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In Silico Study of Pyrazolylaminoquinazoline Toxicity by Lazar, Protox, and Admet Predictor

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ABSTRACT

Pyrazolylaminoquinazoline is obtained from synthetic AZD4547 and can inhibit kinase activity in recombinant fibroblast growth factor receptor (FGFR) in vitro. The objective of this study was to obtain high activity and low toxicity pyrazolylaminoquinazoline derivatives in silico. The 2-dimensional structures were generated using the ChemDraw application. The Lazar application was used to predict endpoint carcinogenicity, maximum daily dose, and mutagenicity. The ProTox application was used for endpoint LD50 and toxicity classes, while the ADMET application was used for endpoint hepatotoxicity, with reproductive system disorders, and endocrine. Based on the scoring from the three software applications, two compounds were identified as being active against FGFR 2, with no carcinogenic or toxic effects on the liver, endocrine system, and the reproductive system, but they were predicted to have mutagenic effects. These compounds were V29 (N-(5-(3,5-dimethoxy phenethyl -1H-pyrazol-3-yl)-7(octahydro-2H-pyrido[1,2-a]pyrazine-2-yl) quinazoline-4-amine), with an IC50 of 0.2 ± 0.1 nM and a toxicity score of 1027, and V32 (N-(5-(3,5-dimethoxy phenethyl)-1H-pyrazol-3-yl)-7-(4-(dimethylamino)piperidine-1-yl)quinazoline-4-amine), with an IC50 of 0.3 ± 0.1 nM and a toxicity score of 1024.

INTRODUCTION

Many bioactive compounds have been shown to have anticancer activity, but their uses are limited due to side effects and high toxic effects (Malchers *et al*., 2017). Nonetheless, toxicity can be assessed using computational resources (computational algorithms, software, and data) to organize, analyze, model, simulate, visualize, or predict chemical toxicity (Raies and Bajic, 2016). Predicted toxicity *in silico* is performed prior to *in vitro* and *in vivo* testing to minimize the number of test compounds and test animals in subsequent tests. Such in silico tests include Lazy Structure-Activity Relationships (Lazar), Prediction of Rodent Oral Toxicity (ProTox), and ADMET Predictor™.

Lazar is a useful tool to predict the toxic properties of chemical structures. It produces predictions for the query structure of the database with experimentally determined toxicity data in the quantitative QSAR (quantitative structure-activity relationship) statistical approach. The performance of the Lazar software model in the external validation dataset has an accuracy of 86% and a sensitivity of 78% in the carcinogenicity test, with 95% accuracy for the mutagenicity test (Helma, 2006).

ProTox is a web server for predicting small molecule oral toxicity in rodents. LD_{50} and toxicity classes are calculated on the basis of chemical compounds similar to those of toxic compounds. Researchers rely on known toxicity data to develop models that can predict the toxicity of new compounds. This web server calculates sensitivity, specificity, and precision for all considered toxicity classes, with values of 76%, 95%, and 75% (Drwal *et al*., 2014).

ADMET Predictor™ uses integrated sequences to examine how the molecular structure of a compound plays a role in absorption, distribution, metabolism, excretion, and toxicology. The classification accuracy qualitatively reaches 85–90%. The program has an intuitive user interface that allows visualization of the data (Hassan *et al*., 2013).

Pyrazolylaminoquinazoline derivative compounds can inhibit the fibroblast growth factor receptor (FGFR). Indeed, pyrazolilaminoquinazoline derivatives synthesized from AZD4547 have been shown to be effective, via targeting

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FGFR, against leukaemia in the KG-1 cell line (Gu *et al.*, 2006), gastric cancer in the KATO III cell line (Kunii *et al.*, 2008), bladder cancer in the RT112 cell line (Wang *et al.*, 2014), and lung cancer in the H1581 cell line (Malchers *et al.*, 2017). The IC_{50} values ranged from 0.2–10 Nm, but their toxicity was not determined. Therefore, this study aimed to predict the toxicity of pyrazolylaminoquinazoline derivatives *in silico* using Lazar, ProTox and ADMET PredictorTM applications. The results will help in the selection of anticancer drugs with high activity, but low toxicity prior to *in vivo* toxicity through preclinical testing. This is particularly important as *in vivo* animal testing is limited by time, ethical considerations, and a financial burden.

