Synthesis and preliminary pharmacological evaluation of aminobenzensulfonamides derivatives of diflunisal as an anti-inflammatory agents

Munther F. mahdi,* Abdul-Rassoul Wars,* Samira Fingan**

* Department of pharmaceutical chemistry, **Department of pharmacology and toxicology, college of pharmacy, university of Baghdad.

Received: ٣.٣.٨٠٠٢ Accepted: ٢.١١.٨٠٠٢

ABSTRACT

Objective: Synthesized of amino derivatives [4-aminobenzenesulfonamide, 4-amino-N-methylbenzenesulfonamide, or N-[4-aminophenylsulfonyl] acetamide] bound to carboxyl group of diflunisal, a well known nonsteroidal anti-inflammatory drugs (NSAIDs), and evaluation as a potential anti-inflammatory agent with expected selectivity against COX-2 enzyme.

Design: Experimental study

Results: In vivo acute anti-inflammatory activity of the final compounds (٣١, ٤١, & ٥١) was evaluated in rat using egg-white induced edema model of inflammation in a dose equivalent to (٠.٥ mg/Kg) of diflunisal. All tested compounds produced significant reduction of paw edema with respect to the effect of propylene glycol ٠.٥% v/v (control group). Moreover, compound (٤١) exhibited comparable anti-inflammatory activity to diclofenac (٣ mg/Kg), while compound (٣١) showed short duration of action, and compound (٥١) exhibited comparable effect to that of diclofenac with slower onset of action.

Conclusion: The result of this study indicate that the incorporation of the 4-aminobenzenesulfonamide pharmacophore & its derivatives into diflunisal enhanced its anti-inflammatory activity & may increased its selectivity toward COX-2 enzyme which can be confirmed in future by assessing COX-2:COX-1 inhibitory ratio using whole blood assay.

الخلاصة

الأهداف: مجموعة من المشتقات الأمينية [٤- أمينوبينزين سلفوناميد، ٤- أمينو-N-ميثيل بنزين سلفوناميد، ٤- أمينوبينزيل سلفوناميد] كمضادات لالتهابات جيدة مع نز�性 ممتازة ضد الكوكس الثاني (COX-2) قد تحسنت قدرة ديفلوينسال (diflunisal) كدواء غير الستيرويدي المعروف جيدا كمضاد للالتهاب، وتمت وحضرة تقييمها كمضادات للالتهاب.

التصميم: دراسة مخبرية

النتائج: في الجسم الحي، تم تقييم فعالية المضادات للالتهابات الحادة للمركبات النهائية (٣١، ٤١، ٥١) في الجرعة (٠.٥ مل/كم) باستخدام مادة الزيت الالطريدة م محلل بجرعة معينة. كل المركبات المختبرية انتجت انخفاضا ملحوظا بالمقارنة مع البروبيلين كلايكول ٥٠% (propylene glycol) كموجبة ضابطة. ان مركب (٤١) أظهر فعالية مضادة لالتهاب مقارنة للدابليوفينكاك (diclofenac) ٣ ملغ/كم، بينما المركب (٣١) أظهر فعالية استمرارية أقصى والمركب (٥١) أظهر فعالية مضادة لالتهاب مقارنة للدابليوفينكاك مع فعالية ابتدائية ابسط.

الاستنتاج: نتائج هذه الدراسة تشير الى ان اندماج الجزء العقاقيري ٤- أمينوبينزين سلفوناميد ومشتقاته مع ديفلوينسال ينشط فعاليتهم المضادة للالتهاب مع احتمال زيادة انتقائيته نحو ازيم الكوكس الثاني والذي يمكن أن تثبت مستقبلا بتحصيل النسبة المنخفضة للكوكس-2 إلى الكوكس-١ باستخدام معايرة الدم لكل.
In 1899, acetyl salicylic acid (aspirin, I) was introduced as the first potent drug to treat rheumatic disease. In the following decades, dozens of non-steroidal anti-inflammatory drugs (NSAIDs) were developed and launched, but the first real progress in our understanding of the mechanism of action of the NSAIDs came in 1971 when Vane revealed that these chemically varied drugs all reduced the formation of prostaglandins (PG). This ability was associated with inhibition of cyclooxygenase (COX) enzyme.

Cyclooxygenase is a rate limiting enzyme for prostaglandin synthesis. The three isoenzymes of COX (COX-1, COX-2 and COX-3) have been identified, though COX-3 activity in human has not been confirmed. COX-1 is constitutively expressed, widely distributed and has "housekeeping" function. It is of particular importance in maintaining gastric mucosal integrity, renal function and homeostasis. COX-2 is highly induced in settings of inflammation by cytokines and inflammatory mediators or physiological stress. However, COX-2 also is constitutively expressed in certain areas of kidney, brain, reproductive tract, the vascular system, in wound healing, lung and bone.

