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CoMFA and CoMSIA Studies of 1,2-dihydropyridine Derivatives as Anticancer Agents

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Abstract

Taking advantage of our in-house experimental data on 3-cyano-2-imino-1, 2-dihydropyridine and 3-cyano-2-oxo-1,2-dihydropyridine derivatives as inhibitors of the growth of the human HT-29 colon adenocarcinoma tumor cell line, we have established a highly significant CoMFA and CoMSIA models ($q^2_{cv}=0.70/0.639$). The models were investigated to assure their stability and predictivity ($r^2_{pred}=0.65/0.61$) and successfully applied to design a new potential cell growth inhibitory agent with IC_{50} s in the submicromolar range.

Keywords

3D-QSAR; CoMFA/CoMSIA model; 3-cyano-2-imino-1,2-dihydropyridine; 3-cyano-2-oxo-1,2-dihydropyridine

1. INTRODUCTION

There is an interest in the 3-cyano-4,6-diaryl-2-(1*H*) imino or oxopyridine series of compounds due to their diverse pharmacological activities, such as antimicrobials against Gram positive and Gram negative microorganisms [1–3], antidepressant [4], cardiotonic through inhibition of PDE3 [5] and anticancer [6–8]. Cross reactivity is a potential possibility e.g. milrinone; is a 3-cyano-2-oxopyridine derivative that has been introduced to the clinic for the treatment of congestive heart failure. Its mechanism of action involves PDE3 inhibition, leading to high levels of cAMP and consequent inotropic effect. Recent studies showed that PDE3, PDE4 and PDE5 are over expressed in cancerous cells compared

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DISCLOSURE

Methods for the generation of structures, exploration of the conformational space and alignment steps were done as previously described in our previous publication *Bioorg. Med. Chem.* **2006**, 19, 5898–5912.

with normal cells. In addition, cross inhibition of PDE3 together with other PDEs may lead to inhibition of tumor cell growth and angiogenesis [9–12].

We have reported 3- cyano –2-iminopyridine and 3-cyano –2-oxopyridine derivatives for their anticancer activity, particularly against leukemia and colorectal HT-29 cell lines.^{6–8} Taking advantage of our in-house data on inhibitors of the growth of the human HT-29 colon adenocarcinoma tumor cell line, we herein present a ligand-based 3D-QSAR approach (Chart 1) in order to improve and refine our understanding of the molecular requirements for optimized anti-cancer activity, as well as to establish a working model that allows to facilitate further drug discovery efforts. We applied a well-established protocol on our training and test set comprising of 30 and 5 compounds, respectively. In addition to several techniques for monitoring the statistical quality and predictivity, we demonstrate the applicability of the resulting model in guiding synthetic efforts toward highly potent anti-cancer. Thus, the study involved the prediction, synthesis, and biological testing of novel 3-cyano-4,6-diaryl-2-(1*H*) iminopyridines (**36,37**). The good correspondence between the obtained experimental log IC₅₀ values and our previous predictions is encouraging. To ensure optimal comparability of the anticancer activity for all ligands, we have obtained all biological data used in this study within our laboratory.

2. METHODS

2.1. Generation of Structures and Exploration of the Conformational Space

Structure building and refinement for the entire set of 1,2-dihydropyridine analogues (1–37, Scheme 1 and Table 1) was accomplished using SYBYLX 1.1 molecular modeling software [13]. Compound 1 has been selected as a template for generating the whole series of 1,2-dihydropyridines.

We performed a grid search, using the Tripos force field [14] with Gasteiger–Marsili charges [15], on the 4,6-phenyl-1,2-dihydropyridine structure, iterating the bonds ,between 1,2-dihydropyridine and the two phenyl rings on 4&6 positions, in steps of 30°. The most reasonable low energy conformer was chosen from the obtained conformations. Using this template structure, all other ligands were derived by modification of the aromatic moiety and the phenyl substituents. Finally, all ligands were optimized with MOPAC using a semiempirical AM1 Hamiltonian [16] to improve the molecular geometries and ensure better comparability of the ligand structures by providing structures based on identical levels of calculation.

2.2. Alignment

In general, the alignment is one of the most challenging aspects of 3D-QSAR. Thus, various approaches involving different degrees of complexity exist in order to address this problem. The spectrum of available techniques ranges from simple atom-based fitting procedures to sophisticated binding-site guided protocols. However, none of these has evolved to be superior over the others. We have found the ligand-based alignment technique ASP to be very useful. Thus, we used the module ASP as implemented in the QSAR package TSAR [17], which allows us to perform an alignment by comparison of steric overlap and

molecular electrostatic potentials. For deriving reasonable electrostatic potentials, first, VESPA charges were calculated using the semiempirical program package VAMP [18]. These atom centered partial charges are obtained by a fit of the electronic wave function to the atomic positions. Compared to other charge schemes such as Coulson or Mulliken, they have the advantage that the anisotropy of the electron distribution around the molecule, especially for aromatic systems, is described in more detail.

