ORIGINAL ARTICLE

INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

Published by JK Welfare & Pharmascope Foundation Journal Home Page: www.ijrps.com

In Silico studies of 4-Anilino Quinazoline derivatives as Anti-tubercular Agents

Hemalatha K*[1], Sujatha K[2], Panneerselvam P[3], Girija K[1]

[1]Department of Pharmaceutical Chemistry, College of Pharmacy, Mother Theresa Post Graduate and Research Institute of Health Sciences, (A Government of Puducherry Institution), Indira Nagar, Gorimedu, Puducherry-06, India
[2]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Sri Ramachandra Institute of Higher Education and Research, (Deemed to be University), Porur, Chennai-116, Tamil Nadu, India
[3]Faculty of Pharmacy, (Medical Campus), Bharath Institute of Higher Education and Research, Chrompet, Chennai-44, Tamil Nadu, India

Article History:

Received on: 03 Oct 2020
Revised on: 06 Nov 2020
Accepted on: 02 Dec 2020

Keywords:

Enoyl-acyl Carrier Protein Reductase, 4-anilino quinazoline, Mycobacterium Tuberculosis, BACTEC MGIT method

ABSTRACT

Inh A, the Enoyl Acyl Carrier protein Reductase from *Mycobacterium tuberculosis* is one of the pivotal enzyme involved in the mycobacterial fatty acid elongation cycle and has been considered as an important target for anti-tubercular screening. Inhibition of Inh A affects the biosynthesis of the mycolic acids, which are the central constituents of the mycobacterial cell wall. In the present research work, 4-anilino quinazoline derivatives were designed based on the quinazoline based drugs by means of lipophilic insertion and Fragment replacement. The designed compounds were synthesized, and molecular docking studies were performed on the human pathogenic bacterial enzyme InhA from its parent domain *Mycobacterium Tuberculosis.* Molecular docking study revealed that compounds SMOQ2, SNAQ3, 4AAQ7, 2AP9, PABAQ10 were found to possess good binding affinity towards the target InhA. With reference to the binding energy obtained from molecular docking study, five compounds were subjected to *in vitro* anti-tubercular activity against *M. tuberculosis* H37Rv and I2487 (Resistant strain) using BACTEC MGIT method. Compound SMOQ2 and 4AAQ7 showed sensitivity in both H37Rv (Sensitive strain) and I2487 (Resistant strain) at the concentration of 250, 500, 1000 and 1500 mcg/mL. *In silico* Pharmacokinetic predictions of the synthesized compounds were determined using SwissADME online web tool. All the synthesized compounds obeyed the Lipinski's rule of five properties.

*Corresponding Author

Name: Hemalatha K
Phone: 9787627805
Email: hemalathampharm@gmail.com

ISSN: 0975-7538

DOI: https://doi.org/10.26452/ijrps.v11i4.3948

Production and Hosted by

IJRPS | www.ijrps.com

INTRODUCTION

Mycobacterium tuberculosis, the causative agent of tuberculosis (TB), responsible for the morbidity and mortality of a large population worldwide (Franco *et al.*, 2009). In 2015, WHO estimated that there were 580,000 new cases of MDR-TB and that 250,000 MDR-TB deaths occurred globally. About 10% of healthy individuals may develop TB in their lifetime due to various genetic factors (Barrett and Barrett, 2003). The development of drug resistance against various anti-tubercular drugs and the influence of HIV epidemic has made the disease remain

a major global public health problem (Jiang *et al.*, 1990). According to WHO, one-third of the world's population have been infected with *Mycobacterium tuberculosis* (MTB) (Xia *et al.*, 2001). Inh A, the Enoyl Acyl Carrier protein Reductase from *Mycobacterium tuberculosis* is one of the enzymes which is involved in the mycobacterial fatty acid elongation cycle. Inh A is considered as the main target for anti-tubercular drug designing. Inhibition of Inh A affects the biosynthesis of the mycolic acids, which is central constituents of the mycobacterial cell wall. Hence the cell wall synthesis gets interrupted.

