



SYNTHESIS, CHARACTERIZATION AND ANTI-MICROBIAL SCREENING OF NOVEL HETEROCYCLIC SYSTEMS CONTAINING BRIDGEHEAD NITROGEN ATOM

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ABSTRACT

The facile synthesis of 3-substituted-2-phenyl-benzimidazo[2, 1b] pyrazolo [3,4 d] [1, 3] thiazole (**4a-j**) has been achieved by the reaction of 2-mercapto benzimidazole with chloroacetic acid. It affords benzimidazol-2-thio acetic acid (**1**), which on cyclization with a mixture of acetic anhydride and pyridine furnishes thiazolo (3, 2-a) benzimidazol-3 (2H)-one (**2**), (**2**) on condensation with different aryl aldehydes furnishes arylidene thiazolidinone (**3a-j**) followed by treatment with phenyl hydrazine in the presence of sodium acetate affords (**4a-j**). All the synthesized compounds have been supported by spectral analysis. The antimicrobial activity of synthesized compounds has also been evaluated.

Key words: Benzimidazole, Thiazolidine-4-one, Benzylidene derivatives, Pyrazolothiazolidine, Anti-microbial activity.

INTRODUCTION

The versatile uses of thiazolidinones as anaesthetics¹, anti-convulsants², amoebicides³, hypotensive⁴ and tuberculostatic agents⁵ have stimulated a considerable interest to explore the possible synthesis of new potential compounds in which the thiazolidinone ring is fused with another biologically active nucleus. With a view in achieving such a system, the pyrazolo thiazolidine was fused with benzimidazole. Benzimidazole nucleus was chosen because certain 2-mercapto benzimidazole were found to possess some anti-viral activity⁶. 3-Substituted-2-phenyl-benzimidazo[2, 1b] pyrazolo [3, 4d]

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[1, 3] thiazole (**4a-j**) has been synthesized by reaction of 2-mercapto benzimidazole with chloroacetic acid to give benzimidazol-2-thio acetic acid (**1**). It underwent cyclization on treatment with acetic anhydride furnishing thiazolo(3, 2-a) benzimidazol-3(2*H*)-one (**2**), which on condensation with different aryl aldehyde yields arylidene thiazolidinone (**3a-j**). It on further treatment with phenyl hydrazine in the presence of sodium acetate affords (**4a-j**). All the synthesized compounds have been supported by their IR and ¹H NMR spectral data and were screened for their antimicrobial activity.

EXPERIMENTAL

Materials and methods

Melting points were determined in open capillaries and are uncorrected. The purity of the compounds was ascertained by TLC on silica gel-G plate. Characterizations of synthesized compounds were done by spectral studies. IR spectra were taken in KBr on a Thermo Nicolet NEXUS-670 Spectrophotometer. ¹H NMR spectra were recorded on AVANCE-300 MHz Spectrophotometer in DMSO-*d*₆ with TMS as internal standard. The chemical shift values are in delta (ppm). Physical data and antimicrobial activities of synthesized compounds were recorded in Table 1 and Table 2, respectively.

Table 1: Physical data of Compounds

Compd.	R	M.P (°C)	Yield (%)	Molecular formula
4a	Phenyl	226-228	63	C ₂₂ H ₁₆ N ₄ S
4b	4-Hydroxyphenyl	234-235	68	C ₂₂ H ₁₆ N ₄ OS
4c	4-Hydroxy,3-methoxy phenyl	276-279	66	C ₂₃ H ₁₈ N ₄ O ₂ S
4d	Furan	237-240	69	C ₂₀ H ₁₄ N ₄ OS
4e	2 -Chlorophenyl	246-248	70	C ₂₂ H ₁₅ N ₄ SCl
4f	2-Nitrophenyl	279-281	65	C ₂₂ H ₁₅ N ₅ O ₂ S
4g	4-Methoxyphenyl	268-270	68	C ₂₃ H ₁₈ N ₄ OS
4h	4-Dimethylaminophenyl	256-258	68	C ₂₄ H ₂₁ N ₅ S
4i	3,4,5-Trimethoxyphenyl	269-271	71	C ₂₅ H ₂₂ N ₄ O ₃ S
4j	2-Hydroxyphenyl	254-257	69	C ₂₂ H ₁₆ N ₄ OS

