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Quercetin Hybrids—Synthesis, Spectral Characterization and Radical Scavenging Potential

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Abstract: New quercetin-based derivatives are synthesized in an easily accessible one-pot manner. The method is based on the reaction of quercetin with in situ formed electrophilic *N*-alkoxycarbonylazolium ions. The position of the newly formed C-C bond and structure were spectrally characterized by 1D, 2D ^{1}H, ^{13}C-NMR, IR, and MS analysis. Thus, in all cases, good regioselectivity in the C-8 position for the obtained products was demonstrated. The obtained compounds were evaluated for their DPPH and ABTS free radical scavenging activity and compared to natural compounds—quercetin and rutin.

Keywords: quercetin; benzazoles; *N*-acyliminium ions; one-pot reaction; antioxidant activity

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1. Introduction

Flavonoids constitute one of the most common groups of plant phenolics. In plants, flavonoids occur as aglycones, their glycosides and methylated derivatives [1,2]. Quercetin (Figure 1) is a flavonoid aglycone abundantly found in almost all edible vegetables and fruits.

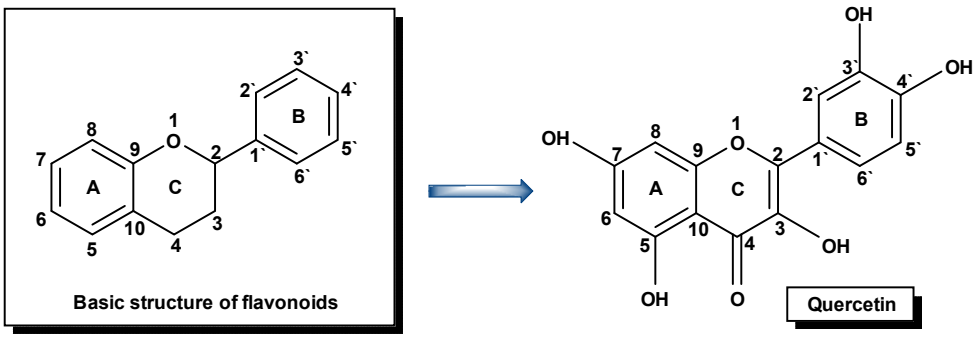


Figure 1. Basic skeleton of flavonoids and structure of quercetin.

Quercetin is a natural compound with a variety of pharmacological properties, such as anti-inflammatory, antioxidant [3,4], and anticancer activities [5,6]. This flavonoid is also known to be a potent inhibitor for several clinically and toxicologically important enzymes, such as NADH-cytochrome b5 reductase [7], NQO1 in HepG2 cells, after activation of nuclear factor E2-related factor 2 (Nrf2) [8]. In addition, quercetin has the ability to enter cells and accumulate in mitochondria. Carrasco-Pozo et al. reported a protective effect against mitochondrial dysfunction produced by indomethacin in Caco-2 cells [9]. It is proved that the lipophilization of quercetin modulates its functional antioxidant properties, increasing hydrophobicity and reducing water solubility [10]. In the last two years, the remarkable benefits of quercetin in the treatment of the COVID-19 virus and the resulting problems have also been established [11–13].

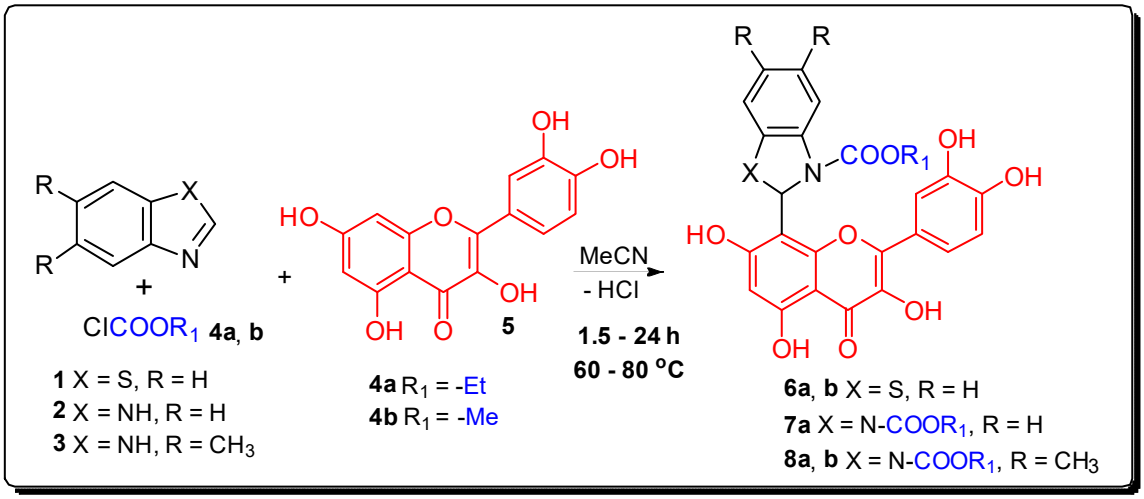
The synthetic derivatives of quercetin have attracted interest nowadays because of their diverse bioactive profile and application as antioxidant and neuroprotective agents [14] and antiviral and cytotoxic agents [15]. The mainly employed method for the preparation of quercetin derivatives is based on the reactions of *O*-alkylation or acylation [14,16]. Another commonly used approach is *C*-aminoalkylation based on the Mannich reaction [17–19]. In this context, Ilkei et al. reported the C-8 regioselective synthesis of three flavonoid alkaloids by the biomimetic transformation of some 5,7-dihydroxylatedflavonoids [20]. However, methods for direct *C*-heteroarylation and azole scafold based alkylation of quercetin are still not found in the literature.

