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Synthesis, screening and QSAR studies of 2,4-disubstituted 1,5-benzodiazepine derivatives

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ABSTRACT

New 2,4-disubstituted 1,5-benzodiazepine derivatives containing different functional groups have been synthesized and screened for their antibacterial activity. The 2,4-disubstituted 1,5-benzodiazepine derivatives were synthesized by reacting substituted chalcones synthesized using aldol condensation with o-phenylenediamine. QSAR studies of synthesized derivatives were performed. QSAR equation show that electronic and liphopholic parameters have good correlation with anti bacterial activity. Lead molecules, which are designed in accordance with QSAR equations, are exhibit improved antibacterial activity.

Key words: 1,5-benzodiazepine, QSAR, antibacterial, aldol condensation.

INTRODUCTION

It is evident from the literature that diazepine derivatives have been found to show various pharmacological activities. Diazepine ring is the backbone of several antibacterial[1-7] antifungal[8] and anti-inflammatory drugs[9]. Considering the scope for further studies on diazepine derivatives we have synthesized some new 2,4-disubstituted -1, 5 benzodiazepine derivatives and screened for antibacterial activity.

In (scheme-I) the 2,4-disubstituted-1,5 benzodiazepine (II) were prepared by condensation of chalcones with o-phenylenediamine. The synthesis was ascertained from spectral and physicochemical analysis. The first step was synthesis of various substituted chalcones, synthesized by using, substituted aromatic acetophenone and benzaldehyde were reacted in presence of sodium hydroxide to form corresponding product. IR and physicochemical data analysis confirmed the products. Physicochemical data of the 2,4 disubstituted -1,5 benzodiazepine derivatives is shown in Table 1. Microwave irraditation was used so as to obtain good yield (93-95%) and the reported modifications also led to decrease in reaction time. In the second step, carbonyl group of chacones was reacted with diamines in presence of glacial acetic acid to form 2,4 disubstituted-1,5 benzodiazepine. The reaction involves acid catalyzed nucleophilic substitution. Results of IR and NMR analysis confirmed formation of the desired product.

IR spectrum of the product in KBr showed peaks in the region 3070-3220 cm^{-1} (N-H stretching), 1645-1655 cm^{-1} (C=N) as diagnostic absorptions. All the synthesized final derivatives were confirmed by NMR spectral analysis.

EXPERIMENTAL

Melting points of synthesized compounds were determined by an open capillary method and are uncorrected. Analytical TLC was performed on Silica gel-G. Spot was detected by using iodine

vapours or under UV light (254 nm). The IR (KBr) spectra were recorded on a Jasco- FTIR 4100 instrument. The ^{1}H NMR spectra of the compounds were recorded on 400 MHz Varian NMR. The solvent used was DMSO. This series was subjected to QSAR studies using MOE 2006.08 running on P-IV processor.

Synthesis of Chalcones[10]

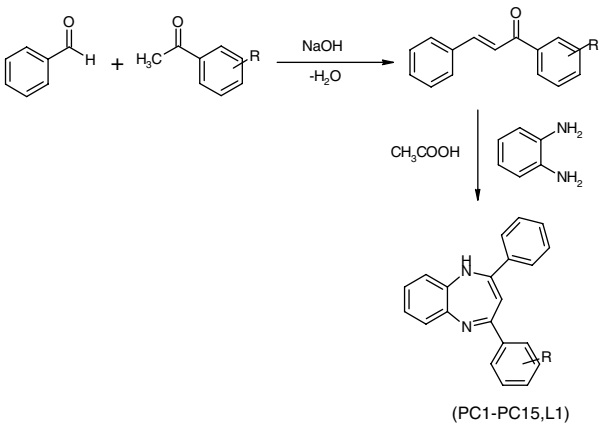
A solution of 22 g sodium hydroxide in 200mL of water and spirit placed in a round bottom flask with magnetic stirrer and immerse the flask in bath of crushed ice, poured in it acetophenone (0.43 mol) and started the stirrer and added the benzaldehyde (0.43 mol). The temperature of mixture kept at about 25°C and stirred the mixture vigorously so that the stirring is no effective. Removed the stirrer and reaction mixture was left in the ice chest over night. Filtered the product and washed with the cold water until the washing are neutral to the litmus recrystalized from the spirit.

