Substituted benzimidazoles: A novel class of anti-tubercular agents

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Abstract
Reaction of O-phenylenediamine with phthalic anhydride gives 2H-benzimidazole -1-yl-3, 8 - benzpyrolidine - 2-one **(I)** This on condensation with anthranilic acid and 4-aminoacetophenone gives II. II on reaction with different aldehydes in presence of sodium hydroxide and ethanol stirred at room temperature for 24 h gives N-[4-(3-Aryl-acryloyl)–phenyl]–2-(1H– benzimidazol–2–yl)–benzamide (III-X**)**. All the compounds have been characterized by elemental analysis, IR and NMR and Mass spectral analysis. All the compounds have been evaluated for their anti-tubercular activity against different stain of mycobacterium species.

Keywords: Benzimidazole, O-phenylenediamine, Tuberculastatics, Antimicrobials, Aminobenzaldehyde.

Introduction
Benzimidazole nucleus has been of great interest to synthetic and medicinal chemists for a long time due to their unique chemical & biological properties. Benzimidazoles & its derivatives have been found to possess biological activity such as anti-viral,[1] anti-baterial,[2] anti-tungal,[3] anti-inflamatory[4] and anti-cancer[5] activity etc. Recently including tuberculastatic activity[6-9] of benzimidazole derivatives was reported. These reports prompted us to synthesize different Benzimidazole derivatives (III-X). Continuous increase in bacterial resistance to existing drugs has been resulted due to wide spread use of antibiotics leading to research on new substances possessing antimicrobial activity[10-17] In the present study the N-[4-(3 - Aryl-acryloyl) – phenyl] –2- (1 H – benzimidazol – 2 – yl) – benzamide (III-X) were prepared by the reaction of O-Phenylenediamine with phthalic anhydride and heated under reflux at 110° C yields compound (I), this on condensing with anthranilic acid and 4-aminoacetophenone in dimethyl formamide heated under reflux at 110° C gives II. The compound II is on treating with different aldehydes in presence of 40% sodium hydroxide and ethanol stirred at room temperature for 24 h gives III-X. The resulted compounds were characterized on the basis of elemental and spectral analysis.

Materials and Methods
Experimental
The purity of the synthesized compounds were ascertained by thin layer chromatography on silica gel G in various solvent systems using iodine vapours as detecting agent. Melting points were determined in open capillary tubes and are uncorrected. Infrared spectra were recorded on perkin - Elmer FT-IR spectrometer (Spectrum RX1) as KBr disks. Proton NMR spectra were recorded on varian AMX 400 instrument (300 MHz) using tetra methyl silane as internal standard.

Synthesis of 1 (2H-{Benzimidazole – lyl} -3, 8-Benzpyrolidine) -2-one (I)
1,2 diamino benzene (0.1mol) and phthalic anhydride (0.1mol) were taken in a 250ml round bottom flask and fused it for 30 minutes. To this, acetic anhydride (10ml) was added and refluxed for 6 hrs, cooled and poured in to ice cold water, filtered through buchner funnel and washed with cold water. The recrystallisation was done using dimethyl formamide m.p. 243^{0}C, yield 72%.

N (4-Carboxy phenyl -4-(IH-{Benzimidazole -2-yl}-benzamide (III)
The compound **I** (0.05mol) and anthranilic acid (0.05mol) was taken in a 250ml round bottom flask. To this dimethyl formamide (10ml) was added and refluxed for 6 hrs, cooled and poured in to ice cold water, filtered and washed with cold water. The recrystallization was done using dimethyl formamide. m.p. 237^{0}C, yield 65%.

N (4-(IH-{Benzimidazole - 2yl} - Benzamide -1- phenyl)-4- propionic ester (IV)
The compound **II** (0.05ml) was dissolved in dimethyl formamide (5ml). To this ethylalcohol (15ml) and concentrated hydrochloric acid (3ml) was added and mix it thoroughly and stir it for one hour on a magnetic stirrer and refluxed for 30 min, cooled filtered and washed with ice-cold water. The recrystallization was done using dimethyl formamide. m.p. 228^0 C, yield 58%.

