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Graphical Abstract

Synthesis, Biological Evaluation and Structure-Activity Relationship of 2-Styrylquinazolones as Anti-tubercular Agents

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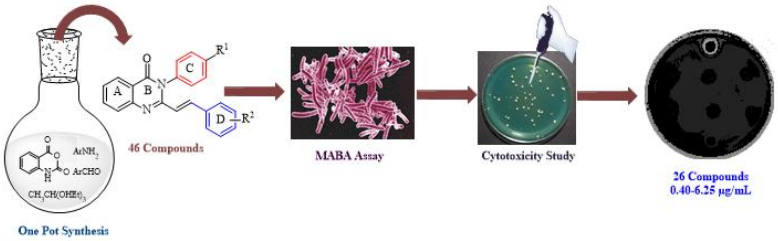

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ABSTRACT

2-Styrylquinazolones are reported as a novel class of potent anti-mycobacterial agents. Forty-six target compounds have been synthesized using one pot reaction involving isatoic anhydride, amine, and triethyl orthoacetate followed by aldehyde to construct the 2-styrylquinazolone scaffold. The anti-mycobacterial potency of the compounds was determined against $H_{37}Rv$ strain. Twenty-six compounds exhibited anti-Mtb activity in the range of 0.40-6.25 µg/mL. Three compounds **8c**, **8d** and **8ab** showed MIC of 0.78 µg/mL and were found to be non-toxic (< 50% inhibition at 50 µg/mL) to HEK 293T cell lines with the therapeutic index >64. The most potent compound **8ar** showed MIC of 0.40 µg/mL with the therapeutic index >125.An early structure activity relationship for this class of compounds has been established. The computational studies indicate the possibility of these compounds binding to the penicillin binding proteins (PBPs).

Human tuberculosis (TB) continues to be a major cause of death every year, and the emergence of drug resistant strains of *Mycobacterium tuberculosis* (Mtb) has reincarnated the demand to discover and develop new drugs to combat with the deadly pathogenic mycobacteria.[1] The current year 2015 marks the 22nd anniversary of the declaration of tuberculosis as a global health emergency by WHO (World Health Organization).[2] Despite enormous efforts have been made in the hunt for new drugs, TB still remains the first bacterial cause of mortality worldwide causing an estimated 9.0 million new cases in 2013 and 1.5 million death, 360 000 of whom were HIV-positive.[3] The first line drugs such as isoniazid (INH), rifampin (R), pyrazinamide (Z), streptomycin (S) and ethambutol (E) are used to treat active TB and latent tuberculosis infection (LTBI) these suffer from one or more serious side effects.[4] For the treatment of drug-sensitive TB, initially patients are treated with first-line four drugs (INH, R, Z and E) for 2 months followed by 4 months of INH plus R through directly observed treatment short course strategy (DOTS) possessing a cure rate of >95%. The treatment of drug-resistant TB requires 18-24 months or longer, involving the use of more toxic and costly second-line medicines such as ciprofloxacin (Cfx), *para*-amino salicylic acid (Pas), kanamycin (Km), cycloserine (Dcs), ethionamide (Eto), amikacin (Amk), capreomycin (Cm), thioacetazone (Thz).[5] Notably, after 40 years a new chemical entity, bedaquiline, has been approved by the U.S. Food and Drug Administration (FDA) with the name Sirturo in the end of 2012,[6] and by the European Medicine Agency (EMA) in 2014 for the treatment of MDR-TB patients.[7] The emergence of multi-drug resistant (MDR) and extensively drug-resistant (XDR) strains of Mtb insists the need for new therapies, which might have novel mechanism of action.[8,9] Furthermore, the resurgence in TB is alarming due to the development of pathogenic synergy with HIV.[5] Thus the discovery and development of new anti-Mtb molecules continues to be the perpetual interest to academia and pharma industry to tackle the TB pandemic.

In continuation of our efforts to search for new anti-Mtb scaffolds[10] we were attracted by the findings on the identification of the styrylquinazolones **1** and **2** (Figure 1) as the potent anti-Mtb agents[11] with high selectivity index (SI) under Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF) Program that encouraged us to synthesize various 2-styrylquinazolones and evaluate their anti-Mtb potential to establish the structure activity relationship (SAR).

