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# Novel 2-(2-(4-aryloxybenzylidene) hydrazinyl)benzothiazole derivatives as anti-tubercular agents

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ABSTRACT

A series of structurally novel, substituted 2-(2-(4-aryloxybenzylidene) hydrazinyl)benzothiazole derivatives incorporating 2-hydrazinyl benzothiazole and 4-(aryloxy)benzaldehyde were designed and synthesized using molecular hybridization approach. All the synthesized compounds exhibited promising activity (MIC 1.5–29.00 μg/ml) against *Mycobacterium tuberculosis* H37Rv strains of using REMA. Five of the evaluated compounds exhibit MIC <3.0 μg/ml. Compound (*E*)-6-chloro-2-(2-(4-(2,4-dichlorophenoxy) benzylidene)hydrazinyl) benzothiazole showed MIC of 1.5 μg/ml. Thus, this compound could act as a potential lead for further development of new anti-tubercular drugs.



Tuberculosis (TB) is a chronic infectious disease caused by mycobacteria of the 'tuberculosis complex', including primarily *Mycobacterium tuberculosis*, but also *Mycobacterium bovis* and *Mycobacterium africanum*. In the last decade, TB has re-emerged as one of the leading causes of death worldwide (nearly 3 million deaths annually).[1] The estimated 8.8 million new cases every year correspond to 52,000 deaths per week or more than 7,000 each day.[2,3] These numbers however, are only a partial depiction of the global TB threat. More than 80% of TB patients are in the economically productive age of 15–49 years, which results in tremendous economic and social problems. It was estimated that nearly 1 billion more people will be infected with TB in the next 20 years. About 15% of that group (150 million) will exhibit symptoms of the disease, and about 3.6% (36 million) will die from TB if new disease prevention and treatment measures are not developed.[4] Tuberculosis is difficult to treat due to residence of bacteria within the macrophages and its unusual cell wall barrier. Moreover, multi-drug resistant strains of TB (MDR-TB) and extensively drug resistant tuberculosis (XDR-TB) have emerged recently.[5,6] The dramatic increase in TB cases observed in the recent years is a result of two major factors. First is the increased susceptibility of people infected with Acquired Immunodeficiency Syndrome (AIDS) to TB, which augments the risk of developing the disease 100-fold.[7] Second is the increase in resistant strains of the disease[8] with some showing cross-resistance to as many as nine drugs.[7] Although one possible long term solution to the problem is a better vaccine, in the short term, the major reliance will be on chemotherapy requiring the development of novel, effective and non-toxic antitubercular agents.[9]

Despite numerous attempts to develop new structural prototype in the search for more effective antimicrobials, benzothiazole still remain as one of the most versatile class of compounds against microbes[10–14] and therefore, are useful substructures for further molecular exploration. Benzothiazole derivatives have attracted continuing interest because of their varied biological activities viz antitumour,[15–18] antitubercular,[19–21] antimalarial,[22] anticonvulsant,[23] antihelmintic,[24] analgesic,[25] anti-inflammtory[26] and antidiabetic.[27]

The diphenyl ether, triclosan 5-chloro-2-(2,4-dichloro-phenoxy) phenol ether,[28] is a broad-spectrum biocide that has been used for over 30 years mainly as a component of antimicrobial wash in consumer products such as toothpastes, mouthwashes, deodorant soaps, lotions, children toys and cutting boards.[29] Subsequently extensive biochemical and structural studies have been performed to confirm that triclosan is a specific inhibitor of *Escherichia coli* ENR Triclosan also directly inhibits ENR from *M. tuberculosis* and *Mycobacterium smegmatis* (encoded by InhA) and *Plasmodium falciparum*, the malarial parasite. The common theme in the inhibition of ENRs by triclosan is the requirement of the NAD+ cofactor. The interaction of triclosan with ENR is stabilized by the π–π stacking interaction between the hydroxyl chloro phenyl ring and the hydroxyl group of a tyrosine from hydrogen bonding interactions with the hydroxyl group of triclosan. Ring B of triclosan makes several hydrophobic contacts with adenoyl acyl carrier protein reductase (ENR). The ether oxygen of triclosan may also be critical in the formation of the stable ENR–triclosan–NAD+ complex.[28–32]