MATERIALS AND METHODS

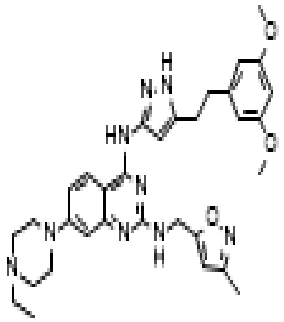
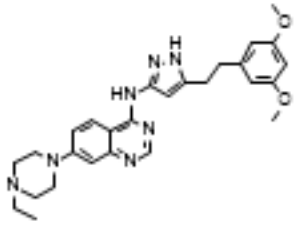
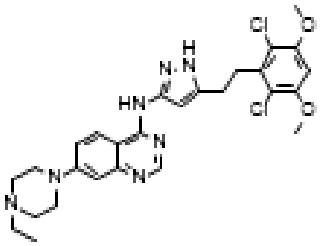
Equipment and materials

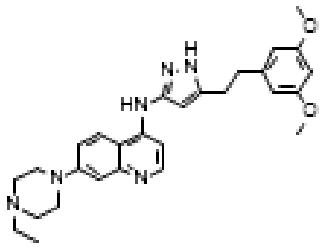
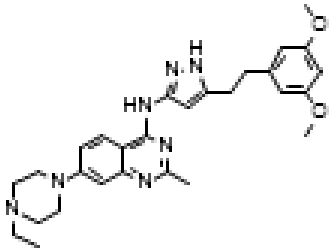
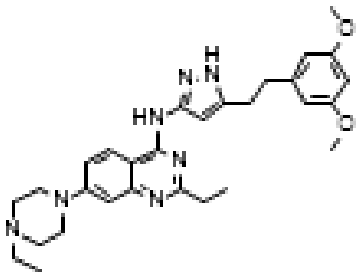
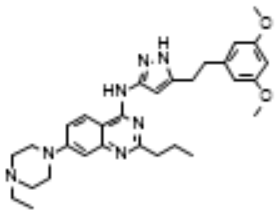
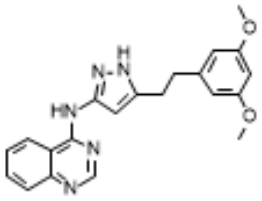
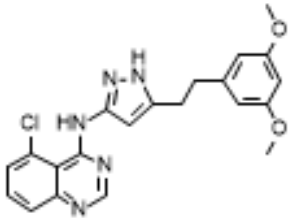
The hardware used in this study was a PC with AMD A8-7410 Quad Core 2.2-2.5 GHz specification, with 4 gigabytes of DDR3 RAM and a Windows 10 Pro 64-bit operating system. The software used were ChemDraw Pro 16.0 (http://scistore.cambridgesoft.com/) under license code: 338-428260-4806, pkCSM (http://biosig.unimelb.edu.au/pkcsm/), Open Babel GUI (http://openbabel.org/wiki/Category:Installation), Lazar (https://lazar.in-silico.ch/predict), ProTox (http://tox.charite.de/tox/), and ADMET Predictor™ v8.0.4.62016 (http://simplusdownloads.com/LicensingInstructions/AP8.html) with activation ID: 537-778-03-08-2017-10-03-11-5095, Node Locked ID: CF9B5E81DD7C, and License Model: FIXED. The pyrazolylaminoquinazoline derivatives analyzed with IC_{50} values according to Fan *et al.*, (2016) are shown in Table 1.

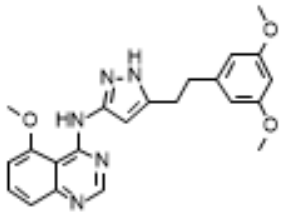
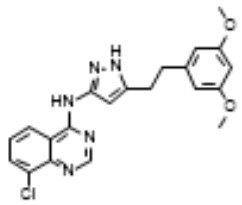
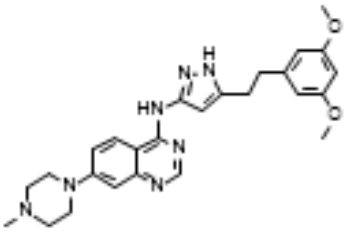
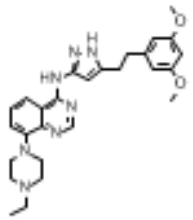
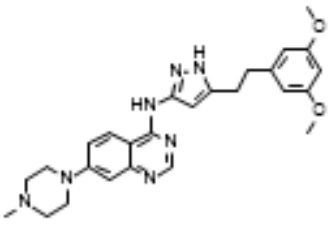
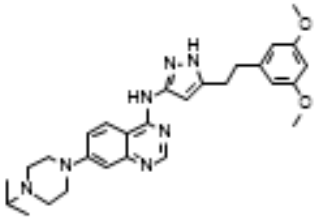
Experimental procedure

