INTERNATIONAL JOURNAL OF PHARMACEUTICAL, CHEMICAL AND BIOLOGICAL SCIENCES

Available online at www.ijpcbs.com

Research Article

SYNTHESIS, SCREENING AND QSAR ANALYSIS OF CHALCONE DERIVATIVES AS POTENTIAL ANTI BACTERIAL AGENTS

Narendra Velagapudi[1]*, Nisha Sharma[2] and Raviteja Bandla[3]

[1]Departmrent of Pharmacy, N.R.I.College of Pharmacy, Vijayawada, Andhra Pradesh, India.
[2]C.S.J.M.University, Kanpur, Uttar Pradesh, India.
[3] M.A.M.College of Pharmacy, Narasaraopet, Andhra Pradesh, India.

ABSTRACT

Chalcones are important starting materials for the synthesis of varies heterocyclic compounds. Most of them are widely used in pharmaceuticals. Keeping this in mind new chalcones are synthesized by conventional method and the structures were confirmed by spectral evidence. Synthesized compounds were screened for their antibacterial activity the molecules were screened for their structural activity relationships by atom based 3D QSAR studies.

Keywords: Chalcones, QSAR, antibacterial activity.

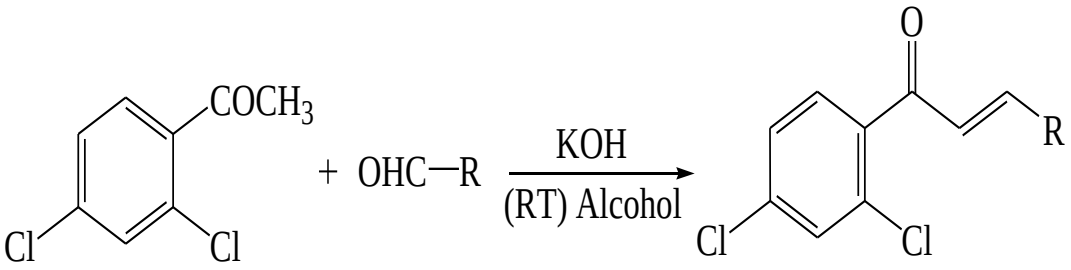
INTRODUCTION

Chalcones, a group of compounds with two aromatic rings connected by a keto-vinyl chain, constitute an important class of naturally occurring flavonoids exhibiting a wide spectrum of biological activities. The presence of a reactive α,β-unsaturated keto functional group is partly responsible for their activity.

General procedure for the synthesis of chalcones

A mixture of 2,4-Dichloroacetophenone (0.001 mole) and the appropriate aryl aldehyde (0.001 mole) was stirred in ethanol (7.5 mL) and to it aqueous solution of KOH (50%, 7.5 mL) was added. The mixture was kept for 24 h and it was acidified with 1:1 mixture of hydrochloric acid and water, then it was filtered under vacuum and the product was washed with water. Characterization of chalcones were given in table1-3.

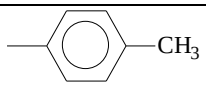
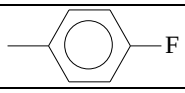
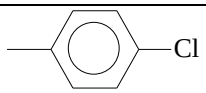
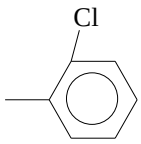
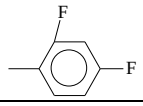
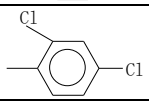
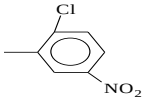
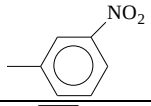
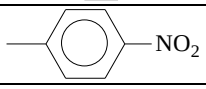
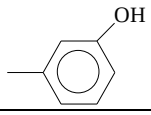
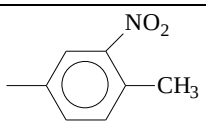
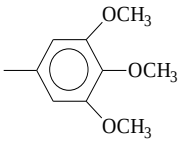
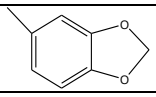
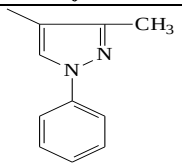
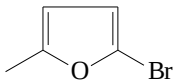
General scheme of reaction



