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Drug design and In-Silico Hepatotoxicity studies of Synthesized Novel 4-Quinolone containing Pyrazolidinedione derivatives

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Abstract: Present research work focuses on *in-silico* drug design studies of the synthesized (n=9) novel substituted 4-quinolone containing pyrazolidinedione derivatives, screening of non hepatotoxic derivatives and finding out the characteristics of the functional groups responsible for hepatotoxicity in molecules by using *in-silico* screening of (n=180) hypothetical and novel substituted 4-quinolone containing pyrazolidinedione derivatives. In the present study, zone of inhibition data for (n=9) synthesized compounds obtained against two gram *+ve* and gram *-ve* organisms were used to develop multiple regression equations using TSAR soft. with r^2>0.8, t-probabilities<0.05. *In-silico* pharmacokinetic studies implied that these derivatives had no CYP450 2D6 inhibitions, low BBB penetration and good oral absorptions. QSTR (Quantitative Structure Toxicity Relationship) studies by using TOPKAT *(v6.1)* in various computational animal models showed considerable safety. This was followed by multiple regression equations development using computational LD_{50}, LC_{50} and LOAEL values for synthesized derivatives with r^2>0.8, t-probabilities<0.05. *In-silico* pharmacokinetic and toxicity studies data for (n=9) synthesized derivatives were validated by comparing them with standard compounds and computational descriptors.

Keywords: QSAR, fluoroquinolone, antibacterial agents, pyrazolidin-3,5-dione, anti-inflammatory agents.

Introduction and Experimental

There has been a biggest problem of bacterial resistance ever since the development of anti-bacterial agents. It was also found from the literature review that it takes nearly 14 years and 800 million dollars to get a new molecule into the market. Considering that 50% of the compounds fails in preclinical study phases, which leaves unsuitable compounds to progress into clinical testing, great interest has been focused on the determining the pharmacokinetic profile of the new molecules developed prior to its sending for animal or human testing[1].

The aim of this experimental study was to carry out computational high through put studies on synthesized (n=9) novel 4-quinolone containing pyrazolidine-3,5-dione derivatives. Quinolones are reported to have anti-bacterial activity, good anti-mitotic action and anti-tumor activity whereas pyrazolidine-3,5-dione

was reported to have anti-inflammatory, anti-bacterial and angiotensin II receptor antagonist[2,3]. The work of clubbing 4-quinolone and pyrazolidine-3,5-dione together and getting broad spectrum antibacterial and anti-inflammatory activity has been recently reported by our group[4].

Rationale for this study was to gain an insight into structural features related to improving anti-bacterial activity, pharmacokinetic and toxicity profile of the synthesized (n=9) derivatives. These *in-silico* data were also validated by comparing them with the data from standard drug molecules. Present research also focuses on the determination of structural features required for reduction of dose dependent hepatotoxicity of (n=9) synthesized derivatives by using (n=180) hypothetical structures and analysing them using high through put in-silico technique. Followed by predicting activity using developed multiple regression equations for synthesized (n=9) derivatives along with pharmacokinetic and toxicity profile for non-hepatotoxic hits.

Materials and Method

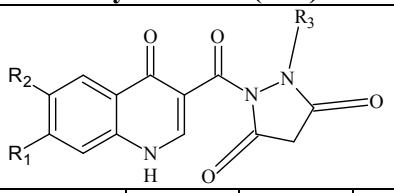
In the present research work high through put screening of synthesized (3A-3I) derivatives (table 1a) and hypothetical structures were carried out. Softwares used for above study were D. S Viewer Pro., TSAR (Oxford Soft.), Accord for Excel *(v6.1)* and TOPKAT *(v6.2)*. All these softwares were obtained from Accelrys Inc. QSAR, QSTR, ADME and Screening studies were carried out using Hewlett Packard computer system.

A set of (n=9) 4-quinolone pyrazolidine-3,5-dione derivatives were synthesized and checked for their anti-bacterial activity using zone of inhibition studies[4]. The biological activity data zone of inhibition in mm (table 1(b)) reported for *S. aureus*, *B. subtilis*, *Proteus vulgaris* and *Klebsiella pneumoniae* were subjected to QSAR, QSTR and ADME screening using Accelrys software modules. All the 2D structures were drawn in ACD/ChemSketch (freeware) and these were transferred to D.S Viewer Pro for the conversion of 2D structures to 3D structures and for energy optimization. The file formats for 3D structures was changed to .smi and were used for QSAR studies using TSAR soft. This software was used to develop multiple regression equations by establishing correlationship between suitable physicochemical parameters and biological activity. Here, different combinations were tried and only those equations which gave best possible values for r^2, r^2(C.V), s, F and t-probabilities were selected.

