Molecular Docking Supported Observed Changes in Anticholinesterase, Antioxidant and α-Glucosidase Inhibitions upon the Bromination of Benzene Sulfonamide

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Summary: Sulfonamide or sulfa drug is a known term used in various classes of medicines. Due to the high efficiency of sulfonamide group in various drugs candidates, it is still used as a vital moiety for the drug syntheses by the medicinal chemists. In this research, we have brominated benzenesulfonamide to get *N, N*-dibromobenzenesulfonamide. Furthermore, we have checked the comparative activities of both the starting sulfonamide and the brominated product. The anticholinesterase activity was determined with Ellman's assay. α-Glucosidase inhibitory potential was determined with chromogenic assay. DPPH and ABTS free radicals were used in antioxidant assay. Both benzene sulfonamide and its brominated product showed activities in various concentrations in the in-vitro assays. The acetylcholinesterase and butyrylcholinesterase (AChE & BChE) inhibitions of the brominated product were prominent, i.e., 63.98±1.51% and 67.98±0.07% at highest concentrations with IC_{50} 192.89 and 120.52 µg/ml respectively. The benzene sulfonamide exhibited 61.40±0.21% and 63.06±0.50% at highest concentrations with IC_{50} 241.85 and 190.44 µg/ml respectively. The activity of the positive control galantamine was75.72±0.35% and 77.05±0.13% with IC_{50} 43.30 and 35.06 µg/ml against AChE and BChE respectively. Similarly, in α-glucosidase assay, the brominated product showed excellent activity. Sulfonamide causing 74.62±0.40% while brominated sulfonamide showed 78.61% enzyme inhibition at 1 milligram per milliliter showing IC_{50} 47.70 and 122.40 microgram per milliliter respectively. The Acarbose standard drug exhibited 86.61±0.43% activity with IC_{50} of 34.39 microgram per milliliter. In the ABTS and DPPH antioxidant assays, the synthesized dibromobenzenesulfonamide exhibited comparable results with sulfonamide i.e., 63.06±0.50% and 67.37±0.26% radicals scavenging at 1 milligram per milliliter having IC_{50} (µg/ml) of 190.44 and 109.03 respectively. Binding poses were explored by the help of docking simulations. Binding affinity data confirmed the in vitro activity. Our results concluded that in anticholinesterase, α-glucosidase inhibitory and antioxidant the activity can be increased by bromination of benzene sulfonamide.

Keywords: Molecular docking, Benzenesulfonamide, *N,N*-dibromobezenesulfonamide, Anticholinesterase, α-glucosidase and Antioxidant.

Introduction

Sulfonamides, a well-known class of biologically active molecules have vital importance in the field of medicinal chemistry [1]. Various derivatives of sulfonamides have been synthesized by the medicinal chemists over several years [2]. More than 30 drugs of this functional group are in the clinical use which includes hypoglycemics, anticonvulsants, diuretics and HIV protease inhibitors [3], antihypertensive agent bosentan [4], antibacterial, antifungal [5], antiprotozoal [6], anti-inflammatory [7], translation initiation inhibitors [8] and nonpeptidic vasopressin receptor antagonists [9]. Various contagious diseases caused by Gram-positive or Gram-negative bacteria are also treated extensively with sulfonamides [10]. These compounds have been found in the literature to possess anticancer and antiviral properties [11]. Due to their ease of use and having no interaction with defense system, several examples are reported with GIT and UTI curing potentials [12]. Recently, sulfonamides drugs such as MZA (metahacetazoamides) and AZA (acetazolamide) are extensively used mostly as anti-glaucoma agents and are also employed for the treatment of some other diseases [13]. Sulfonamides have strong cysteine protease inhibitors, which can probably extend their curative applications to include conditions like Alzheimer's disease, cancer and arthritis [14]. Other derivatives like sulfonylurea including acetohexamide, tolazamide, and chlorpropamide are used for the treatment of diabetes [2, 15]. Based on the sulfonamide literature, we have brominated this and have check the effect in various in-vitro activities.

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Alzheimer's, a common neurological disease mainly happens in elder people [16]. The exact etiology of AD is still mysterious. However, it is believed to possess certain inflammatory processes, oxidation in the body, formation of amyloid plaques as the signs of Alzheimer's [17]. These two well establish hypotheses are the commonly known till date. The most dominant known pathogenesis of Alzheimer is the presence of acetylcholine neurotransmitter in the brain synaptic region [18]. So, based on this concept, targeting the ACh level in the region is one of the vital biochemical targets in the treatment of AD patients [19]. It is one of the challenging goals of medicinal researchers to design specific therapeutic measure for the treatment of AD [20]. The currently available treatment of Alzheimer is only limited to symptomatic treatment [21].

