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MEDICINAL CHEMISTRY RESEARCH

ANTIPYRETIC, ANTINOCICEPTIVE AND ANTI-INFLAMMATORY ACTIVITIES OF CERTAIN γ -OXOBUTANOIC ACID DERIVATIVES

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ABSTRACT: Three chemical entities were synthesized as analogs of fenbufen as well as open ring analog of suldinac and celecoxib. The new compounds were evaluated for antipyretic, antinociceptive and anti-inflammatory activities. Among these compounds, 6-benzo[1,3]dioxol-5-yl-4-oxo-hex-5-enoic acid (**3**) was the most active one.

Non steroidal anti-inflammatory drugs (NSAIDs) are the most widely used medications in the world; worldwide, 300 million people are estimated to use NSAIDs[1-4]. The pharmacological activity of these agents is related to the suppression of prostaglandin H_2(PGH_2) biosynthesis from arachidonic acid by inhibiting the activity of the enzyme cyclooxygenases (COX-1 and COX-2)[5-7]. Recently, it was

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discovered that most of the NSAIDs on the market show greater selectivity for COX-1, which is believed to be responsible for inducing mucosal injury primarily by impairing prostaglandin-dependant mucosal protective mechanisms in the gastrointestinal (GI) tract than COX-2[8]. Therefore, chronic use of these non-selective agents may elicit appreciable gastrointestinal ulceration and bleeding[9-13]. The incidence of clinically significant GI side effects due to NSAIDs is high (over 30%) and causes some patients to abandon their NSAIDs therapy[14]. Synthetic approaches based upon NSAIDs chemical modification have been performed with the aim of improving their safety profile. One such approach has been to mask the NSAID carboxylic acid moiety as ester prodrugs that hydrolyse, *in vivo*, to release the active parent NSAIDs[15-17]. Recently, highly selective COX-2 inhibitors have been developed and marketed as promising gastroprotective agents. Many of these agents have been developed and marketed such as celecoxib and refecoxib, as exceptional anti-inflammatory drugs with reduced GI toxicity. Even so, it has become apparrant that there are potential limitations of long term COX-2 inhibitor therapy, including ulcer exacerbation in high-risk patients, delayed gastroduodenal ulcer healing, thrombosis due to prostacyclin deficiency and kidney toxicity[18,19]. Thus, although COX-2 inhibitors have created excitement, they have not eliminated the need for improved drugs in this area.

Rational and Design

In continuing our efforts to develop a new selective NSAID, we designed some γ-oxobutanoic acid derivatives.

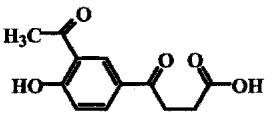
Compounds **1** and **2**[20] were designed and synthesized to posses structural features closely related to fenbufen (I) with replacing the biphenyl moiety with o–hydroxyacetophenone and salicylamide, respectively. Compound **3**[21,22] (4-Oxobutanoic acid derivative) has strucutral feature closely related to sulindac (II). The exocyclic double bond with an oxygen atom at the para position of the benzene ring with unshaired electron pairs, most probably like the sulfur atom in sulindac (II). Also, there is an acidic function at the same distance from the phenyl ring, which is

conjugated as its enol form with the phenyl ring as shown in Chart 1. Moreover, compound **3**[21,22] possesses strucural relationships to celecoxib (**III**) as shown in Chart 2.

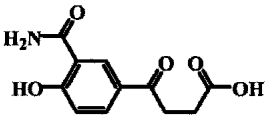
Chemistry

For the synthesis of the target compounds, two pathways were employed. The first utilized a Friedel-Crafts acylation of either salicylamide or o-hydroxyacetophenone with succinic anhydride in the presence of aluminium chloride in DMF to produce compounds **1** and **2**[20]. The second reaction involves condensation of piperonal and levulenic acid under basic catalysis in presence of piperidne to furnish compound **3**[21,22]. The structure of the final products were confirmed by 1H-NMR, EIMS and elemental analyses. The synthetic route is depicted in schemes 1 and 2.

