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

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ABSTRACT

Objective: Synthesized of amino derivatives [٤-aminobenzenesulfonamide, ٤-amino-N-methylbenzenesulfonamide, or N-(٤-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-٢ enzyme.

Design: Expermental study

Results: *In vivo* acute anti-inflammatory activity of the final compounds (١٣, ١٤ & ١٥) was evaluated in rat using egg-white induced edema model of inflammation in a dose equivalent to (٥٠mg/Kg) of diflunisal. All tested compounds produced significant reduction of paw edema with respect to the effect of propylene glycol ٥٠% v/v (control group). Moreover, compound (١٤) exhibited comparable anti-inflammatory activity to diclofenac (٣mg/Kg), while compound (١٣) showed short duration of action, and compound (١٥) exhibited comparable effect to that of diclofenac with slower onset of action.

Conclusion: The result of this study indicate that the incorporation of the ٤-aminobenzenesulfonamide pharamacophore & its derivatives into diflunisal enhanced its anti-inflammatory activity& may increased its selectivity toward COX-٢ enzyme which can be confirmed in future by assessing COX-٢:COX-١ inhibitory ratio using whole blood assay.

الخلاصة

الأهداف: مجموعة من المشتقات الامينية[٤- امينوبنزين سلفونامايد، ٤ - امينو- N-مثيل بنزين سلفونامايد،N -(٤- امينوفنيل سلفونيل)اسيتاميد] مرتبطه بمجموعة الكاربوكسيل للدايفلونيسال (diflunisal) الدواء غير الستيرويدي المعروف جيدا كمضاد للالتهاب، صممت وحضرت لتقييمها كمضادات للالتهاب .

التصميم: دراسة مخبرية

النتائج: في الجسم الحي، اجري تقييم الفعالية المضادة للالتهاب الحاد للمركبات النهائية(١٣، ١٤، ١٥) في الجرذ باستخدام زلال البيض مستحدثة وذمة التهابية تحت الجلد بجرعة مكافئة للدايفلونيسال (٥٠ملغم/كغم).كل المركبات المختبرة انتجت انخفاض مؤثرا للوذمة بالمقارنة مع البروبلين كلايكول ٥٠%(propylene glycol) كمجموعة ضابطة.
ان مركب (١٤) اظهر فعالية مضادة للالتهاب مقارنة للدايكلوفيناك (diclofenac) (٣ملغم/كغم) ، بينما المركب (١٣) اظهر فعالية استمرارية اقصر والمركب (١٥) اظهر فعالية مضادة للالتهاب مقارنة للدايكلوفيناك مع فعالية ابتدائية ابطء.

الاستنتاج: نتيجة هذه الدراسة تشير الى ان اندماج الجزء العقاقيري ٤- امينوبنزين سلفونامايد ومشتقاته مع الدايفلونيسال نشط فعاليته المضادة للالتهاب مع احتمال زيادة انتقائيته تجاه انزيم الكوكس الثاني والذي يمكن ان تثبت مستقبلا بتحصيل النسبة المثبطة للكوكس-٢ الى الكوكس-١ باستخدام معايرة الدم ككل.

In ١٨٩٩, acetyl salicylic acid (aspirin, I) was introduced as the first potent drug to treat rheumatic disease. In the following decades, dozen 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 ١٩٧١ 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.[١]

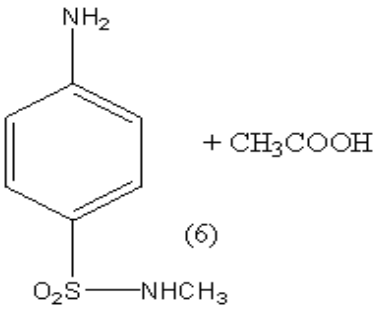
Cyclooxygenase is a rate limiting enzyme for prostaglandin synthesis.[٢] The three isoenzymes of COX (COX-١, COX-٢ and COX-٣) have been identified [٣, ٤] though COX-٣ activity in human has not been confirmed [٥] COX-١ is constitutively expressed, widely distributed and has "housekeeping" function. It is of particular importance in maintaining gastric mucosal integrity, renal function and homeostasis.[٦] COX-٢ is highly induced in settings of inflammation by cytokines and inflammatory mediators or physiological stress. [٧,٨] However, COX-٢ also is constitutively expressed in certain areas of kidney, brain, reproductive tract, [٩] the vascular system, [١٠] in wound healing, lung and bone.[١١]

