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VIRTUALTOXICITYSTUDIESOFNOVELSPIROAZETIDIN-2-ONESTETHEREDWITHFURANS

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ABSTRACT:
Common uses for spiroazetidin-2-ones and furans include anti-inflammatory, anti-fungal, anti-cancer, anti-parkinson, and cardiovascular effects. The researchers in this investigation set out to simulate toxicity tests on the syntheticthe chemicals 1-(substitutedphenyl)-3-chloro-5,9-bis(furan-2-ylmethylidene)The 1a–3h-alkyl azaspiro[3.5]nonan-2-ones list is long.Subjects and procedures:The TOPKAT 6.1 (Toxicity Prediction Komputer Assisted Technology version 6.1) was used to develop computational toxicology and mutagenicity profiles of these substances. After receiving the query molecule's molecular structure as a SMILES string and choosing a TOPKAT predictor, TOPKAT automatically analyses the compound. The results show that chemicals 3a–3h do not undergo aerobic biodegradation and do not cause mutations, as predicted by the TOPKAT 6.1 model. Oral LD50 values for compounds 3a–3h in rats were estimated to be between 1.1 and 115.0 mg/kg. The chemicals seem to be more safe due to their high LD50 values. For all substances, the calculated likelihood of skin irritation was 1.000, and for carcinogenicity, it was determined to be 0.950-1.000. The use of computerized statistical methods allows for the identification of more promising compounds. Based on the results of these investigations, we set out to determine the toxicological profile of new spiroazetidin-2-one bound with furan moieties.

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

INTRODUCTION: Spiroazetidin-2-onesandfurans have emerged as an important class of drugsfor the treatment of a variety of health conditions.The compounds having these nuclei are commonlyused to treat health conditions which include anti-bacterial,anti-fungal,anti-inflammatory,cardiovascular anticancer, antiparkinson agents*etc*.[1-7].

DrugdesignsoftwareslikeTOPKATenablethedis covery of lead molecules, more efficiently andquickly. Conventional synthesis and evaluation ofdrugs require number of animal sacrifices, use ofthissoftwarehelpsinreducingthenumberofanim alsacrificesmadeforthe*invivo*studies.TOPKAT accurately and rapidly assess the toxicityof drugs solely from their 2D molecular structureusingarangeofrobust,cross-validatedQuantitativeStructureToxicityRelation ship(QSTR) models for assessing specific toxicologicalend points, thus providing a detailed data of querymoleculeswhichcanbewidelyanalyzedandc ompared with the existing molecular libraries. UseofQSARsoftwaresundoubtedlyreducesthenu mberofcompoundssynthesizedtherebyproviding withpromisingleadsforfurtherexploration. The rationale behind this work was todevelopextensivetoxicologicalprofileofthederi vativescontaining1-(substitutedphenyl)-3-chloro-5,9-bis(furan-2-ylmethylidene)-1-azaspiro[3.5]nonan-2-ones (3a-3h)[8].

MATERIALS AND METHODS: The molecularstructureofthequerycompoundisgivenas SMILES string and a desired TOPKAT predictor isselected.Ifthe structure is a member of trainingset, the database information for the compound isdisplayed.Ifthequerydoesnotbelongtothetraining set,softwaredisplaystheresultwithnecessarywarni ngs.Underthesecircumstances,cautioninacceptingt heestimateshouldbeexercised. Models which satisfy all the validationcriteria for the query compound are computed andresults arerecorded[9-

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Virtualtoxicitystudieshavebeendoneforthefollowingsetofcompounds,substitutionsforwhichis given in **Table1**.

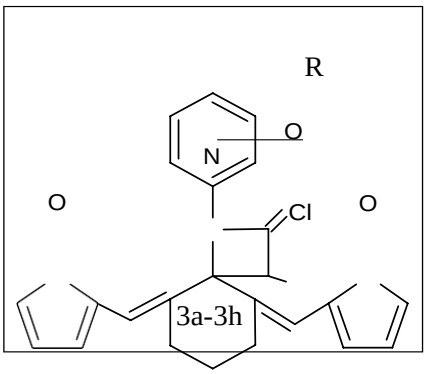

TABLE1:SETOFCOMPOUNDSFORSCREENING

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

Evaluatinganassessment: IfweconsideraTOPKAT assessmentofaquerystructureasahypothesis which states that the model parameterspresent in the query structure are the determinantsof its toxicity, then this hypothesis can be testedagainst similar compounds in the model's database.ThesimilaritysearchfunctioninTOPKAT willautomaticallyrankallthecompoundsintherespectivemodeldatabasebasedontheirQSTRsimilaritytothequerystructure.Informationregarding the actual experimental result, TOPKATpredictedresultandwhetherthecompoundwasused in training set is available for each compound.With this information whether the query structurelies in an information-rich region of the model dataspace and similar compounds are well predicted bythemodel is determined.

