RESEARCH ARTICLE

Synthesis of New Benzimidazole Derivatives Containing Azoles Ring Moiety and Study their Biological Activity

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ABSTRACT

This study provides a new library of benzimidazole derivatives containing azole ring moiety was synthesized *via* 1,3-dipolar cycloaddition. The obtained compounds were characterized by FT-infrared, ^{1}H- and ^{13}C- NMR spectroscopy. The biological activity was tested for most of the synthesized compounds against four types of bacteria two of them are gram-ve [*Escherichia coli* (*E. coli*) and *Pseudomonas aeruginosa* (*P. aeruginosa*)] and two gram+ve [*Bacillus subtilis* (*B. subtilis*) and *Staphylococcus aureus* (*S. aureus*)]. Indeed, the synthesized compounds show a good to moderate effect on both types of bacteria.

Keywords: Benzimidazole, 1,3-dipole cycloaddition, Antibacterial, Isoxazolidine.

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INTRODUCTION

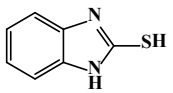
Out of many heterocyclic compounds, benzimidazole is widely used in synthetic organic chemistry. These structures are crucial due to the wide existence in medicinal compounds.[1] It is a heterocyclic compound that exists in nature as an core structure of cyanocobalamin and has been incorporated into pharmaceutical agents to form enzyme inhibitors and DNA intercalators.[2] Benzimidazole is a versatile core ring producing a diverse range of pharmaceutical uses such as anti-inflammatory and analgesic,[3]2,4-triazolo[2,3-a] benzimidazoles were synthesized through the reaction of 1,2-diaminobenzimidazole with carbon disulfide. The resulting 1,2,4-triazolo[2,3-a]benzimidazole-2-thione intermediate reacted with one equivalent of the alkyl halide to give the corresponding 2-alkylthio derivative 3a-g. The latters were acylated to afford the 1-acyl-2-alkylthio-1,2,4-triazolo[2,3-a]-benzimidazole derivatives 4–10 in good yields. Structures of the new compounds were verified on the basis of spectral and elemental methods of analyses. Fourteen of the prepared compounds were tested for their possible antifungal activities. Most of the tested compounds showed activity against Candida albicans and Fusarium oxysporum comparable to that of fluconazole as a reference drug. Compounds 8a, 9a, and 10d are the most active ones against most of the fungi used. Compounds 3e, 4d, 5d, 6d, 7d, 8c, 8d, 9d, and 10d were tested for their anti-inflammatory and analgesic effects; most of these compounds showed potent and significant results compared to indomethacin. Moreover, ulcerogenicity and the median lethal dose (LD50 anti-Tubercular,[4] antifungal,[5] antimicrobial,[6] anthelmintic,[7]and anticancer.[8]

Azoles are a pentatomic heterocyclic which play a crucial role in organic chemistry as constituents of biologically interesting compounds and as valuable intermediates in many synthetic processes. Such heterocyclic rings can be obtained via the 1,3-dipolar cycloaddition, which provides quick access to a huge number of natural compounds. This strategy has been used to generate an isoxazolidine ring that might not be made by classical chemistry. The isoxazolidine moiety rarely exists in the natural compounds but still represents a valuable intermediate in synthesis.[9] Compounds containing isoxazolidine ring possess crucial and interesting pharmacological effects such as antioxidant,[10] antibacterial,[11] antiviral,[12] antifungal,[13] and antiretroviral activities.[14]

MATERIALS AND METHODS

Stuart SMP3 electronic apparatus was used for melting points, FT-IR spectrums were taken using Shimadzu FT-IR spectrophotometer, ^{1}H and ^{13}C –NMR spectrums have obtained from (Brucker 400 MHz) spectrometer, (DMSO) was used as a solvent. The completion of the reaction was followed using TLC silica gel plates and the visualization using $KMnO_4$ dip and UV light.

