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New method for predict biological activity of kinases inhibitors

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Abstract

Computational studies have been applied in order to discover and develop new drugs with the advancer of experimental time reduction. On this way, a group of quinazolines, hypotetical inhibitors of epidermal grow factor (EGFR), has been in one computational models elaborated

to do correlation between experimental values of biological activity and the ability of this quinazolines to inhibit the kinase activity. By conversion of biological activity (IC_{50}) in pIC_{50} we obtained the first group of data for the linear correlation model. The second group of data are computational results obtained. These data were obtained using the computational plataform *Molinspitation*. Using this approach gave a correlation coefficient R^2. This correlation was used for test of new molecules. The capacity of kinase inhibition for each quinazoline was computationally calculated to obtain an estimate pIC_{50}. Three new molecules 1, 2 e 3 has been tested. For molecule 1 the estimate pIC_{50} was 6.61, what is considerate a strong inhibitor. In the other way the molecule 3 had a low pIC_{50} (3.56) and was considerate a weak inhibitor. New methodology like that one present in this work could be used for discovery and screening new molecules for synthesis without the needs of expense biological test.

Keywords: Kinase Inhibitors, Quinazoline, Drug Discovery, EGFR.

Introdution

Protein kinases represent a group of enzymes that catalyze the phosphorylation of other proteins by transferring the phosphate group from adenosine triphosphate (ATP) - or in exceptional cases, guanosine triphosphate (GTP) - for threonine, serine or tyrosine residues [(1)]. Protein phosphorylation, a pos traductional protein modification, is a important event in regulation of many biological process. These processes are responsable for activation of many signaling transduction pathways that control several physiological events. Gene transcription, cell cycle, differentiation and cell death are some examples [(2)]. The knowledge about the large importance of protein kinases to control a multitude of biological process has triggered a large medical research interest [(3)].

The disregulation or overexpression of proteins kinases are associated to various pathologies - such as asthma, cancer, cardiovascular diseases, diabetes, central nervous system disorders, among other conditions - due to the processes of apoptosis, cell proliferation, glycogen metabolism, neurotransmission and oncogenesis triggered by these proteins[1]. In the case of tumors, the disregulated kinases play an important role, since the maintenance of phosphorylation allows permanent activation of signal transduction [(2)].

Proteins kinases inhibitors act in one or more protein kinase by bloking their action. These inhibtors can promote a conformational change in the protein kinase and block the ATP-binding site in the catalytic domain of the enzyme. This new conformation make the protein inable to phosphorylate target protein in one signal transduction pathway, reducing for example, cell proliferation[1]. In this sense, the development of kinase inhibitors drugs with antitumor activity is a reality. At present are available first and second generation inhibitors [2]. The main feature of these inhibitors is the presence in its structure of a nitrogen nuclei able to mimic the adenine ring of ATP substrate. This feature allows the connection of this ring to the catalytic site. The availability of these inhibitors may be associated with the presence of a tertiary amine grouping [2]. The kinase inhibitors commercially available are relatively specific for epidermal growth epidermal factor receptor (EGFR). A good exemple is the Erlotinit a reversible and selective inhibitor of EGFR. The Erlotinit compets with ATP by the same biding site on the intracellular domain of the receptor [3]. In the presence of Erlotinit, the phosphorylation of tyrosine residues in EGFR is not possible and the signal cascades are blocked. In animal experiments, the therapeutic importance of Erlotinib has been showed its antitumor activity in head and neck cancer, breast, colorectal and vulvar carcinoma [4].

The use of molecular models and bioinformatic tools are a new and revolutionary field in the science. Such tools are useful to select new substances with biological activity without the need for extense experimental tests. In this way, if you will start a work to test, a high amount of molecules, using the approach you can easily select ony the most promising compound to be tested [5-6]. A good example of this application is the quantitative structure-activity (QSAR). This method is used for propose new molecules with potential to pharmacotherapy [5]. In this work we applied the QSAR methodology to reporter a new method that could be useful for improvement and development of new molecules, such as kinase inhibitors more specifically, EGFR inhibitors, drugs important in the treatment of cancer [7]. Here we used a set of quinazolines for validate the method.

