Synthesis and biological evaluation of 1,3,5-triazine-substituted ureido benzenesulfonamides as antioxidant, acetylcholinesterase and butyrylcholinesterase inhibitors

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Abstract: A series of twenty 1,3,5-triazine-substituted ureido benzenesulfonamides **2 (a-e)** and **3 (a-o)** were re-synthesized and assayed for antioxidant properties by using several different methods including 1,1-diphenyl-2-picryl hydrazyl (DPPH) free radical scavenging assay, 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) cation radical decolarization assay, metal chelating and cupric reducing antioxidant capacity (CUPRAC) methods. The inhibitory effects of compounds on acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes have been also demonstrated. All compounds showed a greater antioxidant capacity against ABTS assay by having a more potent activity than the standards BHT, BHA and α-TOC. In general, all compounds were non susceptible to against AChE enzyme. On the other hand, several lead compounds were obtained from the current series against BChE enzyme. More specifically, compound **3m** showed great inhibition profile against BChE with % inhibition value of 93.77, which is better than the standard drug galantamine (% inhibition value of 87.86).

Keywords: 1,3,5-triazine; sulfonamide; acetylcholinesterase; butyrylcholinesterase; antioxidant. ©2020 ACG Publications. All rights reserved.

1. Introduction

Sulfonamides are one of the most important pharmacophores that are used in a wide range of pharmaceutical applications such as1,3,5-triazine; sulfonamide, antibacterial, anticancer, anti-inflammatory and antioxidant effective compounds. [1-5] Furthermore, they are also used as a research tool for treatment of Alzheimers' disease with their effect on carbonic anhydrase enzyme inhibition. [6,7] On the other hand, the 1,3,5-triazine group is known as the s-triazine scaffold finds an area of investigation with its antimicrobial, antiviral, diuretic, anticancer and anti-inflammatory effects. [8-11] More specifically, recent studies have found that sulfonamides containing 1,3,5-triazine scaffolds have a selective and potent carbonic anhydrase (CA) inhibitory effect. [12-15] In a recent study, it was determined by us that sulfonamides incorporating 1,3,5-triazine moieties act as potent AChE and BChE inhibitors. [16]

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Alzheimer is a neurodegenerative disease characterized by cognitive impairments. [17] It is estimated that almost 50 million people suffer from this disease, according to current reports. This number is thought to increase gradually. The disease is caused by the accumulation of beta-amyloid. Factors such as oxidative stress, inflammation, tau-protein aggregation, dishomeostasis of bio-metals, acetylcholine (ACh) and butyrylcholine (BCh) deficiencies are included in the pathophysiology of the disease. [18-20]

The enzyme AChE is present in high concentrations in the central and peripheral nervous system, parasympathetic synapses, and neuromuscular junctions. [21] This enzyme is of great importance for the hydrolysis of ACh. In the physiological system, the presence of AChE level outside optimal values causes various diseases. Alzheimer and Parkinson disease are the main neurological diseases that occur with this condition. [22] The enzyme BChE, also known as nonspecific cholinesterase, take a key part in the regulation of BCh. BChE is an enzyme found in the blood, glia cells, central nervous system, liver, pancreas and heart. The BChE and AChE sequences are similar. Therefore, their success in treatment is similar. [23,24] Various cholinesterase inhibitors such as tacrine, galantamine, donepezil and rivastigmin are used in the symptomatic treatment of Alzheimer disease. [25] These drugs used in treatment have gastrointestinal side effects such as nausea, vomiting, diarrhea, novelization, syncope and weight loss. [20-26] It is also thought that antioxidants exhibit protective effects against Alzheimer disease. [27] For the treatment of Alzheimer disease, cholinesterase inhibitors with antioxidant effects and less toxic effects are needed. Therefore, the development of new cholinesterase inhibitors that are effective for the treatment of the disease and have a low side effect profile is of interest.