The search for a clinical replacement for aspirin resulted in the development of the nonacetylating salicylic acid derivative diflunisal (II) that is a more potent anti-inflammatory and analgesic agent with a longer duration of action that is less ulcerogenic than aspirin.

However, since the identification of cyclooxygenase-2 (COX-2), the field of inflammation and particularly the search for effective NSAIDs with fewer adverse effects has greatly intensified. Increasing number of experimental and clinical data support the role of selective COX-2 inhibitor in anti-inflammatory processes and the involvement of COX-1 inhibition in the side effects associated with using NSAIDs. Many of the selective COX-2 inhibitors containing benzene-sulfonamide derivative, like valdecoxib (III), celecoxib (IV), or benzene-N-methyl sulfonamide like compound (V).

In a recent study, it was shown that the incorporation of a para-N-acetysulfonamido substitute on the C-phenyl ring of the rofecoxib (VI) regiosomer provided a highly potent and selective COX-2 inhibitor that has the potential to acetylate the COX-2 isoenzyme.

\[
\text{compound I} \quad (1\text{-amino-N-methylbenzenesulfonamide})
\]

\[
\text{compound II} \quad (1\text{-aminobenzenesulfonamide})
\]
In the view of this background, the present study was conducted to design, synthesize and preliminarily evaluate new diflunisal derivatives as potential NSAIDs and future study to measure their selectivity's on COX-2 enzyme.

**Chemistry**

The general routes outlined in schemes 1 and 7 were used to synthesize all compounds described here. As shown in scheme 1; 1-amino-phenylsulfonamide (1) and 1-amino-N-methylbenzenesulfonamide (7) were prepared as described previously (Vogel) starting from acetonilide.

![Chemical structures](image-url)
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 & BDH, England). Diflunisal was supplied from RAM Company, Jordan.

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 0.25 mm to check the purity and progress of reaction. The identification of compounds was done using iodine vapour and the chromatograms were eluted by Methanol: Acetic acid: Ether (1:1:1) v/v.

IR spectra were recorded on model 250 scientific IR spectrophotometer, Buck Company (USA) as a KBr film. CHN microanalysis was done by using Carlo Erba elemental analyzer 1106, Italy. The synthesis of -acetoxy-\( \%\), -diflourophenethyl-\( \%\) carboxylic acid (\( \%\)).

In a 10 ml conical flask, equipped with reflux condenser, diflunisal (\( \%\)g, \( \%\)mmol) and acetic anhydride (\( \%\)ml, \( \%\)mmol) were placed and 1 drop of concentrated sulfuric acid was added dropwise. The reaction mixture was refluxed gently for 1 hour, and then allowed to cool with occasional stirring. Cold ice-water was then added until precipitate was formed, filtered by using suction pump, washed by cold distilled water several times, the crude product was collected. The recrystallization was carried out by using ethanol 95%, the precipitate was collected and dried to give compound \( \%\) (\( \%\)g, \( \%\)% yield) as white crystal. m.p. \( \%\) C, Rf = 1.8, \% cm\(^{-1}\) of acetate ester, \( \%\) (C=O) of carboxylic acid, \( \%\) & \% cm\(^{-1}\) of \% Synthesis of -acetoxy-\( \%\), -diflourophenethyl-\( \%\)-carboxylic anhydride (11).

Compound \( \%\) (\( \%\)g, \( \%\)mmol) was dissolved in THF (\( \%\) ml), and then Di-Cyclohexyl Carbodiimide (DCC) (\( \%\)g, \( \%\)mmol) was added. The reaction mixture was continuously stirred at room temperature for 2 hours. A white precipitate of di-Cyclohexyl Urea (DCU) was formed which then removed by filtration. The solvent was evaporated under vacuum to give \( \%\) (\( \%\)g, \( \%\)% yield) as a white powder. m.p. \( \%\) C, Rf = 1.8, \% cm\(^{-1}\) of anhydride, \% & \% cm\(^{-1}\) of (C=O) of \% of \( \%\), \( \%\)-diflouro-\( \%\)-(\( \%\)-sulfamoylphenylcarbamoyl) biphenyl-\( \%\)-yl acetate (11).