N (4-Acetylphenyl -4-(IH-{Benzimidazole -2-yl}-benzamide (V)
A solution of 4-aminoaectophenone (0.05 mol) in DMF was added to **I** in DMF. The reaction mixture was refluxed for 6 h. The product was isolated by pouring onto ice anr recrystallized from DMF. Yield 65%, m.p 248° C

N-[4-(3 - Aryl-acryloyl) – phenyl] –2- (1 H –benzimidazol – 2 – yl) – benzamide (VI-X)
A solution of various substituted benzaldehyde (0.05mol) in minimum quantity of ethanol was added separately to a mixture of IV (0.05 mol) in ethanol and 40% NaOH (2-3

drops) was added to make it alkaline. The reaction mixture was then stirred separately for 24 h at room temperature. The reaction mixture was poured onto ice. The product was recrystallised using dioxane.Yield 60%,

Results and Discussion

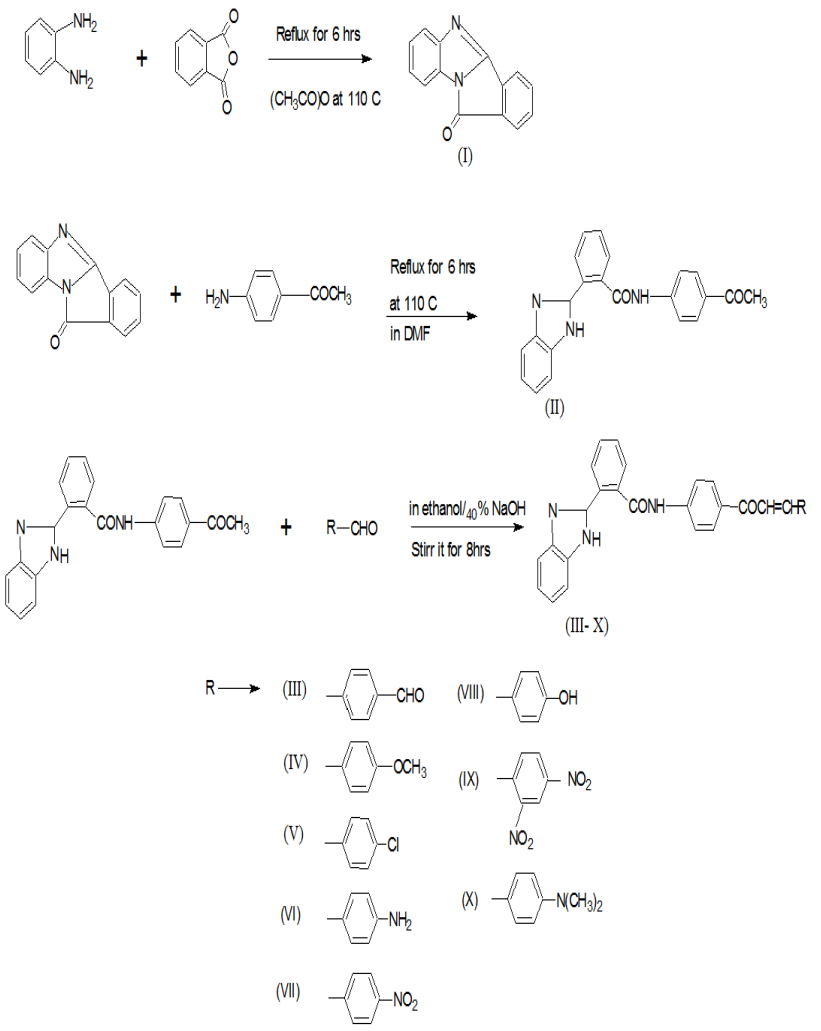
Antimicrobial Activity

All the compounds were screened for their antibacterial activity against gram positive strains (Staphylococcus aures, proteus vulgaris and streptococcus pyogens) and gram negative strains (Escherichia coli, pseudomonas aeroginosa and klebsiella pneumonia) using ciprofloxacin as a standard.

