Bulgarian Chemical Communications, Volume 47, Number 2 (pp. 626 - 630) 2015

Synthesis and anti-inflammatory evaluation of novel piperazine derivatives of mefenamic acid

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Received February 24, 2014; revised May 24, 2014

In order to explore novel anti-inflammatory agents, some novel mefenamic acid derivatives as potential non-steroidal anti-inflammatory agents (NSAIDs) were synthesized and characterized by IR, ^{1}H-NMR, ^{13}C-NMR, mass and elemental analysis. The anti-inflammatory activities of the target compounds were evaluated *via* the croton oil-induced ear oedema test in mice. According to screened results, compounds **I** and **II** show potential anti-inflammatory activity comparable to aspirin.

Keywords: Non-steroidal anti-inflammatory drugs (NSAIDs), Mefenamic acid derivatives, Cyclooxygenase (COX).

INTRODUCTION

Inflammation is defined as a complex series of tissue changes that result in pain and fever [1]. During the last three decades the development of non-steroidal anti-inflammatory drugs (NSAIDs) has shown to be one of the major advancements in chemotherapeutical research [2,3]. These agents are among the most widely used drugs worldwide and represent a mainstay in the therapy of acute and chronic pain, fever and inflammation by blocking the formation of prostaglandins (PGs). PGs are well-known as the mediators of inflammation, pain and swelling. They are produced by the action of cyclooxygenase (COX) enzyme on arachidonic acid. COX is the principal target of NSAIDs. Pharmacological inhibition of COX can provide relief from symptoms of inflammation and pain. NSAIDs, such as aspirin and ibuprofen, exert their effects through inhibition of COX. The three main groups of prostanoids: prostaglandins, prostacyclins and thromboxanes are each involved in the inflammatory response. In the 1990s, researchers discovered that two different COX enzymes existed, now known as COX-1 and COX-2. Cyclooxygenase-1 (COX-1) is known to be present in most tissues. In the gastrointestinal tract, COX-1 maintains the normal lining of the stomach. The enzyme is also involved in kidney and platelet function. Cyclooxygenase-2 (COX-2) is primarily present at sites of inflammation. While both COX-1 and COX-2 convert arachidonic acid to prostaglandin, resulting in pain and inflammation, their other functions make inhibition of COX-1 undesirable while inhibiton of COX-2 is considered desirable [4, 5]. Selective COX-2 inhibitors are still under development [6,7]; they were proposed as drugs with higher selectivity for COX-2 tending to induce cardiovascular disease [8-10]. The present study aimed at design, synthesis and preliminary evaluation of new mefenamic acid derivatives as potential NSAIDs.

EXPERIMENTAL

Material and equipments

All chemicals and solvents were purchased from Merck (Darmstadt, Germany) and Sigma-Aldrich Chemical Co. (U.S.A.). Melting points (uncorrected) were determined with a digital Electrothermal melting point apparatus **(**model 9100**,** Electrothermal Engineering Ltd**.,** Essex, UK**).** ^{1}H and ^{13}C-NMR spectra were recorded with a Bruker 300 MHz (model AMX, Karlsruhe, Germany) spectrometer (internal reference: TMS). IR spectra were recorded with a Thermo Nicolet FT-IR (model Nexus-870, Nicolet Instrument Corp., Madison, Wisconsin, U.S.A.) spectrometer. Mass spectra were recorded with an Agilent Technologies 5973, mass selective detector (MSD) spectrometer (Wilmington, USA). Elemental analyses were carried out using a Perkin-Elmer, CHN elemental analyzer model 2400.

Synthesis of 2-(2, 3-dimethylphenylamino) benzoic anhydride (I)

Mefenamic acid (5.0 g, 0.02 mol) was dissolved in THF (30 ml), and dicyclohexyl carbodiimide (DCCI) (2.06 g, 0.01 mol) was added. The reaction

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mixture was continuously stirred at room temperature for 4 h. The reaction was completed (monitored by TLC) and a white precipitate of dicyclohexylurea (DCU) was formed and separated by filtration. The filtrate was evaporated under vacuum to obtain compound (**I**) [11, 12].

White-gray powder; 73% yield; m.p. 142-144 ^{o}C; IR (KBr, cm^{-1}): 3330, 2921, 2845, 1800, 1752, 1625, 1530, 1522, 1275, 1169.