2,4-Dichloroacetophenone Aromatic/ Heterocyclic aldehyde

Chalcone derivative

Table 1: Physical characterization data of chalcones (B_1-B_{25})

Compound	R	Molecular Formula	Relative Molecular Mass (RMM)	Melting Point (°C)	Yield %
B_1		$C_{16}H_{12}Cl_2O$	291	134-137	89
B_2		$C_{15}H_9FCl_2O$	294	87-90	86
B_3		$C_{15}H_9Cl_3O$	310	121-124	76
B_4		$C_{15}H_9Cl_3O$	310	130-133	82
B_5		$C_{15}H_8F_2Cl_2O$	312	110-113	73
B_6		$C_{15}H_8Cl_4O$	344	93-96	86
B_7		$C_{15}H_8Cl_3NO_3$	356	131-134	77
B_8		$C_{15}H_9F_2NO_3$	322	114-117	83
B_9		$C_{15}H_9Cl_2NO_3$	322	122-125	82
B_{10}		$C_{15}H_{10}Cl_2O_2$	293	132-135	92
B_{11}		$C_{16}H_{11}Cl_2NO_3$	336	126-129	81
B_{12}		$C_{18}H_{16}Cl_2O_4$	367	110-111	82
B_{13}		$C_{16}H_{10}Cl_2O_3$	321	148-151	72
B_{14}		$C_{19}H_{14}Cl_2N_2O$	357	112-115	73
B_{15}		$C_{13}H_7Cl_2BrO_2$	346	126-129	62

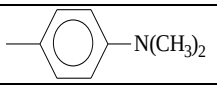
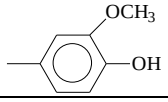
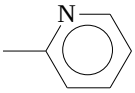
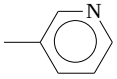
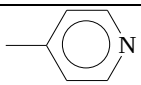
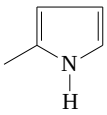
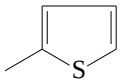
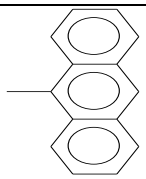
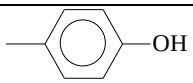
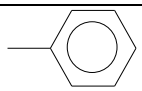
B_{16}		$C_{17}H_{15}Cl_2NO$	320	152-155	81
B_{17}		$C_{16}H_{12}Cl_2O_3$	323	99-102	85
B_{18}		$C_{14}H_9Cl_2NO$	278	91-94	84
B_{19}		$C_{14}H_9Cl_2NO$	278	78-81	86
B_{20}		$C_{14}H_9Cl_2NO$	278	96-99	78
B_{21}		$C_{13}H_9Cl_2NO$	266	101-104	69
B_{22}		$C_{13}H_8Cl_2OS$	283	106-109	79
B_{23}		$C_{23}H_{14}Cl_2O$	377	108-111	76
B_{24}		$C_{15}H_{10}Cl_2O_2$	293	91-94	77
B_{25}		$C_{15}H_{10}Cl_2O$	277	66-69	82

