New 1,3,5-triazine Derivatives Incorporating Aminobenzenesulfonamide, Aminoalcohol, Piperazine, Chalcone or Stilbene Structural Motifs and Evaluation of Their Antioxidative Activity †

Eva Havránková [1,*], Nikola Čalkovská[1], Tereza Padrtová[1], Jozef Csöllei [1], Radka Opatřilová [1] and Pavel Pazdera [2]

[1] Department of Chemical Drugs, Faculty of Pharmacy, University of Veterinary and Pharmaceutical Sciences, Brno, Czech Republic

[2] Department of Chemistry, Faculty of Science, Centre for Syntheses at Sustainable Conditions and Their Management, Masaryk University, Brno, Czech Republic

* Correspondence: havrankovae@vfu.cz

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Abstract: A series of 1,3,5-triazine derivatives, incorporating aminobenzenesulfonamide, aminoalcohol/phenol, piperazine, chalcone, or stilbene structural motifs, were evaluated as potential antioxidants. The compounds were prepared by using step by step nucleophilic substitution of chlorine atoms in starting 2,4,6-trichloro-1,3,5-triazine. Reactions were catalyzed by Cu(I)-supported on a weakly acidic resin. The radical scavenging activity was determined in terms of %inhibition activity and IC_{50}, using the ABTS method. Trolox and ascorbic acid (ASA) were used as standards. In the lowest used concentration 1×10^{-4} M, the %inhibition activity at time 0 min was comparable with both standards at least for 10 compounds. After 60 min compounds **1**, **2**, **9** and **25** showed nearly twice %inhibition (73.44 – 87.09 %) in comparison with standards (Trolox = 41.49 %; ASA = 31.07 %). Values of IC_{50} correlated with %inhibition activity. For compounds **1**, **2**, **9** and **25** values of IC_{50} in time 60 min (17.16 – 27.78 μM) were 5 times lower than IC_{50} of both standards (Trolox = 178.33 μM; ASA = 147.47 μM). Based on these results, the presented 1,3,5-triazine derivatives and their analogs have a high potential in the treatment of illnesses caused or related to oxidative stress.

Keywords: 1,3,5-triazine; 4-aminophenol; hydroxychalcone; hydroxystilbene; antioxidative activity; ABTS method

1. Introduction

Oxidative stress or damage is the imbalance between the capacity of antioxidative protection systems of the organism and the occurrence of reactive oxygen species and/or reactive nitrogen species. [1, 2] Oxygen reactive species, such as free radicals, can be produced by metabolic pathways, UV irradiation, environmental pollutants, and others. [1-6]

Damage of cells caused by oxidative stress is correlated to pathophysiology and pathogenesis of a wide range of diseases, *i.e.*, Alzheimer's, Huntington's, and Parkinson's diseases, inflammatory diseases, cardiovascular diseases, diabetes, and cancer. [1, 3, 4, 7-9]

