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Molecular Modeling of 3-Arylisoquinoline Antitumor Agents Active Against A-549. A Comparative Molecular Field Analysis Study

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Abstract—A series of 58 3-arylisoquinoline antitumor agents were investigated for defining the pharmacophore model using comparative molecular field analysis (CoMFA) program. The studied compounds related to bioisostere of benzophenanthridine alkaloid were synthesized and evaluated for antitumor cytotoxicity against human lung tumor cell (A 549). In order to perform the systematic molecular modeling study of these compounds, the conformational search was carried out based on the single X-ray crystallographic structure of 7,8-dimethoxy-3-phenylisoquinolin-(2*H*)-one (**2**). Interestingly, two types of structures having different dihedral angles between the isoquinoline ring and 3-aryl ring were found in the crystals. Therefore, CoMFA was performed two different, overlapping ways. The alignments of the structures were based on the common isoquinoline ring and 3-aryl ring. The 3-D-quantitative structure–activity relationship study resulted in significant cross-validated, conventional r^2 values equal to 0.715 and 0.927, respectively.

Introduction

New chemotherapeutic agents for a treatment of cancer from natural compounds have been developed over the last decade.[1] With the finding of potent antitumor activity of 7,8-dimethoxy-2-methyl-3-(4,5-methylenedioxy-2-vinylphenyl)isoquinolin-1(2*H*)-one (**1**) which is a synthetic intermediate for natural benzo[*c*]phenanthridine alkaloid, chelerythrine, we tried to study structure–activity relationships of 3-arylisoquinolines against human tumor cell lines.[2] Most of the 3-arylisoquinoline derivatives exhibited potent cytotoxicities against five different human tumor cell lines. The styrene (**1**) is considered to be a bioisostere of benzo[*c*]-phenanthridine which has exhibited potent antitumor activity by inhibiting DNA topoisomerase I or II.[2] From earlier studies, we could obtain the results that the amide carbonyl group of isoquinoline ring and alkyl group on aromatic rings played an important role for exhibiting the activities.[3] In order to increase water solubility of the compound, the amide carbonyl group was modified to various amines which could be converted to salt forms because the water solubility of the drugs should be considered for in vivo or clinical experiments. As we expected, the modification of carbonyl amide group to amines gave relatively potent cytotoxicites against several tumor cell lines as well as good water solubility. Among these compounds, 1-(4-methylpiperazinyl)-3-phenylisoquinoline hydrochloride (CWJ-a-5) was tested in vivo assay using BDF1 mice (P388 leukemia) and resulted in 160 T/C% with low toxicity.[3] Moreover, the high oral bioavailability of CWJ-a-5 indicated the possibility of it being developed into an oral formulation. Also, the pharmacokinetic data served as useful information in future clinical studies of CWJ-a-5.[4]

CoMFA, which is a ligand-based drug design skill, is not only one of the most used 3-D-QSAR methods, but also has been applied to a number of different classes of compounds.[5]

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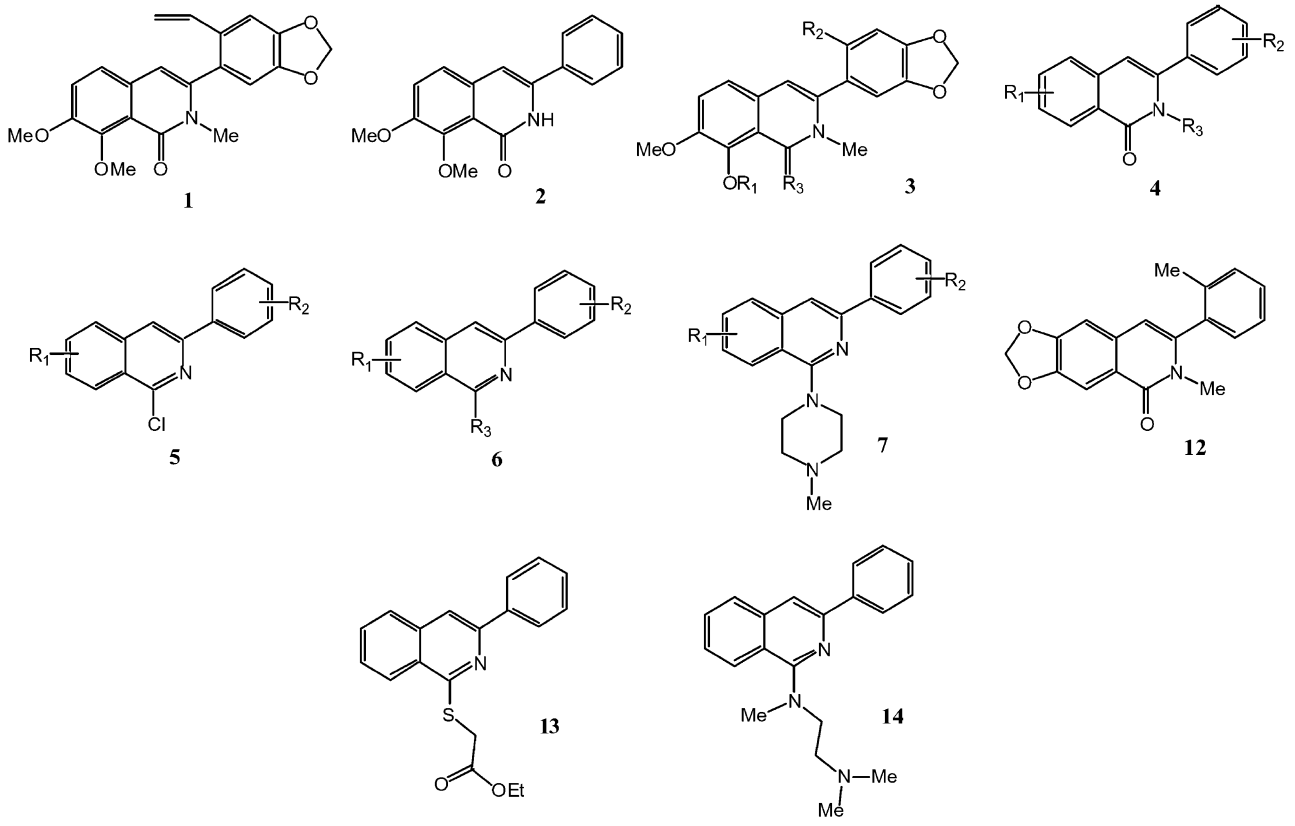


Scheme 1. The structure of 3-arylisoquinolines.

