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ORIGINAL ARTICLE

Synthesis and *in vitro* antimycobacterial potential of novel hydrazones of eugenol

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KEYWORDS

Hydrazone;
Molecular docking;
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Abstract Fifty one hydrazone derivatives of eugenol were designed and docked with 2NSD and 2X22 (enzymes of H37Rv strain) using Schrodinger v7.4. The selective ten hydrazone derivatives (4, 5, 11, 18, 30, 34, 35, 37, 42, and 45) of eugenol were synthesized via esterification, hydrazination and treatment with different aldehydes. Synthesized compounds were characterized by IR, ^{1}H NMR, and LCMS data. The compounds were evaluated for their antitubercular potential against H37Rv using microplate alamar blue assay (MABA). The study revealed that all synthesized compounds were significantly active at concentration 50 and 100 μg/ml, whereas compound 11 exhibited activity at 25 μg/ml. Present study showed that antitubercular activity of novel hydrazone derivatives of eugenol is strongly connected with the position of the substituent on aromatic aldehyde or ketones.

1. Introduction

Current decade noticed tuberculosis as the most common infectious disease in the world that being a leading cause of mortality. Regardless of availability of tuberculosis treatment, yet every year 9 million new cases are reported in those 1.5 million are fatal cases (Pitucha et al., 2019). Tuberculosis (TB) is caused by mycobacterium of the ''tuberculosis complex'', including primarily *Mycobacterium tuberculosis*, *Mycobacterium bovis* and *Mycobacterium africanum* (Sensi and Grass, 1996). Drug discovery and development are complicated, time intense and costly method (Pieczonka et al., 2013). It becomes more expensive when safety, efficacy and other issues are raised. In silicon approach of drug design plays a significant role in all stages of drug development from the initial lead design to final stage of clinical aspect of drug (Abdel-Wahab et al., 2011). Reports suggest eugenol and hydrazones to possess significant anti-tubercular potential (de Almeida et al., 2019; More et al., 2018; Krátký et al., 2017). The chemistry of hydrazones always attract the investigators, as incorporation of these moieties in medicinal compounds due to their biological potential. The hydrazones are known to exhibit wide

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variety of biological activities. They are used as antibacterial agent, anti-tubercular agent, analgesic, anti-inflammatory agent, antiviral agent, antifungal agent, muscle relaxants and antihistamines etc (Yatcheria et al., 2015; Saidugari et al., 2017; Raja et al., 2010; Zheng et al., 2009). In hydrazone, the nitrogen is attached to hydrogen and these hydrazones are stable enough for isolation (March, 1992). However, in some cases, especially with simple alkyl group, they rapidly decompose or polymerizes unless there is at least one aryl group on nitrogen or the carbon (Fuloria et al., 2008). When there is an aryl group the compound are quite stable and these compound are called as Schiff bases. The Schiff reaction is straight forward and proceeds in high yield (Fuloria et al., 2008). Enticed by research evidences, it was thought worthwhile to synthesize some novel hydrazone derivatives of eugenol. We have made an attempt to convert aryloxy moiety into some novel hydrazones via hydrazide and ester intermediates to explore its biological potential. These hydrazones were subjected to molecular docking, synthesis, characterization and antimycobacterial screenings against Mycobacterium tuberculosis.

2. Materials and methods

2.1. General

All chemicals, reagents and solvents were procured from Sigma-Aldrich and Merck Pvt Ltd. and were used without further purification. The reactions were carried out in oven-dried glassware (120 °C) under atmospheric condition. Chemicals and related solvents were procured from Merck Chemicals. From fifty-one in silico docked compounds, the selective ten compounds were subjected to synthesis. The reactions and purity of compounds were monitored by thin layer chromatography (TLC) over percolated plates coated with 0.2 mm Merck 60 F254 silica gel using butanol, acetic acid and methanol (4:3:1, v/v) eluent mixture, and were visualized by UV irradiation (254 nm). The synthesized compounds were purified using column chromatography. The melting points were determined using B-540 melting point apparatus using open capillaries and are uncorrected. The infrared spectra were recorded on a Shimadzu, MIRacle-10, IR Affinity-1 in the range of 400–4000 cm^{-1}. The ^1H NMR spectra were recorded in $CDCl_3$ using Agilent VNMRS 400 instrument at 300 MHz with chemical shift 0–10. The chemical shifts, δ are reported in ppm from 0 to 10 using tetramethylsilane (TMS) as internal standard. The mass spectra were performed using LCMS6103 at m/z values: 0–500.