Computation of toxicity by TOPKAT: TOPKATcomputesaprobablevalueoftoxicityfor asubmitted chemical structure from a QuantitativeStructure-Toxicity Relationship (QSTR) equation.The equation is linear in the structure descriptors.Thecoefficientsareoptimizedduringthedevelopmentoftheequation.Theproductofastructuredescriptorsvalueanditscorrespondingcoefficientisthedescriptorscontributiontotheprobable toxicity. Contributions from the productsmaybeeitherpositiveornegative;apositivecontributionwillincreasetheprobabilityofthechosen property, whereas a negative contributionwill decrease it. Toxicity values are computed bysummingtheindividualcontributions.Forassessing toxicity values such as LD50 or LC50,this sum is transformed into a weight/weight unit(mg/kg)oraweight/volumeunit(mg/1);for2-groupclassifications,suchascarcinogens/non-carcinogens,thissumistransformedintoaprobability value between 0.0 and 1.0.

Probabilityvalues: Probabilityvaluesfrom0.0to 0.30areconsideredlowprobabilities,andchemicals withTOPKAT-computedprobabilityvaluesinthisrangearenotlikely toproduceapositiveresponseinanexperimentalassay;whereasprobabilityvaluesgreaterthan0.70areconsideredhigh,andarelikelytoproduceapositiveresponseinanexperimentalassay.Probabilities greater than 0.30 but less than 0.70 areconsideredindeterminate(i.e.,toonearchance(0.50)foranassessmenttobe meaningful).

Query structure Examination: TOPKAT alwaysoutputs a value of toxicity; however,

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whether theassessmentismeaningfulornotcanonlybeansweredby:

1. A univariate analysis or Coverage Examination,thatis,whetherallofthestructuralfragmentsofthequerystructurearewellrepresentedinthedatabase compoundswhichwereusedtodevelopthemodel (trainingset).
2. A multivariate analysis, or OPS Examination,thatis,whetherthesubmittedstructurefitswithin, or near the periphery of, the OptimumPredictionSpace(OPS) ofthe equation.

These2stepsareaccomplishedautomaticallyinTOPKATandresultsareoutputintermsofaconfidencepercentage.

CoverageExamination: EveryQSTRmodelisassociated with a certain training set of compounds,andthesecompoundscontainalimitedsetofstructuralattributes.AQSTRmodel,whenextrapolatedtochemicalstructurescontainingstructuralattributeswhichare notrepresentedinthetrainingset,mayproduceunreliabletoxicityassessments. Therefore, it is important to determinewhetherthestructuralattributesofthequerymoleculearerepresentedinthecompoundsusedforthedevelopmentofaQSTR.TOPKATautomatically determineswhethertheinputstructurecontainsmolecularsubstructureswhichareforeigntothetrainingset(aunivariateanalysis).Additionally,duringthisprocess,TOPKATcomparesthevaluesofthemodeldescriptors for the query structure to the range ofthevaluesoftherespectivedescriptorsinthetrainingsetcompounds.

Optimumpredictionspace: Aswellasdetermining its coverage, TOPKAT checks whethera query structure is located inside or outside theOptimumPredictionSpace(OPS)ofaQSTR(multivariate analysis). The OPS of a QSTR is amulti-dimensionalspace,thenumber ofdimensionsbeingonemorethanthenumberofmodelparametersofthe QSTR.

AnimportantcharacteristicoftheOPSisthatwithin and nearits periphery the QSTR may beappliedwithconfidence.TheOPSconfidencecontainsinformationaboutboththeOptimumPrediction Space and the fragment coverage. Whenaquerystructureisdeterminedtobeinsideall dimensions of a model OPS, the computed value oftoxicitycanbeconsideredacceptable(unlessevidence exists to refutethe assessment).

However, if a query structure is found outside oneor more dimensions, the computed toxicity may ormay notbe acceptable depending onthe query'sdistancefromOPS.Thedistanceofaquerystructure from the OPS is a complex function of thequery'slocationineachdimension.EveryTOPKAT QSTR model has a permissible limit ofdistancefrom the OPS.

