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GENOTOXIC EVALUATION OF 2,2'-(PIPERAZINE-1,4-DIYL) BIS[*N*-(2,6-DIMETHYLPHENYL)] ACETAMIDE AN IMPURITY OF RANOLAZINE

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ABSTRACT

The prediction of compound toxicities is an important part of the drug design development process. Computational toxicity estimations are not only faster than the determination of toxic doses in animals but can also help to reduce the amount of animal experiments. ProTox, a virtual tool for the prediction of toxicities of small molecules. ProTox incorporates molecular similarity, fragment propensities, most frequent features and (fragment similarity-based CLUSTER cross-validation) machine-learning, based a total of 33 models for the prediction of various toxicity endpoints such as acute toxicity, hepatotoxicity, cytotoxicity, carcinogenicity, mutagenicity, immunotoxicity, adverse outcomes (Tox21) pathways and toxicity targets. This present study explains the genotoxic evaluation of 2,2'-(piperazine-1,4-diyl) bis[*N*-(2,6-dimethylphenyl)] acetamide an impurity of ranolazine and the result concludes that there is no literature report on genotoxicity, mutagenicity or carcinogenicity for this ranolazine impurity 2,2'-(Piperazine-1,4-diyl) bis[*N*-(2,6-dimethylphenyl)] acetamide This compound is potentially less genotoxic based on consensus of structural alert and QSTR models.

KEYWORDS: Carcinogenicity, Cytotoxicity, Genotoxicity, Impurity, Mutagenicity, Ranolazine.

1. INTRODUCTION

Pharmaceutical impurities are those substances which co-exist with the API or they may develop during synthesis or ageing of both API and formulation. The presence of these impurities even in minor amounts can influence the efficacy and safety of drug.[1] Potential genotoxic impurities in pharmaceuticals at trace levels are of increasing concern to both pharmaceutical industries and regulatory agencies due to their possibility for human carcinogenesis.[2] The analysis of potential genotoxic impurities (PGIs) in active pharmaceutical ingredients (APIs) is a challenging task.[3] The prediction of compound toxicities is an important part of the drug design development process. Computational toxicity estimations are not only faster than the determination of toxic doses in animals but can also help to reduce the amount of animal experiments. Genotoxic impurities represent a special case relative to the International Conference on Harmonisation Q3A/Q3B guidance, because genotoxicity tests used to qualify the drug substance may not be sufficient to demonstrate safety of a potentially genotoxic impurity.[3] The term "genotoxic carcinogens" was coined in the late 1980s based on the results of the United States National Toxicology Program (NTP).[4] Different mechanisms of genotoxicity are chromosomal aberration, sister chromatid exchange, micronuclei formation[5,6], D.N.A double strand breaks[7] and dimer formation[8] All these parameters are used to predict the potential of a drug substance to act as a genotoxic one. But merely the presence of one or the other parameter is not enough holding the drug responsible and put the drug on the shelf as there may be other reasons contributing more to the genotoxicity of the drug rather than the drug itself, such as presence of an impurity, its amount in the single administrable dose of the drug, cumulative toxicity of the impurity during full course of therapy with the drug or in case of chronic use of the drug.[9,10]

1.1 Toxic doses and toxicity classes

Toxic doses are often given as LD50 values in mg/kg body weight. The LD50 is the median lethaldose meaning the dose at which 50% of test subjects die upon exposure to a compound.

Toxicity classes are defined according to the globally harmonized system of classification of labelling of chemicals.

LD50 values are given in[mg/kg]

- Class I: fatal if swallowed (LD50 ≤ 5)
- Class II: fatal if swallowed (5 < LD50 ≤ 50)
- Class III: toxic if swallowed (50 < LD50 ≤ 300)
- Class IV: harmful if swallowed (300 < LD50 ≤ 2000)
- Class V: may be harmful if swallowed (2000 < LD50 ≤ 5000)

- Class VI: non-toxic (LD50 > 5000)

2. METHOD

Genotoxicity prediction is a consensus inference derived from three different methodologies.

a) Decision Tree based alerts: It uses the fragment rule base which is validated in accordancewith the results of Joint Research centre's European Bureau hazard estimation based on the Benigni/Bossa rule base for genotoxic carcinogenicity and mutagenicity

