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Original article

Novel Biginelli dihydropyrimidines with potential anticancer activity: A parallel synthesis and CoMSIA study

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ABSTRACT

Novel Biginelli dihydropyrimidines of biological interest were prepared using *p*-toluene sulphonic acid as an efficient catalyst. All the thirty-two synthesised dihydropyrimidines were evaluated for their *in vitro* antioxidant activity using DPPH method. Only, compounds **28** and **29** exhibited reasonably good antioxidant activity. Furthermore, the synthesised Biginelli compounds were subjected for their *in vitro* anticancer activity against MCF-7 human breast cancer cells. The title compounds were tested at the concentration of 10 μg. Compounds exhibited activity ranging from weak to moderate and, from moderate to high in terms of percentage cytotoxicity. Among them, compounds **10** and **11** exhibited significant anticancer activity. In order to elucidate the three-dimensional structure–activity relationships (3D QSAR) towards their anticancer activity, we subjected them for comparative molecular similarity indices analysis (CoMSIA). Illustration regarding their synthesis, analysis, antioxidant activity, anticancer activity and 3D QSAR study is described.

1. Introduction

Drug discovery and development is a very laborious and costly process involving synthesis and screening of diverse organic compounds. In this regard, multicomponent reactions (MCRs) are of increasing importance in the field of medicinal chemistry [1–5]. Currently, attention is put on speed, diversity, and efficiency in the drug discovery process [6]. MCRs can provide products with the diversity needed for the discovery of new lead compounds or lead optimization employing combinatorial chemistry techniques. The search and discovery for new MCRs on one hand [7], and the full exploitation of already known multicomponent reactions on the other hand, are therefore of considerable current interest.

In 1893, Pietro Biginelli has reported on the acid-catalyzed cyclocondensation reaction of ethylacetoacetate, benzaldehyde and urea. The reaction was carried out by simply heating a mixture of the three components dissolved in ethanol with a catalytic amount of HCl at reflux temperature. The product of this novel one-pot, three-component synthesis that precipitated on cooling the reaction mixture was identified correctly by Biginelli as dihydropyrimidin-2-one [8]. The scope of this reaction was gradually extended by the variation of all three building blocks, allowing access to a large number of multifunctionalized dihydropyrimidines of medicinal use [9–12]. Dihydropyrimidines show a diverse range of biological activities. They are known to possess activities such as antibacterial [13] and antiviral (nitractin) [14], antitumor [15–18], analgesic and anti-inflammatory [19], antiplatelet aggregation [20], and antihypertensive activity [21]. Thus development of methodologies for efficient lead structure identification and for pharmacophore variation of dihydropyrimidine motif has always attracted the attention of pharmaceutical industry [22].

Herein, we report a simple protocol for the parallel synthesis of some novel Biginelli dihydropyrimidines using appropriate building blocks and acid catalyst. Also we present anticancer, antioxidant activities of the title compounds along with 3D QSAR study to establish the structure–activity relationships towards their anticancer activity.

2. Results and discussion

2.1. Chemistry

Our strategy for the syntheses of title compounds was designing the suitable and appropriate library. Hence, we identified appropriate building blocks, synthesised and later subjected for Biginelli condensation reaction. *o*-Chloroacetoacetanilide (**1**) was synthesised by refluxing equimolar amounts of *o*-chloroaniline and

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ethylacetoacetate under solvent free conditions according to Scheme 1 [23,24].

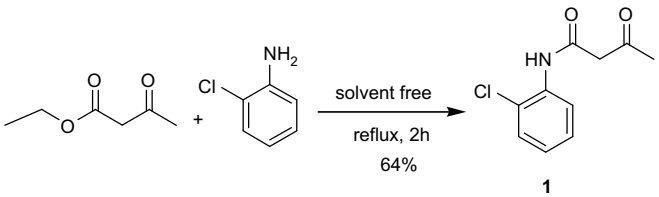
The parallel synthesis of 1,4-dihydropyrimidines was carried out by multicomponent-cyclisation reaction of equimolar amounts of aryl or heteryl aldehyde, *o*-chloroacetoacetanilide or *N*-methyl-acetoacetamide or cyanoguanidine and excess of urea or thiourea in the presence of catalytic amounts of *p*-toluene sulphonic acid (PTSA) in ethanol (Scheme 2). Some of the reaction mixtures needed reflux for 24 h for the synthesis of **2–5**, **8–11**, **18–21** and **33** 1,4-dihydropyrimidines. Whereas, all other 1,4-dihydropyrimidines are obtained just by simple stirring at room temperature for 24–30 h. In all the cases reaction went smoothly to give the corresponding 1,4-dihydropyrimidines' **2–32** (67–81%) in moderate to good yields. The compound **33** was the only compound synthesised using cyanoguanidine and obtained in a very poor yield of 12%. We were unable to improve the yield of **33** even after several trials by varying the acid catalysts. Hence, we could not extend this methodology for the parallel synthesis of 1,4-dihydropyrimidines derivatives using cyanoguanidine. The Biginelli 1,4-dihydropyrimidines **2–33** were synthesised relatively easy using PTSA as an efficient catalyst and it does not demand any anhydrous conditions unlike the other Lewis acid catalysts. The compounds **2–33** were prepared using a simple distilled ethanol without further drying. Alternatively, for those title compounds which required reflux were synthesised by microwave (MW) irradiation technique. By MW method the reaction time was reduced to 30–40 min. However, the yields were found to be similar to that of conventional method. One more advantage of MW method over the conventional method is it's comparatively easy to perform the parallel synthesis. All the synthesised Biginelli dihydropyrimidine compounds are novel and not reported elsewhere. All the synthesised compounds showed satisfactory analytical results. The characteristic peak for symmetric CH proton in HNMR spectrum between 5 and 6 δ ppm, confirmed the formation of dihydropyrimidines.

2.2. In vitro antioxidant activity

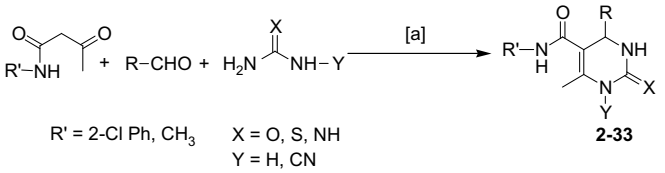
The synthesised compounds were screened for their possible antioxidant activity by DPPH (2,2-diphenyl-1-picrylhydrazyl) method [25]. Among the thirty-two title compounds, compounds **28** and **29** with 3-nitro phenyl moiety at the fourth position of dihydropyrimidine showed good antioxidant activity with IC_{50} value of 58 and 63 μg concentrations, respectively. The remaining thirty compounds failed to exhibit the antioxidant activity when tested, primarily, at 100 μg concentrations.

