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Biological evaluation of aromatic bis-sulfonamide Schiff bases as antioxidant, acetylcholinesterase and butyrylcholinesterase inhibitors

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Abstract

Aromatic/heterocyclic Schiff bases are one of the most investigated and studied scaffold for many pharmaceutical applications. For this reason, in the current work, a series of aromatic bis-sulfonamide Schiff bases (7-15) were re-synthesized by reacting aromatic bis-aldehydes and aromatic sulfonamides in ethanol and assayed for antioxidant properties by using different bioanalytical methods such as DPPH free radical scavenging assay, ABTS cation radical decolarization, cupric reducing antioxidant capacity (CUPRAC) and metal chelating methods. The acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibition profiles were also assessed. In general, the synthesized compounds showed weak antioxidant activity against all tested methods, but two compounds (12 and 15) showed good CUPRAC activity at 10, 25 and 50 µM concentrations. The weak inhibition potency was obtained against AChE and moderate activity was observed against BChE enzymes at 200 µM.

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1. Introduction

Schiff bases (R-C=N-R) are one of the most versatile ligands which are synthesized from the condensation of primary amines with active carbonyl groups [1]. The imine group of the Schiff bases is an interesting core for medicinal chemistry applications. In literature, Schiff base derivatives shown to have broad biological properties such as antimicrobial, antifungal, diuretics and antitumor activities [2-8]. More specifically, aromatic/heterocyclic mono and bis sulfonamide Schiff bases were investigated as carbonic anhydrase inhibitors (CAIs) [9-12] and histamine Schiff bases as carbonic anhydrase activators (CAAs) by several groups and us [13-15]. Although mono type of sulfonamide Schiff bases was extensively investigated for many different biological applications, bis type of sulfonamide Schiff base derivatives was not yet investigated, the best of our knowledge, as an antioxidant and cholinesterase inhibitors.

More recently, our group showed the efficient carbonic anhydrase inhibition profile on aromatic bis-sulfonamide Schiff base derivatives [9]. The nanomolar potency was obtained against human carbonic anhydrase IX and XII (hCA IX and XII), which are tumor-overexpressed membrane-bound isozymes of carbonic anhydrase enzyme [9]. Since the potent inhibition profile of these compounds, in the present study, prompted by these potent biological activities, we re-synthesized and assessed these aromatic bis-sulfonamide Schiff bases as antioxidant and cholinesterase (AChE and BChE) inhibitors.

2. Material and Methods

2.1. Chemistry

General synthetic route for the preparation of aromatic bis-sulfonamide Schiff bases (7-15) were depicted in Figure 1. These compounds were synthesized and characterized previously by us as an efficient carbonic anhydrase inhibitors [9]. As a general procedure for the synthesis of aromatic bis-sulfonamide Schiff bases, the aromatic sulfonamides were conjugated with aromatic bis-aldehydes in ethanol in the presence of a few drops of formic acid as a catalyst and then refluxed. The obtained compounds were washed with ice-cold methanol/ethanol and collected by filtration. The final compounds (7-15) were dried under vacuum and characterized by physicochemical and spectroscopic methods as previously described by us [9].

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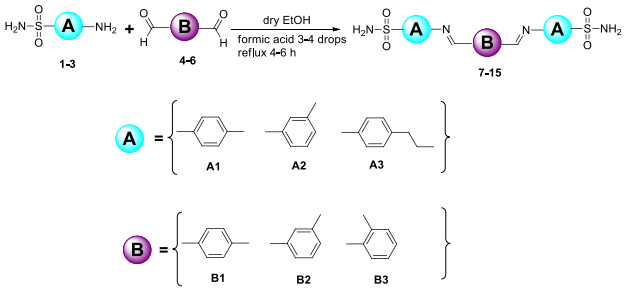

Figure 1. General synthetic route for the synthesis of bis-sulfonamide Schiff bases **(7-15)** [9].

2.2. Determination of antioxidant and anticholinesterase activity of bis-sulfonamide schiff bases (7-15)

2.2.1 DPPH radical scavenging ability

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity of the synthesized compounds was determined by spectrophotometric method based on the reduction of an ethanol solution of DPPH [16]. 2, 5, 10, 20 µL of 1 mM stock solution of each compound was completed to 40 µL with the DMSO and mixed with 160 µL of 0.1 mM of DPPH free radical solution. The mixture was led to stand for 30 min in the dark and the absorbance was then measured at 517 nm against a blank. Inhibition of free radical, DPPH, in percent (I %) was calculated according to the formula:

$$I\ \% = (A_{control} - A_{sample})/A_{control} \times 100;$$

where $A_{control}$ is the absorbance of the control reaction (containing all reagents except for the tested compounds), and A_{sample} is the absorbance of the test compounds. Tests were carried out in triplicate. BHA, BHT and α-Toc were used as positive control.

