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Research Article

ECO-FRIENDLY SYNTHESIS OF N-BENZYLIDENE-4-CHLOROBENZENESULFONAMIDES, THEIR BIOLOGICAL ACTIVITIES AND QSAR STUDIES

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ABSTRACT

This article deals with the synthesis of N-benzylidene-4-chlorobenzenesulfonamides, their antimicrobial and analgesic activities and QSAR studies. The synthesis of N-benzylidene-4-chlorobenzenesulfonamides was carried out by the conventional method and an eco-friendly, microwave (MW) irradiation method. The MW irradiation method proved to be superior to the conventional method of synthesis since it reduced the reaction time and increased the yield of the compounds. An acute oral toxicity of all the synthesized compounds was determined using the OECD guidelines. All the compounds were found to be safe for the administration up to 2000 mg/kg weight of a mouse. The synthesized compounds were screened for the antimicrobial and analgesic activities. The antibacterial activity of the compounds was better as compared to their antifungal activity. The antibacterial and analgesic activities of these compounds were correlated separately, with their physicochemical parameters, and various QSAR models were developed. The best QSAR model was obtained when the antibacterial activity of the synthesized compounds against *E. coli* was correlated with their binding to the human serum albumin (Log k_{hsa}). In case of analgesic activity, the best QSAR model was obtained for the correlation of the analgesic activity with the hydrogen bond donors (donor HB) in the molecule and the predicted IC_{50} values for the blockage of the **H**uman ***E**ther-à-go-go* **R**elated **G**ene channels (HERG K^+ ion channels).

Keywords: N-Benzylidene-4-chlorobenzenesulfonamides, antimicrobial activity, QSAR.

INTRODUCTION

Sulfonamides form the family of broad spectrum synthetic bacteriostatic antibiotics. They have been used against the most gram positive and gram negative organisms, some fungi and certain protozoa[1]. They are also used as anti-inflammatory[2], analgesic[3], antimalarial[4] and anticancer agents[5]. In the search of more potent molecules, possessing antimicrobial and analgesic activities, several new N-benzylidene-4-chlorobenzene sulfonamides have been synthesized and screened for antimicrobial and analgesic activities.

The microwave radiation, falling in the range of 0.3 to 300 GHz[6], has become the source for the eco-friendly synthesis of organic compounds. It provides distinct advantages of the reduced reaction time and increased yield of the products.

The advances in the area of computer software provide guidelines for the rational drug designing and their modifications. The quantitative structure activity relationships

(QSAR) are the models or the mathematical relationships (often a statistical correlation), which correlate biological activity of molecules with their physico-chemical properties. The best QSAR model helps to identify the physico-chemical parameters influencing the biological activities of the compounds[7].

MATERIALS AND METHODS

The chemicals required for the synthesis of N-benzylidene-4-chlorobenzenesulfonamides were purchased from Merck Chemical Co., Spectrochem Laboratories, and Rankem Laboratories. For MW irradiation, the MW synthesizer from CEM Corporation, USA, was used. The characterization of the synthesized compounds was done by recording their melting points on EXPO-HITECH melting point apparatus, by determining their R_f values by thin layer chromatography (TLC), and by recording their IR spectra using JASCO FT/IR- 5300 spectrometer. The structures of N-benzylidene-4-chlorobenzenesulfonamides were confirmed from their ^{1}H-NMR spectra recorded on ^{1}H-NMRS -300 spectrometer.

Synthesis

The reactions involved in the synthesis of N-benzylidene-4-chlorobenzenesulfonamides are represented in **Scheme 1**. Ten N-benzylidene-4-chlorobenzenesulfonamides were synthesized in three steps. The first two steps were carried out by the conventional method and the last step was carried out both by the conventional and MW irradiation methods.

Synthesis of 4-chlorobenzenesulfonyl chloride

Chlorosulfonic acid (30 mL, 0.4 M) was taken in a round bottom flask and cooled to 0 ^{o}C using the freezing mixture of ice and salt. Chlorobenzene (15 mL, 0.1M) was added to it in portions over a period of 1 h, so that the temperature of a well stirred mixture did not raise more than 5 ^{o}C. Further, the reaction mixture was stirred for 4 h and then allowed to stand overnight in refrigerator. The cold liquid was poured on crushed ice. The oily layer was then separated from the aqueous layer and the former was washed several times to remove unreacted chlorosulfonic acid. 4-Chlorobenzenesulfonyl chloride thus obtained was used immediately for the next reaction.

Synthesis of 4-chlorobenzenesulfonamide

4-Chlorobenzenesulfonamide was synthesized by treating equi-molar amounts of 4-chlorobenzenesulfonyl chloride and liquor ammonia. The product was filtered, washed with water and recrystallized using the mixture of water and ethanol (20:30).

Synthesis of N-benzylidene-4-chlorobenzenesulfonamides

a) Conventional method

The mixture of 4-chlorobenzenesulfonamide (1.93 g, 0.01 M), substituted aldehyde (0.02 M) and 40 % NaOH (10 mL) was prepared in the minimum quantity of ethanol in a round bottom flask fitted with a reflux condenser. The mixture was heated to 60 to 80 ^{o}C, till the completion of the reaction, which was monitored by TLC. After the completion of the reaction, the reaction mixture was cooled to room temperature and treated with concentrated HCl. The precipitated product was filtered off and washed with water. Recrystallization was carried out by using the mixture of methanol and water (70: 30) and the product was dried in hot air oven.

b) MW irradiation method

For the synthesis of N-benzylidene-4-chlorobenzenesulfonamides by MW irradiation, the MW synthesizer from CEM Corporation, USA, was used. Exactly the same procedure, as mentioned in the conventional method, was used for their synthesis by MW irradiation. The mixture of 4-chlorobenzenesulfonamide (1.93 g, 0. 01 M), substituted aldehyde (0.02 M) and 40 % NaOH (10 mL) was prepared in the minimum quantity of ethanol in a microwave flask fitted with a reflux condenser. The mixture was exposed to the MW radiation of 450-560 W, till the completion of the reaction. After the completion of the reaction, the reaction mixture was processed exactly as per the conventional method of synthesis.

