



VIRTUAL TOXICITY STUDIES OF NOVEL 1,4-DIHYDROPYRIDINE DERIVATIVES

Asma samaunnisa A^{1*}, C.H.S. Venkataramana¹, V Madhavan²¹Department of Pharmaceutical chemistry, M.S.Ramaiah College of Pharmacy, Bangalore, India²Department of Pharmacognosy, M.S. Ramaiah College of Pharmacy, Bangalore, India

Email: asmasamaunnisa@gmail.com

Article Received on: 10/01/13 Revised on: 07/02/13 Approved for publication: 18/03/13

DOI: 10.7897/2230-8407.04354

IRJP is an official publication of Moksha Publishing House. Website: www.mokshaph.com

© All rights reserved.

ABSTRACT

Virtual toxicity of N³, N⁵-diphenyl-1,4-dihydropyridine-3,5-dicarbohydrazides [2A-2D'] and 2,6-dimethyl-1,4-dihydropyridine-3,5-yl-bis[carbonyl-2-(phenyl)]pyrazolidine-3,5-diones [3A-3D'] were extensively studied. 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. TOPKAT automatically conducts a unique 2-stage analysis of the query compound. Various models were assessed and obtained results were thoroughly discussed.

Keywords: 1,4-Dihydropyridine, Pyrazolidine-3,5-Diones, Toxicity, Mutagenicity, TOPKAT.

INTRODUCTION

1,4-Dihydropyridines and pyrazolidine-3,5-diones have emerged as important classes of drugs for the treatment of variety of health conditions. These nuclei are common to numerous bioactive compounds which include antihypertensive, antitumor, vasodilator, antibacterial, anticonvulsant, anti-inflammatory, antidiabetic agents¹⁻⁶.

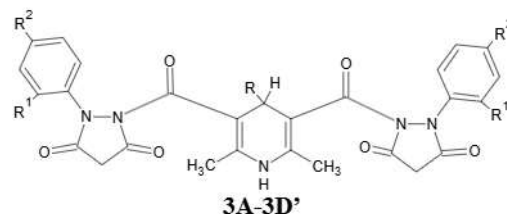
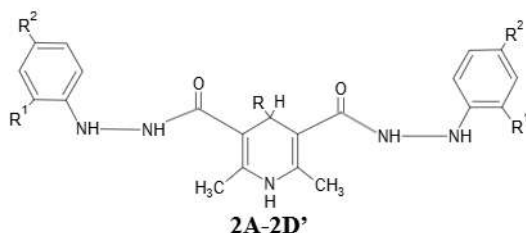
Drug design softwares like TOPKAT enables the discovery of lead molecules, more efficiently and quickly. Conventional synthesis and evaluation of drugs require number of animal sacrifices. Use of this software helps in reducing the number of animal sacrifices made for the *in vivo* studies. TOPKAT accurately and rapidly assesses 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, 4-Dihydropyridines and pyrazolidine-3,5-dione moieties(2A-2D')⁷ and (3A-3D')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.^{9,10}

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



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/l); 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.

Table 1: Set of compounds for virtual toxicity studies

Compound	R ¹	R ²	R
2A	H	H	H
2B	NO ₂	NO ₂	H
2C	H	Cl	H
2D	H	NO ₂	H
2A'	H	H	C ₆ H ₄ OH
2B'	NO ₂	NO ₂	C ₆ H ₄ OH
2C'	H	Cl	C ₆ H ₄ OH
2D'	H	NO ₂	C ₆ H ₄ OH
3A	H	H	H
3B	NO ₂	NO ₂	H
3C	H	Cl	H
3D	H	NO ₂	H
3A'	H	H	C ₆ H ₄ OH
3B'	NO ₂	NO ₂	C ₆ H ₄ OH
3C'	H	Cl	C ₆ H ₄ OH
3D'	H	NO ₂	C ₆ H ₄ OH