Quinazolines are classes of fused heterocyclic ring system with a broad spectrum of pharmacological activities such as anti-cancer, anti-tubercular, antibacterial (Gangwal *et al.*, 2001), anti-fungal (Bartroli *et al.*, 1998), anti-HIV (Alagarsamy *et al.*, 2004), anthelmintic (Gupta *et al.*, 1987), analgesic (Manivel *et al.*, 2010), anti-inflammatory (Chao *et al.*, 1999), anti-hypertensive (Wright *et al.*, 1987), anti-diabetic (Saeedi *et al.*, 2019) and anti-oxidant (Subramaniam *et al.*, 2010) activities.

The present work involves the molecular interactions of the Enoyl-Acyl carrier protein Reductase *Mycobacterium tuberculosis* (InhA), (PDB ID: 4TZK) with the designed ligands using molecular docking tool. With reference to the binding energy, compound SMOQ2, SNAQ3, 4AAQ7, 2APQ9 and PABAQ10 were evaluated for their *in vitro* anti-mycobacterial activity against Mycobacterium tuberculosis H37Rv (Sensitive stain) using BACTEC MGIT method. Compound SMOQ2 and 4AAQ7 showed sensitivity in both H37Rv (Sensitive stain) and I2487 (Resistant strain) at the concentration of 250, 500, 1000 and 1500 mcg/mL. The designed derivatives were predicted for their *in silico* ADME predictions using the SwissADME server.

MATERIALS AND METHODS

Chemistry

A series of 4-Anilino quinazoline derivatives have been designed (Figure 1) and synthesized from anthranilic acid in four steps via benzoxazinones, Quinazolin-4-ones and 4-Chloro quinazolines. The synthetic work has been published. (Hemalatha *et al.*, 2018) The scheme for the synthesis of title compounds were depicted in Figure 1 and their structure were given in Figure 2.

Molecular Docking

Protein Preparation

Molecular docking was performed for 4-Anilino quinazolines using AUTODOCK 4.0 on the Windows 64-bits operation system. The target protein Enoyl-Acyl carrier protein Reductase *Mycobacterium tuberculosis* (InhA), (PDB ID: 4TZK) in complex with 1-cyclohexyl-N-(3,5-dichlorophenyl0-5-oxopyrrolidine-3-carboxamide were retrieved from Protein Data Bank (He *et al.*, 2006).

Ligand Preparation

Chemsketch was used to draw the structures of designed Quinazoline derivatives which acts as a ligands followed by generation of 3D structure in PDB format using Marvin sketch.

The structure with the lowest binding free energy was chosen for the optimum docking conformation and interaction with the target.

Absorption, Distribution, Metabolism and Excretion (ADME) prediction

ADME screening were checked to predict the various physiochemical and pharmacokinetic properties of the designed 4-anilino quinazoline derivatives and also to predict their drug-likeness properties. *In silico* pharmacokinetic screening was done using the online SwissADME web tool. MW- Molecular Weight, RB- No. of Rotatable bonds, TPSA- Topographic Polar Surface Area, ESOL- Water solubility, SOL-Soluble, M.SOL- Moderately Soluble, V. SOL-Very Soluble, GIA- GastroIntestinal Absorption, BBB P- Blood-Brain Barrier Permeability, PgpS- Permeability Glycoprotein Substate, CYP1- CYP9 inhibitor, CYP2, a CYP2D6 inhibitor, LogKp (cm/s)-Skin permeant

Evaluation of Anti-tubercular Activity

Based on the binding energy obtained from the molecular docking study (least energy), 5 compounds SMOQ2, SNAQ3, 4AAQ7, 2APQ9 and PABAQ10 were subjected to *in vitro* anti-tubercular study by MGIT (mycobacteria growth indicator tube) sensitivity method at various concentrations 250, 500, 1000, 1500 mcg/mL against the sensitive strain (H37Rv) and Resistant Strain (I2487). Positive tubes, identified by the BD BACTED MGIT instrument, should be sub-cultured and an acid-fast smear prepared.

