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SHORT COMMUNICATION

SYNTHESIS AND ANTI-INFLAMMATORY EVALUATION OF NOVEL THIADIAZOL DERIVATIVES OF MEFENAMIC ACID

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ABSTRACT. In the search for new potential non-steroidal anti-inflammatory agents, some novel Mefenamic acid derivatives were synthesized and confirmed by spectroscopic data. The anti-inflammatory activities of these compounds were evaluated by the croton oil-induced ear oedema test in mice. The preliminary pharmacological evaluations indicate that these new compounds showed potent anti-inflammatory activities compare to control and Indomethacin groups.

KEY WORDS: Non-steroidal anti-inflammatory drugs, Mefenamic acid derivatives, Cyclooxygenase, Ear oedema test

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDS) such as Ibuprofen, Mefenamic acid and selective cyclo-oxygenase-2 (COX-2) inhibitors are some of the most commonly prescribed medications worldwide, these drugs are used to treat painful inflammatory conditions such as arthritis, traumatic injuries, pain and fever [1]. Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most frequently prescribed drugs for treatment of pain, fever, and inflammatory and rheumatic diseases. Studies in this field, aimed at discovering better tolerated and potent NSAIDs with fewer side effects characteristic of current NSAIDs have been of interest for many years. The non-selective inhibition of the 2-isoforms of COX is considered to have been responsible for the unfavorable side effects associated with the chronic use of NSAIDs. It was assumed that more selective COX-2 inhibitors would have reduced side effects due to the COX-1 inhibition [2, 3]. Therefore, it would be useful to develop COX-2 selective inhibitors, which are demonstrated to possess a significantly enhanced gastric safety compared to non-selective NSAIDs. Celecoxib and rofecoxib are 2 well known selective COX-2 inhibitors belonging to the COXIB class. 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 inhibition of COX-2 is considered desirable [4, 5]. Selective COX-2 inhibitors are still under development [6, 7], they were proposed that drugs with higher selectivity for COX-2 tend to induce cardiovascular disease [8-10]. The objective of this study, to synthesis new anti-inflammatory derivatives of Mefenamic acid as potential selective COX-2 inhibition with less ulcerogenic effect based on drug development. The present study was conducted to design, synthesize and preliminary evaluation of new Mefenamic acid derivatives as potential NSAIDs.

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EXPERIMENTAL

Material and equipments. All of chemicals and solvents were purchased from Merck (Darmstadt, Germany) and Sigma-Aldrich chemical Co. (United State of America). 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) [11].

2-(2, 3-Dimethylphenylamino) benzoic anhydride (A). Mefenamic acid (4.12 g, 17.11 mmol) was dissolved in dichloromethane (50 mL) and dicyclohexylcarbodiimide (DCC) (1.76 g, 8.56 mmol) was added. The reaction mixture was continuously stirred at room temperature for 3 h, then a white precipitate of dicyclohexylurea was formed and removed by filtration, the solvent was evaporated under vacuum, and semisolid product was obtained to yield Mefenamic acid anhydride know as intermediate compound (**A**) [12, 13]. 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 (I-III). Compound (**A**) (6.35 mmol), appropriate thiadiazole (6.35 mmol), zinc dust (0.006 g), glacial acetic acid (0.61 mL, 10.67 mmol) and dioxane (40 mL) were placed in 100 mL rounded bottomed flask, fixed with reflux condenser, boiling stone were added. The reaction mixture was refluxed for about 2.5 h with continuously stirring, and the reaction was checked by TLC to make sure that completion of reaction. The solvent was evaporated under vacuum, and residue was dissolved in ethyl acetate, then the reaction mixture was washed, with $NaHCO_3$ (10%) 3 times, HCl (1 M) 3 times, and 3 times with distilled water, using (20 mL), filtered over anhydrous sodium sulfate. The filtrate was evaporated and the residue was re-dissolved in ethyl acetate and filtered. The re-crystallization was carried out by adding petroleum ether (60-80 ^{o}C) on the filtrate until turbidity occurred and kept in cold place over night. Then the mixture was filtered while it is cold and the crystals were collected to produce compound (**I-III**).

2-(2,3-Dimethyl-phenylamino)-N-(5-methylsulfanyl-[1,3,4]thiadiazol-2-yl)-benzamide ***(I)***. White needle crystals; yield 57.35%; m.p. 190-192 ^{o}C; IR (KBr, Cm^{-1}) 3250 (NH), 1650, 1600, 1550, 1450 (C=C, Ar), 1675 ((-NH-C=O, carbonyl); ^{1}H-NMR ($CDCl_3$) (ppm): 7.15 (2H, d, Ar),7.25 (2H, d, Ar), 2.55 (s, 3H, S-CH_3), 7.05 (s,1H), 2.84 (6H,CH_3); ^{13}C-NMR ($CDCl_3$) (ppm): 5.6, 14.4, 18.4, 114.0, 118.9, 119.5, 120.6, 121.2, 124.3, 126.4, 128.9, 130.1, 131.9, 142.2, 145.7, 165.7; $C_{18}H_{18}N_4OS_2$. MS: m/z (regulatory intensity): 370 (100), 371 (20), 372 (9).

2-(2,3-Dimethyl-phenylamino)-N-(5-methyl-[1,3,4]thiadiazol-2-yl)-benzamide ***(II)***. Faint white crystals; yield 55.2%; m.p. 180-182 ^{o}C; IR (KBr, cm^{-1}) 3280 (NH), 1675 (-NH-C=O, carbonyl), 1450, 1550, 1600, 1650 (C=C, Ar); ^{1}H-NMR ($CDCl_3$) (ppm): 7.15(2H, d), 7.25(2H, d), 7.243 (s,1), 2.48 (9H, s, CH_3); ^{13}C-NMR ($CDCl_3$) (ppm): 4.6, 15.8, 19.7, 116.0, 117.9, 119.9, 121.8, 122.2, 125.3, 127.4, 129.9, 131.1, 133.9, 143.4, 148.7, 168.6; $C_{18}H_{18}N_4OS$. m/z (regulatory intensity): 338 (100), 339 (22.3).

