Synthesis of new Ester Entities of NSAIDs with Nitric Oxide Releasing Properties

Gehan H. Hegazy[1] Gehan M.Kamel[2]

[1] Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Cairo University, Egypt.
[2]Department of Pharmacology, Faculty of Veterinary Medicine, Cairo University, Egypt.
gehan_hegazy@yahoo.com

Abstract: All NSAIDs are suffering from deadlier GIT toxicity. The free carboxylic group is thought to be responsible for this toxicity. In this work, the main motto was to develop new chemical entities as potential anti-inflammatory agents with no gastric toxicity. In this work we esterified some commonly used NSAIDs as ibuprofen, mefenamic acid and indomethacin to p-aminophenol. These esters were then converted to their nitrate derivative to combine the benefits of both esterification and nitrate releasing properties on the GIT. The newly synthesized compounds were biologically evaluated as anti-inflammatory and analgesic. The ulcerogenicity of these compounds was also determined. The new compounds showed similar or enhanced anti-inflammatory and analgesic activities with reduced ulcerogenic potential.
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Key Words: NSAIDs; Esters; Nitric oxide; Gastric toxicity.

1. Introduction

Non steroidal anti-inflammatory drugs (NSAIDs) are among the most widely prescribed drugs worldwide [1-3]. Despite the intensive research that has been aimed at the development of NSAIDs, their clinical usefulness is still restricted by their gastrointestinal side effects like gastric irritation, ulceration, bleeding and in some cases may develop into life threatening condition [4]. The damaging effect of some NSAIDs upon the stomach and intestine is generally believed to be caused by two different mechanisms. The first mechanism involves a local action composed of a direct effect, while the other mechanism has indirect effect on the GIT mucosa. The direct effect can be attributed to a combination of a local irritation produced by acidic group of NSAIDs and local inhibition of prostaglandin synthesis in the GIT tract [5-7]. The use of prodrug to temporarily mask the acidic group of NSAIDs has been postulated as an approach to decrease the GIT toxicity due to direct contact effect [8, 9]. These prodrugs release the parent moieties after absorption by undergoing enzymatic or chemical hydrolysis. Moreover it has repeated that conversion of the carboxylic group of NSAIDs to ester and amide makes them more selective towards COX 2 enzyme [10]. Recent strategies adopted to minimize the side effects of NSAIDs involve the use of hybrid molecules of NSAIDs and nitric oxide donating moiety. This approach is one of the most promising ones because nitric oxide supports endogenous GIT defense mechanism including increase in mucus, increase in mucosal blood flow and inhibition of the activation of proinflammatory cells [11]. In addition to the beneficial effects of NO on cardiovascular system, these drugs are expected to be devoid of the potential cardiovascular adverse effects associated with the use of selective COX 2 inhibitors [12-14]. Nitric oxide is also known to spare the renal system mainly through stimulating the renal blood flow [15]. Promoted with the above mentioned studies the present study aimed to prepare the NO derivatives of NSAIDs esters to combine the three bioactive entities (NSAIDs, p-aminophenol esters and nitric oxide) into one compact structure in order to retain the anti-inflammatory activity and improve the ulcerogenicity.

2. Experimental

2.1 Chemistry

All melting points are uncorrected and determined by the open capillary method using Gallenkamp melting point apparatus (MFB-595-010M; Weiss Gallenkamp, London, UK). IR spectra were recorded on a Shimadzu 435 Spectrometer (IR-435; Shimadzu, Japan) using KBr disks. ^{1}HNMR spectra were recorded on a Perkin-Elmer NMR FXQ-200 MHZ Spectrometer (Tokyo, Japan), using TMS as internal standard. Elemental analyses for C, H, and N were within ±0.4% of the theoretical values and were performed at the Microanalytical Center, Cairo University, and they were of the theoretical values. Progress of the reactions was monitored by TLC using precoated aluminum sheets silica gel MERCK 60 F254 (Merck, Germany) and was visualized by UV lamp.

2.1.1General procedure for the preparation of 4-aminophenyl esters 2a-c.

Appropriate NSAIDs was esterified according to the reported method [16].