2.3. In vitro anticancer activity

Furthermore, the compounds were subjected for *in vitro* anticancer screening against MCF-7 breast cancer cells using trypan blue dye exclusion tests [26,27]. The motive for us to check the anticancer activity of the synthesised compounds was, some reports that have claimed significant anticancer activity for some of the 1,4-dihydropyrimidines [15–18]. The obtained % cytotoxicity



Scheme 1. Synthesis of *o*-chloroacetoacetanilide.



Scheme 2. Reagents and conditions for the parallel synthesis of Biginelli dihydropyrimidines: (a) PTSA, C_2H_5OH, stirred for 24–30 h at room temperature and some of the title compounds which require heating were synthesised by refluxing reaction mixtures for 24 h or by irradiating with microwaves for 30–40 min at 500 W power.

values for the synthesised compounds when tested at the concentration of 10 μg are shown in Table 1. Among them, compounds **10** and **11** showed significant activity against MCF-7 cells with cinnamoyl moiety at the fourth position of 1,4-dihydropyrimidine ring system. Compounds **6**, **7** and **18** also exhibited potential anticancer activity having furan and pyridine moieties at the same position.

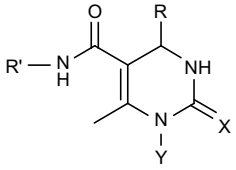
2.4. CoMSIA study

In order to establish 3D structure–activity relationships towards their anticancer activity, we have performed the CoMSIA study. The total set of compounds was initially divided randomly into two groups as a training and test set, with 26 compounds in the training set and 6 compounds in the test set. Test and training set compounds were chosen manually such that low, moderate and high activity compounds were present in approximately equal proportions in both the sets. The % cytotoxicity was converted logarithm to the base 10, as it would give numerically larger values for the active compounds than those of the inactive compounds (pACA). The training set compounds were used to develop the CoMSIA model and the test set compounds were used to validate the developed model.

For the selected or developed CoMSIA model, the cross-validated correlation coefficient (q^2) value of the training set was 0.51 with four principal components. The non-cross-validated r^2 value was 0.933 with a standard error of estimation (SEE) of 0.113 and a Fischer's covariance ratio (F) of 43.856 (significant at the 99% level). The field fit alignment of the dihydropyrimidines and the correlation between the experimental cytotoxicity and the predicted cytotoxicity (pACA) are shown in Figs. 1 and 2, respectively. The statistical results have been indicated the stability of the selected CoMSIA model. The predictive ability of the model was further validated using the external test set of 6 compounds. The test set compounds were predicted closer to their experimental values. Compound **17** was an exception to this statement because of large difference between the predicted and experimental activities. Possible reason could be the structurally closer compound i.e. compound **16** in the training set has showed approximately two folds more activity when compared to the compound **17**. The results authenticated the good prediction ability of the developed 3D-QSAR model. In the developed CoMSIA model, the contributions of steric, electrostatic, hydrophobic, hydrogen-bond acceptor and hydrogen-bond donor fields were found to be 11.7%, 34.2%, 24.7%, 20.4%, and 9.1%, respectively.

The structure–activity relationships based on the above CoMSIA contour maps are as follows. 1,4-Dihydropyrimidine scaffold is the basic requirement for the cytotoxicity, as all the compounds are superimposed and aligned on that part of the substructure (Fig. 3). The dihydropyrimidine ring with anilide portion at the fifth position seems to be the part of pharmacophore (common substructure) for this class of compounds to exhibit the anticancer

Table 1
Biological activity data and calculated values for 1,4-dihydropyrimidine derivatives.



2-33

Cpd no	R	X	% Cytotoxicity	pACA	
				Exp activity	Pred activity
2	Phenyl	S	25	1.3979	1.412
3[a]	Phenyl	O	30	1.4771	1.5839
4	4-Methoxoxyphenyl	S	47	1.6720	1.626
5	4-Methoxoxyphenyl	O	51	1.7075	1.758
6[a]	Furan-2-yl	S	76	1.8808	1.7291
7	Furan-2-yl	O	69	1.8388	1.873
8	2-Hydroxyphenyl	S	49	1.6901	1.632
9	2-Hydroxyphenyl	O	58	1.7634	1.812
10	Cinnamoyl	S	71	1.8512	1.809
11[a]	Cinnamoyl	O	79	1.8976	1.7897
12	3-Nitrophenyl	S	13	1.1139	1.149
13	3-Nitrophenyl	O	29	1.4623	1.417
14	4-Hydroxy-3-methoxyphenyl	S	45	1.6532	1.684
15	4-Hydroxy-3-methoxyphenyl	O	52	1.7160	1.674
16	4-Bromophenyl	S	18	1.25552	1.336
17[a]	4-Bromophenyl	O	07	0.8450	1.4845
18	Pyridin-4-yl	S	72	1.8573	1.748
19	Pyridin-4-yl	O	59	1.7708	1.834
20	Phenyl	S	35	1.5440	1.5440
21	4-Methoxyphenyl	S	48	1.6812	1.6812
22	Furan-2-yl	S	57	1.7558	1.7558
23	2-Hydroxyphenyl	S	45	1.6532	1.6532
24	Furan-2-yl	O	61	1.7853	1.7853
25	Phenyl	O	27	1.4313	1.4313
26	4-Methoxyphenyl	O	41	1.6127	1.6127
27	Cinnamoyl	O	47	1.6720	1.6720
28[a]	3-Nitrophenyl	S	39	1.5910	1.3998
29	3-Nitrophenyl	O	23	1.3617	1.3617
30[a]	4-Hydroxy-3-methoxyphenyl	S	41	1.6127	1.6403
31	4-Hydroxy-3-methoxy-phenyl	O	49	1.6901	1.6901
32	Pyridine-4-yl	O	64	1.8061	1.8061
33	Phenyl	NH	35	1.5440	1.5440

[a] Test set compounds; **R′** = CH_3 for compounds **20**–**32**, whereas **R′** = *o*-chloro phenyl for all the remaining compounds; **Y** = CN for compound **33**, whereas **Y** = H for compounds **1**–**32**.

