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ARTICLE

Design, Synthesis and Evaluation of Aryloxybenzylidene Hydrazinyl-Benzoxazoles/Benzothiazoles Analogs as Antimycobacterial Agents

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ABSTRACT

Substituted 2-(2-(4-aryloxybenzylidene)hydrazinyl)benzothiazole/benzoxazoles series were designed through molecular hybridization and synthesized in condensation reaction of hydrazinylbenzothiazole/benzoxazole with substituted aryloxy benzaldehydes. All the synthesized compounds were assigned structure based on spectral data and were evaluated for antimycobacterial activity. Among both benzothiazole and benzoxazole derivatives, the compounds **8f** and **9e** were found to show most potent antitubercular activity with MIC value of 0.89 and 0.92 μM which are on a par with those of standard antitubercular drugs. In order to know the binding interactions of all the compounds were docked within the mycobacterial pantothenate synthetase, which showed interactions with Asp88, Arg200, Ser196, Asn199, Met 195 and Lys 160 of pantothenate synthetase.

KEYWORDS

Benzoxazole, Benzothiazole, Antitubercular, Pantothenate synthetase, Docking.

INTRODUCTION

Till now more than 30 Mycobacterium species are reported to infect human and *Mycobacterium tuberculosis* (MTB) is one among such species, which causes tuberculosis (TB) [1]. WHO (2019) report states that TB stands one among the top ten cause of human mortality. About 10.4 million cases of TB patients were there worldwide, including 1.2 million (11%) patients living with MTB coinfected with HIV. African and south Asian countries including India are high affected area across the globe. A serious situation of TB is aggrandized by rapid spread of multi drug resistant TB (MDR-TB) [2]. An estimated 4.9 million people worldwide developed MDR-TB. China, India and the Russian Federation (WHO-2016) were the countries with the largest numbers of MDR-TB cases (47% of the global total). The emergence of MDR-TB and extensively drug resistant (XDR-TB) infections are now known to be more fatal for human life. MDR-TB, which do not respond to two potent first-line anti-TB drugs isoniazid and rifampicin. The dosage regimen for MDR-TB involves cocktail of both first line and second line drugs with long duration which makes

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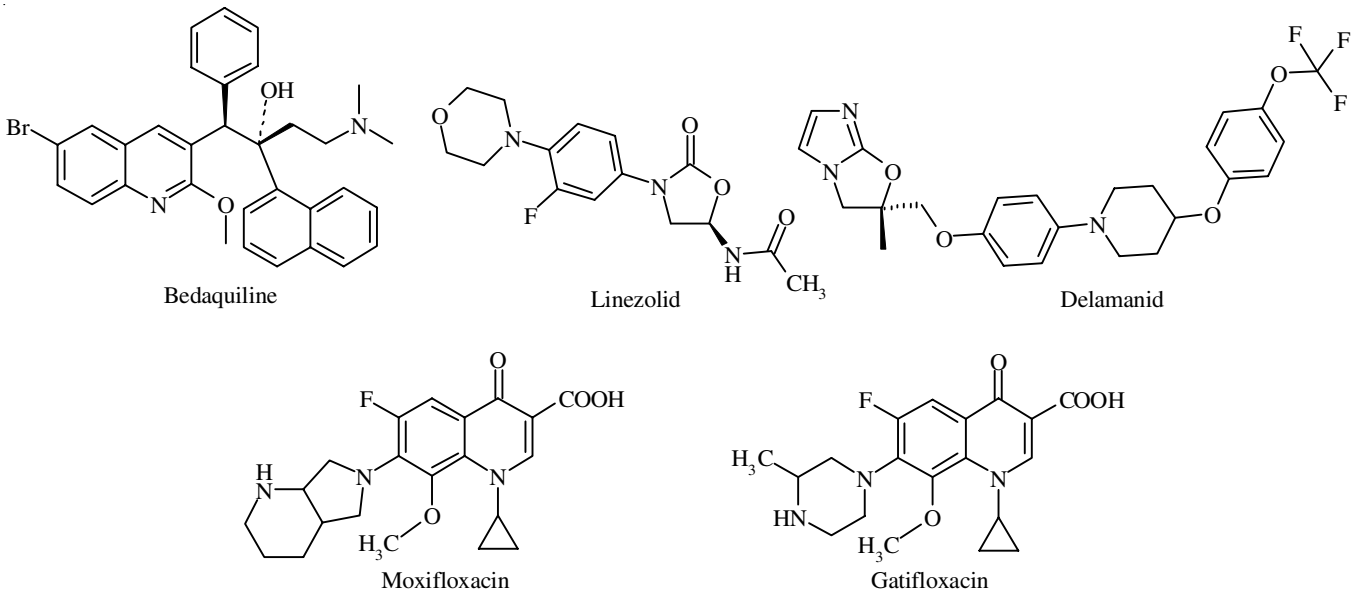


Fig. 1. Structures newer and clinical antitubercular agents

patients uncomfortable and even miss the treatment. XDR-TB is a highly severe type of MDR-TB caused by bacteria which is resistant additionally to the most effective second-line anti-TB drugs taking away patients without any further treatment options [3,4]. Thus coinfection with HIV and rapid rise in incidences of MDR-TB and XDR-TB has caused havoc among the medical fraternity for the treatment [5,6].

