AMINO SUBSTITUTED BENZAMIDE DERIVATIVES AS POTENTIAL ANTIOXIDANTS

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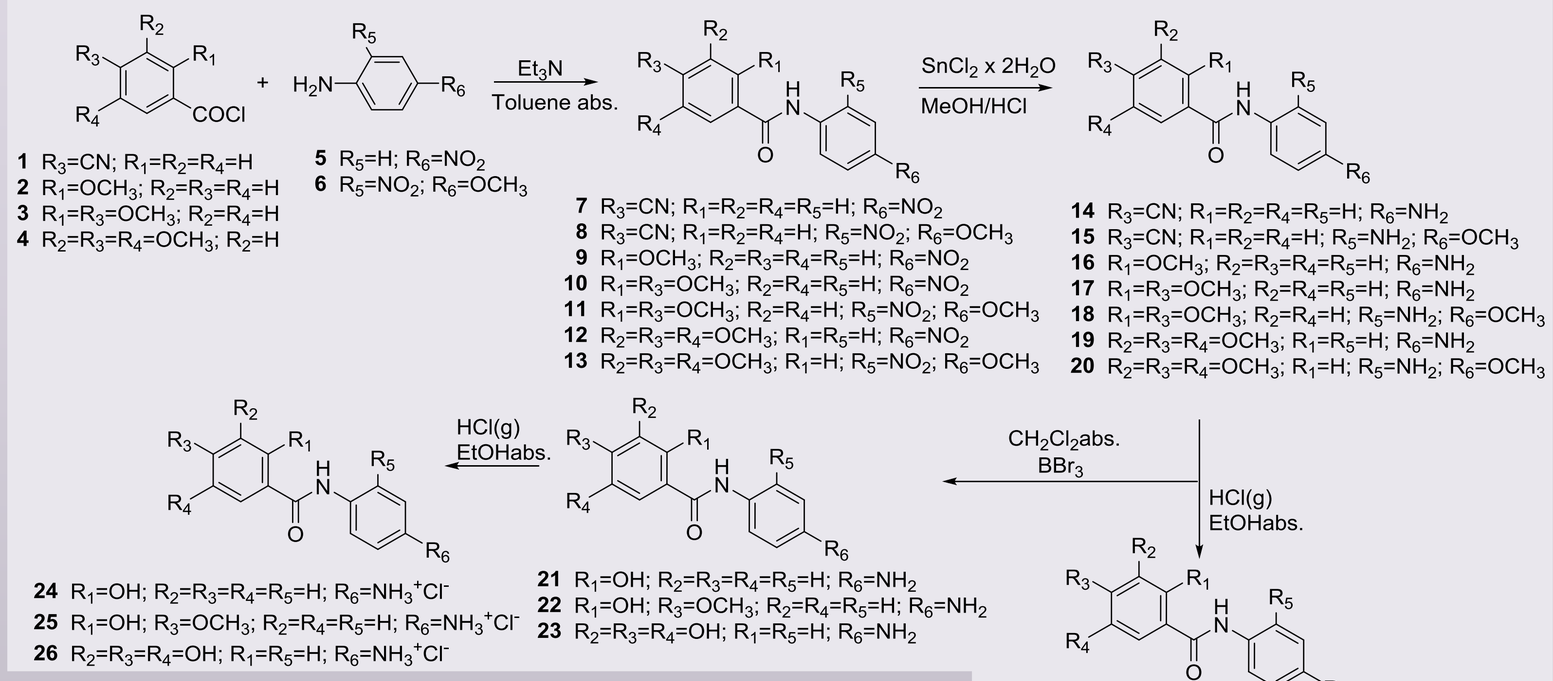
In the last few decades, the search for the novel antioxidants led to the synthesis or isolation of different organic molecules which showed promising and more effective antioxidant activity in comparison with the standard antioxidants, such as vitamins C and A or BHT[1]. We prepared a range of *N*-arylbenzamides with a variable number of methoxy and hydroxy groups, bearing either amino or amino protonated moieties. The purpose of this study was to experimentally and computationally assess the impact of a variable number and type of substituents placed on this simple organic scaffold with an already demonstrated biological potential.



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2 R_1=OCH_3; R_2=R_3=R_4=H
3 R_1=R_3=OCH_3; R_2=R_4=H
4 R_2=R_3=R_4=OCH_3; R_2=H

5 R_5=H; R_6=NO_2
6 R_5=NO_2; R_6=OCH_3

7 R_3=CN; R_1=R_2=R_4=R_5=H; R_6=NO_2
8 R_3=CN; R_1=R_2=R_4=H; R_5=NO_2; R_6=OCH_3
9 R_1=OCH_3; R_2=R_3=R_4=R_5=H; R_6=NO_2
10 R_1=R_3=OCH_3; R_2=R_4=R_5=H; R_6=NO_2
11 R_1=R_3=OCH_3; R_2=R_4=H; R_5=NO_2; R_6=OCH_3
12 R_2=R_3=R_4=OCH_3; R_1=R_5=H; R_6=NO_2
13 R_2=R_3=R_4=OCH_3; R_1=H; R_5=NO_2; R_6=OCH_3