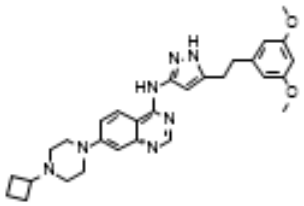
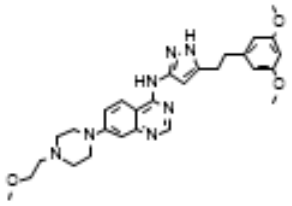
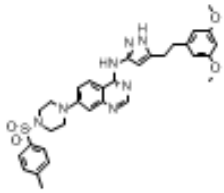
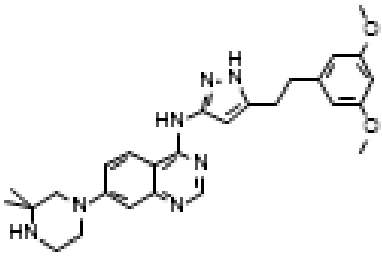
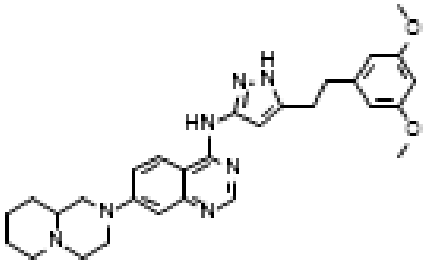
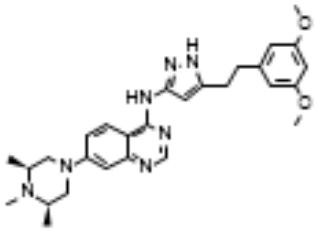
The 2D structure of 37 pyrazolylaminoquinazoline compounds was generated using the ChemDraw 2016 application. All pyrazolylaminoquinazoline compounds were screened using the pkCSM application to determine whether the compounds met Lipinski's Rule of Five. Compounds which did not meet the maximum two endpoints of Lipinski's Rule of Five were eliminated. The toxicity of the screened pyrazolylaminoquinozoline compounds was then predicted using Lazar for the carcinogenic endpoint, maximum daily dose, and mutagenicity, the ProTox application for LD_{50} endpoint and toxicity classes, as well as the ADMET Predictor application for hepatotoxicity endpoint, as well as reproductive system disorders, and endocrine.

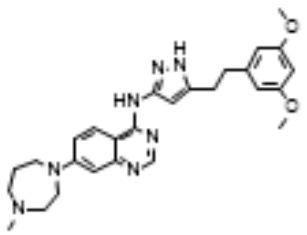
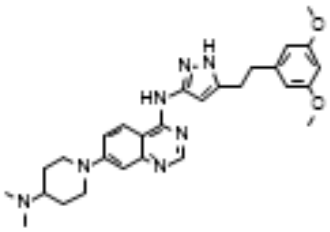
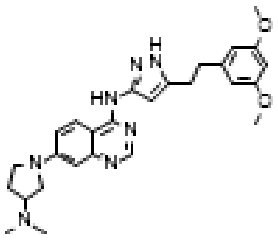
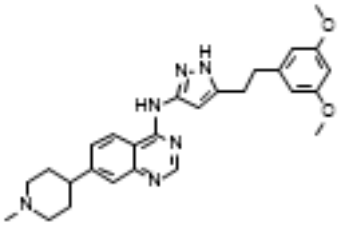
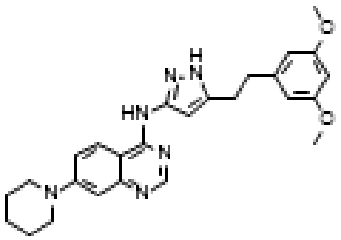
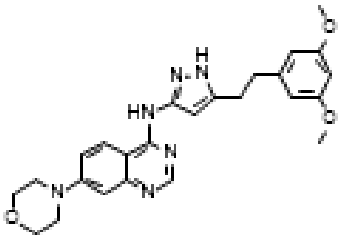
Table 1: Pyrazolylaminoquinazoline derivatives.