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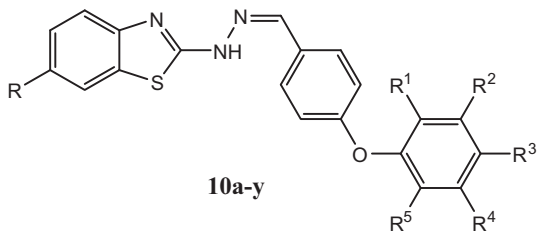



**Figure 1.** General Structure of 2-(2-(4-aryloxybenzylidene)hydrazinyl)benzo[*d*] thiazole.

The purpose of our research is the development of new antituberculotic agents with high activity using molecular hybridization approach which is one of the most valuable structural modification tools useful for the discovery of ligands and prototypes presenting either optimised affinity for one bioreceptor or the ability to modulate more than one bioreceptor associated with the target disease. The growing efforts to discover hybrid drugs resulting from the combination of pharmacophoric moieties of different known lead. In this light, various molecules were designed by molecular hybridization of 2-hydrazinyl benzothiazole and 4-(aryloxy) benzaldehyde. The design based on reason was expected at combining the synergistic activity of 2-hydrazinyl benzothiazole and 4-(aryloxy) benzaldehyde. In addition, the designed molecules could inhibit the adenoyl acyl carrier protein reductase (ENR) enzyme since the molecules contain substituted and unsubstituted diphenyl ether moiety like triclosan. Design of 2-(2-(4-aryloxybenzylidene) hydrazinyl) benzothiazole involved substitution 6-position of the benzothiazole ring with various groups like halogen, methyl, methoxy etc. (Fig 1).

From the QikProp[33] analysis of designed molecules, it was observed that the designed molecules exhibited good drug likeliness (Table 1). Most of the molecules exhibited physicochemical properties which fall in the range of known drugs as evidenced from # stars for compounds being 0 or 2. Molecules also lacked known toxicophores or reactive functional groups in all cases. The partition coefficient exhibited by QPlogPo/w, were within range 4.14–6.09. The next parameter of interest considered by QikProp analysis was QPPCaco. According to QikProp analysis, value of QPPCaco parameter <25 is considered poor and >500 is considered excellent. It was observed that most of the designed first generation molecules exhibited QPPCaco within acceptable limits. Human oral absorption of most of compound is 100, which was considered to be good. The designed molecules also followed the Lipinski's rule of five. Compounds showing good results were further considered for synthesis.

In the present study of benzothiazole derivatives bearing diphenyl ether moiety (**10a–y**) were synthesized as portrayed in the Scheme 1. 2-amino benzothiazole derivatives (**4a–e**) required as the starting Material was prepared according to the literature procedure.[34] 2-aminobenzothiazole derivatives (**4a–e**) which on treatment with hydrazine hydrate in presence of ethylene glycol resulted in the formation of 2-hydrazinyl benzothiazole derivatives (**6a–e**). Compound (**6a–e**) was reacted with substituted / unsubstituted 4-phenoxy aromatic aldehydes (**9a–e**) in ethanol under reflux, to give desired products (**10a–y**) in good yields.[35]

