Bulgarian Chemical Communications, Volume 56, Special Issue D1 (pp. 167-171) 2024 DOI: 10.34049/bcc.56.D.S2P3

Synthesis and antioxidant activity of benzazole-based hybrids

M. Bachvarova[1], D. Kirkova[2], Y. Stremski[1*], E. Suyleyman[1], S. Statkova-Abeghe[1], M. Docheva[2]

[1]*Paisii Hilendarski University of Plovdiv, Faculty of Chemistry, Dept. of Organic Chemistry, 24,"Tzar Assen" Str. Plovdiv 4000, Bulgaria*

[2]*Tobacco and Tobacco Products Institute, Agricultural Academy, Markovo 4108, Bulgaria*

Received: xxxx; Revised: April 11, 2024

The preparation of benzazole derivatives with a phenolic fragment in the second position is of great interest to the synthetic community. These compounds have been applied as antioxidants in medicine but also in the food and cosmetic industry as preservatives. Some phenols such as resorcinol have high toxicity and different methods for their structure modification are often studied and announced. Molecular hybridization with the introduction of a benzazole substituent in the aromatic ring of resorcinol would lead to enhanced oxidative stability and radical scavenging activity. The results showed more active profiles of benzothiazole-containing derivatives than benzimidazole ones.

Keywords: Multifunctional molecules, resorcinol, quercetin, benzazole hybrids, radical scavenging activity, α-amidoalkylation

INTRODUCTION

Benzimidazole ring is an important pharmacophore used widespread in medicine [1]. This privileged structure has an important biological significance based on a varied range of biological properties, such as antioxidant [1-3], antimicrobial [4], anti-inflammatory [5], antifungal [4], etc. The literature reports have shown remarkable antiproliferative [6-8] and antitumor activities [2, 3] of 2-phenylbenzimidazole compounds. The antiproliferative effect of a series of benzimidazole-based compounds has been tested on Colo-38 human skin cancer melanoma cells. Compound *1* (Figure 1), shows the highest antiproliferative effect at low micromolar concentration (IC_{50} = 6.20 μM) [7].

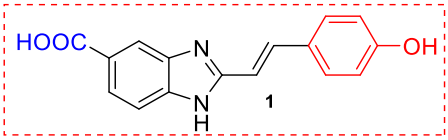


Figure 1. Phenol-containing hybrid *1*

Photoprotectors including various sunscreens are important since they reduce a large number of skin diseases caused by UV radiation [9]. Some synthetic benzazole compounds have proved potential application as UV-protectors in cosmetics [7, 8, 10, 11]. In this regard, 2-phenyl-1*H*-benzimidazole-5-sulfonic acid *2* (PBSA, Figure 2) is one of the UVB filters used most commonly in cosmetics for sun protection, having an excellent safety profile. Another example is Oxisol *3* (Figure 2), as well as the disodium salt of phenyl-dibenzimidazole tetrasulfonate *4* (known as Neo Heliopan, Figure 2), which absorb mainly in the UVA range. Neo Heliopan is water-soluble, photostable and safe, with extremely low skin penetration. In addition, the 2-aryl benzimidazole compounds *5* showed satisfactory SPF values tested *in vitro* compared to the commercial PBSA filter [7].

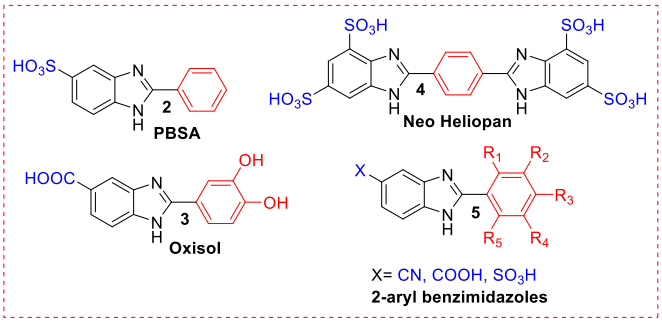


Figure 2. Benzimidazole-based UV-filters

* To whom all correspondence should be sent:
E-mail: *stremski@uni-plovdiv.net*

According to the authors, the most promising 2-arylbenzimidazole-5-sulfonic acids (compounds *6* and *7*), as well as 2-styryl-benzimidazole *1* show broad-spectrum solar protection against UVA and UVB rays [7].

