Synthesis and Preliminary Pharmacological Study of Sulfonamide Conjugates with Ibuprofen and Indomethacin as New Anti-Inflammatory Agents

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Abstract

4-aminobenzenesulfonamide conjugates of ibuprofen (compound 10) and indomethacin (compound 11) have been designed and synthesized by the reaction of sulfanilamide (compound 7) with 2-(4-isobutylphenyl) propanoic acid (ibuprofen) and 2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-yl)acetic acid (indomethacin) for the evaluation as potential anti-inflammatory agents with expected selectivity against COX-2 enzyme. *In vivo* acute anti-inflammatory activity of the synthesized final compounds (10 and 11) was evaluated in rats using egg-white induced edema model of inflammation in a dose equivalent to (10mg/Kg) of ibuprofen and (2mg/kg) of indomethacin. The tested compounds produced significant reduction of the rat paw edema with respect to the effect of propylene glycol 50% v/v (control group). Moreover, compound (10) exhibited comparable anti-inflammatory activity to diclofenac (3mg/Kg) (reference group) while compound (11) exhibited less anti-inflammatory activity than diclofenac. The result of this study indicate that the incorporation of the 4-aminobenzenesulfonamide pharmacophore into ibuprofen and indomethacin maintained their anti-inflammatory activity & may increase their selectivity toward COX-2 enzyme which can be confirmed in future by assessing COX-2:COX-1 inhibitory ratio.

Key words: Cyclooxygenase; Anti-inflammatory activity; Ibuprofen; indomethacin; NSAIDs.

الخلاصة

تم تصميم و تحضير مقترنات [٤-امينوبنزين سلفونامايد] للايبوبروفين (مركب ١٠) و للاندوميثاسين مركب (١١) عن طريق تفاعل السلفانيلامايد (مركب ٧) مع ٢(٤-ايزوبيوتيل فنيل) بروبانويك اسيد (الايبوبروفين) و ٢-(١-(٤-كلوروبنزويل)-٥-ميثوكسي-٢-مثيل-١H-اندول-٣-يل)استيك اسيد (الاندوميثاسين) لتقييمهما كعوامل مضادة للالتهاب جديدة مع احتمالية انتقائية مضادة لانزيم الكوكس الثاني (COX-2) .اجري تقييم الفعالية المضادة للالتهاب في الجسم الحي للمركبان النهائيان (١٠ و ١١) في الجرذ باستخدام زلال البيض مستحدثة وذمة التهابية تحت الجلد بجرعة مكافئة للايبوبروفين (١٠ملغم/كغم) و بجرعة مكافئة للاندوميثاسين (٢ملغم/كغم).المركبات المختبرة انتجت انخفاض مؤثرا للوذمة بالمقارنة مع البروبلين كلايكول ٥٠%(propylene glycol) كمجموعة ضابطة.لقد اظهر المركب (١٠) فعالية مضادة للالتهاب مقارنة للدايكلوفيناك (diclofenac) (٣ملغم/كغم) كمجموعة مرجعية بينما المركب (١١) اظهر فعالية مضادة للالتهاب اقل من الدايكلوفيناك.تشير نتيجة هذه الدراسة الى ان اندماج الجزء العقاقيري ٤- امينوبنزين سلفونامايد مع الايبوبروفين و الاندوميثاسين قد حافظ على فعاليتهما المضادة للالتهاب مع احتمال زيادة انتقائيتهما تجاه انزيم الكوكس الثاني والذي يمكن ان تثبت مستقبلا بتحصيل النسبة المثبطة للكوكس-٢ الى الكوكس-١ .