A relief in terms of new drugs has little eased the combination of drugs for the treatment of all forms of TB after several decades. New drugs like bedaquiline, delamanide and linezolid (Fig. 1) have given extended choice for the combination of drug treatment along with latest fluoroquinolones such as moxifloxacin and gatifloxacin [7-9].

Despite of availability and choice of new drugs, still there persists the need of new drugs with different mechanism of actions to shorten the duration of treatment, effective, safe and well-tolerated simple regimens for drug-resistant and drug-susceptible TB [10].

Compounds bearing benzothiazole scaffold have been disclosed to possess wide range of pharmacological activities such as anticancer, antimicrobial, anti-inflammatory, anti-Alzheimer's and antituberular [11,12]. Also, compounds bearing benzoxazoles have displayed a range of activities including anticancer, analgesic, antimicrobial, DNA topoisomerase inhibitory activity, *etc*. [13-15]. 2-(2-(4-Aryloxybenzylidene)-hydrazinyl)benzothiazole derivatives have been reported to show antituberuclar activity and 2-(substituted benzylamino) linked benzoxazoles and benzothiazoles have been reported to exhibit antibacterial activities [13,16-19]. These reports encouraged us to apply molecular hybridization to modify 2-aminobenzothiazoles and 2-aminobenzoxazoles into their hydrazines and then to hydrazones as depicted in Fig. 2. This work describes the synthesis, antitubercular and docking studies of substituted aryloxybenzylidene-hydrazinyl-benzothiazoles/benzoxazoles analogs containing substituted aryloxyphenyl group at 2nd position of benzothiazole/benzoxazole.

