N'-(Substitutedbenzylidene)-3-(2-nitrophenylamino)-3-oxopropanehydrazide: Synthesis, molecular docking studies and anti-mycobacterial screenings

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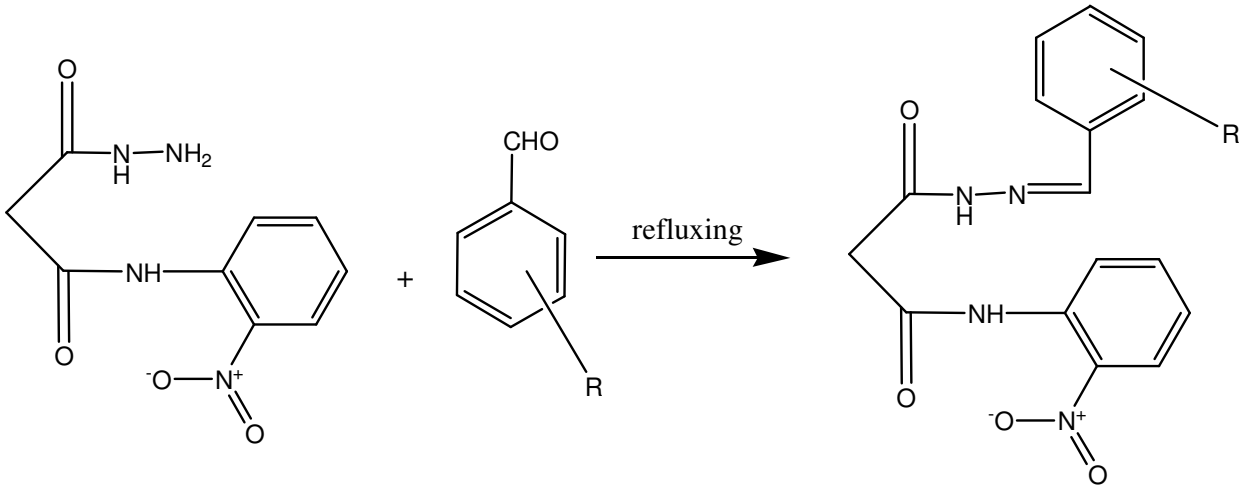
Abstract: The problem of tuberculosis (TB) drug resistance and the continuing rise in the disease incidence has prompted the research on new drug development as well as on increasing the understanding of the mechanisms of drug resistance. The full therapeutic possibilities of hydrazides were realized after the discovery of isonicotinic acid hydrazide (INH). Hydrazides and their derivatives have been described as useful synthons of various heterocyclic rings. Hydrazide-hydrazones have been reported to possess a wide variety of pharmacological activities such as anti-bacterial, anti-convulsant, anti-inflammatory, antitubercular, intestinal antiseptic, anti-depressant, or anti-platelet activity. A survey of literature reveals that extensive work has been done on the condensation products of hydrazides i.e. hydrazones, which exhibits a wide range of biological activity variations. These properties prompted us to synthesize some new malonamic acid hydrazones. Molecular docking was performed to study the binding activity of synthesized hydrazones onto the active site of *Mycobacterium tuberculosis* protein kinase B (PKnB) in an effort to increase the understanding of the action and resistance of synthesized hydrazone in this bacterium. The docking result demonstrated that the binding energies of Hydrazone were in the range of -6.99 kcal/mol to -8.27 kcal/mol, with the minimum binding energy of –8.27 kcal/mol. These newly synthesized compounds were screened for their anti-mycobacterial activities against *Mycobacterium tuberculosis* H37Rv using Resazurin micro-titre assay method.

Keywords: hydrazones, synthesis, molecular docking, Mycobacterium tuberculosis, Resazurin micro titre assay.

