IJPSR (2018), Volume 9, Issue 1 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 06 May, 2017; received in revised form, 19 July, 2017; accepted, 31 July, 2017; published 01 January, 2018

VIRTUAL TOXICITY STUDIES OF NOVEL SPIROAZETIDIN-2-ONES TETHERED WITH FURANS

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Keywords:

Spiroazetidin-2-one, Furan, Toxicity, Mutagenicity, TOPKAT

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ABSTRACT: Background and aim: Spiroazetidin-2-ones and furans are commonly used as anti-bacterial, anti-fungal, anti-inflammatory, cardiovascular activities, anticancer, antiparkinson agents etc. The purpose of this study was to perform virtual toxicity studies of the synthesized compounds 1-(substitutedphenyl)-3-chloro-5,9-bis(furan-2-ylmethylidene)-1-azaspiro[3.5]nonan-2-ones (3a-3h). **Materials and methods:** Computational toxicology and mutagenicity profiles of these compounds were generated by using TOPKAT 6.1 (Toxicity Prediction Komputer Assisted Technology version 6.1). The molecular structure of the query compound was given as a SMILES string and a desired TOPKAT predictor was selected, then TOPKAT automatically conducts analysis of the query compound. **Results:** According to TOPKAT 6.1 model the compounds 3a - 3h are non-mutagenic and devoid of aerobic biodegradability. The computed Rat oral LD_{50} values for the compounds 3a - 3h ranged from 1.1g/kg to 115.0mg/kg. These high LD_{50} values suggest higher safety of these compounds. The computed probability of skin irritation for all compounds was found to be 1.000 and the probability for the carcinogenicity was found to be 0.950-1.000. **Conclusion:** By using computerized statistical methodology, more promising molecules can be identified. These studies provided us information for research to carry out an extensive study of novel spiroazetidin-2-one tethered with furan moieties for their toxicological profile.

INTRODUCTION: Spiroazetidin-2-ones and furans have emerged as an important class of drugs for the treatment of a variety of health conditions. The compounds having these nuclei are commonly used to treat health conditions which include anti-bacterial, anti-fungal, anti-inflammatory, cardio vascular activities, anticancer, antiparkinson agents *etc.* [1 - 7].

QUICK RESPONSE CODE

DOI:
10.13040/IJPSR.0975-8232.9(1).286-91

Article can be accessed online on:
www.ijpsr.com

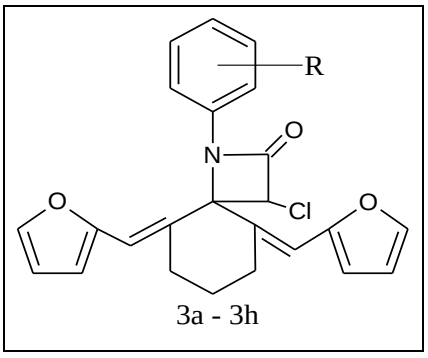
DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.9(1).286-91

Drug design softwares like TOPKAT enable the discovery of lead molecules, more efficiently and quickly. Conventional synthesis and evaluation of drugs require number of animal sacrifices, use of this software helps in reducing the number of animal sacrifices made for the *in vivo* studies. TOPKAT accurately and rapidly assess the toxicity of drugs solely from their 2D molecular structure using a range of robust, cross-validated Quantitative Structure Toxicity Relationship (QSTR) models for assessing specific toxicological end points, thus providing a detailed data of query molecules which can be widely analyzed and compared with the existing molecular libraries. Use of QSAR softwares undoubtedly reduces the

number of compounds synthesized there by providing with promising leads for further exploration. The rationale behind this work was to develop extensive toxicological profile of the derivatives containing 1-(substitutedphenyl)-3-chloro - 5, 9- bis (furan-2-ylmethylidene)-1-azaspiro[3.5]nonan-2-ones (3a - 3h) [8].