The physical and spectral data for all the N-benzylidene-4-chlorobenzenesulfonamides are presented below. In the IR data, the abbreviation 'str' stands for the stretching vibrations.

N-(Benzylidene)-4-chlorobenzene sulfonamide (Compound 1)

M. P. 141-144 ^{o}C; **TLC** [Benzene: Ethyl acetate (1:1)]; **R_f:** 0.82; **Molecular Formula:**

$C_{13}H_{10}O_2ClNS$; **IR** (KBr cm^{-1})**:** 3094 (C-H str), 1583 (C=N str), 1494 (C=C str), 1327, 1107 (SO_2 str), 767 (C-Cl str); **^{1}H NMR** δ (ppm)**:** 7.8 (d, 4H, Ar-H), 7.45 (m, 3H, Ar-H), 7.2 (d, 2H, Ar-H), 3.8 (s, 1H, N=CH)

N-(4-Chlorobenzylidene)-4-chlorobenzene sulfonamide (Compound 2)

M. P. 247-249 °C; **TLC** [Benzene]; **R_f:** 0.52; **Molecular Formula:** $C_{13}H_9O_2Cl_2NS$; **IR** (KBr cm^{-1})**:** 2866 (C-H str), 1587 (C=N str), 1419 (C=C str), 1331, 1176 (SO_2 str), 744 (C-Cl str); **^{1}H NMR** δ (ppm)**:** 7.9 (d, 2H, Ar-H), 7.7 (d, 2H, Ar-H), 7.5 (d, 4H, Ar-H), 3.8 (s, 1H, N=CH)

N-(2-Chlorobenzylidene)-4-chlorobenzene sulfonamide (Compound 3)

M. P. 87-90 °C; **TLC** [Benzene]; **R_f:** 0.33; **Molecular Formula:** $C_{13}H_9O_2Cl_2NS$; **IR** (KBr cm^{-1})**:** 2883 (C-H str), 1685 (C=N str), 1519 (C=C str), 1317, 1170 (SO_2 str), 744 (C-Cl str); **^{1}H NMR** δ (ppm)**:** 8.0 (d, 2H, Ar-H), 7.4 (m, 6H, Ar-H), 3.8 (s, 1H, N=CH)

N-(4-Hydroxybezylidene)-4-chlorobenzene sulfonamide (Compound 4)

M. P. 79-82 °C; **TLC** [Benzene: Ethyl acetate (3:1)]; **R_f:** 0.63; **Molecular Formula:** $C_{13}H_{10}O_3ClNS$; **IR** (KBr cm^{-1})**:** 3447 (O-H str), 2870 (C-H str), 1670 (C=N str), 1591 (C=N str), 1319, 1170 (SO_2 str), 619 (C-Cl str); **^{1}H NMR** δ (ppm)**:** 10.3 (s, 1H, O-H), 7.5 (d, 4H, Ar-H), 6.75 (d, 4H, Ar-H), 3.3 (s, 1H, N=CH)

N-(2-Nitrobezylidene)-4-chlorobenzene sulfonamide (Compound 5)

M. P. 158-160 °C; **TLC** [Benzene: Ethyl acetate (2:2)]; **R_f:** 0.63; **Molecular Formula:** $C_{13}H_9O_4ClN_2S$; **IR** (KBr cm^{-1})**:** 3025 (C-H str), 1712 (C=N str), 1591 (NO_2 str), 1454 (C=C str), 1290, 1157 (SO_2 str), 767 (C-Cl str); **^{1}H NMR** δ (ppm)**:** 7.8 (d, 2H, Ar-H), 7.6 (m, 6H, Ar-H), 3.5 (s, 1H, N=CH)

N-(3, 4, 5, Trimethoxybenzylidene)-4-chlorobenzenesulfonamide (Compound 6)

M. P. 149-151 °C; **TLC** [Benzene: Ethyl acetate (2:2)]; **R_f:** 0.47; **Molecular Formula:** $C_{16}H_{16}O_5ClNS$; **IR** (KBr cm^{-1})**:** 2837 (C-H str), 1685 (C=N str), 1466 (C=C str), 1327, 1124 (SO_2 str), 1269 (C-O-C str), 761 (C-Cl str); **^{1}H NMR** δ (ppm)**:** 7.3 (s, 2H, Ar-H), 7.2 (m, 4H, Ar-H), 3.8 [m, 10H, N=CH & (3×OCH_3)]

N-(2, 4-Dimethoxybenzylidene)-4-chlorobenzenesulfonamide (Compound 7)

M. P. 163-165 °C; **TLC** [Benzene: Ethyl acetate (3:0.3)]; **R_f:** 0.63; **Molecular Formula:** $C_{15}H_{11}O_4ClNS$; **IR** (KBr cm^{-1})**:** 2947 (C-H str), 1595 (C=N str), 1466 (C=C str), 1321, 1107 (SO_2 str), 1259 (C-O-C str), 756 (C-Cl str); **^{1}H NMR** δ (ppm)**:** 7.8 (d, 2H, Ar-H), 7.3 (d, 2H, Ar-H), 7.2 (s, 1H, Ar-H), 6.9 (s, 2H, Ar-H), 3.9 (s, 1H, N=CH), 3.8 [s, 6H, (2×OCH_3)]