A substance which prevents tissue from oxidative damage is called as antioxidant. Antioxidants are important for defense against free radical damage and maintain body healthy [22]. Literature survey revealed that free radicals are responsible for various types of mental, neurological diseases and other factor like gastritis, reperfusion injury of tissues, ischemic heart diseases, and atherosclerosis [16]. The need for antioxidants increases day by day due to increased exposure to free radicals. Currently, an oxidant supplement is also recommended as important part of a balance diet [23, 24]. Moreover, it is well known that a series of human illnesses, such as atherosclerosis, cancer, cerebro and cardio-vascular diseases, immune system destruction, neurodegenerative diseases and diabetes, is related to the harmful action of free radicals [25]. Free radicals are implicated in the progression of numerous disorders including Alzheimer's diseases (AD) and diabetes mellitus (DM). In AD, amyloid beta (Aβ) which is considered as mitochondrial poison, liberate excessive number of free radicals thus disrupt mitochondrial function and readily attack neuronal tissues causing their degeneration [26, 27]. Likewise, generation of excessive free rad006icals cause lipids peroxidation, glycation of proteins (non-enzymatic) as well as cause glucose oxidation thus leading to DM and its complications [28, 29]. Subsequently, supplementation of exogenous antioxidants is extremely necessary in both chronic diseases [30, 31].

The occurrence of diabetes worldwide is rising at an alarming rate, affecting close to five percent of its population and it is severely associated to some other diseases [32]. Diabetes is a syndrome that is mainly characterized by high blood glucose level due to insulin dysfunction [33]. The reactive oxygen species are also supposed to implicate in diabetes. Various natural and synthetic antioxidants are used which can also be helpful in the management of metabolic disorder [34-36]. In conclusion, the aim of this study was to investigate the biological properties, including anti-cholinesterase, antioxidant, and anti-diabetic activity of the synthesized dibromosulfonamide.

Experimental

Bromination of benzene sulfonamide

To a two necked flask were added benzene sulfonamide (1 equiv) followed by potassium hydroxide (2 equiv) with continuous stirring in water (1M). Afterwards, bromine (6 equiv) was added slowly and continuo the stirring at room temperature. The reaction was monitored by TLC. After completion, the product was confirmed by ^{1}H NMR analysis.

In-vitro Anticholinesterase assay

The source of acetylcholinesterase used in this study was electric eel while equine serum was the origin for butyrylcholinesterase. Both of these assays were performed as per the reported method [37]. The enzymes and substrates were determined by spectroscopic methods. In this method, a solution of each enzyme (five microliters) was added to the microplate reader's wells. Then added 205 microliters of the samples to be tested. Finally added 5 microliters of reagent (DTNB). The resultant mixture was first incubated for fifteen minutes at 30 degree Celsius. After incubation, the five microliters substrate solution was added. The values were measured at 412 nanometers of the microplate instrument. Except inhibitor all the reagents were mixed to form negative control. The change in absorption values were monitored for four minutes. The percent inhibition values and its IC50s were calculated as per the reported procedure [38].

In-vitro Alpha glucosidase assay

For the α-glucosidase inhibitory potential previously described protocol was followed with little bit modification [29]. The solution of glucosidase was obtained by mixing of phosphate buffer (120 µL of 0.1 M having pH 6.9) with α-glucosidase enzyme (0.5 u/ml). The substrate solution was prepared in 0.1 M buffer solution having pH 6.9. Various serial dilutions were prepared from samples in concentration ranging from 62.5–1000 µg/mL. Both solutions were then mixed and

incubated (37 ℃ for 20 min). Substrate solutions (20 µL) was then added enzyme and test sample mixture, and again incubated for further 15 minutes at 37 ℃. The reaction was finished byadding80 µl sodium carbonate solution (0.2 M). The absorbance was measured at 405nm via UV-VIS spectrophotometer (UV-1800. Germany). Acarbose served as control drug and the sample without compounds was blank one. The percent inhibition values and its IC50s were calculated as per the reported procedure.

