This result had the same binding energy and kd as the result for the standard (Table 4.1). This suggests that clomocycline could be used for drug repurposing in anti-*Wolbachia* therapy.

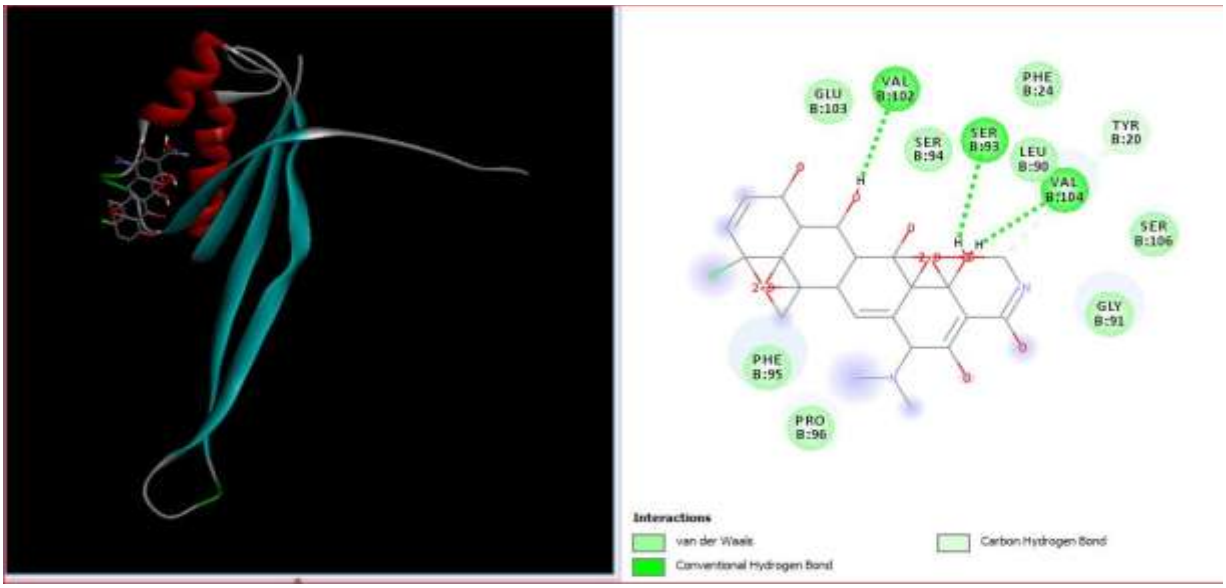

Fig. XIII: Molecular docking result for clomocycline and 30S ribosomal protein S10

Fig. XIII shows the same interaction, but in different formats. The figure by the left is the 3D structure of the result. The one by the right side is a 2D diagram of the result showing the interactions involved. The interactions involved can be identified by their different colours as shown in the figure. This result had a lower binding energy and kd than the result for the standard (Table 4.1). This suggests that clomocycline could be used for drug repurposing in anti-*Wolbachia* therapy.

Table 4.1: Comparism of molecular docking and inhibition constants of tetracycline and *wOo* proteins with other antibiotics and the same proteins that docked with tetracycline for drug repurposing.

S/N	Drug targets	Antibiotics	Binding energy (Kcal/mol)	Dissociation constant	Interactions involved
1	30S ribosomal protein S4	Tetracycline	-6.5	1.70×10^{-5}	Vander waals, conventional hydrogen bond, unfavourable positive-positive.
		Clomocycline	-6.5	1.70×10^{-5}	Vander waals, salt brigde, attractive charge, conventional hydrogen bond, unfavourable positive-positive,unfavourable donor-donor
		Paramomycin	-6.4	2.01×10^{-5}	Vander waals, conventional hydrogen bond, unfavourable positive-positive, unfavourable donor-donor, pi-cation, pi-alkyl
2	30S ribosomal protein S10	Tetracycline	-6.2	2.82×10^{-5}	Vander waals, conventional hydrogen bond, unfavourable donor-donor
		Clomocycline	-6.4	2.01×10^{-5}	Vander waals, conventional hydrogen bond, carbon hydrogen bond
		Paramomycin	-5.5	9.20×10^{-5}	Vander waals, conventional hydrogen bond, carbon hydrogen bond, unfavourable acceptor-acceptor, unfavourable donor-donor, pi-donor hydrogen bond, pi-sigma

Table 4.1 (continued): Comparism of molecular docking and inhibition constants of tetracycline and *wOo* proteins with other antibiotics and the same proteins that docked with tetracycline for drug repurposing.

S/N	Drug targets	Antibiotics	Binding energy (Kcal/mol)	Dissociation constant	Interactions involved
3	30S ribosomal protein S3	Tetracycline	-7.4	3.71×10^{-6}	Vander waals, conventional hydrogen bond, carbon hydrogen bond, unfavourable acceptor-acceptor, unfavourable positive-positive
		Clomocycline	-7.3	4.40×10^{-6}	Vander waals, attractive charge, conventional hydrogen bond, carbon hydrogen bond, unfavourable positive-positive
		Paramomycin	-8.2	9.61×10^{-7}	Vander waals, attractive charge, conventional hydrogen bond, pi-sigma, pi-alkyl
4	30S ribosomal protein S8	Tetracycline	-6.4	2.01×10^{-5}	Vander waals, conventional hydrogen bond, carbon hydrogen bond, unfavourable positive-positive
		Clomocycline	-6.4	2.01×10^{-5}	Vander waals, attractive charge, conventional hydrogen bond, carbon hydrogen bond
		Paramomycin	-6.4	2.01×10^{-5}	Vander waals, conventional hydrogen bond, unfavourable donor-donor, pi-anion, pi-donor hydrogen bond, pi-pi T-shaped, amide-pi-stacked

CHAPTER FIVE

5.0 DISCUSSION

In this study, an *in-silico* approach to identify potential drug targets of *wolbachia* of *Onchocerca ochengi* for bovine onchocerciasis was investigated. Due to the challenge of antibiotic resistance by gram negative bacteria (Ventola, 2015) and *Wolbachia* strains being reported to be resistant to rifampicin (Liu *et al.,* 2014), it was necessary to identify new drug targets for bovine onchocerciasis. Also, the availability of bioinformatics and computational tools has made drug target identification easier and cost effective.

Targeting central metabolism in bacterial pathogens represents a fascinating proposition in the development of new antibacterial drugs (Murima *et al.,* 2014). Metabolism involves chemical reactions that occur within an organism to sustain its life. It is advantageous to use metabolic pathway information for the identification of potential targets as the survival of the bacterium depends on each step in the pathway (Morya *et al.,* 2010). In this study, the metabolic pathways of *wOo* were used in the identification of drug targets for bovine onchocerciasis. 70) metabolic pathways of *wOo* were identified from *KEGG*. The comparison of metabolic pathways of *wOo* and *Bos taurus* (cattle) using KEGG showed only 13 unique pathways and 57 common pathways. A study reported by Sharma and Kumar (2016) showed 65 metabolic pathways in *Wolbachia* endosymbiont of *Brugia malayi* (*WBm*) from KEGG. The difference in pathways of the two *Wolbachia* endosymbionts authenticates the fact that that all *Wolbachia* endosymbionts of filarial nematodes are not of the same strain or specie.

Another important thing to consider in the identification of potential drug targets is whether the drug target is present or absent in the host. Absence of drug targets in the host reduce side effects of drug in the host. Also, the essentiality of proteins to the host, is a good factor to consider in the identification of good drug targets because the identification of proteins that are important for the survival and pathogenicity of a pathogen, is of great importance for disruption of pathogen functions (Chawley *et al.,* 2012). There are two kinds of enzymes/pathways -dispensible and indispensible or non-essential- known to be present in any living system (Morya *et al.,* 2010). Dispensible enzymes/proteins, as the name implies, are enzymes/proteins that the organism can do without while indispensible or essential proteins/enzymes are proteins/enzymes that are needed by the organism for its survival. Only indispensible proteins/enzymes are selected as drug targets. These two parameters- drug targets being non-homologous to host proteins and essentiality of drug targets- have been used in the identification of drug targets in *wBm* (Sharma and Kumar, 2016) and other bacteria (Morya *et al.*,2010, Parvege *et al.,* 2014) and was also used in this study. The choice of good drug targets includes the essentiality of the genes/proteins which is a significant element for the selection of suitable targets. However, this approach has its limitation, which is its incapacity to predict some of the potential drug targets (false negatives) and its inclusion of unsuccessful drug targets (false positives) (Doyle *et al.,* 2010). Not withstanding, this study revealed 204 *wOo* proteins that were non homologous to cattle proteins and 180 essential proteins of *wOo*.

Drug target prioritization processes such as prediction of subcellular localization of proteins, molecular weight identification and assessment of the druggability of proteins were further achieved by other computational tools. Result from this study revealed 58 druggable

targets which have homologs in DrugBank. Some of the metabolic pathways associated with the drug targets include: biosynthesis of amino acids, two component system, arginine biosynthesis, alanine, aspartate and glutamate metabolism, glycoxylate and dicarboxylate metabolism, nitrogen metabolism, amongst others (Appendix VIII).

The assembly of bacterial ribosomes is a possible antibacterial drug target (Maguire, 2009). Six out of the 32 potential drug targets identified in this study are associated with the ribosome. They are: 30S ribosomal protein S3, S4, S8, and S10 and 50S ribosomal protein L10 and L16 (Appendix X).

Also, DNA replication proteins such as replicative DNA helicase, DNA polymerase I, amongst others, have been reported to be potential targets for antimicrobials in drug-resistant bacterial pathogens (Eijk *et al.,* 2017). In this study, replicative DNA helicase, DNA polymerase I, DNA polymerase III subunit gamma/tau, which are involved in DNA replication were revealed to be potential drug targets of *wOo* for bovine onchocerciasis.

Additionally, Aminoacyl-tRNA synthetases (aaRSs) are already known drug targets for bacterial pathogens (Hurdle *et al.,* 2005, Kalidas, 2014). Valine--tRNA ligase was revealed as a potential drug target in this study.

Interestingly, 16 of the potential drug targets revealed in this study were also previously reported by Sharma and Kumar (2016) as potential drug targets for human lymphatic filariasis. These include: N5-carboxyaminoimidazole ribonucleotide synthase, D-alanine-D-alanine ligase, UDP-N-acetylglucosamine 1-carboxyvinyltransferase, D-alanyl-D-alanine carboxypeptidase,

Glutamine synthetase, 3-oxoacyl-(acyl-carrier-protein) synthase 3, N5-carboxyaminoimidazole ribonucleotide mutase, Thioredoxin reductase, 30S ribosomal protein S10, 30S ribosomal protein S8, 30S ribosomal protein S3, DNA polymerase III subunit gamma/tau, replicative DNA helicase, NADH-quinone oxidoreductase subunit K, 50S ribosomal protein L10 and **UDP-N-acetylenolpyruvoylglucosamine reductase.** However, these proteins are not all involed in the same pathways in both humans and cattle. This similar drug targets for both humans and cattle diseases showed that results from cattle-based filariasis studies have important implications for the control of human filariasis (Renz *et al.*, 1995; *Njongmeta et al., 2004;* Bronsvoort *et al.*, 2005). Therefore, this study suggests significant implications for the the development of drugs for human onchocerciaisis.

A number of other drug targets reported by previous studies were also identified in this study. These include: ribonucleoside-diphosphate reductase (Tholander and Sjoberg, 2012), flavin-dependent thymidylate synthase (FDTS) (Choi *et al.*, 2017) and adenylosuccinate lyase (Banejee *et al*., 2014).

This study revealed 23 previously reported drug targets and 9 novel/new drug targets. The identification of previously known targets authenticates the protocol used and shows the robustness of the approach used in this study for the identification of drug targets for bovine onchocerciasis. The 9 novel drug targets revealed in this study are: NADH-quinone oxidoreductase subunit H, ferredoxin--NADP reductase, fumarate hydratase class II (Fumarase C), ribonucleoside-diphosphate reductase subunit beta, flavin prenyltransferase UbiX, GMP synthase, geranylgeranyl pyrophosphate synthase, malic enzyme and ABC-type Fe3+ transport system periplasmic component.

Finally, molecular docking studies of the 58 druggable proteins with 36 drugs from DrugBank database was performed to further substantiate the findings from this study. Docking helps in prediction of binding orientation of small molecule drug candidates with their protein targets in order to predict the affinity and activity of the drugs. The drugs used for molecular docking were drugs retrieved from DrugBank. Only 32 out of the 58 drug targets completed the molecular docking process.

The conformation of both the ligand as well as ligand preparation is important in molecular docking. Ligand preparation has prominent effect on the docking results because the ligand recognition by any biomolecule depends on 3-dimensional orientation and electrostatic interaction (Dar and Mir, 2017). Therefore, the challenge of 26 proteins not completing the molecular docking process could be due to the fact that the ligands were not adequately prepared/designed or due to the inability of the biomolecule to recognise the ligand because of the orientation of the 3 dimensional structure of the ligand, which in turn affected the molecular docking result. However, regardless of the fact that these protein drug targets did not dock with ligands (drugs), they could still be considered as potential drug targets because drugs can be designed and tested against these targets.

In molecular docking, lower energy scores depict better protein-ligand bindings compared to higher energy scores (Thomsen and Christensen, 2006). In order words, the least binding energy is usually preferred for good binding mode while docking small compounds with target protein (Jagadeb *et al.,* 2014; Kumar *et al.,* 2014). Results from this study showed binding energies between -9. 1kcal/mol and -1.3 kcal/mol (Appendix X). Malic enzyme (-9.1kcal/mol)

showed the lowest binding energy and the most favourable protein-ligand binding when docked with NADH (Fig. VII).

Also, the Kd is a useful way to present the affinity of a drug for its biological target. This is because the number Kd shows the concentration of drug that is required to yield a significant amount of interaction with the target protein. (https://faculty.missouri.edu/~gatesk/15_DrugEquilibEnergetics; 2020, March 18). The lower the *Kd* value, the stronger the binding and the higher the affinity. The opposite occurs when a drug has a high *Kd* (Salahudeen and Nishtala, 2017). The results from this study showed that the dissociation constant of each interaction was directly proportional to their binding energies (Appendix X and Table 4.1).

The drugs which completed the molecular docking process with *wOo* proteins were divided into 8 groups namely: co-enzymes, organic acids, antibiotics, enzyme activators, inorganic acids, aminoacids, trace element (zinc) and purine related drugs. Based on binding energy, co-enzymes and an antibiotic (tetracycline) had strong interactions with *wOo* proteins. Organic acids, antibiotics, enzyme activators, inorganic acids, aminoacids, purine related drugs had moderate interactions with *wOo* proteins. One trace element (Zinc) and one organic acid (Formic acid) had weak interactions with *wOo* proteins.

The drugs which docked with the drug targets can be considered as lead compounds for the treatment of bovine onchocerciasis except for drugs in the co-enzymes group such as NADH, flavin adenine dinucleotide and flavine adenine mononucleotide. Generally, co-enzymes help

proteins to function. This means that these co-enzymes facilitate the activity of *wOo* proteins. Since these compounds are not inhibitors but facilitators of the activity of *wOo* proteins, they cannot be regarded as good drugs for bovine onchocerciasis.

Apart from the co-enzymes, which showed strong interactions with *wOo* proteins, drugs in the antibiotics group such as tetracycline, linomycin, clomocycline, amongst others (see Appendix X) were also revealed to interact with a number of *wOo* proteins in this study. Molecular docking of tetracycline and 30S ribosomal protein S8, resulted in a binding energy of -6.4 kcal/mol. Tetracycline has already been reported to bind to the bacterial 30s ribosomal subunit, thereby stopping the binding of aminoacyl tRNA to the ribosome acceptor site (Wishart *et al.,*2006). Also, linomycin bound to 50S ribosomal protein L10 during the molecular docking process with a binding affinity of -5.6kcal/mol. Linomycin has also been previously reported to inhibit protein synthesis in bacteria by binding to the 50S subunit of bacterial ribosomes and preventing peptide bond formation upon transcription (Wishart *et al.,* 2006). Also, Clomocycline docked with 30S ribosomal protein S4, with a binding affinity of -6.5kcal/mol. It has been previously reported that Clomocycline binds to the 30S ribosomal subunit and prevents the amino-acyl tRNA from binding to the A site of the ribosome (Wishart *et al.,* 2006).

Therefore, these further re-emphasize the robustness of the approach used in this study for the identification of drug targets for bovine onchocerciasis, as the results from this study agree with previous findings. An antifungal antibiotic (cerulenin) was also shown to have significant interaction with *wOo* proteins. The antibiotic which showed the strongest interaction with a *wOo* protein- 30S ribosomal protein S3- is tetracycline (-7.4kcal/mol) (Fig VIII).

Similarly, besides the docking results of co-enzymes and antibiotics with *wOo* proteins, molecular docking of cladribine (a purine related drug) and ribonucleoside diphosphate reductase subunit beta (a novel drug target), gave the least binding energy (Appendix X).

From the second molecular docking study, results showed that Paromomycin had stronger interactions with 30S ribosomal protein S3 of *wOo* than tetracycline's interaction with 30S ribosomal protein S3 of *wOo* (Table 4.1). Again, Paromomycin's interaction with 30S ribosomal protein S8 of *wOo* gave the same binding energy and dissociation constant when tetracycline interacted with the same protein (Table 4.1). Also, the binding energy and dissociation constant of paromomycin's interaction with 30S ribosomal protein S4 of *wOo* and tetracycline's interaction with the same 30S ribosomal protein S4 were very close (Table 4.1). This suggests that paromomycin may be used for drug repurposing in anti-*Wolbachia* therapy for bovine onchocerciasis. Paromomycin is known to be used in the treatment of acute and chronic intestinal amebiasis. It is also used with other drugs to help lessen the symptoms of hepatic coma. Paromomycin belongs to the class of drugs known as aminoglycoside antibiotics. It works by killing bacteria or preventing their growth (https://www.mayoclinic.org/drugs-supplements/paromomycin-oral-route/description/drg-20074521, 2020, March 19). Results from this study have shown that paromomycin could also be used in the treatment of bovine onchocerciasis via anti- *Wolbachia* therapy.

Additionally, clomocycline, in the second molecular docking study, showed reasonable interactions with proteins of *wOo* in comparism to the interactions of tetracycline with the same proteins (Table 4.1). Clomocycline showed the same binding energy and kd as the standard when it interacted with 30S ribosomal protein S4 (Table 4.1). Again, clomocycline showed the same

binding energy and kd as the standard when it interacted with 30S ribosomal protein S8 (Table 4.1). Clomocycline is an antibiotic that is commonly prescribed by medical doctors for infections and to treat acne. It may also be used to treat urinary tract infections, gum disease, and other bacterial infections such as gonorrhea and chlamydia. Clomocycline is also used commonly as a prophylactic treatment for infection by Bacillus anthracis (anthrax). It is also effective against Yersinia pestis and malaria and is also prescribed for the treatment of Lyme disease (Wishart *et al.*, 2006). Also, based on the binding energy and kd, clomocycline had a better interaction with 30S ribosomal protein S10 than tetracycline's interaction with the protein. This suggests clomocycline as a better drug for bovine onchocerciasis via anti-*Wolbachia* therapy.

Based on the binding energies and dissociation constants of clomocycline's and paromomycin's interaction with *wOo* proteins when compared with the standard (tetracycline) in this study (Table 4.1), it could be inferred that clomocycline and paromomycin could be used for drug repurposing in combating bovine onchocerciasis through anti-*Wolbachia* therapy.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 Summary

This study had identified 70 metabolic pathways of *wOo* from KEGG; 13 of these pathways being unique to *wOo* while 57 are common to both cattle (*Bos taurus*) and *wOo*. Out of 349 proteins associated with the 70 pathways of *wOo,* only 204 proteins were identified as non-homologous to cattle proteins by NCBI blast search tool. 180 proteins were further identified as essential to the survival of *Wolbachia* of *Onchocerca ochengi* using DEG. Prioritization of the resultant 180 proteins (which was done by identifying the subcellular localization of these proteins, investigating their molecular weights and a local blast search of *wOo* protein sequences against the approved drug targets from DrugBank database), revealed 58 proteins as druggable targets for bovine onchocerciasis. 3D structures of drug targets were predicted using Raptor X server. Molecular docking studies of the three dimensional structures of these 58 proteins with 36 drug structures from DrugBank database resulted in only 32 proteins of *wOo* completing the molecular docking process. Molecular docking results showed that paromomycin and clomocycline had strong interactions with *wOo* proteins.

6. 2 Conclusion

Drug target identification is one of the first steps to drug discovery. These 32 drug targets for bovine onchocerciasis identified in this study can aid in the discovery of drugs to be used in the treatment of bovine onchocerciasis, thereby, proffering alternative approaches to tackle the disease. Also, paromomycin and clomocycine showed strong interactions with some *wOo*

proteins suggesting that they could both be used for drug repurposing in the treatment of bovine onchocerciasis via anti-*Wolbachia* therapy.

6.3 Recommendations

1. Further study, such as wet labourabouratory procedures, is required to validate these research findings. Wet laboratory procedures could be carried out to investigate whether the drugs which showed interactions with *wOo* proteins and had binding energies greater than,equal to or close to the binding energies and dissociation constants of tetracyclines' interaction with *wOo* proteins, qualify as new drugs for the treatment of bovine onchocerciasis. Further study should also be done to validate if paromomycin and clomocycline are actually effective in the treatment of bovine onchocerciasis.

2. Novel drugs could be designed for druggable proteins which did not complete the molecular docking process.

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APPENDIX I

Pathway identification numbers, pathway names and KEGG Identification numbers of proteins of *wOo.*

Pathway id nos	Pathway names	KEGG ID
path:woo00010	Glycolysis/Gluconeogenesis	woo:wOo_00040 woo:wOo_00790 woo:wOo_02910 woo:wOo_04120 woo:wOo_04230 woo:wOo_04840 woo:wOo_05050 woo:wOo_05840 woo:wOo_05880 woo:wOo_08670 woo:wOo_09510 woo:wOo_09770
path:woo00020	Citrate Cycle (TCA cycle)	woo:wOo_00040 woo:wOo_01260 woo:wOo_02330 woo:wOo_02620 woo:wOo_02910 woo:wOo_05140 woo:wOo_05410 woo:wOo_05880 woo:wOo_07720 woo:wOo_08300 woo:wOo_08670 woo:wOo_09270 woo:wOo_09280 woo:wOo_09340 woo:wOo_09510 woo:wOo_09540 woo:wOo_10110 woo:wOo_10120
path:woo00030	Pentose phosphate pathway	woo:wOo_00830 woo:wOo_03930 woo:wOo_05050 woo:wOo_07060 woo:wOo_07810 woo:wOo_09770
path:woo00051	Fructose and mannose metabolism	woo:wOo_04840 woo:wOo_05050 woo:wOo_09770 woo:wOo_10050
path:woo00061	Fatty acid biosynthesis	woo:wOo_02270

		woo:wOo_02760 woo:wOo_03620 woo:wOo_04110 woo:wOo_04980 woo:wOo_07970 woo:wOo_08450
path:woo00130	Ubiquinone and other terpenoid-quinone biosynthesis	woo:wOo_00240 woo:wOo_03170 woo:wOo_06110 woo:wOo_08240 woo:wOo_08560 woo:wOo_09580 woo:wOo_09670
path:woo00190	Oxidative phosphorylation	woo:wOo_00230 woo:wOo_00310 woo:wOo_00320 woo:wOo_00480 woo:wOo_00490 woo:wOo_00500 woo:wOo_00510 woo:wOo_00530 woo:wOo_00840 woo:wOo_00850 woo:wOo_01720 woo:wOo_02070 woo:wOo_02260 woo:wOo_02300 woo:wOo_03340 woo:wOo_04910 woo:wOo_05140 woo:wOo_05410 woo:wOo_05780 woo:wOo_05790 woo:wOo_05800 woo:wOo_06830 woo:wOo_06840 woo:wOo_07730 woo:wOo_07740 woo:wOo_07750 woo:wOo_08060 woo:wOo_08070 woo:wOo_08080 woo:wOo_08090 woo:wOo_08100 woo:wOo_08510 woo:wOo_08520

		woo:wOo_08890 woo:wOo_10110 woo:wOo_10120
path:woo00220	Arginine biosynthesis	woo:wOo_04400 woo:wOo_05300 woo:wOo_06980 woo:wOo_09620
path:woo00230	Purine metabolism	woo:wOo_00170 woo:wOo_00350 woo:wOo_00940 woo:wOo_01240 woo:wOo_01640 woo:wOo_02550 woo:wOo_03490 woo:wOo_03770 woo:wOo_03850 woo:wOo_03930 woo:wOo_04300 woo:wOo_04350 woo:wOo_04530 woo:wOo_04550 woo:wOo_04590 woo:wOo_06430 woo:wOo_06530 woo:wOo_06930 woo:wOo_07350 woo:wOo_07380 woo:wOo_07450 woo:wOo_07820 woo:wOo_08740 woo:wOo_07920 woo:wOo_09130 woo:wOo_09170 woo:wOo_09320 woo:wOo_09640 woo:wOo_10240 woo:wOo_10270
path:woo00240	Pyrimidine metabolism	woo:wOo_00940 woo:wOo_01640 woo:wOo_01760 woo:wOo_02550 woo:wOo_03250 woo:wOo_03380 woo:wOo_03770 woo:wOo_03850

		woo:wOo_04050 woo:wOo_05030 woo:wOo_05160 woo:wOo_05310 woo:wOo_05960 woo:wOo_06050 woo:wOo_06430 woo:wOo_06530 woo:wOo_06570 woo:wOo_06930 woo:wOo_07450 woo:wOo_07820 woo:wOo_08740 woo:wOo_08810 woo:wOo_09170 woo:wOo_09320 woo:wOo_09950 woo:wOo_09990 woo:wOo_10540
path:woo00250	Alanine, aspartate and glutamate metabolism	woo:wOo_04050 woo:wOo_04400 woo:wOo_06980 woo:wOo_08290 woo:wOo_09130 woo:wOo_09620 woo:wOo_09640 woo:wOo_09990 woo:wOo_10540
path:woo00260	Glycine, serine and threonine metabolism	woo:wOo_00040 woo:wOo_03500 woo:wOo_04120 woo:wOo_04430 woo:wOo_04790 woo:wOo_05590 woo:wOo_07600 woo:wOo_09510
path:woo00261	Monobactam biosynthesis	woo:wOo_03500 woo:wOo_07600 woo:wOo_08350 woo:wOo_08720
path:woo00270	Cysteine and methionine metabolism	woo:wOo_03500 woo:wOo_04400 woo:wOo_06470 woo:wOo_07600 woo:wOo_07720 woo:wOo_08860

		woo:wOo_08930 woo:wOo_09490
path:woo00280	Valine, leucine and Isoleucine degradation	woo:wOo_00040 woo:wOo_00540 woo:wOo_02180 woo:wOo_09510
path:woo00300	Lysine biosynthesis	woo:wOo_00610 woo:wOo_03500 woo:wOo_07600 woo:wOo_07780 woo:wOo_08350 woo:wOo_08720 woo:wOo_08850 woo:wOo_09260 woo:wOo_10750
path:woo00310	Lysine degradation	woo:wOo_02620 woo:wOo_09540
path:woo00330	Arginine and proline metabolism	woo:wOo_04400 woo:wOo_08290
path:woo00400	Phenylalanine, tyrosine and tryptophan biosynthesis	woo:wOo_04400
path:woo00450	Selenocompound metabolism	woo:wOo_00800 woo:wOo_06100 woo:wOo_08860 woo:wOo_10770
path:woo00471	D-Glutamine and D-glutamate metabolism	woo:wOo_01420 woo:wOo_04220
path:woo00480	Glutathione metabolism	woo:wOo_02330 woo:wOo_06470 woo:wOo_07480 woo:wOo_08200 woo:wOo_09490
path:woo00520	Amino sugar and nucleotide sugar metabolism	woo:wOo_03240 woo:wOo_06610 woo:wOo_10050
path:woo00550	Peptidoglycan biosynthesis	woo:wOo_01420 woo:wOo_01490 woo:wOo_03200 woo:wOo_03240 woo:wOo_03800 woo:wOo_04220 woo:wOo_06020 woo:wOo_06610 woo:wOo_07780 woo:wOo_08850 woo:wOo_09500

path:woo00561	Glycerolipid metabolism	woo:wOo_04760 woo:wOo_07980 woo:wOo_09360
path:woo00562	Inositol phosphate metabolism	woo:wOo_04840 woo:wOo_05580
path:woo00564	Glycerophospholipid metabolism	woo:wOo_04760 woo:wOo_04790 woo:wOo_04800 woo:wOo_05440 woo:wOo_05450 woo:wOo_0679 woo:wOo_07980 woo:wOo_09360 woo:wOo_09910
path:woo00620	Pyruvate metabolism	woo:wOo_00040 woo:wOo_01260 woo:wOo_02910 woo:wOo_05880 woo:wOo_06800 woo:wOo_07720 woo:wOo_08670 woo:wOo_09510 woo:wOo_09800
path:woo00630	Glyoxylate and dicarboxylate metabolism	woo:wOo_00040 woo:wOo_00540 woo:wOo_02180 woo:wOo_04430 woo:wOo_05670 woo:wOo_07720 woo:wOo_08300 woo:wOo_09340 woo:wOo_09510 woo:wOo_09620
path:woo00640	Propanoate metabolism	woo:wOo_00040 woo:wOo_00540 woo:wOo_02180 woo:wOo_06700 woo:wOo_09270 woo:wOo_09280 woo:wOo_09510
path:woo00650	Butanoate metabolism	woo:wOo_05140 woo:wOo_05410 woo:wOo_10110 woo:wOo_10120
path:woo00670	One carbon pool by folate	woo:wOo_01240

		woo:wOo_03180 woo:wOo_03250 woo:wOo_04430 woo:wOo_04590 woo:wOo_06870 woo:wOo_10560
path:woo00680	Methane metabolism	woo:wOo_04120 woo:wOo_0423 woo:wOo_04430 woo:wOo_05050 woo:wOo_07720 woo:wOo_09770
path:woo00730	Thamine metabolism	woo:wOo_07870 woo:wOo_08310 woo:wOo_10270
path:woo00750	Vitamin B6 metabolism	woo:wOo_02140
path:woo00760	Nicotinate and nicotinamide metabolism	woo:wOo_04090 woo:wOo_06930
path:woo00780	Biotin metabolism	woo:wOo_02270 woo:wOo_03620 woo:wOo_04980 woo:wOo_08050 woo:wOo_08450
path:woo00785	Lipoic acid metabolism	woo:wOo_04290 woo:wOo_07000
path:woo00790	Folate biosynthesis	woo:wOo_06860 woo:wOo_06870 woo:wOo_06880 woo:wOo_06890 woo:wOo_07280 woo:wOo_07580
path:woo00860	Porphyrin and chlorophyll metabolism	woo:wOo_00530 woo:wOo_00560 woo:wOo_04390 woo:wOo_05180 woo:wOo_05590 woo:wOo_05800 woo:wOo_05940 woo:wOo_06380 woo:wOo_06490 woo:wOo_06600 woo:wOo_08340
path:woo00900	Terpenoid backbone biosynthesis	woo:wOo_02950 woo:wOo_04960 woo:wOo_08770 woo:wOo_09920

path:woo00910	Nitrogen metabolism	woo:wOo_06980 woo:wOo_09620
path:woo00920	Sulphur metabolism	woo:wOo_01150
path:woo00970	Aminoacyl-tRNA biosynthesis	woo:wOo_00220 woo:wOo_00300 woo:wOo_00420 woo:wOo_00430 woo:wOo_00560 woo:wOo_00680 woo:wOo_00730 woo:wOo_01000 woo:wOo_01010 woo:wOo_01280 woo:wOo_01570 woo:wOo_01850 woo:wOo_01940 woo:wOo_02130 woo:wOo_02160 woo:wOo_02220 woo:wOo_02250 woo:wOo_02410 woo:wOo_02500 woo:wOo_02530 woo:wOo_02930 woo:wOo_03220 woo:wOo_03270 woo:wOo_03370 woo:wOo_03690 woo:wOo_03700 woo:wOo_03940 woo:wOo_04000 woo:wOo_04520 woo:wOo_05000 woo:wOo_05010 woo:wOo_05040 woo:wOo_05180 woo:wOo_05210 woo:wOo_05230 woo:wOo_05910 woo:wOo_05980 woo:wOo_06030 woo:wOo_06070 woo:wOo_06100 woo:wOo_06400 woo:wOo_07040 woo:wOo_07290