Introduction

Prostaglandins (PGs) are active mediators of inflammatory responses and also provide cytoprotection in the stomach and intestine. The key enzyme of their biosynthesis is prostaglandin H2 synthase (PGHS) or called cyclooxygenase (COX) [(1)]**.** It is now well established that three distinct COX isoforms exist: the constitutive form COX-1 is expressed virtually in all tissues and is involved in the regulation of physiological functions such as in maintaining platelet aggregation and homeostasis of the GI tract and the kidney [(2)]. COX-2 is rapidly induced in inflammatory cells in response to cytokines such as tumor necrosis factor-α (TNF-α), interleukines, growth factors, and so on [(3)]. Recently a third full active isoform, COX-3, and two partial isoforms, pCOX1a and b, were reported to be found in the cerebral cortex and in human heart[(4, 5)]. Non-steroidal anti-inflammatory drugs (NSAIDs) continue to be one of the more widely used groups of therapeutic agents, which inhibit COX-1, COX-2, and tromboxane synthase with a varying degree of selectivityResearchers have recently focused on selective COX-2 inhibitors which are believed to reduce inflammation without influencing normal physiologic functions of COX-1. The first COX-2 selective NSAID approved by Food and Drug Administration (FDA) was celecoxib (1), which was followed by introduction of rofecoxib (2) and valdecoxib (3).

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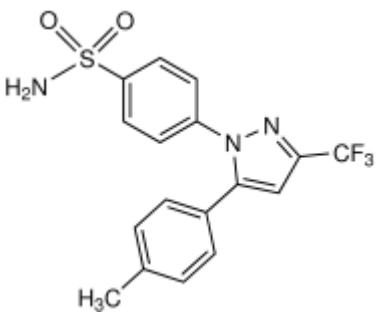
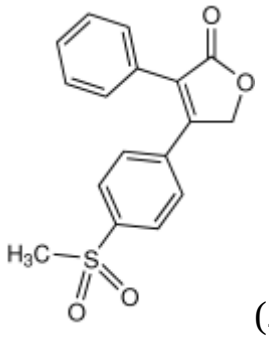
A common structural backbone of most COX-2 selective inhibitors consists of two aryl groups linked to adjacent atoms of a central ring which can be homocyclic or heterocyclic, one of the aryl groups is substituted in the para position with an aminosulfonyl (SO_2NH_2) group [6]. Since their introduction, COX-2 specific inhibitors have become a rapidly growing segment of prescription drug market, especially for osteoarthritis and rheumatoid arthritis patients. However, recently there is a controversy regarding the hepatic toxicity of nimusulide (4) and for the cardiovascular complications of rofecoxib. FDA has banned the use of nimusulide in pediatric patients and rofecoxib in both adults and children. In spite of these facts, there is a growing need for the development of safer COX-2 selective inhibitors [7]. Therefore, in view of the above facts, we report the synthesis and preliminary pharmacological evaluation of an ibuprofen_ and an indomethacin_ sulfonamide conjugate as NSAIDs with expected COX-2 inhibitors.



(1)



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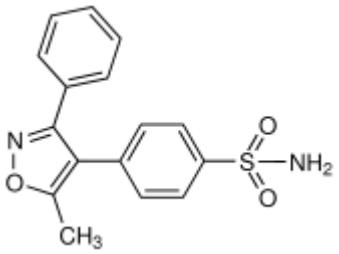

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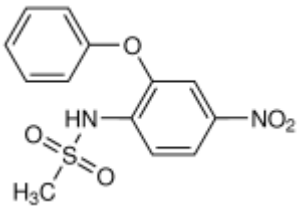
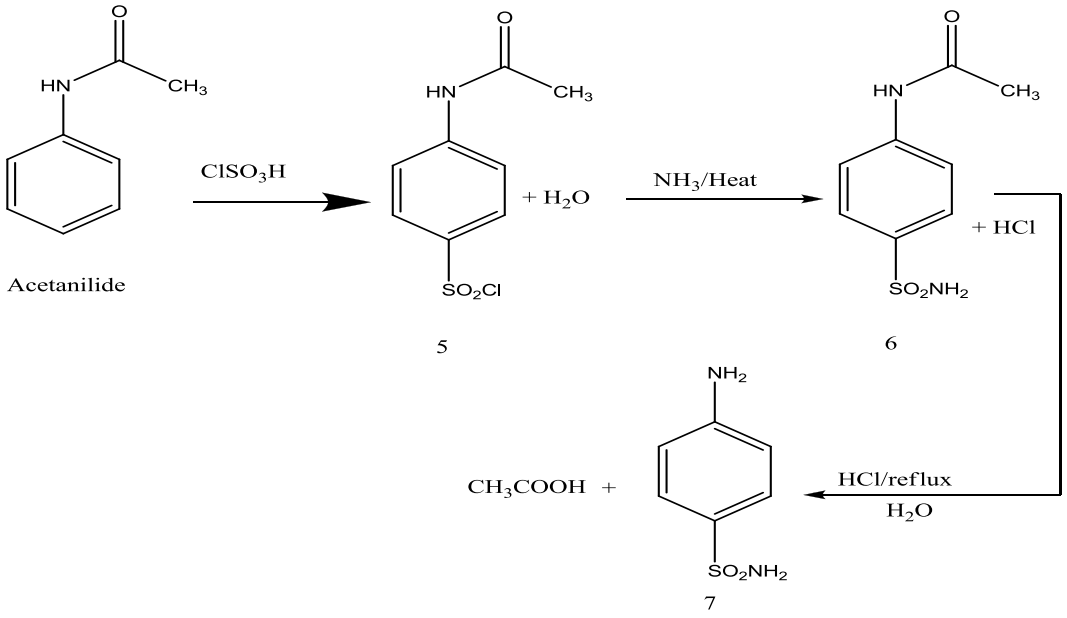