activity. A large green contour at the fourth position of aldehyde substitution of 1,4-dihydropyrimidine ring system, indicates that the substitution favouring the activity. Evidence for this is compounds **10** and **11**, as they possess a lengthy cinnamoyl substitution at that position. A small yellow contour near the third position of same in steric contour map indicates that, there should not be any substitution or steric extension in that region. Evidence for this is most of the active compounds did not had substitution at that position. The blue contour over the dihydropyrimidine nucleus indicates that, electronegative atoms like nitrogen at the first and third positions are essential for the activity. Blue coloured contour over the aldehyde portion indicates that, presence of electronegative atoms like oxygen and nitrogen in heterocyclic ring enhances the activity (compounds **6**, **7**, **18**, **19** and **32**). In Fig. 4, the white contour near the fourth position of aldehyde substitution indicates that, hydrophobic groups like aromatic rings will favour the activity (compounds **10** and **11**). The cyan contour after the white indicates that, further extension with hydrophobic rings will not contribute to the activity. The orange contour near the second position of aldehyde substitution indicates that, presence of hydrogen-bond donors partially contributes to the activity (compounds **8** and **9**). The purple contour at the fourth and second positions indicates that presence of hydrogen-bond acceptors will increase the activity (**5**, **9**, **15**, **18** and **32**). The compounds with bromine at fourth position failed to show anticancer activity (compounds **16** and **17**).

3. Conclusions

We have synthesised a library of novel Biginelli compounds of biological interest. The title compounds were prepared using PTSA as an efficient catalyst. Compounds **28** and **29** showed good antioxidant activity. Compounds **10** and **11** are considered to be the candidate compounds to investigate further, as they exhibited significant anticancer activity against MCF-7 human breast cancer cells. CoMSIA, being one of the important methods of 3D QSAR elucidated the structure–activity relationships for the title compounds towards their anticancer activity. Present 3D-QSAR model can be used to design the new ligands of this class for their anticancer activity.

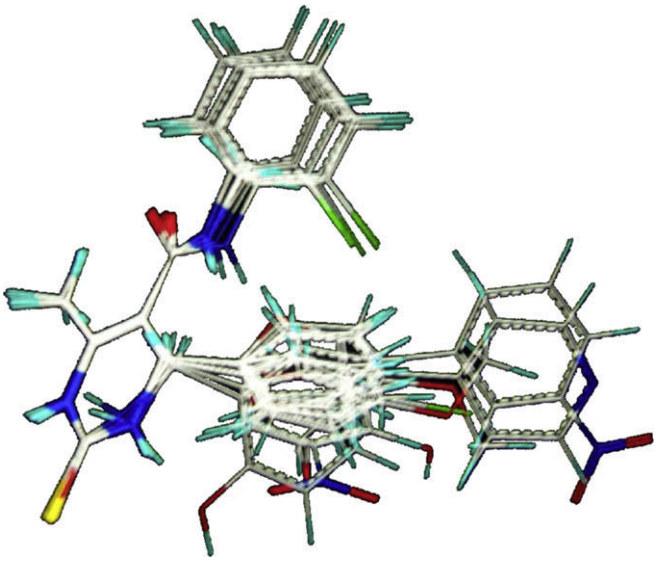


Fig. 1. Training set molecules after alignment by field fit method.

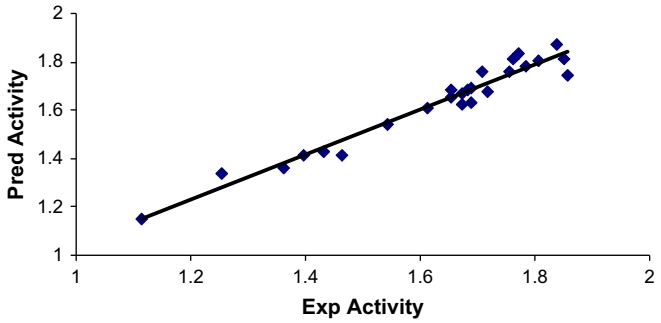


Fig. 2. Correlation between the observed and predicted activities of the developed CoMSIA model.

4. Experimental

4.1. Chemistry

The synthesised compounds were characterised by MP, IR, NMR and MASS spectral analyses. TLC was performed using 2% ethyl acetate in chloroform as a mobile phase on aluminium plates precoated with silica gel GF. The melting points of the compounds were determined using melting apparatus by open capillary method and are uncorrected. The IR spectra of the compounds were recorded on FT-IR spectrometer (Perkin–Elmer Infrared-283) using KBr pellet technique. ^{1}H NMR spectra were recorded on AV-III (400 MHz FT-NMR) using DMSO-d_6 as solvent and TMS as internal standard. The mass spectra were recorded using Shimadzu LCMS 2010A spectrometer under electro spray ionisation technique. Microwave irradiation was carried out using Whirlpool domestic microwave oven.

4.1.1. Synthesis of o-chloroacetoacetanilide (**1**)

Preparation of 2-chloro acetoacetanilide was carried out as per Scheme 1. Ethylacetoacetate (0.01 M) and *o*-chloroaniline (0.01 M) were mixed and refluxed for about 2 h under solvent free conditions. The yellowish liquid formed was then heated on a water bath for 30 min. The reaction mixture was allowed to cool. The crude solid obtained was filtered and washed with ether. The product was recrystallised from aqueous alcohol.

Compound no **1**: White crystals (64%); mp 104–106 °C; IR (KBr, cm^{-1}): 3208 (N–H), 3066 (ArC–H), 2932 (AliC–H), 1704 (C=O), 1538 (C=C), 763 (C–Cl); ^{1}H NMR (DMSO-d_6): δ 2.18 (s, 3H, CH_3), 3.48 (s, 2H, CH_2), 7.11–7.47 (m, 4H, ArH), 9.92 (s, 1H, NH). MS (*m*/*z*): M + 1 calculated 212; found 211.90; mass fragments (*m*/*z*): 127, 89.