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.[١٢]

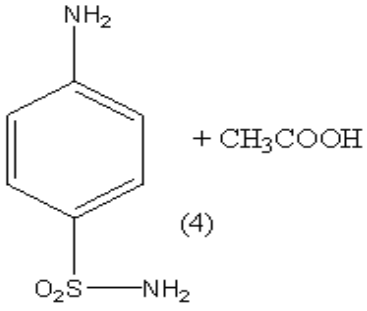
However, since the identification of cyclooxygenase-٢ (COX-٢), the field of inflammation and particularly the search for effective NSAIDs with fewer adverse effects has greatly intensified. Increasing number of experimental and clinical data support the role of selective COX-٢ inhibitor in anti-inflammatory processes and the involvement of COX-١ inhibition in the side effects associated with using NSAIDs.[١٣] Many of the selective COX-٢ inhibitors containing benzene-sulfonamide derivative, like valdecoxib (III), [١٤] ,celecoxib (IV),[١٥] or benzene-N-methyl sulfonamide like compound (V).[١٦]

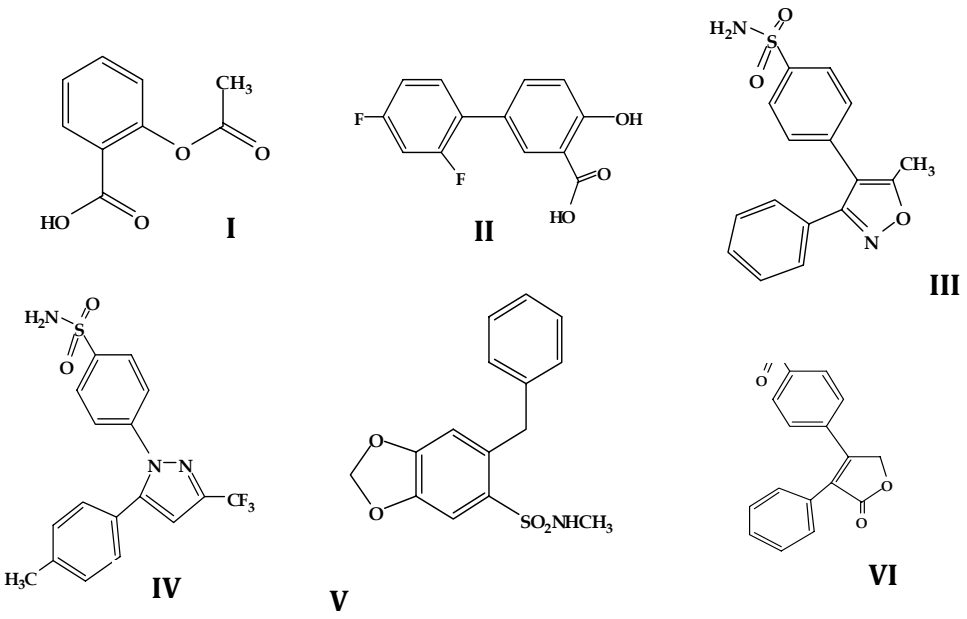
In a recent study, it was shown that the incorporation of a para-N-acetylsulfonamido substitute on the C-٣ phenyl ring of the rofecoxib (VI) regioisomer provided a highly potent and selective COX-٢ inhibitor that has the potential to acetylate the COX-٢ isoenzyme. [١٧]

compound ٦
(٤-amino-N-methylbenzenesulfonamide)



compound ٤
(٤-aminobenzenesulfonamide)



