Synthesis and Preliminary Pharmacological Evaluation of Aminobenzensulfonamides Derivatives of Mefenamic Acid as a Potential Anti-inflammatory Agents

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Abstract

A group of amino derivatives [4-aminobenzenesulfonamide,4-amino-N[1] methylbenzenesulfonamide, or N[1]-(4-aminophenylsulfonyl)acetamide] bound to carboxyl group of mefenamic acid a well known nonsteroidal anti-inflammatory drugs (NSAIDs) were designed and synthesized for evaluation as a potential anti-inflammatory agent. *In vivo* acute anti-inflammatory activity of the final compounds (9, 10 and 11) was evaluated in rat using egg-white induced edema model of inflammation in a dose equivalent to (7.5mg/Kg) of mefenamic acid. All tested compounds produced a significant reduction in paw edema with respect to the effect of propylene glycol 50% v/v (control group). Moreover, the 4-amino-N-methylbenzenesulfonamide derivative (compound 10) exhibited comparable anti-inflammatory activity to diclofenac (3mg/Kg) at times 180-300 minute with the same onset of action. The results of this study indicate that the incorporation of the 4-aminobenzenesulfonamide pharmacophore and its derivatives in to mefenamic acid maintain its anti-inflammatory activity.

Key ward: benzenesulfonamide, anti-inflammatory, paw edema, NSAIDs, mefenamic acid

الخلاصة

مجموعة من المشتقات الامينية [4 -امينوبنزين سلفونامايد , 4-امينو-ان-مثيل بنزين سلفونامايد ,ان-(4-امينوفنيل سلفونيل(اسيتامايد] متحدة بمجموعة الكاربوكسيل للميفانميك اسيد (mefenamic acid) الدواء غير الستيرويدي المعروف جيدا كمضاد للالتهاب ,قد صممت وحضرت لتقييمها كمضادات قوية للالتهاب . في الجسم الحي ,اجري تقييم الفعالية المضادة للالتهاب للمركبات النهائية) 9 ,10 ,11(في الجرذ باستخدام زلال البيض مستحدثة وذمة التهابية تحت الجلد بجرعة مكافئة للميفانميك اسيد (7.5ملغم/كغم(.كل المركبات المختبرة انتجت انخفاض مؤثرا للوذمة بالمقارنة مع البروبلين كلايكول (propylene glycol 50%) كمجموعة ضابطة.علاوة على ذلك مشتق 4-امينو-ان-مثيل بنزين سلفونامايد (مركب 10(اظهر فعالية مضادة للالتهاب مقارنة للدايكلوفيناك)3ملغم/كغم(في اوقات180 -300 دقيقة مع نفس الفعالية الابتدائية للدايكلوفيناك. نتيجة هذه الدراسة تشير الى ان اندماج الجزء العقاقيري 4 -امينوبنزين سلفونامايد ومشتقاته مع الميفانميك اسيد حافظ على فعاليته المضادة.

Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely used to treat acute or chronic inflammation and offer symptomatic pain relief[1,2]. Conventional NSAIDs act by non selective inhibition of cyclooxygenase (COX) enzymes, which catalyze the formation of prostaglandins (PGs) from arachidonic acid[3, 4]. There are three isoenzymes of COX (COX-1, COX-2 and COX-3) have been identified[5,6]. COX-1 is expressed in most tissues of the body and largely governs the homeostatic production of arachidonic acid metabolites necessary to maintain physiologic integrity[7]. COX-2 is highly induced in settings of inflammation by cytokines and inflammatory mediators or physiological stress[8,9]. COX-3 activity in human has not been confirmed[10], but it may be implicated in fever[11]. All classic NSAIDs inhibit COX-2 as well as COX-1 to varying degrees; thus they can be considered nonspecific[12,13]. All classical NSAIDs are associated with an increased risk of gastrointestinal (GI) ulcers and serious upper GI complications, including GI hemorrhage, perforation, and obstruction[14,15].