The structure of molecules with antioxidant activity can be very diverse. Recently, triazine derivatives with desirable antioxidant activity were described. Gonzalez *et al.* proved the antioxidant activity of their 1,3,5-triazine bridged small molecules (Figure 1a). [3] 5,6-Diphenyl-3-oxo-1,2,4-triazine linked piperazine derivatives (Figure 1b) [10] or arylidene hydrazine derivatives of substituted 1,2,4-triazine scaffolds also exhibit attractive radical scavenging effects (Figure 1c). [11] Substituted triazene benzenesulfonamides (Figure 1d), synthesized by Akocak *et al.*, also showed significant antioxidative activity. [12] A promising antioxidant effects can be found even between chalcone derivatives. For example, Nageshi *et al.* synthesized very effective antioxidants based on the structure of chalcones and containing phenolic functional group (Figure 1e). [13] Phenolic compounds are one of the largest groups of antioxidants with high activity. [1, 4, 7-9, 14] An example of phenolic antioxidant, which is used in medicine as an adjuvant treatment of cardiovascular diseases, metabolic syndrome, type 2 diabetes, and inflammatory diseases, is resveratrol (stilbene derivative, Figure 1f). [4]
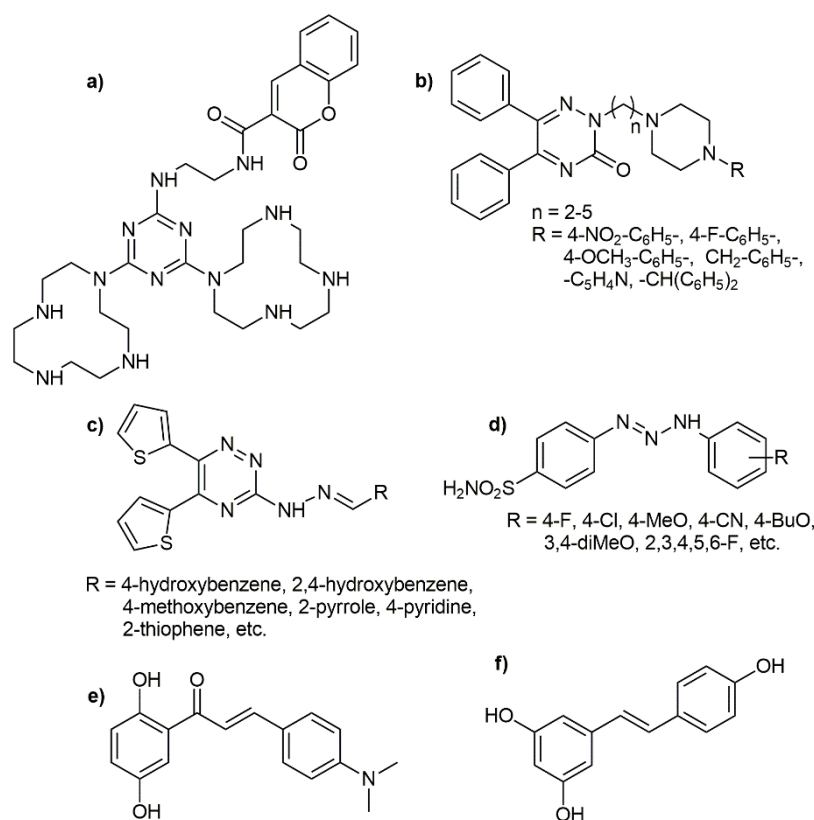

Figure 1. Examples of diversity of antioxidants with high activity: a) 1,3,5-triazine bridged small molecules [3]; b) biphenyl-3-oxo-1,2,4-triazine linked piperazine derivatives [10]; c) arylidene hydrazine derivatives of substituted 1,2,4-triazine [11]; d) substituted aminobenzenesulfonamides [12]; e) chalcone derivatives [13]; f) stilbene derivatives (resveratrol) [4].

Antioxidants possess great potential as drugs suitable for the treatment of various diseases related to oxidative stress. Their structural diversity provides many possibilities for the design and synthesis of new highly effective agents. [1, 2, 4]