Synthesis of 2-mercapto benzimidazole[15]



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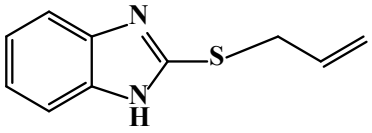
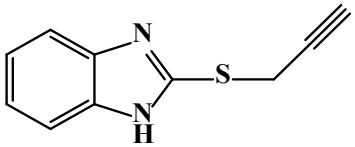
To a mixture of *o*-phenylenediamine (2.0 gm, 18.5 mmol) and potassium hydroxide (1.0 gm, 18.5 mmol) in EtOH (23 mL) and H_2O (4.5 mL), carbon disulfide (18.5 mmol, 1.0 mL) was added. The mixture was heated under reflux for 3 hours, then charcoal (0.4 gm) was added cautiously and the mixture is further refluxed for more 10 minutes. The charcoal was removed by filtration, and the solution was heated at 60-70 °C, 100 mL of warm water was added, the solution was neutralized with dilute acetic acid under vigorous stirring. Glistening white crystals was obtained and stored in a freezer for 3 hours. The product was collected and dried, and recrystallized from EtOH. Light beige crystals, m.p) 300-303 °C(, IR (KBr, cm^{-1}): N-H (3155), C–H (3113), S–H (2571), C=N (1622), C=C (1512, 1463), 80% yield.

Synthesis of 2-(allylthio)-1*H*-benzo[*d*]imidazole (B)[16]



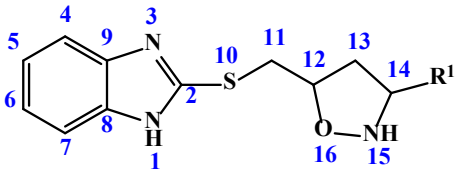
A 2-mercapto benzimidazole (2.0 gm, 12.0 mmol) and allyl bromide (4.2 mL) in EtOH (24 mL) and the reaction left under reflux. After -23 hours, the reaction allowed to cool to room temperature, the completion of the reaction was monitored by TLC plate (petroleum: ethyl acetate 7.5:2.5). The solvent was evaporated under reduced pressure, and the precipitate was triturated from ether (12 mL). Beige in color, m.p) 98-100 °C(, IR (KBr, cm^{-1}): N–H (3250), C–H (3068), C=N (1620), C=C (1517, 1452), 89% yield.

Synthesis of 2-(prop-2-yn-1-ylthio)-1*H*-benzo[*d*]imidazole



A 2-mercapto benzimidazole (2.0 gm, 12.2 mmol) and propargyl bromide (2.6 mL) in ethanol (24 mL) was refluxed for 2-1 h, the reaction monitored by TLC plate (petroleum: ethyl acetate 7.5:2.5). The solution was concentrated, and the residue was triturated with ether (12 mL). Light Brown in colour, m.p) 171-174 °C(, IR (KBr, cm^{-1}): N–H (3304), C–H (3007), C–H (3192), C=N (1620), C=C (1512, 1452), C≡C (2119), 95% yield.

Synthesis of Compounds B$_{(1\text{-}5)}$



Hydroxylamine hydrochloride (0.5 gm, 7.8 mmol) and Et_3N (1.8 mL, 13 mmol) were added to aldehyde (1.0 gm, 7.8 mmol) in PhMe (10 mL). The mixture was refluxed for 30 minutes. Then 2-(allylthio)-1*H*-benzo[*d*]imidazole (1.0 gm, 5.2 mmol) was added and mixture was left under reflux. After 15 h, the mixture allowed to cool to room temperature, the solution was concentrated. The completion of the reaction was checked on Thin-layer chromatography (TLC) plate (petroleum ether: ethyl acetate 8:2).

3-(5-(((1*H*-Benzo[*d*]imidazol-2-yl)thio)methyl) isoxazolidin-3-yl)phenol (B_1)

Beige in colour, m.p: 248-250 °C, IR (KBr, cm^{-1}): O-H (3365), N–H (3267), C–H (3047), C–H (2978, 2945), C=N (1625), C=C (1477, 1575), 70% yield.

1-(5-(((1*H*-Benzo[*d*]imidazol-2-yl)thio)methyl) isoxazolidin-3-yl) naphthalene-2-ol (B_2)

Dark Beige in colour, m.p: 230-233 °C, IR (KBr, cm^{-1}): O-H (3439), N-H (3300), Ar C-H (3057), aliphatic C- H (2978, 2945), C=N (1627), Arc C=C (1477, 1535), S-C (740), 68% yield.