2.2. Molecular docking

Fifty one compounds (Table 1) were docked in Small-Molecule Drug Discovery Suite of Schrodinger. All compounds were targeted on two enzymes such as 2NSD and 2X22 involved in tuberculosis activity. InhA, the enoyl-ACP reductase in Mycobacterium tuberculosis is an attractive target for the development of novel drugs against tuberculosis. The generated lower energy conformers of all ligands were docked into generated grid of active site of enzymes by XP precision of docking inside Glide-v7.4 (Joshi et al., 2016; Rohane and Makwana, 2019).

2.3. Synthesis of ethyl aryloxy acetate **2**

A mixture of compound 1 (eugenol: 0.1 mol), ethyl chloro acetate (0.1 mol) and anhydrous potassium carbonate (0.15 mol) in dried acetone was refluxed for 12 h. Resultant mixture was distilled off and poured on to ice-cold water and stirred. Residue was extracted with ether and the extract was dried over anhydrous sodium sulphate and was purified under reduced pressure to yield compound 2.

2.4. Synthesis of ethylaryloxyacetyl hydrazine **3**

A mixture of compound 2 (0.05 mol) and hydrazine hydrate (0.075 mol) in ethanol was refluxed for 4 h and after distilling off the solvent the residue was recrystallized from methanol to yield compound 3.

2.5. Synthesis of hydrazones **4, 5, 11, 18, 30, 34, 35, 37, 42,** and **45**

A mixture of compound 3 (0.01 mol) and 2, 4-dihydroxy benzaldehyde (0.01 mol) was refluxed for 2 h using acetic acid. The crystals formed were washed with ice-cold water, dried and recrystallized from methanol to yield compound 4. Following the same procedure using respective aldehydes / ketones, other compounds 5, 11, 18, 30, 34, 35, 37, 42, and 45 were synthesized Scheme 1.

2.6. Anti-tubercular activity

The synthesized compounds (4, 5, 11, 18, 30, 34, 35, 37, 42, and 45) were evaluated for their anti-tubercular potential against standard strain of H37Rv. The method used was microplate Alamar Blue assay (MABA). The final drug concentrations tested were 100–0.2 μg/ml. Plates were covered and sealed with parafilm and incubated at 37 °C for five days. After addition of Alamar Blue reagent and incubating for 24 h, the results were observed. A blue color in the well was interpreted as no bacterial growth, and pink color was scored as growth.

Being a non-toxic method, it has several advantages such as thermal stability of the reagent, and good correlation with BACTEC radiometric method.

3. Result and discussion

3.1. Molecular docking

The Insilco study of all fifty-one compounds was performed using Small-Molecule Drug Discovery Suite of Schrödinger. The compound 11exhibited good docking score and predicted interaction with enzymes. The docking result of novel hydrazone revealed that the binding energies were in the range of −6.097 kcal/mol to −10.393 kcal/mol, with the minimum binding energy of −10.393 kcal/mol (Table 2). The molecules were tested for structure analysis by the visualization tool. The entire compounds protein-ligand complex showed H - bond with the active site residue TYR 158 and PHE 149of 2NSD (Fig. 1) and GLY96 and TYR 158 of X22 (Fig. 2).

Table 1 . List of compounds screened for molecular docking.