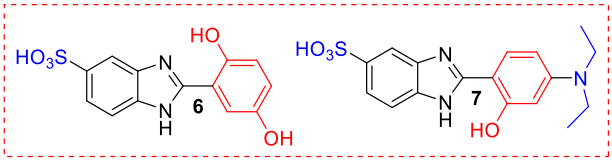


Figure 3. 2-arylbenzimidazole-5-sulfonic acids *6*, *7*

Many polyphenols isolated from plants, including flavonoids, are reported to have significant photoprotective effects on the skin and act as weak UV-filters. In addition, they have antioxidant, anti-inflammatory and anticarcinogenic profiles. The presence of hydroxyl groups and aromatic fragments in the structure improves their capability of absorbing UV light in a wide range of wavelengths, which determines their properties [12]. Recent research suggested that polyphenols may be an effective source of skin protection from the effects of UV rays (UVA и UVB) [13].

A validated and standardized method for *in vitro* assessment of UVA protection is described in ISO 24443:2021 [14], while the assessment of UVB protection is still not clearly investigated. The method proposed by Diffey-Robson for *in vitro* estimation of the SPF parameter in 1989 has been widely and successfully applied [15]. Djuidje *et al.* recently reported for the synthesis of various 2-substituted benzimidazoles, structural analogues of phenylbenzimidazole sulfonic acid (PBSA). The isosteric modification approach was used by the authors to design and investigate the structure-activity relationship of benzimidazole derivatives. The compounds showed good antifungal activity against *M. gypseum, M. canis, T. mentagrophytes, T. tonsurans* and *E. floccosum* with IC_{50} values in the range of 0.97–3.80 μg/ml. The authors concluded that series of 2-heteroaryl benzimidazoles could be privileged for the development of new multifunctional molecules, useful for the treatment of multifactorial diseases [16].

In the last decade, the design and synthesis of novel multifunctional benzazole molecules with antioxidant activity similar to the polyphenols has been of interest among the scientific community [7]. A series of newly synthesized 2-aryl benzimidazoles (*5*, Figure 2) were estimated for their antioxidant potential. DPPH, FRAP and ORAC assays were used for the evaluation of radical scavenging activity of the synthesized compounds and their structure-activity relationship depending on the number and position of hydroxyl groups in the 2-aryl moiety. The presence of diethylamino group or 2-styryl group is associated with good antioxidant potential. Also, it was observed that the presence of sulfonic acid group at the 5-position of the benzimidazole core was the least favourable, while derivatives containing 5-cyano- or 5-carboxy-groups showed moderate to high antioxidant activity [7].

High antioxidant activity for benzazole compounds containing resorcinol in their structure, represented by the general formula (Figure 4) has been also reported [3]. Racane *et al.* compared the DPPH and ABTS radical scavenging assays, in terms of evaluating the antioxidant activity of a series of 2-aryl benzimidazole and benzothiazole compounds. Benzimidazole compounds were found to exhibit higher radical scavenging activity compared to benzothiazole analogues *via* the ABTS method [3].

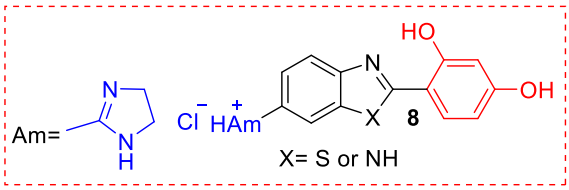


Figure 4. Benzazole-resorcinol hybrids *8*

In recent studies, we reported the synthesis of some 2-hydroxyphenyl benzothiazolines following an accessible reaction of *α*-amidoalkylation [17]. In addition, the results for their antimicrobial activity gave good lead for further structure optimisations. Furthermore, we proposed the synthesis of novel quercetin hybrids with potent radical scavenging activity [18], and also possibility for modification of a benzimidazole ring with resorcinol [19].

In this study, we have applied our efficient protocol to synthesize benzazole-resorcinol hybrids [17, 19], exploring their radical scavenging potential. Assessing their ability to neutralize free radicals is particularly relevant for potential use in cosmetics, addressing both aesthetic concerns and skin health. Positive results could position these compounds as promising candidates for further cosmetic research, contributing to the development of skincare products with enhanced antioxidant and protective properties. This study aligns with the ongoing quest for multifunctional molecules in cosmetic science.

