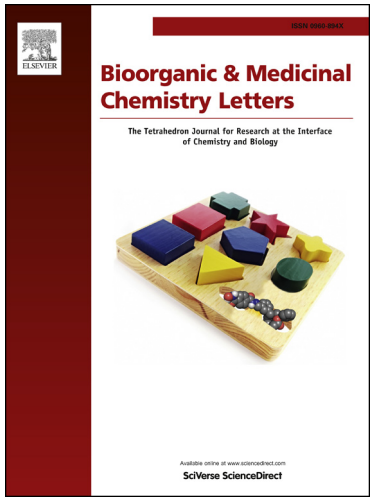

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PII: S0960-894X(15)00761-1
DOI: http://dx.doi.org/10.1016/j.bmcl.2015.07.057
Reference: BMCL 22946

To appear in: *Bioorganic & Medicinal Chemistry Letters*

Received Date: 28 April 2015
Revised Date: 13 July 2015
Accepted Date: 18 July 2015

Please cite this article as: Kamal, A., Subba Reddy, B.V., Sridevi, B., Ravikumar, A., Venkateswarlu, A., Sravanthi, G., Padma Sridevi, J., Yogeeswari, P., Sriram, D., Synthesis and biological evaluation of phaitanthrin congeners as anti-mycobacterial agents, *Bioorganic & Medicinal Chemistry Letters* (2015), doi: http://dx.doi.org/10.1016/j.bmcl.2015.07.057

Synthesis and biological evaluation of phaitanthrin congeners as anti-mycobacterial agents

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Abstract

Natural alkaloid, tryptanthrin (indolo[2,1-*b*]quinazoline-6,12-dione) and its analogues are found to exhibit potent anti-tubercular activity against MDR-TB. A novel class of indolo[2,1-*b*]quinazolinones have been synthesized to evaluate their anti-mycobacterial activity. Enoyl-acyl carrier protein reductase (InhA) of *M. tuberculosis* is one of the key enzymes and has been validated as an effective anti-microbial target. *In silico* molecular docking study demonstrates that the synthesized compounds exhibit high affinity for the *M. tuberculosis* drug target InhA. Phaitanthrin is a natural product, which belongs to a family of tryptanthrin and exhibits structural similarity except at position 6. Phaitanthrin derivatives are prepared by modifying the keto functionality of tryptanthrin. These phaitanthrin congeners are found to display promising anti-tubercular activity.

Keywords: Tryptanthrin, indolo[2,1-*b*]quinazoline-6,12-dione, MDR-TB, Enoyl-acyl carrier protein reductase (InhA), *In silico* molecular docking study.

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Tuberculosis (TB), which is acknowledged as a global health catastrophe, is a chronic bacterial infection caused by *Mycobacterium tuberculosis*. Most infections in humans result in asymptomatic, latent infection and about one in ten latent infections eventually progresses to active disease.[1] Hence, the discovery of new drugs is very much in need to combat

tuberculosis. Multidrug resistant (MDR) and extensively drug resistant (XDR) TB strains are of concern for human society, the solution for growing emergence of resistance in the pathogen is the development of new affordable drugs with high potency and low toxicity.