RESULTS AND DISCUSSION

Molecular Docking Study

Molecular docking study showed that compound SMOQ2 (-9.66 kcal/mol), SNAQ3 (-9.31 Kcal/mol), 2APQ9 (-9.19 kcal/mol), 4AAQ7 (-8.85 Kcal/mol) and PABAQ10 (-8.8 kcal/mol) possess the highest binding affinity towards the target InhA compared to the standard drug Isoniazid (-5.6 Kcal/mol). The compound with least negative binding free energy means a better binding; The free energy of interac-

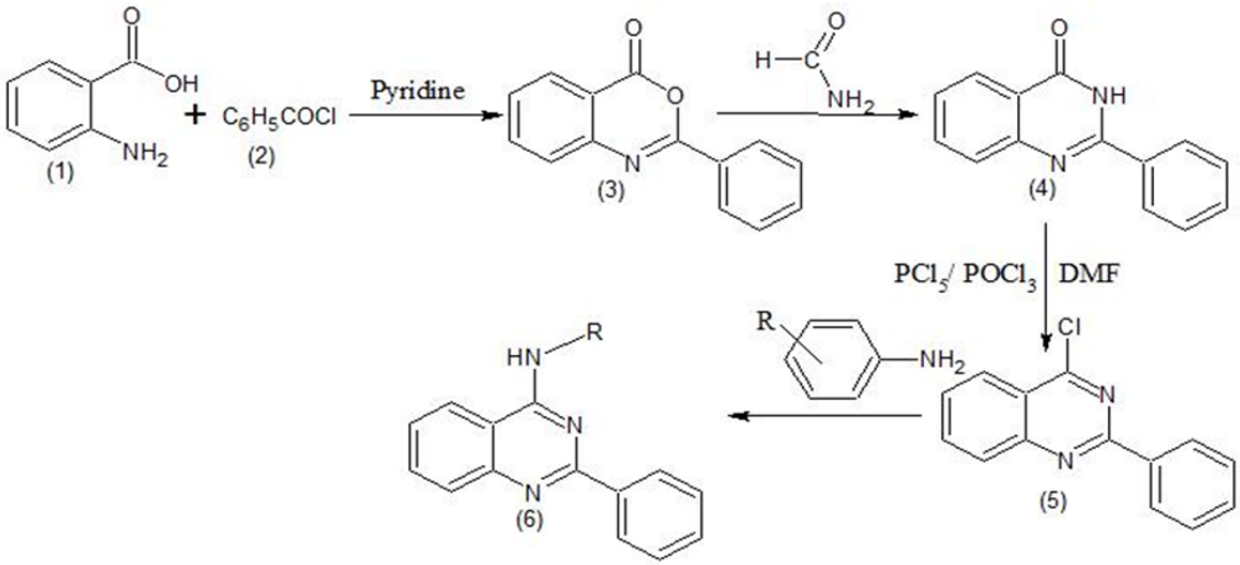

Figure 1: Synthetic Scheme for 4-anilino quinazolines

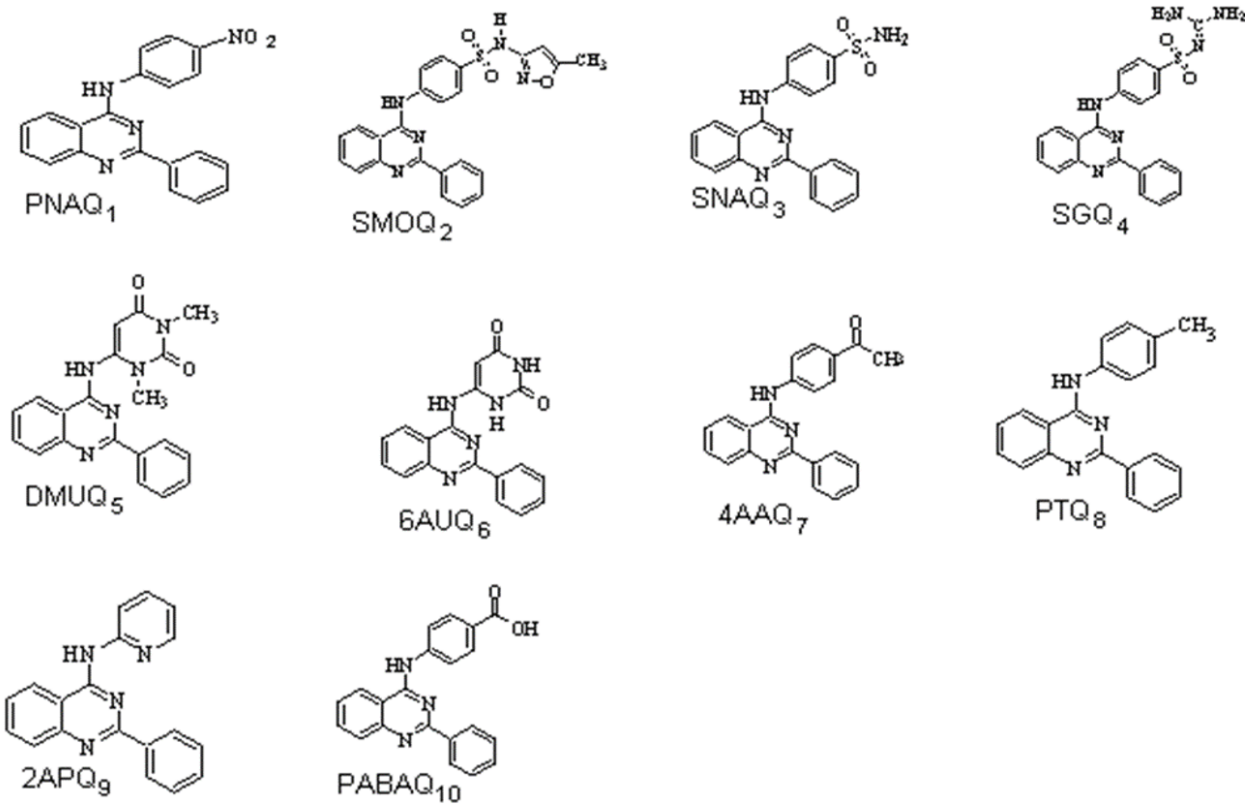

Figure 2: Structure of synthesized compounds and its code

tions/binding for each compound were compared to standard Isoniazid. Based on these results, Isoniazid with the binding free energy of -5.6kcal/mol can form two hydrogen bonds with amino acid residues Val 65 and Gly 14 with the distance of 2.014 A° and 2.061 A° respectively. Besides, Isoniazid showed van der Waals interactions with Ile 95, Thr 39, Leu 63 and Phe 41. Compound SMOQ2 showed the binding energy of -9.66 Kcal/mol and formed one hydrogen bond with amino acid residue Met 98 (NH) with the distance of 2.029 A°, followed by Compound SNAQ3 showed the binding energy of -9.31 Kcal/mol and formed one hydrogen bond with the amino acid residue Ile 194 (NH) and its interatomic distance was 2.22 A°. The docking results were shown in Table 1. The inhibition constant, vander waals desolvation energy and the various amino acid involved in vander waals interactions were given inTable 2. The molecular interactions of designed ligands in the active site of the protein were shown in Figure 3.