In our previous study, we synthesized new molecules by combining the 1,3,5-triazine scaffold and ureido benzenesulfonamide groups as a potent and selective carbonic anhydrase inhibitors. [28] Hence, in the present study, our aim is to further investigation of the antioxidant and anticholinesterase enzyme activity of the 1,3,5-triazine-substituted ureido benzenesulfonamides **2 (a-e)** and **3 (a-o)**.

2. Experimental

2.1. Chemistry

In our previous work, we have successfully synthesized and characterized the target compounds **2 (a-e)** and **3 (a-o)** as a potent and selective carbonic anhydrase inhibitors (Scheme 1). [28]
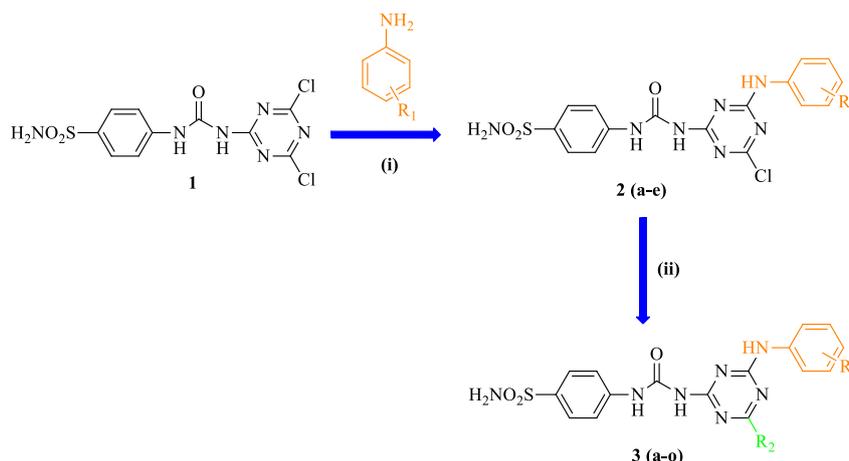
Scheme 1. General synthetic route for the synthesis of 1,3,5-triazine-substituted ureido benzenesulfonamides **2 (a-e)** and **3 (a-o)**. Reagents and conditions: (i) R_1ArNH_2, DMF, 0 to 5 ^{0}C, 1h, then R.T. 4h, (ii) Appropriate amine, DMF, R.T., 1h, then 90 ^{0}C, 2h.

In the current work, these compounds were re-synthesized by reacting of intermediate compound **1** with aromatic and aliphatic amines. In the first step, intermediate compound **1** was N-alkylated with different substituted aromatic amines to produce compounds **2 (a-e)**. After that, the final chloro atom of the second intermediate compounds **2 (a-e)** was reacted with dimethylamine, morpholine

or piperidine to obtain compounds **3 (a-o)**. Experimental details, physicochemical, and spectroscopic characterization of synthesized compounds **2 (a-e)** and **3 (a-o)** have been previously presented by us. [28]

2.2. Determination of Antioxidant and Anticholinesterase Activity *2 (a-e)* and *3 (a-o)*.

2.2.1. DPPH Radical Scavenging Ability

The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity of the re-synthesized compounds were determined by spectrophotometric method. [29,30] Different amounts of each compound were completed to 40 µL with DMSO. 160 µL 0.1 mM DDPH solution was added to it. Absorption was measured at 517 nm after the mixture was left in the dark for 30 minutes. Free radical, DPPH, percent (I %) inhibition calculated according to the formula:

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

$A_{control}$ is the absorbance of the control reaction (it contains all reagents except the compounds tested), and A_{sample} is the absorbance of the test compounds. The tests were done three times. Butylated hydroxyanisol (BHA), butylated hydroxytoluene (BHT) and α-tocopherol (α-Toc) were used as positive control.

