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STRUCTURAL AND ELECTRONIC PROPERTIES OF TYROSINE KINASES INHIBITORS

M.B. SANTILLÁN[1✍], F. TOMÁS-VERT[2], J.M. AULLÓ[2], E.A. JÁUREGUI[1] and G.M. CIUFFO[3]

[1✍] Departamento de Química, Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis, Ejército de los Andes 950, 5700 San Luis, Argentina
Fax: +54 2652 422 644; E-mail: msanti@unsl.edu.ar
[2] Departamento de Quimica Física, Universidad de Valencia, 46100 Burjassot, Valencia, España
[3] Departamento de Bioquímica y Cs. Biológicas, Facultad de Química, Bioquímica y Farmacia, Universidad Nacional de San Luis, Chacabuco y Pedernera, 5700 San Luis. Argentina

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Abstract - Protein tyrosine kinases (TKs) regulate cell proliferation, cell differentiation, and play a fundamental role in signal transduction pathway. Uncontrolled signaling from receptor tyrosine kinases and intracellular tyrosine kinases was related to diseases such as cancer, atherosclerosis and psoriasis. For the present study, we selected a number of structurally related ATP-binding site inhibitors of EGF-receptors of diverse classes. Molecular properties of competitive inhibitors are key features for the action mechanism of these compounds. We performed a theoretical study at the RHF/6-311G* level of theory, in order to correlate the molecular parameters with the biological inhibitory activities. Species stability as evaluated by ionization potentials as well as the E_{HOMO}-E_{LUMO} energy gap, is in very good correlation with higher inhibitory potency. The most active species, **1**, **5**, **6**, **10**, **11** and **12** exhibited strongly negative charged atoms over the C^6 and C^7 positions, the higher IP, higher and higher energy gap. In summary, a good correlation was observed between the molecular parameters, such as ionization potential, dipolar moment and E_{HOMO}-E_{LUMO} energy gap and inhibitory potency, suggesting that these properties play an important role for the interaction at the ATP-binding site of EGF-receptors.

Key words: EGF receptors, tyrosine kinase activity, selective inhibitors, molecular properties

INTRODUCTION

Protein tyrosine kinases (TKs) regulate cell proliferation, cell differentiation, and play a fundamental role in signal transduction pathway (2,8). Uncontrolled signaling from receptor tyrosine kinases and intracellular tyrosine kinases can lead to inflammatory responses and to diseases such as cancer, atherosclerosis and psoriasis (2,8,11). Tyrosine kinases are therefore attractive targets for the design of new therapeutics agents (6,9,11,12).

Tyrosine phosphorylation can be considered the primary biochemical reaction associated with growth of multicellular organisms. Tyrosine kinase receptors (TKRs) participate in transmembrane signaling, whereas the intracellular tyrosine kinases take part in signal transduction within the cell. Because of the topology of receptor tyrosine kinases, the ligand binding domain and the protein kinase activity are separated by the plasma membrane. The family of the epidermal growth factor receptor (EGF-R), a receptor with protein tyrosine kinase activity, belongs to the larger class of the transmembrane growth factor receptors. EGF-R and its ligands have been implicated in numerous tumors of epithelial origin and proliferative disorders (2,6,9,11,12). Moreover, mutated EGF receptors are often introduced into the cells by viruses, thus inducing tumor formation. Over 80% of the oncogenes and the protooncogenes involved in the human cancer are related to PTKs (11). The enhanced activity of PTKs is also implicated in many non-malignant diseases, such as psoriasis, papilloma, restenosis and pulmonary fibrosis (2,5,8,12).

Since a large family of TK members is known, it is very important to obtain selective inhibitors for different tyrosine kinase receptors. During the last years, several classes of compounds have been reported as tyrosine kinase inhibitors. Some of these groups of compounds are inhibitors competing with ATP for binding at the catalytic domain of the enzyme (3,6,11-19). These compounds are of interest as potential anticancer drugs (8,9).

The 4-(phenylamino) quinazolines are known to be

Abbreviations: **EGF-R**: epidermal growth factor receptors; **FGF-R**: fibroblast growth factor receptors; **IP**: **PTK**: protein tyrosine kinase; **TK**: tyrosine kinase

potent and selective inhibitors of the tyrosine kinase activity of the epidermal growth factor receptor (6,12), via competitive binding at the ATP site of the enzyme. Some of these compounds are presently under clinical trial (2,8,9,12) for treatment of diseases such as psoriasis and have been patented by different pharmaceutical companies, such as PD153035 (3) (also named as AG1517 or SU5271), AG1478 (11), PD158780 and CGP59326 (2,12,13,16).

