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Synthesis and Biological Activities of 2-[3-(4-Morpholino)propylthio]-5-(difluromethoxy)benzimidazole Derivatives

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Benzimidazole derivatives exhibit some important pharmacological actions in animals and human beings. The synthesis and pharmacological effects of two novel synthesized compounds, 2-[3-(4-morpholino)propylthio]- 5-(difluromethoxy)benzimidazole (BM1) and 2-[3-(4-morpholino)ethylthio]-5-(difluromethoxy)benzimidazole (BM2), were described in this work. The tests showed that BM1 had better antiinflammatory effect than aspirin and better analgesia activity than indomethacin, while these two compounds had lower side effect of gastric ulcer than aspirin. Therefore, BM1 was an ideal antiinflammatory compound for further development.

Key Words: Benzimidazole derivatives, Synthesis, Antiinflammatory and Analgesia activities, Side effect of gastric ulcer.

INTRODUCTION

Inflammation is a rather complicated physiopathological process, which is often caused by multi inflammatory mediators. Non-steroidal antiinflammatory drugs (NSAIDs), such as aspirin (2-ethanoylhydroxybenzoic acid), indomethacin (1-(4-chlorobenzoyl)-5-methoxy-2-methyl- 3-in-doleacetic acid), all show excellent antiinflammatory, antinociceptive and antifebric effects and are now widely used clinically for the treatments of acute and chronic pains and stepped care of cancer pains.

Benzimidazole derivatives showed multi bioactivities such as anticonvulsion, antibiosis, antiinflammation and anticancer[1-3] and they also could be used as inhibitors of DNA toporase and HIV reverse transcriptase[4,5]. Therefore, the synthesis of new benzimidazole derivatives would be of great significant meaning for the discovery of new drugs and bioscience research.

Ten more benzimidazole derivatives had been designed and successfully synthesized for the further research and development of better antiinflammatory and analgesia activities while lower side effect of gastric ulcer[6], among which the two described in this paper *i.e.*, 2-[3-(4-morpholino)propylthio]-5-(difluromethoxy) benzimidazole (BM1) and 2-[3-(4-morpholino)ethylthio]-5-(difluromethoxy)benzimidazole (BM2) (Fig. 1). Their chemical structure had been elucidated by melting point, IR and NMR, *etc.* At the same time, compared with aspirin and indomethacin, the biological research of antiinflammatory, analgesia activities and side effect of gastric ulcer of BM1 and BM2 were also carried out. The results showed that BM1 and BM2 had obvious antiinflammatory activity, while lower side effect of gastric ulcer than

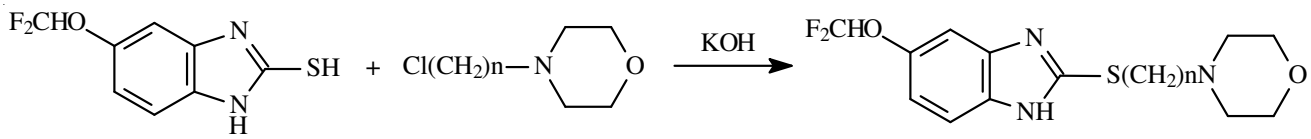

Fig. 1. Synthetic route of benzimidazole derivatives (n = 2 or 3)

aspirin. BM1's analgesia ratio was 81.9 %, better than indomethacin's 67.5 %, revealing that it could be developed into an ideal antiinflammatory drug.

EXPERIMENTAL

Melting point was carried out with the capillary tube method, without the calibration of temperature. NMR data were obtained by Bruker ARX-300. IR was carried out with Bruker IFS-55. And all used reagents were of analytical grade.

Synthesis of BM1: 8.2 g of 2-sulfhydryl-5-(difluorine-methoxy)benzimidazole, 4.3 g of KOH, 10 mL of H_2O and 20 mL of C_2H_5OH were mixed into the condition of refluence, while 10 mL C_2H_5OH solution of 4-(3-chloropropyl)-morpholine (6.2 g) was dropping into the agitated mixture. After refluencing the mixture for more 6 h, C_2H_5OH and H_2O were evaporated and the residue was resolved in H_2O. Extracted the solution with 30 mL $CHCl_3$, then evaporated the $CHCl_3$ layer. The residue was then subjected to C_2H_5OH which was saturated with HCl. Cooled down the temperature of the solution would generate white amorphous powder. The recrystallization of the above in absolute C_2H_5OH gave out the final product BM1 with the yielding rate equaling to 69 %. The relative physicochemical parameters were as the following: m.p. 128-130 ºC; IR (KBr, ν_{max}, cm^{-1}): 2915 (C-H), 2880 (C-H), 1446 (C-H), 1320 (C-H), 3321 (N-H); ^1H NMR ($CDCl_3$) δ: 7.45 (1H, d), 7.36 (1H, s), 7.18 (1H, d), 6.82 (1H, d), 4.98 (1H, s), 3.88 (4H, m), 2.93 (2H, t), 2.72 (4H, m), 2.36 (1H, d), 1.80 (2H, dd).

