Synthesis and Preliminary Pharmacological Evaluation of New Analogues of Diclofenac as Potential Anti-inflammatory Agents

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Abstract

A group of amine derivatives [4-aminobenzenesulfonamide derivatives, 2-aminopyridine and 2-aminothiazole] incorporated to α-carbon of diclofenac a well known non-steroidal anti-inflammatory drug (NSAID) to increase bulkiness were designed and synthesized for evaluation as a potential anti-inflammatory agents with expected COX-2 selectivity. In vivo acute anti-inflammatory activity of the selected final compounds (9, 12 and 13) was evaluated in rats using egg-white induced edema model of inflammation in a dose equivalent to (3 mg/Kg) of diclofenac sodium. 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-aminobenzenesulfonamide derivative (compound 9) exhibited superior anti-inflammatory activity compared to diclofenac sodium at times 180-300 minutes with the same onset of action. The results of this study indicate that the incorporation of the selected aromatic amino groups in to diclofenac maintain its anti-inflammatory activity.

Key words: amine derivatives, anti-inflammatory, diclofenac derivatives, COX-2 selectivity.

الخلاصة

مجموعة من المشتقات الامينية لمشتقات ٤-امينوبنزين سلفونامايد, ٢-امينوبيريدين, ٢-امينوثيازول اندمجت مع الفا كاربون للدايكلوفيناك الدواء غير الستيرويدي المعروف جيدا كمضاد للالتهاب لزيادة الضخامه, قد صممت وخلقت لتقييمها كعوامل مؤثره للألتهاب مع انتقائية متوقعه ل"أنزيم سايكواوكسجنيز-٢". في الجسم الحي للجرذان, تم تقييم الفعالية الحاده المضادة للالتهاب للمركبات النهائية المختاره (٩و١٢و١٣) في الجرذ باستخدام طريقة استحداث وذمه تحت الجلد باستخدام زلال البيض بجرعة مكافئة للصوديوم دايكلوفيناك (٣ ملغ/ كغم). كل المركبات المختبرة اظهرت انخفاضا مؤثرا للوذمة بالمقارنة مع البروبلين كلايكول ٥٠% (كمجموعة ضابطة). علاوة على ذلك المركب ٤-امينوبنزين سلفونامايد اظهر فعالية مضادة للالتهاب اعلى مقارنة بالصوديوم دايكلوفيناك لفترة الأختبار من الدقيقه ١٨٠-٣٠٠ مع نفس الفعالية الابتدائية للصوديوم دايكلوفيناك. اظهرت النتائج الى ان اندماج مجاميع مختاره من المركبات الحلقيه و غير الحلقيه للامين مع الدايكلوفيناك حافظ على فعاليتة المضادة للالتهاب.

Introduction

Non-steroidal anti-inflammatory drugs represent one of the most widely used classes of drugs, and are used primarily for treatment of osteoarthritis, rheumatoid arthritis and other inflammatory disorders; however, the use of NSAIDs is significantly limited by their ability to induce the formation of erosions and ulcers in the gastrointestinal (GI) tract [(1)]. The mechanism of action principally responsible for most of the NSAIDs seems to be inhibition of prostaglandin synthesis by causing almost complete blockade of the activity of the precursor enzymes, *cyclooxygenase*[(2)]. These are the rate-limiting enzymes in the synthesis of the inflammatory prostaglandins PGE2 and PGF2α, the cytoprotective prostaglandin PGE1, and the vasoactive prostanoids thromboxaneA2 (TXA2) and prostacyclin (PGI2). Three COX iso-zymes are known; COX-1, COX-2, and COX-3[(3).] The 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[(4)].In contrast,COX-2 is induced in setting of inflammation by cytokines and inflammatory mediators or physiological stress[(5)]. COX-3 could play a role in fever and pain processes[(6)]. The analgesic effects of NSAIDs are ascribed primarily to COX-2 inhibition, whereas several adverse effects are believed to be mediated by COX-1 inhibition[(7)]. Selective COX-2 inhibitors differ from traditional NSAIDs in that they are less likely to result in NSAID-induced gastropathy [(8)].