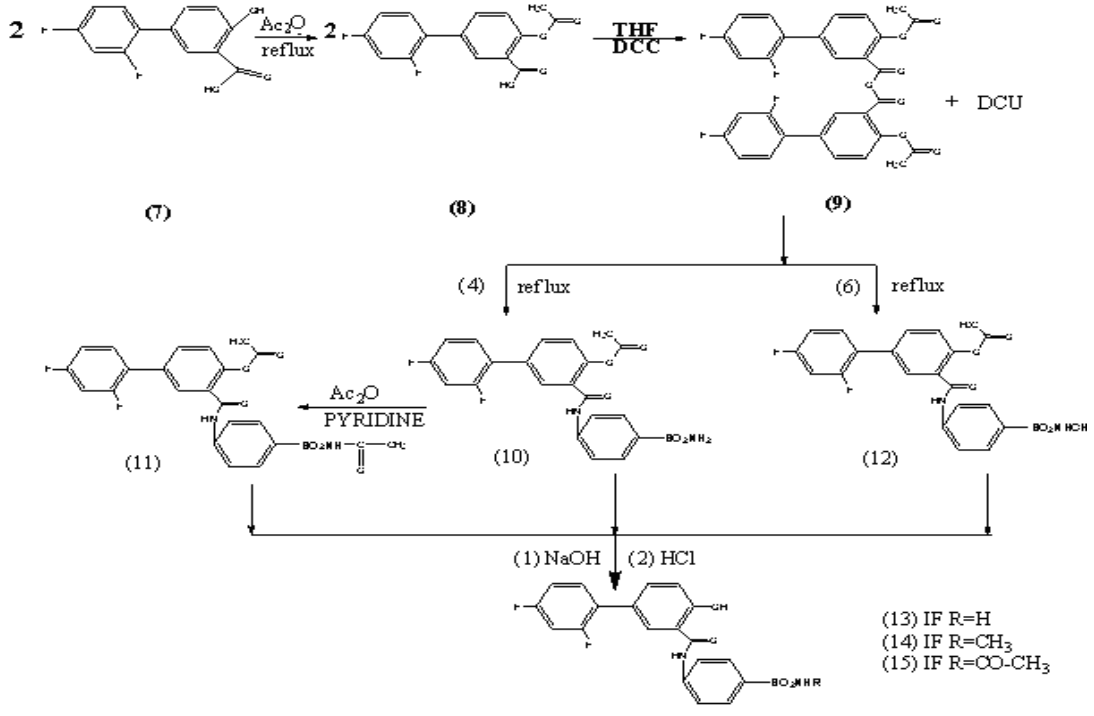


In the view of this background, the present study was conducted to design, synthesize and preliminarily evaluate new diflunisal derivatives as potential NSAIDs and future study to measure their selectivity's on COX-٢ enzyme.

Chemistry

The general routes outlined in schemes ١ and ٢ were used to synthesize all compounds described here.

As shown in scheme ١; ٤-aminobenzene-sulfonamide (٤) and ٤-amino-N-methylbenzenesulfonamide (٦) were prepared as described previously (Vogel) [١٨] starting from acetanilide.



Scheme1:Synthesis of compounds13,14and 15

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 ٠.٢ 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: Benzene.(١:١٨:٦٠:٢٠).

IR spectra were recorded on model ٥٠٠ scientific IR spectrophotometer, Buck Company (USA) as a KBr film.CHN microanalysis was done by using Carlo Erba elemental analyzer ١١٠٦, Italy. ٣.١ Synthesis of ٤-acetoxy-٢´, ٤´-diflourobiphenyl-٣-carboxylic acid (٨).[١٩]

In a ٢٥٠ml conical flask, equipped with reflux condenser, diflunisal (٧) (٥g, ٢٠mmol) and acetic anhydride (١٥ml, ١٥٩mmol) were placed and ٣ drops of concentrated sulfuric acid were added dropwise. The reaction mixture was refluxed gently for ١ hour, and then allowed to cool with occasional stirring. Cold ice-water was then added until precipitate was formed, filtered by using sucking pump, washed by cold distill water several times, the crude product was collected. The recrystallization was carried out by using ethanol ٩٥%, the precipitate was collected and dried to give compound ٨ [١٩] (٥.٣g, ٩١% yield) as white crystal. m.p. ١٧١-١٧٢ ^{o}C. R_f =٠.٨٩. IRυcm$^{-١}$ ١٧٦٨(C=O) of acetate ester, ١٧٠٠(C=O) of carboxylic acid, ١٦١٧, ١٥٩٣&١٤٨٨(Aromatic) cm$^{-١}$. ٣.٢ Synthesis of ٤-acetoxy-٢´, ٤´-diflourobiphenyl-٣-carboxylic anhydride (٩)