Certainly, 2-substituted derivatives of benzothiazole and benzimidazole are important biologically active compounds with diverse medicinal applications [21–23]. Furthermore, the synthesis of hybrid molecules containing two pharmacophore fragments and their evaluation as potent drug candidates have been under constant escalation for the last two decades [24,25]. Additionally, the radical scavenging assays of the new synthetic compounds have been consummated to analyze their effectiveness as antioxidant agents. Many methods have been developed to determine antioxidant activity. They are all based on the generation of various free radicals that interact with antioxidants for a period of time. Some of the most applied are DPPH and ABTS- assay [3,4].

The current direction of our scientific group is developing an accessible approach to various heterocycles [26–28]. The aim of this study is to apply this method for obtaining new quercetin–benzazole hybrids and evaluate their radical scavenging potential.

2. Results and Discussion

Here we report the investigations on the application of adducts obtained from various benzazoles (benzothiazole, benzimidazole, and 5,6-dimethylbenzimidazole) (**1**–**3**) with alkyl chloroformates (**4a**, **b**) for α-amidoalkylation of quercetin (**5**). The above-mentioned reactions lead to products (**6**–**8 a**, **b**, Scheme 1).



Scheme 1. Synthesis of new quercetin hybrids (**6**–**8 a**, **b**) by one-pot reaction of α-amidoalkylation.

The reaction conditions, including the ratio of the reagents, temperature, and time, are presented in Table 1.

Table 1. The reaction conditions and yields of **6**–**8 a**, **b**, prepared according to Scheme 1.

Product	X	R	R_1	Ratio *	T, °C	Time, h	Yield, %
6a	S	H	Et	3:1	80	5	45
6b	S	H	Me	3:1	60	5	37
7a	NH	H	Et	2:1	60	3	59 **
8a	NH	Me	Et	2:1	80	24	61 **
8b	NH	Me	Me	3:1	80	1.5	51 **

* ratio of *N*-alkoxycarbonylazolium ion: quercetin. ** obtained using Et_3N as hydrochloric scavenger.

The one-pot reactions were successfully completed under heating (60–80 °C) for 1.5–24 h. It was found that acetonitrile is a suitable solvent for carrying out the reactions. The best yields of products (**6**–**8 a**, **b**) in the range of 37% to 61% (Table 1) were obtained with a two or threefold excess of benzazole and alkyl chloroformates. Acetonitrile was found to be the optimal solvent for the reactions, while halogenated solvents, such as dichloromethane or 1,2-dichloroethane, were not sufficient due to the low solubility of quercetin. Table 1 indicates the reaction conditions under which the C-8 monosubstituted products were obtained with the highest yields. The yield of the products depended on the stability of the in situ generated *N*-acyliminium adducts. In this regard, ethyl chloroformate performed better than methyl chloroformate for higher-yielding products. The mixture of regioisomers was observed (monitored by TLC) with the equimolar ratio of starting reagents according to our previously published study [28]. For the reactions with benzimidazole and 5,6-dimethylbenzimidazole, the presence of Et_3N as a hydrochloric scavenger was required. The conditions for the amidoalkylation of quercetin with *N*-alkoxycarbonylbenzimidazolium adduct obtained from benzimidazole (**2**) with methyl chloroformate (**4b**) were not established and, therefore, the expected product was not successfully isolated.

Analytically pure samples of only C-8 monosubstituted products were isolated by column chromatography on silica using a mixture of petroleum/diethyl ether as eluents, and the yields are indicated in Table 1. The structure of C-8 alkylated products (**6**–**8 a**, **b**) were confirmed by 1D, 2D ^{1}H-NMR experiments. In particular, the ^{1}H resonances of the quercetin and the obtained hybrids are reported in the text below. Comparing these chemical shifts with the one of quercetin reported by Kyriakou et al. [29] led to the conclusion that substitution occurs in the C-8 position. The absence of signal about 6.40 ppm in ^{1}H-NMR spectra and the C-8 downfield shifts in the range of δ = 97.1–99.0 ppm (^{13}C-NMR spectra) for the compounds (**6**–**8 a**, **b**) correctly support the above-mentioned conclusion. The ^{1}H-NMR spectra of the analyzed compounds indicate singlets in the range of δ = 7.50–7.68 ppm for the proton on sp^3- C-2 atom in benzazole rings. The ^{1}H-, ^{13}C-HSQC measurements proved the exact location of these characteristic signals. The resulting products were structurally characterized by 1D, 2D ^{1}H, ^{13}C-NMR, IR, and MS spectra. Original spectra are available in the Supplementary Materials section (S1).