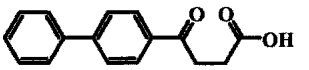
Chart 1:



Compound 1

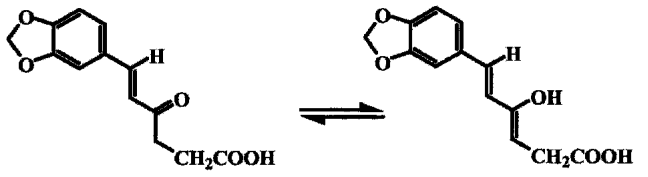


Compound 2

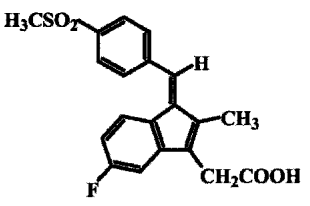


Fenbufen (I)

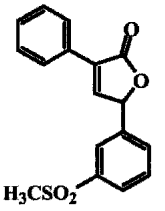
Chart 2:



Compound 3

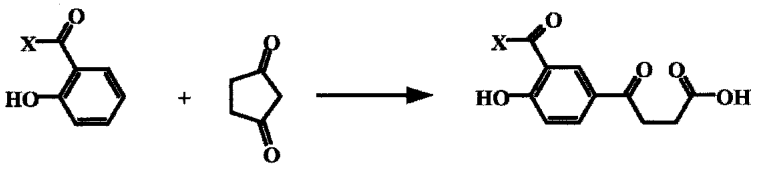


Suldinac (II)



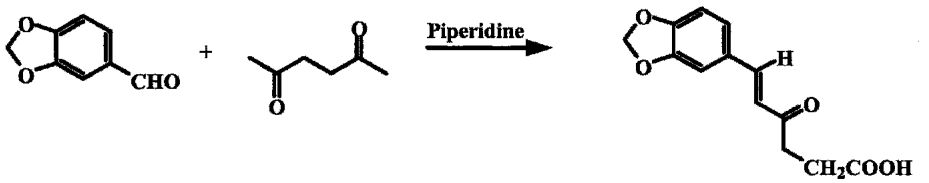
Celecoxib (III)

Scheme 1:



1; X = CH_3 2; X = NH_2

Scheme 2:



Compound 3

Pharmacology

The new compounds were evaluated for antipyretic, antinociceptive and anti-inflammatory activities using reported methods[23-25].

Antipyretic Activity:

Results in Table 1 shows that all test compounds exhibited significant antipyretic activities after oral administration as indicated by the significant reduction in the rectal temperature of the yeast-fevered rats compared with the control group. The percentage of reduction in rectal temperature after oral administration of compound **3** (50 mg/ Kg) after 60, 90 and 120 minutes, were 4.08%, 3.41% and 2.49%, respectively. Similarly, oral administration of compound **2** (50 mg/ Kg), after 60, 90 and 120 minutes, reduced the rectal temperature to 2.11%, 2.71% and 2.44% respectively. However, compound **1** (50 mg/ Kg) significantly reduced the rectal temperature to 2.63% and 2.52% after 90 and 120 minutes from oral administration, respectively. Paracetamol (100mg/ Kg) significantly reduced the rectal temperature. The percentage reduction in rectal temperature induced by paracetamol (100 mg/ Kg) were 4.18%, 5.14%, 5.47% and 6.06% after 30, 60, 90 and 120 minutes from oral administration, respectively. The results reveal that the test compounds possess antipyretic effect.

Antinociceptive Activity

Data of Table 2 show that oral administration of test compounds at a dose of 50 mg/Kg, significantly protect the mice against abdominal constriction induced by intraperitoneal injection of acetic acid.

The percentage protection of compounds **1**, **2** and **3** were 61.47%, 59.24% and 69.65%, respectively and that of Diclofenac Sodium was 72.58 %.

These results reveal the analgesic efficacy of the tested compounds and that compound **3** is the most effective, while compounds **1** and **2** have similar potency in this respect.

Anti-inflammatory Activities

Carrageenan induced rat hind paw edema:

Results recorded in Table 3 show that 7 days of oral administration of compounds at doses 50, 100 mg/Kg, significantly reduced the rat hind paw edema. The percentage reductions of compound **3** were 57.49% and 68.79% respectively. Similarly the high dose of compound **2** (100mg/ Kg) significantly reduced the edema to 27.52% while compound **1** produced a nonsignificant reduction in edema volume compared with the control. The percentage inhibition of edema produced by Diclofenac sodium was 47.64%. These data revealed that compound **3** is the most effective antiinflammatory compound and that compound **2** is less potent, while compound **1** is considered to be not effective at the used dose levels.