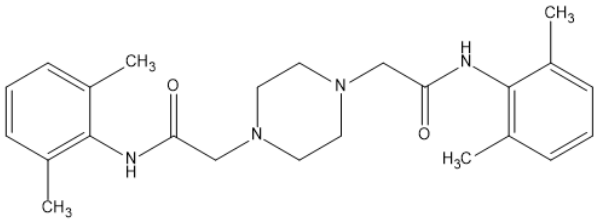
b) Toxicophore significance by ANOVA: This methodology uses database of compounds having TD50 values reported for rats and mouse species from handbook of Carcinogenic Potency and Genotoxicity Database of Gold and Zeiger. Toxicophores carefully collected from literature are used for ANOVA analysis andcorresponding F-ratio and probability are estimated. If the fragment is observed in any ofthe test compound, its significance in terms of contribution to genotoxicity is reported. The significance of toxic fragment helps estimating the extent of toxicity of the compounds.

c) Genotoxicity prediction using SAR /QSAR models: Robust and reliable QSTR models for genotoxicity, mutagenicity and carcinogenicity, are generated using k nearest neighbour method. The classification model is validated as per REACH guidelines as recommended by OECDguidance.

The genotoxicity prediction is based on experimental/literature results obtained either from literature survey and/or databases used to drive the QSTR models. In absence of literature, a consensus is reached using the multiple QSTR model predictions. The QSTR model based on hierarchical clustering method dataset is assigned highest priority followed by CPDB database and COMET assay based QSTR models. The prediction priority is revisedbased on the confidence of prediction, where a 'HIGH' confidence prediction within Applicability Domain over-rules all other predictions. Carcinogenic endpoint is used to access potential of non-genotoxic carcinogenicity.

The term "Allied Compounds" used in the following report refers to "Database compounds with presence of maximum number of structural alerts or functional groups present as in the query molecule".

3. RESULT AND DISCUSSION



Chemical Formula: $C_{24}H_{32}N_4O_2$
Molecular Weight: 408.54
Boiling Point: 1089.5 [K]
Melting Point: 867.41 [K]
Critical Temperature: 1144.74[K
Critical Pressure: 17.08 [Bar]
Critical Volume: 1149.5 [cm^3/mol]
Gibbs Energy: 488.84 [kJ/mol]
Log P: 3.3
MR: 119.74 [cm^3/mol]
Henry's Law: 20
Heat of Form: -93.25 [kJ/mol]
tPSA: 64.68

Oral toxicity prediction results for input compound

Predicted Toxicity Class: 4 [harmful if swallowed (300 < LD50 ≤ 2000)]

Average similarity: 74.43%

Prediction accuracy: 69.26%

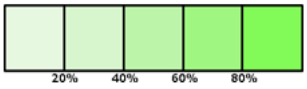


Table 1: Physicochemical properties of compound.

Name	User defined
Mol weight	408.54
Number of hydrogen bond acceptors	36
Number of hydrogen bond donors	2
Number of atoms	59
Number of bonds	61
Number of ratable bonds	7
Molecular refractivity	125.58
Topological Polar Surface Area	64.68
octanol/water partition coefficient log P)	3.73

