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In silico molecular docking studies of di hydro pyrimidinones as MTB thymidylate kinase inhibitors

Girija Sastry Vedula
Department of Pharmaceutical Chemistry, College of Pharmaceutical Sciences, Andhra University, Vishakapatnam, 530003, India

Siva Kumar Nemala
Department of Pharmaceutical Chemistry, College of Pharmaceutical Sciences, Andhra University, Vishakapatnam, 530003, India
Corresponding author email: nemalasivakumar@gmail.com

***Abstract*---**Tuberculosis is the one of the leading cause of death. Efforts are needed to develop new anti-tuberculosis medicines with alternative scaffolds because the current antibiotics are increasingly becoming ineffective against M. tuberculosis. In this study, 21 novel di hydro pyrimidinone derivatives were designed and synthesized to arrive at potentially effective anti-tubercular agents. Molecular docking studies were conducted on Mtb thymidylate kinase of Mycobacterium tuberculosis H37Rv (PDB ID 5NQ5). The enzyme active sites were docked with the title compounds after they were energy-minimized. According to their docking scores and binding energies, the ligands were ordered. The molecular docking results for the title compounds with Mycobacterium TB thymidylate kinase are very promising. Thymidylate kinase appears to be inhibited by 17c and 20c, according to the study.

***Keywords*---**thymidylate kinase, molecular docking, ADMET, Di hydro pyrimidinones.

Introduction

Despite effective drug treatment and the widespread use of the BCG vaccine, *Captain of all these men of death*, Tuberculosis is a scourge since prehistoric times affects more than 9 million and causes about 1.5 million deaths every year. Tuberculosis (TB) remains a threat to the health of people worldwide in terms of morbidity and mortality next to HIV. An infection with Mycobacterium tuberculosis (M.Tb) can lead to a balanced "symbiosis" for survival. About 10% of

infected people will get the active disease, Tuberculosis (TB), while the rest have no symptoms but still carry the long-lived M.Tb. This is called latent tuberculosis infection (LTBI). Latently infected people, estimated to number 7 billion worldwide, offer a massive reservoir for tuberculosis reactivation, which is a common barrier to tuberculosis treatment and control.[1,2]

Dihydropyrimidines (DHPMs) offer a wide range of biological and pharmacological applications due to their molecular diversity in chemical and medicinal chemistry.[4-5] There have been reports of antibacterial, anti-HIV, analgesic, antiarrhythmic, antiinflammatory, anticancer properties for various substituted DHPMs as well as antihypertensive, anticonvulsant, antidiabetic, and antitubercular effects.[6-7]**.** Some drugs on the market, like Methylthiouracil[8], 5-Fluorouracil[9], Emivirine[10], Aminophylline[11], Idoxuridine[12], etc., have a dihydropyrimidine ring**.**

Di hydro pyrimidinones lineages are fascinating class of hetero cyclic molecules. Researchers are doing more work on dihydropyrimidinones because, it has more number of pharmacological activities such as anti-viral, anti- tumor, anti-bacterial and also calcium channel blocker activities. Monastrol, a molecule with a simple structure called DHPM, has been found to be a new cell-permeable molecule that stops the motor activity of mitotic kinesin, a motor protein that is important for spindle bipolarity, and stops the cell cycle in mammalian cells.[13] Calcium channel blockers and antihypertensive medications are examples of substituted dihydropyrimidinone molecules with noteworthy biological characteristics. These chemicals have a wide range of biological effects, including anti-inflammatory, anticancer, antiviral, and antibacterial properties.[14]

In order for Mtb to survive, it needs the enzyme MtTMPK, which helps synthesise the DNA-building compound thymidine triphosphate (TTP). [15] It's an appealing target for drug creation because of its well-known crystal structure and druggable active site. [16-18] According to this paper, our efforts have been made to improve the di hydro pyrimidinone's MtTMPK inhibitory activity via structure-based drug design.[19]

Materials and Methods

Materials

Docking studies were carried out using software Schrodinger 2021-2, in which GLIDE(Grid-based Ligand Docking with Energitics) module was mainly used for molecular modelling ,running on intel(R) Core™ i5-7200U CPU @ 2.50GHz × 4 processor using Linux workstation(Ubuntu 20.04).