Table 2: IR (KBr disc) spectral data of chalcones

Compound	Position of absoption band (cm^{-1})
B_1	1655 (C=O), 1602 (C=C of Ar), 1505(CH=CH), 825 (C-Cl)
B_2	1664 (C=O), 1580 (C=C of Ar), 1524 (CH=CH), 828 (C-Cl)
B_3	1653 (C=O), 1585 (C=C of Ar), 1505 (CH=CH), 835 (C-Cl)
B_4	1652 (C=O), 1583 (C=C of Ar), 1502 (CH=CH), 833 (C-Cl)
B_5	1655 (C=O), 1581 (C=C of Ar), 1510 (CH=CH), 825 (C-Cl), 926 (C-F)
B_6	1663 (C=O), 1578 (C=C of Ar), 1506 (CH=CH), 833 (C-Cl)
B_7	1658 (C=O), 1603 (C=C of Ar), 1515 (CH=CH), 824 (C-Cl),1525 (N=O, asymmetric), 1348 (N=O, symmetric)
B_8	1655 (C=O), 1605 (C=C of Ar), 1508 (CH=CH), 1533 (N=O, asymmetric), 1345 (N=O, symmetric), 829 (C-Cl)
B_9	1652 (C=O), 1610 (C=C of Ar), 1502 (CH=CH), 1541 (N=O, asymmetric), 1346 (N=O, symmetric), 823 (C-Cl)
B_{10}	3520 (O-H), 1648 (C=O), 1612 (C=C of Ar), 1505 (CH=CH), 823 (C-Cl)
B_{11}	1655 (C=O), 1605 (C=C of Ar), 1500 (CH=CH), 1545 (N=O, asymmetric), 1343 (N=O, symmetric), 822 (C-Cl)
B_{12}	1652 (C=O), 1585 (C=C of Ar), 1462 (CH=CH), 1127 (-O-CH_3), 827 (C-Cl)
B_{13}	1643 (C=O), 1574 (C=C of Ar), 1500 (CH=CH), 1240 (O-CH_2-O), 829 (C-Cl)
B_{14}	1663 (C=O), 1610 (C=N), 1588 (C=C of Ar), 1510 (CH=CH), 1391 (C-N), 821 (C-Cl)
B_{15}	1652 (C=O), 1585 (C=C of Ar), 1503 (CH=CH), 829 (C-Cl)
B_{16}	1650 (C=O), 1586 (C=C of Ar),1505 (CH=CH), 1178 (-N(CH_3)$_2$), 821 (C-Cl)
B_{17}	3450 (O-H), 1648 (C=O), 1606 (C=C of Ar), 1510 (CH=CH), 1225 (-OCH_3), 825 (C-Cl)
B_{18}	1653 (C=O), 1605 (C=C of Ar), 1595 (C=N), 1508 (CH=CH), 1385 (C-N), 822 (C-Cl)
B_{19}	1645 (C=O), 1603 (C=C of Ar), 1590 (C=N), 1502 (CH=CH), 1370 (C-N), 923 (C-Cl)
B_{20}	1650 (C=O), 1605 (C=C of Ar), 1581 (C=N), 1505 (CH=CH), 1373 (C-N), 829 (C-Cl)
B_{21}	1652 (C=O), 1605 (C=C of Ar), 1588 (C=N), 1506 (CH=CH), 1375 (C-N), 821 (C-Cl)
B_{22}	1655 (C=O), 1610 (C=C of Ar), 1505 (CH=CH), 624 (C-S), 823 (C-Cl)
B_{23}	1658 (C=O), 1605 (C=C of Ar), 1503 (CH=CH), 823 (C-Cl)
B_{24}	3460 (O-H), 1648 (C=O), 1606 (C=C of Ar), 1505 (CH=CH), 824 (C-Cl)
B_{25}	1650 (C=O), 1605 (C=C of Ar), 1502 (CH=CH), 829 (C-Cl)