Indolo[2,1-*b*]quinazoline-6-12-dione or tryptanthrin is a versatile lead for designing the potential drugs with diverse medical functions. Tryptanthrin is a potent and structurally useful alkaloid isolated from the natural sources such as *Phaius mishmensis and Isatis, Calanthe*, *Wrightia*, *Couroupota* and *Strobilanthus* species.[2] In particular the orchid *Phaius mishmensis* was reported to be a rich source of tryptanthrin based compounds and all those shown in Fig. **1,** have been isolated from this plant.[3] Tryptanthrin consists of both quinazoline and indole core structures and showed a variety of intriguing biological properties such as antibacterial, antifungal, antiprotozoal and antiparasitic activities.[4] Although systematic synthesis and structure-activity relationship studies for anti-tubercular, COX inhibitory and cytotoxic activity have led several promising candidates for further development, none of them has yet been successfully launched for clinical usage.[5] Structure of tryptanthrin is comparatively simple, lacking asymmetric centres, it has a fairly low molecular weight and has a structure which differs significantly from all previously established anti-tubercular agents. Tryptanthrin proved rather more potent against *M tb* H37Rv (1 mg/L) and *M. avium* (4 mg/L) than against *M. smegmatis* (6 mg/L) and exhibit same potency as that of well-known anti-tubercular agents such as INH, streptomycin and ethambutol. One significant implication of this is that tryptanthrin can be proposed to be operating by a molecular mechanism different than that employed by most of the existing anti-tubercular agents and might be useful in cases where existing agents would fail to cure patients.[6]

The natural alkaloid, tryptanthrin represents a potential lead for new tuberculosis drugs since tryptanthrin and its synthetic analogues possess potent *in vitro* activity against *Mycobacterium tuberculosis* (*Mtb*). When tryptanthrin is tested against a panel of multidrug-resistant strains of *M. tuberculosis*, it shows more potency (MICs of 0.5-1µg/mL) than isoniazid (MICs 4-16µg/mL).[7] Though tryptanthrin and its analogues are active against MDR-TB, their cellular target is unknown. Therefore, tryptanthrin is an important class of structural motif for the development of new drugs for the treatment of tuberculosis. As a part of our interest on chemical and pharmacological studies of natural alkaloids, we have been interested in compounds possessing the tryptanthrin skeleton due to their fascinating biological activities.