The pharmacophore requirements at the ATP-binding site have been evaluated on the basis of chemical modifications of series of compounds. Different rationales have been used in the search of the pharmacophore model for the design of the TK inhibitors. Recently, the crystal structure of the TK domain of FGF (fibroblast growth factor) receptors have been solved and the interaction of inhibitors at the ATP-binding site have been modeled (10). Apparently, the ATP is anchored at the active site of the enzyme by two key hydrogen bonds involving the amino group and the N pyrimidine nitrogen of the adenosine moiety. On the basis of molecular modeling and experimental studies it has been proposed that the side chain of the inhibitors form a covalent linkage with the sulfhydryl group of the Cys^{773} of EGFR. The bromoaniline residue lies in an hydrophobic pocket surrounded by Val^{702}, Ala^{719} and Thr^{766} (10).

For the present study, we selected a number of structurally related ATP-binding site inhibitors belonging to diverse classes: phenylaminopyrimidines, phenyl-aminoquinazolines (PD135035), 7-amino4-pyrimidines, (PD158780) and 4-phenyl(-amino)pyrrolopyrimidines (CGP59326) (3,5,11-19). Molecular properties of competitive inhibitors are key features for the understanding of the action mechanism of the compounds under study. We performed a theoretical study at a high level of theory in order to correlate the molecular parameters with the biological activities of these inhibitors.

MATERIALS AND METHODS

Molecular systems

We selected 13 molecules for the present study, belonging to four different classes of related derivatives. Fig. 1 summarizes the chemical structure, designation used in the bibliography, heteroatom numbering and biological activities of the systems under study (3,5,8,11-19). For all the compounds, two free rotations were allowed around the N^{11} atom (enamine portion). For comparison, we studied also an ATP model, where a methyl group replaced the phosphate portion.

Calculations

Conformations were initially optimized by using AM1 calculations. Gaussian 98 (7) package was used to perform RHF calculations on the compounds of interest, where the molecular geometries were fully optimized. RHF/6-311G* level of theory (1,4) was used, since it would be able to provide precise molecular structures and chemical properties of the species under consideration. Physical chemical properties as HOMO LUMO molecular orbital, atomic net Mulliken charges, dipole moment were calculated at RHF/6-311G*.

RESULTS

We selected for our study compounds from four different classes of inhibitors of EGF receptors, including several compounds for each series of analogs derived from the same precursor, where different electron donating or withdrawing groups were introduced (3,6,11-19). The biological activities of the compounds under study were taken from the literature. Thus, IC_{50} values in Fig. 1 are reported values from *in vitro* tyrosine kinase activity assays. Species **1** and **3** belong to the phenylamino quinazoline group, being species **1** the most active one. Species **5** and **10** are the most active compounds from the dimethoxy quinazolines and imidazo quinazoline group, respectively. Species **12** is a highly potent TK inhibitor.

Geometrical aspects

In spite of the initial conformations, most of the inhibitors studied arrive to a completely planar final conformation (Table 1 and Fig. 2). Thus, no chirality was present at N^{11}, the enamine portion of the molecules (Fig. 2) for several of the systems studied, while some of them belong to the **S** type such as species **4**, **5**, **7**, **8**, **10** and **11**. The energy values obtained at the RHF-6-311G* calculation level are also included, thus indicating that final stable conformations were achieved.

Net atomic charges

One of the key parameters to be considered for the design of ATP-binding inhibitors has to be charge distribution over the molecules and particularly those of heteroatoms. Table 2 includes the net atomic charges over the heteroatoms present on the different molecules studied. All the compounds studied have in common atoms N^1, N^3 and N^{11}. The presence of different substituents affect the charge over these atoms within a short range: N^1 (0.4661 to 0.4921 H), N^3 (0.5136 to 0.5645 H) and N^{11} from 0.8962 H to 0.9206 H, being the largest variation that observed for N^3.

For all the species bearing an halogen substitution, similar charges were observed: species **1**, **6** and **13** have Cl at position 17, while species **2**, **5**, **10**, **11**, **13** bears a Br substitution (see Table 2 and Fig. 2).

For better comparisons, we grouped the compounds according to their chemical structure.

Phenylamino quinazolines

A first group of molecules (**1** to **3**) has non-substitution over atoms C^6 and C^7. From these compounds, addition of halogen atoms, such as Cl or Br atoms over the anilino

Fig. 1 Structure, atom numbering and biological activity for compounds of the different series studied.