Synthesis of 2,4 disubstituted 1,5 benzodiazepines

A mixture of chalcone (0.01 mole), o-phenylenediamine (0.01 mole) and glacial acetic

Table 1: Physical data of the 2,4 disubstituted 1,5 benzodiazepine derivatives

Comp.Code	R'	m.p.°C	% Yield	R_f value*
P1	H	77	70	0.48
P2	4-CH_3	100	85	0.57
P3	4-Br	120	80	0.34
P4	4-Cl	126	80	0.68
P5	4-NO_2	140	87	0.48
P6	3-NO_2	142	74	0.48
P7	4-CH_3O	160	76	0.58
P8	2,4 Cl	180	78	0.55
P9	3,4 Cl	178	76	0.58
P10	2,4 CH_3O	160	88	0.57
P11	2,5 CH_3O	156	86	0.54
P12	3,4 CH_3O	168	80	0.48
P13	4-OH	120	78	0.58
P14	2-OH	116	76	0.6
P15	3-OH	114	70	0.55



Scheme 1

acid 5mL in DMF 15mL was taken in a conical flask and placed in a microwave and irradiated for 5 min. The reaction mixture was allowed to attain room temperature and treated with the cold water. The solid separated was filtered, washed with water and recrystalized from methanol. I.R 3316.96 cm^{-1} (NH), 1613 cm^{-1} (CH=CH), 1663.3 cm^{-1} (C=N), N.M.R 3.2 (NH)

Anti-microbial activity[11,12]

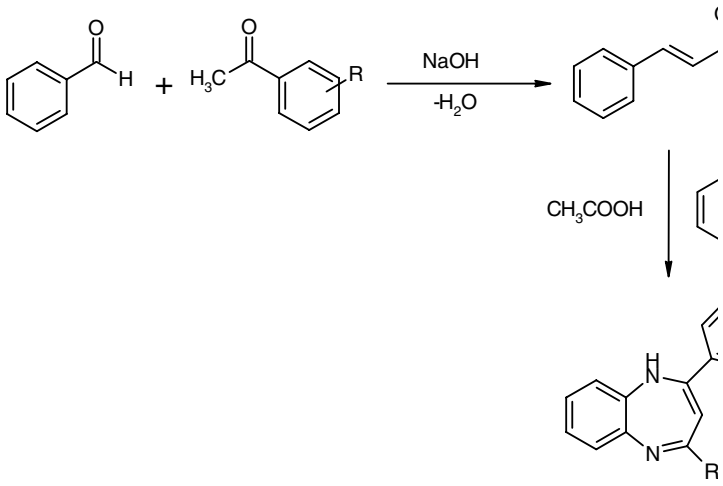
All the compounds were screened for antibacterial activity (Indian Pharmacopoeia, 1996), performed against *S. aureus* (NCIM01065), *P. aeruginosa* (NCIM501) by cup plate agar diffusion method. Ciprofloxacin used as standard drugs for antibacterial activity In each plate, four cavity of 6 mm diameter were made with a sterile-borer. The test solutions at the concentrations 1000, 500, 250, 125, 62, 31.5µg/mL was added to the respective cavity aseptically and labeled accordingly. Standard was added in concentration of 100 µg/mL. The plates were kept undisturbed for at list 2 hour at room temperature to allow diffusions of the solution properly in the nutrient agar medium. After incubation of the plates at 37 ± 1°C for 24 hours, the diameter of zone of inhibition surrounding each of the cavities was measured.