In the previous paper, we reported a comparative molecular field analysis (CoMFA) result of 29 3-arylisoquinolines with human melanoma tumor cell (SK-MEL-2) to produce a hypothetical pharmacophore model.[6] In the present study, the model for A-549 tumor cell line is extended to include various substituents of the aromatic ring as well as C_1 position because it is well recognized that 3-D QSAR study of diverse modified molecules, which show a wide range of activities, affords a reliable pharmcophore model. Therefore, new molecules were designed and synthesized based on the postulated pharmacophore model derived from former CoMFA study. Moreover, we tried to determine the X-ray crystallographic structure of 7,8-dimethoxy-3-phenylisoquinolin-(2*H*)-one (**2**) for getting the information of the torsion angle of 3-aryl ring, which is considered to be crucial for performing CoMFA of the 3-arylisoquinoline derivatives (Scheme 1). Five different CoMFA pharmacophore contour maps obtained using A-549, SK-OV-3, SK-MEL-2, XF 498 and HCT 15 tumor cell lines showed very similar patterns. Although the target sites of action of the compounds were not clarified yet, we assumed that they share the same binding sites because of their closely related structures.[7]

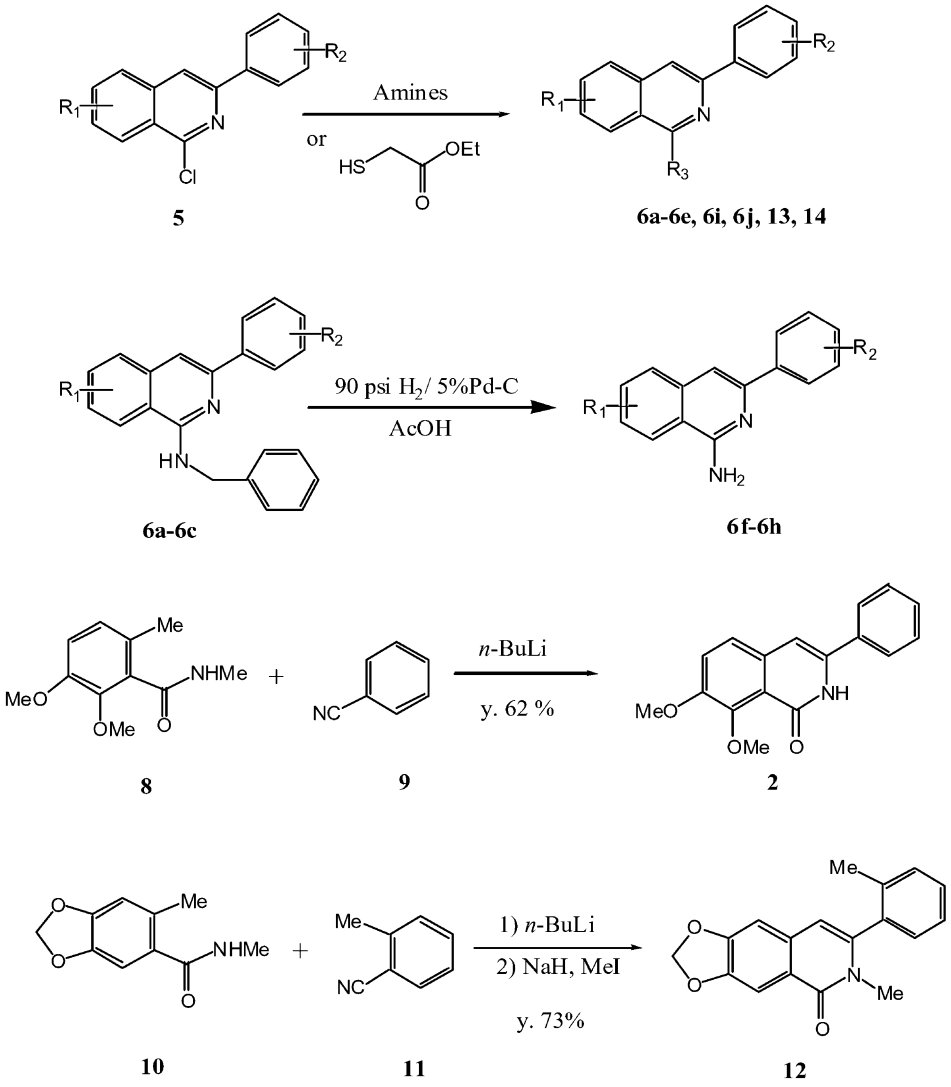
Methods

Synthesis

Synthesis of diverse 3-arylisoquinoline analogues is described in Scheme 2. The imine chloride (**5**)[6] derived from the corresponding amide was treated with various amines or thioacetic acid in refluxing DMF to provide the 1-substituted isoquinolines in good yield. For the preparation of primary amines (**6f–6h**), the benzylamines (**6a–6c**) was performed to a catalytic hydrogenation reaction with 5% Pd/C in 80 psi hydrogen atmosphere to afford the corresponding amines in moderate yield. The coupling reaction of 2,3-dimethoxy-6,*N*-dimethylbenzamide (**8**) with benzonitrile (**9**) was accomplished via dilithio species using *n*-BuLi in 62% yield. The cyclization reaction of 3,4-methylenedioxy-6,*N*-dimethylbenzamide (**10**) and 2-methylbenzonitrile (**11**) was also performed to yield the corresponding isoquinoline which was then treated with MeI to give the *N*-methylated isoquinoline (**12**) in 63% yield.

Biological data

Cytotoxicity (IC_{50} value) was obtained by the following method: All experimental procedures were followed up using the NCI (USA)'s protocol based on the sulforhodamine B (SRB) method.[8] Briefly, tumor cells were cultured to maintain logarithmic growth by changing the medium 24 h before cytotoxicity assays. On the day of the assay, the cells were harvested by trysinization, counted, diluted in media and added to 96-well plates. The concentration of tumor cells (A-549) used was 1×10^4. The cells were then preincubated for 24 h in a 5% CO_2 incubator at 37 °C. The compounds dissolved in DMSO were added to the wells in six 2-fold dilutions starting from the highest concentrations, and incubated for 48 h in a 5% CO_2 incubator at 37 °C. The final



Scheme 2. Synthesis of 3-arylisoquinoline derivatives.

