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Synthesis and characterization of 2-mercaptobenzimidazole derivatives as potential analgesic agents

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ABSTRACT

In seeking broad spectrum pharmacological activities of 2-mercaptobenzimidazole moiety, a series of 2-mercaptobenzimidazole derivatives were synthesized by mannich reaction and screened for analgesic activity by hot plate method and tail flick method. Totally a series of ten new 2-mercaptobenzimidazole derivatives (1A-1J) were synthesized from 2-mercaptobenzimidazole by reaction with compounds having secondary amine and formaldehyde. The purity of the synthesized compounds was checked by melting point and TLC and their structure was established by various analytical techniques such as IR and [1]HNMR spectral studies. All the synthesized compounds were exhibited significant analgesic activity when compared with standard drug, pentazocine.

Key words: 2-Mercaptobenzimidazole, Mannich reaction, Analgesic, Hot plate, Tail flick.

INTRODUCTION

In the present century, due to the advancement and changes in the culture and life style, new diseases are being existed among the human population which indicates that the search for better

drugs is still necessary. Discovery of new drugs that is therapeutically useful and goes in to clinics is a lifetime dream for medicinal chemist. Structure activity relationships of imidazole-containing structures have dominated investigations in medicinal chemistry for active biological entities [1]. Many benzimidazole derivatives are of wide interest because of their diverse biological activity such as anti-HIV [2], anthelmintic [3], antibacterial [4], antifungal [5], CNS depressant [6], analgesic [7] and anti-inflammatory [8] activities and clinical applications. 2-Mercaptobenzimidazole derivatives, one of the most important derivatives of benzimidazole are also known to possess varied biological activities. They exhibit a wide variety of interesting biological activities such as antimicrobial [9], antihistamine [10] and neutropic [11] activities. These observations have been guiding for the development of new 2-mercaptobenzimidazole derivatives that possess varied biological activities.

In recent years, Mannich bases have gained importance because of their pharmaceutical importance [12]. In this investigation, an attempt was made on incorporation of some selected compounds having secondary amine with 2-mercaptobenzimidazole by mannich reaction that this modification would improve the efficacy of the basic moiety. Thus, in this present work, a series of novel 2-mercaptobenzimidazole derivatives were synthesized and tested for their analgesic activity.

EXPERIMENTAL SECTION

Starting materials and reagents used were procured from commercial suppliers. Melting points were determined by open-ended capillary tube on Veego electrical melting point apparatus and were uncorrected. The purity of the compounds were checked by TLC using Silica Gel as stationary phase and chloroform-methanol (9:1) as eluent and the spots were visually detected in an Iodine chamber. The structure of the synthesized compounds was elucidated by IR spectra in υ_{max} (cm^{-1}) on FT-IR (Shizmadu-8400 series) using KBr disc technique and ^1H NMR spectra in δ units (ppm) relative to an internal standard of tetramethylsilane on ^1H NMR (Brucker 400 MHz) in DMSO-d_6. Elemental analyses for final compounds were performed on Carlo Erba 108 and the observed values were within the acceptable limits (±0.4%).

Procedure for the synthesis of novel 2-mercaptobenzimidazole derivatives

2-mercaptobenzimidazole was synthesized by the method described by Maw-Ling Wang *et al* [13]. Equimolar quantities (0.01 mol) of 2-mercaptobenzimidazole and the respective compounds having secondary amine were dissolved in methanol (30 mL) in a beaker under perfect ice-cold condition and stirred constantly. To this solution the respective aldehyde (0.01 mol) was added slowly and heated to reflux for 3 h. The content was kept overnight in the freezer. The corresponding crystals of mannich base of 2-mercaptobenimidazole obtained was recrystallized from alcohol [14]. The physical data and spectral data were recorded in Table 1 and Table 2 respectively. The synthetic method is depicted in Scheme 1.

