PREPARATION AND ANTIMICROBIAL STUDIES OF NOVEL FUSED HETEROCYCLIC COMPOUNDS

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ABSTRACT

The imidazole derivate say 2-(1-methyl-1H-benzo[d]imidazol-2-ylthio)acetohydrazide (1) was synthesized. Various Schiff bases (3a-e) of (1) were prepared by reacting with various benzaldehyde derivatives (2a-e). All the 3a-e compounds reacted with Succinic Anhydride to afford 1-(2-(1-methyl-1H-benzo[d]imidazol-2-ylthio) acetamido)-5-oxo-2-arylpyrrolidine-3-carboxylic acid derivatives (4a-e). These (4a-e) compounds reacted with o-phenylene diamine yields N-(3-(1H-benzo[d]imidazol-2-yl)-5-oxo-2-arylpyrrolidin-1-yl)-2-(1-methyl-1H-benzo[d]imidazol-2-ylthio) acetamide (5a-e). The Compounds (5a-e) reacted with benzaldehyde affords 4-benzylidene derivatives (6a-e), they further reacted with hydrazine hydrate gives N-(4-(1H-benzo[d]imidazol-2-yl)-5-aryl-3-phenyl-4,5-dihydropyrrolo[2,3-c]pyrazol-6(2H)-yl)-2-((1-methyl-1H-benzo[d]imidazol-2-yl)thio)acetamide (7a-e). All the synthesized compounds characterized spectroscopically and tested for antimicrobial activity.

Keywords: Benzimidazole, Pyrrolidine, Isoxazole, Characterization, Antibacterial activity, and Antifungal activities.

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INTRODUCTION

Heterocyclic compounds exhibit pharmaceutical as well as biological activity. Among them, Benzimidazole derivatives have been found to possess various biological as well as pharmacological activities, such as anti-inflammatory, analgesics, antipyretic and antifungal, anticonvulsant, antitumor, antiviral and analgesic activities.¹⁻⁷ Pyrazole display various activity like antibacterial, antifungal, antimicrobial, antidepressant, anti-tumor, anticonvulsant, anti-inflammatory and anti-amoebic activities.⁸⁻¹⁵

The present research paper deals with the synthesis of novel heterocyclic compounds, N-(4-(1H-benzo[d]imidazol-2-yl)-5-aryl-3-phenyl-4,5-dihydropyrrolo[2,3-c]pyrazol-6(2H)-yl)-2-((1-methyl-1H-benzo[d]imidazol-2-yl)thio)acetamide which contains benzimidazole and pyrazole. The synthetic route is as follows.

EXPERIMENTAL

Materials

All chemicals used were of laboratory grade. 2-(1-methyl-1H-benzo[d]imidazol-2-ylthio)acetohydrazide (1) prepared by reported research work.¹⁶

Measurement

Melting points were determined in open capillary tubes and were uncorrected. The IR spectra were recorded in KBr pellets on a Nicolet 400D spectrometer and ¹H NMR spectra were recorded in DMSO with TMS as internal standard on a Bruker spectrometer at 400 MHz.

Preparation of 1-(2-(1-methyl-1H-benzo[d]imidazol-2-ylthio) acetamido)-5-oxo-2-aryl pyrrolidine-3-carboxylic acid derivatives (4a-e)

A stoichiometric amount of 2-(1-methyl-1H-benzo[d]imidazol-2-ylthio)acetohydrazide (1) and substituted benzaldehyde (2a-e) in absolute alcohol was heated on the steam bath for 3 hours. The solid separated was filtered, washed and then reacted with succinic anhydride in p-xylene was heated and on

the steam bath for 7.5 hours. The resultant product was kept aside for 3 days. The obtained product was filtered and recrystallized by using rectified spirit and yielded 1-(2-(1-methyl-1H-benzo[d]imidazol-2-ylthio) acetamido)-5-oxo-2-aryl pyrrolidine-3-carboxylic acid derivatives (4a-e). The yields, melting points and other characterization data of these compounds are given in Table -1.

