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Synthesis and Biological Evaluation of Some Piperazine Derivatives as Anti-Inflammatory Agents

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ABSTRACT

Some 1-((4-methylpiperazin-1yl)methyl)-1H-benzo[d]imidazole & 1-((4-phenylpiperazin-1yl)methyl)-1H-benzo[d]imidazole derivatives were synthesized through reaction of 1-substituted piperazines with different benzimidazole derivatives in methanol yielded the corresponding mannich bases (42-a to 42-i). All the synthesized compounds were elucidated by IR, ^{1}H NMR and MASS spectroscopy. They were tested for anti-inflammatory activity using *in-vivo* (Carrageenan- induced rat paw edema model) method at a dose of 50mg/kg. result showed that compounds 42-c, 42-d and 42-h were found to be most potent in series.

Keywords: 1,4-disubstituted Piperazine, Anti-inflammatory, Mannich Base.

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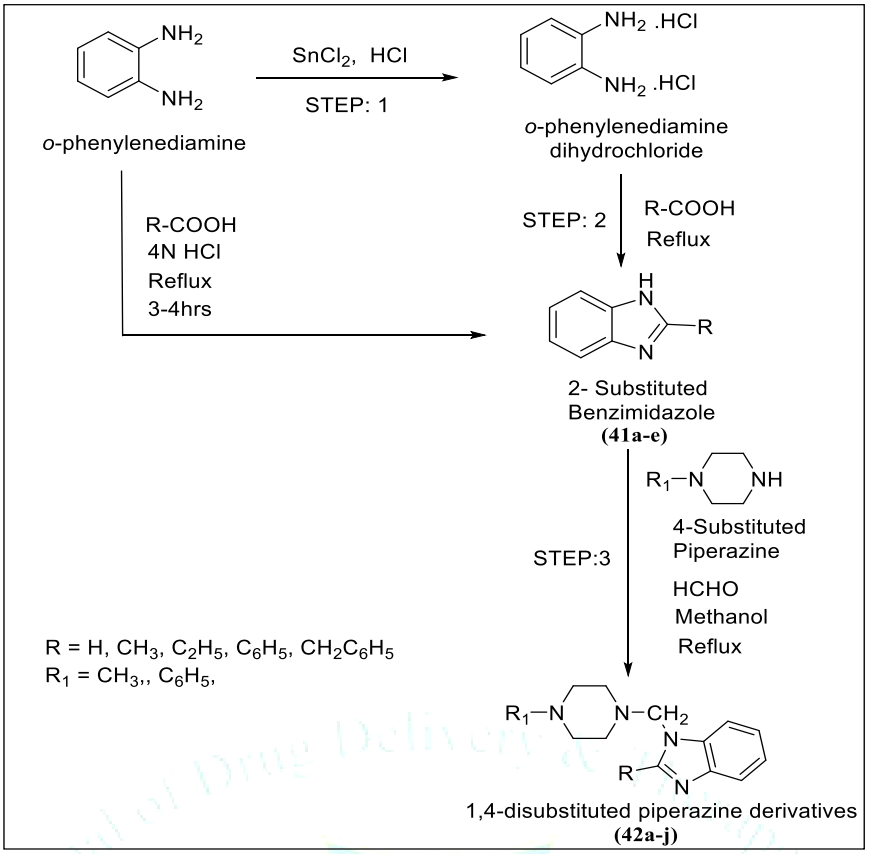
INTRODUCTION

Prostaglandins, prostacyclin (PGI2) and thromboxaneA2 (TXA2) are produced from arachidonic acid by the enzyme cyclooxegenase which exists in a constitutive (COX-1) and an inducible (COX-2) isoforms. NSAIDs acts by blocking PG generation. The classical COX inhibitors are not selective and the main adverse effects of their use are peptic ulceration, nephrotoxicity and dyspepsia.[1] In designing new drug molecules, developing safer anti-inflammatory drugs without such side effects has recently been the goal of many researchers. Piperazine derivatives have been used over the past few decades because of the wide range of activities like antimicrobials, anthelmintics, antihistamines, anti-HIV, hypotensive, anaesthetics, analgesics, anticonvulsants.[2-15]