4.1.2. Synthesis of 1,4-dihydropyrimidines (**2**–**33**)

Preparation of 1,4-dihydropyrimidines by one-pot multicomponent reaction was carried out as per Scheme 2. The mixture of appropriate β-ketoester (0.005 M, 2-chloro acetoacetanilide or *N*-methylacetoacetamide), urea or thiourea or cyanoguanidine (0.0075 M), and appropriate aldehyde (0.005 M) with catalytic amount of PTSA (0.025 g) was transferred to a round bottom flask containing 15 ml of ethanol to serve as a solvent. The round bottom flask was stirred to dissolve the reactants. The mixture was heated at reflux temperature for compounds **2**–**5**, **8**–**11**, **18**–**21** and **33**. The remaining title compounds were synthesised by simple stirring at room temperature for about 24–30 h (**6**, **7**, **12**–**17**, **22**–**32**). The reactions were monitored through TLC. After the completion of reaction, the mixtures were allowed to cool. The solid products formed were filtered, washed with water to remove the unreacted urea or thiourea or cyanoguanidine and dried. Products were further purified by recrystallisation with methanol. Alternatively, the reactions which required reflux were driven by irradiation with microwaves at 500 W power for about 30–40 min under similar conditions using conical flasks with funnel placed over it. Intermittent cooling was given for 5 min after every 5 min of MW irradiation. However, the yields were found to be more or less same. But, microwave method was found to be convenient to perform the parallel synthesis when compared to the conventional method. Fluorescence exhibited by most of the dihydropyrimidines was considered for their detection on thin layer chromatograms.

Compound no **2**: Pale yellowish amorphous solid (71%); mp 158–160 °C; IR (KBr, cm^{-1}): 3625 (N–H), 3026 (ArC–H), 2953 (AliC–H), 1955 (C=S), 1670 (C=O), 1581 (ArC=C), 749 (C–Cl); ^{1}H NMR (DMSO-d_6): δ 2.2 (s, 3H, CH_3), 5.5 (s, 1H, CH), 7.2–7.6 (m, 9H, ArH), 9.3 (s, 1H, NH), 9.5 (s, 1H, NH), 10.0 (s, 1H, NH); MS (*m*/*z*): M + 1 calculated 358; found 358.46; mass fragments (*m*/*z*): 324, 263, 231, 146.

Compound no **3**: Pale yellow crystals (79%); mp 207–209 °C; IR (KBr, cm^{-1}): 3600 (N–H), 3050 (ArC–H), 2925 (AliC–H), 1664 (C=O), 1654 (C=O), 1623 (ArC=C), 750 (C–Cl); ^{1}H NMR (DMSO-d_6): δ 2.2 (s, 3H, CH_3), 5.5 (s, 1H, CH), 7.1–7.6 (m, 9H, ArH), 7.8 (s, 1H, NH), 8.9 (s, 1H, NH), 9.5 (s, 1H, NH); MS (*m*/*z*): M + 1 calculated 342; found 341.87; mass fragments (*m*/*z*): 311, 279, 231, 167.

Compound no **4**: Yellow crystals (80%); mp 236–238 °C; IR (KBr, cm^{-1}): 3443 (N–H), 3012 (ArC–H), 2933 (AliC–H), 1941 (C=S), 1744 (C=O), 1700 (ArC=C), 1211 (C–O), 746 (C–Cl); ^{1}H NMR (DMSO-d_6): δ 2.1 (s, 3H, CH_3), 3.8 (s, 3H, OCH_3), 7.1–7.6 (m, 9H, ArH), 7.8 (s, 1H, NH), 8.9 (s, 1H, NH), 9.5 (s, 1H, NH); MS (*m*/*z*): M − 1 calculated 386; found 385.68; mass fragments (*m*/*z*): 345, 280, 186, 140, 115, 101.

Compound no **5**: Pale yellowish amorphous solid (70%); mp 222–224 °C; IR (KBr, cm^{-1}): 3262 (N–H), 3005 (ArC–H), 2828 (AliC–H), 1677 (C=O), 1631 (C=O), 1609 (ArC=C), 1113 (C–O), 750 (C–Cl); ^{1}H NMR (DMSO-d_6): δ 3.5 (s, 3H, CH_3), 3.8 (s, 3H, OCH_3), 5.4 (s, 1H, CH), 7.0–7.6 (m, 8H, ArH), 7.8 (s, 1H, NH), 8.7 (s, 1H, NH), 9.5 (s, 1H, NH); MS (*m*/*z*): M − 1 calculated 370; found 369.82; mass fragments (*m*/*z*): 345, 331, 317, 164, 155, 98, 83.

Compound no **6**: Pale brownish amorphous solid (68%); mp 178–180 °C; IR (KBr, cm^{-1}): 3240 (N–H), 3005 (Ar–CH), 2972 (AliC–H), 1946 (C=S), 1676 (C=O), 1630 (ArC=C), 1158 (C–O), 748 (C–Cl); ^{1}H NMR (DMSO-d_6): δ 2.1 (s, 3H, CH_3), 5.5 (s, 1H, CH), 6.3 (s, 1H, CH), 6.5 (s, 1H, CH), 7.2–7.8 (m, 4H, ArH), 9.2 (s, 1H, NH), 9.5 (s, 1H, NH), 10.1 (s, 1H, NH). M + 1 calculated 348; found 348.32; mass fragments (*m*/*z*): 315, 311, 307, 220, 156, 79.

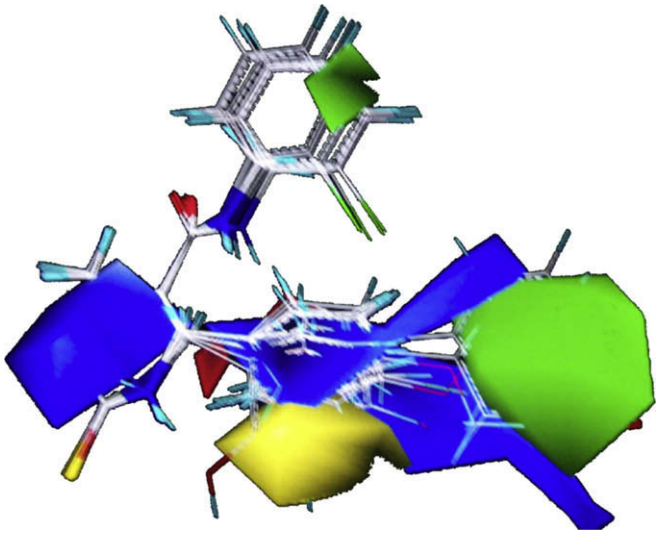


Fig. 3. CoMSIA steric and electrostatic SD × coefficient contour plot; green contours indicate regions where steric bulk is favorable and yellow contours indicate regions where steric bulk is not favored. Blue contours indicate regions where electronegative groups increase activity and red contours indicate regions where electropositive groups increase activity.