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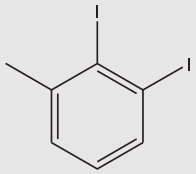
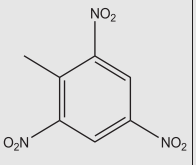
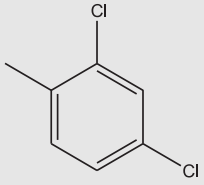
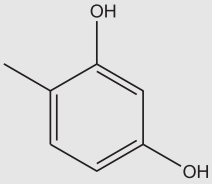
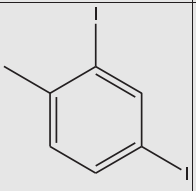
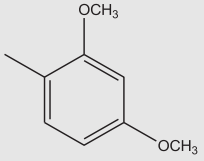
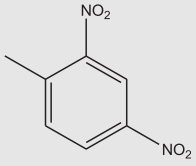
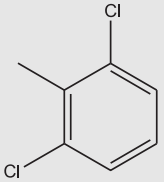
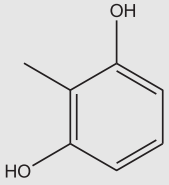
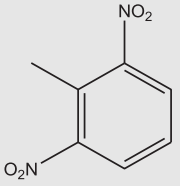
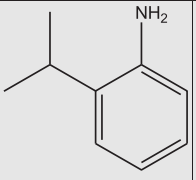
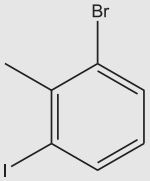
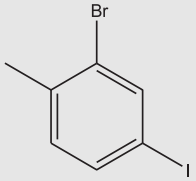
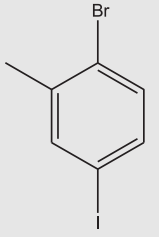
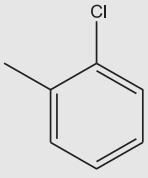
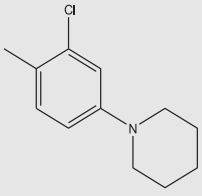
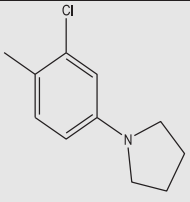
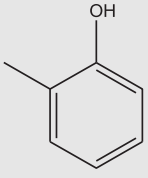
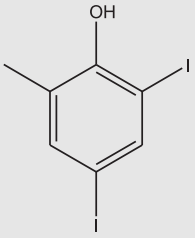
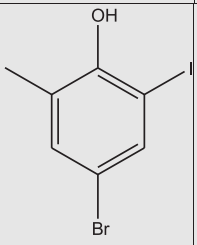
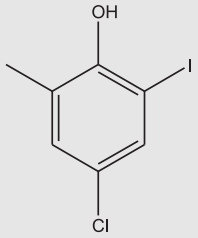
1		2		3	
4		5		6	
7		8		9	
10		11		12	
13		14		15	
16		17		18	
19		20		21	

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Table 1 (*continued*)