2.1.2 General procedure for the preparation of 4-(2-chloroacetamido) phenyl esters 3a-c.

Compounds **2a-c** (10.00 mmol) was dissolved in dry benzene (50 mL). Pyridine (0.02 mL) was added to the solution, and then chloroacetyl chloride (2.26 g, 20.00 mmol) was added. The mixture was then refluxed for 6-7 hrs. The excess solvent was then removed under vacuum and the products were crystallized from ethanol/ether mixture.

2.1.2.1 4-(2-Chloroacetamido) phenyl-2-(4-isobutylphenyl) propanoate. **3a**

Yield 65%; mp: 210 ^{0}C. IR (cm^{-1}) :3320 (NH), 1720,1639 (2CO), ^{1}H-NMR(DMSO-d6) δppm:1.02(d,6H,2 CH_3,J= 7.2Hz), 1.83 (d,3H,CH_3, J=7.1),2.30 (m,1H, $CH(CH_3)_2$), 2.42(d,2H, $\underline{CH_2}$-CH-$(CH_3)_2$,J=6.5), 2.95 (m,1H,CH-CO), 3.40(s,2H,CH_2-Cl), 7.13-7.25(m,8H,Ar), 8.40 (s,1H,NH). Anal. Calcd. for $C_{21}H_{24}ClNO_3$ (373.87): C 67.46, H 6.47 and N 3.75. Found: C 67.51, H 6.12 and N 3.50.

2.1.2.2 4-(2-Chloroacetamido) phenyl 2-(2,4-dimethylphenylamino) benzoate 3b.

Yield 55%; mp: 234 ^{0}C. IR (cm^{-1}) :3320 (2 NH), 1720,1639 (2CO), ^{1}H-NMR(DMSO-d6) δppm:2.20(s,6H, $2CH_3$), 3.20(s,2H,CH_2), 6.90-7.90 (m,11H,Ar),8.20 (s,1H,NH) , 10.00 (s,1H,NH). Anal. Calcd. for $C_{23}H_{21}ClN_2O_3$ (408.88): C 67.56, H 5.18 and N 6.85. Found: C 67.20, H 5.40 and N 6.70.

2.1.2.3 4-(2-Chloroacetamido) phenyl 1-(4-chlorobenzoyl)-2-methyl -5-methoxy indol-3-yl acetate 3c.

Yield 60%; mp: 280 ^{0}C. IR (cm^{-1}) :3320 (NH), 1720,1639 (3CO), ^{1}H-NMR(DMSO-d6) δppm:2.32(s,3H,CH_3), 3.40(s,2H,CH_2-CO),3.70(s, 3H, OCH_3), 4.20 (s,2H,CH_2-Cl), 6.75-7.80 (m,11H,Ar), 10.01(s,1H,NH). Anal. Calcd. for $C_{27}H_{22}Cl_2N_2O_5$ (525.38): C 61.72, H 4.22 and N 5.33. Found: C 61.84, H 4.50 and N 5.50.

2.1.3 General procedure for the preparation of 4-(2-nitrooxyacetamido) phenyl esters 4a-c.

Chloroacetamidophenyl esters **3a-c** (18 mmol) was dissolved in acetonitrile (100 mL) by stirring for 10 minutes. Silver nitrate (3.40 g, 20 mmol) was dissolved in acetonitrile (50 mL), and then added to the previous solution. The mixture was then stirred over night at room temperature. The precipitate formed was filtered and the solution was evaporated to dryness under vacuum to obtain the products. The products were recrystallized from ethanol.

2.1.3.1 4-(2-Nitrooxyacetamido) phenyl 2-(4-isobutylphenyl) propanoate 4a.

Yield 50%; mp: 250 ^{0}C. IR (cm^{-1}) :3350 (NH), 1720,1639 (2CO), ^{1}H-NMR(DMSO-d6) δppm:1.21(d,6H,2CH_3,J= 7.6Hz), 1.92 (d, 3H, CH_3, J= 6.0Hz), 2.00 (m,1H,$CH(CH_3)_2$), 2.30 (d,2H,$\underline{CH_2}$-CH-$(CH_3)_2$, J= 6.5) , 3.84 (m,1H,CH-CO), 5.50 (s,2H,CH_2ONO_2) , 7.01-7.55 (m,8H,Ar) , 8.24 (s,1H,NH).Anal. Calcd. for $C_{21}H_{24}N_2O_6$ (400.43): C 62.99, H 6.04 and N 7.00. Found: C 62.61, H 6.40 and N 6.70.