Table 2: Antimicrobial activity data

Compds. (50 µg/ mL)	Antibacterial activity of inhibition in (mm)			Zone	Antifungal activity Zone of inhibition in (mm)	
	<i>Bacillus cereus</i> ATCC11778	<i>Staphylococcus aureus</i> ATCC9144	<i>Escherichia coli mutant</i> ATCC25922		<i>Candida albicans</i> ATCC2091	<i>Aspergillus niger</i> ATCC9 29
	4a	21	22	20		20
4b	24	23	22		21	17
4c	25	26	24		22	19
4d	22	24	21		20	18
4e	19	20	20		18	16
4f	20	21	19		19	17
4g	25	24	23		22	18
4h	26	25	25		23	20
4i	23	23	22		20	19
4j	20	21	21		19	17
Standard	33	29	31		33	28
Control DMF	-	-	-		-	-

Preparation of benzimidazol-2-thioacetic acid (1)

A mixture of 2-mercapto benzimidazole (0.01 mol), chloro acetic acid (0.01 mol) and potassium hydroxide (2 g) in ethanol (40 mL) was heated under reflux on a steam bath for 4 hr. The reaction mixture was cooled to room temperature, filtered to remove the insoluble impurities, diluted with water, acidified with dil. acetic acid and kept overnight. The solid, thus separated, was filtered, washed well with water and crystallized from ethanol as white flakes, yield 2.4 g (66%).

Preparation of thiazolo (3, 2-a) benzimidazol-3(2H)-one⁷ (2)

To (1) (1 g), pyridine (3 mL) and acetic anhydride (1.0 mL) were added and the mixture was heated on a water bath for 20 min. The reaction mixture was kept overnight and

the solid, thus obtained, was filtered, washed well with water and crystallized from ethanol to give light grey crystals; m.p. 217⁰C; yield 0.6 g (63%)

(2): IR (KBr): 2924.6 cm⁻¹ (Ar-H), 1601.68 cm⁻¹ (C=N), 1735.4 cm⁻¹ (C=O)

¹H NMR (DMSO-*d*₆): 7.2-7.9 (m, Ar-H, benzimidazole-H), 4.5 (s, S-CH₂-).

Preparation of arylidene thiazolo (3, 2-a) benzimidazol-3(2H)-one⁸ (3 a-j)

A mixture of **(2)** (0.005 mol), different aryl aldehyde (0.005 mol) and anhyd. sodium acetate (0.005 mol) in glacial acetic acid (25 mL) was refluxed on a heating mantle for 3 hr. the coloured solid that separated on cooling, was filtered, washed with water and crystallized from ethanol to give bright coloured flakes. The obtained compound was characterized by IR and ¹H NMR spectral data.

3a: IR (KBr): 3025.6 cm⁻¹ (Ar-H), 1678.28 cm⁻¹ (C=O), 1660 cm⁻¹ (C=CH), 1612.0 cm⁻¹ (C = N)

¹H NMR (DMSO-*d*₆): 6.9-7.2 (m, Ar-H, benzimidazole-H), 7.6 (s, benzylidene H).

3b: IR (KBr): 3049.7 cm⁻¹ (Ar-H), 1681 cm⁻¹ (C=O), 1654.96 cm⁻¹ (C=CH), 1621.68 cm⁻¹ (C=N), 3454 cm⁻¹ (OH)

¹H NMR (DMSO-*d*₆): 6.5-7.5 (m, Ar-H, benzimidazole-H), 7.8 (s, benzylidene H), 5.5 (s, Phenolic-OH).

3c: IR (KBr): 3027.05 cm⁻¹ (Ar-H), 1677.08 cm⁻¹ (C=O), 1651.95 cm⁻¹ (C=CH), 1601.6 cm⁻¹ (C=N), 3255 cm⁻¹ (-OH)

¹H NMR (DMSO-*d*₆): 6.2-7.4 (m, Ar-H, benzimidazole-H), 7.8 (s, benzylidene-H), 4.0 (s, O-CH₃), 5.6 (s, Phenolic-OH).