QSAR studies

The QSAR studies were carried out to investigate relationship between various physicochemical parameters and antibacterial activity of 2,4 disubstituted-1,5-benzodiazepine derivatives. The correlation was sought between MIC value and various physicochemical constants. Calculated descriptors included lipophilic descriptors like partition coefficient (LogP), water accessible surface area (ASA), electronic parameters like electronic energy (E), highest occupied molecular orbital (HOMO), lowest unoccupied molecular orbital (LUMO), dipole moment along X-axis (D-X) while steric parameters like molar refractivity (SMR), principle moment of inertia (PMI), principle moment of inertia-X component (PMIX), principle moment of inertia-Y component (PMIY), principle moment of inertia-Z component(PMIZ). Only those parameters having intercorrelation coefficient below 0.5 were considered to select best equation. The correlation plot was plotted between pMIC (observed) and pMIC

Table 2: Physical data of the Lead molecules (scheme-II).

Comp.Code	R'	m.p.°C	% Yield	R_f value*
L1	CH_3	77	70	0.48



Scheme 2

(calculated) in Fig 1. The best model was selected on basis of correlation coefficient(R) and various statistical parameters like squared correlation coefficient (R^2) standard error (SE) and F values. The cross validation coefficient (XR^2) was found to be 0.8122**.** The equation for antibacterial activity

For *S. aureus*

- Root mean square error (RMSE): 0.11697
- Correlation Coefficient (R2) : 0.67337
- pMIC *S. aureus* =0.88775+0.27806 * logS+0.34428 * SMR
- Relative Importance Of Descriptors: 1.00000: logS, 0.891506: SMR
- Inter correlation 0.4460

For *P aeruginosa*

- Root mean square error (rmse): 0.11584
- Correlation Coefficient (R2): 0.67961
- pMIC *P. aeruginosa* =0.36077+0.00449 *

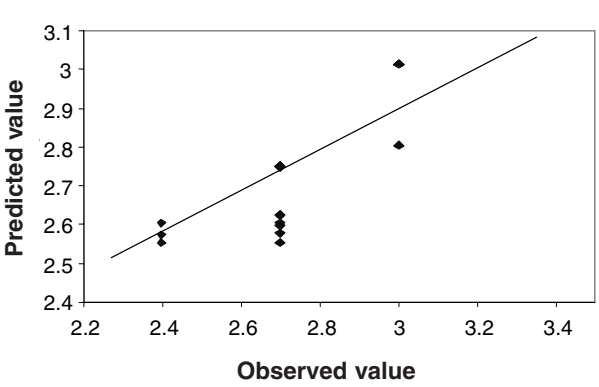


Fig. 1: Plot of observed activity Vs predicted activity against *S.aureus*

Table 3: showing the observed activity and predicted activity (pMIC)

	S. aureus			*P. aeruginosa*		
Compound	**Observed**	**Predicted**	**Residuals**	**Observed**	**Predicted**	**Residuals**
P1	2.6990	2.5969	0.1061	2.6990	2.5980	0.1010
P2	2.6990	2.6242	0.0748	3.000	2.7499	0.2501
P3	2.6990	2.5767	0.1192	2.6990	2.6997	0.0293
P4	2.3979	2.5729	-0.1750	2.3979	2.5626	-1646
P5	2.3979	2.6023	-0.2043	2.3979	2.4780	-0.0801
P6	2.6990	2.6023	0.0967	3.000	2.8148	0.1852
P7	3.000	2.8045	0.1955	3.000	2.9078	0.0922
P8	2.3979	2.5529	-0.1550	2.6990	2.7685	-0.0695
P9	2.6990	2.5529	0.1460	2.6990	2.6956	0.0034
P10	3.000	3.0161	-0.0161	3.000	3.0916	-0.0916
P11	3.000	3.0161	-0.0161	3.000	3.0758	-0.0758
P12	3.000	3.0161	-0.0161	3.000	2.9360	0.0640
P13	2.6990	2.7509	-0.0519	2.6990	2.7365	-0.0375
P14	2.6990	2.7509	-0.0519	2.6990	2.7448	-0.0494
P15	2.6990	2.7509	-0.0519	2.6990	2.8555	-.0.1565