2.2.2. ABTS cation radical decolorization

The percent inhibition of decolorization of ABTS (2,2`-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) cation radical is obtained as a function of time and concentration, and evaluated by comparison with the BHT, BHA and α-Toc compounds used as standard [17, 18]. The tested compounds at different concentrations are added to each well and 160 µL of 7 mM ABTS solution is added. After 6 min at room temperature, the absorbances were measured at 734 nm. ABTS cation radical decolorization activities were determined by using the equation below:

$$\%Inhibition = (A_{control} - A_{sample})/A_{control}\ x100$$

where A is the absorbance. Tests were carried out in triplicate. BHA, BHT and α-Toc were used as positive control.

2.2.3. Metal Chelate

The chelating ability of synthesized compounds was examined according to the method of Dinis et al. [19]. The tested compounds at different concentrations were added to each well and 4 µL of 2 mM ferrous (II) chloride was added. Then 8 µL of 5 mM ferrozine was added and the reaction was started. After 10 min at room temperature, the absorbance was measured at 562 nm against blank. The results were expressed as percentage of inhibition of the ferrozine-Fe^{2+} complex formation. The percentage inhibition of the ferrozine - Fe^{2+} complex formation was calculated using the formula given below:

$$\text{Chelating ability } (\%) = (A_{control} - A_{sample})/A_{control} \times 100$$

where A is the absorbance. Tests were carried out in triplicate. EDTA was used as a positive control.

2.2.4. Cupric reducing antioxidant capacity (CUPRAC) method

CUPRAC method comprises the reduction of Cu(II)-Neocuproine into its colored form Cu(I)-Neocuproine chelate in the presence of antioxidant compounds [20, 21]. The tested compounds at different concentrations were added to each well and 61 µL of $CuCl_2$, Neocuproine and NH_4OAc solutions were added. After 1 hour at room temperature, the absorbance was measured at 450 nm. The absorbance values were compared with the standard molecules BHA, BHT and α-Toc. Each of samples was applied three times.

2.2.5. Anticholinesterase activity of the bis-sulfonamide Schiff bases

The inhibitory effect of bis-sulfonamide Schiff base derivatives (7-15) on AChE and BChE activities was determined according to the slightly modified spectrophotometric method of Ellman et al. [22]. All compounds were dissolved in DMSO to prepare stock solutions at 4 mM concentration. Aliquots of 150 µL of 100 mM sodium phosphate buffer (pH 8.0), 10 µL of sample solution and 20 µL AChE (or BChE) solution were mixed and incubated for 15 min at 25 °C, and DTNB (5,5'-Dithio-bis(2-nitro-benzoic)acid) (10 µL) is added. The reaction was then initiated by the addition of acetylthiocholine iodide (or butyrylthiocholine iodide) (10 µL). The final

concentration of the tested compounds' solution was 200 μM.

$\%Inhibition = (A_{control}-A_{sample})/A_{control}$ x100

where A is the absorbance. Tests were carried out in triplicate. Galantamine was used as positive control. IC_{50} values were calculated from the equation of the curve obtained from the concentration-inhibition graph. The value of IC_{50} is the value found when we write fifty instead of inhibition in the equation of the obtained curve.

2.3. Statistical analysis

The results of the antioxidant and anticholinesterase activity assays are expressed as the mean ± SD of three parallel measurements. The statistical significance was estimated using a Student's t-test, where p-values < 0.05 were considered significant.

3. Results and Discussion

In the current study, we report the antioxidant, acetylcholinesterase and butyrylcholinesterase inhibition activities of a series of bis-sulfonamide Schiff base derivatives (7-15). These compounds were re-synthesized as described in our previously published work. In the present study, the main idea was to assess antioxidant and cholinesterase activities of these potent carbonic anhydrase inhibitors.