3d: IR (KBr): 3026.33 cm⁻¹ (Ar-H), 1678.93 cm⁻¹ (C=O), 1653.63 cm⁻¹ (C=CH), 1614.8 cm⁻¹ (C=N)

¹H NMR (DMSO-*d*₆): 6.2-7.6 (m, furan-H, benzimidazole-H), 7.9 (s, benzylidene-H).

3e: IR (KBr): 2983.19 cm⁻¹ (Ar-H), 1680.78 cm⁻¹ (C=O), 1654.28 cm⁻¹ (C=CH), 1622.4 cm⁻¹ (C=N)

¹H NMR (DMSO-*d*₆): 6.6-7.2 (m, Ar-H, benzimidazole-H), 7.6 (s, benzylidene H).

3f: IR (KBr): 3013.8 cm⁻¹ (Ar-H), 1681.3 cm⁻¹ (C=O), 1651 cm⁻¹ (C=CH), 1609.3 cm⁻¹ (C=N)

- ^1H NMR (DMSO- d_6): 6.5-7.4(m, Ar-H, benzimidazole-H), 7.7(s, benzylidene H).
- 3g:** IR (KBr): 3025.1 cm^{-1} (Ar-H), 1680.2 cm^{-1} (C=O), 1647.96 cm^{-1} (C=CH), 1601.53 cm^{-1} (C=N).
- ^1H NMR (DMSO- d_6): 6.7-7.5 (m, Ar-H, benzimidazole-H), 7.9 (s, benzylidene-H), 4.3 (s, O-CH₃).
- 3h:** IR (KBr): 3018.5 cm^{-1} (Ar-H), 1679.8 cm^{-1} (C=O), 1652.5 cm^{-1} (C=CH), 1617.3 cm^{-1} (C=N).
- ^1H NMR (DMSO- d_6): 6.1-7.5 (m, Ar-H, benzimidazole-H), 7.8 (s, benzylidene-H), 3.2 (s, N-(CH₃)₂),
- 3i:** IR (KBr): 3025.12 cm^{-1} (Ar-H), 1678.44 cm^{-1} (C=O), 1658.14 cm^{-1} (C=CH), 1602.7 cm^{-1} (C=N)
- ^1H NMR (DMSO- d_6): 6.3-7.5 (m, furan-H, benzimidazole-H), 7.8 (s, benzylidene-H), 4.1 (s, O-CH₃).
- 3j:** IR (KBr): 3042.1 cm^{-1} (Ar-H), 1681 cm^{-1} (C=O), 1652.6 cm^{-1} (C=CH), 1611.8 cm^{-1} (C=N), 3342 cm^{-1} (OH)
- ^1H NMR (DMSO- d_6): 6.4-7.4 (m, Ar-H, benzimidazole-H), 7.9 (s, benzylidene-H), 5.3 (s, Phenolic-OH).

Preparation of 3-substituted-2-phenyl-benzimidazo [2,1b] pyrazolo [3, 4 d] [1, 3] thiazole (4a-j)

A mixture of **3a-j** (0.0025 mol), phenyl hydrazine (0.005 mol) and anhydrous sodium acetate (0.005 mol) in glacial acetic acid (50 mL) was refluxed on a heating mantle for 5 hrs. The reaction mixture was concentrated, cooled and poured into ice cold water. The solid thus obtained was filtered, washed with water and recrystallized from ethanol to give crystals. The obtained compound was characterized by IR and ^1H NMR spectral data.