N-(4-Fluorobenzylidene)-4-chlorobenzenesulfonamide (Compound 8)

M. P. 153-155 °C; **TLC** [Benzene: Ethyl acetate (3:0.5)]; **R_f:** 0.52; **Molecular Formula:** $C_{13}H_9O_2FClNS$; **IR** (KBr cm^{-1})**:** 2941 (C-H str), 1687 (C=N str), 1577 (C=C str), 1300, 1105 (SO_2 str), 613 (C-Cl str); **^{1}H NMR** δ (ppm)**:** 8.1 (d, 2H, Ar-H), 8.0 (d, 2H, Ar-H), 6.9 (d, 4H, Ar-H), 3.8 (s, 1H, N=CH)

N-(4-Methoxybenzylidene)-4-chlorobenzenesulfonamide (Compound 9)

M. P. 181-183 °C; **TLC** [Benzene: Ethyl acetate (3:1)]; **R_f:** 0.61; **Molecular Formula:** $C_{14}H_{12}O_3ClNS$; **IR** (KBr cm^{-1})**:** 2932 (C-H str), 1597 (C=N str), 1547, 1415 (C=C str), 1325, 1097 (SO_2 str), 1255 (C-O-C str), 615 (C-Cl str); **^{1}H NMR** δ (ppm)**:** 7.5 (m, 8H, Ar-H), 3.9 (m, 4H, 1×OCH_3 & N=CH)

N-(2-Furylmethylene)-4-chlorobenzenesulfonamide (Compound 10)

M. P. 149-153 °C; **TLC** [Benzene: Ethyl acetate (3:1)]; **R_f:** 0.69; **Molecular Formula:** $C_{11}H_8O_3ClNS$; **IR** (KBr cm^{-1})**:** 2924 (C-H str), 1597 (C=N str), 1498 (C=C str), 1323, 1107 (SO_2 str), 756 (C-Cl str); **^{1}H NMR** δ (ppm)**:** 7.8 (d, 2H, Ar-H), 7.3 (m, 5H, Ar-H), 3.5 (s, 1H, N=CH)

Biological activities

The acute oral toxicity studies and analgesic activity were performed on Swiss albino mice of either sex, weighing between 25 and 30 g. All the animals were purchased from Haffkine Biopharmaceutical Corporation Ltd., Mumbai, India. The animals were maintained at 25 ± 2 °C and 50 ± 5 % relative humidity. The animals were fasted for 24 h prior to the experiments and water provided *ad libidum.* The animal study protocols were approved by the Institutional Animal Ethics Committee of C. U. Shah College of Pharmacy, Mumbai, India.

Acute oral toxicity studies

The acute oral toxicity studies were performed as per the Organization for Economic Co-operation and Development (OECD) guidelines[8]. Before the experimentation, the animals were divided into the *control group* and the *test groups*, consisting of six animals each. The *control group* received a single dose of 10 mL/kg body weight of 1 % w/v sodium carboxymethyl cellulose (Na-CMC) suspension in 0.9 % saline solution. The test compounds (**1-10**), at the dose levels of 500, 1000 and 2000 mg/kg body weight of a mouse, were administered orally to the mice present in the *test groups*. After the administration of the test compounds, the animals were observed for a period of 14 days for any changes in the skin, fur, eyes and behavioral pattern. The mortality of mice in each group was also observed. A dose leading to these changes or mortality was considered as a toxic dose.

Antimicrobial activity

The antibacterial activity of all the synthesized N-benzylidene-4-chlorobenzenesulfonamides (**1-10**) was examined against two gram-positive [*Staphylococcus aureus* (ATCC 6538) and *Pseudomonas aeruginosa* (ATCC 9027)] and two gram-negative [*Escherichia coli* (ATCC 2592) and *Klebsiella pneumoniae* (ATCC 2957)] bacteria. The fungal strains, *Candida albicans* (ATCC 10231) and *Aspergillus niger* (ATCC 26036), were selected for the antifungal activity of the compounds. The antimicrobial activity was performed by the cup plate method at four different concentrations of 100, 250, 500 and 1000 µg/mL, and was expressed in terms of zone of inhibition (ZOI). The *positive control* used for the antibacterial activity was sulfamethoxazole and that for the antifungal activity was miconazole, at concentrations of 100, 250, 500 and 1000 µg/mL. Dimethylsulfoxide was used as the *negative control*.

Analgesic activity[9]

The analgesic activity of N-benzylidene-4-chlorobenzenesulfonamides was determined by the acetic acid induced writhing in mice. Mice were divided into seven groups, each group consisting of six animals. Group 1 was used as the *negative control*, group 2 was used as the *positive control* (standard, aspirin) and groups 3 to 7 were used as *test groups*. Group 1 received saline solution orally (10 mL/kg body weight). Group 2 was administered orally, a dose of 30 mg/kg body weight of aspirin in the form of a suspension in Na-CMC and groups 3 to 7 were treated orally, with a dose of 100 mg/kg body weight of test compounds in the form of suspension in Na-CMC. Thirty min after the administration of these compounds, the mice in all the groups were injected intraperitoneally (i. p.), 0.6 % w/v solution of aqueous acetic acid (10 mL/kg body weight). Ten min after the i. p. injection of acetic acid, the number of writhes (abdominal contractions) in each mouse were counted for 20 min. The lesser the number of writhes, the higher is the analgesic activity of a compound. The per cent inhibition of writhes was calculated by using the following formula.