14 R_3=CN; R_1=R_2=R_4=R_5=H; R_6=NH_2
15 R_3=CN; R_1=R_2=R_4=H; R_5=NH_2; R_6=OCH_3
16 R_1=OCH_3; R_2=R_3=R_4=R_5=H; R_6=NH_2
17 R_1=R_3=OCH_3; R_2=R_4=R_5=H; R_6=NH_2
18 R_1=R_3=OCH_3; R_2=R_4=H; R_5=NH_2; R_6=OCH_3
19 R_2=R_3=R_4=OCH_3; R_1=R_5=H; R_6=NH_2
20 R_2=R_3=R_4=OCH_3; R_1=H; R_5=NH_2; R_6=OCH_3

21 R_1=OH; R_2=R_3=R_4=R_5=H; R_6=NH_2
22 R_1=OH; R_3=OCH_3; R_2=R_4=R_5=H; R_6=NH_2
23 R_2=R_3=R_4=OH; R_1=R_5=H; R_6=NH_2

24 R_1=OH; R_2=R_3=R_4=R_5=H; R_6=$NH_3^+Cl^-$
25 R_1=OH; R_3=OCH_3; R_2=R_4=R_5=H; R_6=$NH_3^+Cl^-$
26 R_2=R_3=R_4=OH; R_1=R_5=H; R_6=$NH_3^+Cl^-$

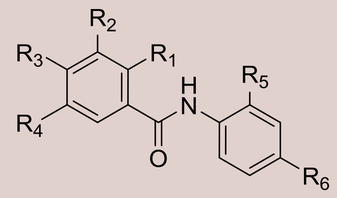
27 R_3=CN; R_1=R_2=R_4=R_5=H; R_6=$NH_3^+Cl^-$
28 R_3=CN; R_1=R_2=R_4=H; R_5=$NH_3^+Cl^-$; R_6=OCH_3
29 R_1=OCH_3; R_2=R_3=R_4=R_5=H; R_6=$NH_3^+Cl^-$
30 R_1=R_3=OCH_3; R_2=R_4=R_5=H; R_6=$NH_3^+Cl^-$
31 R_1=R_3=OCH_3; R_2=R_4=H; R_5=NH_3^+Cl-; R_6=OCH_3
32 R_2=R_3=R_4=OCH_3; R_1=R_5=H; R_6=$NH_3^+Cl^-$
33 R_2=R_3=R_4=OCH_3; R_1=H; R_5=$NH_3^+Cl^-$; R_6=OCH_3

All investigated benzamide derivatives were synthesized according to Scheme 1. Structures of 7–26 were confirmed by both ^1H and ^{13}C NMR spectroscopies and elemental analysis.

Scheme 1.

To determine the antioxidant capacity of the investigated systems, the reducing activity of the stable 1,1-diphenyl-picrylhydrazyl radical (DPPH) and ferric reducing/antioxidant power (FRAP) parameters were evaluated [2].

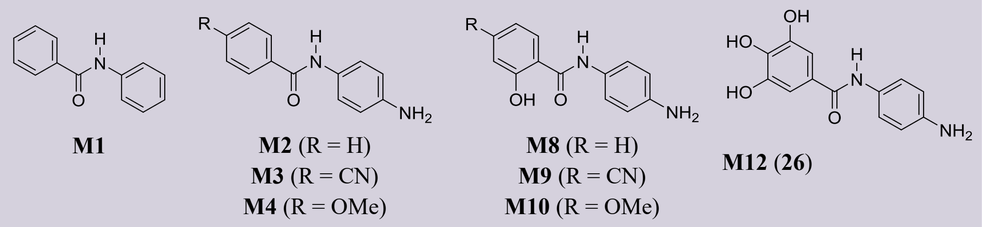
Table 1. IC_{50} values of investigated systems for DPPH free radical scavenging and FRAP activities.



System	R_1	R_2	R_3	R_4	R_5	R_6	DPPH IC_{50} μM	FRAP $mmolFe^{2+}/mmol_C$
21	OH	H	H	H	H	NH_2	23.80 ± 0.3	2283.67 ± 62.12
22	OH	H	OCH_3	H	H	NH_2	24.0 ± 1.5	1530.69 ± 35.87
23	H	OH	OH	OH	H	NH_2	16.85 ± 0.1	3259.93 ± 85.51
24	OH	H	H	H	H	$NH_3^+Cl^-$	22.25 ± 0.9	2328.80 ± 118.03
25	OH	H	OCH_3	H	H	$NH_3^+Cl^-$	10.81 ± 3.3	2174.40 ± 99.85
26	H	OH	OH	OH	H	$NH_3^+Cl^-$	12.93 ± 1.9	4856.15 ± 70.18
27	H	H	CN	H	H	$NH_3^+Cl^-$	30.73 ± 8.1	1763.47 ± 32.66
28	H	H	CN	H	$NH_3^+Cl^-$	OCH_3	18.12 ± 0.3	989.11 ± 85.02
29	OCH_3	H	H	H	H	$NH_3^+Cl^-$	30.02 ± 4.1	2307.42 ± 63.87
30	OCH_3	H	OCH_3	H	H	$NH_3^+Cl^-$	18.4 ± 3.1	1905.99 ± 87.57
32	H	OCH_3	OCH_3	OCH_3	H	$NH_3^+Cl^-$	26.56 ± 1.8	1677.96 ± 93.20
33	H	OCH_3	OCH_3	OCH_3	$NH_3^+Cl^-$	OCH_3	30.45 ± 2.1	1763.47 ± 219.52
BHT							25 ± 4.2[a]	2089.34 ± 55.98

