ISSN: 0975-766X
CODEN: IJPTFI
Available Online through
Research Article
www.ijptonline.com

PHARMACOLOGICAL AND PASS PREDICTION ACTIVITIES OF 2-(4-HYDROXY-3-METHOXY BENZYLIDENEAMINO)BENZOIC ACID SCHIFF BASE

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Received on 28-03-2012 *Accepted on 16-04-2012*

Abstract

The Schiff base 2-(4-hydroxy-3-methoxybenzylideneamino) benzoic acid was prepared from Vanillin and anthranilic acid by condensation method using standard procedure. Analgesic, antipyretic and CNS activities of 2-(4-hydroxy-3-methoxybenzylideneamino) benzoic acid Schiff base were studied using albino rats of both the sexes. Animals were divided into three groups, each consisting of four animals. Group 1 served as control and group 2 received standard drug. Group 3 received 250 mg/kg of 2-(4-hydroxy-3-methoxybenzylidene amino) benzoic acid. For the determination of antipyretic activity, pyrexia was induced by 20% yeast suspension. The analgesic activity was determined by tail immersion method. CNS depressant activity of the compound was measured by placing the rat individually in the actophotometer for 10 min. The results obtained showed that the Schiff base was found to exhibit analgesic, antipyretic and CNS activities. The analgesic activity of the Schiff base compared to the standard drug pentazocine was found to be higher in the first hour and then the activity decreases slowly. The reduction in the rectal temperature for this compound was observed to be less than the standard drug paracetamol. 2-(4-hydroxy-3-methoxybenzylideneamino)benzoic acid Schiff base of 250mg/kg possessed CNS stimulant activity with a probability <0.001. The PASS prediction of 2-(4-hydroxy-3-methoxybenzylideneamino)benzoic acid Schiff base was found to possessed highest Arylacetonitrilase inhibitor activity of 79% and other activities greater than 70% .

Keywords: Vanillin, anthranilic acid, analgesic, antipyretic, CNS and PASS.

Introduction

Schiff bases and their metal complexes are of growing importance in co-ordination chemistry, attributable to recent observations in antibacterial, antifungal and oxygen carrier properties. The investigations of structure and bonding of Schiff base complexes helps to understand the complexes. Among the organic reagents actually used, Schiff bases possess excellent characteristics, structural similarities with natural biological substances, relatively simple preparation procedures and the synthetic flexibility that enables design of suitable structural properties [1-2]. Schiff bases of 2-(4-hydroxy-3-methoxybenzylideneamino)benzoic acid and its complexes have a variety of application in biological, clinical, analytical and pharmacological areas [3-6]. Studies of new chemotherapeutic Schiff bases attract much attention in the field of pharmacology [7-8]. In continuation of our work on pharamacological activity of Schiff bases, the present work deals with 2-(4-hydroxy-3-methoxybenzylideneamino)benzoic acid schiff base analgesic, antipyretic and CNS activities.

PASS provides simultaneous prediction of many types of biological activity based on the structure of organic compounds. The bioactivity of the 2-(4-hydroxy-3-methoxybenzylideneamino) benzoic acid Schiff bases can be predicted by using PASS.

Materials and Methods

Materials used

The chemicals such as Vanillin, anthranilic acid of E.merck grade and distilled ethanol were used. The melting point was determined using melting point apparatus and IR spectra was recorded in FT IR affinity-1 Schimadhu.

Drugs

Chlorpromazine(standard for CNS), Pentazocine(standard for analgesic) and paracetamol(standard for antipyretic) were chosen for our work.

Animals used

For the analgesics, anti-pyretic and CNS depressant activity studies twenty eight albino rats of both sexes of

weight 100-265g for each studies were used. The animals were kept in poly propylene cages in a dark/light cycle, 12hrs/12hrs and animals were fed with pelleted diet and drinking water ad libitum. All the experimental protocols were approved by the committee for the purpose of control and supervision on experiments on animals (CPCSEA), animal ethics committee vide number SBCP/ 2011-2012/ IAEC/ CPCSEA/6.