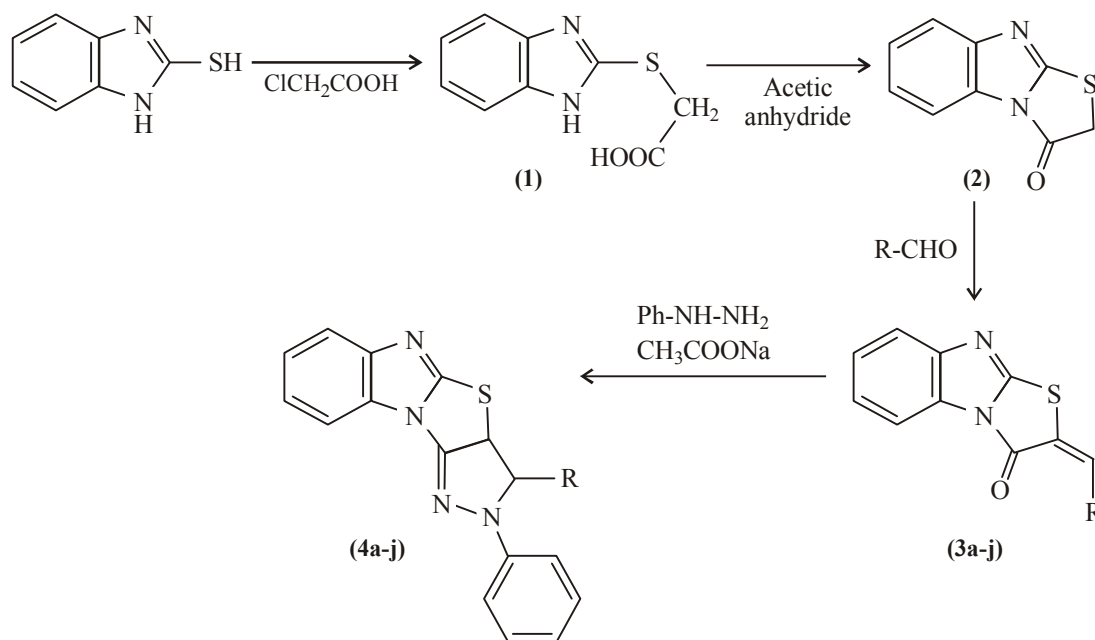
- 4a:** IR (KBr): 3058.2 cm^{-1} (Ar-H), 1604.2 cm^{-1} (C=N)
- ^1H NMR (DMSO- d_6): 7.0-7.4 (m, Ar-H, benzimidazole-H, pyrrole-H), 3.3 (d, 1H, 3'a-CH), 4.30 (d, 1H, 3'-CH).
- 4b:** IR (KBr): 2993.23 cm^{-1} (Ar-H), 1605.03 cm^{-1} (C=N), 3121.2 cm^{-1} (OH)
- ^1H NMR (DMSO- d_6): 6.2-7.5 (m, Ar-H, benzimidazole-H, pyrrole-H), 3.4 (d, 1H, 3'a-CH), 4.10 (d, 1H, 3'-CH), 5.1 (s, Phenolic-OH).