In-vitro Antioxidant assay

DPPH free radical Assay

For the DPPH free radicals inhibition effect previously described procedure with minor changes as per the requirement of our assay [39]. To 100 ml of methanol, 24 mg of DPPH was added. The color of DPPH solution changed to deep violet. Initially, solutions of the compounds having concentration of one milligram per milliliter was made in EtOH. Afterwards, serial dilutions of this solution (500, 250, 125 and 62.5µg/mL) were made. After that, 0.3mL of the serially diluted solution of each vial was added DPPH solution (3.0 mL). This mixture was incubated at 23 ^{o}C in a dark place for 30 min and then the absorbance was measured 517 nanometers. The standard ascorbic acid served as control drug in the experiment. The percent inhibition values and its IC50s were calculated as per the reported procedure.

ABTS free radical assay

We also performed the ABTS free radicals scavenging assay. This assay was based on the same method as our previously reported procedures [40]. The data was obtained with a double beam spectrophotometer and repeated three times. The percent inhibitions and its IC_{50} values were calculated according to the standard formula previously reported.

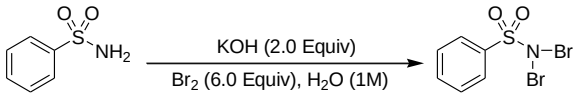
Statistical analysis

The statistical analysis, standard error mean and median inhibitory concentration was calculated as per the previous standard method.

Results and Discussion

N,N-dibromobenzesulfonamide

The *N,N*-dibromobenzene sulfonamide was synthesized with excellent yield as shown in Scheme 1 below. ^{1}H NMR (400 MHz, $CDCl_3$) (ppm): 7.45-7.56 (m, 2H), 7.68-7.72 (m, 1H), 7.76-7.80 (m, 2H).



Scheme-1: Synthesis of *N,N*-dibromobenzenesulfonamide.

Anticholinesterase studies

The results of sulfonamide, dibromosulfonamide and standard drug galantamine against AChE and BChE activities are shown in Table-1. The sulfonamide exhibited 61.40 ±0.21, 56.04 ±0.44, 51.30 ±0.31, 43.28 ±0.11, 39.33 ±0.23% against AChE and 63.06 ±0.50,57.10 ±0.16, 54.62 ±0.70, 47.58 ±0.41, 41.65 ±1.17% against BChE at the concentration from 1000, 500, 250, 125, 62.5 µg/mL respectively. Likewise, dibromosulfonamide showed excellent activity as compared to sulfonamide against AChE and BChE exhibiting IC_{50} values of 192.89 and 120.52 µg/mL respectively. Similarly, the galantamine IC_{50} values were 43.30 and 35.06 µg/mL against AChE and BChE respectively.

Alpha glucosidase studies

The alpha glucosidase inhibition of the sulfonamide, dibromosulfonamide and standard drug acarbose was shown in Table-2. The sulfonamide showed 74.62±0.40, 65.60±0.46, 57.37±0.68, 52.62±0.74 and 41.00±0.30% at concentration ranging from 62.5-1000µg/mL with IC_{50} 122.40 µg/mL. Similarly, dibromosulfonamide exhibited most prominent activity as compared to sulfonamide. The percent inhibition values of the dibromosulfonamide were78.61±0.43, 70.03±0.86, 65.76±0.58, 58.68±0.49 and 53.58±0.77% with IC_{50} 47.70 µg/mL which was almost near to that of positive control acarbose at same concentration. The observed concentration for the control was 86.61±0.43, 77.58±0.70, 70.45±0.49, 63.44±0.51 and 59.73±064% with IC_{50} 34.39 µg/mL.

Anti-radical studies

The antiradical activity is summarized in Table-3.

Table-1: Anticholinesterase activity of the compounds.

Samples	Conc. (µg/mL)	AChE activity		BChE activity	
		Percent inhibition (mean ±SEM)	IC_{50} (µg/ml)	Percent inhibition (mean ±SEM)	IC_{50} (µg/ml)
	1000	61.40±0.21		63.06±0.50	
	500	56.04±0.44		57.10±0.16	
Benzenesulfonamide	250	51.30±0.31	241.85	54.62±0.70	190.44
	125	43.28±0.11		47.58±0.41	
	62.5	39.33±0.23		41.65±1.17	
	1000	63.98±1.51		67.98±0.07	
N,N-Dibromobenzenesulfonamide					
	500	56.75±0.69	192.89	61.95±0.58	120.52
	250	51.58±0.38		56.05±0.13	
	125	46.93±0.81		51.38±0.34	
	62.5	41.08±0.08		43.71±0.66	
	1000	75.72±0.35		77.05±0.13	
	500	71.66±0.43	43.30	71.41±0.37	35.06
Galanthamine	250	65.41±0.37		65.78±0.73	
	125	59.50±0.19		60.35±0.31	
	62.5	52.59±0.26		55.05±0.99	

Table-2: Alpha glucosidase inhibitory potential of compounds.