In contrast many of the selective COX-2 inhibitors containing benzene-sulfonamide derivative, like valdecoxib(I) [16] , celecoxib(II) [17],or benzene-N-methyl sulfonamide like compound (III) [18] and benzene methylsulfonyl

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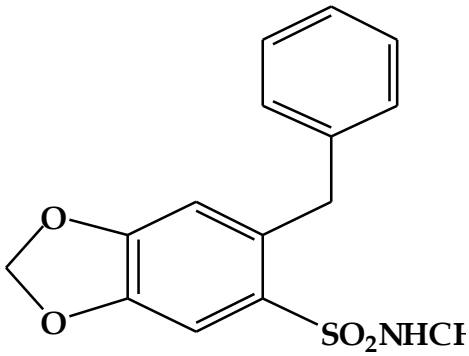
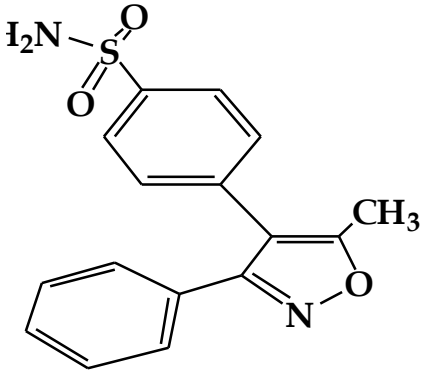
Received : 29 /10 / 2007
Accepted : 15 /3/ 2008

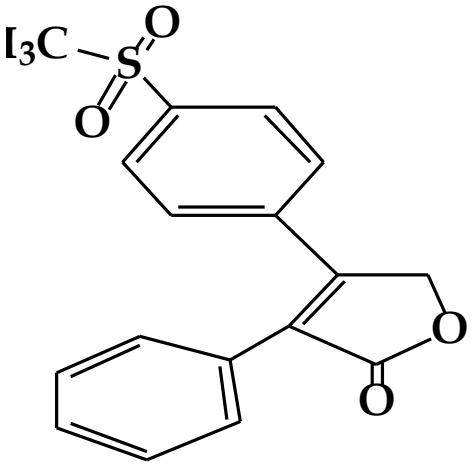
derivative, like Rofecoxib (IV) exert anti-inflammatory and analgesic activity in the clinic with markedly less GI toxicity than traditional NSAIDs[19]. In a recent study, it was shown that the incorporation of a para-N-acetylsulfonamido substitute on the C-3 phenyl ring of the Rofecoxib regioisomer provided a highly potent and selective COX-2 inhibitor (compound V) that has the potential to acetylate the COX-2 isozyme[20]. The improved GI tolerance of COX-2 selective inhibitors not withstanding, there is evidence to suggest that COX-2 selective inhibitors may inhibit COX-1 and induce GI irritation or ulceration with long term use or at higher doses[21,22]. Preclinical cardiovascular and renal liabilities of at least some COX-2 selective inhibitors have also been reported[23]. Thus there is still a need for new anti-inflammatory agents with an improved safety profile.



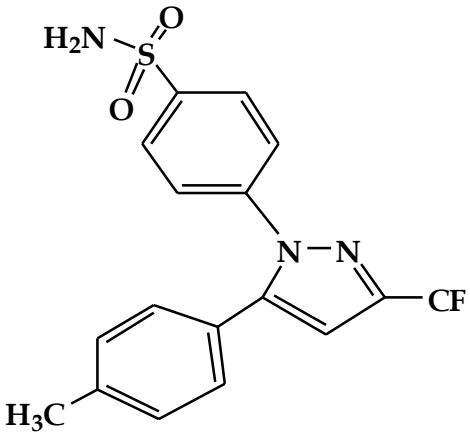
(III)



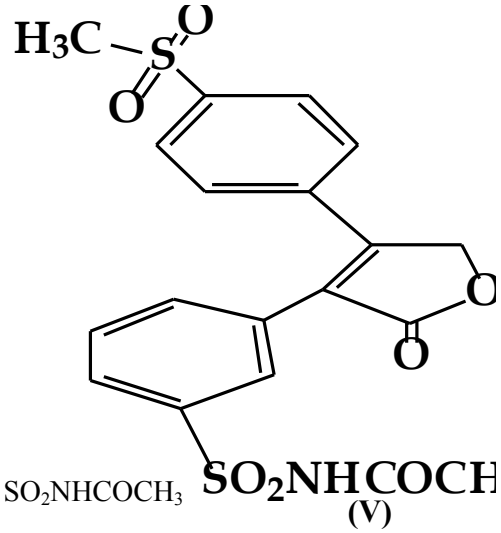
Valdecoxib (I)



Rofecoxib(IV)



Celecoxib (II)

