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In-silico modeling and *in-vitro* studies of 2,1-benzothiazine-2,2-dioxide based hydrazone derivatives as α-glucosidase inhibitors

Shakeel Ahmad[1], Saman Taj[2], Sana Aslam[3], Samreen Gul Khan[1]*, Tahir Farooq[4], Usman Ali Ashfaq[2] and Matloob Ahmad[1]*

[1]Department of Chemistry, Government College University, Faisalabad, Pakistan
[2]Department of Bioinformatics and Biotechnology, Government College University, Faisalabad, Pakistan
[3]Department of Chemistry, Government College Women University, Faisalabad, Pakistan
[4]Department of Applied Chemistry, Government College University, Faisalabad, Pakistan

Abstract: Diabetes mellitus (DM) is a metabolic disorder characterized by frequent urination, hunger and high blood sugar level. α-glucosidase inhibitors are considered as a frontline treatment for the DM. This research article deals with the identification of benzothiazine derivatives as α-glucosidase inhibitors through *in-silico* techniques and then the confirmation through *in-vitro* analysis. Molecular docking studies were carried out to find out the binding interactions of targeted molecules with receptor molecule *i.e.,* α-glucosidase enzyme. The synthetic compounds 1 (a-n), 2 (a-d) and 3 (a-b) were evaluated for *in-vitro* alpha glucosidase inhibitory activities that resulted in the discovery of various potent molecules. Majority of the compounds (1c, 1f, 1g, 1k-n, 2a-d and 3a-b) exhibited good inhibitory activity against α-glucosidase. Compounds 1c, 1g, 1k and 1m appeared as the potent active compounds with the IC_{50} values 17.44, 27.64, 24.43, 42.59 and 16.90 µM respectively. Compounds 1c & 2c were found almost 3-folds more active than the standard acarbose. The study may lead to discover potent drug candidates with less complication for the treatment of the type II diabetes mellitus.

Keywords: 2,1-Benzothiazine 2,2-dioxides, hydrazones, α-glucosidase enzyme, docking studies, cardiovascular (CV), diabetes mellitus (DM).

INTRODUCTION

Diabetes mellitus (DM), also known as a mother of all diseases is caused by improper secretion of insulin by beta cells of pancreas or cells become resistant to use insulin. It was reported for the first time by an Egyptian researcher with symptoms of frequent urination and hunger with high blood sugar level. DM is prolonged illness that damages the other body organs and cause serious complications including neuropathy, nephropathy, retinopathy and cardiovascular diseases (Holman *et al.,* 2017). It is classified into two types based on the availability of insulin; Type I (insulin dependent) and type II (non-insulin dependent). Approximately 366 million peoples are estimated to suffer by this disease up to 2030 (Dinparast *et al.,* 2016).

It is a well-studied fact that the lysis of carbohydrates results in increased blood sugar levels in patients suffering from DM. Different types of enzymes are involved in catabolism and absorption of complex carbohydrates. Alpha amylase found in saliva breaks down the complex sugars into simpler sugars and alpha glucosidase found in brush border of small intestine involved in absorption of monosaccharides into the blood. The progression or serious aspects of the disease could be controlled by inhibiting the absorption and lysis of dietary carbohydrates and it could be achieved by using α-glucosidase and α-amylase inhibitors (Satoh *et al*., 2015). The available α-glucosidase inhibitors for the treatment of diabetes are miglitol, acarbose, and voglibose (Cardullo *et al.,* 2020). However, these drugs have number of adverse effects including weight gain, hepatotoxicity and also triggers the cardiovascular diseases (Chen *et al*., 2020). The available AGIs are associated with a number of side effects, such as, diarrhea, flatulence and abdomen pain (Joshi *et al.*, 2015). Thus, the new α-glucosidase inhibitors should be developed to control the DM with minimal side effects.

Benzothiazine is known for its ability to act as template for a wide range of bioactive molecules such as, antibacterial (Sebbar *et al*., 2016), monoamine oxidase inhibitors (Abid *et al.,* 2017), CD73 inhibitors (Ukrainets *et al.,* 2016) and as neuroprotective agents (Mancini *et al.,* 2017). Various other compounds of this family are found useful in rheumatoid arthritis (Shabbir *et al.,* 2016). Pyrazolobenzothiazine 5,5 dioxide derivatives are reported to have anti-HIV-1 (Ahmad *et al.,* 2014) and anti-diabetic (Taj *et al.,* 2019) activities. In our previously reported work, we have developed and identified benzothiazine derivatives as antiviral agents (Aslam *et al.,* 2014; Khalid *et al.,* 2015), cholinesterase inhibitors (Aslam *et al.,* 2014b) and monoamine oxidase inhibitors (Abid *et al.,* 2017; Ahmad *et al.,* 2018) and as α-Glucosidase inhibitors (Saddique *et al.*, 2019). In this

**Corresponding author:* e-mails: samreengul@gcuf.edu.pk; matloob.Ahmad@gcuf.edu.pk

present work, we report the *in-silico* and *in-vitro* anti-diabetic activity of 2,1-benzothiazine derivatives.