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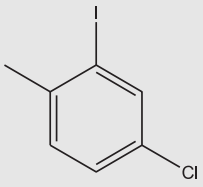
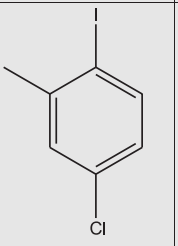
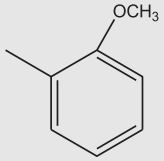
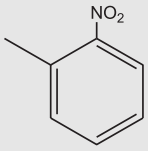
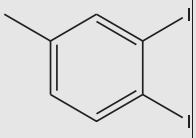
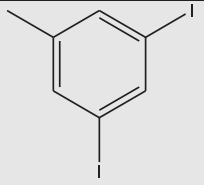
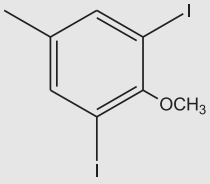
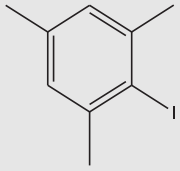
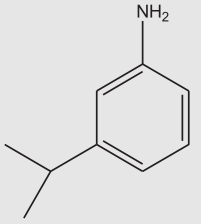
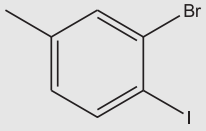
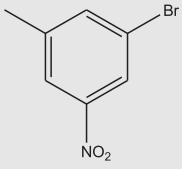
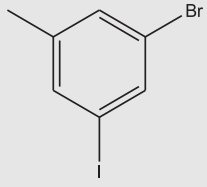
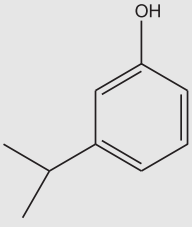
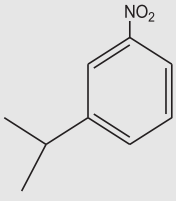
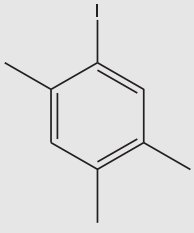
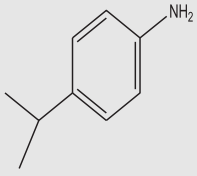
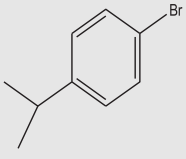
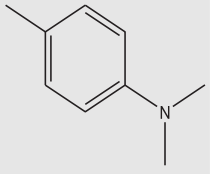
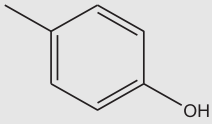
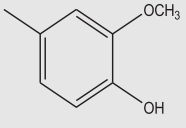
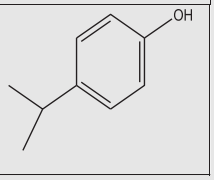
22		23		24	
25		26		27	
28		29		30	
31		32		33	
34		35		36	
37		38		39	
40		41		42	

Table 1 (*continued*)

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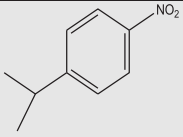
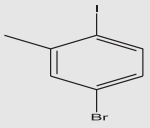
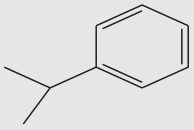
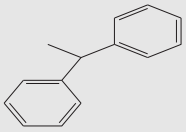
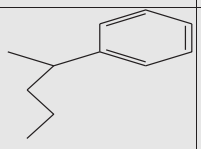
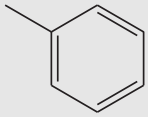
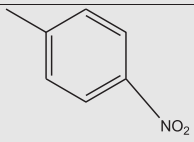
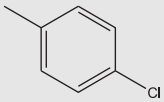
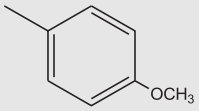
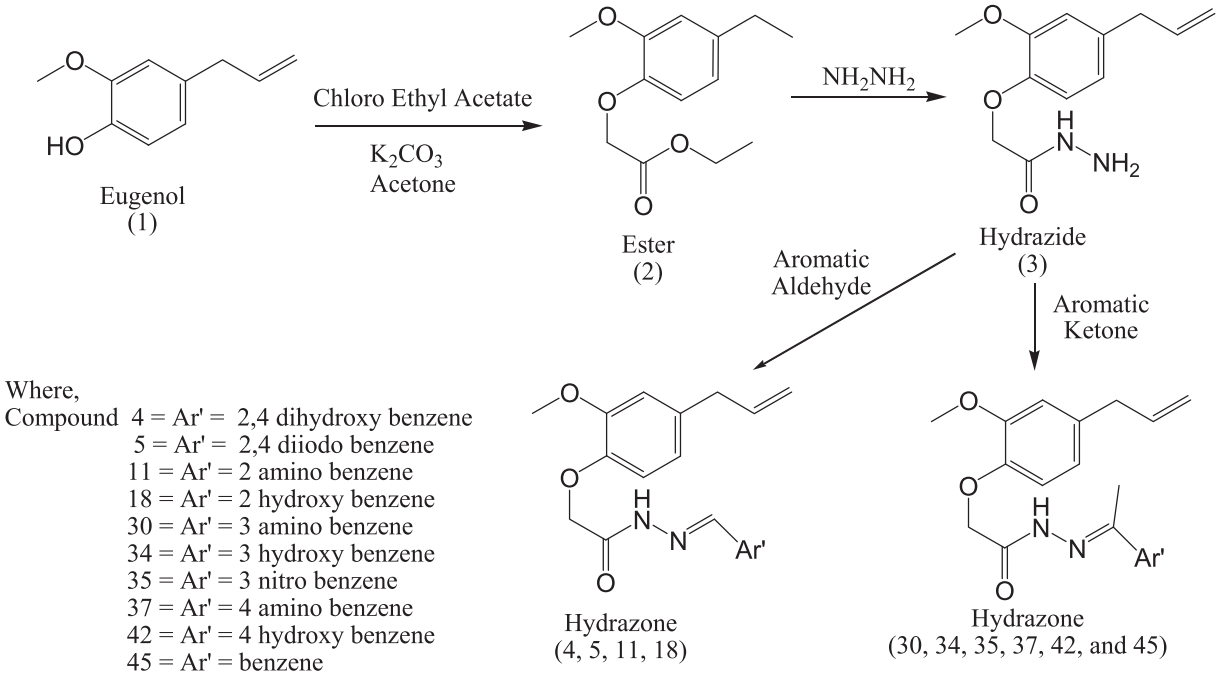
43		44		45	
46		47		48	
49		50		51	