2. Materials and Methods

2.1. Chemistry

Target compounds were prepared according to the methodology published in [15] by step by step nucleophile substitution of chlorine atoms in starting 2,4,6-trichloro-1,3,5-triazine. The appropriate starting compound reacted with a nucleophile and anhydrous potassium carbonate in a molar ratio 1:1:1. Reactions were catalyzed by Cu(I)-supported on a weakly acidic resin. The substitution of first, second, or third chlorine atom was controlled by the temperature mode (Scheme 1).
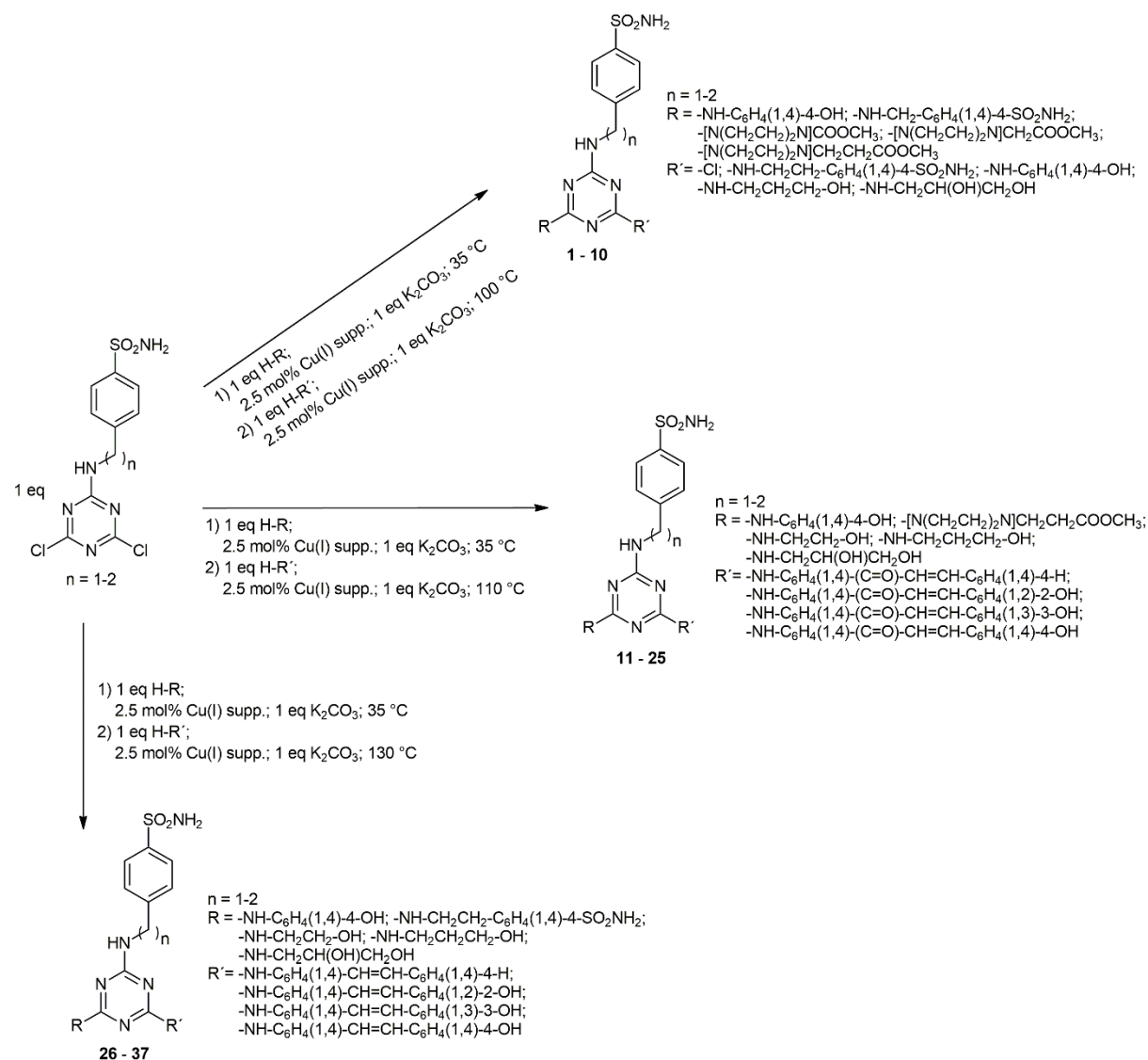
Scheme 1. General synthetic scheme of target compounds.

2.2. Characterization of New Compounds

The characterization of only two representative compounds is given here. The full characterization of all new compounds will be part of the published full manuscript.

(E)-4-[2-({4-[(2-Hydroxyethyl)amino]-6-({4-[3-(3-hydroxyphenyl)acryloyl]phenyl}amino)-1,3,5-triazin-2-yl}amino)ethyl]benzenesulfonamide (**19**): 77.7%; brown solid; mp 256-258 °C; ^{1}H-NMR (500 MHz, DMSO-d$_6$) δ ppm 7.91 (2H, d, *J* = 8.1 Hz, CH), 7.80 (2H, d, *J* = 8.1 Hz, CH), 7.71 (1H, d, *J* = 15.5 Hz, <u>CH</u>=CH-CO), 7.56 (2H, d, *J* = 8.1 Hz, CH), 7.48 (1H, d, *J* = 15.5 Hz, CH=<u>CH</u>-CO), 7.26 (2H, d, *J* = 8.1 Hz, CH), 6.91 (2H, d, *J* = 8.1 Hz, CH), 6.69 (7H,

s, OH, NH, NH₂), 4.56-4.52 (2H, m, CH₂), 3.55-3.53 (2H, m, C$\underline{H}$₂-OH), 3.34-3.32 (2H, m, NH-C$\underline{H}$₂), 2.93-2.91 (2H, m, CH₂); ^{13}C-NMR (125 MHz, DMSO-d_6) δ ppm 189.4, 168.6, 166.3, 165.6, 146.7, 144.5, 144.1, 142.3, 141.8, 141.2, 138.2, 130.4, 128.2, 126.6, 125.9, 119.7, 118.6, 114.2, 58.8, 44.5, 41.9, 36.6; IR ν_{max} (cm^{-1}) 3336, 3222 (OH, NH, NH₂), 2930 (CH$_{alif}$, CH$_{2alif}$), 1698, 1660, 1654, 1635 (C=C, C=N, C=O), 1583, 1345, 1155, (SO₂NH₂), 1076 (C-OH), 1034 (COC)

