Synthesis of Schiff Bases of Benzaldehyde and Salicylaldehyde as Anti-inflammatory Agents

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Abstract

Three Schiff bases from Benzaldehyde and Salicylaldehyde have been synthesized (A, 1and 2) and two of them (1and 2) have been tested for anti-inflammatory activity. The p-aminobenzene sulfonamide has been synthesized from acetanilide through the addition of excess chlorosulfonic acid then concentrated ammonia solution; Schiff base of this derivative (2) exhibited good level of activity against egg-white induced edema in rat hind paw, while the other tested derivative exhibited no activity.

Key words: Schiff bases, sulfonamide derivatives, salicylaldehyde

الخلاصة:

تم في هذه الدراسة تخليق ثلاث قواعد شف من البنزالديهايد والسالسيل الديهايد (A٢,١) وقد اختبرت فعالية اثنتين منها كمضادات للالتهابات. ان البارا امينوبنزين سلفون امايد هو احد الامينات قد تم تخليقه من الاستاناليد من خلال اضافة كمية كبيرة من حامض الكلوروسلفونيك ومن ثم سائل الامونيا المركز. ان قاعدة شف لهذا المشتق قد اظهرت فعالية جيدة كمضاد للالتهابات المستحدثة في الجرذان بواسطة زلال البيض، اما القاعدة الثانية المخلقة فلم تظهر أي فعالية مضادة للالتهابات.

Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used for the treatment of pain, inflammatory conditions and fever [1,2]. Their efficacy has been documented in a number of clinical disorders including osteoarthritis, rheumatoid arthritis, ankylosing spondylitis, gout and dental pain [3]. In the past decades; it has become apparent that there are two separate cyclooxygenase (COX) gene products, COX-1 and COX-2, which can initiate the metabolism of arachidonic acid to prostaglandins (PGs) and related lipid mediators [3]. COX-1 expressed in most tissues of the body and largely governs the hemostatic production of arachidonic acid metabolism; whereas COX-2 is induced in response to inflammatory stimuli or physiologic stress and is responsible for the enhanced production of eicosanoid mediators characteristics of these situations. All classical NSAIDs inhibit COX-2 as well as COX-1 to varying degrees, thus they can be considered non-specific [4, 5]. For a long time Sparatore and co-workers have been described sets of Schiff bases (diaryl- and arylheteroaryl azomethines) endowed with strong and long lasting anti-inflammatory activity against the rat hind paw edema induced by carrageenan [6]. The same compounds reduced, dose dependently, the nitric oxide and PGEs production [7]. All these properties were mainly correlate with the presence of phenolic functions, which can display a generic anti-oxidant and radical scavenging activity, more than with the presence of the azomethine function [7]. On the other hand, the azomethine function is endowed with multiform reactivity and particularly is able to react with thiol groups [8]. Thus it could establish easily some kind of link with enzymatic or receptorial proteins. The diarylazomethines are isosteric with stilbenes and like these can exist in interconvertible *cis* and *trans* forms. Suitable substituted *cis*-stilbene derivatives are characterized by potent inhibitory activity on COX-2, quite similarly with that observed for a variety of vicinal diarylheterocycles, among which important anti-inflammatory drugs, like celecoxib and valdecoxib, are found[9]. In the last class of drugs, the central five member ring may be of very different nature, either heterocyclic or carbocyclic [10,11]; while the nature of substitutents on the two benzene rings is believed to be responsible for COX-2 selectivity by insertion into the secondary pocket of the enzyme, with the p-sulfonamido and p-methylsulfonyl groups playing a key role [12]. Accordingly, we have now designed and synthesized Schiff bases of salicylaldehyde (compounds 1 and 2). Some of them bearing these peculiar substituents, in addition to azomethine function, which could play some peculiar role in the interaction with COX enzymes.

