Synthesis of some novel 2-azetidinone derivatives of 2-methylbenzimidazoles by conventional and microwave assisted and evaluation of their antimicrobial efficacy

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Abstract

The aim of this work was to find out the efficiency of $N^1$-[2-substituted-benzylidene-imino-5'-methylene]-1',3',4'-thiadiazole]-2-methylbenzimidazoles, 4(a-n) and $N^1$-[2'-(4-substituted phenyl-3-chloro-azetidin-2-one-5'-methylene]-1',3',4'-thiadiazole]-2-methylbenzimidazole, 5(a-n) for the synthesis by conventional and greenar approach in terms of yield and reaction time along with antimicrobial activity against Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae and Streptococcus aureus bacteria and Aspergillus niger, Aspergillus flavus, Fusarium oxysporium and Trichoderma viride fungi in vitro at 50 and 100 ppm concentrations. Some of the compounds displayed pronounced biological activity. The structures of all the new compounds were established on the basis of micro analytical and spectral (IR, $^1$HNMR and mass) data.

Keywords: 2-Methylbenzimidazole, aryldiene, 2-azetidinone, antimicrobial activity.

INTRODUCTION

The structure and therapeutic diversity coupled with commercial viability of small heterocyclic molecules has fascinated organic and medicinal chemists. These are numerous biological active molecules whose framework includes a four and five membered ring containing heteroatom. 2-Azetidinone skeleton is well stabilised as the key pharmacophore of β-lactam antibiotics, the most widely employed class of antibacterial agents. The most widely used antibiotics such as the penicillins, cephalosporins, carumonam, aztreonam, thienamycin and the nocardicins all contain β-lactam rings[1]. The important and structural diversity of biologically active β-lactam antibiotics led to the development of many novel methods for the construction of appropriately substituted 2-azetidinones with attendant control of functional group and stereochemistry. Tricycle β-lactam antibiotics, generally referred to as trinems, are a new class of synthetic antibacterial agent featuring good resistance of β-lactamases and dehydropeptidases[2]. Benzimidazole derivatives are of wide interest because of their diverse biological activity and
clinical applications. Recently, some other types of biological activity besides the antibacterial activity have been reported in compounds containing benzimidazole ring [3,4]. Such biological activities include antifungal [5], antitubercular [6], antitumor [7], antiulcer [8], antihypertensive [9], antiviral [10], anti-histaminic [11], anticancer [12], antioxidant [13], cholesterol absorption inhibition and enzyme inhibition activity [14].

We have synthesized of N₁-{[(2-substituted-benzylidene-imino-5'-methylene)-1',3',4'-thiadiazole]-2-methylbenzimidazoles, 4(a-n) and N₁-{2'-(4-substituted-phenyl-3-chloro-azetidin-2-one-5'-methylene)-1',3',4'-thiadiazole]-2-methylbenzimidazole, 5(a-n) synthesis by conventional and green approaches in terms of yield and reaction time along with antimicrobial activity against Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae and Streptococcus aureus bacteria and Aspergillus niger, Aspergillus flavus, Fusarium oxysporium and Trichoderma viride fungi in vitro at 50 and 100 ppm concentrations. Some of the compounds displayed pronounced biological activity. The structures of all the new compounds were established on the basis of micro analytical and spectral data (IR, ¹HNMR and mass) data.

MATERIAL AND METHODS

Melting points were taken in open capillaries. Purity of compounds was monitored on silica gel "G" coated TLC plates. All instrumental analysis was performed at the Central Drugs Research Institute, Lucknow (India). IR spectra were recorded in KBr disc on a Schimadzu 8201 PC, FTIR spectrophotometer (νmax in cm⁻¹) and ¹HNMR spectra were measured on a Brucker DRX-300 spectrometer in CDCl₃ at 300 MHz using TMS as an internal standard. All chemical shifts were reported as δ (ppm) values. The FAB mass spectra were recorded on a Jeol SX–102 mass spectrometer. Elemental analyses were performed on a Carlo Erba–1108 analyzer. The analytical data of all the synthesized compounds were highly satisfactory. For chromatographic purification Merck silica Gel 60 (230-400 Mesh) was used. Microwave assisted reaction were carried out in a Q pro-M-modified microwave oven. The reagent grade chemicals were purchased from Merck and Aldrich Chemical Co. Ltd. Anhydrous silica gel 60 (0.063-0.2 mm) was used as solid support after dehydration under microwave irradiation for 4 minutes.