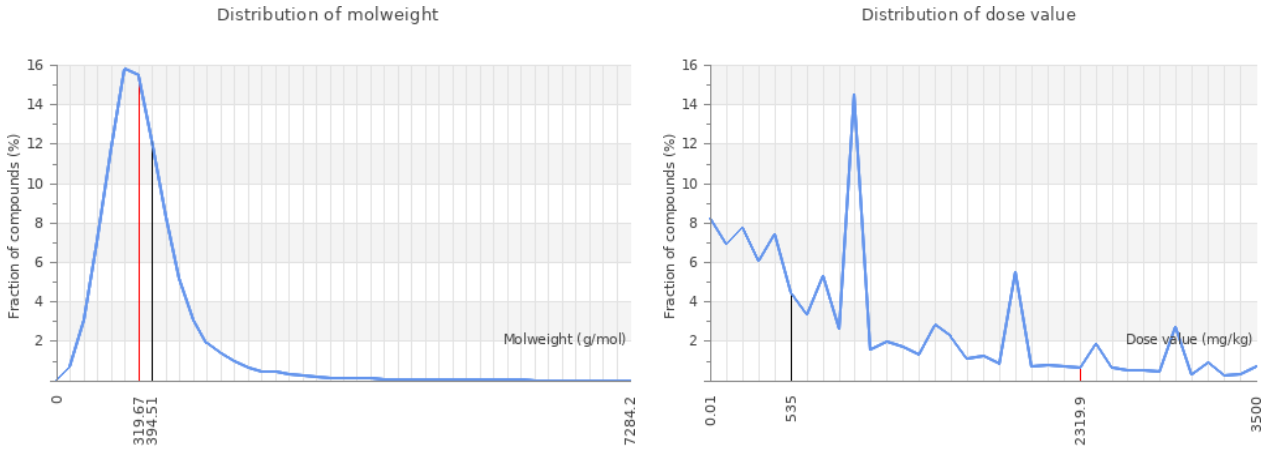


Figure 1: Comparison of input compounds with dataset compounds.

Value of input compound

Mean value of dataset

3.1 Allied compounds or similar compounds

Table 2: Allied compound 1/similar compound 1.

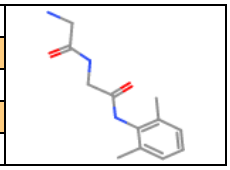
Formula	C12H17N3O2	
Mol weight	235.28	
Endpoint	LD50	
tox class, avg	4	
tox class, min	4	

Table 3: Allied compound 2/similar compound 2.

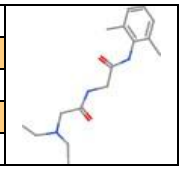
Formula	C16H25N3O2	
Mol weight	291.39	
Endpoint	LD50	
tox class, avg	4	
tox class, min	4	

Table 4: Allied compound 3/similar compound 3.

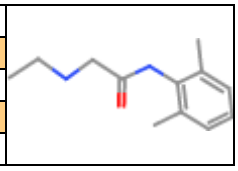
Formula	C12H18N2O	
Mol weight	206.28	
Endpoint	LD50	
tox class, avg	3	
tox class, min	3	

Table 5: Toxicity model report.

Classification	Target	Shorthand	Prediction	Probability
Organ toxicity	Hepatotoxicity	Dili	Inactive	0.85
Toxicity end points	Carcinogenicity	Carcino	Inactive	0.71

Toxicity end points	Immunotoxicity	Immuno	Inactive	0.99
Toxicity end points	Mutagenicity	Mutagen	Inactive	0.75
Toxicity end points	Cytotoxicity	Cyto	Inactive	0.68
Tox21-Nuclear receptor signalingpathways	Aryl hydrocarbon Receptor (AhR)	nr_ahr	Inactive	0.91
Tox21-Nuclear receptor signallingpathways	Androgen Receptor (AR)	nr_ar	Inactive	0.97
Tox21-Nuclear receptor signalingpathways	Androgen Receptor LigandBinding Domain (AR-LBD)	nr_ar_lbd	Inactive	0.98
Tox21-Nuclear receptor signallingpathways	Aromatase	nr_aromatase	Inactive	0.96
Tox21-Nuclear receptor signallingpathways	Estrogen Receptor Alpha (ER)	nr_er	Inactive	0.92
Tox21-Nuclear receptor signallingpathways	Estrogen Receptor LigandBinding Domain (ER-LBD)	nr_er_lbd	Inactive	0.99
Tox21-Nuclear receptor signallingpathways	Peroxisome Proliferator Activated Receptor Gamma (PPAR- Gamma)	nr_ppar_gamma	Inactive	0.97
Tox21-Stress response pathways	Nuclear factor (erythroid-derived2)-like 2/antioxidant responsive element (nrf2/ARE)	sr_are	Inactive	0.97
Tox21-Stress response pathways	Heat shock factor responseelement (HSE)	sr_hse	Inactive	0.97
Tox21-Stress response pathways	Mitochondrial MembranePotential (MMP)	sr_mmp	Inactive	0.82
Tox21-Stress response pathways	Phosphoprotein (TumorSupressor) p53	sr_p53	Inactive	0.95
Tox21-Stress response pathways	ATPase family AAA domain-containing protein 5 (ATAD5)	sr_atad5	Inactive	0.97

a. Mutagenicity Prediction

a. By Consensus method

Prediction results		
Endpoint	**Experimental value**	**Predicted value**
Mutagenicity result	N/A	Mutagenicity Negative

Table 7: Individual Predictions.