DMSO concentration was 0.05%. At the termination of the incubation, the culture medium in each well was removed, and the cells were fixed with cold 10% trichloroacetic acid (TCA) for 1 h at room temperature. The microplates were washed, dried, and stained with 0.4% SRB in 1% acetic acid for 30 min at room temperature. The cells were washed again and the bound stain was solubilized with 10 mM Tris base solution (pH 10.5), and the absorbances were measured spectrophotometrically at 520 nm on a microtiter plate reader (Molecular Devices, Sunnyvale, CA, USA). The data was transformed into Lotus-123 format and survival fractions were calculated by regression analysis (plotting the cell viability versus the concentration of the test compound). The IC_{50} values represent the concentrations of the compounds that inhibit 50% of cell growth. All data represents the average values for a minimum of three wells (Table 1).

X-ray crystallographic structures and superimposition

In order to assign the torsion angle of 3-arylisoquinolines, we carried out a single crystal X-ray analysis of 7,8-dimethoxy-3-phenylisoquinolin-1(2*H*)-one (**2**). Yellowish transparent prisms were obtained from an ethylacetate solution. The crystal was mounted on a MacScience KAPPA goniometer equipped with 18KW rotating anode. Cell constants were determined as follows: monoclinic crystal system, $a = 13.804(2)$, $b = 15.683(4)$, $c = 12.632(2)$ Å, $\beta = 93.87(2)°$, space group; $P2_1/a$, $Z = 8$. A total of 4403 independent reflections were measured. The structure was solved by direct method and refined by full matrix least-squares procedure to the final *R*-value of 0.054 with maXus1.1 software package.[9] There are two independent molecules in an asymmetric unit of the crystal as shown in Fig. 1. Two types of conformers having different dihedral angles between isoquinoline and 3-aryl rings were found in crystal. As depicted, there are three torsion angles on the molecules such as conformer 1 ($\tau 1$: $-150.0°$, $\tau 2$: $95.0°$, $\tau 3$: $-179.4°$) and conformer 2 ($\tau 1$: $174.6°$, $\tau 2$: $99.1°$, $\tau 3$: $2.2°$) shown on the right and left side, respectively, in Figure 1.

As the initial conformations of the compounds, the X-ray crystallographic data of **2** was utilized. Due to the

two different conformations of the molecule **2**, we tried to use both of them as lead templates for determining the conformation of the molecules as shown in Figure 2. The conformations of the compounds seem to be affected by τ1 value of 3-aryl ring rather than others. To investigate the conformation profile, we carried out an extensive conformational search of the compounds using the torsion angle of conformers 1 and 2. First, the torsion angle of 3-aryl ring (τ1) in the compounds was fixed as the value (−150.0°) of conformer 1 and the conformation of each molecule was obtained after performing the energy minimization process. For the optimized coordinates, atomic charges were calculated using Gasteiger–Huckel. Secondly, by performing the above energy minimization process using τ1 (174.6°) of conformer 2, the active conformers of the compounds were also obtained. Each active conformer of the 58 compounds was superimposed with that of the reference molecules (conformer 1 and conformer 2 in Fig. 1) so that each structural component is as close as possible to the corresponding components of the reference. Thus, nine atoms, that is A ring and C3–C4 atoms and the C14 atom of C ring were selected, and the sum of the

Table 1. Observed and calculated cytotoxicity values against A-549 of the 3-arylisoquinoline derivatives

No	Compd	R1	R2	R3	PIC_{50}		
					Obsd	Calcd	Diff
1	**1**[a]	—	—	—	8.05	8.15	−0.10
2	**3a**[a]	Me	Et	H_2	5.89	6.20	−0.31
3	**3b**[a]	Me	Vinyl	H_2	5.72	5.80	−0.08
4	**3c**[a]	Me	$CH_2CH(OMe)_2$	O	6.49	6.52	−0.03
5	**3d**[a]	Me	Et	O	7.57	6.90	0.67
6	**3e**[a]	H	Et	O	4.93	5.27	−0.34
7	**4a**[b]	5-NMe_2	4-OMe	H	4.90	4.53	0.37
8	**4b**[b]	6-Me	4-Cl	H	5.24	4.85	0.39
9	**4c**[b]	H	2-PMB	H	4.12	4.29	−0.17
10	**4d**[b]	H	3-Me	H	4.39	4.45	−0.06
11	**4e**[b]	H	4-Br	H	4.21	4.43	−0.22
12	**4f**[b]	H	4-Me	H	4.52	4.42	0.10
13	**4g**[b]	H	4-OMe	H	3.81	4.32	−0.51
14	**4h**[b]	H	H	H	3.91	3.43	0.48
15	**4i**[c]	H	2-CHO	Me	3.87	3.75	0.12
16	**4j**[c]	H	2-CH_2OH	Me	3.84	3.95	−0.11
17	**4k**[c]	H	2-CH_2OPMB	Me	4.67	4.66	0.01
18	**4l**[c]	H	2-$CH_2CH(OMe)_2$	Me	3.69	3.66	0.03
19	**4m**[c]	H	2-vinyl	Me	4.22	4.15	0.07
20	**4n**[b]	H	H	Me	4.07	4.25	−0.18
21	**5a**[b]	5-NMe_2	4-Cl	—	3.84	3.89	−0.05
22	**5b**[b]	6-Me	2-Cl	—	3.88	3.85	0.03
23	**5c**[b]	6-Me	2-Me	—	3.58	3.86	−0.28
24	**5d**[b]	6-Me	4-Me	—	3.95	4.24	−0.29
25	**5e**[b]	6-Me	H	—	3.80	3.95	−0.15
26	**5f**[b]	H	3-Me	—	3.77	3.75	0.02
27	**5g**[b]	H	4-Br	—	3.95	3.71	0.24
28	**5h**[b]	H	H	—	3.82	3.44	0.38
29	**6a**[d]	6-Me	2-Me	Bn	4.56	4.39	0.17
30	**6b**[d]	H	2-Me	Bn	3.85	3.78	0.07
31	**6c**[d]	H	H	Bn	3.76	3.82	−0.06
32	**6d**[d]	6-Me	3-Me	Bn	6.13	6.28	−0.15
33	**6e**[d]	H	H	morpholine	3.91	3.96	−0.05
34	**6f**[d]	6-Me	2-Me	NH_2	6.76	6.25	0.51
35	**6g**[d]	H	2-Me	NH_2	5.50	5.70	−0.20
36	**6h**[d]	H	H	NH_2	5.56	5.80	−0.24
37	**6i**[d]	H	H	NH-PMB	3.61	3.59	0.02
38	**6j**[d]	H	H	piperidine	3.83	4.25	−0.42
39	**7a**[b]	5-NMe_2	2-Me	—	4.67	4.86	−0.19
40	**7b**[b]	5-NMe_2	3-Me	—	5.03	4.91	0.12
41	**7c**[b]	5-NMe_2	4-Br	—	5.04	5.17	−0.13
42	**7d**[b]	5-NMe_2	4-Cl	—	5.13	5.13	0
43	**7e**[b]	5-NMe_2	4-OMe	—	4.72	4.77	−0.05
44	**7f**[b]	5-NMe_2	H	—	4.83	4.62	0.21
45	**7g**[b]	6-Me	2-Me	—	5.03	4.86	0.17
46	**7h**[b]	6-Me	4-Cl	—	5.41	5.29	0.12
47	**7i**[b]	6-Me	4-Me	—	4.95	5.29	−0.34
48	**7j**[b]	6-Me	H	—	5.13	5.09	0.04
49	**7k**[b]	H	2-Me	—	4.23	4.30	−0.07
50	**7l**[b]	H	3-Me	—	4.67	4.94	−0.27
51	**7m**[b]	H	4-Br	—	4.73	4.65	0.08
52	**7n**[b]	H	4-Cl	—	4.68	4.59	0.09
53	**7o**[b]	H	4-Me	—	4.90	4.68	0.22
54	**7p**[b]	H	4-OMe	—	4.75	4.53	0.22
55	**7q**[b]	H	H	—	4.47	4.38	0.09
56	**12**[d]	—	—	—	4.63	4.52	0.11
57	**13**[d]	—	—	—	3.69	3.70	−0.01
58	**14**[d]	—	—	—	4.60	4.77	−0.17