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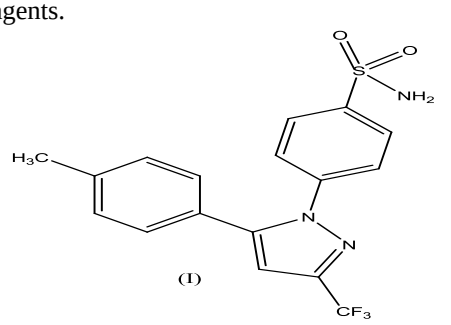
Received : 29/9/2010
Accepted : 3/1/2011

In addition, several other clinical uses of selective COX-2 inhibitors have been investigated[9], some of these indications including the treatment of the neurodegenerative disease like Alzheimer[10] and Parkinson disease[11] are now under clinical trials to validate the therapeutic possibilities of COX-2 inhibitors[12]. More ever studies show that the COX-2 enzyme would be an interesting target in the treatment of some cancers[13], viral infection[14], and endometriosis[15]. Preferential inhibition of COX-2 is thought to be due to the additional space in the COX-2 hydrophobic channel, as well as to the presence of a side pocket in the channel[16].This side pocket can discriminate the selective NSAIDs , like celecoxib (I)[12],valdicoxib (II)[17] which contain amino benzene sulfonamide derivatives that occupied the side pocket[18], from nonselective agents. In the view of this back ground we synthesized and preliminary evaluated new diclofenac derivatives by increase its bulkiness by incorporated the prerequisite (benzene sulfonamide group) for COX-2 selectivity or other aromatic amine derivatives found in potent NSAIDs like Meloxicam (III) and Piroxicam(IV) as potent anti-inflammatory agents.



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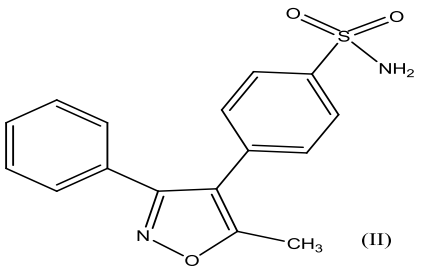

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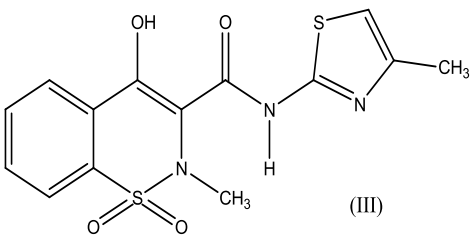

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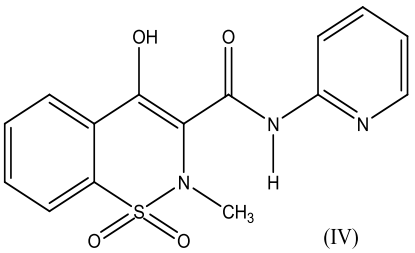


(IV)

Experimental

All reagents and anhydrous solvents were of analar type and generally used as received from the commertial suppliers (Merck-Germany, Riedel-Dehaen-Germany, GCC-U.K,BDH-England and Fluka-Switzerland). Diclofenac was supplied from Cox.Tx.Lx Chem. China. Melting points were determined by capillary method on Thomas Hoover apparatus (England). 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; n-hexane: ethyl acetate: acetic acid (7: 2.5: 0.5 v/v)[19]. FT-IR spectra were recorded at (College of science- Kufa University) by using Shimadzu-Japan spectrophotometer and the determination of the spectra were performed by using KBr discs. CHNOS microanalysis has been done in Cleveland clinical foundation learner research institute-France, by using Carlo Erba elemental analyzer.