Scheme-1

Table-1: Analytical Data and Elemental Analysis of Compounds (4a-e)

Compd.	Molecular formula (Mol.wt.)	Yield %	M.P.* °C	Elemental Analysis						%S	
				%C		%H		%N			
				Found	Calcd.	Found	Calcd.	Found	Calcd.	Found	Calcd.
4a	C ₂₁ H ₂₀ N ₄ O ₄ S (424)	79	208-209	59.4	59.42	4.7	4.75	13.1	13.20	7.5	7.55
4b	C ₂₁ H ₁₉ N ₄ O ₄ SCl (457.5)	74	203-204	54.9	54.96	4.1	4.17	12.2	12.21	6.9	6.99
4c	C ₂₁ H ₁₉ N ₄ O ₄ SBr (502)	69	200-201	50.0	50.11	3.7	3.80	11.1	11.13	6.3	6.37
4d	C ₂₁ H ₁₉ N ₄ O ₄ SF (442)	72	196-198	56.9	57.00	4.3	4.33	12.6	12.66	7.2	7.25
4e	C ₂₁ H ₁₉ N ₅ O ₆ S (469)	70	192-193	53.7	53.73	4.0	4.08	14.9	14.92	6.8	6.83

* Uncorrected LC-MS data of 4a-429,4c-508

Preparation of N-(3-(1H-benzo[d]imidazol-2-yl)-4-benzylidene-5-oxo-2-arylpiperidin-1-yl)-2-(1-methyl-1H-benzo[d]imidazol-2-ylthio)acetamide (6a-e)

A mixture of o-Phenylene diamine and 1-(2-(1-methyl-1H-benzo[d]imidazol-2-ylthio) acetamido)-5-oxo-2-aryl piperidine-3-carboxylic acid derivatives (4a-e) was refluxed thermally. The reaction mixture was cooled and sodium hydroxide solution was added and then the crude product of (5a-e) was washed with ice-cold water and dissolved in boiling water for recrystallization, filtered and dried. The solution of (5a-e) and benzaldehyde in dioxane (50mL) in the presence of sodium ethoxide were refluxed for about 4-5 hrs. The resulting product was recrystallized from R-sprite to yield compound N-(3-(1H-benzo[d]imidazol-2-yl)-4-benzylidene-5-oxo-2-arylpiperidin-1-yl)-2-(1-methyl-1H-benzo[d]imidazol-2-ylthio)acetamide (6a-e). The yields, melting points and other characterization data of these compounds are given in Table -2.

Table-2: Analytical Data and Elemental Analysis of Compounds (6a-e)

Compd.	Molecular Formula (Mol.wt.)	Yield %	M.P.* °C	Elemental Analysis						%S	
				%C		%H		%N			
				Found	Calcd.	Found	Calcd.	Found	Calcd.	Found	Calcd.
6a	C ₃₄ H ₂₈ N ₆ O ₂ S (584)	76	231- 233	69.8	69.84	4.8	4.83	14.3	14.37	5.4	5.48
6b	C ₃₄ H ₂₇ N ₆ O ₂ SCl (617.5)	72	238- 239	65.9	65.96	4.3	4.40	13.5	13.57	5.1	5.18
6c	C ₃₄ H ₂₇ N ₆ O ₂ SBr (662)	66	243- 245	61.5	61.54	4.0	4.10	12.6	12.66	4.8	4.83
6d	C ₃₄ H ₂₇ N ₆ O ₂ SF (602)	70	237- 239	67.7	67.76	4.5	4.52	13.9	13.94	5.3	5.32
6e	C ₃₄ H ₂₇ N ₇ O ₄ S (629)	67	235- 236	64.8	64.85	4.3	4.32	15.5	15.57	5.0	5.09

* Uncorrected LC-MS data of 6b-626,6e-634

Preparation of N-(4-(1H-benzo[d]imidazol-2-yl)-5-aryl-3-phenyl-4,5-dihydropyrrolo[2,3-c]pyrazol-6(2H)-yl)-2-((1-methyl-1H-benzo[d]imidazol-2-yl)thio)acetamide (7a-e)

The reaction mixture of (6a-e) and hydrazine hydrate in glacialacetic acid was refluxed in a magnetic stirrer for 8-9 hrs. The completion of the reaction observed by TLC using ethyl acetate/hexane. The reaction mixture was cooled to room temperature and poured into ice-cold water, then neutralized by dilute HCl. The obtained solid was filtered, washed with water, and recrystallized from ethanol. The yields, melting points and other characterization data of these compounds are given in Table-3.

Antibacterial Activities

The antibacterial activities of all the compounds were monitored against gram-positive and gram-negative bacteria (shown in Table-4). The agar cup plate method¹⁷⁻¹⁹ was adopted. The result was measured as

inhibition zone in mm. and presented in Table-4. Examination of the result reveals that compounds 4b, 6b and 7b were more toxic for all bacteria while other compounds are toxic but less than that at 3b, 6b, and 7b.