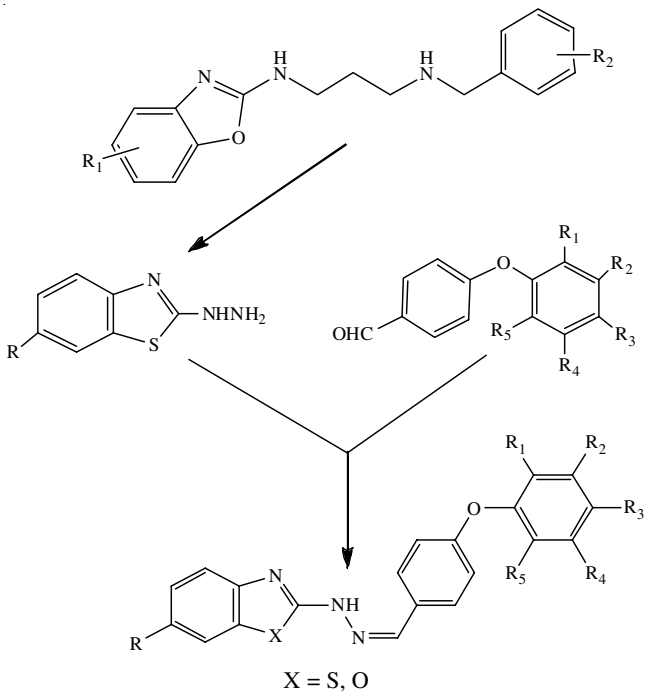


Fig. 2. Design of phenoxy benzylidene hydrazones of benzothiazole/benzoxazoles

EXPERIMENTAL

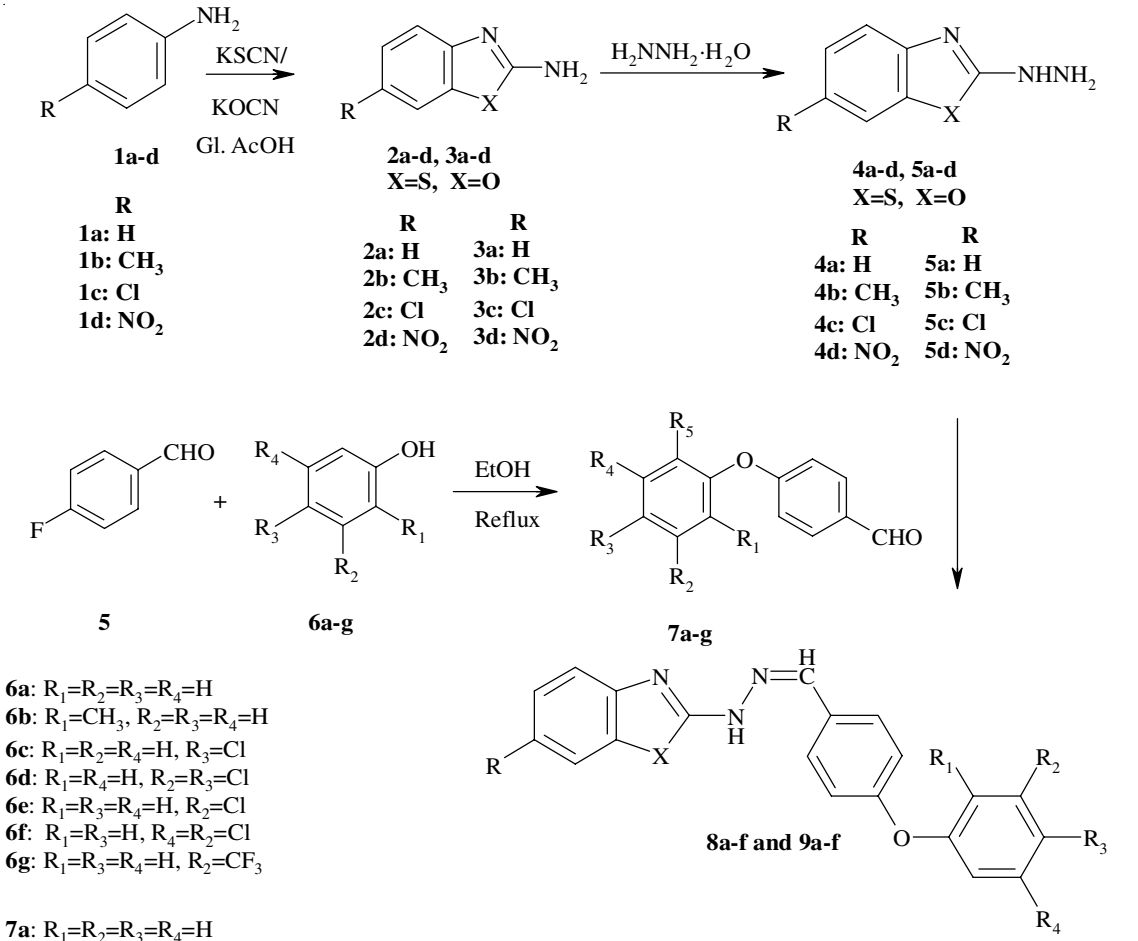
All chemicals, solvents and reagents were acquired from local vendors, Merck and Sigma-Aldrich of reagent grade and solvents were purified whenever required. Electrothermal 1A 9100 (Shimadzu, Japan) apparatus was used for the determination of melting points were taken in open capillaries and are uncorrected. The status of the reaction was monitored on readymade silica-gel plates by eluting with appropriate solvents and spots were visualized by exposure to UV light or chemical reagents. IR spectra were recorded on Bruker ALPHA FTIR spectrometer and ^{1}H NMR spectra were determined on a Bruker

(200, 400 MHz) spectrometer using $CDCl_3$ and DMSO-d_6 as solvent and chemical shifts are reported as parts per million using TMS as an internal standard. Mass spectra were read on EI MS-QP 1000 EX, Shimadzu, Japan.

Synthesis of substituted 2-aminobenzothiazoles (2a-d) and 2-aminobenzoxazoles (3a-d): To a cooled solution of 50 mM of 4-substituted anilines **1a-d** in 100 mL glacial acetic acid was added 60 mM of KSCN or KOCN in an ice bath and stirred for 30 min. To the above cooled mixture, bromine solution in glacial acetic acid 0.6 mM was added dropwise with controlled rate to keep the temperature below 10 °C throughout the addition. The above reaction mixture was further stirred at ambient temperate for 2-4 h which results in formation of hydrogen bromide salt which is filtered and washed with glacial acetic acid. The dried hydrogen bromide salt was dissolved in hot water and alkalined with ammonia (pH = < 10). The precipitate thus formed is filtered, washed with plenty of water and filtered. The crude product was passed through silica bed by eluting with a mixture of hexane and ethyl acetate (5:1) to get the substituted benzothiazoles/benzoxazoles (**2a-d** and **3a-d**) in pure form (**Scheme-I**) [20].

Synthesis of substituted hydrazinobenzothiazoles (4a-d) and hydrazinobenzoxazoles (5a-d): 2-Aminobenzothiazoles (**2a-d**) and 2-aminobenzoxazoles (**3a-d**) (10 mM) were taken in ethylene glycol and added with hydrazine hydrate (20 mM). The reaction mixture was refluxed for 4 h and resultant crystals thus formed were filtered and dried to obtain corresponding hydrazinobenzothiazoles/hydrazinobenzoxazoles (**4a-d/5a-d**) in competitive yields [20].