Methods

Ligand Preparation

The 3D structures of the di hydro pyrimidinone derivatives (Table 1) were generated from the corresponding 2D structures with the software. The ligands were prepared using the “Lig-Prep” module.[20] A set of confirmations is generated

for each ligand. The geometry optimization was performed using the standard OPLS-4 force field and conventional bond lengths and bond angles. Finally the energy minimization was carried out and optimized confirmations are taken up for docking.

Protein Preparation

Docking studies were performed using protein crystal structures retrieved from PDB, which is a part of the Brookhaven Protein Database. The resolution of the protein structures was a deciding factor in their selection., Mtb thymidylate kinase in complex with 5-methyl-1-[(3~{S})-1-[(3-phenoxyphenyl)methyl]piperidin-3-yl]pyrimidine-2,4-dione with Resolution, 2.8 Å (PDB ID 5NQ5);. “Protein preparation wizard”[21] was used to prepare the protein. During protein preparation, we eliminated co-crystallized ligand and water molecules, added H-atoms, formed disulphide linkages, and fixed side chains. An energy refining and energy reduction operation was then carried out on the structure.

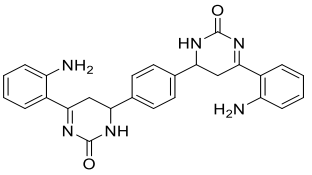
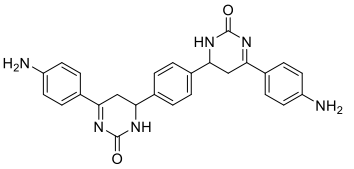
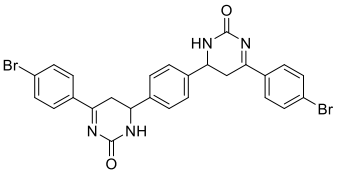
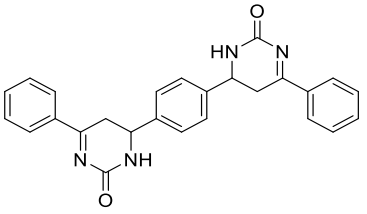
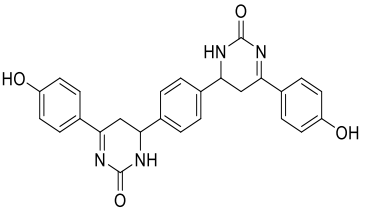
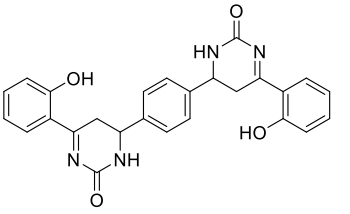
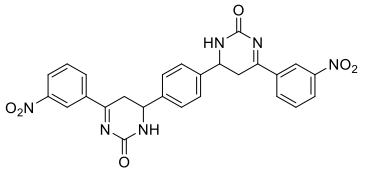
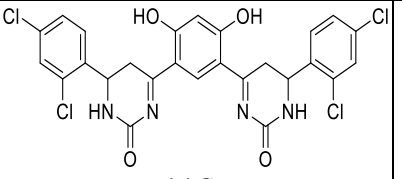
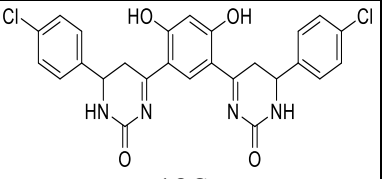
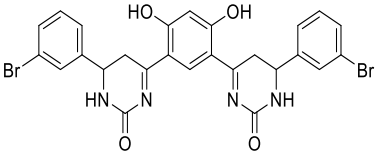
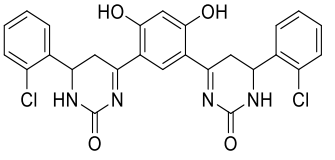
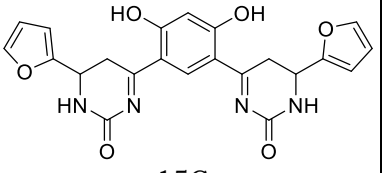
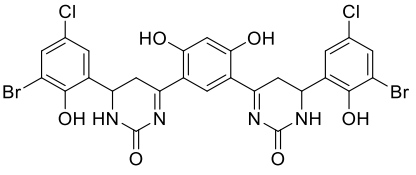
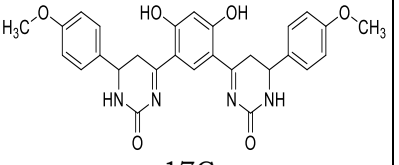
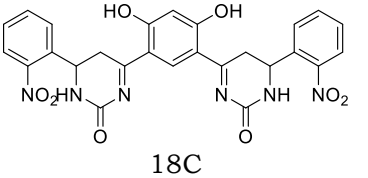
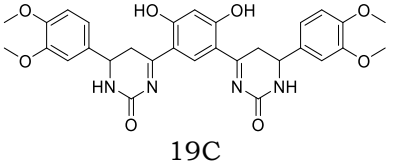
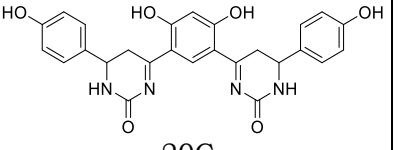
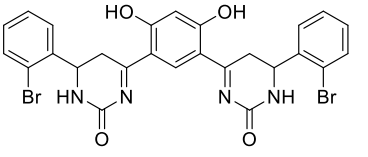
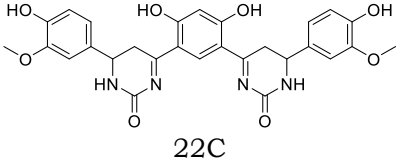
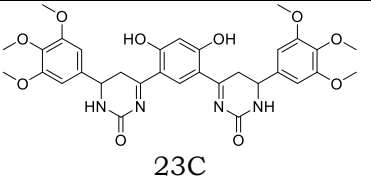
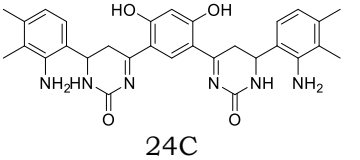
Receptor Grid Generation