Table-3: Analytical Data and Elemental Analysis of Compounds (7a-e)

Compd.	Molecular Formula (Mol.wt.)	Yield %	M.P.* °C	Elemental Analysis						%S	
				%C		%H		%N			
				Found	Calcd.	Found	Calcd.	Found	Calcd.	Found	Calcd.
7a	C ₃₄ H ₂₈ N ₈ OS (597)	73	268- 269	68.4	68.44	4.7	4.73	18.7	18.78	5.3	5.37
7b	C ₃₄ H ₂₇ N ₈ OSCl (631.5)	70	272- 273	64.6	64.70	4.3	4.31	17.7	17.75	5.0	5.08
7c	C ₃₄ H ₂₇ N ₈ OSBr (675)	65	267- 268	60.4	60.44	4.0	4.03	16.5	16.59	4.7	4.75
7d	C ₃₄ H ₂₇ N ₈ OSF (615)	68	271- 272	66.4	66.43	4.4	4.43	18.2	18.23	5.2	5.22
7e	C ₃₄ H ₂₇ N ₉ O ₃ S (642)	63	264- 265	63.6	63.64	4.2	4.24	19.6	19.64	4.9	5.00

* Uncorrected LC-MS data of 7a-605, 7c-679

Table-4: Antibacterial Properties of Compounds (4a-e), (6a-e) and (7a-e)

Compounds	Gram +Ve		Gram -Ve	
	<i>Bacillus subtilis</i>	<i>Staphylococcus Aureus</i>	<i>Klebsiella promioe</i>	<i>E.coli</i>
4a	09	11	10	09
4b	14	15	13	15
4c	11	12	8	11
4d	12	13	12	13
4e	11	11	9	12
6a	12	15	11	11
6b	17	19	14	17
6c	14	16	9	13
6d	15	17	13	15
6e	14	15	10	14
7a	14	17	15	14
7b	19	21	18	20
7c	16	18	13	16
7d	17	19	17	18
7e	16	17	14	17

Antifungal Activities

The antifungal activity of all three series of compounds was monitored for pathogens shown in Table-5. The method¹⁷⁻¹⁹ was adopted by using Potato-Dextrose -Agar (PDA) medium. The percentage of inhibitory growth of the fungus was measured from the area of colony growth and inhibitory. The result is shown in Table-4. The result indicates that compare 4b, 6b and 7b are more toxic due to the presence of chlorine atoms in the molecules. The fungicidal activity of all the compounds was studied at 1000 ppm concentration in vitro.

Table-5: Percentage of Inhibition Growth of Fungi by Compounds (4a-e), (6a-e) and (7a-e)

Compounds	<i>Nigrospora Sp.</i>	<i>Aspergillus Niger</i>	<i>Botrydepladia Thiobromine</i>	<i>Rhizopus Nigricum</i>
4a	54	53	50	55
4b	61	66	69	65
4c	63	65	66	69
4d	56	61	64	56
4e	58	55	53	57
6a	58	55	53	57

6b	65	68	72	67
6c	67	67	69	71
6d	60	63	67	58
6e	62	57	56	59
7a	60	56	54	59
7b	66	68	74	68
7c	66	66	70	65
7d	61	65	69	59
7e	63	58	57	60

RESULTS AND DISCUSSION

It was found that 2-(1-methyl-1H-benzo[d]imidazol-2-ylthio)acetohydrazide (1) undergoes facile condensation with substituted benzaldehyde(2a-e) to afford the corresponding Schiff's bases(3a-e). The (3a-e) react with succinic anhydride to gave 1-(2-(1-methyl-1H-benzo[d]imidazol-2-yl thio) acetamido)-5-oxo-2-aryl pyrrolidine-3-carboxylic acid derivatives (4a-e) , the structure of (4a-e) were confirmed by elemental analysis and IR spectra showing an absorption band at 1735cm^{-1} (C=O of pyrrolidine ring), $3040\text{-}3058\text{ cm}^{-1}$ (C-H, of Ar.), $1650\text{-}1670\text{ cm}^{-1}$ (-CO of -COOH,-CONH) , $1620\text{-}1640\text{ (C=N)}$, $710\text{-}720\text{ (C-S)}$, 1080 (-Cl) , 1555 , $1375\text{ (-NO}_2\text{)}$, 710 (C-Br) , 1255 (C-F) . $^1\text{H NMR}$: $7.28\text{-}7.66$ (9H,m,Ar-H), 5.15 (1H,d,C₂H of the ring), 3.37 (1H,q,C₃H of the ring), $2.55, 2.79$ (2H, d,C₄H of the ring), 12.9 (1H,s,-COOH), 11.80 (1H,s,-CONH), 4.02 (2H,s,-CH₂), 3.84 (3H,s,-CH₃). The C, H, N analysis data of all compounds are presented in Table-1.