[a]From ref 2.
[b]From ref 6.
[c]From ref 13.
[d]Newly synthesized compounds.

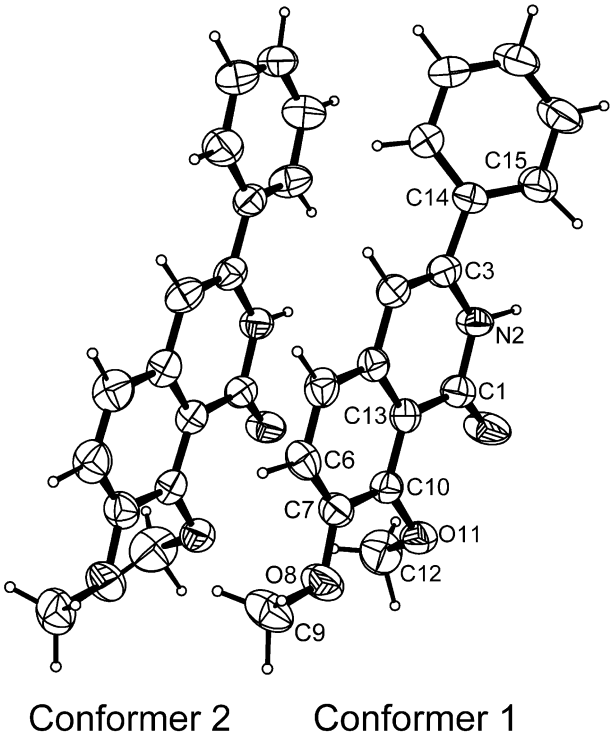


Figure 1. The crystal structure of 7,8-dimethoxy-3-phenylisoquinolin-1(2*H*)-one (**2**) drawn by ORTEP.[10] The thermal ellipsoids are drawn at the 50% probability level. Hydrogen atoms are drawn as small circles of arbitrary radii. Two independent molecules in the asymmetric unit are designated as Conformer 1 and 2, respectively.

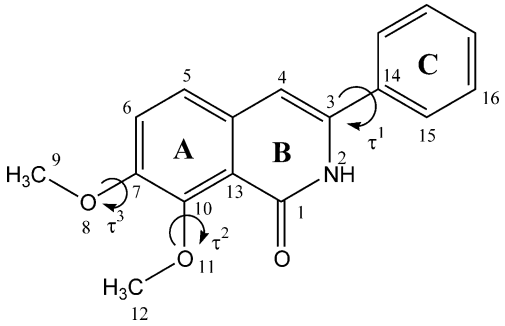


Figure 2. Torsion angles of 7,8-dimethoxy-3-phenylisoquinolin-1(2*H*)-one (**2**).

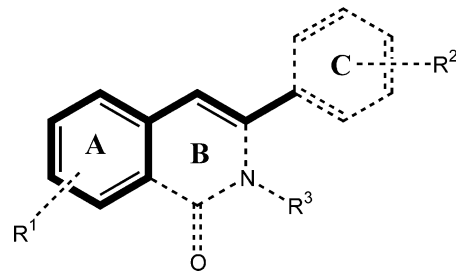


Figure 3. Template molecule for molecular superimposition.

squares of the distance of the nine atomic positions from the corresponding atomic positions of the reference compound was made as small as possible, as shown in Figure 3.

Computer modeling

All computations were done with the molecular modeling software package Sybyl 6.6 version.[11] The coordinates of the substructures to be modified in substitued derivatives were calculated with the Sybyl standard values for bond lengths and angles using the building module. For the conventional CoMFA, the region was first created automatically with the dimension of 18 × 18 × 18 Å ($X=-7$ to 11, $Y=-12$ to 6, $Z=-6$ to 12). When the region was manually defined for the molecules to be embedded in the grid box with dimension of 24 × 22 × 32 Å ($X=-10$ to 14, $Y=-13$ to 9, $Z=-18$ to 14), more reliable statistical values such as r^2_{cv} and SEP were obtained as shown in Table 2. From this result, it is suggested that the grid size should be defined considering that all of the molecules were adequately fitted in the lattice box. The steric and electrostatic potential energies at each lattice point were calculated using the Coulombic and Lennard–Jones potential function, respectively. The lattice spacing was 2.0 Å and the +1 charge and the sp^3 carbon were used as probes to estimate the electronic and steric fields, respectively with an energetic cutoff of 30 kcal/mol. Regression analyses were performed using the Sybyl implementation of the PLS algorithm, initially with cross-validation (the leave-one-out technique) to reduce the probability of obtaining chance correlation. The number of groups of cross-validation was set as equal to the number of components of the training set. The optimal number of latent variables (components) to be used in conventional analyses was chosen on the basis of the highest cross-validated r^2 (r^2_{cv}) value, the smallest standard error of prediction (SEP), and the minimum number of components. All leave-one-out calculations were carried out selecting a 2.0 kcal/mol energy column filter in order to enhance the signal-to-noise ratio. The steric and electrostatic field columns were obtained according to the COMFA-STD default scaling option. In this method, a field is considered as a whole and every CoMFA variable is affected by the overall field mean and standard deviation. Final PLS (non-cross-validated models) calibration equations were then derived using the optimal number of components so identified.