MATERIALS AND METHODS

Reagents and equipment

The starting reagents, solvents and materials were provided by commercial suppliers (Merck).

Melting points were determined on a Kruss M5000 melting point meter. HRMS spectral measurements were performed on a Bruker mass spectrometer. IR spectra were measured on an ALPHA II FT-IR spectrometer. NMR spectra were measured on a Bruker Avance AV600 spectrometer at the Institute of Organic Chemistry with Centre of Phytochemistry-BAS, Sofia. Deuterated dimethyl sulfoxide or chloroform were used as NMR solvents. The absorbance of free radical scavenging assay was measured by a Spectroquant Pharo 300, UV/Vis spectrophotometer.

Materials

A suitable approach for the preparation of benzazole-resorcinol hybrids through one-pot reaction was applied and optimized (Scheme 1) [17, 19]. The reactions completion was monitored by thin layer chromatography (TLC). All synthesized compounds were purified by column chromatography on silica gel with a mixture of petroleum/diethyl ether as eluents. The expected structure of the compounds was spectrally confirmed by 1D ^{1}H, $^{13}C\{^{1}H\}$-NMR, 2D HSQC-NMR, IR and HRMS spectra and published in our previous studies [17, 19].

Methods

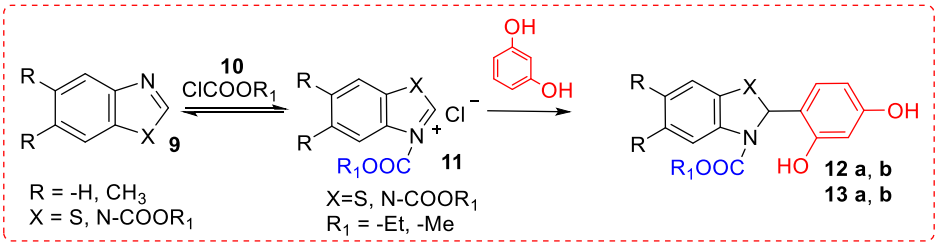
ABTS free radical scavenging assay. The procedure to determine the radical scavenging activity of the new synthesized compounds by using $ABTS^{\bullet+}$, follows the original method of Re *et al.* [20] with slight modifications: 7 mM ABTS and 2.45 mM $K_2S_2O_8$ dissolved in water, were mixed in equal amounts 1:1 (v/v), and the mixture was allowed to stay for 14 – 16 hours for stabilization [20]. The stability of undiluted $ABTS^{\bullet+}$ is at least 1 week, stored in a refrigerator. The resulting free radical cation was diluted with pure methanol to reach an absorbance of 0.7±0.02, which was measured spectrophotometrically at 734 nm. Sample loading relative to ABTS free radical was 0.1:0.9 (0.3 ml analyte + 2.7 ml $ABTS^{\bullet+}$). The time required for the reaction to proceed was 10 min at room temperature in the dark, after which the absorbance of the sample analysed was measured. The stability of the undiluted $ABTS^{\bullet+}$ is a minimum of 1 week in refrigerated storage. In the present studies, stock solutions of the substances were prepared at a concentration of 1 mg/ml. From these, solutions of lower concentrations were made by dilution with methanol. A plot of % inhibition (RSA%) *versus* concentration of each substance was plotted and the IC_{50} values for each substance were found in μg/ml (ppm) and converted to μM.

RESULTS AND DISCUSSION

As a result of molecular hybridization, a benzazole fragment was successfully combined with resorcinol by applying an intermolecular reaction of *α*-amidoalkylation. The electrophilic *N*-acyliminium reagents were generated *in situ* and the subsequent reaction with resorcinol according to Scheme 1 led to final products *12*, *13*.

The main purpose of this work after the obtaining of benzazole-rezorcinol hybrids was to develop an effective method for examination of their radical scavenging activity.