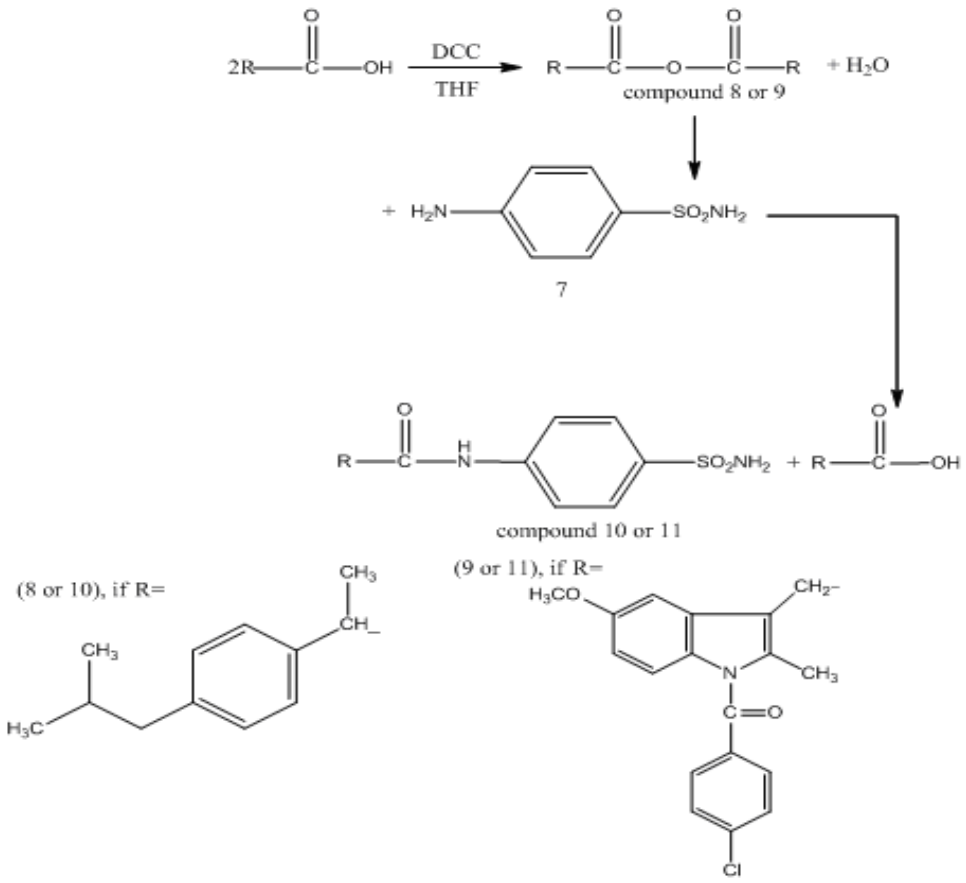
(4)

Chemistry

The general routes outlined in schemes 1, 2 and 3 were used to synthesize all compounds described here.As shown in scheme 1; 4-aminobenzenesulfonamide (7) was prepared as described previously (Vogel) [8] starting from acetanilide.