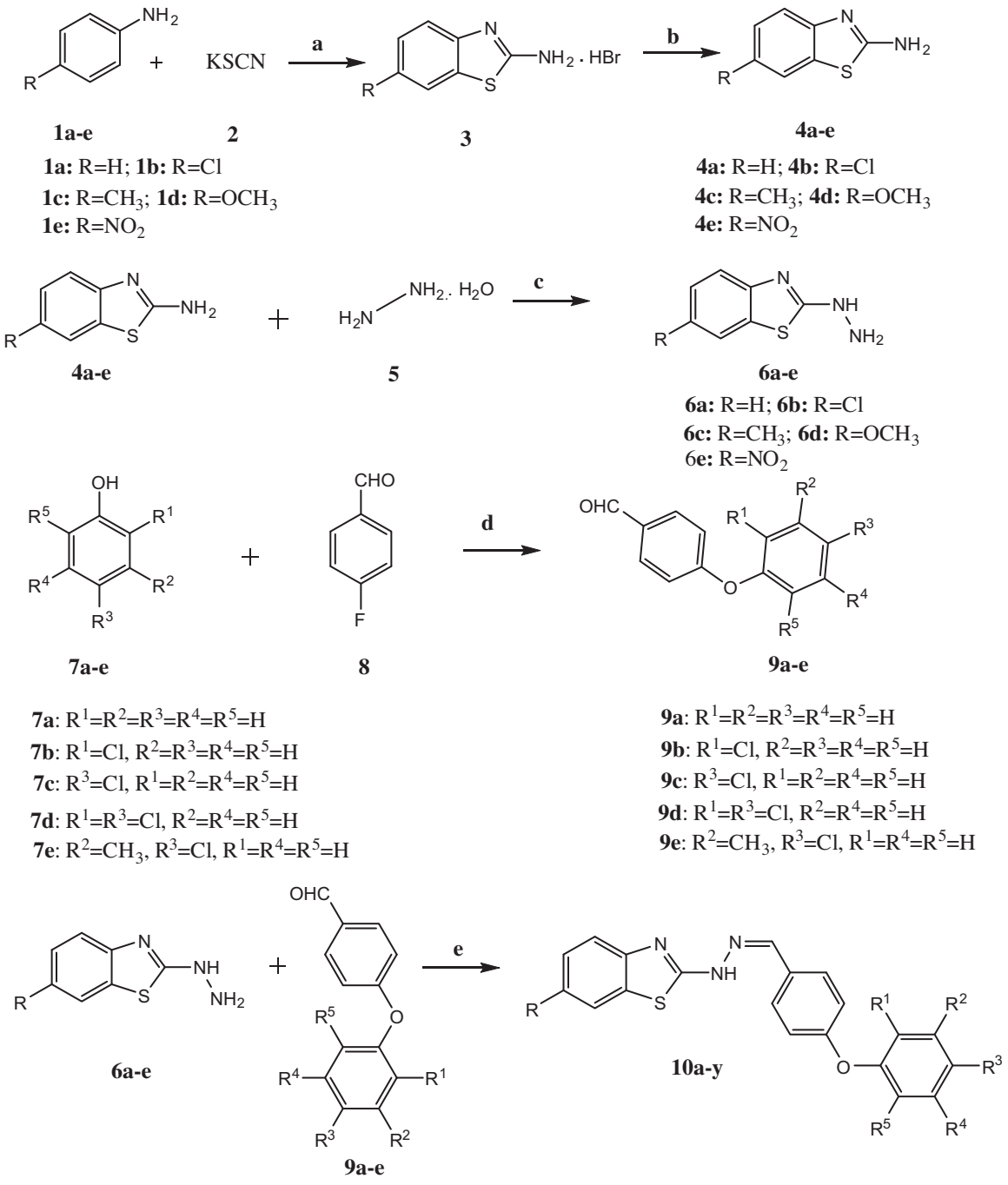
Spectral data (IR, $^1$H NMR and MS) of all synthesized compounds were in agreement with the proposed structures. IR(KBr) spectrum of all the synthesized compounds had small broad N–H absorption at 1600–1660 cm$^{-1}$, 1100–1200 cm$^{-1}$, which are assigned to C=N and C–O stretching, respectively. The $^1$HNMR spectrum exhibited singlets at 7–9 ppm, which were assigned to azomethine –N=CH–. The Mass spectrum showed M+1 peak of all the synthesized compounds.

The synthesized compounds (**10a–y**) were screened against *M. tuberculosis* H37Rv in order to determine the minimum