In Vitro DPPH and ABTS Radical Scavenging Assay

The new quercetin hybrids were screened for their in vitro antioxidant property by DPPH and ABTS radical scavenging assay using quercetin and rutin as the standards. The effectiveness of the compounds to scavenge the free radicals was calculated using Equation (1):

$$\text{DPPH (ABTS) scavenging effect (\%)} = (A_0 - A_1/A_0) \times 100 \quad (1)$$

where A_0 is the absorbance of the blank sample and A_1 is the absorbance in the presence of the analysis compounds.

The free radical scavenging activity of natural antioxidants quercetin and rutin were, respectively, 4.60 ± 0.3 μM and 5.02 ± 0.4 μM for DPPH or 48.0 ± 4.4 μM and 95.3 ± 4.5 μM for ABTS assay. The results correspond to our previous research [30,31].

The free radical scavenging potential of the synthetic quercetin hybrids varied in the range of IC_{50} = 6.17 ± 0.3 μM (**8b**)–7.26 ± 0.3 μM (**8a**) measured with DPPH assay, and IC_{50} = 49.8 ± 3.5 μM (**6a**)–62.4 ± 3.5 μM (**8a**) with ABTS assay. The hybrid molecules had the same high antioxidant activity as natural antioxidants quercetin and rutin investigated by DPPH-assay. The radical scavenging activity of synthetic derivatives was close to the quercetin activity and higher than rutin. The summarized results are presented in Table 2.

Table 2. Free radical scavenging activities of synthetic derivatives **6–8 a**, **b**, compared with natural antioxidants—quercetin and rutin.

Compound	MWg /mol	DPPH IC_{50}, μM	ABTS IC_{50}, μM
Quercetin	302.24	4.60 ± 0.3	48.0 ± 4.4
Rutin	610.52	5.02 ± 0.4	95.3 ± 4.5
6a	509.49	6.95 ± 0.3	49.8 ± 3.5
6b	495.46	6.83 ± 0.3	50.0 ± 3.5
7a	564.50	6.18 ± 0.3	58.3 ± 3.5
8a	592.56	7.26 ± 0.3	62.4 ± 3.5
8b	564.50	6.17 ± 0.3	55.1 ± 3.5

3. Materials and Methods

Quercetin [CAS No. 117-39-5], benzothiazole [CAS No. 95-16-9], benzimidazole [CAS No. 51-17-2], 5,6-dimethylbenzimidazole [CAS No. 582-60-5], triethylamine [CAS No. 121-44-8], ethyl chloroformate [CAS No. 541-41-3], methyl chloroformate [CAS No. 79-21-1], methanol [CAS No. 79-21-1], and synthetic free radicals DPPH (1,1-diphenyl-2-picrylhydrazyl) [CAS No. 79-21-1] and ABTS (2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) [CAS No. 79-21-1] were purchased from Sigma-Aldrich, Merck (Darmstadt, Germany).

Melting points were measured on a Boetius PHMKO5 hot stage apparatus (Carl Zeiss Jena, Germany) and are uncorrected. IR spectra were measured on a VERTEX 70 FT-IR spectrometer (Bruker Optics, Ettlingen, Germany). High resolution mass spectral measurements were performed on a Bruker mass spectrometer. ^{1}H-, ^{13}C-NMR spectra were measured on Bruker Avance AV600 spectrometer (Bruker, Billerica, MA, USA) at BAS-IOCCP - Sofia, and chemical shifts (δ, ppm) are downfield from TMS. To average out the rotamers observed in quercetin-*N*-acyl benzazole hybrids reaching adequate assignment of peaks and structure determination, the spectra were measured at 80 °C in DMSO-d_6, as indicated in the text below. TLC was done on precoated 0.2 mm Merck silica gel 60 plates. Silica gel was used for column chromatography. The absorbance of the antioxidant assay was measured by a Spectroquant Pharo 300, UV/Vis spectrophotometer.

3.1. Synthetic Procedures

3.1.1. Synthesis of Quercetin Hybrids (**6a**, **6b**), General Procedure

The corresponding alkyl chloroformate (ethyl chloroformate—3 mmol, 0.29 mL or methyl chloroformate—3 mmol, 0.24 mL) was added dropwise to a magnetically stirred solution of benzothiazole (3 mmol, 0.33 mL) in acetonitrile (10 mL/mmol), followed immediately by quercetin (0.302 g, 1 mmol). The reaction was continued under the conditions described in Table 1. After completion of the reaction (monitored by TLC), the solvent was evaporated under reduced pressure, and the crude mixture was dry-loaded onto silica gel. The products were isolated by column chromatography on silica gel with mixtures of petroleum/diethyl ether as the eluents and successfully crystallized.

