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COMPUTATIONAL AND BIOLOGICAL EVALUATION OF BENZOTHIAZOLE DERIVATIVES IN STREPTOZOTOCIN INDUCED DIABETES RATS

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ABSTRACT

Pre-ADMET software derived information has significant value in the design of newer potent drug molecules. All these predicted data help us to screen the potent and safe compounds for further their synthesis with its biological evaluation. Benzothiazole is versatile heterocyclic rings associated with multiple biological activities that result to inspire continue developing of Benzothiazole analogues. The antidiabetic activity can be evaluated by inducing the diabetic conditions in the experimental model using streptozotocin (STZ). The present research work was focused on screening of potent Benzothiazole derivatives with the help of Pre ADMET toxicity profile and further evaluating their biological activity in the streptozotocin induced diabetes rat model. Among all the selected compounds 6e and 6f were found more potent for anti-diabetic activity at 350 mg/kg (p. o.).

Keywords: Diabetes Mellitus (DM), Benzothiazole, Streptozotocin, Pre ADMET, Antidiabetic Activity.

Introduction

Pre-clinical ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) research is at the moment a loom used for challenging and screening for development of drugs at an early stage of the drug discovery process (Selick, Beresford, & Tarbit, 2002; Tsaioun & Kates, 2011). In designing of new compounds, ADMET information plays an imperative role. Outcomes of ADMET information can influence the selection to proceed with synthesis of newer drugs (Tam, 2013). The relationships between important ADME parameters and molecular structure and properties with deep understanding, has been used to develop *in silico* models that allow the early assessment of several ADME properties (Selick *et al*., 2002; Van De Waterbeemd & Gifford, 2003). Amongst other main issues, we go to predict properties to get the information concerning dose size and dose frequency, for instance oral absorption, bioavailability, brain penetration, clearance and volume of distribution (van de Waterbeemd, Camenisch, Folkers, Chretien, & Raevsky, 1998).

Streptozotocin (STZ) is a glucosamine-nitrosourea compound that has been most frequently used drug for induction of diabetes in rats (Akbarzadeh *et al*., 2007; Gheibi, Kashfi, & Ghasemi, 2017). It is commonly used economical effective method that offers efficient effects in the most strains of rodents (Deeds *et al*., 2011; Graham, Janecek, Kittredge, Hering, & Schuurman, 2011). Due to intra-venous injection of 60mg/kg dose of streptozotocin in adult wistar rats, pancreas became swell and degeneration of Langerhans islet beta cells induces experimental diabetes mellitus in the 2-4 days.

All the animals became diabetic after three days of beta cells degeneration. Nicotinamide-adenine dinucleotide (NAD) in pancreas islet beta cells and causes histopathological effects in beta cells which probably intermediates induction of diabetes (Engel *et al*., 2019; Mabhida *et al*., 2019; Premilovac *et al*., 2017).

Many side effects have associated with currently available drugs and therefore searching for newer compounds is the essential to overcoming these problems. The heterocycles are the versatile compounds existing in most of the natural products and synthetic organic compounds, usually associated with varied biological activities and of great scientific interest nowadays (Argyropoulou, Geronikaki, Vicini, & Zani, 2009; Kaur, Kumar, Singh, Saxena, & Kumar, 2010). Benzothiazole derivatives containing benzimidazole and imidazoline ring has diverse chemical reactivity along with a broad spectrum biological action. There was the significant interest to synthesise the benzo-thiazole derivatives with an aim to obtain potent biologically active and safe anti-diabetic agents (Gill, Rawal, & Bariwal, 2015; Haroun, 2020; Yurttaş, Kaplancıklı, Göger, & Demirci, 2016).

Materials and Methods

We were already synthesized and reported the different substituted Benzothiazole derivatives 6(a-i) listed in Table 1 using following scheme (Kumar *et al*., 2016; Kurar *et al*., 2017):

Table 1: List of Benzothiazole Derivatives with Different Substitutions .

S. NO.	Compound Code	R_1	R_2	R_3
1.	7a	CH_3	H	C_6H_{5-}
2.	7b	CH_3	H	$pOHC_6H_{4-}$
3.	7c	CH_3	H	$pOCH_3C_6H_{4-}$
4.	7d	CH_3	H	$m\ NO_2C_6H_{4-}$
5.	7e	NO_2	H	C_6H_{5-}
6.	7f	NO_2	H	$pOHC_6H_{4-}$
7.	7g	NO_2	H	$pOCH_3C_6H_{4-}$
8.	7h	NO_2	H	$m\ NO_2C_6H_{4-}$
9.	7i	H	NO_2	C_6H_{5-}
10.	7j	H	NO_2	$pOHC_6H_{4-}$
11.	7k	H	NO_2	$pOCH_3C_6H_{4-}$
12.	7l	H	NO_2	$m\ NO_2C_6H_{4-}$