To determine the radical scavenging activity of the newly synthesized compounds, we applied the ABTS method, building on our previous experience. In earlier studies of the radical scavenging activity of natural phenols [21], we found that gallic acid (IC_{50} = 37.7±1.25 μM) and pyrogallol (IC_{50} = 22.7±1.45 μM) exhibited the highest radical scavenging activity, while catechol (IC_{50} = 111.2±0.5 μM) exhibited the lowest one. The ABTS assay resulted in comparable and reproducible results. In this regard, the radical scavenging activity of the synthetic 2-aryl benzothiazolines was measured under the same conditions in a medium containing free radical cations of ABTS [21]. As a result, benzothiazole derivatives containing pyrogallol (IC_{50} = 45.9±6.21 μM) and resorcinol (*12a* IC_{50} = 55.6±0.51 μM), (*12b* IC_{50} = 46.3±6.21 μM) fragments showed activity similar to the gallic acid and resorcinol (48.2±4.9 μM). Table 1 represents the radical scavenging activity data examined for compounds *12a, b* and *13a, b* and resorcinol.



Scheme 1. Synthesis of 2-(2,4-dihydroxyphenyl)benzazolines

Table 1. Radical scavenging activity of 2-aryl benzazoles by the ABTS-method

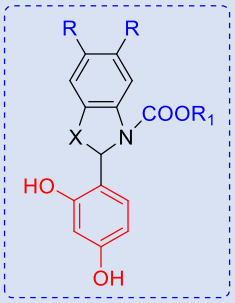
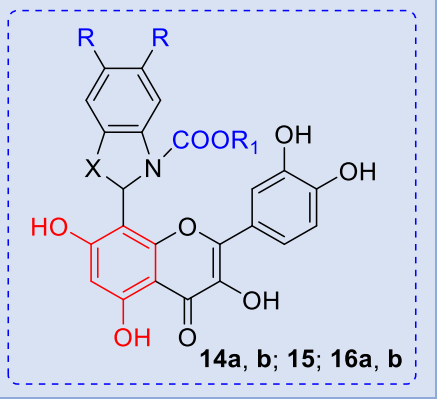
Compound	MW	IC_{50} μM	Structure
Resorcinol	110.11	48.2±4.9	
12a X = -S, R = -H, R_1 = -Et [17]	317.36	55.6±0.5	
12b X = -S, R = -H, R_1 = -Me [17]	303.33	46.3±6.2	
13a X = -NCOOR$_1$, R = -H, R_1 = -Et [19]	372.38	88.9±4.0	
13b X = -NCOOR$_1$, R = -Me, R_1 = -Et [19]	400.43	71.6±4.0	

Table 2. Radical scavenging activity of quercetin hybrids by the ABTS-method [18]

Compound	MW	IC_{50} μM	Structure of Qct-hybrids
Quercetin (Qct)	302.24	48.0±4.4	
Rutin	610.52	95.3±4.5	
14a X = -S, R = -H, R_1 = -Et	509.49	49.8±3.5	
14b X = -S, R = -H, R_1 = -Me	495.46	50.0±3.5	
15 X = -NCOOR$_1$, R = -H, R_1 = -Et	564.50	58.3±3.5	
16a X = -NCOOR$_1$, R = -Me, R_1 = -Et	592.56	62.4±3.5	
16b X = -NCOOR$_1$, R = -Me, R_1 = -Me	564.50	55.1±3.5	

Moreover, we also reported the radical scavenging activity of the novel 2-substituted benzazole-quercetin hybrids (*14a, b - 16a, b*). In the 2-substituted benzazole-quercetin hybrids, no significant differences in the activity of the compounds were observed depending on the hetero part of the molecule (Table 2). The benzimidazole-resorcinol derivatives *13a* and *13b* showed lower activity than 1*2a* and *12b* containing a benzothiazole fragment, but close to the natural compound - rutin.

The activity of *12a* and *12b* was comparable to the quercetin hybrids, pure quercetin and resorcinol. The new benzazole-resorcinol hybrids demonstrated good radical scavenging potential. From them *12b* was with the highest activity (Table 1). The results achieved in this work give lead for future investigations based on the explanation of the mechanism of interactions with the free radical, also the reasons for differences in the activity caused by the presence of hydroxyl groups.

CONCLUSION

A molecular hybridization method was successfully demonstrated for the preparation of resorcinol hybrids containing benzothiazole, benzimidazole and 5,6-dimethylbenzimidazole fragment by applying an earlier introduced *one-pot* intermolecular reaction of *α*-amidoalkylation. The resulting compounds have high radical-scavenging ability. The radical scavenging activity of 2-(2,4-dihydroxyphenyl)-*N*-carboxymethyl-benzothiazol-ines *12b* - IC_{50} - 46.3±6.2 μM is comparable to that of resorcinol and quercetin. The radical scavenging activity of the resorcinol hybrids is higher than that of rutin. Two assays, such as ABTS and DPPH were compared to evaluate the radical scavenging activity of synthetic benzazole compounds. The ABTS method is more sensitive than the DPPH. The results are promising and give good lead for potential application in cosmetics.