Compound ٨ (٥g, ١٧.١mmol) was dissolved in THF (٣٠ml), and then Di-Cyclohexyl Carbodiamide (DCC) (١.٧٥g, ٨.٥٥mmol) was added. The reaction mixture was continuously stirred at room temperature for ٤ hours. A white precipitate of di-Cyclohexyl Urea (DCU) was formed which then removed by filtration. The solvent was evaporated under vacuum to give ٩[٢٠], (٤g, ٨٣% yield) as a white powder. m.p. ١٤٩-١٥١ ^{o}C. R_f =٠.٦٤. IRυcm$^{-١}$ ١٨١٥ &١٧٤٣(C=O) of anhydride, ١٦٢٣, ١٥١٨&١٤٨٨(Aromatic), ١٢٧٧, ١٢١٥&١١٧٣[C - (C=O) – O-(C=O) –C] cm$^{-١}$ of anhydride.٣.٣ Synthesis of ٢´, ٤´-diflouro-٣-(٤-sulfamoylphenylcarbamoyl) biphenyl -٤-yl acetate (١٠)[٢١]

Compound ٩ (٢.١g, ٣.٧mmol), compound ٤ (٠.٦٤g, ٣.٧mmol), zinc dust (٤mg), glacial acetic acid (٠.٣٥ml, ٦.١٣mmol) and dioxane (٢٠ml) were placed in a flask, equipped with reflux condenser. The reaction mixture was refluxed gently for ٩٠ minutes. The solvent was evaporated under vacuum, the residue was dissolved in ethylacetate, washed with $NaHCO_٣$ (١٠%), HCl (١N) and distilled water (three times for each step), filtered over anhydrous magnesium sulfate. The filtrate was evaporated under vacuum to give compound ١٠. Recrystallization was carried out by dissolving the compound in ethyl acetate, petroleum ether (٨٠-١٠٠ ^{o}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 ١٠, (٠.٦g, ٣٦% yield) as white crystals. m.p. ٢١٢-٢١٣^{o}C. R_f =٠.٨٧. IRυcm$^{-١}$ ٣٣٥٢&٣٢٥٩ (N-H) of primary sulfonamide & secondary bonded amide, ١٧٧٨ (C=O) of acetate ester, ١٦٧٠ (C=O) of secondary amide, ١٥٩٣, ١٥٢٧&١٤٨٥(Aromatic), ١٣١٩&١١٦١ (SO٢) cm$^{-١}$ ٣.٤ Synthesis of ٣-(٤-(N-acetylsulfamoyl) phenylcarbamoyl) - ٢´, ٤´-diflouro biphenyl-٤-yl acetate (١١)

Acetic anhydride (٠.٦ml, ٦mmol), was added to a solution of compound ١٠ (٠.٨٩g, ٢mmol) in pyridine (١٠ml) and the reaction was allowed to proceed at ٢٥ ^{o}C with stirring for ٦ hours. Ethyl acetate (١٠٠ml) was added and this solution was washed successively with saturated aqueous ammonium chloride (٢x٢٠ml) and distilled water (٢x٢٠ml). The organic fraction was dried with anhydrous magnesium sulfate and the solvent was removed in vacuum to give ١١ [٢٢], (٠.٤٧g, ٤٩% yield) as a white powder. m.p. ١٨٠-١٨٢ ^{o}C. R_f =٠.٧٨. IRcm$^{-١}$ ٣٣٠٤&٣٢٢٧ (N-H) of secondary amide &sulfonamide, ١٧٥٣ (C=O) of acetate ester, ١٦٦٠ (C=O) of secondary amide, ١٥٩٨ & ١٥٣٠(c=c) &١٣٢٧ &١١٥٧(SO٢) asymmetric and symmetric., respectively . ٣.٥ Synthesis of ٢´, ٤´-diflouro-٣-(٤-(N-methylsulfamoyl)phenylcarbamoyl) biphenyl-٤-yl acetate (١٢)

Compound ٩ (٢.٣٥g, ٤.١٥mmol), compound ٦ (٠.٧٧g, ٤.١٥mmol), zinc dust (٤mg), glacial acetic acid (٠.٤ml, ٧mmol) and dioxan (٢٥ml)

were placed in flask, equipped with reflux condenser, boiling stones were added. The reaction mixture was refluxed gently for ٩٠ minutes, and then worked up as prescribed in section٣.٣ to liberate ١٢(٠.٥٣g, ٢٢% yield) as white crystals. m.p. ١٨٥-١٨٦ ^{o}C. R_f =٠.٨. IRcm$^{-١}$ ٣٣٢٦&٣١٩٠ (N-H) of secondary amide &sulfonamide, ١٧٥٣ (C=O) of acetate ester, ١٦٨٧ (C=O) of secondary amide, ١٥٩٣, ١٥٣٧ & ١٤٩٤(c=c) &١٣٢١ &١١٥٣(SO٢) . ٣.٦ General procedure to liberate the final compounds (١٣, ١٤&١٥)[٢٣]