(E)-Methyl 3-(4-{4-({4-[3-(4-hydroxyphenyl) acryloyl]phenyl}amino)-6-[(4-sulfamoyl-phenethyl)amino] -1,3,5-triazin-2-yl)piperazin-1-yl)propanoate (**25**): 78.1%; orange solid; mp 101-103 °C; ^{1}H-NMR (500 MHz, DMSO-d₆) δ ppm 7.97 (2H, d, *J* = 8.1 Hz, CH), 7.94 (2H, d, *J* = 8.1 Hz, CH), 7.8 (1H, d, *J* = 15.5 Hz, $\underline{CH}$=CH-CO), 7.42 (1H, d, *J* = 15.5 Hz , CH=$\underline{CH}$-CO), 7.31 (2H, d, *J* = 8.1 Hz, CH), 7.28 (2H, d, *J* = 8.1 Hz, CH), 6.99 (2H, d, *J* = 8.1 Hz, CH), 6.68 (2H, d, *J* = 8.1 Hz, CH), 3.91-3.86 (4H, m, CH₂), 3.74-3.71 (2H, m, NH-C$\underline{H}$₂), 3.65 (3H, s, CH₃), 2.99-2.87 (4H, m, CH₂), 2.76-2.74 (2H, m, CH₂), 2.59 (2H, t, *J*=7.8 Hz, N-CH₂), 2.11 (2H, t, *J*=7.8 Hz, CH₂-COO); ^{13}C-NMR (125 MHz, DMSO-d_6) δ ppm 186.7, 171.9, 167.8, 167.1, 162.6, 159.9, 158.4, 154.2, 150.9, 143.8, 142.7, 144.3, 119.2, 131.3, 130.6, 126.8, 124.9, 116.3, 113.1, 66.4, 51.3, 51.1, 44.3, 43.7, 35.1, 31.6; IR ν_{max} (cm^{-1}) 3332, 3218 (OH, NH, NH₂), 2950 (CH$_{alif}$, CH$_{2alif}$) 1698, 1683, 1669, 1651, 1646, 1630, 1588 (C=C, C=N, COO), 1349 (SO₂NH₂), 1227 (OH), 1162 (SO₂NH₂), 1105, 1028 (COC)

2.3. Determination of antioxidant activity by ABTS method

BioTekTM CytationTM 3 Cell Imaging Multi-Mode Reader (BioTek USA) equipment was used for measurement.

A stock solution containing free cation radicals ABTS$^{·+}$ was prepared as follows: 10 mL of an aqueous solution of ABTS (7.4 mmol) and 10 mL of an aqueous solution of potassium peroxodisulfate (2.6 mmol) were mixed and allowed to stand at room temperature in the dark for 24 h. After the end of the incubation period, a 50 mL volumetric flask was charged with 1.1 mL of ABTS stock solution and filled up with ethanol. This solution of ABTS$^{·+}$ was used for the determination of antioxidant activity.

Tested compounds were dissolved in DMSO. Solutions of concentration 1x10^{-2} M, 1x10^{-3} M, 5x10^{-4} M, 1x10^{-4} M, 5x10^{-5} M, 1x10^{-5} M and 1x10^{-6} M were prepared.

Antioxidant activity of tested compounds was determined as follows: 200 μl of a solution of ABTS$^{·+}$ and 10 μl of a solution of the tested compound of appropriate concentration were mixed. Antioxidant activity was determined by measurement of absorbance at 754 nm (max absorbance of ABTS$^{·+}$) after 0, 5, 30, and 60 minutes. Pure DMSO was used as a blank. Trolox and ascorbic acid were used as standards (solutions were prepared at the same concentrations as tested compounds).

ABTS cation radical scavenging ability of tested compounds expressed as %inhibition was calculated using the following equation (1): [14]

$$\% = \frac{A_B - A}{A_B}\,100 \tag{1}$$

Where: A_B means absorbance of blank; A means absorbance of the sample.

The values of IC₅₀ were determined as follows: The values of %inhibition of ABTS$^{·+}$ radical in time (0 min, 5 min, 30 min, and 60 min) at concentrations of 1x10^{-2} M, 1x10^{-3} M, 5x10^{-4} M, 1x10^{-4} M, 5x10^{-5} M, 1x10^{-5} M, and 1x10^{-6} M were determined; a plot of concentration versus %inhibition was obtained from the data; a number 50 was substituted for the unknown x (50% inhibition) in the trendline equation, and value IC₅₀ was calculated.

All calculated results are shown in Table 1 and Table 2.