Methods used

Preparation of Schiff bases

The Vanillin and anthranilic acid were taken in a equimolar ratio of 0.01mol and refluxed with ethanol for 2hours. After refluxing, the product obtained was filtered, dried and recrystallized using ethanol. The synthesized compound was used to study the analgesic, antipyretic and CNS activities. The structure of the compound was proved by melting point determination and IR spectral studies.

The melting point was recorded using melting point apparatus. The melting point of 2-(4-hydroxy-3-methoxybenzylideneamino)benzoic acid Schiff base observed as 232^{o}C and the IR spectral studies reveals the presence of OH, C-N, C=N & C-O functionalities and showed the following stretching frequencies. O-H Stretching at υ3471cm^{-1}, C-N Stretching at υ1157cm^{-1}, C=O stretching at υ1851cm^{-1}, C=N stretching at υ1680cm^{-1} and C-O stretching was υ1211 cm^{-1}.

Determination of Analgesic Activity [9]

The tail immersion test was carried out as described by standard procedure [9] . The albino rats were selected and last 3.5 cm of their tail was immersed in hot water thermo-statistically maintained at 55° C, a procedure that caused them to rapidly withdraw their tail. Three groups of animals were held in position in a suitable restrainer with the tail extending out. The latency to withdraw the tail was recorded with a stopwatch, and a cut-off maximum latency of 15 sec was established in order to prevent tissue damage. Group I served as control, which received only vehicle (5 mg/kg, i.p). Other groups of animals received one of the following in a similar manner: Pentazocine (4 mg/kg,i.p) and 2-(4-hydroxy-3-methoxy benzylidene amino) benzoic acid Schiff base (250mg/kg,p.o). The initial

reading was taken immediately before administration of test samples and then at 1, 2, 3 and 4 hours after the administration and the recorded data were listed in **Table-1.**

Antipyretic Activity Determination [10,11]

The rectal temperature determination can be described by using standard procedure[10&11].Three groups of four animals of albino rats of both sexes of weight 100-265g were used for the study. The animals were kept in polypropylene cages in a room maintained under controlled atmospheric conditions. The animals were fed with standard diet (Hindustan liver, Mumbai, India) and had free access to clean drinking water. Antipyretic activity was measured by Brewer's induced pyrexia model in rats. Rats were fasted overnight with water ad lib before the experiments. Pyrexia was induced by subcutaneously injecting 20% w/v brewer's yeast suspension (10 ml/kg) into the animals' dorsum region. Eighteen hours after the injection, the rectal temperature of each rat was measured using a digital thermometer (Sato Keiryoki Mfg. Co., Ltd., Japan). Only rats that showed an increase in temperature of at least 0.9°C were used for the experiments. Animals were divided in to 3 groups, each containing four animals. Group I served as control (received distilled water), Group II received the standard drug (received paracetamol 33mg/kg, p.o) .Group III received 2-(4-hydroxy-3-methoxy benzylidene amino) benzoic acid schiff base (250mg/kg, p.o). The temperature was measured at 1, 2, 3 and 4hr after drug administration. The recorded values were listed in **Table-2.**

Determination of CNS Activity [9]

CNS depressant activity-determination

Locomotor methods were employed to determine the CNS depressant activity.

Locomotor activity

Locomotor activity was recorded with using Actophotometer (digital activity cage). The animals were divided into three groups (n = 4). Each rat was individually placed in the actophotometer for 10 min. Animals of group 1 were intraperitoneally treated with Caffeine (30 mg/kg) (i.p). Group 2 was treated orally with Chlorpramazine (3 mg/kg, i.p.). Group 3 was treated orally with 250 mg/kg dose levels of drugs. Basal reaction time was noted before and 30 min after the administration of treatment. Account is recorded when the beam of light falling on the photocell of

actophotometer is cut off by rat Sample 1 received reference standard Chlorpramazine at a dose of 3 mg/kg (i.p.) 30 min before the test. Mean change in the locomotor activity was recorded for each group and were listed in **Table-3.**

Chemdraw ultra11.0 software

The structure of 2-(4-hydroxy-3-methoxybenzylideneamino)benzoic acid schiff base was drawn in chemultra11.0 appear as given in **Fig.1.** and their structure was saved as molfiles**(*.mol)**.