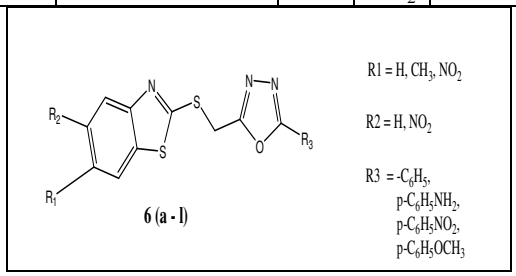

Fig. 1: Basic Nucleus of Substituted Compounds (6a-6l)

Computational Study

The Molinspiration online property calculation toolkit was used to calculate the molecular properties of synthesized compounds like TPSA, number of rotatable bonds (n-ROTB), molecular weight (MW), molecular volume (MV), number of hydrogen donor (n-OHNH), acceptor atoms (n-ON) and violations of Lipinski's rule of five (Lipinski, Lombardo, Dominy, & Feeney, 1997). All these various parameters were calculated and reported in Table 2. A Topological polar surface area (TPSA) is one of the descriptor used to predict the passive transportation of molecule through membranes. It helps to determine the kinetics of molecule with crossing the intestine and blood-brain barrier (BBB) (Ertl, Rohde, & Selzer, 2000). The Absorption percentage (%ABS) was calculated using a formula: %ABS = 109-(0.345 x TPSA) (Tripathi, Singh, & Stables, 2011).

The predicted properties of all compounds showed the non violation of Lipinski's rule and further they were selected for the prediction of ADME and toxicity profile. The pharmacokinetics properties and toxicity prediction of all the selected compounds were analyzed were reported in Table 3 and Table 4 and also presented with help of bar graph in the Fig. 2.

Table 2: Physicochemical Parameters for Good Oral Bioavailability of Synthesized Compounds (6a-6l)

S. No	Compound Code	Mol. Wt.	Log P	HBD[a]	HBA[b]	Molar refractivity	TPSA[c]	%ABS	Lipinski's Violation
1.	6a	339.43	1.85	3	2	51.67	44.15	84.77	0
2.	6b	354.45	1.92	3	2	55.22	52.37	81.93	0
3.	6c	384.43	1.95	3	3	62.637	52.18	82.00	0
4.	6d	369.46	1.89	3	3	54.51	55.13	80.98	0
5.	6e	370.41	**2.90**	3	2	42.56	46.82	83.85	0
6.	6f	385.42	**2.91**	3	2	43.58	41.87	85.55	0
7.	6g	415.40	**2.82**	3	3	62.61	55.19	80.96	0
8.	6h	400.43	1.98	3	3	50.22	52.11	82.02	0
9.	6i	370.41	1.89	2	2	41.53	59.81	79.37	0
10.	6j	385.42	**1.96**	3	2	42.52	49.81	82.82	0
11.	6k	415.40	**2.03**	3	2	61.62	52.11	82.02	0
12.	6l	400.43	1.97	2	3	52.52	55.16	80.97	0
13.	Glibenclamide*	494.004	**4.17**	4	3	70.98	56.65	80.46	0

Table 3: Predicted ADME Profile of Selected Compounds (6a-6l)

Compound Code	BBB	Human intestinal absorption level	Aq. Solubility _mg_L	*Caco-2 cell [d]permeability assay*	CYP2D6 Inhibition	Plasma protein binding
6a	0.983351	94.6573	49.6478	29.4431	Non	89. 1321
6b	0.717584	84.0943	61.54634	23.4738	Inhibitor	75.4132
6c	1.268976	96.5470	117.5427	29.0236	Non	75. 7499
6d	0.955345	91.7589	88.6647	17.7493	Non	89.6749
6e	1.163352	95.11324	1228.657	28.4324	Non	95.6489
6f	1.26333	95.11324	1738.9478	28.4324	Non	84.6415
6g	0.976437	92.15453	1217.2312	22.7365	Non	84.379
6h	0.946478	94.78398	429.1467	22.5678	Inhibitor	82.3569
6i	0.983436	93.64783	349.839	13.2542	Non	78.4670
6j	1.929884	97.67488	787.467	19.518	Inhibitor	89.87423
6k	1.183935	94.3672	625.62	14.3782	Non	97.29672
6l	1.281926	94. 5453	423.10	29. 1781	Non	87.86721
Glibenclamide*	2.354679	99.9764	1942.24	49.152	Non	99.15655

[d]Caco2-cell - heterogeneous human epithelial colorectal adenocarcinoma cell lines; Caco2-cells permeability (nm/s): Low(less than 4),

Moderate (4-70), High (more than 70); % human intestinal absorption: Well absorbed (70-100 %), Moderately absorbed (20-70%), Poorly absorbed(0-20%-); % plasma protein binding: Strongly bound (>90),weakly bound (<90%);*Standard Antidiabetic drug.