3. Results and discussion

In this paper, a series of 1,3,5-triazine derivatives, incorporating aminobenzenesulfonamide, aminoalcohol/ phenol, piperazine, chalcone, or stilbene structural motifs were evaluated as potential antioxidants. These 1,3,5-derivatives were primarily designed as inhibitors of hCA IX (isozyme of human carbonic anhydrase), and the activity of this enzyme is correlated with tumor growth. Since

the synthesized compounds contain one or more structural motifs in their structure that already demonstrated antioxidant properties (chapter 1), the screening of their antioxidant activity was made. The ABTS assay was used for the evaluation of an antioxidant activity. The results were compared to commercially available standards Trolox and ascorbic acid. The ABTS method was performed because tested compounds contain either 4-aminophenol or hydroxystilbene or hydroxychalcone structural moieties. Currently, the ABTS method is one of the most widely used for the determination of the antioxidative properties of phenols. [14] We assumed that the mechanism of action is probably very similar to resveratrol and analogous compounds.

We were pleased to find that almost half of the tested compounds had exhibited excellent antioxidative activity. The results of the (cation)radical scavenging activity are shown in Table 1 (%inhibition activity) and Table 2 (values of IC_{50}).

Over twenty derivatives fully captured the cation radical $ABTS^{.+}$ after a few seconds at the highest used concentration of tested compounds 1×10^{-2} M. At the lowest concentration of 1×10^{-4} M at time 0 min, for ten derivatives was %inhibition of the cation radical $ABTS^{.+}$ comparable or better than both used standards (Trolox and ascorbic acid). Furthermore, derivatives **1**, **2**, **9**, **24,** and **25** showed after 60 min almost double %inhibition activity (73.44 – 87.09 %) in comparison to standard Trolox (41.09 %) and ascorbic acid (31.07 %).

From the results presented in Table 2, some general statements can be made. Compounds with the highest values of %inhibition activity contain the phenolic fragment in their structure. The hydroxy group can inhibit (scavenge) the cation radical $ABTS^{.+}$ probably by the single-electron transfer mechanism (SET). The resulting cation radical formed after scavenging is stabilized by the delocalization of the electron across the molecule. The presence of the oxo group (C=O) and the unsaturated double bond in the chalcone structural motif enhanced the antioxidative properties of the phenolic hydroxy group. This is caused by a very high electron delocalization of the formed cation radical, which is supported by the electron-withdrawing character of the carbonyl group. In fact, the compounds containing the hydroxychalcone moieties are, in general, the most active of tested compounds. On the other hand, the compounds incorporating the stilbene structural motif exhibit lower %inhibition of the cation radical $ABTS^{.+}$. The reason is less effective conjugation of the cation radical in comparison with the chalcone derivatives. In general, a higher level of electron delocalization of formed cation radical increases the energy of HOMO orbital, which means the better oxidation-reduction potential of the compound and, therefore, the better oxidative activity.

The ability of the tested compounds to donate the hydrogen proton also effects the %inhibition of the cation radical $ABTS^{.+}$. Compounds containing the hydroxychalcone structural moiety, which is more willing to donate the hydrogen proton, showed significantly better antioxidative activity than compounds containing hydroxystilbene structural motif, which has a much lower ability to donate the hydrogen proton.

Table 1. ABTS cation radical scavenging ability of tested compounds expressed as % inhibition in different times (0 min, 5 min, 30 min, and 60 min) and different concentrations (1×10^{-2} M and 1×10^{-4} M). Significant results are shown in bold.