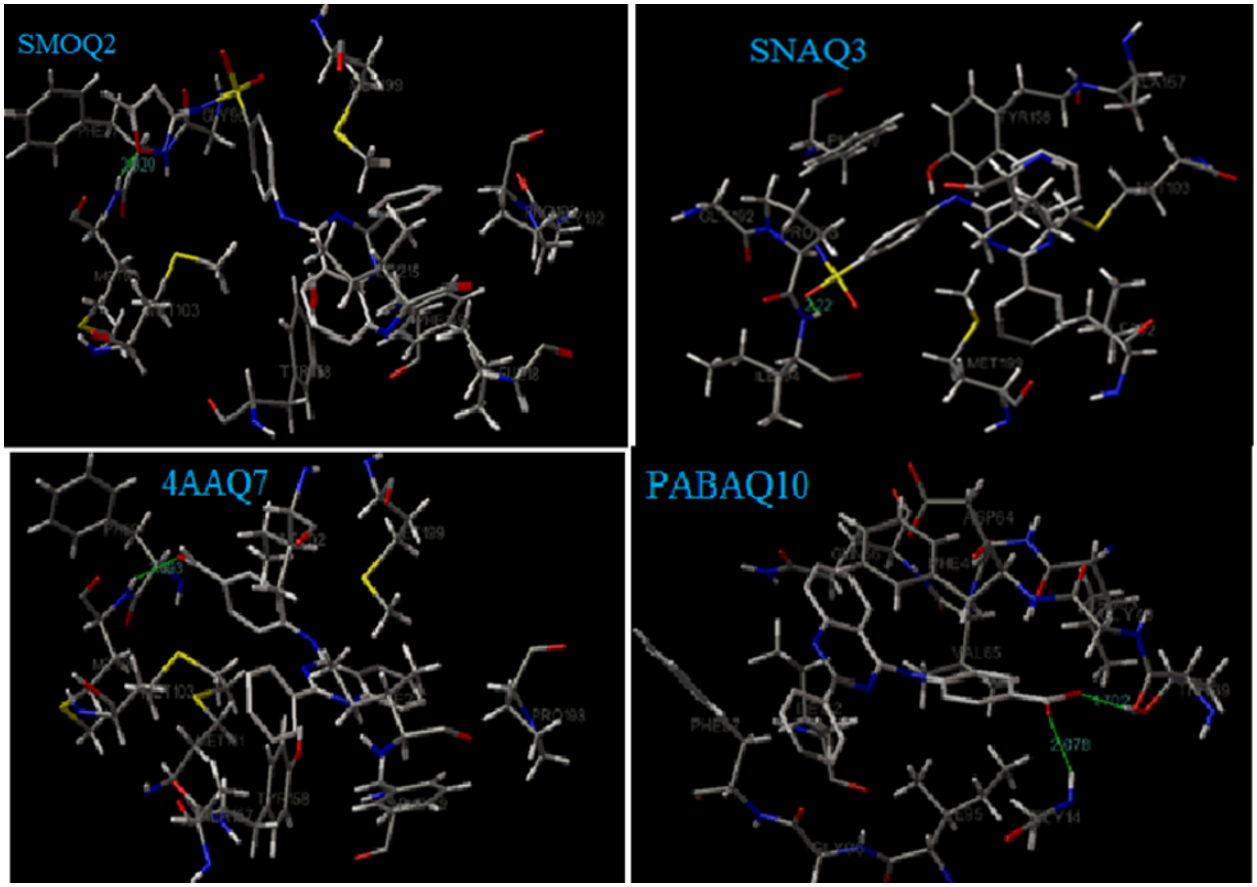

Figure 3: Interaction of Compounds SMOQ2, SNAQ3, 4AAQ7, 2APQ9 and PABAQ10 in the active site of the protein.

Table 1: Molecular Interactions of designed compounds in the active site of the protein, (InhA) (PDB ID: 4TZK)

Sl.No.	Compound code	No. of hydrogen bond formed	Amino acid involved in Hydrogen bond interactions	Distance between Donor and Acceptor (Å)	Binding Energy
1	PNAQ1	1	Thr 158	2.173	-8.51
2	SMOQ2	1	Met 98 (NH)	2.029	-9.66
3	SNAQ3	1	Ile 194 (NH)	2.22	-9.31
4	SGQ4	1	Pro 156	2.868	-8.64
5	DMUQ5	1	Gly 14(NH)	2.119	-6.13
6	6AUQ6	2	Ile 15 Gly 14(NH)	2.21 2.075	-8.64
7	4AAQ7	1	Met 98 (NH)	2.093	-8.85
8	PTQ8	1	Met 98 (NH)	2.088	-8.16
9	2APQ9	2	Pro 156 (O) Pro 156 (O)	2.694 2.994	-9.19
10	PABAQ10	2	Gly 14 Thr 39	2.078 1.702	-8.8
11	Standard (Isoniazid)	2	Val 65 (NH) Gly 14 (NH)	2.014 2.061	-5.6