As the basic template onto which the other ligands are to be superimposed, we have chosen compound **1**, which shows considerably high growth inhibitory activity on HT-29 colon adenocarcinoma tumor cell line. To quantify the relative orientation of two molecules, the combined similarity index based on the Carbo index for electrostatics and the shape similarity index to account for steric differences was evaluated (with both indices weighted equally) using three Gaussian functions for integration. This parameter was then optimized by overlaying the centroids of the molecules and performing a full translational and orientational search of each rigid comparison molecule relative to the lead compound **1** by systematically rotating around the Cartesian x-, y-, and z-axes in 10° steps. For each new orientation, a Simplex algorithm in combination with Simulated Annealing directs the six degrees of freedom to an alignment with optimal similarity [19]. Finally, the orientation and placement of each ligand on the template **1** featuring the highest score related to this search algorithm was chosen to yield the TSAR-based alignment.

2.3. CoMFA and CoMSIA

To ensure best comparability between molecules, all pharmacological data of the ligands used in this study have been measured within our laboratory. We performed a Comparative Molecular Field Analysis (CoMFA) evaluating the typically used steric and electrostatic fields implemented in SYBYL. All CoMFA calculations were accomplished using an sp³ carbon atom with a charge of +1, a cutoff value of 30 kcal/mol for the Lennard-Jones and Coulomb-type potential, and a constant dielectric function. The dimension of the surrounding lattice (1.0 Å grid spacing) was selected with a sufficiently large margin to enclose all aligned molecules by at least 4 Å. Putative problems of the analysis can arise from the absolute orientation of the molecules within the grid space. A useful protocol to address this problem is the AOS/APS script of Wang *et al.*, [20] which automatically rotates/translates the entire dataset within the lattice without changing the relative orientation or alignment of the molecules. We applied the APS protocol varying the ligands by steps of 0.1 Å in all three dimensions within a 1.0 Å grid. After each of 1000 translational steps, the PLS analysis was repeated. This procedure gives detailed information about the translational dependence of the CoMFA, helps to ensure that no artificial effects are included, and provides evidence of the robustness of the model. In the CoMSIA, all five physicochemical descriptors (electrostatic, steric, hydrophobic, and hydrogen-bond donor and acceptor) were evaluated using a common probe atom placed within a 3D grid. The atom was set up with a radius of 1.0 Å and charge, hydrophobic interaction, and hydrogen-bond donor and acceptor properties all equal to +1. Like in the CoMFA, the grid was extended beyond the molecular dimensions by 4.0 Å in the x, y, and z directions and the spacing between probe points within the grid was set at 1.0 Å. For the attenuation factor α controlling the steepness of the Gaussian function the standard value of 0.3 was accepted.

2.4. Partial Least Squares (PLS) Analysis

The PLS method [21] was used to linearly correlate the CoMFA and CoMSIA fields to biological activity values. The cross-validated analysis was performed using the leave-one-out (LOO) method in which one compound is removed and its activity is predicted using the model derived from the rest of the dataset. Complementing the results obtained from the leave-one-out cross-validation, the more robust leave-many-out procedure was performed to ensure the reproducibility of q^2 [14].

The cross-validated q^2 that resulted in minimal number of components and lowest standard error of prediction was accepted. To speed up the analysis and reduce noise, column filtering values ($\sigma_{\min}$) were iterated between 1.0 and 5.0 kcal/mol. Thus, only columns with a standard deviation of more than $\sigma_{\min}$ were used for the cross-validation, resulting in approximately 5.2- 15.3% of the original data to be used in the CoMFA and 4.0–12.6% used in the CoMSIA. A final non-cross-validated analysis was performed using the optimal number of previously identified components. After obtaining the models, the CoMFA and CoMSIA results were graphically interpreted by field contribution maps.

2.5. Calculation of the Predictive Correlation Coefficient (r^2_{pred}) and Prediction of Novel Compounds

The predictive ability of the 3D-QSAR model was determined from a set of 5 compounds (**31–35**) that were not included in the training set (Table 1). The compounds were manually assigned to the training or test set ensuring a reasonable range of log IC₅₀ units and structural diversity in both sets. These molecules were aligned using the same method as described before, and their activities were predicted using the model generated by the training set. Accompanying a synthetic strategy to design some analogues of the 1,2-dihydropyridine ligands used in the training and test set, we also predicted two ligands (**36 & 37**), which were subsequently synthesized and tested. In addition to the classical test set, we also included these compounds in the calculation of r^2_{pred} . Based on the test set molecules; this predictive correlation coefficient (r^2_{pred}) is defined as $r^2_{\text{pred}} = (\text{SD} - \text{PRESS})/\text{SD}$; where SD is the sum of the squared deviations of each biological property value from their mean and PRESS is the sum of squared differences between the predicted and actual logIC₅₀ values for every molecule in test set.