		woo:wOo_07400 woo:wOo_07630 woo:wOo_07800 woo:wOo_08370 woo:wOo_08630 woo:wOo_08940 woo:wOo_09010 woo:wOo_09120 woo:wOo_09470 woo:wOo_09860 woo:wOo_09980 woo:wOo_10520 woo:wOo_10530 woo:wOo_10560
path:woo01040	Biosynthesis of unsaturated fatty acid	woo:wOo_08450
path:woo01100	Metabolic pathways	woo:wOo_00040 woo:wOo_00170 woo:wOo_00240 woo:wOo_00310 woo:wOo_00320 woo:wOo_00350 woo:wOo_00480 woo:wOo_00490 woo:wOo_00500 woo:wOo_00510 woo:wOo_00530 woo:wOo_00540 woo:wOo_00560 woo:wOo_00610 woo:wOo_00790 woo:wOo_00830 woo:wOo_00840 woo:wOo_00850 woo:wOo_00940 woo:wOo_01150 woo:wOo_01180 woo:wOo_01240 woo:wOo_01260 woo:wOo_01420 woo:wOo_01490 woo:wOo_01640 woo:wOo_01720 woo:wOo_01740 woo:wOo_01760 woo:wOo_02070

		woo:wOo_02140 woo:wOo_02180 woo:wOo_02260 woo:wOo_02270 woo:wOo_02300 woo:wOo_02330 woo:wOo_02550 woo:wOo_02620 woo:wOo_02760 woo:wOo_02910 woo:wOo_03170 woo:wOo_03180 woo:wOo_03240 woo:wOo_03250 woo:wOo_03340 woo:wOo_03380 woo:wOo_03490 woo:wOo_03500 woo:wOo_03620 woo:wOo_03770 woo:wOo_03800 woo:wOo_03850 woo:wOo_03930 woo:wOo_03940 woo:wOo_04050 woo:wOo_04090 woo:wOo_04110 woo:wOo_04120 woo:wOo_04220 woo:wOo_04230 woo:wOo_04290 woo:wOo_04300 woo:wOo_04340 woo:wOo_04350 woo:wOo_04390 woo:wOo_04400 woo:wOo_04430 woo:wOo_04530 woo:wOo_04550 woo:wOo_04590 woo:wOo_04760 woo:wOo_04790 woo:wOo_04800 woo:wOo_04840 woo:wOo_04910 woo:wOo_04960

		woo:wOo_04980 woo:wOo_05000 woo:wOo_05030 woo:wOo_05050 woo:wOo_05140 woo:wOo_05160 woo:wOo_05180 woo:wOo_05300 woo:wOo_05310 woo:wOo_05410 woo:wOo_05440 woo:wOo_05580 woo:wOo_05590 woo:wOo_05670 woo:wOo_05780 woo:wOo_05790 woo:wOo_05800 woo:wOo_05840 woo:wOo_05880 woo:wOo_05940 woo:wOo_05960 woo:wOo_06020 woo:wOo_06050 woo:wOo_06110 woo:wOo_06380 woo:wOo_06430 woo:wOo_06470 woo:wOo_06490 woo:wOo_06530 woo:wOo_06570 woo:wOo_06600 woo:wOo_06610 woo:wOo_06700 woo:wOo_06790 woo:wOo_06800 woo:wOo_06830 woo:wOo_06840 woo:wOo_06870 woo:wOo_06880 woo:wOo_06890 woo:wOo_06930 woo:wOo_06980 woo:wOo_07000 woo:wOo_07060 woo:wOo_07280 woo:wOo_07350

		woo:wOo_07380 woo:wOo_07450 woo:wOo_07580 woo:wOo_07600 woo:wOo_07720 woo:wOo_07730 woo:wOo_07740 woo:wOo_07750 woo:wOo_07780 woo:wOo_07810 woo:wOo_07870 woo:wOo_07970 woo:wOo_07980 woo:wOo_08050 woo:wOo_08060 woo:wOo_08070 woo:wOo_08080 woo:wOo_08090 woo:wOo_08100 woo:wOo_08200 woo:wOo_08240 woo:wOo_08290 woo:wOo_08300 woo:wOo_08310 woo:wOo_08340 woo:wOo_08350 woo:wOo_08450 woo:wOo_08510 woo:wOo_08520 woo:wOo_08560 woo:wOo_08670 woo:wOo_08720 woo:wOo_08740 woo:wOo_08770 woo:wOo_08810 woo:wOo_08860 woo:wOo_08890 woo:wOo_08930 woo:wOo_09010 woo:wOo_09130 woo:wOo_09170 woo:wOo_09260 woo:wOo_09270 woo:wOo_09280 woo:wOo_09320 woo:wOo_09340

		woo:wOo_09360 woo:wOo_09490 woo:wOo_09500 woo:wOo_09510 woo:wOo_09540 woo:wOo_09580 woo:wOo_09620 woo:wOo_09640 woo:wOo_09670 woo:wOo_09770 woo:wOo_09800 woo:wOo_09910 woo:wOo_09950 woo:wOo_09990 woo:wOo_10050 woo:wOo_10110 woo:wOo_10120 woo:wOo_10190 woo:wOo_10240 woo:wOo_10270 woo:wOo_10540 woo:wOo_10750
path:woo01110	Biosynthesis of secondary metabolites	woo:wOo_00040 woo:wOo_00240 woo:wOo_00350 woo:wOo_00530 woo:wOo_00560 woo:wOo_00790 woo:wOo_00830 woo:wOo_01180 woo:wOo_01240 woo:wOo_01260 woo:wOo_01740 woo:wOo_02330 woo:wOo_02620 woo:wOo_02910 woo:wOo_02950 woo:wOo_03170 woo:wOo_03490 woo:wOo_03500 woo:wOo_03930 woo:wOo_04120 woo:wOo_04230 woo:wOo_04300 woo:wOo_04340 woo:wOo_04350

		woo:wOo_04390 woo:wOo_04400 woo:wOo_04430 woo:wOo_04530 woo:wOo_04590 woo:wOo_04760 woo:wOo_04790 woo:wOo_0480 woo:wOo_04840 woo:wOo_04960 woo:wOo_0505 woo:wOo_05140 woo:wOo_05180 woo:wOo_05300 woo:wOo_05410 woo:wOo_05450 woo:wOo_05590 woo:wOo_05670 woo:wOo_05800 woo:wOo_05840 woo:wOo_05880 woo:wOo_05940 woo:wOo_06110 woo:wOo_06380 woo:wOo_06490 woo:wOo_06530 woo:wOo_06600 woo:wOo_06930 woo:wOo_07060 woo:wOo_07280 woo:wOo_07350 woo:wOo_07380 woo:wOo_07600 woo:wOo_07720 woo:wOo_07810 woo:wOo_07980 woo:wOo_08240 woo:wOo_08290 woo:wOo_08300 woo:wOo_08340 woo:wOo_08350 woo:wOo_08560 woo:wOo_08670 woo:wOo_08720 woo:wOo_08770 woo:wOo_08860

		woo:wOo_08930 woo:wOo_09130 woo:wOo_09260 woo:wOo_09270 woo:wOo_09280 woo:wOo_09340 woo:wOo_09360 woo:wOo_09510 woo:wOo_09540 woo:wOo_09580 woo:wOo_09670 woo:wOo_09770 woo:wOo_09910 woo:wOo_09920 woo:wOo_10050 woo:wOo_10110 woo:wOo_10120 woo:wOo_10270
path:woo01120	Microbial metabolism in diverse environments	woo:wOo_00040 woo:wOo_00540 woo:wOo_00560 woo:wOo_00610 woo:wOo_00790 woo:wOo_00830 woo:wOo_01150 woo:wOo_01260 woo:wOo_02140 woo:wOo_02180 woo:wOo_02330 woo:wOo_02620 woo:wOo_02910 woo:wOo_03170 woo:wOo_03180 woo:wOo_03500 woo:wOo_03930 woo:wOo_04120 woo:wOo_04230 woo:wOo_04430 woo:wOo_04840 woo:wOo_05050 woo:wOo_05140 woo:wOo_05180 woo:wOo_05410 woo:wOo_05840 woo:wOo_05880 woo:wOo_06380

		woo:wOo_06600 woo:wOo_06800 woo:wOo_07060 woo:wOo_07600 woo:wOo_07720 woo:wOo_07810 woo:wOo_08300 woo:wOo_08340 woo:wOo_08350 woo:wOo_08670 woo:wOo_08720 woo:wOo_09260 woo:wOo_09270 woo:wOo_09280 woo:wOo_09340 woo:wOo_09510 woo:wOo_09540 woo:wOo_09620 woo:wOo_09770 woo:wOo_09800 woo:wOo_10110 woo:wOo_10120 woo:wOo_10750
path:woo01130	Biosynthesis of antibiotics	woo:wOo_00040 woo:wOo_00540 woo:wOo_00790 woo:wOo_00830 woo:wOo_01240 woo:wOo_01260 woo:wOo_01740 woo:wOo_02180 woo:wOo_02270 woo:wOo_02330 woo:wOo_02620 woo:wOo_02910 woo:wOo_03490 woo:wOo_03500 woo:wOo_03930 woo:wOo_04110 woo:wOo_04120 woo:wOo_04230 woo:wOo_04300 woo:wOo_04340 woo:wOo_04350 woo:wOo_04400 woo:wOo_04430

		woo:wOo_04530 woo:wOo_04590 woo:wOo_04840 woo:wOo_04960 woo:wOo_05050 woo:wOo_05140 woo:wOo_05300 woo:wOo_05410 woo:wOo_05670 woo:wOo_05840 woo:wOo_05880 woo:wOo_06530 woo:wOo_07060 woo:wOo_07350 woo:wOo_07380 woo:wOo_07600 woo:wOo_07720 woo:wOo_07810 woo:wOo_08290 woo:wOo_08300 woo:wOo_08350 woo:wOo_08450 woo:wOo_08670 woo:wOo_08720 woo:wOo_08770 woo:wOo_09130 woo:wOo_09260 woo:wOo_09270 woo:wOo_09280 woo:wOo_09340 woo:wOo_09510 woo:wOo_09540 woo:wOo_09770 woo:wOo_10050 woo:wOo_10110 woo:wOo_10120 woo:wOo_10270
path:woo01200	Carbon metabolism	woo:wOo_00040 woo:wOo_00540 woo:wOo_00790 woo:wOo_00830 woo:wOo_01260 woo:wOo_02180 woo:wOo_02330 woo:wOo_02620 woo:wOo_02910

		woo:wOo_03180 woo:wOo_03930 woo:wOo_04120 woo:wOo_04230 woo:wOo_04430 woo:wOo_04840 woo:wOo_05050 woo:wOo_05140 woo:wOo_05410 woo:wOo_05840 woo:wOo_05880 woo:wOo_06800 woo:wOo_07060 woo:wOo_07720 woo:wOo_07810 woo:wOo_08300 woo:wOo_08670 woo:wOo_09270 woo:wOo_09280 woo:wOo_09340 woo:wOo_09510 woo:wOo_09540 woo:wOo_09770 woo:wOo_09800 woo:wOo_10110 woo:wOo_10120
path:woo01210	2-oxocarboxylic acid metabolism	woo:wOo_02330 woo:wOo_03500 woo:wOo_04400 woo:wOo_05300 woo:wOo_07600 woo:wOo_08300 woo:wOo_09340
path:woo01212	Fatty acid metabolism	woo:wOo_02270 woo:wOo_02760 woo:wOo_03620 woo:wOo_04110 woo:wOo_04980 woo:wOo_07970 woo:wOo_08450
path:woo01230	Biosynthesis of aminoacids	woo:wOo_00610 woo:wOo_00790 woo:wOo_00830 woo:wOo_02330 woo:wOo_0350 woo:wOo_03930

		woo:wOo_04120 woo:wOo_04230 woo:wOo_04400 woo:wOo_04430 woo:wOo_04840 woo:wOo_05050 woo:wOo_05300 woo:wOo_05840 woo:wOo_07060 woo:wOo_07600 woo:wOo_07810 woo:wOo_08300 woo:wOo_08350 woo:wOo_08720 woo:wOo_08860 woo:wOo_08930 woo:wOo_09260 woo:wOo_09340 woo:wOo_09620 woo:wOo_10750
path:woo01501	Beta-lactam resistance	woo:wOo_00120 woo:wOo_03200
path:woo01502	Vacomycin resistance	woo:wOo_01490 woo:wOo_03800 woo:wOo_07780 woo:wOo_09500
path:woo01503	Cationic antimicrobial peptide (CAMP) resistance	woo:wOo_00120 woo:wOo_06310
path:woo02010	ABC transporters	woo:wOo_00150 woo:wOo_00690 woo:wOo_00860 woo:wOo_02200 woo:wOo_03470 woo:wOo_04060 woo:wOo_04310 woo:wOo_06540 woo:wOo_06550 woo:wOo_07900 woo:wOo_08150 woo:wOo_08710 woo:wOo_08750 woo:wOo_10080 woo:wOo_10220
path:woo02020	Two component system	woo:wOo_00120 woo:wOo_00530 woo:wOo_01150

		woo:wOo_03340 woo:wOo_06310 woo:wOo_06830 woo:wOo_06840 woo:wOo_06950 woo:wOo_06960 woo:wOo_09610 woo:wOo_09620 woo:wOo_10220
path:woo02024	Quorum sensing	woo:wOo_01580 woo:wOo_02570 woo:wOo_03000 woo:wOo_03050 woo:wOo_03590 woo:wOo_03650 woo:wOo_03870 woo:wOo_07150 woo:wOo_07280 woo:wOo_08800 woo:wOo_09300 woo:wOo_10300
path:woo03010	Ribosome	woo:wOo_00100 woo:wOo_00110 woo:wOo_00910 woo:wOo_00920 woo:wOo_01530 woo:wOo_01540 woo:wOo_01600 woo:wOo_01610 woo:wOo_01620 woo:wOo_01630 woo:wOo_01880 woo:wOo_02960 woo:wOo_03680 woo:wOo_04100 woo:wOo_04320 woo:wOo_07010 woo:wOo_07050 woo:wOo_07830 woo:wOo_08960 woo:wOo_08970 woo:wOo_09040 woo:wOo_09140 woo:wOo_09150 woo:wOo_09160 woo:wOo_09290

		woo:wOo_09350 woo:wOo_09970 woo:wOo_10130 woo:wOo_10230 woo:wOo_10250 woo:wOo_10260 woo:wOo_10310 woo:wOo_10320 woo:wOo_10330 woo:wOo_10340 woo:wOo_10350 woo:wOo_10360 woo:wOo_10370 woo:wOo_10380 woo:wOo_10390 woo:wOo_10400 woo:wOo_10410 woo:wOo_10420 woo:wOo_10430 woo:wOo_10440 woo:wOo_10450 woo:wOo_10460 woo:wOo_10470 woo:wOo_10480 woo:wOo_10490 woo:wOo_10500 woo:wOo_10570 woo:wOo_10580
path:woo03018	RNA-degradation	woo:wOo_02280 woo:wOo_04230 woo:wOo_06190 woo:wOo_07700 woo:wOo_07820 woo:wOo_08210 woo:wOo_09020 woo:wOo_09030 woo:wOo_09720
path:woo03020	RNA-degradation	woo:wOo_01640 woo:wOo_01760 woo:wOo_10240
path:woo03030	DNA-degradation	woo:wOo_03770 woo:wOo_03810 woo:wOo_03850 woo:wOo_05810 woo:wOo_06080 woo:wOo_06120

		woo:wOo_06430 woo:wOo_06920 woo:wOo_07450 woo:wOo_08360 woo:wOo_08740 woo:wOo_09170 woo:wOo_09320
path:woo03060	Protein export	woo:wOo_00710 woo:wOo_01580 woo:wOo_02570 woo:wOo_03000 woo:wOo_03050 woo:wOo_03590 woo:wOo_03870 woo:wOo_04620 woo:wOo_05830 woo:wOo_06590 woo:wOo_07120 woo:wOo_07150 woo:wOo_07460 woo:wOo_08800 woo:wOo_09300 woo:wOo_10300
path:woo03070	Bacterial secretion system	woo:wOo_00120 woo:wOo_00710 woo:wOo_01290 woo:wOo_01300 woo:wOo_01310 woo:wOo_01320 woo:wOo_01340 woo:wOo_01350 woo:wOo_01580 woo:wOo_02080 woo:wOo_02570 woo:wOo_02790 woo:wOo_03000 woo:wOo_03590 woo:wOo_03820 woo:wOo_03870 woo:wOo_05830 woo:wOo_06590 woo:wOo_07120 woo:wOo_07150 woo:wOo_07230 woo:wOo_07240 woo:wOo_07250

		woo:wOo_07260 woo:wOo_07270 woo:wOo_08800 woo:wOo_09300 woo:wOo_10300
path:woo03410	Base excision repair	woo:wOo_00360 woo:wOo_01160 woo:wOo_02150 woo:wOo_02310 woo:wOo_04680 woo:wOo_06370 woo:wOo_06430 woo:wOo_06920
path:woo03420	Nucleotide excision repair	woo:wOo_00970 woo:wOo_02480 woo:wOo_04730 woo:wOo_06430 woo:wOo_06850 woo:wOo_06920
path:woo03430	Mismatch repair	woo:wOo_01030 woo:wOo_01160 woo:wOo_01730 woo:wOo_02480 woo:wOo_03770 woo:wOo_03850 woo:wOo_06920 woo:wOo_07450 woo:wOo_08360 woo:wOo_08740 woo:wOo_09170 woo:wOo_09320
path:woo03440	Homologous recombination	woo:wOo_01160 woo:wOo_03770 woo:wOo_03850 woo:wOo_05130 woo:wOo_06430 woo:wOo_07450 woo:wOo_08360 woo:wOo_08740 woo:wOo_09170 woo:wOo_09320
path:woo04122	Sulphur relay system	woo:wOo_05750 woo:wOo_07870 woo:wOo_08310

APPENDIX II

Pathway identification numbers and pathway names of *Bos taurus*

Pathway id no	Pathway name
Path:bta00010	Glycolysis/Gluconeogenesis
Path:bta00020	Citrate cycle (TCA cycle)
Path:bta00030	Pentose phosphate pathway
Path:bta00040	Pentose and glucuronate interconversions
Path:bta00051	Fructose and mannose metabolism
Path:bta00052	Galactose metabolism
Path:bta00053	Ascorbate and aldarate metabolism
Path:bta00061	Fatty acid biosynthesis
Path:bta00062	Fatty acid elongation
Path:bta00071	Fatty acid degradation
Path:bta00072	Synthesis and degradation of ketone bodies
Path:bta00100	Steriod biosynthesis
Path:bta00120	Primary bile acid biosynthesis
Path:bta00130	Ubiquinone and other terpenoid-quinone biosynthesis
Path:bta00140	Steriod hormone biosynthesis
Path:bta00190	Oxidative phosphorylation
Path:bta00220	Arginine biosynthesis
Path:bta00230	Purine metabolism
Path:bta00232	Caffeine metabolism
Path:bta00240	Pyrimidine metabolism
Path:bta00250	Alanine aspartate and glutamate metabolism
Path:bta00260	Glycine, serine and threonine metabolism
Path:bta00270	Cysteine and methione metabolism
Path:bta00280	Valine, leucine and isoleucine degradation
Path:bta00290	Valine, leucine and isoleucine biosynthesis
Path:bta00310	Lysine degradation
Path:bta00330	Arginine and proline metabolism
Path:bta00340	Histidine metabolism
Path:bta00350	Tyrosine metabolism
Path:bta00360	Phenylalanine metabolism
Path:bta00380	Tryptophan metabolism
Path:bta00400	Phenylalanine tyrosine
Path:bta00410	Beta-alanine metabolism
Path:bta00430	Taurine and hypotaurine metabolism
Path:bta00440	Phosphonate and phosphinate metabolism
Path:bta00450	Selenocompound metabolism
Path:bta00471	D-glutamine and D-glutamate metabolism
Path:bta00472	D- Arginine and D-orinthine metabolism
Path:bta00480	Glutathione metabolism
Path:bta00500	Starch and sucrose metabolism

Path:bta00510	N-Glycan biosynthesis
Path:bta00511	Other glycan degradation
Path:bta00512	Mucin type Oglycan biosynthesis
Path:bta00514	Other types of O-glycan biosynthesis
Path:bta00515	Mannose type O-glycan biosynthesis
Path:bta00520	Amino sugar and nucleotide sugar metabolism
Path:bta00524	Neomycin, Kanamycin and gentamicin biosynthesis
Path:bta00531	Glycosaminoglycan degradation
Path:bta00532	Glycosaminoglycan biosynthesis-chondrotin sulphate/dermatan sulfate
Path:bta00533	Glycosaminoglycan biosynthesis-keratan sulfate
Path:bta00534	Glycosaminoglycan biosynthesis-heparan sulphate/heparan
Path:bta00561	Glycerolipid metabolism
Path:bta00562	Inositol phosphate metabolism
Path:bta00563	Glycosylphosphatydyl inositol (GPI)- anchor biosynthesis
Path:bta00564	Glycerophospholipid metabolism
Path:bta00565	Ether lipid metabolism
Path:bta00590	Arachidonic acid metabolism
Path:bta00591	Linoleic acid metabolism
Path:bta00592	Alpha-linolenic acid metabolism
Path:bta00600	Sphingolipid metabolism
Path:bta00601	Glycosphingolipid biosynthesis- lacto and neolacto series
Path:bta00603	Glycosphingolipid biosynthesi- globo and isoglobo series
Path:bta00604	Glycosphingolipid biosynthesis- ganglio series
Path:bta00620	Pyruvate metabolism
Path:bta00630	Glyoxylate and dicarboxylate metabolism
Path:bta00640	Propanoate metabolism
Path:bta00650	Butanoate metabolism
Path:bta00670	One carbon pool by folate
Path:bta00730	Thiamine metabolism
Path:bta00740	Riboflavin metabolism
Path:bta00750	Vitamin B6 metabolism
Path:bta00760	Nicotinate and nicotinamide metabolism
Path:bta00770	Pantothenate and CoA biosynthesis
Path:bta00780	Biotin metabolism
Path:bta00785	Lipoic acid metabolism
Path:bta00790	Folate biosynthesis
Path:bta00830	Retinol metabolism
Path:bta00860	Porphyrin and chlorophyll metabolism
Path:bta00900	Terpenoid backbone biosynthesis
Path:bta00910	Nitrogen metabolism
Path:bta00920	Sulphur metabolism
Path:bta00970	Aminoacyl-tRNA biosynthesis
Path:bta00980	Metabolism of xenobiotics by cytochrome P450
Path:bta00982	Drug metabolism-Cytochome P450

Path:bta00983	Drug metabolism-other enzymes
Path:bta01040	Biosynthesis of unsaturated fatty acids
Path:bta01100	Metabolic pathways
Path:bta01200	Carbon metabolism
Path:bta01210	2-oxocarboxylic acid metabolism
Path:bta01212	Fatty acid metabolism
Path:bta01230	Biosynthesis amino acids
Path:bta01521	EGFR tyrosine Kinase inhibitor resistance
Path:bta01522	Endocrine resistance
Path:bta01523	Antifolate resistance
Path:bta01524	Platinum drug resistance
Path:bta02010	ABC transporters
Path:bta03008	Ribosome biosynthesis in euksryotes
Path:bta03010	Ribosome
Path:bta03013	RNA transport
Path:bta03015	mRNA surveillance pathway
Path:bta03018	RNA degradation
Path:bta03020	RNA Polymerase
Path:bta03022	Basal transcription factors
Path:bta03030	DNA replication
Path:bta03040	Spliceosome
Path:bta03050	Proteasome
Path:bta03060	Protein export
Path:bta03320	PPAR signalling pathway
Path:bta03410	Base excision repair
Path:bta03420	Nucleotide excision repair
Path:bta03430	Mismatch repair
Path:bta03440	Homologous recombination
Path:bta03450	Non homologous end-joining
Path:bta03460	Faconi anemia pathway
Path:bta04010	MAPK signalling pathway
Path:bta04012	ErbB signalling pathway
Path:bta04014	Ras signalling pathway
Path:bta04015	Rap 1 signalling pathway
Path:bta04020	Calcium signalling pathway
Path:bta04022	cGMP-PKG signalling pathway
Path:bta04024	cAMP signalling pathway
Path:bta04060	Cytokine-cytokine receptor interaction
Path:bta04062	Chemokine signalling pathway
Path:bta04064	NF-Kappa B signalling
Path:bta04066	HIF-1 signalling pathway
Path:bta04068	Fox 0 signalling pathway
Path:bta04070	Phosphatidylinositol signalling system
Path:bta04071	Shingolipid signalling pathway
Path:bta04072	Phospholipase D signalling pathway

Path:bta04080	Neuroactive ligand-receptor interaction
Path:bta04110	Cellcycle
Path:bta04114	Oocyte meiosis
Path:bta04115	P53 signalling pathway
Path:bta04120	Ubiquitin mediated proteolysis
Path:bta04122	Sulphur relay system
Path:bta04130	SNARE interaction in vesicular transport
Path:bta04136	Autophagy – other
Path:bta04137	Mitophagy – animal
Path:bta04140	Autophagy – animal
Path:bta04141	Protein processing in endoplasmic reticulum
Path:bta04142	Lysosome
Path:bta04144	Endocytosis
Path:bta04145	Phagosome
Path:bta04146	Peroxisome
Path:bta04150	m TOR signalling pathway
Path:bta04151	P13K – AKT signalling pathway
Path:bta04152	AMPK signalling pathway
Path:bta04210	APOPTOSIS
Path:bta04211	Longevity regulating pathway
Path:bta04213	Longevity regulating pathway – multiple species
Path:bta04215	Apoptosis – multiple species
Path:bta04216	Ferroptosis
Path:bta04217	Necroptosis
Path:bta04218	Cellular senescence
Path:bta04260	Cardiac muscle contraction
Path:bta04261	Adrenergic signalling in cardiomycytes
Path:bta04270	Vascular smooth muscle contraction
Path:bta04310	Wat signalling pathway
Path:bta04330	Notch signalling pathway
Path:bta04340	Hedgehog signalling pathway
Path:bta04350	T9F – beta signalling pathway
Path:bta04360	Axon guidance
Path:bta04370	VEGF signalling pathway
Path:bta04371	Apelin signalling pathway
Path:bta04380	Osteoclast differentiation
Path:bta04390	Hippo signalling pathway
Path:bta04392	Hippo signalling pathway – multiple species
Path:bta04510	Focal adhesion
Path:bta04512	ECM – receptor interaction
Path:bta04514	Cell adhesion molecules (CAMs)
Path:bta04520	Adherens junction
Path:bta04530	Light junction
Path:bta04540	Gap junction
Path:bta04550	Signalling pathway regulating pluripoterey of stem cells

Path:bta04610	Complement and coagulation cascades
Path:bta04611	Platelet activation
Path:bta04612	Antigen processing and presentation
Path:bta04614	Renin – angiotensin system
Path:bta04620	Toll – like receptor signalling pathway
Path:bta04621	NOD – like receptor signalling pathway
Path:bta04622	RIG – I – like receptor signalling pathway
Path:bta04623	Cytosolic DNA – sensing pathway
Path:bta04630	Jak – STAT signalling pathway
Path:bta04640	Hematopoietic cell linage
Path:bta04650	Natural killer cell mediated cytotoxicity
Path:bta04657	IL – 17 signalling pathway
Path:bta04658	Th 1and Th 2 cell differentiation
Path:bta04659	Th 17 cel differentiation
Path:bta04660	T cell receptor signalling pathway
Path:bta04662	B cell receptor signalling pathway
Path:bta04664	Fe epilson R1 signalling pathway
Path:bta04666	Fe gamma R – mediated phagocytosis
Path:bta04668	TNF signalling pathway
Path:bta04670	Leukocyte trans endothelial migration
Path:bta04672	Intestinal immune network for IgA production
Path:bta04710	Greadian rhythm
Path:bta04713	Greadian entrainment
Path:bta04720	Long – term potentiation
Path:bta04721	Synaptic vesicle cycle
Path:bta04722	Neurotrophin signalling pathway
Path:bta04723	Retrograde endocannabinoid signalling
Path:bta04724	Glutamatergic synapse
Path:bta04725	Cholinergic synapse
Path:bta04726	Serotonergic synapse
Path:bta04727	GABAergic synapse
Path:bta04728	Dopaminergic synapse
Path:bta04730	Long –term depression
Path:bta04740	Olfactory transduction
Path:bta04742	Taste transduction
Path:bta04744	Photo transduction
Path:bta04750	Inflammatory mediator regulation of TRP channels
Path:bta04810	Regulation of actin cytoskeleton
Path:bta04910	Insulin signalling pathway
Path:bta04911	Insulin secretion
Path:bta04912	GnRH signalling pathway
Path:bta04913	Ovarian steroidogenesis
Path:bta04914	Progesterone – mediated oocyte maturation
Path:bta04915	Estrogen signalling pathway
Path:bta04916	Melanogenesis

Path:bta04917	Prolactin signalling pathway
Path:bta04918	Thyroid hormone synthesis
Path:bta04919	Thyroid hormone signalling pathway
Path:bta04920	Adipocytokine signalling pathway
Path:bta04921	Oxytocin signalling pathway
Path:bta04922	Glucagon signalling pathway
Path:bta04923	Regulation of lipolysis in adipocytes
Path:bta04924	Renin secretion
Path:bta04925	Aldosterone synthesis and secretion
Path:bta04926	Relaxin signalling pathway
Path:bta04930	Type II diabetes mellitus
Path:bta04931	Insulin resistance
Path:bta04932	Non – alcoholic fatty liver disease (NAFLD)
Path:bta04933	AGE –RAGE signalling pathway in diabetic complications
Path:bta04940	Type I diabetes mellitus
Path:bta04950	Maturity onset diabetes of the young
Path:bta04960	Aldosterone – regulated sodium reabsorption
Path:bta04961	Endocrine and other factor – regulated calcium reabsorption
Path:bta04962	Vasopressin – regulated water reabsorption
Path:bta04964	Proximal tubule bicarbonate reclamation
Path:bta04966	Collecting duct acid secretion
Path:bta04970	Salivary secretion
Path:bta04971	Gastric acid secretion
Path:bta04972	Pancreatic secretion
Path:bta04973	Carbohydrate digestion and absorption
Path:bta04974	Protein digestion and absorption
Path:bta04975	Fat digestion and absorption
Path:bta04976	Bile secretion
Path:bta04977	Vitamin digestion and absorption
Path:bta04978	Mineral absorption
Path:bta04979	Cholesterol metabolism
Path:bta05010	Alzheimer’s disease
Path:bta05012	Parkinson’s disease
Path:bta05014	Amyotropic lateral sclerosis (ALS)
Path:bta05016	Huntington’s disease
Path:bta05020	Prion disease
Path:bta05030	Cocaine disease
Path:bta05031	Amphetamine addiction
Path:bta05032	Morphine addiction
Path:bta05033	Nicotine addiction
Path:bta05034	Alcoholism
Path:bta05100	Bacterial invasion of epithelial cells
Path:bta05132	Salmonella infection
Path:bta05133	Pertussis
Path:bta05134	Legionellosis

Path:bta05140	Leishmaniasis
Path:bta05142	Chagas disease
Path:bta05143	African trypanosomiasis
Path:bta05144	Malaria
Path:bta05145	Toxoplasmosis
Path:bta05145	Ameebiasis
Path:bta05150	Staphylococcus aureus infection
Path:bta05152	Tuberculosis
Path:bta05160	Hepatitis C
Path:bta05161	Hepatitis B
Path:bta05162	Measles
Path:bta05164	Influenza A
Path:bta05165	Human papillomavirus infection
Path:bta05166	HTLV – I infection
Path:bta05167	Kaposi's sarcoma – associated herpes virus infection
Path:bta05168	Herpes simplex infection
Path:bta05169	Epstein – Barr virus infection
Path:bta05200	Pathways in cancer
Path:bta05202	Transcriptional misregulation in cancer
Path:bta05203	Viral carcinogenesis
Path:bta05204	Chemical carcinogenesis
Path:bta05205	Proteoglycans in cancer
Path:bta05206	microRNAs in cancer
Path:bta05210	Colorectal cancer
Path:bta05211	Renal cell carcinoma
Path:bta05212	Pancreatic cancer
Path:bta05213	Endometrial cancer
Path:bta05214	Glioma
Path:bta05215	Prostrate cancer
Path:bta05216	Thyroid cancer
Path:bta05217	Basal cell carcinoma
Path:bta05218	Melanoma
Path:bta05219	Bladder cancer
Path:bta05220	Chronic myeloid leukaemia
Path:bta05221	Acute myeloid leukaemia
Path:bta05222	Small cell lung cancer
Path:bta05223	Non – small cell lung cancer
Path:bta05224	Breast cancer
Path:bta05225	Hepatocellular carcinoma
Path:bta05226	Gastric cancer
Path:bta05230	Central carbon metabolism in cancer
Path:bta05231	Choline metabolism in cancer
Path:bta05310	Asthma
Path:bta05320	Autoimmune thyroid disease
Path:bta05321	Inflammatory bowel disease (IBD)