MATERIAL AND METHODS

Synthetic Route

Schematic representation of novel piperazine derivatives is given following figure;



SCHEME-1

Figure 1: Schematic representation of novel piperazine derivatives

Synthetic Procedure

Synthetic Procedure for Preparation of Benzimidazole Derivatives (41a-e)

Synthesis of benzimidazole (41-a)[16]

0.025mol of *o*-phenylenediamine was placed in a 250ml RBF and 0.034mol of 90% formic acid was added. The mixture was heated on water bath for 2hrs. Cooled, 10% NaOH solution was added slowly with constant rotation of flask until the mixture is just alkaline to litmus. The crude benzimidazole was filtered off at the pump, washed with ice-cold water, drained well and washed again with 5ml of cold water. The crude product was dissolved in 40ml of boiling water, 0.2g of decolorizing carbon and digested for 15min. The product was filtered of rapidly at the pump through a preheated Buchner funnel and flask. The filtrate was cooled to about 10°C, benzimidazole was filtered off, washed with 5ml of cold water and dried at 100°C.

Synthesis of 2-methyl benzimidazole (41-b) & 2-benzyl benzimidazole (41-e)[16]

A mixture of 0.03mol of *o*-phenylenediamine dihydrochloride, 20ml of water 0.09mol of acetic acid/phenylacetic acid was refluxed for 2hrs.The reaction mixture was cooled and made it distinctly basic by gradual addition of concentrated ammonia solution, the precipitated product was collected and recrystallized it from 10% aqueous methanol.

Synthesis of 2-ethyl benzimidazole (41-c) [14]

A mixture of 0.03mol of *o*-phenylenediamine dihydrochloride, 20ml of water and 0.09mol of propionic acid was refluxed for 2hrs.The reaction mixture was cooled and made it distinctly basic by gradual addition of concentrated ammonia solution, the precipitated product was collected and recrystallized it from 10% aqueous methanol.

Synthesis of 2-phenyl benzimidazole (41-d) [17]

0.01mol *o*-phenylenediamine and 0.01mol of benzaldehyde were mixed in 10ml DMF, 0.003mol of sodium bisulphate was added and the mixture was stirred at 80°C for 6hr. The mixture was cooled to room temperature and added dropwise to cold water under vigorous stirring. The product separated as gummy material was extracted with ethylacetate. The extract was washed with water, brine solution, dried over sodium sulphate and evaporated. The residue thus obtained was recrystallized from ethanol to afford compound.

General procedure for the synthesis of Mannich bases (42-a-j)[14,18]

0.01mol of benzimidazole derivatives (41a-e) in 20ml of methanol was stirred at room temperature with 0.01mol of 1-methyl piperazine/1-phenyl piperazine, l ml formaldehyde solution (40%w/v) and 1ml concentrated HCl. Then the reaction mixture was refluxed for 10hrs at 70-75°C. The hot mixture was filtered and kept in refrigerator overnight. The product precipitated out and was filtered, dried and recrystallized from ethanol to give solid compound.

Biological Evaluation[19]

Albino wistar rats of either sex (150-200 g) were divided into six groups of three animals each. Animals were deprived of food for 12 hours prior to the experiment and only water was given. First group was used as a control and received 1

ml of 1 % w/v PVP suspension in saline, the second group received PVP suspension of Aspirin (50 mg/kg) orally and the remaining four groups received PVP suspension of test compounds at a dose of 50 mg/kg orally. One hour after the administration of the compounds, carrageenan suspension (0.1 ml of 1% w/v suspension in 0.9% saline solution) was injected into the sub planter region of right hind paw of the animals. Immediately, the paw thickness was measured from the ventral to the dorsal surface using a digital vernier caliper (initial paw thickness, Tc). Thereafter, the paw thickness was measured after 1 and 3hrs after carrageenan administration. Data was shown as increase in paw thickness and % inhibition of paw edema produced by treated groups.

