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Original Article

Synthesis and Antidepressant Activity of N-Substituted Imidazole-5-Carboxamides in Forced Swimming Test Model

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Abstract

Moclobemide is a selective and reversible monoamine oxidase-A inhibitor, which is used as an antidepressant. Three moclobemide analogues were synthesized by replacing moclobemide phenyl ring with substituted imidazoles. So, N-[(4-morpholinyl) ethyl)]-1-benzyl-2-(alkylthio)-1H-imidazole-5-carboxamides (7a-c) were synthesized and studied for the antidepressant activity using forced swimming test in mice. Analogues 7a-c were found to be more potent than moclobemide. Minimum effective doses for moclobemide and analogues 7a-c were found to be 20, 2.5, 1.25 and 2.5 mg/kg i.p. respectively.

Keywords: Imidazolecarboxamides; MAOIs; Antidepressant; Forced swimming model.

Introduction

Depression is a potentially life-threatening disorder that affects should be typed connected of people all over the world. It can occur at any age from childhood to late life and is a tremendous cost to society as it causes severe distress and disruption of life and, if left untreated, it can be fatal (1).

The pharmacotherapy of depression started in the 1950s, with prominent publications and discoveries that still govern the manner in which we treat depression. There are currently 10 to 20 different drugs marketed as antidepressants, depending on the country. Tricyclic antidepressants and monoamine oxidise inhibitors (MAOIs) were called first generation antidepressants, and selective serotonin reuptake inhibitors (SSRI) and reversible and selective inhibitors of monoamine oxidase A (RIMA) were called second generation antidepressants. Third-generation antidepressants include more recent drugs, such as mirtazapine, nefazodone, milnacipran, and reboxetine.

In the field of the antidepressant drugs, today efforts are focused towards the development of selective and reversible MAO-A inhibitors. The MAO-A inhibitors such as moclobemide are effective in the treatment of depression.

A few structure activity relationship studies have been previously reported regarding moclobemide (2, 3). In our previous work, we studied the effect of replacing morpholine ring with other heterocyclic rings (4). Recently, synthesis of the 4-arylpiperazine derivatives of moclobemide has also been reported (5). In another work, the pyrrole-2-carboxamidesincluding moclobemide analogue, N-[2-(4-morpholinyl)ethyl)] pyrrole-2-carboxamide, has been reported as as

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monoamine oxidase inhibitor (6).

Our interest in the chemistry of nitrogen containing rings and specially imidazole, which provides more sites for binding to amino acids within the proteins and enzymes, motivated us to design similar structures to moclobemide by replacing moclobemide phenyl ring with substituted imidazoles. So, N-[2-(4-morpholinyl)ethyl)-1-benzyl-2-(alkylthio)-1H-imidazole-5-carboxamides (7a-c) were synthesized and tested as antidepressant by forced swimming test model in mice.

Experimental

Chemistry

Melting points were determined by using Capillary Electrothermal apparatus and were uncorrected. The IR spectra were obtained using a Unicam SP-1100 spectrophotometer, using samples prepared as KBr disks. ^{1}H-NMR spectra were obtained using a Bruker Ac-80 spectrophotometer and chemical shifts (d) were reported as ppm relative to internal tetramethylsilane. Elemental microanalyses were carried out with a Perkin-Elmer instrument a temperature of 240 °C, and were within±0.4% of the theoretical values for C, H, and N. All solvents and reagents were purchased from the Fluka, Aldrich or Merck Chemical Company. Compounds 1-6 were synthesized as reported previously.

N-[2-(4-morpholinyl)ethyl)]-1-benzyl-2-(methylthio)-1H-imidazole-5-carboxamide (7a)

To a stirred solution of 4-(2-aminoethyl) morpholine (260 mg, 2 mmo1), in 25 ml of dry THF (tetrahydrofuran) at 0℃, 2-methylthio-1-benzylimidazole-5-carbonyl chloride 6a (800 mg, 2 mmol) was successively added under nitrogen atmosphere. The system was then stirred for 24 h, after which the precipitate was filtered. The filtrate was dried and the crude material was purified by silica gel thin layer chromatography ($CHCl_3$/EtOH = 70:30) to obtain (7a) (420 mg, 55%) with melting point of 194-197 °C. Calculated analyses for $C_{18}H_{24}N_4O_2S$ were as follows: C, 59.97; H, 6.71; N15.54. and the found results were: C, 56.73; H, 6.55; N, 15.37%; IR ν_{max}: 3432 (N-H), 1648 (CONH). ^{1}H-NMR ($CDCl_3$) δ: 8.2 (s, 1H, H-C_4 imidazole), 7.5-7.2 (m, 5H, arom), 7.0 (br, 1H, CONH), 5.7 (s, 2H, CH_2N), 5.5-5.0 (m, 6H, CH_2), 4.7-4.2 (m, 6H, CH_2), 2.8 (s, 3H, CH_3S).