Material and methods

The new method proposed in this work was elaborated using a set of quinazolines that are kinase inhibitors. The quinazolines represents a group with similar structure. The molecules were designed using ChemSketch software (ACD ChemSketch versão 8.17, 2005) and were

previously test by biological experiments for its half maximum inhibitory concentrations (IC_{50}) (www.acdlabs.com/products/draw_nom/draw).

This new methodology makes the linear correlation between two important data: an experimental data (pIC_{50}) and a computational data (predict bioactivity).

Computational and experimental data:

The ChemSketch software generates a SMILE formula for each molecule of quinazoline designed. We introduced this formula in the Molinspiration computer plataform (www.molinspiration.com/cgi-bin/properties) to obtain the predict bioactivity values using the quantitative structure-activity relationship (QSAR) methodology. This methodology use a data base from Molinspiration to do correlation between structural information of current drugs, drugs in development, and molecules with known biological activity (www.molinspiration.com/cgi-bin/properties).

The experimental data of IC_{50} used in this work were generated by Bridges *et al,* 1996. These values were converted in pIC_{50}, i.e, – Log IC_{50}, for each quinozaline molecule.

The linear correlation model:

The linear correlation was made using the computational and experimental data. To do the correlation are necessary computational and experimental values in the same proportion. For this ration all pIC_{50} values are multiplied for ten. The linear correlation equation gives a linear equation with a coefficient of correlation (R^2). The linear equation and R^2 are using to obtain the estimate pIC_{50} from new molecules that have a predict bioactivity computationally.

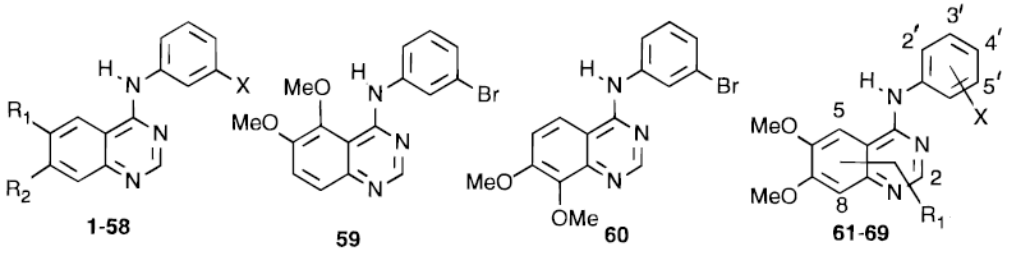
Results and Discussion

The pIC_{50} values and probability of quinazolines to inhibit kinase

The ChemSketch (www.acdlabs.com/products/draw_nom/draw) software was used to design molecules with their different substituents. Using the experimental IC_{50} values obtained experimentally by Bridges *et al,* 1996 we did the calcule of pIC_{50} for each quinazoline (Table 1). The Molinspiration software (www.molinspiration.com/cgi-bin/properties) was used for

molecules insertion and to calcule the probability of kinase inhibiting (Table 1 as "Kinase Inhibitor").

Table 1 - The quinolones series and their respective *IC_{50}* values, calculated *pIC_{50}* and the probability of inhibiting kinases.



N°.	R_1	R_2	X	IC_{50}[a] (nM)	pIC_{50}[a] ($-logIC_{50}$[a])	Kinase Inhibitor
1	H	H	H	344	6,46344156	0,59
2	H	H	F	56	7,25181197	0,69
3	H	H	Cl	23	7,63827216	0,60
4	H	H	Br	27	7,56863624	0,60
5	H	H	I	80	7,09691001	0,61
6	H	H	CF_3	577	6,23882419	0,72
7	OMe	H	H	55	7,25963731	0,62
8	OMe	H	H	30	7,52287875	0,61
9	NH_2	H	H	770	6,11350927	0,79
10	NH_2	H	CF_3	574	6,24108811	0,85
11	NH_2	H	Br	0,78	9,1079054	0,78
12	NO_2	H	H	5000	5,30103	0,49
13	NO_2	H	Br	900	6,04575749	0,48
14	NO_2	H	CF_3	$>10^4$	5	0,53
15	H	MeO	H	120	6,92081875	0,64
16	H	MeO	Br	10	8	0,63
17	H	NH_2	H	100	7	0,77
18	H	NH_2	F	2,0	8,69897	0,84
19	H	NH_2	Cl	0,25	9,60205999	0,76
20	H	NH_2	Br	0,1	10	0,76
21	H	NH_2	I	0,35	9,45593196	0,77

