ISSN 0975 - 6299 Vol 2 / Issue 4 / Oct - Dec 2011

International Journal of Pharma and Bio Sciences

RESEARCH ARTICLE

MEDICINAL CHEMISTRY

IN SILICO ADME AND TOXCITY STUDIES OF SOME SYNTHSIZED NOVEL ISATIN DERIVATIVES

JUDY JAYS[1]*, ANKIT ROCHANI[1], AMIT KUMAR[1], B.V. SUMA[1], CHS VENKATARAMANA[1] AND V. MADHAVAN[2]

[1]Department of Pharmaceutical Chemistry, M.S. Ramaiah College of Pharmacy, Bangalore-12.
[2]Department of Pharmacognosy, M.S. Ramaiah College of Pharmacy, Bangalore-12.

JUDY JAYS
Department of Pharmaceutical Chemistry, M.S. Ramaiah College of Pharmacy, Bangalore-12.

ABSTRACT

Due to ever growing need of lead modification and repositioning of various drug molecules; *in-silico* drug design techniques are being considered as the most economical and high through put screening methods. The present study shows not only the importance of Isatins as antimicrobials but also tries to identify and filter useful data pertaining to structural peculiarities which are in turn useful for pharmacological and medicinal predictions. From the present study, it is observed that all the compounds are predicted to have oral bioavailability, considerable chances of human intestinal absorption, and has no BBB penetrations. Available LD_{50}, LC_{50}, LOAEL values suggest that the molecules are safe for the further evaluations. Also from the available data generated by *in-silco* experimentation, it is predicted that compound 3B have considerable pharmacological profile for further developments.

KEYWORDS

In-silico ADME, Isatin, *In-silico* toxicity studies.

INTRODUCTION[1,2,3,4,5]

The synthetic versatility of isatin has led to the extensive use of this compound in organic synthesis. It's a metabolite of adrenaline. Isatin derivatives posses a wide range of biological activities such as antibacterial, anthelmintic, amoebicidal, antifungal agent, antifertility, anti-HIV, CNS-depressant, analgesic, anti-inflammatory, anxiogenic, sedative and also act as a potent antagonist on atrial natriuretic peptide receptors *in vitro*. Isatin derivatives of mannich bases have fibrinolytic, muscle relaxant, antiallergic, immunosuppressant, antithrombotic activity and also show hypotensive, respiratory depression, antidiuretic effects and cardio inhibitory effect on frog heart. Substituted benzyloxy group show broad spectrum local anaesthetics, antileishmanial and cysticidal activities. Isatin hydrazones have been reported to possess anticonvulsant activity. Substituted imino derivatives show kinase inhibitory properties against three serine/threonine kinases namely CDK1/cyclin B, CDK5/p25 and GSK3α/β and in vitro antitumor properties showed against MCF7(breast), NCI-H460(lung) and SF268(CNS) cancer cell lines.

In view of these observations, we thought that it would be of interest to undertake the synthesis of some novel substituted derivatives of isatin (1H-indole-2,3-dione) moiety as possible antimicrobial and anti-inflammatory agents. The synthesis route and biological activities had already been published by Judy Jays *et al*[6]. Authors of this paper also published QSAR, QSTR and ADME studies of 2-Indolinone derivatives for anticancer activity[7].

In the present paper we show mathematical values in support of optimization of Isatin for antibacterial and anti-fungal activity with respect to its toxicity parameters. We carried out *in-silico* toxicity and ADME studies in order to understand the pharmacological behavior of lead for rationalized medicinal chemistry studies.

MATERIALS AND METHODS

Software used for the above studies are D.S Viewer Pro, Accord for Excel (v 6.1) and TOPKAT (v 6.2). All these software were obtained from Accelrys Inc and data acquisition was carried on Hewlett Packard computer systems.

IN-SILICO ADME STUDIES

The structures were drawn from Chemsketch software and imported into Accord for Excel. It is a Microsoft Excel based software. After importing the structures to software we examined the quantitative figures for the following descriptors: FPSA, LogP98, HIA, Protein Binding, BBB distribution descriptors, ADME AQ. SOL.LOG.LEV, CYP2D6 PROB and HEPATOXICITY PROB. Data for the descriptors are presented in **table-3**.