Schiff bases and their metal complexes were extensively studied as a potential antioxidant regeants [23, 24]. Specifically, a histamine Schiff bases were recently evaulated as an antioxidant and cholineterase inhibitors by us [20]. Among the series, most of the compounds showed moderate antioxidant activity with IC_{50} values ranging from 249.63 to 945.23 μM for DPPH activity, from 89.18 to 868.89 μM for ABTS activity, and from 70.34 to 296.25 μM for metal chelating activity. Another study from the Khan et al. [25], demonstrated the IC_{50} values within the range of 15.16-48.26 μM for in vitro free radical scavenging activity by using Schiff bases of 4-amino-1,5-dimethyl-2-phenylpyrazolones. Aslam et al. [26], showed also DPPH antioxidant activity of Schiff bases that synthesized from the condensation of 2-aminophenol and various chloro- and nitro-benzaldehydes. The good antioxidant activity was observed from these compounds with IC_{50} values ranging from 17.2 to 33.1 μM having much better potential when compared with the standard BHA [26].

The antioxidant capacities of synthesized bis-sulfonamide Schiff base derivatives (7-15) are determined by using four different antioxidant methods, including DPPH free radical scavenging, ABTS cation radical decolorization, cupric reducing (CUPRAC) and metal chelating methods. Acetylcholinesterase and butyrylcholinesterase inhibition activities were also investigated.

The results revealed that bis-sulfonamide Schiff base derivatives (7-15) showed no significant activity in case of DPPH free radical scavenging method with IC_{50} values of >1000 μM. In the present work, all compounds were less active than the standard BHA (61.72 μM), BHT (232.11 μM), and α-Toc (56.86 μM) (Table 1) in case of DPPH free radical scavenging method. The ABTS cation radical scavenging activities of synthesized compounds was assayed and compared with BHT, BHA, and α-Toc used as standards and IC_{50} values of compounds were summarized in Table 1. All compounds showed weak activity with IC_{50} values of <1000 μM, except the compounds 12 (A2B3) and 13 (A3B1) displayed moderate activity with IC_{50} values of 964.82 and 355.77 μM, respectively (Table 1).

The metal chelating effect of the bis-sulfonamide Schiff base derivatives on iron (II) ions was presented in Table 1 and compared with standard EDTA. It was considered that compounds 13 (A3B1) and 15 (A3B3) were the most active compounds with IC_{50} values of 92.31 and 70.32 μM, respectively. Interestingly, both of these compounds have 4-(2-aminoethyl) benzenesulfonamide moiety.These compounds showed close chelating activity to standard EDTA (IC_{50} =52.35 μM). The remaining compounds showed moderate chelating activity with IC_{50} values ranging from 101.43 to 268.56 μM, except the compound 12 (A2B3), which displayed no chelating activity with IC_{50} value of >1000 μM.

The cupric reducing antioxidant capacity (CUPRAC) method was also applied to identify the antioxidant activity of the prepared bis-sulfonamide Schiff base derivatives (7-15). As expected, the activity of the compounds increased with increasing concentration (10 to 100 μM) as shown in Table 2. The results of the CUPRAC test of the synthesized compounds at 10, 25, 50 and 100 μM were compared with standards BHT, BHA and α-Toc. In the current study, the compounds 12 (A2B3) and 15 (A3B3) showed a better CUPRAC activity than standard α-Toc at 10, 25 and 50 μM. Specifically, the compound 12 had better activity at 10 μM of concentration than all three standards (BHT, BHA and α-Toc). Interestingly, these two active compounds (12 and 15) have 1,2-disubstituted phenyl (B3) with different sulfonamides (A2 and A3).

Table 1. DPPH radical scavenging, ABTS cation radical decolorization and metal chelating activities of aromatic bis-sulfonamide Schiff base derivatives (7-15) and controls BHA, BHT, α-Toc, and EDTA.

Comp.	A	B	IC_{50} (μM)[a]		
			DPPH Free Radical Scavenging Activity	ABTS Cation Radical Scavenging Activity	Metal Chelating Activity
7	A1	B1	>1000	>1000	101.43±1.24
8	A1	B2	>1000	>1000	144.51±1.91
9	A1	B3	>1000	>1000	120.55±0.06
10	A2	B1	>1000	>1000	268.56±1.04
11	A2	B2	>1000	>1000	109.90±1.14
12	A2	B3	>1000	964.82±0.94	>1000
13	A3	B1	>1000	355.77±0.50	92.31±0.26
14	A3	B2	>1000	>1000	181.34±1.78
15	A3	B3	>1000	>1000	70.32±2.26
BHA[b]	--	--	61.72±0.85	45.40±1.08	-
BHT[b]	--	--	232.11±3.01	26.54±0.18	-
α-TOC[b]	--	--	56.86±0.77	34.12±0.41	-
EDTA[b]	--	--	-	-	52.35±1.15

[a] IC_{50} values represent the means (standard deviation of three parallel measurements ($p < 0.05$).
[b] Reference compounds.