Path:bta05322	Systemic lupus erythrematosus
Path:bta05323	Rheumatoid arthritis
Path:bta05330	Allograft rejection
Path:bta05332	Graft – virus – host disease
Path:bta05340	Primary immunodeficiency
Path:bta05410	Hypertrophic cardiomyopathy (HCM)
Path:bta05412	Arrhymogenic right ventricular cardiomyopathy (ARVC)
Path:bta05414	Dilated cardiomyopathy (DCM)
Path:bta05416	Viral myocarditis
Path:bta05418	Fluid shear stress and atherosclerosis

APPENDIX III

Unique and common metabolic pathways of *wolbachia* of *Onchocerca ochengi* in relation to cattle (*Bos taurus).*

UNIQUE PATHWAYS

1. Path:woo00261: Monobactam biosynthesis
2. Path:woo00300: Lysine biosynthesis
3. Path:woo00550: Peptidoglycan biosynthesis
4. Path:woo00680: Methane metabolism
5. Path:woo01110: Biosynthesis of se
6. Path:woo01120: Microbial metabolism in diverse environments
7. Path:woo01130: Biosynthesis of antibiotics
8. Path:woo01501: Beta-lactam resistance
9. Path:woo01502: Vacomycin resistance
10. Path:woo01503: Cationic anti microbial pepetide (cAMP) resistance
11. Path:woo02020: Two component system
12. Path:woo02024: Quorum sensing
13. Path:woo03070: Bacterial secretion system

COMMON PATHWAYS

1. Path:woo00010: Glycolysis/ Gluconeogenesis
2. Path:woo00020: Citrate cycle (TCA cycle)
3. Path:woo00030: Pentose phosphate pathway
4. Path:woo00051: Fructose and mannose metabolism
5. Path:woo00061: Fatty acid
6. Path:woo00130: Ubiquinone and other terpenoid-quinone biosynthesis
7. Path:woo00190: Oxidative phosphorylation
8. Path:woo00220: Arginine biosynthesis
9. Path:woo00230: Purine metabolism
10. Path:woo00240: Pyrimidine metabolism
11. Path:woo00250: Alanine, aspartate and glutamate metabolism
12. Path:woo00260: Glycine, serine and threonine metabolism
13. Path:woo00270: Cysteine and methionine metabolism
14. Path:woo00280: Vailne, leucine and isoleucine degradation
15. Path:woo00310: Lysine degradation

16. Path:woo00330: Arginine and proline metabolism
17. Path:woo00400: Phenylalanine, tyrosine and tryptophan biosyhthesis
18. Path:woo00450: Selenocompound metabolism
19. Path:woo00471: D-glutamine and D-glutamate metabolism
20. Path:woo00480: Glutathionine metabolism
21. Path:woo00520: Amino sugar and nucleotide sugar metabolism
22. Path:woo00561: Glycerolipid metabolism
23. Path:woo00562: Inositol phosphate metabolism
24. Path:woo00564: Glycerophospholipid metabolism
25. Path:woo00620: Pyruvate metabolism
26. Path:woo00630: Glycoxylate and dicarboxylate metabolism
27. Path:woo00640: Propanoate metabolism
28. Path:woo00650: Butanoate metabolism
29. Path:woo00670: One carbon pool by folate
30. Path:woo00730: Thamine metabolism
31. Path:woo00750: Vitamin B6 metabolism
32. Path:woo00760: Nicotinate and nicotinamide metabolism
33. Path:woo00780: Biotin metabolism
34. Path:woo00785: Lipopoic acid metabolism
35. Path:woo00790: Folate biosynthesis
36. Path:woo00860: Porphyrin and chlorophyll metabolism
37. Path:woo00900: Terpenoid backbone biosynthesis
38. Path:woo00910: Nitrogen metabolism
39. Path:woo00920: Sulfur metabolism
40. Path:woo00970: Amino-acyl tRNA biosynthesis
41. Path:woo01040: Biosynthesis of unsaturated fatty acid
42. Path:woo01100: Metabolic pathways
43. Path:woo01200: Carbon metabolism
44. Path:woo01210: 2-oxocarboxylic acid metabolism
45. Path:woo01212: Fatty acid metabolism
46. Path:woo01230: Biosynthesis of Aminoacids
47. Path:woo02010: ABC transporters
48. Path:woo03010: Ribosome
49. Path:woo03018: RNA degradation
50. Path:woo03020: RNA polymerase
51. Path:woo03030: DNA replication
52. Path:woo03060: Protein export
53. Path:woo03410: Base excision repair
54. Path:woo03420: Nucleotide excision repair
55. Path:woo03430: Mismatch repair
56. Path:woo03440: Homologous recombination
57. Path:woo04122: Sulfur relay system

APPENDIX IV

List of 349 proteins involved with all the pathways of *wOo*

S/N	KEGG ID	Protein name
1	woo:wOo_00040	Dihydrolipoyl dehydrogenase
2	woo:wOo_00100	30S ribosomal protein S9
3	woo:wOo_00110	50S ribosomal protein L13
4	woo:wOo_00120	Outer membrane protein
5	woo:wOo_00150	ABC-type transport system involved in cytochrome c biogenesis permease component
6	woo:wOo_00170	Guanylate kinase
7	woo:wOo_00230	Inorganic pyrophosphatase
8	woo:wOo_00240	Ubiquinone biosynthesis protein COQ7
9	woo:wOo_00310	NADH-quinone oxidoreductase subunit H
10	woo:wOo_00320	NADH-quinone oxidoreductase
11	woo:wOo_00350	Inosine-5'-monophosphate dehydrogenase
12	woo:wOo_00360	Uracil-DNA glycosylase
13	woo:wOo_00420	Leucine--tRNA ligase
14	woo:wOo_00480	ATP synthase subunit a
15	woo:wOo_00490	ATP synthase subunit c
16	woo:wOo_00500	F0F1-type ATP synthase subunit B
17	woo:wOo_00510	F0F1-type ATP synthase subunit B
18	woo:wOo_00530	Heme A synthase (HAS)
19	woo:wOo_00540	Acetylpropionyl-CoA carboxylase alpha subunit
20	woo:wOo_00560	Glutamate--tRNA ligase
21	woo:wOo_00610	2,3,4,5-tetrahydropyridine-2,6-dicarboxylate N-succinyltransferase
22	woo:wOo_00690	ABC-type spermidineputrescine transport system AT Pase component
23	woo:wOo_00710	Sec-independent protein translocase protein TatA
24	woo:wOo_00730	Cysteine--tRNA ligase
25	woo:wOo_00790	Phosphoglycerate kinase
26	woo:wOo_00800	Ferredoxin--NADP reductase
27	woo:wOo_00830	Transaldolase
28	woo:wOo_00840	F0F1-type ATP synthase epsilon subunit
29	woo:wOo_00850	ATP synthase subunit beta
30	woo:wOo_00860	Phosphate transport system permease protein
31	woo:wOo_00940	Ribonucleoside-diphosphate reductase
32	woo:wOo_00970	UvrABC system protein C
33	woo:wOo_01000	Glycine--tRNA ligase beta subunit
34	woo:wOo_01010	Glycine--tRNA ligase alpha subunit
35	woo:wOo_01030	DNA mismatch repair protein MutS
36	woo:wOo_01150	Cytochrome c2

37	woo:wOo_01160	Single-stranded DNA-specific exonuclease RecJ
38	woo:wOo_01180	tRNA dimethylallyltransferase
39	woo:wOo_01240	Bifunctional purine biosynthesis protein PurH
40	woo:wOo_01260	Fumarate hydratase class II
41	woo:wOo_01280	Lysine--tRNA ligase
42	woo:wOo_01290	Type IV secretory pathway VirB3 components
43	woo:wOo_01300	Type IV secretory pathway VirB4 components
44	woo:wOo_01310	Type IV secretory pathway VirB6 components
45	woo:wOo_01320	Type IV secretory pathway VirB6 components
46	woo:wOo_01340	Type IV secretory pathway VirB6 components
47	woo:wOo_01350	Type IV secretory pathway VirB6 components
48	woo:wOo_01420	UDP-N-acetylmuramoylalanine--D-glutamate ligase
49	woo:wOo_01490	D-alanine--D-alanine ligase
50	woo:wOo_01530	30S ribosomal protein S12
51	woo:wOo_01540	30S ribosomal protein S7
52	woo:wOo_01580	Protein translocase subunit SecE
53	woo:wOo_01600	50S ribosomal protein L11
54	woo:wOo_01610	50S ribosomal protein L1
55	woo:wOo_01620	50S ribosomal protein L10
56	woo:wOo_01630	50S ribosomal protein L7/L12
57	woo:wOo_01640	DNA-directed RNA polymerase subunit beta'
58	woo:wOo_01720	ATP synthase gamma chain
59	woo:wOo_01730	DNA mismatch repair protein MutL
60	woo:wOo_01740	N5-carboxyaminoimidazole ribonucleotide mutase
61	woo:wOo_01760	DNA-directed RNA polymerase subunit omega
62	woo:wOo_01880	50S ribosomal protein L25
63	woo:wOo_01940	Phenylalanine--tRNA ligase alpha subunit
64	woo:wOo_02070	NADH-quinone oxidoreductase subunit D
65	woo:wOo_02080	Type IV secretory pathway VirB9 component
66	woo:wOo_02140	Pyridoxine/pyridoxamine 5'-phosphate oxidase
67	woo:wOo_02150	Formamidopyrimidine-DNA glycosylase
68	woo:wOo_02180	Acetyl-CoA carboxylase carboxyltransferase component
69	woo:wOo_02200	Cytochrome c biogenesis ATP-binding export protein CcmA
70	woo:wOo_02220	Aspartate--tRNA(Asp/Asn) ligase
71	woo:wOo_02250	Threonine--tRNA ligase
72	woo:wOo_02260	NADH-quinone oxidoreductase subunit I
73	woo:wOo_02270	Enoyl-[acyl-carrier-protein] reductase [NADH]
74	woo:wOo_02280	Metallo-beta-lactamase superfamily hydrolase
75	woo:wOo_02300	NADH-quinone oxidoreductase subunit F
76	woo:wOo_02310	Endonuclease III
77	woo:wOo_02330	Isocitrate dehydrogenase
78	woo:wOo_02480	DNA helicase
79	woo:wOo_02550	Ribonucleoside-diphosphate reductase subunit beta

80	woo:wOo_02570	Protein translocase subunit SecA
81	woo:wOo_02620	Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex
82	woo:wOo_02760	Trans-2-enoyl-ACP reductase FabK
83	woo:wOo_02790	Type IV secretory pathway VirB4 components
84	woo:wOo_02910	Acetyltransferase component of pyruvate dehydrogenase complex
85	woo:wOo_02930	Tryptophan--tRNA ligase
86	woo:wOo_02950	Geranylgeranyl pyrophosphate synthase
87	woo:wOo_02960	30S ribosomal protein S16
88	woo:wOo_03000	Signal recognition particle receptor FtsY
89	woo:wOo_03050	Signal peptidase I
90	woo:wOo_03170	Flavin prenyltransferase UbiX
91	woo:wOo_03180	Bifunctional protein FolD
92	woo:wOo_03200	Cell division protein FtsI
93	woo:wOo_03220	Proline--tRNA ligase
94	woo:wOo_03240	UDP-N-acetylglucosamine 1-carboxyvinyltransferase
95	woo:wOo_03250	Flavin-dependent thymidylate synthase (FDTS)
96	woo:wOo_03340	Ubiquinol-cytochrome c reductase iron-sulfur subunit
97	woo:wOo_03380	Thymidylate kinase
98	woo:wOo_03470	ABC-type Fe3+ transport system permease component
99	woo:wOo_03490	N5-carboxyaminoimidazole ribonucleotide synthase
100	woo:wOo_03500	Aspartate-semialdehyde dehydrogenase
101	woo:wOo_03590	Signal recognition particle protein
102	woo:wOo_03620	3-hydroxyacyl-[acyl-carrier-protein] dehydratase FabZ
103	woo:wOo_03650	Zinc metalloprotease
104	woo:wOo_03680	30S ribosomal protein S1
105	woo:wOo_03770	Beta sliding clamp
106	woo:wOo_03800	Phospho-N-acetylmuramoyl-pentapeptide-transferase
107	woo:wOo_03810	Ribonuclease HII
108	woo:wOo_03820	Type IV secretory pathway component VirB8
109	woo:wOo_03850	ATPase involved in DNA replication HolB
110	woo:wOo_03870	Preprotein translocase subunit YajC
111	woo:wOo_03930	Phosphoribosylpyrophosphate synthetase
112	woo:wOo_03940	Asp-tRNAAsnGlu-tRNAGln amidotransferase C subunit
113	woo:wOo_04050	Aspartate carbamoyltransferase
114	woo:wOo_04060	ABC-type Fe3+ transport system periplasmic component
115	woo:wOo_04090	NAD kinase
116	woo:wOo_04100	50S ribosomal protein L31
117	woo:wOo_04110	Malonyl CoA-acyl carrier protein transacylase
118	woo:wOo_04120	2,3-bisphosphoglycerate-independent phosphoglycerate mutase
119	woo:wOo_04220	UDP-N-acetylmuramate-alanine ligase

120	woo:wOo_04230	Enolase
121	woo:wOo_04290	Octanoyltransferase
122	woo:wOo_04300	Phosphoribosylformylglycinamidine synthase domain-containing protein
123	woo:wOo_04310	Phosphate transport system permease protein PstA
124	woo:wOo_04320	50S ribosomal protein L19
125	woo:wOo_04340	Phosphoribosylaminoimidazole-succinocarboxamide synthase
126	woo:wOo_04350	Phosphoribosylformylglycinamidine cyclo-ligase
127	woo:wOo_04390	Uroporphyrinogen decarboxylase
128	woo:wOo_04400	Aminotransferase
129	woo:wOo_04430	Serine hydroxymethyltransferase
130	woo:wOo_04520	Serine--tRNA ligase
131	woo:wOo_04530	Phosphoribosylamine--glycine ligase
132	woo:wOo_04550	GMP synthase
133	woo:wOo_04590	Phosphoribosylglycinamide formyltransferase
134	woo:wOo_04620	Lipoprotein signal peptidase
135	woo:wOo_04680	Putative 3-methyladenine DNA glycosylase
136	woo:wOo_04730	UvrABC system protein A
137	woo:wOo_04760	Glycerol-3-phosphate acyltransferase
138	woo:wOo_04790	Phosphatidylserine synthase
139	woo:wOo_04800	Phosphatidylserine decarboxylase proenzyme
140	woo:wOo_04840	Triosephosphate isomerase
141	woo:wOo_04910	NADH dehydrogenase subunit E
142	woo:wOo_04960	Geranylgeranyl pyrophosphate synthase
143	woo:wOo_04980	3-oxoacyl-[acyl-carrier-protein] synthase 2
144	woo:wOo_05000	Glutamyl-tRNA(Gln) amidotransferase subunit A
145	woo:wOo_05030	Dihydroorotate dehydrogenase
146	woo:wOo_05050	Fructose-bisphosphate aldolase
147	woo:wOo_05130	Primosomal protein N'
148	woo:wOo_05140	Succinate dehydrogenase flavoprotein subunit
149	woo:wOo_05160	Dihydroorotase
150	woo:wOo_05180	Glutamate--tRNA ligase
151	woo:wOo_05210	Isoleucine--tRNA liga
152	woo:wOo_05230	Alanine--tRNA ligase
153	woo:wOo_05300	Acetylglutamate kinase
154	woo:wOo_05310	Deoxyuridine 5'-triphosphate nucleotidohydrolase
155	woo:wOo_05410	Succinate dehydrogenase iron-sulfur subunit
156	woo:wOo_05440	Phosphatidylglycerophosphatase A
157	woo:wOo_05450	Glycerol-3-phosphate dehydrogenase
158	woo:wOo_05580	Fructose-16-bisphosphatase
159	woo:wOo_05590	5-aminolevulinate synthase
160	woo:wOo_05670	Phosphatase
161	woo:wOo_05750	tRNA-specific 2-thiouridylase MnmA
162	woo:wOo_05780	Cytochrome c oxidase subunit 2

163	woo:wOo_05790	Cytochrome c oxidase subunit 1
164	woo:wOo_05800	Protoheme IX farnesyltransferase
165	woo:wOo_05810	Ribonuclease H
166	woo:wOo_05830	Sec-independent protein translocase protein TatC
167	woo:wOo_05840	Glyceraldehyde-3-phosphate dehydrogenase GapA
168	woo:wOo_05880	Pyruvate dehydrogenase E1 component subunit beta
169	woo:wOo_05940	Coproporphyrinogen oxidase
170	woo:wOo_05960	Orotidine 5'-phosphate decarboxylase
171	woo:wOo_05980	Valine--tRNA ligase
172	woo:wOo_06020	D-alanyl-D-alanine carboxypeptidase
173	woo:wOo_06030	Phenylalanine--tRNA ligase beta subunit
174	woo:wOo_06050	dCTP deaminase
175	woo:wOo_06080	Replicative DNA helicase
176	woo:wOo_06100	Methionine--tRNA ligase
177	woo:wOo_06110	Ubiquinone biosynthesis O-methyltransferase
178	woo:wOo_06120	DNA primase
179	woo:wOo_06190	60 kDa chaperonin
180	woo:wOo_06310	Periplasmic serine endoprotease DegP-like
181	woo:wOo_06370	Exonuclease III
182	woo:wOo_06380	Uroporphyrinogen-III synthase
183	woo:wOo_06430	DNA polymerase I
184	woo:wOo_06470	Glutamate-cysteine ligase
185	woo:wOo_06490	Ferrochelatase
186	woo:wOo_06530	Nucleoside diphosphate kinase
187	woo:wOo_06540	ABC-type Zn2+ transport system periplasmic component
188	woo:wOo_06550	ABC-type Zn2+ transport systems ATPase component
189	woo:wOo_06570	Orotate phosphoribosyltransferase
190	woo:wOo_06590	Protein translocase subunit SecD
191	woo:wOo_06600	Hydroxymethylbilane synthase
192	woo:wOo_06610	UDP-N-acetylenolpyruvoylglucosamine reductase
193	woo:wOo_06700	Malonyl-CoA decarboxylase
194	woo:wOo_06790	Phosphatidylglycerophosphate synthase
195	woo:wOo_06800	Malic enzyme
196	woo:wOo_06830	Cytochrome b
197	woo:wOo_06840	Cytochrome c1
198	woo:wOo_06850	UvrABC system protein B
199	woo:wOo_06860	DNA polymerase III beta clamp subunit
200	woo:wOo_06870	Phosphoribosylaminoimidazole synthetase
201	woo:wOo_06880	Dihydropteroate synthase putative
202	woo:wOo_06890	Cytosineadenosine deaminase
203	woo:wOo_06920	DNA ligase
204	woo:wOo_06930	5'-nucleotidase SurE
205	woo:wOo_06950	Response regulator PleD
206	woo:wOo_06960	Response regulator PleD

207	woo:wOo_06980	NAD-specific glutamate dehydrogenase
208	woo:wOo_07000	Lipoyl synthase
209	woo:wOo_07010	50S ribosomal protein L28
210	woo:wOo_07050	30S ribosomal protein S4
211	woo:wOo_07060	Transketolase
212	woo:wOo_07120	Protein-export membrane protein SecF
213	woo:wOo_07150	Membrane protein insertase YidC
214	woo:wOo_07230	Type IV secretory pathway VirD4 component
215	woo:wOo_07240	Type IV secretory pathway VirB11 component
216	woo:wOo_07250	Type IV secretory pathway VirB10 component
217	woo:wOo_07260	Type IV secretory pathway component VirB9
218	woo:wOo_07270	Type IV secretory pathway component VirB8
219	woo:wOo_07280	GTP cyclohydrolase II
220	woo:wOo_07350	Phosphoribosylformylglycinamidine synthase subunit PurL
221	woo:wOo_07380	Amidophosphoribosyltransferase (ATase)
222	woo:wOo_07400	Histidine--tRNA ligase
223	woo:wOo_07450	DNA polymerase III subunit gamma/tau
224	woo:wOo_07480	Glutathione S-transferase
225	woo:wOo_07580	UDP-N-acetylmuramoylalanine-D-glutamate ligase
226	woo:wOo_07600	Aspartokinase
227	woo:wOo_07630	Tyrosine--tRNA ligase
228	woo:wOo_07700	RNA pyrophosphohydrolase
229	woo:wOo_07720	Malate dehydrogenase
230	woo:wOo_07730	NADH-quinone oxidoreductase subunit C
231	woo:wOo_07740	NADH-quinone oxidoreductase subunit B
232	woo:wOo_07750	NADH-quinone oxidoreductase subunit A
233	woo:wOo_07780	UDP-N-acetylmuramoyl-tripeptide--D-alanyl-D-alanine ligase
234	woo:wOo_07810	Ribulose-phosphate 3-epimerase
235	woo:wOo_07820	Polyribonucleotide nucleotidyltransferase
236	woo:wOo_07830	30S ribosomal protein S15
237	woo:wOo_07870	Cysteine sulfinate desulfinasecysteine desulfurase
238	woo:wOo_07900	Peptide ABC transporter ATPase
239	woo:wOo_07920	Deoxyguanosinetriphosphate triphosphohydrolase-like protein
240	woo:wOo_07970	3-oxoacyl-[acyl-carrier-protein] synthase 3
241	woo:wOo_07980	Phosphate acyltransferase
242	woo:wOo_08050	Biotin-acetyl-CoA carboxylase-ligase
243	woo:wOo_08060	NADH-quinone oxidoreductase subunit N
244	woo:wOo_08070	NADH ubiquinone oxidoreductase chain M
245	woo:wOo_08080	NADH ubiquinone oxidoreductase chain L
246	woo:wOo_08090	NADH-quinone oxidoreductase subunit K

247	woo:wOo_08100	NADH ubiquinone oxidoreductase chain J
248	woo:wOo_08150	Heme exporter protein C
249	woo:wOo_08200	Probable cytosol aminopeptidase
250	woo:wOo_08210	Transcription termination factor Rho
251	woo:wOo_08240	4-hydroxybenzoate octaprenyltransferase
252	woo:wOo_08290	Bifunctional protein PutA
253	woo:wOo_08300	Aconitate hydratase
254	woo:wOo_08310	Cysteine desulfurase IscS
255	woo:wOo_08340	Delta-aminolevulinic acid dehydratase
256	woo:wOo_08350	4-hydroxy-tetrahydrodipicolinate synthase
257	woo:wOo_08360	Single-stranded DNA-binding protein
258	woo:wOo_08450	Short-chain alcohol dehydrogenase family enzyme
259	woo:wOo_08510	ATP synthase subunit alpha
260	woo:wOo_08520	ATP synthase subunit delta
261	woo:wOo_08560	Ubiquinone/menaquinone biosynthesis C-methyltransferase UbiE
262	woo:wOo_08630	Arginine--tRNA ligase
263	woo:wOo_08670	Pyruvate dehydrogenase E1 component subunit alpha
264	woo:wOo_08710	ABC-type phosphate transport system ATPase component
265	woo:wOo_08720	4-hydroxy-tetrahydrodipicolinate reductase
266	woo:wOo_08740	DNA polymerase III delta subunit
267	woo:wOo_08750	ABC-type Mn2+Zn2+ transport system permease component
268	woo:wOo_08770	4-diphosphocytidyl-2-C-methyl-D-erythritol kinase
269	woo:wOo_08800	Preprotein translocase subunit SecG
270	woo:wOo_08810	CTP synthase
271	woo:wOo_08850	UDP-N-acetylmuramoyl-L-alanyl-D-glutamate--2,6-diaminopimelate ligase
272	woo:wOo_08860	Cystathionine beta-lyase
273	woo:wOo_08890	Cytochrome c oxidase subunit 3
274	woo:wOo_08930	S-adenosylmethionine synthetase
275	woo:wOo_08960	50S ribosomal protein L27
276	woo:wOo_08970	50S ribosomal protein L21
277	woo:wOo_09010	Aspartyl/glutamyl-tRNA
278	woo:wOo_09020	Chaperone protein DnaK
279	woo:wOo_09030	Ribonuclease RneRng family protein
280	woo:wOo_09040	50S ribosomal protein L33
281	woo:wOo_09130	Adenylosuccinate lyase
282	woo:wOo_09140	50S ribosomal protein L9
283	woo:wOo_09150	30S ribosomal protein S18
284	woo:wOo_09160	30S ribosomal protein S6
285	woo:wOo_09170	DNA-directed DNA polymerase
286	woo:wOo_09260	Diaminopimelate epimerase
287	woo:wOo_09270	Succinate--CoA ligase [ADP-forming] subunit alpha
288	woo:wOo_09280	Succinate--CoA ligase [ADP-forming] subunit beta

289	woo:wOo_09290	30S ribosomal protein S21
290	woo:wOo_09300	Protein-export protein SecB
291	woo:wOo_09320	DNA polymerase III subunit epsilon
292	woo:wOo_09340	Citrate synthase
293	woo:wOo_09350	30S ribosomal protein S20
294	woo:wOo_09360	1-acyl-sn-glycerol-3-phosphate acyltransferase
295	woo:wOo_09490	Glutathione synthetase
296	woo:wOo_09500	UDP-N-acetylglucosamine--N-acetylmuramyl-(pentapeptide) pyrophosphoryl-undecaprenol N-acetylglucosamine transferase
297	woo:wOo_09510	Dihydrolipoyl dehydrogenase
298	woo:wOo_09540	Alpha-ketoglutarate decarboxylase
299	woo:wOo_09580	3-polyprenyl-4-hydroxybenzoate decarboxylase UbiD
300	woo:wOo_09610	Chromosomal replication initiator protein DnaA
301	woo:wOo_09620	Glutamine synthetase
202	woo:wOo_09640	Adenylosuccinate synthetase
303	woo:wOo_09670	2-polyprenyl-6-methoxyphenol hydroxylase FAD-dependent oxidoreductase
304	woo:wOo_09720	tRNA nucleotidyltransferasepolyA-polymerase
305	woo:wOo_09770	Fructose-1,6-bisphosphatase
306	woo:wOo_09800	Pyruvate, phosphate dikinase
307	woo:wOo_09910	Phosphatidate cytidylyltransferase
308	woo:wOo_09920	Isoprenyl transferase
309	woo:wOo_09950	Uridylate kinase (UK)
310	woo:wOo_09970	30S ribosomal protein S2
311	woo:wOo_09990	Carbamoyl-phosphate synthase large chain
312	woo:wOo_10050	Phosphomannomutase
313	woo:wOo_10080	ABC-type transport system involved in lipoprotein release permease component
314	woo:wOo_10110	Succinate dehydrogenase subunit D sdhD hydrophobi c membrane anchor protein
315	woo:wOo_10120	Succinate dehydrogenase subunit C sdhC
316	woo:wOo_10190	Cytochrome c oxidase assembly protein CtaG
317	woo:wOo_10220	ABC-type phosphate transport system periplasmic component
318	woo:wOo_10230	50S ribosomal protein L17
319	woo:wOo_10240	DNA-directed RNA polymerase subunit alpha
320	woo:wOo_10250	30S ribosomal protein S11
321	woo:wOo_10260	30S ribosomal protein S13
322	woo:wOo_10270	Adenylate kinase (AK)
323	woo:wOo_10300	Protein translocase subunit SecY
324	woo:wOo_10310	50S ribosomal protein L15
325	woo:wOo_10320	30S ribosomal protein S5
326	woo:wOo_10330	50S ribosomal protein L18
327	woo:wOo_10340	50S ribosomal protein L6
328	woo:wOo_10350	30S ribosomal protein S8

329	woo:wOo_10360	30S ribosomal protein S14
330	woo:wOo_10370	50S ribosomal protein L5
331	woo:wOo_10380	50S ribosomal protein L24
332	woo:wOo_10390	50S ribosomal protein L14
333	woo:wOo_10400	30S ribosomal protein S17
334	woo:wOo_10410	50S ribosomal protein L29
335	woo:wOo_10420	50S ribosomal protein L16
336	woo:wOo_10430	0S ribosomal protein S3
337	woo:wOo_10440	50S ribosomal protein L22
338	woo:wOo_10450	30S ribosomal protein S19
339	woo:wOo_10460	50S ribosomal protein L2
340	woo:wOo_10470	50S ribosomal protein L23
341	woo:wOo_10480	50S ribosomal protein L4
342	woo:wOo_10490	50S ribosomal protein L3
343	woo:wOo_10500	30S ribosomal protein S10
344	woo:wOo_10540	Carbamoyl-phosphate synthase small chain
345	woo:wOo_10560	Methionyl-tRNA formyltransferase
346	woo:wOo_10570	50S ribosomal protein L20
347	woo:wOo_10580	50S ribosomal protein L35
348	woo:wOo_10750	Succinyl-diaminopimelate desuccinylase
349	woo:wOo_10770	Thioredoxin reductase

APPENDIX V

Non-homologous proteins to cattle proteins

S/N	KEGG ID	Name of protein
1	wOo_00120	Outer membrane protein
2	wOo_00150	ABC-type transport system involved in cytochrome c biogenesis permease component
3	wOo_00230	**Inorganic pyrophosphatase**
4	wOo_00310	**NADH-quinone oxidoreductase subunit H**
5	wOo_00360	Uracil-DNA glycosylase
6	wOo_00420	**Leucine--tRNA ligase**
7	wOo_00500	F0F1-type ATP synthase subunit B
8	wOo_00510	F0F1-type ATP synthase subunit B
9	wOo_00610	**2,3,4,5-tetrahydropyridine-2,6-dicarboxylate N-succinyltransferase**
10	wOo_00710	**Sec-independent protein translocase protein TatA**
11	wOo_00800	**Ferredoxin--NADP reductase, FNR, Fd-NADP(+) reductase**
12	wOo_00840	F0F1-type ATP synthase epsilon subunit
13	wOo_00860	**Phosphate transport system permease protein**

14	wOo_00940	**Ribonucleoside-diphosphate reductase**
15	wOo_00970	**UvrABC system protein C**
16	wOo_01000	**Glycine--tRNA ligase beta subunit**
17	wOo_01010	**Glycine--tRNA ligase alpha subunit**
18	wOo_01160	Single-stranded DNA-specific exonuclease RecJ
19	wOo_01180	**tRNA dimethylallyltransferase**
20	wOo_01260	**Fumarate hydratase class II**
21	wOo_01280	**Lysine--tRNA ligase**
22	wOo_01290	Type IV secretory pathway VirB3 components
23	wOo_01300	Type IV secretory pathway VirB4 components
24	wOo_01310	Type IV secretory pathway VirB6 components
25	wOo_01320	Type IV secretory pathway VirB6 components
26	wOo_01340	Type IV secretory pathway VirB6 components
27	wOo_01350	Type IV secretory pathway VirB6 components
28	wOo_01420	**UDP-N-acetylmuramoylalanine--D-glutamate ligase**
29	wOo_01490	**D-alanine--D-alanine ligase**
30	wOo_01580	**Protein translocase subunit SecE**
31	wOo_01610	50S ribosomal protein L1
32	wOo_01620	**50S ribosomal protein L10**
33	wOo_01730	**DNA mismatch repair protein MutL**
34	wOo_01740	**N5-carboxyaminoimidazole ribonucleotide mutase**
35	wOo_01760	**DNA-directed RNA polymerase subunit omega, RNAP omega subunit**
36	wOo_01880	**50S ribosomal protein L25**
37	wOo_01940	**Phenylalanine--tRNA ligase alpha subunit**
38	wOo_02080	Type IV secretory pathway VirB9 component
39	wOo_02150	**Formamidopyrimidine-DNA glycosylase, Fapy-DNA glycosylase**
40	wOo_02280	Metallo-beta-lactamase superfamily hydrolase
41	wOo_02480	**DNA helicase**
42	wOo_02550	**Ribonucleoside-diphosphate reductase subunit beta**
43	wOo_02570	**Protein translocase subunit SecA**
44	wOo_02760	Trans-2-enoyl-ACP reductase FabK
45	wOo_02790	Type IV secretory pathway VirB4 components
46	wOo_03050	**Signal peptidase I**
47	wOo_03170	**Flavin prenyltransferase UbiX**
48	wOo_03200	Cell division protein FtsI
49	wOo_03220	**Proline--tRNA ligase**
50	wOo_03240	**UDP-N-acetylglucosamine 1-carboxyvinyltransferase**
51	wOo_03250	**Flavin-dependent thymidylate synthase, FDTS**
52	wOo_03380	**Thymidylate kinase**
53	wOo_03470	ABC-type Fe3+ transport system permease component
54	wOo_03490	**N5-carboxyaminoimidazole ribonucleotide synthase, N5-CAIR synthase**
55	wOo_03500	**Aspartate-semialdehyde dehydrogenase, ASA dehydrogenase**
56	wOo_03620	**3-hydroxyacyl-[acyl-carrier-protein] dehydratase FabZ**