After optimising the protein with co-crystallized ligand, the typical process of glide manual was followed to create an active site 3D grid. Afterward, the prepared ligand is docked into the co-crystallized ligand molecule. For ligand docking, receptor grid creation provides an outline of the protein active site size and location**.**

Docking Protocol

GLIDE module of Maestro was used to study the docking of the Di hydro pyrimidinone derivatives on the target proteins.[21-23] Glide was run on flexible docking mode where the protein is rigid and the ligand is flexible. The binding of the ligands were estimated using a variety of scoring functions that have been compiled into a single Glidescore (GScore).[24] Since it's based on empirical evidence, The final score is based on terms like "lipophilic-lipophilic," "hydrogen bonding," "rotatable-bond penalty," and "protein-ligand coulomb vdW energy contributions." Hydrophobic enclosure, or the displacement of water molecules by a ligand from locations containing many proximal lipophilic protein atoms, is also taken into account in GlideScore's scoring system. Protein-ligand hydrogen bonds formed within hydrophobic enclosing areas are essential for binding. Using the Emodel[25] scoring function and the GlideScore ranking function, Glide distinguishes between proteins with ligand complexes of a particular ligand and those with weak or no binding (inactive). “The Emodel scoring function is primarily defined by the protein-ligand coulomb-vdW energy with a small contribution from GlideScore”.