2.2.2. ABTS Cation Radical Decolorization

The percent inhibition of decolonization of the ABTS (2,2`-azino-bis (3-ethylbenzotiazolin-6-sulfonic acid)) cation radical is achieved as a function of concentration and time. As standard; BHT, BHA and α-Toc compounds are used. [31,32] Compounds synthesized in different concentrations and a 160 µL 7 mM ABTS solution are added to each well. Compounds held 6 minutes at room temperature. Then, absorbance values are measured at 734 nm. ABTS cation radical decolonization activities were determined using the following equation:

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

The tests were done three times. BHA, BHT and α-Toc compounds have been used as positive control.

2.2.3. Metal Chelate

Chelating ability of synthesized compounds were determined by the method of Dinis et al. [33] Different concentrations of the compounds tested were added to each well. Then, each sample was mixed with 4 µL 2 mM of iron (II) chloride. The reaction was initiated by adding 8 µL 5 mM ferrozine. The resulting mixture is kept at room temperature for 10 minutes. Subsequently, absorbance values were measured at 562 nm. The results were expressed as a percentage of inhibition of ferrozin-Fe^{2+} complex formation. EDTA has been used as a positive control. Percent inhibition of ferrozin-Fe^{2+} complex formation was calculated using the formula given below:

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

Tests were repeated 3 times.

2.2.4. Cupric Reducing Antioxidant Capacity (CUPRAC)

The method involves the reduction of Cu(II)-Neocuproin to the colored form Cu(I)-Neocuproin chelate in the presence of antioxidant compounds. [34] The compounds synthesized were added to each well in different concentrations. 61 µL solutions of $CuCl_2$, Neocuproine and NH_4OAc were added to it. After waiting at room temperature for 1 hour, absorption values were measured at 450 nm. The measured

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absorbance values were compared with the BHA, BHT and α-TOC molecules used as standard. Tests were repeated 3 times.

2.2.5. Anticholinesterase Activity

The inhibitory effect of synthesized compounds on BChE and AChE activities was applied according to the spectrophotometric method of Ellman et al. [35] Compounds to be tested were dissolved in DMSO in order to prepare stock solutions at a concentration of 4 mM. Aliquots of 150 µL 100 mM sodium phosphate buffer (pH 8.0), sample solution and AChE (or BChE) solution were mixed. After incubating at 15 °C for 25 minutes, DTNB (5,5'-dithio-bis (2-nitro-benzoic acid)) was added. 10 µL acetylthiocoline iodide (or butyrylthiocoline iodide) was added to the reaction. 30 minutes after the addition of substrates (acetylthiocoline iodide or butyrylthiocoline iodide) and absorption values were measured at 412 nm.

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

Tests were repeated 3 times. Galantamine was preferred as a positive control. IC_{50} value was determined by a concentration-inhibition graph.

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 present study, a large series of 1,3,5-triazine-substituted ureido benzenesulfonamides were re-synthesized as a general synthetic route shown in Scheme 1. The current study focused on the in vitro antioxidant activity of the target compounds **2 (a-e)** and **3 (a-o)** by using several assays, including DPPH free radical scavenging, ABTS cation radical scavenging, cupric reducing (CUPRAC) and metal chelating methods. The acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) activities of these compounds were also investigated.

