



Synthesis and pharmacological screening of derivatives of benzimidazole linked with quinoline and tetrazole

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ABSTRACT

Benzimidazolyl acetamide synthesised by the reaction of acetamide group containing acid with the ortho phenylene diamine in presence of polyphosphoric acid which on treatment with Vilsmeier–Haack reagent (DMF+ POCl₃) gave the fused pyridine ring by cyclization, which gave compound 2-chloroquinoline 3-carbaldehyde. Further treatment with sodium azide gave quinoline ring fused with tetrazole. These compounds were containing the free aldehyde group in their structure which form Schiff base on treatment with the different substituted aniline. Elemental analysis, IR, ¹H NMR, ¹³C NMR, and Mass spectral data were used to elucidate the structures of all newly synthesized compounds. In vitro antimicrobial activities of synthesized compounds were investigated against Gram-positive *B. subtilis*, *S. aureus* Gram-negative, *K. pneumoniae*, *P. Aeruginosa* and fungi *Candida albicans*, in comparison with standard drugs. Some of the tested compounds showed significant antimicrobial activity.

Keywords: Benzimidazole, Quinoline, Tetrazole, Antibacterial and Antifungal.

INTRODUCTION

Benzimidazole is among the important heterocyclic compounds found in several natural and non-natural products such as Vitamin B12, marine alkaloid, Benzimidazole nucleosides [1, 2]. Some of their derivatives are marketed as anti-fungal agent (Carbendazim), anthelmintic agent (Mebendazole and thiabendazole), anti-psychotic drug (Pimozide) [3, 4, 5] and other derivatives have been found to possess some interesting bioactivities such as anti-tubercular, anti-cancer and antihypertensive, [6, 7]. Nitrogen containing heterocycles are one of the most extensively synthesized and screened compounds as they show diverse pharmacological activities. The development of tetrazole chemistry has been largely associated with wide scale of applications of these classes of compounds in medicine, biochemistry, agriculture and also large number of medicinally important tetrazole heterocyclic incorporated drugs approved by the FDA [8]. Quinoline and their derivatives are important constituents of pharmacologically active synthetic compounds. The quinoline nucleus also occurs in the structure of numerous naturally occurring alkaloids which have been associated with a broad spectrum of biological activities [9]. The fusion of quinoline to the tetrazole ring is known to increase the biological activity [10]. The tetrazole group, which is considered as a carboxylic group pharmacore, possesses a wide range of biological activities. Several substituted tetrazoles have been shown to possess anti-inflammatory [11, 12], antimalarial [13], anticancer [14], antifungal [15, 16], anticonvulsant [17, 18], antibacterial [19, 20], vasorelaxing [21, 22], antiviral [23] and CNS dispersant activities [24]. The different substituted schiff bases were prepared to enhance the antimicrobial activity [18].

EXPERIMENTAL SECTION

Melting points were determined by Veego melting point apparatus and were uncorrected. Silica gel G plates were used for TLC and the solvent systems were benzene: ethyl acetate (7:3) for compounds. The purity of the compounds was checked by TLC and the spots were visualized by iodine vapours. Infrared spectra were obtained

on a Shimadzu IR Prestige-21 FTIR spectrophotometer using potassium bromide discs. Nuclear magnetic resonance spectra were recorded on Mercury Plus 400 MHz NMR Spectrometer.

1. Synthesis of Para acetamido benzoic acid

P- Amino benzoic acid (5 gm) was taken in beaker containing 125 ml water, with constant stirring, 5 ml HCl was added for dissolution of Para amino benzoic acid. To this solution sodium acetate (8.3 gm in 40 ml water) and 5ml acetic anhydride was added simultaneously. The solution was stirred vigorously for five minutes, when white precipitate is formed which is filtered and dried [25, 26].

2. Synthesis of benzimidazolyl acetamide

Equimolar quantity of 4-acetamido benzoic acid (4 mM, 0.716 gm) and o-phenylene diamine (4 M, 0.432 gm) was taken in round bottom flask to it 5 ml polyphosphoric acid was added as cyclising agent. Reaction mixture refluxed for 20 hours end point was detected by TLC. After completion of the reaction, mixture was poured in crushed ice and to it sodium bicarbonate was added for neutralization of unreacted polyphosphoric acid, salt of acid is formed which is soluble in water, creamish yellow product is formed which is filtered and dried [27, 28].

3. Synthesis of 6-(1H-benzo[d]imidazol-2-yl)-2-chloroquinoline-3-carbaldehyde

The Vilsmeier-Haack reagent was prepared by drop wise addition of POCl₃ (98.28 mM, 9 ml) to DMF (34.65 mM, 2.7 ml) in a round bottom flask maintaining the temperature at 0–5°C. The mixture was allowed to stir for about 5 min. Benzimidazolyl acetamide was then added to the reaction mixture individually and heated for 8 hrs on water bath at constant temperature 80°C. After completion of reaction, the reaction mixture was poured in ice cooled water, neutralised with ammonia solution and stirred well to get yellowish precipitate which was filtered, dried and recrystallized by ethanol [29].