N-[2-(4-morpholinyl)ethyl)]-1-benzyl-2-(ethylthio)-1H-imidazole-5-carboxamide (7b)

It was prepared from 6b**,** similar to 7a with a 60% yield and m.p of 177-179 °C; Calculated analyses for $C_{19}H_{26}N_4O_2S$ were as follows: C,

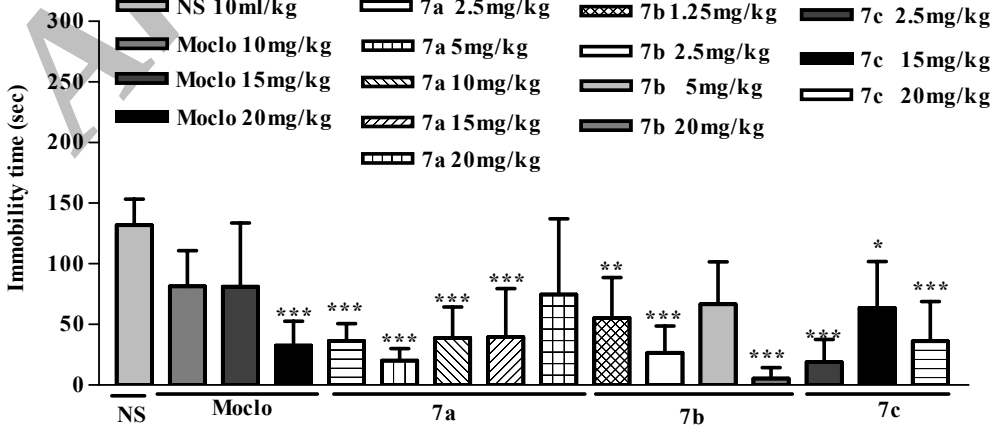


Figure 1. The effect of moclobemide (Moclo) and its analogues (**7a-c**) on immobility time in the forced swimming test. Substances were administered to mice intraperitoneally 23.5, 5 and 1h prior to test. Values are the mean ± S.E.M. for 6 mice [*P<0.05 , **P<0.01 and ***P<0.001, (by Tukey-Kramer)].

60.94; H, 7.00; N. and the found result were: C, 60.83; H, 6.84; N, 14.90%; IR ν_{max}: 3432 (N-H), 1648 (CONH). ^{1}H-NMR ($CDCl_3$) δ: 7.5 (s, 1H, H-C_4 imidazole), 7.4-7.0 (m, 5H, arom), 6.5(br, 1H, CONH), 5.6 (s, 2H, CH_2N), 3.9-2.9 (m, 8H, CH_2), 2.7-2.2 (m, 6H, CH_2N), 1.3 (t, 3H, CH_3S).

*N-[2-(4-morpholinyl)ethyl)]-1-benzyl-2-(benzylthio)-1H-imidazole-5-carboxamide (7**c**)*

It was prepared from 6c**,** similar to 7a with a 50% yield and m.p. 275-280 ℃; Calculated analyses for $C_{24}H_{28}N_4O_2S$ were as follows: C, 66.03; H, 6.46; N, 12.83. and the found result were: C, 65.92; H, 6.62; N, 12.76%; IR ν_{max}: 3430 (N-H), 1645 (CONH).^{1}H-NMR ($CDCl_3$) δ: 8.2 (s, 1H, H-C_4 imidazole), 7.6-7.1 (m, 10H, arom), 7.0 (br, 1H, CONH), 5.7 (s, 2H, CH_2N), 5.5-5.0 (m, 8H, CH_2), 4.7-4.2 (m, 6H, CH_2).

Pharmacology

Animals

Male BALB/c mice, weighing 22-25 g, were kept in the animal house of Mashhad University of Medical Sciences, in colony rooms with a 12/12 h light/dark cycle at 21±2℃. The animals had free access to food and water.

Preparation of samples

Test chemicals were dissolved in normal saline. The solutions were injected intraperitoneally in a constant volume of 10 ml/kg. Doses used are shown in Figure 1. Control animals were given the some volume of a 0.9% NaCl solution. Six mice were used for each dose.

Forced swimming test

This test was performed in a manner essentially bassed on what had previously been described (8). Groups of mice ($n = 6$) were individually introduced into a cylinder (13 cm in diameter) containing water (13 cm deep, 25℃) and left there for 15 min (habituation). The mice were then dried and returned to their home cage. Twenty four hours later, they were again placed in the cylinders containing water (17 cm deep, 25℃) and left there for 6 min; the total duration of immobility in each mouse was measured during the last 4 min (test). The mice were judged to be immobile when they ceased struggling and remained floating motionless in the water, making only those movements necessary to keep their heads above water. Test compounds were administered i.p. 23.5, 5 and 1 h before the test session. The effect of drug was evaluated by the doses that produced a statistically significant reduction in the duration of immobility as compared with control.

Acute toxicity

Different doses of extracts were injected intraperitoneally into different groups of six mice. The number of deaths were counted at 24 h after treatment. LD_{50} values and the corresponding confidence limits were determined by the Litchfield and Wilcoxon method (PHARM/PCS Version 4). The LD_{50} values are reported with 95% confidence intervals in parenthesis.