(A)   **(1)**

(2)
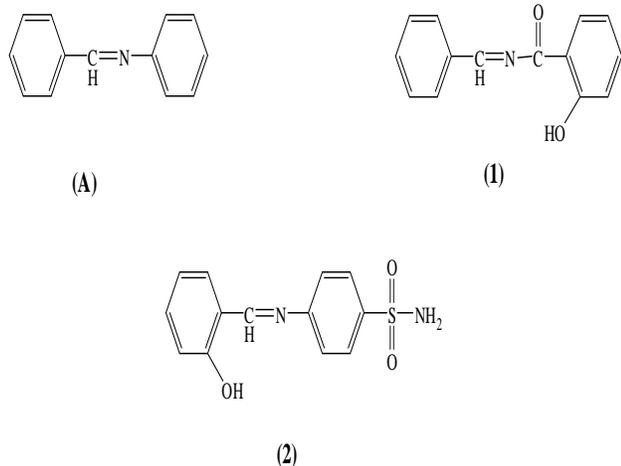
Experimentals
A. Chemistry

Materials: Acetanilide (Riedel-Dehaen, Germany), ammonia solution, benzaldehyde, salicylaldehyde, chlorosulfonic acid, absolute ethanol and ether (BDH, England), all solvents and materials used were of analar type and used without further purification.

General procedure: Melting points were determined by capillary method on Thomas Hoover apparatus (England) and IR spectra were recorded on model 500 scientific IR spectrophotometry, Buck Company (USA). 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 (1:1:6:2) [13]

Method for Preparation of p-aminobenzene sulfonamide

(a) Preparation of p-acetamidobenzene sulfonyl chloride

Acetanilide (6.67gm, 49.4mmol) was placed in 250ml flask and melted in the flask over a free flamed and caused the compound to solidify over the lower part of the flask by swirling the liquid formed and immersion in an ice bath momentarily. The chlorosulfonic acid (17ml, 262mmol) was added all at once with continuous shaking, then the reaction mixture was heated on a water bath for 90 minutes in order to complete the reaction. The mixture was cooled and the oily substance was poured with stirring. This suspension was filtered off with suction, pumped and washed with a little cold water and dried to give crude product, which was used immediately in the next step without further purification [13].

(b) Preparation of p-acetamidobenzene sulfonamide

The crude p-acetamidobenzene sulfonyl chloride was transferred to the rinsed reaction flask, and a mixture of concentrated ammonia solution (24 ml) and water (24 ml) was added to the flask. The contents of the flask were mixed thoroughly and heated with occasional swirling to just below the boiling point for about 20 minutes. The sulfonyl chloride will be converted into a pasty suspension of the corresponding sulfonamide. The suspension was cooled in an ice bath and then dilute sulfuric acid was added until the mixture was just acid to Congo red paper. The product was collected in Buchner funnel, washed with a little cold water and drained as completely as possible to give 53% yield of faint yellow crystals with melting pint of 213-214 °C and R_f values of 0.45. IR (in KBr disk): 3376cm^{-1} and 3304cm^{-1} (N-H stretching vibration of primary sulfonamide); 3227cm^{-1} (N-H stretching vibration of secondary amide); 1660cm^{-1} (C=O stretching vibration of secondary amide); 1598cm^{-1} and 1530cm^{-1} (C=C stretching vibration of aromatic ring); 1327cm^{-1} and 1157cm^{-1} (S=O stretching vibration of sulfonamide).

(c) Preparation of p-aminobenzene sulfonamid

The crude p-acetamidobenzene sulfonamide was transferred to a flask contain a 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 and activated charcoal (2 gm) was added. The mixture was boiling gently under reflux for 90 minutes. Cooled to room temperature and activated charcoal 92gm) was added. The mixture was heated to boiling and filtered with suction through a hardened filter paper. The filtrate (a solution of 4-aminobenzene sulfonamide hydrochloride) was placed in a beaker and sodium bicarbonate was added in portions with stirring until the suspension become neutral by testing with litmus paper. The mixture was cooled in ice bath and filtered by suction and dried to give 51% yield of the white crystals with melting point of 160-161 °C (reported 163-165 °C) [14] and R_f value of 0.75. IR (in KBr disk): 3461cm^{-1} and 3373cm^{-1} (N-H stretching vibration of primary amine); 3247cm^{-1} (N-H stretching vibration of sulfonamide); 1639cm^{-1} (N-H bending of primary amine); 1600cm^{-1}, 1571 cm^{-1} and

1504cm^{-1} (C=C stretching vibration of aromatic); 1309cm^{-1} and 1145cm^{-1} (S=O stretching vibration of sulfonamide).