MATERIALS AND METHODS

Chemical synthesis

The titled 2,1-benzothiazine based hydrazones derivatives were prepared by our reported methodology (Ahmad *et al.,* 2018). Methyl anthranilate was used as a starting material and was coupled with methane sulfonyl chloride. The product was subsequently *N*-benzylated and cyclized to get the benzothiazine precursor, 2,1-benzothiazine-4-one 2,2-dioxide which was treated with hydrazine, followed by the reaction with various benzaldehydes to get the titled compounds.

In-vitro α-glucosidase inhibition assay

α-Glucosidase inhibition assay of benzothiazine derivatives was performed according to previously reported protocols with slight modifications (Şöhretoğlu *et al*., 2017; Mohammadi-Khanaposhtani *et al.,* 2018) as well as in our work (Taj *et al*., 2019). The in-vitro results are described in table 1.

Molecular docking

Molecular docking study was carried-out using MOE software (MOE 2014) according to the reported methodology (Saddique *et al*., 2019). Enzyme structure was accessed from protein data bank (PDB: 2QMJ). The active site of the enzyme containing Asp(A203), Asp(A327), Arg(A526), Asp(A542) and His(A600) were selected. The standard parameters were set for docking.

RESULTS

The benzothiazine derivatives were subjected to *in vitro* and *in silico* studies.

In vitro screening

The benzothiazine derivatives were screened to evaluate their *in vitro* α-glucosidase inhibitory activity. Acarbose, a standard α-glucosidase inhibitor was used as a reference and the results are presented in table 1. Following formula is used to calculate the IC_{50} values of the compounds.

$IC_{50} = X_1 + M (50 - Y_1)$

Where $M = (X_2-X_1) / (Y_2-Y_1)$; X_2= Just above 50 (Dose Response), Y_2 = Just below 50 (Dose response), X_1= Just above 50% inhibition, Y_1= Just below 50% inhibition.

STATISTICAL DATA

All experiments were conducted in replicate. *Mean of experiments are presented, $n = 3$ and $p < 0.05$ were considered statistically significant (Ms. Excel 2010).

In silico screening

Molecular docking was carried out by MOE, used to check the theoretical binding mode among the most active compounds and α-glucosidase enzyme. The results are presented in table 2.

DISCUSSION

Chemistry

The synthesis of targeted molecules was done by reported method (Ahmad *et al.,* 2018). Mesylation of methyl anthranilate resulted targeted compound, which was further *N*-methylation/*N*-benzylation reactions to form the cyclized product**,** which was further converted into relevant hydrazone, resulted relevant benzylidene and ethylidene derivatives (Ahmad *et al.,* 2018).

α-Glucosidase inhibition

Previously we have reported various benzothiazine derivatives as inhibitors of α-Glucosidase enzyme (Taj *et al.,* 2019). In this study, the compounds 1c, 1f, 1g, 1k-n, 2a-d and 3a-b, reflect the potent inhibitory potential along with IC_{50} values of 17.44, 230.07, 27.64, 24.43, 90.26, 42.59, 321.5, 95.82, 140.92, 16.90, 394.02, 214.47 and 289.75μM respectively. The compounds 1k and 1m (IC_{50} = 24.43 and 42.59 μM), containing methoxy (OCH_3) and methyl (CH_3) groups at second (2) & third (3) positions of the benzylidene derivatives respectively, were found to be the most potent compounds. The remaining compounds including 1a, 1b, 1d, 1e, 1h, 1i and 1j reflected steady state decrease in α-glucosidase inhibition as shown in (table 1). The study was analyzed in-depth to establish the structure-activity relationship. So the substitution of electron donating group on benzylidene and phenyl ethylidene derivatives respectively proceeded the increase in inhibitory activity, especially in compound 1k (IC_{50} = 24.43μM) along with (OCH_3) methoxy substitution at the second (2) position of benzylidene derivatives and was evaluated to be much more active compound. Furthermore, the compounds 1a, 1b, 1e, 1h, 1i and 1j also showed inhibition but not as effective due to substitution of electron withdrawing groups at benzylidene derivatives. The excellent binding interactions in most active molecules along with excellent active site of α-glucosidase were verified using molecular docking studies.

Molecular docking

Compounds were screened out on the base of rmsd, docking score and interacting residues. Compound 1k and 1m shows 1.092 and 1.41 rmsd respectively (table 2). Both the compounds show interaction with Asp542, Asp203.