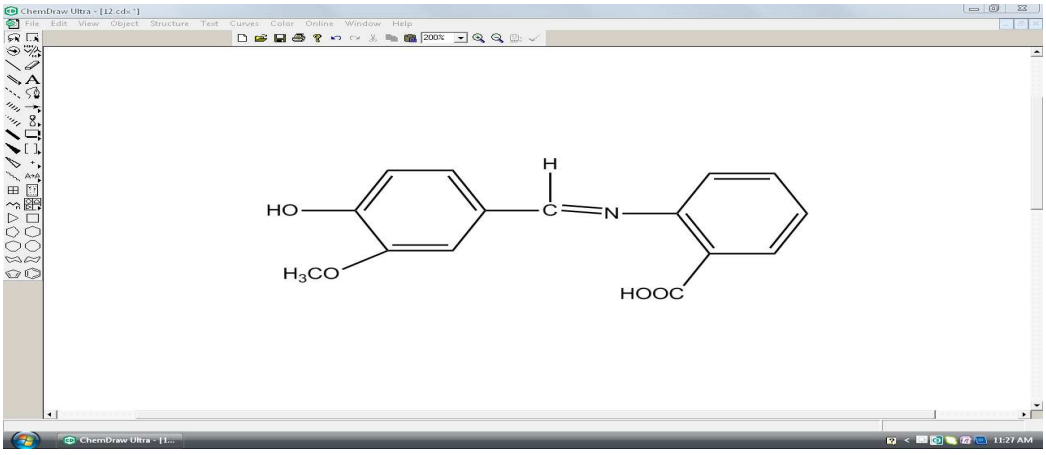

Fig 1: **Structure of 2-(4-hydroxy-3-methoxybenzylideneamino)benzoic acid Schiff base**.

Docking

Their possible bioactivities with PASS software (V.Poroikov et al, version 1.917) was predicted as given in **Fig.2** and the result was given as **Table-4.**

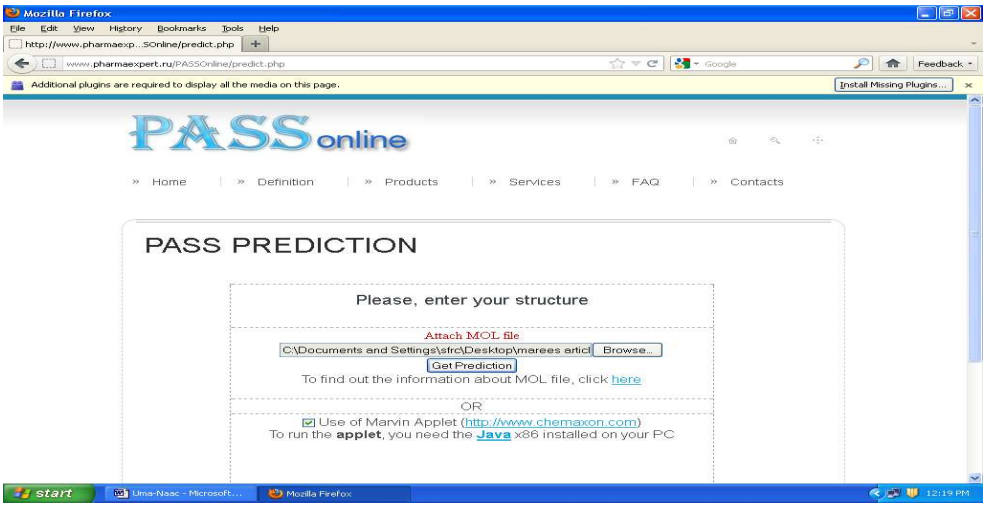

***Fig.2*. PASS Prediction window.**

Result and Discussion

Table-1: Effect of 2-(4-hydroxy-3-methoxybenzylideneamino)benzoic acid Schiff base on reaction time (in sec) in albino rats.