The DPPH activity of the re-synthesized compounds was tested and compared with standards BHT, BHA and α-TOC (Table 1). In general, compounds showed moderate DPPH activity with IC_{50} values ranging from 91.46 to 730.61 µM. The most active compound was **3i** (R_1= 4-Cl and R_2= piperidine) with IC_{50} values of 91.46 µM. On the other hand, best biological activities among the tests that they were conducted in the current work were obtained against ABTS cation radical scavenging assays. All compounds from the current series showed better ABTS cation radical scavenging activity than standards BHT (IC_{50}: 26.54 µM), BHA (IC_{50}: 45.40 µM) and α-TOC (IC_{50}: 34.12 µM) with IC_{50} values of 8.60 to 20.59 µM (Table 1). Compounds **3e**, **3i**, **3k**, **3l**, **3n,** and **3o** showed <10 µM activity. Interestingly, these compounds (**3e**, **3i**, **3k**, **3l**, **3n,** and **3o**) substituted on one site with morpholine or piperidine. More specifically, compounds **3e**, **3k** and **3n** substituted one one side with morpholine and on the other side with 4-F, 3,4-diCl and 4-SO$_2$NH$_2$, and shows very close activity to each other with IC_{50} values of 8.60, 9.83, and 8.65 µM, respectively. Similarly, compounds **3i**, **3l**, and **3o** substituted on one side with piperidine and on the other side with 4-Cl, 3,4-diCl and 4-SO$_2$NH$_2$, exhibits great antioxidant activity with IC_{50} values of 9.42, 8.72, and 9.08 µM , respectively. The metal chelating effect of 1,3,5-triazine-substituted ureido benzenesulfonamides on iron (II) ions was also summarized in Table 1 and compared with standard EDTA. Only compound **3a** (R_1= -H and R_2= -N(Me)$_2$) showed better activity than standard EDTA (IC_{50}: 52.35 µM) with an IC_{50} value of 36.33 µM. The compound **3k** (IC_{50}: 54.88 µM) was also indicated to close activity to standard EDTA. Compounds **2e**, **3i**, **3j**, **3l** and **3n** showed moderate activity with IC_{50} values spanning between 81.34 to 95.94 µM.

Table 1. DPPH radical scavenging, ABTS cation radical decolorization and metal chelating activities of 1,3,5-triazine-substituted ureido benzenesulfonamides **2 (a-e) and 3 (a-o)** and controls BHA, BHT, α-Toc, and EDTA

Comp.	R_1	R_2	IC_{50} (µM)[a]		
			DPPH Free Radical Scavenging Activity	ABTS Cation Radical Scavenging Activity	Metal Chelating Activity
2a	-H	-Cl	359.28±1.00	12.00±0.56	>1000
2b	4-F	-Cl	311.14±0.40	20.07±0.03	>1000
2c	4-Cl	-Cl	730.61±0.85	18.96±0.66	195.36±0.15
2d	3,4-diCl	-Cl	459.77±0.63	13.38±0.06	164.74±0.05
2e	4-SO$_2$NH$_2$	-Cl	665.24±0.71	14.08±0.30	81.34±0.22
3a	-H	-N(Me)$_2$	371.64±0.50	13.50±0.15	36.33±0.49
3b	-H	Morpholine	152.77±0.33	11.26±0.07	162.49±0.68
3c	-H	Piperidine	163.91±0.45	11.21±0.60	>1000
3d	4-F	-N(Me)$_2$	107.54±0.93	10.30±0.21	>1000
3e	4-F	Morpholine	165.47±0.41	8.60±0.13	>1000
3f	4-F	Piperidine	395.72±0.55	13.07±0.85	>1000
3g	4-Cl	-N(Me)$_2$	200.29±0.32	10.51±0.10	>1000
3h	4-Cl	Morpholine	204.33±0.70	10.38±0.89	>1000
3i	4-Cl	Piperidine	91.46±0.06	9.42±0.38	85.33±0.24
3j	3,4-diCl	-N(Me)$_2$	322.89±0.65	20.59±0.51	95.72±0.07
3k	3,4-diCl	Morpholine	193.38±0,39	9.53±0.28	54.88±0.43
3l	3,4-diCl	Piperidine	306.49±0,45	8.72±0.14	95.55±0.81
3m	4-SO$_2$NH$_2$	-N(Me)$_2$	297.48±0.16	13.16±0.86	125.64±0.14
3n	4-SO$_2$NH$_2$	Morpholine	185.81±0.26	8.65±0.01	95.94±0.91
3o	4-SO$_2$NH$_2$	Piperidine	134.04±0.31	9.08±0.57	230.43±0.93
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±041	------
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.

