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Synthesis, characterization and *in vitro* anti *Mycobacterium tuberculosis* evaluation of some novel phthalimide derivatives

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ABSTRACT

In the present study, a series of novel phthalimide derivatives synthesized because of its potent anti-mycobacterial activity. The title compounds (A1a, A1b, A1 c, A1d, A2a, A2b, A2 c, A2d) have been characterized by TLC, Infrared spectroscopy, H^1 NMR and Mass spectroscopy and were evaluated for their anti-mycobacterial activity through MABA (Microplate Alamar blue assay) method. A total of 8 compounds were synthesized and screened for their anti-mycobacterial activity against Mycobacterium tuberculosis H37Rv bacteria. The minimal inhibitory concentrations (MIC) of the compounds were determined. These compounds A1a, A1b, A1c, A1d and A2b showed to be more active than the Nitro phthalimide A2a, A2c, A2d derivatives in inhibition of Mycobacterium tuberculosis growth so these compounds could be considered new lead compounds in the treatment of tuberculosis.

Keywords: Phthalimide derivatives, anti-mycobacterial, lipophilicity

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INTRODUCTION

Tuberculosis is a chronic granulomatous disease that results from infection with *Mycobacterium tuberculosis* (MTB). This aerobic bacillus has the cell wall with a high lipid content which results in a high degree of lipophilicity [1].

The ever increasing drug resistance, toxicity, and side effects of currently used antitubercular drugs, and the absence of their bactericidal activity highlight the need for new, safer, and more effective antimycobacterial compounds. The potential activities of several natural and synthetic compounds have been described against *M. Tuberculosis*. Among these, compounds containing phthalimide subunit have been described as a scaffold to design new prototypes drug candidates with different biological activities [2-4].

Cyclic imides possess a structural feature –CO-N(R)-CO- and an imide ring which helps them to be biologically active and pharmaceutically useful. [5, 6] Phthalimide & its derivatives have received much attention due to their antimicrobial [7, 8], analgesic & anti-inflammatory [9-11], antitumor [12, 13], anticonvulsant [14, 15], anxiolytic [16] and antiHIV-1 activities. [17] It is known that cyclic imide derivatives possess a structural feature –CO-N(R)-CO and an imide ring which confer on them potent biological activities [18].

Chemistry

Pthalimide and their derivatives have been found to be an important moiety in the creation of novel medical, polymeric, photonic, and electronic materials [19]. Often, these pthalimide are oxidative stable, heat retardant,

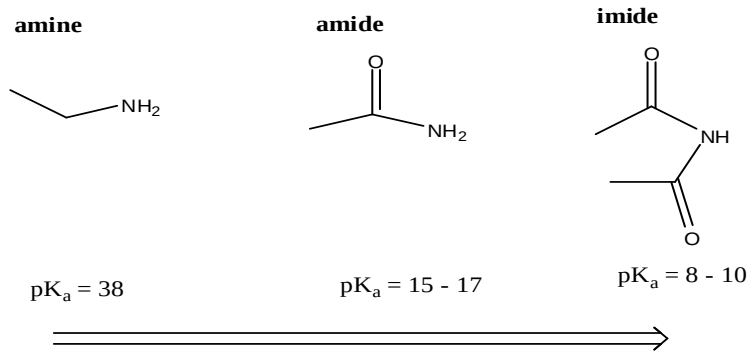
solvent resistant, and have superior mechanical properties. The specific reactivity of imides is a result of the relative acidity of the NH group, a direct consequence of the presence of the two carbonyl groups. It is known that cyclic imide derivatives possess a structural feature –CO-N(R)-CO and an imide ring which confer on them potent biological activities [20].

In N-Benzyl phthalimide the benzene and phthalimide groups are planar and make a dihedral angle of 74.2 (1) ° with one another. There are three weak C-H. . .O hydrogen bonds, forming a two-dimensional network structure. Most of the imides are cyclic compounds derived from dicarboxylic acids and their names reflect the parent acid. Examples are succinimide derived from succinic acid and phthalimide derived from phthalic acid [21].

As imide has the formula NH, being highly polar, imides exhibit good solubility in polar media. The N-H center for imides derived from ammonia is acidic and can participate in hydrogen bonding. Unlike the structurally related acid anhydrides, they resist hydrolysis and some can even be recrystallized from boiling water.