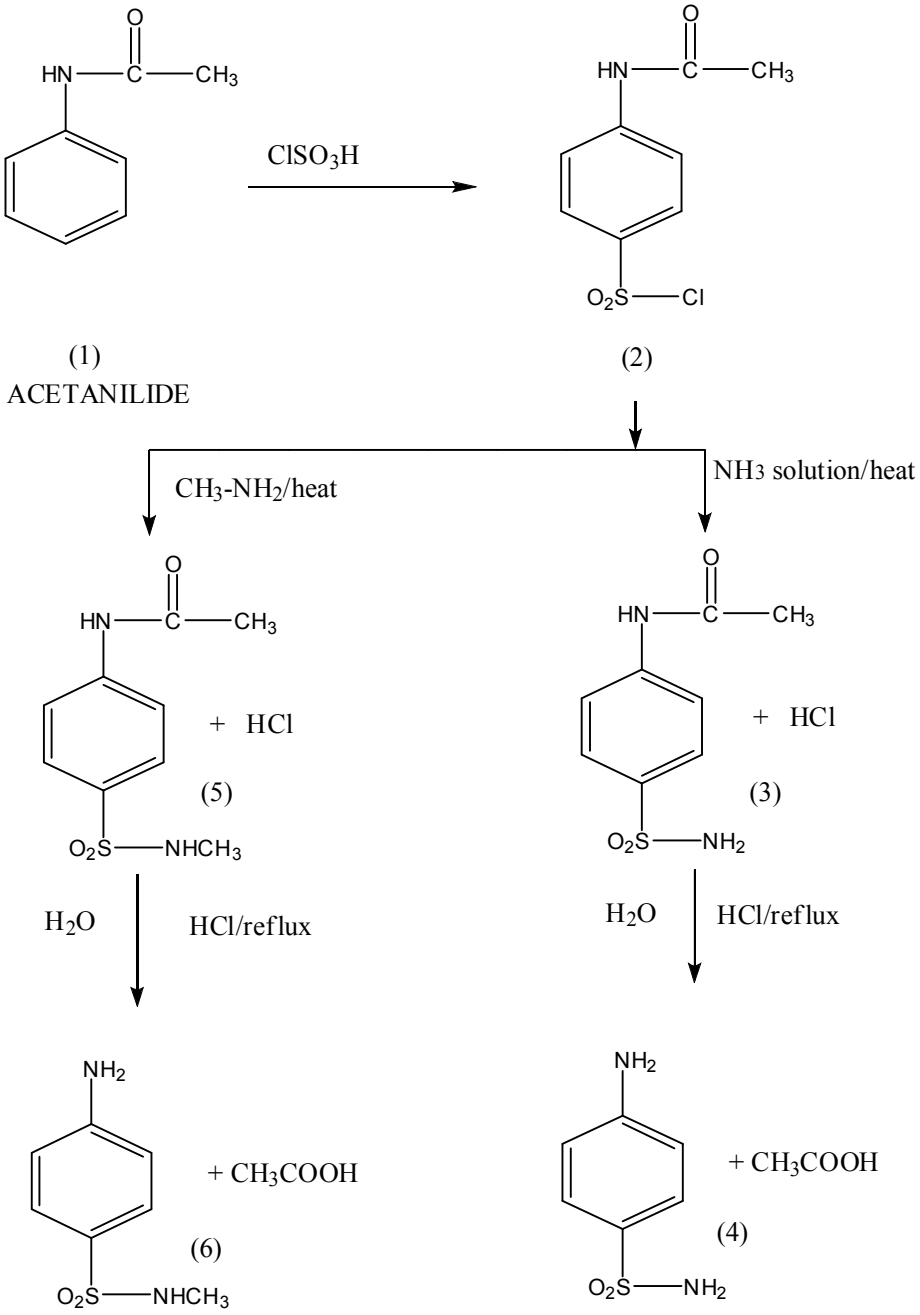


(V)