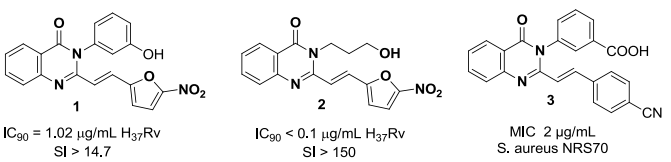


Figure 1. Literature reported styryl quinazolones having anti-tubercular and anti-bacterial activity.

In the present study we planned to explore the 2-styrylquinazolones having the general structure as represented in Figure 2, for evaluating their anti-Mtb potential. The goal of this

study was to synthesize the diverse library of 2-styrylquinazolones through variations in the C and D ring and to establish the early SAR for this class of compounds.

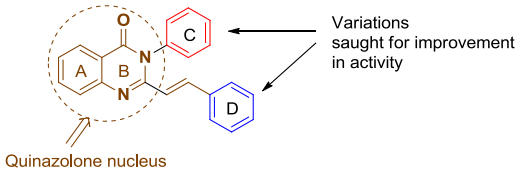
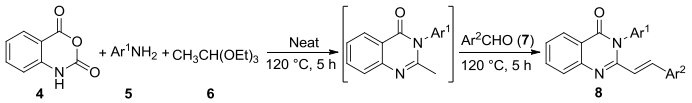


Figure 2.Designed 2-styrylquinazolones.

During the progress of this work we were delighted to observe that the styrylquinazolone **3** (Figure 1) has been found to be potent antibacterial antibiotic.[12]

Recently we reported a convenient synthesis of 2-styrylquinazolones **8** through one-pot reaction involving isatoic anhydride **4**, aryl amine **5** and triethyl orthoacetate **6** at 120 ^{0}C to form the intermediate 2-methyl-3-aryl-quinazolone followed by treatment with aromatic aldehydes **7** (Scheme 1) to afford the desired products.[13]



Scheme 1. Synthesis of 2-styryl quinazolones (**8**).

The forty-six target compounds **8** having various substitutions (electron donating/withdrawing) on C and D ring were synthesized in the 54-79% yields (Table 1) following this reported procedure.

Table 1. One pot synthesis of 2-stryrylquinazolones (**8**)[a]