After molecular docking, compounds with low docking score, rmsd<3 and maximum no of interacting residues were chosen for *in-vitro* ant diabetic analysis. Synthetic chemicals 1m and 1k showed significant bonding interaction with Asp (203) and Asp542 residues and also showed low docking score and rmsd value as compared other compounds.

Table 1: α-glucosidase inhibitory activity of 2,1-Benzothiazine-2,2-dioxide derivatives.

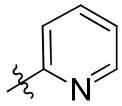
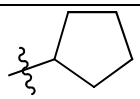
Sr. No.	Compd.	R_1	R_2	R_3	R	% Inhibition	$IC_{50\ (\mu M)}$
1	1a	Cl	H	H	H	29±0.217	-
2	1b	H	H	Cl	H	40±0.008	-
3	1c	H	H	$N(CH_3)_2$	H	56±1.409	17.44±0.03
4	1d	H	OCH_3	OH	H	29±0.02	
5	1e	NO_2	H	H	H	30±0.000	-
6	1f	H	OH	H	H	78±0.001	230.07±0.10
7	1g	H	H	OH	H	54±0.003	27.64±0.11
8	1h	H	H	NO_2	H	32±0.025	-
9	1i	Br	H	H	H	27±0.005	-
10	1j	H	H	Br	H	32±0.008	-
11	1k	OCH_3	H	H	H	78±0.032	24.43±0.05
12	1l	OH	OCH_3	H	H	55±0.00	90.26±0.02
13	1m	H	CH_3	H	H	64±0.007	42.58±0.06
14	1n	H	H	CH_3	H	66±0.009	321.50±0.05
15	2a	H	H	F	H	57±0.02	95.82±0.08
16	2b	Cl	H	Cl	H	70±0.002	140.92±0.75
17	2c	H	H	Cl	H	57±0.004	16.90±0.03
18	2d	H	H	H	H	71±0.002	394.02±0.70
19	3a	CH_3		-	-	60±0.000	214.47±0.04
20	3b	H		-	-	60±0.001	289.75±0.35
	Acarbose					100.00	58±0.015

Table 2: Molecular docking parameters of synthetic compounds

Sr. No	Compound	Docking score	rmsd	Interacting residues
1	1a	-9.107	2.160	Asp542, Asp203
2	1b	-9.698	2.377	Asp542, Asp327
3	1c	-9.953	0.902	Asp327
4	1d	-1.585	1.343	Asp542, Asp203
5	1e	-10.033	1.994	Asp203, Asp327, Arg526
6	1f	-9.952	1.763	Asp327
7	1g	-10.140	2.778	Asp542
8	1h	-9.37	5.194	Asp542, Asp327
9	1i	-10.840	1.293	Asp542, Arg526
10	1j	-1.590	2.394	Asp542, Asp203
11	1k	-9.655	1.092	Asp542, Asp203
12	1l	-1.489	0.900	Asp 542
13	1m	-10.667	1.41	Asp203, Asp542
14	1n	-10.006	1.659	Asp542, Asp203, Arg526
15	2a	-10.596	1.764	Asp542
16	2b	-2.029	1.657	Asp203
17	2c	-9.559	5.24	Asp542, Arg526
18	2d	-8.960	1.492	Asp542, Arg526
19	3a	-2.172	2.066	Asp542
20	3b	-9.850	1.939	Asp203

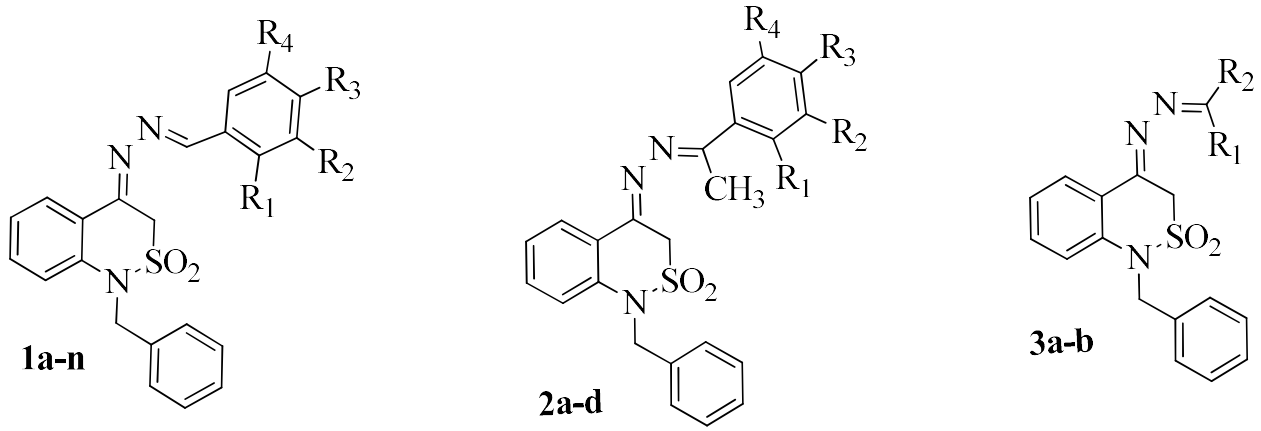


Fig. 1: The chemical structures of benzothiazine derivatives subjected for α-glucosidase inhibition activity.