2-(2,3-Dimethyl-phenylamino)-N-(5-ethyl-[1,3,4]thiadiazol-2-yl)-benzamide ***(III)***. White-light yellow crystals, m.p. 182-184 ^{o}C; 63% yield; KBr, cm^{-1}) 3270 (NH), 1678 (-NH-C=O, carbonyl), 1455, 1545, 1610, 1660 (C=C, Ar); ^{1}H-NMR ($CDCl_3$) (ppm): 7.18 (2H, d), 7.26 (2H, d), 7.24 (s,1),1.13 (3H, s, CH_3), 2.46 (6H, s, CH_3), 2.73 (2H, s, CH_2); ^{13}C-NMR ($CDCl_3$) (ppm):

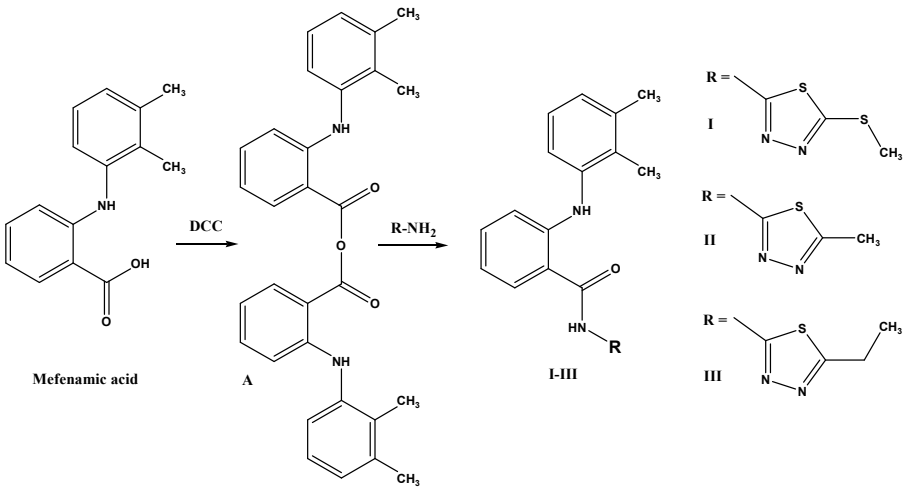
6.5, 14.3, 16.5, 22.7, 115.0, 118.9, 119.1, 123.8, 124.1, 126.3, 128.7, 129.1, 132.8, 133.4, 145.1, 149.8, 167.1; $C_{19}H_{20}N_4OS$. m/z (regulatory intensity): 352 (100), 353 (22.3).

Pharmacological methods. The topical anti-inflammatory activity was evaluated as inhibition of the croton oil-induced ear oedema in Swiss mice [14]. 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 the experiments were conducted between 7 a.m. and 7 p.m., under a normal room light and 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), respectively. Inflammation was induced always in the late morning (10 a.m. to 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 a 2% croton oil suspended in 42% aqueous ethanol. Control animals received only the irritant, whereas 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 the 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.

RESULTS AND DISCUSSION

Chemistry. The synthetic pathway to give the target compounds was carried out according to Scheme 1. In order to prepare the key intermediates (Compound **A**) of Mefenamic acid the carboxyl group react with DCC as showed in Scheme-1 to liberate very good reactive anhydride intermediate compound (**A**) ,this intermediate has a good characteristics like carbonyl carbon with electron deficiency which increased with zinc dust (catalyst). The coupling of the key intermediate **A** with amino group of heterocyclic compounds. This procedure is analogous to that reported by Vogel for preparation of amide linkage [15]. The acylation of anhydride with amino group of heterocyclic compounds were faster than the using of obnoxious acylchloride. The presence of zinc dust as catalyst acts to accelerate the reaction. This reaction is an example of nucleophilic reaction in which the nucleophile ($-NH_2$) is added to carbonyl carbon of anhydride in slightly acidic media (by adding glacial acetic acid) and presence of zinc as catalyst.

The conversion of carboxylic acid group of Mefenamic acid to benzamide group by conjugating the selected moiety of heterocyclic compounds may produce new non-steroidal anti-inflammatory agents. Spectroscopic (IR, ^{1}H and ^{13}C-NMR, Mass) data confirmed the structure of the synthesized compounds. Preliminary pharmacological evaluation has been done for the designed compounds.



Scheme 1. Scheme of synthesis for intermediate **A** and final compounds **I-III**.

Biological evaluation

The anti-inflammatory effects of target compounds have been investigated by croton oil-induced ear oedema test, and the results show some target compounds induced oedema reductions and it has been found that these compound exhibit anti-inflammatory effects compared to that with 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 *	12.41±7.13		
Indomethacin	6.97±2.95	46.18	<0.01
I	10.61±4.88	27.29	<0.05
II	10.76±5.78	24.37	<0.05
III	11.19±4.22	12.68	

*0.5% sodium carboxymethyl cellulose aqueous solution.

In particular, compounds **I** and **II** showed the significant effects ($p < 0.05$) with 27.29% and 24.37% oedema inhibition, respectively, at the administered dose.

CONCLUSION

In conclusion, some novel thiadiazol derivatives of Mefenamic acidwere synthesized and characterized. Then some target compounds were evaluated for anti-inflammatory activities by the croton oil ear oedema test in mice as a model of acute inflammation. Compounds **I** and **II** showed the significant effects compare to control and indomethacine groups.

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