% inhibition of paw edema by using given formula:

% Inhibition = [1- (Tt/Tc)] × 100

RESULTS

Synthesis of piperazine derivatives (42-a-j) done as per given scheme. The physical data of intermediates (41-a-e) & synthesized derivatives (42-a-j) are given in table 1 & table 2 respectively. Structures of synthesized compounds were characterized by NMR, IR, MASS spectroscopy. In the same way, the result of the biological evaluation of the prepared derivatives is also shown in table 3.

Physical characteristics of 2-substituted benzimidazole (41a-e)

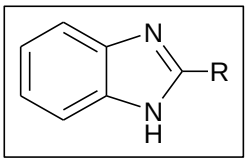


Table 1 Physical characteristics of 2-substituted benzimidazole

Code no.	R	Molecular formula	Molecular weight (g/mol)	Melting point (°C)	Yield (%)	R_f *
41-a	H	$C_7H_6N_2$	118.14	168-170	59.65	0.52
41-b	CH_3	$C_8H_8N_2$	132.16	172-174	54.75	0.56
41-c	C_2H_5	$C_9H_{10}N_2$	146.19	170-172	55.80	0.59
41-d	C_6H_5	$C_{13}H_{10}N_2$	194.23	286-288	52.68	0.61
41-e	$CH_2C_6H_5$	$C_{14}H_{12}N_2$	208.26	186-188	55.86	0.65

*Solvent system: n-hexane: ethyl acetate (3:2)

Physical characteristics of targeted compounds (42a-j)

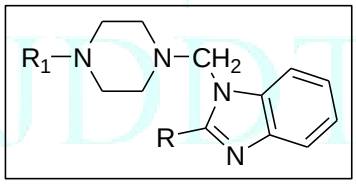


Table 2 Physical characteristics of targeted compounds

Code no.	R	R_1	Molecular formula	Molecular weight (g/mol)	Melting point (°C)	Yield (%)	R_f *
42-a	H	CH_3	$C_{13}H_{18}N_4$	230.31	230-232	47.68	0.60
42-b	CH_3	CH_3	$C_{14}H_{20}N_4$	244.34	238-240	45.66	0.62
42-c	C_2H_5	CH_3	$C_{15}H_{22}N_4$	258.36	234-236	52.86	0.65
42-d	C_6H_5	CH_3	$C_{19}H_{22}N_4$	306.40	>300	40.52	0.66
42-e	$CH_2C_6H_5$	CH_3	$C_{20}H_{24}N_4$	320.43	240-242	43.25	0.69
42-f	H	C_6H_5	$C_{18}H_{20}N_4$	292.38	236-238	44.15	0.63
42-g	CH_3	C_6H_5	$C_{19}H_{22}N_4$	306.40	242-244	42.50	0.65
42-h	C_2H_5	C_6H_5	$C_{20}H_{24}N_4$	320.43	238-240	53.56	0.67
42-i	C_6H_5	C_6H_5	$C_{24}H_{24}N_4$	368.47	>300	41.52	0.70
42-j	$CH_2C_6H_5$	C_6H_5	$C_{25}H_{26}N_4$	382.50	246-248	51.86	0.72

*Solvent system: benzene: methanol (3:2)

Spectral characteristics of targeted compounds

1-((4-methylpiperazin-1yl)methyl)-1H-benzo[d]imidazole (42-a)

(KBr) cm^{-1}: 3058 (aromatic CH stretch), 2967(aliphatic CH stretch), 1182 (C-N) 1656(C=N) ^{1}H NMR (δ, ppm): 2.15 (s, 3H, N-CH_3), 2.30 (s, 8H, 4CH_2 of piperazine), 4.81 (s, 2H, N-CH_2-N), 7.19-7.27 (m, 2H, Ar-H), 7.62 (d, 2H, Ar-H), 8.18 (s, 1H, N-CH_2=N). EIMS(m/z): 230 (M+). Elemental analysis: (found/calculated) C, 67.71/67.80; H, 7.91/7.88; N, 24.38/24.33

2-methyl-1-((4-methylpiperazin-1yl)methyl)-1H-benzo[d]imidazole (42-b)