SCHEME-1

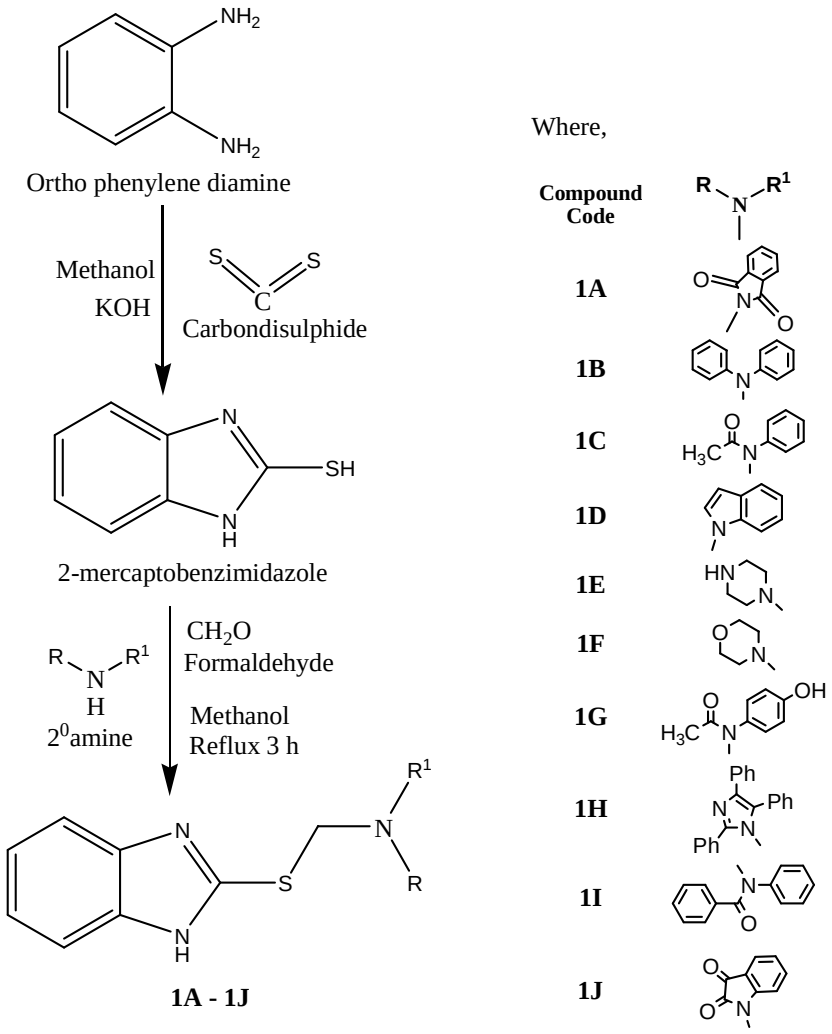

Table 1. Physical data of newly synthesized 2-mercaptobenzimidazole derivatives

Compound code	Molecular formula	M.W.	m.p.	R_f value	% yield	Elemental analysis % calculated (% found)				
						C	H	N	S	O
1A	$C_{16}H_{11}N_3O_2S$	309	143^0C	0.4836	69	62.12 (62.10)	3.58 (3.56)	13.58 (13.56)	10.37 (10.34)	10.34 (10.32)
1B	$C_{20}H_{17}N_3S$	331	135^0C	0.6211	72	72.48 (72.42)	5.17 (5.14)	12.68 (12.64)	9.67 (9.66)	-
1C	$C_{16}H_{15}N_3OS$	297	164^0C	0.3288	74	64.62 (64.58)	5.08 (5.06)	14.13 (14.10)	10.78 (10.72)	5.38 (5.34)
1D	$C_{16}H_{13}N_3S$	279	113^0C	0.5434	72	68.79 (68.74)	4.69 (4.66)	15.04 (14.98)	11.48 (11.44)	-
1E	$C_{12}H_{16}N_4S$	248	184^0C	0.7128	78	58.04 (58.00)	6.49 (6.44)	22.56 (22.52)	12.91 (12.88)	-
1F	$C_{12}H_{15}N_3OS$	249	130^0C	0.6624	76	57.81 (57.78)	6.06 (6.02)	16.85 (16.82)	12.86 (12.84)	6.42 (6.38)
1G	$C_{16}H_{15}N_3O_2S$	313	173^0C	0.4221	68	61.32	4.82	13.41	10.23	10.21