5-(((1*H*-Benzo[*d*]imidazol-2-yl)thio)methyl)-3-(thiophen-2-yl)isoxazolidine (B_3)

Light Beige in colour, m.p: 97-100 °C, IR (KBr, cm^{-1}): N-Hstr (3234) aromtic C-Hstr (3065), aliphatic C- Hstr (2978, 2941), C=Nstr (1622), aromatic C=Cstr (1477, 1535), S-Cstr (740), ^{1}H-NMR (DMSO-d6) δ ppm: 3.92 (s, 2H, CH_2-) (C_{11}), 5.04 (t, 2H, CH_2-) (C_{13}), 5.30 (t, 1H, CH-) (C_{14}), 5.96 (p, 1H, CH-) (C_{12}), 10.77 (s, 1H, NH)(N_{15}), 11.88 (s, 1H, NH) (N_1), 7.84-7.11 (m, 7H, Ar-H), ^{13}C-NMR (DMSO-d6) δ ppm: 33.86 (C_{11}), 39.23 (C_{13}), 70.36 (C_{14}), 82.84 (C_{12}), 159.39 (C_2), 114.25-144.31(aromatic C), 85% yield.

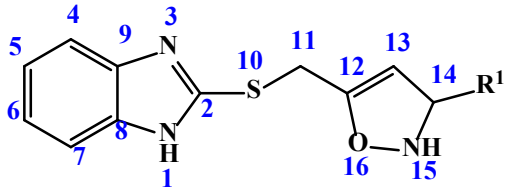
5-(((1*H*-Benzo[*d*]imidazol-2-yl)thio)methyl)-3-(4-nitrophenyl)isoxazolidine (B_4)

Beige in colour, m.p: 257-258 °C, IR (KBr, cm^{-1}): N–H (3307), C–H (3033), C–H (2978, 2943), C=N (1625), C=C (1475, 1521), NO (1398, 1562), yield: 80%.

5-(((1*H*-Benzo[*d*]imidazol-2-yl)thio)methyl)-3-(4-chlorophenyl)isoxazolidine (B_5)

Bright Whit in colour, m.p: 250-252 °C, 70% yield; IR (KBr, cm^{-1}): N–H (3433), C–H (3035), C–H (2978, 2941), C=N (1624), C=C (1477, 1537), C–Cl (713), ^{1}H-NMR (DMSO-d6) δ ppm: 3.53 (d, 2H, CH_2), 4.67 (t, 2H, CH_2), 4.87 (t, 1H, CH), 5.57 (p, 1H, CH), 10.95 (s, 1H, NH), 12.36 (s, 1H, NH), 6.68-7.72 (m, 8H, ArH), ^{13}C-NMR (DMSO-d6) δ ppm: 22.05, 35.94, 67.88, 80.22, 155.09, 119.45, 138.44.

Synthesis of the Compounds C$_{(1\text{-}5)}$



Hydroxylamine hydrochloride (0.5 gm, 7.8 mmol) and Et_3N (1.8 mL, 13 mmol) were added to aldehyde (1.0 gm, 7.8 mmol) in PhMe (10 mL) and the mixture was heated to 110°C. After 30 minutes, 2-(prop-2-yn-1-ylthio)-1*H*-benzo[*d*]imidazole (1.0 gm, 5.2 mmol) was added, then refluxed for 15 h. The solution allowed to cool to room temperature, and solvent evaporated. The reaction was monitored using TLC plate (petroleum ether: ethyl acetate 8:2).

3-(5-(((1*H*-Benzo[*d*]imidazol-2-yl)thio)methyl)-2,3-dihydroisoxazol-3-yl)phenol (C_1)

Pale Brown in colour, m.p: 242-245 °C, IR (KBr, cm^{-1}): O–H and N–H (3450), C–H (3040), C–H (2974, 2939), C=N (1635), C=C (1475, 1534), yield 76%.

