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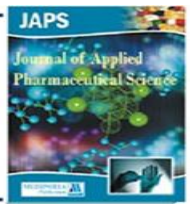

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In-silico ADME and toxcity studies of some novel indole derivatives

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ABSTRACT

Drug design is a process which begins with a compound that displays an interesting biological profile and ends with optimizing both the activity profile for the molecule. The process is initiated when the chemist conceives a hypothesis which relates the chemical features of the molecule (or series of molecules) to the biological activity. Study of Quantitative structural activity relationships (QSAR) is an important aspect of computational chemistry for optimizing the structural features to obtain better activity. Present research work focuses on *in-silico* drug design studies of novel indole derivatives containing pteridine and benzimidazole moieties. These studies include QSAR (Quantitative structure activity relationship) and QSTR (Quantitative Structure Toxicity Relationship) and are carried out using different software's namely DS Viewer Pro suite, Accord for Excel *(v6.1)* and TOPKAT *(v6.2)*. All the software's were obtained from Accelrys Discovery studio. *In-silico* pharmacokinetic studies implied that these derivatives had no CYP4502D6 inhibitions, no BBB penetration and good oral absorptions. QSTR (Quantitative Structure Toxicity Relationship) studies by using TOPKAT *(v6.1)* in various computational animal models showed high LD50 values and the compounds are found to be noncarcenogenic.

Keywords: Indoles, pteridines, QSAR, QSTR, LD50.

INTRODUCTION

Indole, an important heterocyclic ring, present in a large number of biologically active molecules of different pharmacological classes and is known to posses fungicidal, bactericidal, antitubercular, antiparkinsons, antithyroid and anticonvulsant activities (Lather and Chowdary, 2003). The synthetic versatility of isatin (1H-indole-2, 3-Dione) has led to the extensive use of this compound in organic synthesis. The interest in this compound has stemmed from the wide spread biological and pharmacological properties of its derivatives. Isatin derivatives of mannich bases had found to have fibrinolytic, muscle relaxant, antiallergic, immunosuppressant and antithrombotic activity. They also showed cardio inhibitory effect on frog's heart apart from respiratory depression and antidiuertic effects (Rastogi *et al*., 2009; Rastogi *et al*., 2009; Pandeya *et al*., 2005). The pteridine system is a combination of a pyrazine ring and pyrimidine ring. A number of compounds with pteridine ring system have diverse pharmacological activities such as antibacterial, antitumor, cardiotonic, hepatoprotective antihypertensive, antibronchitis, antiallergic, antimalarial, analgesic and antifungal activities (Deb and Bhuyan, 2006). The benzimidazole derivatives are of wide interest because of their diverse biological activity and clinical applications. This ring system is present in numerous antiparasitic, fungicidal, antihelmintic and antiinflammatory drugs. Apart from the anti-hypertensive, anti-viral, anti-fungal, anti-tumor and anthelmintic activity substituted benzimidazoles are potent inhibitors of parietal cell proton pump,

the H^+/K^+ ATPase (Gringanz, 1997; Kazimierczuk *et al*., 2002). In view of these observations and the ever increasing importance of these biologically active heterocycles in medicine, we have attempted to design some novel heterocyclic derivatives combining indole, Pteridine and benzimidazole moieties and to study their QSAR and QSTR profiles.

A Quantitative structure activity relationship (QSAR) can be expressed in its most general form by, Biological activity= *f* (physicochemical and/or structural parameters). The present computational studies include DS VIEWER PRO SUITE, DS Accord for Excel and TOPKAT studies. DS VIEWER PRO SUITE is used to examine the 3D structure of the molecule and for energy minimization of the molecule. DS Accord for Excel helps to estimate the aqueous solubility, Blood brain barrier (BBB) penetration, Cytochrome P450 (CYP4502D6) inhibition, hepatotoxicity, human intestinal absorption (HIA) and plasma protein binding for a given set of ligands. TOPKAT 6.1 (Toxicity Prediction Komputer Assisted Technology version 6.1) accurately and rapidly assesses the toxicity of chemicals solely from their 2D molecular structure. It uses a range of robust, cross-validated Quantitative Structure-Toxicity Relationship (QSTR) models for assessing specific toxicological endpoints. TOPKAT currently supports assessment of Developmental Toxicity Potential (DTP), Mutagenicity (Ames test), Rodent Carcinogenicity, Skin Sensitization (GPMT), Rat Oral LD_{50}, Daphnia magna $EC_{50,}$ Fathead Minnow $LC_{50,}$ Inhalational LC_{50}, Aerobic biodegradability and Ocular irritation. In the present study ADME and toxicity profiles of all the novel derivatives was studied.