The antifungal activity was performed against candida albicans and Cryptococcus informans using griseofulvin as a standard. The MIC of the compounds tested in this study was determined according to the method of Goto *et al*[18] by a serial dilution technique. The inoculum size was approximately 10^6 colony forming units (cFu/m).

The reference standard ciprofloxacin inhibits the gram negative bacteria viz., E.coli, pseudomonas and klebsiella pneumonia at a MIC of 0.15µg/ml and 0.2µg/ml respectively whereas against gram positive bacteria viz., S. aures, P. Vulgaris and St.Pyogens at a MIC of 10µg/ml and 15µg/ml respectively. The screening results revealed that the compound III, VI, VII**,** IX and **X** showed good activity against gram positive strains (S. aures) and gram negative strain (E.Coli) than other compound**.** The screening results are given in Table III. The majority of the active compounds possessed phenyl substituted derivatives.

Scheme of Synthesized Compounds



The physical data of the synthesized compounds recorded in Table I.

Table 1: Characterization data of compounds prepared (I-III)

Compound	M.P(^{0}C)	Yield(%)	R_f(Value)	Molecular formula	Found (calcd)(%)N	Found (calcd)(%)C	Found (calcd)(%)H
I	243	72	0.52	$C_{14}H_8N_2O$	12.71(12.72)	75.36(76.36)	3.61(3.63)
II	237	65	0.59	$C_{21}H_{15}N_3O_3$	11.62(11.76)	70.54(70.58)	6.10(6.18)
III	228	58	0.47	$C_{23}H_{19}N_3O_3$	10.81(10.90)	71.32(71.68)	8.51(8.63)
IV	248	65	0.58	$C_{22}H_{17}N_3O_2$	11.70(11.83)	74.02(74.36)	4.61(4.78)
V	250	60	0.54	$C_{30}H_{23}N_3O_3$	8.78(8.87)	75.98(76.10)	4.80(4.86)
VI	246	60	0.62	$C_{29}H_{20}N_3O_2Cl$	8.68(8.80)	72.80(72.95)	4.10(4.19)
VII	254	60	0.57	$C_{29}H_{22}N_4O_2$	12.10(12.22)	75.82(75.98)	4.76(4.80)
VIII	240	60	0.41	$C_{29}H_{20}N_4O_4$	11.38(11.47)	71.11(71.31)	4.01(4.09)
IX	268	61	0.64	$C_{29}H_{18}N_4O_6$	10.80(10.81)	67.15(67.18)	3.45(3.47)
X	251	56	0.52	$C_{29}H_{19}N_3O_5$	8.48(8.50)	71.09(71.11)	3.85(3.88)
XI	249	62	0.62	$C_{29}H_{18}N_5O_8$	12.82(12.86)	63.90(63.97)	3.25(3.30)
XII	257	63	0.58	$C_{31}H_{24}N_4O_4$	10.82(10.85)	72.03(72.09)	4.61(4.65)

Table 2: Anti- tubercular activity of compounds I-XII

Compound	MIC µg /ml		
	$H_{37}Rv$	Drug-resistant strain	Drug-susceptible strain
	100	100	100
II	12.5	25	12.5
III	12.5	25	50
IV	12.5	12.5	25
V	6.5	12.5	12.5
VI	6.5	6.5	12.5
VII	50	25	12.5
VIII	25	50	12.5
IX	6.5	12.5	12.5
X	6.5	12.5	6.5