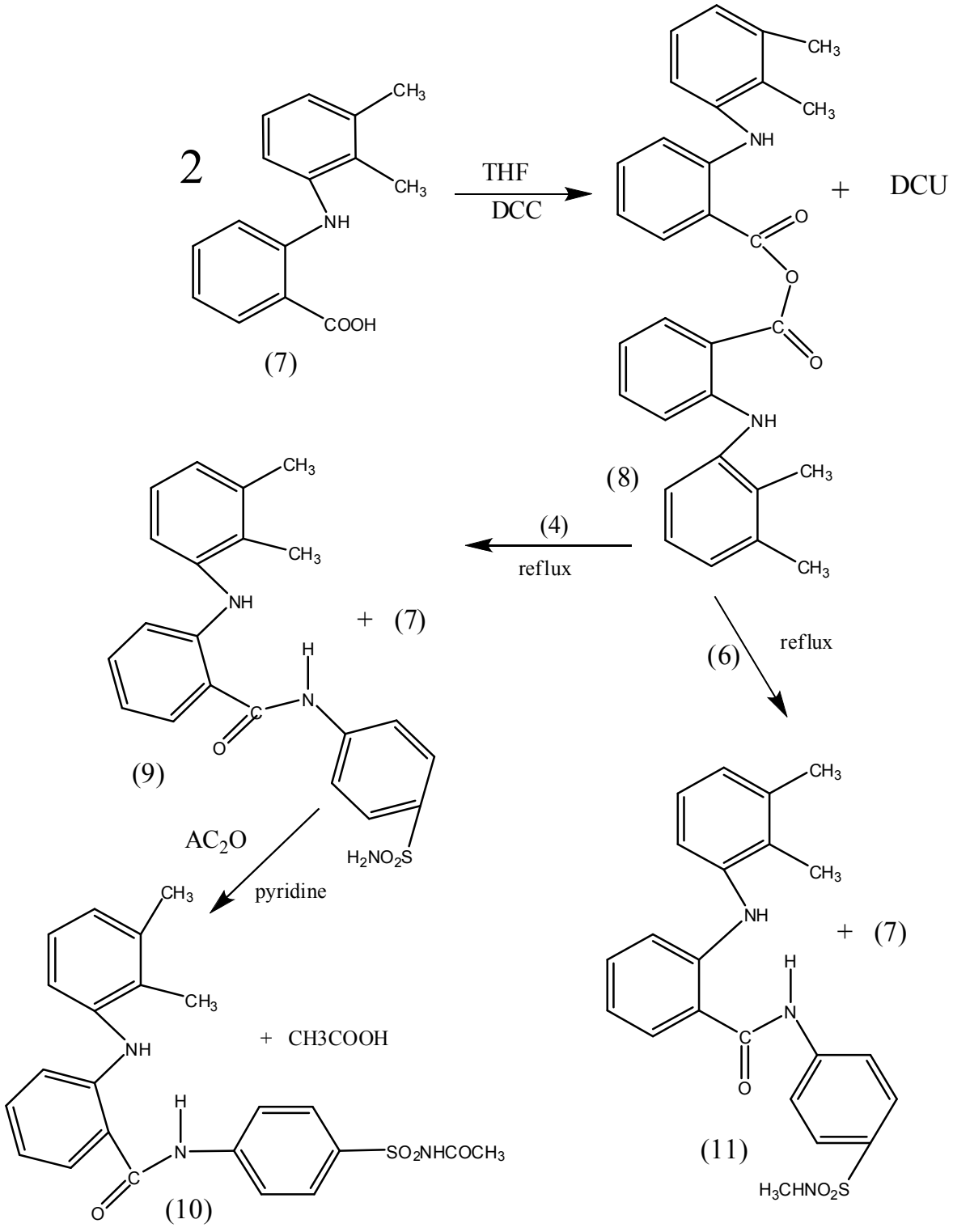
In the view of this background, the present study was conducted to design, synthesize and preliminarily evaluate new mefenamic acid derivatives as potential NSAIDs. [Future study: to measure their selectivity's on COX-2 enzyme.]

Chemistry

The general routes outlined in schemes 1 and 2 were used to synthesize all compounds described here. 4-aminobenzene-sulfonamide (4) and 4-amino-N-methylbenzene sulfonamide (6) was prepared as described by Vogel [24] starting from acetanilide as shown in scheme 1.Their characterization and physical data are presented in the table1.



Scheme 1: Synthesis of 4-aminobenzene sulfonamide (4) & 4-amino-N[1]-methylbenzene sulfonamide (6)



DCU: dicyclohexyl urea

Scheme 2: Synthesis of compounds 9, 10, and 11

Table (1): The characterization and physical data of the compounds (3-6 and 8-11).