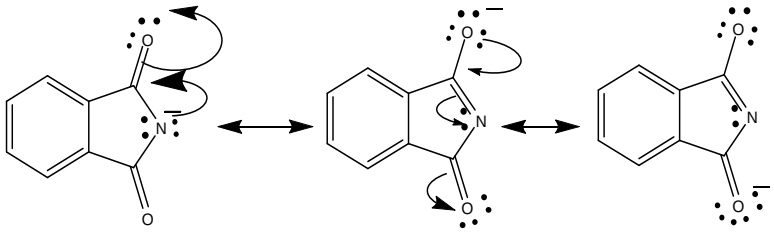
Effect of neighboring carbonyl groups on acidity of N-H bond
Imides such as phthalimide readily dissolve in aqueous NaOH as water-soluble salts.

Imides are more acidic than amides.



Increasing Order Of N-H acidity

Imides are more acidic than amides because
1. The electron-withdrawing inductive of the two adjacent C=O groups weakens the N-H bond a
2. More resonance delocalization of the negative charge.



A Resonanance- stabilized anion

The phthalimide moiety serves as a ‘protected’ form of ammonia. The phthalimide carbonyls increase the acidity of the nitrogen (thus allowing formation of its conjugate base).Most importantly, the phthalimide carbonyls protect the nitrogen form ‘over alkylation’ thus preventing the formation of secondary and tertiary amines (and quaternary ammonium salts) [22].

MATERIAL AND METHODS

Pharmacology

A Protocol for antimycobacterial activity testing in Vitro

The anti - mycobacterial activity of phthalimide derivatives were evaluated in vitro at the Microbiological Divison of CDRI, Lucknow by the method shown below:

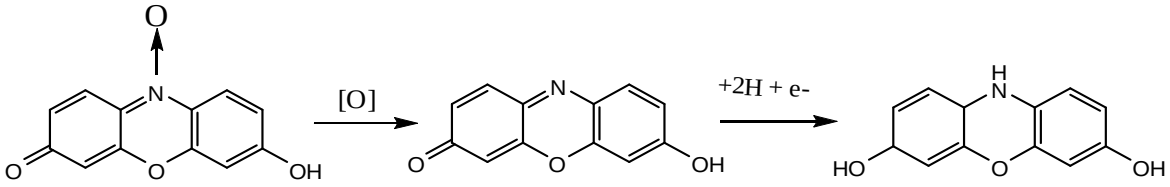
Anti-mycobacterial activity was evaluated against *Mycobacterium tuberculosis* H37Ra using a Microplate Alamar blue assay (MABA) method. Anti-mycobacterial susceptibility test was performed in black, clear bottomed, 96-well micro plates in order minimize background fluorescence.

Principle

Microplate Alamar Blue Assay (MABA) is a non-toxic rapid, inexpensive and high throughput assay for antitubercular drug screening. The principle behind Microplate Alamar Blue Assay is that, in the presence of cellular metabolism resazurin (oxidized form of Alamar blue,) which is non fluorescent blue in color is converted to resorufin (reduced form of Alamar blue) which is fluorescent pink in color.

Resazurin is purple in color. Resazurin reduces in two steps, irreversibly to resorufin and then reversibly to dihydroresorufin, providing color changes from purple to pink to colorless. The tubercle bacteria reduce resazurin which causes disappearance in purple color. The synthetic compounds cause oxidation, which is pink in color. The color intensity is measured in fluorometer. The percentage inhibition is defined as

$$\% \text{ inhibition} = \frac{\text{Test well fluroscence}}{\text{Mean fluroscence of triplicate wells}}$$



Resazurin Resorufin

Mechanism of MABA method

Preparation of Medium

Middle brook 7H9 Broth Base and Middle brooks OADC growth supplement was prepared by mixing the ingredients mentioned Table 1 & Table 2.

Table 1. Middlebrook 7H9 Broth Base

S.No.	Ingredients	Quantity (grams/liter)
1.	Ammonium sulphate	0.50
2.	Disodium phosphate	2.50
3.	Mono potassium phosphate	1.00
4.	Sodium citrate	0.10
5.	Magnesium sulphate	0.05
6.	Calcium chloride	0.0005
7.	Zinc chloride	0.001
8.	Copper sulphate	0.001
9.	Ferric ammonium citrate	0.04
10.	L-glutamic acid	0.50
11.	Pyridoxine	0.001
12.	Biotin	0.0005