Table 2: Inhibitory constant, VdW desolvation energy and its interactions

Sl. No.	Compoun code	Inhibitory constant (μM)	Vdw. Des-olvation Energy	Aminoacid involved in van der waals interactions
1	PNAQ1	577.61	-9.35	Leu 218, Phe 149, Pro 193, Tyr 158, Ile 215, Met 161, Ala 167, Gly 104, Met 103, Leu 207, Met 98, Phe 97, Ile 202, Met 199
2	SMOQ2	125.61	-11.2	Leu 207, Ile 202, Met 199, Met 103, Met 161, Ala 157, Tyr 158, Ile 216, Pro193, Phe 149, Asp 148, Met 155, Leu 216, Ala 191
3	SNAQ3	148.77	-10.52	Ile 194, Gly 192, Pro 193, Phe 149, Met 199, Tyr 158, Ile 215, Ile 202, Ala 157, Met 103
4	SGQ4	632.78	-9.17	Ile 95, Val65, Thr 39, Phe 97, Gly 96, Gly 40, Ile 47, Phe 41
5	DMUQ5	348.22	-6.55	Thr 39, Ser 94, Gly 96, Ile 95, Val 65, Phe 97, Leu63
6	6AUQ6	461.45	-9.53	Ile95, Val 65, Thr 39, Phe 97, Gly 96, Gly 40, Ile 47
7	4AAQ7	327.05	-9.99	Phe 97, Met -9.04199, Ile 202,-10.11 Met 103, Ile 21-9.215, Pro 193, He 149, Tyr 158, Ala 157, Met 161
8	PTQ8	1.05	-9.04	Phe 149, Pro 193, Gly 192, Met 199, Met 161, Ile 215, Phe 145, Leu 218, Tyr 158, Met 103
9	2APQ9	183.42	-10.11	Pro 193, Phe 149, Met 199, Ile 202, Ile 215, Leu 207, Leu 218, Gln 214, Ala 157, Met 155, Thr 158, Ala 157, Met 103
10	PABAQ10	353.45	-9.21	Gln 66, Asp 64, Phe 41, Leu 63, Gly 40, Ile 95, Val 65, Phe 97, Ile 122, Gly 96, Ile 95
11	Standard (Isoniazid)	78.99	-5.89	Ile 95, Thr 39, Leu 63, Phe 41

Table 3: Physicochemical properties prediction of synthesized compounds

Sl.No	Compound code	Physicochemical Properties			
		MW	RB	TPSA (Å)	ESOL Class
1	PNAQ1	342.36	4	83.63	M.Sol
2	SMOQ2	457.51	6	118.39	M.Sol
3	SNAQ3	376.44	4	106.35	M.Sol
4	SGQ4	432.51	5	144.73	M.Sol
5	DMUQ5	359.39	3	81.81	M.Sol
6	AUQ6	331.33	3	103.53	Sol
7	4AAQ7	339.40	4	54.88	M.Sol
8	PTQ8	311.39	3	37.81	M.Sol
9	APQ9	298.35	3	50.7	M.Sol
10	PABAQ10	341.37	4	75.11	M.Sol

Table 4: Pharmacokinetics properties prediction of synthesized compounds

Sl.No.	Compound Code	Pharmacokinetics Properties					
		GIA	BBB P	Pgp S	CYP1	CYP2	Log Kp (cm/s)
1	PNAQ1	High	No	No	Yes	Yes	-4.69
2	SMOQ2	Low	No	No	No	Yes	-5.7
3	SNAQ3	High	No	No	Yes	Yes	-5.79
4	SGQ4	Low	No	No	Yes	Yes	-6.41
5	DMUQ5	High	No	No	Yes	No	-6.53
6	AUQ6	High	No	No	No	No	-6.62
7	4AAQ7	High	Yes	No	Yes	Yes	-4.77
8	PTQ8	High	Yes	Yes	Yes	Yes	-4.12
9	APQ9	High	Yes	No	Yes	Yes	-4.82
10	PABAQ10	High	No	No	Yes	Yes	-4.9

Table 5: Lipophilicity of synthesized compounds

Sl.No.	Compound Code	Lipophilicity				
		I Log P	X Log P	W Log P	M Log P	Cons. Log P
1	PNAQ1	2.89	5.21	5.47	3.52	3.89
2	SMOQ2	2.83	4.78	6.03	2.43	3.83
3	SNAQ3	2.51	3.95	4.77	2.23	3.14
4	SGQ4	2.07	3.44	4.08	2.43	2.73
5	DMUQ5	3.26	2.76	2.44	2.25	2.59
6	AUQ6	2.2	2.39	2.42	1.79	2.42
7	4AAQ7	3.17	5.07	5.24	3.58	4.33
8	PTQ8	3.42	5.75	5.35	4.28	4.7
9	APQ9	2.98	4.65	4.44	2.98	3.74
10	PABAQ10	2.76	4.91	4.74	2.23	3.65