Table 1 Diedhral angles, energies and chiralities obtained for all the species studied with RHF/6-311G*

Compound	^{1} **(3,4,11,12)**	^{2} **(5,4,11,12)**	Energy (Hartree) E_{RHF}	Chirality
1	-0.0111	179.9831	-1158.98140345	Planar
2	-0.0411	-179.9085	-3271.8426871	Planar
3	-2.3698	-153.0735	-739.101548423	Planar
4	-2.1485	-147.9536	-966.9123177	S
5	-1.6301	-152.5218	-3499.6534482	S
6	0.0140	179.9930	-1386.7921659	Planar
7	-4.81	-125.95	-1058.6565894	S
8	-2.48	-143.76	-999.862875	S
9	-0.03	-179.79	-924.9423701	Planar
10	-1.69	-150.34	-3418.6408586	S
11	-0.2	-174.849	-3381.9185364	S
12	0.0	-179.98	-1215.1951962	Planar
13	0.0	179.99	-3328.0564529	Planar
	(4-5-6-7)	**(18-6-7-8)**		
ATP model	-120.4	-105.9	-997.185921	

Table 2 Net atomic charges of heteroatoms for the different inhibitors obtained on fully optimized *ab initio* calculations at the RHF/6-311G* level of theory

	Net Atomic Charges (H)						
Compounds	**N^{1}**	**N^{3}**	**N^{11}**	**Cl, Br^{17}**	**X^{19}**	**X^{20}**	**X**
1	-0.4790	-0.5507	-0.9107	-0.1059			
2	-0.4798	-0.5524	-0.9108	-0.0453			
3	-0.4838	-0.5311	-0.9023				
					O^{19}	**O^{20}**	
4	-0.4870	-0.5254	-0.9039		-0.4424	-0.4372	
5	-0.4835	-0.5323	-0.9033	-0.0462	-0.4412	-0.4365	
6	-0.4835	-0.5535	-0.9182	-0.1077	-0.4413	-0.4364	
7	-0.4921	-0.5136	-0.9007		-0.4436	-0.4383	**N^{23}** -0.7774
					N^{19}	**N^{20}**	
8	-0.4894	-0.5207	-0.8987		-0.8541	-0.6316	**O^{22}** -0.4899
9	-0.4918	-0.5558	-0.9150		-0.4490	-0.5623	
10	-0.4784	-0.5300	-0.8962	-0.0443	-0.7803	-0.4442	
11	-0.4661	-0.5421	-0.9109	-0.0440	-0.7395		**N^{8}** -0.4693
12	-0.4861	-0.5642	-0.9203	-0.1113			**N^{8}** -0.8013
13	-0.4862	-0.5645	-0.9206	-0.0517			**N^{8}** -0.8013
ATP model	-0.4862	-0.4602			-0.9679		**N^{13}** -0.5187

portion increases the affinity of the compounds (3). Both, Cl of Br exhibited rather low charges, being lower for the last substituent. The presence of these substituents modifies the atomic charge over N^{11}, thus indicating that the electronic contribution of the halogen atoms is redistributed over the molecule. This observation is in agreement with experimental data which indicates that the bromoanilino portion is interacting with an electrophobic pocket of the enzyme (13-19).

6,7-dimethoxy-4-(phenylamino) quinazolines

The utility of electron-donating substituents at position 6- and 7-, such as oxymethyl groups was used in the design of the compounds **4** to **7** (18), species with higher activity compared to quinazoline derivatives **1** to **3**. Previous studies (3) pointed out the importance of substitutions over atoms C^{6} and C^{7}, providing hydrophilic groups which favored the interaction at the binding site (18). The $-OCH_3$ substitution over atoms C^{6} and C^{7} on compounds **4**, **5**, **6** and **7** clearly affects the charge over atoms C^{6} and C^{7}. All the species showed an important charge over atoms O^{19} and O^{20} (see Table 2).

The most active compound on this group of analogues is species **5** (PD135053), which bears a bromo anilino

Fig. 2 Spatial view of the lowest-energy conformations for species **1,5,10,11,12** and **ATP** model, at the RHF/6-311G* calculation level.

portion. Over the last atom, a very low charge is present, however, this atom provides a highly polarizable electronic cloud (see Fig. 2). The combination of both properties explains the allocation of bromide within the hydrophobic pocket. Species **6** (AG1478), one of the compounds under clinical trial, which is the Cl substituted analogue of species **5,** shows comparable atomic charge distributions.

Species **7**, where the halogen substituent over the anilino portion was replaced by a pyrrolo ring including N^{23}, looses activity (Fig. 1). In this case, N^{23} has a highly negative charge (-0.7774 H) as opposed to the low negative charge of Br (-0.0462) in species **5** or Cl in species **6** (-0.1077). Thus this highly charged atom would interfere with the interaction at the hydrophobic pocket of the enzyme.