						(61.28)	(4.78)	(13.42)	(10.20)	(10.18)
1H	$C_{29}H_{22}N_4S$	458	189^0C	0.6711	66	75.95 (75.92)	4.84 (4.82)	12.22 (12.18)	6.99 (6.96)	-
1I	$C_{21}H_{17}N_3OS$	359	118^0C	0.6268	78	70.17 (70.14)	4.77 (4.76)	11.69 (11.66)	8.92 (8.88)	4.45 (4.42)
IJ	$C_{16}H_{11}N_3O_2S$	309	160^0C	0.3128	72	62.12 (62.10)	3.58 (3.56)	13.58 (13.54)	10.37 (10.34)	10.34 (10.32)

Table 2. Spectral data of newly synthesized 2-mercaptobenzimidazole derivatives

Comp. code	IR (KBr disc) υ_{max} (cm^{-1})	^{1}H NMR (DMSO-d_6) δ (ppm)
1A	3366 NH, 3061 ArCH, 1751 C=O, 1601 C=N, 1463 CH_2, 1351 C-N	5.29 (s, NH, 1H), 5.78 (s, CH_2, 2H), 7.26 - 8.06 (m, ArH, 8H)
1B	3354 NH, 3113 ArCH, 1605 C=N, 1461 CH_2, 1354 C-N	4.72 (s, CH_2, 2H), 5.12 (s, NH, 1H), 6.76 – 7.98 (m, ArH, 14H)
1C	3356 NH, 3021 ArCH, 1663 C=O, 1598 C=N, 1463 CH_2, 1423 CH_3, 1352 C-N	2.38 (s, CH_3, 3H), 5.14 (s, CH_2, 2H), 5.32 (s, NH, 1H), 7.16 – 7.76 (m, ArH, 9H)
1D	3339 NH, 3063 ArCH, 1616 C=N, 1461 CH_2, 1347 C-N	5.18 (s, NH, 1H), 5.48 (s, CH_2, 2H), 6.64 – 7.82 (m, ArH, 10H)
1E	3352 NH, 2996 ArCH, 2910 CH, 1616 C=N, 1457 CH_2, 1339 C-N	1.94 (s, NH, 1H), 2.34 (t CH_2, 4H), 2.77 (t CH_2, 4H), 4.31 (s CH_2, 2H), 5.11 (s NH 1H), 7.16 – 7.88 (m, ArH, 4H)
1F	3324 NH, 3060 ArCH, 2950 CH, 1610 C=N, 1448 CH_2, 1345 C-N	3.70 (s CH_2, 2H), 5.13 (s NH 1H), 2.76 (t CH_2, 4H), 3.70 (t CH_2, 4H), 7.27 – 7.36 (m, ArH, 4H)
1G	3453 OH, 3283 NH, 3010 ArCH, 1750 C=O, 1226 ArOH, 1359 C-N, 1447 CH_3, 1459 CH_2, 1613 C=N	2.38 (s, CH_3, 3H), 5.22 (s, OH, 1H), 5.65 (s, CH_2, 2H), 5.08 (s, NH, 1H), 6.78 – 7.42 (m, ArH, 8H)
1H	3283 NH, 3063 ArCH, 1457 CH_2, 1589 C=N, 1357 C-N	5.08 (s, NH, 1H), 5.77 (s, CH_2, 2H), 7.12 – 8.28 (m, ArH, 19H)
1I	3285 NH, 3019 ArCH, 1751 C=O, CH_2 1460, 1359 C-N, 1599 C=N	4.86 (s, CH_2, 2H), 5.75 (s, NH, 1H), 6.92 - 7.98 (m, ArH, 14H)
IJ	3349 NH, 3012 ArCH, 1729 C=O, 1462 CH_2, 1615 C=N, C-N 1333	4.88 (s, NH, 1H), 5.32 (s, CH_2, 2H), 6.94 - 8.26 (m, ArH, 8H)

Analgesic Activity

Animals

Adult albino mice of either sex (20 – 30 g) were used for this study. All the animals were housed in standard cages, at room temperature (25 ± 3°C), with 12 h dark/12 h light cycles and were fed with standard pellets and water was provided *ad libitum*. All animal experiments were conducted under the standard conditions of the Animal Scientific Procedures. The experimental protocol for animal study was approved by the institutional animal ethical committee (AKCP/CPCSEA/509/F(1h)/2007).