Synthesis of substituted aryloxy bezaldehydes (7a-g): Suspension of sodium hydride (4.7g, 60% w/w dispersion in oil) in 20 mL DMF was added by substituted phenols (**6a-g**, 50 mM) in dry DMF under nitrogen atmosphere with constant stirring. A solution of 4-fluorobenzaldehyde (**5**, 55 mM) in dry DMF was added in portions in 10 min with constant stirring. The resultant mixture was heated at 90 °C for 15-18 h till the completion of the reaction as monitored by TLC and then quenched by pouring in ice cold water. Subsequently the solution was extracted with ethylacetate three times and the combined organic extracts were washed with brine, dried over anhydrous sodium sulphate. Crude substituted aryloxybenzaldehydes (**7a-g**) were obtained upon removal of volatilities,



6a: $R_1=R_2=R_3=R_4=H$
6b: $R_1=CH_3$, $R_2=R_3=R_4=H$
6c: $R_1=R_2=R_4=H$, $R_3=Cl$
6d: $R_1=R_4=H$, $R_2=R_3=Cl$
6e: $R_1=R_3=R_4=H$, $R_2=Cl$
6f: $R_1=R_3=H$, $R_4=R_2=Cl$
6g: $R_1=R_3=R_4=H$, $R_2=CF_3$

7a: $R_1=R_2=R_3=R_4=H$
7b: $R_1=CH_3$, $R_2=R_3=R_4=H$
7c: $R_1=R_2=R_4=H$, $R_3=Cl$
7d: $R_1=R_4=H$, $R_2=R_3=Cl$
7e: $R_1=R_3=R_4=H$, $R_2=Cl$
7f: $R_1=R_3=H$, $R_4=R_2=Cl$
7g: $R_1=R_3=R_4=H$, $R_2=CF_3$

8a: X=S, $R=R_1=R_2=R_3=R_4=H$
8b: X=S, $R_1=CH_3$, $R=R_2=R_3=R_{41}=H$
8c: X=S, R=Cl, $R_1=R_2=R_4=H$, $R_3=Cl$
8d: X=S, $R=NO_2$,$R_1=R_2=R_4=H$, $R_3=Cl$
8e: X=S, $R=CH_3$, $R_1=R_2=R_3=R_4=H$
8f: X=S, R=Cl, $R_1=R_4=H$, $R_2=R_3=Cl$

9a: X=O, $R=R_1=R_3=H$, $R_2=R_4=Cl$
9b: X=O, $R=R_2=R_3=R_4=H$, $R_1=CH_3$
9c: X=O, $R=R_2=Cl$, $R_1=R_3=R_4=H$
9d: X=O, $R=R_3=Cl$, $R_1=R_2=R_4=H$
9e: R=Cl, $R_1=R_3=R_4=H$, $R_2=CF_3$
9f: X=O, $R=NO_2$, $R_1=R_3=H$, $R_2=R_4=Cl$

Scheme-I: Synthesis of Substituted benzothiazole hydrazones (**8a-f**) and benzoxazole hydrazones (**9a-f**)

which were purified over silica gel using a mixture of hexane ethylacetate to afford compounds **7a-g**.

Synthesis of 2-(2-(4-substituted aryloxybenzylidene)-hydrazinyl)benzothiazole/benzoxazoles (8a-f and 9a-f): A mixture of 2-hydrazinobenzothiazole/2-hydrazinobenzoxazole (**4a-d/5a-d**, 10 mM) and desired 4-aryloxy benzaldehydes (**7a-g**, 10 mM) was refluxed at 80 °C for 6-10 h along with few drops of glacial acetic acid. Upon completion of reaction, the cooled reaction mixture was then concentrated under reduced pressure and partitioned between ethylacetate for thrice. The accumulated organic part was washed with brine, dried over anhydrous sodium sulfate and concentrated under vacuum to afford targeted compounds **8a-f** and **9a-f**. The crude products were purified by column chromatography using 20% hexane ethylacetate eluent to afford pure 2-(2-(4-aryloxybenzylidene)-hydrazinyl)benzothiazole/benzoxazoles [20].

Antitubercular activity: Stock solution of test compounds were prepared in DMSO and subsequently diluted with water to attain concentrations used for screening from 1000 to 2 µg/mL. Similarly, standard drug, isoniazid (INH), ethambutol and streptomycin dilutions were prepared.

Middle brook 7H9 medium and with OADC supplement was employed during the screening of test compounds for antitubercular activity. Specific concentrations of selected compounds were added individually to the freshly prepared sterile media and mixed well. A loop of diluted Mycobacterial H37Rv subculture and control were inoculated into sterile medium separately and mixed properly using vortex. Microtiter tubes were incubated at 37 °C for three weeks and the growth inhibition was recorded for on 15th and 21st days respectively. The MIC value represents the lowest dilution of the compound at which no bacterial growth (turbidity) was detected [21].