Table 3
ADME Values for n=8 synthesized derivatives

S. No.	Descriptor	3a	3b	3c	3d	3e	3f	3g	3h
1	ALOGP98	-0.01	0.47	0.73	0.56	0.65	0.194	0.194	-0.11
2	FPSA	130.9	130.9	130.9	130.9	130.9	130.9	130.9	130.9
3	AQ.SOL.LOG	-2.79	-2.68	-3.61	-3.46	-3.27	-2.87	-2.87	-2.78
4	AQ.SOL.LOG.LEV	3	3	3	3	3	3	3	3
5	BBB.LOG.LVL	3	4	3	3	3	3	3	4
6	CYP2D6	1	0	0	0	0	0	0	0
7	CYP2D6.PROB	0.53	0.23	0.26	0.34	0.35	0.10	0.10	0.34
8	HEPATOTOX	1	0	1	1	1	1	1	1
9	HEPATOTOX.PROB	0.80	0.82	0.82	0.82	0.78	0.84	0.76	0.79
10	HIA.FABS.LEV	0	1	0	0	0	0	0	1
12	PROT.BIND.LEV	2	2	2	2	2	2	2	2
13	HBOND.ACCEPTOR	6	6	6	6	6	6	6	6
14	ALERT	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE
15	HBOND.DONOR	2	2	2	2	2	2	2	2
16	ALERT	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE
17	LOGP	0.96	1.43	1.75	2.22	1.48	1.105	1.105	1.05
18	MLOGP.ALERT	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE
19	WEIGHT.ALERT	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE
20	RULE.OF.FIVE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE	FALSE

IN-SILICO TOXICITY STUDIES

All the structures were drawn from D.S. Viewer Pro and converted smiles notation. The smiles notations for all the structures were imported to TOPKAT software and subjected to *in-silico* animal model based toxicity studies. The output of the study was structure similarity search by software. Here, in this study, we evaluated the structures for their toxicity profile using *in-silico* virtual animal models and determined Rat LD_{50}, Rat inhalational LC_{50} and LOAEL values were calculated in the form of a dose along with 95% confidence limits. We had also subjected these compounds for following *in- silico* model models: mutagenicity, developmental toxicity potential, skin irritation, various skin sensitization reactions, ocular irritancy models, aerobic biodegradation model, Rat maximum tolerated dose, Daphnia EC_{50}. Data for all the models are presented in **table: 4a- 4e**.

Table 4a
RatLD_{50}, Rat Inhalational LC_{50} and LOAEL values for synthesized n=8 derivatives

Comp.	Rat LD_{50}		Rat Inhalational LC_{50}		LOAEL	
	Computed values (mg/kg)	95% confidence limit (mg/kg)	Computed values ($mg/m^3/H$)	95% confidence limit ($mg/m^3/H$)	Computed values (mg/kg)	95% confidence limit (mg/kg)
3A	779.4	105.8 &	380.6	46.3 & 3100	1.9	0.3472 & 9.9

		5700				
3B	1000	136.9 & 7700	437.0	52.9 and 3600	1.9	0.3504& 10.0
3C	175.1	22.7 & 1400	483.0	64 & 4300	2.3	0.420 & 12.4
3D	346.6	45.5 & 2600	530.3	64.6 & 4400	2.6	0.471 & 14.0
3E	187.6	24.7 & 1400	575.9	71.8 & 4600	5.621	0.160.1 & 4.7
3F	446.3	58.6 & 3400	>10,000	1000 & >10,000	5.276	0.336 & 10.0
3G	131.5	17.5 & 987.5	10.5	1.4 & 78.4	4.741	1.4 & 28.7
3H	381.3	49.1 & 3000	521.2	60.7 & 4500	5.534	0.1919 & 6.2

Table 4b
Toxicity Data

Comp.	MUTAGENICITY		DTP		SKIN IRRITATION		SKIN SEN. NEG V SENS (V 6.1)	
	Prob.	Discri. Score	Prob.	Discri. Score	Prob.	Discri. Score	Prob.	Discri. Score
3A	0.999	6.677	0.000	-19.038	0.000	-34.508	0.999	7.339
3B	0.999	7.130	0.000	-17.489	0.000	-36.434	0.998	6.442
3C	0.999	6.665	0.000	-18.780	1.000	-37.318	0.620	0.490
3D	0.000	-10.596	0.000	-19.038	0.000	-37.860	0.987	4.317
3E	0.999	6.531	0.000	-18.780	0.000	-36.146	0.951	2.959
3F	0.173	-1.565	0.000	-18.780	0.000	-32.797	0.000	-31.406
3G	0.998	6.302	0.000	-15.251	0.000	-32.804	0.000	-37.261
3H	0.998	6.037	0.000	-18.866	0.000	-55.393	0.983	4.040