General procedure for the synthesis of (II-IV)

To a solution of the appropriate 1-(2-substituted phenyl) piperazine (0.01 mol) in dioxane (20 ml) were added: compound **I** (2.5 g, 0.005 mol), zinc powder (catalyst, 0.01 g), glacial acetic acid (0.5 ml) and 10 mg benzyltriethylammonium chloride as phase transfer catalysts. The reaction mixture was gently refluxed for 90 min. After reaction completion (monitored by TLC), the reaction mixture was neutralized, extracted with ethyl acetate or brine, dried over $MgSO_4$ and evaporated under vacuum. Re-crystallization from ethyl acetate-petroleum ether (60-80 ^{o}C) mixture was carried out to obtain the desired compounds (II-IV).

[2-(2,3-Dimethyl-phenylamino)-phenyl]-(4-phenyl-piperazin-1-yl)-methanone (II)

White-light brown powder, m.p. 202-204 ^{o}C; 68% yield; IR (KBr, cm^{-1}): 3340, 3080, 2850, 2851, 1690, 1600, 1524, 1468, 1389, 1316, 1250, 973, 790. ^{1}H-NMR ($CDCl_3$) (ppm): 2.15 (6H, m, CH_3), 3.39 (4H, m, CH_2), 3.85 (4H, m, CH_2), 6.3-7.7 (12H, m, phenyl), 10.57 (br, s, NH). ^{13}C-NMR ($CDCl_3$) (ppm): 6.6, 12.4, 48.4, 54.6, 57.5, 113.0, 117.9, 118.5, 118.6, 121.2, 122.3, 126.4, 127.9, 130.1, 131.9, 145.2, 145.7, 168.7. Anal. Calcd. for $C_{25}H_{27}N_3O$: C, 77.89; H, 7.06; N, 10.90. Found: C, 77.75; H, 7.10; N, 10.83. MS: m/z (regulatory intensity): 385 (100), 386 (30).

[2-(2,3-Dimethyl-phenylamino)-phenyl]-(4-pyridin-2-yl-piperazin-1-yl)-methanone (III)

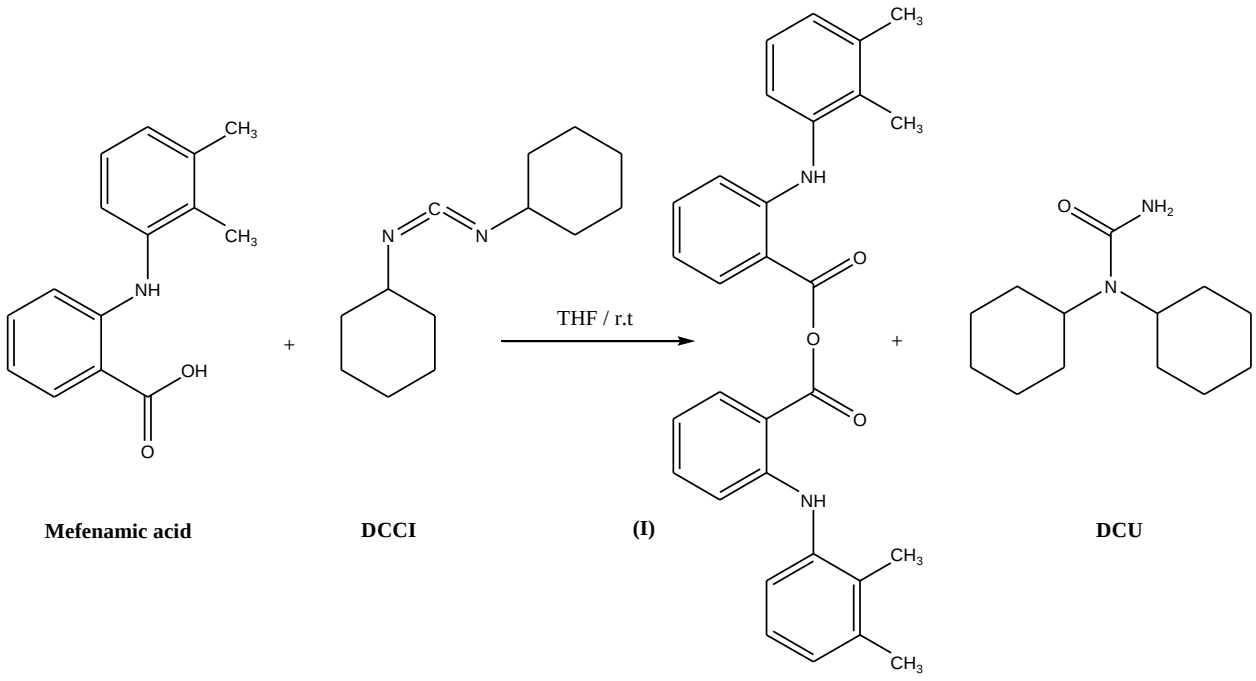
White-light yellow powder, m.p. 192-194 ^{o}C; 63% yield; IR (KBr, cm^{-1}): 3330, 3095, 2978, 2851, 1698, 1625, 1554, 1458, 1389, 1316, 1266, 1118, 780. ^{1}H-NMR ($CDCl_3$) (ppm): 2.10 (6H, m, CH_3), 3.41 (4H, m, CH_2), 3.77 (4H, m, CH_2), 6.45-8.4 (11H, m, phenyl), 10.62 (br, s, NH). ^{13}C-NMR ($CDCl_3$) (ppm): 5.9, 13.3, 49.4, 55.6, 60.5, 115.0, 119.9, 121.5, 123.6, 124.2, 125.3, 126.4, 127.9, 133.1, 135.9, 146.2, 149.7, 170.1. Anal. Calcd. for $C_{24}H_{26}N_4O$: C, 74.58; H, 6.78; N, 14.50. Found: C, 74.65; H, 6.80; N, 14.43. MS: m/z (regulatory intensity): 386 (100).