The smiles notations for (n=9) synthesize derivatives were transferred to TOPKAT (v 6.2) for carrying out toxicity studies in various virtual animal models. This software was also used determine carcinogenicity calls (as per FDA and NTP norms) for these (n=9) synthesized derivatives. The 2D structures for (n=9) derivatives were also introduced into Accord for Excel (v 6.1) for carrying out ADME screening. The descriptor values for all the molecules were calculated using "Calculate" module of the program. Later the pharmacokinetic descriptors were used for plotting HIA (Human Intestinal Absorption) and BBB (Blood Brain Barrier) plots.

Apart from the drug design studies of (n=9) synthesized derivatives an analysis of n=180 hypothetical molecules were carried out. Here, a search in the product catalogue of Sigma Aldrich was carried out and recorded which provided us 15 substituted anilines and 12 aliphatic/aromatic hydrazines. And using these two types of reagents, 180 hypothetical compounds were determined. These compounds were chosen for high through put screening of non dose dependent hepatotoxic hits and to understand nature of effect of these substituents on biological activity, pharmacokinetic behaviour and toxicity profile by using all the above mentioned drug design software by Accelrys Inc.

Table 1(a): Structures of synthesized (n=9) 3A-3I compounds



	3A	3B	3C	3D	3E	3F	3G	3H	3I
R1	Chloro	H	H	H	H	H	H	H	Chloro
R2	Fluoro	H	F	Cl	Br	I	CH_3	NO_2	H
R3	H	H	H	H	H	H	H	H	Ph

Table 1(b): Zone of Inhibition of 3A-3I compounds in mm

S. No.	Compound	Antibacterial activity zone of inhibition in (mm)			
		S.aureus **(gram-positive)**	***B. subtilis*** **(gram-positive)**	***Klebsiella pneumonieae*** **(gram-negative)**	***Proteus vulgaris*** **(gram-negative)**
1.	3A	17	18	21	29
2.	3B	17	20	24	26
3.	3C	19	17	20	30
4.	3D	22	18	22	28
5.	3E	20	18	22	29
6.	3F	19	19	23	27
7.	3G	18	20	25	28
8.	3H	20	17	21	25
9.	3I	19	18	20	23
10.	Ciprofloxacin	37	40	35	31
11.	Amoxicillin	43	39	35	41
14.	Control (DMF)	NI	NI	NI	NI

Note: All the values are mean of triplicates, NI: no inhibition

Table 2(a): Multiple Regression Equation for Bacterial Zone of Inhibition

Bacterial Cell Culture	Equation
S. aureus	$B.A= 0.35614482*X_1 - 0.14444731*X_2 + 1.7785262*X_3 + 28.727625$
B. subtilis	$B.A= 0.023674089*X_1 - 0.23717964*X_2 - 0.22033824*X_3 + 12.942339$
Klebsiella	$B.A = 0.043455075*X_1 - 0.83600259*X_2 - 5.2747588*X_3 + 59.067047$
Proteus Vulgaris	$B.A = 0.0011343086*X_1 - 3.0570893*X_2 + 3.4513075*X_3 + 33.979237$

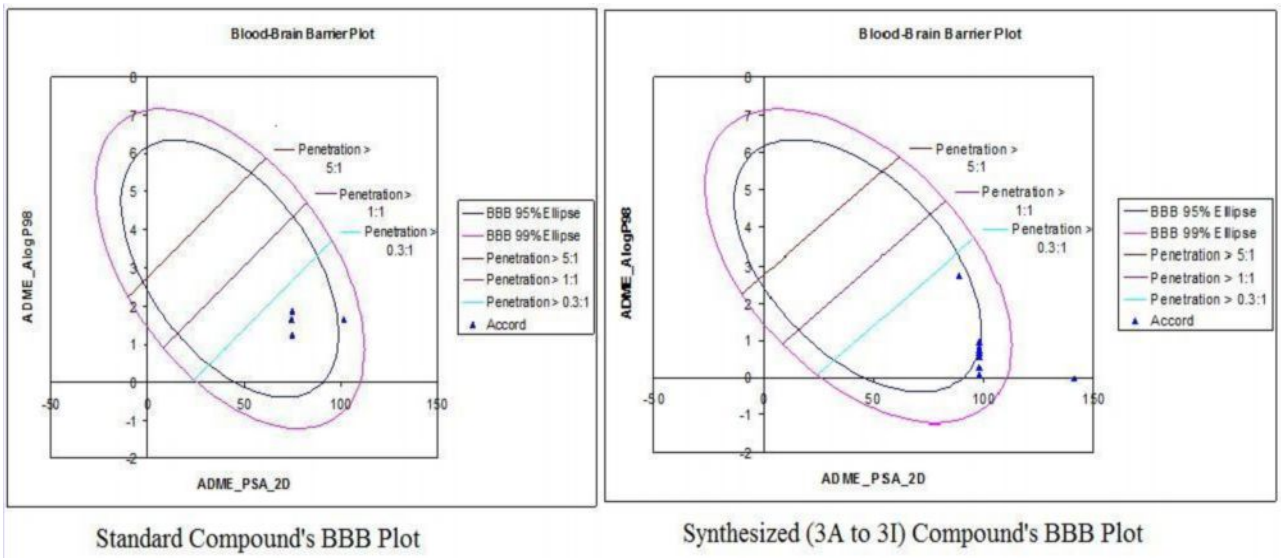
Table 2(b): Statistical values for validation of QSAR multiple regression equation