3.RESULTS AND DISCUSSION

3.1. CoMFA

Using our properly selected training set of 30 1,2-dihydropyridine derivatives (Scheme 1 and Table 1), we obtained statistically significant QSAR models (Table 2). The initial PLS analysis of our aligned training set applying a default $\sigma_{\min}$ data filter of 2 kcal/mol yielded a crossvalidated q^2 of 0.7 with $S_{c/v} = 0.465$ using 4 components (Table 2, Panel B). Increasing the minimum level of field variation $\sigma_{\min}$ to purpose a more efficient reduction of noise, only a negligible further improvement of the statistics of the CoMFA model is found for $\sigma_{\min} = 3.0$ and 4.0 kcal/mol (Table 2, Panels C, D). Likewise, reduction of $\sigma_{\min}$ to 1.0 kcal/mol shows also only negligible effects on the $q^2_{c/v}$ (Table 2, Panel A). In contrast, increasing $\sigma_{\min}$ further to 5.0 kcal/mol results even in a reduction of the cross validated q^2 to

0.68 (Table 2, Panel E), which presumably reflects that some statistically relevant descriptors have been filtered out at this $\sigma_{\min}$ value. With increasing $\sigma_{\min}$ the number of remaining descriptors is noticeably decreased from 3544 to 1231, while at the same time the electrostatic contribution is raised from 27.7% to 36.1%. Thus, these results demonstrate that the obtained CoMFA is stable at a highly significant level (>0.7) and not prone to deviate noticeably with varying numbers of descriptors.

As q^2_{cv} is dependent on the grid spacing and the absolute position of the aligned molecules within the lattice to the shape and steepness of the hyperbolic Lennard-Jones and Coulomb potentials used during the analysis [22]. They stated that in the case of a 2.0 Å lattice, important contributions to the correlation analysis could be lost due to the required arbitrary cutoff values. Despite the fact that changing from a 2.0 to 1.0 Å lattice spacing results in an increase in computing time by a factor of 8, we performed all analyses using the smaller increment. Quantifying the translational dependence of q^2_{cv} helps to ensure that no artificial effects are included in the final CoMFA and gives evidence of the robustness of the model. Thus, we applied a procedure published by Wang *et al.* [20] which systematically translates the aligned dataset in space, followed by a PLS analysis after every retranslation. The histogram plot of the resulting 1000 model variants showed to be approximately corresponding to a normal distribution of the obtained q^2 values (Fig. 1). The range of the values 0.685–0.720 is quite narrow, indicating that our CoMFA model is rather stable toward different grid placements.

The highest q^2_{cv} (0.720) was found for 5 components. Thus, it should be noted in this context that the increase of q^2_{cv} values by less than 5% with the use of an additional component is regularly considered inappropriate due to the ‘parsimony-principle’ [23]. Thus, we used the original models, which are in line with this rule, to derive the graphical CoMFA maps. Modification of other CoMFA parameters, such as changing the cutoff values for steric/electrostatic energies, did not give any improvement of the model. The final predicted/cross-validated versus experimental $\log IC_{50}$ values for model B and their residuals ($\log IC_{50}$) are given in Table 1.

3.2. CoMSIA

CoMSIA computes the steric and electrostatic fields (as in CoMFA), but it also calculates additional hydrophobic, hydrogen-bond donor, and hydrogen-bond acceptor fields. The resulting contour maps are easier to interpret than those in CoMFA because a Gaussian function is used to determine the distance-dependence. Therefore, the similarity indices can also be calculated at the grid points inside the molecules, not just outside, as it is in CoMFA [23]. Several CoMSIA models were generated using the combinations of different fields (Table 3). The purpose of using several combinations of different fields is not only to increase the significance and predictive power of the 3D-QSAR models, but also to partition the various properties into spatial locations where they play a decisive role in determining the activity. CoMSIA, in most instances, performs similarly to CoMFA in terms of predictive ability. The results of the initial CoMSIA models for different combinations are summarized in Table 3.

The combined use of the steric, electrostatic, hydrophobic, and hydrogen-bond donor descriptors produced the best model ($q^2=0.639$ and $r^2=0.965$ with four components).

In analogy to the CoMFA, already the initial PLS analysis of our aligned training set applying a default $\sigma_{\min}$ data filter of 2.0 kcal/mol yielded a highly significant cross-validated q^2 of 0.639 with $S_{c/v} = 0.529$ using four components (Table 2, Panel G). Variation of the column filtering parameter $\sigma_{\min}$ shows that for increasing $\sigma_{\min}$ values from 1.0 to 5.0 kcal/mol, the number of descriptors is strongly reduced from 6581 to 2110, while the q^2_{cv} is steadily increased from 0.637 (F) to 0.655 (J). Although the q^2 values of the CoMSIA models are slightly reduced compared to those of the CoMFA models, they are still indicating stable analyses of high quality. Furthermore, the CoMSIA models comprise valuable complementary information, as they offer additional explanation for the molecules different inhibitory activities by introducing three auxiliary field types, the hydrophobic field, the hydrogen bond acceptor field, and the hydrogen bond donor field. Interestingly, while the noise reduction in the CoMFA model decreased the fraction of the steric contribution from 72.3% down to 63.9%, it was increased by the noise reduction in the CoMSIA model from 14.8% up to 21.5%. The other CoMSIA field types typically contribute to the full model in the order: hydrophobic (30.6–34.1%) > electrostatic (25.5–26.4%) > donor (24.1–25.6%). The final predicted/cross-validated versus experimental pKi values for model G and their residuals ($-\log IC_{50}$) are given in Table 3.