Table 4c
Toxicity Data

Comp.	SKIN SENSITIZATION MLD/MOD V SEV (V 6.1)		OCCULAR IRRI. SEV/ MOD VS MLD/NON (V 5.1)		OCCULAR IRRI. SEV VS MOD (5.1)	
	Prob.	Discri. Score	Prob.	Discri. Score	Prob.	Discri. Score
3A	0.976	3.725	0.000	-9.795	1.000	17.612
3B	0.000	-16.429	0.000	-10.637	1.000	15.588
3C	0.998	5.995	1.000	15.999	1.000	33.380
3D	0.999	6.711	0.000	-7.775	1.000	16.815
3E	0.988	4.448	0.001	-7.034	1.000	14.235
3F	0.507	0.029	0.004	-5.586	1.000	9.196
3G	0.002	-6.026	0.110	-2.092	1.000	12.184
3H	0.971	3.517	0.160	-1.657	1.000	12.375

Table 4d
Toxicity Data

Comp.	OCCULAR IRRI. MLD VS NON (V 5.1)		RAT MTD FEED/ WATER		AEROBIC BIO. DEGRADABILITY (V 6.1)	
	Prob.	Discri. Score	Computed Values (mg/kg)	95% confidence limit (mg/kg)	Prob.	Discri. Score
3A	1.000	32.967	36.8	5.1 &263.3	0.000	-20.675
3B	1.000	38.902	44.8	6.3 & 320.7	0.000	-26.360
3C	1.000	43.486	46.3	4.6 & 472.7	0.873	1.932
3D	1.000	33.037	24.8	3.4 & 181.1	0.000	-23.336
3E	1.000	34.121	15.3	2.1 & 113.5	0.000	-28.267
3F	1.000	36.238	20.1	2.8 & 145.6	0.000	-37.897
3G	1.000	36.238	29.1	3.7 & 227.9	0.000	-40.458
3H	1.000	37.741	17.7	2.4 & 129.8	0.000	-19.620

Table 4e
Toxicity Data

Comp.	R.MTD. GAVAGE		DAPHNIA EC_{50} (V 3.1)	
	Computed values	95% confidence limit	Computed values	95% confidence limit
3A	101.9 mg/kg	13.1 mg/kg & 791.2 mg/kg	1.3 mg/l	1.6 g/l & 133.6 g/l
3B	49.5 mg/kg	6.3 mg/kg & 367.5 mg/kg	2.4 g/l	288.8 mg/l &20.2 g/l
3C	44.3 mg/kg	5.8 mg/kg & 358.6 mg/kg	2.2 g/l	260.0 mg/l & 19.2 g/l
3D	68.7 mg/kg	8.8 mg/kg & 533.6 mg/kg	2.5 g/l	290.0 mg/l & 21.4 g/l
3E	42.5 mg/kg	5.4 mg/kg & 332.8 mg/kg	2.0 g/l	231.6 mg/l & 17.1 g/l
3F	20.1 mg/kg	2.8 mg/kg & 145.6 mg/kg	1.9 g/l	221.1 mg/l & 16.4 g/l
3G	>10 g/m3/H	3.9 g/m3/H & >10 g/m3/H	2.0 g/l	226.4 mg/l & 16.9 g/l
3H	48.9 mg/kg	6.3 mg/kg and 381.5 mg/kg	6.9 g/l	627.2 mg/l & 76.4 g/l

RESULTS AND DISCUSSION

All the compounds were synthesized in desired yield and checked for their activity in micro organism. It was found that all the compounds had considerable biological activity against gram *+ve* and gram-*ve* bacteria and fungus. *In-silico* ADME and Toxicity studies suggest that compound **3B** is not having dose dependent hepatotoxicity. Compound **3A** show CYP2D6 inhibition with probability value of 0.536. **HIA** (Human Intestinal Absorption) descriptors and ellipse **(figure 1)** suggest that all the compounds are having good chance of oral bioavailability. Compounds **3B** and **3H** have been predicted to poorly soluble. All the compounds obey Lipinski Rule of Five. Hence, all the compounds have been predicted to be having good oral bioavailability. All the

derivatives fell outside the ellipse constructed for AlogP98 vs FPSA suggest that no compound crosses BBB and are predicted to be devoid of any major CNS side effects **(figure 2).**