57	wOo_03650	**Zinc metalloprotease**
58	wOo_03680	30S ribosomal protein S1
59	wOo_03770	**Beta sliding clamp**
60	wOo_03800	**Phospho-N-acetylmuramoyl-pentapeptide-transferase**
61	wOo_03810	**Ribonuclease HII, RNase HII**
62	wOo_03820	Type IV secretory pathway component VirB8
63	wOo_03850	ATPase involved in DNA replication HolB
64	wOo_03870	Preprotein translocase subunit YajC
65	wOo_03940	Asp-tRNAAsnGlu-tRNAGln amidotransferase C subunit
66	wOo_04050	**Aspartate carbamoyltransferase**
67	wOo_04060	ABC-type Fe3+ transport system periplasmic component
68	wOo_04090	**NAD kinase**
69	wOo_04100	**50S ribosomal protein L31**
70	wOo_04110	**Malonyl CoA-acyl carrier protein transacylase**
71	wOo_04120	**2,3-bisphosphoglycerate-independent phosphoglycerate mutase, BPG-independent PGAM, Phosphoglyceromutase, iPGM**
72	wOo_04220	UDP-N-acetylmuramate-alanine ligase
73	wOo_04290	**Octanoyltransferase**
74	wOo_04300	Phosphoribosylformylglycinamidine synthase domain-containing protein
75	wOo_04310	**Phosphate transport system permease protein PstA**
76	wOo_04320	**50S ribosomal protein L19**
77	wOo_04340	**Phosphoribosylaminoimidazole-succinocarboxamide synthase**
78	wOo_04390	**Uroporphyrinogen decarboxylase**
79	wOo_04550	**GMP synthase (glutamine-hydrolyzing)**
80	wOo_04620	**Lipoprotein signal peptidase**
81	wOo_04680	**Putative 3-methyladenine DNA glycosylase**
82	wOo_04730	**UvrABC system protein A**
83	wOo_04760	**Glycerol-3-phosphate acyltransferase**
84	wOo_04790	Phosphatidylserine synthase
85	wOo_04800	**Phosphatidylserine decarboxylase proenzyme**
86	wOo_04960	Geranylgeranyl pyrophosphate synthase
87	wOo_05050	Fructose-bisphosphate aldolase
88	wOo_05130	**Primosomal protein N'**
89	wOo_05160	**Dihydroorotase, DHOase**
90	wOo_05210	**Isoleucine--tRNA ligase**
91	wOo_05230	**Alanine--tRNA ligase**
92	wOo_05300	Acetylglutamate kinase
93	wOo_05310	**Deoxyuridine 5'-triphosphate nucleotidohydrolase**
94	wOo_05440	**Phosphatidylglycerophosphatase A**
95	wOo_05580	Fructose-16-bisphosphatase
96	wOo_05670	Phosphatase
97	wOo_05750	**tRNA-specific 2-thiouridylase MnmA**
98	wOo_05800	**Protoheme IX farnesyltransferase**
99	wOo_05810	**Ribonuclease H, RNase H**
100	wOo_05830	**Sec-independent protein translocase protein TatC**

101	wOo_05940	**Coproporphyrinogen oxidase**
102	wOo_05960	**Orotidine 5'-phosphate decarboxylase**
103	wOo_05980	**Valine--tRNA ligase**
104	wOo_06020	D-alanyl-D-alanine carboxypeptidase
105	wOo_06030	**Phenylalanine--tRNA ligase beta subunit**
106	wOo_06050	**dCTP deaminase**
107	wOo_06080	**Replicative DNA helicase**
108	wOo_06120	**DNA primase**
109	wOo_06380	Uroporphyrinogen-III synthase
110	wOo_06430	**DNA polymerase I**
111	wOo_06470	Glutamate-cysteine ligase
112	wOo_06540	ABC-type Zn2+ transport system periplasmic component
113	wOo_06590	**Protein translocase subunit SecD**
114	wOo_06610	**UDP-N-acetylenolpyruvoylglucosamine reductase**
115	wOo_06700	Malonyl-CoA decarboxylase
116	wOo_06790	Phosphatidylglycerophosphate synthase
117	wOo_06800	Malic enzyme
118	wOo_06850	**UvrABC system protein B, Protein UvrB**
119	wOo_06860	DNA polymerase III beta clamp subunit
120	wOo_06870	Phosphoribosylaminoimidazole synthetase
121	wOo_06880	Dihydropteroate synthase putative
122	wOo_06890	Cytosineadenosine deaminase
123	wOo_06920	**DNA ligase**
124	wOo_06930	**5'-nucleotidase SurE**
125	wOo_06950	Response regulator PleD
126	wOo_06960	Response regulator PleD
127	wOo_06980	NAD-specific glutamate dehydrogenase
128	wOo_07010	**50S ribosomal protein L28**
129	wOo_07050	**30S ribosomal protein S4**
130	wOo_07120	**Protein-export membrane protein SecF**
131	wOo_07150	**Membrane protein insertase YidC**
132	wOo_07230	Type IV secretory pathway VirD4 component
133	wOo_07240	Type IV secretory pathway VirB11 component
134	wOo_07250	Type IV secretory pathway VirB10 component
135	wOo_07260	Type IV secretory pathway component VirB9
136	wOo_07270	Type IV secretory pathway component VirB8
137	wOo_07280	GTP cyclohydrolase II
138	wOo_07350	**Phosphoribosylformylglycinamidine synthase subunit PurL, FGAM synthase**
139	wOo_07450	**DNA polymerase III subunit gamma/tau, EC 2.7.7.7**
140	wOo_07600	**Aspartokinase**
141	wOo_07630	**Tyrosine--tRNA ligase**
142	wOo_07700	**RNA pyrophosphohydrolase**
143	wOo_07780	**UDP-N-acetylmuramoyl-tripeptide--D-alanyl-D-alanine ligase**
144	wOo_07810	**Ribulose-phosphate 3-epimerase**

145	wOo_07820	**Polyribonucleotide nucleotidyltransferase**
146	wOo_07830	**30S ribosomal protein S15**
147	wOo_07920	**Deoxyguanosinetriphosphate triphosphohydrolase-like protein**
148	wOo_07970	**3-oxoacyl-[acyl-carrier-protein] synthase 3**
149	wOo_07980	**Phosphate acyltransferase, EC 2.3.1.274**
150	wOo_08050	Biotin-acetyl-CoA carboxylase-ligase
151	wOo_08090	**NADH-quinone oxidoreductase subunit K**
152	wOo_08100	**NADH-quinone oxidoreductase subunit J**
153	wOo_08150	**Heme exporter protein C**
154	wOo_08210	**Transcription termination factor Rho**
155	wOo_08720	**4-hydroxy-tetrahydrodipicolinate reductase, HTPA reductase**
156	wOo_08740	DNA polymerase III delta subunit
157	wOo_08750	ABC-type Mn2+Zn2+ transport system permease component
158	wOo_08770	**4-diphosphocytidyl-2-C-methyl-D-erythritol kinase, CMK**
159	wOo_08800	**Protein-export membrane protein SecG**
160	wOo_08850	**UDP-N-acetylmuramoyl-L-alanyl-D-glutamate--2,6-diaminopimelate ligase**
161	wOo_08970	**50S ribosomal protein L21**
162	wOo_09010	**Aspartyl/glutamyl-tRNA(Asn/Gln) amidotransferase subunit B, Asp/Glu-ADT subunit B**
163	wOo_09030	Ribonuclease RneRng family protein
164	wOo_09040	**50S ribosomal protein L33**
165	wOo_09130	**Adenylosuccinate lyase, ASL**
166	wOo_09140	**50S ribosomal protein L9**
167	wOo_09160	**30S ribosomal protein S6**
168	wOo_09170	**DNA-directed DNA polymerase**
169	wOo_09260	**Diaminopimelate epimerase, DAP epimerase**
170	wOo_09290	30S ribosomal protein S21
171	wOo_09300	**Protein-export protein SecB**
172	wOo_09320	**DNA polymerase III subunit epsilon**
173	wOo_09350	**30S ribosomal protein S20**
174	wOo_09490	**Glutathione synthetase**
175	wOo_09500	**UDP-N-acetylglucosamine--N-acetylmuramyl-(pentapeptide) pyrophosphoryl-undecaprenol N-acetylglucosamine transferase**
176	wOo_09580	3-polyprenyl-4-hydroxybenzoate decarboxylase UbiD
177	wOo_09610	**Chromosomal replication initiator protein DnaA**
178	wOo_09620	Glutamine synthetase
179	wOo_09720	tRNA nucleotidyltransferasepolyA-polymerase
180	wOo_09770	**Fructose-1,6-bisphosphatase**
181	wOo_09800	**Pyruvate, phosphate dikinase**
182	wOo_09920	**Isoprenyl transferase**
183	wOo_09950	**Uridylate kinase, UK**
184	wOo_09990	**Carbamoyl-phosphate synthase large chain**
185	wOo_10050	Phosphomannomutase
186	wOo_10080	ABC-type transport system involved in lipoprotein release permease

		component
187	wOo_10110	Succinate dehydrogenase subunit D sdhD hydrophobi c membrane anchor protein
188	wOo_10220	ABC-type phosphate transport system periplasmic component
189	wOo_10240	**DNA-directed RNA polymerase subunit alpha, RNAP subunit alpha**
190	wOo_10300	**Protein translocase subunit SecY**
191	wOo_10320	**30S ribosomal protein S5**
192	wOo_10330	**50S ribosomal protein L18**
193	wOo_10340	**50S ribosomal protein L6**
194	wOo_10350	**30S ribosomal protein S8**
195	wOo_10400	**30S ribosomal protein S17**
196	wOo_10410	**50S ribosomal protein L29**
197	wOo_10420	**50S ribosomal protein L16**
198	wOo_10430	**30S ribosomal protein S3**
199	wOo_10440	**50S ribosomal protein L22**
200	wOo_10470	**50S ribosomal protein L23**
201	wOo_10500	**30S ribosomal protein S10**
202	wOo_10540	**Carbamoyl-phosphate synthase small chain**
203	wOo_10580	**50S ribosomal protein L35**
204	wOo_10770	**Thioredoxin reductase**

APPENDIX VI

Results from GnegmPloc, CELLO server and molecular weight of proteins

S/N	KEGG ID	Name of protein	Subcellular localisation of proteins (Gneg mPloc)	Subcellular localisation of proteins (CELLO server)	Molecular Weight (kd)
1	wOo_00230	**Inorganic pyrophosphatase**	**Cytoplasm**	**Cytoplasm**	18. 14
2	wOo_00310	**NADH-quinone oxidoreductase subunit H**	**Cell inner membrane**	**Inner membrane**	**37.99**
3	wOo_00360	Uracil-DNA glycosylase	Cytoplasm	Cytoplasm	29.71
4	wOo_00420	**Leucine--tRNA ligase**	**Cytoplasm**	**Cytoplasm**	**97.32**
5	wOo_00500	F0F1-type ATP synthase subunit B	Cell inner membrane	Cytoplasm	18.61
6	wOo_00610	**2,3,4,5-tetrahydropyridine-2,6-dicarboxylate N-succinyltransferase**	**Cytoplasm**	**Cytoplasm**	**30.58**
7	wOo_00710	**Sec-independent protein translocase protein TatA**	**Cell inner membrane**	**Cytoplasm/periplasm**	**6.03**
8	wOo_00800	**Ferredoxin--NADP reductase, FNR, Fd-NADP(+) reductase**	**Cytoplasm**	**Cytoplasm/ periplasm/inner membrane**	**37.74**
9	wOo_00840	F0F1-type ATP synthase epsilon subunit	Cell inner membrane	Cytoplasm	12.69
10	wOo_00860	**Phosphate transport system permease protein**	Cell inner membrane	Inner membrane	39.98
11	wOo_00940	**Ribonucleoside-diphosphate reductase**	**Cytoplasm**	**Cytoplasm**	**68.99**
12	wOo_00970	**UvrABC system protein C**	**Cytoplasm**	**Outer membrane/Cytoplasm**	**63.46**
13	wOo_01000	**Glycine--tRNA ligase beta subunit**	**Cytoplasm**	**Cytoplasm**	**68.24**
14	wOo_01010	**Glycine--tRNA ligase alpha subunit**	**Cytoplasm**	**Cytoplasm**	31.55
15	wOo_01160	Single-stranded DNA-specific exonuclease RecJ	**Cytoplasm**	**Inner membrane**	**63.8**1
16	wOo_01180	**tRNA dimethylallyltransferase**	**Cell inner membrane**	**Cytoplasm**	**37.73**

17	wOo_01260	**Fumarate hydratase class II**	**Cytoplasm**	**Cytoplasm**	**50.75**
18	wOo_01280	**Lysine--tRNA ligase**	**Cytoplasm**	**Cytoplasm**	**59.93**
19	wOo_01420	**UDP-N-acetylmuramoylalanine--D-glutamate ligase**	**Cytoplasm**	**Cytoplasm**	**49.4**
20	wOo_01490	**D-alanine--D-alanine ligase**	**Cytoplasm**	**Cytoplasm**	**34.** 1
21	wOo_01580	**Protein translocase subunit SecE**	**Cell inner membrane**	**Cytoplasm**	**7.55**
22	wOo_01610	50S ribosomal protein L1	Cell inner membrane/ **Cytoplasm**	**Cytoplasm**	**24.** 1
23	wOo_01620	**50S ribosomal protein L10**	**Cell inner membrane/ Cytoplasm**	**Periplasm/Inner membrane/ Cytoplasm**	19.31
24	wOo_01730	**DNA mismatch repair protein MutL**	**Cell inner membrane/ Cytoplasm**	**Cytoplasm**	19.39
25	wOo_01740	**N5-carboxyaminoimidazole ribonucleotide mutase**	**Cell inner membrane/ Cytoplasm**	**Inner membrane/ Cytoplasm**	17.22
26	wOo_01760	**DNA-directed RNA polymerase subunit omega, RNAP omega subunit**	**Cell inner membrane/ Cytoplasm**	**Extracellular/ Cytoplasm**	14.44
27	wOo_01880	**50S ribosomal protein L25**	**Cell inner membrane**	**Cytoplasm**	**22.6**
28	wOo_01940	**Phenylalanine--tRNA ligase alpha subunit**	**Cytoplasm**	**Cytoplasm**	**40.** 1
29	wOo_02150	**Formamidopyrimidine-DNA glycosylase, Fapy-DNA glycosylase**	**Cell inner membrane/ Cytoplasm**	**Cytoplasm**	**3**1.08
30	wOo_02280	Metallo-beta-lactamase superfamily hydrolase	Cytoplasm	Cytoplasm	60.79
31	wOo_02480	**DNA helicase**	**Cell inner membrane**	**Cytoplasm**	**73.36**
32	wOo_02550	**Ribonucleoside-diphosphate reductase subunit beta**	**Cell inner membrane/ Cytoplasm**	**Cytoplasm**	**37.98**
33	wOo_02570	**Protein translocase subunit SecA**	**Cytoplasm**	**Cytoplasm**	101.89
34	wOo_02760	Trans-2-enoyl-ACP reductase FabK	**Cytoplasm**	Inner membrane	37.27
35	wOo_03050	**Signal peptidase I**	**Cell inner membrane**	**Cytoplasm**	**29.46**
36	wOo_03170	**Flavin prenyltransferase UbiX**	**Cell inner membrane**	**Cytoplasm**	**22.92**

37	wOo_03200	Cell division protein FtsI	Cell inner membrane	Outer membrane	59.05
38	wOo_03220	**Proline--tRNA ligase**	**Cytoplasm**	**Cytoplasm**	**48.56**
39	wOo_03240	**UDP-N-acetylglucosamine 1-carboxyvinyltransferase**	**Cytoplasm**	**Cytoplasm**	**46.** 17
40	wOo_03250	**Flavin-dependent thymidylate synthase, FDTS**	Cell inner membrane/ Cytoplasm	Cytoplasm	34. 15
41	wOo_03380	**Thymidylate kinase**	**Cytoplasm**	**Cytoplasm**	**23.5**
42	wOo_03470	ABC-type Fe3+ transport system permease component	Cell inner membrane	Inner membrane	59.74
43	wOo_03490	**N5-carboxyaminoimidazole ribonucleotide synthase, N5-CAIR synthase**	Cell inner membrane/ Cytoplasm	Cytoplasm	40.04
44	wOo_03500	**Aspartate-semialdehyde dehydrogenase, ASA dehydrogenase**	**Cytoplasm**	**Cytoplasm**	**38.56**
45	wOo_03620	**3-hydroxyacyl-[acyl-carrier-protein] dehydratase FabZ**	**Cytoplasm**	**Cytoplasm**	16.21
46	wOo_03650	**Zinc metalloprotease**	Cell inner membrane	Inner membrane	42.78
47	wOo_03680	30S ribosomal protein S1	Cytoplasm	Cytoplasm	61.5
48	wOo_03770	**Beta sliding clamp**	**Cytoplasm**	**Cytoplasm**	**42.76**
49	wOo_03800	**Phospho-N-acetylmuramoyl-pentapeptide-transferase**	Cell inner membrane	Inner membrane	36.63
50	wOo_03810	**Ribonuclease HII, RNase HII**	Cell inner membrane/ Cytoplasm	Periplasm/ Cytoplasm	22.25
51	wOo_03850	ATPase involved in DNA replication HolB	Cytoplasm	Cytoplasm	31.33
52	wOo_03870	Preprotein translocase subunit YajC	Cell inner membrane	Periplasm/Cytoplasm	15.84
53	wOo_03940	Asp-tRNAAsnGlu-tRNAGln amidotransferase C subunit	Cell inner membrane	Cytoplasm	13.43
54	wOo_04050	**Aspartate carbamoyltransferase**	**Cytoplasm**	**Cytoplasm**	**33.38**
55	wOo_04060	ABC-type Fe3+ transport system periplasmic component	Periplasm	Outer membrane	38.32

56	wOo_04090	**NAD kinase**	Cell inner membrane/ Cytoplasm	Outer membrane/ Cytoplasm	29.88
57	wOo_04100	**50S ribosomal protein L31**	Cell inner membrane/ Cytoplasm	Cytoplasm	7.96
58	wOo_04110	**Malonyl CoA-acyl carrier protein transacylase**	**Cytoplasm**	**Outer membrane**	**35.88**
59	wOo_04120	**2,3-bisphosphoglycerate-independent phosphoglycerate mutase, BPG-independent PGAM, Phosphoglyceromutase, iPGM**	**Cytoplasm/Periplasm**	**Cytoplasm**	**55.72**
60	wOo_04220	UDP-N-acetylmuramate-alanine ligase	Cytoplasm	Cytoplasm	53.2
61	wOo_04290	**Octanoyltransferase**	**Cytoplasm**	**Cytoplasm**	**23.85**
62	wOo_04300	Phosphoribosylformylglycinamidine synthase domain-containing protein	Cytoplasm	Cytoplasm	29.9
63	wOo_04310	**Phosphate transport system permease protein PstA**	Cell inner membrane	Inner membrane	46.62
64	wOo_04320	**50S ribosomal protein L19**	Cell inner membrane/ Cytoplasm	Cytoplasm	14.4
65	wOo_04340	**Phosphoribosylaminoimidazole-succinocarboxamide synthase**	Cell inner membrane/ Cytoplasm	Cytoplasm	27.76
66	wOo_04390	**Uroporphyrinogen decarboxylase**	**Cell inner membrane/ Cytoplasm**	**Cytoplasm**	**37.85**
67	wOo_04550	**GMP synthase (glutamine-hydrolyzing)**	**Cytoplasm**	**Cytoplasm**	**58.** 16
68	wOo_04620	**Lipoprotein signal peptidase**	Cell inner membrane	Inner membrane	17.77
69	wOo_04730	**UvrABC system protein A**	Cell inner membrane	Outer membrane	104.91
70	wOo_04760	**Glycerol-3-phosphate acyltransferase**	Cell inner membrane	Inner membrane	21.26
71	wOo_04790	Phosphatidylserine synthase	Cell inner membrane	Inner membrane	29.26
72	wOo_04800	**Phosphatidylserine**	Cell inner membrane	Outer membrane, periplasm,	26. 12

		decarboxylase proenzyme		cytoplasm	
73	wOo_04960	Geranylgeranyl pyrophosphate synthase	Cytoplasm	Periplasm, Cytoplasm	27.22
74	wOo_05130	**Primosomal protein N'**	**Cytoplasm**	**Cytoplasm, Inner membrane**	**84.99**
75	wOo_05160	**Dihydroorotase, DHOase**	**Cytoplasm**	**Cytoplasm**	**48.55**
76	wOo_05210	**Isoleucine--tRNA ligase**	**Cytoplasm**	**Cytoplasm**	127.42
77	wOo_05230	**Alanine--tRNA ligase**	**Cytoplasm**	**Cytoplasm**	102.6
78	wOo_05300	Acetylglutamate kinase	Cytoplasm	Cytoplasm	33.95
79	wOo_05310	**Deoxyuridine 5'-triphosphate nucleotidohydrolase**	Cell inner membrane	Cytoplasm	17.03
80	wOo_05440	**Phosphatidylglycerophosphatase A**	Cell inner membrane	Inner membrane	18.03
81	wOo_05580	Fructose-16-bisphosphatase	Cell inner membrane/ Cytoplasm	Cytoplasm	27.89
82	wOo_05670	Phosphatase	Cytoplasm	Outermembrane/Cytoplasm	25. 17
83	wOo_05750	**tRNA-specific 2-thiouridylase MnmA**	**Cytoplasm**	**Cytoplasm**	41.26
84	wOo_05800	**Protoheme IX farnesyltransferase**	Cell inner membrane	Inner membrane	33.25
85	wOo_05810	**Ribonuclease H, RNase H**	Cell inner membrane	**Cytoplasm**	16.55
86	wOo_05830	**Sec-independent protein translocase protein TatC**	Cell inner membrane	Inner membrane	29.48
87	wOo_05940	**Coproporphyrinogen oxidase**	**Cytoplasm**	**Cytoplasm**	**32**
88	wOo_05960	**Orotidine 5'-phosphate decarboxylase**	Cell inner membrane/ Cytoplasm	Cytoplasm	24.33
89	wOo_05980	**Valine--tRNA ligase**	**Cytoplasm**	**Cytoplasm**	**98.68**
90	wOo_06020	D-alanyl-D-alanine carboxypeptidase	Cell inner membrane	Inner membrane/ Cytoplasm	42.26
91	wOo_06030	**Phenylalanine--tRNA ligase beta subunit**	**Cytoplasm**	**Cytoplasm/Outer membrane**	**88.6**
92	wOo_06050	**dCTP deaminase**	Cell inner membrane/Cytoplasm	Periplasm/Cytoplasm	20.7
93	wOo_06080	**Replicative DNA helicase**	**Cytoplasm**	**Cytoplasm**	**54.7**

94	wOo_06120	**DNA primase**	Cell inner membrane/ Cytoplasm	Outer membrane/ Cytoplasm	68.52
95	wOo_06380	Uroporphyrinogen-III synthase	Cell inner membrane	Outer membrane/ Cytoplasm	26.64
96	wOo_06430	**DNA polymerase I**	**Cytoplasm**	**Cytoplasm**	**98.34**
97	wOo_06540	ABC-type Zn2+ transport system periplasmic component	Periplasm	Periplasm/Outermembrane/Cytoplasm	32. 14
98	wOo_06590	**Protein translocase subunit SecD**	Cell inner membrane	Inner membrane	55.21
99	wOo_06610	**UDP-N-acetylenolpyruvoylglucosamine reductase**	**Cytoplasm**	**Outer membrane**	**32.59**
100	wOo_06790	Phosphatidylglycerophosphate synthase	Cell inner membrane	Inner membrane	20.23
101	wOo_06800	Malic enzyme	Cell inner membrane/ Cytoplasm	Cytoplasm	49.35
102	wOo_06850	**UvrABC system protein B, Protein UvrB**	**Cytoplasm**	**Cytoplasm**	**73.56**
103	wOo_06870	Phosphoribosylaminoimidazole synthetase	Cell inner membrane/ Cytoplasm	Inner membrane/ Cytoplasm	18.41
104	wOo_06880	Dihydropteroate synthase putative	Cell inner membrane/ Cytoplasm	Outer membrane/ Cytoplasm	48.8
105	wOo_06890	Cytosineadenosine deaminase	Cell inner membrane/ Cytoplasm	Cytoplasm	15.44
106	wOo_06920	**DNA ligase**	Cell inner membrane/ Cytoplasm	**Outer membrane**	**77.05**
107	wOo_06930	**5'-nucleotidase SurE**	Cell inner membrane/ Cytoplasm	Extracellular/ Outer membrane/ Cytoplasm	27.34
108	wOo_06950	Response regulator PleD	Cytoplasm	Cytoplasm	36. 13
109	wOo_06960	Response regulator PleD	Cytoplasm	Cytoplasm	15. 13
110	wOo_06980	NAD-specific glutamate dehydrogenase	Cell inner membrane	Cytoplasm	181.56
111	wOo_07010	**50S ribosomal protein L28**	Cell inner membrane	Cytoplasm	14.45
112	wOo_07050	**30S ribosomal protein S4**	**Cytoplasm**	**Cytoplasm**	**23.66**
113	wOo_07120	**Protein-export membrane**	Cell inner membrane	Inner membrane	32.58

		protein SecF			
114	wOo_07150	**Membrane protein insertase YidC**	Cell inner membrane	Inner membrane/ Outer membrane	65.79
115	wOo_07240	Type IV secretory pathway VirB11 component	Cytoplasm	Cytoplasm	37.05
116	wOo_07280	GTP cyclohydrolase II	Cell inner membrane/ Cytoplasm	Cytoplasm	41.69
117	wOo_07350	**Phosphoribosylformylglycinamidine synthase subunit PurL, FGAM synthase**	**Cytoplasm**	**Cytoplasm**	113.87
118	wOo_07450	**DNA polymerase III subunit gamma/tau, EC 2.7.7.7**	**Cytoplasm**	**Inner membrane/Cytoplasm**	**55.63**
119	wOo_07600	**Aspartokinase**	**Cytoplasm**	**Cytoplasm**	**42.62**
120	wOo_07630	**Tyrosine--tRNA ligase**	**Cytoplasm**	**Cytoplasm**	**47.79**
121	wOo_07700	**RNA pyrophosphohydrolase**	**Cytoplasm**	**Cytoplasm**	19.33
122	wOo_07780	**UDP-N-acetylmuramoyl-tripeptide--D-alanyl-D-alanine ligase**	**Cytoplasm**	**Cytoplasm**	**55.47**
123	wOo_07810	**Ribulose-phosphate 3-epimerase**	**Cell inner membrane/ Cytoplasm**	**Cytoplasm**	**24.62**
124	wOo_07820	**Polyribonucleotide nucleotidyltransferase**	**Cytoplasm**	**Cytoplasm**	**84.** 16
125	wOo_07830	**30S ribosomal protein S15**	Cell inner membrane	Cytoplasm	10.66
126	wOo_07920	**Deoxyguanosinetriphosphate triphosphohydrolase-like protein**	Cell inner membrane/ Cytoplasm	Cytoplasm	46.28
127	wOo_07970	**3-oxoacyl-[acyl-carrier-protein] synthase 3**	**Cytoplasm**	**Cytoplasm**	**35.** 13
128	wOo_07980	**Phosphate acyltransferase, EC 2.3.1.274**	Cell inner membrane/ Cytoplasm	Outer membrane/Inner membrane/ Cytoplasm	37.62
129	wOo_08050	Biotin-acetyl-CoA carboxylase-ligase	Cytoplasm	Cytoplasm	29.67
130	wOo_08090	**NADH-quinone oxidoreductase subunit K**	**Cell inner membrane**	**Inner membrane**	11. 18

131	wOo_08100	**NADH-quinone oxidoreductase subunit J**	Cell inner membrane	Inner membrane	22.81
132	wOo_08150	**Heme exporter protein C**	Cell inner membrane	Inner membrane	28.38
133	wOo_08210	**Transcription termination factor Rho**	Cell inner membrane	Cytoplasm	52.47
134	wOo_08720	**4-hydroxy-tetrahydrodipicolinate reductase, HTPA reductase**	**Cytoplasm**	**Cytoplasm**	**28.74**
135	wOo_08740	DNA polymerase III delta subunit	Cell inner membrane	Cytoplasm	38.07
136	wOo_08750	ABC-type Mn2+Zn2+ transport system permease component	Cell inner membrane	Inner membrane	29.39
137	wOo_08770	**4-diphosphocytidyl-2-C-methyl-D-erythritol kinase, CMK**	Cell inner membrane	Cytoplasm	31.89
138	wOo_08850	**UDP-N-acetylmuramoyl-L-alanyl-D-glutamate--2,6-diaminopimelate ligase**	Cytoplasm	Cytoplasm	52.65
139	wOo_08970	**50S ribosomal protein L21**	Cell inner membrane/ Cytoplasm	Cytoplasm	11.85
140	wOo_09010	**Aspartyl/glutamyl-tRNA(Asn/Gln) amidotransferase subunit B, Asp/Glu-ADT subunit B**	**Cytoplasm**	**Cytoplasm**	**53.5**
141	wOo_09030	Ribonuclease RneRng family protein	Cytoplasm	Outermembrane	67. 11
142	wOo_09040	**50S ribosomal protein L33**	**Cytoplasm**	**Cytoplasm**	**7.78**
143	wOo_09130	**Adenylosuccinate lyase, ASL**	**Cytoplasm**	**Cytoplasm**	**50.3**
144	wOo_09140	**50S ribosomal protein L9**	**Cell inner membrane/ Cytoplasm**	**Cytoplasm**	21. 16
145	wOo_09160	**30S ribosomal protein S6**	Cell inner membrane	Cytoplasm	29.64
146	wOo_09170	**DNA-directed DNA polymerase**	**Cytoplasm**	**Outermembrane/Cytoplasm**	126.68
147	wOo_09260	**Diaminopimelate epimerase, DAP epimerase**	**Cytoplasm**	**Cytoplasm**	**33.0**1
148	wOo_09300	**Protein-export protein SecB**	Cell inner membrane	Cytoplasm	18.68