Table 2. Middlebrook Oadc Growth Supplement

S.No.	Ingredients	Quantity
1.	Bovine albumin fraction V	2.50 gm
2.	Dextrose	1.00 gm
3.	Catalase	0.002 gm
4.	Oleic acid	0.025 gm
5.	Sodium chloride	0.425 gm
6.	Distilled water	50.00 ml

Prepared media were stored below 8˚C at pH 6.6 +/-0.2, protected from direct light. 2.35 gm of Middle Brook 7H9TB broth base was suspended in 450ml distilled water, which contains 5 ml glycerol sterilized by autoclaving at 15 psi pressure at 121˚C for 15 minutes. Cooked to 400˚C and enriched with dextrose to a final concentration of 0.5% of bovine albumin fraction V.

Method

Test compounds were suspended in 10% (v/v) DMSO. Two fold serial dilutions of compounds were made in Middlebrook 7H9 medium supplemented with 10% (v/v) OADC, in 96- well micro plates in duplicate. An inoculum of 10^5 CFU/ml was prepared and 200 µL was added per well. Growth controls containing no drug and a sterile control without bacteria were also prepared for each assay. In positive control rifampicin was added in 2µgm/ml and was added in the medium containing a bacterial culture. Plates were incubated at 37º C for 5 days before adding 20 µL of sterile 0.01% resazurin to all the wells and then incubated for a further 24 hrs at 37ºC. A change in color from blue (oxidized state) to pink (reduced state) indicated growth of bacteria. After 24 hrs, the MIC was determined as the lowest drug concentration that prevented the growth (i.e., the color change from blue to pink) and, therefore the occurrence of the color change was observed and fluorescence was measured in a microplate fluorometer in bottom reading mode with excitation at 530 nm and emission at 590 nm. The MIC_{90} data are given in table 3:

Table 3. MIC_{90} data of synthesized compounds

Sample Code	A1a	A1b	A1c	A1d	A2a	A2b	A2c	A2d	Rifampicin
MIC_{90} (µg/ml)	9.76	9.34	13.21	11.87	>18.52	10.69	>24.26	>22.04	0.20

Experimental

General procedure for synthesis of 3-benzylideneisoindolin-1-one and its derivatives (2 (a-b))

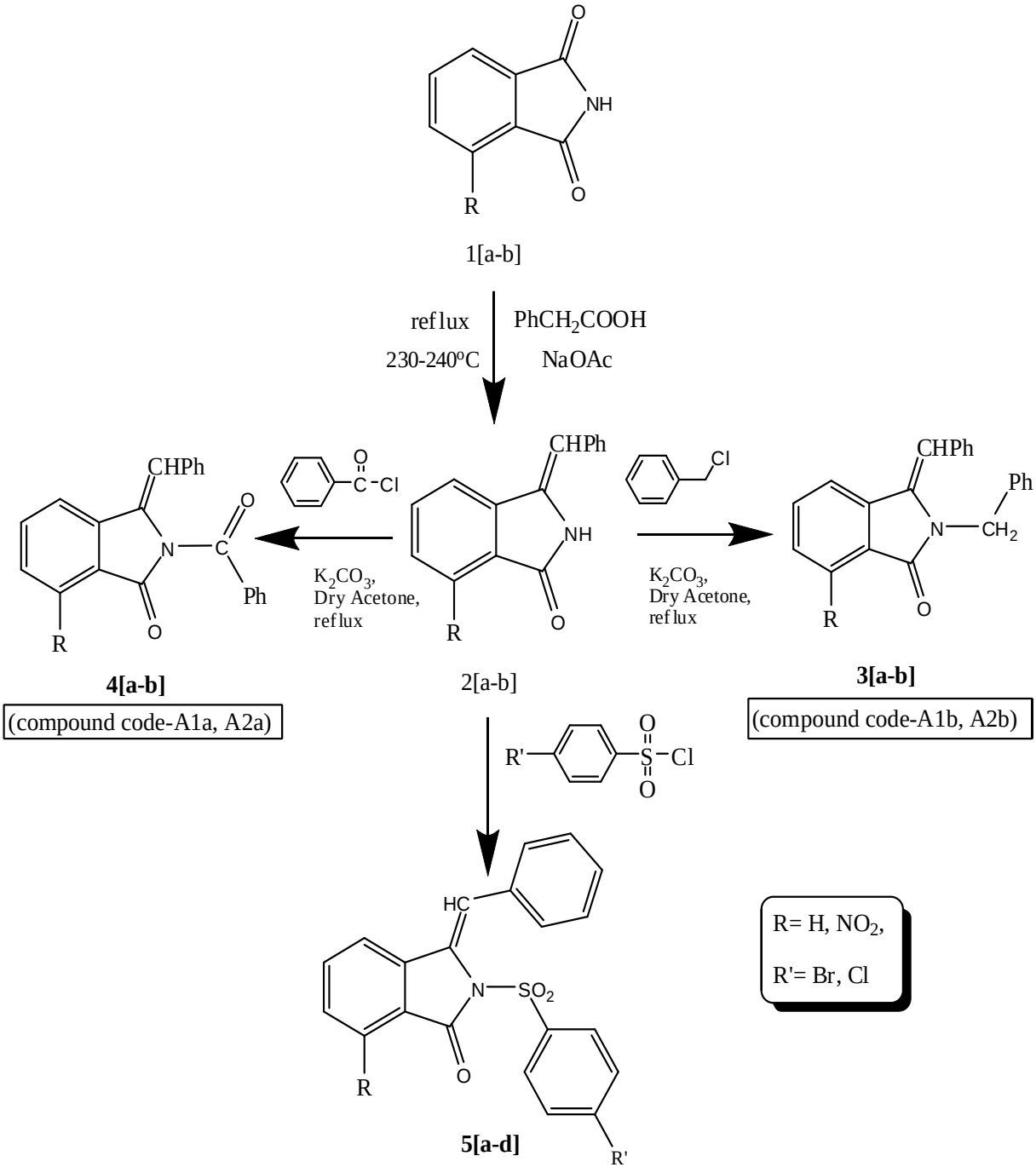
In a 500 ml round-bottomed flask with a short neck (not longer than 3 cm.) are placed 15gm of phthalimide **(1a)** or nitropthalimide **(1b)**, 16.5 gm of phenylacetic acid and 0.39 gm of freshly fused sodium acetate. A few chips of porous plate are added, and the flask is provided with a cork bearing a thermometer, which reaches almost to the bottom, and a wide, bent glass tube leading to a condenser. The tube ends just at the lower edge of the cork and does not protrude into the neck of the flask. The