Acute toxicity study

The acute toxicity study described by Miller *et al* [15] was employed in the determination of the LD_{50} for the test substance. The animals were treated intraperitoneally with test compounds at a dose of 200, 400, 600, 800 and 1000 mg/kg [15]. The animals were then observed for 24 h for any behavioral effects such as nervousness, excitement, dullness, in-coordination or even death. The LD_{50} was found to be 200 mg/kg.

Hot plate method

All the newly synthesized compounds were tested for analgesic activity by Eddy's hot plate method [16]. Swiss albino mice were divided into 12 groups of six mice each. One group served as control and was administered 0.5 % (V/V) Tween 80 (0.5 mL) suspension. One group was administered the standard drug pentazocine intraperitoneally at a dose of 5 mg/kg and Tween 80

suspensions (0.5 % V/V) of test compounds (1A-1J) was administered intraperitoneally at a dose of 20 mg/kg to the remaining groups respectively, 15 min before the analgesic activity evaluation. The reaction time in seconds was noted for all the groups on Eddy's hot plates at 30, 60, 120 and 240 min. and tabulated in Table 3.

Tail flick method

The synthesized compounds were also tested for analgesic activity by Tail flick method [17]. The animals were divided into 12 groups of six mice each. The control group of animals was administered 0.5 % V/V Tween 80 (0.5 mL) suspension. The standard drug pentazocine was administered intraperitoneally at a dose of 5 mg/kg. Tween 80 suspensions (0.5% V/V) of test compounds (1A-1J) were administered intraperitoneally at a dose of 20 mg/kg to the remaining groups respectively, 15 min before the analgesic activity evaluation. The animals were held in position by a suitable restrained with the tail extending out and the tail up to 5 mm was then dipped in a beaker of water maintained at 55 ± 5^0C. The time in seconds taken to withdraw the tail clearly out of water was taken as the reaction time. A cut off point of 10 sec was observed to prevent the tail damage. The reading was recorded at 30, 60, 120 and 240 min. and tabulated in Table IV.

Statistical analysis

All the results are expressed as mean ± SEM. The values obtained for the above parameters in synthesized compounds were compared with control group using One-Way ANOVA followed by students " t " test [18]. The values of $P < 0.01$ and $P < 0.001$ were considered to indicate a significant difference between the groups.

Table 3. Evaluation of Analgesic activity of newly synthesized titled compounds by Hot plate method

Treatment	Basel Reaction Time (sec) before Treatment (Mean±SEM)	Reaction Time (sec) after administration (Mean ± SEM)			
		30 min	60 min	120 min	240 min
Control	3.33 ± 0.26	3.37 ± 0.22	3.24 ± 0.28	3.46 ± 0.32	3.42 ± 0.62
Standard	3.81 ± 0.27	10.25 ± 0.47**	12.15 ± 0.22**	13.20 ± 0.24**	13.82 ± 0.36**
1A	3.52 ± 0.38	5.86 ± 0.42*	8.32 ± 0.56**	10.48 ± 0.32**	11.64 ± 0.32**
1B	3.72 ± 0.54	5.37 ± 0.39*	7.22 ± 0.24**	9.78 ± 0.36**	10.55 ± 0.52**
1C	3.53 ± 0.45	5.02 ± 0.63*	6.38 ± 0.48*	7.82 ± 0.63**	10.22 ± 0.42**
1D	3.88 ± 0.49	5.71 ± 0.47*	7.44 ± 0.52**	8.36 ± 0.24**	9.36 ± 0.46**
1E	4.69 ± 0.56	5.92 ± 0.39*	6.08 ± 0.52*	8.56 ± 0.26**	9.68 ± 0.32**
1F	4.94 ±0.62	5.56 ± 0.34*	7.42 ± 0.42*	8.24 ± 0.42**	9.84 ± 0.28**
1G	2.18 ± 0.33	6.02 ± 0.73*	7.86 ± 0.42*	12.44 ± 0.23**	13.33 ± 0.42**
1H	3.12 ± 0.49	5.05 ± 0.41*	7.42 ± 0.44**	8.08 ± 0.32**	10.22 ± 0.42**
1I	3.03 ± 0.73	5.25 ± 0.33*	6.84 ± 0.32*	8.24 ± 0.24**	9.20 ± 0.32**
IJ	3.88 ± 0.40	4.98 ± 0.61*	5.42 ± 0.45*	8.84 ± 0.32**	9.66 ± 0.54**