Cons. Log P- Consensus Log P

Table 6: Drug likeness properties of synthesized compounds

Sl.No.	Compound Code	Drug Likeness					
		No. of Violations					BAS
		L	G	V	E	M	
1	PNAQ1	0	0	0	0	1	0.55
2	SMOQ2	0	1	0	1	0	0.55
3	SNAQ3	0	0	0	0	0	0.55
4	SGQ4	0	0	1	1	0	0.55
5	DMUQ5	0	0	0	0	0	0.55
6	AUQ6	0	0	0	0	0	0.55
7	4AAQ7	0	0	0	0	1	0.55
8	PTQ8	1	0	0	0	1	0.55
9	APQ9	0	0	0	0	0	0.55
10	PABAQ10	0	0	0	0	0	0.56

L-Lipinski, G-Ghose, V-Veber, E-Egan, M-Muegge rule, BAS- Bioavailability Score

Table 7: Evaluation of In-vitro anti-tubercular activity

Sl.No.	Sample code	H37Rv stain (sensitive Strain)				I2487 Stain (Resistant Strain)			
		250	500	1000	1500	250	500	1000	1500
1	SMOQ2	S	S	S	S	S	S	S	S
2	SNAQ3	R	R	S	S	R	R	S	S
3	4AAQ7	S	S	S	S	S	S	S	S
4	2APQ9	R	R	S	S	R	R	R	S
5	PABAQ10	R	R	S	S	R	R	R	S

S: Sensitive R:Resistant

Pharmacokinetic Prediction

ADME screening was checked to predict the various physiochemical and pharmacokinetic properties of the designed 4-anilino quinazoline derivatives and also to predict their drug-likeness properties. The Lipinski's rule of five is a rule, to evaluate the drug-likeness of a molecule or compound. The rule states that an orally active drug can be less than 5 hydrogen bond donors, less than 10 hydrogen bond acceptors, MW should be less than 500 daltons, and the octanol-water partition coefficient (log P) does not exceed 5. Based on these calculations, the designed ligands satisfies the Lipinski's rule of five properties. The results were given in Tables 3, 4, 5 and 6.

Anti-tubercular Activity

Compound SMOQ2 and 4AAQ7 showed sensitivity in both H37Rv (Sensitive stain) and I2487 (Resistant strain) at the concentration of 250, 500, 1000 and 1500 mcg/mL. (Table 7).

CONCLUSION

In the present study, a series of novel 4-anilino Quinazoline derivatives were designed and docked into the active site of the protein Enoyl-Acyl carrier protein Reductase *Mycobacterium tuberculosis* (InhA) (PDB ID: 4TZK) as an anti-tubercular target. Molecular docking study showed that compound SMOQ2 (-9.66 kcal/mol), SNAQ3 (-9.31 Kcal/mol), 2APQ9 (-9.19 kcal/mol), 4AAQ7 (-8.85 Kcal/mol) and PABAQ10 (-8.8 kcal/mol) possess the highest binding affinity towards the target InhA compared to the standard drug Isoniazid (-5.6 Kcal/mol). The results of anti-tubercular activity revealed that quinazoline compounds containing oxazole ring and acetophenone group exhibited significant activity.

ACKNOWLEDGEMENT

The authors are thankful to DR.Muthuraj.M, Department of Microbiology, Government Hospital for Chest Diseases, (Intermediate Research Laboratory), Govt. of Puducherry, Gorimedu, Puducherry-06 for providing necessary facilities and support to carry out the anti-tubercular work.

Conflict of Interest

The authors declare that they have no conflict of interest for this study.

Funding Support

The authors declare that they have no funding support for this study.

REFERENCES

Alagarsamy, V., Revathi, R., Meena, S., Ramaseshu, K. V., Rajasekaran, S., Clercq, E. 2004. AntiHIV, antibacterial and antifungal activities of some 2,3-disubstituted quinazolin-4(3H)-ones. *Indian Journal of Pharmaceutical Sciences*, 66:459–462.

Barrett, C. T., Barrett, J. F. 2003. Antibacterials: are the new entries enough to deal with the emerging resistance problems? *Current Opinion in Biotechnology*, 14(6):621–626.

Bartroli, J., Turmo, E., Algueró, M., Boncompte, E., Vericat, M. L., Conte, L., Ramis, J., Merlos, M., García-Rafanell, J., Forn, J. 1998. New Azole Antifungals. 3. Synthesis and Antifungal Activity of 3-Substituted-4(3H)-quinazolinones1,2. *Journal of Medicinal Chemistry*, 41(11):1869–1882.