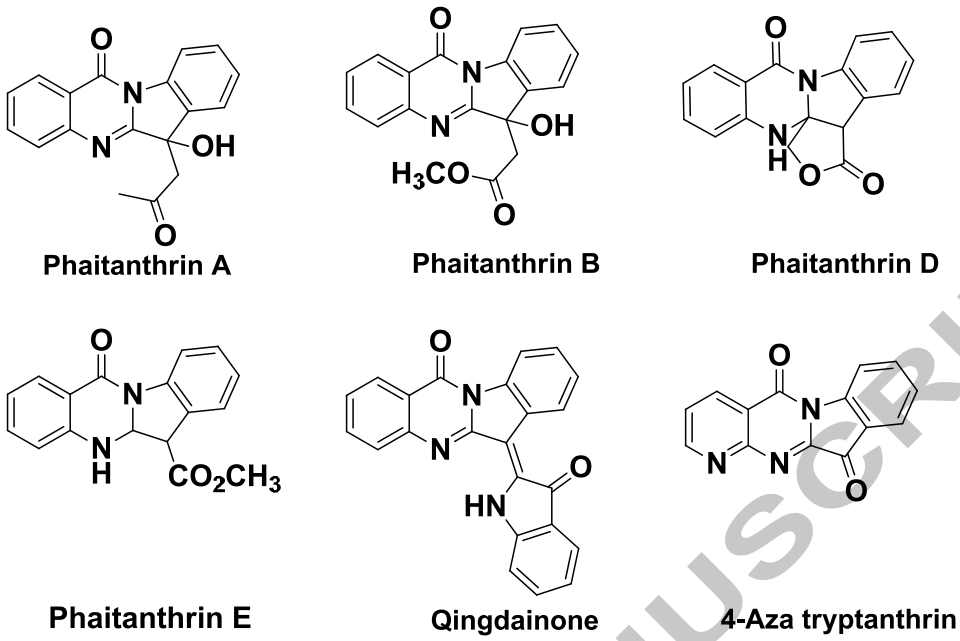


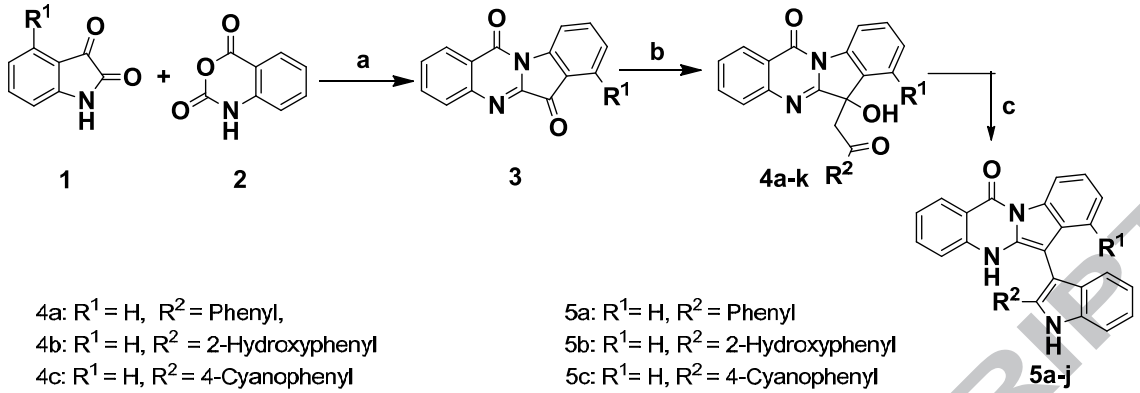
Figure 1: Tryptanthrin derivatives isolated from natural sources

We designed and synthesized a new series of tryptanthrin based molecules in which the keto group at position 6 of the indolo[2,1-*b*]quinazoline core was replaced by other functional groups. In this manuscript we report the synthesis and activity studies performed.

Results and Discussions

In this endeavor, we designed and synthesized a series of novel anti-tubercular agents, based on indolo[2,1-*b*]quinazoline-6,12-dione and 11*H*-indeno[1,2-*b*]quinoxalin-11-one derived natural products.

Scheme 1. Synthesis of indolo[2,1-*b*]quinazoline analogues. Reagents and conditions: a) *N*-ethylpiperidine, DIC, pyridine, 24 h, 100 ^{o}C; b) dimethylamine, ketone, neat, 30 ℃, 12 h; c) $PhNHNH_2$, glacial AcOH, EtOH, 80 ^{o}C, 12 h.



4a: R^1 = H, R^2 = Phenyl,
4b: R^1 = H, R^2 = 2-Hydroxyphenyl
4c: R^1 = H, R^2 = 4-Cyanophenyl
4d: R^1 = H, R^2 = 4-Fluorophenyl
4e: R^1 = H, R^2 = 4-Bromophenyl
4f: R^1 = H, R^2 = 2-Aminophenyl
4g: R^1 = H, R^2 = Thiophenyl
4h: R^1 = H, R^2 = 4-Ethyl
4i: R^1 = H, R^2 = 4-Aminophenyl
4j: R^1 = Br, R^2 = 4-Bromophenyl
4k: R^1 = H, R^2 = 1-(4-nitrophenyl)-3-phenylthiourea

5a: R^1= H, R^2 = Phenyl
5b: R^1= H, R^2 = 2-Hydroxyphenyl
5c: R^1= H, R^2 = 4-Cyanophenyl
5d: R^1= H, R^2 = 4-Fluorophenyl
5e: R^1= H, R^2 = 4-Bromophenyl
5f: R^1= H, R^2 = 2-Aminophenyl
5g: R^1= H, R^2 = Thiophenyl
5h: R^1=H, R^2 = 4-Ethyl
5i: R^1= H, R^2 = 4-Aminophenyl
5j: R^1=Br, R^2 = 4-Bromophenyl

The synthesis of indolo[2,1-*b*]quinazoline-6,12-dione is shown in **Scheme 1**. Accordingly, treatment of isatin (**1**) with isatoic anhydride (**2**) in the presence of *N*-ethyl piperidine and diisopropylcarbodiimide(DIC) in dry pyridine at 100 ^{o}C afforded the desired product **3** in 93% yield. Aldol condensation of **3** with ketones in the presence of dimethyl amine furnished the β-hydroxyketones (**4a-k**). Finally, the condenstion of **4a-k** with phenylhydrazine in glacial acetic acid at 80 ^{o}C afforded the corresponding indole derivatives (**5a-j**).