Table -1: Structures of novel di hydro pyramidinone derivatives

 1C	 2C	 3C
 4C	 5C	 6C
 7C	 11C	 12C
 13C	 14C	 15C
 16C	 17C	 18C
 19C	 20C	 21C
 22C	 23C	 24C

Results and Discussion

Molecular docking of the title compounds with potential targets of Mtb thymidylate kinase was performed. Glide combines a powerful sampling protocol with a value of a custom scoring function designed to identify ligand poses. Individual poses were examined to identify the binding interactions at the active site of respective protein and the ligands were evaluated in terms of Glide score and Emodel. The docked poses were ranked according to their docking scores. The ranking of the compounds was based on their binding energy with the enzyme. If the binging energy is less, the compound is more active.

Docking of Title Compounds with MTB Thymidylate Kinase of Mycobacterium Tuberculosis H37Rv

Table 2 shows the docking data for the ligands with thymidylate kinase. Good glide and Emodel scores have been achieved in XP mode. Compound 17C with Glide Score -8.314, Emodel -82.877 was found with the highest score. The phenyl groups of 17C have formed pi-pi stacking interactions with ASP 94, TYR 103, ARG 14 at the active site of the protein (Figure 1). The compound 20C with GScore -6.949 and Emodel -75.989 has also exhibited similar interaction with ARG 167, ARG 74, PHE 70. Most of the analogues have good GScores and show common binding interactions with PHE 70 and TYR 39 at the active site. The study suggests that pi-pi stacking interaction with PHE 70 and TYR 39 at the active site might be essential for thymidylate kinase inhibition.

Table 2: Molecular Docking results of Di hydro pyrimidinone derivatives with protein 5NQ5 (Thymidylate Kinase)

Code	Xp Mode			
	Gscore	Dscore	Emodel	Interactions At Active Site
5-methyl-1-[(3~{S})-1-[(3-phenoxyphenyl)methyl]piperidin-3-yl]pyrimidine-2,4-dione	-8.314	-8.314	-82.877	ASN 100, ARG 74, PHE 70, TYR 103
17C	-7.791	-7.588	-75.989	ASP 94, TYR 103, ARG 14
20C	-6.949	-6.738	-80.831	ARG 167, ARG 74, PHE 70
22C	-6.729	-6.015	-73.403	PHE 70, TYR 96, ALA 48, ARG 167
13C	-6.029	-5.814	-66.566	PHE 70, ARG 167, ARG 74
5C	-5.559	-5.553	-62.161	TYR 39, ARG 74, TYR 39
6C	-5.276	-5.258	-62.982	PRO 37
16C	-5.432	-5.134	-73.658	ASP 168, GLY 10, ARG 95, PHE 70
15C	-5.131	-4.947	-66.455	TYR 39, ALA 48, ARG 107,

				PHE 70
23C	-5.130	-4.920	-61.450	ASP 168, VAL 8, ARG 107
19C	-5.011	-4.801	-71.272	ALA 48, PHE 70
21C	-4.945	-4.732	-70.456	TYR 39, ARG 95
14C	-5.301	-4.594	-65.763	ARG 95, LYS 13
2C	-4.500	-4.500	-58.304	ASN 100, TYR 39
7C	-4.373	-4.373	-67.428	ARG 107, ARG 74, PHE 36
11C	-5.052	-4.345	-68.966	LYS 13, PHE 39, ARG 74, PHE 39
4C	-4.330	-4.330	-58.074	TYR 39
18C	-4.260	-4.041	-71.215	TYR 39, ARG 95
3C	-4.035	-4.035	-60.394	ARG 107, PHE 70, ARG 74
15C	-4.684	-3.903	-60.773	TYR 39, ALA 48, ARG 107, PHE 70
24C	-4.505	-3.781	-61.831	LYS 13, ARG 95, ASN 100, ARG 107
1C	-3.008	-3.008	-53.068	TYR 39, PHE 70