N°.	**R_1**	**R_2**	**X**	**IC_{50}[a] (nM)**	**pIC_{50}[a] (-logIC_{50}[a])**	**Kinase Inhibitor**
22	H	NH_2	CF_3	3,3	8,48148606	0,83
23	H	NO_2	H	1,2 x 10^4	4,92081875	0,47
24	H	NO_2	F	6100	5,21467016	0,54
25	H	NO_2	Cl	810	6,09151498	0,47
26	H	NO_2	Br	1000	6	0,46
27	H	NO_2	I	540	6,26760624	0,47
28	H	NO_2	CF_3	>10^4	5	0,51
29	OMe	OMe	H	29	7,537602	0,71
30	OMe	OMe	F	3,8	8,4202164	0,76
31	OMe	OMe	Cl	0,31	9,50863831	0,69
32	OMe	OMe	Br	0,025	10,60206	0,68
33	OMe	OMe	I	0,89	9,05060999	0,70
34	OMe	OMe	CF_3	0,24	9,61978876	0,62
35	NHMe	H	Br	4	8,39794001	0,77
36	NMe_2	H	Br	84	7,07572071	0,70
37	$NHCO_2Me$	H	Br	12	7,92081875	0,70
38	H	OH	Br	4,7	8,32790214	0,72
39	H	NHAc	Br	40	7,39794001	0,56
40	H	NHMe	Br	7,0	8,15490196	0,75
41	H	NHEt	Br	12	7,92081875	0,67
42	H	NMe_2	Br	11	7,95860731	0,68
43	NH_2	NH_2	Br	0,12	9,92081875	0,82
44	NH_2	NHMe	Br	0,69	9,16115091	0,89
45	NH_2	NMe_2	Br	159	6,79860288	0,81
46	NH_2	OMe	Br	3,8	8,4202164	0,81
47	NH_2	Cl	Br	6,5	8,18708664	0,78
48	NO_2	NH_2	Br	53	7,27572413	0,59
49	NO_2	NHMe	Br	68	7,16749109	0,53
50	NO_2	NMe_2	Br	2000	5,69897	0,46
51	NO_2	NHAc	Br	28	7,55284197	0,34

N°.	R_1	R_2	X	IC_{50}[a] (nM)	pIC_{50}[a] (-logIC_{50}[a])	Kinase Inhibitor
52	NO_2	OMe	Br	15	7,82390874	0,51
53	NO_2	Cl	Br	25	7,60205999	0,47
54	OC	H_2O	Br	15	7,82390874	0,66
55	OH	OH	Br	0,17	9,76955108	0,76
56	OEt	OEt	Br	0,006	11,2218487	0,58
57	OPr	OPr	Br	0,17	9,76955108	0,55
58	OBu	OBu	Br	105	6,9788107	0,54
59	5,6di-OME			1367	5,86423149	0,68
60	5,6di-OME			$>10^4$	5	0,56
61	2-Me		3'-Br	$>10^4$	5	0,46
62	2-NH_2		3'-Br	463	6,33441901	0,71
63	4N-Me		3'-Br	152	6,81815641	0,69
64	5-OMe		3'-Br	0,67	9,1739252	0,70
65	8-OMe		3'-Br	$>10^4$	5	0,56
66	H		2'-Br	128	6,89279003	0,61
67	H		4'-Br	0,96	9,01772877	0,65
68	H		3',4'-diBr	0,072	10,1426675	0,70
69	H		3',5'-diBr	113	6,94692156	0,66