3.3. Advanced Cross-Validation and Assessment of Model Predictivity

Despite the good results obtained for CoMFA and CoMSIA cross-validation, of course, we are aware that there is always the danger to overemphasize the usefulness of cross-validation and q^2 as measures of the predictive performance. High values of q^2_{LOO} can be regarded as a necessary, but not a sufficient, condition for a model to possess significant predictive power. Thus; we also performed the more critical and widely accepted leave-10%-out and leave-20%-out cross-validations. With $q^2_{L10\%O}$ -values (mean $\pm$ SD) of 0.696 ± 0.013 and 0.639 ± 0.017 for the CoMFA and CoMSIA, respectively, we have shown that even after discarding every tenth compound from the training set the predictivity of the models is hardly impaired. Repeating this calculation $n = 20$ times for each model assesses the liability of the respective model to random effects caused by different assignment of the ligands to the 10 groups. The negligible standard deviation (SD) is a further indicator of the model quality. Also the $q^2_{L20\%O}$ -values of 0.68 ± 0.025 and 0.618 ± 0.032 for CoMFA and CoMSIA, respectively, show only a small decrease of the mean values, although disregarding every fifth compound. Likewise, there is only a marginal increase in the standard deviation. Therefore, we conclude that leave-10%-out and leave-20%-out cross-validation clearly corroborates the good stability and robustness of the models.

The predictive power of the CoMFA (Table 2, Panel B) and CoMSIA (Table 2, panel G) analysis was further examined using a test set of 5 compounds (31–35, Scheme 1 and Table 1) that had been omitted from the training set. In addition to these test set ligands, we also included two 1,2-dihydropyridine derivative (36&37) in the calculation of the r^2_{pred} value, which were synthesized accompanied by these QSAR predictions as described subsequently.

The calculation was performed and gave better results for the CoMFA with $r^2_{\text{pred}} = 0.651$ than for the CoMSIA model with $r^2_{\text{pred}} = 0.613$.

3.4. 3D Contour Maps

To visualize the information content of the derived 3D-QSAR models, CoMFA and CoMSIA contour maps were generated. The field energies at each lattice point were calculated as the scalar results of the coefficient and the standard deviation associated with a particular column of the data table (“stdev*coeff”), which was always plotted as the percentage of the contribution to the CoMFA or CoMSIA equation. In Figs. (2 and 3) discussed below, the isocontour diagrams of the field contributions (“stdev*coeff”) for different properties calculated by the CoMFA and CoMSIA analysis are illustrated with exemplary ligands. The contour plots may help to identify important regions where any change may affect the binding preference. Furthermore, they may be helpful in identifying important features contributing to interactions between the ligand and the active site of an enzyme.

3.4.1. CoMFA—Favored and disfavored cutoff energies were set at the 80th and 20th percentiles for both the steric and the electrostatic contributions. The presence of a substituent near a green region, the absence of a substituent near a yellow region, the increase of a negative charge near a red region, or a positive charge near a blue region increases the inhibitory activity of the ligands, while the presence of a substituent near a yellow region, the absence of a substituent near a green region, the increase of a negative charge near a blue region, or a positive charge near a red region decreases the inhibitory activity. Such fields could, in principle, suggest the way a leading structure should be modified for gaining higher activity. Because it is a representative example of the most active ligands, compound **2** is shown embedded as a guide into the steric (A) and electrostatic fields (C) (Fig. 2) resulting from a non-cross-validated CoMFA run ($\sigma_{\text{min}} = 2.0$ kcal/mol). Likewise, compound **17** is shown enclosed in the steric (B) and compound **15** is displayed in panel (D) as a representative of lower activity ligands. In panel (A), The large green isopleth in the middle indicate that substituents near this region increase inhibitory activity of the ligands. The yellow isopleths at left near the (hetero)aryl rings in position 4 of 1,2-dihydropyridine ring reflect a strict decrease in activity for all of these ‘anchor moieties’ being dislocated into this area. This yellow isopleths reveal that (hetero)aryl rings with no substituents are preferentially recognized to yield high $\log\text{IC}_{50}$ values, while any ogized to yield high $\log\text{IC}_{50}$ values, while any substituents, as well as sterically demanding systems, is detrimental for activity (exemplified by **17** in (Panel B)). This, for instance, applies to **12** & **13**, which both bear an ethoxy substituent in position 4 of phenyl ring and have lower activity compared to compound **2**.

In panel (C), the electrostatic contour map shows a large region of red polyhedrals and a small blue area below phenyl ring in position 6 of 1,2-dihydropyridines, indicating that electron-rich substituents in ortho position of phenyl ring are beneficial for the activity as shown for **2** in panel (C), This is also the case with **3**, **5**, **10** and **11**. While electron rich substituent separated from meta position by one c-c bond exerts a negative impact on the inhibitory activity as shown for **15** in panel (D). The red maps positioned on the right side

are due to phenyl rings in position 6 of 1,2-dihydropyridine with electron rich substituents in p-position have very high activity, this is the case with **6,8** and **12**.