Ester compounds (١٠,١٢,١١), each (١.٢g, ٢.٦mmol) was dissolved in a minimum volume of ethanol ٩٩%: THF (٣:١) mixture. The solution was cooled to ١٨ ^{o}C, and then sodium hydroxide (٢N, ١.٦ml, ٣.٢٣mmol) was added drop wise with continuous stirring over a period of ٣٠ minutes. Stirring was continued at ١٨^{o}C for additional three hours. The reaction mixture was acidified with HCl (٢N, ١.٧ml, ٣.٤٤mmol), excess of cold water was added and the precipitate was filtered and dried to give the final compound(١٣, ١٤ or ١٥).[٢٣]

Compound ١٣: (٥٩% yield) as white crystals. m.p. ٢٥٣-٢٥٤ ^{o}C. R_f =٠.٩٢. IR ٣٣٥٩ (O-H) of H-bonded phenol, ٣٢٧٤ (N-H) of secondary amide, ٣١٢٢ (N-H) of amine salt, ١٦٦٣(C=O) of secondary amide, ١٥٩٣, ١٥٥٢ & ١٤٩٢(Aromatic) &١٣٣٨ &١١٥٥(SO٢) cm$^{-١}$ .CHN Calculated (C١٩H١٤N٢O٤SF٢): C, ٥٦.٤٣; H, ٣.٤٩; N, ٦.٩٣. Found: C, ٥٦.٩٢; H, ٣.١١; N, ٦.٥٣.

Compound ١٤: (٥٧% yield) as white crystals. m.p. ١٩٠-١٩٢ ^{o}C. R_f =٠.٩٠. IR ٣٣٢٦ (O-H) of H-bonded phenol, ٣١٩٠ (N-H) of secondary sulfonamide, ١٦٨٥(C=O) of secondary amide, ١٥٩٣, ١٥٤١&١٤٩٢(Aromatic) &١٣٠٩ &١١٥٣(SO٢) cm$^{-١}$ .CHN Calculated (C٢٠H١٦N٢O٤SF٢): C, ٥٧.٤١; H, ٣.٨٦; N, ٦.٧٠. Found: C, ٥٧.٧٩; H, ٣.٤٥; N, ٦.٤٤.

Compound ١٥: (٥٥% yield) as a faint yellow crystals. m.p. ١٩٩-٢٠١^{o}C. R_f =٠.٨٢. IR ٣٣٣٤ (O-H) of H-bonded phenol, ٣٢٠١ (N-H) of secondary sulfonamide, ١٦٨٨(C=O) of secondary amide, ١٥٩٦, ١٥٤٠&١٤٩٥(Aromatic) &١٣١١ &١١٥٣(SO٢) cm$^{-١}$ .CHN Calculated (C٢١H١٦N٢O٥SF٢): C, ٥٦.٧٥; H, ٣.٦٠; N, ٦.٣٠. Found: C, ٥٦.٥٧; H, ٣.٧٥; N, ٦.٢٢.

Pharmacology

Albino rats of either sex weighing (١٥٠ ± ١٠ 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 (١٢ light-١٢ dark cycle) for ٧ days for acclimatization. Animals were fed commercial chaw and had free access to water ad libitum. Animals were brought ١ hour before the experiment to the laboratory, and were divided into five groups (each group consist of ٦ rats) as follow**:** group A**:** served as control and treated with the vehicle (propylene glycol ٥٠% v/v in water); group B: treated with sodium diclofenac (reference agent) in a dose of ٣mg/kg suspended in propylene glycol ٥٠% v/v in water [٢٤]; group C, D and E: treated with tested compounds ١٣, ١٤ and ١٥ respectively in a dose equivalent to ٥٠ mg/kg of diflunisal as finely homogenized suspension in ٥٠% v/v propylene glycol in water (The doses were chosen as being equivalent to ١٢.٥, ٢٥, ٥٠ and ١٠٠mg/kg diflunisal. According to preliminary results the decision was made to choose the dose that equivalent to ٥٠mg/kg diflunisal).