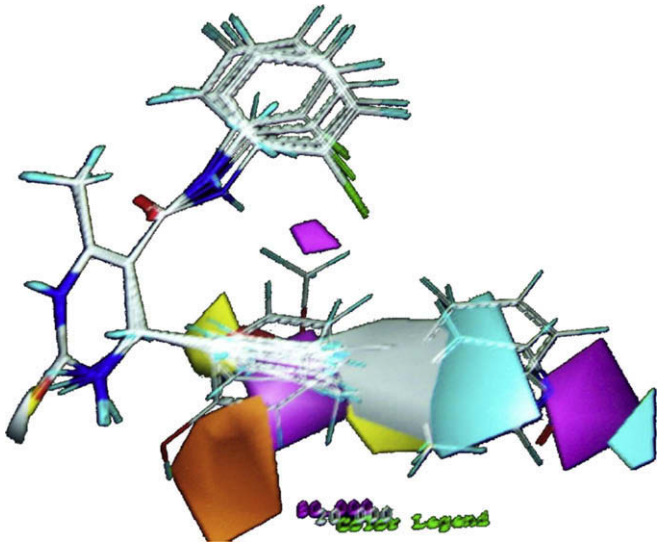


Fig. 4. CoMSIA hydrophobic and hydrogen-bond donor SD × coefficient contour plot; white contours indicate regions where hydrophobicity is favorable and cyan contours indicate regions where hydrophobicity is not favored. Orange contours indicate regions where hydrogen-bond donors increase activity and yellow contours indicate regions where hydrogen-bond donors decrease activity. Purple contours indicate regions where hydrogen-bond acceptors increase activity and green contours indicate regions where hydrogen-bond donors decrease activity (not contributed in the above contour).

Compound no **7**: Pale brownish amorphous solid (79%); mp 146–148 °C; IR (KBr, cm^{-1}): 3290 (N–H), 3117 (ArC–H), 2910 (AliC–H), 1663 (C═O), 1639 (C═O), 1590 (ArC═C), 1103 (C–O), 744 (C–Cl); ^{1}H NMR (DMSO-d_6): δ 2.2 (s, 3H, –CH_3), 4.3 (s, 1H, CH), 4.5 (s, 1H, CH), 5.6 (s, 1H, CH), 7.2–7.8 (m, 4H, ArH), 9.7 (s, 1H, NH), 9.8 (s, 1H, NH), 10.1 (s, 1H, NH). M − 1 calculated 330; found 329.79; mass fragments (*m*/*z*): 310, 301, 233, 117.

Compound no **8**: Yellowish amorphous solid (81%); mp 200–203 °C; IR (KBr, cm^{-1}): 3200 (N–H), 3397 (O–H), 3067 (ArC–H), 2932 (AliC–H), 1950 (C═S), 1671 (C═O), 1601 (ArC═C), 753 (C–Cl); ^{1}H NMR (DMSO-d_6): δ 1.7 (s, 3H, CH_3), 3.5 (s, 1H, CH), 4.7 (s, 1H, CH), 7.1–7.8 (m, 8H, ArH), 9.0 (s, 1H, NH), 9.4 (s, 1H, NH), 9.9 (s, 1H, NH); MS (*m*/*z*): M − 1 calculated 372; found 371.77; mass fragments (*m*/*z*): 328, 219, 195, 74.

Compound no **9**: Pale yellowish amorphous solid (75%); mp 199–204 °C; IR (KBr, cm^{-1}): 3399 (N–H), 3500 (O–H), 2920 (AliC–H), 1676 (C═O), 1657 (C═O), 1607 (Ar–C═C), 751 (C–Cl); ^{1}H NMR (DMSO-d_6): δ 2.5 (s, 3H, CH_3), 4.2 (s, 1H, OH), 5.3 (s, 1H, CH), 7.0–7.7 (m, 8H, ArH), 9.0 (s, 1H, NH), 9.6 (s, 1H, NH), 9.8 (s, 1H, NH). M + 1 calculated 358; found 358.19; mass fragments (*m*/*z*): 345, 287, 231, 220, 92.

Compound no **10**: Yellowish crystals (70%); mp 227–230 °C; IR (KBr, cm^{-1}): 3399 (N–H), 3063 (ArC–H), 1950 (C═S), 1606 (ArC═C), 1673 (C═O), 751 (C–Cl); ^{1}H NMR (DMSO-d_6): δ 2.5 (s, 3H, CH_3), 4.5 (s, 1H, CH), 4.6 (s, 1H, CH), 5.5 (s, 1H, CH), 7.2–7.6 (m, 9H, ArH), 7.8 (s, 1H, NH), 8.0 (s, 1H, NH), 8.6 (s, 1H, NH). M − 1 calculated 382; found 381.88; mass fragments (*m*/*z*): 357, 310, 256, 120.

Compound no **11**: Pale yellowish amorphous solid (72%); mp 211–215 °C; IR (KBr, cm^{-1}): 3231 (N–H), 3025 (ArC–H), 2930 (AliC–H), 1689 (C═O), 750 (C–Cl), 1640 (C═O); ^{1}H NMR (DMSO-d_6): δ 2.5 (s, 3H, CH_3), (s, 1H, CH), 5.5 (s, 1H, CH), 7.2–7.8 (m, 8H, ArH), 8.6 (s, 1H, NH), 10.2 (s, 1H, NH). M + 1 calculated 368; found 368.21; mass fragments (*m*/*z*): 348, 264, 120.

Compound no **12**: Yellow crystals (77%); mp 193–196 °C; IR (KBr, cm^{-1}): 3390 (N–H), 3012 (ArC–H), 2919 (AliC–H), 1884 (C═S), 1644 (C═O), 1512 (NO_2), 750 (C–Cl); ^{1}H NMR (DMSO-d_6): δ 2.5 (s, 3H, CH_3), 7.2–7.8 (m, 9H, ArH), 10.4 (s, 1H, Ar–NH). M + 1 calculated 403; found 402.79; mass fragments (*m*/*z*): 389, 312, 219, 134.