(KBr) cm^{-1}: 3098 (aromatic CH stretch), 2971(aliphatic CH stretch), 1267 (C-N), 1615(C=N) ^{1}H NMR (δ, ppm): 2.14 (s, 3H, N-CH_3), 2.31 (s, 8H, 4CH_2 of piperazine), 2.52 (s, 3H, CH_3 of 2-methyl benzimidazole) 4.80 (s, 2H, N-CH_2-N), 7.18-7.27 (m, 2H, Ar-H), 7.52-7.64 (m, 2H, Ar-H). EIMS(m/z): 244 (M+). Elemental analysis: (found/calculated) C, 68.78/68.82; H, 8.20/8.25; N, 23.02/22.93

2-ethyl-1-((4-methylpiperazin-1yl)methyl)-1H-benzo[d]imidazole (42-c)

(KBr) cm^{-1}: 3053 (aromatic CH stretch), 2920(aliphatic CH stretch), 1255 (C-N), 1638(C=N). ^{1}H NMR (δ, ppm): 1.27 (t, 3H, CH_3 of 2-ethyl benzimidazole), 2.13 (s, 3H, N-CH_3), 2.33 (s, 8H, 4CH_2 of piperazine), 2.81 (m, 2H, CH_2 of 2-ethyl benzimidazole), 4.79 (s, 2H, N-CH_2-N), 7.17-7.26 (m, 2H, Ar-H), 7.53-7.64 (m, 2H, Ar-H) EIMS(m/z): 258 (M+). Elemental analysis: (found/calculated) C, 69.69/69.73; H, 8.60/8.58; N, 21.71/21.69

1-((4-methylpiperazin-1yl)methyl)- 2-phenyl-1H-benzo[d]imidazole (42-d)

(KBr) cm^{-1}: 759 (aromatic CH out-of-plane bend), 2925(aliphatic CH stretch), 1165 (C-N), 1626(C=N). ^{1}H NMR (δ, ppm): 2.13 (s, 3H, N-CH_3), 2.32 (s, 8H, 4CH_2 of piperazine), 4.81 (s, 2H, N-CH_2-N), 7.25-7.27 (t, 2H, Ar-H), 7.49 (t, 3H, Ar-H), 7.64-7.70 (m, 2H, Ar-H), 8.26 (d, 2H, Ar-H). EIMS(m/z): 306 (M+). Elemental analysis: (found/calculated) C, 74.44/74.48; H, 7.23/7.24; N, 18.33/18.29

2-benzyl-1-((4-methylpiperazin-1yl)methyl)-1H-benzo[d]imidazole (42-e)

(KBr) cm^{-1}: 3061 (aromatic CH stretch), 2943(aliphatic CH stretch), 1235 (C-N), 1648(C=N). ^{1}H NMR (δ, ppm): 2.14 (s, 3H, N-CH_3), 2.30 (s, 8H, 4CH_2 of piperazine), 4.00 (s, 2H, CH_2 of 2-benzyl benzimidazole), 4.80 (s, 2H, N-CH_2-N), 7.15-7.28 (m, 9H, Ar-H), 7.51 (d, 1H, Ar-H), 7.65 (d,1H, Ar-H) EIMS(m/z): 320 (M+). Elemental analysis: (found/calculated) C, 74.92/74.97; H, 7.56/7.55; N, 17.52/17.48

1-((4-phenylpiperazin-1yl)methyl)-1H-benzo[d]imidazole (42-f)

(KBr) cm^{-1}: 749 (aromatic CH), 2924(aliphatic CH stretch), 1122 (C-N), 1612(C=N). ^{1}H NMR (δ, ppm): 2.45 (t, 4H, 2CH_2 of piperazine), 3.45 (t, 4H, 2CH_2 of piperazine), 4.81 (s, 2H, N-CH_2-N), 6.80 (t, 1H, Ar-H of phenyl piperazine), 6.93 (d, 2H, Ar-H of phenyl piperazine), 7.20-7.27 (m, 4H, Ar-H), 7.61 (d, 2H, Ar-H), 8.19 (s, 1H, N-CH_2=N) EIMS(m/z): 292(M+). Elemental analysis: (found/calculated) C, 73.98/73.94; H, 6.85/6.89; N, 19.17/19.16

2-methyl-1-((4-phenylpiperazin-1yl)methyl)-1H-benzo[d]imidazole (42-g)