Table 1: Synthesis of indolo[2,1-*b*]quinazoline analogues (**4a-4k**) and (**5a-5j**):

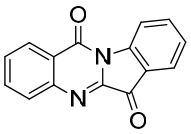
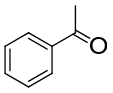
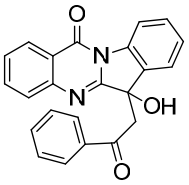
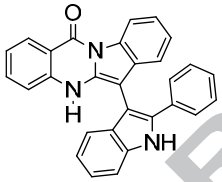
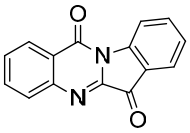
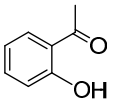
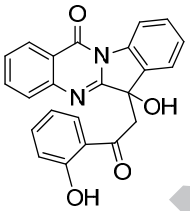
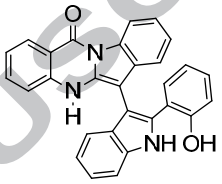
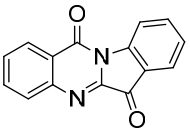
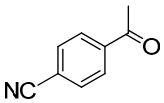
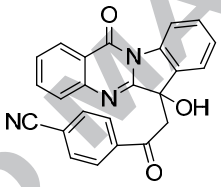
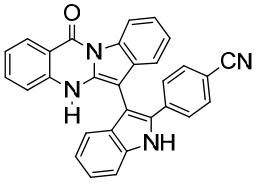
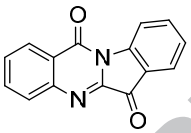
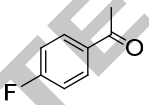
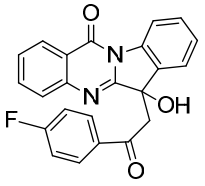
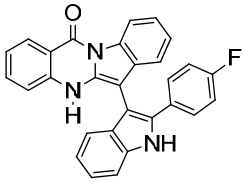
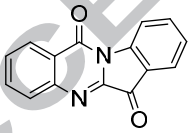
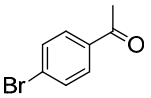
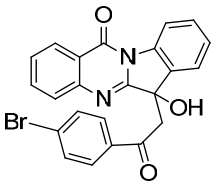
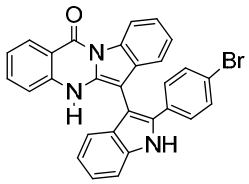
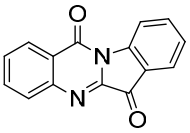
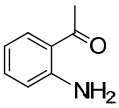
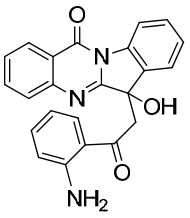
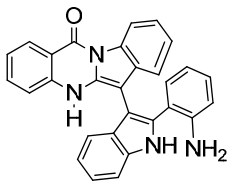
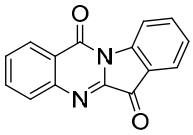
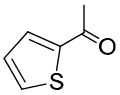
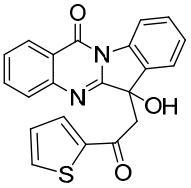
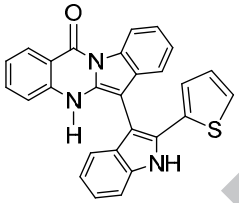
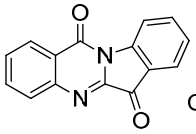
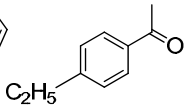
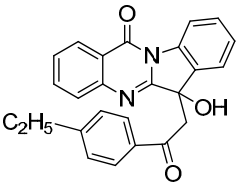
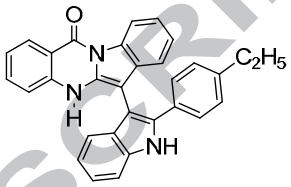
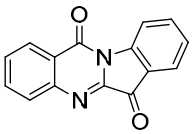
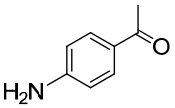
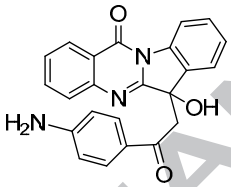
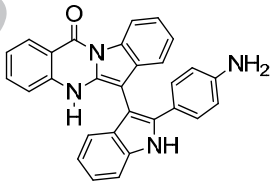
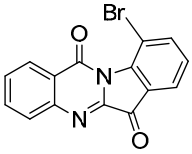
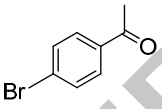
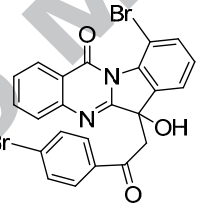
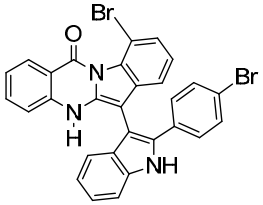
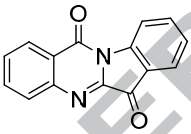
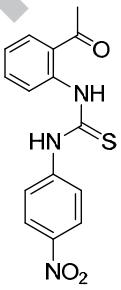
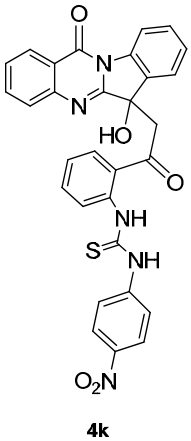
Entry	Tryptanthrin	ketone	Hydroxyketone 4	Yield(%)	Indoloquinazoline product 5	Yield(%)
1			 **4a**	92	 **5a**	88
2			 **4b**	90	 **5b**	86
3			 **4c**	84	 **5c**	92
4			 **4d**	80	 **5d**	85
5			 **4e**	91	 **5e**	84
6			 **4f**	92	 **5f**	86