2.1.3.2 4-(2-Nitrooxyacetamido) phenyl 2-(2,4-dimethylphenylamino) benzoate 4b.

Yield 52%; mp: 230 ^{0}C. IR (cm^{-1}) :3350 (2NH), 1720,1639 (2 CO), ^{1}H-NMR(DMSO-d6) δppm: 2.28 (s, 6H, $2CH_3$), 5.43 (s, 2H, CH_2ONO_2) , 6.87-7.56 (m, 11H,Ar),8.10 (s,1H,NH) 10.00 (s ,1H ,NH). Anal. Calcd. for $C_{23}H_{21}N_3O_6$ (435.43): C 63.44, H 4.86 and N 9.65. Found: C 63.51, H 4.50 and N 9.70.

2.1.3.3 4-(2-Nitrooxyacetamido) phenyl 1-(4-chlorobenzoyl)-2-methyl -5-methoxy indol-3-yl acetate 4c.

Yield 51%; mp: 195 ^{0}C. IR (cm^{-1}) :3350 (NH), 1720,1639 (3 CO), ^{1}H-NMR(DMSO-d6) δppm: 2.35 (s, 3H, CH_3), 3.30 (s, 2H, CH_2-CO),3.70(s,3H,OCH_3),4.47 (s, 2H, CH_2ONO_2) , 7.60-7.80 (m, 11H,Ar),10.01 (s,1H,NH). Anal. Calcd. for $C_{27}H_{22}ClN_3O_8$ (551.93): C 58.76, H 4.02 and N 7.61. Found: C 58.41, H 4.30 and N 7.52.

2.2 Nitric oxide releasing measurements

A solution of the appropriate compound (20 µL) in dimethylsulfoxide (DMSO) was added to 2 mL of 1:1 v/v mixture of 50 mM phosphate buffer (pH 7.4) with MeOH, containing 5× 10^{-4} M L-cysteine. The final concentration of drug was 10^{-4} M. After 1 hr at 37 ^{0}C, 1 mL of the reaction mixture was treated with 250 µL of Griess reagent [sulfanilamide (4 g), N-naphthylethylenediamine dihydrochloride (0.2 g), 85% phosphoric acid (10 mL) in distilled water (final volume was100 mL)]. After 10 min at room temperature, the absorbance was measured at 540 nm. Sodium nitrite standard solutions (10–80 nmol/mL) were used to construct the calibration curve.The results were expressed as the percentage of NO released (n=2) relative to a theoretical maximum release of 1 mol NO/mol of test compound (Table 4).

2.3 Biological activity

2.3.1Materials and Methods

2.3.1.1 Animals

Adult rats of both sexes weighing 180-200 gm and adult mice weighing 25-30 gm were used in the present study. The animals were kept under natural conditions for light, temperature, ventilation, water and food. Experiments were carried out on groups of 5 animals each .All experimental procedures used in the present study followed the Institutional Animal Ethics Committee regulations. All experiments were performed in the morning according to the guidelines for the care of laboratory animals.

2.3.1.2 Drugs and chemicals

Formaldehyde, sod. CMC and acetic acid were obtained from Sigma Chemical Co. (St.Louis, MO, USA), while indomethacin (Indocid), was obtained from Kahira Pharmaceutical company(Cairo, Egypt).

2.3.1.3 Statistical analysis

Results of anti-inflammatory and analgesic activity were represented as mean ± S.E "standard error". The significance difference between groups was tested using one way ANOVA followed by Dunnett's test at $p \leq 0.05, 0.01, 0.001$.

2.3.2 Anti-inflammatory activity

Wister albino rats of either sex were divided into 5 groups of 5 animals each. They were treated via oral rout. The first group was given sod.CMC (1% w/v) watery suspension and kept as control. The second group was administered indomethacin (10 mg/kg b.wt) as standard drug, the tested compounds **4a-c**, in the form of CMC suspensions, were given at a dose of 100 mg/kg b.wt. to the last three groups. The initial paw thickness was measured for each animal using caliber before induction of edema. After one hour 0.1 ml of 2% formaldehyde was injected into the foot pad of the left hind paw of each rat for induction of paw edema. The increase in this thickness was determined after 30 min, 1, 2, 3 and 24 hrs after formaldehyde injection .The anti-inflammatory activity was expressed as inhibition percent in paw thickness in treated groups compared to the control one using the formula

Edema inhibition percent = $T_c - T_t / T_c \times 100$

Where, T_c and T_t represent the average paw thickness in the control and treated groups, respectively.