Entry	Compd No.	2-Styrylquinazolones (**8**)	Yield[b] (%)
1	**8a**	Ar^1 = Ph, Ar^2 = Ph	65
2	**8b**	Ar^1 = 4-Me-C_6H_4, Ar^2 = Ph	72
3	**8c**	Ar^1 = 4-SMe-C_6H_4, Ar^2 = Ph	79
4	**8d**	Ar^1 = 2,3,4-tri-OMe-C_6H_4, Ar^2 = Ph	66
5	**8e**	Ar^1 = Ph, Ar^2 = 4-F-C_6H_4	74
6	**8f**	Ar^1 = 4-Me-C_6H_4, Ar^2 = 4-F-C_6H_4	68
7	**8g**	Ar^1 = 2,3,4-tri-OMe-C_6H_4, Ar^2 = 4-F-C_6H_4	79
8	**8h**	Ar^1 = 4-SMe-C_6H_4, Ar^2 = 4-F-C_6H_4	70
9	**8i**	Ar^1 = Ph, Ar^2 = 4-OMe-C_6H_4	60
10	**8j**	Ar^1 = 2,3,4-tri-OMe-C_6H_4, Ar^2 = 4-OMe-C_6H_4	65
11	**8k**	Ar^1 = 4-SMe-C_6H_4, Ar^2 = 4-OMe-C_6H_4	64
12	**8l**	Ar^1 = Ph, Ar^2 = 4-F-C_6H_4	67
13	**8m**	Ar^1 = 4-Me-C_6H_4, Ar^2 = 4-Cl-C_6H_4	71
14	**8n**	Ar^1 = 2,3,4-tri-OMe-C_6H_4, Ar^2 = 4-Cl-C_6H_4	76
15	**8o**	Ar^1 = 4-SMe-C_6H_4, Ar^2 = 4-Cl-C_6H_4	76
16	**8p**	Ar^1 = 4-SMe-C_6H_4, Ar^2 = 4-N,N-di-Me-C_6H_4	66
17	**8q**	Ar^1 = 4-SMe-C_6H_4, Ar^2 = 2-thiazolyl	73
18	**8r**	Ar^1 = Ph, Ar^2 = 2,6-di-OH-C_6H_4	54
19	**8s**	Ar^1 = Ph, Ar^2 = 4-Phenylmethoxy-C_6H_4	68
20	**8t**	Ar^1 = 4-Cl-C_6H_4, Ar^2 = 4-F-C_6H_4	65
21	**8u**	Ar^1 = 4-OMe-C_6H_4, Ar^2 = 4-OMe-C_6H_4	60
22	**8v**	Ar^1 = 4-Me-Ph-C_6H_4, Ar^2 = 4-Cl-C_6H_4	67
23	**8w**	Ar^1 = 4-SMe-C_6H_4, Ar^2 = 4-OCOCH$_3$-C_6H_4	71
24	**8x**	Ar^1 = 4-SMe-C_6H_4, Ar^2 = 2-Furanyl	75
25	**8y**	Ar^1 = 2,3,4-tri-OMe-C_6H_4, Ar^2 = 2-Furanyl	74
26	**8z**	Ar^1 = 2,3,4-tri-OMe-C_6H_4, Ar^2 = 2-thiazolyl	75
27	**8aa**	Ar^1 = Ph, Ar^2 = 4-SMe-C_6H_4	68
28	**8ab**	Ar^1 = Ph, Ar^2 = 2,3,4-tri-OMe-C_6H_4	61
29	**8ac**	Ar^1 = 4-OMe-C_6H_4, Ar^2 = Ph	70
30	**8ad**	Ar^1 = 4-OMe-C_6H_4, Ar^2 = 4-Cl-C_6H_4	75
31	**8ae**	Ar^1 = 4-OMe-C_6H_4, Ar^2 = 2-thiazolyl	74
32	**8af**	Ar^1 = 4-OMe-C_6H_4, Ar^2 = 2-Furanyl	71
33	**8ag**	Ar^1 = 4-Me-C_6H_4, Ar^2 = 4-OMe-C_6H_4	69
34	**8ah**	Ar^1 = Ph, Ar^2 = 2-thiazolyl	70
35	**8ai**	Ar^1 = Ph, Ar^2 = 2-Furanyl	66
36	**8aj**	Ar^1 = 4-Cl-C_6H_4, Ar^2 = Ph	64
37	**8ak**	Ar^1 = 4-Cl-C_6H_4, Ar^2 = 4-Cl-C_6H_4	66
38	**8al**	Ar^1 = 4-F-C_6H_4, Ar^2 = Ph	64
39	**8am**	Ar^1 = 4-F-C_6H_4, Ar^2 = 4-Cl-C_6H_4	68
40	**8an**	Ar^1 = 4-Br-C_6H_4, Ar^2 = Ph	71
41	**8ao**	Ar^1 = 4-Br-C_6H_4, Ar^2 = 4-F-C_6H_4	66
42	**8ap**	Ar^1 = 4-F-C_6H_4, Ar^2 = 4-Me- C_6H_4	61
43	**8aq**	Ar^1 = 4-F-C_6H_4, Ar^2 = 4-F-C_6H_4	64
44	**8ar**	Ar^1 = 2,3,4-tri-OMe-C_6H_4, Ar^2 = 4-SMe-C_6H_4	63
45	**8as**	Ar^1 = 4-SMe-C_6H_4, Ar^2 = 2,3,4-tri-OMe-C_6H_4	69
46	**8at**	Ar^1 = 4-Me-C_6H_4, Ar^2 = 4-Me-C_6H_4	67

[a]The mixture of isatoic anhydride **4** (2.5 mmol), amine/NH_4OAc (2.5 mmol) and triethyl orthoacetate **6** (2.5 mmol) was heated under neat condition at 120 °C for 5 h followed by addition of aldehyde **7** (2.5 mmol, 1 equiv) and continued stirring for further 5 h.
[b]Isolated yield of the 2-styrylquinazolone (**8**).

The forty-six synthesized 2-styrylquinazolones (**8a-8at)** were subjected to in vitro anti-Mtb activity test against *M. tuberculosis* $H_{37}Rv$ (ATCC 27294 strain).[14] The minimum inhibitory concentration (MIC), minimum concentration in µg/mL of the compound required for 99% inhibition of bacterial growth, of **8a** to **8at** and those of the standard drugs (INH, R, E, Z and Cfx) were determined in triplicate at pH 7.4 (Table 2). All of the synthesized compounds showed MIC values in the micromolar range (0.40 – >25 µg/mL). Twenty-two compounds exhibited MIC in the range of 1.56–6.25 µg/mL, and the compounds **8c, 8d** and **8ab** were found to be potent (MIC 0.78 µg/mL). The most potent compound **8ar** showed MIC of 0.40 µg/mL.