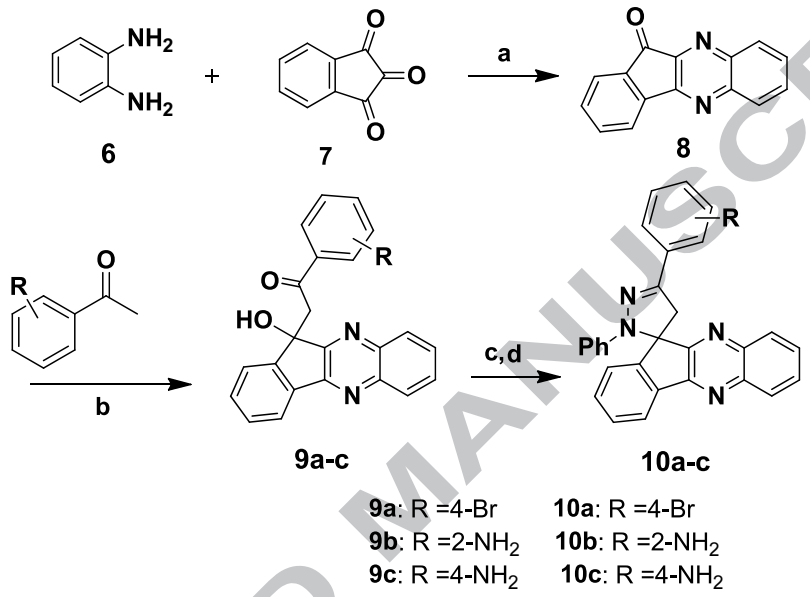
Table 1.....