2.3.3 Analgesic activity

2.3.3.1 Acetic acid induced writhing test:

Five groups of mice (5/group) were used in this test. The first group of animals was treated orally with sod.CMC watery solution (2% w/v) (5ml/kg), served as control. The second group of animals was treated with indomethacin (10 mg/kg) as standard. The tested compounds **4a-c** in the form of CMC watery suspensions (100 mg/kg bwt) were given to the animals of the rest groups. Muscle contractions were induced in mice by intraperitoneal injection of 0.6% solution of acetic acid(10ml/kg) after 30 minutes of drug administration. Immediately after administration of acetic acid, each animal was placed separately in a glass cage, and the number of stretching per animal was recorded during the following 15 min. Writhing movement was accepted as contraction of the abdominal muscles accompanied by stretching of hind limbs. The percentage inhibition of writhing was calculated.

2.3.3.2 Hot plate test:

The animals were divided into control, standard and tested groups of 5 mice each. In the first group animals were orally administered sod.CMC watery solution (2% w/v) (5ml/kg) served as control one. Indomethacin (10 mg/kg bwt) was administered as standard to the second group .The tested compounds **4a-c** in the form of CMC watery suspensions (100 mg/kg bwt) were given to the animals of the rest groups. The hot plate (Model 7280, Ugo Basile, Italy) was maintained at $55.0 \pm 0.2\ ^0$ C and each animal was placed into a glass beaker on the heated surface. The time to discomfort reaction (licking paws or jumping) was recorded as a response latency time at, 30 min, 1hr and 2hrs after administration in each group. A cut-off period of 15 sec was considered as maximum latency time to avoid injury to the paws. The pain inhibition percentage (PIP) was calculated according to the following formula:

Pain inhibition percentage (PIP) = $(T_t - T_c) / T_c \times 100$

Where T_t is drug latency time and T_c is control latency time.

2.3.4 Ulcerogenic liability

Rats of either sex were divided into five groups of five animals each. The animals were fasted 18 hrs before drug administration. One group was treated with 5ml /kg sod. CMC watery suspension (2% w/v) as a control group. Another one was treated with indomethacin (10 mg/kg b.wt) as a standard one. The tested compounds **4a-c** in the form of CMC watery suspensions (100 mg/kg b.wt) were administered each in one of the rest three groups. Treatment was continued once daily for 3 successive days in all groups. One hour after the last dose, the animals were sacrificed and the stomach was removed, opened along the greater curvature and rinsed with saline. The gastric mucosa was examined with a magnifying lens (10^x) for the presence of lesions in the form of hemorrhages or linear breaks and erosions. The ulcer index was calculated and the degree of ulcerogenic effect was expressed in terms of:

1. Percentage incidence of ulcer divided by 10.
2. Average number of ulcers per stomach.
3. Average severity of ulcers.

The ulcer index is the value that resulted from the summation of the above three values.

2.3.5 Acute toxicity and lethality test

In the first stage of the test, three groups (of 9 animals each) were used for each tested compound, each one of these compounds was administered orally in the form of sod. CMC watery suspension (1% w / v) at a dose of 10, 100, and 1000 mg/kg (n = 3). Animals were observed continuously for the first three hours for any toxic symptoms after administrations and number of deaths within 24hrs. No death occurred in any of these groups for each compound. A second stage of the test was conducted in which 1500, 2000 and 3000 mg/kg doses of each compound were administered to a fresh groups of animals (n = 1) and no death was recorded within 24 hrs. Thus, the oral LD_{50} in mice were found to be greater than 3000 mg/kg for each tested compound.

3. Results and Discussion

3.1 Chemistry

Compounds **2a-c** were synthesized by esterification of NSAIDs with p-aminophenol using N, N` dicyclohexylcarbodiimide. Reaction of these esters with chloroacetyl chloride in benzene using pyridine as catalyst yields compounds **3a-c.** Treatment of **3a-c** with silver nitrate in acetonitrile affords the corresponding nitrate derivatives **4a-c**. The structures of the prepared compounds were confirmed on the basis of their IR,^{1}HNMR Mass spectra and elemental analyses. The reaction sequences are outlined in scheme 1.