***P < 0.001 and *P < 0.01 statistically (mean ±SEM) significant from control group (n=6)*

Table 4. Evaluation of Analgesic activity of newly synthesized titled compounds by Tail flick method

Treatment	Basel Reaction Time (sec) before Treatment (Mean ± SEM)	Reaction Time (sec) after administration (Mean ± SEM)			
		30 min	60 min	120 min	240 min
Control	3.33 ± 0.26	3.37 ± 0.22	3.24 ± 0.28	3.46 ± 0.32	3.42 ± 0.62
Standard	3.81 ± 0.27	8.25 ± 0.32**	9.86 ± 0.32 **	10.42 ± 0.41**	11.28 ± 0.41**
1A	3.52 ± 0.38	4.96 ± 0.43*	5.32 ± 0.44*	7.48 ± 0.32**	9.48 ± 0.21**
1B	3.72 ± 0.54	5.37 ± 0.32*	5.22 ± 0.23*	8.78 ± 0.22**	10.55 ± 0.33**
1C	3.53 ± 0.45	5.02 ± 0.23*	6.38 ± 0.51*	8.82 ± 0.28**	9.22 ± 0.39**
1D	3.88 ± 0.49	5.71 ± 0.42*	6.44 ± 0.36*	9.36 ± 0.32**	10.36 ± 0.21**

1E	3.69 ± 0.56	4.92 ± 0.36*	5.08 ± 0.24*	6.56 ± 0.41*	7.68 ± 0.65**
1F	3.44 ±0.62	5.06 ± 0.32*	5.42 ± 0.32*	6.24 ± 0.32*	8.84 ± 0.33**
1G	3.18 ± 0.33	5.02 ± 0.43*	6.86 ± 0.26**	7.44 ± 0.28**	9.64 ± 0.28**
1H	3.12 ± 0.49	5.16 ± 0.32*	7.42 ± 0.36**	7.08 ± 0.32**	9.22 ± 0.41**
1I	3.14 ± 0.73	5.75 ± 0.33*	6.84 ± 0.32**	8.24 ± 0.63**	10.20 ± 0.22**
1J	3.88 ± 0.40	4.98 ± 0.69*	7.42 ± 0.43**	8.84 ± 0.22**	11.16 ± 0.58**

***P < 0.001 and *P < 0.01 statistically (mean ±SEM) significant from control group (n=6)*

RESULTS AND DISCUSSION

Totally a series of 10 novel mannich bases of 2-mercaptobenzimidazole derivatives were synthesized. The melting points and R_f value of the synthesized compounds indicated the formation of new chemical analogues. The structure of the synthesized compounds was established by spectral (IR and ^{1}H NMR) as well as elemental analysis data. The IR and ^{1}H NMR spectrum of all the synthesized compounds showed the presence of NH band (3283 - 3356 cm^{-1}) and NH proton signal (4.88 – 5.75 ppm) of 2-mercaptobenzimidazole respectively which confirmed the reaction was not taken at 1*H* position. The IR and ^{1}H NMR spectrum of all the compounds showed the presence of CH_2 stretching (1448 - 1463 cm^{-1}) and CH_2 proton signal (3.70 – 5.78 ppm) respectively together with the absence of SH proton of 2-mercaptobenzimidazole confirmed the formation of the titled compounds.