Systems having the protonated amino moiety showed significant improvement in the antioxidative capacity relative to their unionized analogues. Also, the presence of the electron-donating methoxy and hydroxy moieties yielded a considerable positive impact on the measured data. Trihydroxy derivative with the $-NH_3^+$ group 26 was elucidated as the most active system based on both assays and proposed as a lead compound for further optimization of the investigated scaffold towards more efficient antioxidants.

Computational analysis at the B3LYP/6–311++G(2df,2pd)//(SMD)/B3LYP/6–31+G(d) level offered interpretation of the measured data through the hydrogen atom transfer (HAT) mechanism, related with bond dissociation energies (BDE) calculated as Gibbs free energies for M–H → M$^\bullet$ + H$^\bullet$, while utilizing model systems M1–M12.



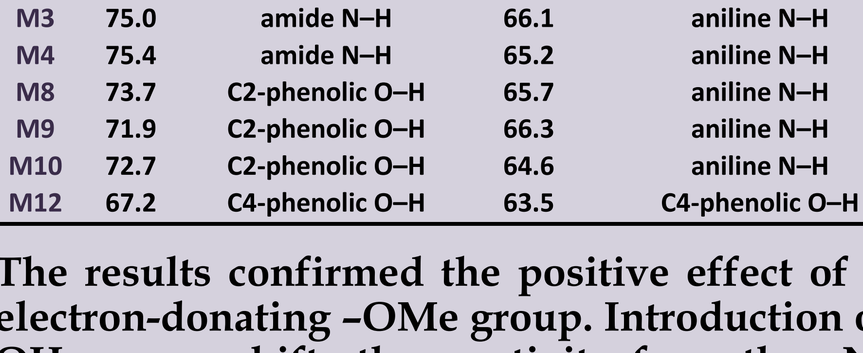
M1 **M2** (R = H) **M3** (R = CN) **M4** (R = OMe) **M8** (R = H) **M9** (R = CN) **M10** (R = OMe) **M12 (26)**

Figure 3. Schematic representation of molecules studied computationally. In the cationic forms, all systems are most preferably protonated at the aniline amino moiety.

Table 2. Bond dissociation energies (BDE) in ethanol solution. The corresponding site of the hydrogen atom abstraction is denoted next to a BDE value.

system	neutral molecules M*n*		cationic protonated molecules M*n*H$^+$	
	BDE	site of the X–H cleavage	BDE	site of the X–H cleavage
M1	85.3	amide N–H		
M2	75.6	amide N–H	65.8	aniline N–H
M3	75.0	amide N–H	66.1	aniline N–H
M4	75.4	amide N–H	65.2	aniline N–H
M8	73.7	C2-phenolic O–H	65.7	aniline N–H
M9	71.9	C2-phenolic O–H	66.3	aniline N–H
M10	72.7	C2-phenolic O–H	64.6	aniline N–H
M12	67.2	C4-phenolic O–H	63.5	C4-phenolic O–H

The results confirmed the positive effect of the electron-donating –OMe group. Introduction of –OH groups shifts the reactivity from the $-NH_2$ moiety towards this fragment, and promotes the antioxidative properties. This is particularly enhanced in the trihydroxy derivative M12, due to the stabilizing [O$^\bullet$ ····H–O] hydrogen bonding between the created phenoxyl radical and the neighboring –OH groups.



DPPH (IC_{50}) 12.93 ± 1.9 μM
FRAP ($mmolFe_2^+/mmolc$) 4856.15 ± 70.18

Figure 2. The most promising antioxidant 26.

[1] N. Tailor and M. Sharma, *Mini-Rev. Med. Chem.* 13 (2013) 280.
[2] M. Tireli, K. Starčević, T. Martinović, S. Kraljević Pavelić, G. Karminski-Zamola and M. Hranjec, *Mol. Divers.* 21 (2017) 201.
[3] N. Perin, P. Roškarić, I. Sović, I. Boček, K. Starčević, M. Hranjec and R. Vianello, *Chem. Res. Toxicol* (2018) **DOI:** 10.1021/acs.chemrestox.8b00175

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