The cupric reducing antioxidant capacity (CUPRAC) analyses was also applied to determine the antioxidant capacity of the re-synthesized 1,3,5-triazine-substituted ureido benzenesulfonamides **2 (a-e)** and **3 (a-o)** and the obtained results were compared with the standards BHT, BHA and α-TOC as depicted in Table 2. As expected, the antioxidant activity of the compounds increased with increasing concentration (10 to 100 µM). In the current study, the compounds **2a** (R_1= -H and R_2= -Cl), **3d** (R_1= 4-F and R_2= -N(Me)$_2$), and **3e** (R_1= 4-F and R_2= morpholine) showed better activity than the all standards at 10 µM. The activity of all compounds were less than the standards BHT and BHA at 100 µM, but several of them (such as **2a**, **2d**, **3d**, **3e**, and **3h**) were more potent than the standard α-TOC.

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Table 2. Absorbance values for the cupric ion reducing antioxidant capacity (CUPRAC), of 1,3,5-triazine-substituted ureido benzenesulfonamides **2 (a-e) and 3 (a-o)** and controls BHA, BHT, and α-Toc.

Comp.	R₁	R₂	Absorbance Values[a]			
			10 µM	25 µM	50 µM	100 µM
2a	-H	-Cl	0.315±0.098	0.496±0.109	0.877±0.071	1.453±0.008
2b	4-F	-Cl	0.254±0.036	0.421±0.034	0.584±0.044	0.700±0.025
2c	4-Cl	-Cl	0.207±0.019	0.295±0.023	0.431±0.049	0.612±0.047
2d	3,4-diCl	-Cl	0.227±0.037	0.389±0.034	0.597±0.027	0.941±0.030
2e	4-SO₂NH₂	-Cl	0.171±0.026	0.257±0.005	0.391±0.004	0.527±0.032
3a	-H	-N(Me)₂	0.235±0.017	0.404±0.031	0.533±0.058	0.650±0.044
3b	-H	Morpholine	0.232±0.025	0.387±0.016	0.636±0.024	0.935±0.011
3c	-H	Piperidine	0.275±0.039	0.456±0.019	0.656±0.029	0.826±0.023
3d	4-F	-N(Me)₂	0.298±0.045	0.435±0.028	0.780±0.035	0.918±0.007
3e	4-F	Morpholine	0.393±0.061	0.503±0.047	0.753±0.035	0.989±0.059
3f	4-F	Piperidine	0.241±0.044	0.337±0.015	0.457±0.014	0.832±0.008
3g	4-Cl	-N(Me)₂	0.232±0.009	0.348±0.031	0.467±0.009	0.672±0.015
3h	4-Cl	Morpholine	0.246±0.036	0.375±0.026	0.615±0.015	0.992±0.032
3i	4-Cl	Piperidine	0.209±0.012	0.346±0.022	0.503±0.042	0.856±0.080
3j	3,4-diCl	-N(Me)₂	0.177±0.039	0.194±0.005	0.271±0.024	0.274±0.023
3k	3,4-diCl	Morpholine	0.217±0.006	0.321±0.005	0.490±0.016	0.643±0.039
3l	3,4-diCl	Piperidine	0.175±0.061	0.311±0.015	0.442±0.003	0.639±0.028
3m	4-SO₂NH₂	-N(Me)₂	0.144±0.008	0.201±0.005	0.279±0.012	0.427±0.012
3n	4-SO₂NH₂	Morpholine	0.188±0.007	0.306±0.017	0.441±0.003	0.541±0.042
3o	4-SO₂NH₂	Piperidine	0.212±0.006	0.323±0.017	0.516±0.039	0.686±0.037
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]	------	------	0.296±0.012	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 large series of re-synthesized 1,3,5-triazine-substituted ureido benzenesulfonamides **2 (a-e) and 3 (a-o)** were also assessed to anticholinesterase (AChE and BChE) activity (Table 3). None of the compounds from the series showed any activity against AChE, except the compounds **3n** (% inhibition of 52.61)and **3o** (% inhibition of 4.38), which both have –SO₂NH₂ substitution on their structures. On the other hand, potent inhibition was observed against BChE with some of the compounds. More specifically, compound **3m** (R₁= 4-SO₂NH₂ and R₂= -N(Me)₂) showed great BChE inhibition with % inhibition of 93.77 at 200 µM concentration which is better than the standard drug galantamine (% inhibition 87.86). Also, the compounds **3c**, **3g**, **3i** and **3l** exhibited good inhibition with % inhibition values are ranging between 74.89 to 80.85 at 200 µM concentration, which