7			 **4g**	82	 **5g**	88
8			 **4h**	84	 **5h**	78
9			 **4i**	80	 **5i**	72
10			 **4j**	64	 **5j**	74
11			 **4k**	62		

Next, we prepared a new series of spiroindenoquinoxalines in good yields through a condensation of β-hydroxyketones (**9a-c**) with phenylhydrazine under acidic conditions

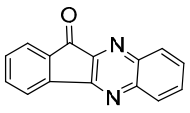
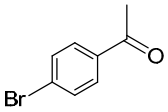
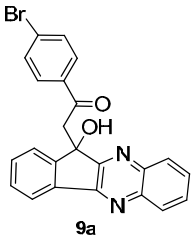
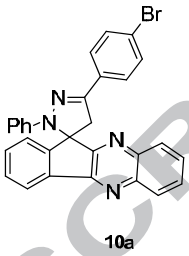
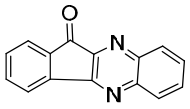
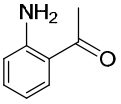
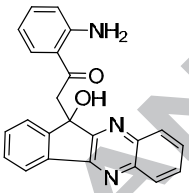
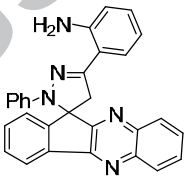
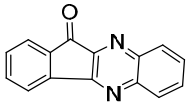
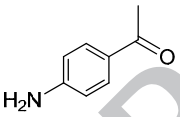
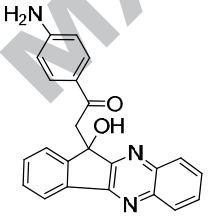
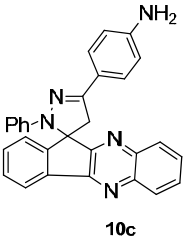
(**Scheme 2**)**.** Accordingly, treatment of *o*-phenylenediamine with ninhydrin in the presence of acetic acid in ethanol gave the 11*H*-indeno[1,2-*b*]quinoxalin-11-one **8**, which was then

Scheme 2: Synthesis of indenoquinoxaline derivatives. Reagents and conditions**:** a) glacial AcOH, EtOH, 60 ^{o}C, 12 h, 90%; b) dimethylamine, arylketone, neat, 25^{o}C, 30 min; c) glacial AcOH, HCl, 80 ^{o}C, 30 min; d)) $PhNHNH_2$, EtOH, 80 ^{o}C, 12 h.



subjected to aldol condensation with aryl ketones in the presence of dimethyl amine to afford the β-hydroxyketones (**9a-c**). Upon treatment of β-hydroxyketone with phenylhydrazine in the presence of catalytic amount of HCl in acetic acid at 80 ^{o}C afforded the corresponding pyrazole derivatives (**10a-c**).

Table2. Synthesis of indenoquinoxaline derivatives **(9a-9c)** and **(10a-10c):**

Entry	11H-indeno[1,2-b] quinoxalin-11-one	Acetophenone	Hydroxyketone 9	Yield (%)	Quinoxaline-pyrazole 10	Yield (%)
1			 **9a**	78	 **10a**	86
2			 **9b**	92	 **10b**	84
3			 **9c**	80	 **10c**	76

***In-vitro* MTB screening**

These indolo[2,1-*b*]quinazoline-6,12-dione and 11*H*-indeno[1,2-*b*]quinoxalin-11-one based molecules were screened for their *in vitro anti-mycobacterial activity* against *M. tuberculosis* H37Rv (*Mtb*) by Agar dilution method for determination of the MIC values along with standard drugs ethambutol and ciprofloxacin. Based on the results presented in Table **3**, some preliminary structure-activity relationship aspects were deduced. The substituent present on aromatic ring of the phenacyl group had shown certain effects on biological activity. Of various compounds, 2-aminophenacyl derivative **4f** showed an excellent anti-tubercular activity with MIC 3.99 µM, which is higher than ethambutol (MIC 15.3 µM) and ciprofloxacin (MIC 12.6 µM). Conversely, 4-aminophenacyl derivative **4i** is less effective than **4f**. Furthermore, thiourea derivative **4k** is less potent than free amino