Table 4: Toxicity Profile of Selected Compounds (6a-6l) using Toxicity Prediction .

S. No	Compound Code	AMES Mutagenicity	Carcino_Mouse	Carcino_Rat	hERG_inhibition
1.	6a	Mutagen	Negative	Negative	Medium_Risk
2.	6b	Non Mutagen	Negative	Negative	Medium_Risk
3.	6c	Non Mutagen	Negative	Positive	Medium_Risk
4.	6d	Non Mutagen	Negative	Negative	Medium_Risk
5.	6e	Non Mutagen	Negative	Negative	Low Risk
6.	6f	Non Mutagen	Negative	Negative	Low Risk
7.	6g	Mutagen	Positive	Negative	Medium_Risk
8.	6h	Mutagen	Positive	Negative	Medium_Risk
9.	6i	Non Mutagen	Negative	Negative	Low Risk
10.	6j	Mutagen	Positive	Positive	High_Risk
11.	6k	Non Mutagen	Positive	Negative	Medium_Risk
12.	6l	Non Mutagen	Negative	Positive	Low risk
13.	Glibenclamide*	Mutagen	Negative	Negative	Low risk

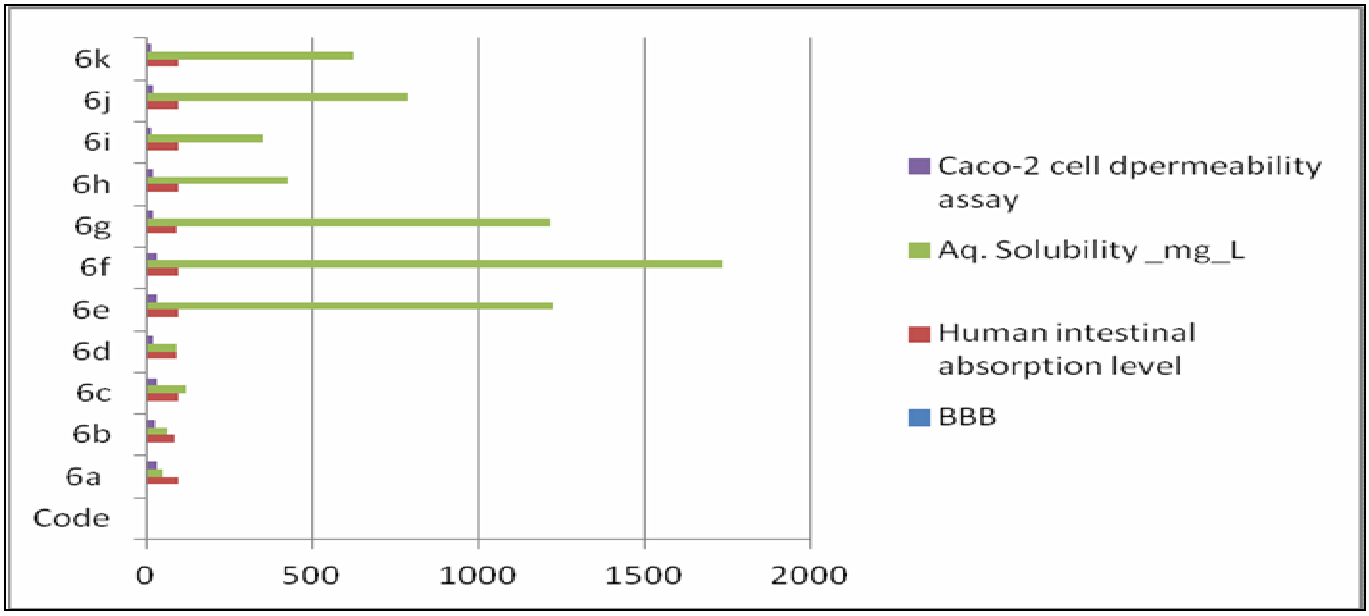

Fig. 2: Predicted ADMET Profile of Selected Compounds (6a-6l).