If the query structures distance from the OPS isgreater than this permissible limit, the TOPKAT-assigned toxicity value is considered unacceptable.The permissible limits of distance from the OPS forallQSTRmodelshavebeenprecalculatedandstoredinTOPKAT.Foreveryquerystructureoutside the OPS, TOPKAT reports the location of aquery structure with respect to the permissible limitofdistancefrom the OPS.

RESULTSAND DISCUSSION: All the 8 derivativescontaining1-(substitutedphenyl)-3-chloro-5,9-bis(furan - 2 -ylmethylidene)-1-azaspiro[3.5]nonan-2-ones(3a-3h)wereextensivelystudied byTOPKAT6.1.

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TABLE2:RATORALLD$_{50}$ANDLOGPDATA

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

TABLE3:AEROBICBIODEGRADABILITYANDDEVELOPMENTALTOXICITYPOTENTIALDATA

Compound	AerobicBiodegradability (v6.1)		DevelopmentalToxicityPotential(DTP)(v3.1)	
	ComputedProbability	DiscriminantScore	ComputedProbability	DiscriminantScore
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

TABLE4:MUTAGENICITYANDSKINIRRITATION DATA

Compound	Mutagenicity(v3.1)		SkinIrritation(v6.1)	
	Probability ofBiodegradability	DiscriminantScore	Probability ofMOD/SEV	DiscriminantScore
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

TABLE5:CARCINOGENICITYMALEMOUSEAND CARCINOGENICITYFEMALEMOUSEDATA

Compound	CarcinogenicityMaleMouse(v3.2)		CarcinogenicityFemaleMouse(v3.2)	
	ComputedProbability	DiscriminantScore	ComputedProbability	DiscriminantScore
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

TABLE6:CARCINOGENICITYMALERATANDCARCINOGENICITYFEMALERATDATA

Compound	CarcinogenicityMaleRat(v3.2)		CarcinogenicityFemaleRat(v3.2)	
	ComputedProbability	DiscriminantScore	ComputedProbability	DiscriminantScore
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 computedRatoralLD50valuesforthecompounds3a-3hrangedfrom1.1g/kgto115.0mg/kg.ThesehighLD$_{50}$valuessuggesthighersafetyofthesecompounds. And Log P values of all 8 derivativesare well below 5.6. So Log P parameter of all thederivatives obey Lipinski's rule and fall well withintherangeof -0.4 to +5.6 (**Table 2**).

Thecompounds(3a-3h)weredevoidofaerobicbiodegradability.Thestructuredescriptorscontributenegativelytotheassessmentofconfidencelimits.Allthederivativesresultedinverylowcomputedprobabilityandnegativediscriminantscorevalues forDevelopmentalToxicity Potential (V 3.1).Data is given in **Table3**.

Allthe8compounds(3a-3h)arenon-mutagenicand the computed probability of skin irritation forall compounds was found to be 1.000, there wouldbeprobabilityofskinirritationontopicalapplication(**Table 4**).

Allthederivativesshowedthecomputedprobability value 1.0anddiscriminantscoresarenotlikelytoproduceapositiveresponseinanexperimental assay and positive contribution to theincreaseintheprobabilityofchosenpropertywhich is carcinogenicity of male mouse (V 3.2) andfemalemouse(3.2).Dataisgivenin**Table5**.Computed probability values of all the derivativesrange from 0.96 to 0.99 for the carcinogenicity ofmale rat (V 3.2) are greater than 0.7, which impliesthattheyarelikelytoproduceapositiveresponseinan experimental assay.

All the derivatives except 3b and 3e showed thecomputedprobabilityvaluelessthan0.7andnegativediscriminantscoresarenotlikelytoproduceapositiveresponseinanexperimentalassay and negative contribution to the increase intheprobabilityofchosenpropertywhichiscarcinogenicity of female rat (V 3.2). Data is givenin **Table 6**.

CONCLUSION: Thesuccessofpreviousgeneration of drugsandvaccines has lead to anincrease in human life expectations. But it does notmean that all the molecules developed each year byrandomscreeningmethodologyaresuccessfultoprovidenewblockbusterdrugs.Soitistimetochange the methodology for research from randomscreening to rationalized approach of drug design.Byfollowingthecomputerizedstatisticalmethodology,morepromisingmoleculescanbeidentified.Thishasprovideduswithsomedirectionforresearch tocarryoutanextensivestudy of novel derivatives of Spiroazetidin-2-onestethered with furans (3a-3h) for their toxicologicalprofile.