Compound	Empirical formula	Molecular weight	Description	% yield	Melting point Observed	Melting point reported	R_f value
3	$C_8H_{10}N_2O_3S_1$	214	Faint yellow crystals	53	213-214	216 [25]	0.45
4	$C_6H_8N_2O_2S_1$	172	White crystals	51	160-161	163-164 [24]	0.75
5	$C_9H_{12}N_2O_3S_1$	228	White crystals	62	179-181		0.52
6	$C_7H_{10}N_2O_2S_1$	186	White powder	44	107-108		0.68
8	$C_{30}H_{28}N_2O_3$	464	White powder	80	141-143		0.69
9	$C_{21}H_{21}N_3O_3S_1$	395	White crystals	40	198-199		0.82
10	$C_{23}H_{23}N_3O_4S_1$	437	White powder	48	169-171		0.76
11	$C_{22}H_{23}N_3O_3S_1$	409	White crystals	35	180-181		0.8

Solvent system: Methanol: Acetic acid: Ether: Benzene (2:18:60:20**)**

Experimental

All reagents and anhydrous solvents were of analar type and generally used as received from the commercial supplier(Merk-Germany,Reidel-Dehean-Germany ,Sigma-Aldrich-Germany and BDH-England).Mefenamic acid was supplied from Micro Company - Indian.Melting points were determined by capillary method on Thomas Hoover apparatus (England) and ascending thin layer chromatography (TLC) was run on DC-Kartan SI Alumina 0.2 mm to check the purity and progress of reaction. The identification of compounds was done using iodine vapor and the chromatograms were eluted by: **Methanol: Acetic acid: Ether: Benzene (2:18:60:20)**.

IR spectra were recorded on model 500 scientific IR spectrophotometer, Buck Company (USA) as a **KBr film**.**CHN** microanalysis has been done using exter TE micro-analyzer (Germany).The analysis was done in the micro analytical center faculty of science –University of Cairo.

Synthesis of 2-(2, 3-dimethylphenylamino) benzoic anhydride (8):

Mefenamic acid (comp.7) (5g, 20.7mmol) was dissolved in THF (30ml), and then DCC (2.12g, 10.35mmol) was added. The reaction mixture was continuously stirred at room temperature for 4 hours. A white precipitate of DCU was formed which then removed by filtration. The solvent was evaporated under vacuum to give comp.8[26] . The percent yield, physical data and R_f value were given in table (1). IR 3330(NH) of secondary amine 1814 and 1743 (C=O) of anhydride, 1618, 1515 and 1488 (C=C st.v.), 1274, 1215 and 1172[C-(C=O) – O-(C=O) –C] cm^{-1} of anhydride.

Synthesis of 2-(2, 3-dimethylphenylamino)-N-(4-sulfamoylphenyl) benzamide (9):

Compound 8 (2.5g, 5.4mmol), compound 4 (0.93g, 5.4mmol), zinc dust (6mg), glacial acetic acid (0.5ml, 8.75mmol) and dioxane (20ml) were placed in a flask, equipped with refluxed condenser, boiling stones were added. The reaction mixture was refluxed gently for 90 minutes. The solvent was evaporated under vacuum, the residue was dissolved in ethyl acetate, washed with $NaHCO_3$ (10%, 3*10ml), HCl (1N, 3*10ml) and distilled water (3*10ml), filtered over anhydrous magnesium sulfate. The filtrate is evaporated under vacuum to give the product. The crystallization is carried out by dissolving the compound in ethyl acetate and petroleum ether (80-100 ^oC) is added to the filtrate until turbidity take place and it is kept in cold place over night. The mixture is filtered while it is cold and the precipitate is collected to give comp.9[27] . The percent yield, physical data and R_f value were given in table (1). IR 3376and3304 (N-H) of primary sulfonamide, 3227 (N-H) of secondary amine, 1660 (C=O) of secondary amide, 1598and1530(C=C st.v.), 1327and1157 (SO2) cm^{-1} .

CHN Calculated (C21H21N3O3S1): C, 63.78; H, 5.35; N, 10.36; S, 8.11. Found: C, 62.55; H, 5.44; N, 10.51; S, 8.25.