3.1.2. Synthesis of Quercetin Hybrids (**7a**, **8a**, **8b**), General Procedure

The corresponding alkyl chloroformate (ethyl chloroformate—2 mmol, 0.19 mL or methyl chloroformate—2 mmol, 0.16 mL) was added dropwise to a magnetically stirred at 0 °C solution of benzimidazole (2 mmol, 0.236 g) or 5,6-dimethylbenzimidazole (2 mmol, 0.292 g) and triethylamine (2 mmol, 0.28 mL) in acetonitrile (10 mL/mmol). After 20 min. another 2 mmol alkyl chloroformate were added, followed immediately by quercetin (0.302 g, 1 mmol). The reaction was then continued under the conditions described in Table 1. After completion of the reaction (monitored by TLC), the solvent was evaporated under reduced pressure, the residue was dissolved in chloroform (20 mL) and washed twice with water (40 mL). The organic layer was dried (Na_2SO_4), and the crude mixture was dry-loaded onto silica gel. The products were isolated by column chromatography on silica gel with mixtures of petroleum/diethyl ether as the eluents and successfully crystallized.

3.2. Radical Scavenging Activities

3.2.1. DPPH Free Radical Scavenging Assay

The DPPH free radical scavenging activities were measured as previously reported by Docheva et al. [30]: 0.12 mM DPPH was dissolved in methanol. The absorbance change was measured at 515 nm on a UV-Vis spectrophotometer within 30 min. The total DPPH radical scavenging activity within 30 min was measured in triplicate in the absence of light. The blank sample was prepared as above by replacing the test sample with equivalent methanol. The radical scavenging activity (RSA%) was calculated. IC_{50} value determined the effective concentration at which 50% of DPPH radicals were scavenged, and it was obtained by interpolation from linear regression analysis. A lower IC_{50} value indicates a higher antioxidant activity.

3.2.2. ABTS Free Radical Scavenging Assay

The ABTS free radical was prepared by the method of Re et al. [32] with some modification. ABTS radical cation ($ABTS^{\bullet+}$) was produced by 7 mM ABTS and 2.45 mM $K_2S_2O_8$ dissolved in deionized H_2O (the mixture stayed in the dark at room temperature for 12–16 h before use). A mixture of the reagents as 1:1 (*v*/*v*) $ABTS^{\bullet+}$ solution was diluted with methanol to an absorbance of 0.70 ± 0.02 at 734 nm. The ABTS radical scavenging activity within 10 min was measured in triplicate in the absence of light at room temperature. The percentage inhibition (%) of radical scavenging activity was calculated according to the corresponding equation of the method.

The stock solution of all compounds was prepared in a concentration of 1 mg/mL. The working solutions were prepared by dissolving aliquot parts of the stock solution with methanol. The final concentration of IC_{50} was calculated according to the dilution factor. Statistical analysis from the antioxidant activity was analyzed using SPSS. Data were mean ± S.D. The experiments were performed three times.

Quercetin: ^{1}H-NMR (600 MHz, 80 °C, DMSO-d_6, δ ppm, *J* Hz): 6.20 (d, *J* = 2.4, 1H, C-6), 6.40 (d, *J* = 1.8, 1H, C-8), 6.90 (d, *J* = 8.2, 1H, C-5′), 7.55 (dd, *J* = 2.4, 6.4, 1H, C-6′), 7.67 (d, *J* = 1.8, 1H, C-2′), 8.97 (brs, 3H, 3xOH, C-3′, C-3, C-4′), 10.25 (brs, 1H, OH, C-7), 12.35 (brs, 1H, OH, C-5);

^{13}C-NMR (150 MHz, 80 °C, DMSO-d_6, δ ppm, *J* Hz): 93.9, 98.8, 103.6, 115.8, 116.2, 120.6, 122.7, 136.2, 145.6, 147.5, 148.2, 156.8, 161.3, 164.5, 176.4;

Compound **6a** (Ethyl 2-(2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4-oxo-4H-chromen-8-yl)benzo[d]thiazole-3(2H)-carboxylate): pale yellow solid, isolated by column chromatography on silica gel with mixtures of petroleum/diethyl ether (8:1, increasing polarity to 2:1), yield: 45%, m.p. = 158–161 °C;

^{1}H-NMR (600 MHz, 80 °C, DMSO-d_6, δ ppm, *J* Hz): 0.9 (t, *J* = 7.0, 3H, $CO_2CH_2\underline{CH_3}$), 3.99–4.04 (m, 2H, $CO_2\underline{CH_2}CH_3$), 6.21 (s, 1H, C-6), 6.85 (d, *J* = 8.2, 1H, C-5′), 6.97 (t, *J* = 7.6, 1H, CH-Benzothiazole), 7.07 (t, *J* = 7.6, 1H, CH-Benzothiazole), 7.18 (d, *J* = 7.6, 1H, CH-Benzothiazole), 7.49 (m, 1H, C-6′), 7.52 (s, 1H, *CH), 7.72 (d, *J* = 2.4, 1H, C-2′), 7.78 (d, *J* = 8.2,