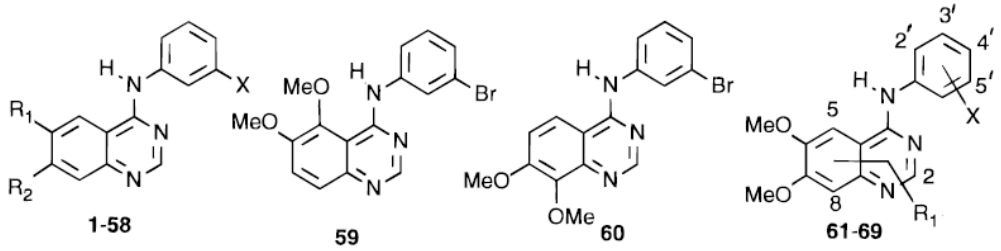
A series of quinazolines (Table 1) candidates of EGFR-inhibitors were used to create two important variables: ability to kinase inhibition and biological activity. Using the experimental data obtained by Bridges, *et al.*, 1996 a set of quinazolines were synthesized and their biological activities were determined by obtaining the experimental IC_{50} values.

According to other studies of molecular modeling [8] it was agreed to replace the experimental value of IC_{50} by their respective values of pIC_{50}. The need for this conversion implies better comparative analysis of the molecules under study and their biological activities. A set of quinolones (Table 1) was subjected to analysis for ability to inhibit kinase. This was possible because the number of quinazolines in this study represent a kinase example, *i.e.*, the epidermal growth factor receptor inhibitors (EGFR) belongs to a family of protein kinases [9].

New values for the probability of kinase inhibition correlated with *pIC$_{50}$*

The *pIC$_{50}$* values and the probability of each molecule to inhibit kinase were calculated. To do the correlation between boths valules, the probability values needed to be multiplied by 10 (Table 2). It was necessary to put the different values in the same order of magnitude.

Table 2 - The correlation of *pIC$_{50}$* with the new values of Kinase inhibition probability



N°.	R_1	R_2	X	pIC_{50}[a] (-logIC_{50}[a])	Kinase Inhibitor x10
1	H	H	H	6,46344156	5,9
2	H	H	F	7,25181197	6,9
3	H	H	Cl	7,63827216	6
4	H	H	Br	7,56863624	6
5	H	H	I	7,09691001	6,1
6	H	H	CF_3	6,23882419	7,2
7	OMe	H	H	7,25963731	6,2
8	OMe	H	H	7,52287875	6,1
9	NH_2	H	H	6,11350927	7,9
10	NH_2	H	CF_3	6,24108811	8,5
11	NH_2	H	Br	9,1079054	7,8
12	NO_2	H	H	5,30103	4,9
13	NO_2	H	Br	6,04575749	4,8
14	NO_2	H	CF_3	5	5,3
15	H	MeO	H	6,92081875	6,4
16	H	MeO	Br	8	6,3
17	H	NH_2	H	7	7,7
18	H	NH_2	F	8,69897	8,4
19	H	NH_2	Cl	9,60205999	7,6

Nº.	**R_1**	**R_2**	**X**	**pIC_{50}[a] (-logIC_{50}[a])**	**Kinase Inhibitor x10**
20	H	NH_2	Br	10	7,6
21	H	NH_2	I	9,45593196	7,7
22	H	NH_2	CF_3	8,48148606	8,3
23	H	NO_2	H	4,92081875	4,7
24	H	NO_2	F	5,21467016	5,4
25	H	NO_2	Cl	6,09151498	4,7
26	H	NO_2	Br	6	4,6
27	H	NO_2	I	6,26760624	4,7
28	H	NO_2	CF_3	5	5,1
29	OMe	OMe	H	7,537602	7,1
30	OMe	OMe	F	8,4202164	7,6
31	OMe	OMe	Cl	9,50863831	6,9
32	OMe	OMe	Br	10,60206	6,8
33	OMe	OMe	I	9,05060999	7
34	OMe	OMe	CF_3	9,61978876	6,2
35	NHMe	H	Br	8,39794001	7,7
36	NMe_2	H	Br	7,07572071	7
37	$NHCO_2Me$	H	Br	7,92081875	7
38	H	OH	Br	8,32790214	7,2
39	H	NHAc	Br	7,39794001	5,6
40	H	NHMe	Br	8,15490196	7,5
41	H	NHEt	Br	7,92081875	6,7
42	H	NMe_2	Br	7,95860731	6,8
43	NH_2	NH_2	Br	9,92081875	8,2
44	NH_2	NHMe	Br	9,16115091	8,9
45	NH_2	NMe_2	Br	6,79860288	8,1
46	NH_2	OMe	Br	8,4202164	8,1
47	NH_2	Cl	Br	8,18708664	7,8
48	NO_2	NH_2	Br	7,27572413	5,9
49	NO_2	NHMe	Br	7,16749109	5,3