3.4.2. CoMSIA—In Fig. (3), as well as in the following discussion, the CoMSIA contour plots are exemplified by ligands of low and high affinity. It becomes obvious from a direct comparison of Figs. (2 and 3) that steric and electrostatic properties in CoMFA and CoMSIA show a high degree of similarity, however, a certain degree of complementary information can be found.

3.4.2.1. Steric Contributions: In panel (A), green and yellow isopleths are drawn at a contribution level of 80% and 20%. Areas indicated by green contours correspond to regions where steric occupancy with bulky groups should increase anticancer activity. Areas encompassed by yellow isopleths should be sterically avoided; otherwise, reduced anticancer activity can be expected.

As in the CoMFA, the green isocontour in the middle found in Fig. (3A and B) is located very near to substituent in *ortho* position of phenyl group located in C-4 of 1,2-dihydropyridines such as compound **2**. The green isopleth positioned on the right side can also be explained by a number of high activity ligands (**1–3, 5, 7, 10 & 11**) bearing an *ortho* or *meta* substituent in this region. The low activity of the 2-nitrofuryl derivative **17** could be due to the orientation of its nitro group to the left sided lower yellow region. Accordingly, the ethoxy groups of **12** and **13** are pointing toward the upper yellow area.

3.4.2.2. Electrostatic Contributions: Red and blue isopleths (contribution level 90%: 20%) enclose regions favorable for negative and positive charge, respectively. In panels C and D, the electrostatic property maps include **2** and **12** as examples for high and low activity ligands, respectively. A large, red isopleth is located at the *ortho* and *meta* positions of the right-sided phenyl rings of ligands showing high anticancer activity (**2 & 3**) (Fig. 3C). The small blue map positioned on the left side indicates where electron-deficient substructures should be placed. For **12 & 13** the blue mesh is completely buried in the transparent red electrostatic potential at the oxygen of ethoxy groups. The red isopleth positioned on the left side exhibits the influence of the oxygen of methoxy groups of the active ligands.

3.4.2.3. Hydrophobic Contributions: Yellow and orange isopleths (contribution level 80%: 20%) enclose regions favorable for hydrophobic and hydrophilic groups, respectively. The hydrophobic effect on the activity can be drawn from panels E and F, suggesting that occupation of the *ortho* or *meta* positions of the phenyl ring on C-6 of 1,2-dihydropyridines by a hydrophobic group is crucial for a highly active ligand, as illustrated by compounds **2, 3** and **5** and **1&7**, respectively, while the orange mesh on the left is due to the methoxy group of **2, 3** and **6**. The ligands, which have very reduced activity, have 4-bromo substituents in the small orange isopleth on the right side, which is exemplified by ligands **16–18**.

3.4.2.4. Hydrogen Bond Donor Contributions: The graphical interpretation of the field contributions of the H-bond donor (from CoMSIA) is shown in panels G and H. Cyan isopleth contour maps (contribution level 80%) are representing the position of H-bond donor groups which favor biological activity, while purple isopleths (20%) are outlining the

location of biologically unfavored donors. In principle, they should highlight the areas beyond the ligands where putative partners in the target can form a hydrogen bond that will influence the biological activity significantly. For the active anticancer ligand **2**, the cyan transparent area of the NH of 1,2-dihydropyridines is positioned on the cyan mesh, however for less active ligands, the cyan transparent near OH of **16**, **19**, **22** and **24** is aligned on the purple mesh.

3.5. Prediction of Novel, Anticancer Compounds

Based on our initial approach toward the development of anticancer compounds^{6,8} this work focused on the rational prediction of novel anticancer ligands with improved biological activity. Consequently, we chose the highly potent **2** (logIC₅₀ 0.39 μ M) as an interesting lead compound for the structural design of potential anticancer agents. We examined further substitution patterns by replacing ortho-methoxy by an ortho & para dichloro substituents, and also transferring bromine from ortho to meta position of phenyl nucleus, to obtain the 1,2-dihydropyridine derivative **36** and only transferring bromine from ortho to meta position of phenyl nucleus to obtain **37**.

When the target compounds **36** and **37** were predicted employing the CoMFA and CoMSIA model, **36** was suggested to have a logIC₅₀ of -0.96/-0.77 as predicted from CoMFA/CoMSIA, respectively. The other derivative **37** was supposed to give a logIC₅₀ of 0.25/ 0.5 by CoMFA/CoMSIA prediction, respectively. At this point, we were ready to proceed with the synthesis of the two novel ligands **36** and **37** to verify our aforementioned predictions by in vitro biological testing of the ligands.

3.6. Chemistry

The general synthesis of the two compounds **36** and **37**, using the in-solution one pot synthesis approach. Briefly, the aromatic ketone 3-bromoacetophenone, the respective aromatic aldehyde, ammonium acetate and malononitrile were refluxed in ethanol for 15–24 hours. The afforded compounds were purified by recrystallization from a mixture of DMF-Ethanol in different ratios.