Compound	n	R₁	R₂	1×10^{-2} M; (%)²				1×10^{-4} M; (%)²			
				0 min	5 min	30 min	60 min	0 min	5 min	30 min	60 min
1 [1]	1	NH-C₆H₄(1,4)-4-OH	Cl	**92.05**	**92.00**	**91.88**	**91.88**	**31.52**	**38.51**	**44.71**	**73.51**
2 [1]	2	NH-C₆H₄(1,4)-4-OH	Cl	**91.38**	**91.26**	**91.21**	**91.21**	**45.38**	**49.84**	**63.65**	**73.44**
3 [1]	1	NH-CH₂-C₆H₄(1,4)-4-SO₂NH₂	NH-CH₂CH₂-C₆H₄(1,4)-4-SO₂NH₂	35.58	47.98	58.35	66.69	21.82	24.81	27.23	35.57
4 [1]	2	NH-CH₂-C₆H₄(1,4)-4-SO₂NH₂	NH-CH₂CH₂-C₆H₄(1,4)-4-SO₂NH₂	51.53	74.64	79.54	90.36	31.46	35.24	40.99	41.83
5 [1]	1	[N(CH₂CH₂)₂N]COOCH₃	NH-CH₂CH(OH)CH₂OH	**90.70**	**92.84**	**92.95**	**92.90**	24.02	31.01	39.92	52.99
6 [1]	1	[N(CH₂CH₂)₂N]CH₂COOCH₃	NH-CH₂CH(OH)CH₂OH	63.98	82.13	87.15	91.77	25.37	35.07	34.73	50.06
7 [1]	1	[N(CH₂CH₂)₂N]CH₂COOCH₃	NH-CH₂CH₂-C₆H₄(1,4)-4-SO₂NH₂	79.20	86.47	88.33	88.39	24.81	34.28	42.90	64.38
8 [1]	1	[N(CH₂CH₂)₂N]CH₂CH₂COOCH₃	NH-C₆H₄(1,4)-4-OH	**89.63**	**89.69**	**89.69**	**89.69**	**38.28**	**39.41**	**41.89**	**60.66**
9 [1]	1	NH-C₆H₄(1,4)-4-OH	NH-CH₂CH(OH)CH₂OH	**89.40**	**89.29**	**89.18**	**89.12**	**33.55**	**36.31**	**48.71**	**78.24**
10 [1]	1	NH-C₆H₄(1,4)-4-SO₂NH₂	NH-CH₂CH₂CH₂OH	43.81	62.80	75.54	82.19	26.39	29.09	30.67	49.16
11	1	NH-CH₂CH₂CH₂OH	-NH-C₆H₄(1,4)-(C=O)-CH=CH-C₆H₄(1,4)-4-H	52.03	68.04	75.88	81.57	24.36	23.68	25.88	42.62
12	2	NH-CH₂CH₂CH₂OH	-NH-C₆H₄(1,4)-(C=O)-CH=CH-C₆H₄(1,4)-4-H	52.60	70.01	85.29	89.12	18.33	23.97	31.63	46.34
13	1	NH-CH₂CH₂CH₂OH	-NH-C₆H₄(1,4)-(C=O)-CH=CH-C₆H₄(1,4)-3-OH	**89.57**	**89.57**	**89.63**	**89.63**	**24.70**	**31.91**	**34.62**	**50.79**
14	2	NH-CH₂CH₂CH₂OH	-NH-C₆H₄(1,4)-(C=O)-CH=CH-C₆H₄(1,4)-3-OH	**90.47**	**90.59**	**90.25**	**90.14**	19.01	18.55	23.18	38.39
15	1	NH-CH₂CH₂CH₂OH	-NH-C₆H₄(1,4)-(C=O)-CH=CH-C₆H₄(1,4)-2-OH	**90.70**	**90.36**	**89.97**	**89.74**	**34.28**	**35.41**	**37.32**	**57.56**
16	2	NH-CH₂CH₂CH₂OH	-NH-C₆H₄(1,4)-(C=O)-CH=CH-C₆H₄(1,4)-2-OH	**91.43**	**91.32**	**91.04**	**90.76**	23.46	25.04	24.13	40.03
17	1	NH-CH₂CH₂OH	-NH-C₆H₄(1,4)-(C=O)-CH=CH-C₆H₄(1,4)-2-OH	**90.59**	**90.19**	**89.80**	**89.52**	24.02	28.87	30.90	49.39
18	2	NH-CH₂CH₂OH	-NH-C₆H₄(1,4)-(C=O)-CH=CH-C₆H₄(1,4)-2-OH	**90.08**	**89.40**	**88.84**	**89.07**	22.50	23.63	24.70	47.07
19	2	NH-CH₂CH₂OH	-NH-C₆H₄(1,4)-(C=O)-CH=CH-C₆H₄(1,4)-3-OH	**89.12**	**89.01**	**89.01**	**89.12**	**25.49**	**34.79**	**47.81**	**65.28**