2-(5-(((1*H*-Benzo[*d*]imidazol-2-yl)thio)methyl)-2,3-dihydroisoxazol-3-yl)naphthalen-1-ol (C_2)

Black in colour, m.p: 248-250 °C, IR (KBr, cm^{-1}): O–H and N–H (3452), C–H (3099), C–H (2976, 2941), C=N (1616), C=C (1477, 1554), yield 78%.

5-(((1H-Benzo[d]imidazol-2-yl)thio)methyl)-3-(thiophen-2-yl)-2,3-dihydroisoxazole (C_3)

Beige in colour, m.p: 246-248 °C, IR (KBr, cm^{-1}): N–H (3415), C–H (3010), C–H (2976, 2941), C=N (1620), C=C (1477, 1530), yield 80%.

5-(((1*H*-benzo[*d*]imidazol-2-yl)thio)methyl)-3-(4-nitrophenyl)-2,3-dihydroisoxazole (C_4)

Dark Brown in colour, m.p: 217-220 °C, 62% yield; IR (KBr, cm^{-1}): N–H (3271) C–H (3062), C–H (2976, 2941), C=N (1645), C=C (1475, 1540), ^{1}H-NMR (DMSO-d6) δ ppm: 3.82 (s, 2H, CH_2), 4.15 (d, 1H, CH), 10.05 (s, 1H, NH), 11.83 (s, 1H, NH), 6.88-8.37 (m, 9H, ArH and CH), ^{13}C-NMR (DMSO-d6) δ ppm: 44.64, 70.29, 94.80, 146.74, 141.31, 118.97-139.94.

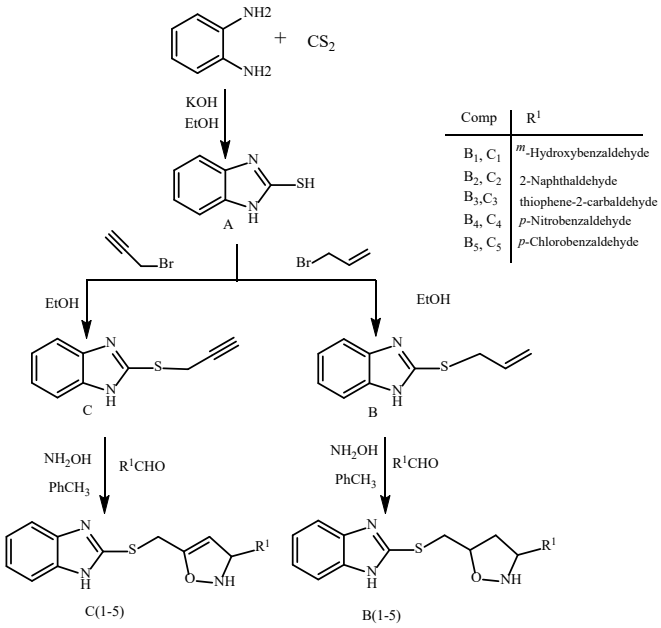
5-(((1*H*-benzo[d]imidazol-2-yl)thio)methyl)-3-(4-chlorophenyl)-2,3-dihydroisoxazole (C_5)

Beige in colour, m.p: 247-249 °C, IR (KBr, cm^{-1}): N–H (3469), C–H (3022), C–H (2976, 2941), C=N (1640), C=C (1475, 1545), 75% yield.

RESULT AND DISCUSSION

New derivatives of benzimidazole were prepared containing a ring of isoxazoline and isoxazolidine; the first step was treating 1,2-Phenylenediamine with carbon disulfide (CS_2) and NaOH in alcohol to give benzimidazole (A). Then this was reacted with allyl bromide and propargyl bromide to obtain compounds (B and C). Having compounds B and C will allow us to examine the cycloaddition reaction. To do so, treating aldehyde and hydroxylamine hydrochloride in toluene gave nitrone in situ, to this compound B and C were added, to give compounds B(1-5) and C(1-5) see Scheme 1.