In ¹H-NMR, successful formation of the two compounds has been indicated by the appearance of a singlet peak around 6.90 ppm corresponding to the aromatic proton at position 5. Compound **37** showed a singlet peak with a chemical shift ranging between 3.82 ppm due to the methoxy substituent at position 4 of the 1,2-dihydropyridine ring.

Mass Spectrometry of the synthesized compounds showed the molecular ion peaks were also the base peaks indicating the stable nature of these compounds. As the synthesized compounds possess a 3-bromophenyl group, accordingly their mass spectrometry displays a common feature involving the molecular ion peak [M⁺] and [M⁺ +2] indicating the isotopic nature of the bromine atom. Infrared spectra showed a band at 3320–3480 cm⁻¹ for the NH stretching and a band at 2210–2220 cm⁻¹ for the CN stretching.

3.7. Biological Testing

The two synthesized compounds were tested for their in vitro ability to inhibit the growth of human HT-29 colon adenocarcinoma tumor cells (Table 4). The two compounds were evaluated for the growth inhibitory activity in two steps. The first step was determination of the percentage of inhibition at 50 μ M performed in triplicate. For compounds displaying a percentage of inhibition greater than 70 % at the screening dose, the exact IC_{50} was determined by testing a range of 10 concentrations with at least two replicate per concentration.

The obtained experimental results showed to be in very good consistence with our previous CoMFA/CoMSIA predictions. All deviations were found to be below 0.5 log IC_{50} units.

4. CONCLUSION

In the current work, we have successfully established CoMFA and CoMSIA models based on a training set of 30 ligands. We have carefully evaluated the statistical significance of the models and found high q^2_{cv} values of 0.70 (4 components) and 0.639 (4 components) for CoMFA and CoMSIA, respectively, when using the standard leave-one-out cross-validation method. Even for the more critical leave-20%-out method, q^2_{cv} was only slightly reduced yielding still highly significant mean values ($n = 20$ runs) of 0.696 for CoMFA and 0.639 for CoMSIA. The models were verified to be stable and robust against the variation of the underlying parameters. Thus, an all placement search (APS) yielded only CoMFA models in the rather narrow range between 0.685–0.720. Using a test set of 5 ligands, we were able to demonstrate that the models are also predictive for new compounds. This was extended to the successful application of our models guiding the synthesis of novel, active derivatives (36 & 37). The theoretical investigations presented in this study provide a valuable tool for predicting the affinity of novel compounds and, thus, for guiding and evaluating further structural modifications.

5. EXPERIMENTAL

5.1. Methods and Materials

All reactions were performed with commercially available reagents and they were used without further purification. Solvents were dried by standard methods and stored over molecular sieves. All reactions were monitored by thinlayer chromatography (TLC) carried out on precoated silica gel plates (ALUGRAM SIL G/UV254) and detection of the components was made by short and long UV light. Melting points were determined in open capillaries using a Buchi Melting Point B-540 apparatus and are uncorrected. 1H NMR spectra were recorded on Varian spectrometer at 300 MHz using tetramethylsilane (TMS) as internal reference. Chemical shift values are given in ppm at room temperature using DMSO- d_6 as a solvent; chemical shifts (δ) were reported in parts per million (ppm) downfield from TMS; multiplicities are abbreviated as: s: singlet; d: doublet; q: quartet; m: multiplet; dd: doublet of doublet; br s: broad singlet. Yields are not optimized. Elemental analyses were performed by Institute of Organic Chemistry, Jena University; and the Micro-

analytical Unit, Faculty of Science, Cairo University; found values were within $\pm 0.4\%$ of the theoretical ones, unless otherwise indicated.

5.2. 3-Cyano-4-(2,4-dichlorophenyl)-6-(3-bromophenyl)-2-imino-1,2-dihydropyridine (36)

A mixture of 3-bromoacetophenone (0.5 g, 2.5 mmol), 2,4 dichlorobenzaldehyde (2.5 mmol), malononitrile (0.16 g, 2.5 mmol) and ammonium acetate (1.54 g, 20 mmol) in absolute ethyl alcohol (30 ml) was heated under reflux, with stirring for 15–24 h. The reaction mixture was cooled and the formed precipitate was filtered, washed with cold ethyl alcohol, allowed to dry, and crystallized from DMF/ethanol 1:2.

Yield 56%, pale brown powder; mp 189–91 °C; IR (cm^{-1}) 3321 (–NH), 2224 (–C=N); $^1\text{H-NMR}$: δ 6.95 (s, 1H, aromatic), 7.22–7.93 (m, 4H, aromatic +–NHs), 8.04 (s, 1H, aromatic), 8.09 (s, 1H, aromatic), 8.16–8.19 (d, 1H, aromatic), 8.30–8.33 (d, 1H, aromatic), 8.37 (s, 1H, aromatic); MS-EI m/z 420 (M^+ ; 100%), m/z 422 (M^++2), m/z 424 (M^++4); Anal ($\text{C}_{18}\text{H}_{10}\text{BrCl}_2\text{N}_3$) calcd. C 51.58, H 2.40, N 10.03; found C 51.88; H 2.69; N 10.27

5.3. 3-Cyano-4-(2-methoxyphenyl)-6-(3-bromophenyl)-2-imino-1,2-dihydropyridine (37)

A mixture of 3-bromoacetophenone (0.5 g, 2.5 mmol), 2-methoxy benzaldehyde (2.5 mmol), malononitrile (0.16 g, 2.5 mmol) and ammonium acetate (1.54 g, 20 mmol) in absolute ethyl alcohol (30 ml) was heated under reflux, with stirring for 15–24 h. The reaction mixture was cooled and the formed precipitate was filtered, washed with cold ethyl alcohol, allowed to dry, and crystallized from DMF/ethanol 1:2.