MATERIALS AND METHODS

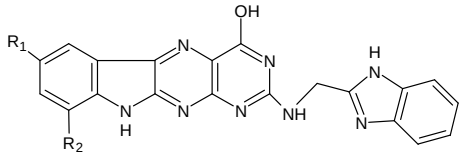
Softwares used for the study were DS Viewer Pro Suite, Accord for Excel *(v6.1)* and TOPKAT *(v6.2)*. All these softwares were obtained from Accelrys Discovery studio. QSTR and ADME studies were carried out using Hewlett Packard computer system.

ADME STUDIES

Computer aided ADME studies have been done by using the software Accord for Excel (Accelrys Discovery studio software). These studies are solely based on the chemical structure of the molecule. Some of the parameters that are calculated using Accord for Excel includes Atom based Log P98 (A LogP 98), ADME 2D Fast polar surface area (ADME 2D FPSA), Aqueous solubility (AQ SOl), Aqueous solubility level (AQ SOl LEV), Blood Brain Barrier Logarithmic value (BBB LOG), Blood Brain Barrier Level (BBB LEV), Cytochrome P4502D6 (CYP2D6), Cytochrome P450 2D6 Probability (CYP PROB), Hepatotoxicity Level (HEPATOX LEV), Hepatotoxicity Probability (HEPATOX PROB), Plasma protein binding logarithmic value (PPB LOG) and Plasma protein binding logarithmic level (PPB LEV).

The following structures are drawn using DS Viewer Pro Suite software and are appended into Accord for Excel software and the parameters have been calculated. The structures of the novel compounds are shown in Table-1.

Table 1 Novel compounds.



S.NO	COMPOUND	R_1	R_2
01	A	-H	-H
02	B	-Cl	-H
03	C	-Br	-H
04	D	-I	-H
05	E	$-NO_2$	-H
06	F	-F	-H
07	G	-H	-F
08	H	$-CH_3$	-H

VIRTUAL TOXICITY STUDIES

The molecular structure of the query chemical is given as a SMILES string and a desired TOPKAT predictor is selected. If the structure is a member of the training set, the database information for the compound is displayed. If the query structure does not belong to the training set, the software displays the results with necessary warnings. Under these circumstances caution in accepting the estimate should be exercised. The various models that can be calculated using TOPKAT are NTP carcinogenicity call (Male rat) (v3.2), NTP carcinogenicity call (Female rat) (v3.2), NTP carcinogenicity call (Female mouse) (v3.2), NTP carcinogenicity call (Male mouse) (v3.2), FDA carcinogenicity Male Rat Non vs. Carc (v3.1), FDA carcinogenicity Female Rat Non vs. Carc (v3.1), FDA carcinogenicity Male Mouse Non vs. Carc (v3.1), FDA carcinogeni- city Female Mouse Non vs. Carc (v3.1), FDA carcinogenicity Male Rat Single vs. Mult (v3.1), FDA carcinogenicity Female Rat Single vs. Mult (v3.1), FDA carcinogenicity Male Mouse Single vs. Mult (v3.1), FDA carcinogenicity Female Mouse Single vs. Mult (v3.1), Weight of Evidence carcinogenicity call (v5.1), Ames Mutagenicity (v3.1), Developmental Toxicity Potential (v3.1), Rat Oral LD50 (v3.1), Skin irritation (v6.1), Fathead Minnow LC50 (v3.2), Daphnia EC50 (v3.1), Rat Inhalational LC50 (v6.1), Fathead Minnow LC_{50}, Daphnia magna EC_{50}, Aerobic Biodegradability (v6.1) and Rat Inhalational Lc50 (v6.1).

Models which satisfy all the validation criteria for the query compound are computed and results are recorded.

RESULT AND DISCUSSION

ADME Studies

All the parameters calculated are tabulated in Table 2. The ADME-Plot is a 2D plot using calculated FPSA_2D and A logp98 properties. The graph was plotted against Alogp98 vs. FPSA. Blood Brain Barrier (BBB) plot (Fig 1) and Human Intestinal Absorption (HIA) plot (Fig 2) was drawn using all the compounds. In BBB plot all the compounds were fallen outside the 99% ellipse (undefined). Hence the compounds may not be able to penetrate the blood brain barrier. So, the chances of CNS side effects are low or absent. In HIA plot compounds A, C have fallen inside the 99%

Table 2 COMPUTER AIDED ADME SCREENING OF THE NOVEL COMPOUNDS.

