Synthesis and Evaluation of N-substituted Imidazole Derivatives for Antimicrobial Activity

NAMITA GUPTA* AND D. P. PATHAK
Department of Pharmaceutical Chemistry, Delhi Institute of Pharmaceutical Sciences and Research (DIPSAR), Sector-3, Pushp Vihar, New Delhi-110 017, India

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***Address for correspondence**
E-mail: namitagupta12@gmail.com

A series of N-substituted imidazole derivatives was synthesized. Imidazole nucleus was reacted with ethylchloroacetate to form imidazole ester. Reaction of the imidazole ester (I) with different amines yields the desired products (1a- 1e). The compounds were characterized by FT-IR, ^{1}H-NMR and mass spectra. The synthesized compounds were evaluated for the antimicrobial activity against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Candida albicans* and *Aspergillus niger* by determination of MIC (minimum inhibitory concentration) using tube dilution method. Compound (1b) was found to be the most active antimicrobial compound amongst others in the series.

Key words: ^{1}H-NMR, FT-IR, imidazole, minimum inhibitory concentration, tube dilution method

Development of drug resistance in microorganisms is a tussle between science and nature. An increasing public health problem is disease-causing microbes that have become resistant to antibiotic drug therapy. The current antimicrobial therapy is increasingly compromised by the emergence and spread of bacteria resistant to commonly used antimicrobial agents[1].

There is growing evidence of high levels of antibiotic resistance among important pathogens, including vancomycin resistant enterococci (VRE), methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-intermediate/resistant *S. aureus* (VISA/VRSA), and multidrug-resistant (MDR) *Pseudomonas aeruginosa*, which have emerged in the past 20 years[2]. Since 2000 for the treatment of Gram-positive infections only few new classes have been introduced; these are the oxazolidinones (linezolid), cyclic lipopeptides (daptomycin), and glycylcyclines (tigecycline)[2].

Various nuclei which have been studied for antimicrobial activity are benzothiazepine[3,4], triazole[5,6], benzoxazole[7,8], indazole[9,10], quinazoline[11,12], pyrazole[13,14], imidazole[15-19], benzimidazole[20-24] etc.

As healthcare providers we have a responsibility to acknowledge the issue of increasing resistance and to develop strategies for combating this continuing challenge to the management and treatment of the infectious diseases. Thus, in the present study we aim to synthesize a new chemical class of heterocyclic compounds to overcome the problem of microbial resistance.

The incorporation of imidazole nucleus is an important strategy in drug discovery. The high therapeutic properties of the imidazole related drugs have encouraged the medicinal chemists to synthesize a large number of novel chemotherapeutic agents based on imidazole nucleus[19].

The melting points were taken in open glass capillary tubes using Lab India visual melting point apparatus and are uncorrected. IR spectra of compounds were recorded on Jasco-410 FTIR spectrometer. ^{1}H-NMR spectra of compounds were recorded on Bruker 300 MHz NMR spectrophotometer using $CDCl_3$ as a solvent and TMS (tetramethylsilane) as an internal standard. Mass spectra of compounds were recorded on Waters micromass Q-Tof micro spectrometer. The purity of the compounds was checked by thin layer chromatography using silica gel G as adsorbent and spots were visualized by exposure to iodine vapours.

Synthesis of ethyl 1H-imidazol-1-yl acetate I: Ethylchloroacetate (0.075 mol) was added to a solution of imidazole (0.05 mol) in dry acetone (50 ml). To the mixture anhydrous K_2CO_3 (0.05 mol) was added and the reaction mixture was refluxed till the starting material disappeared. Acetone was evaporated in vacuo and the residue extracted with carbon tetrachloride. Organic layer was separated, dried over sodium sulphate and solvent evaporated to obtain semi liquid product. The product obtained was used for next step without further purification, yield 82%.

General procedure for the synthesis of compounds 1a-1e: The mixture of compound I (0.02 mol) and desired amine (0.03 mol) was heated till the reactants disappeared. The reaction mixture was extracted with chloroform. Organic layer was separated, dried over sodium sulphate and solvent evaporated to obtain solid product which was recrystallized using chloroform/hexane.