General procedure for the synthesis of compounds

Preparation of N₁-Ethylacetate-2-methylbenzimidazole (1):

Conventional Method: A mixture of 2-methylbenzimidazole (0.30 mole, 39.60 g) and ethylchloroacetate (0.30 mole, 36.74 g) with K₂CO₃ (6.168 g) in methanol (250 ml) was kept overnight at room temperature. The reaction mixture was refluxed on a steam bath for about 3 hrs. It was cooled filtered and solvent was distilled off under reduced pressure and the solid thus obtained was passed through a column of silica gel using chloroform: methanol (5:5 v/v) mixture as eluent. The eluate (250 ml) was concentrated to give a product which was recrystallised with ethanol to furnish colourless needles of compound 1. Yield 83%, m.p. 94-96°C. Anal. Calcd for C₁₂H₁₄N₂O₂S : C, 66.05, H, 6.42, N, 12.84%; found C, 65.97, H, 6.38, N, 12.79%; IR: 2866, 1470, 1270 (-NCH₂), 2912, 2875, 1427, 710 (-CH₂ and -CH₃), 1720 (>C=O of ester), 1050 (C-O-C), 3012, 2842, 1598, 1392, 744 (benzimidazole ring), 2816(-CH₃); ¹HNMR : 1.90 (t, 3H, J=7.0 Hz, -COOCH₂CH₃), 4.19 (q, 2H, J= 7.0 Hz, -CH₂CH₃), 2.64 (s,1H, -CH₃), 7.30 -7.65 (m, 4H, ArH), 3.63 (s, 2H, -NCH₂); MS : 218(M⁺).

Microwave Method: A mixture of 2-methylbenzimidazole (0.30 mole, 39.60 g) and ethylchloroacetate (0.30 mole, 36.74 g) with K₂CO₃ (6.168g) was added and mixed thoroughly. The mixture was air dried and subjected to microwave irradiation for 3 minutes (completion of reaction as indicated by TLC). The reaction mixture was cooled to room temperature and the
separated solid was extracted with ethanol. On standing the filtrate afforded colourless crystalline solid. The product was purified by column chromatography and recrystallised from ethanol, yield 94%. Spectral and analytical data were found to similar as reported for conventional method.

**Preparation of N\textsuperscript{1}-Acetylthiosemicarbazide-2-methylbenzimidazole (2):**

**Conventional Method:** The compound 1 (0.15 mole, 32.70 g) and thiosemicarbazide (0.15 mole, 30.67 g) in methanol (200 ml) was refluxed on a steam bath for about 8 hrs. It was then cooled, filtered and excess of solvent was removed which gave a product. It was purified over the column of silica gel using acetone: methanol 6:4 (v/v) mixture as an eluent. The eluate (200 ml) was concentrated and product was recrystallised with ethanol to give compound 2. Yield 73%, m.p. 146-48ºC. Anal. Calcd for C\textsubscript{11}H\textsubscript{13}N\textsubscript{5}OS: C, 50.19, H, 4.91, N, 26.61%; found C, 49.97, H, 4.91, N, 26.56%; IR: 3400, 3275 (\(-\text{NH}_2\)), 3352 (\(-\text{NH}\)), 1128 (\(>\text{C}=\text{S}\)), 2864, 1471, 1274 (\(-\text{NCH}_2\)), 1668 (\(>\text{CO}\)), 2822 (\(-\text{CH}_3\)), 3018, 2844, 1601, 1408, 742 (benzimidazole ring); \textsuperscript{1}HNMR: 8.12 - 8.35 (m, 4H, \(-\text{NHNHCSNH}_2\)), 2.65 (s,1H, \(-\text{CH}_3\)), 3.68(s, 2H, \(-\text{NCH}_2\)) 7.28-7.64 (m, 4H, Ar-H); Mass(FAB): 262(M\textsuperscript{+}).

**Microwave Method:** A mixture compound 1 (0.15 mole, 32.70 g) and thiosemicarbazide (0.15 mole, 30.67 g) was ground in a mortar using a pestle for uniform mixing. The mixture was kept inside a microwave oven operating at 160 w for 5 min. The completion of the reaction was checked by TLC. The product was purified by column chromatography and recrystallised from ethanol. Spectral and analytical data were found to similar as reported for conventional method.