Table 3: ^{1}H NMR spectral data of chalcones

Compound	Chemical shift (δ) in ppm
B_1	2.40 (3H, s, Ar-CH_3), 7.23 (1H, d,*J* = 17 Hz, -CO-CH=), 7.73 (1H, d, *J* =17 Hz, =CH-Ar), 7.20-7.78 (7H, Ar-H)
B_2	7.15 (1H, d, *J* = 17 Hz, -CO-CH=), 7.62 (1H, d, *J* =17 Hz, =CH-Ar), 7.05-7.71 (7H, Ar-H)
B_3	7.45 (1H, d, *J* = 17 Hz, -CO-CH=), 7.82 (1H, d, *J* =17 Hz, =CH-Ar), 7.38-8.20 (7H, Ar-H)
B_4	7.43 (1H, d, *J* = 17 Hz, -CO-CH=), 7.80 (1H, d, *J* =17 Hz, =CH-Ar), 7.36-8.21 (7H, Ar-H)
B_5	7.40 (1H, d, *J* = 17 Hz, -CO-CH=), 7.73 (1H, d, *J* =17 Hz, =CH-Ar), 7.15-8.10 (6H, Ar-H)
B_6	7.68 (1H, d, *J* = 17 Hz, -CO-CH=), 7.85 (1H, d, *J* =17 Hz, =CH-Ar), 7.42-8.20 (6H, Ar-H)
B_7	7.49 (1H, d, *J* = 17 Hz, -CO-CH=), 7.65 (1H, d, *J* =17 Hz, =CH-Ar), 7.12-8.60 (6H, Ar-H)
B_8	7.40 (1H, d, *J* = 17 Hz, -CO-CH=), 7.62 (1H, d, *J* =17 Hz, =CH-Ar), 7.20-8.55 (7H, Ar-H)
B_9	7.43 (1H, d, *J* = 17 Hz, -CO-CH=), 7.68 (1H, d, *J* =17 Hz, =CH-Ar), 7.21-8.59 (7H, Ar-H)
B_{10}	7.38 (1H, d, *J* = 17 Hz, -CO-CH=), 7.52 (1H, d, *J* =17 Hz, =CH-Ar), 6.89 (1H, s, Ar-OH), 7.18-7.79 (7H, Ar-H)
B_{11}	2.50 (3H. s, Ar-CH_3), 7.40 (1H, d, *J* = 17 Hz, -CO-CH=), 7.65 (1H, d, *J* =17 Hz, =CH-Ar),7.15-8.53 (6H, Ar-H)
B_{12}	7.15 (1H, d, *J* = 17 Hz, -CO-CH=), 7.64 (1H, d, *J* =17 Hz, =CH-Ar), 7.12-7.58 (5H, Ar-H), 3.78 (3H,s,Ar-OCH_3), 3.88 (6H,s,2x Ar-OCH_3)
B_{13}	6.10 (2H,s,-O-CH_2O-), 6.88 (1H, d, *J* = 17 Hz, -CO-CH=), 7.69 (1H, d, *J* =17 Hz, =CH-Ar), 7.10-7.29 (6H, Ar-H)
B_{14}	2.45 (3H, s, Ar-CH_3), 6.85 (1H, d, *J* = 17 Hz, -CO-CH=), 7.65 (1H, d, *J* =17 Hz, =CH-Ar), 6.58-7.90 (8H, Ar-H)
B_{15}	7.23 (1H, d, *J* = 17 Hz, -CO-CH=), 7.71 (1H, d, *J* =17 Hz, =CH-Ar), 7.18-7.95 (5H, Ar-H)
B_{16}	3.10 (6H,s,-$N(CH_3)_2$, 6.88 (1H, d, *J* = 17 Hz, -CO-CH=), 7.75 (1H, d, *J* =17 Hz, =CH-Ar), 6.65-7.90 (7H, Ar-H)
B_{17}	7.21 (1H, d, *J* = 17 Hz, -CO-CH=), 7.68 (1H, d, *J* =17 Hz, =CH-Ar), 7.20-7.93 (6H, Ar-H), 6.75 (1H.s, Ar-OH), 3.82 (3H,s,Ar-OCH_3)
B_{18}	7.15 (1H, d, *J* = 17 Hz, -CO-CH=), 7.65 (1H, d, *J* =17 Hz, =CH-Ar), 6.30-8.15 (7H, Ar-H)
B_{19}	7.18 (1H, d, *J* = 17 Hz, -CO-CH=), 7.70 (1H, d, *J* =17 Hz, =CH-Ar), 7.12-8.20 (7H, Ar-H)
B_{20}	7.15 (1H, d, *J* = 17 Hz, -CO-CH=), 7.75 (1H, d, *J* =17 Hz, =CH-Ar), 7.20-8.15 (7H, Ar-H)
B_{21}	7.10 (1H, d, *J* = 17 Hz, -CO-CH=), 7.70 (1H, d, *J* =17 Hz, =CH-Ar), 6.35-7.90 (7H, Ar-H)
B_{22}	7.12 (1H, d, *J* = 17 Hz, -CO-CH=), 7.70 (1H, d, *J* =17 Hz, =CH-Ar), 6.62-8.10 (6H, Ar-H)
B_{23}	7.35 (1H, d, *J* = 17 Hz, -CO-CH=), 7.60 (1H, d, *J* =17 Hz, =CH-Ar), 7.20-8.90 (12H, Ar-H)
B_{24}	7.28 (1H, d, *J* = 17 Hz, -CO-CH=), 7.59 (1H, d, *J* =17 Hz, =CH-Ar), 6.85 (1H,s,Ar-OH),7.21-7.89 (7H, Ar-H)
B_{25}	7.21 (1H, d, *J* = 17 Hz, -CO-CH=), 7.62 (1H, d, *J* =17 Hz, =CH-Ar), 7.11-7.90 (8H, Ar-H)