Equation	n	r	r^2	r^2(C.V)	S	f
1	9	0.945731	0.894407	0.32434	0.464628	14.1172
2	9	0.979312	0.959052	1.00000	0.286175	39.0354
3	9	0.981791	0.963913	0.905753	0.416196	44.5176
4	9	0.913086	0.833725	0.474322	1.14692	8.35692

Table 2(c): t-probability values for corresponding parameter

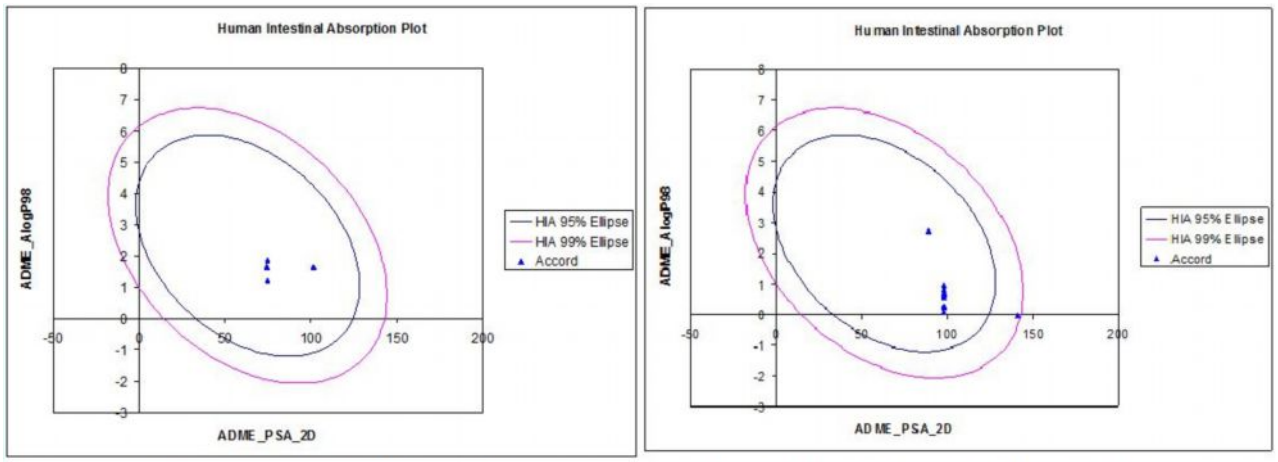
Equation	Bacterial Cell Culture	Parameters And Corresponding t-probability values		
		X_1	X_2	X_3
1.	S. Aureus	LogP	Molecular Refractivity	Vander Waal's Energy
	t-probability values	0.61408	0.074165	0.0032897
2.	B. Subtilis	Molecular Mass	Rotatable Bonds	Ionisation Potential
	t-probability values	0.000137367	0.318828	0.784561
3.	Klebsiella	Molecular Mass	Shape flexibility Index	Ionisation Potential
	t-probability values	0.000604814	0.224927	0.00518482
4.	Proteus Vulgaris	Molecular Mass	Rotatable Bonds	LUMO
	t-probability values	0.904695	0.0224091	0.165323

Figure 1(a): BBB Plot of Standard Vs Synthesized (3A to 3I) Compounds



Standard Compound's BBB Plot — Synthesized (3A to 3I) Compound's BBB Plot

Figure 1(b): HIA Plot of Standard Vs Synthesized (3A to 3I) Compounds



Standard Compound's HIA Absorption Plot — Synthesized (3A-3I) Compound's HIA Absorption Plot

Results and Discussion

A) QSAR: QSAR studies on synthesized compounds (n=9) 3A-3I was carried out and multiple regression equations of these compounds obtained for zone of inhibition (in mm) data for these compounds on four microorganisms are summarized in table: 2a. Various physicochemical descriptors and validation parameters for each equation are presented **table: 2b and 2c**. These four equations gave a considerable correlationship between the respective physicochemical parameters under study with r^2>0.8 which indicates a linear correlationship between predicted and actual biological activity values. Equation for *S. aureus* showed a good correlationship between thermodynamic (LogP & Vander Waal's energy), steric (Molecular refractivity) parameter and biological activity. According to t-probability values (presented in the table: 2c) order of significance can be defined as Vander Waal's Energy > Molecular Refractivity > LogP. Equation for *B. subtilis* showed good correlationship between thermodynamic (molecular mass), steric (rotatable bonds), electronic (ionisation potential) parameters and biological activity. As per t-probability values (presented in the **table: 2c**) it can be stated that the order of significance can be defined as Molecular mass > Rotatable bonds > Ionisation Potential. Equation for *Proteus vulgaris* showed good correlationship between thermodynamic (molecular mass), steric (rotatable bonds), electronic (LUMO) parameters and biological activity. But the order of significance by t-probability values (presented

in the table: 2c) can be defined as Rotatable bonds > LUMO > Molecular mass. Equation for *Klebsiella pneumoniae* showed good correlationship between thermodynamic (molecular mass), topological (shape flexibility index), electronic (ionisation potential) parameters and biological activity. But by the t-probability values (presented in the **table: 2c**) order of significance can be defined as Molecular mass > Ionisation Potential > Shape flexibility index.