Table 2. Absorbance values for the cupric ion reducing antioxidant capacity (CUPRAC), of the aromatic bis-sulfonamide Schiff base derivatives **(7-15)** and controls BHA, BHT, and α-Toc.

Comp.			Absorbance Values[a]			
	A	B	10 μM	25 μM	50 μM	100 μM
7	A1	B1	0.076±0.001	0.083±0.005	0.090±0.012	0.097±0.002
8	A1	B2	0.086±0.002	0.177±0.002	0.288±0.001	0.398±0.005
9	A1	B3	0.085±0.002	0.090±0.004	0.117±0.004	0.222±0.004
10	A2	B1	0.084±0.004	0.085±0.003	0.087±0.003	0.101±0.003
11	A2	B2	0.088±0.002	0.178±0.003	0.281±0.001	0.399±0.005
12	A2	B3	0.277±0.001	0.391±0.005	0.494±0.003	0.512±0.002
13	A3	B1	0.121±0.085	0.277±0.002	0.383±0.001	0.395±0.006
14	A3	B2	0.082±0.003	0.077±0.002	0.084±0.002	0.099±0.003
15	A3	B3	0.377±0.004	0.479±0.000	0.583±0.002	0.791±0.003
BHA[b]	--	--	0.288±0.015	0.572±0.046	1.026±0.013	1.984±0.035
BHT[b]	--	--	0.303±0.010	0.610±0.010	1.167±0.024	2.000±0.173
α-TOC[b]	--	--	0.179±0.001	0.296±0.012	0.482±0.017	0.912±0.065

[a]Values expressed are means ± SD of three parallel absorbance measurements ($p<0.05$)
[b] Reference compounds

In this study, a series of aromatic bis-sulfonamide Schiff bases were assessed against cholinesterase (AChE and BChE) enzyme. None of the compounds from the series showed better activity than standard drug galantamine (Table 3). Specifically, only three compounds 13, 14 and 15, showed some activity against AChE with % inhibitions about 16.19, 5.42 and 24.00, respectively. Interestingly, these three compounds have A3 (4-(2-aminoethyl)benzenesulfonamide) substitution with different linker types (B1, B2 and B3). Slightly better activity was observed against BChE, which all compounds had some activity with % inhibitions ranging from 5.44 to 26.19 at 200 μM. Specifically, compounds 7, 8 and 15 showed moderate activity against BChE with % inhibition rates 26.19, 24.40 and 21.17, respectively. The remaining compounds showed weak inhibition activity against BChE enzyme with % inhibition values ranging from 5.44 to 13.69 depending on the nature of the compounds (Table 3).

Table 3. Anticholinesterase activity of the aromatic bis-sulfonamide Schiff base derivatives **(7-15)** at 200 μM and standard drug galantamine.

Comp.	A	B	AChE (Inhibition %)[a]	BChE (Inhibition %)[a]
7	A1	B1	NA	26.19±0.33
8	A1	B2	NA	24.40±0.25
9	A1	B3	NA	6.97±0.24
10	A2	B1	NA	7.40±0.32
11	A2	B2	NA	6.12±0.20
12	A2	B3	NA	10.20±0.72
13	A3	B1	16.19±0.57	13.69±0.37
14	A3	B2	5.42±0.79	5.44±0.92
15	A3	B3	24.00±0.78	21.17±0.36
Galantamine[b]	--	--	84.20±0.74	87.86±0.24

[a] 200 μM
[b] Standart madde
NA: Not Active

4. Conclusions

In the current study, a series of aromatic bis-sulfonamide Schiff bases (7-15) were re-synthesized from the condensation reaction of aromatic sulfonamides (A1, A2, and A3) and aromatic bis-aldehydes (B1, B2 and B3). The antioxidant properties of the bis-sulfonamide Schiff base derivatives were investigated by DPPH free radical scavenging assay, ABTS cation decolarization, cupric reducing antioxidant capacity (CUPRAC) and metal chelating methods. The acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibition profiles were also determined. In general, weak DPPH, ABTS and metal chelating activity were observed. On the other hand, two compounds (12 and 15) showed good CUPRAC activity at 10, 25 and 50 μM concentrations. Some of the compounds did not show any activity against AChE (compounds 7-12). In general, a weak to moderate activity were obtained against AChE and BChE enzymes with % inhibition values ranging from 5.42 to 26.9 at 200 μM concentration.

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Conflicts of interest

The authors state that did not have conflict of interests

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