$$\text{Per cent inhibition of writhes} = \frac{(N - N_t)}{N} \times 100$$

where, N = Average number of writhes in the negative control group and N_t = Average number of writhes in a test or the control group

Quantitative structure activity relationships studies

The QSAR studies involved the correlation between the physicochemical parameters of the synthesized compounds and their biological activities. "*Maestro*" – the molecular modeling software from *Schrödinger Inc*, USA, was used to develop the quantitative structure activity relationships models. The software "*LigPrep*" was used to get the correct conformational structures of the synthesized compounds. The software "*QikProp*" was used to obtain different physicochemical parameters of the synthesized compounds. The correlations between the biological activities and physicochemical parameters of N-benzylidene-4-chlorobenzenesulfonamides were studied using the program "*Strike*" from *Schrödinger*. Various QSAR models were developed by correlating antibacterial activity of N-benzylidene-4- chlorobenzene sulfonamides, against *E. coli* at 1000 µg/mL, with their physicochemical parameters by simple linear regression analysis**,** i. e. only one parameter was correlated at a time with the antibacterial activity. The antibacterial activity was expressed in terms of logarithm of zone of inhibition (Log ZOI). The analgesic activity was expressed in terms of the logarithm of the

per cent inhibition of writhes. Various QSAR models were developed by the multiple regression analysis, i.e. by correlating more than one parameter at a time, with the analgesic activity of N-benzylidene-4-chlorobenzenesulfonamides. In both the cases, the best QSAR models were chosen based on the statistically significant values of the square of the correlation coefficient (r^2), Fischer's value (F) and standard deviation (SD). The best QSAR model was validated by the *internal* and the *external* validation methods. For this purpose, the data set of 10 sulfonamides was divided into a *training set* (5 compounds) and a *test set* (5 compounds), randomly.

RESULTS AND DISCUSSION

Synthesis

Ten N-benzylidene-4-chlorobenzene sulfonamides were synthesized by the conventional and microwave irradiation methods. The reaction time and the yield of the products by both the methods are listed in **Table 1**. Compared to the conventional heating, the microwave irradiation method was found to improve the yield of benzylidene sulfonamides by 2-16 %. The reaction time was reduced from h to min. The maximum reduction in time from 7 h to 6 min was observed for **compound 6**. The maximum increase in the yield from 40 % in the conventional method to 55.6 % in the microwave irradiation method was observed in case of **compound 8**. The MW irradiation was found to be eco-friendly and efficient as compared to the conventional method.

Acute oral toxicity studies

The synthesized N-benzylidene-4-chlorobenzenesulfonamides were tested for their toxic effects at different dose levels of 500, 1000 and 2000 mg/kg in mice. None of the synthesized compounds showed significant changes in the skin, fur and eyes at any of the tested dose levels up to 14 days. No mortality was observed in the control and the test groups. Thus, these compounds were considered safe for the administration up to the dose of 2000 mg/kg body weight.

Antimicrobial activity

The results of the antibacterial and antifungal activities of N-benzylidene-4-chlorobenzenesulfonamides are tabulated in **Tables 2** and **3**, respectively. The antibacterial activity against *E. coli, P. aeruginosa* and *K. pneumoniae* and antifungal activity against *A. niger* were significant when compared with the standards, sulfamethoxazole and miconazole, respectively. The graphical presentation of these activities is shown in **Figures 1, 2, 3** and **4**, respectively. **Table 4** enlists the compounds having antimicrobial activity greater than that of the respective standards, sulfamethoxazole and miconazole. **Table 5** represents the compounds having antimicrobial activity equivalent to that of the respective standards, sulfamethoxazole and miconazole. Many compounds showed good antibacterial activity against *P. aeruginosa* and *K. pneumoniae*. The antifungal activity of N-benzylidene-4-chlorobenzenesulfonamides was better against *A. niger* as compared to that of *C. albicans*.

Analgesic activity

The analgesic activity of all the synthesized compounds, the positive control (aspirin) and the negative control (0.6 % acetic acid solution), is listed in **Table 6**. Aspirin produced 84.5 % reduction in the writhes in mice, whereas **compound 4** showed 87 % reduction in writhes. Thus, it was superior to aspirin, as far as its analgesia was concerned. The per cent reduction in writhes for four compounds was in the range of 71-76 and for the other five compounds, it was in the range of 51-66.

Quantitative structure activity relationships (QSAR) studies for antibacterial activity

The QSAR studies of ten N-benzylidene-4-chlorobenzenesulfonamides were carried out by correlating their antibacterial activity with one physicochemical parameter (simple linear regression analysis). Various QSAR models were developed. The best QSAR model, represented by **equation 1**, was selected based on the statistical parameters.

$$\text{Log (ZOI)} = 0.93640 - 0.45306 \text{ Log } k_{hsa} \quad \text{..... Eq. 1}$$

$$n = 10,\ r^2 = 0.785,\ SD = 0.0945,\ F = 29.2$$

where, ZOI is the zone of inhibition for N-benzylidene-4-chlorobenzenesulfonamides against *E. coli* at 1000 µg/mL. It is a measure of antibacterial activity of compounds. The term k_{hsa} is the rate of binding of compounds to the human serum albumin[10]. The negative sign associated with Log k_{hsa} indicated that the lesser the binding of N-benzylidene-4-chlorobenzenesulfonamides to the human

serum albumin, the higher would be their antibacterial activity.