Tricyclic quinazolines

Species **8**, **9** and **10** belong to the group of fused tricyclic compounds. The third ring was obtained by introducing amino groups bridged to form a third ring at positions C^6 and C^7 (imidazo quinazolines) (14). The third ring introduced on these compounds does not cause major charge variation over atoms N^1 and N^3. Atoms N^{19} and N^{20} exhibited for all these compounds a high negative atomic charge, in agreement with the above mentioned hydrophilic property and experimental data. Introduction of Br on the anilino portion sensibly improves biological activity, lowering atomic charges over N^{11}.

Species **8**, a 2-oxoimidazolino quinazoline derivative, has the lower affinity among the tricyclic compounds studied. Incorporation of O^{22} in compound **8**, disrupts the electronic delocalization of the 5- membered ring, delocalization maintained in compounds **9** and **10** (see Fig. 3) as well as modifies charge distribution.

Compound **10**, which has a bromoaniline portion and lacks of the methyl substituent over the pyrrol ring, is the compound with the highest inhibitory capability.

Fig. 3 shows a representation of charge densities and. extended delocalization can be observed in some of the imidazo-quinazolines derivatives, such as species **10**. The oxo-substitution in species **8** disrupt delocalization, observation that could explain its lower biological activity 35 nM compared to 0.08 nM of compound **10**.

For the ATP model, Table 2 shows charges of N atoms of the adenine portion with positions equivalent to that of the studied compounds. Comparable charges were obtained. Dipolar moment was not considered because in our model we replaced the phosphate group by a methyl group in order to simplify the calculation.

Imidazo and pyrrolo-pyrimidines

Although chemically different, species **11**, **12** and **13** shares some structural similarities and were compared. The N^8 atom of the imidazo or pyrrolo portion for the three compounds have an important negative charge (see Table 2). Thus, introduction of the N atom on the ring could replace the hydrophilic substituents (oxymethyl) present in species **4** to **10**.

Compound **11** (PD158780) an imizadopyrimidine and compound **12** (CGP59326) a pyrrolo-pyrimidine, are highly potent TK inhibitors. Fig. 3 represents charge densities and extended delocalization is observed for species **11** and **12**.

Ionization potentials and electron affinities

The HOMO orbital is directly related to the ionization potential and characterizes the susceptibility of the molecule toward attack by electrophyllic groups. Within each structurally related group of species, compounds **1**, **5**, **10** and **11** exhibited the highest IP, with values very close to the one obtained for the ATP model (0.30741H).

For the quinazolines derivatives, compounds **1** and **5**

Table 3

Compounds	E_{HOMO}- E_{LUMO}	IP (H)	EA (H)	(D)
1	0.23171	0.30354	0.07183	5.457
2	0.23127	0.30328	0.07201	5.390
3	0.21547	0.29498	0.07951	3.2414
4	0.19324	0.28640	0.09316	3.1045
5	0.20881	0.29456	0.08575	5.3465
6	0.20785	0.29327	0.08542	5.5858
7	0.17457	0.27791	0.10334	4.408
8	0.21227	0.29168	0.07941	2.1551
9	0.20654	0.28178	0.07524	4.1010
10	0.2306	0.29513	0.06453	8.4501
11	0.22404	0.28636	0.06232	5.8645
12	0.17304	0.28171	0.10866	6.5446
13	0.17302	0.28159	0.10850	2.8117
ATP model	0.17613	0.30741	0.13128	ND

Fig. 3 Charge density maps and delocalization obtained for species **1,5,10,11,12** and **ATP** model at the final conformation obtained at the RHF/6-311G* calculation level.

bearing an halogen atom (Cl or Br, respectively), are the most potent analogs of the corresponding family of molecules. Both exhibited the largest ionization potential, a property that makes these species to be more reactive to electrophylic attack.

Molecules exhibiting higher IP values correlate with an increased electron acceptor capability of the species under study. Species **10** from the imidazoquinazolines, has the highest IP and consequently, the lower polarizability. Apparently, these properties correlate very well with the highest biological activity.

For each group of molecules studied, a common trend was observed: compounds with the highest ionization potential correspond to those with higher biological activity.

Dipolar moment

Once again, compounds with high biological activity are the ones with higher dipoles, as follows: Compound **1**: = 5.457 D; Compound **5**: = 5.346 D; Compound **6**: = 5.5858 D; Compound **10**: = 8.45 D; Compound **11**: = 5.86 D; Compound **12**: = 6.544 D. Similarly to the trend observed for IP, species with the highest biological activity within each series are the ones with higher dipolar moment.