**Preparation of N\textsuperscript{1}-(2-amino-5-methylene)-1', 3', 4'-thiadiazole-2-methylbenzimidazole (3):**

**Conventional Method:** Equimolar solution of compound 2 (0.10 mole, 26.30 g) and concentrated H\textsubscript{2}SO\textsubscript{4} (0.10 mole, 9.80 g, AR grade) in methanol (150 ml) was kept over night at room temperature. It was then refluxed on a steam bath for about 10 hr. After cooling the solution was neutralized with concentrated liq. ammonia and filtered. The solvent was removed \textit{in vivo} and the solid thus obtained was dried and purified over the column of silica gel using chloroform: methanol (5:5 v/v) mixture as eluent. The eluate (180 ml) was concentrated to give a product which was recrystallised from ethanol to give compound 3. Yield, 68%, m.p. 126-28ºC. Anal. Calcd. for C\textsubscript{11}H\textsubscript{11}N\textsubscript{5}S: C, 53.87, H, 4.48, N, 28.57%; found C, 53.79, H, 4.43, N, 28.51%; IR: 3396 (\(-\text{NH}_2\)), 2829, 1463, 1279 (\(-\text{NCH}_2\)), 1630, 1196, 1132, 1068, 624 (thiadiazole), 3016, 2846, 1603, 1408, 740 (benzimidazole ring) 2820 (\(-\text{CH}_3\)) ; \textsuperscript{1}HNMR: 4.81 (s, 1H, \(-\text{NH}_2\)), 2.64(s,1H,\(-\text{CH}_3\)), 7.25-7.69(m, 4H, Ar-H). MS : 245(M\textsuperscript{+}).

**Microwave Method:** The compound 2 (0.10 mole, 26.30g) dissolved in chloroform and concentrated H\textsubscript{2}SO\textsubscript{4} (0.10 mole, 9.80g) was kept over night at room temperature. Anhydrous transparent inorganic solid support silica gel was added and the solvent was removed under vacuum. The adsorbed reaction mixture was introduced in an open quartz tube which was subjected to microwave irradiation in the resonance cavity of the microwave power system for 1.30 minutes. The initial and the final sample temperature was measured. The sample was cooled in an ice bath and the irradiation was repeated several times. TLC was used to monitor the reaction progress. The product was extracted with ethanol and filtered. After filtration of the solution, it was neutralized with concentrated liq.ammonia and solvent was removed \textit{in vacuo}. The product was purified by column of silica gel and recrystallised from ethanol to give compound 3 yield 91%. Spectral and analytical data were found to similar as reported for conventional method.
Preparation of $N^1$-[(2-Benzylidene-imino-5'-methylene)-1',3',4'-thiadiazole]-2-methylbenzimidazole (4a):

Conventional Method: The equimolar solution of compound 3 (0.0085 mole, 2.08 g) and benzaldehyde (0.0085 mole, 0.902 g) in methanol (50 ml) with 4-5 drops of glacial acetic acid was refluxed on a water bath for about 3 hr. The solvent was distilled off under reduced pressure and the solid thus obtained was purified over the column of silica gel using chloroform: methanol (6:4 v/v) mixture as eluent. The eluate (60 ml) was concentrated and the product was recrystallised with ethanol to give compound 4a. Yield 74%, m.p. 172-74ºC. Anal. Calcd. for C$_{18}$H$_{15}$N$_5$S: C, 64.86, H, 4.50, N, 21.02%, found: C, 64.83, H, 4.47, N, 20.96%; IR: 1546 (-N=CH), 2824 (-CH$_3$), 2861, 1467, 1276 (-NCH$_2$), 1632, 1191, 1139, 1070, 634 (thiadiazole ring), 3018, 2842, 1610, 1411, 739 (benzimidazole ring); $^1$HNMR: 7.20-7.68 (m, 9H, Ar-H), 2.66 (s, 1H, -CH$_3$) 3.65 (-NCH$_2$), 4.91 (s, 1H, -N=CH); MS: 333(M$^+$).

Microwave Method: Equimolar solution of compound 3 (0.0085 mole, 2.08 g) and benzaldehyde (0.0085 mole, 0.902 g) in methanol (20 ml) with 4-5 drops of glacial acetic acid was kept at room temperature. Anhydrous microwave transparent solid support silica gel was added and the solvent was removed under vacuum. The adsorbed reaction mixture was introduced in an open quartz tube which was then subjected to microwave irradiation in the resonance cavity of the microwave power system for 1.30 minutes. The sample was cooled in an ice bath and TLC was used to monitor the reaction progress. The reaction product was extracted with methanol, filtered and dried over anhydrous sodium sulphate and then the solvent was removed. The product was purified by column of silica gel and recrystallised with ethanol to give compound 4a. Yield 89%.