Compound \( \%\) (\( \%\)g, \( \%\)mmol), compound \( \%\) (\( \%\)g, \( \%\)mmol), zinc dust (\( \%\)mg), glacial acetic acid (\( \%\)ml, \( \%\)mmol) and dioxane (\( \%\)ml) were placed in a flask, equipped with reflex condenser. The reaction mixture was refluxed gently for 1 hour. The solvent was evaporated under vacuum, the residue was dissolved in ethyl acetate, washed successively with saturated aqueous sodium bicarbonate, and distilled water (three times for each step), filtered over anhydrous magnesium sulfate. The filtrate was evaporated under vacuum to give compound \( \%\). Recrystallization was carried out by dissolving the compound in ethyl acetate, petroleum ether (\( \%\):\( \%\):\( \%\) C) was then added on the filtrate until turbidity took place and it was kept in cold place over night. The mixture was filtered while cold and the precipitate was collected to give compound \( \%\) (\( \%\)g, \( \%\)% yield) as white crystals. m.p. \( \%\) C, Rf = 1.8, \% cm\(^{-1}\) of \% Synthesis of \% (\( \%\)-diflourophenethyl-\( \%\)-yl acetate (11).

Acetic anhydride (\( \%\)ml, \( \%\)mmol), was added to a solution of compound \( \%\) (\( \%\)g, \( \%\)mmol) in pyridine (\( \%\)ml) and the reaction was allowed to proceed at \( \%\) C with stirring for 2 hours. Ethyl acetate (\( \%\)ml) was added and this solution was washed successively with saturated aqueous ammonium chloride (\( \%\):\( \%\):\( \%\) ml) and distilled water (\( \%\):\( \%\):\( \%\) ml). The organic fraction was dried with anhydrous magnesium sulfate and the solvent was removed in vacuum to give \( \%\) (\( \%\)g, \( \%\)% yield) as a white powder. m.p. \( \%\) C, Rf = 1.8, \% cm\(^{-1}\) of (\( \%\):\( \%\):\( \%\) N-H) of primary sulfonamide & secondary bonded amide, \% (C=O) of acetate ester, \% C (C=O) of secondary amide, \% \% (Aromatic), \% \% (SO\(_4\)) cm\(^{-1}\) of Synthesis of \% (\( \%\)-(N-acetysulfamoyl) phenylcarbamoyl) - \%, \%-diflouro biphenyl-\( \%\)-yl acetate (11).
were placed in flask, equipped with reflux condenser, boiling stones were added. The reaction mixture was refluxed gently for 90 minutes, and then worked up as prescribed in section 6 to liberate \( \text{C} \) (1, 1g, 1% yield) as white crystals. m.p. \( 184.6 \pm 1.8 \)C, Rf = 0.8, IR cm\(^{-1}\): 2951, 2925, 1777 & 1551 (N-H) of secondary amide & sulfonamide, 1710 (C=O) of acetate ester, 1647 (C=O) of secondary amide, 1093, 1037 & 1013 (C=c) & 1113 & 1135(SO\(^{1}\)) cm\(^{-1}\). 

General procedure to liberate the final compounds (11, 12 & 13). 

Ester compounds (11, 12, 11), each (1.2g, 7.3 mmol) was dissolved in a minimum volume of ethanol 96%: THF (1:1) mixture. The solution was cooled to 18°C, and then sodium hydroxide (1N, 1.2ml, 7.3 mmol) was added drop wise with continuous stirring over a period of 20 minutes. Stirring was continued at 18°C for additional three hours. The reaction mixture was acidified with HCl (1N, 1.3ml, 7.4 mmol) excess of cold water was added and the precipitate was filtered and dried to give the final compound (11, 12 or 13).

Compound 11 (% yield) as white crystals. m.p. \( 34.2 \pm 0.4 \)C, Rf = 0.65, IR \( \text{cm}^{-1} \)s (O-H) of H-bonded phenol, \( 3324 \) (N-H) of secondary amide, \( 1457 \) (N-H) of amine salt, \( 1689 \) (C=O) of secondary amide, \( 1647 \) & 1627 (Aromatic) \( \text{cm}^{-1} \). CHN Calculated (C\(^{1}\)H\(^{1}\)N\(^{1}\)O\(^{1}\)S\(^{1}\)): C, 59.5; H, 4.5; N, 9.7. Found: C, 59.4; H, 4.6; N, 9.5.

Compound 12 (% yield) as white crystals. m.p. \( 34.2 \pm 0.4 \)C, Rf = 0.65. IR \( \text{cm}^{-1} \)s (O-H) of H-bonded phenol, \( 3324 \) (N-H) of secondary sulfonamide, \( 1689 \) (C=O) of secondary amide, \( 1647 \) & 1627 (Aromatic) \( \text{cm}^{-1} \). CHN Calculated (C\(^{1}\)H\(^{1}\)N\(^{1}\)O\(^{1}\)S\(^{1}\)): C, 59.6; H, 4.6; N, 9.7. Found: C, 59.5; H, 4.6; N, 9.5.