149	wOo_09320	**DNA polymerase III subunit epsilon**	**Cytoplasm**	**Cytoplasm**	**26.69**
150	wOo_09350	**30S ribosomal protein S20**	Cell inner membrane	**Cytoplasm**	**9.96**
151	wOo_09490	**Glutathione synthetase**	**Cytoplasm**	**Cytoplasm**	**35.9**1
152	wOo_09500	**UDP-N-acetylglucosamine--N-acetylmuramyl-(pentapeptide) pyrophosphoryl-undecaprenol N-acetylglucosamine transferase**	Cell inner membrane	**Outer membrane/Cytoplasm**	**38.75**
153	wOo_09580	3-polyprenyl-4-hydroxybenzoate decarboxylase UbiD	Cell inner membrane/ Cytoplasm	Cytoplasm	56.62
154	wOo_09610	**Chromosomal replication initiator protein DnaA**	**Cytoplasm**	**Outer membrane/Cytoplasm**	**52.8**
155	wOo_09620	Glutamine synthetase	Cytoplasm	Cytoplasm	27.5
156	wOo_09720	tRNA nucleotidyltransferasepolyA-polymerase	Cell inner membrane/ Cytoplasm	Cytoplasm	48.78
157	wOo_09770	**Fructose-1,6-bisphosphatase**	**Cytoplasm**	**Cytoplasm**	**33.45**
158	wOo_09800	**Pyruvate, phosphate dikinase**	**Cytoplasm**	**Cytoplasm**	**98.25**
159	wOo_09920	**Isoprenyl transferase**	Cell inner membrane/ Cytoplasm	Cytoplasm	26.94
160	wOo_09950	**Uridylate kinase, UK**	**Cytoplasm**	**Cytoplasm**	**27.** 1
161	wOo_09990	**Carbamoyl-phosphate synthase large chain**	**Cytoplasm**	**Cytoplasm**	120.65
162	wOo_10050	Phosphomannomutase	Cell inner membrane/ Cytoplasm	Cytoplasm	51.62
163	wOo_10080	ABC-type transport system involved in lipoprotein release permease component	Cell inner membrane	Inner membrane	45. 19
164	wOo_10110	Succinate dehydrogenase subunit D sdhD hydrophobi c membrane anchor protein	Cell inner membrane	Inner membrane	14.64
165	wOo_10240	**DNA-directed RNA polymerase subunit alpha, RNAP subunit**	Cell inner membrane	Outer membrane	39.23

		alpha			
166	wOo_10300	**Protein translocase subunit SecY**	Cell inner membrane	Inner membrane	41. 12
167	wOo_10320	**30S ribosomal protein S5**	Cell inner membrane/ Cytoplasm	Cytoplasm	18.44
168	wOo_10330	**50S ribosomal protein L18**	Cell inner membrane	Outer membrane/ periplasm/ cytoplasm	14.56
169	wOo_10340	**50S ribosomal protein L6**	Cell inner membrane/ Cytoplasm	Cytoplasm	20.34
170	wOo_10350	**30S ribosomal protein S8**	Cell inner membrane	**Cytoplasm**	15.01
171	wOo_10400	**30S ribosomal protein S17**	Cell inner membrane/ Cytoplasm	Cytoplasm	8.86
172	wOo_10410	**50S ribosomal protein L29**	Cell inner membrane	**Cytoplasm**	7.4
173	wOo_10420	**50S ribosomal protein L16**	Cell inner membrane	Cytoplasm	15.33
174	wOo_10430	**30S ribosomal protein S3**	Cell inner membrane	Cytoplasm	23.25
175	wOo_10440	**50S ribosomal protein L22**	Cell inner membrane	Cytoplasm	12.97
176	wOo_10470	**50S ribosomal protein L23**	Cell inner membrane/ Cytoplasm	Cytoplasm	11. 17
177	wOo_10500	**30S ribosomal protein S10**	Cell inner membrane/ Cytoplasm	Cytoplasm	13.78
178	wOo_10540	**Carbamoyl-phosphate synthase small chain**	**Cytoplasm**	**Cytoplasm**	**43.09**
179	wOo_10580	**50S ribosomal protein L35**	Cell inner membrane	**Periplasm/Cytoplasm**	**7.68**
180	wOo_10770	**Thioredoxin reductase**	**Cytoplasm**	**Cytoplasm**	**34.54**

APPENDIX VII

Drug targets for bovine onchocerciasis

S/N	KEGG id no	Protein name	Pathway name	Subcellular localisation	Experimentally solved 3D structures	Calculated 3D structures	M/W (KDal)
1	*wOo_00310*	NADH-quinone oxidoreductase subunit H	Oxidative phosphorylation Metabolic pathways	Inner membrane	None	available	37.99
2	*wOo_00420*	Leucine--tRNA ligase	Aminoacyl tRNA biosynthesis	Cytoplasmic	None	available	97.32
3	*wOo_00800*	Ferredoxin--NADP reductase	Selenocompound metabolism	Cytoplasmic Periplasmic Inner embrane	None	available	37.74
4	*wOo_00940*	Ribonucleoside-diphosphate reductase	Purine metabolism Pyrimidine metabolism	Cytoplasmic	None	available	68.99

			Metabolic pathways				
5	*wOo_01260*	Fumarate hydratase class II (Fumarase C)	Metabolic pathways Biosynthesis of secondary metabolites Microbial metabolism in diverse environments Biosynthesis of antibiotics Carbon metabolism	Cytoplasmic	None	available	50.75

6	*wOo_01490*	D-alanine--D-alanine ligase	Citrate cycle (TCA Cycle) Peptidoglycan biosynthesis Metabolic pathways Vacomycin resistance	Cytoplasmic	None	available	34. 1
7	*wOo_01620*	50S ribosomal protein L10	Ribosome	Periplasmic Inner membrane Cytoplasmic	None	available	19.31
8	*wOo_01730*	DNA mismatch repair protein MutL	Mismatch repair	Cytoplasmic	None	available	19.39
9	*wOo_01740*	N5-carboxyaminoimidazole ribonucleotide mutase	Purine metabolism Metabolic pathways Biosynthesis of secondary metabolites Biosynthesis of antibiotics	Inner membrane Cytoplasmic	None	available	17.22
10	*wOo_01940*	Phenylalanine--tRNA ligase alpha subunit	Aminoacyl tRNA biosynthesis	Cytoplasmic	None	available	40. 1
11	*wOo_02550*	Ribonucleoside-diphosphate reductase subunit beta	Purine metabolism Pyrimidine metabolism	Cytoplasmic	None	available	37.98

			Metabolic pathways				
12	*wOo_03170*	Flavin prenyltransferase UbiX	Ubiquinone and other terpenoid-quinone biosynthesis Metabolic pathways Biosynthesis of secondary metabolites Microbial metabolism in diverse environments	Cytoplasmic	None	available	22.92
13	*wOo_03200*	Cell division protein FtsI	Peptidoglycan biosynthesis Beta lactam resistance	Outer membrane	None	available	59.05
14	*wOo_03220*	Proline--tRNA ligase	Aminoacyl tRNA biosynthesis	Cytoplasmic	None	available	48.56
15	*wOo_03240*	UDP-N-acetylglucosamine 1-carboxyvinyltran-sferase	Amino sugar and nucleotide sugar metabolism Peptidoglycan biosynthesis Metabolic pathways	Cytoplasmic	None	available	46. 17
16	*wOo_03250*	Flavin-dependent thymidylate synthase (FDTS)	Pyrimridine metabolism One carbon pool by folate	Cytoplasmic	None	available	34. 15

			Metabolic pathways				
17	*wOo_03490*	N5-carboxyaminoim-idazole ribonucleotide synthase	Purine metabolism Metabolic pathways Biosynthesis of secondary metabolites Biosynthesis of antibiotics	Cytoplasmic	None	available	40.04
18	*wOo_04050*	Aspartate carbamoyltransfe-rase	Metabolic pathways Pyrimidine metabolism Alanine, aspartate and glutamate metabolism	Cytoplasmic	None	available	33.38
19	*wOo_04060*	ABC-type Fe3+ transport system periplasmic component	ABC transporters	Outermembran e	None	available	38.32
20	*wOo_04300*	Phosphoribosylf-ormylglycinamid-ine synthase domain-containing protein	Metabolic pathways Biosynthesis of secondary metabolites Biosynthesis of antibiotics Purine metabolism	Cytoplasmic	None	available	29.9
21	*wOo_04340*	Phosphoribosyl-aminoimidazole-succinocarboxam-ide	Metabolic pathways Biosynthesis of	Cytoplasmic	None	available	27.76

		synthase	secondary metabolites Biosynthesis of antibiotics Purine metabolism				
22	*wOo_04550*	GMP synthase (glutamine-hydrolyzing)	Metabolic pathways Purine metabolism	Cytoplasmic	None	available	58. 16
23	*wOo_04730*	**UvrABC system protein A**	Nucleotide excision repair	Outer membrane	None	available	104.91
24	*wOo_04960*	Geranylgeranyl Pyrophosphate synthase	Metabolic pathways Biosynthesis of secondary metabolites Biosynthesis of antibiotics Terpenoid backbone biosynthesis	Cytoplasmic	None	available	27.22
25	*wOo_05160*	Dihydroorotase (DHOase)	Metabolic pathways Pyrimidine metabolism	Cytoplasmic	None	available	48.55
26	*wOo_05230*	Alanine--tRNA ligase	Aminoacyl-trna biosynthesis	Cytoplasmic	None	available	102.6
27	*wOo_05580*	Fructose-16-bisphosphatase	Metabolic pathways Inositol phosphate metabolism	Cytoplasmic	None	available	27.89
28	*wOo_05980*	Valine--tRNA ligase	Aminoacyl-tRNA	Cytoplasmic	None	available	98.68

			biosynthesis				
29	*wOo_06020*	D-alanyl-D-alanine carboxypeptidase	Metabolic pathways Peptidoglycan biosynthesis	Cytoplasmic Inner membrane	None	available	42.26
30	*wOo_06030*	Phenylalanine--tRNA ligase beta subunit	Aminoacyl-tRNA biosynthesis	Outer membrane Cytoplasmic	None	available	88.6
31	*wOo_06080*	Replicative DNA helicase	DNA replication	Cytoplasmic	None	available	54.7
32	*wOo_06430*	DNA polymerase I	Metabolic pathways DNA replication Base excision repair Nucleotide excision repair Homologous recombination Purine metabolism Pyrimidine metabolism	Cytoplasmic	None	available	98.34
33	*wOo_06610*	**UDP-N-acetylenolpyruvo-ylglucosamine reductase**	Amino sugar and nucleotide sugar metabolism	Outer membrane	None	available	32.59

			Peptidoglycan biosynthesis Metabolic pathways				
34	*wOo_06800*	Malic enzyme	Metabolic pathways Microbial metabolism in diverse environments Carbon metabolism Pyruvate metabolism	Cytoplasmic	None	available	49.35
35	*wOo_06850*	UvrABC system protein B	Nucleotide excision repair	Cytoplasmic	None	available	73.56
36	*wOo_06870*	Phosphoribosyl-aminoimidazole synthetase	Metabolic pathways Microbial metabolism in diverse environments Folate biosynthesis	Inner-membrane Cytoplasmic	None	available	18.41
37	*wOo_06880*	Dihydropteroate synthase putative	Metabolic pathways Folate biosynthesis	Outer membrane Cytoplasmic	None	available	48.8
38	*wOo_07050*	30S ribosomal protein S4	Ribosome	Cytoplasmic	None	available	23.66

39	*wOo_07450*	DNA polymerase III subunit gamma/tau	Metabolic pathways DNA replication Mismatch repair Homologous recombination Purine metabolism Pyrimidine metabolism	Cytoplasmic Inner-membrane	None	available	55.63
40	*wOo_07630*	Tyrosine--tRNA ligase	Aminoacyl-tRNA biosynthesis	Cytoplasmic	None	available	47.79
41	*wOo_07970*	3-oxoacyl-[acyl-carrier-protein] synthase 3	Metabolic pathways Fatty acid metabolism Fatty acid biosynthesis	Cytoplasmic	None	available	35. 13
42	*wOo_08050*	Biotin-acetyl-CoA carboxylase-ligase	Metabolic pathways Biotin metabolism	Cytoplasmic	None	available	29.67
43	*wOo_08090*	NADH-quinone oxidoreductase subunit K	Metabolic pathways Oxidative phosphorylation	Inner-membrane	None	available	11. 18

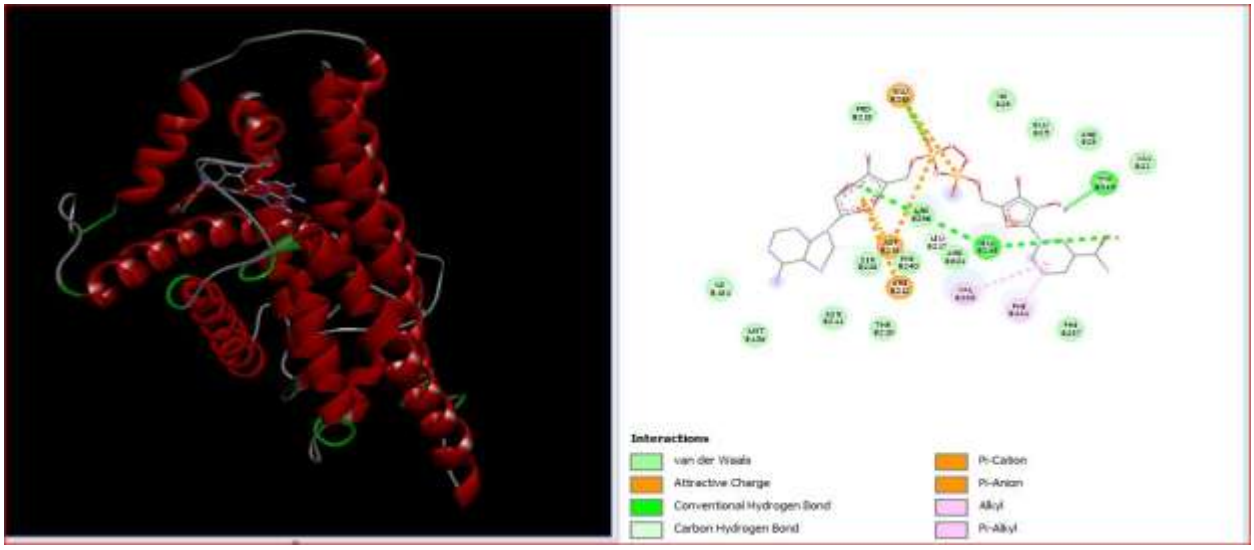
44	*wOo_08210*	Transcription termination factor Rho	RNA degradation	Cytoplasmic	None	available	52.47
45	*wOo_09010*	Aspartyl/glutamyl-tRNA(Asn/Gln) amidotransferase subunit B	Metabolic pathways Amino acyl-tRNA biosynthesis	Cytoplasmic	None	available	53.5
46	*wOo_09130*	Adenylosuccinate lyase (ASL)	Metabolic pathways Biosynthesis of secondary metabolites Biosynthesis of antibiotics Purine metabolism Alanine aspartate and glutamate metabolism	Cytoplasmic	None	available	50.3
47	*wOo_09620*	Glutamine synthetase	Metabolic pathways Microbial metabolism in diverse environments Biosynthesis of aminoacids Two component system Arginine biosynthesis	Cytoplasmic	Nones	available	27.5

			Alanine, aspartate and glutamate metabolism Glycoxylate and dicarboxylate metabolism Nitrogen metabolism				
48	*wOo_10240*	**DNA-directed RNA polymerase subunit alpha**	Purine metabolism Pyrimidine metabolism Metabolic pathways RNA polymerase	Outer membrane	None	available	39.23
49	*wOo_10320*	30S ribosomal protein S5	Ribosome	Cytoplasmic	None	available	18.44
50	*wOo_10350*	30S ribosomal protein S8	Ribosome	Cytoplasmic	None	available	15.01
51	*wOo_10410*	50S ribosomal protein L29	Ribosome	Cytoplasmic	None	available	7.4
52	*wOo_10420*	50S ribosomal protein L16	Ribosome	Cytoplasmic	None	available	15.33
53	*wOo_10430*	30S ribosomal protein S3	Ribosome	Cytoplasmic	None	available	23.25
54	*wOo_10440*	50S ribosomal protein L22	Ribosome	Cytoplasmic	None	available	12.97
55	*wOo_10470*	50S ribosomal protein L23	Ribosome	Cytoplasmic	None	available	11. 17
56	*wOo_10500*	30S ribosomal protein S10	Ribosome	Cytoplasmic	None	available	13.78
57	*wOo_10540*	Carbamoyl-phosphate	Metabolic pathways	Cytoplasmic	None	available	43.09

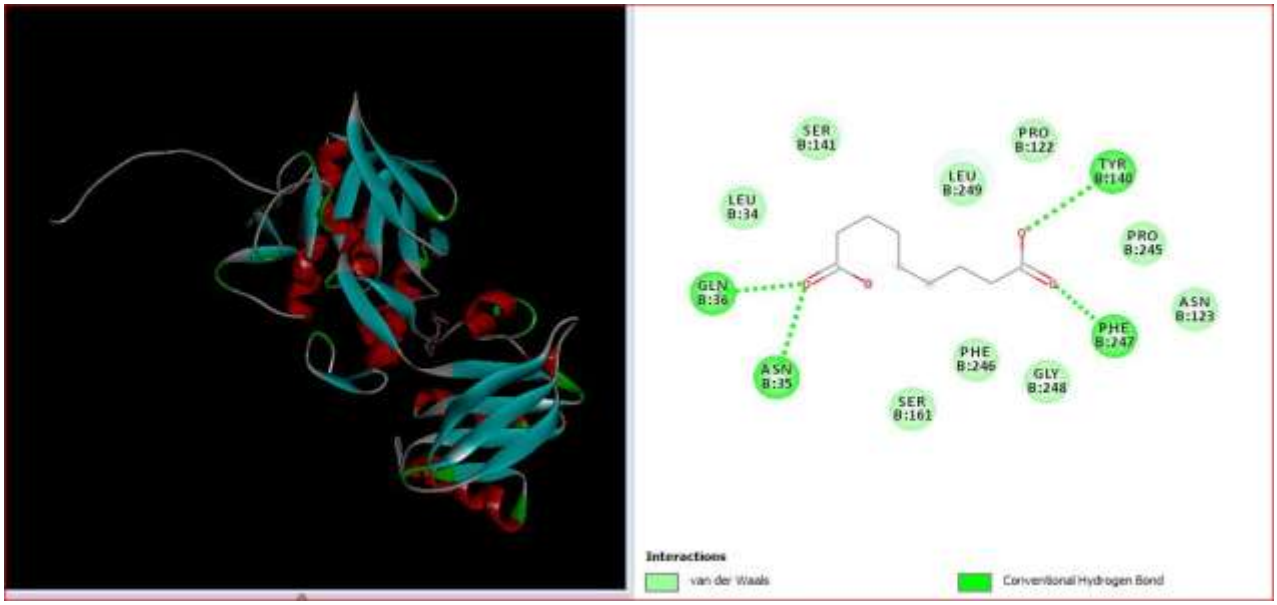
		synthase small chain	Pyrimidine metabolism Alanine, aspartate and glutamate metabolism				
58	*wOo_10770*	Thioredoxin reductase	Seleno-compound metabolism	Cytoplasmic	None	available	34.54

APPENDIX VIII

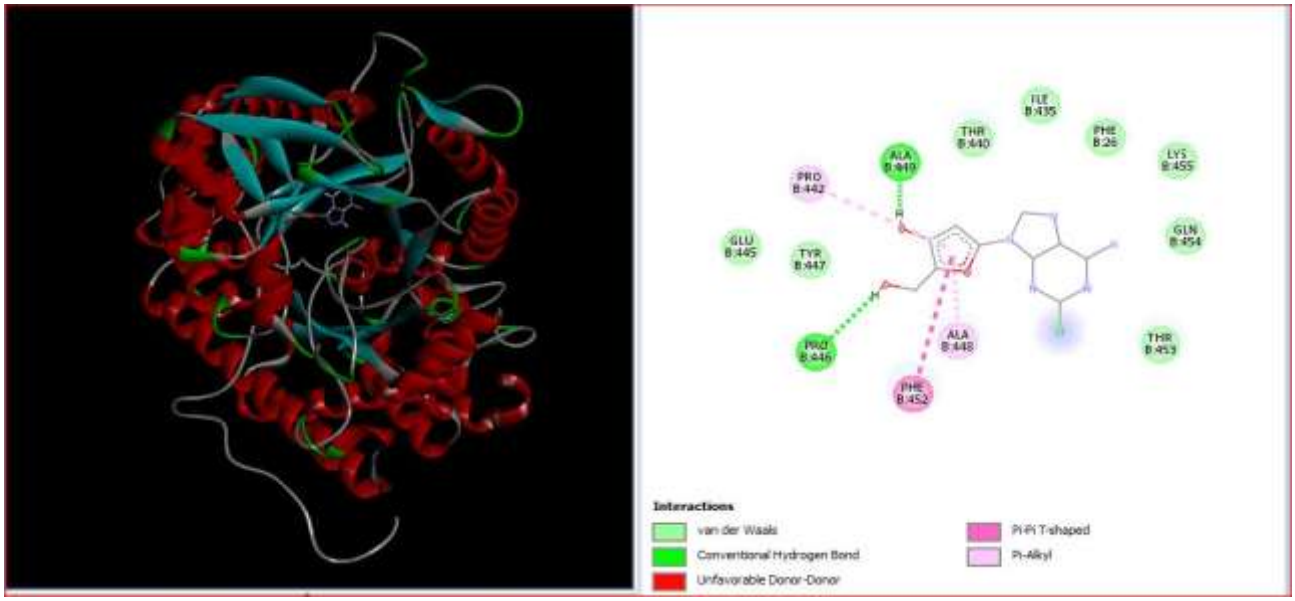
DOCKING RESULTS



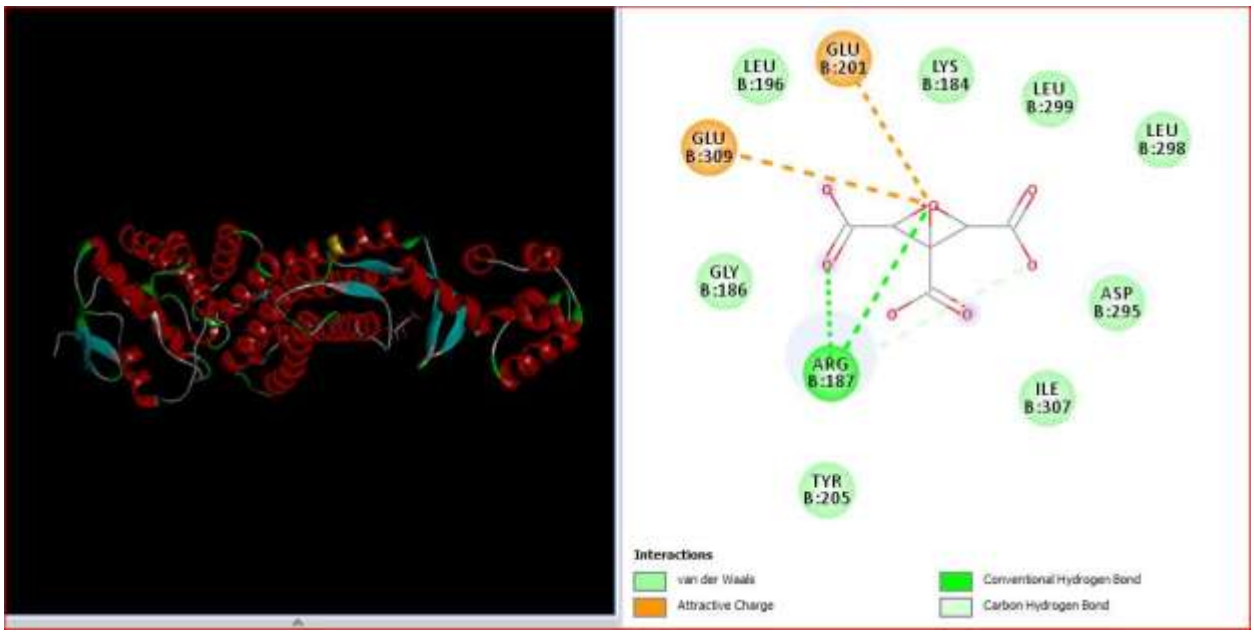
Molecular docking result for NADH-quinone oxidoreductase subunit H and NADH



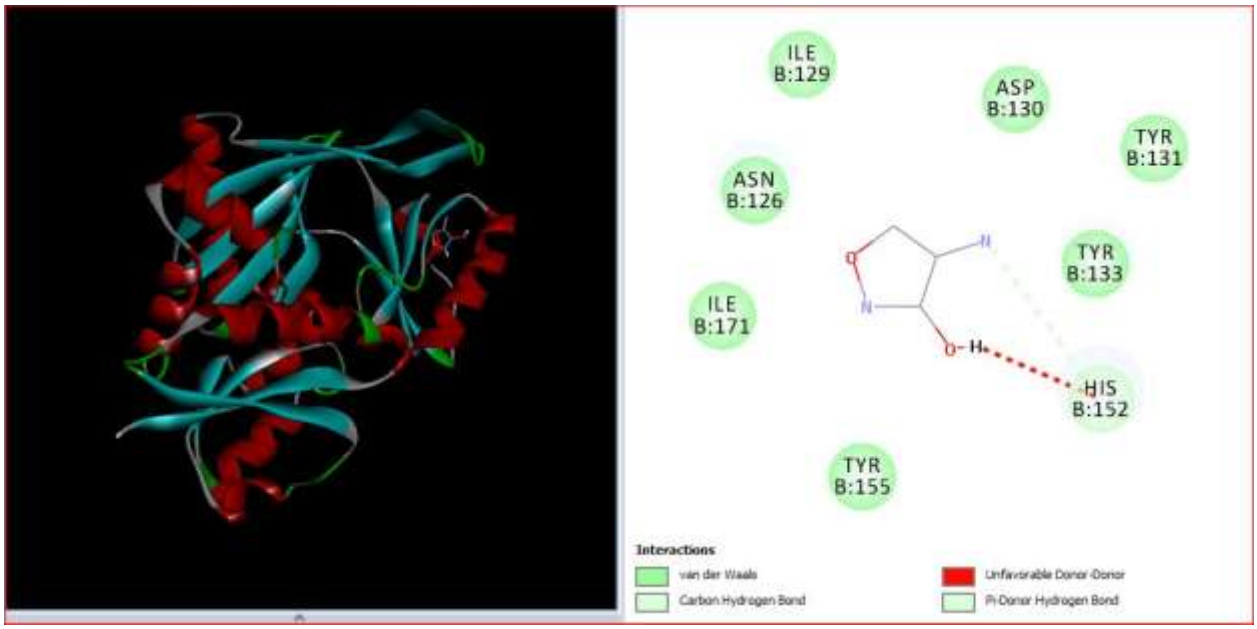
Molecular docking result for Ferredoxin—NADP reductase and Azelaic acid/organic acid



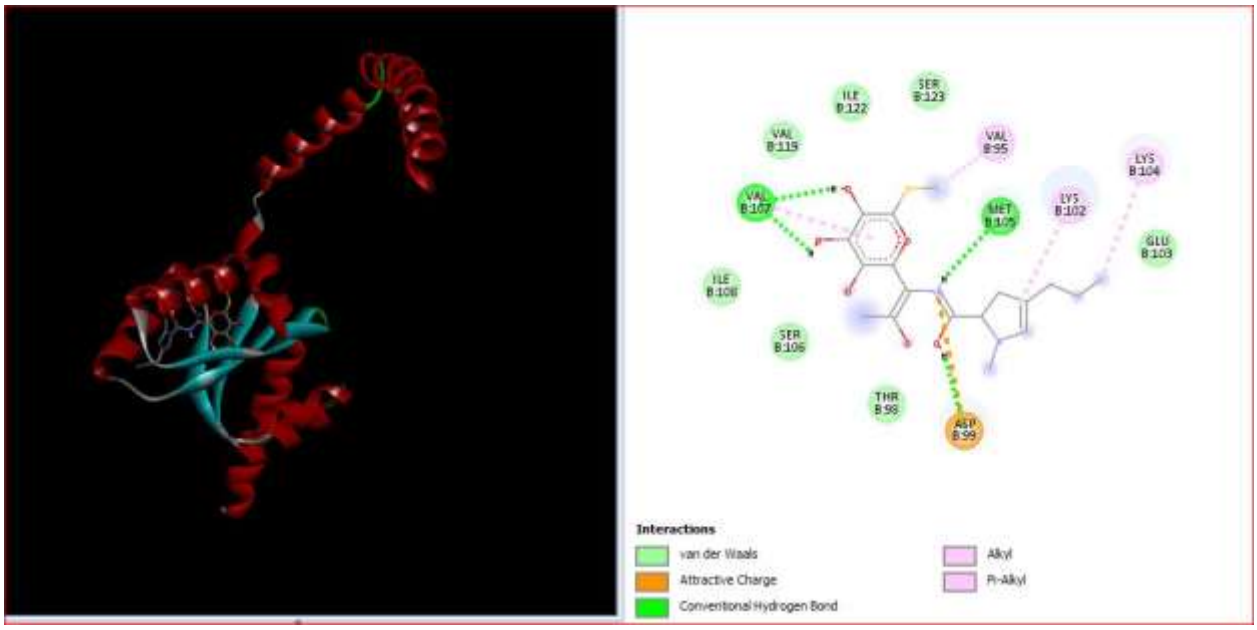
Molecular docking result for Ribonucleoside diphosphate reductase and cladribine



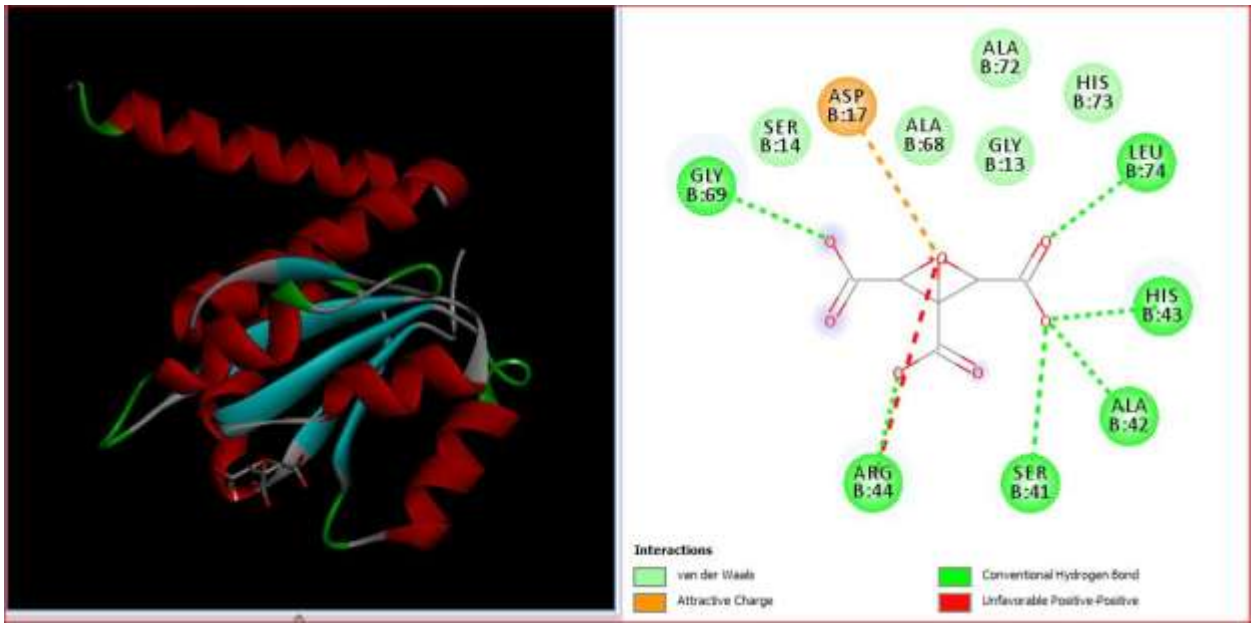
Molecular docking result for Fumarate hydratase class II and citric acid



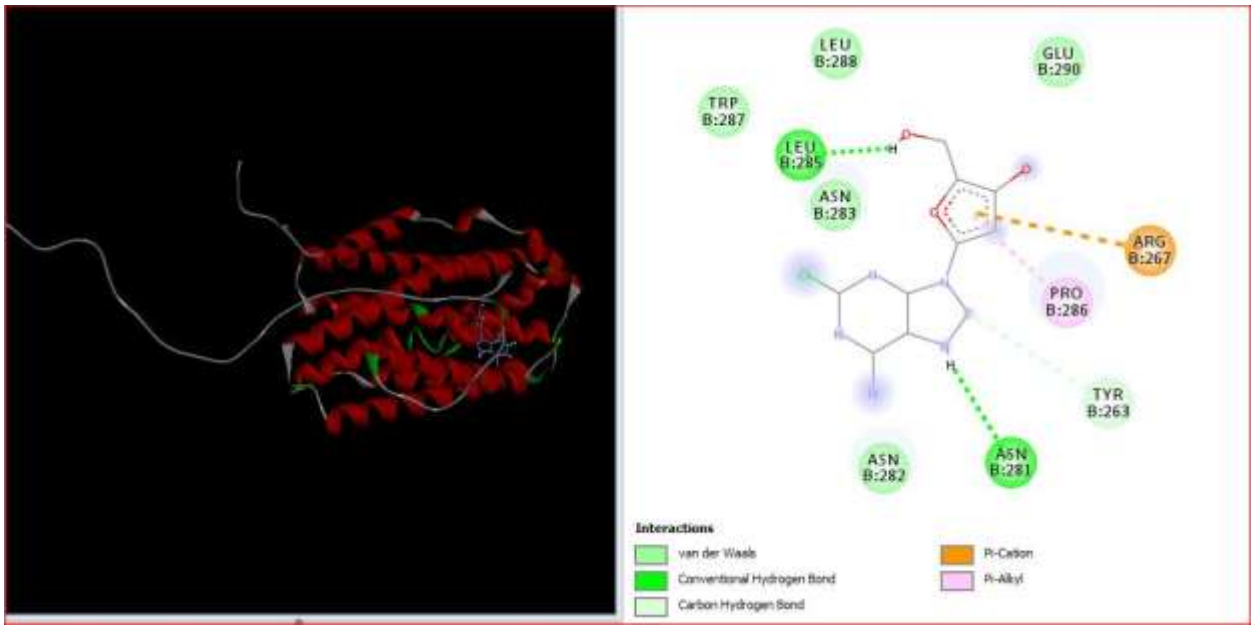
Molecular docking result for D-alanine—D-alanine ligase and cycloserine