3.2 Nitric oxide releasing measurements

The nitric oxide releasing properties of such class of compounds were assessed in phosphate buffer of pH 7.4 with Griess reagent. The reaction was carried out in the presence of L-cysteine as a source of SH group. The amount of NO released from the tested compounds was measured relative to nitric oxide released from standard sodium nitrite solution [17].

3.3 Biological activity

3.3.1 Anti-inflammatory activity

The newly synthesized compounds **4a-c** were evaluated for their anti-inflammatory activity using formalin induced rat paw edema described by Dharmasiri *et al.* [18]. Rats were administered indomethacin orally (10 mg/kg b.wt) as standard. The tested compounds **4a-c** were given at a dose of 100 mg/kg bwt. Paw volume was determined after 30 minutes, 1, 2, 3 and 24 hrs after formaldehyde injection. The results are listed in table 1 and illustrated in figure 1. It was clear that all tested compounds possess anti-inflammatory activity compared to the standard indomethacin.

3.3.2 Analgesic activity

3.3.2.1 Acetic acid induced writhing test:

This test was done using the method described by Collier *et al.* [19]. Muscle contractions were induced in mice by intraperitoneal injection of acetic acid 10 ml/kg b.wt. The tested compounds were given orally in a dose of 100 mg/kg b.wt while indomethacin was given in a dose of 10 mg/kg b.wt as standard. The number of stretching per animal was recorded. The results are listed in table 2 and illustrated in figure 2. All compounds showed good analgesic activity in this test.

3.3.2.2 Hot plate test:

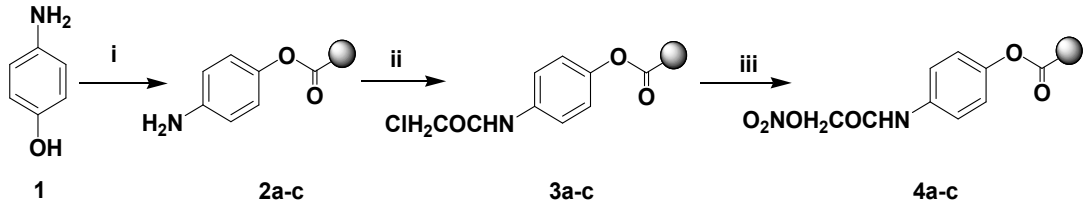
Analgesic activity was determined in mice by hot plate method described by Hosseinzadeh *et al.* [20]. Indomethacin (10 mg/kg b. wt) was used as standard, while the tested compounds **4a-c** were used orally in a dose of 100 mg/kg b. wt. Each animal was placed into a glass beaker on the heated surface and the time for licking paws or jumping was recorded as a response latency time. The results were represented in table 3 and figure 3 as pain inhibition percent. It was found that all the tested compounds possess good analgesic activity under this study.

3.3.3 Ulcerogenic liability

Being many of the compounds tested have pronounced anti-inflammatory and analgesic activity compared to the standard (indomethacin), therefore the ulcerogenic liability for all the compounds was evaluated in albino rats following the reported method [21]. As shown in table 4 and figure 4, the ulcerogenic liability was decreased for all compounds compared by indomethacin. The potential medicinal value of these compounds as anti-inflammatory and analgesic agents is that they have higher safety margin on gastric mucosa than NSAIDs.

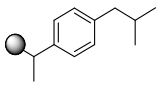
3.3.4 Acute toxicity and lethality test

The acute toxicity and lethality (LD_{50}) of the tested compounds was estimated in mice using the method described by Klimmek *et al* [22]. It was found that LD_{50} for these compounds in mice is greater than 3000 mg/kg bwt.