A. Chemistry

Synthesis of methyl 2- [2-(2,6-dichlorophenylamino) phenyl]acetate(2) [20]

Suspension of diclofenac (1g, 3.37mmol) in absolute methanol, was cooled down to -15^{o} C, then thionyl chloride (0.25ml, 3.37mmol) was added drop wise. (The temperature should be kept below -10^{o} C). The reaction mixture was kept at 40 ^{o} C for three hours, followed by refluxing for three hours, and left at room temperature over night. The solvent was evaporated to dryness, re-dissolved in methanol and evaporated. The process was repeated several times to ensure complete removal of thionyl chloride. The residue was collected and re-crystallized from methanol-ether. The percent yield, physical data and R_f values were given in table (1).

Synthesis of methyl 2-bromo-2-[2-(2,6-dichlorophenylamino)phenyl]acetate(3) [17]

Compound 2 (1g, 3.23mmol) was dissolved in methylene chloride (15ml), then NBS (0.57g, 3.23mmol) was added gradually with continuous stirring. The reaction was

allowed to proceed at room temperature with stirring for three hours, then the solvent was evaporated. Ether was added to the residue, then filtered, the filtrate was dried to give compound 3 . The percent yield, physical data and R_f values were given in table (1).

Synthesis of methyl 2- [2- (2, 6-dichlorophenylamino) phenyl]- 2- (4-sulfamoylphenylamino) acetate(4) [21]

Compound 3 (1g, 2.57mmol), sulfanilamide (0.44g, 2.57mmol) were placed in round flask, then dissolved with ethanol 99%:DMF (50:50) mixture (30ml). The reaction mixture was refluxed gently for three hours. The solvent was evaporated, the residue was dissolved in ethyl acetate, washed with NaOH (5%, 3X), filtered over anhydrous magnesium sulfate, The filtrate was evaporated to give compound 4. The percent yield, physical data and R_f values were given in table(1).

Synthesis of methyl 2 - [4- (N-acetylsulfamoyl) phenylamino] -2 [2- (2, 6-dichloro phenylamino) phenyl]acetate (5) [21]

Compound 3 (1g, 2.57mmol) and sulfacetamide (0.55g, 2.57mmol) were placed in round flask, then dissolved in ethanol: DMF (50:50) mixture (40ml). The reaction mixture was refluxed gently for three hours, then it was worked up as prescribed for compound 4 to liberate compound 5. The percent yield, physical data and R_f values were given in table(1).

Synyhesis of methyl 2- [2- (2, 6-dichlorophenylamino) phenyl] -2- [4- (N-methyl sulfamoyl) phenylamino] acetate (6) [21]

Compound 3 (1g, 2.57mmol), 4-amino-N-methylbenzene sulfonamide (0.47g, 2.57mmol) were placed in round flask, then dissolved in ethanol: DMF (50:50) mixture (40ml). The reaction mixture was refluxed gently for three hours, then it was worked up as prescribed for compound 4 to liberate compound 6.The percent yield, physical data and R_f values were given in table (1).

Synthesis of methyl 2-[2-(2,6-dichlorophenylamino) phenyl]-2-(pyridine-2-ylamino)acetate(7) [21]

Compound 3 (0.5g, 1.28mmol) and 2-aminopyridine (0.12g, 1.28mmol) were placed in round flask, then dissolved with ethanol: DMF (50:50) mixture (40ml). The reaction mixture was refluxed gently for three hours, then it was worked up as prescribed for compound 4 to liberate compound 7.The percent yield, physical data and R_f values were given in table (1).

Synthesis of methyl 2-[2-(2,6-dichlorophenylamino) phenyl]-2-(thiazol-2-ylamino)acetate (8) [21]

Compound 3 (0.5g, 1.28mmol) and 2-aminothiazol (0.128g, 1.28mmol) were placed in round flask, then dissolved with ethanol 99%:DMF (50:50) mixture (20ml). The reaction mixture was refluxed gently for three hours, then it was worked up as prescribed for compound 4 to liberate compound 8. The percent yield, physical data and R_f values were given in table (1).