B) ADME: In present research work of lead optimisation; ADME parameters plays a significant role in new drug discovery. Data acquired for synthesized (n=9) 3A-3I derivatives is presented in table 3. Interpretation of the values was done using standards provided by Accelrys Inc. It was observed that they have slight aqueous solubility and showed good human intestinal absorption under 95% confidence limits along with nitro derivative which showed absorption values in 99% confidence limit (**figure: 1b**). Lipinski rules of five also supported that all the synthesized derivatives were orally active molecules. BBB penetration descriptor values showed that all the compounds had low penetration (**figure: 1a**). Here, seven compounds fell in 99% and one in 95% confidence limits of level 3 (low penetration region). But nitro compound fell outside ellipse. The data for HIA and BBB was validated by having comparison of data from standard derivatives (Ciprofloxacin, Lomefloxacin, Ofloxacin and Sparfloxacin in **table: 9**) and data from synthesized compounds (**figure: 1a and 1b).** Hence it can be concluded that the molecular safety of synthesized derivative should be very much similar to fluoro quinolones. It was further observed that none of the (3A-3I) compounds has any CYP450 2D6 inhibition probabilities. All the compounds showed probabilities for dose dependent hepatotoxicity. Protein binding level showed that all the compounds have level 0 with Binding is < 90%. Compound 3H showed level 1 with Binding is >= 90% (**table3).**

Table 3: Descriptor values for synthesized compounds [3A-3I] of ADME functions

S. No.	Descriptor	3A	3B	3C	3D	3E	3F	3G	3H	3I
1	ALOGP98	0.93	2.71	0.06	0.27	0.73	0.81	0.64	0.55	-0.03
2	FPSA	98.1	88.7	98.1	98.1	98.1	98.1	98.1	98.1	140.9
3	AQ.SOL.LOG	-3.30	-4.79	-2.05	-2.47	-2.87	-2.95	-2.80	-2.55	-2.46
4	AQ.SOL.LOG.LEV	3	2	3	3	3	3	3	3	3
5	BBB.LOG.LVL	3	3	3	3	3	3	3	3	3
6	CYP2D6	0	0	0	0	0	0	0	0	0
7	CYP2D6.PROB	0.29	0.34	0.21	0.13	0.39	0.29	0.36	0.40	0.32
8	HEPATOTOX	1	1	1	1	1	1	1	1	1
9	HEPATOTOX.PROB	0.71	0.93	0.80	0.76	0.73	0.76	0.76	0.62	0.83
10	HIA.FABS.LEV	0	0	0	0	0	0	0	0	1
11	HIA.FABS.T2	2.04	1.39	3.16	2.82	2.23	2.15	2.32	2.43	9.13
12	PROT.BIND.LEV	0	2	2	0	0	0	0	1	0
13	PROT.BIND.LEV.LOG	0	0	0	0	0	0	0	0	0
14	HBOND.ACCEPTOR	7	7	7	7	7	7	7	7	10
15	ALERT	False	False	False	False	False	False	False	False	False
16	HBOND.DONOR	2	1	2	2	2	2	2	2	2
17	ALERT	False	False	False	False	False	False	False	False	False
18	MLOGP.ALERT	False	False	False	False	False	False	False	False	False
19	WEIGHT.ALERT	False	False	False	False	False	False	False	False	False
20	RULE.OF.FIVE	0	0	0	0	0	0	0	0	0
21	ALERT	False	False	False	False	False	False	False	False	False

Table 4(e): Descriptors for X_1, X_2 and X_3

S. No	Descriptors	Parameters		
		X_1	X_2	X_3
1.	LD_{50}	Molecular Surface Area	Molecular Volume	Vander Waal's Energy
	t-probability values	0.0357175	0.061696	0.3246
2.	LC_{50}	LogP	Molecular Refractivity	Total Dipole
	t-probability values	0.529064	0.646931	0.00975224
3.	LOAEL	Molecular Surface Area	Molecular Volume	Total Energy
	t-probability values	0.025855	0.017742	0.018051

Table 5(a): Toxicity probability & discriminant scores for synthesized compounds (3A-3I)