Tuberculostatic Activity

The compounds obtained were tested towards the standard Mycobacterium tuberculosis H37Rv strain as well as two strains isolated from tuberculosis patients: one resistant to the isonicotinic acid (INH), ethambutal (ETB) and rifampicin (RFP), the other fully susceptible to the tuberculostatics administered. Tuberculostatic activity was determined *in vitro* by classical test tube method with Youman's liquid medium containing 10% of bovine serum. On the ground of the minimum inhibiting concentration (MIC) values obtained one may conclude that some of the compounds tested exhibited a high tuberculostatic activity. The most active compounds were III, VI, VII, VIII, IX and X as their MIC values were within limits of 6-50µg/ml (Table 2).The majority of the compounds possessed amide substituted phenyl substitutents of benzimidazole system. The presence of benzimidazole alone did not show the activity.

Spectral data of the title compounds (I- X)

1. IR (cm^{-1}, KBr): 1560(aromatic ring), 1670(C=0), 1601(C=C & C=N), 1071(C-C), 2965 (C-H); 'HNMR ($CDCl_3$):δ, 6.5-8.2 (8H, m, aromatic protons).
2. IR (KBR): 3372 (N-H of imidazole ring), 1728(aromatic -COOH), 1640(CONH), 1620(C=C & C=N), 1060(C-C), 1560(aromatic - benzene), 1698(amide C=0), 3642(C-OH), 2972(C-H); 'HNMR ($CDCl_3$): δ 7.4-8.2 (13H, m, aromatic proton), 11.0 (IH,S, carboxylic acid), 8.2-8.8 (IH,NH).
3. IR (KBr): 3365(CONH), 1610(C=C & C=N), 1730(aromatic ester), 1670(amido C=0), 3410(N-H of imidazole ring), 1106 (C-C), 1270 (C-N), 1565 (aromatic benzene);'HNMR ($CDCl_3$): δ 7.3 -8.2 (12H,m,Ar-H), 0.8 -1.3 (3H, t -CH_3), 1.4-2.6 (2H,q,methylene proton of ester moiety), 8.5-8.8 (IH, NH), 6.79 – 7.31(d, J = 8.0 Hz,4H, Ar-H),7.30 (d, J =7.0 Hz, 2H, Ar-H), 7.60 (2H, Ar-H), 8.10 (d, J = 8.0 Hz,2H, Ar-H).
4. IR (KBr): 3346(N-H), 1679(-COCH3), 1662(CONH), 1178(C-O-C); ^{1}HNMR ($CDCl_3$): δ 2.7 (s 3H, CH3), 7.4 (8H, m, Ar-H), 8.35 (s, 1H, NH), 6.79 – 7.31(d, *J = 8.0 Hz*,4H, Ar-H), 7.30 (d, *J =7.0 Hz*, 2H, Ar-H), 7.60 (2H, Ar-H), 8.10 (d, *J = 8.0 Hz*,2H, Ar-H).
5. IR (KBr): 3306(NH), 1666(-CONH), 1650(C=O), 1170(C-O-C): ^{1}HNMR ($CDCl_3$):δ: 3.81 (t, 3H, OCH3), 8.33(m, 16H, Ar-H), 8.35 (s, 1H, NH), 6.79 – 7.31(d, *J = 8.0 Hz*,4H, Ar-H),7.30(d, *J =7.0 Hz*, 2H, Ar-H), 7.60(2H, Ar-H), 8.10 (d, *J = 8.0 Hz*,2H, Ar-H).
6. IR (KBr): 3311 (NH), 1664 (-CONH), 1650 (C=O), 1170 (C-O-C), 750 (C-Cl): ^{1}HNMR ($CDCl_3$):δ: 8.33(m, 16H, Ar-H), 8.35 (s, 1H, NH), 6.79 – 7.31(d, *J = 8.0 Hz*,4H, Ar-H),7.30 (d, *J =7.0 Hz*, 2H, Ar-H), 7.60 (2H, Ar-H), 8.10 (d, *J = 8.0 Hz*,2H, Ar-H).