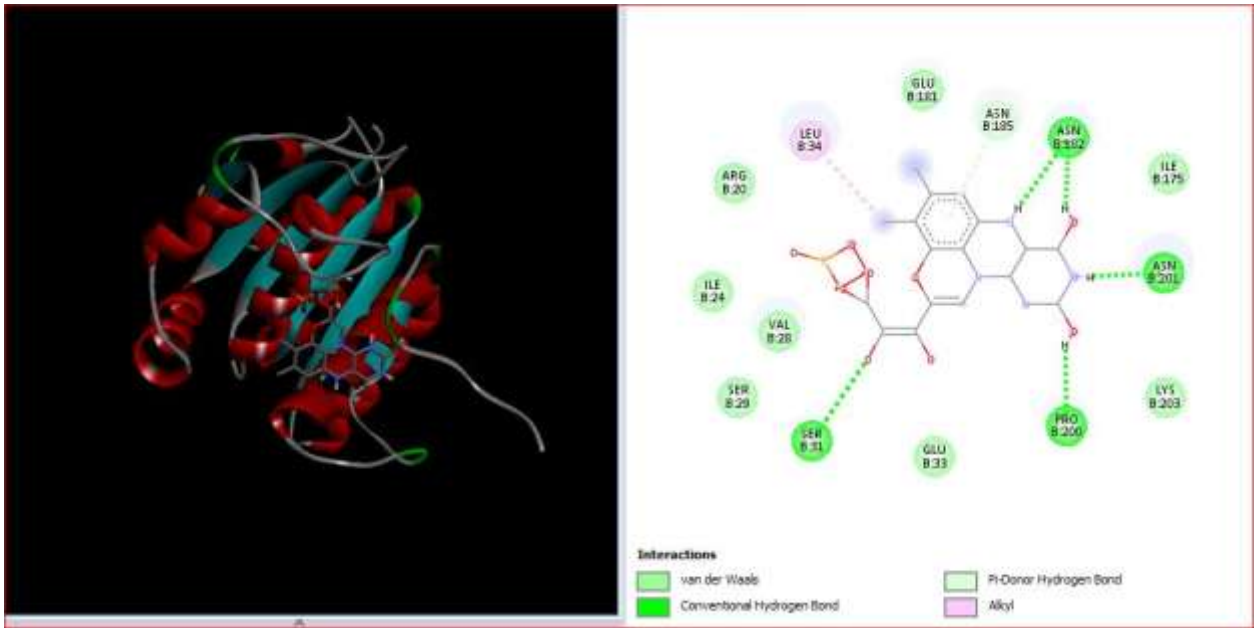
Molecular docking result for 50S ribosomal protein L 10 and linomycin



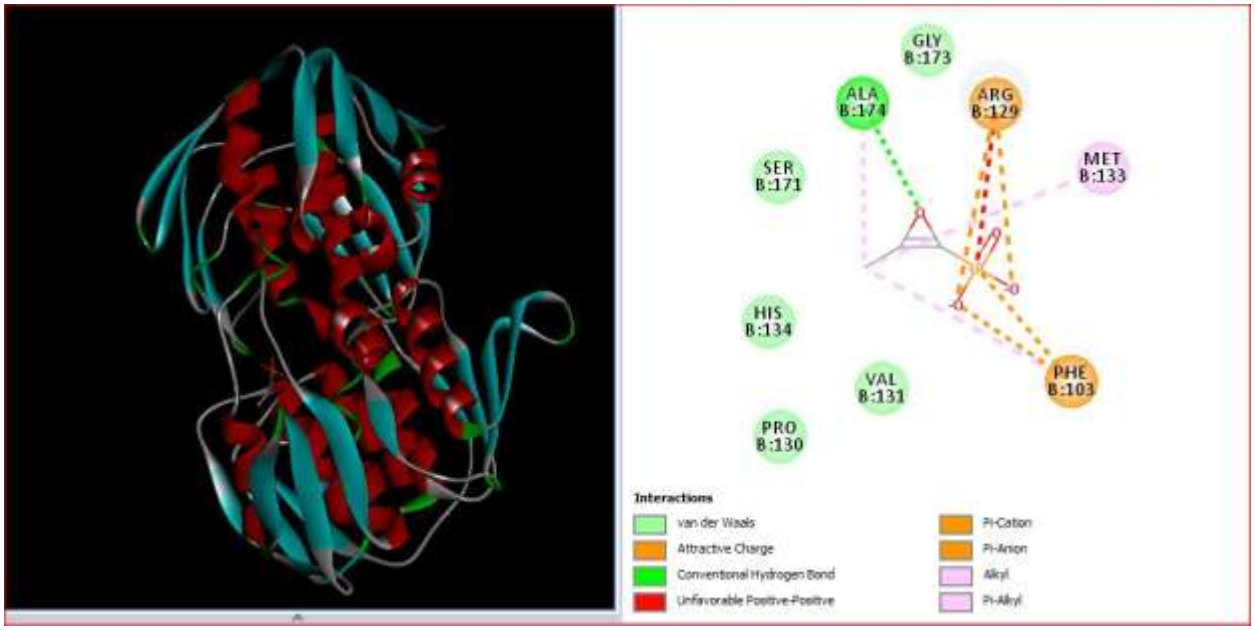
Molecular docking result for N5-carboxyaminoimidazole ribonucleotide mutase and citric acid



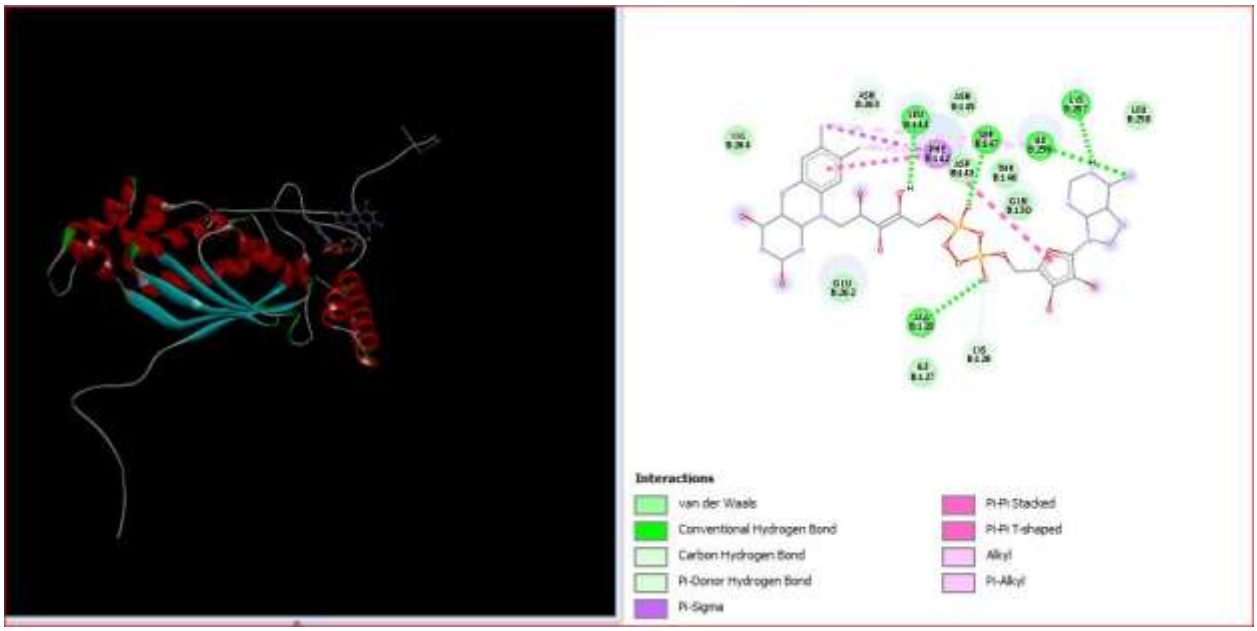
Ribonucleoside-diphosphate reductase subunit beta and cladribine



Molecular docking result for Flavin prenyltransferase UbiX and flavin mononucleotide



Molecular docking result for UDP-N-acetylglucosamine 1 carboxyvinyl transferase and and fosfomycin



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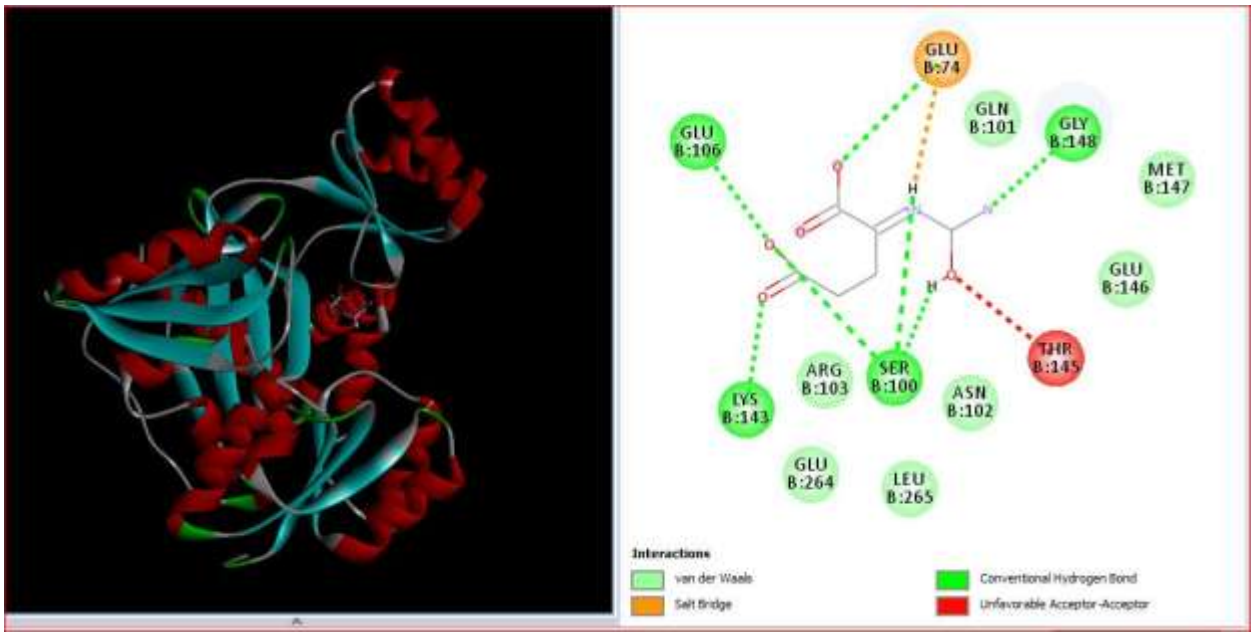
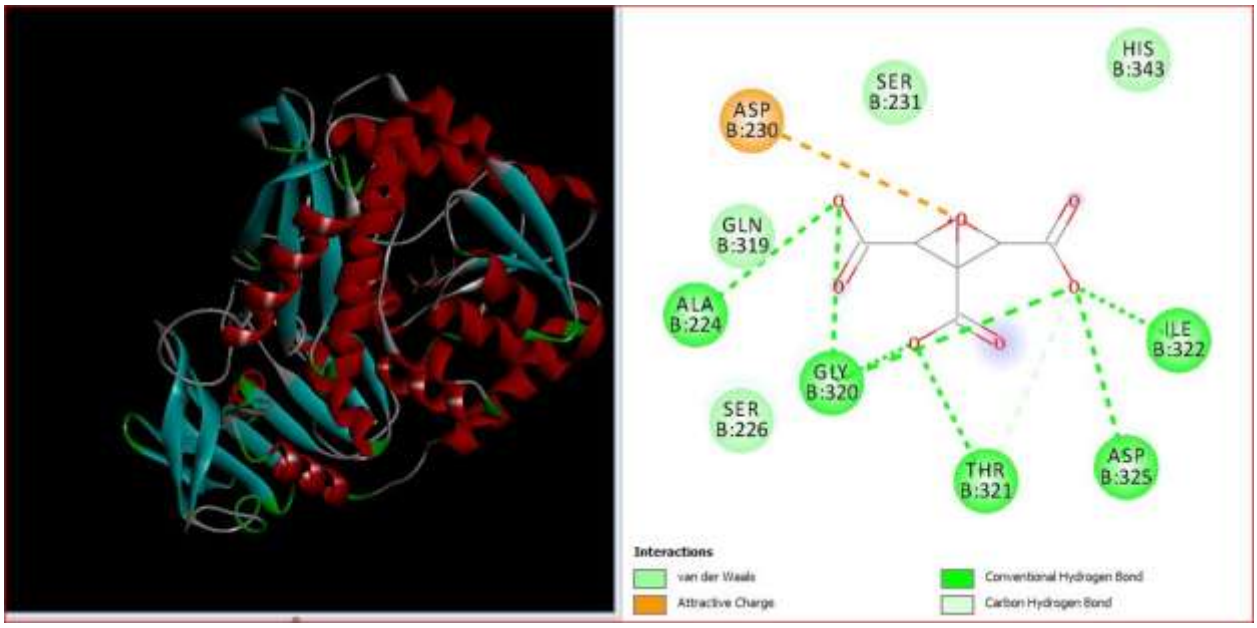
Molecular docking result for Flavin dependent thymidylate synthase,FDTS and flavine adenine dinucleotide

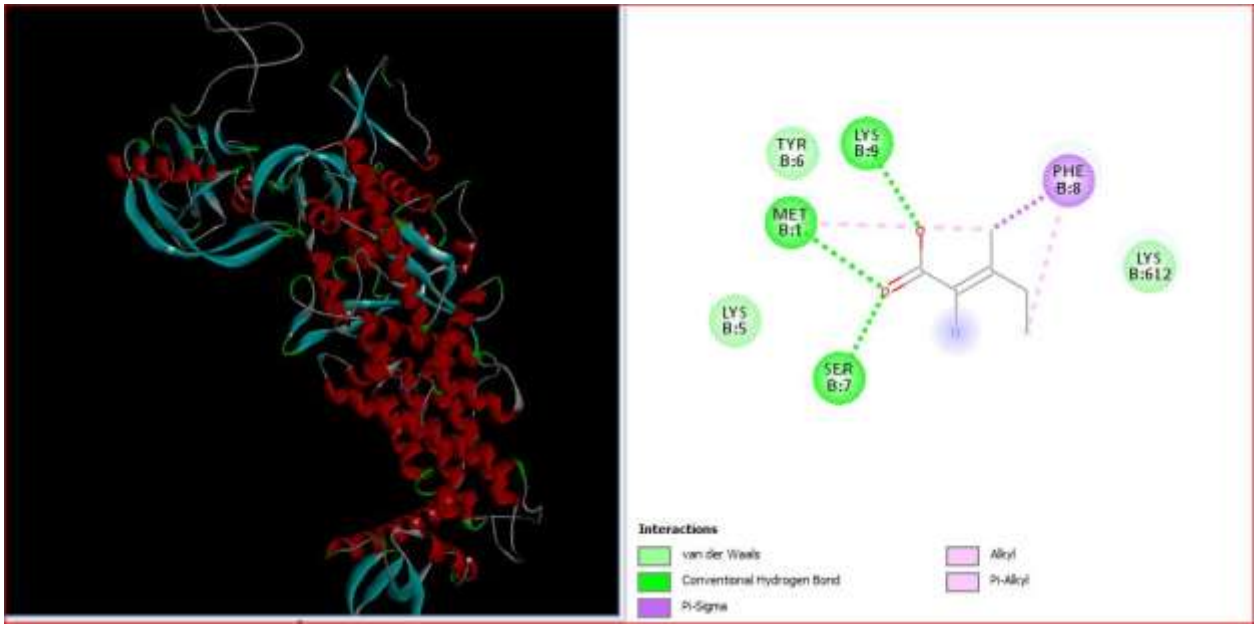


Molecular docking result for N- 5 carboxyaminoimidazole ribonucleotide synthase, n-5 CAIR synthase and carglumic acid

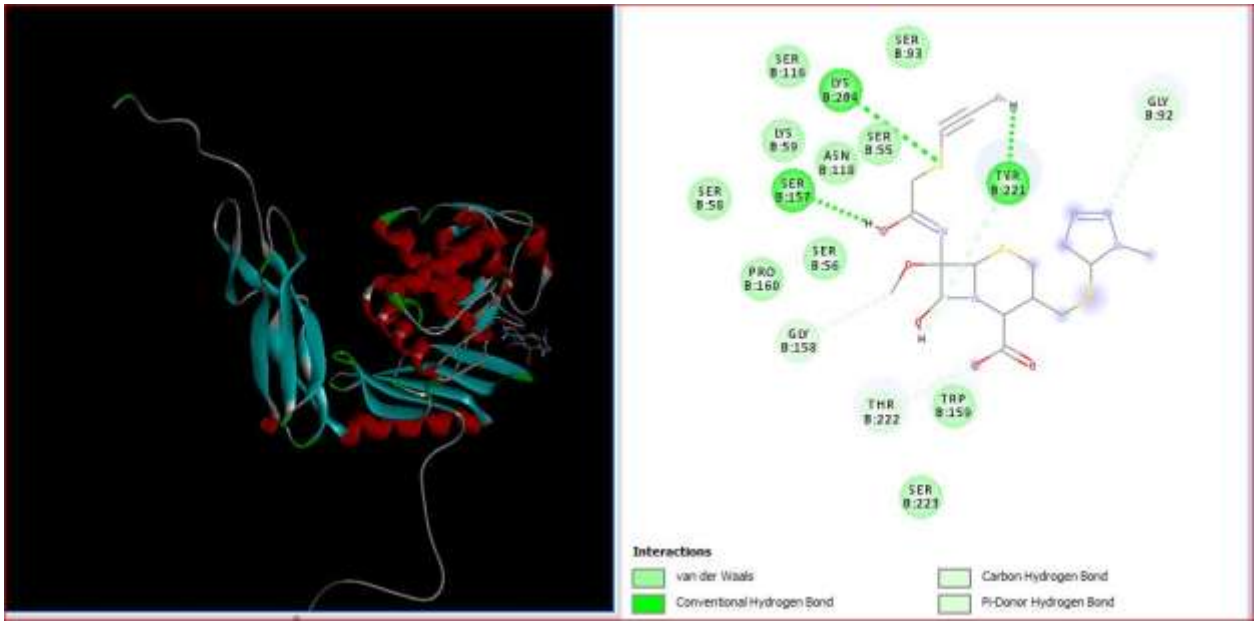


Molecular docking result for GMP synthase (glutamine hydrolyzing) and citric acid

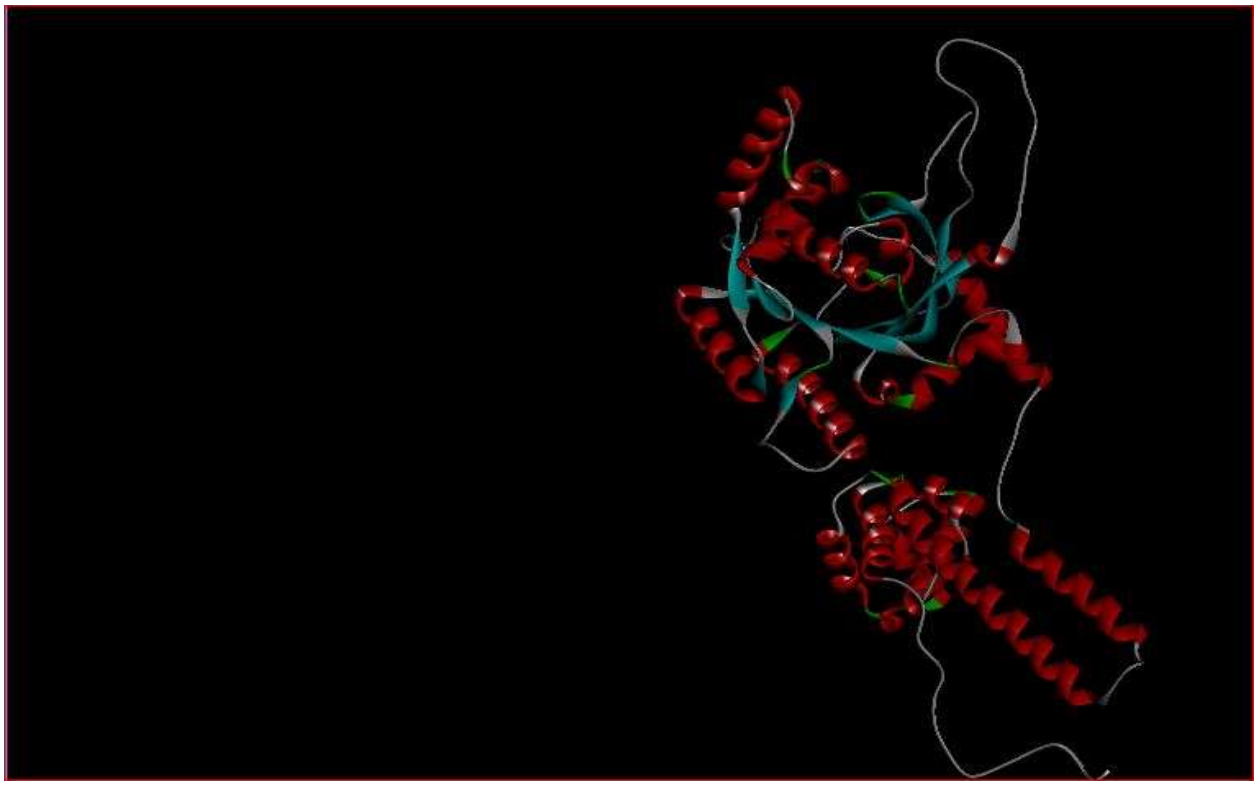
Molecular docking result for geranyigeranyl pyrophosphate synthase and pyrophosphoric acid



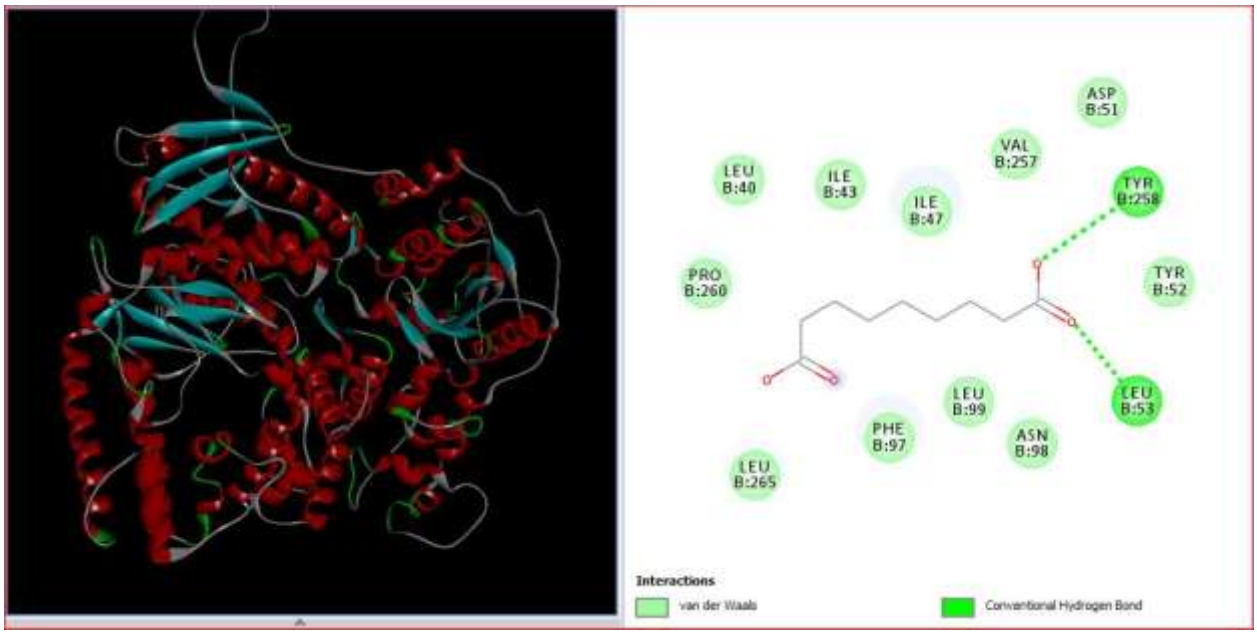
Molecular docking result for valine t-RNA ligase and L-isoleucine



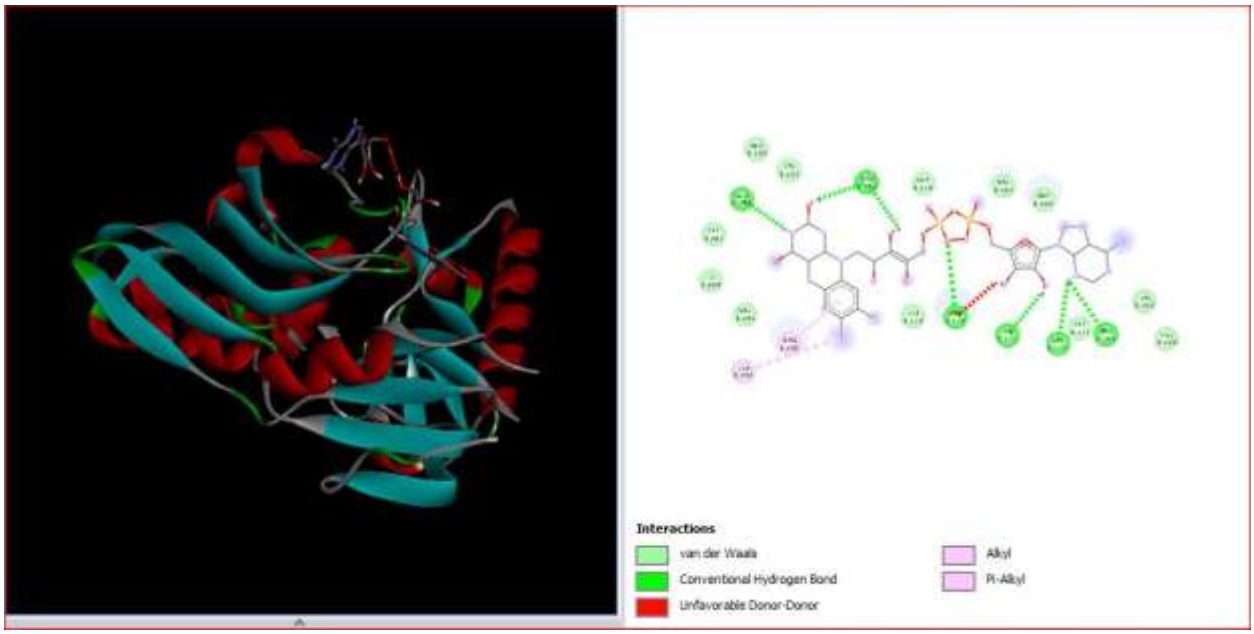
Molecular docking result for D-alanyl-D-alanine carboxypeptidase and cefmatazole



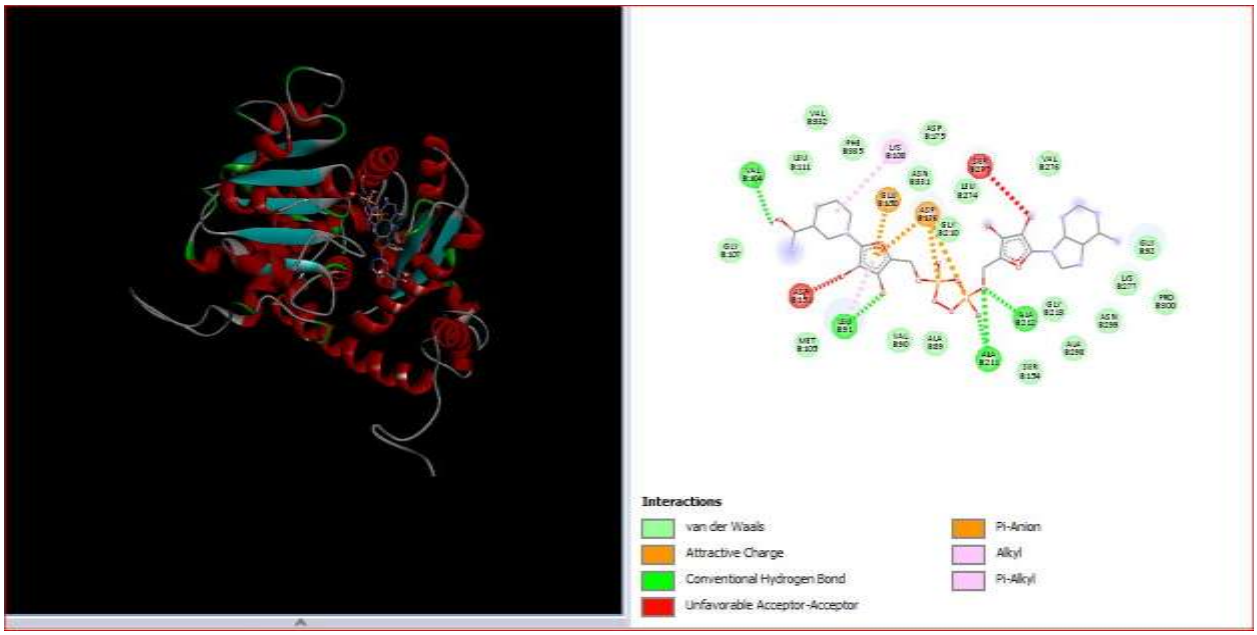
Molecular docking result for replicative DNA helicase and zinc



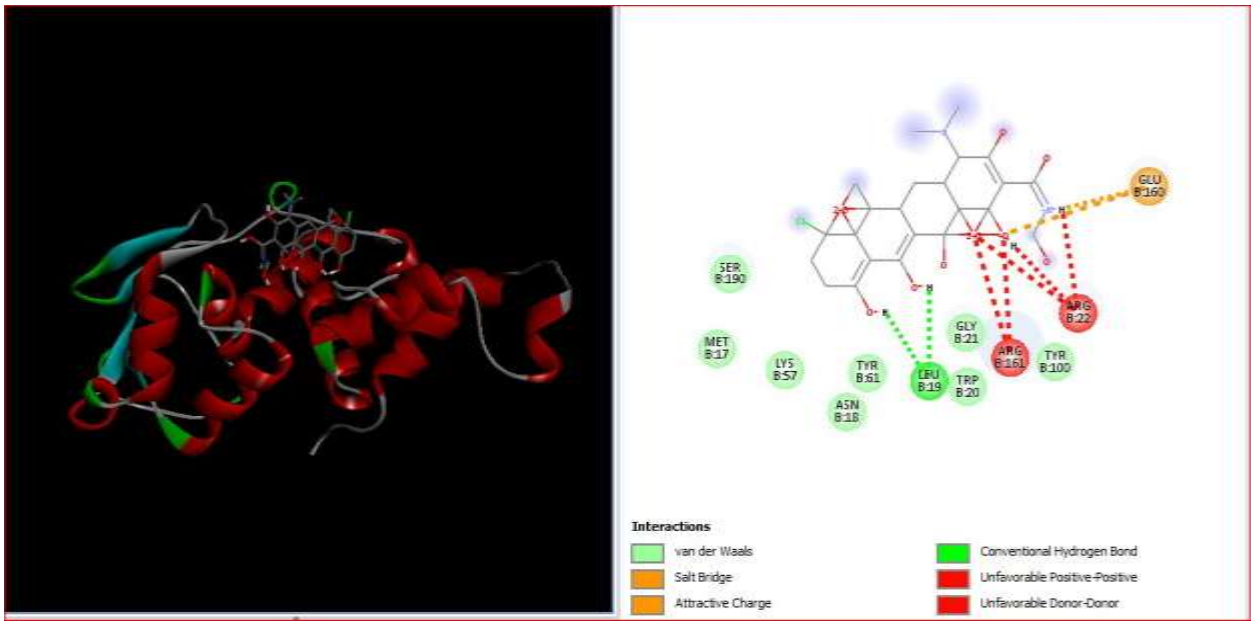
Molecular docking result for DNA polymerase I and azelaic acid