Compound	Conc.(µg/ml)	Percent inhibition	IC_{50} (µg/ml)
	1000	74.62±0.40	
	500	65.60±0.46	
Benzene sulfonamide	250	57.37±0.68	122.40
	125	52.62±0.74	
	62.5	41.00±0.30	
	1000	78.61±0.43	
N,N-dibromobenzenesulfonamide	500	70.03±0.86	
	250	65.76±0.58	47.70
	125	58.68±0.49	
	62.5	53.58±0.77	
	1000	86.61±0.43	
	500	77.58±0.70	
Acarbose	250	70.45±0.49	34.39
	125	63.44±0.51	
	62.5	59.73±064	

Table-3: Antioxidant activity of the compounds.

Compound	Conc (µg/ml)	Percent ABTS (mean ±SEM)	ABTS IC_{50} (µg/ml)	Percent DPPH (mean ± SEM)	DPPH IC_{50} (µg/ml)
	1000	64.55±0.31		67.62±0.37	
	500	57.82±0.51		62.86±1.43	
Benzenesulfonamide	250	52.84±1.08	155.46	54.48±0.78	151.48
	125	45.70±0.11		47.79±0.17	
	62.5	39.83±0.62		41.48±0.11	
	1000	63.06±0.50		67.37±0.26	
	500	56.10±0.16		61.52±0.95	
N,N-Dibromobenzenesulfonamide	250	51.62±0.70	190.44	55.90±0.46	109.03
	125	47.58±0.41		51.02±0.18	
	62.5	41.65±1.17		46.04±0.14	
	1000	84.51±0.30		87.66±0.45	
	500	77..84±0.27	23.20	81.64±0.42	17.47
Gallic acid	250	73..50±2.26		76.01±1.61	
	125	65..74±0.16		70.46±0.32	
	62.5	61.56±0.28		64.50±0.02	

Results of DPPH assay

In this, the synthesized compound exhibited high percent activity with concentration dependent manner. The percent antioxidant activity of the tested samples at concentration ranging from the 62.5-1000 (µg/ml) were 67.37±0.26, 61.52±0.95, 55.90±0.46, 51.02±0.18 and 46.04±0.14% with IC_{50} 109.03 µg/mL while percent scavenging of sulfonamide was 67.62±0.37, 62.86±1.43, 54.48±0.78, 47.79±0.17 and 41.48±0.11% with IC_{50} 151.48 µg/mL. The standard drug (Gallic acid) showed 87.66±0.45, 81.64±0.42, 76.01±1.61, 70.46±0.32 and 64.50±0.02% with IC_{50} 17.47 µg/mL.

Results of ABTS assay

The results are provided in Table-3. The sulfonamide exhibited high percent ABTS activity with the inhibition value 64.55±0.31, 57.82±0.51, 52.84±1.08, 45.70±0.11 and 39.83±0% with IC_{50} 155.46 µg/mL. Similarly, dibromosulfonamide causing 63.06±0.50, 56.10±0.16, 51.62±0.70, 47.58±0.41 and 41.65±1.17% with IC_{50} value 190.44 µg/mL. The positive control exhibited IC_{50} value 23.20 at same concentration.

During the metabolic degradation, free radicals are produced in the human body and are related with

various types of pathological disorders such as neurodegenerative diseases, cancer, coronary heart disorder, diabetes and immune suppression [28]. The free radicals are hydroxyl, superoxide, nitric oxide, lipid peroxyl and non-free radicals which generally contain singlet oxygen and hydrogen peroxide [41]. Naturally the body defense system abolish the toxic effect of the free radicals by different mechanistic system that may be beneficial in various physiological and neurodegenerative disorders such as chain breaking antioxidants generation and protective antioxidant [42]. When the limits of naturally occurring scavenging mechanisms exceed the limit, then the excessive free radicals in the body cause tissue injury [43]. The sulfonamide type of compounds can protect tissue injury from radicals' generation. Noteworthy, anti-radical potential of synthesized compound was revealed by DPPH (IC_{50} of 109.03 μg/mL) and ABTS (IC_{50} of 190.44 μg/mL) free radical scavenging assays.