Chao, Q., Deng, L., Shih, H., Leoni, L. M., Genini, D., Carson, D. A., Cottam, H. B. 1999. Substituted Isoquinolines and Quinazolines as Potential Antiinflammatory Agents. Synthesis and Biological Evaluation of Inhibitors of Tumor Necrosis Factor α. *Journal of Medicinal Chemistry*, 42(19):3860–3873.

Franco, B. E., Martínez, M. A., Rodríguez, M. A. S., Wertheimer, A. I. 2009. The determinants of the antibiotic resistance process. *Infection and drug resistance*, 2:1–11.

Gangwal, N. A., Kothawade, U. R., Galande, A. D., Pharande, D. S., Dhake, A. S. 2001. Synthe-

sis of 1-substituted-2-chloromethyl-4-(1H)-quinazolinones as antimicrobial agents. *Indian journal of heterocyclic chemistry*, 10(4):291–294.

Gupta, S., Kumar, M., Ahmad, S. 1987. An algorithm for automatic prober movement control for better coverage of test sites on a wafer during measurement. *Microelectronics Reliability. Elsevier BV*, 27:281–282.

He, X., Alian, A., Stroud, R., de Montellano, P. R. O. 2006. Pyrrolidine Carboxamides as a Novel Class of Inhibitors of Enoyl Acyl Carrier Protein Reductase fromMycobacterium tuberculosis. *Journal of Medicinal Chemistry*, 49(21):6308–6323.

Hemalatha, K., Selvin, J., Girija, K. 2018. Synthesis, In silico Molecular Docking Study and Anti-bacterial Evaluation of some Novel 4-Anilino Quinazolines. *Asian Journal of Pharmaceutical Research*, 8(3):125.

Jiang, J. B., Hesson, D. P., Dusak, B. A., Dexter, D. L., Kang, G. J., Hamel, E. 1990. Synthesis and biological evaluation of 2-styrylquinazolin-4(3H)-ones, a new class of antimitotic anticancer agents which inhibit tubulin polymerization. *Journal of Medicinal Chemistry*, 33(6):1721–1728.

Manivel, A., Naveenraj, S., Kumar, P. S. S., Anandan, S. 2010. CuO-TiO2 Nanocatalyst for Photodegradation of Acid Red 88 in Aqueous Solution. *Science of Advanced Materials*, 2:51–57.

Saeedi, M., Mohammadi-Khanaposhtani, M., Pourrabia, P., Razzaghi, N., Ghadimi, R., Imanparast, S., Faramarzi, M. A., Bandarian, F., Esfahani, E. N., Safavi, M., Rastegar, H., Larijani, B., Mahdavi, M., Akbarzadeh, T. 2019. Design and synthesis of novel quinazolinone-1,2,3-triazole hybrids as new anti-diabetic agents: In vitro α-glucosidase inhibition, kinetic, and docking study. *Bioorganic Chemistry*, 83:161–169.

Subramaniam, R., Pai, S., Vigra, G. K., Sodhi, G. S. 2010. Synthesis and in-vitro study of biological activity of 2,3-substituted quinazolin-4(3H)-ones. *Journal of Chemical and Pharmaceutical Research*, 2(2):462–468.

Wright, W. B., Tomcufcik, A. S., Chan, P. S., Marsico, J. W., Press, J. B. 1987. Thromboxane synthetase inhibitors and antihypertensive agents. 4. N-[(1H-imidazol-1-yl)alkyl] derivatives of quinazoline-2,4(1H,3H)-diones, quinazolin-4(3H)-ones, and 1,2,3-benzotriazin-4(3H)-ones. *Journal of Medicinal Chemistry*, 30(12):2277–2283.

Xia, Y., Yang, Z.-Y., Hour, M.-J., Kuo, S.-C., Xia, P., Bastow, K. F., Nakanishi, Y., Nampoothiri, P., Hackl, T., Hamel, E., Lee, K.-H. 2001. Antitumor Agents. Part 204: Synthesis and Biological Evaluation of Substituted 2-Aryl Quinazolinones. *Bioorganic and Medicinal Chemistry Letters*, 11(9):1193–1196.