Table 2. Statistical comparison of the region defined method and Auto CoMFA with conformer 1 superposition

Principal components	Alignment I[a]		Alignment II[b]	
	r^2_{cv}	SEP	r^2_{cv}	SEP
1	0.023	0.855	0.001	0.865
2	0.331	0.714	0.281	0.741
3	0.537	0.600	0.517	0.613
4	0.624	0.546	0.540	0.604
5	0.697	0.495	0.616	0.558
6	0.715	0.485	0.632	0.552
7	0.685	0.491	0.639	0.552

[a]Region defined method; X, −7 to 11; Y, −12 to 6; Z, −6 to 12.
[b]Auto CoMFA method; X, −10 to 14; Y, −13 to 9; Z, −18 to 14.

pK_a values of amines

To validate whether or not amines are protonated under the physiological condition, we calculated their pK_a values using the empirical correlation eqs 1 and 2 for aliphatic amines ($NR_1R_2R_3$) reported by Takayama et al. and Hall, respectively.[12] Eq. 1 is for the primary and secondary amines, while eq 2 is for the tertiary amines.

$$pK_a = -3.606\ \Sigma\ \sigma^* + 0.335\left[E_s^c(R_2) + E_s^c(R_3)\right] + 1.427\ n\mathrm{H} + 9.545 \quad (1)$$

$$pK_a = -3.30\ \Sigma\ \sigma^* + 9.61 \quad (2)$$

In these equations, $\Sigma\ \sigma^*$ is the summation of the Taft values of three *N*-substituents. $E_s^c(R_2) + E_s^c(R_3)$ are, respectively, the Hancock corrected steric constants of two bulkier substituents R_2 and R_3 among three *N*-substituents, in which $R_1 = H$ and $E_s^c(R_2) \geq E_s^c(R_3)$, and nH is the number of hydrogen atoms in respective conjugate ammonium ions. σ^* and E_s^c values used in the calculations were taken from the literature.[12] The calculation with eqs (1) and (2) estimated the pK_a value as follows: **7a**–**7q**, 10.23; **6j**, 11.34. Thus, all the amines containing the *N*-methylpiperazinyl group, or primary amine, are reasonably considered to exist mostly in their protonated forms under the physiological pH conditions. Therefore, molecular modeling study of amines was done in their protonated forms.

CoMFA results

The CoMFA statistical results for the cytotoxic activities on A-549 are summarized in Table 3. In these equations, *m* indicates the number of latent variables and SEP is the standard deviation obtained from the leave-one-out cross-validation. RC refers to the relative contribution of steric and electrostatic effects to variations in antitumor activity. To determine whether the hydrophobic parameter is significantly involved, cLogP values were inserted in the CoMFA analyses. However, their contribution for the equation was less than 2% in

Table 3. CoMFA correlation statistics for 3-arylisoquinolines ($n = 58$)

Type	m^a	N[b]	S[c]	F	r^2	Cross-valid[d]		RC[e]		
						SEP	q^2	Steric[f]	Elec[g]	Eq no.
Conformer 1 Superposition/ defined region	6	59	0.251	96.804	0.924	0.485	0.715	40.1	59.9	(3)
Conformer 2 Superposition/ defined region	6	59	0.237	109.752	0.932	0.652	0.486	46.2	53.8	(4)
Conformer 1 Superposition/ auto CoMFA	7	59	0.251	96.804	0.924	0.552	0.639	43.2	56.8	(5)

[a]Number of components.
[b]Number of compounds.
[c]Standard deviation.
[d]Obtained from the leave-one-out cross-validation.
[e]Relative contribution.
[f]Steric field descriptors.
[g]Electrostatic field descriptors.

most cases. So, we initially analyzed for 58 compounds and obtained eqs 3–5. Among these equations, eq 3, which was derived from the overlapping of the compounds on X-ray conformer 1 as for a lead template, was the most significant. On the other hand, eq 4 was obtained from X-ray conformer 2 which gave poorer statistical results. Therefore, we assumed that conformer 1 is the active form for showing the cytotoxicity and the molecules should have a $-150°$ torsion angle of 3-aryl ring. Therefore, the observed pIC_{50} values in Table 1 were calculated from eq 3. Figure 4 represents the major steric and electrostatic potential contour maps drawn according to eq 3 after CoMFA. The green zone is more important and located around the benzene ring of the isoquinoline skeleton according to the higher activity of the molecules. That area indicates regions where submolecular bulk is well accommodated with an increase in cytotoxicity, whereas the yellow areas indicate regions where the submolecular bulk is unfavourable for activity. The red area indicates regions where the more negative electrostatic interaction with the receptor increases the activity, whereas the blue areas show regions where the reverse is the case.

In a contour map, a positive electrostatic-potential region, favoring to activity, appears around the nitrogen atoms of the *N*-methylpiperazinyl group or primary amines, whereas a negative electrostatic region, favorable to the activity, is located at the oxygen atoms of the carbonyl group and 7,8-dimethoxy group. A sterically forbidden region exists at the C-5 position which includes the dimethylamino group and *N*-methylpiperazinyl group at C-1. The compounds which have substituents on C-5 position are less active because their residues are extended away from the favored region of interaction. On the other hand, a sterically permissible region appears at the C-6 position. It is well explained that methyl substitution at the C-6 position contributes to the increase of activity.

Discussion

The application of the comparative molecular field analysis to a structurally varied set of 58 antitumor agents resulted in a significant 3-D QSAR model. For getting higher statistical data, a grid box was constructed without using the Auto CoMFA method. This work extends our previous analysis performed on a congeneric set of isoquinoline analogues and constitutes a further step towards a comprehensive 3-D QSAR model of the cytotoxic activities against A 549 tumor cell line.