Figure 1
***HIA plot for n=8 synthesized* compounds**

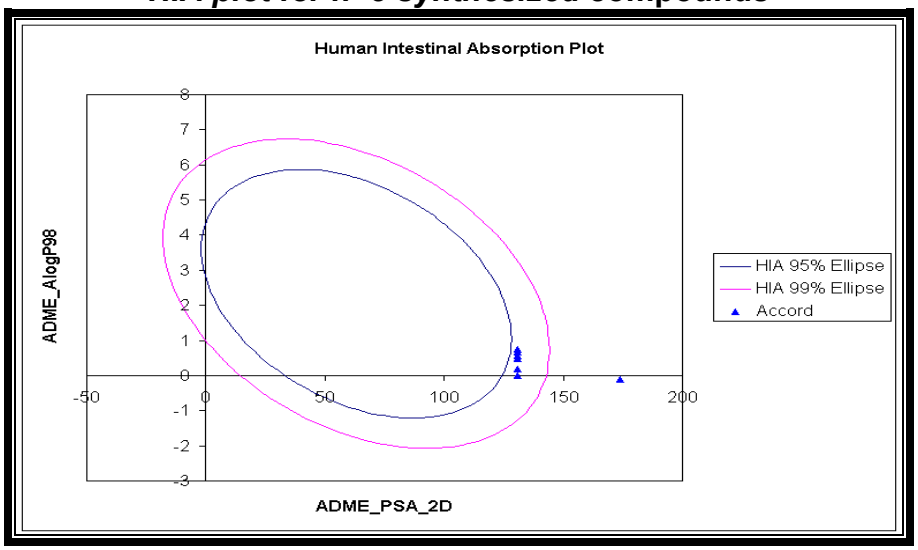
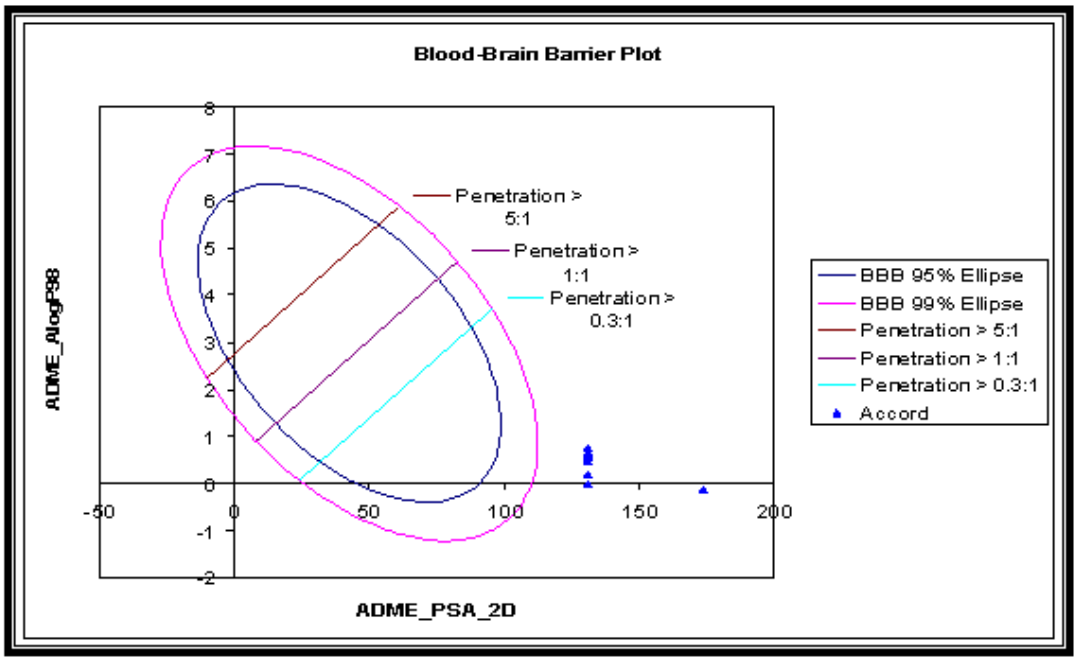


Figure 2
BBB plot for n=8 synthesized compounds



Toxicity data against mice rat models gave us some predictions regarding LD_{50} and LC_{50} values **(table 4a).** From LD_{50}, LC_{50} and LOAEL values, it can be suggested that doses required for killing is more compared to quantities required for providing zone of inhibition values. This suggests the safety profile for the molecule for further investigations. Toxicity data **(table 4b-4e)** provide information about chances of having toxicities in skin, eyes, mutagenicity and developmental toxicity potential for individual derivative.