The in vitro cell viability of the compounds with MIC ≤ 6.25 µg/mL were evaluated against HEK-293T (human embryonic kidney) cell lines at 50 µg/mL concentration by using [(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide)] MTT assay. The % inhibitory cytotoxicity data are summarized in Table 2 and graphically represented in Figure 3, along with the MIC values of the respective compounds. In general, most of the active compounds were found to be non-toxic (<50% inhibition) and **8c**, **8d**, **8ab** and **8ar** turned out to be the most active

compounds and promising anti-Mtb leads from this series with therapeutic index of > 64 and >125 respectively.

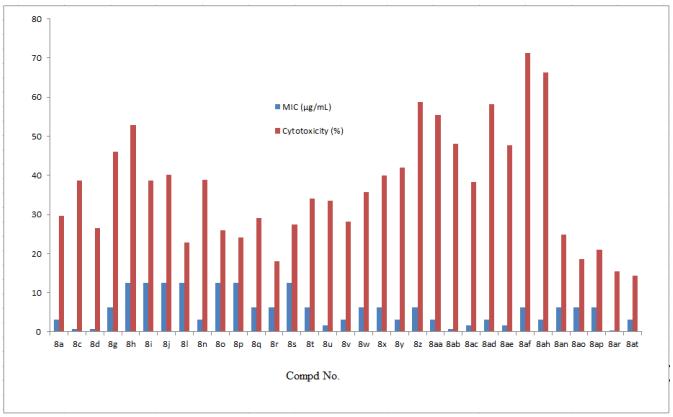


Figure 3. Graphical representation of the anti-mycobacterial activity and cytotoxicity profile of the synthesized compounds with MIC ≤ 12.5 µg/mL.

A complete SAR can be drawn considering the MIC values and cytotoxicity data (Table 2).

Table 2. Biological evaluation of **8a-8at** and a few standard anti-TB drugs.

Compd No.	MIC[a] (µg/mL)	HEK 293T Inhibition[b]	Compd No.	MIC[a] (µg/mL)	HEK 293T Inhibition[b]
8a	3.125	29.70	**8aa**	3.125	55.47
8b	>25	ND	**8ab**	0.78	47.99
8c	0.78	38.70	**8ac**	1.56	38.36
8d	0.78	26.54	**8ad**	3.125	58.14
8e	25	ND	**8ae**	1.56	47.68
8f	>25	ND	**8af**	6.25	71.33
8g	6.25	46.12	**8ag**	12.5	ND
8h	12.5	ND	**8ah**	3.125	66.29
8i	12.5	ND	**8ai**	12.5	ND
8j	12.5	ND	**8aj**	12.5	ND
8k	>25	ND	**8ak**	>25	ND
8l	12.5	ND	**8al**	25	ND
8m	25	ND	**8am**	12.5	ND
8n	3.125	38.90	**8an**	6.25	24.86
8o	12.5	ND	**8ao**	6.25	18.66
8p	12.5	ND	**8ap**	6.25	21.06
8q	6.25	29.00	**8aq**	>25	ND
8r	6.25	18.12	**8ar**	0.40	15.41
8s	12.5	ND	**8as**	>25	ND
8t	6.25	34.00	**8at**	3.125	14.27
8u	1.56	33.60	**INH**	0.098	ND
8v	3.125	28.20	**R**	0.197	ND
8w	6.25	35.80	**E**	1.56	ND
8x	6.25	40.00	**Z**	6.25	ND
8y	3.125	42.07	**Cfx**	1.56	ND
8z	6.25	58.77			

[a] 99% inhibition of growth of *M. tuberculosis* $H_{37}Rv$ (ATCC 27294 strain).
[b] % Inhibition at 50 µg/mL concentration determined against HEK 293T cell lines. ND: Not Determined.

The systematic variations on the C-ring keeping the quinazolone nucleus and D ring intact have varying effects on the anti-Mtb activity of the compounds. The substitution of thiomethyl and methoxy groups at the 4-position of the C-ring resulted in the active compounds **8c**and **8ac** having MIC of 0.78 µg/mL and 1.56 µg/mL respectively, more than its un-substituted counterpart **8a** (MIC of 3.125 µg/mL). While the substitution of halides (**8aj**, **8al and 8an**) and methyl (**8b**) group at the 4-position gave less active compounds. The 3,4,5-trimethoxy substitution on the C ring also resulted in the compound (**8d**) with activity of 0.78 µg/mL. All the active compounds were found to be safe with the selectivity index (SI) ranging from 16 to >64 (Table 3, Figure 4).