Molecular docking studies: A molecular docking study was performed in order to apprehend the binding interactions of tested molecules within active site of mycobacterial pantothenate synthatase [22]. The crystal structure of mycobacterial pantothenate synthetase 3IVX with crystal ligand 2-(2-(benzofuran-2-ylsulfonylcarbamoyl)-5-methoxy-1*H*-indol-1-yl)acetic acid) was downloaded. All the docking modeling operations were done on Desktop with windows operating system. Structure of molecules was drawn using draw model of Sybyl 6.7, Gasteiger-Huckel charges were added and minimized with Steapest Descent in the initial followed by Conjugate gradient 1000 iteration to attain gradient of 0.001 kcal/mol using Powel method. Further docking calculations were performed on Autodock 4.2 [23].

Upon fetching the cocrystal structure on AutoDock, water molecules were removed followed by addition of hydrogens and protein structure was saved as PDBQT. The test molecules were then loaded to set their torsions and saved in PDBQT. As Autodock is grid based docking tool, a grid box with the dimensions 60 × 60 × 60 and selected coordinates of X:15.137, Y:17.850 and Z: 3.573 Å was created. AMBER force field utilized Lamarckian genetic algorithm as scoring function to calculate binding energy.

Spectral data:

2-(2-(4′-Phenoxybenzylidene)hydrazinyl)benzo[*d*]-thiazole (8a): Brown solid: IR (KBr, ν_{max}, cm^{-1}): 1590 (C=N), 1533 (C=C), 1306 (C-O), 1485 (N=CH). ^{1}H NMR (DMSO-d_6, 400 MHz): 6.62-7.92 (m, 14H, Ar-H, 4,5,6,7,2′,3′,5′, 6′, 2″,3″,4″,5″,6″, CH), 8.67 (bs, 1H, NH), D_2O Exchangeable. FAB MS *m/z*: 346 [M+1]$^+$.

2-(2-(4′-(*o*-Tolyloxy)benzylidene)hydrazinyl)benzo[*d*]-thiazole (8b): Light brown solid: IR (KBr, ν_{max}, cm^{-1}). 2924 (aliphatic, C-H), 1590 (C=N), 1533 (C=C), 1307 (C-O), 1485 (N=CH). ^{1}H NMR (DMSO-d_6, 400 MHz): 2.38 (s, 3H, CH_3, H-2″), 6.952-6.99 (d, 4H, *J* = 8, Ar-H, (H-2′, 3′, 5′, 6′), 7.13-7.17 (t, 4H, *J* = 8, Ar-H, H-5, 6, 4″, 5″), 7.29-7.42 (d, 5H, *J* = 8, Ar-H, H-4, 7, 3″, 6″, CH), 10.70 (bs, 1H, N-H). FAB MS *m/z*: 360.1 [M+1]$^+$. Elemental analysis calcd. (found) (%): C 69.54 (69.62), H 4.38 (4.42), N 12.17 (12.19), O 4.63 (4.72), S 9.28 (9.32).

2-(2-(4′-(4″-Chlorophenoxy)benzylidene)hydrazinyl)-6-nitrobenzo[*d*]thiazole (8d): Creame colour solid. IR (KBr, ν_{max}, cm^{-1}): 3303 (NH), 3127 (arom. C-H), 1609 (C=N), 1595 (C-O). ^{1}H NMR (DMSO-d_6, 400 MHz): 7.003-7.069 (m, 3H, Ar-4, 2′,6), 7.425-7.511 (m, 5H, Ar-3′,5′,2″,6″, CH), 7.898-7.919 (d, 3H, *J* = 8, Ar-5,3″,5″), 8.31 (s, 1H, Ar-7), 8.70 (bs, 1H, N-H). FAB MS *m/z*: 427.86 [M+2]$^+$.

6-Chloro-2-(2-(4′-(3″,4″-dichlorophenoxy)benzylidene)hydrazinyl)benzo[*d*]thiaz′ole (8f): II-7S-2m (m.w. 449). Brown solid: IR (KBr, ν_{max}, cm^{-1}): 3367 (NH), 2921 (aliph. C-H), 1593 (C=N), 1537 (C=C), 747 (C-Cl). ^{1}H NMR (DMSO-d_6, 300 MHz): 7.306-7.431 (m, 4H, Ar-H, 5,2″,5″, CH), 7.51-7.69 (m, 6H, Ar-H, 4,2′,3′,5′,6′,6″), 8.70 (s, 1H, Ar-7), 10.10 (bs, 1H, NH,). D_2O Exchange: 10.10 (s, 1H, NH, benzothiazole N-H). FAB-MS *m/z*: 455.1 [M+6]$^+$.