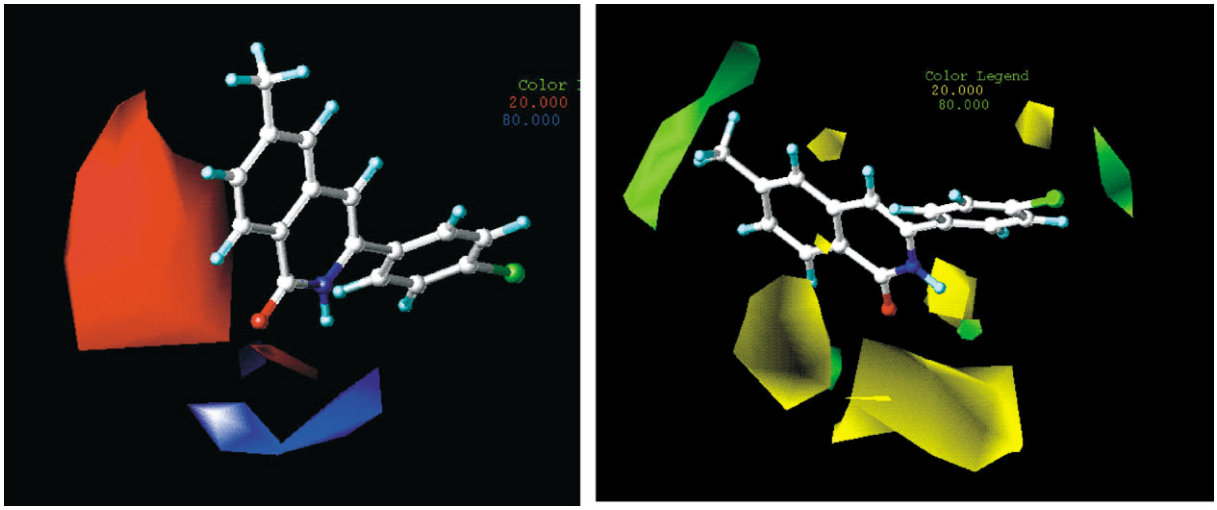


Figure 4. Steric and electrostatic contour map from the CoMFA model for antitumor 3-arylisoquinolines. Favoring activity: green, bulky group; yellow, less bulky group; blue, positive charge; red, negative charge.

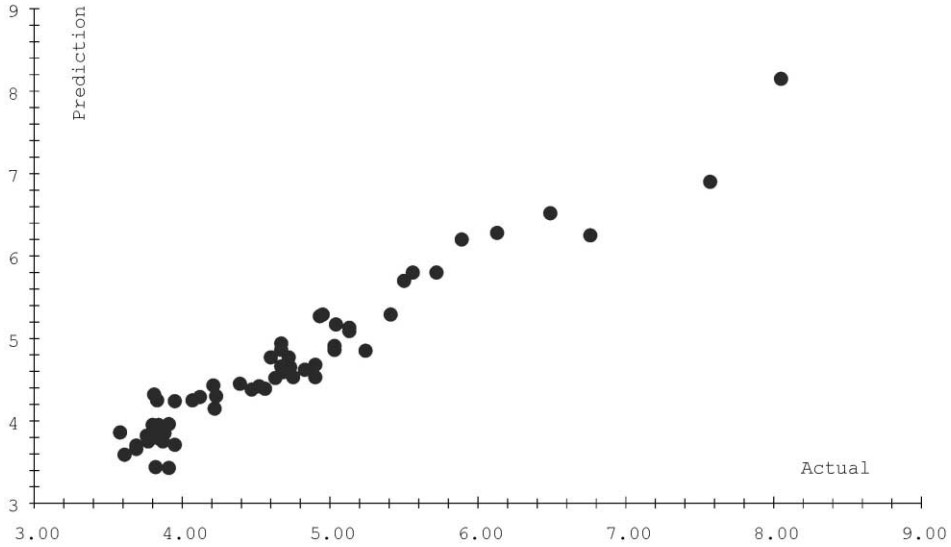


Figure 5. Plot of the calculated versus observed pIC_{50} for the CoMFA analysis of the 58 compounds aligned according to eq 3.

The descriptive characteristics of the present model are good and the plot of the calculated versus the observed pIC_{50} for the CoMFA of the 58 compounds is shown in Figure 5.

As regards the steric contours, they show a main region of positive (green) contribution to the activity that surrounds the 8-methyl group area and this is in agreement with the cytotoxic activities of the compounds. The most active compounds such as **1**, **3d** and **6f** could also indicate favorable steric interactions of these structural elements with a large hydrophobic pocket in the active site. The 7,8-dimethoxy group and the amide carbonyl group at the C-1 position were indicated as suitable for electronegative substitution. The reliability of a QSAR model is usually evaluated by testing its ability to predict the biological activity of newly designed molecules acting on the same biological system. From this study the CoMFA model not only exhibited that the cytotoxicities of the 58 isoquinoline derivatives had an excellent correlation with the electrostatic and steric field, but also provided us with an important pharmacophore model which could be useful to consider the receptor sites three dimensionally.

Experimental

Melting points were determined on an Electrothermal IA9200 melting point apparatus and are uncorrected. Nuclear magnetic resonance spectra (^{1}H NMR) were recorded on Varian 300 spectrometers, using TMS as the internal standard ; chemical shifts are reported in parts per million (δ) and signals are quoted as s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). IR spectra were recorded on a Perkin-Elmer 783 spectrometer and a Nicolet instrument using KBr pellets. Elemental analyses were performed on a CaHo Erba elemental analyser. Anhydrous tetrahydrofuran (THF) was distilled from sodium-benzophenon ketyl. Solvents were routinely distilled prior to use. Column chromatography was performed on Merck silica gel 60 (70–230 mesh). TLC was carried out using plates coated with silicagel 60F 254 purchased from Merck Co. Reagents were obtained from commercial suppliers and were used without purification.

Benzyl(6-methyl-3-*o*-tolylisoquinolin-1-yl)amine (6a)

A mixture of 1-chloro-6-methyl-3-*o*-tolylisoquinoline[6] (460 mg, 1.72 mmol), benzylamine (402 mg, 3.75 mmol), and potassium carbonate (400 mg, 2.85 mmol) in DMF was refluxed at 6 h. The reaction mixture was cooled to room temperature, diluted with water, and extracted with CH_2Cl_2. The combined organic extracts were washed with water, dried, and concentrated. The residue was purified by column chromatography on silica gel with CH_2Cl_2/MeOH (200:1 to 25:1) to give **6a** (420 mg, 72%) as a yellow solid; mp 99–101 °C; IR KBr cm^{-1} 3400; ^{1}H NMR ($CDCl_3$) δ 8.00–7.01 (13H, m, Ar–H), 5.40 (1H, s, NH), 4.96 (2H, d, $J = 5.2$ Hz, –CH_2–), 2.48, 2.43 (each 3H, each s, Me × 2); MS, *m*/*e* (%) 338 (M^+, 39), 340 (27), 336 (23). Anal. $C_{24}H_{22}N_2$ (C, H, N) calcd: 85.17, 6.55, 8.28; found: 85.06, 6.33, 8.56.