Scheme 1: Synthesis of compound ٧



Scheme 2: Synthesis of compounds 8, 9, 10 and 11

Experimental

All reagents and solvents were of analar type and generally used as received from the commercial supplier (E Merck-Germany, Reidel-Dehean-Germany, Sigma-Aldrich-Germany & BDH-England). Ibuprofen and indomethacin were supplied from SAFA Company-Iraq.Melting points were determined by capillary method on Gallenkamp apparatus (England) and ascending thin layer chromatography (TLC) was run on silica gel to check the purity and progress of reaction. The identification of compounds was done using iodine vapor and the chromatograms were eluted by: Ethyl acetate: Methanol: Ammonia (85:10:5). IR spectra were recorded on Shimadzu FTIR spectrophotometer (Japan) as a KBr film in the College of Science/University of Basrah. CHN microanalysis was done by using Euro Vector EA3000A elemental analyzer, Italy, in the AL-Albait University /Jordan.

Synthesis of 4-acetamidobenzene-1-sulfonyl chloride (5)

Acetanilide (1.35g, 10mmol) was placed in a 100ml flask and melted in it over a free flame that caused the compound to solidify over the lower part of the flask when swirling the liquid formed after immersion in an ice bath momentarily. Chlorosulfonic acid (3.5ml, 53mmol) was added all at once with continuous shaking, and then the reaction mixture was heated on a water bath for 90 minutes. The mixture was cooled and poured with stirring onto crushed ice (or ice water), and after stirring for about 5 minutes an even suspension of the granular solid was obtained. This suspension was filtered off under vacuum, washed with a little cold water, and pressed dried to give compound (5) which was immediately used in the next step without further purification [8].

Synthesis of N-(4-sulfamoylphenyl) acetamide (6)

Compound (5) (2.34g, 10mmol) was added to a mixture of concentrated ammonia solution (20 ml) and water (20 ml). The contents of the flask were heated with occasional swirling to just below the boiling point for about 20 minutes. The formed suspension was cooled in an ice bath and then acidified with diluted sulfuric acid and the formed precipitate was filtered under vacuum then washed with cold water to give the intermediate product (6) [8]. (53% yield) as a faint yellow crystals. m.p. 213-215^{o}C. R_f =0.61.

Synthesis of 4-aminobenzenesulfonamide (7)

Compound (6) (2.14g, 10mmol) was transferred to a flask containing mixture of concentrated hydrochloric acid (10 ml) and water (30 ml). The mixture was boiled gently under reflex for 90 minutes. Cooled to room temperature, then activated charcoal (2 gm) was added. The mixture was heated to boiling and filtered with suction through hardened filter paper. The filtrate was placed in a beaker and sodium bicarbonate was added in portions with stirring until the suspension become neutral to litmus. The mixture was cooled in ice bath and filtered by suction and dried to give compound (7) [8], (45% yield) as white crystals. m.p. 160-161^{o}C. R_f =0.75. **IR** 3477& 3375 (N-H) stretching of primary amine, 3367&3296 (N-H) stretching of sulfonamide, 1593, 1562&1502 (C=C) stretching of aromatic, 1301&1149 cm^{-1} (SO2) stretching.

Synthesis of 2-(4-isobutylphenyl) propanoic anhydride (8)

Ibuprofen (2.06g, 10mmol) was dissolved in tetrahydrofuran (THF) (10 ml), and then dicyclohexyl carbodiimide (DCC) (1g, 5mmol) was added. The reaction mixture was stirred continuously at room temperature for 3.5 hours, where a white precipitate of dicyclohexylurea (DCU) was formed which was then removed by filtration. The solvent was evaporated under vacuum to yield compound (8) [9], (80% yield) as a white powder. m.p. 208-213 ^{o}C. R_f =0.67.