For the validation of the best QSAR model, ten N-benzylidene-4-chlorobenzenesulfonamides were placed in two different sets, a *training set* consisting of 5 compounds and a *test set* consisting of 5 compounds. **Equation 2** shows the best QSAR model obtained by the simple linear regression analysis for the *training set* compounds, and is expressed as:

Log (ZOI) = 0.91714 - 0.45963 Log k_{hsa} …… Eq. 2
***n* = 5, r^2= 0.8189, *SD* = 0.0574, *F* = 13.6**

For the *internal validation* of this model (**Equation 2**), the Log k_{hsa} values for the *training set* compounds were substituted in it and their antibacterial activity (Log ZOI) was predicted. The antibacterial activity of these compounds was also determined experimentally. The experimentally obtained and the predicted antibacterial activities of the *training set* compounds are listed in **Table 7.** The experimentally obtained antibacterial activity of these compounds was then correlated with their predicted antibacterial activity. This correlation is represented graphically in **Figure 5**. Good correlation between the observed and the predicted antibacterial activities was obtained for the *training set* compounds, as the square of the correlation coefficient (r^2) was 0.8088. Thus, **equation 2** provided good internal predictivity.

For the *external validation* of the best QSAR model (**equation 2**), the compounds from the*test set* were used. The antibacterial activity of the *test set* compounds was predicted from **equation 2**. The antibacterial activity of these compounds was also determined experimentally. The observed and the predicted antibacterial activities of the *test set* compounds are listed in **Table 7**. The correlation between the observed and the predicted antibacterial activities for the *test set* compounds is represented graphically in **Figure 6**. Good correlation between the observed and the predicted antibacterial activities was obtained in this case also, as the square of the correlation coefficient was 0.8056. Thus, **equation 2** can be used successfully for the prediction of the antibacterial activity of the compounds from the series of N-benzylidene-4-chlorobenzenesulfonamides against *E. coli*.

Quantitative structure activity relationships (QSAR) studies for analgesic activity

The QSAR studies of ten benzylidenesulfonamides were carried out by correlating their analgesic activity with two physicochemical parameters simultaneously (multiple regression analysis). Various QSAR models were developed. The best QSAR model was obtained when the analgesic activity, expressed as per cent reduction in writhes produced by acetic acid, was correlated with the terms donor HB and Log HERG, and is represented by **equation 3**.

Log (% Reduction in writhes) = 0.11931 + 0.0783733 donor HB –0.34720 Log HERG …….. Eq. 3
***n* = 10, r^2 = 0.7597, *SD* = 0.0393, *F* = 11.1**

where, the term donor HB represents the number of hydrogen bonds that would be formed by the solute with water molecules in an aqueous solution. The positive sign associated with the parameter, donor HB, indicated that the higher the number of hydrogen bonds formed by the compounds, the higher would be their analgesic activity. Thus, the presence of polar functional groups like OH, COOH, NH_2, etc., in N-benzylidene-4-chlorobenzenesulfonamides would enhance their analgesic activity. In equation 3, the term HERG stands for the **H**uman ***E**ther-à-go-go* **R**elated **G**ene and plays an important role in electrical activity of the heart which coordinates the beating of the heart[11]. Log HERG indicates the predicted IC_{50} value for the blockage of HERG potassium ion channel that codes for a protein known as K_v11.1 potassium ion channel. The negative value of Log HERG indicated that the lower the value of Log HERG, lesser is the blockage of potassium ion channels and therefore, higher is the analgesic activity of the compounds.

For the validation of the best QSAR model, the ten N-benzylidene-4-chlorobenzene sulfonamides were placed in two different sets, a *training set* consisting of 5 compounds and a *test set* consisting of 5 compounds. **Equation 4** shows the best QSAR model obtained by the simple linear regression analysis for the *training set* compounds, and is expressed as:

Log (% Reduction in writhes) = 0.91473 - 0.4836 Log HERG …….. Eq. 4
***n* = 5, r^2 = 0.869, *SD* = 0.026, *F* = 19**

For the *internal validation* of this model, the Log HERG values for the *training set* compounds were substituted in **equation 4** and their analgesic activity (% reduction in writhes) was predicted. The analgesic activity of these compounds was also determined experimentally. The experimentally obtained and the predicted analgesic activities of the *training set* compounds are listed in **Table 8**. The experimentally obtained analgesic activity of these compounds was then correlated with their predicted analgesic activity. This correlation is represented graphically in **Figure 7**. Good correlation between the observed and the predicted analgesic activities was obtained for the *training set* compounds as the square of the correlation coefficient (r^2) was 0.8683. Thus, **equation 4** provided good internal predictivity.

For the *external validation* of the best QSAR model (**equation 4**), the compounds from the *test set* were used. The analgesic activity of the *test set* compounds was predicted from **equation 4**. The analgesic activity of the test set compounds was also determined experimentally. The observed and the predicted analgesic activities of the *test set* compounds are listed in **Table 8** and the correlation between the observed and the predicted analgesic activities is represented graphically in **Figure 8**. Good correlation between the observed and the predicted analgesic activities was obtained in this case also, as the square of the correlation coefficient was 0.7451. Thus, **equation 4** can be used successfully for the prediction of the analgesic activity of the compounds from the series of N-benzylidene-4-chlorobenzenesulfonamides.

CONCLUSIONS

An eco-friendly microwave irradiation method for the synthesis of N-benzylidene-4-chlorobenzenesulfonamides was established in the laboratory. Compared to the conventional heating, the microwave irradiation method increased the yield of N-benzylidene-4-chlorobenzenesulfonamides by 2-16 % and reduced the reaction time from h to min.