Yield 80%, red powder; mp 274–76 °C; IR (cm^{-1}) 3482.3 (–NH), 2205.8 (–C $\equiv$ N); $^1\text{H-NMR}$: δ 3.89 (s, 3H, –OCH₃), 7.06 (s, 1H, aromatic), 7.20–7.54 (m, 5H, aromatic), 7.61–7.64 (d, 1H, aromatic), 7.72–7.75 (d, 1H, aromatic), 7.81 (s, 1H, aromatic); MS-EI m/z 379 (M^+ ; 100%), m/z 381 (M^++2); Anal. ($\text{C}_{19}\text{H}_{14}\text{BrN}_3\text{O}$) calcd. C 60.02, H 3.71, N 11.05; found C 60.33; H 4.04; N 11.23.

5.4. Biology

5.4.1. Cell Cultures—HT-29 tumor cells were obtained from ATCC. They were grown under standard cell culture conditions at 37°C in a humidified atmosphere with 5% CO₂. Cells were grown in RPMI 1640 containing 5% fetal bovine serum. Cell count and viability was determined by Trypan blue staining followed by hemocytometry. Only cultures displaying >95% cell viability were used for experiments.

5.4.2. Growth Assays—Tissue culture treated microtiter 96-well plates were seeded at a density of 5000 cells/well. The plates were incubated for 18–24 h prior to any treatment. Cell viability was measured 72 h after treatment by the Cell Titer Glo Assay (Promega), which is a luminescent assay that is an indicator of live cells as a function of metabolic activity and ATP content. The assay was performed according to manufacturer's specifications.

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Fig. (1).

Histogram showing the distribution of q^2 values calculated by SAMPLS²⁵ leave-one-out cross-validation after systematic translation of aligned molecules within the lattice by an all placement search (APS).

Fig. (2).

Stdev*coeff contour plots illustrating steric (A and B) and electrostatic features (C and D) as obtained by the final CoMFA. In (A) and (B), regions where steric bulk will enhance affinity are shown enclosed by green contours (contribution level: 80%), whereas regions which should be kept unoccupied to prevent decrease of affinity are contoured in yellow (20%). This is exemplified by the high activity ligand **2** in (A) and the lower activity ligand **17** in (B). In (C) and (D), red contours (contribution level: 20%) encompass regions where electron-rich fragments with negative partial charges will improve activity. Blue contours (80%) indicate regions where reduced electron density (positive partial charges) is predicted to increase activity. Again, compounds **2** (C) and **15** (D) are used to exemplify the plots for a high and lower affinity ligand, respectively.

Fig. (3).

Stdev*coeff contour plots illustrating steric (A + B), electrostatic (C + D), hydrophobic (E + F), and hydrogen bond donor (G + H) properties revealed by the final CoMSIA. For all features, one ligand with high (A: 2, C:2, E: 2, G: 2) and another with low activity (B: 17, D: 12, F: 17, H: 22) are shown in comparison. The mesh fields represent the stdev*coeff plots, whereas the transparent surfaces indicate the fields of the particular ligand, thus, facilitating the recognition of matching or mismatching features.

1-3, 5-7, 10-12, 14-16 and 18-37

4 and 9

13

Scheme 1.

8 and 17

3-cyano-4,6-diaryl-2-(1H)imino or oxo-1,2-dihydropyridines

Table 1

Data Set of 30 Compounds in the Training Set and 5 Compounds in the Test Set and their Experimental and Predicted Activity ^a

Cpd.	R1	R2	R3	R4	X	logIC ₅₀ (Exp.)	logIC ₅₀ ^a	
							CoMFA	CoMSIA
Training set								
1	3-Br	-	Cl	4-Cl	NH	0.329	-0.027	0.308
2	2-Br	-OCH ₃	-	-	NH	0.397	-0.304	-0.561
3	2-Br	-OCH ₃	-	-	O	0.436	-0.439	-0.212
4	4-OCH ₃	-	-	-	-	0.438	0.482	0.531
5	2-Br	-	-	4-Cl	NH	0.491	-0.348	0.536
6	4-Br	-OCH ₃	-	5-OCH ₃	NH	0.600	-0.247	-0.653
7	3-Br	-	-	4-OCH ₃	NH	0.633	0.217	0.043
8	4-Br		-	-	-	0.780	-0.666	-0.452
9	2-OCH ₂ CH ₃	-	-	-	-	0.807	-0.533	0.546
10	2-Br	Cl	-	-	NH	1.016	0.091	0.031
11	2-Br	Cl	-	-	NH	1.017	0.463	0.483
12	4-Br	-	-	4-OCH ₂ CH ₃	NH	1.060	0.102	-0.369
13					-	1.080	0.293	-0.129
14	3-Br	Cl	-	-	NH	1.114	-0.319	0.303
15	3-CF ₃	Cl	-	5-Cl	O	1.113	0.004	0.119
16	4-Br	-OH	-	-	O	1.322	0.526	0.446
17	4-Br		-	-	-	1.430	-0.424	0.044
18	4-Br	-OCH ₃	-		NH	1.700	0.356	-0.151