General Method for preparation of azomethines (Schiff bases)

To a solution of 10 mmol aniline (compound A), salicylamide (compound 1), or p-aminobenzene sulfonamide (compound 2)in 50 ml of absolute ethanol, 12 mmol of benzaldehyde (compound 1) or salicylaldehyde (compound 2) were added and the mixture was refluxed for a reliable time; 6 hr for compound 1, 18 for the remaining compounds 1 and 2. After cooling the precipitate was collected, the solution was concentrated and a second part of the product was obtained. The joined fractions were washed with dry ether to remove some unreacted aldehyde and then crystallized by dissolution in dimethylformamide (DMF) and gradual addition of absolute ethanol.

Compound 1: melting point (151-152 ^{o}C), yield (55% of the yellow crystals), Rf value (0.64); IR in KBr disk: 3346 cm^{-1} (O-H stretching vibration of phenol); 1655cm^{-1} (C=O stretching vibration); 1620cm^{-1} (C=N stretching vibration of imine).

Compound 2: melting point (193-195 ^{o}C), yield (49% of the orange crystals), Rf value (0.86); IR in KBr disk: 3342 cm^{-1} (O-H stretching vibration of phenol); 3246cm^{-1} (N-H stretching vibration of sulfonamide); 1617cm^{-1} (C=N stretching vibration of imine); 1313cm^{-1} and 1163cm^{-1} (S=O stretching vibration of sulfonamide)

B. Pharmacology

Albino rats weighing (150 ± 10 gm) were supplied by the National Center for Quality Control and Drug Research. Animals were fed commercial chew and had free access to water *add libitum*, and were divided into four groups (each group consist of 6 rats) as follow: **group A**: served as control and treated with the vehicle (propylene glycol 50% v/v); **group B**: treated with indomethacin (reference agent) in a dose of 2mg/kg suspended in propylene glycol [15]; **group C** and **D**: treated with tested compounds 1

and 2 respectively in a dose of 200mg/kg and 100mg/kg respectively as finely homogenized suspension in 50% v/v propylene glycol (initial dose of 200mg/kg was trialed and compounds which exhibited a statistically significant activity at this dose were further tested at doses decreasing by a factor of 2).

Anti-inflammatory activity

The anti-inflammatory activity of the tested compounds was studied using egg-white induced edema model [16]. 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; 15 minutes after i.p. administration of the drugs or their vehicle. The paw thickness was measured by vernier at eight time intervals (0, 15, 30, 60, 120, 180, 240 and 300 minutes) after vehicle or drug administration. Statistical significance versus control group was evaluated by Student's t-test and P-values less than 0.05 were considered significant.

Results and Discussion

Compounds 1 and 2 were screened for anti-inflammatory activity and their results together with indomethacin and control groups are summarized in table(1). Compound 2 exhibited significant inhibition of the egg-white-induced rat paw edema at the i.p. dose of 100mg/kg; which may resulted mainly from the nature of sulfonamide constituents on the aromatic ring. In Conclusion, the previously observed strong anti-inflammatory activity of Schiff bases has been now confirmed in compound 2. This activity may be attributed mainly to the incorporation of sulfonamide group substituent to the aromatic ring with only secondary contribution from the azomethine double bond. However, this issue deserves further investigations and further recommendations are warranted to demonstrate their selectivity towards COX-2 isoenzyme , As shown in fig(1) .

Table (1): The anti-inflammatory activity of the indomethacin and tested compounds.