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	0.003	-5.820	0.831	1.590	1.000	8.130	0.000	-30.151
3B	0.000	-10.297	-	-	0.000	-11.590	0.000	-29.625
3C	0.000	-36.658	0.019	-3.932	1.000	51.012	1.000	22.782
3D	0.000	-17.854	0.999	7.362	1.000	9.822	0.000	-28.278
3E	0.985	4.164	0.999	7.362	0.997	5.901	0.473	-0.109
3F	1.000	9.209	0.999	7.362	0.989	4.529	0.010	-4.574
3G	0.003	-5.941	0.999	7.362	0.980	3.894	0.151	-1.729
3H	0.000	-15.848	1.000	11.570	1.000	13.427	0.220	-1.269
3I	0.973	3.581	0.997	5.702	1.000	13.427	0.999	7.564

Table 5(b): Toxicity Probability & discriminant scores for synthesized (3A-3I) compounds

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	0.001	-6.988	0.995	5.349	1.000	10.502
3B	0.814	1.480	0.728	0.984	1.000	27.685
3C	1.000	27.951	1.000	82.561	0.000	-18.983
3D	0.100	-2.196	1.000	8.051	1.000	18.319
3E	0.991	4.684	1.000	8.882	1.000	24.048
3F	0.999	7.092	1.000	9.173	1.000	26.053
3G	1.000	8.207	1.000	9.307	1.000	26.981
3H	0.029	-3.517	1.000	11.640	1.000	34.002
3I	0.722	0.955	1.000	12.182	1.000	17.156

Table 5(c): Toxicity probabilities & discriminant scores for synthesized (3A-3I) compounds

Comp	OCCULAR IRRI. SEV VS MOD (5.1)		OCCULAR IRRI. MLD VS NON (V 5.1)		AEROBIC BIO. DEGRADABILITY (V 6.1)		RAT MTD FEED/ WATER	
	Prob.	Discri. Score	Prob.	Discri. Score	Computed Values (mg/kg)	95% confidence limit (mg/kg)	Prob.	Discri. Score
3A	0.995	5.349	0.000	-27.443	21.3	1.5 & 310	1.000	10.502
3B	0.728	0.984	0.000	-31.716	14.2	1.0 & 194.3	1.000	27.685
3C	1.000	82.561	0.115	-2.044	62.6	14.5 & 270	0.000	-18.983
3D	1.000	8.051	0.000	-15.799	7.5	0.6105 & 92.7	1.000	18.319
3E	1.000	8.882	0.000	-9.472	6.5	0.5391& 0.0791	1.000	24.048
3F	1.000	9.173	1.000	19.424	7.5	0.619 & 89.9	1.000	26.053
3G	1.000	9.307	0.002	-6.233	11.5	954 & 139.6	1.000	26.981
3H	1.000	11.640	0.002	-6.181	25.4	2.4 & 265.2	1.000	34.002
3I	1.000	12.182	0.997	5.908	3.5	0.2738 & 45.5	1.000	17.156

Table 5(d): Toxicity values and confidence limits for synthesized (3A-3I) compounds

Comp.	Fat Head Minnow LC_{50}		DAPHNIA EC_{50} (V 3.1)	
	Computed values (µg/l)	95% confidence limit (µg/l)	Computed values (mg/l)	95% confidence limit(mg/l)
3A	247.5	54.7 & 1100	6.4	1.1 & 39.1
3B	50.9	10.8 & 241.0	1.3	0.1839l & 8.6
3C	2500	556.3 & 11300	0.021	0.0013 & 0.3399
3D	535.6	118.3 & 2400	48.7	7.7 & 308.7
3E	395.0	87.3 & 1800	51.4	8.1 & 326.3
3F	390.1	86.2 & 1800	58.0	9.3 & 373.7
3G	381.4	84.3 & 1700	66.8	10.5 & 423.9
3H	448.5	99.0 & 2000	3.6	489.7 & 26.5
3I	386.2	85.4 & 1700	183.0	23 & 1400

C) QSTR: Rat LD_{50}, LC_{50}, and LOAEL values along with 95% confidence limits for synthesized (3A-3I) derivatives are presented in table: 4a. The multiple regression equations for $logLD_{50}$, $logLC_{50}$ and Log LOAEL values along with necessary statistical values are presented in **table: 4c, 4d and 4e**. All the equations showed good correlationship. Toxicity profiles for these molecules were also reported (along with probability and discriminant values) in **table: 5a to 5d and table: 6a to 6c**. Following is the interpretation of the results

a) Mutagenicity: **3A, 3B, 3C, 3D 3G** and **3H** did not show probability of mutagenicity.

b) Skin Irritation: **3B, 3E, 3F** and **3G** showed zero probabilities for having skin irritation.

c)Skin Sensitisation: Here two sub-models were selected for carrying obtaining the results (as shown in table: 5a and 5b). **3A, 3D, 3F and 3H** did not show positive signs for skin sensitisation.

d) Occular Irritation: Here three sub-models were selected (table: 5b and 5c) for obtaining the results. Out of three, two models suggested positive signs for ocular irritation for all the compounds.