REFERENCES:

1. One, Srivastava SD, Pushkal S, Sharma R, and Srivastava SK. Phenothiazine 2-oxo-azetidine derivatives: synthesis and biological action. The organic communication was published in 2011 with the number 43.

2. 2. Karanj Parikh, Parikh AR, Oza PS, Bhatt SB. Possible antitubercular drugs derived from a novel class of 2-azetidinones synthesized. Publication: 2000 in the Indian Journal of Chemistry, Section B: Organic Chemistry, Incorporated Medical Chemistry, 39, 716–718.

3. 3. Shaheed M. Sreenivasa, Jayachandran E., Yognanda R.

4. H. New N-substituted-3-chloro-2-azetidiones: synthesis and characterisation for anticonvulsant drug development. [Published in 2009] in the Journal of Biomedical Science and Research.

5. Four authors: Singh, Sharma, Srivastava, and Kumar. A few substituted pyridine derivatives: synthesis and insecticidal actions. Journal of Organic Chemistry, Part B: Industrial and Medical Chemistry, 2006, 45: 1557–1563.

6. Kumar S, Kaur H, and Kumar K. *Kumari, Saxena, Sharma, and Vishwakarma *P. Novel adamantylthiazolidinonyl/azetidinonylindole derivatives: synthesis and antiparkinsonian efficacy. Article published in 2010 in the Indian Journal of

Chemistry, Section B: Organic Chemistry, Including Medical Chemistry, 49: 1398–1405.

7. 6. The authors are Goel RK, Mahajan MP, and Kulkarni SK. Several new monocyclic beta lactams were tested for their ability to prevent hyperglycemia. Proceedings of the Journal of Pharmaceutical Science, 2004; 7: 80-3.

8. Kumar HK, Banerjee M., and Banerjee R. Medicinal importance of furan derivatives: A Review. Global Journal of Life Sciences. 2012; 2: 7-16.

9. Rajikala R, Babu MN, 8. New Spiroazetidin-2-ones attached to furans: synthesis and characterization for

10. antimicrobial and anti-inflammatory purposes. Published in 2017 in the World Journal of Pharmaceutical Research, volume 6, pages 681–690.

11. Virtual toxicity investigations of new 1,4-dihydropyridine compounds. Asma SA, Venkataramana CHS, Madhavan V., etc. "International Research Journal of Pharmaceuticals" (2013), 4, 249–255.

12. This is the tenth publication by Swain, Samanthula, Bhagat, Bharatam, PV, Akula, and Sinha. Sofosbuvir: A new inhibitor of the human hepatitis C virus NS5B polymerase and its forced degradation products; in silico toxicity prediction. The citation for this article is Jan Pharm Biomed Anal 2016: 120: 352-63.

13. 11. Ismail IS,Wai LK, and RadhakrishnanNarayanaswamy AI. Investigation of some compounds in Clinacanthusnutans using molecular docking as inhibitors of xanthine oxidase, nitric oxide synthase, human neutrophil elastase, matrix metalloproteinase 2, matrix metalloproteinase 9, and squalene synthase. Pharmaceutical Magazine, 2016; 12: S21.

14. 12. The authors are Manral, Saini, Meena, and Tiwari. New diallyl disulfide (DADS) compounds

15. that have several functions, including decreasing β-amyloid, acting as cholinergic agents, acting as antioxidants, and chelating metals, are being developed for the treatment of Alzheimer's disease. The article was published in Bioorg Med Chem in 2015 and can be found on page 6394-003.

16. 13. The authors of the study are GonellaDiaza, Manganelli, Esposito, Roncaglioni, Manganaro, and Benfenati. 7. We compare in silico methods for assessing acute toxicity in rats when given oral poisoning. Environmental Research (SAR QSAR) 2015; 26: 1-27.

17. 14. A Singh, Srivastava R, and RK Singh. Innovative Heterocyclic Arylsulphonamide Derivatives: Design, Synthesis, and Antimicrobial Activities. Overarching Subject Sci: Comput Life Sci. 2017; 13: 1-4. 15. Srikala R, Babu MN. Adverse event and toxicity evaluation of new spiroazetidin-2-ones attached to furans. Screening of newly synthesized spiroazetidin-2-ones for oral action. In World Journal of Pharmaceutical Sciences, 2017; 6: 1351-7. 16. Srikala R, Babu MN. Published in 2017 in the World Journal of Pharmaceutical Research, volume 6, pages 995–999.