BIOLOGICAL EVALUATION

Antibacterial activity

The antibacterial activity of the chalcones (**B_1** to **B_{25}**) was assessed by determining the MIC, which is defined as the lowest concentration of the compound that completely inhibited the growth of each strain after overnight incubation. MIC was determined using serial tube dilution technique. In this technique the tubes of broth medium containing graded doses of compounds were inoculated with the test organisms. After suitable incubation, growth occurred in those tubes where the concentration of the compound was below the inhibitory level and the culture become turbid. No growth was noticed above the inhibitory level and the tubes remained clear results were given in table-4.

Table 4: Antibacterial activity of chalcones (compounds B_1 to B_{12}):(Expressed as MIC in µg/mL)

Compound	R	*B.subtilis*	*S.aureus*	*E.coli*	*P.vulgaris*
B_1	4"-methylphenyl	128	128	64	64
B_2	4"-fluorophenyl	64	128	64	128
B_3	4"-chlorophenyl	64	128	128	64
B_4	2"-chlorophenyl	64	128	128	64
B_5	2",4"-difluorophenyl	32	64	32	32
B_6	2",4-dichlorophenyl	64	64	32	128
B_7	2"-chloro-5"-nitrophenyl	32	128	128	128
B_8	3"-nitrophenyl	128	256	128	256
B_9	4"-nitrophenyl	128	256	128	128
B_{10}	3"-hydroxyphenyl	256	256	128	256
B_{11}	3"-nitro-4"-methylphenyl	128	64	128	128
B_{12}	3",4",5"-trimethoxyphenyl	64	64	64	32
B_{13}	3",4"-methylendioxyphenyl	256	128	256	128
B_{14}	1"-phenyl-3"methylpyrazole-4"-yl	128	128	128	256
B_{15}	5"-bromofuran-2"-yl	64	64	32	128
B_{16}	4"-dimethylaminophenyl	64	128	64	64
B_{17}	3"-methoxy-4"-hydroxyphenyl	128	128	128	128
B_{18}	2"-pyridinyl	128	256	128	256
B_{19}	3"-pyridinyl	128	256	256	256
B_{20}	4"-pyridinyl	128	128	128	128
B_{21}	2"-pyrrolyl	256	256	64	64

B_{22}	2"-thienyl	128	64	128	128
B_{23}	9"-anthracenyl	256	128	128	256
B_{24}	4"-hydroxyphenyl	264	128	64	64
B_{25}	Phenyl	256	256	256	256
Standard (Ampicillin)		< 1	< 1	< 1	< 1

RESULTS AND DISCUSSION

From the above results it is evident that all the chalcones synthesized, showed antibacterial activity with different MIC values against the tested organisms, but not comparable with that of the standard. Among the compounds tested, B_5 with difluorophenyl moiety was found to be the most potent against *B.subtilis*, *E.coli* and *P.vulgaris* having a MIC value of 32 µg/mL in each case. The chalcones, B_6 having a dichlorophenyl substitution, B_7 having 2-chloro-5-nitrophenyl substitution and B_{15} having bromofuran substitution were also found to be equipotent with a MIC value of 32 µg/mL against *E.coli*, *B.subtilis* and *E.coli* respectively.