N-benzyl-2-(1H-imidazol-1-yl)acetamide (1a). The following spectral data were recorded for the compound 1a: IR (KBr cm^{-1}): 3212 (N-H str.), 3030

(Ar C-H str.), 2940 (C-H str.), 1683 (C=O str.), 1597 (C=N str.). ^{1}H-NMR ($CDCl_3$): 7.462 (s, -N=CH-, 1H), 7.333-7.348 (t, -NH-, 1H), 7.183-7.294 (m, Ar-H, 5H), 6.952-7.076 (d, imidazole-H, 2H), 4.672 (s, -N-CH_2-, 2H), 4.412-4.431 (d, -CH_2-, 2H). MS-ES: 216 (M^+ + 1).

N-cyclohexyl-2-(1H-imidazol-1-yl)acetamide (1b). The following spectral data were recorded for the compound 1b: IR (KBr cm^{-1}): 3297 (N-H str.), 2854 (C-H str.), 1654 (C=O str.), 1550 (C=N str.). ^{1}H-NMR ($CDCl_3$): 7.537 (s, -N=CH-, 1H), 7.179- 7.269 (d, -NH-, 1H), 6.964-7.179 (d, imidazole-H, 2H), 4.635 (s, -N-CH_2-, 2H), 3.729- 3.827 (m, -NH-CH, 1H), 1.164-1.854 (m, -CH_2-, 10H). MS-ES: 208 (M^++1).

2-(1H-imidazol-1-yl)-N-(naphthalen-1-ylmethyl) acetamide (1c). The following spectral data were recorded for the compound 1c: IR (KBr cm^{-1}): 3279 (N-H str.), 3041 (Ar C-H str.), 2926 (C-H str.), 1650 (C=O str.), 1556 (C=N str.). ^{1}H-NMR ($CDCl_3$): 7.875-7.916 (t, -NH-, 1H), 7.261-7.572 (m, Ar-H, 7H), 7.484 (s, -N=CH-, 1H), 6.892-7.080 (d, imidazole-H, 2H), 4.889-4.908 (d, -CH_2-, 2H), 4.702 (s, -N-CH_2-, 2H). MS-ES: 266 (M^++1).

2-(1H-imidazol-1-yl)-N-phenylacetamide (1d). The following spectral data were recorded for the compound 1d: IR (KBr cm^{-1}): 3267 (N-H str.), 3080 (Ar C-H str.), 2926 (C-H str.), 1671 (C=O str.), 1544 (C=N str.). ^{1}H-NMR ($CDCl_3$): 7.644 (s, -NH-, 1H), 7.127-7.418 (m, Ar-H, 5H), 7.151 (s, -N=CH-, 1H), 7.035-7.072 (d, imidazole-H, 2H), 4.824 (s, -N-CH_2-, 2H), 4.412-4.431 (d, -CH_2-, 2H). MS-ES: 202 (M^++1).

2-(1H-imidazol-1-yl)-1-(piperidin-1-yl)ethanone (1e). The following spectral data were recorded for the compound 1e: IR (KBr cm^{-1}): 3127 (Imidazole C-H str.), 2853(C-H str.), 1643 (C=O str.), 1509 (C=N str.), 1255 (C-N str.). ^{1}H-NMR ($CDCl_3$): 7.522 (s, -N=CH-, 1H), 6.963-7.099 (d, imidazole-H, 2H), 4.768 (s, -N-CH_2-, 2H), 3.391-3.579 (t, piperidine-H, 4H), 1.256-1.908 (m, piperidine-H, 6H). MS-ES: 194 (M^+ + 1).

The antimicrobial activity was performed against Gram-positive bacteria: *S. aureus, B. subtilis;* Gram-negative bacteria: *E. coli, P. aeruginosa* and fungal strains: *C. albicans and A. niger*. The standard and test samples were dissolved in DMSO (dimethyl sulphoxide) to give a concentration of 100 µg/ml. The minimum inhibitory concentration (MIC) was determined by tube dilution method. Two fold dilutions of test and standard compounds were prepared in double strength nutrient broth IP (bacteria) or Sabouraud dextrose broth IP (fungi). The samples were incubated at 37° (bacteria) for 24 h, 25° for 7 days (*A. niger*) and 37° for 48 h (*C. albicans*), respectively, and the results were recorded in terms of MIC (the lowest concentration of test substance which inhibited the growth of microorganism). The procedure was repeated twice.