- 4c:** IR (KBr): 2992.45 cm^{-1} (Ar-H), 1605.0 cm^{-1} (C=N), 3092.65 cm^{-1} (-OH)
 ^1H NMR (DMSO- d_6): 6.8-7.7 (m, Ar-H, benzimidazole-H, pyrrole-H), 3.3 (d, 1H, 3' a-CH), 4.30 (d, 1H, 3'-CH), 3.7 (s, O-CH₃), 5.1 (s, Phenolic-OH).
- 4d:** IR (KBr): 2992.45 cm^{-1} (Ar-H), 1608.5 cm^{-1} (C=N)
 ^1H NMR (DMSO- d_6): 6.5-7.6 (m, furan-H, benzimidazole-H, pyrrole-H), 3.3 (d, 1H, 3'a-CH), 4.2 (d, 1H, 3'-CH).
- 4e:** IR (KBr): 3058.27 cm^{-1} (Ar-H), 1586.0 cm^{-1} (C=N)
 ^1H NMR (DMSO- d_6): 6.5-7.3 (m, Ar-H, benzimidazole-H, pyrrole-H), 3.2 (d, 1H, 3'a-CH), 4.31 (d, 1H, 3'-CH).
- 4f:** IR (KBr): 3026.83 cm^{-1} (Ar-H), 1602.93 cm^{-1} (C=N)
 ^1H NMR (DMSO- d_6): 6.9-7.4 (m, Ar-H, benzimidazole-H, pyrrole-H), 3.4 (d, 1H, 3' a-CH), 4.40 (d, 1H, 3'-CH).
- 4g:** IR (KBr): 2992.45 cm^{-1} (Ar-H), 1605.0 cm^{-1} (C=N).
 ^1H NMR (DMSO- d_6): 6.8-7.5 (m, Ar-H, benzimidazole-H, pyrrole-H), 3.3 (d, 1H, 3' a-CH), 4.40 (d, 1H, 3'-CH). 3.8 (s, O-CH₃).
- 4h:** IR (KBr): 2990.2 cm^{-1} (Ar-H), 1602.8 cm^{-1} (C=N).
 ^1H NMR (DMSO- d_6): 6.8-7.6 (m, Ar-H, benzimidazole-H, pyrrole-H), 3.3 (d, 1H, 3' a-CH), 4.27 (d, 1H, 3'-CH), 2.8 (s, N-(CH₃)₂),
- 4i:** IR (KBr): 2998.8 cm^{-1} (Ar-H), 1579.3 cm^{-1} (C=N)
 ^1H NMR (DMSO- d_6): 6.9-7.5 (m, Ar-H, benzimidazole-H, pyrrole-H), 3.31 (d, 1H, 3'a-CH), 4.31 (d, 1H, 7-CH), 3.9 (s, O-CH₃).
- 4j:** IR (KBr): 2992.0 cm^{-1} (Ar-H), 1589.6 cm^{-1} (C=N), 3078.8 cm^{-1} (OH)
 ^1H NMR (DMSO- d_6): 6.9-7.7 (m, Ar-H, benzimidazole-H, pyrrole-H), 3.38 (d, 1H, 3'a-CH), 4.31 (d, 1H, 3'-CH), 5.1 (s, Phenolic-OH).

Antibacterial activity

All the compounds were tested for their antibacterial activity against *Bacillus cereus* ATCC11778, *Staphylococcus aureus* ATCC9144 (Gram + ve) and *Escherichia coli* ATCC25 922 (Gram -ve) using disc diffusion method^{9,10}. DMF was run as a control and test was performed at different concentrations using a solvent DMF. Ciprofloxacin was used as a standard drug. All the pyrazolothiazolidine derivatives (**4a-j**) showed antibacterial activity.

Scheme



Anti-fungal activity

All the compounds were tested for their antifungal activity against *Candida albicans* ATCC 2091 and *Aspergillus niger* ATCC 9029 by disc diffusion method^{9,10}. DMF was run as a control and test was performed at different concentrations using a solvent DMF. Ketoconazole was used as a standard drug. All the compounds (4a-j) showed antifungal activity.

RESULTS AND DISCUSSION

The structures of the new compounds were firmly established on the basis of their IR and ^1H NMR analysis. Compounds (4a-j) showed two doublets at 3.3 ppm and 4.2 ppm, respectively for the protons at 3'a and 3' positions corroborated the cyclic structure and cis configuration. In the IR spectra of the thiazolidinones, the characteristic C=O bands appeared in the region of $1680\text{-}1640\text{ cm}^{-1}$. The strong sharp bands at $1610\text{-}1590\text{ cm}^{-1}$ corresponding to initial azomethines were absent, which was the most characteristic evidence of the cyclocondensation. In compounds (4a-j), amide carbonyl band was absent, which clearly confirmed that a cyclocondensation with phenyl hydrazine had taken place. In general, all the compounds showed antibacterial and antifungal activity. The maximum

activity was obtained, when R was substituted by a hydroxyl and methoxy group in the phenyl ring of thiazolidine nucleus (26 mm). The thiazolidine derivatives having chlorine atom showed minimum activity (16 mm). Rest of the compounds showed moderate activity. It was found that the compounds possessing electron releasing groups considerably enhanced the antimicrobial activity, when compared to the electron withdrawing substituents on the phenyl ring. In summary, a new series of potentially bioactive substituted pyrazole fused with thiazolidinyl moieties have been synthesized, having antibacterial and antifungal activities.

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