2-(2-(4′-(3″,5″-Dichlorophenoxy)benzylidene)hydrazinyl)benzo[*d*]oxazole (9a): Brown solid: ^{1}H NMR (DMSO-d_6, 300 MHz): 6.759-7.644 (m, 8H, Ar- 4,7,3′,5′,2″,4″,6″, CH), 7.8-7.892 (m, 4H, Ar-5,6,2′,6′), 8.751 (bs, 1H, NH). FAB-MS *m/z*: 402 [M+4]$^+$.

6-Chloro-2-(2-(4′-(3″-chlorophenoxy)benzylidene)-hydrazinyl)benzo[*d*]oxazole (9c): Yellow solid: IR (KBr, ν_{max}, cm^{-1}): 3355 (NH), 1609 (C=N), 1308 (C-O), 699 (C-Cl). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 5.8 (s, 1H, N-H, benzooxazole N-H), 6.69-6.76 (m, 4H, Ar-H, H-2″,4″,5″,6″), 7.30-7.34 (d, 2H, *J* = 12, Ar-H, H-4, 5), 7.61-7.64 (d, 4H, *J* = 9, H-2′,3′,5′,6′), 7.80 (s, 1H, Ar-H, H-7), 8.5 (s, 1H, CH). FAB-MS *m/z*: 402.1 [M+4]$^+$

6-Chloro-2-(2-(4′-(4″-chlorophenoxy)benzylidene)-hydrazinyl)benzo[*d*]oxazole (9d): Brown solid: IR (KBr, ν_{max}, cm^{-1}): 3366 (NH), 2920 (aliphatic, C-H), 1632 (C=N). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 4.7 (s, 1H, N-H, benzooxazole N-H), 6.90-7.02 (m, 5H, Ar-H, H-2″,3″,5″,6″,7), 7.25-7.35 (d, 4H, *J* = 8.7, Ar-H, H-2′,3′,5′,6′), 7.48 -7.51 (d, 1H, *J* = 8.7, Ar-H, H-4), 7.60-7.63 (d, 1H, *J* = 8.7, Ar-H, H-5), 8.48 (s, 1H, C-H). FAB-MS *m/z*: 402 [M+4]$^+$. ^{13}C NMR: 118.483, 118.639, 118.719, 118.921, 119.027, 119.635, 119.713, 120.572, 121.281, 126.042, 128.206, 128.940, 129.720, 129.917, 130.862, 138.135, 140.283, 152.985, 155.051, 157.344.

6-Chloro-2-(2-(4′-(3″-trifluoromethyl)phenoxy)benzylidene)hydrazinyl)benzo[*d*]oxazole (9e): Light brown solid: IR (KBr, ν_{max}, cm^{-1}): 3369 (NH), 2929 (aliph., C-H), 1631 (C=N), 1092 (C-F). 616 (C-Cl). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 3.86 (bs, 1H, N-H, benzooxazole N-H), 6.91-7.07 (m, 2H, Ar-H, H-2″,5″), 7.42-7.66 (m, 6H, Ar-H, H-4″,6″,2′,3′,5′,6′),

7.93-8.26 (m, 4H, Ar-H, C-H, H-4,5,7, CH). FAB-MS *m/z*: 440.80 $[M+8]^+$. ^{13}C NMR: 108.558, 111.120,117.412, 119.120, 119.615, 121.512, 124. 412, 124.862, 128.343, 130.212, 134.412, 136.120, 138.110, 138.610, 139.572, 139.818, 148.741, 151.441, 155.841, 156.572, 157.841

RESULTS AND DISCUSSION

Substituted anilines in cyclization reaction were converted into 2-aminobenzothiazoles and 2-aminobenzoxazoles in competitive yields by reaction with potassium thiocyante or potassium isocyanate in presence of bromine. The aforementioned compounds were characterized by FTIR (presence of both asymmetric and symmetric frequencies) and compared with reported literature melting points. Substituted 2-aminobenzoxazoles and 2-aminobenzothiazoles were converted in nucleophilic substitution reaction into their corresponding hydrazines by the reaction with hydrazine hydrate. As numerous hydrazines of benzothiazole and benzoxazole are reported, the characterization of these compounds has been done on the basis of FTIR data (presence of both asymmetric and symmetric peaks for primary amine) and melting points. The required substituted phenoxybenzaldehydes were synthesized in nucleophilic substitution reaction between 4-fluorobenzaldyde and substituted phenols in dry DMF and sodium hydride as base. 2-Hydrazinobenzothiazoles and 2-hydrazinobenzoxazoles were further treated with substituted phenoxy benzaldehydes in typical aldehyde nucleophilic addition reactions to afford desired hydrazones in good yields. All the compounds were purified over silica and eluted with mixture of hexane and ethyl acetate further characterization. All the hydrazones were characterized by FTIR which showed presence of NH band of secondary amine in the range of 3350-3200 cm^{-1}. Similarly, C=N stretching was found in the range of 1600-1575 cm^{-1} and aromatic CH stretching vibration were found around 3100 cm^{-1}. ^1H NMR of hydrazones showed broad NH peak at ~8.0 ppm and N=CH proton resonated in the range of 6.0-7.5 δ values and all aromatic hydrogens resonated in between 6.5-8.0 ppm. Finally, mass spectrometric data authenticated and complemented other spectral data, the physical constants of all the final compounds are listed in Table-1.