Benzyl(6-methyl-3-phenylisoquinolin-1-yl)amine (6b). The same procedure as described in the preparation of **6a** to give **6b** (75%) as a yellow solid; mp 68–69 °C; IR KBr cm^{-1} 3370; ^{1}H NMR ($CDCl_3$) δ 8.18–6.91 (14H, m, aromatic–H), 5.33 (1H, s, NH), 4.86 (2H, d, $J = 4.5$ Hz, –CH_2–), 2.36 (3H, s, Me); MS, *m*/*e* (%) 324 (M^+, 39), 321 (19). Anal. $C_{23}H_{20}N_2$ (C, H, N) calcd: 85.15, 6.21, 8.63; found: 85.43, 6.03, 8.78.

Benzyl(3-phenylisoquinolin-1-yl)amine (6c). The same procedure as described in the preparation of **6a** to give **6c** (68%) as a yellow solid; mp 122–128 °C; IR KBr cm^{-1} 3400, 1520; ^{1}H NMR ($CDCl_3$) δ 8.20–7.21 (15H, m, aromatic–H), 5.43 (1H, s, NH), 4.96 (2H, d, $J = 5.0$ Hz, –CH_2–); MS, *m*/*e* (%) 310 (M^+, 100), 301 (46). Anal. $C_{22}H_{18}N_2$ (C, H, N) calcd: 85.13, 5.85, 9.03; found: 85.42, 5.63, 9.28.

Benzyl(6-methyl-3-*m*-tolylisoquinolin-1-yl)amine (6d). The same procedure as described in the preparation of **6a** to give **6d** (65%) as a yellow solid; mp 67–68 °C; IR KBr cm^{-1} 3340; ^1H NMR ($CDCl_3$) δ 8.00–7.01 (13H, m, aromatic–H), 5.45 (1H, s, NH), 4.90 (2H, d, $J = 5.0$ Hz, $–CH_2–$), 2.54, 2.45 (each 3H, each s, Me × 2); MS, *m*/*e* (%) 338 (M^+, 100), 305 (76), 258 (54). Anal. $C_{24}H_{22}N_2$ (C, H, N) calcd: 85.17, 6.55, 8.28; found: 85.42, 6.63, 8.55.

1-Morpholin-4-yl-3-phenylisoquinoline (6e). The same procedure as described in the preparation of **6a** to give **6e** (95%) as a yellow solid; mp 113–114 °C; IR KBr cm^{-1} 3150, 1650, 1475; ^1H NMR ($CDCl_3$) δ 8.20–7.39 (9H, m, aromatic–H), 7.23 (1H, s, C_4–H), 4.01 (4H, t, $J = 4.5$ Hz, $–CH_2–$), 3.54 (4H, t, $J = 4.8$ Hz, $–CH_2–$); MS, *m*/*e* (%) 290 (M^+, 100), 289 (87), 259 (54). Anal. $C_{19}H_{18}N_2O$ (C, H, N) calcd: 78.59, 6.25, 9.65; found: 78.38, 6.46, 9.58.

6-Methyl-3-*o*-tolylisoquinolin-1-ylamine (6f). The reaction mixture of the benzylamine (**6a**, 1.14 g, 3.37 mmol) and 5% Pd/C (300 mg) in acetic acid (15 mL) was shaken at 66 °C under H_2 (90 psi) using Parr apparatus for 3 days. The reaction mixture was cooled down to room temperature and neutralized with satd $NaHCO_3$ solution. The extration was done with ethyl acetate and the combined organic layer was washed with water, brine and dried over sodium sulfate. The solvent was evaporated off to give the residue which was purified by column chromatography on silica gel with CH_2Cl_2/MeOH (100:3) to afford the free amine as an oil (600 mg, 72%). The free amine was dissolved in acetone and was added several drops of *c*-HCl to afford the hydrochloric acid salt of the amine. The resulting precipitate was collected and dried in vacuo; mp 269.1–270.2 °C; IR KBr cm^{-1} 3300, 1650; ^1H NMR (DMSO-d_6) δ 13.84, 9.40 (1H and 2H, each s, NH_3), 8.79–7.57 (7H, m, aromatic–H), 7.38 (1H, s, C_4–H), 2.75, 2.61 (each 3H, each s, Me × 2); ^{13}C NMR (DMSO-d_6) δ 153.4, 144.4, 137.0, 135.9, 134.8, 131.6, 129.4, 128.7, 128.5, 128.4, 125.7, 124.8, 124.0, 113.0, 109.5; MS, *m*/*e* (%) 248 (M^+, 100), 232 (87), 230 (54). Anal. $C_{17}H_{17}ClN_2$ (C, H, N) calcd: 71.70, 6.02, 9.84; found: 71.58, 6.27, 9.58.

3-*o*-Tolylisoquinolin-1-ylamine (6g). The same procedure as described in the preparation of **6f** to give **6g** (63%) as a yellow oil; mp 232–234 °C (HCl salt); IR $CHCl_3$ cm^{-1} 3350, 1650, 1475; ^1H NMR ($CDCl_3$) δ 7.90–6.85 (9H, m, aromatic–H), 8.93 (2H, s, NH_2), 2.42 (3H, s, Me); MS, *m*/*e* (%) 234 (M^+, 100), 232 (87), 215 (54). Anal. $C_{16}H_{15}ClN_2$ (C, H, N) calcd: 70.98, 5.58, 10.35; found: 70.88, 5.56, 10.56.

3-Phenylisoquinolin-1-ylamine (6h). The same procedure as described in the preparation of **6f** to give **6h** (62%) as a yellow oil; mp 242–245 °C (HCl salt); IR $CHCl_3$ cm^{-1} 3500; ^1H NMR ($CDCl_3$) δ 7.90–6.85 (9H, m, aromatic–H), 8.92 (2H, s, NH_2), 2.40 (3H, s, Me); MS, *m*/*e* (%) 220 (M^+, 100), 210 (87), 168 (54). Anal. $C_{15}H_{13}ClN_2$ (C, H, N) calcd: 70.18, 5.10, 10.91; found: 70.34, 5.09, 10.66.

(4-Methoxybenzyl)-(3-phenylisoquinolin-1-yl)amine (6i). The same procedure as described in the preparation of **6a** to give **6i** (62%) as a yellow solid; mp 130–131 °C; IR KBr cm^{-1} 3300, 1640, 1476; ^1H NMR ($CDCl_3$) δ 8.20–6.81 (13H, m, aromatic–H), 5.30 (1H, s, NH), 4.87 (2H, d, $J = 5.0$ Hz, $–CH_2–$), 3.79 (3H, s, OMe); MS, *m*/*e* (%) 340 (M^+, 100), 339 (32). Anal. $C_{23}H_{20}N_2O$ (C, H, N) calcd: 81.15, 5.92, 8.23; found: 81.35, 5.88, 8.46.