The flask is embedded up to the neck in a sand bath and is heated rapidly until the thermometer reaches 230°, then the temperature is raised slowly until the water produced in the reaction and some entrained organic matter pass out through the exit tube. The water is collected in a small vessel and its quantity noted from time to time in order to follow the progress of the reaction. The operation should be conducted so that the temperature rises from 230 to 240° in the course of about two hours. The reaction is maintained at 240° until the distillation of water ceases; this requires about one additional hour. The flask now contains a brown mass covered with a film. The stopper is removed and a test portion is taken out by means of a glass rod. The test portion is placed in a test tube or small beaker, treated with a little alcohol, and heated to boiling. When the reaction is complete, the material dissolves readily in the hot alcohol and crystallizes on cooling. When this test has been found to be satisfactory, the flask is allowed to cool to 90–95°and the product is dissolved in 60 ml of boiling alcohol. The solution is filtered from insoluble matter and allowed to cool. The yellow crystals of 3-benzylideneisoindolin-1-one or its Nitro derivative (3-benzyliden-7-Nitro-isoindoline-1-one) filter with suction and washed with 10 ml of cold alcohol. The synthesized compounds were analyzed by TLC using chloroform: methanol (9:1) ratio as a solvent system [23**].**

General procedure for synthesis of 2-benzyl-3- benzylideneisoindolin-1-one or its nitro derivative (2-benzyl-3-benzyliden-7-nitro-isoindoline-1-one) (3(a-b))

A mixture of 1.5 gm of 3- benzylideneisoindolin-1-one or 3-benzyliden-7-nitro-isoindoline-1-one (**2a-b)** and 1.002 gm of benzyl chloride was refluxed in dry acetone (10.41 ml) containing 1.04 gm of potassium carbonate for 6 hrs.

The mixture was filtered and the filterate was evaporated at 60^0 C under reduced pressure to yield crystallized product. The synthesized compounds were analyzed by TLC using chloroform: methanol (9:1) ratio as a solvent system.