[2-(2,3-Dimethyl-phenylamino)-phenyl]-(2,3,5,6-tetrahydro-[1,2']bipyrazinyl-4-yl) methanone (IV)

White-light orange powder, m.p. 188-190 ^{o}C; 59 % yield; IR (KBr, cm^{-1}): 3430, 3065, 2958, 2833, 1675, 1625, 1502, 1458, 1378, 1330, 1226, 1118, 750. ^{1}H-NMR ($CDCl_3$) (ppm): 2.18 (6H, m, CH_3), 3.44 (4H, m, CH_2), 3.69 (4H, m, CH_2), 6.35-7.95 (10H, m, phenyl), 10.70 (br, s, NH). ^{13}C-NMR ($CDCl_3$) (ppm): 6.1, 12.3, 50.1, 54.9, 61.7, 116.3, 119.3, 122.3, 123.8, 124.1, 125.8, 126.7, 127.1, 134.2, 137.1, 147.1, 148.3, 169.5. Anal. Calcd. for $C_{23}H_{25}N_5O$: C, 71.29; H, 6.50; N, 18.07. Found: C, 71.35; H, 6.55; N, 17.95. MS: m/z (regulatory intensity): 387 (100).

Pharmacological Methods

The topical anti-inflammatory activity was evaluated as inhibition of the croton oil-induced ear oedema in Swiss mice [13]. Male mice (18-22 g), at the beginning of the experiment, were randomly housed in a temperature-controlled colony room under 12 h light/dark cycle. Rats were given free access to water and standard laboratory rat chow. All experiments were conducted between 7 a.m. and 7 p.m., under normal room light at 25^{o}C. Groups (each group containing 10 mice) were used in all tests. The tested compounds and the reference drug were suspended in 0.5% sodium carboxymethyl cellulose (CMC). Inflammation was always induced in the late morning (10 a.m.-12 p.m.). Mice were anaesthetized with ketamine hydrochloride (145 mg/kg, intra-peritoneally) and inflammatory response was induced on the inner surface of the right ear (surface: about 1 cm^2) by application of 20 μL of 2% croton oil suspended in 42% aqueous ethanol. Control animals received only the irritant, whereas the other animals received the irritant together with the tested substances. At the maximum of the oedematous response, 6 h later, mice were sacrificed and a plug (φ=8 mm) was removed from both the treated (right) and the untreated (left) ears. Oedema was measured as the mass difference between the two plugs. The anti-inflammatory activity was expressed as the percentage of oedema reduction in treated mice compared to that in the control mice. As reference, the non-steroidal anti-inflammatory drug ibuprofen was used. The results were expressed as mean±SD and statistical analysis was performed by means of Student's t-test or by one-way analysis of variance followed by the Dunnett's test for multiple comparisons of unpaired data. Statistically, a P value of less than 0.05 was considered to be significant and a P value of less than 0.01 was considered to be very significant.