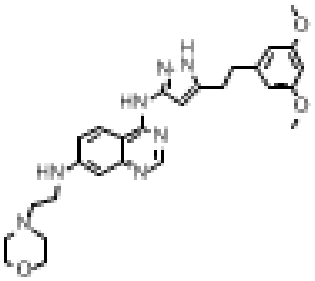
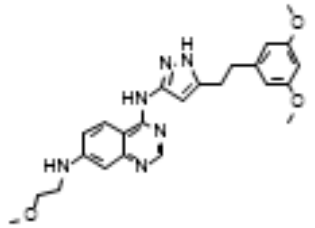
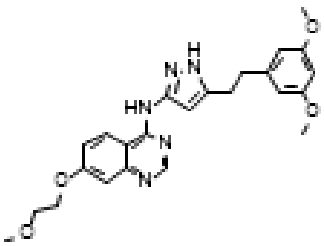
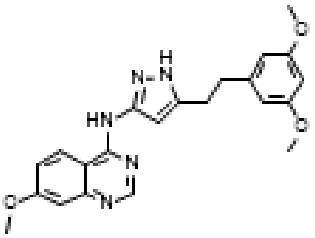
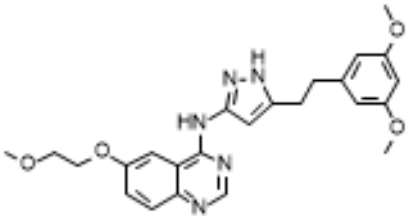
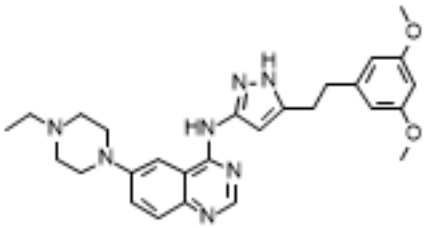
No.	Comp. Code	Structure	IC50 (nM)	Compound name
1	V2		<10	N4-(5-(3,5-dimethoxy phenethyl)-1H-pyrazol-3-yl)-7-(4-ethylpiperazine-1-yl)-N2-((3-methylisoxazol-5-yl) methyl)quinazoline-2,4-diamine
2	V3		0.8 ± 0.2	N-(5-(3,5-dimethoxy phenethyl) -1H-pyrazol-3-yl)-7-(4-ethylpiperazine-1-yl) quinazoline-4-amine
3	V12		0.3 ± 0.1	N-(5-(2,6-dichloro-3,5-dimeth oxyphenethyl)-1H-pyrazol-3-yl)-7-(4-ethylpiperazine-1-yl) quinazoline-4-amine

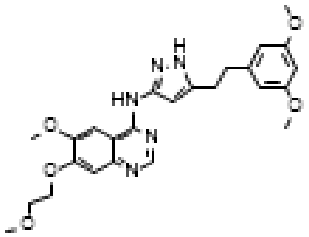
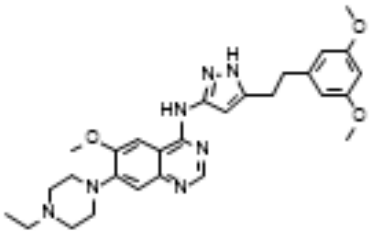
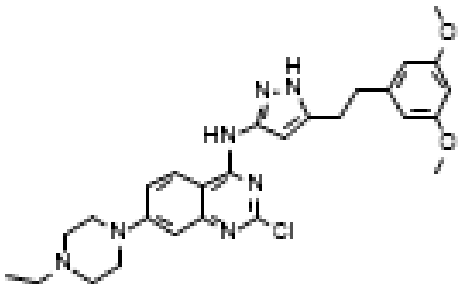
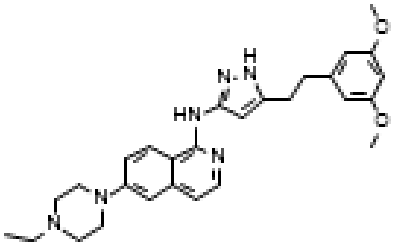
4	V13		29.9 ± 0.2	N-(5-(3,5-dimethoxy phenethyl) -1H-pyrazol-3-yl)-7-(4-ethylpipera-zine-1-yl) quinoline-4-amine
5	V14		0.6 ± 0.2	N-(5-(3,5-dimethoxy phenethyl) -1H-pyrazol-3-yl)-7-(4-ethylpiper-azine-1-yl)-2-methylquinazoline-4-amine
6	V15		0.5 ± 0.1	N-(5-(3,5-dimethoxy phenethyl) -1H-pyrazol-3-yl)-2-ethyl-7-(4-ethylpiperazine-1-yl) quinazoline-4-amine
7	V16		0.7 ± 0.2	N-(5-(3,5-dimethoxy phenethyl) -1H-pyrazol-3-yl)-7-(4-ethyl piper-azine-1-yl)-2-propyl quinazoline-4-amine
8	V17		3.2 ± 0.5	N-(5-(3,5-dimethoxy phenethyl)-1H-pyrazol-3-yl) quinazoline-4-amine
9	V18		16.9 ± 0.2	5-chloro-N-(5-(3,5-dimethoxy phenethyl)-1H-pyrazol-3-yl) quinazo-line-4-amine