Antitubercular activity: Twelve synthesized compounds consisting of six bezothiazole and benzoxazole derivatives have been tested for antitubercular activity and potency has been reported as minimum inhibitory concentration (μM) presented in Table-2, which showed that antitubercular activity was in the range of 0.89-144.75 μM. Unsubstituted compound **8a**, showed MIC of 139.1 μM and insertion of methyl group at R_1 led to increase in the activity with MIC of 1.07 μM as in **8e**. A dichloro ($R=R_3=Cl$) substituted compound **8c** and the nitro and chloro substituted compound **8d** ($R=NO_2$ and $R_3=Cl$) failed to show significant antitubercular. Introduction of additional chloro group as in **8f** ($R=R_2=R_3=Cl$)) resulted in most

TABLE-1
PHYSICAL PROPERTIES OF **8a-f** AND **9a-f**

Compd.	X	R	R_1	R_2	R_3	R_4	m.w.	R_f	m.p. (°C)	Yield (%)
8a	S	H	H	H	H	H	345	0.68	210-212	71.9
8b	S	H	CH_3	H	H	H	359	0.62	210-212	76.2
8c	S	Cl	H	H	Cl	H	414	0.73	95-98	72.3
8d	S	NO_2	H	H	Cl	H	425	0.71	100-102	73.8
8e	S	CH_3	H	H	H	H	359	0.38	198-200	74.1
8f	S	Cl	H	Cl	Cl	H	449	0.31	> 360	67.9
9a	O	H	H	Cl	H	Cl	398	0.87	129-131	72.3
9b	O	H	CH_3	H	H	H	343	0.62	212-214	76.2
9c	O	Cl	H	Cl	H	H	398	0.31	180-182	71.6
9d	O	Cl	H	H	Cl	H	398	0.57	110-112	72.7
9e	O	Cl	H	CF_3	H	H	432	0.69	110-112	73.3
9f	O	NO_2	H	Cl	H	Cl	443	0.75	135-137	70.0

TABLE-2
ANTITUBERCULAR ACTIVITY OF **8a-f** AND **9a-f**

Compd.	X	R	R_1	R_2	R_3	R_4	$H_{37}RV$ MIC (μM)
8a	S	H	H	H	H	H	139.10
8b	S	H	CH_3	H	H	H	144.75
8c	S	Cl	H	H	Cl	H	120.68
8d	S	NO_2	H	H	Cl	H	117.68
8e	S	CH_3	H	H	H	H	1.07
8f	S	Cl	H	Cl	Cl	H	0.89
9a	O	H	H	Cl	H	Cl	65.26
9b	O	H	CH_3	H	H	H	1.21
9c	O	Cl	H	Cl	H	H	125.55
9d	O	Cl	H	H	Cl	H	125.55
9e	O	Cl	H	CF_3	H	H	0.92
9f	O	NO_2	H	Cl	H	Cl	32.78
Isoniazid	–	–	–	–	–	–	0.28
Streptomycin	–	–	–	–	–	–	2.0
Ethambutol	–	–	–	–	–	–	4.0

active compound which inhibited the growth of organism with MIC value of 0.89 µM.

Two dicloro compounds in benzoxazole series **9c** (R=R_2= Cl) and **9d** (R=R_3=Cl) were found to be inferior during the antitubercualar activity studies as like **8c** with MIC of 125 µM. Replacement of position of one chloro group at R_3 to R_4 as in **9a** resulted in two fold increase in the potency (MIC of 65.26 µM) while insertion of a nitro group at R as in **9f** further enhanced the potency by two fold with MIC value of 32.78 µM. Astonishingly, a methyl group at R **9b** drastically improved the activity with MIC 1.21 µM. Introduction of trifluoro group at R_2 as in compound **9e** resulted in further increase in the antitubercular activity (MIC = 0.92 µM). Compounds **8e, 8f, 9b** and **9e** were found to be more potent than standard drugs streptomycin and ethambutol.

Molecular docking studies: All the docking calculations were studied using Autodock 4.2, which is grid based docking protocol uses Lamarckian genetic algorithm for the calculations of binding energies. The binding interaction energies of docked molecules and their tentative hydrogen bond interactions within active site of pantothenate synthetase are listed in Table-3. The binding mode and orientations at active site were analyzed for all molecules and numerous molecules in best docked conformations demonstrated close hydrogen bond and other weak interactions. Close steric interactions were observed with aminoacid residues such as Asp88, Arg200, Ser196, Asn199, Met195, Lys160, Asp88, Ser196, Arg200, His44, Gln72 and Val187. The docking poses of the compounds, **8c**, **8d**, **9a** are depicted in Figs. 3 and 4.