MATERIALS AND METHODS: The molecular structure of the query compound is given as SMILES string and a desired TOPKAT predictor is selected. If the structure is a member of training set, the database information for the compound is displayed. If the query does not belong to the training set, software displays the result with necessary warnings. Under these circumstances, caution in accepting the estimate should be exercised. Models which satisfy all the validation criteria for the query compound are computed and results are recorded [9 - 14].

Virtual toxicity studies have been done for the following set of compounds, substitutions for which is given in **Table 1**.



3a - 3h

TABLE 1: SET OF COMPOUNDS FOR SCREENING

Compound	R
3a	H
3b	4-NO_2
3c	4-Cl
3d	4-Br
3e	2-NO_2
3f	3,4-diCl
3g	4-F
3h	2,6-diCl

Evaluating an assessment: If we consider a TOPKAT assessment of a query structure as a hypothesis which states that the model parameters present in the query structure are the determinants of its toxicity, then this hypothesis can be tested against similar compounds in the model's database. The similarity search function in TOPKAT will automatically rank all the compounds in the respective model database based on their QSTR similarity to the query structure. Information regarding the actual experimental result, TOPKAT predicted result and whether the compound was used in training set is available for each compound. With this information whether the query structure lies in an information-rich region of the model data space and similar compounds are well predicted by the model is determined.

Computation of toxicity by TOPKAT: TOPKAT computes a probable value of toxicity for a submitted chemical structure from a Quantitative Structure-Toxicity Relationship (QSTR) equation. The equation is linear in the structure descriptors. The coefficients are optimized during the development of the equation. The product of a structure descriptors value and its corresponding coefficient is the descriptors contribution to the probable toxicity. Contributions from the products may be either positive or negative; a positive contribution will increase the probability of the chosen property, whereas a negative contribution will decrease it. Toxicity values are computed by summing the individual contributions. For assessing toxicity values such as LD50 or LC50, this sum is transformed into a weight/weight unit (mg/kg) or a weight/volume unit (mg/1); for 2-group classifications, such as carcinogens/non-carcinogens, this sum is transformed into a probability value between 0.0 and 1.0.

Probability values: Probability values from 0.0 to 0.30 are considered low probabilities, and chemicals with TOPKAT-computed probability values in this range are not likely to produce a positive response in an experimental assay; whereas probability values greater than 0.70 are considered high, and are likely to produce a positive response in an experimental assay. Probabilities greater than 0.30 but less than 0.70 are considered indeterminate (i.e., too near chance (0.50) for an assessment to be meaningful).

Query structure Examination: TOPKAT always outputs a value of toxicity; however, whether the assessment is meaningful or not can only be answered by:

1. A univariate analysis or Coverage Examination, that is, whether all of the structural fragments

of the query structure are well represented in the database compounds which were used to develop the model (training set).

2. A multivariate analysis, or OPS Examination, that is, whether the submitted structure fits within, or near the periphery of, the Optimum Prediction Space (OPS) of the equation.

These 2 steps are accomplished automatically in TOPKAT and results are output in terms of a confidence percentage.

Coverage Examination: Every QSTR model is associated with a certain training set of compounds, and these compounds contain a limited set of structural attributes. A QSTR model, when extrapolated to chemical structures containing structural attributes which are not represented in the training set, may produce unreliable toxicity assessments. Therefore, it is important to determine whether the structural attributes of the query molecule are represented in the compounds used for the development of a QSTR. TOPKAT automatically determines whether the input structure contains molecular substructures which are foreign to the training set (a univariate analysis). Additionally, during this process, TOPKAT compares the values of the model descriptors for the query structure to the range of the values of the respective descriptors in the training set compounds.

Optimum prediction space: As well as determining its coverage, TOPKAT checks whether a query structure is located inside or outside the Optimum Prediction Space (OPS) of a QSTR (multivariate analysis). The OPS of a QSTR is a multi-dimensional space, the number of dimensions being one more than the number of model parameters of the QSTR.