3-Phenyl-1-piperidin-1-yl-isoquinoline (6j). The same procedure as described in the preparation of **6a** to give **6j** (88%) as a yellow solid; mp 100–101 °C; IR KBr cm^{-1} 3250, 1650; ^1H NMR ($CDCl_3$) δ 8.20–7.34 (10H, m, aromatic–H), 3.50 (4H, t, $J = 4.0$ Hz, CH_2), 1.87 (4H, t, $J = 5.6$ Hz, CH_2), 1.74 (2H, q, $J = 4.0$ Hz, CH_2); MS, *m*/*e* (%) 288 (M^+, 100), 287 (87), 259 (84). Anal. $C_{20}H_{20}N_2$ (C, H, N) calcd: 83.30, 6.99, 9.71; found: 83.45, 6.78, 9.66. The solid was dissolved in acetone and c-HCl was added to this mixture to afford the precipitate which was collected and dried in vacuo to give the hydrochloric acid salt; mp 198–201 °C.

7,8-Dimethoxy-3-phenyl-2*H*-isoquinolin-1-one (2). To a solution of 2,3-dimethoxy-6,*N*-dimethylbenzamide (10.25 g, 36.5 mmol) in THF (250 mL) was added *n*-BuLi (2.5 M in hexane, 43 mL, 107.5 mmol) at −20 °C maintaining the reaction mixture not exceeding 0 °C under nitrogen. After adding the *n*-BuLi, the mixture was stirred for 1 h at the same temperature. Then, the color of reaction mixture was turned to red-purple. The cooling bath was removed and the mixture was moved to −50 °C cooling bath. Benzonitrile (6.13 g, 59.5 mmol) in THF (20 mL) was added to the reaction mixture and then the cooling bath was removed and the mixture was stirred overnight at room temperature. The reaction mixture was quenched with excess water at room temperature and the organic layer was separated, washed with water and brine, dried, and concentrated to dryness to give the crude isoquinoline as a yellow solid. The residue was recrystallized from ethyl acetate to give **2** as a pale yellow needles (6.36 g, 62%); mp 226.4–228.5 °C; IR KBr cm^{-1} 1651; ^1H NMR ($CDCl_3$) δ 11.07 (1H, s, NH), 7.76–7.42 (7H, m, Ar–H), 6.79 (1H, s, C_4–H), 3.86, 3.77 (each 3H, each s, OMe × 2); MS, *m*/*e* (%) 281 (M^+, 100), 264 (32). Anal. $C_{17}H_{15}NO_3$ (C, H, N) calcd: 72.58, 5.37, 4.98; found: 72.74, 5.42, 4.83.

6-Methyl-7-*o*-tolyl-6H-[1,3]dioxolo[4,5-g]isoquinolin-5-one (12). To a solution of 6-methylbenzo[1,3]dioxole-5-carboxylic acid methylamide (5.25 g, 27.2 mmol) in THF (100 mL) was added *n*-BuLi (2.5 M in hexane, 22 mL, 54.5 mmol) at −20 °C maintaining the reaction mixture not exceeding 2 °C under nitrogen. After adding the *n*-BuLi, the mixture was stirred for 4 h at the same temperature. The mixture was cooled to −50 °C. 2-Methylbenzonitrile (3.05 g, 29.5 mmol) in THF (10 mL) was added to the reaction mixture and then the cooling bath was removed and the mixture was stirred overnight at room temperature. The reaction mixture was quenched with excess water at room temperature and the organic layer was separated, washed with water and brine, dried, and concentrated to dryness to give the crude isoquinoline as a yellow solid. The residue was dissolved in THF (20 mL) and NaH (60% dispersion in oil, 8.7 g, 35 mmol) was added at 0 °C with MeI (4.98 g,

35 mmol) under nitrogen. The mixture was stirred for 4 h at the same temperature. The reaction mixture was quenched with water and extracted with ethyl acetate the combined organic layer was washed with water, brine and dried over sodium sulfate. The solvent was evaporated off to afford the residue which was purified by column chromatography on silica gel with CH_2Cl_2/MeOH (100:2) to afford the desired compound **12** (5.82 g, 73%) as a pale yellow solid; mp 148.5–150 °C; IR KBr cm^{-1} 1650; ^1H NMR ($CDCl_3$) δ 7.81, 6.81 (each 1H, each s, Ar–H), 7.51–7.20 (4H, m, Ar–H), 6.27 (1H, s, C_4–H), 6.01 (2H, s, –OCH_2O–), 3.27 (3H, s, Me), 2.18 (3H, s, –NMe).; MS, *m*/*e* (%) 293 (M^+, 70), 268 (100). Anal. $C_{18}H_{15}NO_3$ (C, H, N) calcd: 73.71, 5.15, 4.78; found: 73.45, 5.38, 4.75.

(3-Phenylisoquinolin-1-ylsulfanyl)acetic acid ethyl ester (13). The same procedure as described in the preparation of **6a** to give **13** (85%) as a yellow solid; mp 69–70 °C; IR KBr cm^{-1} 1730, 1640; ^1H NMR ($CDCl_3$) δ 8.14–7.37 (10H, m, aromatic–H), 4.18 (2H, s, –SCH_2–), 4.17 (2H, q, $J = 7.2$ Hz, –$O\underline{CH_2}CH_3$), 1.23 (3H, t, $J = 7.2$ Hz, –$OCH_2\underline{CH_3}$); MS, *m*/*e* (%) 323 (M^+, 100), 257 (67), 249 (50). Anal. $C_{19}H_{17}NO_2S$ (C, H, N) calcd: 70.56, 5.30, 4.33; found: 70.35, 5.38, 4.47.

***N,N,N′*-Trimethyl-*N′*-(3-phenylisoquinolin-1-yl)ethane-1,2-diamine (14).** The same procedure as described in the preparation of **6a** to give **14** (86%) as a yellow solid; mp 75–77 °C; IR KBr cm^{-1} 1690, 1350; ^1H NMR ($CDCl_3$) δ 8.21–7.26 (10H, m, aromatic–H), 3.86 (2H, t, $J = 6.5$ Hz, –$N\underline{CH_2}CH_2$–), 3.21 (3H, s, NMe), 3.00 (2H, t, $J = 6.8$ Hz, –$CH_2\underline{CH_2}$–), 2.51 (6H, s, NMe_2); MS, *m*/*e* (%) 305 (M^+, 45), 256 (69), 249 (100). Anal. $C_{20}H_{23}N_3$ (C, H, N) calcd: 78.65, 7.59, 13.76; found: 78.45, 7.38, 13.66.

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