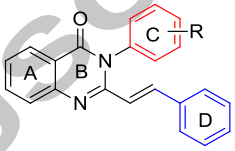


Figure 4. Ring C substitution on 2-styrylquniazolones.

Table 3. Effect of C-ring substitution on anti-TB activity.

Entry	Compd No.	R	MIC (µg/mL)[b]	% Inhibition at 50 µg/mL HEK 293T	SI
1	**8a**	H	3.125	29.70	>16
2	**8b**	4-Me	> 25	ND[a]	-
3	**8c**	4-SMe	0.78	38.70	>64
4	**8d**	3,4,5-tri-OMe	0.78	26.54	>64
5	**8ac**	4-OMe	1.56	38.36	>32
6	**8aj**	4-Cl	12.5	ND[a]	-
7	**8al**	4-F	25	ND[a]	-
8	**8an**	4-Br	6.25	24.86	>8

[a]ND: Not determined.

The systemic variations of the above seven compounds by varying the D ring substitution was next studied to check the effect on the activity of the compounds. Substitutions were introduced on **8a**, **8c**, **8d** and **8ac** which have shown activity in the range of 0.78 to 3.125 µg/mL to get more potent and selective compounds (Figure 5).

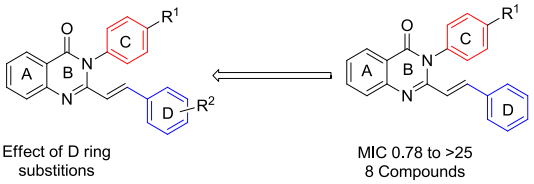


Figure 5. Ring D substitutions on 2-styrylquniazolones.

The substitution by halide (**8e** and **8l**) or alkoxy group (**8i** and **8s**) at the 4 position resulted in decrease in activity (Table 4, Figure 6). 4-Thiomethtyl substitution resulted in the compound (**8aa**) with equal activity. 3,4,5-Trimethoxy substitution gave the more potent compound (**8ab**) with MIC of 0.78 µg/mL and selectivity >64. The 2,6-di-hydroxy substitution yielded compound (**8r**) with MIC value of 6.25 µg/mL and is less potent than the parent compound **8a**. Replacement of D-ring with 5-

membered heterocyclic rings like furan-2-yl (**8ai**) and thiofuran-2-yl (**8ah**) did not improve the activity. However, **8ah** has shown activity equal to the parent compound **8a**.

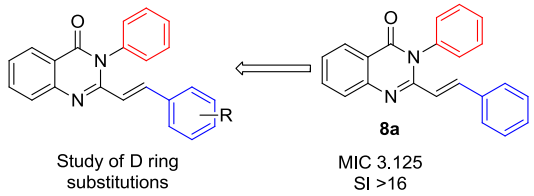


Figure 6. Study of ring D substitution on **8a**.

Table 4. Effect of D-ring substitution on anti-TB activity of **8a**.

Entry	Compd No.	R	MIC (μg/mL)[b]	% Inhibition at 50 μg/mL HEK 293T	SI
1	**8e**	4-F	25	ND[a]	-
2	**8l**	4-Cl	12.5	ND[a]	-
3	**8i**	4-OCH_3	12.5	ND[a]	-
4	**8s**	4-OCH_2Ph	12.5	ND[a]	-
5	**8aa**	4-SMe	3.125	55.47	<16
6	**8ab**	3,4,5-tri-OMe	0.78	47.99	>64
7	**8r**	2,6-di-OH	6.25	18.12	>8
8	**8ai**		12.5	ND[a]	-
9	**8ah**		3.125	66.29	<16

[a]ND: Not determined.

The substitution by halogen atom (**8f** and **8m**) at the 4 position did not improve the activity (Table 5, Figure 7). Alkoxy group (**8ag**) at the 4 position resulted in some improvement in the activity with MIC value of 12.5 μg/mL. While the methyl substitution (**8at**) increased the potency to 3.125 μg/mL. Overall the substitution tried on **8b** didn't significantly alter the activity.

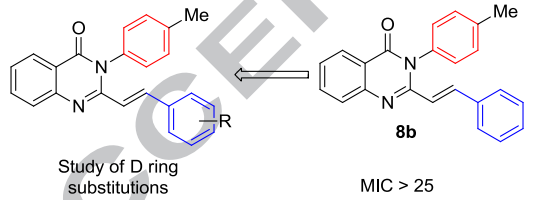


Figure 7. Study of ring D substitution on **8b**.