1H, CH-Benzothiazole), 9.01 (brs, 2H, 2xOH, C-3′, C-3), 9.33 (s, 1H, OH, C-4′), 10.86 (brs, 1H, OH, C-7), 12.58 (brs, 1H, OH, C-5);

^{13}C-NMR (150 MHz, 80 °C, DMSO-d_6, δ ppm, *J* Hz): 12.1, 56.2, 60.1, 97.1, 101.1, 104.7, 113.8, 114.2, 114.4, 118.7, 119.3, 121.6, 121.7, 123.2, 123.9, 134.3, 137.8, 143.7, 145.9, 146.4, 150.8, 151.0, 158.8, 161.3, 174.6;

HRMS m/z (ESI): calcd for $C_{25}H_{18}NO_9S^-$ $[M-H]^-$ 508.0708, found 508.0708;

IR (KBr, cm^{-1}): 3298 (O-H), 1695 (C=O), 1647 (C=O), 1602, 1559, 1514, 1472 (C=C), 1379 (O-H), 1339 (C-N), 1304 (C-H), 1270 (C-O-C), 1246 (C-O), 1198 (C-CO-C), 1046, 1005 (C-N), 864, 829 (C-H), 745 (C-S-C), 650, 601, 521 (C-N-C);

Compound **6b** (Methyl 2-(2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4-oxo-4H-chromen-8-yl)benzo[d]thiazole-3(2H)-carboxylate): pale yellow solid, isolated by column chromatography on silica gel with mixtures of petroleum/diethyl ether (8:1, increasing polarity to 2:1), yield: 37%, m.p. = 160–162 °C;

^{1}H-NMR (600 MHz, 80 °C, DMSO-d_6, δ ppm, *J* Hz): 3.60 (s, 3H, CO_2CH_3), 6.19 (s, 1H, C-6), 6.85 (d, *J* = 8.2, 1H, C-5′), 6.98 (t, *J* = 7.6, 1H, CH-Benzothiazole), 7.08 (t, *J* = 7.6, 1H, CH-Benzothiazole), 7.18 (d, *J* = 7.6, 1H, CH-Benzothiazole), 7.48 (m, 1H, C-6′), 7.50 (s, 1H, *CH), 7.71 (d, *J* = 2.4, 1H, C-2′), 7.73 (d, *J* = 8.2, 1H, CH-Benzothiazole), 9.03 (brs, 2H, 2xOH, C-3′, C-3), 9.21 (brs, 1H, OH, C-4′), 10.83 (brs, 1H, OH, C-7), 12.57 (s, 1H, OH, C-5);

^{13}C-NMR (150 MHz, 80 °C, DMSO-d_6, δ ppm, *J* Hz): 53.3, 58.4, 99.0, 100.5, 103.1, 115.7, 116.3, 116.4, 120.6, 121.2, 121.3, 122.6, 123.7, 125.1, 136.2, 139.6, 145.7, 147.9, 148.3, 152.9, 153.4, 160.7, 163.1, 174.5;

HRMS m/z (ESI): calcd for $C_{24}H_{16}NO_9S^-$ $[M-H]^-$ 494.0551, found 494.0550;

IR (KBr, cm^{-1}): 3337 (O-H), 1676 (C=O), 1652 (C=O), 1601, 1559, 1515, 1473 (C=C), 1370 (O-H), 1320 (C-N), 1316 (C-H), 1271 (C-O-C), 1253 (C-O), 1191 (C-CO-C), 1048, 1003 (C-N), 814, 787 (C-H), 748 (C-S-C), 648, 599, 519 (C-N-C);

Compound **7a** (Diethyl 2-(2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4-oxo-4H-chromen-8-yl)-1H-benzo[d]imidazole-1,3(2H)-dicarboxylate): pale yellow solid, isolated by column chromatography on silica gel with mixtures of petroleum/diethyl ether (8:1, increasing polarity to 4:1), yield: 59%, m.p. = 172–174 °C;

^{1}H-NMR (600 MHz, 80 °C, DMSO-d_6, δ ppm, *J* Hz): 0.90 (brs, 6H, 2x$CO_2CH_2\underline{CH_3}$), 3.98 (brs, 4H, 2x$CO_2\underline{CH_2}CH_3$), 6.14 (s, 1H, C-6), 6.91 (d, *J* = 8.2, 1H, C-5′), 6.98–7.01 (m, 2H, 2xCH-Benzimidazole), 7.61 (brs, 2H, 2xCH-Benzimidazole), 7.68 (s, 1H, *CH), 7.73 (brs, 1H, C-6′), 7.84 (s, 1H, C-2′), 9.01 (brs, 3H, 3xOH, C-3′, C-3, C-4′), 10.67 (brs, 1H, OH, C-7), 12.62 (s, 1H, OH, C-5);

^{13}C-NMR (150 MHz, 80 °C, DMSO-d_6, δ ppm, *J* Hz): 14.0, 61.9, 69.9, 98.8, 102.7, 104.3, 113.1, 115.7, 116.4, 120.9, 123.0, 133.6, 136.1, 145.7, 148.3, 151.1, 154.4, 161.1, 163.2, 176.6;