Atom based 3D-QSAR model for antibacterial activity of chalcones against *B.subtilis*

In atom based 3D-QSAR analysis of chalcones, the Correlation Coefficient (R^2) = 0.7922, Cross validation Coefficient (Q^2) = 0.4647 and Standard Deviation (S.D) = 0.1406 were established. From the it was found that the aromatic ring substitution with hydrogen bond donor or electron withdrawing group or hydrophobic group and a conjugated carbonyl system essential for increasing the antibacterial activity, , as such regions showed blue cubes characteristic of positive effect on the antibacterial activity. Results of the statistical analysis are shown in the following tables and figures.

Table 5: Experimental and predicted MIC (µg/mL) values of training set and test set molecules based on atom based 3D-QSAR model (Antibacterial activity)

Compound code	*B.subtilis* MIC(µg/mL)	Experimental -log(MIC)	Predicted -log(MIC) (Training set)	Predicted -log(MIC) (Test set)
B_1	128	-2.10721	-1.99786	
B_2	64	-1.80618		-1.8522
B_3	64	-1.80618	-1.78244	
B_4	64	-1.80618	-1.83539	
B_5	32	-1.50515	-1.77385	
B_6	64	-1.80618		-1.61438
B_7	32	-1.50515	-1.4236	
B_8	128	-2.10721	-2.13336	
B_9	128	-2.10721	-2.08065	
B_{10}	256	-2.40824	-2.43568	
B_{11}	128	-2.10721		-2.11693
B_{12}	64	-1.80618	-1.84143	
B_{13}	256	-2.40824		-2.24238
B_{14}	128	-2.10721	-2.20928	
B_{15}	64	-1.80618	-1.87677	
B_{16}	64	-1.80618	-1.79833	
B_{17}	128	-2.10721	-2.18291	
B_{18}	128	-2.10721	-2.16989	
B_{19}	128	-2.10721	-2.19334	
B_{20}	128	-2.10721	-2.123	
B_{21}	256	-2.40824	-2.33018	
B_{22}	128	-2.10721	-2.0912	
B_{23}	256	-2.40824	-2.34953	
B_{24}	264	-2.4216		-2.05839
B_{25}	256	-2.40824	-2.01035	

Table 6: Summary of atom based 3D QSAR results

PLS Factors	SD	R^2	F	P	RMSE	Q-squared	Pearson-R
4	0.1406	0.7922	14.3	5.28e-05	0.2	0.4647	0.8391

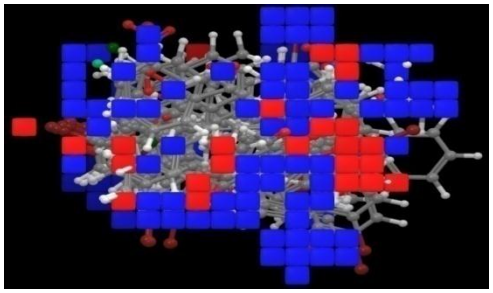


Fig. 1: Atom based 3D-QSAR Model of chalcones along with alignment of structures (Blue cubes indicate favorable regions while red cubes indicate unfavorable region for the activity) against *B.subtilis.*

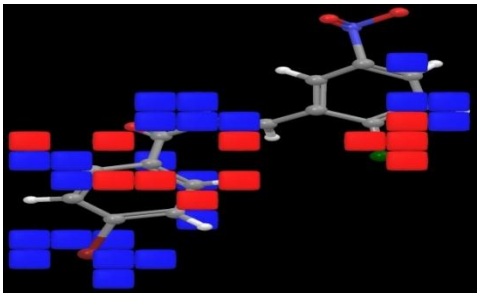


Fig. 2: Atom based 3D QSAR model visualized in the context of highest active compound B_7 against *B.subtilis*.