Table 2: Rat oral LD50 and LogP data

Compound	Rat oral LD50 (V 3.1)		LogP (V 3.1)	
	Computed Rat LD ₅₀	95% confidence limit	Assessment of LogP	95% confidence limit
2A	937.5mg/kg	154mg/kg & 5.7g/kg	5.413	4.876 & 5.949
2B	1g/kg	1.8g/kg & >10g/kg	1.183	0.627 & 1.738
2C	1.2g/kg	198.3mg/kg & 6.9g/kg	6.527	6.003 & 7.052
2D	2.5g/kg	448.5mg/kg & >10g/kg	3.349	2.844 & 3.854
2A'	381.7mg/kg	65.1mg/kg & 2.2g/kg	5.991	5.436 & 6.547
2B'	625.4mg/kg	108.7mg/kg & 3.8g/kg	0.9361	0.3 & 1.572
2C'	400.5mg/kg	69.9mg & 2.3g/kg	6.866	6.6317 & 7.414
2D'	475.7mg/kg	84.9mg/kg & 2.7g/kg	3.605	3.059 & 4.151
3A	>10g/kg	3.5 g/kg & 10g/kg	0.554	0.011 & 1.197
3B	>10 g/kg	>10g/kg & 10g/kg	-2.775	-3.529 & -2.022
3C	>10 g/kg	3.7g/kg & 10g/kg	1.635	1.091 & 2.179
3D	>10 g/kg	>10g/kg & 10g/kg	-1.174	-1.782 & -0.566
3A'	1.2 g/kg	167.9mg/kg & 9.1g/kg	0.146	-0.473 & -3.132
3B'	2.3 g/kg	157.2mg/kg & 10g/kg	-4.032	-4.932 & -3.132
3C'	1.2 g/kg	128.1mg/kg & 10g/kg	-2.032	-1.312 & -2.239
3D'	1.6 g/kg	179.5mg/kg & >10g/kg	-1.922	-2.637 & -1.208

Table 3: Rat inhalational LC50 and Developmental Toxicity Potential data

Compound	Rat inhalational LC50(V 3.1)		Developmental Toxicity Potential (V 3.1)	
	Computed values mg or g/m ³ /H	95% confidence limit mg or g/m ³ /H	Computed Probability	Discriminant Score
2A	812.1mg	110.6mg & 6g	1	27.999
2B	1g	61.9mg & >10g	1	28.737
2C	1.3g	181.4mg & 9.3g	1	29.379
2D	362mg	35mg & 3.7g	1	28.502
2A'	984.9mg	110.6mg & 8.8g	1	23.899
2B'	1.4g	76.5mg & >10g	1	35.944
2C'	1.6g	183.1mg & >10g	1	28.271
2D'	447.2mg	37.7mg & 5.3g	1	28.927
3A	18.6mg	1.4mg & 250.4mg	0.610	0.448
3B	1.6g	132.8mg & 10g	0	-13.959
3C	40.2mg	3.2mg & 499.7mg	0	-8.821
3D	34.4mg	2.9mg & 408.1mg	0	-13.513
3A'	27.3mg	1.8mg & 410.3mg	0.958	3.124
3B'	2.6mg	203.8mg & 10g	0	-24.142
3C'	2.3mg	192.8mg & >10g	0	11.12
3D'	52.2mg	4mg & 682.4mg	0.002	-6.391

Table 4: Carcinogenicity Male Mouse and Carcinogenicity Female Mouse data

Compound	Carcinogenicity Male Mouse (V 3.2)		Carcinogenicity Female Mouse (V 3.2)	
	Computed Probability	Discriminant Score	Computed Probability	Discriminant Score
2A	0	-20.690	0.010	-4.567
2B	0.3651	-0.554	0.959	3.140
2C	0.004	-5.517	1	9.423
2D	0.063	-2.707	0.984	4.091
2A'	0	-19.415	0.945	2.839
2B'	0.175	-1.549	1	8.441
2C'	0.005	-5.301	1	15.8
2D'	0.066	-2.647	1	10.286
3A	0.002	-6.434	0.914	2.367
3B	0	-58.853	0.925	2.506
3C	0.794	1.352	1	14.760
3D	0	-16.839	1	8.885
3A'	0	-13.215	1	8.572
3B'	0	-68.103	0.999	7.571
3C'	0	-59.01	0.989	6.321
3D'	0	-24.854	1	14.711

Table 5: Carcinogenicity Male Rat, Carcinogenicity Female Rat and Mutagenicity data

Compound	Carcinogenicity Male Rat (V 3.2)		Carcinogenicity Female Rat (V 3.2)		Mutagenicity (V 3.2)	
	Computed Probability	Discriminant Score	Computed Probability	Discriminant Score	Computed Probability	Discriminant Score
2A	0.022	-3.815	0	-8.643	0.990	4.578
2B	0.813	1.469	1	61.396	1	51.031
2C	0.058	-2.779	0	-8.742	1	11.344
2D	0.563	0.254	1	26.319	1	30.380
2A'	0.078	-2.473	0	-11.596	1	11.139
2B'	0.963	3.251	1	55.575	1	54.66
2C'	0.203	-1.366	0	-12.860	1	17.211
2D'	0.853	1.760	1	22.097	1	35.701
3A	0.002	-6.409	1	21.953	0	-8.651
3B	0.349	-0.623	1	17.021	1	36.543
3C	0.005	-5.287	0.277	-0.958	0.193	-1.430
3D	0.104	-2.287	1	27.633	1	16.81
3A'	0.009	-4.743	1	8.187	0.006	-5.197
3B'	0.784	1.292	0.596	0.387	1	37.149
3C'	0.684	-0.923	0.491	0.293	1	17.14
3D'	0.402	-0.396	1	12.597	1	19.056