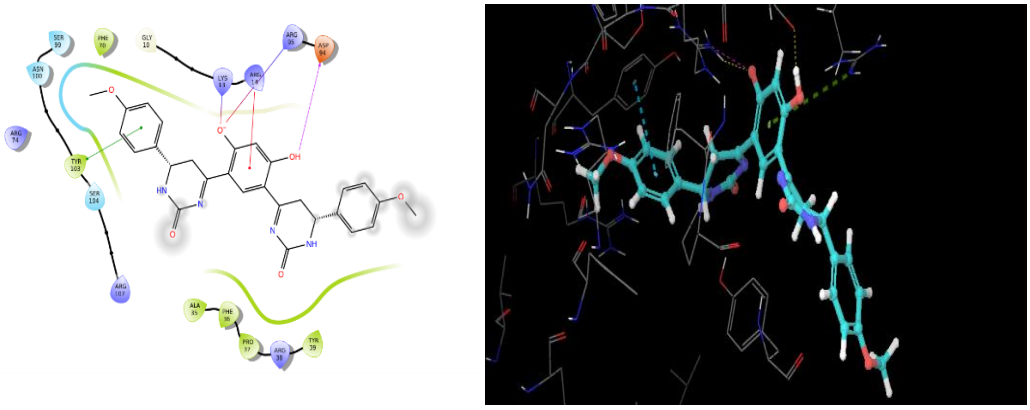


Figure 1: XP Docked pose of Compound 17C with thymidylate kinase of Mycobacterium tuberculosis H37Rv (PDB ID 5NQ5)

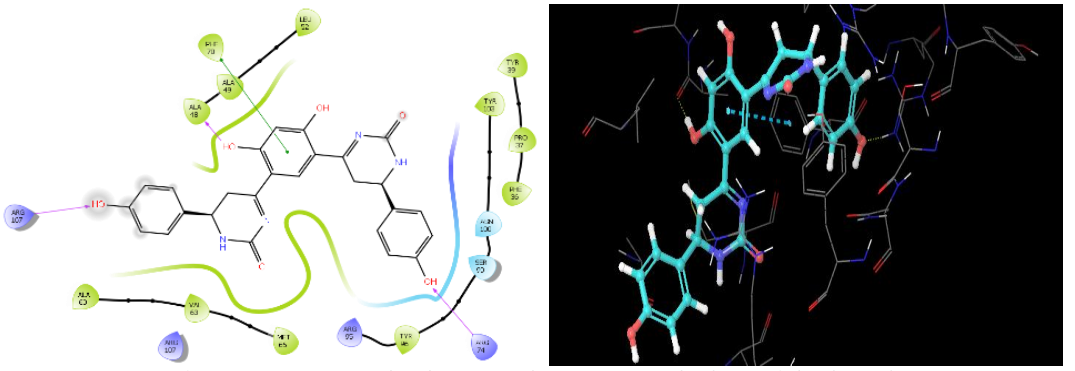


Figure 2: XP Docked pose of Compound 17C with 5NQ5

Figure 3: Thymidylate kinase of Mycobacterium tuberculosis H37Rv (PDB ID 5NQ5) complexed with ligand.

Evaluation of ADME Properties

Evaluation of ADMET and drug-likeness Candidates for drugs should have positive ADME qualities and, ideally, be non-toxic. As a result, using the SwissADME module available in the SIB webserver, (https://www.sib.swiss) the developed compounds were analysed for their ADME profile, [26] which included drug-likeness, partition coefficient, solubility, and numerous other parameters. Table 3 showed that most of the compounds were passed through drug likeliness criteria, namely Total Polar Surface Area (TPSA), Lipophilicity, water solubility and Lipinski rules.

Table 3: Swiss ADME predicted results showing various physicochemical descriptors of the compounds