4. Synthesis of 7 (1H-benzo[d]imidazol-2-yl) tetrazole [1, 5-a] quinoline 4 carbaldehyde

To solution of 6-(1H-benzo[d]imidazol-2-yl)-2-chloroquinoline-3-carbaldehyde in dimethylsulphoxide (200ml) & acetic acid (4ml), a solution of sodium azide (0.03 M) in H₂O (10 ml) was added portion wise. The reaction mixture was stirred at 400°C for 3 hrs. Stirring was continued further for 5 days at ambient temperature. The precipitate was formed was filtered, washed with water & recrystallized from DMF [30].

5. General method for synthesis of substituted N-((7-(1H-benzo[d]imidazol-2-yl) tetrazolo [1,5-a]quinolin-4-yl) methylene) methanamine

Substituted aniline (10 mmole), 3.14 gm of 7-(1H-benzo[d]imidazol-2-yl)-tetrazolo[1,5-a]-quinoline-4-carbaldehyde (10 m mole) and 10 ml ethanol, 2ml glacial acetic acid were taken in round bottom flask and refluxed on water bath for 4 hrs (reaction was monitored with TLC). After completion of reaction, the reaction mixture was poured in ice cold water. The precipitate formed was filtered, washed with cold water and dried. The product was recrystallized with ethanol [31].

Spectral data for Synthesised compounds

Compound 1: IR- N-H (3317), C=O(carboxylic acid)(1708), -OH (carboxylic acid) (3180), C-H (aromatic stretching) (3103), C-N stretch (1128).

Compound 2: IR- C=O stretch (1712), N-H (3475), C=N stretch (1641), C=C stretch (1516), C-H stretch (CH₃) (2920), C-H stretch (aromatic) (3061), C-N stretch (1089).

Compound 3: IR- C-N stretch (1276), C-Cl (748), C=O (aldehyde) (1695), C-H (aldehyde) (2850).

Compound 4: IR- N-H (3340), N=N=N- (1186), C-N stretch (aryl) (1313), C=N stretch (1650), C=O (Aldehyde) (1691), C-H (Aldehyde) (2848). ¹H NMR (DMSO) δ: 8.062 [d, 1H, CH (aromatic)], 3.652 [s, 1H, -NH], 10.59 [s, 1H, CH (aldehyde)]. LC-MS *m/z*: 313 (calculated for C₁₇H₁₀N₆O: 314)

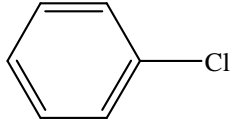
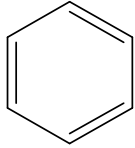
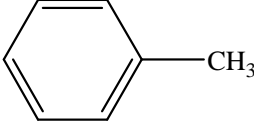
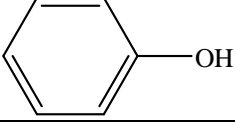
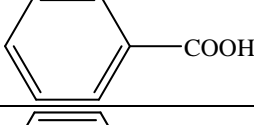
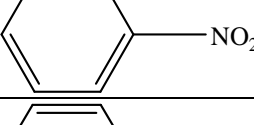
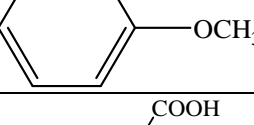
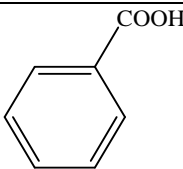
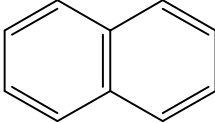
Compound 5a: IR- N-H (3215), C-Cl (746), C=C stretch (2115), N=N=N- (1184), C-H stretch (3059), C-N stretch (1095), C-N stretch (aryl) (1317). ¹H NMR (DMSO) δ: 8.03 [d, 1H, CH (aromatic)], 3.472 [s, 1H, -NH]

Compound 5b: IR- Aromatic stretching (3062), N-H (3442), N=N=N- (1184), C-N stretch (1112), C-N stretch (aryl) (1313). LC-MS *m/z*: 388 (calculated for C₂₃H₁₅N₇: 389)

Compound 5c: IR- N-H (3348), C=N (Imines) (1674), N=N=N- (1184), Stretching of C-N (1047), C-N stretch (aryl) (1309), C=C stretch (1541), C-H (2920).

Compound 5d: IR- N-H (3230), N=N=N- (1182), C-N stretch (aryl) (1267), C=N (Imines) (1612), C-O stretch (1010), O-H (3348).