Introduction: The full therapeutic possibilities of acid hydrazides were realized after the discovery of Isonicotinic acid hydrazide (INH). Investigations of other heterocyclic hydrazides having mono-cyclic nuclei such as furan, thiophene, pyrrole and dicyclic nuclei such as quinoline and idoquinoline was stimulated due to the remarkable clinical value of INH[1]. A large number of such substances have been synthesized in pure form having differing ranges of curative effects. Yale et al[2] reported the synthesis of a number of hydrazides of the nicotinic acid hydrazide type, with a view to establish the structural requirements for antitubercular activity. Hydrazides and their derivatives have been described as useful synthons of various heterocyclic rings [3]. Hydrazide-hydrazones have been reported to possess a wide variety of pharmacological activities such as anti-bacterial [4-5], anti-convulsant [6], anti-inflammatory [7], antitubercular [8], intestinal antiseptic [4], anti-depressant [9], or anti-platelet activity [10]. The aroylhydrazone chelator, 2-hydroxy-1-naphthylaldehyde isonicotinoyl hydrazone, showed greater antimalarial activity than desferrioxamine against chloroquine-resistant and sensitive parasites [11]. 3- and 5-methylthiophene-2-carboxaldehyde-α-(*N*)-heterocyclic hydrazone derivatives exhibited tumor growth inhibition activity against various cell lines at GI50 values between 1.63 and 26.5 μM [12]. Hydrazones are often mentioned among the most effective charge transporting low molecular weight materials used in electrophotography, due to their excellent hole-transporting properties and relatively simple synthesis [13-16]. These properties prompted us to synthesize these novel malonamic acid hydrazones (**1-10**). Protein kinases B (PKnB) plays an important role in mammalian cellular signaling. Mycobacterium tuberculosis PknB is an essential receptor-like protein kinase involved in cell growth control. M. tuberculosis PKnB is a trans-membrane Ser/Thr protein kinase (STPK) highly conserved in Gram-positive bacteria and apparently essential for mycobacterial viability. We have attempted with the help of a docking approach to elucidate the extent of specificity of protein kinase B towards synthesized compound, as anti-tubercular agent. The synthesized hydrazones was screened for anti-tubercular activity against H_{37}Rv employing REMA (Resazurin microtitre assay) method[17].

Experimental Section:
Melting points were determined in open capillary tubes and are uncorrected. The purity of the compound was checked on silica-gel-coated Al plates (Merck). The structures of the compounds are confirmed on the basis of their Infra red spectra (IR) using KBr discs, on a Perkin Elmer Spectrum RX1 infra red spectrophotometer. ^{1}H NMR spectra were recorded in DMSO on Bruker DRX-300 (300 MHz) and Jeol AL300 FT-NMR (300 MHz) systems; chemical shift (δ) are reported in ppm using TMS as an internal reference. Elemental analysis was performed on Elementor Vario EL III. All the compounds gave satisfactory microanalysis. Synthesized molecules were docked into the nucleotide-binding pocket of the M. tuberculosis PKnB structure (PDB ID 2FUM)[18] using the program AutoDock4 [19]. Anti-tubercular screening of compounds was carried out using REMA method.

General procedure of synthesis: N-(2-nitro)phenyl malonamic acid hydrazide was synthesized according to our reported method [20]. N-(2-nitro)phenyl malonamic acid hydrazide (0.001 mol) and substituted aldehydes (0.001 mol) in absolute ethanol (10 ml) were gently refluxed for two hours. On cooling, crystalline solid was obtained and it was purified by recrystallization from hot ethanol (Scheme 1)



1-10

SCHEME 1

Molecular Docking: Molecular Docking of hydrazones was carried out using Lamarckian Genetic Algorithm [21] on the basis of calculated ligand-protein pair wise interaction energies. Study was carried out on 10 molecules and the grid maps representing the protein were calculated using auto grid and grid size was set to 60*60*60 points with grid spacing of 0.375 Å. Docking was carried out with standard docking protocol on the basis of a population size of 150 randomly placed individuals; a maximum number of 2.5 *107 energy evaluations, a mutation rate of 0.02, a crossover rate of 0.80 and an elitism value of 1. Fifteen independent docking runs were carried out for each ligand and results were clustered according to the 1.0 Å rmsd criteria.

Antimycobacterial Activity: The synthesized compounds were tested for their anti-tubercular activity in vitro against M.TB(H37Rv) by REMA method, in middle brook 7H9 supplemented with OADC(Hi-Media) using double dilution technique. A 100µL volume of middle brook (difco USA) was dispensed in each well of a 96 well cell culture plate (nune, Denmark), the compounds were tested against M.TB at different drug concentration (i.e. 3.25, 6.26, 12.5, 25, 50, 100, 200, 400, 500, 1600µg/ml) for the determination of minimum inhibitory concentration (MIC). The MIC was defined as the minimum concentration of the compounds required to inhibit the complete bacterial growth. Rifampicin & Ethambutol were used as standard drug.