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 16 derivatives (8 derivatives are of 2A-2D' series and 8 derivatives are of 3A-3D' series) were extensively studied by TOPKAT 6.1. Rat oral LD50 (V 3.1) and Rat inhalational LC50 (V 3.1) values for all the 16 derivatives shows positive computed probability values and 95% confidence limits which implies that their contribution increases the probability of the chosen property when calculated by TOPKAT 6.1. Compounds 3B, 3D, 3B', 3C' and 3D' has shown negative assessment and 95% confidence limit values for LogP (V 3.1). The structure descriptors contribute negatively for the assessment of confidence limits. Derivatives 3B – 3D and

3B'-3D' resulted in very low computed probability and negative discriminant score values for Developmental Toxicity Potential (V 3.1). Descriptors of 3C' although are not likely to give a positive response in an experimental assay but there is an increase in contribution towards the chosen property. Data is given in Tables 2 and 3.

All the derivatives except 3C 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 male mouse (V 3.2). For carcinogenicity of female mouse (3.2), all the derivatives except 2A shows the values of computed probability greater than 0.7 and are likely to produce a positive response. Only 2A has negative discriminant score, where as all the other derivatives contribute positively for the increase in the probability of chosen property. Data is given in Table 4.

Table 6: Skin Irritation, Skin Sensitization Mild/Moderate VS Severe data

Compound	Skin Irritation(V 6.1)		Skin Sensitization Mild/Moderate VS Severe (V 6.1)	
	Computed Probability	Discriminant Score	Computed Probability	Discriminant Score
2A	0.969	3.456	0	-57.431
2B	0	-69.666	0	-14.277
2C	0.967	3.377	0	-58.306
2D	0	-22.453	0	-37.510
2A'	0.003	-5.887	0	-8.170
2B'	0	-76.207	1	51.233
2C'	0.004	-5.419	0.001	-7.280
2D'	0	-30.463	1	-18.092
3A	1	12.886	1	12.735
3B	0	-62.060	1	53.05
3C	1	12.025	1	12.223
3D	0	-12.539	1	31.866
3A'	0.981	3.943	1	80.767
3B'	0	-73.487	1	137.173
3C'	0	-62.48	1	112.37
3D'	0	-22.236	1	106.312

Table 7: Ocular irritancy Severe VS Moderate and Aerobic Biodegradability data

Compound	Ocular irritancy Severe VS Moderate (V 5.1)		Aerobic Biodegradability (V 6.1)	
	Computed Probability	Discriminant Score	Computed Probability	Discriminant Score
2A	1	27.411	1	33.572
2B	1	45.355	1	30.153
2C	1	27.625	1	17.798
2D	1	37.837	1	47.373
2A'	0	-8.298	0	-11.156
2B'	0.999	7.595	0	-25.001
2C'	0	-9.062	0	-22.842
2D'	0.825	1.553	0.494	-0.025
3A	1	56.2	1	14.614
3B	1	42.603	0	-31.180
3C	1	53.656	0.167	-1.610
3D	1	55.337	1	14.865
3A'	1	15.607	0	-44.75
3B'	0.761	1.160	0	-102.074
3C'	0.671	0.961	0	-100.01
3D'	1	14.567	0	-48.194

Computed probability values of derivatives 2B, 2B', 2D', 3B', 3C' 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 when compared to other derivatives of the class. Positive discriminant score of 2B, 2D, 2B', 2D', 3B' suggests that their positive contribution increases the probability of chosen property. Derivatives 2B, 2D, 2B', 2D', 3A, 3B, 3D, 3A', 3D' shows computed probability values greater than 0.7 to produce a positive response in an experimental assay for

carcinogenicity of female rat (V 3.2). Derivatives other than 2A, 2C, 2A', 2C' and 3C shows a positive discriminant score there by increasing the probability of chosen property by contributing positively. Derivatives 3A, 3C, 3A' has shown negative discriminant scores and almost very low probability values for mutagenicity (V 3.2). Others of the same class has shown probability values of almost 1 and positive discriminant scores. Data given in Table 5.