Synthesis of N-(4-(N-acetylsulfamoyl)-2-(2, 3-dimethylphenylamino) benzamide (10):

Acetic anhydride (0.6ml, 6mmol), was added to a solution of compound 9 (0.79g, 2mmol) in pyridine (10ml) and the reaction was allowed to proceed then at 25 ^{o}C with stirring for 6 hours. Ethyl acetate (100ml) was added and this solution was washed successively with saturated aqueous ammonium chloride (2x20ml) followed by distilled water (2x20ml). The organic fraction was dried with anhydrous magnesium sulfate and the solvent was removed in vacuum to give comp.10 [28] .The percent yield, physical data and R_f value were given in table (1). IR 3350and3292 (N-H) of secondary amide andsulfonamide respectively, 1670 (C=O) of secondary amide, 1595, 1533, and1450(C=C st.v.) and1332 and1157(SO2) cm^{-1} . **CHN** Calculated (C23H23N3O4S1): C, 63.14; H, 5.30; N, 9.60; S, 7.33. Found: C, 62.25; H, 5.40; N, 9.83; S, 7.48.

Synthesis of 2-(2, 3dimethylphenylamino)-N-(4-(N-methylsulfamoyl) benzamide (11):

Compound 8 (2.5g, 5.4mmol), compound 6 (1g, 5.4mmol), zinc dust (6mg), glacial acetic acid (0.5ml, 8.75mmol) and dioxane (25ml) were placed in flask, equipped with reflux condenser, boiling stones were added. The reaction mixture was refluxed gently for 90 minutes, and then it was worked up as prescribed in section3.2 to liberate comp.11. The percent yield, physical data and R_f value were given in table (1). IR 3334and3201 (N-H) of secondary amide andsulfonamide, 1664 (C=O) of secondary amide, 1591, 1529 and 1496 (C=C st.v.) and1321 and1159(SO2) cm^{-1} . **CHN** Calculated (C22H23N3O3S1): C, 64.53; H, 5.66; N, 10.26; S, 7.83. Found: C, 65.20; H, 5.58; N, 10.45; S, 8.01.

Pharmacology:

Albino rats of either sex weighing (150 ± 10 g) were supplied by the National Center for Quality Control and Drug Research and were housed in the animal house of the College of Pharmacy, University of Baghdad under standardized conditions (12 light-12 dark cycle) for 7 days for acclimatization. Animals were fed commercial chaw and had free access to water ad *libitum*. Animals were brought 1 hour before the experiment to the laboratory, and were divided into five groups (each group consist of 6 rats) as follows: **group A**: served as control and treated with the vehicle (propylene glycol 50% v/v in water); **group B**: treated with sodium diclofenac (reference agent) in a dose of 3mg/kg suspended in propylene glycol [29]; **group C**, **D** and **E**: treated with tested compounds 9, 10 and 11 respectively in a dose equivalent to 7.5 mg/kg of mefenamic acid as finely homogenized suspension in 50% v/v propylene glycol[30] .

Anti-inflammatory activity:

The anti-inflammatory activity of the tested compounds was studied using egg-white induced edema model [31]. The drugs or the vehicle were administered i.p. at time zero and acute inflammation was induced by a subcutaneous injection of 0.05ml of undiluted egg-white into the planter side of the left hind paw of the rats at time 15 minutes. The paw thickness was measured by vernier at eight time intervals (0, 15, 30, 60, 120, 180, 240 and 300 minutes) after vehicle or drugs administration. The data are expressed as mean ± S.E.M. and results were analyzed for statistical significance using Student *t*-test (Two-Sample Assuming Equal Variances) for comparisons between mean values. While comparisons between different groups were made using ANOVA: Two-Factor Without Replication. Probability (P) value of less than 0.05 was considered significant.

Results and Discussion

The most widely used primary test to screen new anti-inflammatory agents is based on the ability of a compound to reduce local edema induced in the rat paw following injection of an irritant agent [32]. When egg-white is injected into the paw of rats, a substantial induction of COX-2 is observed at 2 hours coinciding with enhanced PGs and local edema [33]. Tables 2 and 3 show the effect of tested compounds on egg-white induced edema as an indicator for their anti-inflammatory activity. The intraplanter injection of egg-white into rat hind paw induces a progressive edema, which was reached maximum (measured by millimeter) after 2 hours of injection. Table 2 showed the effect of tested compounds (9, 10 and11) in respect to control group. All tested compounds were effectively limited the increase in paw edema, with the effect of compounds 9 and 10 started at time 30 minute (significantly difference compared to control), while compound 11 started at time 120 minute. However, the effect of all tested compounds continued till the end of the experiment with statistically significant ($p > 0.05$) reduction in paw edema. The differences among the

compounds started at time 30 minute in which the compounds 9 and 10 significantly difference at time 30 and 60 minute compared to compound 11. However, the differences among the compounds continued from the time 180 to 300 minute with statistically significant ($p > 0.05$) reduction in paw edema in the following orders 10, 11, and 9 respectively.