Result and Discussion:

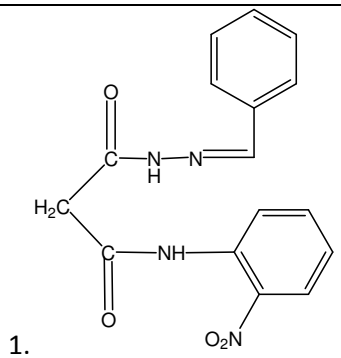
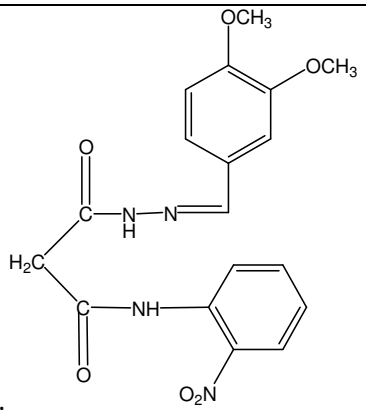
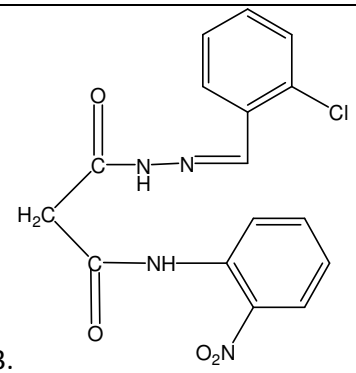
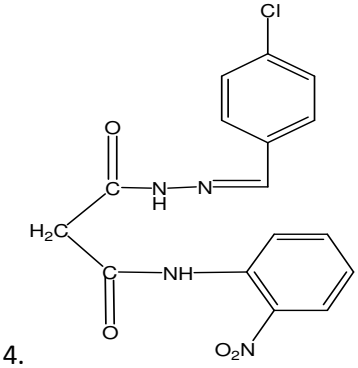
Physical and analytical data is given in table 1 and characterization (IR and ^{1}H NMR) data of compounds is given in table 2. The docking result demonstrated that the binding energies of Hydrazone were in the range of -6.99 kcal/mol to -8.27 kcal/mol, with the minimum binding energy of –8.27 kcal/mol (Table 3). The molecules were then tested for structure analysis by the visualization tool. The coordinate of the docked protein along with the ligand was visualized using UCSF chimera [22] within 6.5 Å region. Out of 10 molecules 3 protein-ligand complex showed H - bond with the active site residue VAL 95 (Table 3). None of the compound was found active against *Mycobacterium tuberculosis* H37Rv at any of the prepared concentrations i.e. 3.25, 6.26, 12.5, 25, 50, 100, 200, 400, 500, 1600µg/ml.

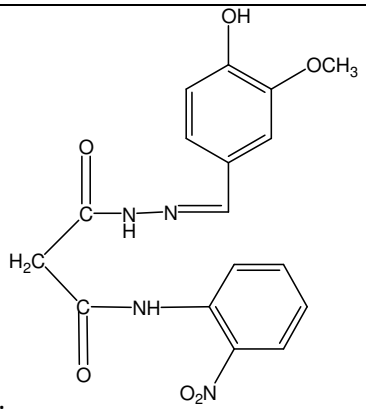
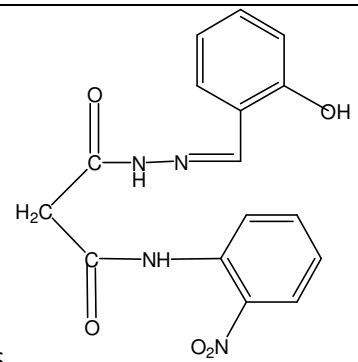
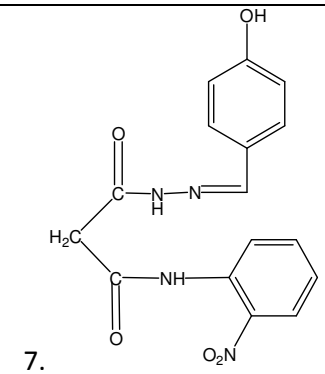
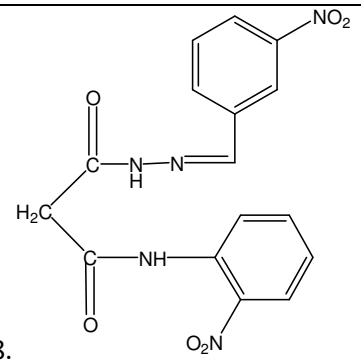
S. No.	Benzaldehyde (BD)	M.P (^{0}C)	% Yield	Mol. Formula	% Nitrogen	
					Found	Calc.
1	BD	143	78	$C_{16}H_{14}N_4O_4$	17.13	17.17
2	3,4-Dimethoxy BD	125	81	$C_{18}H_{18}N_4O_6$	14.44	14.50
3	2-Chloro BD	178	83	$C_{16}H_{13}ClN_4O_4$	15.50	15.53
4	4-Chloro BD	169	80	$C_{16}H_{13}ClN_4O_4$	15.49	15.53
5	4-Hydroxy-3-methoxy BD	134	75	$C_{17}H_{16}N_4O_6$	14.98	15.05
6	2-Hydroxy BD	119	70	$C_{16}H_{14}N_4O_5$	16.33	16.37
7	4-Hydroxy BD	121	79	$C_{16}H_{14}N_4O_5$	16.35	16.37
8	3-Nitro BD	128	69	$C_{16}H_{13}N_5O_6$	18.85	18.86
9	4-Nitro BD	130	72	$C_{16}H_{13}N_5O_6$	18.83	18.86
10	4-Dimethyl amino BD	118	76	$C_{18}H_{19}N_5O_4$	18.92	18.96