Drug treatment	Dose (mg/kg)	Basal reaction time after drug administration(in sec)			
		1hr	2hr	3hr	4hr
Control saline	5mg/kg	1.25±0.2886	1.25±0.2886	1.25±0.2886	1.5±0.3331
Standard Pentazocine	4mg/kg	2.25±0.5527 (44.44%)	6.75±0.7264 (81.48%)	7±1.9436 (82.14%)	8±0.4711 (81.25%)
2-(4-hydroxy-3-methoxy benzylideneamino)benzoic acid Schiff base	250mg/kg	2.7±0.2886 (53.70%)	2.75±0.2886 (54.54%)	4.25±0.2886 (70.58%)	4.75±0.5527 (68.42%)

One way ANOVA

F	4.9411	46.56	8.3873	66.1304
df	(2,9)	(2,9)	(2,9)	(2,9)
P	-	<0.01	<0.5	<0.05

Data are expressed as Mean±SEM, n=3 in each group, statistical analysis done by one way ANOVA followed by Dunnett's test. P<0.01. The value in the parenthesis indicates the percentage of analgesic activity.

parameter	2-(4-hydroxy-3-methoxy benzylidene amino) benzoic acid schiff base			
	1 hour	2 hour	3 hour	4 hour
t values	0.4330	4.6843	0.9217	2.6819
P-values	-	<0.01	<0.5	<0.05

Analgesic Activity

The increase in the basal reaction time from 2.25 to 8 seconds for 4 mg/kg of pentazocine and 2.7 to 4.75 seconds of 2-(4-hydroxy-3-methoxybenzylideneamino)benzoic acid Schiff base were observed. The 2-(4-hydroxy-3-methoxybenzylideneamino)benzoic acid Schiff base was found to possessed higher activity at the first hour and the

activity decreases slowly at second, third and fourth hour and among within their compound the activity increases at third hour when compared to the standard with a probability <0.01.

Table -2: Effect of 2-(4-hydroxy-3-methoxybenzylideneamino)benzoic acid Schiff base on rectal temperature(^{0}C) in albino rats.

Drug treatment	Dose (mg/kg)	Rectal temperature after yeast administration(^{o}C)		Rectal temperature after administration of drug(^{o}C)				Reduction in temperature(^{o}C)
		Normal	18 hr	1 hr	2 hr	3 hr	4 hr	
Control saline	1 ml/kg	36.6± 0.283	37.57± 0.300	37.57± 0.300	37.57± 0.300	37.47± 0.300	37.37± 0.300	-
Standard paracetamol	33 mg/kg	36.5± 0.2000	37.75± 0.219	37.45± 0.197	37.05± 0.197	36.75± 0.238	36.5± 0.2000	1.2
2-(4-hydroxy-3-methoxy benzylidene amino)benzoic acid Schiff base	250 mg/kg	36.4± 0.094	37.5± 0.115	37.3± 0.115	37.05± 0.110	36.85± 0.110	36.65± 0.110	0.9

One way ANOVA

F	0.5342	2.6094	3.8931	6.1702
df	(2,9)	(2,9)	(2,9)	(2,9)
P	<0.5	-	-	-

Data are expressed as Mean±SEM, n=3 in each group, statistical analysis done by one way ANOVA followed by Dunnett's test. P<0.5. The value in the parenthesis indicates the percentage of analgesic activity.

parameter	2-(4-hydroxy-3-methoxybenzylideneamino)benzoic acid Schiff base			
	1 hour	2 hour	3 hour	4 hour
t values	0.8130	0	0.3075	0.4878
P-values	<0.5	-	-	-

Antipyretic Activity

The reduction in temperature from 37.75 to 36.5 for 33mg/kg for paracetamol and 37.5 to 36.65 for 250mg/kg of 2-(4-hydroxy-3-methoxybenzylideneamino)benzoic acid Schiff base were observed. The reduction in temperature for 2-(4-hydroxy-3-methoxy benzylidene amino) benzoic acid Schiff base was (0.9%) slightly lower than that of the standard (1.2%).

Table -3: Effect of 2-(4-hydroxy-3-methoxybenzylideneamino) benzoic acid Schiff base on locomotor activity (in min) in albino rats.