Scheme 1 Synthesis of novel hydrazones.

3.2. Synthesis and characterization of synthesized compounds

The ten hydrazone compounds 4, 5, 11, 18, 30, 34, 35, 37, 42 and 45 were selected for synthesis based on their in-silico docking results. The derivatives were synthesized by condensation of arylhydrazide with various aromatic aldehydes or ketones using ethanol. Physical data of all synthesized compounds are given in Table 3 and characterization data mentioned in supplementary file. In the IR spectra, all hydrazone derivatives displayed characteristic band from 1700 to 1650 cm^{-1} attributed to C=O stretching vibration. The N–H stretching vibration of the compounds exhibited a band at near 3150 cm^{-1}. The stretching bands for C=C and C=N groups were observed at 1610–1490 cm^{-1}. In general, the IR stretching frequencies for –OH groups varied for the compounds 4, 18, 34 and 42 in the region 3200–3650 cm^{-1}. In the ^1H NMR spectra of all the compounds,

Table 2 Docking score of compounds.

Title	2NSD			Title	2X22		
	XP GScore	Title	XP GScore		XP GScore	Title	XP GScore
Isoniazid	−3.682	26	−8.15	Isoniazid	−5.451	26	−4.74
1	−8.747	27	−7.37	1	−5.959	27	–
2	−6.847	28	−8.101	2	−4.009	28	−7.486
3	–	29	−6.097	3	−8.007	29	−2.37
4	−10.393	30	−9.021	4	−8.426	30	−7.769
5	−7.919	31	−7.804	5	−6.09	31	−6.092
6	−7.247	32	−6.384	6	–	32	−4.919
7	−7.179	33	−6.955	7	−6.67	33	−6.071
8	−8.387	34	−10.13	8	−5.981	34	−7.448
9	−8.525	35	−9.813	9	−7.538	35	–
10	−7.099	36	−6.538	10	–	36	−6.226
11	−9.5	37	−9.632	11	−7.726	37	−9.092
12	−6.942	38	−9.587	12	−4.965	38	−7.932
13	−8.921	39	−6.818	13	–	39	−5.289
14	−8.216	40	−6.921	14	−8.073	40	−7.674
15	−7.428	41	−6.98	15	−6.721	41	–
16	−7.815	42	−9.747	16	−2.124	42	−8.527
17	−7.266	43	−9.256	17	−6.04	43	−6.551
18	−7.632	44	−8.898	18	−6.625	44	−5.356
19	−7.681	45	−9.049	19	−5.954	45	−6.262
20	−7.466	46	−9.593	20	−4.423	46	−7.758
21	−6.799	47	−9.485	21	−7.037	47	−7.556
22	−7.005	48	−6.469	22	−5.353	48	−4.896
23	−7.867	49	−8.072	23	–	49	−5.725
24	−7.883	50	−6.338	24	−4.742	50	−6.666
25	−8.657	51	−6.175	25	−6.098	51	−4.293

Note-: Sign ' – ' indicates compound does not show any Gscore.