The structures assigned to N-(3-(1H-benzo[d]imidazol-2-yl)-4-benzylidene-5-oxo-2-arylprrrolidin-1-yl)-2-(1-methyl-1H-benzo[d]imidazol-2-ylthio)acetamide (6a-e) were supported by the elemental analysis and IR spectra showing an absorption bands at 1735cm^{-1} (C=O of pyrrolidine ring), $3040\text{-}3058\text{ cm}^{-1}$ (C-H, of Ar.), $3200\text{-}3220\text{ cm}^{-1}$ (N-H), 1625cm^{-1} (-C=CH-Ar), $1650\text{-}1670\text{ cm}^{-1}$ (-CO of -CONH), $1620\text{-}1640\text{ (C=N)}$, $710\text{-}720\text{ (C-S)}$, 1080 (-Cl) , $1555, 1375\text{ (-NO}_2\text{)}$, 710 (C-Br) , 1255 (C-F) . $^1\text{H NMR}$: $7.28\text{-}7.68$ (14H,m,Ar-H), 5.15 (1H,d,C₂H of the ring), 4.80 (1H,q,C₃H of the ring), 11.80 (1H,s,-CONH), 4.02 (2H,s,-CH₂), 3.84 (3H,s,-CH₃), $7.30, 7.52$ (2H,s,=CH), 5.48 (1H,s,-NH). The C, H, N, S analysis data of all compounds are presented in Table-2. The structures assigned to N-(4-(1H-benzo[d]imidazol-2-yl)-5-aryl-3-phenyl-4,5-dihydro pyrrole [2,3-c]pyrazol-6(2H)-yl)-2-((1-methyl-1H-benzo[d]imidazol-2-yl)thio)acetamide (7a-e) were supported by the elemental analysis and IR spectra showing an absorption bands at 1735 cm^{-1} (C=O of pyrrolidine ring), $3040\text{-}3058\text{ cm}^{-1}$ (C-H, of Ar.), $3200\text{-}3220\text{cm}^{-1}$ (N-H), 1625 cm^{-1} (-C=CH), $1650\text{-}1670\text{ cm}^{-1}$ (-CO of -CONH), $1620\text{-}1640\text{ (C=N)}$, $710\text{-}720\text{ (C-S)}$, 1080 (-Cl) , 1555 , $1375\text{ (-NO}_2\text{)}$, 710 (C-Br) , 1255 (C-F) . $^1\text{H NMR}$: $7.28\text{-}8.16$ (18H,m,Ar-H), $4.75\text{-}4.80$ (2H,d, CH of the ring), 11.80 (1H,s,-CONH), $7.90, 5.48$ (2H,s,-NH), 4.02 (2H,s,-CH₂), 3.84 (3H,s,-CH₃). The C, H, N analysis data of all compounds are presented in Table-3.

The examination of elemental analytical data reveals that the elemental contents are consistent with the predicted structure shown in Scheme-1. The IR data are also directing for the assignment of the predicted structure.

CONCLUSION

In present research work, we synthesis novel fused heterocyclic compound say, N-(4-(1H-benzo[d]imidazol-2-yl)-5-aryl-3-phenyl-4,5-dihydropyrrolo[2,3-c]pyrazol-6(2H)-yl)-2-((1-methyl-1H-benzo[d]imidazol-2-yl)thio)acetamide (7a-e) from 4-benzylidene derivatives of N-(3-(1H-benzo[d]imidazol-2-yl)-5-oxo-2-arylprrrolidin-1-yl)-2-(1-methyl-1H-benzo[d]imidazol-2-ylthio)acetamide (5a-e) and hydrazine hydrate. All the novel synthesized compounds characterized spectroscopically, which assign the structure of synthesized compounds and tested for antimicrobial activity shows moderate to good toxicity.

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