General procedure for synthesis of 2-benzoyl-3- benzylideneisoindolin-1-one or its nitro derivative (2-benzoyl-3-benzyliden-7-nitro-isoindoline-1-one) (4(a-b))

A mixture of 1.5 gm of 3-benzylideneisoindolin-1-one or 3-benzyliden-7-nitro-isoindoline-1-one (**2a-b)** and 1.4 gm of benzyl chloride was refluxed in dry acetone (50 ml) containing 5 gm of potassium carbonate for 6 hrs. After that, TLC monitoring showed the absence of starting material; the mixture was filtered and the filterate was evaporated at 60^0 C under reduced pressure. The residue was dissolved in ethyl acetate, washed with water (40 ml), dried ($MgSO_4$) and concentrated at 60^0 C under reduced pressure to give the crystalline product. It was then recrystallsed by absolute ethanol to give the desired product.

General procedure for synthesis of 3-benzylidene-2-(4-bromo/chlorophenylsulphonyl) isoindoline-1-one or its nitro derivative (3-benzylidene-2-(4-bromo/chlorophenylsulphonyl)-7-nitroisoindoline-1-one) (5(a-d))

Take 1 gm 3-benzylideneisoindolin-1-one or 3-benzyliden-7-nitro-isoindoline-1-one (**2a-b)** and 20 ml of 10% NaOH solution in a securely corked conical flask. Then added 1.5 gm of aryl sulphonyl chloride (4-chloro/4-bromo substituted) either finally powdered or in concentrated acetone solution until shaking the mixture vigorously in the securely corked boiling tube gives no further separation of the semi solid sulphonyl derivative. Then continue the shaking for 5 min. and finally filtered off the solid, washed well with water and recrystallized from methylated spirit. The synthesized compounds were analyzed by TLC using chloroform: methanol (9:1) ratio as a solvent system.

Analytical data:

(E)- 2-benzoyl-3-benzylideneisoindolin-1-one ***(A1a)***

IR (KBr, cm^{-1}): 1530 (-C=C-, Ar), 3174 (Aromatic, C-H, st), 793 (Aromatic, C-H, bending), 1730 (-C=O, amide), 1375(C-N, amide), 1774 (-C=O, anhydride), 712 (monosubstituted phenyl), 1050 (C-N, anhydride) . ^{1}H NMR (DMSO d6): 7.62-8.06 (m, 5H, Ar-H), 7.30-7.72(m, 5H, Ar-H), 6.75 (s, 1H, =CH), 7.51-7.69 (m, 4H, Ar-H). MS (m/z): M^+ 325.13.

(E)- 2-benzyl-3-benzylideneisoindolin-1-one ***(A1b)***

IR (KBr, cm^{-1}): 1504 (-C=C-), 3050 (Aromatic, C-H, st), 802 (Aromatic, C-H, bending), 1680 (-C=O, amide), 1385(C-N, amide), 1716 (-C=O, anhydride), 2955 (-CH_2-), 716 (monosubstituted phenyl), 1089 (C-N, anhydride) . ^{1}H NMR (DMSO d6): 7.08-7.36 (m, 5H, Ar-H), 7.30-7.74 (m, 5H, Ar-H), 6.75 (s, 1H, =CH), 4.85 (d, 2H, -CH_2), 7.56-7.65 (m, 4H, Ar-H). MS (m/z): M^+ 311.25.

(Z)- 3-benzylidene-2-(4-bromophenylsulfonyl) isoindolin-1-one ***(A1c)***

IR (KBr, cm^{-1}): 1615 (-(C=C-), 3058 (Aromatic, C-H, st), 665 (Aromatic, C-H, bending), 1699 (-C=O, anhydride), 1301 (-SO_2-), 762 (S-N), 650 (C-Br), 696 (p-disubstituted phenyl), 1146 (C-N anhydride).^{1}H NMR (DMSO d6): 7.82 -8.06 (m, 4H, Ar-H),7.30-7.77 (m, 5H, Ar-H), 6.75 (s, 1H, =CH), 8.50-8.66 (m, 4H, Ar-H). MS (m/z): M^+ 441.07.