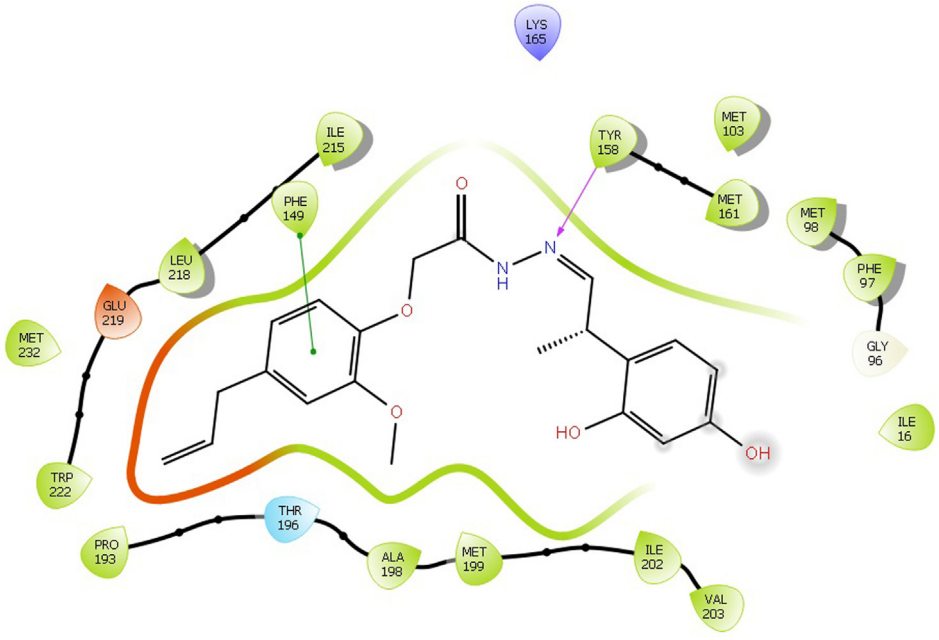


Fig. 1 The Interaction between the compound 4 with the active site of PDB 2NSD.Abbreviation: PDB, protein data bank.

the aromatic and aliphatic protons were observed at the specified ppm scale. Aromatic protons were observed at about δ 6.15–7.78 ppm. Synthesized hydrazones (4, 5, 11, 18, 30, 34, 35, 37, 42 and 45) displayed characteristic NMR signals for —OH, —NH, —CH═N— protons as coupled peaks at δ value of 4.90–5.10 ppm, 7.10–6.90, and 3.35–2.53 respectively.