derivative **4f** but more effective than other derivatives having 4-ethylphenacyl **4h** and 4-fluorophenacyl **4d** groups. In addition **4j, 5a, 5c, 5f, 5g, 9c** and **10c** also showed significant MIC values, which are lower than that of ciprofloxacin, whereas the remaining compounds showed moderate activity with MIC value similar to that of ethambutol. The presence of bromo substituent on phenacyl ring (**4e**) drastically reduced the activity than the fluoro substituent on phenacyl ring (**4d)** may be due to the lipophilicity of fluorine. Overall, the phenacyl derivatives of tryptanthrin are considerably active than indole substituted derivatives. However, the corresponding 4-cyano phenyl substituent on indole moiety **5c** found to exhibit potent activity than 4-cyano phenacyl substitutent **4c.** Among indenoquinoxaline derivatives, **9c** and **10c** are more active than others in the series of compounds (**9a-c** and **10a-c**). Among them, **10c** is more active than **9c** due to rigidity in spirocyclic system. In case of indenoquinoxaline derivatives, 4-aminophenacyl **9c** is more active than 2-aminophenacyl **9b**. Similarly, **10c** showed significant activity than **10b**. The synthesis of several analogs demonstrates that the activity totally depends on the substitution pattern of the aromatic ring.

Table 3. Anti-mycobacterial activity of compounds **4a-k, 5a-j, 9a-c** and **10a-c** against *M. tuberculosis* H37Rv strain (ATCC-27294).

Compound	ClogP	MIC(μM)
4a	2.55	16.91
4b	2.88	16.23
4c	2.16	15.90
4d	2.77	8.85
4e	3.49	111.89
4f	2.49	3.99
4g	2.32	16.69
4h	4.35	7.88
4i	1.83	16.32
4j	4.35	11.86
4k	3.59	5.98
5a	3.57	8.58
5b	4.87	29.38
5c	3.91	7.06
5d	4.31	27.74
5e	5.02	56.43
5f	5.74	6.18

5g	3.65	7.30
5h	3.91	12.32
5i	3.65	14.16
9a	4.23	14.43
9b	4.23	17.01
9c	1.96	8.45
10a	2.57	12.41
10b	7.06	56.85
10c	5.51	7.17
Ethambutol		15.3
Ciprofloxacin		12.6

ClogP was calculated using QikProp model of version 6.0, Schrödinger, **2013**.

2.3 Molecular docking studies:

In order to gain more insight, these new series of phaitanthrin congeners were docked to validate whether the synthesized compounds have comparable binding mode in active site of enoyl-acyl carrier protein reductase (InhA) of *M .tuberculosis*, which is one of the key enzymes and has been validated as an effective anti-microbial target. The synthesized compounds were docked within the active site of InhA crystal structure (PDB code: 4U0J) [8] using Glide 6.0(Schr dinger, 2013).[9] As all the synthesized compounds are racemic mixtures, docking studies were carried out for both the isomers. During the binding studies it was observed that all synthesized compounds exhibit hydrogen bonding interaction with Tyr 158. As depicted in Figure **2**, Compound **4f** showed interactions with active site residues Ile 21, Gly 96, Phe 97, Met 98, Met 103, Phe 149, Met 155, Pro 156, Ala 157, Met 161, Gly 192, Pro 193, Thr 196, Ala 198, Met 199, Ile 194, Ile 202, Leu 207, Ile 215, Leu 218. While in compound **4k,** apart from π-cation interactions of side chain phenyl ring with Lys 165 and -NH- group of thiourea in the side chain formed H-bonding with Gly 14 was additionally observed.