Other compounds 4(b-n) were synthesized in the similar manner using compound 3 and various aromatic aldehydes. Characterization data are presented in Table-1.

Preparation of $N^1$-[(2'--(4-Phenyl-3-chloro-azetidin-2-one-5'-methylene)-1',3',4'-thiadiazole]-2-methylbenzimidazole (5a)
The compound 4a (0.004 mole, 1.33g) and triethylamine (0.004 mole, 0.40g) in methanol (20 ml) with chloroacetyl chloride (0.004mole,0.45g) was first stirred for about 3 hrs. followed by refluxing on a steam bath for about 10 hrs. It was cooled, filtered, dried and passed through a column of silica gel using chloroform: methanol (7: 3 v/v) mixture as eluent. The eluate was concentrated to give a product which was recrystallised from ethanol compound 5a. Yield 74% m.p. 139-41 ºC.Anal. calcd. for C$_{20}$H$_{16}$N$_5$OSCl: C, 58.60, H, 3.90, N, 17.09% found: C,52.54, H, 3.88, N, 16.96 %; IR: 1776(-CO), 776(-CHCl), 2819(-CH$_3$), 2862,1468, 1276(-NCH$_2$), 1630, 1193, 1143,1077,642 (thiadiazole ring), 3014, 2847, 1608, 1417, 734 (benzimidazole ring); $^1$HNMR: 3.64(s,2H, -NCH$_2$), 2.67(s,1H, -CH$_3$),7.22-7.70 (m, 9H, ArH), 5.16 (d $J$=5.0Hz, 1H,-CHCl), 5.18 (d $J$=5.0Hz, 1H,-NCHAr); MS: (FAB): 409(M$^+$).

Microwave Method: The compound 4a (0.004 mole, 1.339) with equimolar solution of triethylamine (0.004 mole, 0.40g) and chloroacetyl chloride (0.004 mole, 0.45g) in methanol (30 ml) in an open quartz tube which was then subjected to microwave irradiation for 8 minutes. Initial and final sample temperature was increased. The sample was cooled in an ice bath, and the irradiation was repeated 4 times. TLC was used to monitor the reaction progress. The solvent was removed under vacuum and the product was purified by column chromatography and recrystallized with ethanol to give compound 5a, yield 86%.

Other compounds 5(b-n) were synthesized in the similar manner using compounds 4(b-n). Characterization data are presented in Table -1.
Table-1: Characterization data of the compounds 4(b-n), and 5(b-n)

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<th>Comp</th>
<th>Ar</th>
<th>% Yield (Reaction time)</th>
<th>M.W. (mins)</th>
<th>Conv. (hrs.)</th>
<th>M.P. (°C)</th>
<th>Molecular formula</th>
<th>MS (FAB)</th>
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MW: Microwave; Conv.: Conventional

Antimicrobial activity

Antibacterial activity: All the synthesized compounds were evaluated in vitro for antibacterial activity by using filter paper disc method [15-16] against different strains of bacteria viz. B. subtilis, E. coli, S. aureus and K. pneumoniae. All the compounds along with standard antibacterial Streptomycin were used at 50 and 100 ppm concentrations.

Procedure: Solution of known concentration (50 and 100 ppm) of the test sample were made by dissolving in DMSO. Dried and sterilized filter paper discs (6mm in diameter) soaked with known amount of test agents were placed on the nutrient agar media solidified in petridishes (120 mm diameter) and inoculated with the test organisms. These plates were then kept at low temperature (4°C) for 24 hours to allow maximum growth of the organisms. The antibacterial activity was determined by measuring the diameter of zone of inhibition in mm.
Antifungal activity: All the compounds were also assayed in vitro for antifungal activity against A. niger, A. flavus, F. oxysporium and T. viride fungi employing the filter paper disc method [17] by measuring inhibition zone in mm. All the tested compounds along with standard fungicide Griseofulvin were used at 50 and 100 ppm concentrations.

Procedure: The test samples were dissolved in DMSO to make 50 and 100 ppm concentration solutions. Sterilized symmetrical filter paper discs of 6 mm diameter were taken in a blank petridishes. Sample solution 10 µl/discs were applied on the discs with the help of a micropipette in an aseptic condition. The discs were left for a few minutes in the aseptic condition for complete removal of the solvent. Isolated spore (4-6 similar) of pure fungus was inoculated in screw capped tube containing equal amount of potato dextrose agar (PDA) media and incubated at 28ºC for 5-7 days for development of new pure culture that was used as inoculum. PDA medium was steamed to dissolve and dispersed 4 ml amount of it into a petridish. It was then autoclaved at 121ºC for 15 minutes. It was allowed to cool to 30ºC until the media became solid. Each petridish was inoculated with different types of inoculums removed from a seven days old culture fungus. Dried and sterile sample discs and standard (Fungal) disc were placed on nutrient agar plates seeded with the test organism. These were then kept at low temperature (4ºC) for 24 hours to allow maximum diffusion. Finally the petridishes were inoculated at 27-28ºC for 5-7 days. The activity was justified by measuring the diameter of zone of inhibition in mm.