For the management of neurodegeneration, selected inhibitors like acetyl- and butyrylcholinesterase play a vital role. These enzymes is an attractive target for the rational drug design as well as for the discovery of mechanism based inhibitors because of its role in the breakdown of acetylcholine, hence having beneficial key role in the management of AD and other neurological diseases [44]. Memory loss, behavior changes and other cognitive disorders are associated with AD [21]. Thus, by inhibiting AChE we can near to treat and manage Alzheimer's disease cognitive symptoms and many other neurological disorders like senile dementia, Parkinson disease, myasthenia gravis and ataxia [42]. The existing drugs manufactured by various pharmaceutical companies are associated with various undesirable effects, therefore it is necessary to explore new therapeutic agents which would be safer [45, 46]. The natural products research is an easy tool to explore safer bioactives for various biological activities [47, 48]. Organic and medicinal chemists have explored the potential of synthetic drugs for their therapeutic effectiveness [49-52]. Herein, we demonstrated dibromosulfonamide as potential acetyl- and butyrylesterase moiety. Interestingly, in anti-AChE and BChE activities of dibromosulfonamide were going positively, in the selected concentrations, the percent inhibition values with IC_{50} 192.89 and 120.52 significantly closer to the positive control (galantamine) as compared to sulfonamide.

In type 2 diabetes mellitus, elevated secretion of insulin causes postprandial hyperglycemias after meal. The researchers have evidence that ROS are implicated in hyperglycemia [53]. In our diet, carbohydrates are digested by the body via glucose metabolizing enzymes, like glucosidase. As we know that acarbose, voglibose and miglitol is the key inhibitor of glucosidase but these inhibitors have been found to possess gastrointestinal side effects like abdominal discomfort, flatulence and diarrhea. As a result of this, there is growing interest in discovering new and effective α-glucosidase inhibitors [54]. Current treatment for Type 2 diabetes is not clear, available modern medicines such as SGLT2 inhibitors, Sulfonylureas, Metformin, Thiazolidinediones, and DPP-4 inhibitors can treat diabetes, but it gave various undesired effects [29]. Literature survey revealed that little bit modification in the structure of compound can result qualitative as well as quantitative changes in the activity [55-57]. We have brominated benzene sulfonamide to get *N*, *N*-dibromobenzenesulfonamide. Our observations reveal the possible inhibition of α-glucosidase via dibromosulfonamide.

Docking studies

Docking studies on α-glucosidase

Docking studies were carried out on our already reported homology modeled α-glucosidase using Molecular Operating Environment (MOE 2016.08) software [58, 59]. The best docking pose of both the synthesized compounds are shown in Fig. 1a-b. The lowest-energy docking pose of benzene sulfonamide shows two hydrogen bonding interactions. Catalytic triad residue Asp214 establishes hydrogen bonding interaction (HBI) with $-NH_2$. While, sulfonyl oxygen interacts with Arg439 (Fig. 1a). The binding affinity for benzene sulfonamide is -4.3775 kcal/mol. Dibromo sulfonamide binding strongly into the binding site and shows three HBIs. Sulfonyl oxygen atoms interacts with Phe157 and Arg439. Catalytic triad residueAsp349 shows interaction with bromine atom (Fig. 1b). The computed binding affinity for N-dibromo derivative is 4.9596 kcal/mol.

Docking studies on acetylcholinesterase

Docking studies on AChE target were also carried out. Crystal structure of *Tc*AChE (PDB code: 1EVE) in complex with donepezil as native ligand was selected for these studies.The docking reliability was validated by using re-dock method. Native ligand donepezil was re-docked into the binding site of AChE and Root Mean Square Deviation (RMSD) was computed. It was found within the acceptable criteria i.e. < 2 Å. Docking poses of both the compound are shown Fig. 2a-b. Benzene sulfonamide shows π-H interactions with Trp84. While, $-NH_2$ establishes HBI with Glu199 (Fig. 2a). The binding affinity for benzene sulfonamide is –4.6852 kcal/mol. Dibromosulfonamide binding strongly (binding affinity=-5.2505 kcal/mol). Ser122 interacts with bromine atom. Phenyl ring forms π-H interaction with His440 (Fig. 2b).