S.NO	DESCRIPTOR	A	B	C	D	E	F	G	H
01	ADME.2D.FPSA	148.31	136.07	120.18	126.48	114.58	176.07	176.07	156.53
02	A LOG P98	4.815	4.5371	4.6211	4.4509	4.4509	4.0782	4.0782	4.8151
03	HIA	2	2	2	1	1	1	1	2
04	AQ Sol LOG	-7.565	-7.746	-7.853	-7.719	-7.238	-7.251	-7.174	-7.565
05	AQ Sol LEV	2	2	2	1	2	2	0	0
06	BBB LEV	4	4	4	4	4	4	4	4
07	BBB LOG LEV	-100	-100	-100	-100	-100	-100	-100	-100
08	CYP 2D6	0	0	0	0	0	0	0	0
09	CYP PROB	0.128	0.237	0.237	0.178	0.207	0.128	0.128	0.148
10	HEPATOX	1	1	1	1	1	1	1	1
11	HEPATOX PROB	0.947	0.973	0.980	0.980	0.973	0.980	0.960	0.947
12	PPB LEV	1	1	1	1	1	1	1	1

confidence ellipse, where as the remaining compounds have fallen outside the 99% ellipse (undefined). Hence the compounds F and G are expected to posses good human intestinal absorption. ADME Aqueous solubility logarithmic level of most of the compounds was found to be 2, 1 or 0 which indicates very little aqueous solubility. The CYP2D6 score predicts the inhibitory and non-inhibitory character of the given query chemical structure on Cytochrome P450 2D6 enzyme. The closer the CYP2D6 scores to either 0 or 1, the more closely the predictions agree with each other. All compounds are predicted as noninhibitors of CYP2D6. The ADME.CYP2D6.PROB values of all the compounds lies in the range of 0.1-0.23 so the results are reliable. Hence the side effects (i.e., liver dysfunction) are not expected upon administration of these compounds. The hepatotoxicity score predicts the hepatotoxic nature of the chemical compounds. The

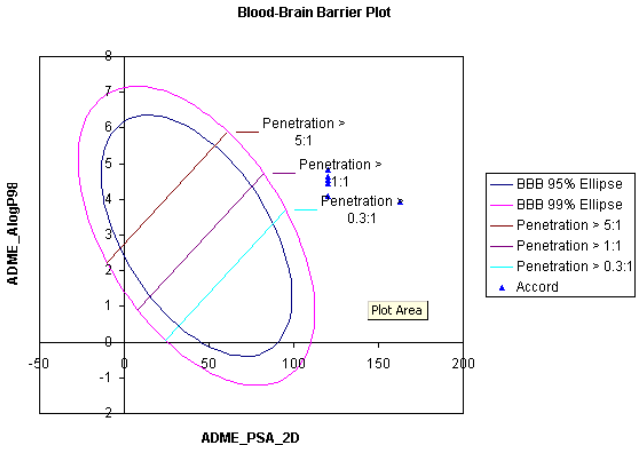


Fig 1 Blood Brain Barrier (BBB) Plot for the newly designed compounds

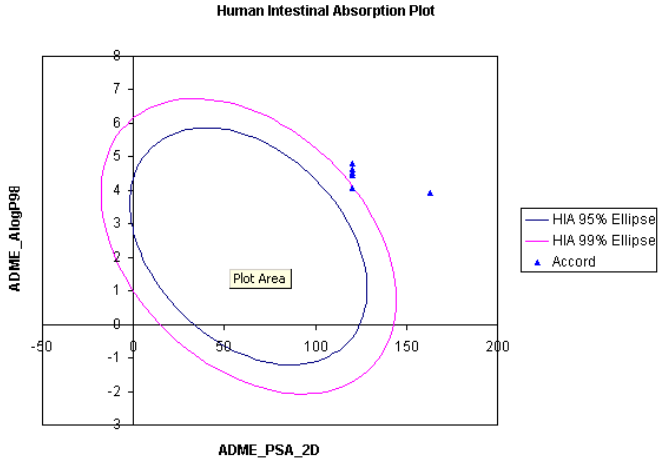


Fig 2 Human Intestinal Absorption (HIA) plot for the newly designed compounds.

ADME.HEPATOTOX.PROB values of the compounds lie in the range of 0.94-0.98. Hence the compounds are likely to posses hepatotoxicity. Further studies are necessary to determine the hepatotoxic dose levels. The plasma protein binding model predicts whether a compound is likely to be highly bound to carrier proteins in the blood. Hence there is high probability that these compounds can reach the desired targets.

Virtual toxicity studies

The toxicity profiles calculated for all the compounds are tabulated in Table 3.