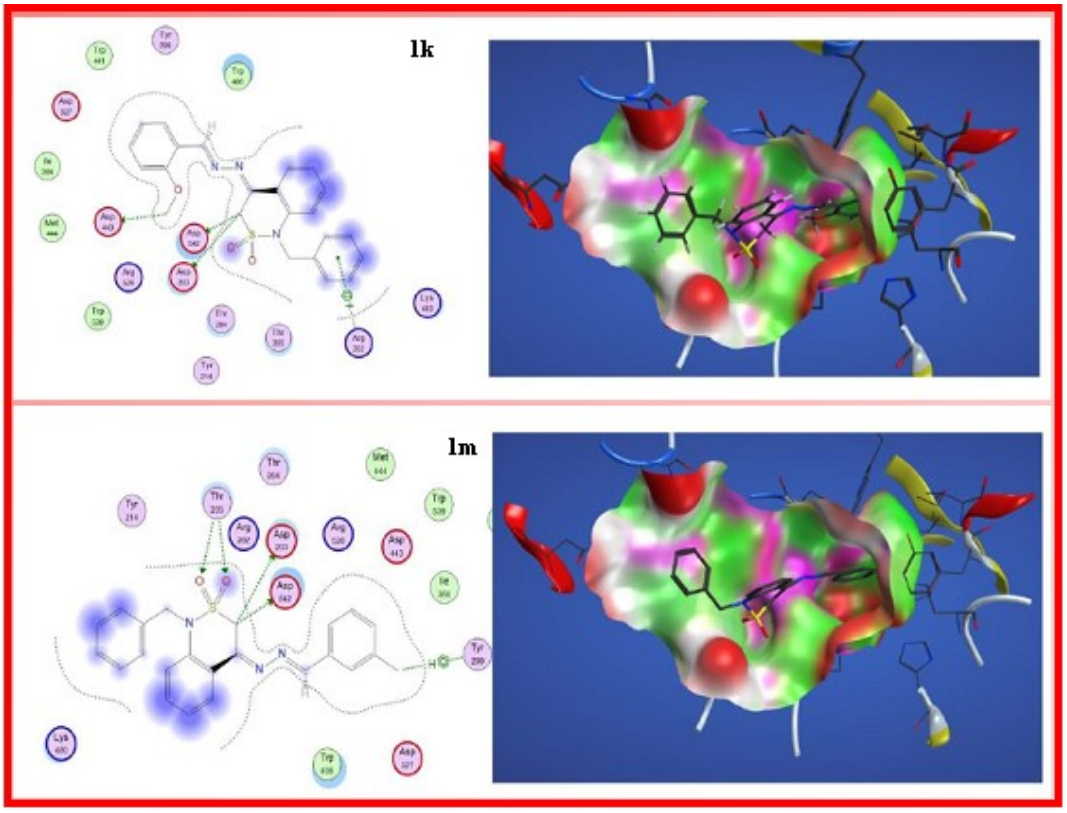


Fig. 2: Compounds 1k and 1m were docked to the binding pocket of Saccharomyces cerevisiae α-glucosidase

CONCLUSION

We have reported an efficient synthetic plan for *N*-benzylation of products through mesylation of methyl anthranilate. The structural diversity of the All synthesized derivatives were checked for their structure activity relationship by addition of substituent on the aromatic ring of benzylidene and phenyl ethylidene derivatives. In conclusion, a series of synthesized novel conjugates 1 (a-n), 12 (a-d) and 13 (a-b) were assayed for their α-glucosidase inhibition potential where most of the compounds were found to be highly active, while 1k and 1m (IC_{50} = 24.4303 & 42.586 µM) were analyzed to be most inhibitory activity against α-glucosidase with low rmsd and more interacting residues with enzyme due to possessing, methoxy (OCH_3) and methyl (CH_3) at second and third positions at the phenyl ring. So, rest of the compounds reflect very low α-glucosidase inhibitory activity as compared to the standard drug acarbose (IC_{50} = 817.38 ± 6.27 µM). Finally, the molecular docking studies were also used to confirm about binding interaction between most active compounds and α-glycosidase inhibitor.

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