Nº.	R_1	R_2	X	pIC_{50}[a] ($-logIC_{50}$[a])	Kinase Inhibitor x10
50	NO_2	NMe_2	Br	5,69897	4,6
51	NO_2	NHAc	Br	7,55284197	3,4
52	NO_2	OMe	Br	7,82390874	5,1
53	NO_2	Cl	Br	7,60205999	4,7
54	OC	H_2O	Br	7,82390874	6,6
55	OH	OH	Br	9,76955108	7,6
56	OEt	OEt	Br	11,2218487	5,8
57	OPr	OPr	Br	9,76955108	5,5
58	OBu	OBu	Br	6,9788107	5,4
59	5,6di-OME			5,86423149	6,8
60	5,6di-OME			5	5,6
61	2-Me		3'-Br	5	4,6
62	2-NH_2		3'-Br	6,33441901	7,1
63	4N-Me		3'-Br	6,81815641	6,9
64	5-OMe		3'-Br	9,1739252	7
65	8-OMe		3'-Br	5	5,6
66	H		2'-Br	6,89279003	6,1
67	H		4'-Br	9,01772877	6,5
68	H		3',4'-diBr	10,1426675	7
69	H		3',5'-diBr	6,94692156	6,6

The results (Table 2) were expressed as a decimal scale ten times larger than the scale obtained for the *pIC_{50}*. It was made to correct the distortion of scale, between the values. The correction does not interfere with evaluation of kinase inhibition ability but allowed to enter the values of *pIC_{50}* in the same ratio of kinase inhibition. Thus, for example, the result of the ability to inhibit kinase to molecules **1** was **0.59** and when using the correction factor was **5.9**. The result obtained for the molecule **2** was **0.69** and after using the correction factor was **6.9**. To molecule **3** obtained a result of **0.6** and after using the correction factor was **6.0**. This correction was made to all molecules. We can observe that after multiplying the results for ten, the ability to inhibit kinase for each molécule was not changed, *i.e.* the molecule **2** showed the

greatest ability to inhibit kinase followed by molecule 3 and 1. This result was equal before multiplying for ten.

The values obtained to predict bioactivity were multiplied by 10 and this is important because allowed to enter the *pIC50* values obtained for the same ratio values. The corrected values are shown in Table 2.

The linear correlation between calculated pIC_{50} and predict bioactivity

The pIC_{50} values are experimental data and the values obtained for kinase inhibiting probability (predict bioactivity) is computational data. Using the pIC_{50} and predicity bioactivity values of each molecule we obtained a graph of linear correlation between these two variables with a $R^2 = 0.706$ (Figure 1).