Cotton pellets granuloma:

The results recorded in Table 4 showed that the percent of inhibition of granuloma growth of compound **3** was (53.28%) a value which is more potent than that of the used standard Diclofenac sodium (45.37%), while the percent of inhibition of compound **2** was 34.44% and that of compound **1** was 5.2%. These data indicated that compound **3** is the most potent anti-inflammatory compound, while compound **2** is less potent and compound **1** is nearly not effective at the used dose level.

Table 1: Effect of test compounds on brewer's yeast induced hyperthermia in rats #

Treatment	Dose mg/ Kg	Mean rectal temperature °C ± SE at respective time in minutes				
		0	30	60	90	120
Control (saline)	—	38.25 ± 0.14	38.50 ± 0.09	38.44 ± 0.12	38.37 ± 0.14	38.12 ± 0.21
Paracetamol	100	38.26 ± 0.04	36.89 ± 0.17* (4.18)	36.56 ± 0.29* (5.14)	36.27 ± 0.25* (5.47)	35.81 ± 0.23* (6.06)
Compound 1	50	38.24 ± 0.07	37.89 ± 0.28 (1.58)	37.76 ± 0.26 (1.80)	37.36 ± 0.18* (2.63)	37.16 ± 0.17* (2.52)
Compound 2	50	38.56 ± 0.22	38.17± 0.20 (0.86)	37.63 ± 0.24* (2.11)	37.33 ± 0.16* (2.71)	37.19 ± 0.18* (2.44)
Compound 3	50	38.54 ± 0.16	38.12 ± 0.12 (0.99)	36.87 ± 0.17* (4.08)	37.06 ± 0.21* (3.41)	37.17 ± 0.19* (2.49)

values are mean ± SE of n = 6 rats per group

* significantly different from the corresponding control value at P≤0.05

Values between parentheses are percent reduction in rectal temperature compared with the corresponding control value at the same time intervals

Table 2: Antinociceptive effects of orally administered test compounds on acetic acid induced writhing in mice

Treatment	Dose (mg/ Kg)	Number of writhing # $\overline{X} \pm SE$	% Protection
Control	—	22.5 ± 1.71	—
	25	6.17 ± 0.087*	72.58
Compound 1	50	8.67 ± 0.99*	61.47
Compound 2	50	9.17 ± 1.01*	59.24
Compound 3	50	6.83 ± 1.19*	69.65

each value represents the mean ± SE, number of animals in each group = 6 mice

* significantly different from control group at P≤0.05

Table 3: Effect of 7 days oral administration of test compounds on rat hind paw edema induced by carrageenan#

Treatment	Dose mg/ Kg	Edema volume (g) Mean ± SE	% inhibition
Control (saline)	—	0.487 ± 0.042	—
Diclofenac Sodium	25	0.255 ± 0.031*	47.64
Compound 1	50	0.467 ± 0.043	4.11
	100	0.378 ± 0.037	22.38
Compound 2	50	0.421 ± 0.057	13.55
	100	0.353 ± 0.032	27.52
Compound 3	50	0.207 ± 0.045*	57.49
	100	0.152 ± 0.031*	68.79

#number of animals in each group are six
*significantly different from control group at $P \leq 0.05$

Table 4: Effect of test compounds on growth of granuloma tissues in subcutaneously implanted cotton pellets in rats

Treatment	Dose mg/kg	Dry weight of granuloma	
		Mean (mg ± SE)	% inhibition
Control (saline)	—	19.22 ± 2.8	—
Diclofenac Sodium	25	10.50 ± 1.5*	45.37
Compound 1	50	18.22 ±2.5	5.20
Compound 2	50	12.6 ± 2.3*	34.44
Compound 3	50	8.98 ± 2.6*	53.28

number of animals in each group are six
* significantly different from control group at $P \leq 0.05$

Conclusion

In conclusion all the prepared compounds may be considered equally effective as antipyretic and antinociceptive agents. However, compound **3** showed more significant anti-inflammatory effect than compound **2**.

Since compound **3** possess some common features similar to Sulindac, this might explain the relatively higher activity of this lead compound than the two others. The exocyclic double bond with an oxygen atom at the para position of the benzene ring with unshaired electron pairs, most probably like the sulfur atom in sulindac (**II**). Also, there is an acidic function at the same distance from the phenyl ring, which is conjugated as its enol form with the phenyl ring as shown in Chart 1.