e) Aerobic Biodegradability: Compound **3F** showed signs of aerobic biodegradability.

f) Carcinogenicity calls (according to NTP or FDA norms):

None of the compounds showed any NTP female rat model carcinogenicity. **3C** did not show carcinogenicity in NTP male mouse model. **3A, 3B,**

3C and **3D** showed carcinogenicity in NTP female mouse model. **3C, 3D and 3I** did not show carcinogenicity in FDA MALE RAT NON VS CARC model. n=9 compounds **[3A-3I]** showed carcinogenicity probabilities in FDA MALE RAT SINGLE vs MULT. model. n=9 compounds **[3A-3I]** did not show carcinogenicity probabilities in FDA FEMALE RAT NON vs CARC. model. **3A, 3C, 3E, 3G, 3H and 3I** compounds did not show carcinogenicity probabilities in FDA FEMALE RAT SINGLE NON vs CARC. model. **3C** compound did not show carcinogenicity probabilities in FDA MALE MOUSE NON vs CARC. model. Also, n=9 compounds **[3A-3I]** did not show carcinogenicity probabilities in FDA MALE MOUSE SINGLE vs MULTI & FDA FEMALE MOUSE NON vs CARC. models. **3B** compound did not show carcinogenicity probabilities in FDA FEMALE MOUSE SINGLE vs MULT. model.

Table 6(a): Carcinogenicity probabilities for synthesized (3A-3I) compounds

Comp.	**NTP MALE RAT**		**NTP FEMALE RAT (V 3.2)**		**NTP MALE MOUSE (V 3.2)**		**NTP FEMALE MOUSE (V 3.2)**	
	Prob.	Discri. Score	Prob.	Discri. Score	Prob.	Discri. Score	Prob.	Discri. Score
3A	1.000	11.047	0.000	-29.584	1.000	28.286	1.000	11.205
3B	1.000	12.425	0.000	-21.517	1.000	35.235	1.000	7.612
3C	0.013	-4.323	0.000	-20.727	0.000	-20.447	1.000	14.547
3D	1.000	10.972	0.000	-27.347	1.000	15.027	1.000	10.591
3E	1.000	10.974	0.000	-26.304	1.000	12.722	0.959	3.149
3F	1.000	10.972	0.000	-25.939	1.000	11.916	0.633	0.544
3G	1.000	10.972	0.000	-25.771	1.000	11.542	0.340	-0.661
3H	1.000	11.172	0.000	-11.202	0.997	5.745	0.995	5.365
3I	1.000	13.248	0.000	-8.841	1.000	20.918	0.984	4.116

Table 6(b): Carcinogenicity probabilities for synthesized (3A-3I) compounds

Comp.	**FDA MALE MOUSE NON VS CARC (V 3.1)**		**FDA MALE MOUSE SINGLE VS MULTI (V 3.1)**		**FDA FEMALE MOUSE NON VS CARC (V 3.1)**		**FDA FEMALE MOUSE SINGLE VS MULT (V 3.1)**	
	Prob.	Discri. Score	Prob.	Discri. Score	Prob.	Discri. Score	Prob.	Discri. Score
3A	0.616	0.471	0.000	-25.578	0.000	-26.799	1.000	12.263
3B	0.461	-0.155	0.000	-26.082	0.000	-26.082	0.000	-13.971
3C	0.001	-6.544	0.000	-11.666	0.000	-20.270	1.000	28.197
3D	0.935	2.648	0.000	-25.364	0.000	-22.843	1.000	10.494
3E	0.980	3.875	0.000	-24.879	0.000	-13.589	1.000	12.102
3F	0.925	2.510	0.000	-24.709	0.000	-10.351	1.000	12.664
3G	0.867	1.879	0.000	-24.631	0.000	-8.852	1.000	12.925
3H	0.990	4.565	0.000	-24.563	0.001	-6.932	1.000	29.035
3I	1.000	9.189	0.000	-25.927	0.000	-25.989	1.000	7.604

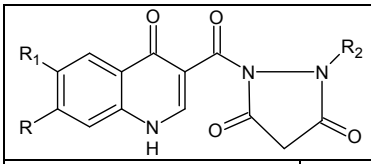
Table 6(c): Carcinogenicity probabilities for synthesized (3A-3I) compounds