Synthesis of 2-[2-(2,6-dichlorophenylamino) phenyl]-2-(4-sulfamoyl phenyl amino) acetic acid (9) [22]

Compound 4 (0.3g, 0.62mmol) was dissolved in minimum volume of ethanol 99%:THF (7:1) mixture. The solution was cold to 18^o C, and then sodium hydroxide (2N, 0.37ml, 0.75mmol) was added drop wise, with continuous stirring over a period of 30 minutes. Stirring was continued at 18^o C for an additional three hours. The reaction mixture was acidified with HCl (2N, 0.37ml, 0.75mmol), excess of cold water was added and the acidic compound was precipitated, then filtered and dried to give compound 9. The percent yield, physical data and R_f values were given in table (1).CHNOS Calculated: C,51.51; H,3.67; N, 9.01; O, 13.72; S, 6.88. Found: C, 51.85; H, 3.7; N, 9.36; O, 14.39; S, 7.13.

Synthesis of 2-[4-(N-acetylsulfamoyl) phenylamino]-2-[2-(2,6-dichloro phenyl amino) phenyl]acetic acid (10) [22]

Compound 5 (0.25.g, 0.47mmol) was dissolved in minimum volume of ethanol 99%:THF (20:1) mixture. The solution was cold to 18^o C, and then sodium hydroxide (2N, 0.28ml, 0.56mmol) was added drop wise, with continuous stirring over a period of 30 minutes. Then the reaction mixture was worked up as prescribed for compound 9 to liberate compound 10. The percent yield, physical data and R_f values were given in table (1).CHNOS Calculated: C,51.98; H,3.77; N,8.27; O, 15.74; S,6.31. Found: C,52.21; H, 3.85; N,8.57; O,16.01; S,6.54.

Synthesis of 2-[2-(2,6-dichlorophenylamino) phenyl]-2-[4-(N-methylsulfamoyl) phenylamino] acetic acid (11) [22]

Compound 6 (0.5.g, 1.24mmol) was dissolved in minimum volume of ethanol 99%:THF (20:1) mixture. The solution was

cold to 18° C, and then sodium hydroxide (2N, 0.74ml, 1.49mmol) was added drop wise, with continuous stirring over a period of 30 minutes. Then the reaction mixture was worked up as prescribed for compound 9 to liberate compound 11. The percent yield, physical data and R_f values were given in table (1). CHNOS Calculated: C, 52.51; H,3.99; N, 8.75;O,13.32; S, 6.68. Found: C, 53.01; H, 4.12; N, 9.07; O,13.56; S, 6.62.