Table 1a
***Compounds synthesized*[6]**

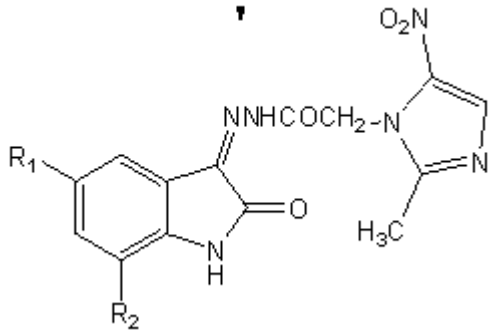
S. No.	COMPOUND	R_1	R_2
1	03A	H	H
2	03B	CH_3	H
3	03C	Br	H
4	03D	I	H
5	03E	Cl	H
6	03F	F	H
7	03G	H	F
8	03H	NO_2	H

Table 1b
***Anti Bacterial activities for n=8 synthesized isatin derivatives*[6]**

S. No.	Compound	Antibacterial activity zone of inhibition in (mm)			
		S. aureus (Gram +ve)	*B. subtilis (Gram +ve)*	*Klebsiella pneumoniae (Gram -ve)*	*Proteus Vulgaris (Gram -ve)*
1	3A	15	19	22	20
2	3B	17	16	20	24
3	3C	22	20	23	23
4	3D	15	17	24	22
5	3E	17	19	23	20
6	3F	20	18	20	23
7	3G	16	17	25	29
8	3H	15	19	20	24
9	Ciprofloxacin	35	41	34	35
10	Amoxycillin	40	38	32	38
11	Fluconazole	-	-	-	-
12	Amphotericin B	-	-	-	-
13	Control (DMF)	NI	NI	NI	NI

Table 1c
Anti-fungal activities of synthesized n=8 isatin derivatives [6.]

S. No.	Compounds	Antifungal activity zone of inhibition in (mm)	
		Aspergillus niger	***Candida Albicans***
1	3A	12	11
2	3B	17	15
3	3C	14	09
4	3D	17	06
5	3E	20	18
6	3F	12	12
7	3G	10	12
8	3H	17	20
9	Ciprofloxacin	-	-
10	Amoxycillin	-	-
11	Fluconazole	30	28
12	Amphotericin B	25	24
13	Control (DMF)	NI	NI



CONCLUSION

All the compounds have some considerable biological activity and toxicity profile for further evaluations and improvements. From all the n=8 compound studies it is found that compound 3B has comparatively more favorable pharmacological profile when compared to other synthesized and characterized derivatives. This data can play an important role in further research for these derivatives and in improving its efficacy from pharmacological point of view.

REFERENCES

1) Hewawasam P, Meanwell NA. A general method for the synthesis of isatins: preparation of regiospecifically functionalized isatins from anilines. *Tetrahedron Lett*, 35(40): 7303-7306, (1994).
2) Tiwari RK, Rastogi N, Sethi R, Shukla S. Synthesis and antileishmanial activity of 1-aminomethyl-5-substituted-3-{$4^{/}$-($3^{//}$-chlorobenzyloxy)-benzoylhydrazone}-2-indolinones. *J Indian Chem Soc*, 85: 85-88, (2008 Jan).
3) Abadi AH et al. Synthesis of 3-substituted-2-oxoindole analogues and their evaluation as kinase inhibitors, anticancer and antiangiogenic agents. *Eur J Med Chem* 2006; 41: 296-305.
4) Nagarajan G et al. Synthesis and *in vitro* cytotoxic activity of isatin derivatives. *Asian J Chem*; 20(3): 1665-1669, (2008).
5) Padhy AK, Panda PK, Sahu SK, Misro PK. Synthesis and pharmacological studies of 1-p- nitrobenzoyl-3-(3-substituted-2-hydroxy- propyloximino) indole-2,3-diones. *Indian J Chem*; 40B: 258-261, (2001 Mar).
6) Judy Jays, Amit Kumar, C H S Venketaramana, B V Suma, V. Madhavan. Synthesis and Biological Evaluation of some New 2-(2-Methyl-5-Nitro-1H-Imidazol-1-yl)- N'-[(3Z)-2-Oxo-1, 2-Dihydro-3H-Indol-3-ylidene]Acetohydrazide derivatives. *International Journal of Chemtech Research,* 3(2):772-777, (April-June 2011).
7) Ankit K. Rochani, B.V.Suma, Surendar Kumar, Judy Jays and V. Madhavan. QSAR, ADME AND QSTR Studies of Some Synthesized Anti-Cancer 2-Indolinone Derivatives. *International Journal of Pharma and Bio Sciences*, 1: 208-218, (2010).