20	2	NH-CH₂CH(OH)CH₂OH	-NH-C₆H₄(1,4)-(C=O)-CH=CH-C₆H₄(1,4)-2-OH	**91.15**	**91.04**	**90.76**	**90.53**	**36.25**	**38.85**	**40.59**	**58.97**
21	2	NH-C₆H₄(1,4)-4-OH	-NH-C₆H₄(1,4)-(C=O)-CH=CH-C₆H₄(1,4)-4-H	**91.21**	**91.15**	**91.15**	**91.09**	22.56	22.61	21.32	39.07
22	2	NH-C₆H₄(1,4)-4-OH	-NH-C₆H₄(1,4)-(C=O)-CH=CH-C₆H₄(1,4)-4-OH	**89.85**	**88.61**	**89.23**	**90.76**	22.22	22.89	23.46	42.73
23	2	NH-C₆H₄(1,4)-4-OH	-NH-C₆H₄(1,4)-(C=O)-CH=CH-C₆H₄(1,4)-3-OH	**90.53**	**90.42**	**90.31**	**90.31**	25.60	27.57	32.31	36.31
24	2	NH-C₆H₄(1,4)-4-OH	-NH-C₆H₄(1,4)-(C=O)-CH=CH-C₆H₄(1,4)-2-OH	**89.40**	**87.32**	**88.56**	**90.19**	**37.72**	**39.47**	**42.28**	**69.34**
25	2	[N(CH₂CH₂)₂N]CH₂CH₂COOCH₃	-NH-C₆H₄(1,4)-(C=O)-CH=CH-C₆H₄(1,4)-4-OH	**90.47**	**91.83**	**91.77**	**92.11**	**40.03**	**46.96**	**55.25**	**87.09**
26	1	NH-CH₂CH₂-C₆H₄(1,4)-4-SO₂NH₂	-NH-C₆H₄(1,4)-CH=CH-C₆H₄(1,4)-4-H	39.92	59.70	81.96	89.40	16.92	16.53	18.78	38.51
27	1	NH-CH₂CH₂-C₆H₄(1,4)-4-SO₂NH₂	-NH-C₆H₄(1,4)-CH=CH-C₆H₄(1,4)-3-OH	**88.67**	**89.97**	**90.19**	**90.25**	24.08	27.46	32.65	43.41
28	1	NH-CH₂CH₂-C₆H₄(1,4)-4-SO₂NH₂	-NH-C₆H₄(1,4)-CH=CH-C₆H₄(1,4)-4-OH	75.43	79.31	83.99	87.37	26.61	26.84	28.19	41.95
29	1	NH-CH₂CH₂CH₂OH	-NH-C₆H₄(1,4)-CH=CH-C₆H₄(1,4)-3-OH	55.92	65.05	74.47	82.92	16.69	21.71	28.98	39.63
30	2	NH-CH₂CH₂CH₂OH	-NH-C₆H₄(1,4)-CH=CH-C₆H₄(1,4)-3-OH	83.37	**90.19**	**91.66**	**91.71**	1.70	3.67	7.23	43.19
31	2	NH-CH₂CH₂OH	-NH-C₆H₄(1,4)-CH=CH-C₆H₄(1,4)-4-OH	79.20	85.91	88.33	88.39	16.98	18.22	21.99	30.73
32	2	NH-CH₂CH(OH)CH₂OH	-NH-C₆H₄(1,4)-CH=CH-C₆H₄(1,4)-3-OH	36.37	56.15	76.89	85.74	14.95	15.12	23.80	59.64
33	2	NH-CH₂CH(OH)CH₂OH	-NH-C₆H₄(1,4)-CH=CH-C₆H₄(1,4)-4-OH	52.54	55.98	76.72	85.12	16.92	17.31	24.36	48.65
34	2	NH-C₆H₄(1,4)-4-OH	-NH-C₆H₄(1,4)-CH=CH-C₆H₄(1,4)-4-H	**90.36**	**90.31**	**90.02**	**89.85**	18.27	19.34	27.40	48.20
35	2	NH-C₆H₄(1,4)-4-OH	-NH-C₆H₄(1,4)-CH=CH-C₆H₄(1,4)-2-OH	71.03	75.09	81.96	87.83	18.78	19.63	23.68	44.14
36	2	NH-C₆H₄(1,4)-4-OH	-NH-C₆H₄(1,4)-CH=CH-C₆H₄(1,4)-3-OH	**90.08**	**89.91**	**89.80**	**89.57**	28.53	31.97	34.28	48.26
37	2	NH-C₆H₄(1,4)-4-OH	-NH-C₆H₄(1,4)-CH=CH-C₆H₄(1,4)-4-OH	**91.04**	**90.98**	**90.81**	**90.64**	23.51	25.21	32.31	29.55
Trolox	-	-	-	89.29	89.23	88.18	89.23	34.96	35.41	37.61	41.49
Ascorbic acid	-	-	-	88.33	88.45	88.33	88.45	29.09	28.81	29.83	31.07