HRMS m/z (ESI): calcd for $C_{28}H_{23}N_2O_{11}^-$ $[M-H]^-$ 563.1307, found 563.1320;

IR (KBr, cm^{-1}): 3325 (O-H), 1703 (C=O), 1690 (C=O), 1650 (C=O), 1600, 1559, 1515, 1505, 1440 (C=C), 1383 (O-H), 1319 (C-N), 1310 (C-H), 1284 (C-O-C), 1262 (C-O), 1195 (C-CO-C), 1050, 1003 (C-N), 825, 798, 757 (C-H), 706, 651, 604, 534 (C-N-C);

Compound **8a** (Diethyl 2-(2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4-oxo-4H-chromen-8-yl)-5,6-dimethyl-1H-benzo[d]imidazole-1,3(2H)-dicarboxylate): pale yellow solid, isolated by column chromatography on silica gel with mixtures of petroleum/diethyl ether (8:1, increasing polarity to 4:1), yield: 61%, m.p. = 163–165 °C;

^{1}H-NMR (600 MHz, 80 °C, DMSO-d_6, δ ppm, *J* Hz): 0.84 (brs, 6H, 2x$CO_2CH_2\underline{CH_3}$), 2.22 (s, 6H, 2xCH_3-5,6-dimethylbenzimidazole), 3.97 (brs, 4H, 2x$CO_2\underline{CH_2}CH_3$), 6.11 (s, 1H, C-6), 6.90 (d, *J* = 8.2, 1H, C-5′), 7.41 (brs, 2H, 2xCH-5,6-dimethylbenzimidazole), 7.63 (s, 1H, *CH), 7.67 (m, 1H, C-6′), 7.82 (s, 1H, C-2′), 8.99 (brs, 1H, OH, C-3′), 9.02 (brs, 1H, OH, C-3), 9.19 (brs, 1H, OH, C-4′), 10.67 (brs, 1H, OH, C-7), 12.63 (s, 1H, OH, C-5);

^{13}C-NMR (150 MHz, 80 °C, DMSO-d_6, δ ppm, *J* Hz): 14.0, 19.8, 61.7, 69.9, 98.8, 102.7, 104.3, 114.5, 114.6, 114.7, 115.7, 116.3, 120.9, 122.8, 130.1, 141.9, 145.7, 148.3, 151.0, 154.4, 161.1, 163.1, 176.5;

HRMS m/z (ESI): calcd for $C_{30}H_{27}N_2O_{11}^-$ $[M-H]^-$ 591.1620, found 591.1624;

IR (KBr, cm^{-1}): 3359 (O-H), 1720 (C=O), 1684 (C=O), 1653 (C=O), 1601, 1515, 1409 (C=C), 1383 (O-H), 1319 (C-N), 1296 (C-O-C), 1261 (C-O), 1199 (C-CO-C), 1080, 1002 (C-N), 806, 786, 754 (C-H), 704, 658, 600, 533 (C-N-C);

Compound **8b** (Dimethyl 2-(2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4-oxo-4H-chromen-8-yl)-5,6-dimethyl-1H-benzo-[d]imidazole-1,3(2H)-dicarboxylate): pale yellow solid, isolated by column chromatography on silica gel with mixtures of petroleum/diethyl ether (8:1, increasing polarity to 4:1), yield: 51%, m.p. = 160–163 °C;

^{1}H-NMR (600 MHz, 80 °C, DMSO-d_6, δ ppm, J Hz): 2.22 (s, 6H, 2xCH_3-5, 6-dimethylbenzimidazole), 3.55 (s, 6H, 2x$CO_2\underline{CH_3}$), 6.10 (s, 1H, C-6), 6.89 (d, J = 8.2, 1H, C-5′), 7.36 (brs, 2H, 2xCH-5,6-dimethylbenzimidazole), 7.62 (s, 1H, *CH), 7.64 (brs, 1H, C-6′), 7.80 (s, 1H, C-2′), 8.99 (brs, 2H, OH, 2xOH, C-3′, C-3), 9.18 (brs, 1H, OH, C-4′), 10.67 (brs, 1H, OH, C-7), 12.65 (s, 1H, OH, C-5);

^{13}C-NMR (150 MHz, 80 °C, DMSO-d_6, δ ppm, J Hz): 19.8, 52.9, 70.1, 98.7, 102.8, 104.0, 114.7, 115.7, 116.3, 120.9, 122.8, 130.2, 131.4, 136.1, 144.5, 145.7, 147.8, 148.3, 151.7, 161.1, 163.2, 176.6;

HRMS m/z (ESI): calcd for $C_{28}H_{23}N_2O_{11}^-$ $[M-H]^-$ 563.1307, found 563.1309;

IR (KBr, cm^{-1}): 3353 (O-H), 1689 (C=O), 1653 (C=O), 1602, 1515, 1447 (C=C), 1385 (O-H), 1304 (C-N), 1304 (C-O-C), 1262 (C-O), 1201 (C-CO-C), 1128, 1002 (C-N), 807, 786, 752 (C-H), 699, 668, 601, 534 (C-N-C).