The FT-IR spectrums of the compounds B(1-5) and C(1-5) shows that the aldehydic C–H peak at (1725 cm^{-1}) was disappeared. For compound B_3 the band at (3234 cm^{-1}) was referred to N–H and (1622 cm^{-1}) assigned for C=N group. In addition, ^{1}H-NMR spectra show peaks as singlet at δ 3.92 ppm and triplet at 5.04 ppm, which are related to two methylene (CH_2), and peaks as triplet at 5.30 ppm and quintet at 5.96 ppm assigned for two (CH), peak at δ 7.11-7.84 as multiplet referred to aromatic. The two N-Hs have appeared at δ 10.77 at 11.88 ppm both as singlet. Further, ^{13}C-NMR spectra of the compound B_3 indicate that all the peaks match the expected value see section method. Table 1 illustrate all the data for the synthesized compounds.



Scheme 1: General steps reactions

Table 1: Data of the Compounds

No.	*R1*	*M.p (°C)*	*M.wt (g/mole)*	*Formula*	*Colour*	*Yield %*
A		300-303	150.20	$C_7H_6N_2S$	Light beige	80%
B		98-100	190.06	$C_{10}H_{10}N_2S$	beige	89%
C		171-174	188.08	$C_{10}H_8N_2S$	Light Brown	95%
B1	m-Hydroxybenzaldehyde	248-250	327.40	$C_{17}H_{17}N_3O_3S$	Beige	70%
B2	2-Naphthaldehyde	230-233	377.12	$C_{21}H_{19}N_3O_2S$	Dark Beige	68%
B3	thiophene-2-carbaldehyde	97-100	317.43	$C_{15}H_{15}N_3OS_2$	Light Beige	85%
B4	p-Nitrobenzaldehyde	257-258	356.40	$C_{17}H_{16}N_4O_3S$	Beige	80%
B5	p-Chlorobenzaldehyde	250-252	345.85	$C_{17}H_{16}ClN_3OS$	Bright Whit	70%
C1	m-Hydroxybenzaldehyde	242-245	325,39	$C_{17}H_{15}N_3O_2S$	Pale Brown	76%
C2	2-Naphthaldehyde	248-250	375.45	$C_{21}H_{17}N_3O_2S$	Black	78%
C3	thiophene-2-carbaldehyde	246-248	315.41	$C_{15}H_{13}N_3OS_2$	Beige	80%
C4	p-Nitrobenzaldehyde	217-220	354.38	$C_{17}H_{14}N_4O_3S$	Dark Brown	62%
C5	p-Chlorobenzaldehyde	247-249	343.83	$C_{17}H_{14}ClN_3OS$	Beige	75%

Table 2: Data of antibacterial test

No.	Concentration (mg/mL)	Zone of inhibition (in mm)			
		Gram-positive		Gram-negative	
		B. subtilis	S. aureus	E. Coli	P. aeruginosa
B_5	100	-	-	-	-
	10	-	-	9	-
C_1	100	-	11	12	17
	10	-	-	-	-
C_4	100	21	18	11	11
	10	-	15	12	-
C_5	100	-	-	12	12
	10	-	-	-	-
Amoxicillin	25	15	17	-	-
Erythromycin	10	21	-	-	-
DMSO		-	-	-	-

Antibacterial Activity

The big challenging problem is the treatment of infectious diseases, and this because of important factors, including the resistance to bacteria therapy. The biological effect of the synthesized compound has screened against gram –ve and gram +ve. As shown in Table 2, some compounds showed moderate to good antibacterial activity compared with erythromycin and amoxicillin.

The antibacterial activities of the synthesized compounds were tested against (*E. coli, P. aeruginosa*) gram-ve bacteria and (*B. subtilis, S. aureus*) gram+ve bacteria using disc diffusion method. Here, Amoxicillin and Erythromycin are tested as reference antibiotics to compare the activity towards the bacteria.[16]

Clearly, Table 1 shows that the chosen strains of prepared compounds' activities have enhanced the antibacterial activity against the selected bacteria.

CONCLUSION

New benzimidazole derivatives containing isoxazoline and isoxazolidine rings were successfully synthesized using cycloaddition 1,3-dipole. This strategy includes the generation of heteroatom ring systems in one step, which saves time and effort. The compounds were confirmed by FT-IR, ^{1}H- and ^{13}C- NMR spectroscopy. The antibacterial activities of some synthesized compounds gave good results towards the selected bacteria.

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