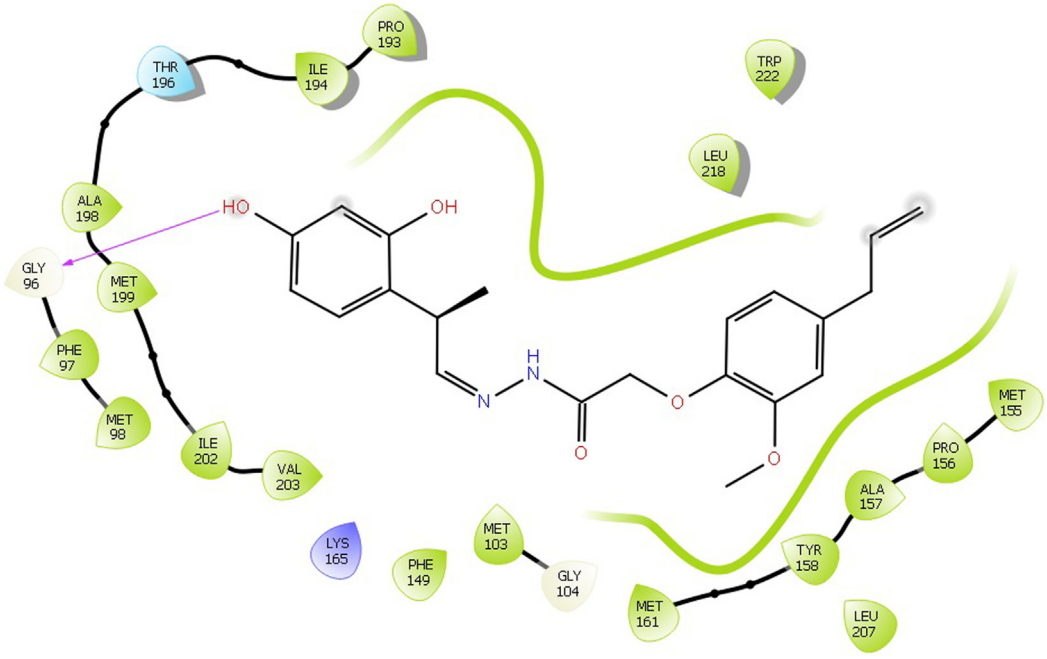
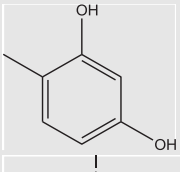
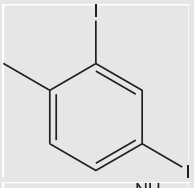
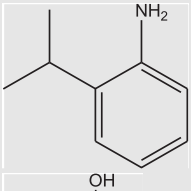
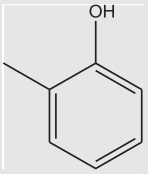
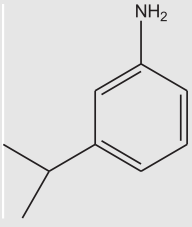


Fig. 2 The Interaction between the compound 4 with the active site of PDB 2X22.

Table 3 Physical characteristics of synthesized hydrazones.

Compound- No	Ar'	Mol. Formula	Mol. Weight	MP °C	Yield %
4		$C_{21}H_{24}N_2O_5$	384.19	220–221	73.08
5		$C_{19}H_{18}I_2N_2O_3$	576.18	235–236	62.00
11		$C_{21}H_{25}N_3O_3$	367.44	191–192	72.03
18		$C_{19}H_{20}N_2O_4$	340.37	229–230	75.00
30		$C_{21}H_{25}N_3O_3$	367.44	190–191	59.15

(*continued on next page*)

Table 3 (*continued*)

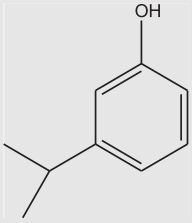
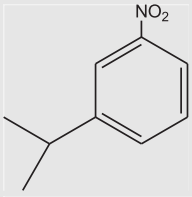
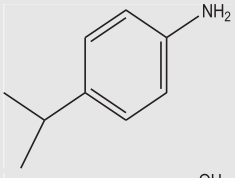
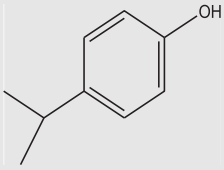
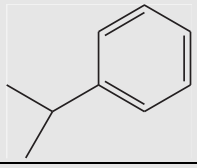
Compound- No	Ar'	Mol. Formula	Mol. Weight	MP °C	Yield %
34		$C_{21}H_{24}N_2O_4$	368.43	217–218	74.08
35		$C_{21}H_{23}N_3O_5$	397.42	235–236	60.08
37		$C_{21}H_{25}N_3O_3$	367.44	190–191	72.03
42		$C_{21}H_{24}N_2O_4$	368.43	219–220	76.90
45		$C_{21}H_{24}N_2O_3$	352.43	184–185	65.55

Table 4 Antimycobacterial activity of novel hydrazones.

Compound	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml
4	S	S	R	R	R	R	R	R
5	S	S	R	R	R	R	R	R
11	S	S	S	R	R	R	R	R
18	S	S	R	R	R	R	R	R
30	S	R	R	R	R	R	R	R
34	S	S	R	R	R	R	R	R
35	S	S	R	R	R	R	R	R
37	S	S	R	R	R	R	R	R
42	S	S	R	R	R	R	R	R
45	S	S	R	R	R	R	R	R
Isoniazid	S	S	S	S	R	R	R	R

NOTE: S - Sensitive R- Resistant.