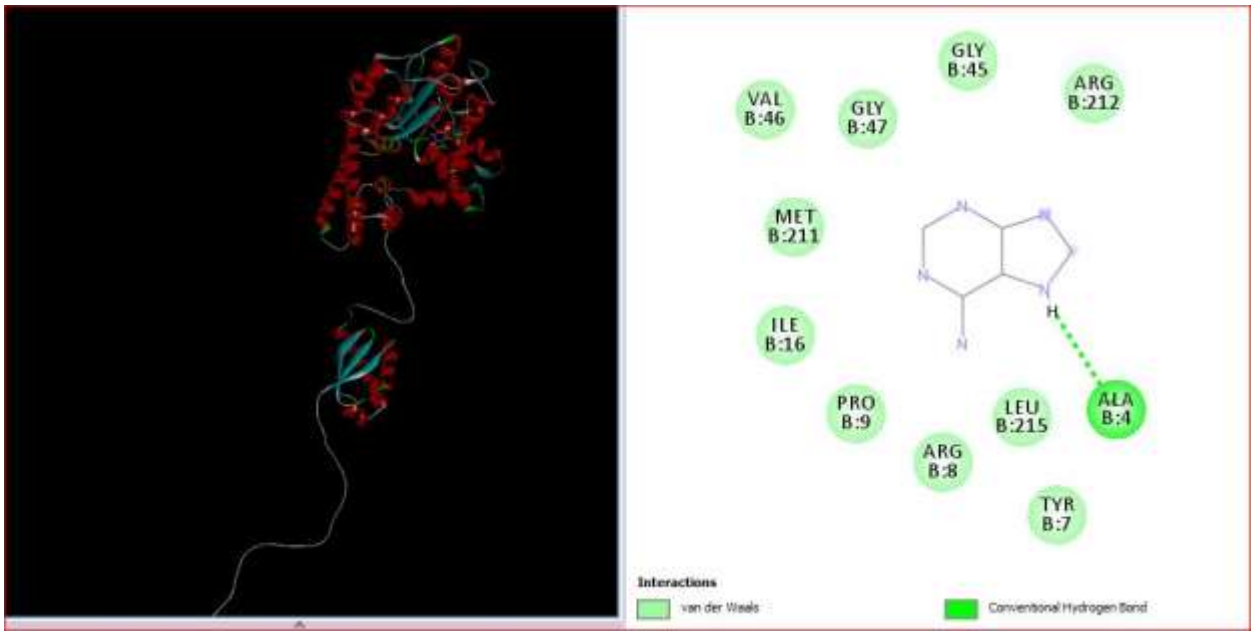
Molecular docking for UDP-N acetylenolpyruvoylglucosamine reductase and flavin adenine dinucleotide



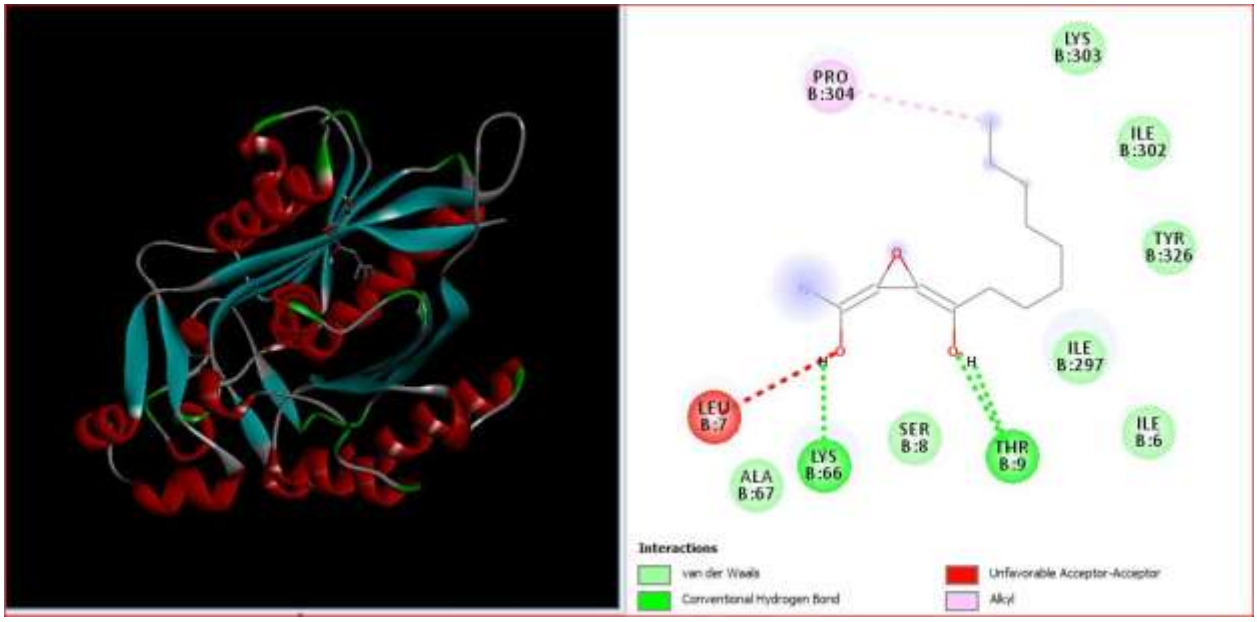
Molecular docking for malic enzyme and NADH



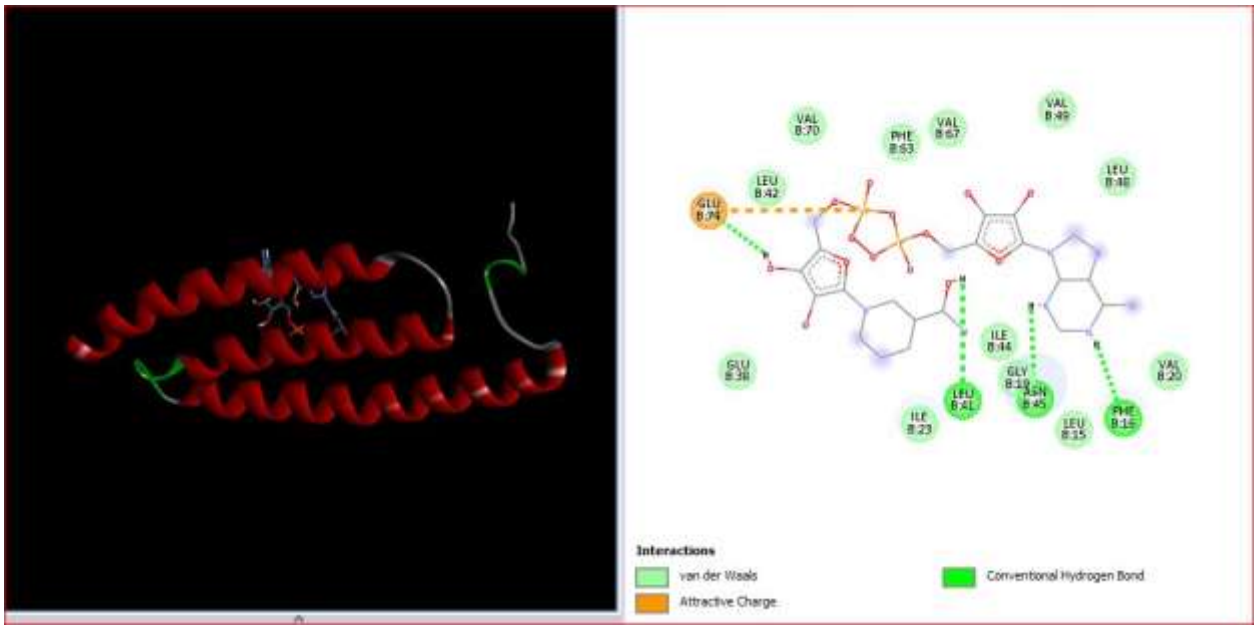
Molecular docking result for 30S ribosomal protein S4 and clomocycline



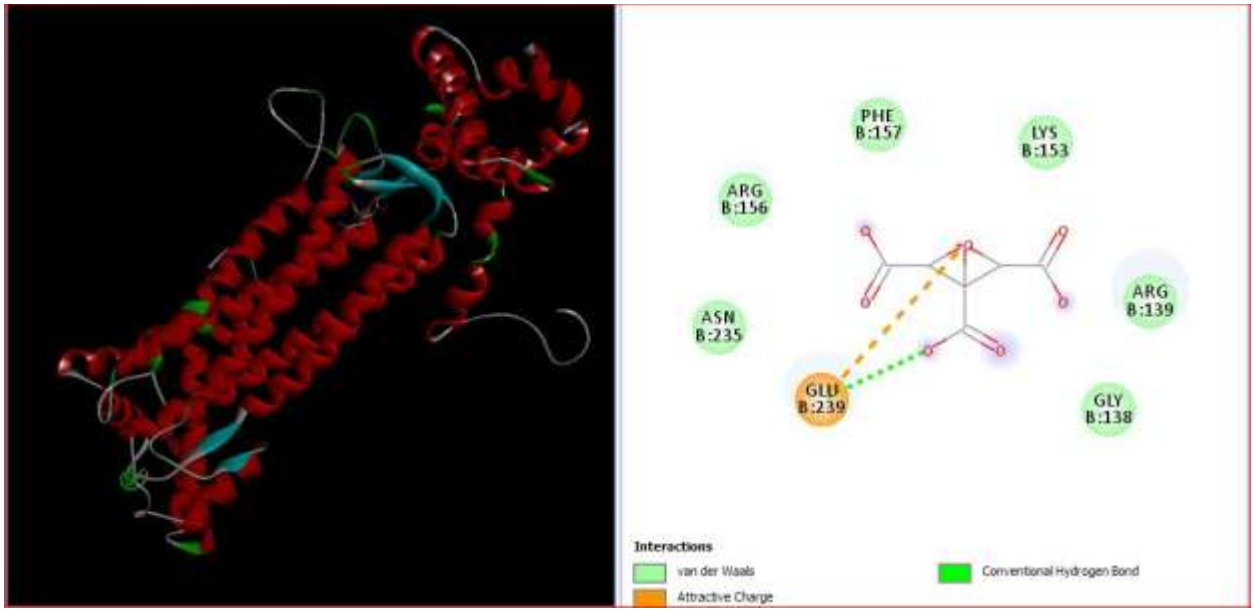
Molecular docking result for DNA polymerase III subunit gamma/tau and adenine



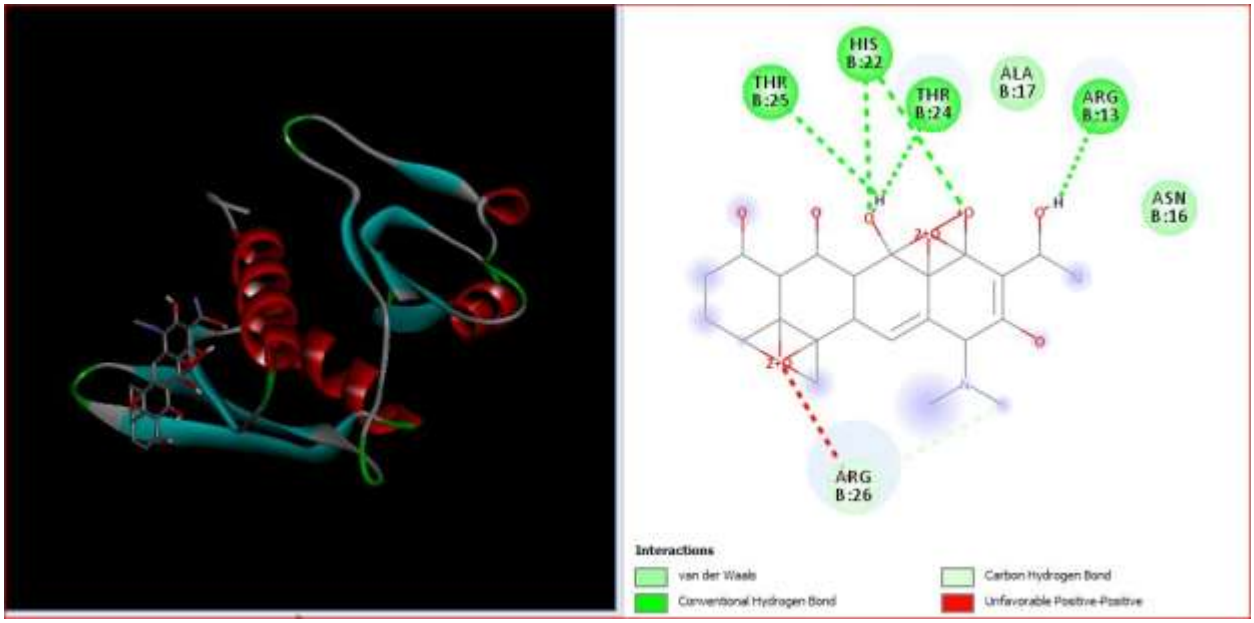
Molecular docking result for 3-oxoacyl-(acyl-carrier-protein) synthase 3 and cerulenin



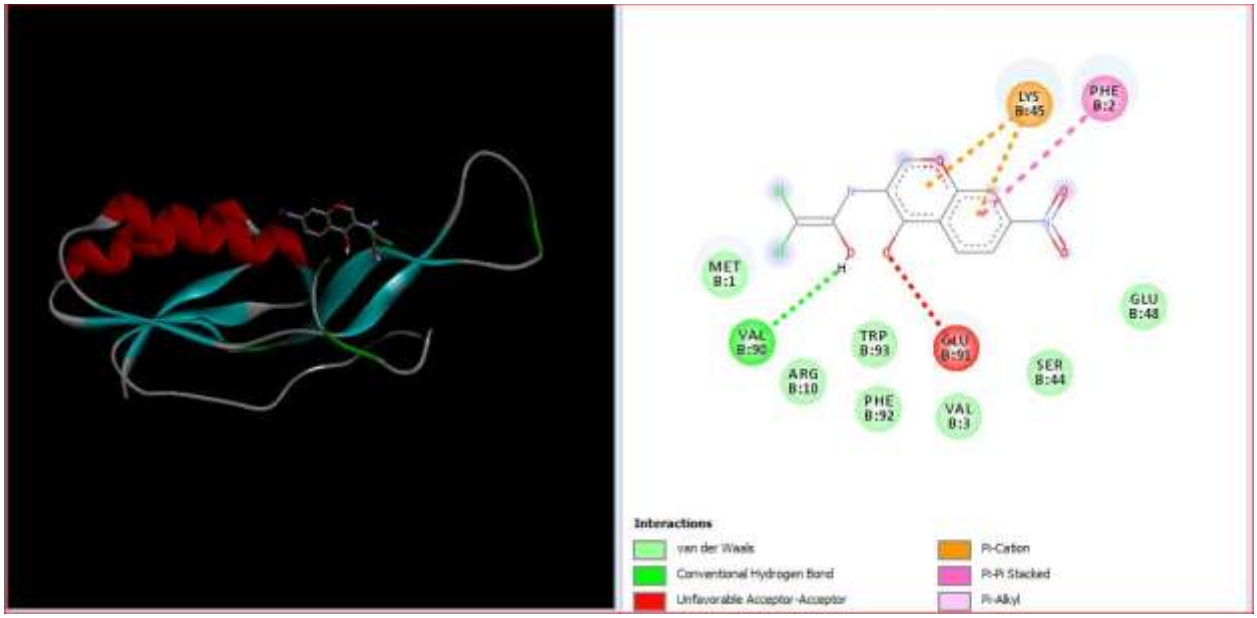
Molecular docking result for NADH quinine oxidoreductase subunit K and NADH



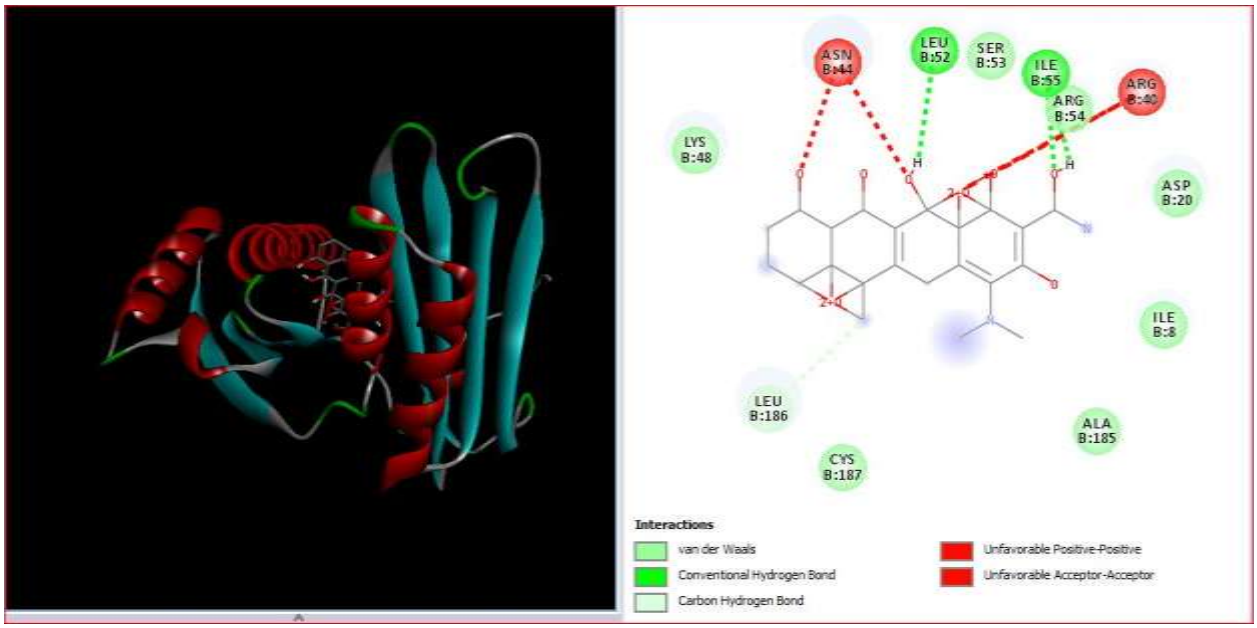
Molecular docking result for adenylosuccinate lyase ASL and citric acid



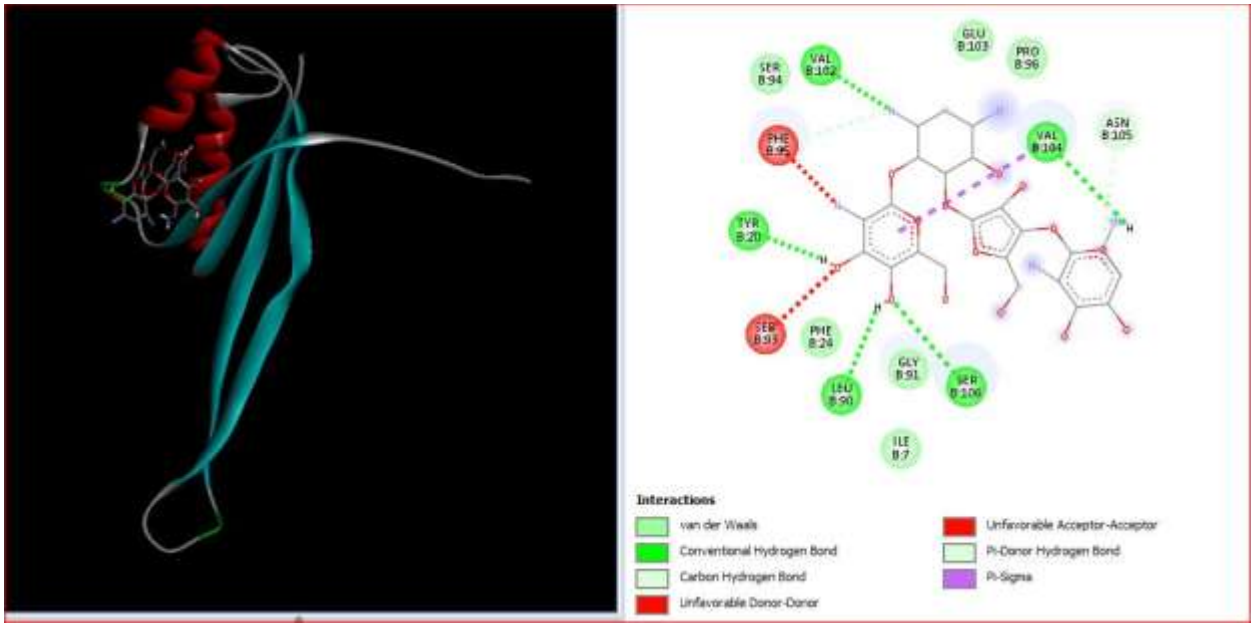
Molecular docking result for 30S ribosomal protein S8 and tetracycline



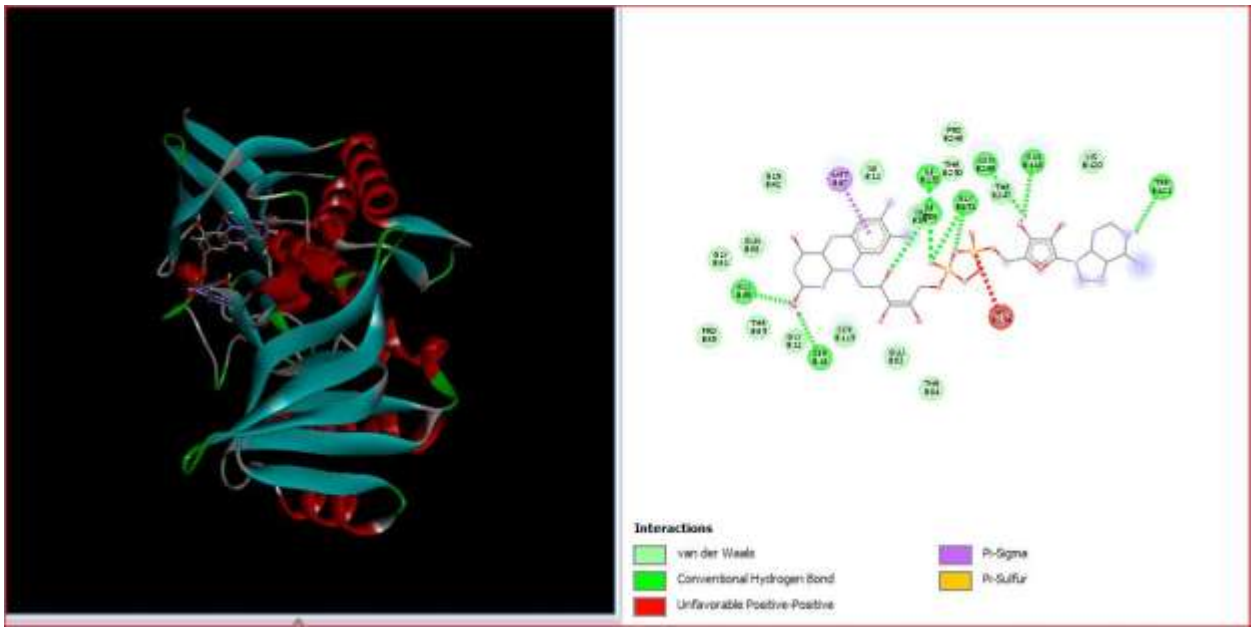
Molecular docking result for 50S ribosomal protein L16 and chlorampenicol



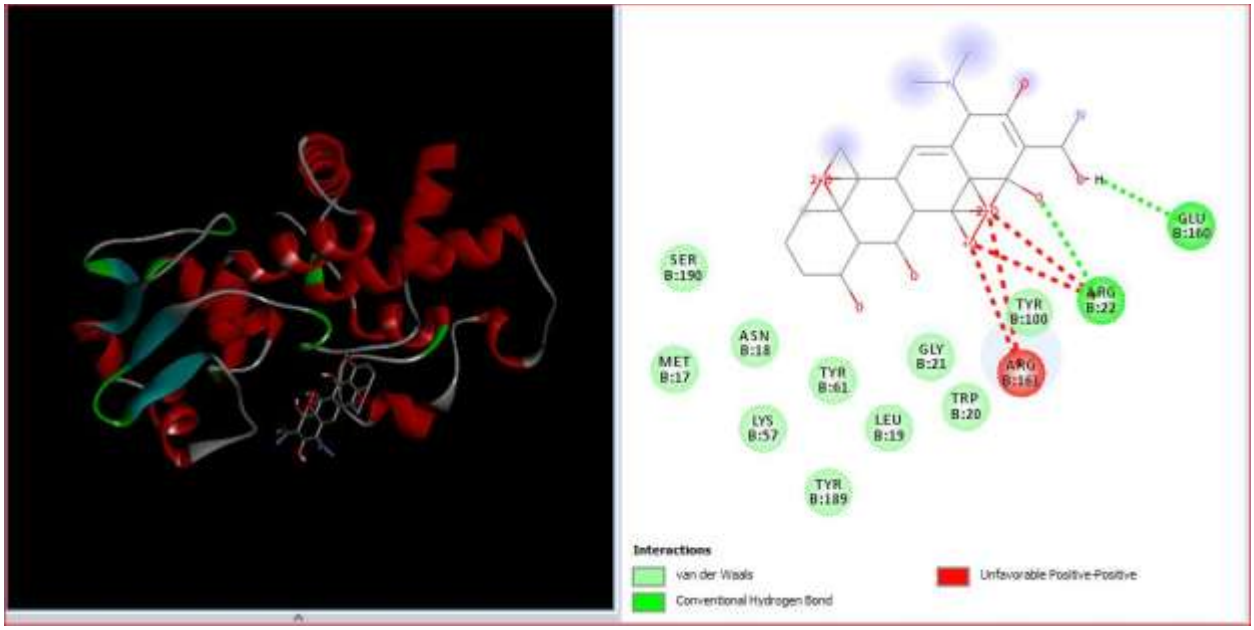
Molecular docking result for 30S ribosomal protein S3 and tetracycline



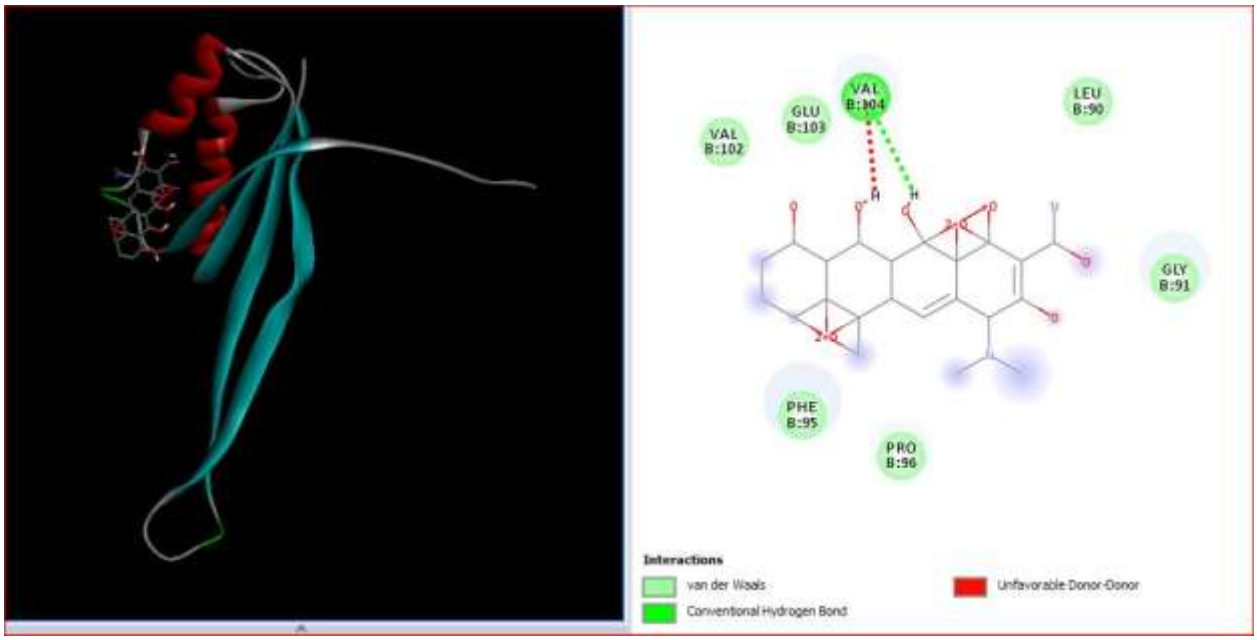
Molecular docking result for 30S ribosomal proteinS10 and paromomycin



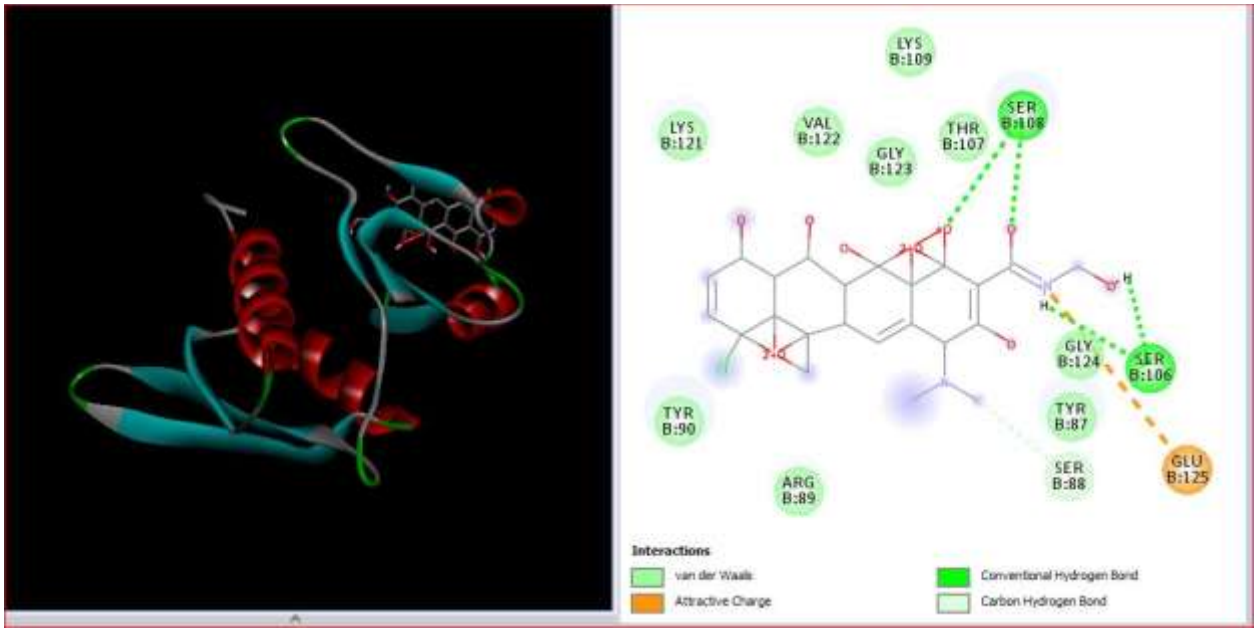
Molecular docking result for thioredoxin reductase and flavine adenine dinucleotide



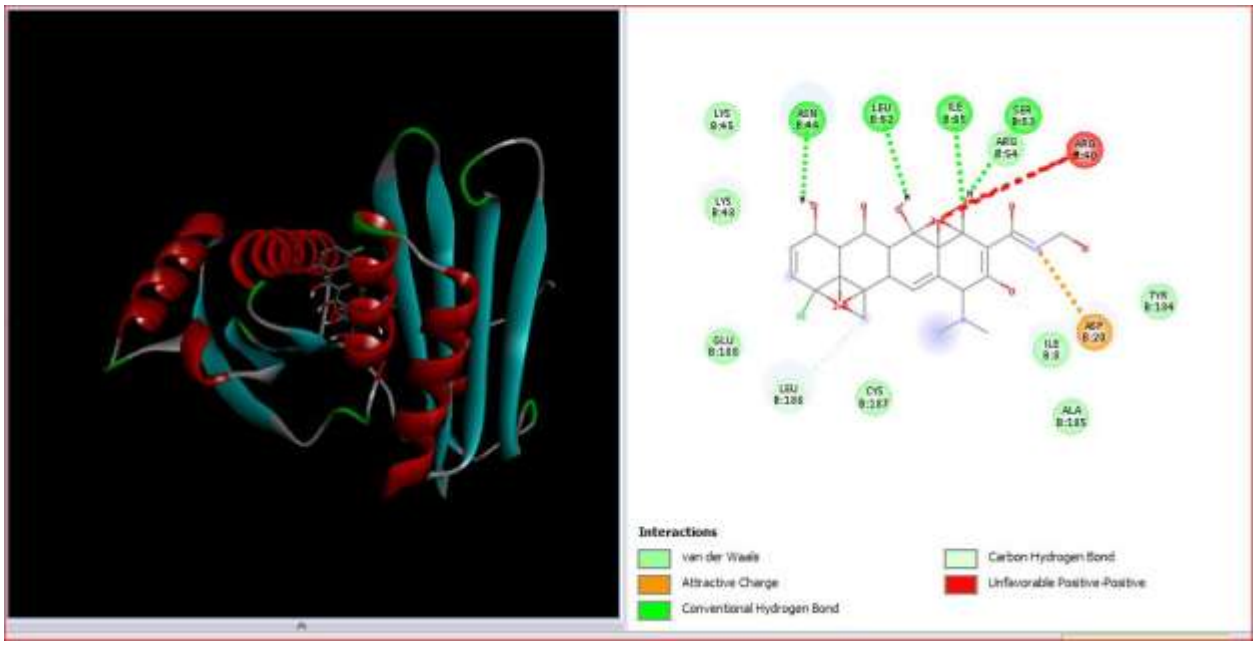
Molecular docking result for tetracycline and 30S ribosomal protein S4