Cpd.	R1	R2	R3	R4	X	logIC ₅₀ (Exp.)	logIC ₅₀ ^a	
							CoMFA	CoMSIA
19	4-F	-OH	-	-	O	1.281	0.000	0.320
20	4-F	-OCH ₃	-	-	NH	1.965	0.256	0.018
21	3-F	-OCH ₃	-	-	O	1.868	0.325	0.404
22	3-F	-OH	-	-	NH	1.164	-0.467	0.130
23	4-F	OCH ₂ CH ₃	-	-	NH	1.972	0.028	0.364
24	2-F	-OH	-	-	O	0.591	-0.444	-0.317
25	3-F	OCH ₂ CH ₃	-	-	NH	1.996	-0.062	0.293
26	2-F	OCH ₂ CH ₃	-	-	NH	1.972	0.432	0.401
27	2-F	-OCH ₃	-	-	O	1.352	0.229	-0.132
28	3-F	OCH ₃	-	-	NH	1.994	0.256	0.084
29	2-F	-	Cl	4-Cl	NH	0.76	0.276	0.458
30	3-F	-	Cl	4-Cl	NH	0.597	0.014	0.289
Test set								
31	4-Br	Cl	-	4-Cl	O	0.681	-0.359	-0.190
32	3-Br	Cl	-	-	O	1.113	-0.160	-0.466
33	2-F	OH	-	-	NH	0.813	0.179	-0.301
34	3-F	-OCH ₂ CH ₃	-	-	O	1.779	-0.749	-0.368
35	2-F	OCH ₃	-	-	NH	1.969	-0.041	0.059

^a logIC₅₀ is the error of fitted activities = [logIC₅₀ experimental - logIC₅₀ fitted].

Table 2
Summary of the Results from Several PLS Runs after Applying Different Levels of Noise Reduction by Column Filtering ($\sigma_{\min}$)

PLS Run	CoMFA						CoMSIA					
	A	B	C	D	E		F	G	H	I	J	
$\sigma_{\min}$	1	2	3	4	5		1	2	3	4	5	
q^2_{cv}	0.700	0.700	0.702	0.704	0.680		0.637	0.639	0.642	0.648	0.655	
S _{PRESS}	0.479	0.465	0.455	0.443	0.428		0.533	0.529	0.528	0.524	0.515	
r^2	0.945	0.947	0.947	0.947	0.932		0.964	0.965	0.966	0.966	0.967	
S	0.267	0.265	0.262	0.263	0.292		0.233	0.232	0.229	0.227	0.226	
F	208.0	211.9	215.6	215.1	170.2		141.1	142.3	146.4	150.0	151.4	
Components	4	4	4	4	4		4	4	4	4	4	
Descriptors	3544	2241	1682	1393	1231		6581	4626	3503	2740	2110	
Fraction												
Steric	0.723	0.704	0.671	0.645	0.639		0.148	0.148	0.160	0.187	0.215	
Electrostatic	0.277	0.296	0.329	0.355	0.361		0.255	0.255	0.256	0.264	0.264	
Hydrophob							0.341	0.341	0.334	0.316	0.306	
Donor							0.256	0.256	0.252	0.241	0.241	

Table 3Summary of the CoMSIA Models^a

PLS Statistic	SEHDA	SEHD	SED
q^2_{cv}	0.629	0.639	0.655
S_{PRESS}	0.512	0.529	0.515
r^2	0.965	0.965	0.967
S	0.232	0.232	0.226
F	187.6	142.3	151.4
Components	4	4	4
Descriptors	5427	4626	2907
Fraction			
Steric	0.118	0.148	0.158
Electrostatic	0.259	0.255	0.267
Hydrophob.	0.189	0.341	
Donor	0.232	0.256	0.222
Acceptor	0.201		

^aS, steric; E, electrostatic; H, hydrophobic; D, donor; A, acceptor

Table 4

Inhibitory Effect of the Synthesized Compounds (36 and 37) on HT-29 Cells

<div> <chem>N#Cc1nc(N)c(c1C2=CC=C(C=C2)Br)C3=CC(=CC=C3)Cl</chem> 36</div> <div> <chem>N#Cc1nc(N)c(c1C2=CC=C(C=C2)Br)C3=CC(=CC=C3)OC</chem> 37</div>		
Cpd.	% Growth Inhibition at 50 μ M	Growth Inhibition IC ₅₀ (μ M)
36	99.5	0.24
37	31.14	2.6