Statistical analysis

The data were expressed as mean ± SEM and tested by analysis of variance followed by the multiple comparison test of Tukey-Kramer.

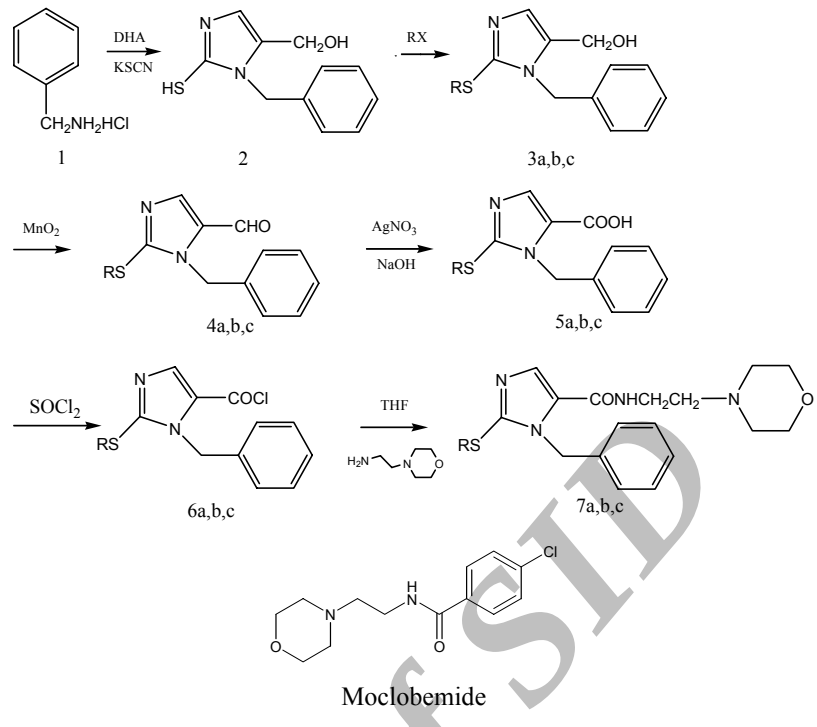
Results and Discussion

Chemistry

Compounds 2-6 were synthesized as previously reported (7). Benzylamine hydrochloride (1) was stirred with 1, 3-dihydroxyacetone dimmer and potassium thiocyanate to give 5-hydroxymethyl-2-mercapto-1-benzylimidazole (2). Subsequent alkylation of compound 2 with alkyl halides resulted in 2-alkylthio-1-benzyl-5-hydroxymethylimidazole (3). Oxidation of 3 with manganese dioxide gave 4, which was further oxidized by being boiled in alkaline solution of silver nitrate to give 2-alkylthio-1-benzylimidazole-5-carboxylic acid (5). Compound **5** was converted to its acid halide (6). 2-Morpholinoethylamie was added dropwise to a solution of 6 in dry THF (tetrahydrofuran) to give N-[2-(4-morpholinyl)ethyl)]-1-benzyl-2-(alkylthio)-1H-imidazole-5-carboxamides (7a-c) (scheme 1).

Pharmacology

The forced swim ming test (FST) was



Scheme 1. a) R = CH_3 b) R = C_2H_5 c) R = $CH_2C_6H_5$

developed by Porsolt and colleagues (8) in the rat and, subsequently, in the mouse. This test is the most widely used tool for assessing antidepressant activity preclinically (9).

The compounds were tested by the porsolt forced swimming test in order to identify potential antidepressant activity. Moclobemide was used as a standard antidepressant. The results are shown in Figure 1. Moclobemide reduced the duration of immobility at 20 mg/kg i.p., and was inactive at lower doses of 10 and 15 mg/kg i.p. According to previous reports moclobemide is inactive in this test at doses of lower than 30 mg/kg i.p. and is active at higher doses(10)**.** Analogue 7a was effective at doses of 2.5, 5, 10 and 15 mg/kg i.p. and had no significant effect at 20 mg/kg i.p. Analogue 7b reduced immobility at 1.25, 2.5 and 20 mg/kg i.p. and was ineffective at 5 mg/kg i.p. Analogue 7c was found to be effective at all doses, i.e. 2.5, 15 and 20 mg/kg i.p.

The present investigation demonstrated that analogues 7a-c were more potent than moclobemide in forced swimming test model.

Acute toxicity

LD_{50} values of moclobemide and analogues 7a,b were 522.6 mg/kg, i.p. (447.1- 610.7), 149.1 mg/kg, i.p. (134.5-165.3), and 196.0 mg/kg, i.p. (180.7-212.6), respectively. LD_{50} for 7c was not determined. According to previous reports LD_{50} of moclobemide is 730 mg/kg in mice. So, with respect to LD_{50} values, analogues 7a, b were more toxic than moclobemide.

In conclusion, replacement of electron deficient 4-chlorophenyl in moclobemide with substituted electron-deficient ring imidazole in analogues 7a-c increased antidepressant potency and also toxicity.

Acknowledgement

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