In acute oral toxicity studies, no significant changes and no deaths were observed at any of the tested dose levels of the synthesized sulfonamides after 14 days of the observation period. The compounds showed good antimicrobial activity against *P. aeruginosa, K. pneumoniae* and *A. niger*. The antibacterial activity of N-benzylidene-4-chlorobenzenesulfonamides was better as compared to their antifungal activity. All the compounds showed good analgesic activity. Out of ten N-benzylidene-4-chlorosulfonamides, one compound was found to be superior to aspirin as far as its analgesia was concerned.

The QSAR studies showed that the antibacterial activity of N-benzylidene-4-chlorobenzenesulfonamides was well correlated with their rate of binding to human serum albumin, whereas the analgesic activity was well correlated with the capacity of molecules to form hydrogen bonds with water molecules and also with the degree of blockage of HERG potassium ion channels.

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Table 1: Comparison of the conventional (CON) and microwave (MW) irradiation methods for the synthesis of N-benzylidene-4-chlorobenzenesulfonamides

Comp. No.	Reaction time		% Yield	
	CON (h)	MW (min)	CON	MW
1	12	45	52.9	66.2
2	17	85	68.3	73.5
3	12	50	36.0	39.0
4	17	80	61.5	67.2
5	18	40	62.6	70.1
6	07	06	42.6	55.6
7	19	135	43.6	53.3
8	17	70	40.0	55.6
9	17	90	51.6	53.4
10	17	70	53.9	63.9

Table 2: Antibacterial activity of N-benzylidene-4-chlorobenzenesulfonamides

Comp. No.	Zone of Inhibition (in mm) against bacteria															
	S. aureus				*P. aeruginosa*				*E. coli*				*K. pneumoniae*			
	Conc. µg/mL				Conc. µg/mL				Conc. µg/mL				Conc. µg/mL			
	100	250	500	1000	100	250	500	1000	100	250	500	1000	100	250	500	1000
1	3	8	10	14	8	**10**	**12**	**14**	7	8	12	12	3	11	12	13
2	6	8	10	13	**10**	**12**	**14**	**15**	**12**	12	13	15	8	9	10	13
3	10	**12**	13	15	7	**12**	**15**	**16**	10	**14**	14	16	**12**	**14**	**16**	**19**
4	**14**	**16**	**18**	**20**	**13**	**16**	**18**	**20**	**12**	**14**	**16**	17	**12**	**13**	**15**	**18**
5	9	**13**	15	17	9	**12**	**14**	**19**	9	11	**17**	17	**10**	**13**	**14**	**16**
6	5	6	13	16	**12**	**13**	**17**	**21**	9	10	**18**	**18**	7	10	12	13
7	11	**14**	15	**19**	9	**10**	**12**	**15**	**12**	**14**	**19**	**19**	**10**	**13**	**15**	**20**
8	9	**12**	14	16	**11**	**14**	**16**	**18**	**12**	**14**	**21**	**22**	**12**	**15**	**17**	**18**
9	8	9	11	13	7	8	9	**15**	4	6	**26**	**26**	4	7	7	10
10	5	7	9	13	4	5	8	10	5	7	**24**	**24**	7	9	10	11
Std*	**12**	**12**	**16**	**18**	**10**	**10**	**10**	**12**	**12**	**14**	**16**	**18**	**10**	**12**	**14**	**16**

***Std: Sulfamethoxazole**

Table 3: Antifungal activity of N-benzylidene-4-chlorobenzenesulfonamides

Comp. No.	Zone of Inhibition (in mm)							
	C. albicans				***A. niger***			
	Conc. µg/mL				Conc. µg/mL			
	100	250	500	1000	100	250	500	1000
1	7	14	14	16	**10**	**11**	**12**	15
2	**19**	20	21	22	**9**	**11**	**12**	14
3	12	15	18	21	**10**	**11**	**12**	15
4	13	15	17	19	**8**	9	11	14
5	11	16	18	19	**10**	**11**	**12**	13
6	12	15	18	21	**10**	**12**	**12**	**16**
7	**21**	23	24	26	**10**	**11**	11	**16**
8	**20**	22	24	26	**10**	**12**	**12**	**16**
9	5	6	9	11	6	9	9	10
10	6	8	9	11	5	7	7	10
Std*	**16**	**28**	**30**	**34**	**8**	**10**	**12**	**16**

***Std = Miconazole**

Table 4: Compounds showing antimicrobial activity higher than that of the respective standards, at various concentrations

Micro-organisms	Compound numbers and concentrations			
	100 µg/mL	**250 µg/mL**	**500 µg/mL**	**1000 µg/mL**
S. aureus	-	4	4	4, 7
P. aeruginosa	4, 6, 8	2, 3, 4, 5, 6, 8	1 to 8	1 to 9
K. pneumoniae	-	3, 4, 5, 7, 8	3, 4, 7, 8	3, 4, 5, 7, 8
E. coli	-	-	5 to 10	7, 8, 9, 10
A. niger	1, 2, 3, 5, 6, 7, 8, 9, 10	1, 2, 3, 5, 6, 7, 8	-	-

Table 5: Compounds showing antimicrobial activity equivalent to that of the respective standards, at various concentrations

Micro-organisms	Compound numbers and concentrations			
	100 µg/mL	**250 µg/mL**	**500 µg/mL**	**1000 µg/mL**
S. aureus	-	3, 8	-	-
P. aeruginosa	2	1, 7	-	-
K. pneumoniae	5, 7	-	5	5
E. coli	2, 4, 7, 8	3, 4, 7, 8	4	6
C. alibicans	2, 7, 8	-	-	-
A. niger	4	-	1, 2, 3, 5, 6, 8	6, 7, 8