TABLE-3
DOCKING INTERACTIONS ENERGIES AND AMINOACIDS

Comp.	Docking energy	Interacting aminoacids
8a	-6.59	Asp 88, Arg 200
8b	-6.35	Asp 88
8c	-8.23	–
8d	-8.09	Met 195
8e	-6.35	Asp 88
8f	-7.12	Met 195
9a	-7.63	Tyr82, Met195
9b	-6.72	Ser199, Asn199
9c	-6.38	Asp88
9d	-6.69	Ser196, Asn199
9e	-6.46	–
9f	-6.15	Ser196, Asn 199

Conclusion

In conclusion, the targeted compounds 2-(2-(4-aryloxybenzylidene)hydrazinyl)-benzo[*d*]thiazoles and 2-(2-(4-aryloxybenzylidene)hydrazinyl)benzo[*d*]oxazoles have been synthesized in multi-step reactions in encouraging yields. The structures of synthesized compounds were ascertained based on their respective spectral data and screened for antitubercular activity. Two benzothiazole analogues **8e** and **8f** and two compounds from benzoxazole series **9b** and **9e** demonstrated potent and satisfactory *in vitro* antitubercular activity comparable to those of the standards employed. Molecular docking studies revealed close hydrogen bond interactions with numerous aminoacids like Asp88 and Arg200. Further synthesis of few more compounds and evaluation could probably aid to the development of new lead compounds.

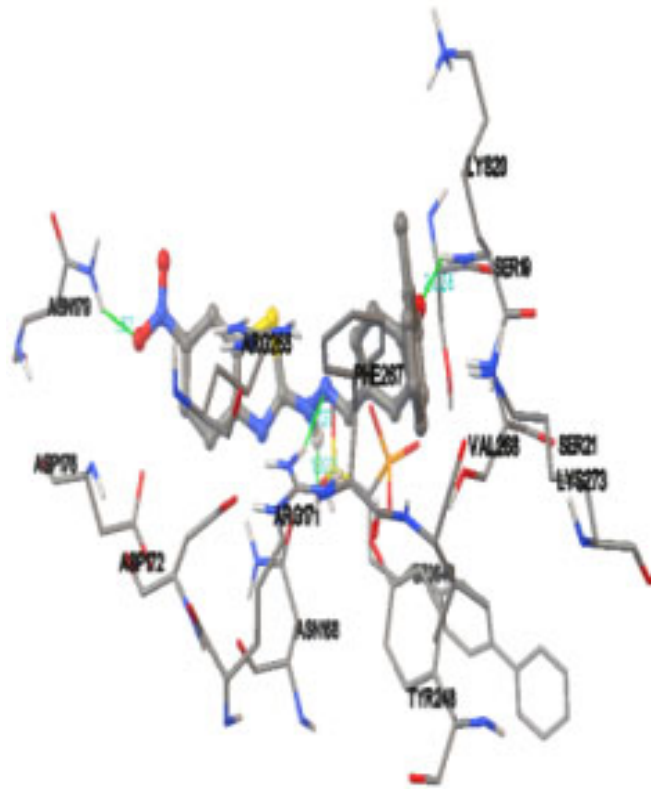


Fig. 3. Docking pictures of **8c**

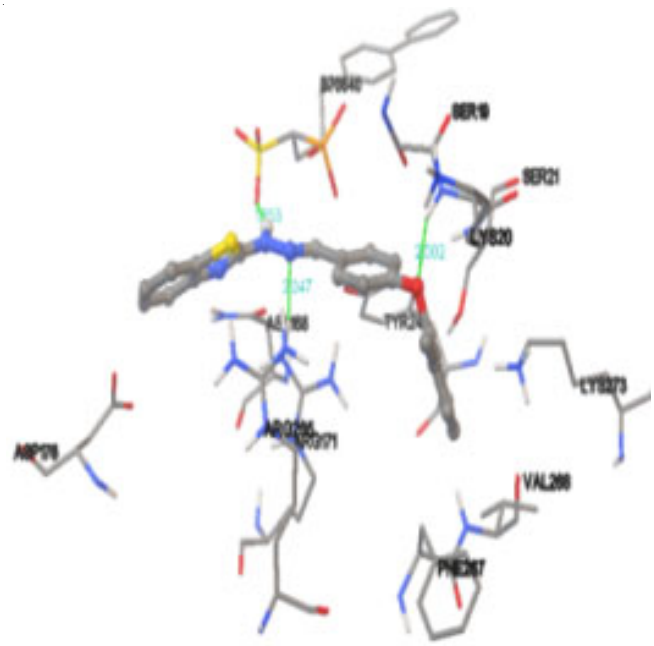


Fig. 4. Docking pictures of **8d**

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