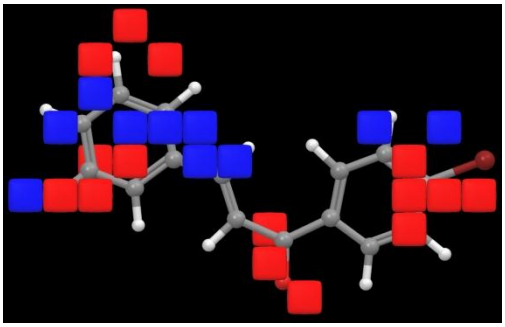


Fig. 3: Atom based 3D QSAR model visualized in the context of lowest active compound B_{25} against *B.subtilis*.

Atom based 3D-QSAR model for antibacterial activity of chalcones against *S.aureus*

In atom based 3D-QSAR analysis of chalcones, the Correlation Coefficient (R^2) = 0.9031, Cross validation Coefficient (Q^2) = 0.4858 and Standard Deviation (S.D) = 0.0765 (Table35) were established. From the results shown in figures. it was found that the aromatic ring substitution with hydrogen bond donor or electron withdrawing group or hydrophobic group and a conjugated carbonyl system essential for increasing the antibacterial activity, as such regions showed blue cubes characteristic of positive effect on the antibacterial activity. Results of the statistical analysis are shown in the following tables and figures.

Table 7: Experimental and predicted MIC (µg/mL) values of training set and test set molecules based on atom based 3D-QSAR model (Antibacterial activity)

Compound code	*S.aureus* MIC(µg/mL)	Experimental -log(MIC)	Predicted -log(MIC) (Training set)	Predicted -log(MIC) (Test set)
B_1	128	-2.10721	-2.03134	
B_2	128	-2.10721	-2.02153	
B_3	128	-2.10721	-2.03373	
B_4	128	-2.10721	-2.03405	
B_5	64	-1.80618	-1.97364	
B_6	64	-1.80618		-1.94537
B_7	128	-2.10721		-2.10334
B_8	256	-2.40824	-2.49357	
B_9	256	-2.40824	-2.38831	
B_{10}	256	-2.40824		-2.25866
B_{11}	64	-1.80618	-1.85607	
B_{12}	64	-1.80618	-1.80874	
B_{13}	128	-2.10721	-2.12386	
B_{14}	128	-2.10721	-2.09377	
B_{15}	64	-1.80618	-1.92587	
B_{16}	128	-2.10721	-2.06055	
B_{17}	128	-2.10721		-2.03624
B_{18}	256	-2.40824	-2.39162	
B_{19}	256	-2.40824	-2.37156	
B_{20}	128	-2.10721	-2.15377	
B_{21}	256	-2.40824	-2.38607	
B_{22}	64	-1.80618	-1.74819	
B_{23}	128	-2.10721	-2.1409	
B_{24}	128	-2.10721	-2.10705	
B_{25}	256	-2.40824		-2.11896

Table 8: Summary of atom based 3D QSAR results

PLS Factors	SD	R^2	F	P	RMSE	Q-squared	Pearson-R
4	0.0765	0.9031	35	1.94e-07	0.16	0.4858	0.8799

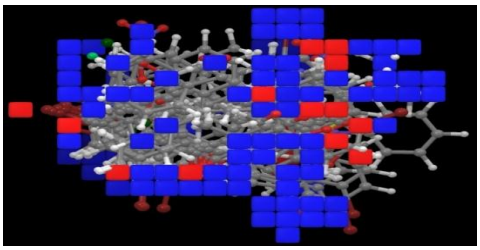


Fig. 4: Atom based 3D-QSAR Model of chalcones along with alignment of structures (Blue cubes indicate favorable regions while red cubes indicate unfavorable region for the activity) against *S.aureus.*

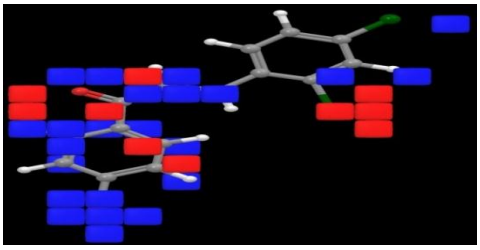


Fig. 5: Atom based 3D QSAR model visualized in the context of highest active compound B_6 against *S.aureus*.