Examination on rat stomach, showed that all compounds do not possess ulcerogenic activity which may be attributed to selective COX2 inhibition. Th*Xin vitro* COX1 and COX2 effects will be performed and published elsewere.

Experimental Section

Chemistry

Melting points were obtained on a Graffin apparatus and are uncorrected. Microanalyses for C, H,and N were carried out at the Microanalytical Center, Cairo University. IR spectra were recorded on a Shimadzu 435 Spectrometer, using KBr discs.^{1}H-NMR spectra were performed on a Jeol NMR FXQ-200 MHZ Spectrometer, using TMS as internal standard. Mass spectra were recorded on a GCMS-QP 1000 EX, Mass Spectrometer. Progress of the reactions was monitored by TLC using precoated aluminium sheets silica gel MERCK 60 F 254 and was visualized by UV lamp.

4-(3-Methylcarbonyl-4-hydroxyphenyl)-4-oxobutanoic acid derivatives (1)

To a stirred slurry of anhydrous $AlCl_3$ (50 g, 0.36 mol) in DMF (20 ml), both succinic anhydride (3.6 g, .036 mol) and o-hyroxyacetophenone (5 g, 0.036 mol) was added portionwise under nitrogen with stirring, while keeping the temperature at 70-75 °C. Stirring was continued for 1 hr at 75 °C and the reaction mixture was poured onto (~250g) crushed ice, then concentrated HCl (25 ml) was added to the resulting solution. After stirring for 1 hr, the formed precipitate was filtered, washed with water and dried. The solid obtained was recrystallized from aqueous ethanol. yield 74%. m.p. 160-162 °C. ^{1}H-NMR: δ 2.54-2.61 (t, 2H, *J*=7 H_2, CH_2), 2.71 (s, 3H, CO-CH_3), 3.22-3.28 (t, 2H, *J*=7 H_2, CH_2), 7.06-7.11 (d, 1H, *J*=8.5 H2, aromatic at C6), 8.10-8.14 (d, 1H, J=8.5 H_2 aromatic at C5), 8.42 (s, 1H aromatic at C3), 12.1 (s, 1H, phenolic OH, D_2O exchangable). IR (KBr) γ (cm^{-1}): 1675 , 1710 (2 C=O, ketonic), 1695 (COOH), 3200, 3350.

4-(3-Aminocarbonyl-4-hydroxyphenyl)-4-oxobutanoic acid derivatives (2)

To a stirred slurry of anhydrous $AlCl_3$ (100 g, 0.73 mol) in DMF (15 ml), a mixture of succinic anhydride (7.3 g, .073 mol) and salicylamide (10 g, .073 mol) (finely powdered) was added portionwise with stirring, while keeping the temperature at 70-75 °C. After the addition was completed, stirring was continued for 1.5 hr at 75 °C. The resulted liquid was poured carefully onto (~500 g) crushed ice, then concentrated HCl (50 ml) was added to the resulting solution. After stirring for 1 hr, the formed precipitate was filtered, washed with water and dried. The solid obtained was treated with hot acetonitrile and the insoluble solid was recrystallized twice from acetone affording the 4-hydroxy derivative. Yield 56 % m.p. 207-209°C.

^{1}H-NMR (DMSO-d_6): δ 2.57 (t, 2H, *J*=6, CH_2), 3.22 (t, 2H, *J*=6, CH_2), 6.99 (d, 1H, *J*=8, aryl), 8.02 (d, 1H, aryl), 8.14 (s, 2H, NH_2), 8.6 (s, 1H, aryl), 8.79 (s, 1H, OH). IR (KBr) γ (cm^{-1}): 1590, 1650 (C=O, amidic), 1675 (C=O, ketonic), 1690 (COOH), 3200, 3400.