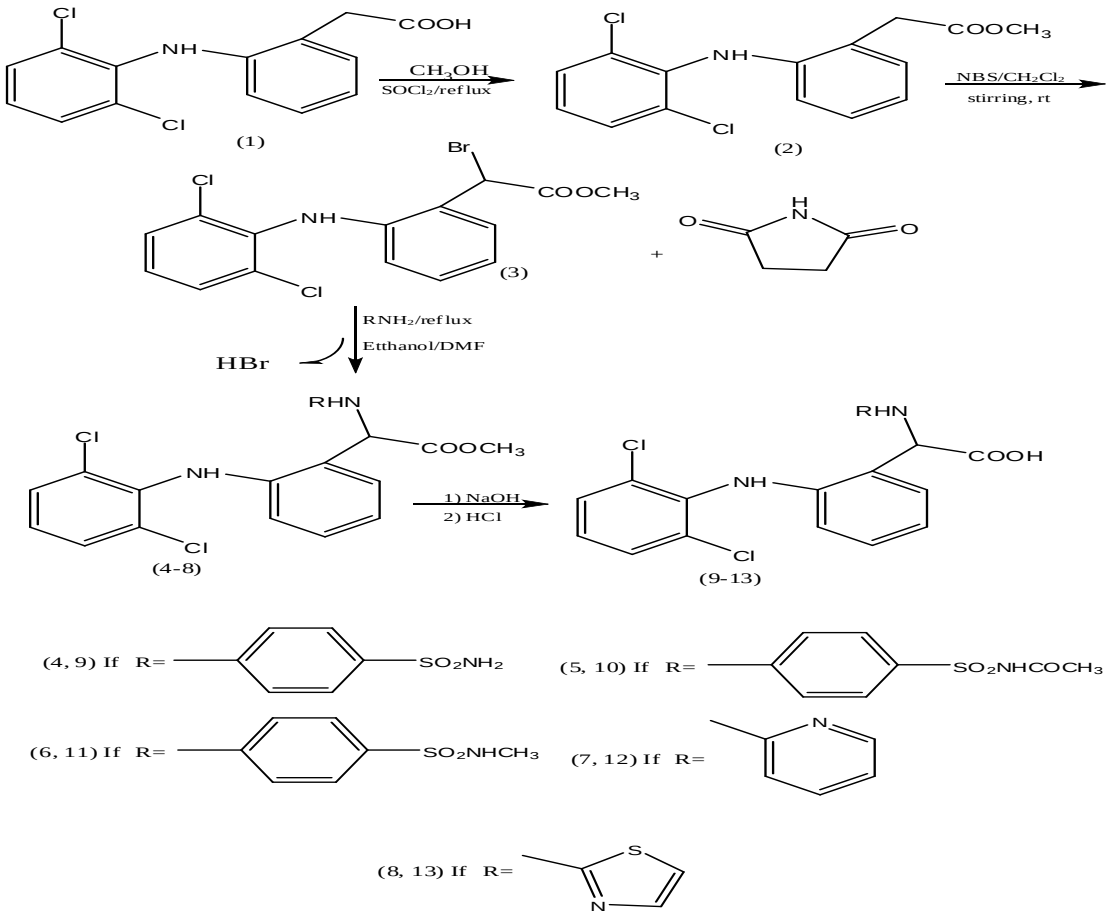
Synthesis of 2- [2- (2,6-dichlorophenylamino) phenyl] -2- (pyridine-2-ylamino) acetic acid(12)[22]

Compound 7 (0.5.g, 1.24mmol) was dissolved in minimum volume of ethanol 99%:THF (20:1) mixture. The solution was cold to 18° C, and then sodium hydroxide (2N, 0.74ml, 1.49mmol) was added drop wise, with continuous stirring over a period of 30 minutes. Then the reaction mixture was worked up as prescribed for compound 9 to liberate compound 12. The percent yield, physical data and R_f values were given in table (1). CHNO Calculated: C, 58.78; H, 3.89; N, 10.82; O, 8.24. Found: C,59.21;H,4.01;N,11.28;O,8.61.

Synthesis of 2-[2-(2,6-dichlorophenylamino) phenyl] -2- (thiazol-2-ylamino) acetic acid (13)[22]

Compound 8 (0.17g, 0.42mmol) was dissolved in minimum volume of ethanol 99%:THF (15:1) mixture. The solution was cold to 18° C, and then sodium hydroxide (2N, 0.25ml,0.5mmol) was added drop wise, with continuous stirring over a period of 30 minutes. Then the reaction mixture was worked up as prescribed for compound 9 to give compound 13. The percent yield, physical data and R_f values were given in table (1). CHNOS Calculated: C,51.79; H, 3.32; N, 10.66; O, 8.12; S, 8.13. Found: C, 52.23;H,3.49;N,10.2;O,8.47;S,8.49.

The general routes outlined in scheme 1 were used to synthesized the target compounds and their intermediates, their characterization and physical data are presented in table 1.