An important characteristic of the OPS is that within and near its periphery the QSTR may be applied with confidence. The OPS confidence contains information about both the Optimum Prediction Space and the fragment coverage. When a query structure is determined to be inside all dimensions of a model OPS, the computed value of toxicity can be considered acceptable (unless evidence exists to refute the assessment).

However, if a query structure is found outside one or more dimensions, the computed toxicity may or may not be acceptable depending on the query's distance from OPS. The distance of a query structure from the OPS is a complex function of the query's location in each dimension. Every TOPKAT QSTR model has a permissible limit of distance from the OPS.

If the query structures distance from the OPS is greater than this permissible limit, the TOPKAT-assigned toxicity value is considered unacceptable. The permissible limits of distance from the OPS for all QSTR models have been precalculated and stored in TOPKAT. For every query structure outside the OPS, TOPKAT reports the location of a query structure with respect to the permissible limit of distance from the OPS.

RESULTS AND DISCUSSION: All the 8 derivatives containing 1-(substitutedphenyl)-3-chloro-5,9-bis(furan - 2 -ylmethylidene)-1-azaspiro [3.5]nonan-2-ones (3a - 3h) were extensively studied by TOPKAT 6.1.

TABLE 2: RAT ORAL LD_{50} AND LOG P DATA

Compound	Rat Oral LD_{50} (v3.1)		Log P (v3.1)	
	Computed Rat Oral LD_{50}	95% Confidence Limits	Assessment of Log P	95% Confidence Limits
3a	708.9 mg/kg	110.7 mg/kg & 4.5 g/kg	3.575	3.144 & 4.006
3b	386.6 mg/kg	62.0 mg/kg & 2.4 g/kg	2.641	2.209 & 3.074
3c	385.5 mg/kg	61.9 mg/kg & 2.4 g/kg	4.076	3.647 & 4.506
3d	330.4 mg/kg	51.8 mg/kg & 2.1 g/kg	4.249	3.818 & 4.680
3e	353.8 mg/kg	57.4 mg/kg & 2.2 g/kg	2.433	1.997 & 2.868
3f	115.0 mg/kg	17.9 mg/kg & 737.5 mg/kg	4.467	4.035 & 4.900
3g	1.1 g/kg	174.2 mg/kg & 6.8 g/kg	3.406	2.977 & 3.835
3h	158.6 mg/kg	25.4 mg/kg & 992.0 mg/kg	4.103	3.658 & 4.548

TABLE 3: AEROBIC BIODEGRADABILITY AND DEVELOPMENTAL TOXICITY POTENTIAL DATA

Compound	Aerobic Biodegradability (v6.1)		Developmental Toxicity Potential (DTP) (v3.1)	
	Computed Probability	Discriminant Score	Computed Probability	Discriminant Score
3a	0.000	-32.704	0.000	-18.738
3b	0.000	-28.347	0.000	-20.378
3c	0.000	-41.558	0.000	-19.894
3d	0.000	-11.896	0.000	-19.894
3e	0.000	-35.526	0.000	-18.535
3f	0.000	-46.264	0.000	-15.177
3g	0.000	-50.663	0.000	-19.894
3h	0.000	-35.821	0.000	-12.636

TABLE 4: MUTAGENICITY AND SKIN IRRITATION DATA

Compound	Mutagenicity (v3.1)		Skin Irritation (v6.1)	
	Probability of Biodegradability	Discriminant Score	Probability of MOD/SEV	Discriminant Score
3a	0.000	-25.709	1.000	58.610
3b	0.000	-14.440	1.000	44.446
3c	0.000	-27.855	1.000	53.970
3d	0.000	-28.208	1.000	53.090
3e	0.000	-16.493	1.000	43.507
3f	0.000	-31.181	1.000	51.567
3g	0.000	-34.760	1.000	56.984
3h	0.000	-33.709	1.000	48.272