Method	**Predicted value**
Hierarchical clustering	0.21
Nearest neighbor	0.67

a. By Hierarchical clustering method

Table 8: Prediction results.

Endpoint	**Experimental value**	**Predicted value**
Mutagenicity result	N/A	Mutagenicity Negative

Table 9: Cluster model predictions and statistics.

Cluster model	**Test chemical descriptor values**	**Predicted value**	**Concordance**	**Sensitivity**	**Specificity**	**Chemicals**	**Applicability Domain**
8683	Descriptors	-0.04	0.950	1.000	0.909	20	OK
8729	Descriptors	0.14	1.000	1.000	1.000	17	OK
9001	Descriptors	0.09	0.895	0.727	0.963	38	OK
9024	Descriptors	0.27	0.935	0.954	0.919	139	OK
9079	Descriptors	0.51	0.943	0.985	0.907	140	OK
9081	Descriptors	0.28	0.930	0.923	0.936	143	OK
9089	Descriptors	0.07	0.931	0.908	0.949	144	OK
9119	Descriptors	0.38	0.917	0.952	0.884	169	OK

Table 10: Cluster models with applicability domain violation.

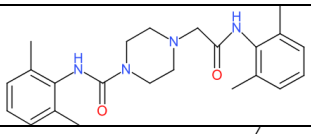
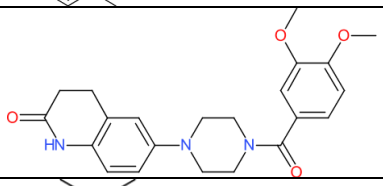
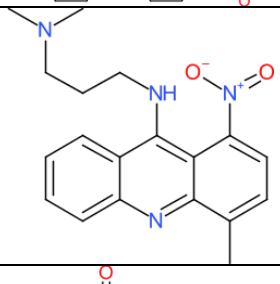
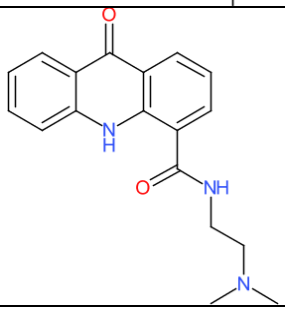
Cluster model	Test chemical descriptor values	Predicted value	Concordance	Sensitivity	Specificity	Chemicals	Applicability Domain
8216	Descriptors	-0.03	1.000	1.000	1.000	14	Rmax constraint notmet
8418	Descriptors	-0.07	1.000	1.000	1.000	16	Rmax constraint notmet
8882	Descriptors	-0.95	0.966	1.000	0.857	58	Model ellipsoid constraint not Met
8902	Descriptors	1.28	1.000	1.000	1.000	26	Model ellipsoid constraint notmet
9101	Descriptors	-0.16	0.919	0.909	0.927	148	Model ellipsoid constraint notmet

a. Nearest neighbor method

Table 11: Prediction results.

Endpoint	Experimental value	Predicted value
Mutagenicity result	N/A	Mutagenicity Positive

Table 12: Nearest neighbors from the training set.

CAS / Molecular ID	Structure	Experimental value	Similarity Coefficient
C_MNDSZMSVQQGNJN-UHFFFAOYSA-N (Test chemical)		N/A	N/A
81840-15-5		0.00	0.77
24400-01-9		1.00	0.75
103554-58-1		1.00	0.74

4. CONCLUSION

There is no literature report on genotoxicity, mutagenicity or carcinogenicity for this ranolazine impurity 2,2'-(Piperazine-1,4-diyl) bis[*N*-(2,6-dimethylphenyl)] acetamide This compound is potentially less genotoxic based on consensus of structural alert and QSTRmodels.

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