Table 1: Characterization data of compounds 5

No.	R	M.F. M.W.	% Yield	M.P.(0°C)
5a		C ₂₃ H ₁₄ N ₇ Cl 423	84	152.3
5b		C ₂₃ H ₁₅ N ₇ 389	86	240
5c		C ₂₄ H ₁₇ N ₇ 403	81	150.2
5d		C ₂₃ H ₁₅ N ₇ O 405	78	210
5e		C ₂₄ H ₁₅ N ₇ O ₂ 433	84	225
5f		C ₂₃ H ₁₄ N ₈ O ₂ 434	77	160.4
5g		C ₂₄ H ₁₇ N ₇ O 419	73	123.6
5h		C ₂₄ H ₁₅ N ₇ O ₂ 433	78	215.7
5i		C ₂₇ H ₁₇ N ₇ 439	76	134

Compound 5e: IR- C=N (Imines) (1614), C-O stretch (carboxylic acid) (1188), -OH (carboxylic acid) (3340), C-H stretch (2920), O-H bend (1433), C-N stretch (aryl) (1260).

Compound 5f: IR- N-H (3387), C=N (Imines) (1614), N=N=N- (1190), N-O (1456), C-H stretch (2918), C-N stretch (aryl) (1303), C-N stretch (1109).

Compound 5g: IR- N-H (3262), C-O-C (phenyl alkyl ether) (1050), C=N (Imines) (1645), N=N=N- (1186), C-H stretch (CH₃) (2924).

Compound 5h: IR- C-O (carboxylic acid) (1184), O-H (carboxylic acid) (3348), C=N (Imines) (1606), O-H bend (1438), C-N stretch (1047), C=C (1541).

Compound 5i: IR- N-H (3360), N-N=N- (1186), C-N stretch (aryl) (1313), C=N (Imines) (1629), Aromatic stretching (3045).