3.3. Antitubercular activity

The compounds 4, 5, 11, 18, 30, 34, 35, 37, 42 and 45 were evaluated for *in vitro* antimycobacterial activity against *M. tuberculosis* H37Rv by microplate alamar blue assay (MABA) method, using isoniazid as standard. The results expressed in minimum inhibitory concentration (MIC) are given in Table 4 and the colour change observed during assay method is shown in Fig. 3. The obtained results indicate the biological potential and varying activity depending on the type of substituent on

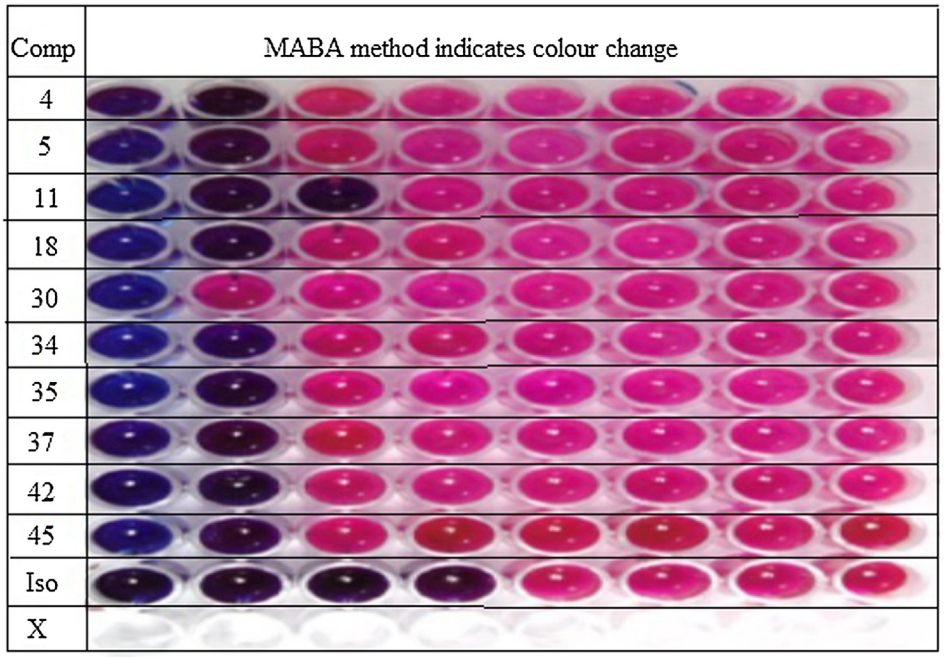


Fig. 3 Assessment of Microplate Alamar blue assay result.

hydrazide nucleus. The results of anti-tubercular activity revealed all newer hydrazones possess inhibitory potential against *Mycobacterium tuberculosis*. Whereas compound 11 possess highest sensitivity against *M. tuberculosis* H37Rv at 25 μg/ml level. The antibacterial activity is strongly connected with the position of the electronegative substituent on phenyl ring of aldehydes in relation to the hydrazone skeleton (Fuloria et al., 2017).

4. Conclusion

The molecular docking studies investigating hydrazone derivatives using the enzyme 2NSD and 2X22 as their potential biological target indicate that the amino, azide, hydroxyl and phenyl nucleus of hydrazone derivatives spacer play an important role in interactions with the active site such as TYR 158, ILE 215, GLU 219, PHE 97 and PHE 149 as the most active amino acid residues. Present study establishes the synthesis of newer hydrazone derivatives 4, 5, 11, 18, 30, 34, 35, 37, 42 and 45 by Schiff's reaction of aryloxy hydrazide with appropriate aldehyde or ketone. The structures of synthesized compounds were confirmed by spectroscopic methods. In the prepared hydrazone all compounds showed significant anti-tubercular action where as compound 11, exhibited highest anti-tubercular activity.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.arabjc.2019.09.004.

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