A series of amides of imidazole were synthesized by reaction of ester of imidazole (fig. 1) with corresponding amines. The ester of imidazole was prepared by reacting it with ethylchloroacetate in the presence of anhydrous potassium carbonate. The physicochemical characteristics of synthesized derivatives are given in the Table 1. Compounds were synthesized in moderate to good yield. Purity of the compounds was determined by TLC on silica gel G plates. The spots were detected by exposure to iodine vapours. Synthesized compounds were characterized by spectral analysis (FT-IR, ^{1}H-NMR and Mass spectra). The spectra were found to be in agreement with the assigned molecular structure.

The synthesized compounds (1a-1e) were evaluated for *in vitro* antimicrobial activity against *Staphylococcus aureus, Bacillus subtilis* (Gram positive); *Escherichia coli, Pseudomonas aeruginosa*

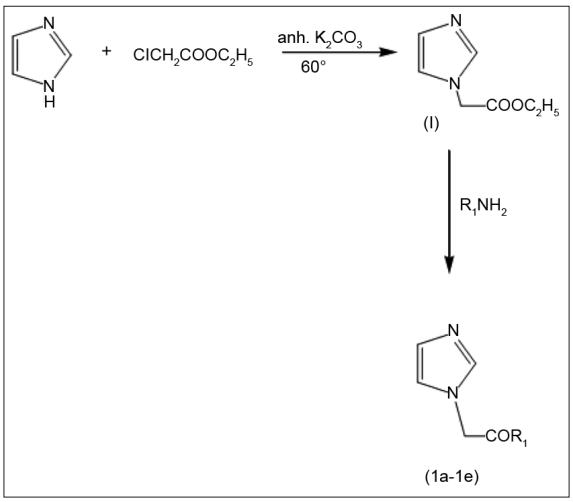


Fig. 1: Synthesis of N-substituted imidazole derivatives
Figure shows the reaction between imidazole and ethylchloroacetate in the presence of potassium carbonate at 60°. The resulting intermediate (I) reacts with different amines to form the products (1a-1e).

TABLE 1: PHYSICOCHEMICAL PROPERTIES OF THE COMPOUNDS

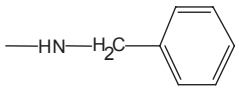
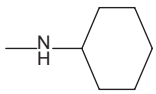
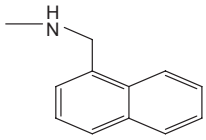
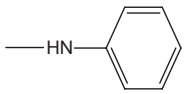
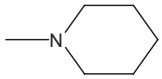
Compound	R_1	Molecular formula	Molecular weight	Melting point (°)	Yield (%)
1a		$C_{12}H_{13}N_3O$	215.25	120-122	69
1b		$C_{11}H_{17}N_3O$	207.27	124-127	74
1c		$C_{16}H_{15}N_3O$	265.31	120-123	64
1d		$C_{11}H_{11}N_3O$	201.22	135-138	62
1e		$C_{10}H_{15}N_3O$	193.25	128-130	55

Table shows the properties like structure of the substituent present, molecular formula, molecular weight, melting point and their percentage yield

TABLE 2: MIC (μg/ml) OF THE SYNTHESIZED COMPOUNDS

Compound	*E. coli*	*P. aeruginosa*	*S. aureus*	*B. subtilis*	*C. albicans*	*A. niger*
1a	25	50	25	25	25	25
1b	50	50	12.5	12.5	25	25
1c	25	50	25	25	50	50
1d	50	25	25	25	25	25
1e	50	25	25	12.5	25	25
Std	0.78[a]	0.78[a]	0.78[a]	0.78[a]	1.56[b]	1.56[b]

Miminum inhibitory concentration of the synthesized compounds against *Staphylococcus aureus, Bacillus subtilis* (Gram positive); *Escherichia coli, Pseudomonas aeruginosa* (Gram negative) and *Candida albicans* and *Aspergillus niger* using standards as a: Ciprofloxacin; b: Fluconazole

(Gram negative) and *Candida albicans* and *Aspergillus niger* by tube dilution method. MIC (μg/ml) was the concurrent reading obtained from the experiment which was done thrice and is given in Table 2. N-cyclohexyl-2-(1H-imidazol-1-yl)acetamide (1b) was found to be more active antibacterial compound than other compounds.

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