(KBr) cm^{-1}: 3053 (aromatic CH stretch), 2974(aliphatic CH stretch), 1271(C-N), 1622(C=N) ^{1}H NMR (δ, ppm): 2.43 (t, 4H, 2CH_2 of piperazine), 2.51 (s, 3H, CH_3 of 2-methyl benzimidazole), 3.45 (t, 4H, 2CH_2 of piperazine), 4.82 (s, 2H, N-CH_2-N), 6.77 (t, 1H, Ar-H of phenyl piperazine), 6.92 (d, 2H, Ar-H of phenyl piperazine), 7.20-7.27 (m, 4H, Ar-H), 7.51-7.63 (d, 2H, Ar-H) EIMS(m/z): 306(M+). Elemental analysis: (found/calculated) C, 74.41/74.48; H, 7.25/7.24; N, 18.34/18.29

2-ethyl-1-((4-phenylpiperazin-1yl)methyl)-1H-benzo[d]imidazole (42-h)

(KBr) cm^{-1}: 3050 (aromatic CH stretch), 2988(aliphatic CHV stretch), 1222 (C-N), 1623(C=N) ^{1}H NMR (δ, ppm): 1.28 (t, 3H, CH_3 of 2-ethyl benzimidazole), 2.45 (t, 4H, 2CH_2 of piperazine), 2.80 (m, 2H, CH_2 of 2-ethyl benzimidazole), 3.44 (t, 4H, 2CH_2 of piperazine), 4.80 (s, 2H, N-CH_2-N), 6.80 (t, 1H, Ar-H), 6.94 (d, 2H, Ar-H), 7.21-7.27 (m, 4H, Ar-H), 7.53 (d, 1H, Ar-H), 7.64 (d,1H, Ar-H) EIMS(m/z): 320(M+). Elemental analysis: (found/calculated) C, 74.88/74.97; H, 7.57/7.55; N, 17.55/17.48

2-phenyl- 1-((4-phenylpiperazin-1yl)methyl)-1H-benzo[d]imidazole (42-i)

(KBr) cm^{-1}: 3004 (aromatic CH stretch), 2835(aliphatic CH stretch), 1268(C-N), 1622(C=N) ^{1}H NMR (δ, ppm): 2.45 (t, 4H, 2CH_2 of piperazine), 3.45 (t, 4H, 2CH_2 of piperazine), 4.76 (s, 2H, N-CH_2-N), 6.83 (t, 1H, Ar-H), 6.96 (d, 2H, Ar-H), 7.20-7.27 (m, 4H, Ar-H), 7.52 (d, 3H, Ar-H), 7.63-7.69 (m, 2H, Ar-H), 8.29 (d, 2H, Ar-H) EIMS(m/z): 368(M+). Elemental analysis: (found/calculated) C, 78.18/78.23; H, 6.59/6.57; N, 15.23/15.21

2-benzyl-1-((4-phenylpiperazin-1yl)methyl)-1H-benzo[d]imidazole (42-j)

(KBr) cm^{-1}: 3063 (aromatic CH stretch), 2942(aliphatic CH stretch), 1286 (C-N), 1668(C=N). ^{1}H NMR (δ, ppm): 2.44 (t, 4H, 2CH_2 of piperazine), 3.42 (t, 4H, 2CH_2 of piperazine), 4.00 (s, 2H, CH_2 of 2-benzyl benzimidazole), 4.82 (s, 2H, N-CH_2-N), 6.80 (t, 1H, Ar-H), 6.94 (d, 2H, Ar-H), 7.16-7.28 (m, 9H, Ar-H), 7.51 (d, 1H, Ar-H), 7.65 (d,1H, Ar-H) EIMS(m/z): 382(M+). Elemental analysis: (found/calculated) C, 78.43/78.50; H, 6.87/6.85; N, 14.70/14.65

Anti-inflammatory Activity

The anti-inflammatory activity of the target compounds (42-a to 42-j) were tested by carrageenan induced inflammation in rat paw edema.