(Z)- 3-benzylidene-2-(4-chlorophenylsulfonyl) isoindolin-1-one ***(A1d)***

IR (KBr, cm^{-1}): 1615 (-C=C-), 3058 (Aromatic, C-H, st), 665 (Aromatic, C-H, bending), 1699 (-C=O, anhydride), 1306 (-SO_2-), 796 (S-N), 757 (C-Cl), 696 (p-disubstituted phenyl), 1142 (C-N anhydride). ^{1}H NMR (DMSO d6): 7.41-7.50 (m, 4H, Ar-H), 7.01-7.20 (m, 5H, Ar-H), 6.99 (s, 1H, =C-H), 7.39-7.43 (m, 4H, Ar-H). MS (m/z): M^+ 395.04

(E)- 2-benzoyl-3-benzylidene-7-nitroisoindolin-1-one ***(A2a)***

IR (KBr, cm^{-1}): 1591 (-C=C-), 3063 (Aromatic, C-H, st), 1680 (-C=O, amide), 1713 (-C=O, anhydride), 730 (monosubstituted phenyl), 1249 (-NO_2), 1057 (C-N, anhydride), 1381 (C-N, amide), MS m/z: M^+ 370.10

(E)- 2-benzyl-3-benzylidene-7-nitroisoindolin-1-one ***(A2b)***

IR (KBr, cm^{-1}): 1535 (-C=C-), 3264 (Aromatic, C-H, st), 692 (Aromatic -CH- bending), 1769 (-C=O, anhydride), 818 (monosubstituted phenyl), 2935 (-CH_2-), 1352 (-NO_2), 1263 (C-N, ter. amine), 1020 (C-N, anhydride). ^{1}H NMR (DMSO d6): 7.28-7.46 (m, 5H, Ar-H), 7.30-7.76 (m, 5H, Ar-H), 7.97-8.15 (t, 3H, Ar-H), 5.34 (d, 2H, -CH_2), 5.41 (s, 1H, =C-H). MS m/z: M^+ 356.13.

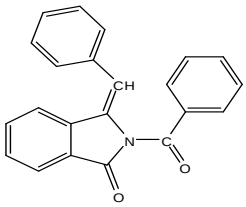
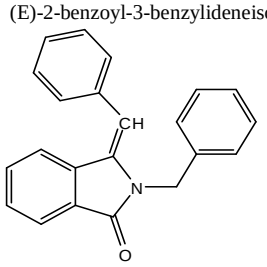
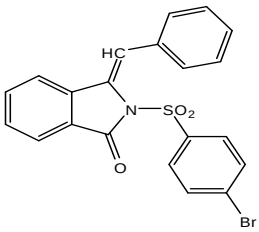
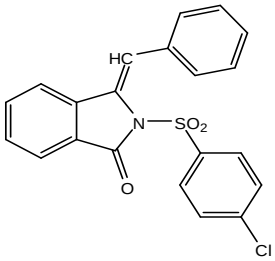
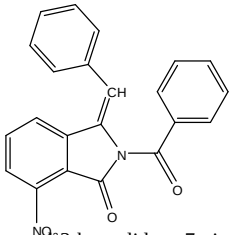
(Z)- 3-benzylidene-2-(4-bromophenyl-sulfonyl)-7-nitroisoindolin-1-one ***(A2c)***

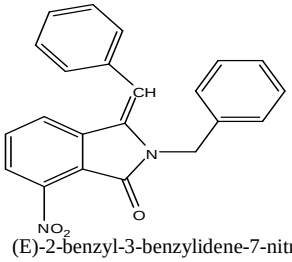
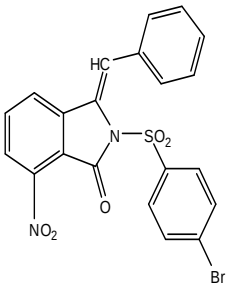
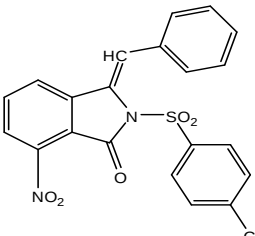
IR (KBr, cm^{-1}): 1610 (-C=C-), 3152 (Aromatic, C-H, st), 1807 (-C=O, anhydride), 748 (p-bromo-disubstituted

benzene), 1371 (-NO_2), 1024 (C-N, anhydride), 1357 (-SO_2-), 950 (S-N), 696 (C-Br). ^{1}H NMR (DMSO d6): 7.52-7.94 (m, 4H, Ar-H), 7.23-7.47 (m, 5H, Ar-H), 8.03-8.23 (t, 3H, Ar-H), 6.58 (s, 1H, =C-H). MS m/z: M^+ 485.97