Biological Evaluation and Assessment of Potent Synthesized Derivative (Nambirajan *et al.*, 2018)

Streptozotocin (STZ) induced diabetes experimental model is the commonly used for biological evaluation of antidiabetic agents. Albino rats of Wister strain of either sex between the age of 2-3 months and weighing 150-200 grams were procured for the present study. They were acclimatized for the seven days by providing standard rat pellet diet with water ad libitum prior to start the study. The animals were administered with the single dose of Streptozotocin (35 mg/kg) in normal saline by intra peritoneal injection for the induction of diabetes. The animals showing blood glucose range of 200-400 mg dL^{-1} were used for the experiment and the hyperglycemia was confirmed after 72 hours of Streptozotocin monohydrate administration (i.p.). All the animal experiment protocols were approved by CPCSEA, Institutional Animal Ethics Committee, and Sapience Bio-analytical Laboratory Bhopal, Madhya Pradesh, India reg. no. 1447/PO/a/11 /CPCSEA.

Blood glucose level was monitored by tail dipping method. The blood glucose concentration was checked on dextrostrix reagent pad using microprocessor digital blood glucometer (Sugerchek Glucometer, Wockhardt manufacture, India). A single dose of (6a-6l) was administered in 350 mg/kg body weight p.o. respectively for 14 days. The blood glucose level was monitored at 0^{th} day, 7^{th} day, 14^{th} day, and 21^{st} day respectively. The antidiabetic activities of potent synthesized compounds on diabetic rats were reported in Table 5.

All the data were analyzed statistically using One-way ANOVA followed by Tukey-Kramer test. The values were considered to be significant at $p<0.05$ and $p<0.01$ level.

Table 5: Antidiabetic activity of synthesized Compounds (6a-6l) on diabetic rats

S. No.	Treatment	Blood Glucose Level (mg/dl)				% Reduction in Blood Glucose
		0th day	7th day	14th day	21st day	
1.	Normal Control	106 ± 0.98	104 ± 1.30	104 ± 0.98	101 ± 0.90	4.08
2.	Diabetic Positive control	338 ± 10.17	357 ± 2.41	344 ± 3.11	336± 6.42	0.65
3.	Glibencl-amide 10 mg/kg	352 ± 2.52	348 ± 3.16	243 ± 4.33	119 ± 6.59	67.30
	Each Test Group receives 350 mg/kg (p.o.) as effective dose.					
4.	6e	371 ± 1.08	353 ± 2.77	258 ± 1.35	142 ± 5.24	61.58
5.	6f	362 ± 1.33	348 ± 1.66	245 ± 2.34	126 ± 6.10	65.15
6.	6i	331 ± 2.23	313 ± 4.99	244 ± 7.80	163 ± 6.60	50.73

[e]Normal control group: diabetic animals received normal saline solution; [f]Positive diabetic control group: diabetic animals received 1 ml of 0.5% carboxy methyl cellulose.

Results and Discussion

All the synthesized compounds have shown the appropriate positive value of logP. Among all the compounds 6f (2.91) have shown maximum score followed by 6e (2.90) and 6g (2.82). The predicted properties of all compounds showed the non violation of Lipinski's rule. Further, these all compounds were selected for the prediction of their pharmacokinetics and toxicity as orally active drugs. The selected compounds have revealed appropriate values of BBB and human intestinal absorption. They also have shown reasonable Caco2-cells permeability with comparable value of plasma protein binding with standard. Most of the selected compounds are non inhibitor of CYP2D6 and thus reflects fewer chances of interactions with other drugs. Compounds 6e, 6f, 6g have shown maximum aqueous solubility and were in comparable range with standard glibenclamide. Toxicity analysis of 6a, 6b, 6d, 6e, 6f, 6i yielded negative carcinogenicity results in both mouse and rat models. Most of the selected compounds predict medium risk due to hERG inhibition and these results are comparable to that of standard Glibenclamide. In Ames test, 6a, 6g, 6h and 6j have shown mutagenecity. In amongst all selected compounds 6e, 6f and 6i can be predicted least toxic molecules.

On the basis of toxicity prediction results, compounds 6e, 6f and 6i were further screened for antidiabetic activity on diabetic rats. This study reveals the result of test groups when significantly compared with positive control (streptozotocin 60 mg/kg) i.v. and standard Glibenclamide 10 mg/kg (p. o.). The antidiabetic activity results exhibited significant antidiabetic response at the end of twenty first day of experimental period.

Conclusions

The pharmacokinetic and toxicity profile of Benzothiazole derivatives were predicted using Pre ADMET software. With the help of these predicted toxicity data, the potent compounds were short listed and determined the anti-diabetic activity of selected compounds in a streptozotocin induced diabetic rat model. The streptomycin was used to induce the diabetic hyperglycemia condition characterized with elevation of glucose level in plasma. It also considered as significant marker of renal dysfunction. Amongst these selected derivatives compound 6e and 6f were found as more potent anti-diabetic agents at dose of 350 mg/kg (p. o.) and would be of better use in drug development to combat the metabolic disorder in future.

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