10	V19		100.9 ± 0.2	N-(5-(3,5-dimethoxy phenethyl) -1H-pyrazol-3-yl)-5-methoxy-quinazoline-4-amine
11	V20		4.8 ± 0.9	8-chloro-N-(5-(3,5-dimethoxy phenethyl)-1H-pyrazol-3-yl) quinazo-line-4-amine
12	V21		3.9 ± 0.1	N-(5-(3,5-dimethoxy phenethyl) -1H-pyrazol-3-yl)-8-methoxy-quinazoline-4-amine
13	V22		4.3 ± 0.1	N-(5-(3,5-dimethoxy phenethyl) -1H-pyrazol-3-yl)-8-(4-ethylpipera-zine-1-yl) quinazoline-4-amine
14	V23		1.0 ± 0.2	N-(5-(3,5-dimethoxy phenethyl) -1H-pyrazol-3-yl)-7-(4-methylpipera-zine-1-yl)quinazoline-4-amine
15	V24		0.6 ± 0.0	N-(5-(3,5-dimethoxy phenethyl)-1H-pyrazol-3-yl)-7-(4-iso propylpipera-zine-1-yl) quinazoline-4-amine

16	V25		0.6 ± 0.1	7-(4-cyclobutylpiperaz-in-1-yl)-N-(5-(3,5-dimethoxy phenethyl)-1H-pyrazol-3-yl) quinazo-line-4-amine
17	V26		0.7 ± 0.2	N-(5-(3,5-dimethoxy phenethyl)-1H-pyrazol-3-yl)-7-(4-(2-methoxyethyl) piperazine-1-yl)-2-quinazoline-4-amine
18	V27		4.18 ± 0.1	N-(5-(3,5-dimethoxy phenethyl)-1H-pyrazol-3-yl)-7-(4-tosylpiperazine-1-yl) quinazoline-4-amine
19	V28		0.9 ± 0.1	N-(5-(3,5-dimethoxy pheneth-yl) -1H-pyrazol-3-yl)-7-(3,3-di methylpiperazine-1-yl) quinazoline-4-amine
20	V29		0.2 ± 0.1	N-(5-(3,5-dimethoxy pheneth-yl)-1H-pyrazol-3-yl)-7(octahy-dro-2H-pyrido[1,2-a]pyrazine-2-yl) quinazoline-4-amine
21	V30		0.2 ± 0.1	N-(5-(3,5-dimethoxy phenethyl)-1H-pyrazol-3-yl)-7-((3S,5R)-3,4,5-trimethyl piperazine-1-yl) quinazo-line-4-amine

22	V31		0.7 ± 0.2	N-(5-(3,5-dimethoxy phenethyl)-1H-pyrazol-3-yl)-7-(4-methyl-1,4-diazepan-1-yl)quinazoline-4-amine
23	V32		0.3 ± 0.1	N-(5-(3,5-dimethoxy phenethyl) -1H-pyrazol-3-yl)-7-(4-(dimethyl-amino) piperidin -1-yl) quinazoline-4-amine
24	V33		0.4 ± 0.2	N-(5-(3,5-dimethoxy phenethyl)-1H-pyrazol-3-yl)-7-(3-(dimethyl-amino) pyrrolidin-1-yl)quinazoline-4-amine
25	V34		0.8 ± 0.1	N-(5-(3,5-dimethoxy phenethyl) -1H-pyrazol-3-yl)-7-(1-methylpiperidin-4-yl) quinazoline-4-amine
26	V35		0.87 ± 0.1	N-(5-(3,5-dimethoxy phenethyl) -1H-pyrazol-3-yl)-7-(piperidin-1-yl) quinazoline-4-amine
27	V36		0.4 ± 0.1	N-(5-(3,5-dimethoxy phenethyl) -1H-pyrazol-3-yl)-7-morpholino-quinazoline-4-amine

28	V37		1.3 ± 0.3	N4-(5-(3,5-dimethoxy pheneth-yl)-1H-pyrazol-3-yl)-N7-(2-morphol-inoethyl) quinazoline-4,7-diamine
29	V38		2.7 ± 0.2	N4-(5-(3,5-dimethoxy phenethyl)-1H-pyrazol-3-yl)-N7-(2-methoxyeth-yl) quinazoline-4,7-diamine
30	V39		0.9 ± 0.2	N-(5-(3,5-dimethoxy phenethyl) -1H-pyrazol-3-yl)-7-(2-methoxye-thoxy) quinazoline-4-amine
31	V40		0.73	N-(5-(3,5-dimethoxy phenethyl) -1H-pyrazol-3-yl)-7-methoxy-quinazoline-4-amine
32	V41		43.9	N-(5-(3,5-dimethoxy phenethyl) -1H-pyrazol-3-yl)-6-(2-methoxye-thoxy) quinazoline-4-amine
33	V42		29.9	N-(5-(3,5-dimethoxy phenethyl) -1H-pyrazol-3-yl)-6-(4-ethylpiperaz-in-1-yl) quinazoline-4-amine