Compound no **13**: White amorphous solid (80%); mp 187–190 °C; IR (KBr, cm^{-1}): 3294 (N–H), 3016 (ArC–H), 2917 (AliC–H), 1594 (ArC═C), 1660 (C═O), 1666 (C═O), 1527 (NO_2), 753 (C–Cl); ^{1}H NMR (DMSO-d_6): δ 2.2 (s, 1H, CH_3), 5.4 (s, 1H, CH), 6.9–7.1 (m, 8H, ArH), 8.9 (s, 1H, NH), 9.3 (s, 1H, NH); MS (*m*/*z*): M − 1 calculated 385; found 385.60; mass fragments (*m*/*z*): 358, 263.

Compound no **14**: Pale yellowish amorphous solid (80%); mp 196–199 °C; IR (KBr, cm^{-1}): 3208 (N–H), 3500 (O–H), 3090 (ArC–H), 2930 (AliC–H), 1964 (C═S), 1678 (C═O), 1255 (C–O), 765 (C–Cl); ^{1}H NMR (DMSO-d_6): δ 1.5 (s, 3H, CH_3), 3.7 (s, 3H, OCH_3), 4.8 (s, 1H, NH), 8.9 (s, 1H, NH), 6.7–7.2 (m, 6H, ArH), 9.4 (s, 1H, OH), 8.9 (s, 1H, NH). M + 1 calculated 404; found 404.12; mass fragments (*m*/*z*): 389, 211, 123.

Compound no **15**: Pale yellowish amorphous solid (75%); mp 179–182 °C; IR (KBr, cm^{-1}): 3190 (N–H), 3420 (O–H), 3090 (ArC–H), 2976 (AliC–H), 1676 (C═O), 1658 (C═O), 1612 (C═C), 1212 (C–O), 751 (C–Cl); ^{1}H NMR (DMSO-d_6): δ 1.4 (s, 3H, CH_3), 3.7 (s, 3H, OCH_3), 4.7 (s, 1H, NH), 6.6–7.0 (m, 6H, ArH), 8.8 (m, 1H, Ar–OH), 8.7 (s, 1H, NH), 8.9 (s, 1H, NH), 9.3 (s, 1H, NH); MS (*m*/*z*): M − 1 calculated 386; found 386.11; mass fragments (*m*/*z*): 273, 153, 127, 100, 83.

Compound no **16**: Pale yellowish amorphous solid (74%); mp 202–205 °C; IR (KBr, cm^{-1}): 3213 (N–H), 3091 (ArC–H), 3008 (AliC–H), 1950 (C═S), 1671 (C═O), 1009 (C–Br), 748 (C–Cl); ^{1}H NMR (DMSO-d_6): δ 2.2 (s, 3H, CH_3), 5.4 (s, 1H, CH), 7.1–7.6 (m, 8H, ArH), 9.3 (s, 1H, NH), 9.6 (s, 1H, NH), 10.1 (s, 1H, NH). M + 1 calculated 437; found 436.63; mass fragments (*m*/*z*): 416, 378, 254, 111.

Compound no **17**: Colourless crystals (81%); mp 195–198 °C; IR (KBr, cm^{-1}): 3282 (N–H), 2962 (AliC–H), 1679 (C═O), 1647 (C═O), 1586 (ArC═C), 1025 (C–Br), 753 (C–Cl); ^{1}H NMR (DMSO-d_6): δ 2.1 (s, 3H, CH_3), 5.3 (s, 1H, CH), 7.2–7.6 (m, 8H, Ar–H), 8.8 (s, 1H, NH), 9.2 (s, 1H, NH), 10.2 (s, 1H, NH). M + 1 calculated 421; found 420.81; mass fragments (*m*/*z*): 405, 372, 297, 103.

Compound no **18**: Yellow crystals (76%); mp 139–143 °C; IR (KBr, cm^{-1}): 3600 (N–H), 3002 (ArC–H), 1604 (C═O), 1473 (C═N), 1824 (C═S), 730 (C–Cl); M − 1 calculated 357; found 346.58; mass fragments (*m*/*z*): 331, 303, 217, 98.

Compound no **19**: Yellowish amorphous solid (78%); mp 137–140 °C; IR (KBr, cm^{-1}): 3178 (N–H), 3032 (ArC–H), 2976 (AliC–H),

1728 (C=O), 1604 (ArC=C), 1681 (C=O), 1537 (C=N), 749 (C–Cl). M + 1 calculated 343; found 343.27; mass fragments (*m*/*z*): 327, 289, 167, 120.

Compound no **20**: Colourless crystals (80%); mp 156–158 °C; IR (KBr, cm^{-1}): 3511 (N–H), 3021 (ArC–H), 2850 (AliC–H), 1812 (C=S), 1686 (C=O), 1245 (C–N); ^{1}H NMR (DMSO-d_6): δ 2.0 (s, 3H, CH_3), 2.5 (s, 3H, CH_3), 5.3 (s, 1H, CH), 7.1–7.4 (m, 5H, ArH), 7.8 (s, 1H, NH), 9.3 (s, 1H, NH), 9.8 (s, 1H, NH); MS (*m*/*z*): M − 1 calculated 260; found 259.71; mass fragments (*m*/*z*): 228, 184.

Compound no **21**: Pale yellow crystals (75%); mp 201–204 °C; IR (KBr, cm^{-1}): 3380 (N–H), 3076 (ArC–H), 2910 (AliC–H), 1844 (C=S), 1714 (C=O), 1245 (C–N), 1195 (C–O); ^{1}H NMR (DMSO-d_6): δ 2.0 (s, 3H, CH_3), 2.4 (s, 3H, CH_3), 2.8 (s, 3H, OCH_3), 5.2 (s, 1H, CH), 6.8–7.3 (m, 4H, ArH), 7.6 (s, 1H, NH), 9.2 (s, 1H, NH), 9.8 (s, 1H, NH). M + 1 calculated 292; found 291.69; mass fragments (*m*/*z*): 250, 198, 126.

Compound no **22**: Pale brownish amorphous solid (75%); mp 230–233 °C; IR (KBr, cm^{-1}): 3332 (N–H), 3395 (OH), 3020 (ArC–H), 2958 (AliC–H), 1932 (C=S), 1713 (C=O), 1240 (C–N), 1195 (C–O); ^{1}H NMR (DMSO-d_6): δ 2.0 (s, 3H, CH_3), 2.6 (s, 3H, CH_3), 5.2 (s, 1H, CH), 6.1 (s, 1H, CH), 6.3 (s, 1H, CH), 7.7 (s, 1H, NH), 9.3 (s, 1H, NH), 9.9 (s, 1H, NH); MS (*m*/*z*): M − 1 calculated 250.30; found 249.85; mass fragments (*m*/*z*): 216, 203.