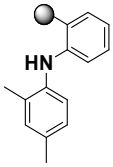


Scheme 1. General method for the preparation of (**2a-c**) , (**3a-c**), and (**4a-c**). Reagent and conditions: (**i**) esterification; (**ii**) chloroacetylation by chloroacetyl chloride; (**iii**) silver nitrate.

a = Ibuprofen



b =Mefenamic acid



c =Indomethacin

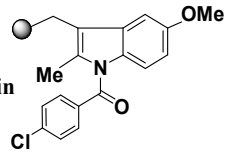


Table (1): Anti-inflammatory activity of the tested compounds (100 mg/kg bwt, P.O) using formalin induced paw edema method.

compound	Paw edema thickness (m.m)					Inhibition %				
	30 min	1hr	2hrs	3hrs	24hrs	30min	1hr	2hrs	3hrs	24hrs
Control	0.53 ± 0.013	0.57 ± 0.014	0.57 ± 0.014	0.58 ± 0.012	0.44 ± 0.018	0	0	0	0	0
Indomethacin	0.23 ± 0.009 [a]	0.22 ± 0.006 [a]	0.15 ± 0.005 [a]	0.03 ± 0.002 [a]	0	56.60	70.18	74.14	88.64	100.00
4a	0.24 ± 0.011 [a]	0.22 ± 0.013 [a]	0.17± 0.009 [a]	0.15 ± 0.006 [a]***	0.05± 0.002 [a]***	54.72	56.60	70.18	74.14	88.64
4b	0.17 ± 0.008 [a]***	0.15 ± 0.009[a]***	0.15 ± 0.007 [a]	0.15 ± 0.007[a]***	0.12 ± 0.005[a]***	67.93	73.68	73.68	74.14	72.73
4c	0.18 ± 0.009[a]***	0.17 ± 0.008[a]***	0.17 ± 0.006[a]*	0.15 ± 0.008[a]***	0.08 ± 0.003[a]***	66.04	70.18	70.18	74.14	80.00

a Significantly different from the control value at $p \leq 0.001$, * significantly different from the indomethacin value at $p \leq 0.05$, *** significantly different from the indomethacin value at $p \leq 0.001$,results are means of five experiments ± S.E.

Table (2): Analgesic activity of the tested compounds (100 mg /kg bwt ,p.o) on acetic acid writhing abdominal contractions .

Treatment	No of contractions/15 min	Inhibition %
Control	45.70± 2.09	0
Indomethacin	12.18 ± 0.27 [a]	73.35
4a	20.23 ± 0.85 [a]***	55.73
4b	14.68± 0.58 [a]**	67.88
4c	17.49 ± 0.64 [a]***	61.73

a Significantly different from the control value at $p \leq 0.001$, ** Significantly different from the indomethacin value at at $p \leq 0.01$, *** Significantly different from the indomethacin value at $p \leq 0.001$, results are means of five experiments ± S.E.

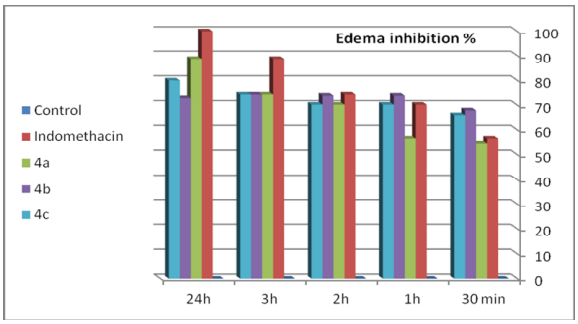


Figure 1: Anti-inflammatory activity of the tested compounds (100 mg/kg bwt,P.O) in formalin induced paw edema method .

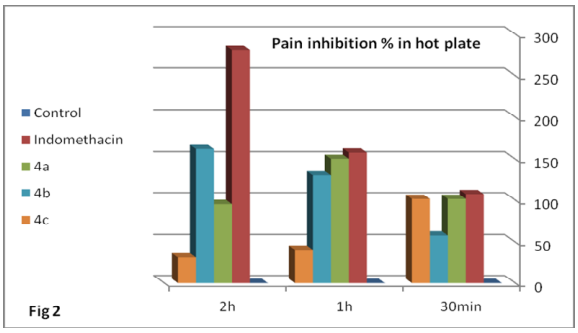


Figure 3: Analgesic activity of the tested compounds (100 mg/kg bwt, P.O) in hot plate method .