34	V43		58.8	N-(5-(3,5-dimethoxy phenethyl) -1H-pyrazol-3-yl)-6-methoxy-7-(2-methoxy ethoxy)quinazoline-4-amine
35	V44		66.7	N-(5-(3,5-dimethoxy phenethyl)-1H-pyrazol-3-yl)-7-(4-ethylpiperazin-1-yl)-6-methoxy quinazoline-4-amine
36	V46		0.6 ± 0.2	2-chloro-N-(5-(3,5-dimethoxy phenethyl)-1H-pyrazol-3-yl)-7-(4-ethylpiperazine-1-yl) quinazoline-4-amine
37	V50		1.2 ± 0.2	N-(5-(3,5-dimethoxy phenethyl) -1H-pyrazol-3-yl)-6-(4-ethylpipera-zine-1-yl) isoquinazoline-4-amine

Data analysis

The predictions were in the form of quantitative and qualitative data. Qualitative data were expressed in positive and negative statements, then expressed in the form of scoring, where a positive toxic score is 1 and a negative toxic score is 2. The data were scaled by summing all endpoints of the Lazar, ProTox, and ADMET predictions to obtain five compounds with the lowest toxicity, that is, the largest score. Five pyrazolylaminoquinazolin compounds were then selected which possessed high activity based on the *in vitro* test of Fan *et al.* (2016). The best compound was then obtained through the selected scoring model by comparing each compound with a low toxic effect, followed by the highest number of toxic negative endpoints. The next step selected two compounds with the highest activity and the lowest toxicity, by comparing the highest scores and the smallest IC_{50} value among the five compounds.

RESULTS AND DISCUSSION

Lipinski's Rule of Five

Lipinski's Rule of Five helps to determine the level of absorption or permeability of lipid bilayers present in the human body, demonstrating the oral bioavailability of a compound. Good bioavailability will satisfy the Lipinski rule, where the maximum molecular weight of the compound is 500, the log P is not more than 5, the donor hydrogen bond is not more than 5, and the number of hydrogen bond acceptor is less than 10 (Lipinski *et al.*, 2001). The results of the Lipinski's Rule of Five calculations using pkCSM are presented in Table 2.

According to Table 2, all pyrazolylaminoquinazoline compounds met the Lipinski rule, so it can be predicted that all compounds have good absorptivity for oral medication. Veber *et al.* (2002) concluded that the lower molecular weight, log P,

hydrogen bond donors, and hydrogen bond acceptor, the higher the bioavailability of a candidate drug.

Toxicity prediction

Based on the results of Lazar, carcinogenicity test prediction of Carcinogenic Potency Database (CPDB) with Leave One Out (LOO) cross-validation of the compounds V14, V15, V18, V21, V25-V26, V29, V31, V34, V39-V43, and V46 is non-carcinogenic, but V40 has the highest non-carcinogenic probability, with probability values 0.0895 for hamster, 0.102 for house mouse and 0.108 for mouse. The higher the non-carcinogen probability value, the higher the non-carcinogenic nature of a compound (Helma, 2006). Ranked from the highest to the lowest non-carcinogen probability values, the compounds are V40, V46, V41, V21, V31, V39, V42, V29, V14, V15, V43, V26, V18, V34, and V25, while compounds V30, V32, V35, and V36 are carcinogens. Regarding the maximum daily dose prediction, the smaller the maximum dose, the more toxic the compound. The maximum daily dose could not be predicted for most compounds due to the lack of similar structures, except for compound V13, which was 7.57 mg/kg BW/day. According to the *in vitro* mutagenicity prediction (Ames test) from the Kazius/Bursi dataset using LOO cross-validation in the CPDB application domain, 35 compounds were predicted to have a risk of a mutagen. However, compound V29 had the lowest mutagen probability with a value of 0.0988. The lower the probability value of mutagen, the lower the mutagen property of a compound (Helma, 2006).

Table 2: Lipinski's Rule of Five Analysis Results.

Comp. code	BM (<500)	LogP (<5)	Hydrogen Bond Acceptor	Hydrogen Bond Donor	Comp. code	BM (<500)	LogP (<5)	Hydrogen Bond Acceptor	Hydrogen Bond Donor
V2	597.724	4.949	11	3	V29	513.646	4.573	8	2
V3	487.608	4.041	8	2	V30	501.635	4.428	8	2
V12	556.498	5.348	8	2	V31	487.608	4.041	8	2
V13	486.62	4.646	7	2	V32	501.635	4.429	8	2
V14	501.635	4.349	8	2	V33	487.608	4.039	8	2
V15	515.662	4.603	8	2	V34	472.593	4.708	7	2
V16	529.689	4.993	8	2	V35	458.566	4.889	7	2
V17	375.432	3.899	6	2	V36	460.538	3.736	8	2
V18	409.877	4.552	6	2	V37	503.607	3.643	9	3
V19	405.458	3.908	7	2	V38	448.527	3.957	8	3
V20	409.877	4.552	6	2	V39	449.511	3.924	8	2
V21	405.458	3.908	7	2	V40	405.458	3.908	7	2
V22	487.608	4.041	8	2	V41	449.511	3.924	8	2
V23	473.581	3.651	8	2	V42	487.608	4.041	8	2
V24	501.635	4.429	8	2	V43	479.537	3.933	9	2
V25	513.646	4.573	8	2	V44	517.634	4.049	9	2
V26	517.634	3.667	9	2	V46	522.053	4.694	8	2
V27	613.744	4.718	9	2	V50	486.62	4.646	7	2
V28	487.608	4.087	8	3					