Compound no **23**: Pale yellowish amorphous solid (71%); mp 220–222 °C; IR (KBr cm^{-1}): 3395 (O–H), 3332 (N–H), 3082 (ArC–H), 2948 (AliC–H), 1950 (C=S), 1717 (C=O), 1357 (C–N); ^{1}H NMR (DMSO-d_6): δ 1.7 (s, 3H, CH_3), 2.6 (s, 3H, CH_3), 4.3 (s, 1H, CH), 4.5 (s, 1H, OH), 7.1–7.3 (m, 4H, ArH), 8.0 (s, 1H, NH), 8.9 (s, 1H, NH), 9.1 (s, 1H, NH). M + 1 calculated 278; found 277.79; mass fragments (*m*/*z*): 216, 185, 120.

Compound no **24**: Pale brownish amorphous solid (74%); mp 253–256 °C; IR (KBr, Cm^{-1}): 3451 (N–H), 3086 (ArC–H), 2980 (AliC–H), 1726 (C=O), 1638 (C=O), 1182 (C–N); ^{1}H NMR (DMSO-d_6): δ 2.1 (s, 3H, CH_3), 2.6 (s, 3H, CH_3), 3.9 (s, 1H, CH), 5.3 (s, 1H, OH), 5.7 (s, 1H, CH), 6.8 (s, 1H, NH), 7.5 (s, 1H, NH), 8.0 (s, 1H, NH). M + 1 calculated 236; found 235.81; mass fragments (*m*/*z*): 210, 187, 119.

Compound no **25**: Pale yellowish amorphous solid (79%); mp 216–219 °C; IR (KBr, cm^{-1}): 3296 (N–H), 3019 (ArC–H), 2939 (AliC–H), 1697 (C=O), 1648 (C=O); ^{1}H NMR (DMSO-d_6): δ 2.3 (s, 3H, CH_3), 2.5 (s, 3H, CH_3), 5.2 (s, 1H, CH), 7.2–7.6 (m, 5H, Ar–H), 7.8 (s, 1H, NH), 8.0 (s, 1H, NH), 8.6 (s, 1H, NH). M + 1 calculated 246; found 245.93; mass fragments (*m*/*z*): 221, 176, 96.

Compound no **26**: Yellowish crystals (76%); mp 180–184 °C; IR (KBr, cm^{-1}): 3468 (N–H), 3108 (ArC–H), 2962 (AliC–H), 1717 (C=O), 1654 (C=O), 1220 (C–N), 1245 (C–O); ^{1}H NMR (DMSO-d_6): δ 2.2 (s, 3H, CH_3), 2.5 (s, 3H, CH_3), 3.7 (s, 3H, OCH3), 5.3 (s, 1H, CH), 7.0–7.4 (m, 4H, Ar–H), 7.5 (s, 1H, NH), 7.9 (s, 1H, NH), 8.5 (s, 1H, NH). M + 1 calculated 276; found 276.25; mass fragments (*m*/*z*): 250, 178, 99.

Compound no **27**: Yellowish amorphous solid (72%); mp 170–173 °C; IR (KBr, cm^{-1}): 3285 (N–H), 3082 (ArC–H), 2941 (AliC–H), 1647 (C=O), 1655 (C=O), 1194 (C–N), 1620 (C=C); ^{1}H NMR (DMSO-d_6): δ 2.1 (s, 3H, CH_3), 2.5 (s, 3H, CH_3), 3.5 (s, 1H, CH), 5.0 (s, 1H, CH), 7.0–7.4 (m, 5H, Ar–H), 7.5 (s, 1H, NH), 7.8 (s, 1H, NH), 8.0 (s, 1H, NH), 8.2 (s, 1H, NH); MS (*m*/*z*): M − 1 calculated 270; found 269.82; mass fragments (*m*/*z*): 214, 203, 173, 102.

Compound no **28**: Yellowish amorphous solid (80%); mp 170–173 °C; IR (KBr, cm^{-1}): 3440 (N–H), 3098 (ArC–H), 1840 (C=S), 2972 (AliC–H), 1652 (C=O), 1350 (NO_2); ^{1}H NMR (DMSO-d_6): δ 2.1 (s, 3H, CH_3), 2.5 (s, 3H, CH_3), 5.3 (s, 1H, CH), 7.7–8.0 (m, 4H, ArH), 8.2 (s, 1H, NH), 9.5 (s, 1H, NH), 10.1 (s, 1H, NH); MS (*m*/*z*): M − 1 calculated 305; found 305.09; mass fragments (*m*/*z*): 267, 143, 115, 102.

Compound no **29**: Pale yellow crystals (76%); mp 194–197 °C; IR (KBr, cm^{-1}): 3362 (N–H), 3006 (ArC–H), 2932 (AliC–H), 1711 (C=O), 1675 (C=O), 1220 (C–N), 1344 (NO_2); ^{1}H NMR (DMSO-d_6): δ 2.0 (s, 3H, CH_3), 2.6 (s, 3H, CH_3), 5.3 (s, 1H, CH), 7.7 (s, 1H, NH), 7.8 (m, 4H, ArH), 8.1 (s, 1H, NH), 8.8 (s, 1H, NH); MS (*m*/*z*): M − 1 calculated 289; found 288.91; mass fragments (*m*/*z*): 247, 123, 100, 83.

Compound no **30**: Colourless crystals (73%); mp 243–246 °C; IR (KBr, cm^{-1}): 3628 (O–H), 3388 (N–H), 3096 (ArC–H), 2968 (AliC–H), 1958 (C=S), 1666 (C=O), 1200 (C–N), 1168 (C–O); ^{1}H NMR (DMSO-d_6): δ 2.0 (s, 3H, CH_3), 2.5 (s, 3H, CH_3), 3.8 (s, 3H, OCH_3), 5.1 (s, 1H, CH), 6.6 (s, 1H, OH), 6.7–6.9 (m, 3H, ArH), 7.7 (s, 1H, NH), 9.0 (s, 1H, NH), 9.2 (s, 1H, NH); MS (*m*/*z*): M − 1 calculated 306; found 306.36; mass fragments (*m*/*z*): 255, 216.