The analgesic activity of the synthesized compounds was evaluated in albino mice by eddy's hot plate method and tail flick method. All the compounds exhibited significant analgesic activity at the dose of 20 mg/mL, i.p. when compared to standard drug pentazocine 5 mg/mL, i.p. All the tested compounds showed onset of action for analgesic activity at 30 min. and the maximum activity attained at 120 min which extended upto 240 min. in both methods evaluated. The findings of the present study revealed that the considerable variation of these effects were seen with each structural change, varying from agents that had less activity to those with high potency, and significant changes in potency resulted even from minor change in chemical structure.

CONCLUSION

By choosing proper experimental conditions, the present investigation was made to synthesize 2-mercaptobenzimidazole derivatives and investigated for their analgesic activity with the hope of discovering new structure leads serving as potential pharmacological agents. The results so far obtained with compounds as analgesic agents are very promising, since they broaden the knowledge of the activity of these versatile derivatives of 2-mercaptobenzimidazole.

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REFERENCES

[1] GW Gribble; TL Gilchrist. Progress in Heterocyclic Chemistry, Vol. 12, Elsevier, United Kingdom, **2000**: 170.
[2] EE Swayze; SM Peiris; LS Kucera; EL White; DS Wise; JC Drach; LB Townsend. *Bioorg. Med. Chem. Lett.*, **1993**, 3(4), 543-546.
[3] SB Wagh; NM Gour; SV Patil; VK Mourya. *Indian. J. Heterocycl. Chem.*, **2000**, 9(1), 227.
[4] HD Patel; AK Bhatt; HG Karadia; PS Shah; MP Parmar. *Indian. J. Heterocycl. Chem.,* **2004**, 13(1), 281.
[5] AR Mishra; RM Mishra; A. Wahab. *Indian. J. Heterocycl. Chem.*, **2003**, 13(3), 29.
[6] S Pratibha; A Mandlai; S Pritmani. *Indian J. Chem.B*, **1999**, 38(11), 1289.
[7] SA Khan; AM Nandan. *Indian. J. Heterocycl. Chem.*, **1997**, 7(3), 55.
[8] ES Lazer; MR Matteo; GJ Possanza. *J. Med. Chem.*, **1987**, 30(4), 726–729.
[9] VA Mauro; HC Silvia; VA Joao; VNS Marcus. *J. Sulfur Chem.*, **2007**, 28(1), 17–22.
[10] M Marco; B Fabrizio S Claudia; S Rivara; Z Valentina; V Federica; R Mirko; B Elisabetta; B Simona; B Vigilio; M Francesca; I Mariannina; VP Pier. *Bioorg. Med. Chem.*, **2004**, 12(4), 663-674.
[11] EV Bakhareva; MG Voronkov; MS Sorokin; VA Lopyrev; SB Seredenin; GM Gaidarov. *Pharmaceut. Chem. J.*, **1996**, 30(2), 89-91,
[12] JR Dimmock; P Kumar. *Curr. Med. Chem.*, **1997**, 4: 1-22.
[13] LW Maw; LL Biing. *J. Chin. Inst. Chem. Eng.*, 2007, 38(2), 161-167.
[14] V Rajamanickam; A Rajasekaran; M Palanivelu; K Anandarajagopal; E Ashik; N Umarani. *Int. J. Chem. Sci.*, **2008**, 6(3), 1669.
[15] LC Miller; ML Tainter. *Proc. Soc. Exp. Biol. Med.*, **1944**, 57, 261-264.
[16] RH Udupi; B Sudheendra; N Srinivasulu; V Rajesh; P Puranik. *Bull. Korean Chem.*, Soc. **2007**, 28(12), 2235-2240.
[17] SK Sahu; M Banerjee; A Samantray; C Behera; MA Azam. *Trop. J. Pharm. Res.*, **2008**; 7(2), 961-968.
[18] SK Kulkarni. Handbook of Experimental Pharmacology, Vallabh Prakashan, New Delhi, India, **1999**, 172.