Derivatives 2A, 2C, 3A, 3C, 3A' has shown probability values greater than 0.7, thus likely to give a positive response

and the same derivatives offer positive contribution for the probability of skin irritation (V 6.1). Studies for skin sensitization (V 6.1), all the derivatives other than 2A-2A' and 2C' has shown value of 1 and so are most likely to produce a positive response. The foretold derivatives showed negative discriminant scores leading to a decreased contribution of descriptors. In this study derivatives 2B' and 2D' exhibited peculiar properties of greater computed probability with negative discriminant scores, which implies that the descriptors of these derivatives contribute negatively to obtain a likely positive response in an experimental assay. Data given in Table 6.

All the derivatives other than 2A' and 2C' has shown computed probability values greater than 0.7, thereby likely to produce a positive response in an experimental assay for ocular irritancy (V 5.1) and has shown positive discriminant scores. Derivatives 2A-2D, 3A, 3D exhibited positive discriminant scores and greater computed probability values. Thus increasing their contribution to the chosen property and are likely to show positive response in an assay respectively for aerobic biodegradability (V 6.1). Data given in Table 7.

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 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 1,4-Dihydropyridines and pyrazolidine-3,5-diones for their toxicological profile.

ACKNOWLEDGEMENTS

I am grateful to express my sincere thanks to the GEF (medical), the Management and staff of M.S.Ramaiah College of Pharmacy, Bangalore for providing all the facilities and encouragement for carrying out the work.

REFERENCES

- Pattan SR, Bhat AR, Taranill AD, Purohit SS, Reddy VVK. Synthesis of new 1,4-dihydropyridine derivatives as antihypertensive agents. *Indian J Heterocyclic Chem* 2005 July-Sep;15:65-6.
- Ashraf AH, Ibrahim TM, Khaled AM, Lehmann J, Tinsley HN, Gary BD, et al. Design, synthesis and biological evaluation of novel pyridine derivatives as anticancer agents and phosphodiesterase 3 inhibitors. *Bioorg & med chem*. 2009; 17: 5974-5982. <http://dx.doi.org/10.1016/j.bmc.2009.06.063> PMID:19628397
- Shashikant PR, Purohit SS, Rasal VP, Malliya S, Marihal SC, Khade AB, et al. Synthesis and pharmacological screening of some 1,4-dihydropyridine and their derivatives for anticonvulsant activity. *Indian J Chem* 2008 April;47B:626-9.
- Rakesh K, sakshi M, Ramesh C. Synthesis and antimicrobial activity of 4-[5-chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl]-dihydropyridines and 4-[5-chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl]-3,4-dihydropyrimidin-2-ones. *Indian J Chem* 2009 May; 48B: 718-724.
- Bertrand LB, Emmanuelle M, Said Y, Jean FG, Raymond H, Jean PH. Synthesis and pharmacological evaluation of new pyrazolidine-3,5-diones as AT1 angiotensin II receptor antagonists. *J. Med Chem* 2000; 43: 2685-2697. <http://dx.doi.org/10.1021/jm9904147>
- Kornet MJ, Thorstenson JH, Lubawy WC. Anticonvulsant Activity of 1-Alkyl-4-substituted 3,5-pyrazolidinediones. *J. P'ceutical sciences* 1974; 63: 1090-1093.
- Asma Samaunnisa A, Venkataramana C.H.S, Madhavan V. Synthesis, characterization and biological evaluation of novel N³, N⁵-diphenyl-1,4-dihydropyridine-3,5-dicarbohydrazide derivatives. *International Journal of Research in Pharmacy and Chemistry* 2013;3(1): 160-7
- Asma samaunnisa A, Venkataramana C.H.S , Madhavan V. Synthesis, characterization and biological evaluation of novel derivatives of bis pyrazolidine-3,5-dione tethered with 1,4-dihydropyridine moiety. *CIOP* 2013; 2(2): 36-42.
- Help files\ Accelrys\ Accord for Excel 6.1, TSAR 3.3
- M.pharm thesis : Asma Samaunnisa A. Design, synthesis and Biological evaluation of novel bis pyrazolidine dione derivatives containing pyridine moiety 2011.

Cite this article as:

Asma samaunnisa A, C.H.S. Venkataramana, V Madhavan. Virtual toxicity studies of novel 1,4-dihydropyridine derivatives. *Int. Res. J. Pharm.* 2013; 4(3):249-253

Source of support: Nil, Conflict of interest: None Declared