Scheme1: Synthesis of target compounds (9-13) and their intermediates.

Table 1 : The characterization and physical data of the final compounds and their intermediates.

Compounds and intermediates	Empirical formula	Molecular weight	Description	% yield	Melting point °C	R_f value
2	$C_{15}H1_3Cl_2NO_2$	310	White crystals	94.9	72-75	0.88
3	$C_{15}H1_2BrCl_2NO_2$	389	Faint pink crystals	75	86-88	0.93
4	$C_{21}H_{19}Cl_2N_3O_4S$	480	Yellowish powder	75.9	95-97	0.83
5	$C_{23}H_{21}Cl_2N_3O_5S$	522	Faint orange powder	74.5	166-168	0.92
6	$C_{22}H_{21}Cl_2N_3O_4S$	494	Faint yellow powder	74.4	71-73	0.93
7	$C_{20}H_{17}Cl_2N_3O_2$	402	White powder	68.9	95-98	0.76
8	$C_{18}H_{15}Cl_2N_3O_2S$	408	Black powder	50.6	51-53	0.92
9	$C_{20}H_{17}Cl_2N_3O_4S$	466	Yellow powder	25.7	100-101d	0.77
10	$C_{22}H_{19}Cl_2N_3O_5S$	508	Faint yellow powder	24.6	182-184	0.87
11	$C_{21}H_{19}Cl_2N_3O_4S$	480	white powder	40.2	168-170	0.9
12	$C_{19}H_{15}Cl_2N_3O_2$	388	Gray powder	23	172-173d	0.65
13	$C_{17}H_{13}Cl_2N_3O_2S$	394	Black powder	31.9	161-163d	0.87

d: decomposition

B. Pharmacology

Albino rats of either sex weighing (220 ± 10 g) were supplied by the animal house of the College of Pharmacy, University of Baghdad. and were housed in the same location under standardized conditions. Animals were fed commercial chaw and had free access to water ad *libitum*. Animals were divided into five groups (each group consist of 6 rats) as follow:

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

Group B: six rats treated with diclofenac sodium in a dose of 3mg/ kg[23,24].suspended in propylene glycol 50%.

Group C-E: six rats/group treated with the tested compounds (9, 12 and 13) respectively in doses that determined below. (Suspended in propylene glycol 50%).

Anti-inflammatory activity

The anti-inflammatory activity of the tested compounds was studied using egg-white induced edema model[25]. Acute inflammation was produced by a subcutaneous injection of 0.05ml of undiluted egg-white into the planter side of the hind paw of the rats.; 30 minutes after i.p. administration of the drugs or their vehicle. The paw thickness was measured by vernea at seven time intervals (0, 30, 60, 120, 180, 240,300 minutes) after vehicle or drugs administration , the results were represented in table 2. The data are expressed as a percent of inhibition of edema thickness at each time interval, which can be calculated from the mean effect in control and treated animals according to the equation:

$$\% \text{ inhibition} = 100\ [(Vc-Vt)/Vc],$$

Where Vc and Vt represents mean paw thickness in control and tested groups (at time t-time zero) respectively[26], the results were represented in table 3.

Results and Discussion

The most widely used primary test to screen new anti-inflammatory agents measure the ability of a compound to reduce local edema induced in the rat paw by injection of an irritant agent [27]. The thickness of the injected paw was measured at zero time of i.p. injection of drug or vehicle) and immediately after egg-white injection (at time 30 min) and after 60, 120, 180, 240 and 300 min.

Table 2 : Effect of compounds 9, 12 and 13, diclofenac (reference) and propylene glycol (control) on egg-white induced paw edema in rats.