these compounds interestingly have piperidine substitution on one site, except the compound **3g** ($R_1 =$ 4-Cl and $R_2 = -N(Me)_2$). Some of the compounds from the series (such as **2a**, **2b**, **2e**, **3b**, **3e**, **3h**, **3j** and **3k**) displayed non activity against BChE. Interestingly, most of these non active compounds have morpholine or –Cl substitution on their one side.

Table 3. Anticholinesterase activity of 1,3,5-triazine-substituted ureido benzenesulfonamides **2 (a-e)** and **3 (a-o)** at 200 µM and standard drug galantamine.

Comp.	R_1	R_2	AChE (Inhibition %)[a]	BChE (Inhibition %)[a]
2a	-H	-Cl	NA	NA
2b	4-F	-Cl	NA	NA
2c	4-Cl	-Cl	NA	52.01±1.43
2d	3,4-diCl	-Cl	NA	
				NA
2e	4-SO_2NH_2	-Cl	NA	
				39.60±1.04
3a	-H	-$N(Me)_2$	NA	37,63±0.73
3b	-H	Morpholine	NA	NA
3c	-H	Piperidine	NA	78.47±1.75
3d	4-F	-$N(Me)_2$	NA	43.57±1.35
3e	4-F	Morpholine	NA	NA
3f	4-F	Piperidine	NA	61.22±1.86
3g	4-Cl	-$N(Me)_2$	NA	74.89±0.57
3h	4-Cl	Morpholine	NA	NA
3i	4-Cl	Piperidine	NA	76.81±1.58
3j	3,4-diCl	-$N(Me)_2$	NA	NA
3k	3,4-diCl	Morpholine	NA	NA
3l	3,4-diCl	Piperidine	NA	80.85±0.61
3m	4-SO_2NH_2	-$N(Me)_2$	NA	93.77±1.47
3n	4-SO_2NH_2	Morpholine	52.61±1.03	48.07±0.43
3o	4-SO_2NH_2	Piperidine	4.38±0.03	62.74±1.70
Galantamin[b]	-----	-----	84.20±0.74	87.86±0.24

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

4. Conclusion

In the current study, we report a series of twenty 1,3,5-triazine-substituted ureido benzenesulfonamides **2 (a-e)** and **3 (a-o),** which were successfully re-synthesized by condensation reaction of various aromatic/aliphatic amines on main intermediate compound 4-(3-(4,6-dichloro-1,3,5-triazin-2-yl)ureido) benzenesulfonamide (**1**). These compounds were investigated as an antioxidant by using several assays such as DPPH, ABTS, metal chelating and CUPRAC methods. Anticholinesterase (AChE and BChE) activities were also determined. In general, all compounds showed moderate to weak DPPH, metal chelating and CUPRAC activity. Importantly, all compounds were susceptible to ABTS assay, which all compounds were more active then the standards BHT, BHA and α-TOC. On the other hand, all compounds were non active against AChE, except the compounds **3n** and **3o**, which they have some activity against AChE. Some of the compounds from the series showed moderate to good inhibition against BChE enzyme. More specifically, great inhibition was observed with the compound **3m** which is more active than the standard drug galantamine against BChE enzyme.

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