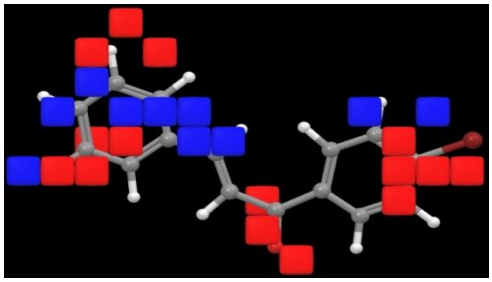


Fig. 6: Atom based 3D QSAR model visualized in the context of lowest active compound B_{25} against *S.aureus*.

DISCUSSION

The structure-activity relationship study based on the above results clearly indicated the importance of electron withdrawing groups in enhancing the antibacterial activity. When more than one such group present on the phenyl ring, a cumulative effect was observed as seen in the case of B_5 and B_6 having difluoro and dichloro substitution respectively. However, compounds with electron releasing substituents as seen in the case of B_{12} and B_{16} also enhanced the activity. Compounds with more number of electron releasing or electron with drawing substituents on the aromatic or heteroaromatic ring at different positions can be synthesized to draw meaningful conclusions with respect to the influence of electronic effects on the antimicrobial activity.

REFERENCES

1. Maayan S, Ohad N and Soliman K. Bioorg Med Chem. 2005;13:433.
2. Wilson CW. J Asian chem Soc. 1938;61:2303.
3. Algar J and Flynn JP. Proc Roy Irish Acad. 1937;42B:1.
4. Claisen L and Claparede A Ber. 1881;14:2463.
5. Van Kostanekki ST and Szabranki W Ber. 1904;37:2634.
6. Datta SC, Murthi VVS and Seshadri TR. Indian JChem. 1971;9:614.
7. Reichel L and Muller K Ber. 1941;74:1741.
8. Shindo J and Sato S. JPharm Soc Japan. 1928;48:791.
9. Mabry TJ, Markham KR and Thomas MB. in : The systematic identification of flavonoids, Springer-Verlag, New York. 1970;227.
10. Hegert HL and Kurth EF. J Am Chem Soc. 1953;75:1622.
11. Dhar DN and Gupta VN. Indian J Chem. 1971;9:818.
12. Mabry TJ, Markham KR and Thomas MB. in : The systematic identification of flavonoids, Springer-Verlag, New York. 1970;267.
13. Pelter A, Ward RS and Ian Greg T. J Chem Soc. Perkin Trans. 1976;1:2475.
14. Sthothers JB. in: ^{13}C NMR Spectroscopy, Academic Press, New York, 1972.
15. Hu N, Tu YP, Liu Y, Jiang K and Pan Y. J Org Chem. 2008;73:3369.
16. Royane J, Williams DH and Bowie JH. J Amer Chem Soc. 1966;88:4980.
17. Van de Sande C, Serum JW and Vandervalle M. Org Mass Spectrometry. 1972;6:1333.
18. Grutzmacher HF. Org Mass Spectrom. 1993;28:1375.
19. Tai Y, Pei S, Wan J, Cao X and Pan Y. Rapid commun Mass spectrom. 2006;20:994.
20. Debattista NB, Devia CM and Pappano NB. Rev Micrebiol. 1998;29:307.
21. Kromann H, Nielsen SF, Boesen T, Larsen M and Schonning K. Bioorg Med Chem. 2004;12:3047.