Comp.	FDA MALE RAT NON VS CARC (V 3.1)		FDA MALE RAT SINGLE VS MULT (V3.1)		FDA FEMALE RAT NON VS CARC (V 3.1)		FDA FEMALE RAT SINGLE NON VS CARC (V 3.1)	
	Prob.	Discri. Score	Prob.	Discri. Score	Prob.	Discri. Score	Prob.	Discri. Score
3A	0.983	4.056	1.000	31.384	0.000	-14.334	0.034	-3.338
3B	0.979	3.836	1.000	28.248	0.015	-4.163	1.000	12.082
3C	0.000	-20.533	1.000	32.001	0.000	-29.627	0.000	-14.084
3D	0.020	-3.892	1.000	30.431	0.000	-14.434	0.905	2.255
3E	0.765	1.183	1.000	28.027	0.000	-17.152	0.000	-19.825
3F	0.935	2.662	1.000	25.701	0.000	-11.891	1.000	20.270
3G	0.988	4.935	1.000	23.609	0.000	-11.585	0.000	-14.896
3H	0.992	4.803	1.000	24.175	0.000	-10.920	0.000	-14.144
3I	0.000	-15.051	1.000	37.054	0.000	-17.121	0.036	-3.295

D) Screening Studies:

After getting results for n=180 compounds using Accelrys software modules these compounds (n=180) were classified as a) Compounds which showed to have dose dependent hepatotoxicity (170 derivatives) and b) Compounds not having dose dependent hepatotoxicity (10 derivatives) presented in table 7. Data for the compounds not having dose dependent hepatotoxicity values are summarized in table: 8(a-d). Compounds coded as **C-1, C-8, C-9, L-3, L-10, L-11, M-10, F-2, F-9 and F-10** showed no dose dependent hepatotoxicity. **L-11** showed least hepatotoxicity probability. Data for other ADME values for this series of non-hepatotoxic compounds are summarized in table: 8(d). From this data **F-9** and **F-10** were found to be crossing BBB upto level 2 (that is medium level BBB penetration). Compounds **L-10** showed low intestinal absorption and **L-11** showed moderate absorption while rest of the compounds showed considerable human intestinal absorption levels. But Lipinski rule of five suggested that **M-10** can also have low intestinal absorption value as its molecular weight > 500 Daltons and LogP value >5. By looking at HIA.FABS.T2 values compound **M-10** has its value on the border. Hence there are strong chances of this compound to be orally inactive in nature whereas all other compounds were found to be orally active in nature.

Table 7: Novel derivatives which were found to be devoid of dose dependent hepatotoxicity.



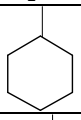
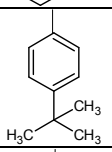
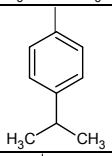
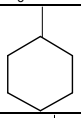
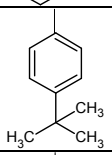
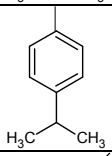
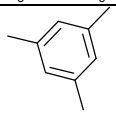
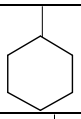
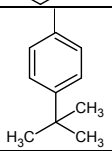
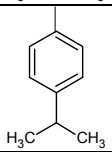
S. No.	Comp.	R	R_1	R_2
1	C-1	H	H	
2	C-8	H	H	
3	C-9	H	H	
4	L-3	H	$-OCF_3$	
5	L-10	H	$-OCF_3$	
6	L-11	H	$-OCF_3$	
7	M-10	$-CF_3$	-Cl	
8	F-2	H	$-CF_3$	
9	F-9	H	$-CF_3$	
10.	F-10	H	$-CF_3$	

Table 8(a): Compounds Not Showing Hepatotoxicity

S. No.	Comp	Descriptor Values for Above Compounds			
		LogP	Molecular Refractivity	Vander Waal's Energy	Molecular Mass
1.	C-1	1.1245	93.1767	0.988872	353.41
2.	C-8	2.8483	110.607	3.43872	403.47
3.	C-9	2.4151	106.132	1.71033	389.44
4.	F-10	3.2979	112.105	0.836532	457.44
5.	F-2	2.0073	99.1504	-0.030573	421.41
6.	F-9	3.7311	116.58	2.62652	471.47
7.	L-10	4.3409	117.149	2.28726	487.47
8.	L-11	3.9077	112.674	1.10294	473.44
9.	L-3	2.6171	99.7189	-1.05867	437.41
10.	M-10	4.2491	121.385	1.98654	505.91

Table 8(b): Descriptor values for Non Hepatotoxic Compounds

S. No.	Comp	Descriptor Values for Above Compounds			
		Rotatable Bonds	Ionisation Potential	Shape flexibility Index	LUMO
1.	C-1	2	9.08508	4.35585	-0.6055
2.	C-8	6	8.80641	4.81511	-0.62754
3.	C-9	5	8.84231	4.79336	-0.62415
4.	F-10	6	8.95715	5.60504	-1.0906
5.	F-2	3	9.50462	5.16499	-1.0719
6.	F-9	7	8.99152	5.62994	-1.0899
7.	L-10	8	8.95337	6.05495	-1.009
8.	L-11	7	8.92409	6.04094	-0.90853
9.	L-3	4	9.34309	5.60182	-0.99644
10.	M-10	7	9.01644	6.04624	-1.3223