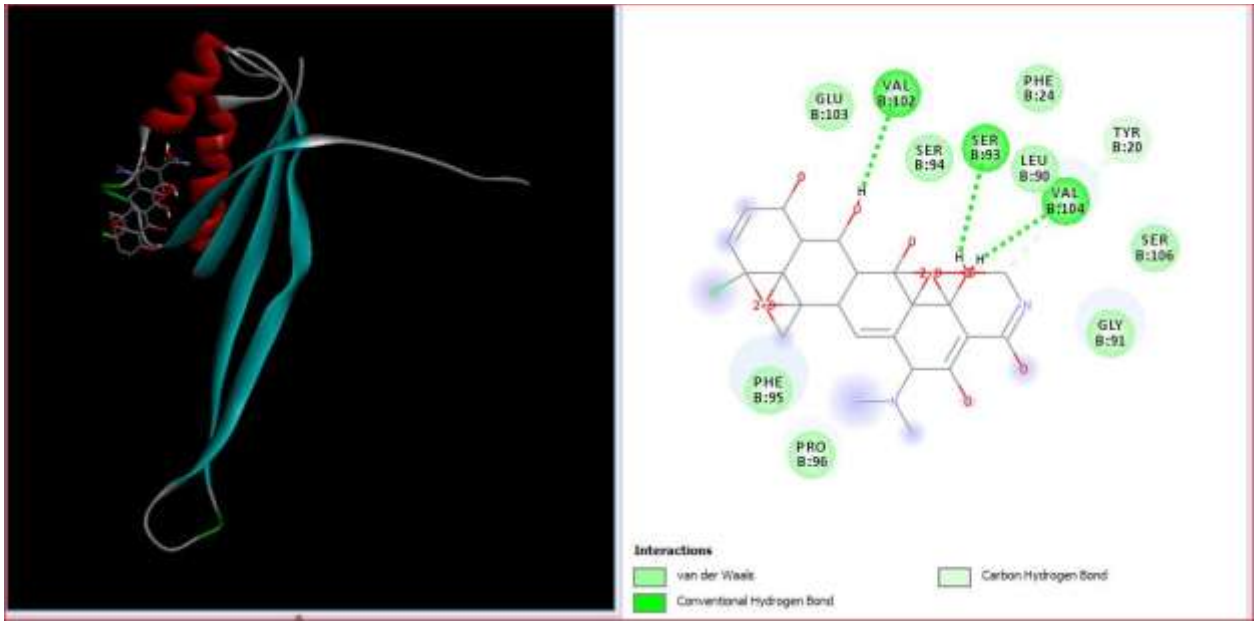
Molecular docking results for tetracycline and 30S ribosomal protein S10



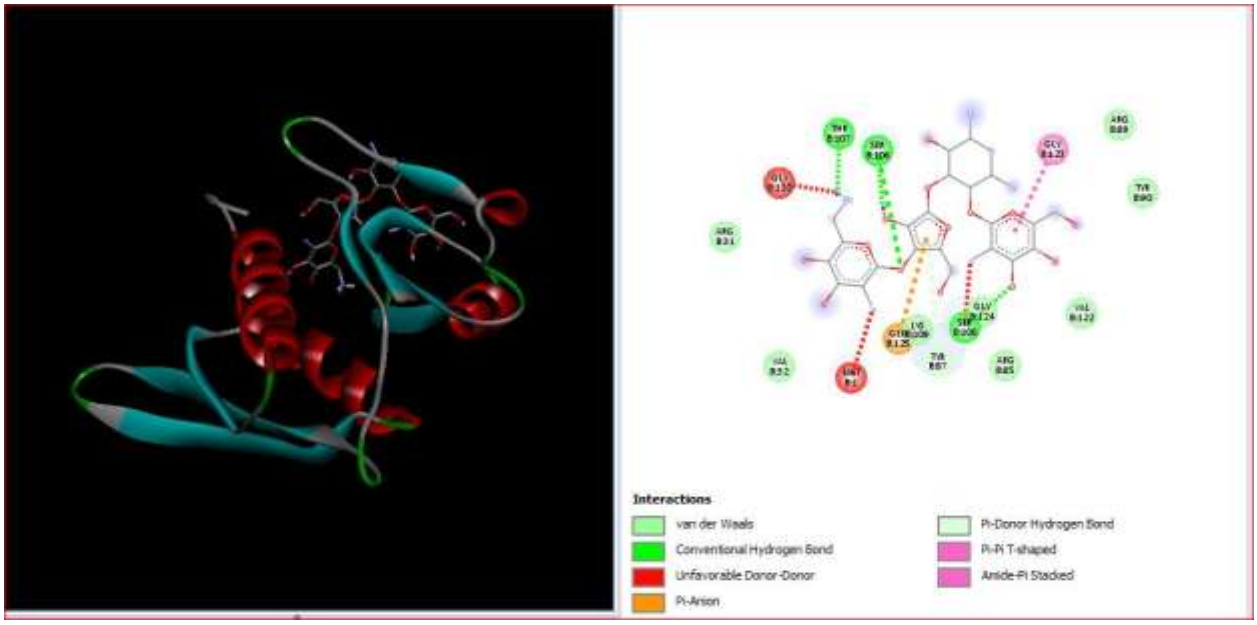
Molecular docking result for Clomocycline and 30S ribosomal protein S8



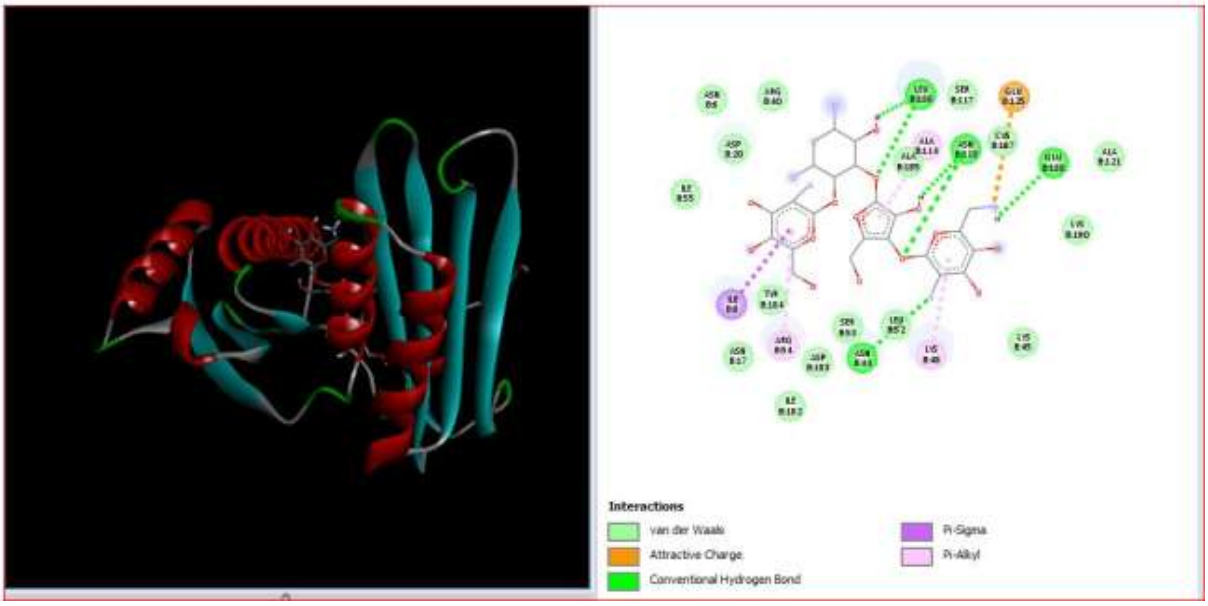
Molecular docking result for 30S ribosomal protein S3 and clomocycline



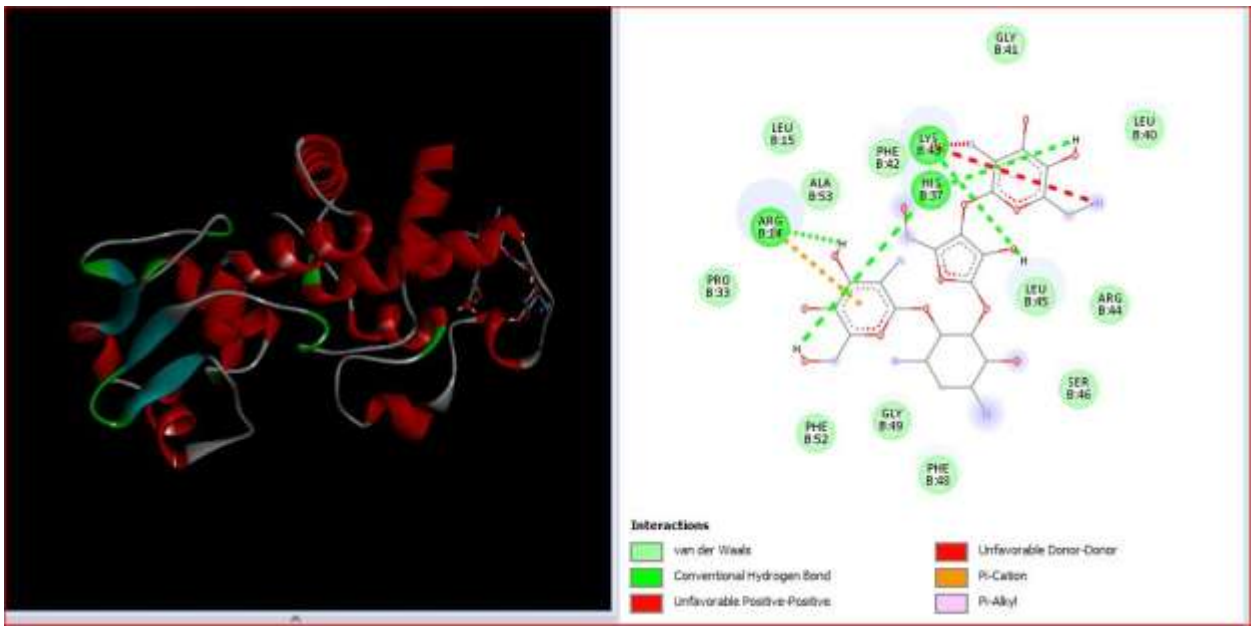
Molecular docking result for clomocycline and 30S ribosomal protein S10



Molecular docking result for paromomycin and 30S ribosomal protein S8



Molecular docking result for Paromomycin and 30S ribosomal protein S3



Molecular docking result for paromomycin and 30S ribosomal protein S4

APPENDIX IX

Essential proteins of *wOo*

S/N	KEGG ID	Name of protein
1	wOo_00230	**Inorganic pyrophosphatase**
2	wOo_00310	**NADH-quinone oxidoreductase subunit H**
3	wOo_00360	Uracil-DNA glycosylase
4	wOo_00420	**Leucine--tRNA ligase**
5	wOo_00500	F0F1-type ATP synthase subunit B
6	wOo_00610	**2,3,4,5-tetrahydropyridine-2,6-dicarboxylate N-succinyltransferase**
7	wOo_00710	**Sec-independent protein translocase protein TatA**
8	wOo_00800	**Ferredoxin--NADP reductase, FNR, Fd-NADP(+) reductase**
9	wOo_00840	F0F1-type ATP synthase epsilon subunit
10	wOo_00860	**Phosphate transport system permease protein**
11	wOo_00940	**Ribonucleoside-diphosphate reductase**
12	wOo_00970	**UvrABC system protein C**
13	wOo_01000	**Glycine--tRNA ligase beta subunit**
14	wOo_01010	**Glycine--tRNA ligase alpha subunit**
15	wOo_01160	Single-stranded DNA-specific exonuclease RecJ
16	wOo_01180	**tRNA dimethylallyltransferase**
17	wOo_01260	**Fumarate hydratase class II**
18	wOo_01280	**Lysine--tRNA ligase**
19	wOo_01420	**UDP-N-acetylmuramoylalanine--D-glutamate ligase**
20	wOo_01490	**D-alanine--D-alanine ligase**
21	wOo_01580	**Protein translocase subunit SecE**
22	wOo_01610	50S ribosomal protein L1
23	wOo_01620	**50S ribosomal protein L10**
24	wOo_01730	**DNA mismatch repair protein MutL**
25	wOo_01740	**N5-carboxyaminoimidazole ribonucleotide mutase**
26	wOo_01760	**DNA-directed RNA polymerase subunit omega, RNAP omega subunit**
27	wOo_01880	**50S ribosomal protein L25**
28	wOo_01940	**Phenylalanine--tRNA ligase alpha subunit**
29	wOo_02150	**Formamidopyrimidine-DNA glycosylase, Fapy-DNA glycosylase**
30	wOo_02280	Metallo-beta-lactamase superfamily hydrolase
31	wOo_02480	**DNA helicase**
32	wOo_02550	**Ribonucleoside-diphosphate reductase subunit beta**
33	wOo_02570	**Protein translocase subunit SecA**
34	wOo_02760	Trans-2-enoyl-ACP reductase FabK
35	wOo_03050	**Signal peptidase I**
36	wOo_03170	**Flavin prenyltransferase UbiX**
37	wOo_03200	Cell division protein FtsI
38	wOo_03220	**Proline--tRNA ligase**

39	wOo_03240	**UDP-N-acetylglucosamine 1-carboxyvinyltransferase**
40	wOo_03250	**Flavin-dependent thymidylate synthase, FDTS**
41	wOo_03380	**Thymidylate kinase**
S/N	KEGG ID	Name of protein
42	wOo_03470	ABC-type Fe3+ transport system permease component
43	wOo_03490	**N5-carboxyaminoimidazole ribonucleotide synthase, N5-CAIR synthase**
44	wOo_03500	**Aspartate-semialdehyde dehydrogenase, ASA dehydrogenase**
45	wOo_03620	**3-hydroxyacyl-[acyl-carrier-protein] dehydratase FabZ**
46	wOo_03650	**Zinc metalloprotease**
47	wOo_03680	30S ribosomal protein S1
48	wOo_03770	**Beta sliding clamp**
49	wOo_03800	**Phospho-N-acetylmuramoyl-pentapeptide-transferase**
50	wOo_03810	**Ribonuclease HII, RNase HII**
51	wOo_03850	ATPase involved in DNA replication HolB
52	wOo_03870	Preprotein translocase subunit YajC
53	wOo_03940	Asp-tRNAAsnGlu-tRNAGln amidotransferase C subunit
54	wOo_04050	**Aspartate carbamoyltransferase**
55	wOo_04060	ABC-type Fe3+ transport system periplasmic component
56	wOo_04090	**NAD kinase**
57	wOo_04100	**50S ribosomal protein L31**
58	wOo_04110	**Malonyl CoA-acyl carrier protein transacylase**
59	wOo_04120	**2,3-bisphosphoglycerate-independent phosphoglycerate mutase, BPG-independent PGAM, Phosphoglyceromutase, iPGM**
60	wOo_04220	UDP-N-acetylmuramate-alanine ligase
61	wOo_04290	**Octanoyltransferase**
62	wOo_04300	Phosphoribosylformylglycinamidine synthase domain-containing protein
63	wOo_04310	**Phosphate transport system permease protein PstA**
64	wOo_04320	**50S ribosomal protein L19**
65	wOo_04340	**Phosphoribosylaminoimidazole-succinocarboxamide synthase**
66	wOo_04390	**Uroporphyrinogen decarboxylase**
67	wOo_04550	**GMP synthase (glutamine-hydrolyzing)**
68	wOo_04620	**Lipoprotein signal peptidase**
69	wOo_04730	**UvrABC system protein A**
70	wOo_04760	**Glycerol-3-phosphate acyltransferase**
71	wOo_04790	Phosphatidylserine synthase
72	wOo_04800	**Phosphatidylserine decarboxylase proenzyme**
73	wOo_04960	Geranylgeranyl pyrophosphate synthase
74	wOo_05130	**Primosomal protein N'**
75	wOo_05160	**Dihydroorotase, DHOase**
76	wOo_05210	**Isoleucine--tRNA ligase**
77	wOo_05230	**Alanine--tRNA ligase**
78	wOo_05300	Acetylglutamate kinase
79	wOo_05310	**Deoxyuridine 5'-triphosphate nucleotidohydrolase**

80	wOo_05440	**Phosphatidylglycerophosphatase A**
81	wOo_05580	Fructose-16-bisphosphatase
82	wOo_05670	Phosphatase
83	wOo_05750	**tRNA-specific 2-thiouridylase MnmA**
84	wOo_05800	**Protoheme IX farnesyltransferase**
85	wOo_05810	**Ribonuclease H, RNase H**
S/N	KEGG ID	Name of protein
86	wOo_05830	**Sec-independent protein translocase protein TatC**
87	wOo_05940	**Coproporphyrinogen oxidase**
88	wOo_05960	**Orotidine 5'-phosphate decarboxylase**
89	wOo_05980	**Valine--tRNA ligase**
90	wOo_06020	D-alanyl-D-alanine carboxypeptidase
91	wOo_06030	**Phenylalanine--tRNA ligase beta subunit**
92	wOo_06050	**dCTP deaminase**
93	wOo_06080	**Replicative DNA helicase**
94	wOo_06120	**DNA primase**
95	wOo_06380	Uroporphyrinogen-III synthase
96	wOo_06430	**DNA polymerase I**
97	wOo_06540	ABC-type Zn2+ transport system periplasmic component
98	wOo_06590	**Protein translocase subunit SecD**
99	wOo_06610	**UDP-N-acetylenolpyruvoylglucosamine reductase**
100	wOo_06790	Phosphatidylglycerophosphate synthase
101	wOo_06800	Malic enzyme
102	wOo_06850	**UvrABC system protein B, Protein UvrB**
103	wOo_06870	Phosphoribosylaminoimidazole synthetase
104	wOo_06880	Dihydropteroate synthase putative
105	wOo_06890	Cytosineadenosine deaminase
106	wOo_06920	**DNA ligase**
107	wOo_06930	**5'-nucleotidase SurE**
108	wOo_06950	Response regulator PleD
109	wOo_06960	Response regulator PleD
110	wOo_06980	NAD-specific glutamate dehydrogenase
111	wOo_07010	**50S ribosomal protein L28**
112	wOo_07050	**30S ribosomal protein S4**
113	wOo_07120	**Protein-export membrane protein SecF**
114	wOo_07150	**Membrane protein insertase YidC**
115	wOo_07240	Type IV secretory pathway VirB11 component
116	wOo_07280	GTP cyclohydrolase II
117	wOo_07350	**Phosphoribosylformylglycinamidine synthase subunit PurL, FGAM synthase**
118	wOo_07450	**DNA polymerase III subunit gamma/tau, EC 2.7.7.7**
119	wOo_07600	**Aspartokinase**
120	wOo_07630	**Tyrosine--tRNA ligase**
121	wOo_07700	**RNA pyrophosphohydrolase**
122	wOo_07780	**UDP-N-acetylmuramoyl-tripeptide--D-alanyl-D-alanine ligase**

123	wOo_07810	**Ribulose-phosphate 3-epimerase**
124	wOo_07820	**Polyribonucleotide nucleotidyltransferase**
125	wOo_07830	**30S ribosomal protein S15**
126	wOo_07920	**Deoxyguanosinetriphosphate triphosphohydrolase-like protein**
127	wOo_07970	**3-oxoacyl-[acyl-carrier-protein] synthase 3**
128	wOo_07980	**Phosphate acyltransferase, EC 2.3.1.274**
S/N	KEGG ID	Name of protein
129	wOo_08050	Biotin-acetyl-CoA carboxylase-ligase
130	wOo_08090	**NADH-quinone oxidoreductase subunit K**
131	wOo_08100	**NADH-quinone oxidoreductase subunit J**
132	wOo_08150	**Heme exporter protein C**
133	wOo_08210	**Transcription termination factor Rho**
134	wOo_08720	**4-hydroxy-tetrahydrodipicolinate reductase, HTPA reductase**
135	wOo_08740	DNA polymerase III delta subunit
136	wOo_08750	ABC-type Mn2+Zn2+ transport system permease component
137	wOo_08770	**4-diphosphocytidyl-2-C-methyl-D-erythritol kinase, CMK**
138	wOo_08850	**UDP-N-acetylmuramoyl-L-alanyl-D-glutamate--2,6-diaminopimelate ligase**
139	wOo_08970	**50S ribosomal protein L21**
140	wOo_09010	**Aspartyl/glutamyl-tRNA(Asn/Gln) amidotransferase subunit B, Asp/Glu-ADT subunit B**
141	wOo_09030	Ribonuclease RneRng family protein
142	wOo_09040	**50S ribosomal protein L33**
143	wOo_09130	**Adenylosuccinate lyase, ASL**
144	wOo_09140	**50S ribosomal protein L9**
145	wOo_09160	**30S ribosomal protein S6**
146	wOo_09170	**DNA-directed DNA polymerase**
147	wOo_09260	**Diaminopimelate epimerase, DAP epimerase**
148	wOo_09300	**Protein-export protein SecB**
149	wOo_09320	**DNA polymerase III subunit epsilon**
150	wOo_09350	**30S ribosomal protein S20**
151	wOo_09490	**Glutathione synthetase**
152	wOo_09500	**UDP-N-acetylglucosamine--N-acetylmuramyl-(pentapeptide) pyrophosphoryl-undecaprenol N-acetylglucosamine transferase**
153	wOo_09580	3-polyprenyl-4-hydroxybenzoate decarboxylase UbiD
154	wOo_09610	**Chromosomal replication initiator protein DnaA**
155	wOo_09620	Glutamine synthetase
156	wOo_09720	tRNA nucleotidyltransferasepolyA-polymerase
157	wOo_09770	**Fructose-1,6-bisphosphatase**
158	wOo_09800	**Pyruvate, phosphate dikinase**
159	wOo_09920	**Isoprenyl transferase**
160	wOo_09950	**Uridylate kinase, UK**
161	wOo_09990	**Carbamoyl-phosphate synthase large chain**
162	wOo_10050	Phosphomannomutase
163	wOo_10080	ABC-type transport system involved in lipoprotein release permease

		component
164	wOo_10110	Succinate dehydrogenase subunit D sdhD hydrophobi c membrane anchor protein
165	wOo_10240	**DNA-directed RNA polymerase subunit alpha, RNAP subunit alpha**
166	wOo_10300	**Protein translocase subunit SecY**
167	wOo_10320	**30S ribosomal protein S5**
S/N	KEGG ID	Name of protein
168	wOo_10330	**50S ribosomal protein L18**
169	wOo_10340	**50S ribosomal protein L6**
170	wOo_10350	**30S ribosomal protein S8**
171	wOo_10400	**30S ribosomal protein S17**
172	wOo_10410	**50S ribosomal protein L29**
173	wOo_10420	**50S ribosomal protein L16**
174	wOo_10430	**30S ribosomal protein S3**
175	wOo_10440	**50S ribosomal protein L22**
176	wOo_10470	**50S ribosomal protein L23**
177	wOo_10500	**30S ribosomal protein S10**
178	wOo_10540	**Carbamoyl-phosphate synthase small chain**
179	wOo_10580	**50S ribosomal protein L35**
180	wOo_10770	**Thioredoxin reductase**

APPENDIX X

Molecular docking of antibiotic drugs with their drug targets

S/N	Drug targets	Drugs/ Drug group	Binding energy (Kcal/mol)	Dissociation constant (Kd)	Interactions involved
1	NADH-quinone oxidoreductase subunit H	NADH (Nicotinamide adenine dinucleotide)/ Co-enzyme	-8.2	9.61×10^{-7}	Van der Waals Attractive charge Conventional Hydrogen Bond Carbon Hydrogen Bond Pi-Cation (electrostatic)) Pi-Anion (electrostatic) Alkyl (hydrophobic) Pi-Alkyl (hydrophobic)
2	Ferredoxin--NADP reductase	Azelaic acid/ organic acid	-4.4	5.90×10^{-4}	Van der Waals Conventional Hydrogen Bond
3	Ribonucleoside-diphosphate reductase	Cladribine/ purine related drug	-5.9	4.68×10^{-5}	Van der Waals Conventional Hydrogen Bond Unfavourable donor donor Pi-pi T shaped Pi-Alkyl (hydrophobic)
4	Fumarate hydratase class II (Fumarase C)	Citric acid/ organic acid	-4.2	8.28×10^{-4}	Van der Waals Attractive charge Conventional Hydrogen Bond Carbon Hydrogen Bond

Molecular docking of Antibiotic drugs with their drug targets

S/N	Drug targets	Drugs/ Drug group	Binding energy (Kcal/mol)	Dissociation constant (Kd)	Interactions involved
5	D-alanine--D-alanine ligase	Cycloserine/ antibiotic	-3.9	1.37×10^{-3}	Van der Waals Carbon Hydrogen Bond Unfavourable donor donor Pi Donor Hydrogen Bond
6	50S ribosomal protein L10	Linomycin/ antibiotic	-5.6	7.77×10^{-5}	Van der Waals Attractive charge Conventional Hydrogen Bond Alkyl (hydrophobic) Pi-Alkyl (hydrophobic)
7	N5-carboxyaminoimidazole ribonucleotide mutase	Citric acid/organic acid	-4.5	4.99×10^{-4}	Van der Waals Attractive charge Conventional Hydrogen Bond Unfavourable positive positive
8	Ribonucleoside-diphosphate reductase subunit beta	Cladribine/ Purine related drug	-6. 1	3.34×10^{-5}	Van der Waals Conventional Hydrogen Bond Carbon Hydrogen Bond Pi-Cation (electrostatic) Pi-Alkyl (hydrophobic)

Molecular docking of Antibiotic drugs with their drug targets

S/N	Drug targets	Drugs/ Drug group	Binding energy (Kcal/mol)	Dissociation constant (Kd)	Interactions involved
9	Flavin prenyltransferase UbiX	Flavine mononucleotide / co-enzyme	-6.9	8.64×10^{-6}	Van der Waals Conventional Hydrogen Bond Pi-Donor Hydrogen Bond Alkyl
10	UDP-N-acetylglucosamine 1-carboxyvinyltransferase	Fosfomycin/ antibiotic	-3.9	1.37×10^{-3}	Van der Waals Attractive charge Conventional Hydrogen Bond Unfavourable positive positive Pi-Cation (electrostatic)) Pi-Anion (electrostatic) Alkyl (hydrophobic) Pi-Alkyl (hydrophobic)
11	Flavin-dependent thymidylate synthase (FDTS)	Flavine adenine dinucleotide/ co-enzyme	-7.4	3.71×10^{-6}	Van der Waals Conventional Hydrogen Bond Carbon Hydrogen Bond Pi- Donor Hydrogen Bond Pi- sigma Pi-pi stacked Pi-pi T shaped Alkyl (hydrophobic) Pi-Alkyl (hydrophobic)

Molecular docking of Antibiotic drugs with their drug targets

S/ N	Drug targets	Drugs/ Drug group	Binding energy (Kcal/mol)	Dissociation constant (Kd)	Interactions involved
12	N5-carboxyaminoimidazole ribonucleotide synthase	Carglumic acid/ enzyme activator	-5.2	1.53×10^{-4}	Van der Waals Salt Bridge Conventional Hydrogen Bond Unfavourable acceptor acceptor
13	ABC-type Fe3+ transport system periplasmic component	Formic acid/ Organic acid	-2.7	1.04×10^{-2}	Van der Waals Conventional Hydrogen Bond
14	GMP synthase (glutamine-hydrolyzing)	Citric acid/ Organic acid	-4.7	3.56×10^{-4}	Van der Waals Attractive charge Conventional Hydrogen Bond Carbon Hydrogen Bond
15	Geranylgeranyl Pyrophosphate synthase	Pyrophosphoric acid/ inorganic acid	-4.5	4.99×10^{-4}	--
16	Valine--tRNA ligase	L-isoleucine/ amino acid	-4.4	5.90×10^{-4}	Van der Waals Conventional Hydrogen Bond Pi- sigma Alkyl (hydrophobic) Pi-Alkyl (hydrophobic)

Molecular docking of Antibiotic drugs with their drug targets

S/N	Drug targets	Drugs/ Drug group	Binding energy (Kcal/mol)	Dissociation constant (Kd)	Interactions involved
17	D-alanyl-D-alanine carboxypeptidase	Cefmatazole/ antibiotic	-5. 1	1.81×10^{-4}	Van der Waals Conventional Hydrogen Bond Carbon Hydrogen Bond Pi- Donor Hydrogen
18	Replicative DNA helicase	Zinc/ trace element	-1.3	1.11×10^{-1}	--
19	DNA polymerase I	Azelaic acid/ organic acid	-5. 1	1.81×10^{-4}	Van der Waals Conventional Hydrogen Bond
20	**UDP-N-acetylenolpyruvo-ylglucosamine reductase**	Flavine adenine dinucleotide/ co-enzyme	-8.3	8.12×10^{-7}	Van der Waals Conventional Hydrogen Bond Alkyl (hydrophobic) Pi-Alkyl (hydrophobic) Unfavourable donor-donor
21	Malic enzyme	NADH/ co-enzyme	-9. 1	2.10×10^{-7}	Van der Waals Attractive charge Conventional Hydrogen Bond Unfavourable acceptor acceptor Pi-Anion (electrostatic) Alkyl (hydrophobic) Pi-Alkyl (hydrophobic)

Molecular docking of Antibiotic drugs with their drug targets

S/N	Drug targets	Drugs/ Drug group	Binding energy (Kcal/mol)	Dissociation constant (Kd)	Interactions involved
22	30S ribosomal protein S4	Clomocycline/ antibiotic	-6.5	1.70×10^{-5}	Van der Waals Salt Bridge Attractive charge Conventional Hydrogen Bond Unfavourable positive positive Unfavourable donor donor
23	DNA polymerase III subunit gamma/tau	Adenine/ purine related drug	-5. 1	1.81×10^{-4}	Van der Waals Conventional Hydrogen Bond
24	3-oxoacyl-[acyl-carrier-protein] synthase 3	Cerulenin/ antibiotic	-4.9	2.54×10^{-4}	Van der Waals Conventional Hydrogen Bond Unfavourable acceptor acceptor Alkyl
25	NADH-quinone oxidoreductase subunit K	NADH/ co-enzyme	-5.9	4.68×10^{-5}	Van der Waals Attractive charge Conventional Hydrogen Bond
26	Adenylosuccinate lyase (ASL)	Citric acid/ organic acid	-4. 1	$9.80 \times 10^{--4}$	Van der Waals Attractive charge Conventional Hydrogen Bond

Molecular docking of Antibiotic drugs with their drug targets

S/N	Drug targets	Drugs/ Drug group	Binding energy (Kcal/mol)	Dissociation constant (Kd)	Interactions involved
27	Glutamine synthetase	Citric acid/ organic acid	-4.3	6.99×10^{-4}	Van der Waals Conventional Hydrogen Bond Carbon Hydrogen Bond Pi-donor Hydrogen Bond
28	30S ribosomal protein S8	Tetracycline/ antibiotic	-6.4	2.01×10^{-5}	Van der Waals Conventional Hydrogen Bond Carbon Hydrogen Bond Unfavourable positive positive
29	50S ribosomal protein L16	Chloramphenicol/ antibiotic	-5.5	9.20×10^{-5}	Van der Waals Conventional Hydrogen Bond Unfavourable acceptor acceptor Pi- Cation Pi-pi stacked Pi-alkyl
30	30S ribosomal protein S3	Tetracycline/ antibiotic	-7.4	3.71×10^{-6}	Van der Waals Conventional Hydrogen Bond Carbon Hydrogen Bond Unfavourable acceptor acceptor Unfavourable positive positive

Molecular docking of Antibiotic drugs with their drug targets

S/N	Drug targets	Drugs/ Drug group	Binding energy (Kcal/mol)	Dissociation constant (Kd)	Interactions involved
31	30S ribosomal protein S10	Paromomycin/ antibiotic	-5.5	9.20×10^{-5}	Van der Waals Conventional Hydrogen Bond Carbon Hydrogen Bond Unfavourable acceptor acceptor Unfavourable donor donor Pi- donor Hydrogen Bond Pi- sigma
32	Thioredoxin reductase	Flavin adenine dinucleotide/ co-enzyme	-8.7	4.13×10^{-7}	Van der Waals Conventional Hydrogen Bond Unfavourable positive positive Pi-sulfur Pi- sigma