Table 6: Analgesic activity of N-benzylidene-4-chlorobenzenesulfonamides

Comp. No.	% Reduction in writhes
1	76
2	66
3	66
4	**87**
5	66
6	51
7	71
8	76.9
9	74
10	53.8
Aspirin	84.5

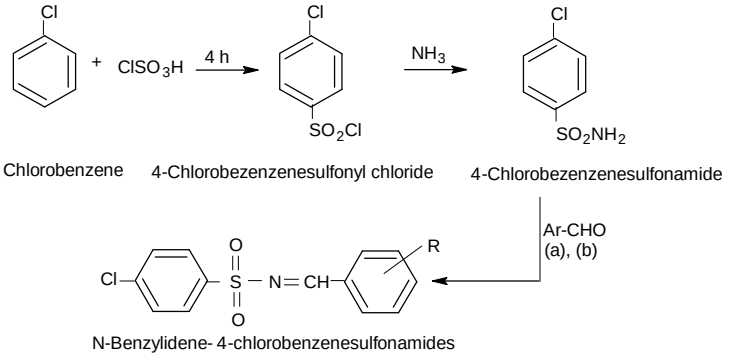
Table 7: Observed and the predicted antibacterial activities (Log ZOI) of the *training* and *test* set compounds against *E. coli* at 1000 µg/mL using the best QSAR model (Equation 2)

Compounds (Training Set)	Log ZOI		Compounds (Test Set)	Log ZOI	
	Observed	Predicted		Observed	Predicted
1	1.0791	1.1688	**2**	1.1139	1.1156
3	1.1461	1.1070	**5**	1.2304	1.1428
4	1.2041	1.1823	**6**	1.2552	1.2411
7	1.2787	1.2825	**8**	1.3222	1.1428
10	1.3802	1.3909	**9**	1.3424	1.2733

Table 8: Observed and the predicted analgesic activities (Log % Reduction in writhes) of the *training* and *test set* compounds using the best QSAR model (Equation 4)

Compounds (Training Set)	Log % Reduction in writhes		Compounds (Test Set)	Log % Reduction in writhes	
	Observed	Predicted		Observed	Predicted
1	1.8808	1.8738	**4**	1.9395	1.72195
2	1.8195	1.7843	**7**	1.8512	1.81675
3	1.8195	1.8434	**8**	1.8859	1.8182
5	1.8195	1.8230	**9**	1.8692	1.79837
6	1.7075	1.7072	**10**	1.7307	1.6344

Scheme 1: Synthesis of N-benzylidene-4-chlorobenzenesulfonamides



(a) = Reflux for conventional synthesis

(b) = Expose to 560 W for MW irradiation

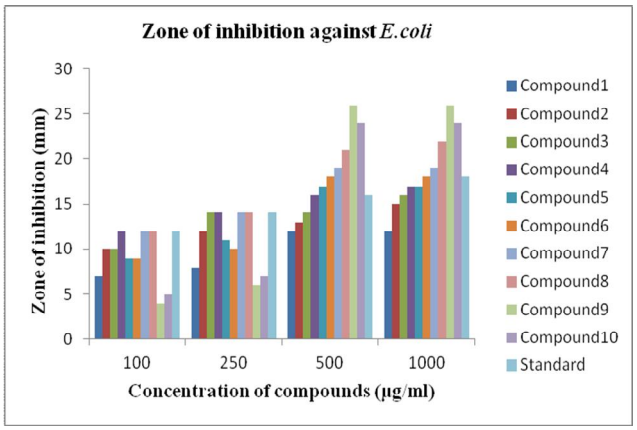


Fig. 1: Zone of inhibition of N-benzylidene-4-chlorobenzenesulfonamides and sulfamethoxazole at various concentrations against *E. coli*

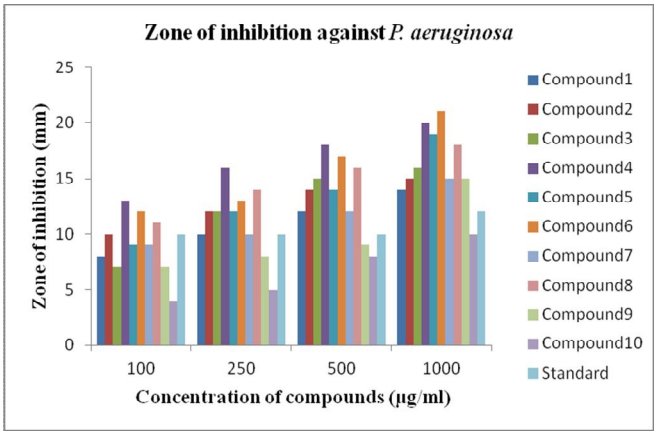


Fig. 2: Zone of inhibition of N-benzylidene-4-chlorobenzenesulfonamides and sulfamethoxazole at various concentrations against *P. aeruginosa*

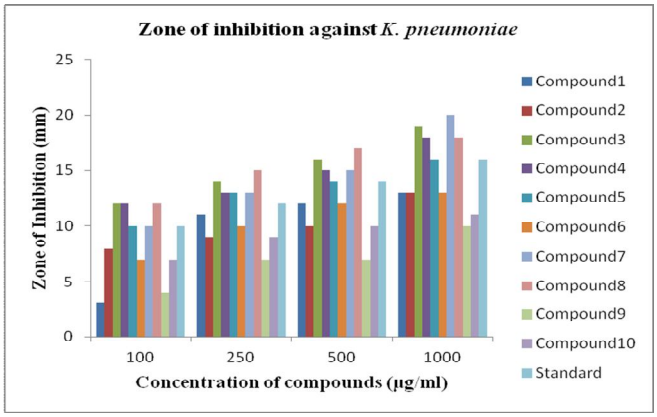


Fig. 3: Zone of inhibition of N-benzylidene-4-chlorobenzenesulfonamides and sulfamethoxazole at various concentrations against *K. pneumonia*