RESULTS AND DISCUSSION

Reaction of ethylchloroacetate with 2-methylbenzimidazole followed by thiosemicarbazide resulted in the formation of N\textsuperscript{1}-acetylthiosemicarbazide-2-methylbenzimidazole, 2. The compound 2 on dehydrative annulation by mineral acid afforded N\textsuperscript{1}–(2-amino-5'-methylene)-1',3',4'-thiadiazole- 2-methylbenzimidazole 3 which on condensation with various substituted aromatic aldehydes furnished N\textsuperscript{1}–[(2- substituted- benzylidene- imino- 5'-methylene)-1', 3', 4'- thiadiazole] -2-methylbenzimidazoles 4(a-n). The compounds 4(a-n) on reaction with chloroacetyl chloride in the presence of triethyl amine afforded N\textsuperscript{1}–[2'-(4- phenyl-3-chloro-azetidin-2-one-5'-methylene)-1',3',4'-thiadiazole]-2-methylbenzimidazole, 5(a-n)
The structures of new compounds were confirmed by elemental analysis, IR, $^1$HNMR and mass spectral data.

Table-2: Antibacterial activity of the compounds 4(a-n) and 5(a-n) against various bacteria at different concentrations (ppm)

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<th>Comp.</th>
<th>B. subtilis</th>
<th>E. coli</th>
<th>K. pneumoniae</th>
<th>S. aureus</th>
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$SM = Streptomycin$, inhibition diameter in mm (-) < 6, (+) 6-10, (+ +) 10-16, (+ + +) 16-25, (+ + + +) 25-30.

All the synthesized compounds 4(a-n) and 5(a-n) have been screened in vitro for their antibacterial activity against B. subtilis (Bs), E. coli (Ec), S. aureus (Sa) and K. pneumoniae (Kp) at two concentrations (50 and 100 ppm) and antifungal activity against A.niger (An), A. flavus (Af), F. oxysporum (Fo) and T. viride (Tv) at two concentrations (50 and 100 ppm). Standard antibacterial Streptomycin and fungicide Griseofulvin were also screened under the similar conditions for comparison. The following compounds were found active against the tested bacteria: 4d (Ec,Sa), 4f(Bs,Kp), 4g(Ec), 4h(Bs,Ec,Sa), 4i,4j(Kp), 5b, 5c, 5d, 5e ,5f, 5g (Bs, Ec, Kp, Sa), 5i (Ec,Kp,Sa), 5h(Kp), 5k (Ec), 5n(Kp,Sa) and fungi :5f(Af,Tv), 5g(An), 5n(Fo), 5b(Tv), 5c (Af, Fo), 5d(An), 5e, 5f, 5g(An, Af, Fo,Tv), 5i(Af, Fo), 5j (An,Af), 5h(Fo,Tv), 5m(Tv), 5n(SM, Af, Fo,Tv), 5o(An,Af), 5p(Fo,Tv), 5q(Tv), 5r(Fo,Tv), 5s(An,Af), 5t(Fo,Tv), 5u(Af,Tv), 5v(Af,Tv), 5w(Af,Tv), 5x(Af,Tv), 5y(Af,Tv), 5z(Af,Tv).
5k(An,Af), 5l, 5m, 5n(An). On the basis of structural activity relationship it has been observed that among the substituents present on the phenyl ring, halo derivatives were found to be highly active against in the series. Further study reveals that bromo derivatives are highly active.

Table 3: Antifungal activity of the compounds 4(a-n) and 5(a-n) against various fungi at different concentrations (ppm)

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<th>A. flavus 100</th>
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GF = Griseofulvin, inhibition diameter in mm (-) < 4, (+) 4-12, (++) 12-18, (+++) 18-27, (++++) 27-30.

Acknowledgement

The authors are thankful to SAIF, CDRI, Lucknow(India), for providing spectral and analytical data of the compounds. We are also grateful Head, Department Chemistry of this University for giving the facilities to carry out the work.
REFERENCES