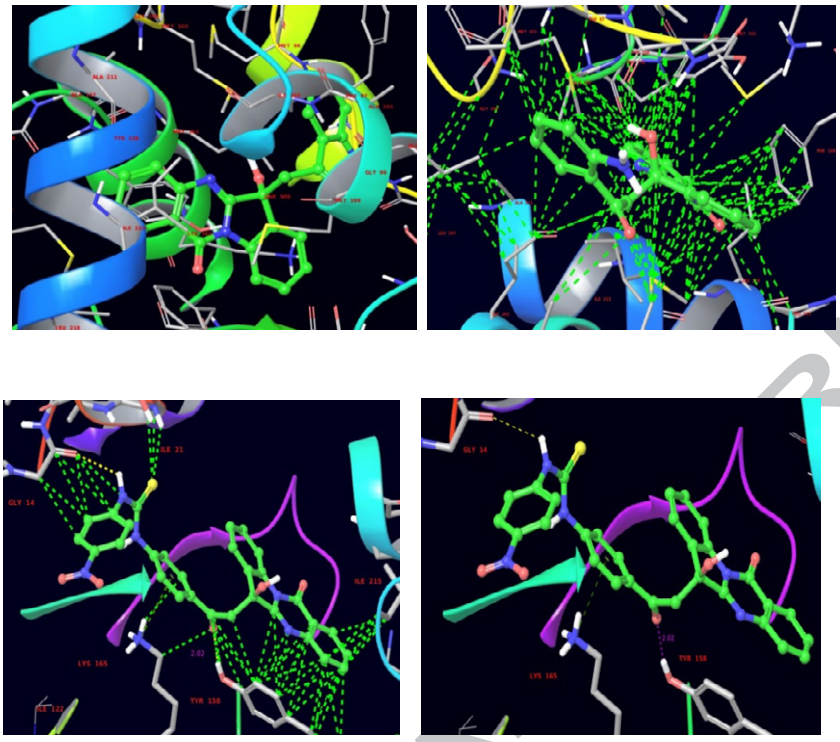


Figure 2.a) Three-dimensional diagram displays the binding poses for compound **4f** interactions at active site of InhA. b) Key residues within a 4.5 Å sphere of **4f** in the binding pocket are shown. c) Compound **4k** interactions within the active site of InhA. d) Hydrogen bonding of **4k** with Gly 14, Tyr 158 and π- cation with Lys 165 (dark green dashed lines) in active site. Pink and yellow dashed lines represent H bonding with amino acid side chain, H bonding with amino acid backbone respectively.

In summary, a new series of indolo[2,1-*b*]quinazolinedione and indenoquinoxaline derivatives were synthesized and screened for their *in vitro* anti-mycobacterial activity. A number of compounds have shown good *in vitro* anti-mycobacterial activity. Among various congeners of phaitanthrin, 2-aminophenyl **4f** and its thiourea **4k** are identified as lead molecules for TB. In addition, **5f, 5c, 10c, 5g, 4h, 9c, 5a** and **4d** have also shown promising activity thus paving the way for the discovery of newer anti-tubercular potential agent. *In silico* molecular modelling studies were performed to examine the interactions of phaitanthrin congeners with InhA of *M. tuberculosis* and these interactions indicated the stability of phaitanthrin congeners in the active residue. Thus, enhanced affinity of phaitanthrin

congeners to binding site of InhA may lead to the synthesis of anti-tubercular agents that are likely to be capable of combating MDR strains of *M. tuberculosis*.

Acknowledgements

The authors, B.S and A.R acknowledge the National Institute of Pharmaceutical Education and Research (NIPER) -Hyderabad, Department of Pharmaceuticals, Ministry of Chemicals and Fertilizers, Govt. of India for the award of research fellowship.

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Graphical Abstract

Synthesis and biological evaluation of phaitanthrin congeners as anti-mycobacterial agents