Table 5. Effect of D-ring substitution on anti-TB activity of **8b**.

Entry	Compd No.	R	MIC (μg/mL)[b]	% Inhibition at 50 μg/mL HEK 293T	SI
1	**8f**	4-F	>25	ND[a]	-
2	**8m**	4-Cl	25	ND[a]	-
3	**8ag**	4-OCH_3	12.5	ND[a]	-
4	**8at**	4-Me	3.125	14.27	>16

[a]ND: Not determined.

The substitution by halogen atom (**8h** and **8o**), alkoxy group (**8k** and **8as**), and *N*,*N*-dimethylamine group (**8p**) at the 4 position resulted in decrease in activity (Table 6, Figure 8). Replacement of D-ring with 5-membered heterocyclic rings like furan-2-yl (**8x**) and thiofuran-2-yl (**8q**) did not improve the activity. 4-Acetyloxy substitution yielded compound (**8w**) with the activity of 6.25 μg/mL less than that of the parent compound **8c**. None of the substituents was able to increase the activity of the parent compound **8c**.

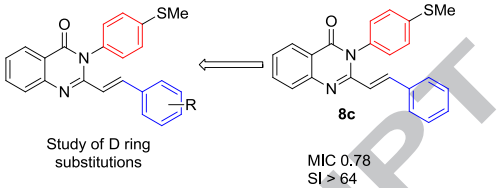


Figure 8. Study of ring D substitution on **8c**.

Table 6. Effect of D-ring substitution on anti-TB activity of **8c**.

Entry	Compd No.	R	MIC (μg/mL)[b]	% Inhibition at 50 μg/mL HEK 293T	SI
1	**8h**	4-F	12.5	ND[a]	-
2	**8o**	4-Cl	12.5	ND[a]	-
3	**8k**	4-OCH_3	>25	ND[a]	-
4	**8p**	4-*N*, *N*-di-Me	12.5	ND[a]	-
5	**8x**		6.25	40.00	>8
6	**8q**		6.25	29.00	>8
7	**8w**	4-$OCOCH_3$	6.25	35.80	-
8	**8as**	3,4,5-tri-OMe	>25	ND[a]	-

[a]ND: Not determined.

Effect of D-ring substitution on **8ac** (Figure 9) on the anti-TB activity and selectivity of the resultant compounds is demonstrated in Table 7. The substitution by halogen atom (**8ad**) and alkoxy group (**8u**) at the 4 position resulted in compounds with MIC values of 3.125 μg/mL and 1.56 μg/mL respectively. Replacement of D-ring with 5-membered heterocyclic rings like furan (**8af**) and thiofuran (**8ae**) did not improve the activity. None of the substituents was able to increase the activity of the parent compound **8ac** as all the substitutions tried gave equipotent or less potent compounds compared to the parent compound.

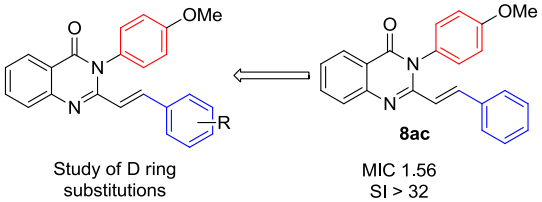


Figure 9. Study of ring D substitution on **8ac**.

Table 7. Effect of D-ring substitution on anti-TB activity of **8ac**.

Entry	Compd No.	R	MIC (μg/mL)[b]	% Inhibition at 50 μg/mL HEK 293T	SI

1	**8ad**	Cl	3.125	58.14	<16
2	**8u**	OCH_3	1.56	33.60	>32
3	**8af**		6.25	71.33	<8
4	**8ae**		1.56	47.68	>32

[a]ND: Not determined.

Effect of D-ring substitution on **8aj** and **8al** (Figure 10) on the anti-TB activity and selectivity is shown in Table 8. Halogen substitution (**8am** and **8t**) led to some improvement in activity.

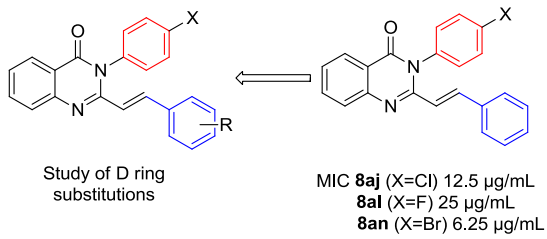


Figure 10. Study of ring D substitution on **8aj**, **8al** and **8am**.