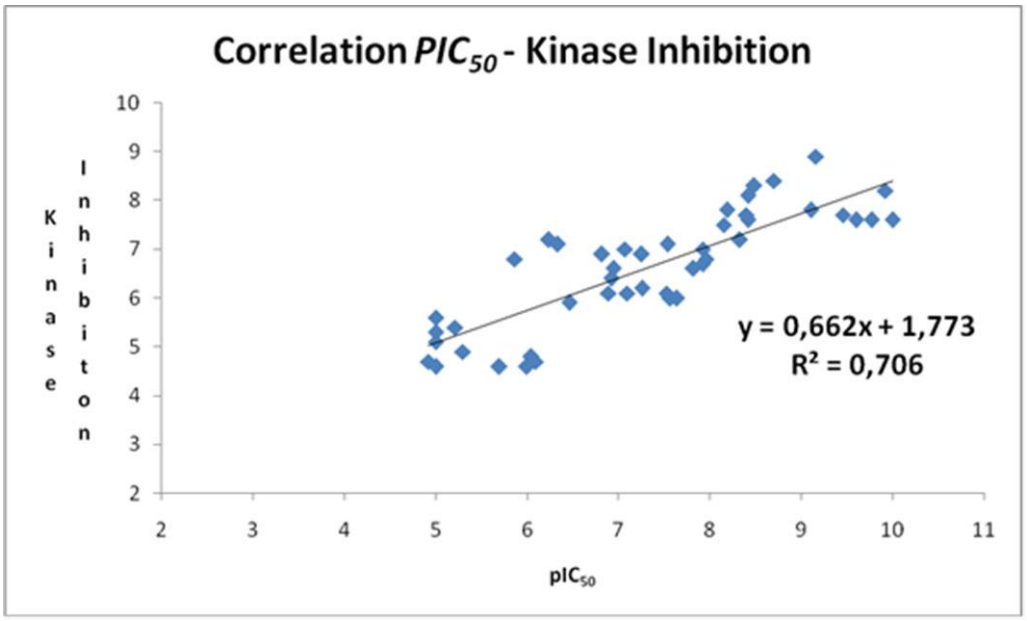


Figure 1 - The linear correlation between *pIC50* and calculated data for computationally inhibiting kinases.

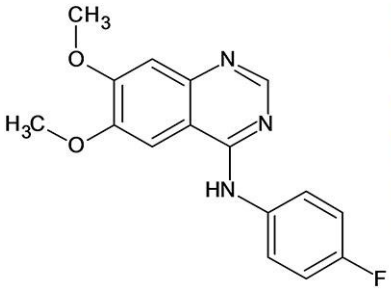
Elaboration of mathematical models can allow correlate variables. Such models are widely used in medicinal chemistry to develop new molecules with potential capacity to be new drugs [10]. A model able to make a correlation between the ability of kinase inhibition and biological activity (pIC_{50}) was built with a R^2 above of **0.7** (Figure 1). According to Dancey *et al.*, 2006[11] a R^2 above 0.7 can be considered strong in terms of score, an important feature to calibrate the model in study [12]. In this way, a model for correlation between two variables: pIC_{50} and predict bioativity was obtained. The predict bioactivity for each quinazoline was obtained using the computing software *Molinspiration* (www.molinspiration.com/cgi-bin/properties). This platform uses correlations between these two variables for QSAR models

calibrated and validated (www.acdlabs.com/products/draw_nom/draw), thus, increases the acurace of the created model. The ability of kinase inhibition was obtained for each quinazoline from Table 1.

Proposals of new molecules and their respective *pIC$_{50}$* values estimated by the method

The results obtained in this work was gerated by correlation between the two variables (*pIC$_{50}$* and predict bioactivity) what allowed to do the estimation of *pIC$_{50}$* or biological activity for three new molecules proposed as EGFR inhibitors (Figure 2 and molecules XYZ Table 1 and 2).

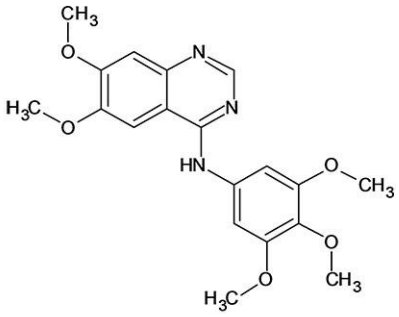
A) miSMILES COc3cc2ncnc(Nc1ccc(F)cc1)c2cc3OC



Molinspiration bioactivity score	v2014.03
GPCR ligand	0.06
Ion channel modulator	-0.03
Kinase inhibitor	0.73
Nuclear receptor ligand	-0.35
Protease inhibitor	-0.42
Enzyme inhibitor	0.14