Drug treatment	Dose (mg/kg)	Before treatment	After treatment	% change in activity
caffeine	3 mg/kg(i.p)	109±4.3462	129.75±4.5796	19.05
chlorpromazine	30 mg/kg(p.o.)	79.25±1.9650	14.25±1.5899	82.05
2-(4-hydroxy-3-methoxy benzylideneamino)benzoic acid Schiff base	(250 mg/kg) p.o	92.5±5.5077	77±1.4906	16.55

One way anova

F	16.7394	519.9233
df	(2,9)	(2,9)
P	<0.05	<0.001

Data are expressed as Mean±SEM, n=3 in each group, statistical analysis done by paired t test. P<0.001, compared to caffeine.

	2-(4-hydroxy-3-methoxy benzylideneamino)benzoic acid Schiff base	
parameter	Before	After
t values	2.2273	15.1533
P-values	<0.05	<0.001

CNS depressant activity

The dose dependent depression in the locomotor activity was measured for caffeine, chlorpromazine and 2-(4-hydroxy-3-methoxybenzylideneamino)benzoic acid Schiff base . Lower stimulant and depressant activity were observed for 250mg/kg of 2-(4-hydroxy-3-methoxy benzylidene amino)benzoic acid Schiff base than that of standard

caffeine, chlorpromazine with a probability <0.001.

Table-4: PASS Prediction of 2-(4-hydroxy-3-methoxy benzylideneamino)benzoic acid Schiff base.

S.No	Pa	Pi	Activity
1	0.799	0.033	Arylacetonitrilase inhibitor
2	0.780	0.035	Feruloyl esterase inhibitor
3	0.766	0.049	Benzoate-CoA ligase inhibitor
4	0.763	0.005	4-Methoxybenzoate monooxygenase (O-demethylating) inhibitor
5	0.762	0.028	Glutathione thiolesterase inhibitor
6	0.761	0.016	Apoptosis agonist
7	0.756	0.004	Antipyretic
8	0.752	0.034	Superoxide dismutase inhibitor
9	0.750	0.048	Taurine dehydrogenase inhibitor
10	0.745	0.028	Alkane 1-monooxygenase inhibitor

The PASS prediction of 2-(4-hydroxy-3-methoxy benzylideneamino)benzoic acid Schiff base showed the Arylacetonitrilase inhibitor activity as (Pa=0.799), Feruloyl esterase inhibitor activity as (Pa=0.780), Benzoate-CoA ligase inhibitor activity was found to possessed (Pa=0.766). Pa=0.763 for 4-Methoxybenzoate monooxygenase (O-demethylating) inhibitor activity, Glutathione thiolesterase inhibitor activity was found to have Pa values as 0.762. Apoptosis agonist activity shows (Pa=0.761), Pa=0.756 for antipyretic activity, Superoxide dismutase inhibitor activity showed to be Pa value 0.752, Pa value 0.750 was found to be for Taurine dehydrogenase inhibitor activity and Alkane 1-monooxygenase inhibitor activity was found to possessed Pa=0.745.

Conclusion

The analgesic activity of 2-(4-hydroxy-3-methoxybenzylideneamino) benzoic acid Schiff base, the was found to be higher in the first hour and then the activity decreases slowly compared to the standard drug pentazocine. The reduction in the rectal temperature for this compound was observed to be less than the standard drug paracetamol.CNS stimulant as well as depressant activities of (250mg/kg) of this compound were found to be lower than that of caffeine and chlorpromazine. The PASS prediction of bioactivity indicated that 2-(4-hydroxy-3-methoxy benzylideneamino)benzoic acid Schiff base was found to exhibit as Arylacetonitrilase inhibitor, Feruloyl esterase inhibitor, Benzoate-CoA ligase inhibitor, 4-Methoxybenzoate monooxygenase (O-demethylating) inhibitor, Glutathione thiolesterase inhibitor , Apoptosis agonist, antipyretic, Superoxide dismutase inhibitor, Taurine dehydrogenase inhibitor activities greater than 75% and Alkane 1-monooxygenase inhibitor activity was observed to be > 70%.

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