TABLE 5: CARCINOGENICITY MALE MOUSE AND CARCINOGENICITY FEMALE MOUSE DATA

Compound	Carcinogenicity Male Mouse (v3.2)		Carcinogenicity Female Mouse (v3.2)	
	Computed Probability	Discriminant Score	Computed Probability	Discriminant Score
3a	1.000	104.21	1.000	23.503
3b	1.000	118.636	1.000	29.191
3c	1.000	110.701	1.000	27.277
3d	1.000	109.731	1.000	24.590
3e	1.000	114.986	1.000	26.679
3f	1.000	112.927	1.000	21.776
3g	1.000	113.473	1.000	34.954
3h	1.000	107.591	1.000	24.806

TABLE 6: CARCINOGENICITY MALE RAT AND CARCINOGENICITY FEMALE RAT DATA

Compound	Carcinogenicity Male Rat (v3.2)		Carcinogenicity Female Rat (v3.2)	
	Computed Probability	Discriminant Score	Computed Probability	Discriminant Score
3a	0.969	3.452	0.058	-25.709
3b	0.998	6.464	1.000	-14.440
3c	0.980	3.911	0.000	-27.855
3d	0.980	3.911	0.000	-28.208
3e	0.999	6.505	0.924	-16.493
3f	0.987	4.351	0.000	-31.181
3g	0.980	3.911	0.000	-34.760
3h	0.986	4.257	0.000	-33.709

According to TOPKAT 6.1 model, the computed Rat oral LD50 values for the compounds 3a-3h ranged from 1.1g/kg to 115.0mg/kg. These high LD_{50} values suggest higher safety of these compounds. And Log P values of all 8 derivatives are well below 5.6. So Log P parameter of all the derivatives obey Lipinski's rule and fall well within the range of -0.4 to +5.6 (**Table 2**).

The compounds (3a-3h) were devoid of aerobic biodegradability. The structure descriptors contribute negatively to the assessment of confidence limits. All the derivatives resulted in very low computed probability and negative discriminant score values for Developmental Toxicity Potential (V 3.1). Data is given in **Table 3**.

All the 8 compounds (3a-3h) are non-mutagenic and the computed probability of skin irritation for all compounds was found to be 1.000, there would be probability of skin irritation on topical application (**Table 4**).

All the derivatives showed the computed probability value 1.0 and discriminant scores are not likely to produce a positive response in an experimental assay and positive contribution to the increase in the probability of chosen property which is carcinogenicity of male mouse (V 3.2) and female mouse (3.2). Data is given in **Table 5**. Computed probability values of all the derivatives range from 0.96 to 0.99 for the carcinogenicity of male rat (V 3.2) are greater than 0.7, which implies that they are likely to produce a positive response in an experimental assay.

All the derivatives except 3b and 3e showed the computed probability value less than 0.7 and negative discriminant scores are not likely to produce a positive response in an experimental assay and negative contribution to the increase in the probability of chosen property which is carcinogenicity of female rat (V 3.2). Data is given in **Table 6**.

CONCLUSION: The success of previous generation of drugs and vaccines has lead to an increase in human life expectations. But it does not mean that all the molecules developed each year by random screening methodology are successful to provide new blockbuster drugs. So it is time to change the methodology for research from random screening to rationalized approach of drug design. By following the computerized statistical methodology, more promising molecules can be identified. This has provided us with some direction for research to carry out an extensive study of novel derivatives of Spiroazetidin-2-ones tethered with furans (3a-3h) for their toxicological profile.

ACKNOWLEDGEMENT: The authors are thankful to the management of Faculty of Pharmacy, M.S. Ramaiah University of Applied Sciences, Bangalore, India for providing necessary facilities.

CONFLICT OF INTEREST: Conflict of interest declared none.

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How to cite this article:
Srikala R and Babu MN: Virtual toxicity studies of novel spiroazetidin-2-ones tethered with furans. Int J Pharm Sci & Res 2018; 9(1): 286-91. doi: 10.13040/IJPSR.0975-8232.9(1).286-91.