[1] Compounds previously reported in [16].; ² Errors in the range of 1 – 5 % of the reported value (Mean from three different assays).

Table 2. ABTS cation radical scavenging ability of the most active compounds (the highest %inhibition) expressed as IC_{50} (µM) in times 0 min and 60 min.

Compound	n	R_1	R_2	IC_{50} (µM) [2]	
				0 min	**60 min**
1 [1]	1	$NH-C_6H_4(1,4)-4-OH$	Cl	**103.89**	**26.59**
2 [1]	2	$NH-C_6H_4(1,4)-4-OH$	Cl	**107.20**	**17.16**
7 [1]	1	$[N(CH_2CH_2)_2N]CH_2COOCH_3$	$NH-CH_2CH_2-C_6H_4(1,4)-4-SO_2NH_2$	317.78	43.84
8 [1]	1	$[N(CH_2CH_2)_2N]CH_2CH_2COOCH_3$	$NH-C_6H_4(1,4)-4-OH$	180.98	51.47
9 [1]	1	$NH-C_6H_4(1,4)-4-OH$	$NH-CH_2CH(OH)CH_2OH$	**127.98**	**27.78**
18	2	$NH-CH_2CH_2OH$	$-NH-C_6H_4(1,4)-(C=O)-CH=CH-C_6H_4(1,4)-2-OH$	1800.00	153.70
19	2	$NH-CH_2CH_2OH$	$-NH-C_6H_4(1,4)-(C=O)-CH=CH-C_6H_4(1,4)-3-OH$	**288.06**	**20.16**
24	2	$NH-C_6H_4(1,4)-4-OH$	$-NH-C_6H_4(1,4)-(C=O)-CH=CH-C_6H_4(1,4)-2-OH$	147.43	45.78
25	2	$[N(CH_2CH_2)_2N]CH_2CH_2COOCH_3$	$-NH-C_6H_4(1,4)-(C=O)-CH=CH-C_6H_4(1,4)-4-OH$	**53.72**	**17.22**
Trolox	-	-	-	293.46	178.33
Ascorbic acid	-	-	-	169.24	147.47

[1] Compounds previously reported in [16].

[2] Errors in the range of 1 – 5 % of the reported value (Mean from three different assays).

The numbers of IC_{50} were obtained as described in the experimental section. The values of IC_{50} correlated with the results of the determination of the %inhibition of cation radical $ABTS^{.+}$. From results presented in Table 2, it is clear that IC_{50} values of the most active compounds after 60 minutes (IC_{50} = 17.16 – 79.34 µM) were nearly ten times lower than IC_{50} values of both used standards (Trolox IC_{50} = 178.33; ascorbic acid IC_{50} = 147.47). The structure of the three derivatives with the highest antioxidant activity (the lowest values of IC_{50}) is very diverse: compound **2** is a disubstituted derivative containing aminobenzenesulfonamide and aminophenol structural motive. Hit **19** contains aminobenzenesulfonamide, aminoethanol, and 3-hydroxy-aminochalcone structural fragments, and compound **25** contains aminobenzenesulfonamide, piperazine, and 4-hydroxy-chalcone motive. All three compounds have the common structural fragment, phenolic hydroxyl group, which is probably responsible for their antioxidative activity.

Tested compounds showed great potential as antioxidants useful in the treatment of diseases caused by oxidative stress. Besides, the antioxidant effect is a great benefit for compounds that are excellent inhibitors of hCA IX (compound **1**) [16] since oxidative stress is associated with tumor development and progression in several tumors. Also, the antioxidative properties may have a beneficial effect in suppressing the side effects associated with the treatment using some classic cytostatics. [17, 18]

4. Conclusion

In this paper, a series of 1,3,5-triazine derivatives, incorporating aminobenzenesulfonamide, aminoalcohol/ phenol, piperazine, chalcone, or stilbene structural motifs were evaluated as potential antioxidants. Target compounds were prepared by using step by step nucleophilic substitution of chlorine atoms in starting 2,4,6-trichloro-1,3,5-triazine in the presence of anhydrous potassium carbonate. Cu(I)-supported on a weakly acidic resin catalyzed the reaction. The antioxidant activity was determined in terms of %inhibition activity and IC_{50}, using the ABTS method. Trolox and ascorbic acid were used as standards. At the lowest concentration 1×10^{-4} M at time 0 min, %inhibition of the cation radical $ABTS^{.+}$ was comparable or better than both used standards for ten derivatives. Furthermore, after 60 min compounds **1**, **2**, **9** and **25** showed nearly twice %inhibition (73.44 – 87.09

%) in comparison with standards (Trolox = 41.49 %; ASA = 31.07 %). Values of IC_{50} correlated with %inhibition activity. For compounds **1**, **2**, **9** and **25** values of IC_{50} in time 60 min (17.16 – 27.78 μM) were 5 times lower than IC_{50} of both standards (Trolox = 178.33 μM; ASA = 147.47 μM). From results, it is clear that more than half of the tested compounds showed great potential as antioxidants useful in the treatment of diseases caused by oxidative stress.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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