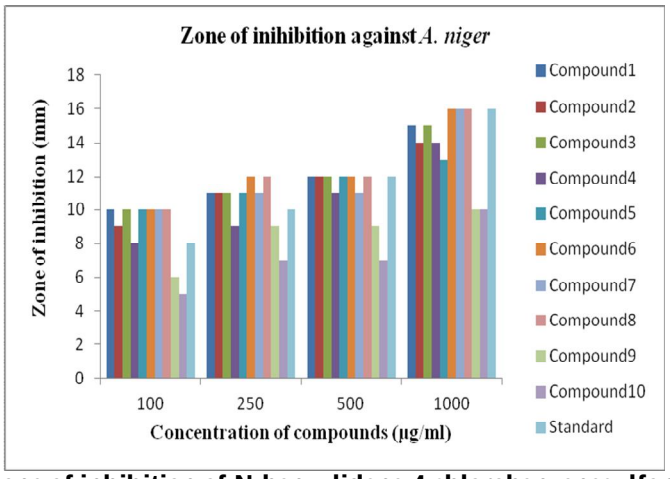


Fig. 4: Zone of inhibition of N-benzylidene-4-chlorobenzenesulfonamides and miconazole at various concentrations against *A. niger*

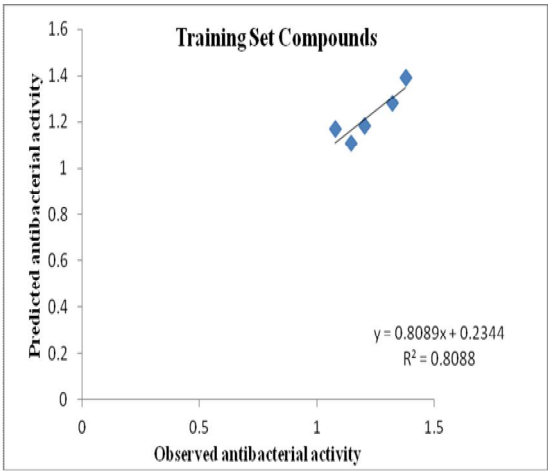


Fig. 5: Correlation of the observed and the predicted antibacterial activities of the *training set* compounds (1000 µg/mL) against *E. coli* using the best QSAR model (Equation 2)

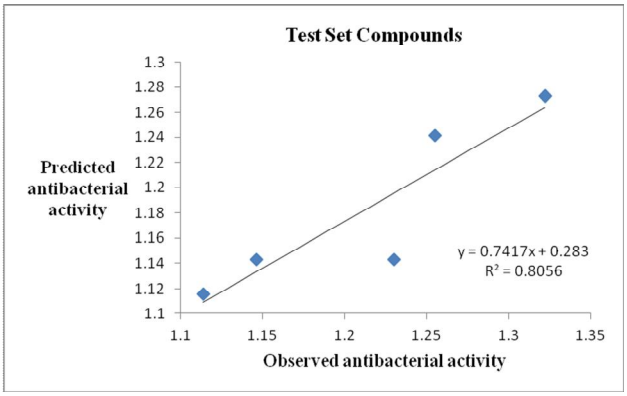


Fig. 6: Correlation of the observed and the predicted antibacterial activities of the *test set* compounds (1000 µg/mL) against *E. coli* using the best QSAR model (equation 2)

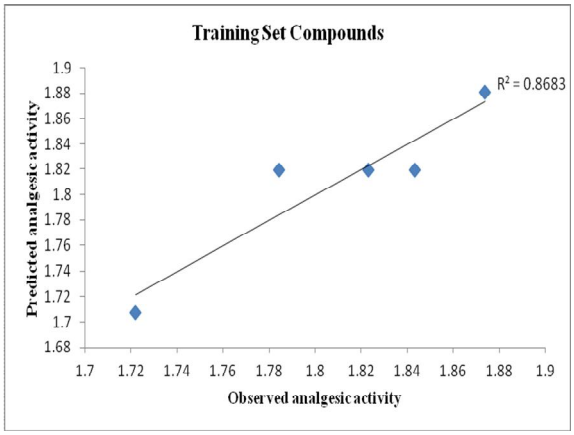


Fig. 7: Correlation of the observed and the predicted analgesic activities of the *training set* compounds using the best QSAR model (equation 4)

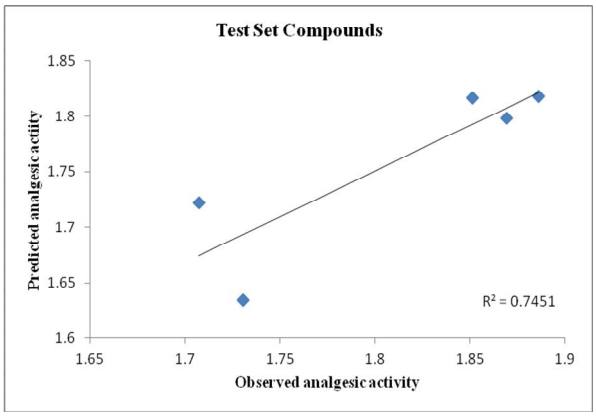


Fig. 8: Correlation of the observed and the predicted analgesic activities of the *test set* compounds using the best QSAR model (equation 4)

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