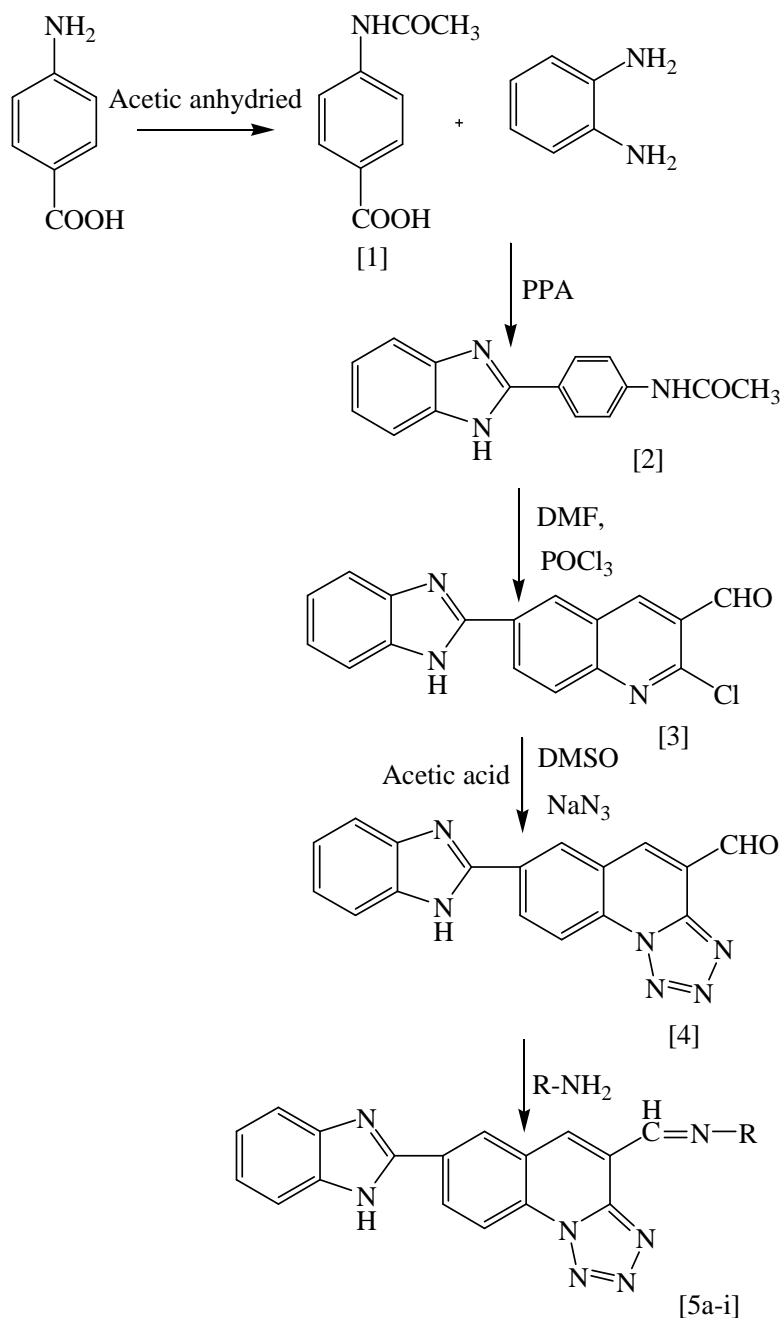


Figure 1: Scheme 1

6. Antimicrobial Activity

The synthesized compounds were tested for antimicrobial activity by disc diffusion method. They were dissolved in DMF. Final inoculums of 20 μ l suspension of each bacterium and fungus used. Nutrient agar (antibacterial activity) and sabouraud's dextrose agar medium (antifungal activity) was prepared and sterilized by an autoclave (121°C and 15 lbs for 20 min) and transferred to previously sterilized petridishes. After solidification, petriplates were inoculated with bacterial organisms in sterile nutrient agar medium at 45 °C, and fungal organisms in sterile sabouraud's dextrose agar medium at 45 °C in aseptic condition. The solidified plates were then seeded with 20 μ l bacterial suspensions (freshly prepared in saline). Cups were cut in the solidified medium using sterile cork borer about 10 mm diameter. The cut agar disk was removed by a splayed-out pen nib. Sample solution (50 μ l) of 125 μ g/ml concentration (calculated from tube dilution method) was loaded in each cup under sterile condition. The plates were left for 30 min to allow the diffusion of compounds at room temperature. Antibiotic of ciprofloxacin (50 μ l) and fluconazole (50 μ l) were used as positive control, while DMF used as negative control. Then the plates were

incubated for 24 h at $37 \pm 1^\circ\text{C}$ for antibacterial activity and 48 h at $37 \pm 1^\circ\text{C}$ for antifungal activity. The zone of inhibition was calculated by measuring the minimum dimension of the zone of no microbial growth around the each disc [32].

RESULTS AND DISCUSSION

All the compounds synthesized were well characterized by spectral (^1H NMR, IR and MS) data. The presence of N-H group, tetrazole ring, imines group were indicated by the appearance of frequencies in the range of $3100\text{-}3500\text{ cm}^{-1}$, $1103\text{-}1309\text{ cm}^{-1}$ and $1600\text{-}1690\text{ cm}^{-1}$ respectively, and also presence of carboxylic acid -OH, -Cl, phenyl alkyl ether, phenolic OH, and $-\text{NO}_2$ groups in derivatives were confirmed from the frequency ranges $3200\text{-}3600$, $1040\text{-}1250$, $3200\text{-}3400$, and $1350\text{-}1520\text{ cm}^{-1}$ respectively, in the IR spectra. In mass spectra, m/z (%) was obtained at 313 on the basis of which we can predict that this was molecular ion peak for the parent compound and for derivative molecular ion peak at 388. The ^1H NMR spectra showed a singlet for NH proton, doublet for aromatic proton and singlet for aldehydic proton at chemical shift of 3-5, 6.5-8 and 8-10 ppm ranges respectively.

All the synthesized compounds have shown positive antibacterial activity against *S. aureus*, *B. subtilis*, *K. pneumoniae*, and *P. Aeruginosa* but they are less active as compared to standard ciprofloxacin. The compounds 5f & 5g, 5e-g and 5c, 5f and 5g shows greatest activity against *S. aureus* and *K. pneumoniae*, *B. subtilis*, *P. Aeruginosa* respectively and show positive activity against *C. albicans*. The compounds 5b, 5f, and 5g showed the greatest activities among the synthesized compounds and they have less activity as compared to standard fluconazole.

Table 2: Zone of inhibition of Synthesised Compounds

Sr. no.	Compound	Zone of inhibition (mm)				
		<i>S. aureus</i>	<i>K.pneumoniae</i>	<i>B. subtilis</i>	<i>P. aeru</i>	<i>C. albicans</i>
1	5a	10	6	11	9	9
2	5b	12	8	15	16	10
3	5c	14	6	17	17	9
4	5d	9	10	12	10	7
5	5e	16	14	18	15	8
6	5f	18	19	20	17	10
7	5g	17	16	21	17	12
8	5h	16	9	13	14	6
Std.	Ciprofloxacin	28	22	25	30	-
Std.	Fluconazole	-	-	-	-	14
Control	DMF	00	00	00	00	00

CONCLUSION

In conclusion, we synthesized a series substituted Schiff's bases attached with Benzimidazole linked with quinoline and tetrazole. All the synthesised compounds characterised for IR, ^1H NMR spectroscopy and Mass spectrometry and screened for antimicrobial activity against Gram-positive *B. Subtilis*, *S. aureus*, Gram-negative *K. Pneumoniae*, *P. Aeruginosa* and fungi *Candida albicans*. Some compound shows greatest activity.

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