Anti-inflammatory activity

The anti-inflammatory activity of the tested compounds was studied using egg-white induced edema model..[٢٥] Acute inflammation was induced by a subcutaneous injection of ٠.٠٥ml of undiluted egg- white into the planter side of the left hind paw of the rats; ١٥ minutes after i.p. administration of the drugs or their vehicle. The paw thickness was measured by vernier at eight time intervals (٠, ١٥, ٣٠, ٦٠, ١٢٠, ١٨٠, ٢٤٠ and ٣٠٠ 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 ٠.٠٥ 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.[٢٦] When egg-white is injected into the paw of rats, a substantial induction of COX-٢ is observed at ٢ hours coinciding with enhanced PGs and local edema [(٢٧)]. Tables ١ & ٢ 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 ٢ hours of injection.

Table ١ showed the effect of tested compounds (١٣, ١٤ &١٥) in respect to control group. All tested compounds were effectively limited the increase in paw edema, with the effect of compounds ١٣ & ١٤ started at time ٣٠ minute (significantly different compared to control), while compound ١٥ started at time ١٢٠ 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>٠.٠٥) reduction in paw edema. At time ٣٠٠ minute compounds ١٤, ١٥ significantly different compared to ١٣.

Table ١: Effect of Control,Diclofenac & Compounds ١٣, ١٤ & ١٥ on egg-white induced paw edema in rats.

	Treatment groups					
	Time (min)	Control (n=٦)	Diclofenac (n=٦)	Compound ١٣ (n=٦)	Compound ١٤ (n=٦)	Compound١٥ (n=٦)
Paw thickness (mm)	٠	٤.٤٦ ± ٠.٠٥	٤.٣٨±٠.١٤	٤.٤٤±٠.٣٣	٤.٤٣±٠.١١	٤.٤٥±٠.٠٦
	١٥	٥.٤١ ± ٠.١٨	٥.٣٧±٠.٤١	٥.٤٢±٠.٤٣	٥.٤٠±٠.٤٠	٥.٤٣±٠.٢٧
	٣٠	٦.٠٥ ± ٠.١٦	٥.٥٨±٠.١١	٥.٥٠±٠.١٠ *a	٥.٣٨±٠.٣٠ *a	٦.٠٥±٠.١٠ b
	٦٠	٦.٣٥ ± ٠.٠٧	٥.٧٠± ٠.١٠	٥.٧٨±٠.١٠ *a	٥.٦٦±٠.١٠ *a	٦.٣٨±٠.٣٧ b
	١٢٠	٦.٥٠ ± ٠.٠٩	٥.٤٨±٠.١٠	٥.٦٣±٠.١٠ *a	٥.٥٥±٠.١٠ *a	٥.٦٦±٠.١٠ *a
	١٨٠	٥.٩٣ ± ٠.١١	٥.١٣±٠.١٠	٥.٣٣±٠.١٠ *a	٥.١٤±٠.١٠ *a	٥.١٥±٠.١٠ *a
	٢٤٠	٥.٣٨ ± ٠.٠٩	٤.٨٧±٠.١٠	٤.٩٣±٠.٠٦ *a	٤.٧٣±٠.١٠ *a	٤.٨٠±٠.١٠ *a
	٣٠٠	٥.٢٠ ± ٠.١	٤.٦٠±٠.١٠	٤.٩٢±٠.١٠ *a	٤.٤٨±٠.١٠ *b	٤.٥٣±٠.١٠ *b

Non-identical superscripts (a, & b) among different tested compounds are considered significantly different (P<٠.٠٥)
* Significantly different compared to control (P<٠.٠٥).

Table ١ showed the effect of tested compounds (١٣, ١٤ &١٥) in respect to reference group (diclofenac). As seen in this table; at time ٠ and ١٥ minute there are no differences among different groups; at time ٣٠ and ٦٠ compound ١٥ is significantly lower effect than diclofenac, compound ١٣, and compound ١٤ .

However, it appears that all the tested compounds had a comparable effect to that of diclofenac at times of ١٢٠-٣٠٠ minute of experiment except compound ١٣ which showed statistically significant (P>٠.٠٥) lower effect than other tested compounds (١٤, ١٥ & diclofenac) at time ٣٠٠ minute.

Conclusion

In vivo anti-inflammatory study showed that the incorporation of ٤-aminobenzenesulfonamide, ٤-amino-N-methylbenzenesulfonamide, or N-(٤-aminophenylsulfonyl)acetamide into well known anti-inflammatory drug (diflunisal) potentially increase its anti-inflammatory activity since diclofenac more potent than other NSAIDs [٢٩].

Finally compound ١٣ showed lower effect comparing with compound ١٤ and ١٥ and that CH_3 and $COCH_3$ may play important roles in reduction of paw thickness.

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