Table 8(c): Activity Predictions for the non hepatotoxic hits

S. No.	Comp.	Predicted Biological Activity for Hypothetical Compounds			
		S. Aureus	B.Subtilis	Klebsiella	Proteus Vulgaris
1.	C-1	17.42	23.83	22.86	26.12
2.	C-8	19.96	19.12	26.12	13.92
3.	C-9	17.29	19.02	25.33	16.98
4.	F-10	15.92	20.37	27.01	12.37
5.	F-2	15.06	20.11	22.92	21.58
6.	F-9	17.89	22.22	27.39	9.35
7.	L-10	17.42	20.61	27.93	6.59
8.	L-11	15.80	20.39	27.52	9.98
9.	L-3	13.37	20.28	24.10	25.68
10.	M-10	16.22	21.27	28.41	8.02

Table 8(d): ADME profile of non hepatotoxic hits

	C-1	C-8	C-9	L-3	L-10	L-11	M-10	F-2	F-9	F-10
Descriptor										
ALOGP98	2.13	3.24	3.04	4.25	5.37	5.16	4.85	3.07	4.19	3.98
FPSA	88.71	88.71	88.71	97.6	97.6	97.6	88.72	88.7	88.7	88.7
AQ.SOL.LOG. LEV	2	2	2	1	1	1	1	2	1	2
BBB.LOG.LVL	3	3	3	4	4	4	4	3	2	2
CYP2D6	0	0	0	0	0	0	0	0	0	0
CYP2D6.PROB	0.40	0.36	0.23	0.27	0.28	0.2	0.26	0.26	0.26	0.26
HEPATOTOX	0	0	0	0	0	0	0	0	0	0
HEPATOTOX. PROB	0.40	0.50	0.40	0.39	0.40	0.36	0.48	0.38	0.39	0.371
HIA.FABS. LEV	0	0	0	0	2	1	0	0	0	0
HIA.FABS.T2	1	2.1	1.8	5.5	9.5	8.7	6.11	1.81	4.09	3.52
PROT.BIND. LEV	1	2	2	1	2	2	1	1	2	2
PROT.BIND. LEV.LOG	0	0	0	1	2	2	1	0	1	0
HBOND. ACCEPTOR	7	7	7	8	8	8	7	7	7	7
ALERT	False	False	False	False	False	False	False	False	False	False
HBOND.DONOR	1	1	1	1	1	1	1	1	1	1
ALERT	False	False	False	False	False	False	False	False	False	False
MLOGP.ALERT	False	False	False	False	False	False	True	False	False	False
WEIGHT. ALERT	False	False	False	False	False	False	True	False	False	False
RULE.OF.FIVE	False	False	False	False	False	False	True	False	False	False
ALERT	0	0	0	0	0	0	2	0	0	0

Table 9: Standard Drug ADME Profile

	Ciprofloxacin	Lomefloxacin	Ofloxacin	Sparfloxacin
Descriptor				
ALOGP98	1.230002	1.877601	1.661402	1.649402
FPSA	74.9323	74.9323	74.4047	101.4723
AQ.SOL.LOG.LEV	3	3	3	2
BBB.LOG.LVL	3	3	3	3
CYP2D6	0	0	0	0
CYP2D6.PROB	0.386139	0.39604	0.465347	0.39604
HEPATOTOX	0	0	0	0
HEPATOTOX. PROB	0.231788	0.311258	0.344371	0.324503
HIA.FABS. LEV	0	0	0	0
HIA.FABS.T2	0.625367	0.227126	0.295176	2.12015
PROT.BIND. LEV	0	0	0	0
PROT.BIND.LEV.LOG	0	0	0	0
HBOND. ACCEPTOR	6	6	7	7
ALERT	False	False	False	False
HBOND. DONOR	2	2	1	4
ALERT	False	False	False	False
MLOGP. ALERT	False	False	False	False
WEIGHT. ALERT	False	False	False	False
RULE.OF.FIVE	0	0	0	0
ALERT	False	False	False	False

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Abbreviations:
ALOGP98: Hydrophobhicity Parameter, FPSA: Fast Polar Surface Area, AQ.SOL.LOG: Log value of Aqueous solubility, AQ.SOL.LOG.LEV: Predicts Aqueous solubility level, BBB.LOG.LVL: Predicts blood-brain-barrier penetration level, CYP2D6: Predicts inhibition or non inhibition of CYP450 2D6 enzyme, CYP2D6.PROB: A scoring function that is a sum of predicted values and CYP2D6 model, HEPATOTOX: Predicts hepatotoxicity or non-hepatotoxicity, HEPATOTOX.PROB: A scoring function that is sum of predicted values of hepatotoxicity model, HIA.FABS.LEV: Predicts passive human intestinal absorption level, HIA.FABS.T2: The Mahalanobis distance for the compound in the FPSA, ALogP98 plane, PROT.BIND.LEV: Predicts Plasma protein binding levels, RULE.OF.FIVE: It's a Lipinski Rule (turns "True" for orally inactive molecules and "False" for orally active molecules in the software).

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