Scheme 1. Scheme of the synthesis of intermediate (**I**)

RESULTS AND DISCUSSION

Chemistry

New piperazine derivatives of mefenamic acid (**II-IV**) were successfully synthesized as shown in Schemes 1 and 2. The conversion of the carboxylic acid group of mefenamic acid to benzamide group by conjugating the selected moiety of the heterocyclic compounds may produce new non-steroidal anti-inflammatory agents. Spectroscopic (IR, ^{1}H and ^{13}C-NMR, mass) and elemental (CHN) data confirmed the structure of the synthesized compounds. The purity of every compound was checked by TLC with ethyl acetate-hexane as the eluent. Preliminary pharmacological evaluation was done for the designed compounds.

Biological Evaluation

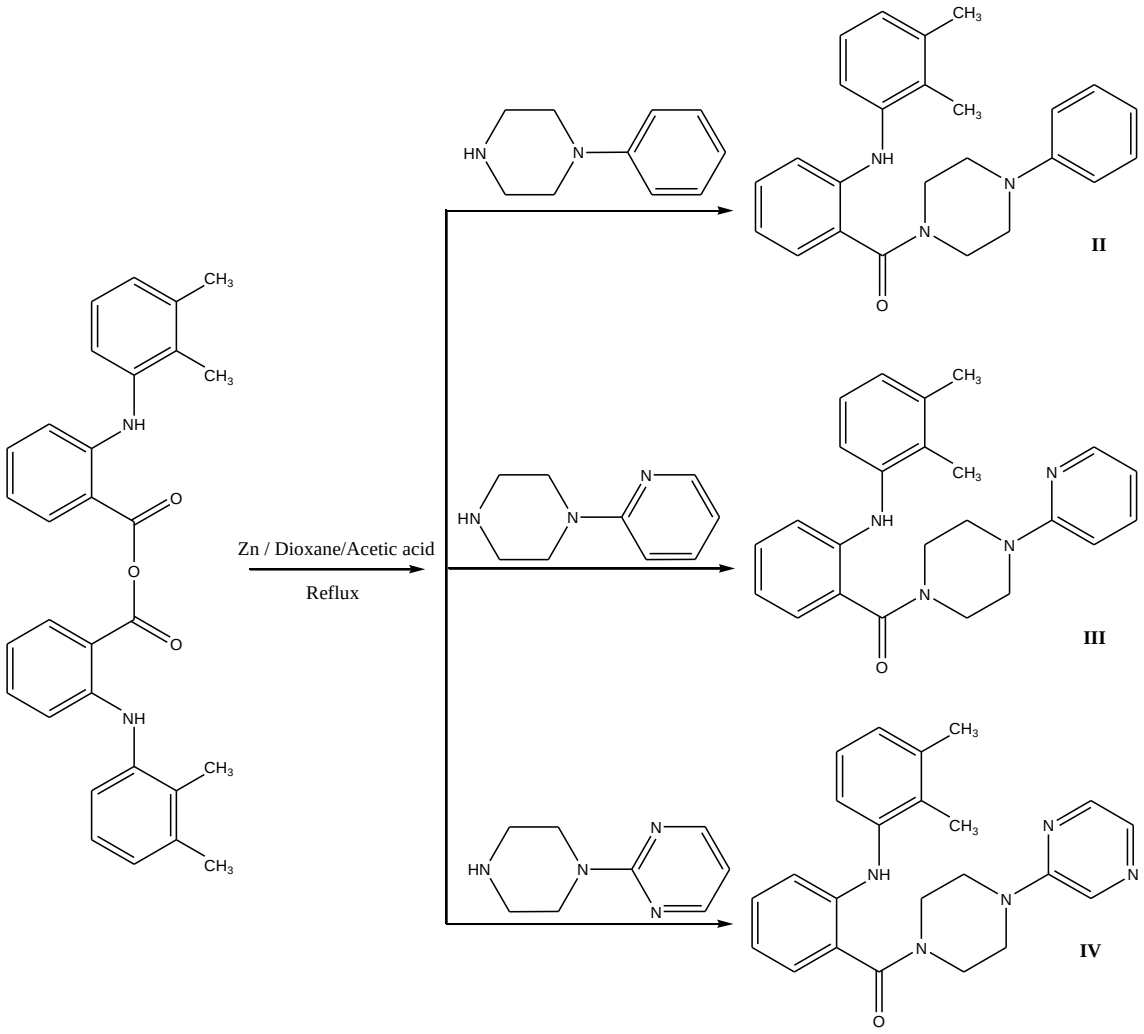
The anti-inflammatory effects of the target compounds were investigated by the croton oil-induced ear oedema test, and the results show that some target compounds induced oedema reduction. It was found that these compounds exhibit anti-inflammatory effects comparable to that of aspirin (Table 1).