(Z)- 3-benzylidene-2-(4-chlorophenyl-sulfonyl)-7-nitroisoindolin-1-one (A2d)
IR (KBr, cm^{-1}): 1615 (-C=C-), 3156 (Aromatic, C-H, st.), 1769 (-C=O, anhydride), 701 (p-bromo-disubstituted benzene), 1376 (-NO_2), 1029 (C-N, anhydride), 1360 (-SO_2-), 932 (S-N). ^{1}H NMR (DMSO d6): 7.64-7.85 (m, 4H, Ar-H), 7.32-7.69 (m, 5H, Ar-H), 7.94-8.28 (t, 3H, Ar-H), 6.89 (s, 1H, =C-H). MS m/z: M^+ 440.03

Table 4. List of synthesized compounds

Code	Structure & IUPAC Name	Mol. formula & M.W.	M.P. (^{0}C)	R_f-value	% yield	Log P[a]
A1a	 (E)-2-benzoyl-3-benzylideneisoindolin-1-one	$C_{22}H_{15}NO_2$ 325.36	140	0.38	54	4.83
A1b	 (E)-2-benzyl-3-benzylideneisoindolin-1-one	$C_{22}H_{17}NO$ 311.38	138	0.86	42	4.95
A1c	 (Z)-3-benzylidene-2-(4-bromophenylsulfonyl)isoindolin-1-one	$C_{21}H_{14}BrNO_3S$ 440.31	133	0.44	66	5.26
A1d	 (Z)-3-benzylidene-2-(4-chlorophenylsulfonyl)isoindolin-1-one	$C_{21}H_{14}ClNO_3S$ 395.86	135	0.46	60	4.99
A2a	 (E)-2-benzoyl-3-benzylidene-7-nitroisoindolin-1-one	$C_{22}H_{14}N_2O_4$ 370.36	130	0.54	65	–

A2b	 (E)-2-benzyl-3-benzylidene-7-nitroisoindolin-1-one	$C_{22}H_{16}N_2O_3$ 356.37	141	0.81	69	4.88
A2c	 (Z)-3-benzylidene-2-(4-bromo-phenyl-sulfonyl)-7-nitroisoindolin-1-one	$C_{21}H_{13}BrN_2O_5S$ 485.31	145	0.58	48	–
A2d	 (Z)-3-benzylidene-2-(4-chloro-phenyl-sulfonyl)-7-nitroisoind-olin-1-one	$C_{21}H_{13}ClN_2O_5S$ 440.86	143	0.56	55	–

[a] *Calculated on CHEM DRAW ULTRA 10 software*

RESULTS AND DISCUSSION

The synthesized compounds were screened for their anti-mycobacterial activity against *in-vitro Mycobacterium tuberculosis* H37Ra by M.A.B.A method. The minimal inhibitory concentrations (MIC_{90}) of the compounds were determined (Table 3). As per results substituted phthalimide derivatives were found to be more active as compared to substituted nitro-phthalimide derivatives. From the results we can conclude that by replacing the carbonyl group present in the 3rd position of phthalimide by benzylidene group anti-mycobacterial activity increases. The reason for this might be due the introduction of double bond in position 3 makes the lateral moiety more rigid. Benzylidene system instead of phthalimide occupies the aromatic area of the pharmacophore and also enhances the lipophilicity of compounds, concluded on the basis of LogP data of compounds (Table 4). Substitution of benzyl group and a benzoyl group at N-2 position make the molecule more lipophilic due to which compound easily crosses the mycobacterial cell wall and anti-mycobacterial activity of compound increases. An electron withdrawing group present at para position of the distal aryl moiety attached to N-H group of phthalimide at 2nd position enhances the activity.

CONCLUSION

All compounds were obtained with good yields (42–69%) from commercially available materials. These compounds A1a, A1b, A1 c, A1d and A2b shown to be more active than the nitrophthalimide A2a, A2c, A2d derivatives in inhibition of *M. tuberculosis* growth with MIC_{90} of 9.76, 9.34, 13.21, 11.87 and 10.69µg/ml respectively. The lipophilicity is an important physicochemical property related to the capacity of the compounds across the membrane. The lipophilicies of the synthesized compounds were calculated using CHEMDRAW ultra 10 (Table 4). Compounds A1a, A1b, A1 c, A1d and A2b presented lipophilicity higher than drug refampicin (log P = 3.7) used in

the treatment. But the compounds A2a, A2c and A2d were not shown lipophicity on the basis of log P data, so they were active at MIC_{90} above 18.52, 24.26 and 22.04 µg/ml respectively. These compounds could be considered new lead compounds in the treatment of tuberculosis.

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