Acknowledgement: *We acknowledge the financial support from the Fund for Scientific Research of the Plovdiv University, project № МУПД23-ХФ-003 and by the National Program of Ministry of Education and Science "Young Scientists and Postdoctoral Students – 2 - 2022".*

REFERENCES

1. B. Narasimhan, D. Sharma, P. Kumar, *Med. Chem. Res.*, **21**, 269 (2012).
2. L. Racané, K. Butković, I. Martin-Kleiner, M. Kralj, G. Karminski-Zamola, M. Hranjec, *Croatica Chemica Acta*, **92**(2), 181 (2019).

3. M. Racané, N. Sedić, M. Ilić, S. Aleksić, G. Kraljević Pavelić, *Med. Chem.,* **17**, 57 (2017).
4. N. Phan, T. Huynh, H. Nguyen, Q. Le, Q. T. Nguyen, K. Ngo, T. Nguyen, K. Ton, K. Thai, T. Hoang, *ACS Omega*, **8**(31), 28733 (2023).
5. R. Veerasamy, A. Roy, R. Karunakaran, H. Rajak, *Pharmaceuticals, MDPI*, **14**(7), (2021).
6. M. Karpińka, J. Matysiak, A. Niewiadomy, *Archives of Pharmacal Research,* **34**(10), 1639 (2011).
7. A. Baldisserotto, M. Demurtas, I. Lampronti, M. Tacchini, D. Moi, G. Balboni, S. Pacifico, S., Vertuani, S. Manfredini, V. Onnis, *Bioorganic Chemistry*, **94**, (2020).
8. R. Barbari, C. Tupini, E. Durini, E. Gallerani, F. Nicoli, I. Lampronti, A. Baldisserotto, S. Manfredini, *Molecules*, **28**(1), (2023).
9. D. Fivenson, N. Sabzevari, S. Qiblawi, J. Blitz, B. Norton, S. Norton, *Int. J. Women's Dermatol.*, **7**, 45 (2021).
10. G. Nitulescu, D. Lupuliasa, I. Adam-Dima, G. Nitulescu, *Multidisciplinary Digital Publishing Institute (MDPI).* **10**(4), (2023).
11. A. Bino, A. Baldisserotto, E. Scalambra, V. Dissette, D. Vedaldi, A. Salvador, E. Durini, S. Manfredini, S. Vertuani, *Journal of Enzyme Inhibition and Medicinal Chemistry*, **32**(1), 527 (2017).
12. J. Nichols, S. Katiyar, *Arch. Dermatol. Res.,* **302**, 71 (2010).
13. H. Sies, W. Stahl, *Annu. Rev. Nutr.,* **24**, 173 (2004).
14. ISO 24443:2021, Cosmetics. Determination of sunscreen UVA photoprotection *in vitro*, (2021).
15. B. Diffey, J. Robson. *J. Soc. Cosmet. Chem.,* **40**, 127 (1989).
16. E. Djuidje, E. Durini, S. Sabrina Sciabica, E. Serra, J. Balzarini, S. Liekens, S. Manfredini, S. Vertuani, A. Baldisserotto, *Molecules*, **25**, 4324 (2020).
17. Y. Stremski, D. Kirkova, S. Statkova-Abeghe, P. Angelov, I. Ivanov, D. Georgiev, *Synthetic Communications*, 3007 (2020).
18. D. Kirkova, Y. Stremski, S. Statkova-Abeghe, M. Docheva, *Molbank*, (1), M1329, 202 (2022).
19. Y. Stremski, M. Bachvarova, D. Kirkova S. Statkova-Abeghe, *Molbank*, (1), M1602 (2023).
20. R. Re, N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, C. Rice-Evans, *Free Radicals in Biology and Medicine*, **26**, 1231 (1998).
21. D. Kirkova, S. Statkova-Abeghe, M. Docheva, Y. Stremski, S. Minkova, *Bulg. Chem. Commun.,* **52** (Special issue D), 196 (2020).