Table 1. Anti-inflammatory activity of target compounds on ear oedema induced by croton oil after topical administration at a dose of 200 mg/kg in mice.

Compound	Swelling (mg, X±SD)	Inhibition (%)	*P*
Control *	13.44±8.25		
Aspirin	7.87±3.05	47.58	<0.01
II	9.81±5.18	28.21	<0.05
III	9.76±6.08	26.87	<0.05
IV	11.89±4.50	11.56	

*0.5% sodium carboxymethyl cellulose aqueous solution.

Acknowledgements: *The author gratefully acknowledges financial support of Islamic Azad University for this research project.*



Scheme 2. Scheme of the synthesis of the final compounds (**II-IV**)

REFERENCES

1. A. C. Guyton, J. E. Hall, Resistance of body to infection: Leucocytes, granulocytes, the monocyte macrophage system and inflammation. In: Textbook of medical physiology (10th ed.). Harcourt Asia PTELTD, Philadelphia, 2000, p. 397
2. R. A. Harvey, P. C. Champe, Lippincott illustrated reviews: pharmacology (3rd ed.). Lippincott Williams and Wilkins, Philadelphia, 2006, p. 495.
3. B. G. Katzung, Basic and clinical pharmacology (9th ed.). Mc-Graw-Hill, New York, 2004, p.298.
4. R. N. Dubois, S. B. Abramson, L. Grofferd, *FASEB J.*, **12**, 1063 (1998).
5. C. J. Hawkey, *Lancet*, **353**, 307 (1999).
6. C. Velazquez, P. N. Rao, R. Mcdonald, E. E. Knausdi, *Bioorg. Med. Chem.*, **13**, 2749 (2005).
7. Q. H. Chen, P. N. Rao, E. E. Knaus, *Bioorg. Med. Chem.*, **13**, 2459 (2005).
8. J. M. Dogne, C. T. Supuran, D. Pratico, *J. Med. Chem.*, **7**, 2251 (2005).
9. L. A. Garcia Rodriques, H. Jick, *Lancet*, **343,** 769 (1994).
10. L. A. Garcia Rodriques, C. Gattaruzzi, M. G. Troncon, L. Agostins, *Arch. Intern. Med.*, **158**, 33 (1998).
11. K. Pradip, J. Baner, L. Gordon, *J. Pharm. Sci.*, **70**, 1299 (1981).
12. M. F. Mahdi, *Iraqi. J. Pharmaceut. Sci.*, **17,** 7 (2008).
13. N. Erdemoglu, E. K. Akkol, E. Yesilada, I. Caliş, *J. Ethnopharmacol.*, **119,** 172 (2008).

СИНТЕЗА И ПРОТИВО-ВЪЗПАЛИТЕЛНО ДЕЙСТВИЕ НА НОВИ ПРОИЗВОДНИ НА ПИПЕРАЗИНА НА МЕФЕНАМИНОВАТА КИСЕЛИНА

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Постъпила на 24 февруари, 2014 г., коригирана на 24 май, 2014 г.

(Резюме)

Синтезирани са нови производни на мефенаиновата киселина и е изследвана тяхната противо-възпалителна активност като не-стероидни агенти (NSAIDs). Те са охарактеризирани чрез IR, ^{1}H-NMR, ^{13}C-NMR, масов и елементен анализ. Противо-възпалителната активност на целевите съединения е ценена върху ушен едем при мишки, индуциран от кротоново масло. Според резултатите от скрийнинга съединения **I** и **II** показват потенциален противо-възпалителен ефект сравним с аспирина.