Table 1: Physical and analytical data of compounds

S. No.	IR (cm^{-1}) (KBr)	^{1}H NMR (δppm)
2	1058, 1279, 1339, 1515, 1660, 1725, 2819, 2945, 3445	3.31 (s, 2H, CH_2), 3.57 (s, 3H, OCH_3), 3.79 (s, 3H, OCH_3), 6.88-7.91 (m, 7H, Ar-H), 8.62 (s, 1H, CONH), 8.11 (s, 1H, N=CH), 8.59 (s, 1H, CONH).
4	670, 1511, 1537, 1647, 2932, 3022, 3301	3.35 (s, 2H, -CH_2), 7.49-7.97 (m, 8H, Ar-H), 8.37 (s, 1H, -CONH), 8.71 (s, 1H, N=CH), 9.04 (s, 1H, -CONH).
5	1506, 1532, 1654, 2935, 2941, 3321	2.51 (DMSO), 3.36 (s, 3H, OCH_3), 3.54 (s, 2H, CH_2), 5.21 (s, 1H, OH), 7.24-7.55(m, 7H, Ar-H), 8.54(s, 1H, -CH=N-), 9.21 (s, 1H, CONH).

Table 2: Characterization data of compounds.

STRUCTURE	Min. Binding Energy	H-Bond
 1.	-7.19	VAL 95 2.675 Å
 2.	-7.20	VAL 95 3.465 Å
 3.	-7.03	ASP 96 2.879 Å
 4.	-7.46	

 5.	-8.27	GLU 93 3.157 Å ASN 67 3.082 Å
 6.	-7.19	TYR 94 3.385 Å ASP 96 3.500 Å
 7.	-7.30	
 8.	-6.99	

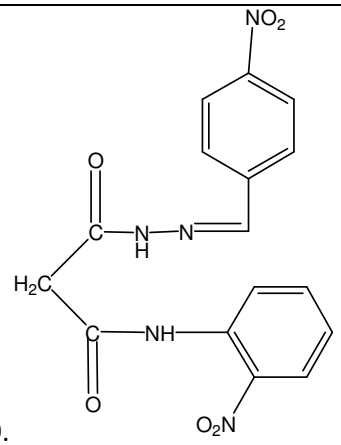
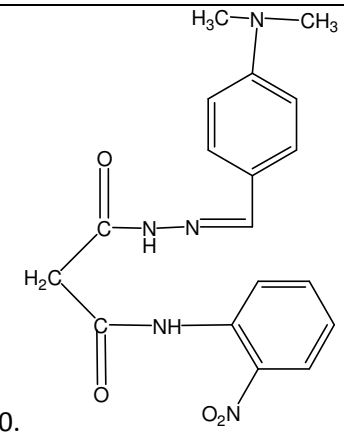
 9.	-7.08	VAL 95 2.806 Å
 10.	-7.22	ASP 96 3.091 Å TYR 94 3.315 Å

Table 3: Binding energies (kcal/mol) of synthesized hydrazones.

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