4. Conclusions

We have successfully synthesized five new quercetin–benzazole hybrids, employing an efficient one-pot approach. The applied reaction of amidoalkylation offers a convenient synthetic route for modification of quercetin by introducing a heterocyclic moiety. The radical scavenging activity of the newly obtained quercetin derivatives gives a good lead for further structural optimization.

Supplementary Materials: S1.PDF—Processed ^{1}H-, ^{13}C-NMR, HSQC, MS, and IR spectra. Quercetin hybrids—**6**–**8 a**, **b** mol files.

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Data Availability Statement: The data presented in this study are available in this article and supporting information.

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References

1. Panche, A.N.; Diwan, A.D.; Chandra, S.R. Flavonoids: An overview. *J. Nutr. Sci.* **2016**, *5*, e47. [CrossRef] [PubMed]
2. Tapas, A.R.; Sakarkar, D.M.; Kakde, R.B. Flavonoids as Nutraceuticals: A Review. *Trop. J. Pharm. Res.* **2008**, *7*, 1089–1099. [CrossRef]
3. Lesjak, M.; Beara, I.; Simin, N.; Pintać, D.; Majkić, T.; Bekvalac, K.; Orčić, D.; Mimica-Dukić, N. Antioxidant and anti-inflammatory activities of quercetin and its derivatives. *J. Funct. Foods* **2018**, *40*, 68–75. [CrossRef]
4. Tian, C.; Liu, X.; Chang, Y.; Wang, R.; Lv, T.; Cui, C.; Liu, M. Investigation of the anti-inflammatory and antioxidant activities of luteolin, kaempferol, apigenin and quercetin. *S. Afr. J. Bot.* **2021**, *137*, 257–264. [CrossRef]
5. Tomečková, V.; Reháková, M.; Mojžišová, G.; Magura, J.; Wadsten, T.; Zelenáková, K. Modified natural clinoptilolite with quercetin and quercetin dihydrate and the study of their anticancer activity. *Microporous Mesoporous Mater.* **2012**, *147*, 59–67. [CrossRef]