7. IR (KBr): 3318 (NH), 1657 (-CONH), 1650(C=O), 1170 (C-O-C): ^{1}HNMR ($CDCl_3$):δ: 8.33(m, 16H, Ar-H), 8.35 (s, 1H, NH) 6.79 – 7.31(d, *J = 8.0 Hz*,4H, Ar-H),7.30(d, *J =7.0 Hz*, 2H, Ar-H), 7.60(2H, Ar-H), 8.10 (d, *J = 8.0 Hz*,2H, Ar-H).
8. IR (KBr): 3302(NH), 1661(-CONH), 1180(C-N), 1610(C=C), 1708(C=O), 3020(C-H), 1170(C-O-C): ^{1}HNMR ($CDCl_3$): δ 8.33(m, 16H, Ar-H), 8.35(s, 1H, NH), 6.79 – 7.31(d, *J = 8.0 Hz*, 4H, Ar-H), 7.30(d, *J =7.0 Hz*, 2H, Ar-H), 7.60 (2H, Ar-H), 8.10 (d, *J = 8.0 Hz*, 2H, Ar-H).
9. (KBr): 3311(NH), 1666(-CONH), 1650(C=O), 1180(C-N), 1610(C=C) 1170(C-O-C): ^{1}HNMR ($CDCl_3$): δ 8.33(m, 16H, Ar-H), 8.35 (s, 1H, NH), 6.79 – 7.31(d, *J = 8.0 Hz*,4H, Ar-H),7.30 (d, *J =7.0 Hz*, 2H, Ar-H), 7.60(2H, Ar-H), 8.10 (d, *J = 8.0 Hz*,2H, Ar-H)
10. (KBr): 3311(NH), 1666(-CONH), 1650(C=O), 1180 (C-N), 1610 (C=C) 1170(C-O-C): ^{1}HNMR ($CDCl_3$): δ 8.33(m, 16H, Ar-H), 8.35(s, 1H, NH), 6.79 – 7.31(d, *J = 8.0 Hz*, 4H, Ar-H), 7.30(d, *J =7.0 Hz*, 2H, Ar-H), 7.60 (2H, Ar-H), 8.10(d, *J = 8.0 Hz*,2H, Ar-H)
11. (KBr): 3311 (NH), 1666(-CONH), 1650(C=O), 1180(C-N), 1610(C=C) 1170 (C-O-C): ^{1}HNMR ($CDCl_3$): δ 8.33(m, 16H, Ar-H), 8.35(s, 1H, NH), 6.79 – 7.31(d, *J = 8.0 Hz*,4H, Ar-H), 7.30(d, *J =7.0 Hz*, 2H, Ar-H), 7.60(2H, Ar-H), 8.10 (d, *J = 8.0 Hz*,2H, Ar-H)
12. (KBr): 3311(NH), 1666(-CONH), 1650(C=O), 1180(C-N), 1610 (C=C) 1170(C-O-C): ^{1}HNMR ($CDCl_3$): δ 8.33(m, 16H, Ar-H), 8.35(s, 1H, NH), 2.46(d, 6H, CH_3), 6.79 – 7.31(d, *J = 8.0 Hz*,4H, Ar-H), 7.30(d, *J =7.0 Hz*, 2H, Ar-H), 7.60 (2H, Ar-H), 8.10(d, *J = 8.0 Hz*, 2H, Ar-H).

Conclusion

All the compounds were evaluated for antitubercular activity against standard Mycobacterium tuberculosis H37Rv strain as well as two strains isolated from tuberculosis patients. The compounds such as III, VI, VII, VIII, IX and X exhibited good antitubercular activity as their MIC values were within limits of 6-50μg/ml (Table II). Among the synthesized one, the compound VI (P-amino phenyl moiety) and **X** (4-dimethylamino moiety) showed good antitubercular activity when compared to other substituent's.

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Conflict of Interest: None.

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