Table 2: Effect of Control and Compounds 9, 10 and 11 on egg-white induced paw edema in rats.

	Treatment groups				
	Time (min)	Control (n=6)	Compound9 (n=6)	Compound 10 (n=6)	Compound11 (n=6)
Paw thickness (mm)	0	4.46 ± 0.16	4.39±0.10	4.41±0.08	4.38±0.13
	15	5.41 ± 0.18	5.45±0.07	5.42±0.12	5.35±0.11
	30	6.05 ± 0.16	5.80±0.05^{*a}	5.76±0.13^{*a}	6.01±0.10^{b}
	60	6.35 ± 0.07	6.00±0.05^{*a}	6.00±0.13^{*a}	6.33±0.09^{b}
	120	6.50 ± 0.09	5.73±0.05^{*a}	5.66±0.08^{*a}	5.70±0.10^{*a}
	180	5.93 ± 0.11	5.40±0.05^{*a}	5.09±0.05^{*b}	5.30±0.07^{*c}
	240	5.38 ± 0.09	5.13±0.05^{*a}	4.86±0.07^{*b}	4.95±0.07^{*c}
	300	5.20 ± 0.10	5.05±0.04^{*a}	4.56±0.08^{*b}	4.68±0.05^{*c}

Non-identical superscripts (a, b, and c) among different groups are considered significantly different ($P<0.05$).

* significantly different compared to control ($P<0.05$).

Table 3 shows the effect of tested compounds (9, 10 and11) with respect to the reference group (diclofenac). As seen in this table; at time 0 and 15 minute there are no differences among different groups; at time 30, only compound 11 is significantly different than diclofenac; at time 60 and 120 all compounds are significantly different than diclofenac; while at time 180 to 300 compounds 9 and 11 are significantly different than diclofenac. The differences among the compounds started at time 30 minute in which the compounds 9 and 10 significantly difference at time 30 and 60 minute compared to compound 11 while at time 120 compound 10 is significantly different than compounds 9 and 11. However, the differences among the compounds continued from the time 180 to 300 minute with statistically significant ($p > 0.05$) reduction in paw edema in the following orders 10, 11, and 9 respectively.

Table 3: Effect of Diclofenac and Compounds 9, 10 and 11 on egg-white induced paw edema in rats.

		Treatment groups			
	Time (min)	Diclofenac (n=6)	Compound9 (n=6)	Compound 10 (n=6)	Compound11 (n=6)
Paw thickness (mm)	0	4.38±0.14	4.39±0.10	4.41±0.08	4.38±0.13
	15	5.37±0.41	5.45±0.07	5.42±0.12	5.35±0.11
	30	5.78±0.11	5.80±0.05 [a]	5.76±0.13 [a]	6.01±0.10 [*b]
	60	5.60± 0.10	6.00±0.05 [*a]	6.00±0.13 [*a]	6.33±0.09 [*b]
	120	5.35±0.10	5.73±0.05 [*a]	5.66±0.08 [*b]	5.70±0.10 [*a]
	180	5.07±0.10	5.40±0.05 [*a]	5.09±0.05 [b]	5.30±0.07 [*c]
	240	4.87±0.10	5.13±0.05 [*a]	4.86±0.07 [b]	4.95±0.07 [*c]
	300	4.61±0.10	5.05±0.04 [*a]	4.56±0.08 [b]	4.68±0.05 [*c]

Non-identical superscripts (a, b, and c) among different groups are considered significantly different ($P<0.05$).
* Significantly different compared to control ($P<0.05$).

Conclusion

The *in vivo* anti-inflammatory study showed that the incorporation of 4-aminobenzenesulfon-amide, 4-amino-N-methylbenzenesulfonamide, or N-(4-aminophenylsulfonyl) acetamide into well known anti-inflammatory drug (mefenamic acid) maintains its anti-inflammatory activity. Compound 10 showed more potent anti-inflammatory effect than compound 9 or 11 and have a comparable effect to that of diclofenac at time 180 to 300 minute with the same onset of action.

Acknowledgments

We are grateful to the staff members and Colleagues of the Department of Pharmaceutical Chemistry and the Department of Pharmacology and Toxicology .Also we wish to express grateful thanks to M.Sc. Sabah Jawad for his help and support.

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