**Table 1**
QikProp analysis data of 2-(2-(4-aryloxybenzylidene)hydrazinyl)benzo[*d*]thiazoles

| Title | #Stars[a] | QPlogPo/w[b] | QPPCaco[c] | % Human oral absorption[d] | No of violations from rule of five[e] |
|---|---|---|---|---|---|
| **10a** | 1 | 4.851 | 3942.074 | 100 | 0 |
| **10b** | 1 | 5.271 | 3942.061 | 100 | 0 |
| **10c** | 1 | 5.345 | 3942.008 | 100 | 0 |
| **10d** | 1 | 5.707 | 3941.729 | 100 | 0 |
| **10e** | 1 | 5.599 | 3941.610 | 100 | 0 |
| **10f** | 1 | 5.346 | 3942.738 | 100 | 0 |
| **10g** | 1 | 5.766 | 3942.930 | 100 | 0 |
| **10h** | 1 | 5.841 | 3942.908 | 100 | 0 |
| **10i** | 1 | 6.003 | 3942.575 | 100 | 0 |
| **10j** | 1 | 6.092 | 3942.428 | 100 | 0 |
| **10k** | 1 | 5.162 | 3941.549 | 100 | 0 |
| **10l** | 1 | 5.580 | 3941.314 | 100 | 0 |
| **10m** | 1 | 5.660 | 3941.314 | 100 | 0 |
| **10n** | 1 | 6.020 | 3941.586 | 100 | 0 |
| **10o** | 1 | 5.909 | 3941.076 | 100 | 0 |
| **10p** | 1 | 4.950 | 3940.414 | 100 | 0 |
| **10q** | 1 | 5.364 | 3940.131 | 100 | 0 |
| **10r** | 1 | 5.443 | 3939.151 | 100 | 0 |
| **10s** | 1 | 5.803 | 3939.171 | 100 | 0 |
| **10t** | 1 | 5.691 | 3939.641 | 100 | 0 |
| **10u** | 2 | 4.149 | 471.476 | 100 | 0 |
| **10v** | 2 | 4.568 | 471.540 | 100 | 0 |
| **10w** | 2 | 4.643 | 471.490 | 100 | 0 |
| **10x** | 2 | 5.061 | 471.505 | 91.47 | 0 |
| **10y** | 2 | 4.895 | 471.460 | 100 | 0 |

[a] #Stars: #stars are MW, dipole, IP, EA, SASA, FOSA, FISA, PISA, WPSA, PSA, volume, donorHB, accptHB, QPlogPoct, QPlogPw, QPlogPo/w, logS, QPLogKhsa, QPlogBB. The range predicted for this parameter using QikProp is 0–5; where 0–1 indicates no violation or best candidate.
[b] QPlogPo/w: This gives the predicted octanol/water partition coefficient. The range predicted for this parameter using QikProp is −2.0–6.5.
[c] QPPCaco: QikProp predictions are for nonactive transport, where <25 is considered poor and >500 is considered excellent.
[d] % Human-Oral Absorption: This gives the predicted human oral absorption on 0–100% scale where >80% is considered high and <25% is considered poor.
[e] Rule of Five: This property denotes the number of violations of Lipinski's rule of five.




**Scheme 1.** Synthetic route of 2-(2-(4-aryloxybenzylidene)hydrazinyl)benzo[*d*]thiazoles.

inhibitory concentration (MIC) with Resazurin microtiter assay (REMA).[36,37] Homogenous mycobacterial (H37Rv) culture suspension was seeded in microtitre plates at density of $10^5$ cells per well in 100 μL of the Middlebrook 7H9 broth (Difco laboratories, Detroit, MI, USA) and the test compounds were serially diluted directly on the plate. The control received equivalent amount of DMSO. The plates were incubated at 37 °C for 7 days. Freshly prepared resazurin dye (0.02%) was added and plates were again incubated for 48 h. MIC is the lowest concentration at which complete inhibition was observed and was determined by visual inspection (colour change from blue to pink) (Table 2). Isoniazid was used as the reference drug.

Structure–activity relationship reveals that compounds with halogen substituent on benzothiazole ring of 2-(2-(4-aryloxybenzylidene) hydrazinyl) benzothiazole, **10f–j**, showed good activity. Further, introduction of methyl, methoxy and nitro group at 6-position on the benzothiazole ring, **10k–y**, results in unfavourable effect on the antitubercular activity. However, 2,4-dichloro diphenyl ether containing benzothiazole molecules (**10d**, **10i**, **10o** and **10x**) are more active compared to other molecules. Further, to validate the design hypothesize, the MIC's of intermediates 2-hydrazinyl benzothiazoles (**6a**, **6b** and **6c**) and 4-(aryloxy)benzaldehydes (**9a**, **9b** and **9c**), were determined. The MIC's obtained for 2-hydrazinyl benzothiazoles **6a**; 287.50 μg/ml, **6b**; 232.80 μg/ml, **6c**; 221.87 μg/ml and 4-(aryloxy)benzaldehydes **9a**; 132.81 μg/ml, **9b**; 123.44 μg/ml, **9c**; 117.19 μg/ml were $\geqslant$15 and $\geqslant$8-folds compared to their molecular hybrids **10a**, **10g** and **10m** which has MIC of 12.69 μg/ml, 2.00 μg/ml and 15.00 μg/ml, respec-