Time (min.)	Control	Indomethacin	Compound 1	Compound 2
0	4.46 ± 0.05	4.40 ± 0.17	4.45 ± 0.50	4.43 ± 0.15
15	5.41 ± 0.18	5.41 ± 0.1	5.40 ± 0.26	5.43 ± 0.10
30	6.05 ± 0.16	6.06 ± 0.13	6.07 ± 0.10	5.82 ± 0.07
60	6.35 ± 0.07	6.20 ± 0.14	6.30 ± 0.15	6.05 ± 0.09
120	6.5 ± 0.09	5.75 ± 0.10	6.29 ± 0.05	5.73 ± 0.12
180	5.93 ± 0.11	5.40 ± 0.10	5.75 ± 0.20	5.39 ± 0.07
240	5.38 ± 0.09	5.11 ± 0.04	5.24 ± 0.45	5.13 ± 0.05
300	5.2 ± 0.1	5.01 ± 0.01	5.13 ± 0.13	5.05 ± 0.04

Data are expressed as means ± SEM.
n = 6.

Anova Test:

The data illustrated in table (2), shows that there are highly significant differences between the action of prepared drugs and between Indomethacin and control, also the time intervals shows highly significant between each its zones.

Table (2): Anova Test

| Source of Variation	SS	df	MS	F calc.	F tab. 0.01	F tab.0.05
Rows	9.98395	7	1.426278571	73.487947[*]	3.6395896	2.4875777
Columns	0.424025	3	0.141341667	7.282524689[**]	4.8740462	3.072467
Error	0.407575	21	0.019408333			
Total	10.81555	31				