Calculated by MOE2006.08

ASA-0.49024 * dipoleX

- Relative Importance Of Descriptors: 0.888260: ASA, 1.000000: dipoleX
- Intercorrelation 0.05

The QSAR parameters and the results of calculated biological activity and their residuals are shown in Table 3

RESULTS

The synthesized compounds were screened for antibacterial activity by serial dilution method, compared with ciprofloxacin. All the compounds produced good activity and the compounds P4and P5 were found to be most active. All the other compounds showed good activity. The QSAR studies show that the antibacterial activity is dependant on the electronic and lipophillic parameters. In case of *S. aureus,* liphophillic factor log solubility showed positive correlation while steric parameter Molar Refractivity showed positive correlation with biological activity. The QSAR equations reveal that decrease in log solubility and Molar Refractivity lead to increase activity. In case of *P. aeruginosa* The Dipole moment along X-axis showed negative correlation while liphophillic factor i.e. water assessable surface area showed positive correlation with biological activity. The QSAR equations reveal that increase in dipole moment will lead to increase activity. The dependence of activity on water assessable surface area shows that the penetration of the molecules inside the microorganism is important for the activity and that decrease in would water assessable surface area show increase in activity. Keeping in this mind we designed two lead molecules, 4-methyl-2-phenyl-1H 1,5 benzodiazepine and 2-phenyl-1H 1,5

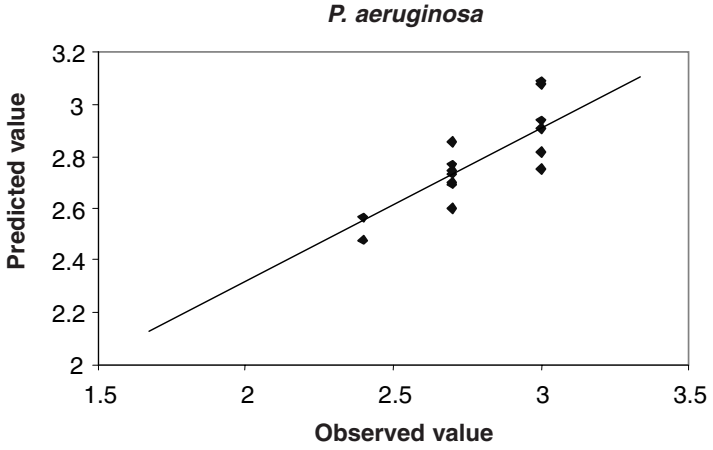


Fig. 2: Plot of observed activity Vs predicted activity against *P. aeruginosa*

benzodiazepine where in we produce sufficient increase in the lipophilic character. These molecules produced improved antimicrobial activity. Thus the QSAR model generated could be successfully used to improve antimicrobial activity.

DISCUSSION

Results of antimicrobial activity of all the synthesized diazepine derivatives indicate that derivatives with substituents at 4–position are relatively more active than those with substituents at 3-position. 4-(4-chlorophenyl)-2-phenyl-1H-1,5-benzodiazepines and 4-(4-chlorophenyl)-2-phenyl-1H-1,5-benzodiazepines have produced good antimicrobial activity which is comparable to that of ciprofloxacin. Specifically 4'-(4 phenyl) substituted benzodiazepine ring produced maximum antimicrobial activity. Hence further quantitative study of steric and lipophilic contributions of various substituents on 4 position of benzodiazepine ring may yield potentially useful compounds. Keeping in this mind we designed two lead molecules, 4-methyl-2-phenyl-1H 1,5 benzodiazepine and 2-phenyl-1H

1,5-benzodiazepine where in we produce sufficient increase in the liphophilic character. These molecules produced improved antimicrobial activity. Thus the QSAR model generated could be successfully used to improve antimicrobial activity.

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