E-6-benzo[1,3]dioxol-5-yl-4-oxo-hex-5-enoic acid (3)

A mixture of 3,4-methylenedioxybenzaldehyde (piperonal) (10 g, 0.067 mol), levulinic acid (7g, 0.067 mol) and piperidine (3 ml) in toluene (200 ml) was heated at reflux using a Dean-Stark apparatus until the theoretical amount of water had been collected (~6 hr). The reaction mixture was cooled and the supernatant toluene layer was decanted and the remaining red oil layer was washed with ether then dissolved in excess 2-propanol. The solution was concentrated and allowed to cool and the separated solid was dissolved in dilute sodium carbonate solution (10%) and filtered. The filtrate was acidified with dilute HCl and the formed solid was dried in vacuum oven and crystallized from aqueous ethanol. Yield 54 % m.p. 132-134°C. ^{1}H-NMR (DMSO-d_6): δ 2.41 (t, 2H, *J*=7.2, CH_2), 2.89 (t, 2H, *J*=7.2, CH_2), 6.1 (s, 2H, CH_2O-CH_2-O), 6.75-6.81 (d, 1H, *J*=16.47, CH-CO), 6.96-6.98 (d, 1H, *J*=6.33, H_2, aromatic at C5), 7.2-7.23 (d, 1H, *J*=7.4 H_2, aromatic H at C6), 7.39 (s, 1H, aromatic H at C2), 7.52-7.58 (d, 1H, *J*=16.42 H_2, CH-CH= benzylic). IR (KBr) γ (cm^{-1}): 1600, 1620 (C=C), 1665 (C=O, ketonic), 1680 (COOH), 3200, 3400.

Pharmacology

Materials and Methods

Adult male Swiss mice (15-20g) and male Sprague Dawley rats (150-200 g) obtained from the animal house of King Saud University were used in the experiments. All animals were housed in a room with controlled temperature (22 ± 2 °C) under 12 hours light/dark cycle. The animals were fed on a standard certified rodents diet and tap water. Carrageenan, Paracetamol (Sigma, St. Louis, USA), Diclofenac sodium (Novartis Pharma AG, Basel, Switzerland.

Antipyretic Activity

Hyperthermia was induced in rats by subcutaneous injection of sterilized 15% active dry yeast aqueous suspension (15ml /Kg) [23]. Seventeen hours later, either Paracetamol 100mg /Kg or test compounds (50 mg/ Kg) were administered orally only to animals showing a minimum rectal temperature above 38°C. Rectal temperature was

measured with a digital thermometer one hour before, as a predrug value as well as after 30, 60, 90 and 120 minutes after treatment.

Antinociceptive Effect

The antinociceptive effect of the test compounds was evaluated using acetic acid induced abdominal constriction method[1]. Acetic acid (0.6 v/v) was injected i.p. in a volume of 0.1 ml/10g in mice. Saline or test compounds (50 mg/ Kg) were administered orally 1 hour before acetic acid injection. Diclofenac Sodium (25 mg / Kg) was used as a reference standard and administered orally in a similar manner. The number of abdominal constrictions in each group were counted for 20 minutes following acetic acid injection and the antinociceptive activity was evaluated in terms of percentage protection from writhes compared with control group.

Anti-inflammatory activity

Carrageenan induced rat hind paw edema:

Inflammation was produced in rats by injecting 0.05 ml of 1% Carrageenan Sodium into the subplanter region of right hind foot of each rat[24]. Rats are divided into 8 groups with each consisting of six rats. The test compounds were orally administered at two dose levels (50, 100 mg/ Kg) for 7 days. The standard group was administered Diclofenac Sodium (25 mg / Kg) orally, while the control group received only saline. Carrageenan was injected in all rats at day 7, one hour after the last treatment dose. Measurements of foot weight were performed by cutting the feet of the rats immediately after 2 hours of Carrageenan injection, and the percentage inhibition of edema volume was calculated and compared with the control group.

Cotton pellet-induced granuloma:

The anti-inflammatory activity of compounds **1**, **2**, and **3** was screened *in vivo* using the cotton-pellet-induced granuloma method[25]. Inflammation was induced by implanting sterile cotton pellets weighing 50.0 ± 1.0 mg in the groin region of the rats under light ether anesthesia. The animals were divided into four groups with each consisting of 6 rats. The control group was given the vehicle (saline) orally. The

animals of the treated groups were given the test compounds at dose of 50 mg/ Kg, orally for 7 consecutive days whereas, animals of the standard group were administered Diclofenac Sodium (25 mg/ Kg orally). On the last day of treatment, the pellets were dissected out under light anesthesia, dried overnight at 70 °C weighed after cooling and compared with those from the control. The effect of the test compounds on the growth of granuloma induced by cotton pellets was taken as the activity parameter.

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