Synthesis of 2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-yl)acetic anhydride (9)

Indomethacin (3.58g, 10mmol) was dissolved in tetrahydrofuran (THF) (10 ml), and then dicyclohexyl carbodiimide (DCC) (1g, 5mmol) was added. The reaction mixture was stirred continuously at room temperature for 3.5 hours, where a white precipitate of dicyclohexylurea (DCU) was formed which was then removed by filtration. The solvent was evaporated under vacuum to yield compound (9) [9], (77% yield) as an oily substance.

Synthesis of 2-(4-isobutylphenyl)-N-(4-sulfamoylphenyl) propanamide (10)

Compound (8) (3.94g, 10mmol), compound (7) (1.72g, 10mmol), zinc dust (0.010g), glacial acetic acid (1.1ml, 20mmol) and dioxan (30ml) were placed in a flask equipped with reflux condenser. The reaction mixture was refluxed gently for 90 minutes, the solvent was evaporated under vacuum, the residue was dissolved in ethyl acetate and washed with 5ml. $NaHCO_3$ (10%, 3X), HCl (1N, 3X), and distilled water (3X),then filtered over anhydrous magnesium sulfate. The filtrate

was evaporated under vacuum to give compound (10). The recrystallization was carried out by dissolving the compound in ethyl acetate and addition of petroleum ether on the filtrate until turbidity occurred and kept in cold place over night. The mixture was filtered while it was cold and the precipitate was collected to give compound (10) [10]. (63% yield) as an off white crystals. m.p. 128-130 ^{o}C. R_f =0.8. **IR:** 3296 (N-H) stretching of sulfonamide, 1662 (C=O) stretching of secondary amide, 1593, 1523&1456 (C=C) stretching of aromatic & 1328&1159 cm^{-1}(SO2) stretching. **CHN** Calculated ($C_{19}H_{24}N_2O_3S$): C, 63.31; H, 6.71; N, 7.77; S, 8.9. Found: C, 63.11; H, 6.93; N, 7.36; S, 8.78.

Synthesis of 2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indol-3-yl)-N-(4-sulfamoylphenyl)acetamide (11)

Compound (9) (6.97g, 10mmol), compound (7) (1.72g, 10mmol), zinc dust (0.010g), glacial acetic acid (1.1ml, 20mmol) and dioxan (30ml) were placed in a flask equipped with reflux condenser. The reaction mixture was refluxed gently for 90 minutes, the solvent was evaporated under vacuum, the residue was dissolved in ethyl acetate and washed with 5ml. $NaHCO_3$ (10%, 3X), HCl (1N, 3X), and distilled water (3X), then filtered over anhydrous magnesium sulfate. The filtrate was evaporated under vacuum to give compound (11). The recrystallization was carried out by dissolving the compound in ethyl acetate and addition of petroleum ether on the filtrate until turbidity occurred and kept in cold place over night. The mixture was filtered while it was cold and the precipitate was collected to give compound (11) [10]. (50% yield) as an oily substance. **IR** 3325 (N-H) stretching of sulfonamide, 1687 (C=O) stretching of secondary amide, 1600, 1523 & 1475 (Aromatic) & 1361&1151 cm^{-1} (SO2) stretching. **CHN** Calculated ($C_{25}H_{22}ClN_3O_5S$): C, 58.65; H, 4.33; N, 8.21; S, 6.26. Found: C, 57.89; H, 4.1; N, 8.16; S, 6.35.

Pharmacology

Rats weighing (160 ±10g) were supplied by the College of Veterinary Medicine/ University of Basrah and were housed in the animal house of the same college. Animals were fed commercial chaw and had free access to water. Animals were brought to the laboratory, and were divided into four groups (each group consist of six rats) as follow:

Group A: Six rats served as control; and treated with the vehicle (propylene glycol 50% v/v);

Group B: treated with diclofenac (reference agent) in a dose of 3mg/kg suspended in propylene glycol 50% v/v [11];

Group C: treated with tested compound 10 in a dose equivalent to 10 mg/kg of ibuprofen as suspension in 50% v/v propylene glycol [12];

Group D: treated with tested compound 11 in a dose equivalent to 2 mg/kg of indomethacin as suspension in 50% v/v propylene glycol [13].