Table 8. Effect of D-ring substitution on anti-TB activity of **8aj**, **8al** and **8an**.

Entry	Compd No.	R	X	MIC (µg/mL)[b]	% Inhibition at 50 µg/mL HEK 293T	SI
1	**8am**	4-Cl	F	12.5	ND[a]	-
2	**8t**	4-F	Cl	6.25	34.0	>8
3	**8ak**	4- Cl	Cl	>25	ND[a]	-
4	**8ap**	4-F	4-Me	6.25	21.06	>8

[a]ND: Not determined.

Effect of D-ring substitution on **8d** (Figure 11) on the anti-Mtb activity and selectivity is shown in Table 9.The substitution by halogen atom (**8g** and **8n**) and alkoxy group (**8j**) at the 4 position resulted in decrease in activity. Replacement of D-ring with 5-membered heterocyclic rings such as furan (**8y**) and thiofuran (**8z**) did not improve the activity. Substitution by methylthio group (**8ar**) gave the most potent compound from the series with MIC of 0.40 µg/mL.

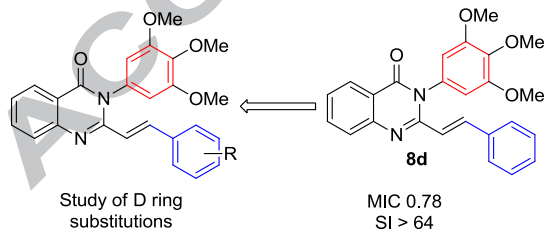


Figure 11. Study of ring D substitution on **8d**.

Table 9. Effect of D-ring substitution on anti-TB activity of **8d**.

Entry	Compd No.	R	MIC (µg/mL)[b]	% Inhibition at 50 µg/mL HEK 293T	SI
1	**8g**	4-F	6.25	46.12	>8
2	**8n**	4- Cl	3.125	38.90	>16
3	**8j**	4- OCH_3	12.5	ND[a]	-
4	**8y**		3.125	42.07	>16
5	**8z**		6.25	58.77	<8
6	**8ar**	4-SMe	0.40	15.41	>125

[a]ND: Not determined.

Recently styrylquinazolines have been reported as new antibiotics and their site of action has been identified to be the PBPs (Pbp2a and Pbp1) present in *S. aureus*.[12] The PBPs are required for the final stages of cell-wall formation in bacteria and are also molecular targets for β-lactam antibiotics.[15,16] The high resolution (1.8 Å) *S. aureus* PBP2a crystal structure (1VQQ) was reported.[17] Active site of this crystal structure was used to identify the styrylquinazoline antibiotics.[12] In order to get further insight on the Mtb activity of the styrylquinazolines described under our investigation in this report we planned to understand the putative targets of these styrylquinazolines thorough computational studies. We performed blast search FASTA sequence of *S. aureus* PBP2a (1VQQ) against the PDB protein and found that the *M. tuberculosis* PBPa (3UN7) has 22% sequence identity, 51% sequence similarity, and an E- value (expectation value) of 1e-12. Although the sequence identity is low, the active site of these PBPs are conserved.[12,16] The protein 3UN7 is in apo form and its co-crystallised structures with antibiotics imipenem [3UPN], penicillin G[3UPO], and ceftriaxone [3UPP] are reported in the PDB reflecting that these antibiotics are bound to its active site.[16] To validate the similarity of the active sites, we have performed the sequence alignment (Figure 12) of these two proteins and compared their active sites.[17,18]

A comparison[19] of the key nine amino acid residues of the active site of PBP2a with that of PBPa revealed that seven of these amino acid residues are identical, while the Met641 in PBP2a is replaced by Leu467 in PBPa which is considered to be similar. The only dissimilarity is that the Tyr446 in PBP2a is replaced by Glu268 in PBPa (Figure 12). Thus, out of the nine amino acid residues of the active site, seven are identical, one is similar, and one is dissimilar. This suggested that their active sites can be considered to be similar. Therefore we have used the active site of *M. tuberculosis* PBPa for docking our compounds.