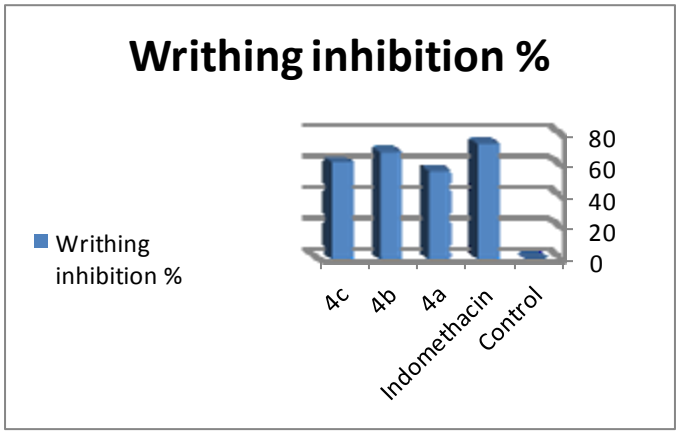


Figure 2: Analgesic activity of the tested compounds (100 mg /kg bwt ,p.o) on acetic acid writhing abdominal contractions .

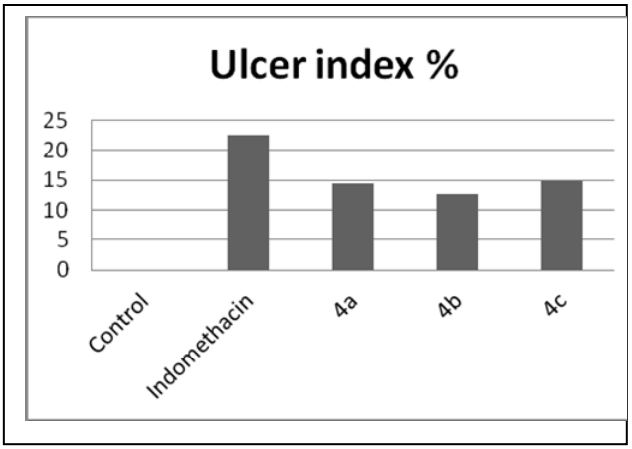


Figure 4: Ulcer index % of the synthesized compounds

Table (3): Analgesic activity of the tested compounds (100mg/kg bwt, P.O) using hot plate method .

	Latency time (min)			Pain inhibition percent (PIP)		
	30 min	1hr	2hrs	30 min	1hr	2hrs
Control	2.74 ± 0.12	2.9 ± 0.13	2.73 ± 0.12	0	0	0
Indomethacin	5.68 ± 0.25 [a]	7.48 ± 0.44 [a]	10.32 ± 0.68 [a]	107.29	157.93	280.95
4a	5.52 ± 0.38 [a]	7.26 ± 0.33 [a]	5.33 ± 0.32 [a]***	101.46	150.34	95.24
4b	4.33 ± 0.32 [a]*	6.67 ± 0.43 [a]	7.16 ± 0.36 [a]***	58.03	130.00	162.27
4c	5.52 ± 0.29 [a]	3.84 0.19 [a]***	3.80 ± 0.17 [a]***	101.46	40.66	31.03

a Significantly different from the control value at $p \leq 0.001$, * significantly different from the indomethacin value at $p \leq 0.05$, *** significantly different from the indomethacin value at $p \leq 0.001$,results are means of five experiments ± S.E.

Table (4): Ulcerogenic liability and % NO release of the synthesized compounds

Compound	Number of animals with ulcers	% incidence divided by 10	Number of ulcers	Severity of ulcers	Ulcer index	% NO release
Control	0	0	0	0	0	0
Indomethacin	5/5	10	10.6 ± 0.71 [a]	2.06 ± 0.224 [a]	22.66 ± 1.89 [a]	0
4a	4/5	8	4.7 ± 0.38***	1.7 ± 0.183	14.4 ± 0.93**	0.51
4b	4/5	8	3.5 ± 0.29***	1.25 ± 0.158*	12.75 ± 0.66**	0.60
4c	5/5	10	3.8 ± 0.26***	1.1 ± 0.05[b]**	14.9 ± 0.89**	0.54

a Significantly different from the control value at $p \leq 0.001$, * significantly different from the indomethacin value at $p \leq 0.05$ ** significantly different from the indomethacin value at $p \leq 0.01$, *** significantly different from the indomethacin value at $p \leq 0.001$,results are means of five experiments ± S.E.

Percentage of NO released(n=2) relative to a theoretical maximum release of 1 mol NO/mol of tested compound; determined by Griess reagent in the presence of 5m M L- cysteine at PH 7.4

4. Conclusion

Oral dosage forms of NSAIDs suffer from the limitation of gastric injuries caused by their free carboxylic group. Therefore in this work we found that it is interesting to modify NSAIDs structure in a way that would lead to great reduction in acidic characters by converting the free carboxylic acid group to ester. Also we intended to retain the benefits of presence of NO-NSAIDs in the same structure.