As expected, a strong effect due to substituent over the anilino portion of the molecules or modifications at the quinazoline ring was observed. The higher value was obtained for compound **10**, the most active of all the studied compounds. Bromide substitution in the meta position of the anilino ring as well as the charged N over the imidazolo fused ring are the most probable cause of charge separation and the reason for the high dipolar moment obtained for species **10**.

E_{HOMO}-E_{LUMO} energy gap

The difference in energy between the HOMO and LUMO, is an important stability index (1,4), a large HOMO-LUMO gap implies high stability for the molecule in the sense of its lower reactivity in chemical reactions (1). Table 3 shows the E_{HOMO}-E_{LUMO} gap for the compounds studied. As it can be observed, compounds with higher biological inhibitory potency, species **1**, **5**, **6**, **10** and **11** are the most stable species within the different series of molecules studied.

The ATP model used in the present study showed a considerably lower E_{HOMO}-E_{LUMO} gap (0.176 H). In our model compound we avoid the phosphates, highly charged groups, and replaced them by a methyl group. This replacement can strongly affect properties of the molecule. Alternatively, and considering that inhibitors are supposed not to progress with the enzyme catalyzed reaction, the lower E_{HOMO}-E_{LUMO} gap might explain the higher reactivity of the modeled ATP.

DISCUSSION

Due to the fact that the catalytic domain of most tyrosine protein kinases have significant amino acid sequence homology and a conserved core structure, it was believed for a long time that compounds interacting with the ATP-binding site will not result in selective inhibition. However, different classes of compounds have proved to be highly potent and selective ATP-competitive tyrosine kinase inhibitors for EGF-receptors.

In the present paper, we performed a theoretical study of the electronic and molecular properties of several EGF-R inhibitors at the ATP-binding site. In spite of the different structure-activity relationships studies used on the rational for design of these inhibitors, very little attention has been paid to the molecular parameters of the inhibitors. In order to achieve precise values, a high level of theory was used, RHF-6-311G*, to study the different molecular systems. Recently, the crystal structure of the TK domain of FGF receptors have been solved and the interaction of inhibitors at the ATP-binding site have been modeled (10,16). Apparently, the ATP is anchored at the active site of the enzyme by two key hydrogen bonds involving the amino group and the pyrimidine nitrogen of the adenosine moiety. Molecular modeling studies suggested and experimental results confirmed that the Michael acceptor side chain of the inhibitors forms a covalent linkage with the sulfhydryl group of the Cys^{773} of EGF-R (16). According to these studies, the bromoaniline residue lies in a hydrophobic pocket surrounded by Val^{702}, Ala^{719} and Thr^{766}.

From *ab initio* calculations, we showed that compounds selected for the present study with different substitutions at the C^6 and C^7 could accomplish these requirements. The most active species, **5**, **6**, **10**, **11** and **12** exhibited strongly negatively charged atoms over the C^6 and C^7 positions. Thus, O^{19}, O^{20} for species **5** and **6**, N^{19}, N^{20} for species **10** and N^8 for species **11** are potential sites for a hydrophilic interaction at the ATP-binding site. N^8 and N^1 can simulate the requirement of two potential sites for hydrogen bond for compounds **11** and **12**, where an imidazo- or pyrrolo- pyrimidine ring was present. Similar charges were present on atoms with a comparable position on our ATP model. The lower activity of compounds bearing a highly charged group over the anilino portion such as species **7**, can also be explained from the present study.

From all the compounds studied, those bearing a Br substituent at position 17 have been reported as the ones with highest inhibitory potency. The Br atom bears a very weak charge, however, Br itself is a highly polarizable atom (see Fig. 2) and this feature could be a requirement for the interaction at the hydrophobic pocket as shown on experimental data.

It has been suggested that planarity and/or aromaticity

of the molecules also appear to be important. For compound 8, with lower biological activity, we showed that aromaticity is disrupted.

Species stability as evaluated by ionization potentials as well as the E_{HOMO}-E_{LUMO} energy gap, is in very good correlation with higher inhibitory potency. Compounds **1**, **5**, **6**, **10** and **11** exhibited the higher IP, higher and higher energy gap. Coincidentally, these compounds correspond to species described as the ones with higher biological activity.

Theoretical studies can be conducted before synthesis of compounds, and taken together with experimental data could be highly informative about molecular properties and a helpful tool in a rational drug design. In synthesis, a good correlation was observed between the molecular parameters, such as ionization potential, dipolar moment and E_{HOMO}-E_{LUMO} energy gap for the most active compounds, thus suggesting that these properties play an important role at the interaction at the ATP-binding site of EGF-receptors.

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