The 3D QSAR techniques such as CoMFA/CoMSIA and molecular docking provide a deeper understanding of the structure activity correlations that would serve as a predictive model for further optimization of the lead structure.[20] Docking analysis was performed on our active molecules to identify key amino acid residues involved in making interactions with the PBPa active site of 3UPN. The GOLD program[21] was used to carry out this analysis. The docked pose of the most potent molecule 8d is shown in Figure 13. The hydrogen bonding interactions were found with the key active site residues Ser222, Gly221 and Asn283 with high gold score of 58 (Figure 13). Methoxy group of 8d interacts with the Ser222 and Gly221; whilst the quinazolone oxygen interacts with the Asn283. This analysis indicates the possibility of these molecules binding to the PBPs. However, further experimental proofs are needed to confirm this.

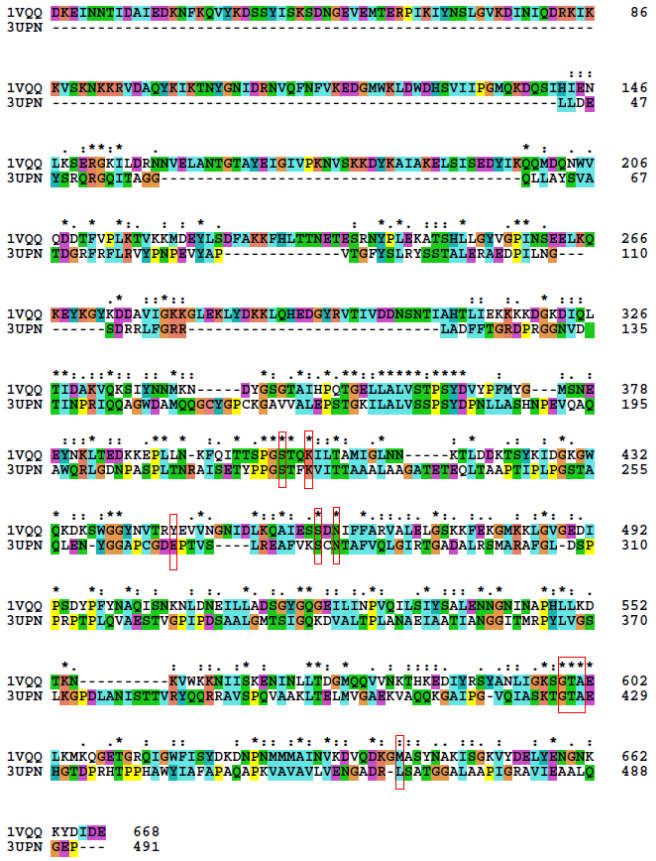


Figure 12.(a) Sequence alignment of Mtb PBPa (3UPN) with template *S. aureus* PBP2a (1VQQ). Red boxes indicate active site residues among PBP proteins. Colors indicate amino acids with their similar characteristics, stars identical amino acids, colons similar amino acids, single dots almost similar amino acids.[17,18]

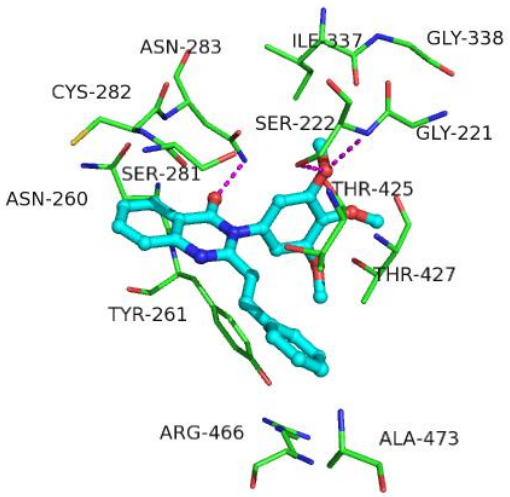


Figure 13. The docking pose of styrylquinazolone antibiotic in PBPa active site. Figure is generated and rendered using PyMOL.

The present work reveals new 2-styrylquinazolones with potent anti-Mtb activity. Forty-six compounds were synthesized using one pot reaction of isatoic anhydride, amine and triethyl orthoacetate followed by treatment of aromatic aldehyde. These were evaluated for in vitro anti-Mtb activity. Twenty-six compounds displayed good in vitro anti-mycobacterial activity ranging from 0.40-6.25 µg/mL. The most potent compounds **8c**, **8d**, **8ab** (MIC, 0.78 µg/mL) and **8ar** (MIC,0.40 µg/mL) were highly selective with therapeutic index >64 and >125 respectively. These compounds have shown potency better than that of the standard drugs E, Z and Cfx. The early SAR for this class of compounds has been also established. The computational study indicates that these compounds might act through PBPs as that of styrylquinazolone antibiotics.

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