**Table 2**
In vitro Anti-tubercular activity of 2-(2-(4-aryloxybenzylidene)hydrazinyl)benzo[*d*]thiazoles

| Compound | R | $R^1$ | $R^2$ | $R^3$ | $R^4$ | $R^5$ | MIC (μg/ml) |
|---|---|---|---|---|---|---|---|
| **10a** | H | H | H | H | H | H | 12.69 |
| **10b** | H | Cl | H | H | H | H | 6.00 |
| **0c** | H | H | H | Cl | H | H | 2.94 |
| **10d** | H | Cl | H | Cl | H | H | 2.63 |
| **10e** | H | H | $CH_3$ | Cl | H | H | 6.63 |
| **10f** | Cl | H | H | H | H | H | 5.50 |
| **10g** | Cl | Cl | H | H | H | H | 2.00 |
| **10h** | Cl | H | H | Cl | H | H | 2.25 |
| **10i** | Cl | Cl | H | Cl | H | H | 1.50 |
| **10j** | Cl | H | $CH_3$ | Cl | H | H | 4.50 |
| **10k** | $CH_3$ | H | H | H | H | H | 9.13 |
| **10l** | $CH_3$ | Cl | H | H | H | H | 4.75 |
| **10m** | $CH_3$ | H | H | Cl | H | H | 15.00 |
| **10n** | $CH_3$ | Cl | H | Cl | H | H | 12.56 |
| **10o** | $CH_3$ | H | $CH_3$ | Cl | H | H | 15.00 |
| **10p** | $OCH_3$ | H | H | H | H | H | 16.50 |
| **10q** | $OCH_3$ | Cl | H | H | H | H | 19.50 |
| **10r** | $OCH_3$ | H | H | Cl | H | H | 8.00 |
| **10s** | $OCH_3$ | Cl | H | Cl | H | H | 27.50 |
| **10t** | $OCH_3$ | H | $CH_3$ | Cl | H | H | 27.00 |
| **10u** | $NO_2$ | H | H | H | H | H | 29.00 |
| **10v** | $NO_2$ | Cl | H | H | H | H | 29.50 |
| **10w** | $NO_2$ | H | H | Cl | H | H | 26.50 |
| **10x** | $NO_2$ | Cl | H | Cl | H | H | 23.50 |
| **10y** | $NO_2$ | H | $CH_3$ | Cl | H | H | 21.50 |
| **Isoniazid** | — | — | — | — | — | — | 0.40 |
| **Triclosan** | — | — | — | — | — | — | 5.00 |

tively, thus confirm our design hypothesis. Furthermore, the molecular hybrid 10 g has significantly enhanced activity as compared to 4-(aryloxy)benzaldehyde and triclosan (MIC: 5.00 μg/ml.[38]) Thus, these additional evaluations indicate the value of molecular hybridization in this instance.

In summary, a series of novel, 2-(2-(4-aryloxybenzylidene) hydrazinyl)benzothiazole was designed based on the molecular hybridization of 2-hydrazinylbenzothiazole and 4-(aryloxy)benzaldehyde. Synthesized compounds were evaluated for activity against *M. tuberculosis* H37Rv. Few of these compounds exhibit promising antitubercular activity and could serve as leads for further development of antitubercular agents.

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## Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.10.064.

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35. General procedure for the synthesis of 2-(2-(4-phenoxybenzylidene) hydrazinyl) benzo[d]thiazole (**10a**–**y**): A mixture of 2-hydrazinylbenzothiazole (**6a**–**e**) (10 mM), 4-phenoxybenzaldehyde (**9a**–**e**), (10 mM) were suspended in absolute ethanol (25–35 mL) and the mixture was subjected for reflux at 80 °C for 6–10 h. The reaction mixture was then cooled to room temperature; concentrated under reduced pressure and partitioned between EtOAc (50 mL) and water (50 mL). The organic layer was separated, and the aqueous layer was extracted further with EtOAc (2 × 25 mL). The combined organic layers were washed with brine (50 mL), dried over sodium sulphate ($Na_2SO_4$), and concentrated under vacuum. Purification of compound was carried out by column chromatography (Silica gel, 6 g, 20% EtOAc/hexane) to obtain pure 2-(2-(4-aryloxybenzylidene) hydrazinyl)benzothiazole (**10a**–**y**) in yields ranging from 62–87%.
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