Treatment groups						
	Time (min)	**Control (n=6)**	**Diclofenc (n=6)**	**Compoud 9 (n=6)**	**Compoud 12 (n=6)**	**Compoud 13 (n=6)**
Paw thickness (mm)	0	4.07±0.05	3.98±0.06	4.06±0.04	4.09±0.1	4.10±0.04
	30	5.83±0.12	5.78±0.11	5.78±0.12	5.85±0.06	5.87±0.12
	60	5.85±0.06	5.42±0.12*	5.26±0.13*[a]	5.23±0.12*[a]	5.80±0.11[b]
	120	5.5±0.11	5.11±0.04*	4.94±0.1*	4.97±0.05*	5.13±0.03*
	180	5.25±0.03	4.95±0.1*[a]	4.59±0.05*[b]	4.83±0.05*[a]	4.96±0.05*[a]
	240	5.03±0.1	4.69±0.12*[a]	4.28±0.0*[b]	4.6±0.11*[a]	4.70±0.02*[a]
	300	4.91±0.02	4.54±0.13*[a]	4.15±0.1*[b]	4.49±0.02*[a]	4.56±0.06*[a]

Data are expressed in mm paw thickness as mean ± SEM
Time (0) is the time of i.p. injection of tested compounds and propylene glycol.
Time (30) is the time of injection of egg-white (induction of paw edema).
* significantly different compared to control ($p<0.05$).
n=number of animals
Non-identical superscripts (a and b) among different groups are considered significantly different ($p<0.05$).

Table 3 show the percent of inhibition of paw edema of the three tested compounds and diclofenac (reference) with respect to the control group. All the tested compounds were effectively limited the increase in paw edema. Diclofenac, compounds 9 and 12 showed inhibition that started from the first hour after drug administration, while compound 13 show delay in its onset as its inhibition effect started from the second hour after drug injection. The percent of inhibition of compound 9 is significantly differ from the reference, compounds 12 and 13 at the third, fourth and fifth hour of the experiment which indicate the superior anti-inflammatory effect, as illustrates in figure 1.

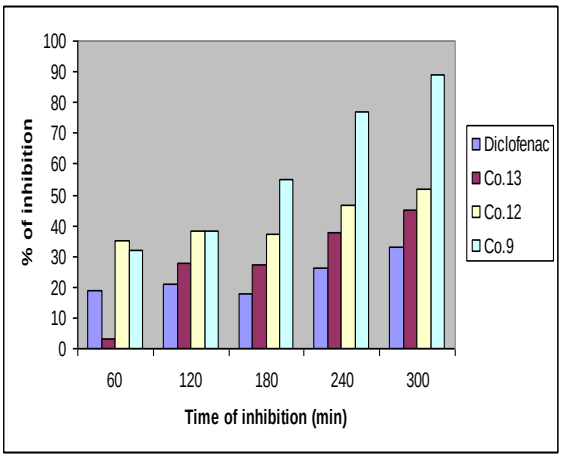


Figure 1: Percent inhibition produced by diclofenac sodium, compounds 9, 12 and 13.

Table 3: Percent inhibition of diclofenac, compounds 9, 12, and 13 on egg-white induced paw edema in rats.

Treatment groups					
	Time (min)	**Diclofenac sodium (n=6)**	**Compound 9 (n=6)**	**Compound 12 (n=6)**	**Compound 13 (n=6)**
Percent inhibition	60	19%	31.8%	35%	3.4%
	120	20.9%	38.4%	38.4%	27.9%
	180	17.7%	55%	37%	27%
	240	26%	77%	46.8%	37.5%
	300	33%	89%	52%	45.2%

Conclusion

In vivo anti-inflammatory study showed that the incorporation of 4-aminobenzenesulfonamide, 2-aminopyridine and 2-aminothiazole into a well known anti-inflammatory drug (diclofenac) maintain its anti-inflammatory activity. More over compound 9 showed more potent anti-inflammatory activity than diclofenac, compounds 12 and 13 at time interval 180-300 min. with the same onset of action.

Acknowledgment

We are grateful to the staff members and colleagues of the department of Pharmaceutical Chemistry and the department of Pharmacology and Toxicology.

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