Compound no **31**: Pale yellowish amorphous solid (70%); mp 200–202 °C; IR (KBr, cm^{-1}): 3261 (N–H), 3016 (ArC–H), 2932 (AliC–H), 1699 (C=O), 1620 (C=O), 1221 (C–N), 1085 (C–O); ^{1}H NMR (DMSO-d_6): δ 2.0 (s, 3H, CH_3), 2.5 (s, 3H, CH_3), 3.7 (s, 3H, OCH_3), 5.1 (s, 1H, CH), 6.7–6.9 (m, 3H, ArH), 7.5 (s, 1H, NH), 8.4 (s, 1H, –NH), 8.9 (s, 1H, –NH). M + 1 calculated 292; found 291.84; mass fragments (*m*/*z*): 279, 186, 101.

Compound no **32**: Light greenish amorphous solid (78%); mp 146–149 °C; IR (KBr, cm^{-1}): 3432 (N–H), 3050 (ArC–H), 2930 (AliC–H), 1668 (C=O), 1238 (C–N), 1295 (C–N). M + 1 calculated 247; found 246.63; mass fragments (*m*/*z*): 219, 158, 120, 88.

Compound no **33**: Pale yellowish amorphous solid (12%); mp 246–248 °C; IR (KBr, cm^{-1}): 3421 (N–H), 3025 (ArC–H), 2913 (AliC–H), 1722 (C=O), 1592 (ArC=C), 526 (C–Cl); ^{1}H NMR (DMSO-d_6): δ 2.1 (s, 3H, CH_3), 6.2 (s, 1H, CH), 7.0–7.7 (m, 9H, ArH), 9.2 (s, 1H, NH), 9.4 (s, 1H, NH), 9.7 (s, 1H, NH); MS (*m*/*z*): M − 1 calculated 364.0; found 363.94; mass fragments (*m*/*z*): 328, 312, 257, 231.

4.2. In vitro antioxidant activity

The DPPH free radicals formed in this assay will be reduced to a corresponding hydrazine when it reacts with hydrogen donors. The DPPH radical is purple in colour and upon reaction with hydrogen donors of the antioxidant changes to yellow colour. It is a discolouration assay, which is evaluated by the addition of the antioxidant or test compound to a DPPH solution in ethanol and the decrease in absorbance was measured.

The assay was carried out in a 96-well microtitre plate. To 200 μl of each of DPPH ethanolic solution, 10 μl of each of the test compound (100 μg) or standard (ascorbic acid, 10 μg) solution was added separately to wells of the microtitre plate. The plates were incubated at 37 °C for 30 min and the absorbance of each solution was measured at 490 nm, using ELISA reader. The IC_{50} values (concentration which inhibits 50% of free radicals) for the compounds **28** and **29** were determined by serial dilution method at the concentrations below 100 μg.

4.3. In vitro anticancer activity

Anticancer activity of the compounds was evaluated by determining the percentage viability of MCF-7, human breast cancer cells using the trypan blue dye exclusion technique. MCF cells were cultured in the peritoneal cavity of healthy albino mice weighing 25–30 g by injecting a suspension of MCF cells (1×10^6 cells/ml) intraperitoneally. The cells were aspirated aseptically from the peritoneal cavity of the mice on day 15. The cells were washed with Hank's balanced salt solution (HBSS) and centrifuged for 10–15 min in the cooling centrifuge. The pellet was resuspended with HBSS and the process was repeated three times. Finally, the cells were suspended in a known quantity of HBSS and the cell count was adjusted to 1×10^6 cells/ml. 0.1 ml of the diluted cell suspension was distributed into eppendorf tubes and exposed to 0.1 ml of the test compound (10 μg) and incubated at 37 °C under 5% CO_2 for 3 h. After 3 h, a trypan blue dye exclusion test was performed to determine the percentage viability. The pooled cells were mixed with 0.4% yield trypan blue in a ratio of 1:1 and the number of

stained, non-stained and total number of cells was counted using a haemocytometer. Cell count taken from cells grown in the absence of the test compound was taken as 100% cell survival (control). Percentage cytotoxicity was calculated by using the formula for triplicate samples:

$$\%\,\text{Cytotoxicity} = (\%\,\text{viability of control} - \%\,\text{viability of test})/(\%\,\text{viability of control}) \times 100.$$

4.4. CoMSIA study

CoMSIA is a powerful and established tool for building 3D-QSAR models that can be applied to drug design [28,29]. Three-dimensional structure building and all the modeling were carried out using the SYBYL 6.7 (SGI work station, SYBYL computer program, version 6.7. St. Louis, MO: Tripos Inc., USA) program package and the conformations of the compounds in the training and test sets were generated using the systematic conformational search method implemented in SYBYL 6.7. Energy minimization was affected using the Tripos force field with a distance-dependent dielectric and the Powell conjugate gradient algorithm with a convergence criterion of 0.001 kcal/mol. Partial atomic charges were calculated by the Gasteiger–Huckel method [30]. Consequently, the dihydropyrimidines were aligned according to their common substructure and using most active compound **11** as a template. Molecular alignment was affected with the field fit alignment method function of SYBYL [31]. After consistently aligning the molecules within a lattice that extended 4 Å units beyond the aligned molecules in all directions with a grid step size of 2 Å, a probe sp^3 carbon atom with a net charge of +1 and Van der Waals radius of 1.52 Å was employed. The five similarity indices in CoMSIA, i.e., steric, electrostatic, hydrophobic, H-bond donor, and H-bond acceptor descriptors were calculated and the fields generated were scaled by the CoMSIA-STD method in SYBYL 6.7. Here, steric indices are related to the third power of the atomic radii, the electrostatic descriptors are derived from the atomic partial charges, the hydrophobic fields are derived from the atom-based parameters, and the H-bond donor and acceptor indices are obtained by a rule-based method based on the experimental results. In optimizing the CoMSIA performance, the most important parameter is how to combine the five fields in the CoMSIA model. To choose the optimal result, we systematically altered the combination of fields and chose the value that gave the best non-cross-validation, the smallest errors, and the largest *F* value. Finally, the model generated by combining the steric, electrostatic, hydrophobic, and hydrogen-bond acceptor and hydrogen-bond donor fields was selected as the best CoMSIA model, and the contours were analyzed using this model. To derive the 3D-QSAR models, the CoMSIA descriptors were used as independent variables with the pACA activity value as a dependent variable. Partial least squares (PLS) regression analyses were conducted with the standard implementation in the SYBYL package. The predictive ability of the models was evaluated by leave-one-out (LOO) cross-validation. The developed model was further evaluated by predicting activities of the external test set compounds.

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