Table 3: Anti-inflammatory activity of the piperazine derivatives (42-a to 42-j)

Treatment	Dose (mg/kg)	Carrageenan induced paw edema			
		Increase in paw edema (mm)	% inhibition	Increase in paw edema (mm)	% inhibition
		After 1 hour		After 3 hour	
1	control	1.40±0.17	-	1.98±0.14	-
2 (Aspirin)	50	0.92±0.18	34.28	0.52±0.16	73.73
42-a	50	1.10±0.15	21.42	1.38±0.17	30.30
42-b	50	1.20±0.15	14.28	1.49±0.18	24.74
42-c	50	1.03±0.16	26.42	1.13±0.13	42.92
42-d	50	1.08±0.13	22.85	1.22±0.17	38.38
42-e	50	1.15±0.11	17.86	1.41±0.16	28.78
42-f	50	1.13±0.18	19.28	1.34±0.17	32.32
42-g	50	1.18±0.14	15.71	1.37±0.15	30.80
42-h	50	1.01±0.16	27.85	1.15±0.13	41.91
42-i	50	1.07±0.15	23.57	1.28±0.15	35.35
42-j	50	1.18±0.11	15.71	1.45±0.14	26.26

Number of Animals used n = 3

Dose of the test compounds and standard drug = 50 mg/kg

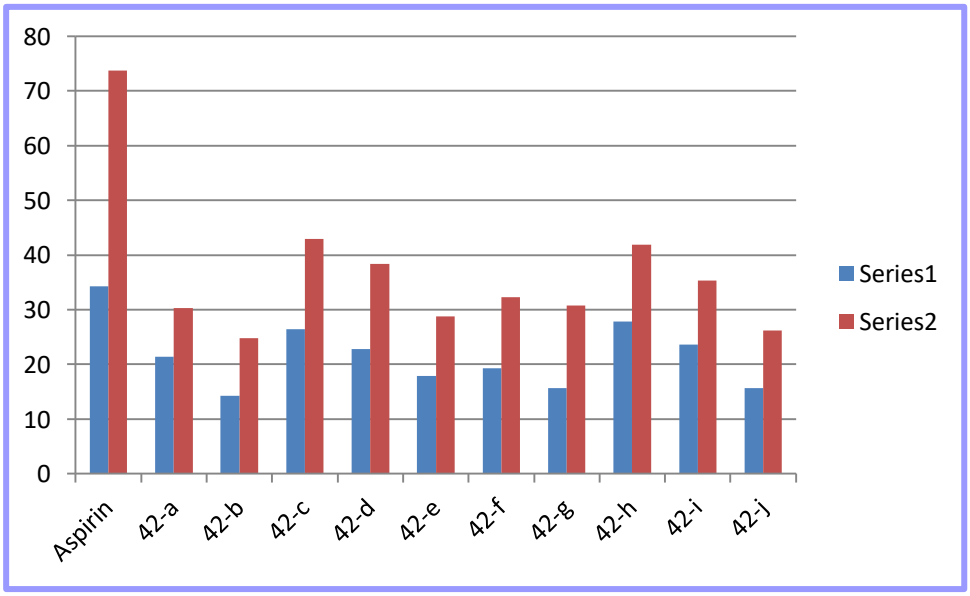


Figure 2: Column chart showing % inhibition

DISCUSSION

Compounds (42-a to 42-j) were screened for anti-inflammatory activity by inhibition of carrageenan induced rat paw edema method at the dose of 10 mg/kg. Significant anti-inflammatory activity was observed with inhibition in the range of after 3 h. Aspirin showed 73.73% inhibition after 3 h. Among all the screened compounds, 42-c & 42-hwere found to be the most potent in the series with inhibition after 3 h. Among all the screened compounds, 42-b & 42-j were found least potent in the series with respect to standard after 3 h.

CONCLUSION

In conclusion, we reported here a series of 1,4-disubstituted piperazine derivatives as anti-inflammatory agents. Synthesized compounds (42-c, 42-d &42-h) exhibit good anti-inflammatory activity but two of them (42-b and 42-i) are less potent, while another five (42-a, 42-e, 42-f, 42-g and 42-j) have significant activity. Compound (42-c) was most potent among the series of synthesized compounds as anti-inflammatory agents.

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