Y = 0,662X + 1,773 **R^2 = 0,706**
pIC_{50} = 0,662 x (0,73 x10) + 1,773
pIC_{50} = 6,61

B) miSMILES COc3cc2ncnc(Nc1cc(OC)c(OC)c(OC)c1)c2cc3OC



Molinspiration bioactivity score	v2014.03
GPCR ligand	0.04
Ion channel modulator	-0.03
Kinase inhibitor	0.59
Nuclear receptor ligand	-0.35
Protease inhibitor	-0.30
Enzyme inhibitor	0.12

Y = 0,662X + 1,773 **R^2 = 0,706**
pIC_{50} = 0,662 x (0,59 x10) + 1,773
pIC_{50} = 5, 68

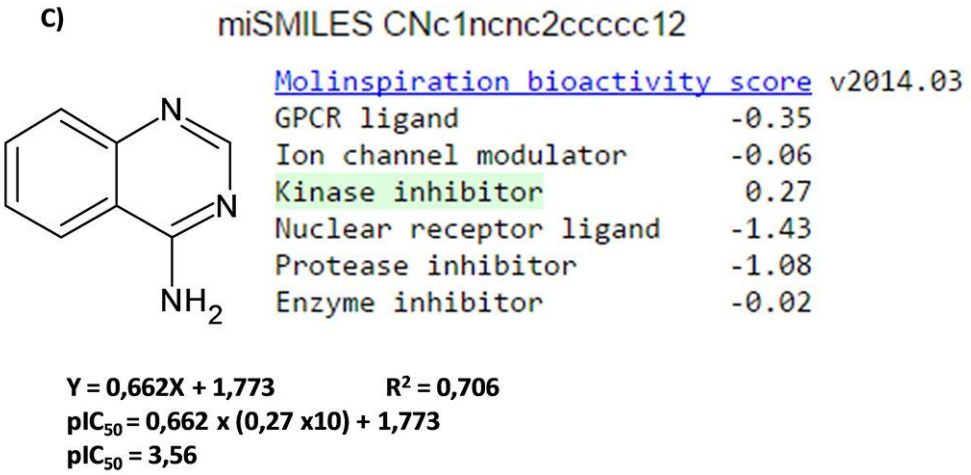


Figure 2 - Molecule 1, 2 and 3 have been proposed to estimate *pIC$_{50}$* values using the test model. (a) Molecule 1 with a higher *pIC$_{50}$* value (6.61). (b) Molecule 2 with a moderate *pIC$_{50}$* (5.68) and (c) Molecule 3 with a low estimated *pIC$_{50}$* (3.56).

Several models has been used for correlation of biological activity and structure known. As exemple the QSAR has been created and contributed to the development of new drug candidates [13]. After creating these models, new molecules are tested and their biological activities, e.i, their *pIC$_{50}$* are estimated without the need to synthesize and to experimental test for biological inhibition. This strategy can reduce time and cost in the development of new drug candidates [10, 14]. Using this methodology, three new molecules were proposed to test the model created in this study (Figure 2). The bioactivity for three molecules were computationally estimated using this model and the values obtained were used for *pIC$_{50}$* estimation. As a result we obtain the estimated values for one molecule was **6.61** (Figure 2a) and **5.68** for the molecule 2 (Figure 2b). These results may be considered that these molecules are strong inhibitors. The molecule **3** exhibited a low pIC50 3.56 (Figure 2c). At last, we can conclude that molecule 1 (Figure 2a) would be the bast candidate proposed for the synthesis as a EGFR-inhibitor.

Conclusion

The rational development of new drugs is a process of high cost and long time consume. Methodologies to reduce time and improve a screening of promising molecules can be created and optimized. Accordingly, the model created in this work correlate biological activity and the ability of molecules to inhibit kinases by the calcule of *pIC$_{50}$* to new candidates of EGFR-inhibitors. This result can be seen for molecules **1** and **2** (Figure 2). Thus, this new method

can be considered as good example of computational studies used for to development of new drugs.

Conflict of Interest

They are no conflict interests were involved in this research.

Acknowledgments

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