Compound code	Hbond Donor	Hbond Acceptor	TPSA Å²	LogPo/w	Drug likeliness (Lipinski)	GI absorption	BBB permeant	Pgp Substrate
1C	4	4	134.96	2.72	Yes	High	No	Yes
2C	4	4	134.96	2.16	Yes	High	No	Yes
3C	2	4	82.92	3.63	2	High	No	No
4C	2	4	82.92	2.74	Yes	High	No	Yes
5C	4	6	123.38	2.23	Yes	High	No	Yes
6C	4	6	123.38	2.92	Yes	High	No	Yes
7C	2	8	174.56	2.21	2	Low	No	No
11C	4	6	123.38	2.34	2	Low	No	No
12C	4	6	123.38	2.66	1	High	No	Yes
13C	4	6	123.38	2.75	2	High	No	No
14C	4	6	123.38	2.67	2	High	No	Yes
15C	4	8	149.66	1.77	Yes	Low	No	Yes
16C	6	8	163.84	2.62	2	Low	No	No
17C	4	8	141.84	2.99	1	Low	No	Yes
18C	4	10	215.02	1.72	2	Low	No	Yes
19C	4	10	160.3	2.92	2	Low	No	Yes
20C	6	8	163.84	1.58	1	Low	No	No
21C	4	6	123.38	2.65	2	High	No	No
22C	6	10	182.3	2.36	3	Low	No	No
23C	4	12	178.76	2.81	2	Low	No	Yes
24C	6	6	175.42	2.55	2	Low	No	No

Evaluation of Toxicity Studies

Toxicity studies were carried out to check if the designed compounds had any functional groups that could cause toxicity, mutagenicity, or metabolic instability. The toxicity of the suggested chemical was also predicted using the ProTox website. [27] (https://tox.charite.de). Most of the compounds are free from hepatotoxicity, cytotoxicity, mutagenicity, or carcinogenicity. Predicted LD50 values of all the compounds were shown in Table 4

Table 4: Toxicity prediction of derived compounds

Compound Code	LD50 (mg/kg)	Toxicity class	Hepatotoxic	Carcinogenic	Mutagenic	Cytotoxic
1C	1000	4	-	-	-	-
2C	1500	4	-	-	-	-
3C	1500	4	-	-	--	-
4C	1500	4	-	-	-	--
5C	1500	4	-	-	--	--
6C	1500	4	-	-	--	--
7C	1000	4	+	+	++	-
11C	1500	4	-	-	--	--
12C	1500	4	-	-	--	--

13C	1000	4	-	-	--	--
14C	1000	4	-	-	--	--
15C	1000	4	-	-	-	--
16C	1000	4	-	+	--	--
17C	705	4	-	-	-	--
18C	1000	4	+	-	++	--
19C	1875	4	-	-	-	--
20C	1000	4	-	+	--	--
21C	1000	4	-	+	--	--
22C	1875	4	-	-	-	--
23C	1875	4	-	-	-	--
24C	1000	4	-	-	-	--

++ Toxic **+** Less toxic **--** Non-toxic **-** safe

Acknowledgment

Authors express special thanks to Ms. Shelvia Malik, Koushik Kasavajhala and Dr. Prajwal Nandekar on providing a free license trial of Schrodinger € for conducting the virtual screening and docking work.

Conclusion

Molecular docking experiments were performed on Mtb thymidylate kinase of Mycobacterium tuberculosis H37Rv for all new di hydro pyrimidinone derivatives. The title compounds' energy was minimised, the protein was optimised and minimised, a 3-dimensional grid was constructed at the active site, and molecular docking was performed using the Glide module's SP and XP docking modes. The ligands were docked into the corresponding proteins' active sites. The molecules were ranked based on the docking simulation findings, including their docking scores (GScore) and binding energy with the enzyme (Emodel). Low Glide scores usually indicate good ligand affinity for the receptor. Compounds with lower binding energy are more active. The results of molecular docking of title compounds with Mycobacterium TB H37Rv thymidylate kinase are highly promising. The enzyme was best inhibited by compound 17c (4-methoxy derivative). As a result, it is expected to have potent antitubercular properties. According to Glide's research, all of the ligands fit nicely inside the enzyme's binding pocket. The residues TYR 146 and PHE 94 play a key role in the receptor's function, according to conformational analysis of the docked complexes. We may extrapolate from the docked poses that pi-pi stacking interactions between the ligand and the receptor are crucial for successful docking. The in silico predictions reveal that title compounds could be potential anti-tubercular agents with specificity in blocking the Mycobacterium tuberculosis H37Rv thymidylate kinase enzyme.

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