Anti-inflammatory activity

Acute inflammation induced by formaldehyde results from cell damage, which provokes the production of endogenous mediators, such as, histamine, serotonin, prostaglandins, and bradykinin. It is well known that inhibition of edema induced by formalin in rats is one of the most suitable test procedures to screen anti-arthritic and anti-inflammatory agents as it closely resembles human arthritis. Compound **4a** possessed significant anti-inflammatory activity beginning after 30 min of formalin injection and increased stepwise reaching to 88.64 % inhibition of edema after 24hrs . On the other hand this activity was significantly lesser than the standard drug at 3hrs after formalin injection which may be attributed to lake of a good kinetic profile for this compound. Compound **4b** showed good anti-inflammatory activity beginning after 30 min with edema inhibition of 67.93 % which increased to 74.14 % after 3hrs. Percent inhibition of this compound after 30 min and 1hr was more potent than the reference indomethacin, as it resulted in a significant more reduction in paw edema thickness compared to the reference drug. Also it possessed nearly equal activity with 73.68% inhibition of edema after 2hrs.

Compound **4c** showed significant anti-inflammatory activity compared to the control group. It induced significant more reduction in paw edema thickness compared to the reference drug after 30 min and equal activity after 1 hr, its maximum effect was achieved after 24hrs with 80.00% edema inhibition. All the tested compounds showed good anti-inflammatory activity confirming that NO-NSAIDs retain the anti-inflammatory activity of original NSAIDs. From these results, it is clear that all the tested compounds exhibited their activity starting from 30 minutes which increased till reach maximum at 3 or 24hrs after formalin injection. All compounds were found to release NO which may contribute to their low ulcerogenic effect (Table 4).

Analgesic activity

Analgesic effects were assessed in two models of nociception, chemical model using acetic acid induced writhing method and thermal model using hot plate test. These methods were selected to evaluate both peripherally and centrally mediated effects of the tested compounds respectively. In writhing test the results elucidated that oral doses of the tested compounds induced significant reduction in the number of abdominal contractions in treated mice compared to indomethacin. Compound **4b** showed the most potent analgesic activity among the tested compounds, with 67.88% inhibition in writhing response. It has been postulated that acetic acid acts indirectly by inducing the release of endogenous mediators, such as PGE2 and PGE2α in peritoneal fluids as well as lipooxygenase products, which stimulate the nociceptive neurons sensitive to NSAIDs. Therefore, the results of the acetic acid induced writhing strongly suggest the inhibition of these compounds to lipooxygenase and cyclooxygenase in peripheral tissues. In hot plate test significant increase in latency time against heat stimuli suggests contribution of central mechanism in the anti-nociceptive effect for these compounds. Compound **4a** showed promising analgesic activity after 30min (101.46% inhibition) and 1hr (150.34%) which was nearly equivalent to the reference indomethacin at the same time interval. Meanwhile its activity was promptly lowered to 95.24% after 2hrs which might be correlated to its kinetic pathway. Compound **4b** exhibited considerable long lasting activity beginning from 58.03% inhibition after 30min then increased to 130.00% and 162.27% after 1 and 2hrs, respectively. Compound **4c** exhibited remarked activity only after 30min (101.46%) which lowered to 40.66 and 31.03% after 1 and 2hrs respectively.

The results of the present study demonstrated that the tested compounds possessed analgesic activity in both nociceptive models suggesting the involvement of both central and peripheral mediated activities. As these compounds are NO- NSAIDs esters, nitric oxide shares a part of this activity as it was previously reported to diminish hyperalgesia via acting at peripheral nociceptors as well as in the spinal pain perception pathway. In this concern nitric oxide has been shown to interact with and reduce transmission via NMDA receptor in the spinal cord. Stimulation of this receptor with some excitatory neurotransmitters like glutamate was found to promote spinal pain perception so inhibition of transmission through this NMDA receptor would be expected to reduce hyperalgesia. In addition all the tested compounds although possess anti-inflammatory and analgesic activities but yet they

are less irritant to GIT than their parent NSAIDs as shown by ulcer index.

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