Anti-inflammatory activity

The anti-inflammatory activity of the tested compounds was studied using egg-white induced edema model. Acute inflammation was produced by a subcutaneous injection of 0.05ml of undiluted egg white into the planter side of the left hind paw of the rats; 15 minutes after i.p. administration of the drugs or the tested compounds or their vehicle. The paw thickness was measured by vernea at six time intervals (0, 1, 2, 3, 4 and 5 hours) after drug administration. The results were analyzed for statistical significance using paired student *t*-test for comparisons between mean values with respect to their baseline, while comparisons between different groups were made using Analysis of Variance (ANOVA) test. P value of less than 0.05 was considered significant.

Results and Discussion

Among the many methods used for screening of anti-inflammatory drugs, one of the most commonly employed techniques is based upon the ability of such agents to inhibit the edema produced in the hind paw of the rat after injection of an irritant agent [14]. The intra-planter injection of egg white into rat hind paw induces a progressive edema, which was reached maximum (measured by millimeters) after 2 hours of injection. Table 1 showed the effect of tested compounds (10&11) in respect to control group. All tested compounds were effectively limited the increase in paw edema, with the effect of compound 10 started at 1 hour (significantly different compared to control), while compound 11 started at 3 hours, which mean it has slower onset of action than the other tested compounds. However, the effect of all tested compounds continued till the end of the experiment with statistically significant ($P>0.05$) reduction in paw edema.

Table 1: Effect of Control, Compounds 10 & 11 on egg-white induced paw edema in rats.

Treatment Group				
	Time	**Control (n=6)**	**Compound 10 (n=6)**	**Compound 11 (n=6)**
Paw Thickness (mm)	Baseline	4.48±0.08	4.35±0.07	4.45±0.16
	0	5.41±0.11	5.40±0.04	5.41±0.11
	1 hr.	6.03±0.08	5.47±0.04^{*a}	5.81±0.07^{b}
	2 hrs.	6.62±0.18	5.69±0.11^{*a}	6.37±0.29^{b}
	3 hrs.	5.87±0.11	5.28±0.06*	5.36±0.09*
	4 hrs.	5.65±0.11	4.97±0.05*	5.00±0.23*
	5 hrs.	5.33±0.11	4.90±0.08*	4.81±0.28*

Non-identical superscripts (a & b) among different tested compounds are considered significantly different (P<0.05)

* Significantly different compared to control (P<0.05).

Table 2 showed the effect of tested compounds (10 & 11) in respect to reference group (diclofenac). As seen in this table; at baseline and time 0 there are no differences among different groups; at time 1 and 2 hours compound 11 has significantly lower effect than diclofenac and compound 10. However, it appears that all the tested compounds had a comparable effect to that of diclofenac at times of 3-5 hours of experiment.

Table 2: Effect of Diclofenac & Compounds 10&11 on egg-white induced paw edema in rats.

Treatment Groups				
	Time	**diclofenac (n=6)**	**Compound 10 (n=6)**	**Compound 11 (n=6)**
Paw Thickness (mm)	Baseline	4.42±0.14	4.35±0.07	4.45±0.16
	0	5.43±0.14	5.40±0.04	5.41±0.11
	1hr.	5.51±0.10	5.47±0.04^{a}	5.81±0.07^{*b}
	2hr.	5.71±0.12	5.69±0.11^{a}	6.37±0.29^{*b}
	3hr.	5.43±0.27	5.28±0.06	5.36±0.09
	4hr.	5.14±0.13	4.97±0.05	5.00±0.23
	5hr.	4.78±0.08	4.90±0.08	4.81±0.28

Non-identical superscripts (a & b) among different tested compounds are considered significantly different (P<0.05)

*Significantly different compared to diclofenac (P<0.05).

Conclusion

In vivo, the anti-inflammatory study showed that the incorporation of 4-aminobenzenesulfonamide into well known anti-inflammatory drugs (ibuprofen & indomethacin) retained the anti-inflammatory activity, when compared with that of diclofenac, compound (10) showed a comparable effect, whereas that of compound (11) showed a lower effect. Similar work in future will be done to find the extent of activity variation of compounds (10) and (11) relative to their parent agents (ibuprofen and indomethacin), respectively, and their COX2:COX1 selectivity ratio.

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