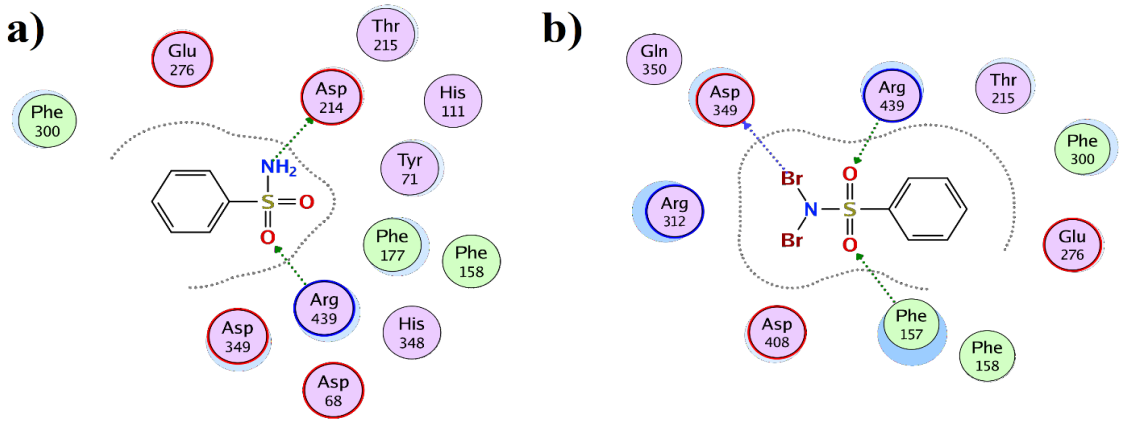


Fig. 1: Two-dimensional interaction plot of synthesized compound (a) benzene sulfonamide (b) dibromo sulfonamide; into the binding pocket of homology modeled α-glucosidase.

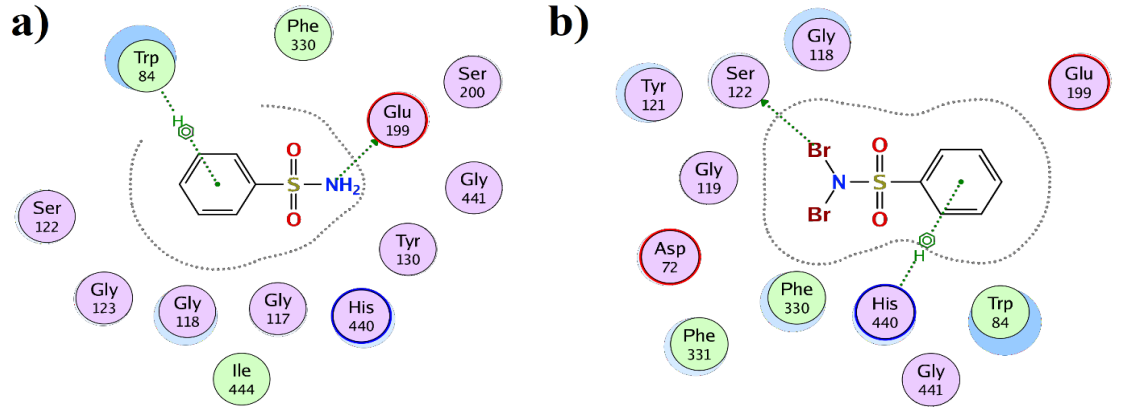


Fig. 2: Two-dimensional interaction plot of synthesized compound (a) benzene sulfonamide (b) dibromo sulfonamide; into the binding pocket of AChE (PDB code=1EVE).

Conclusions

This study is a comparative enzymes inhibition analysis of benzene sulfonamide and its dibrominated form. Our enzyme inhibitions show that the brominated product is more potent inhibitor of acetyl & butyrylcholinesterase, alpha glucosidase and DPPH. It may be inferred that the dibromobenzenesulfonamide is more potent than the benzene sulfonamide in terms of these enzymes assays. Docking studies on two targets i.e. α-glucosidase and acetylcholinesterase were carried out that confirm the change in in-vitro analysis with bromination by comparing the docking energies.

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List of abbreviations

DPPH; 2,2-diphenyl-1-picrylhydrazyl, ABTS; 2, 2'-Azino-Bis-3-Ethylbenzothiazoline-6-Sulfonic Acid, AChE; Acetylcholinesterase, BChE; Butyrylcholinesterase, AD; Alzheimer's disease, DM; Diabetes mellitus, TLC; Thin layer chromatography, NMR; Nuclear magnetic resonance spectroscopy, DTNB; 5,5'-Dithio-bis-(2-nitrobenzoic acid, MeOH; Methanol, SEM; Standard error mean, HBI; Hydrogen bonding interaction.

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