(*) Highly significant differences between time intervals with the probability of ≥ 0.01 type 1 error.
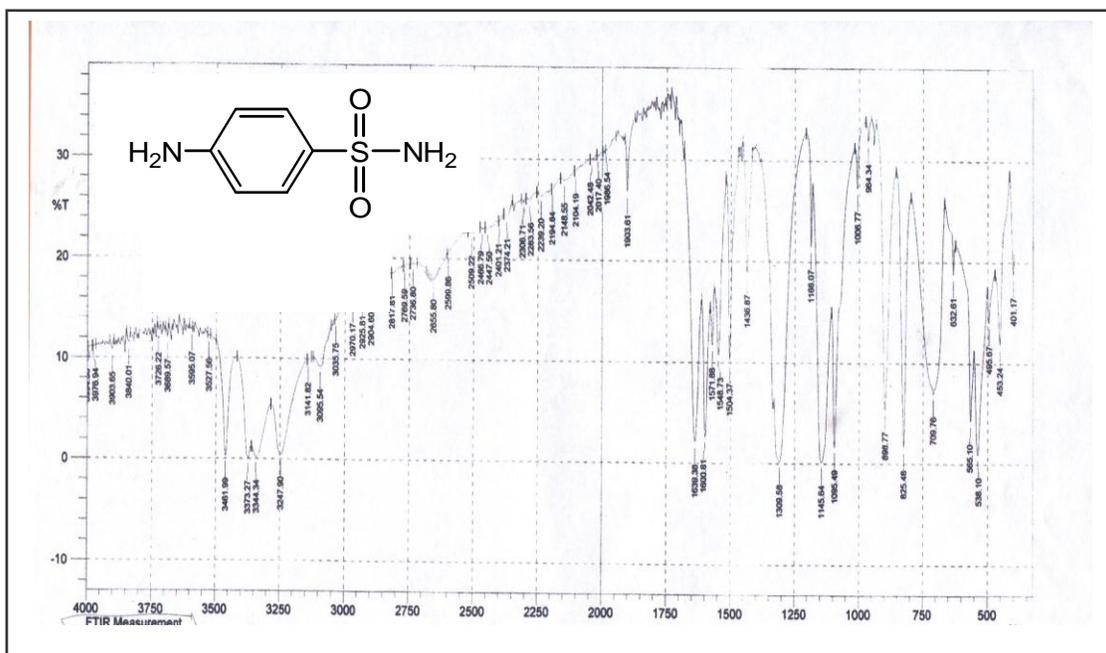(**) Highly significant differences between drugs action with the probability of ≥ 0.01 type 1 error.
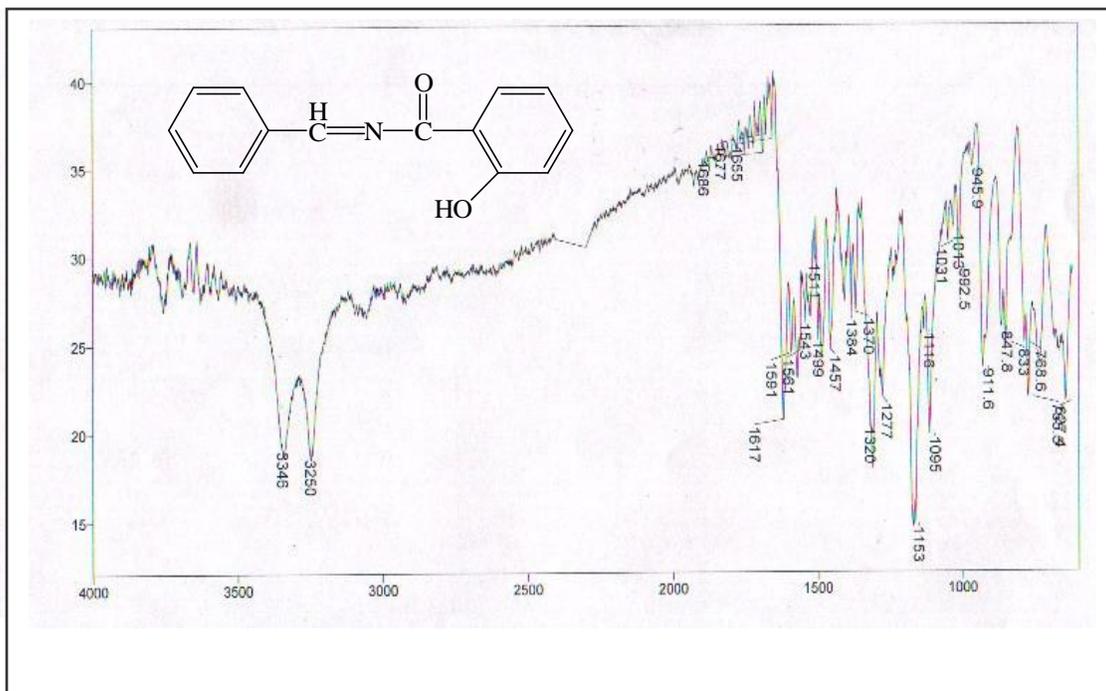
IR spectrum of compound 1 in Kbr disk.