Table 3 Virtual toxicity studies of the compounds

Table 3a

S.NO	Compound	MODEL			
		NTP Carcinogenicity Call (Male Rat)		NTP Carcinogenicity Call (Female Mouse)	
		Dis Score	Computed Probability	Dis Score	Computed Probability
01	A	0.001	-6.942	0.000	-13.273
02	B	0.002	-6.259	0.000	-13.789
03	C	0.002	-6.259	0.000	-16.517
04	D	0.002	-6.259	0.000	-17.780
05	E	0.006	-5.057	0.000	-12.177
06	F	0.002	-6.259	0.000	-5.994
07	G	0.002	-6.259	0.000	-5.994
08	H	0.001	-6.952	0.000	-13.823

Dis Score- Discriminant Score

Table 3b

S.NO	Compound	MODEL			
		Ames Mutagenicity (v3.1) Submodel: Ames Heteroaromatics Model		Developmental toxicity potential	
		Dis Score	Computed Probability	Dis Score	Computed Probability
01	A	1.000	9.887	0.000	-39.546
02	B	1.000	7.789	0.000	-41.096
03	C	1.000	7.738	0.000	-41.096
04	D	0.000	-9.040	0.000	-41.096
05	E	0.776	1.242	0.000	-42.005
06	F	0.482	0.071	0.000	-41.096
07	G	0.482	0.071	0.000	-41.096
08	H	1.000	9.982	0.000	-39.556

Dis Score- Discriminant Score

Table 3c

S.NO	Compound	Model: Rat Oral LD50 (v3.1) Submodel: Rat Oral LD50 Heteroaromatic Model	
		Computed Rat Oral LD50	95% Confidence Limits
01	A	498.7 mg/kg	75.1 mg/kg and 3.3 g/kg
02	B	637.6 mg/kg	98.5 mg/kg and 4.1 g/kg
03	C	576.9 mg/kg	86.8 mg/kg and 3.8 g/kg
04	D	1.2g/kg	185.2 mg/kg and 7.7 g/kg
05	E	777.0 mg/kg	118.2 mg/kg and 5.1 g/kg
06	F	1.6g/kg	238.3 mg/kg and >10 g/kg
07	G	1.6g/kg	238.3 mg/kg and >10 g/kg
08	H	10g/kg	213.2 g/kg and >10 g/kg

Rat oral LD50- Rat oral lethal dose 50

Table 3d

S.NO	Compound	Model: Daphnia EC_{50} (v3.1)	
		Computed EC50 values	95% Confidence Limits
01	A	31.5 ug/l	3.7 ug/l and 265.4 ug/l
02	B	1.3 ug/l	140.8 ng/l and 11.9 ug/l
03	C	1.4 ug/l	147.5 ng/l and 12.4 ug/l
04	D	1.5 ug/l	161.0 ng/l and 13.6 ug/l
05	E	247.5 ug/l	33.7 ug/l and 1.8 mg/l
06	F	1.6 ug/l	168.4 ng/l and 14.5 ug/l
07	G	1.6 ug/l	168.4 ng/l and 14.5 ug/l
08	H	0.0 pg/l	0.0 pg/l and 0.0 pg/l

Daphnia EC_{50} – Daphnia effective concentration 50

TOPKAT carcinogenicity predictor estimation process starts with the identification of the structural features common to submitted structure and the TOPKAT carcinogenicity predictor. According to the three models NTP Carcinogenicity Call (Female Mouse), NTP Carcinogenicity Call (Male Rat) all the compounds are devoid of any carcinogenicity, as all the compounds have got negative discriminant score and the probability for the carcinogenicity is in the range of 0.000 – 0.006. In developmental toxicity potential model all the compounds are devoid of any carcinogenicity, as all the compounds have got negative discriminant score and the probability for the carcinogenicity is 0.000. In TOPKAT mutagencity predictor few compounds are found to be mutagenic in nature as the compounds have got positive discriminant score and the probability for the mutagencity is in the range of 0.071-9.982. However compound D is decisively nonmutagenic. The values obtained from daphnia EC_{50} model show remarkable EC_{50} values. The Rat Oral LD50 values of all the compounds are within the Optimum Prediction Space (OPS). And the LD50 values range from 235.6mg/kg – 4.1g/kg. These high LD_{50} values suggest higher safety of these compounds.

CONCLUSION

The ADME and toxicity profiles of the newly designed compounds were studied. These compounds appear to be safer even at high doses as indicated by the computational studies. With these encouraging results, all the compounds can be further explored for structural modification and detailed investigations to arrive at possibly newer potent agents with better therapeutic activity.

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