6. Mateus, P.G.; Wolf, V.G.; Borges, M.S.; Ximenes, V.F. Quercetin: Prooxidant Effect and Apoptosis in Cancer. *Stud. Nat. Prod. Chem.* **2018**, *58*, 265–285. [CrossRef]
7. Çelik, H.; Koşar, M. Inhibitory effects of dietary flavonoids on purified hepatic NADH-cytochrome b5 reductase: Structure-activity relationships. *Chem. Biol. Interact.* **2012**, *197*, 103–109. [CrossRef]
8. Hur, W.; Gray, N.S. Small molecule modulators of antioxidant response pathway. *Curr. Opin. Chem. Biol.* **2011**, *15*, 162–173. [CrossRef]
9. Carrasco-Pozo, C.; Mizgier, M.L.; Speisky, H.; Gotteland, M. Differential protective effects of quercetin, resveratrol, rutin and epigallocatechin gallate against mitochondrial dysfunction induced by indomethacin in Caco-2 cells. *Chem. Biol. Interact.* **2012**, *195*, 199–205. [CrossRef]
10. Fernández, M. Effect of lipophilization on the antioxidant activity of carvacrol, quercetin and vanillin with conjugated linoleic acid. *Rev. Mex. Ing. Química* **2019**, *18*, 637–645. [CrossRef]
11. Antonio, A.S.; Wiedemann, L.S.M.; Veiga-Junior, V.F. Natural products' role against COVID-19. *RSC Adv.* **2020**, *10*, 23379–23393. [CrossRef]
12. Diniz, L.R.L.; Souza, M.T.S.; Diarte, A.B.S.; Sousa, D.P. Mechanistic Aspects and Therapeutic Potential of Quercetin against COVID-19-Associated Acute Kidney Injury. *Molecules* **2020**, *25*, 5772. [CrossRef] [PubMed]
13. Pierro, F.D.; Iqtadar, S.; Khan, A.; Mumtaz, S.U.; Chaudhry, M.M.; Bertuccioli, A.; Derosa, G.; Maffioli, P.; Togni, S.; Riva, A.; et al. Potential clinical benefits of quercetin in the early stage of COVID-19: Results of a second, pilot, randomized, controlled and open-label clinical trial. *Int. J. Gen. Med.* **2021**, *14*, 2807–2816. [CrossRef] [PubMed]
14. Kim, S.H.; Kumar, C.N.; Kim, H.J.; Kim, D.H.; Cho, J.; Jin, C.; Lee, Y.S. Glucose-containing flavones-their synthesis and antioxidant and neuroprotective activities. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6009–6013. [CrossRef] [PubMed]
15. Gansukh, E.; Nile, A.; Kim, D.H.; Oh, J.W.; Nile, S.H. New insights into antiviral and cytotoxic potential of quercetin and its derivatives—A biochemical perspective. *Food Chem.* **2021**, *334*, 127508. [CrossRef] [PubMed]
16. Mazzotta, S.; Carullo, G.; Sciubba, F.; Di Cocco, M.E.; Aiello, F. 7-Docosahexaenoyl-Quercetin. *Molbank* **2021**, *2021*, M1203. [CrossRef]
17. Kukhareva, T.S.; Krasnova, V.A.; Koroteev, M.P.; Kaziev, G.Z.; Kuleshova, L.N.; Korlyukov, A.A.; Antipin, M.Y.; Nifant'ev, E.E. Electrophilic substitution in the dihydroquercitin system. Aminomethylation. *Russ. J. Org. Chem.* **2004**, *40*, 1190–1193. [CrossRef]
18. Zask, A.; Nakanishi, K.; Ellestad, G.; Sparrow, J.R. Antioxidant Flavonoid Derivatives. Patent WO2014110551A1. Available online: https://patents.google.com/patent/WO2014110551A1 (accessed on 17 July 2014).
19. Hoang, T.K.D.; Huynh, T.K.C.; Do, T.H.T.; Nguyen, T.D. Mannich aminomethylation of flavonoids and anti-proliferative activity against breast cancer cell. *Chem. Pap.* **2018**, *72*, 1399–1406. [CrossRef]
20. Ilkei, V.; Spaits, A.; Prechl, A.; Müller, J.; Konczol, A.; Levai, S.; Riethmüller, E.; Szigetvari, A.; Beni, Z.; Dekany, M.; et al. C8-selective biomimetic transformation of 5,7-dihydroxylated flavonoids by an acid-catalysed phenolic Mannich reaction: Synthesis of flavonoid alkaloids with quercetin and (–)-epicatechin skeletons. *Tetrahedron* **2017**, *73*, 1503–1510. [CrossRef]
21. Dhadda, S.; Raigar, A.K.; Saini, K.; Manju; Guleria, A. Benzothiazoles: From recent advances in green synthesis to anti-cancer potential. *Sustain. Chem. Pharm.* **2021**, *24*, 100521. [CrossRef]
22. Keri, R.S.; Patil, M.R.; Patil, S.A.; Budagupi, S. A comprehensive review in current developments of benzothiazole-based molecules in medicinal chemistry. *Eur. J. Med. Chem.* **2015**, *89*, 207–251. [CrossRef] [PubMed]
23. Pathare, B.; Bansode, T. Review- biological active benzimidazole derivatives. *Results Chem.* **2021**, *3*, 100200. [CrossRef]
24. Gümüş, M.; Yakan, M.; Koca, I. Recent advances of thiazole hybrids in biological applications. *Future Med. Chem.* **2019**, *11*, 1979–1998. [CrossRef]
25. El-Meguid, E.A.A.; Moustafa, G.O.; Awad, H.M.; Zaki, E.R.; Nossier, E.S. Novel benzothiazole hybrids targeting EGFR: Design, synthesis, biological evaluation and molecular docking studies. *J. Mol. Struct.* **2021**, *1240*, 130595. [CrossRef]
26. Stremski, Y.; Statkova-Abeghe, S. (E)-2-(2-oxo-4-phenylbut-3-en-1-yl)benzo[d]thiazole- 3(2H)-carboxylates. *Molbank* **2021**, *2021*, M1236. [CrossRef]
27. Stremski, Y.; Ahmedova, A.; Dołęga, A.; Statkova-Abeghe, S.; Kirkova, D. Oxidation step in the preparation of benzocamalexin: The crystallographic evidence. *Mendeleev Commun.* **2021**, *31*, 824–826. [CrossRef]
28. Stremski, Y.; Kirkova, D.; Statkova-Abeghe, S.; Angelov, P.; Ivanov, I.; Georgiev, D. Synthesis and antibacterial activity of hydroxylated 2-arylbenzothiazole derivatives. *Synth. Commun.* **2020**, *50*, 3007–3015. [CrossRef]
29. Kyriakou, E.; Primikyri, A.; Charisiadis, P.; Katsoura, M.; Gerothanassis, I.P.; Stamatis, H.; Tzakos, A.G. Unexpected enzyme-catalyzed regioselective acylation of flavonoid aglycones and rapid product screening. *Org. Biomol. Chem.* **2012**, *10*, 1739–1742. [CrossRef]
30. Docheva, M.; Dagnon, S.; Statkova-Abeghe, S. Flavonoid content and radical scavenging potential of extracts prepared from tobacco cultivars and waste. *Nat. Prod. Res.* **2014**, *28*, 1328–1334. [CrossRef]
31. Kirkova, D.; Statkova-Abeghe, S.; Docheva, M.; Stremski, Y.; Minkova, S. Structure-Activity Relationship of in Vitro Radical-Scavenging Activity of 2-(Hydroxyphenyl) Benzothiazole Derivatives. *Bulg. Chem. Commun.* **2020**, *52*, 196–200.
32. Re, R.; Pellegrini, N.; Proteggente, A.; Pannala, A.; Yang, M.; Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic. Biol. Med.* **1999**, *26*, 1231–1237. [CrossRef]