IR spectrum of p-aminobenzene sulfonamide in KBr disk.
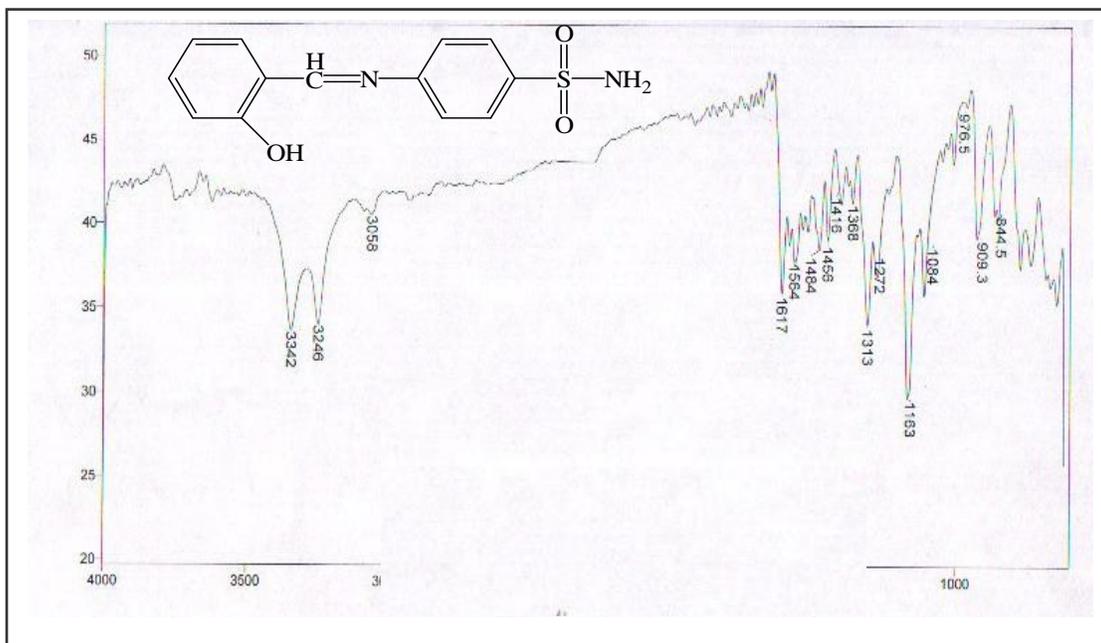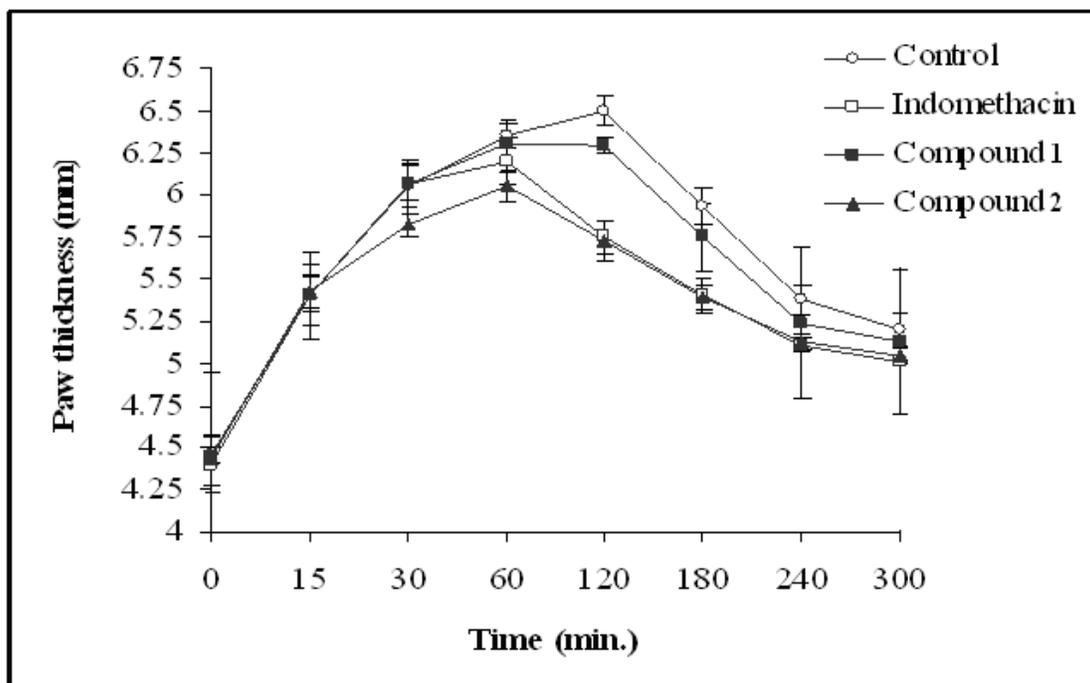
IR spectrum of compound 2 in KBr disk.

Fig. (1): Paw thickness of rats treated with indomethacin, compound 1 and compound 2 with respect to control. Results are expressed as means ± SEM (n=6).

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