Regarding acute oral toxicity, based on the ProTox results, V37 compound was of moderate toxicity (Hodge and Sterner, 2005), with a LD_{50} value of 300 mg/kgBB and in class III Global Harmoni System (GHS) indicating that it could be toxic if swallowed (Drwal *et al.*, 2014). Compound V34 had an LD_{50} value of 3,550 mg/kgBW and in class V GHS, so harmful if swallowed (Drwal *et al.*, 2014). It belongs to class IV (500–5.000 mg/kgBB) according to Hodge and Sterner (2005), so it is mildly toxic. The thirty-three other compounds had LD_{50} values between 380–1130 mg/kgBW and were class IV GHS IV toxicity class, indicating that they are dangerous if swallowed (Drwal *et al.*, 2014). Furthermore, they were also class III (50–500 mg/kgBW) to grade IV (500–5000 mg/kgBW), which means they had moderate to mild toxicity (Hodge and Sterner, 2005).

Based on the results of ADMET Predictor, hepatotoxicity test, endocrine system toxicity, and repro toxicity, it can be seen that compounds V3, V14, V15, V23-V33, V35, V36, and V46 are predicted to have no toxic risk to liver function, the endocrine system, and the reproduction system. Hepatotoxicity predicts five increased serum enzymes for the diagnosis of liver damage, namely alkaline phosphatase (ALP), gamma glutamyl transferase (GGT), lactate dehydrogenase (LDH), aspartate transaminase/serum glutamate oxaloacetate transferase (AST/SGOT), and alanine transaminase/serum glutamate pyruvate transferase (ALT/SGPT). Hepatotoxicity prediction is issued by the Food and Drug Administration (FDA) on the side effects for human liver, based on two databases, the Spontaneous Reporting System (SRS) and the Adverse Event Reporting System (AERS). SRS data distinguishes three classes of compounds: inactive (RI < 3.0), slightly active ($3.0 \leq RI < 4.0$), and active ($RI \geq 4.0$). The ADMET Predictor sets the RI cut-off value at 3.0, therefore, the molecule with an RI < 3.0 is categorized as negative (normal) and with $RI \geq 3.0$ as positive (not normal) in each enzyme (Hassan *et al.*, 2013; Simulations Plus, 2016).

Table 3: Toxicity prediction results from Lazar, ProTox, and ADMET predictor.