Compound 13 (% yield) as a faint yellow crystals. m.p. \( 189.1 \pm 1.2 \)C, Rf = 0.65. IR \( \text{cm}^{-1} \)s (O-H) of H-bonded phenol, \( 3324 \) (N-H) of secondary sulfonamide, \( 1689\) (C=O) of secondary amide, \( 1647 \) & 1627 (Aromatic) \( \text{cm}^{-1} \). CHN Calculated (C\(^{1}\)H\(^{1}\)N\(^{1}\)O\(^{1}\)S\(^{1}\)): C, 59.6; H, 4.6; N, 9.7. Found: C, 59.5; H, 4.6; N, 9.5.

Pharmacology

Albino rats of either sex weighing (10.0 ± 1.0 gm) 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 (14 light-14 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 10 rats) as follow: group A: served as control and treated with the vehicle (propylene glycol 0.5% v/v in water); group B: treated with sodium diclofenac (reference agent) in a dose of 2 mg/kg suspended in propylene glycol 0.5% v/v in water (11); group C, D and E: treated with tested compounds 11, 12 and 13 respectively in a dose equivalent to 50 mg/kg of diflunisal as finely homogenized suspension in 0.5% v/v propylene glycol in water (The doses were chosen as being equivalent to 11.0, 22.0, 55.0 and 110 mg/kg diflunisal. According to preliminary results the decision was made to choose the dose that equivalent to 50 mg/kg diflunisal).

Anti-inflammatory activity

The anti-inflammatory activity of the tested compounds was studied using egg-white induced edema model. Acute inflammation was induced by a subcutaneous injection of 0.2 ml of undiluted egg-white into the planter side of the left hind paw of the rats; 10 minutes after i.p. administration of the drugs or their vehicle. The paw thickness was measured by vernier at eight time intervals (1, 5, 10, 15, 30, 60, 120 and 240 minutes) after vehicle or drug 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. When egg-white is injected into the paw of rats, a substantial induction of COX is observed at hours coinciding with enhanced PGs and local edema. Tables & 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 hours of injection.

Table 1 showed the effect of tested compounds ( & •) in respect to control group. All tested compounds were effectively limited the increase in paw edema, with the effect of compounds • & • started at time minute (significantly different compared to control), while compound • started at time minute, which mean it has later 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<••••) reduction in paw edema. At time • minute compounds •, • significantly different compared to •.

### Table 1: Effect of Control, Diclofenac & Compounds •, • & • on egg-white induced paw edema in rats.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Control (n=7)</th>
<th>Diclofenac (n=7)</th>
<th>Compound 1* (n=7)</th>
<th>Compound 1** (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>0.1 ± 0.1</td>
<td>0.1 ± 0.1</td>
<td>0.1 ± 0.1</td>
<td>0.1 ± 0.1</td>
</tr>
<tr>
<td>30</td>
<td>0.3 ± 0.3</td>
<td>0.3 ± 0.3</td>
<td>0.3 ± 0.3</td>
<td>0.3 ± 0.3</td>
</tr>
<tr>
<td>60</td>
<td>0.5 ± 0.5</td>
<td>0.5 ± 0.5</td>
<td>0.5 ± 0.5</td>
<td>0.5 ± 0.5</td>
</tr>
<tr>
<td>120</td>
<td>0.7 ± 0.7</td>
<td>0.7 ± 0.7</td>
<td>0.7 ± 0.7</td>
<td>0.7 ± 0.7</td>
</tr>
<tr>
<td>180</td>
<td>0.9 ± 0.9</td>
<td>0.9 ± 0.9</td>
<td>0.9 ± 0.9</td>
<td>0.9 ± 0.9</td>
</tr>
<tr>
<td>240</td>
<td>1.1 ± 1.1</td>
<td>1.1 ± 1.1</td>
<td>1.1 ± 1.1</td>
<td>1.1 ± 1.1</td>
</tr>
</tbody>
</table>

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

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

Table 1 showed the effect of tested compounds (•, • & •) in respect to reference group (diclofenac). As seen in this table; at time • and • minute there are no differences among different groups; at time • and • compound • is significantly lower effect than diclofenac, compound •, and compound •.
However, it appears that all the tested compounds had a comparable effect to that of diclofenac at times of 2-3 minutes of experiment except compound 16 which showed statistically significant (P>0.05) lower effect than other tested compounds (14, 10 & diclofenac) at time 30 minutes.

**Conclusion**

In vivo anti-inflammatory study showed that the incorporation of diaminobenzensulfonamide, amino-N-methylbenzenesulfonamide, or N-(diaminophenyl)sulfonyl)acetamide into well known anti-inflammatory drug (diflunisal) potentially increase its anti-inflammatory activity since diclofenac more potent than other NSAIDs (19).

Finally compound 16 showed lower effect comparing with compound 14 and 10 and that CH- and COCH- may play important roles in reduction of paw thickness.

**References**