Synthesis of BM2: 8.6 g of 2-sulfhydryl-5-(difluorine-methoxy)benzimidazole, 4.5 g of KOH, 10 mL of H_2O and 20 mL of C_2H_5OH were mixed into the condition of refluence, while 10 mL C_2H_5OH solution of 4-(2-chloropropyl)-morpholine (6 g) was dropping into the agitated mixture. After refluencing the mixture for more 5 h, C_2H_5OH and H_2O were evaporated and the residue was resolved in H_2O. Then redoing the 1.2 procedure would generate BM2 with the yielding rate equaling to 69 %. The relative physico-chemical parameters were as the following: m.p.: 128-130 ºC; IR (KBr, ν_{max}, cm^{-1}): 2914 (C-H), 2882 (C-H), 1346 (C-H), 1325 (C-H), 3320 (N-H); ^1H NMR ($CDCl_3$) δ: 7.46 (1H, d), 7.36 (1H, s), 7.19 (1H, d), 6.81 (1H, d), 4.99 (1H, s), 3.86 (4H, m), 3.04 (2H, t), 2.75 (2H, t), 2.68 (4H, m).

Pharmacological experiments[7-9]

Reagents and experimental animals: Kunming mice, male, 21-25 g of body weight, were provided by the Animal Centre of Shenyang Pharmaceutical University. Positive drugs, *e.g.*, aspirin and indomethacin, were provided by the Department of Pharmacology of Shenyang Pharmaceutical University. Other reagents were of the analytical grade. The target compounds were suspended in 1 % carboxymethylcellulose sodium solution.

Antiinflammation experiment: Effects of *para*-xylene model: 96 male mice, 54 on day 1 and 42 on day 2, had been on a fast while free to water before 12 h of the experiment. Had been grouped randomly, the mice were given different subjects intragastricly. After 0.5 h of the administration, 50 μL of xylene was applied to the back of right ear of the mice. And after more 1 h, execute the mice for the two ears, at the same time the stomachs were also cut out and dropped in 1 % formaldehyde for further use. A 9 mm diameter auricle was then made at the symmetrical place of the two ears for the torsion balance. Swelling degree was indicated by the difference of auricles of two ears. X ± SD was used for the expression of inhibition rate and t Test was used for the determination of P value. The results were shown in Table-1.

TABLE-1
INHIBITION EFFECTS OF BM1 AND BM2 TOWARDS XYLENE-INDUCED SWELLING OF MOUSE EAR

Compound	Dosage (mg/kg)	No. of animal	Swelling degree (X ± SD, mg)	Inhibition rate (%)
Control	–	6	29.20 ± 1.47	–
Indomethacin	50	6	20.33 ± 5.13**	30.4
BM-1	200	6	20.75 ± 8.59*	28.9
BM-2	200	6	27.33 ± 4.24	6.4
Aspirin	250	6	23.40 ± 4.51*	19.9

*p < 0.05; **p < 0.01

The inhibition rate could be calculated by the following equation:

$$\text{Inhibition rate} = (A-B)/A \times 100\ \%$$

A = swelling degree of the control group; B = swelling degree of the sample group.

The results showed that BM1 and BM2 had significant antiinflammatory activities when at the dosage of 200 mg/kg.

Analgesia experiment

Effects of acetic acid stretching model: 62 Female mice had been subjected to a fast while free to water before 12 h of the experiment. The mice were given different subjects intragastricly. After 0.5 h of the administration, 0.7 % acetic acid was ip into each mouse (0.1 mL/10 g). The stretching behaviours were recorded in the following 15 min. X ± SD was used for the determination of P value. The results were shown in Table-2.

TABLE-2
INHIBITION EFFECTS OF BM1 AND BM2 TOWARDS ACETIC ACID-INDUCED STRETCHING

Compound	Dosage (mg/kg)	No. of animal	Time of stretching (X ± SD, mg)	Inhibition rate (%)
Control	–	8	13.25 ± 11.17	–
Indomethacin	50	10	4.30 ± 5.44*	67.5
BM-1	200	10	2.40 ± 4.86*	81.5
BM-2	200	10	4.40 ± 5.42*	63.8
Aspirin	250	8	0.75 ± 0.89**	94.3

*p < 0.05; **p < 0.01.

The results showed that BM1 and BM2 had obvious antinociceptive activities, while BM1 was much better than indomethacin with the analgesia percent of 81.9 to 67.5 %.

Side effect of gastric ulcer: Having dropped the stomachs got from the 2.2 experiment in 1 % formaldehyde for 24 h, the dissection and observation was made. Sliding caliper was used for the determination of length and width of each ulcer part for the ulcer index and then situation comparison of the above ulcer with the aspirin-ulcer (ig 250 mg/kg) was made (Table-3).

TABLE-3
SIDE EFFECT OF GASTRIC ULCER OF BM1 AND BM2

Compound	No. of animal	Ulcer index/mm^2 (X ± SD)	Inhibition rate (%)*
Indomethacin	6	13.83 ± 21.80	2.7
BM-1	6	157.05 ± 109.45	31.0
BM-2	6	272.26 ± 206.40	53.8
Aspirin	6	506.32 ± 120.78	–

*Rate of ulcer index of compound to aspirin.

The results showed that BM1 and BM2 had the ulcer index rate of 31.8 and 53.8 % against aspirin, respectively. This showed that BM1 had the lower side effect of gastric ulcer, which could be developed into a good antiinflammatory drug.

Conclusion

In this paper, two new benzimidazole derivatives, 2-[3-(4-morpholino)propylthio]- 5-(difluromethoxy)benzimidazole (BM1) and 2-[3-(4-morpholino) ethylthio]-5-(difluromethoxy) benzimidazole (BM2), were reported. They both possessed obvious bioactivities, while BM1 had higher inhibition rate of antiinflammation than aspirin, higher analgesia rate than indomethacin and lower side effect of gastric ulcer, reviewing that it had more perspective for further development into an antiinflammatory drug.

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