Comp. Code	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	Total	Average
V12	1	2	2	0	1	500	4	2	2	2	2	2	2	2	2	526	35.06
V13	1	2	2	7.57	1	500	4	2	2	1	2	2	1	2	2	531	35.43
V14	2	2	2	0	1	1000	4	2	2	2	2	2	2	2	2	1027	68.46
V15	2	2	2	0	1	1000	4	2	2	2	2	2	2	2	2	1027	68.46
V16	1	2	2	0	1	1000	4	2	2	2	2	2	2	1	2	1025	68.33
V17	1	1	2	0	1	1060	4	2	1	1	2	2	2	2	2	1083	72.20
V18	2	2	2	0	1	1000	4	2	1	1	2	2	2	2	2	1025	68.33
V19	1	1	2	0	1	1130	4	2	1	1	2	2	2	2	2	1153	76.87
V20	1	1	2	0	1	1130	4	2	1	1	2	2	2	2	2	1153	76.86
V21	2	2	2	0	1	1000	4	2	1	1	2	2	2	2	2	1025	68.33
V22	1	1	2	0	1	625	4	2	2	1	2	2	2	2	2	649	43.26
V23	1	1	2	0	1	500	4	2	2	2	2	2	2	2	2	525	35.00
V24	1	1	2	0	1	500	4	2	2	2	2	2	2	2	2	525	35.00
V25	2	2	2	0	1	500	4	2	2	2	2	2	2	2	2	527	35.13
V26	2	2	2	0	1	380	4	2	2	2	2	2	2	2	2	407	27.13
V27	1	1	2	0	1	1000	4	2	2	2	2	2	2	2	2	1025	68.33
V28	1	1	2	0	1	500	4	2	2	2	2	2	2	2	2	525	35.00
V29	2	2	2	0	1	1000	4	2	2	2	2	2	2	2	2	1027	68.46
V30	1	1	1	0	1	500	4	2	2	2	2	2	2	2	2	524	34.93
V31	2	2	2	0	1	500	4	2	2	2	2	2	2	2	2	527	35.13
V32	1	1	1	0	1	1000	4	2	2	2	2	2	2	2	2	1024	68.26
V33	1	1	2	0	1	500	4	2	2	2	2	2	2	2	2	525	35.00
V34	2	2	2	0	1	3550	5	2	2	1	2	2	2	2	2	3577	238.46
V35	1	1	1	0	1	1000	4	2	2	2	2	2	2	2	2	1024	68.26
V36	1	1	1	0	1	500	4	2	2	2	2	2	2	2	2	524	34.933
V37	1	2	2	0	1	300	3	2	2	1	2	2	2	2	2	324	21.60
V38	1	1	2	0	1	500	4	2	2	1	2	2	2	2	2	524	34.93
V39	2	2	2	0	1	1060	4	2	2	1	2	2	2	2	2	1086	72.40
V40	2	2	2	0	1	1130	4	2	1	1	2	2	2	2	2	1155	77.00
V41	2	2	2	0	1	1060	4	2	2	1	2	2	2	2	2	1086	72.40
V42	2	2	2	0	1	500	4	2	2	1	2	2	2	2	2	526	35.06
V43	2	2	2	0	1	1060	4	2	2	1	2	2	2	2	2	1086	72.40
V44	1	1	2	0	1	500	4	2	2	1	2	2	2	2	2	524	34.93
V46	2	2	2	0	1	500	4	2	2	2	2	2	2	2	2	527	35.13
V50	1	1	2	0	1	740	4	2	2	1	2	2	2	2	2	764	50.93

where A: Hamster Carcinogenicity Test, B: House mouse Carcinogenicity Test , C: Mouse Carcinogenicity Test, D: Maximal Daily Dosage, E: Mutagenicity Test, F: LD_{50}, G: Toxicity Class (Class 1-6), H: ALP Test, I: GGT Test, J: LDH Test, K: AST Test, L: ALT Test, M: Oestrogen Test, N: Androgen Test, O: Reprocytocity Test, 0: unknown, 1: Positive Toxicity, 2: Negative Toxicity.

Based on the results of the scoring calculations of the three software applications in Table 3, the compound with the lowest toxicity has the highest average scores, which is V34, predicted to cause toxicity to LDH enzymes and V19, V20, and V40 predicted to be toxic to GGT and LDH enzymes. V43 is less effective than the best compound due to its high LD_{50} value

and predicted to be toxic to the liver. Therefore, further analysis is required by comparing the number of non-toxic endpoints for each compound.

From the analysis results, it is predicted that V14, V15, V25, V26, V29, V31 and V46 compounds have no carcinogenic, toxic effects on the liver, endocrine systems, and reproductive systems, but they are predicted to have mutagenic effects. The higher the LD_{50} of a compound, the lower the toxic effect. V14, V15, and V29 compounds have an LD_{50} of 1.000 mg/kgBW, V25, V31, and V46 have an LD_{50} of 500 mg/kgBW, while V26 has an LD_{50} of 380 mg/kgBW, so V26 compound was not selected for the lowest toxic effect.

The lowest mutagen effect has the smallest mutagenic probability value. V14, V15, V25, V29, V31 and V46 compounds have mutagenic probability values of 0.129, 0.125, 0.107, 0.0988, 0.159 and 0.127 respectively, so V31 was not selected for the lowest toxic effect. V14, V15, V25, V29, and V32 have the lowest toxicity with IC_{50} values of 0.6 nM, 0.5 nM, 0.6 nM, 0.2 nM and 0.3 nM respectively.

CONCLUSION

The *in silico* applications, Lazar, ProTox, and ADMET, were used to predict the toxicity of anticancer pyrazolylaminoquinazolin compounds, revealing that the two compounds with the highest activity and the lowest toxicity were V29 (N-(5-(3,5-dimethoxy phenethyl)-1H-pyrazol-3-yl)-7(octahydro-2H-pyrido [1,2-a] pyrazine-2-yl) quinazoline-4-amine), with a IC50 of 0.2 ± 0.1 nM and a toxicity score of 1027, and V32 (N-(5-(3,5-dimethoxy phenethyl)-1H-pyrazol-3-yl)-7-(4-(dimethylamino)piperidine-1-yl)quinazoline-4-amine) with an IC50 of 0.3 ± 0.1 nM and a toxicity score of 1024.

AUTHORS CONTRIBUTIONS

All authors contributed equally.

CONFLICTS OF INTERESTS

All authors have none to declare.

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