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Synthesis and biological properties of novel 1-methyl-2-(2-(prop-2-yn-1-yloxy)benzylidene) hydrazine analogues

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Abstract: 1-Methyl-2-(2-(prop-2-yn-1-yloxy)benzylidene)hydrazine analogues were readily prepared in good yields by the reaction of 2-(prop-2-yn-1-yloxy)benzaldehydes and methyl hydrazine. The reaction tolerates a variety of substituents on the 2-hydroxybenzaldehyde to form nitro-, halo-, methoxy-, and naphthyl-substituted 1-methyl-2-(2-(prop-2-yn-1-yloxy)benzylidene)hydrazines. The in vitro antioxidant capacity measurements revealed that among all the analyzed hydrazine analogues that surpassed the Trolox standard, 1-(2-(but-3-ynyl)-5-nitrobenzylidene)-2-methylhydrazine had the maximum value, which was approximately 1.7 times that of Trolox.

Key words: Hydrazines, hydrazones, ABTS, antioxidant capacities, biological properties

1. Introduction

Hydrazones and hydrazines have gained considerable interest in recent years owing to their wide variety of biological and pharmacological properties.[1–5] Hydrazones are important sources for the synthesis of heterocyclic compounds[6–8] and they are members of Schiff base family, comprehensively studied because of their catalytic properties in various fields and partly for their biological activity.[9–14] Those versatile organic compounds have a general formula of $R_1R_2C = NNR_3R_4$ in which there are two amino-type nucleophilic nitrogen atoms and a carbon atom with nucleophilic and electrophilic character.[15] Both of them constitute the active centers of hydrazines; they generate the characteristic physical and chemical properties of hydrazones.[16,17]

Hydrazones have some common properties of being easy to prepare, a tendency toward crystallinity, and better hydrolytic stability relative to imines. Their synthesis is generally performed by the reaction of hydrazine with aldehydes, ketones, and other such carbonyl compounds in solvents like ethanol, methanol, and butanol.[18–20] Heteroaryl Schiff bases possess additional donor sites with respect to hydrazone Schiff bases and this brings a versatility that makes them good chelating agents.[21] Moreover, they have a relatively high tendency to yield stereochemistry of higher coordination numbers, neutral or deprotonated donor properties, and flexibility providing different conformations.[12,16]

The synthesis and the evaluation of possible antimicrobial,[22,23] antifungal,[24] antitumoral,[25,26] antiinflammatory,[27,28] analgesic,[29] antiproliferative,[30] and antitubercular[31–33] activities of hydrazone derivatives have

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growing popularity for organic chemists and pharmaceutical experts. Those compounds perform their actions in diverse biochemical ways mainly based on their being effective chelating agents,[34] metal ion scavengers,[9] and pharmacophoric inhibitors of some key enzymes in metabolic pathways with factors leading to cytotoxicity that could be used in pharmacological strategies against many diseases and disorders.[35] No matter which mode of operation they have, most efforts spent on these molecules to offer alternative drugs with less toxicity and higher activity lead researchers to first evaluate the basic properties of new derivatives, such as antioxidant capacities.[36,37]

Free radicals are biochemically reactive molecules generated during normal metabolic processes; however, their amounts reach critical levels under oxidative stress conditions. In such circumstances, reactive oxygen species (ROS) are produced. Natural or synthetic antioxidant compounds prevent the formation of those compounds or neutralize them, and they are also used in repair of the damage caused by ROS.[38,39]

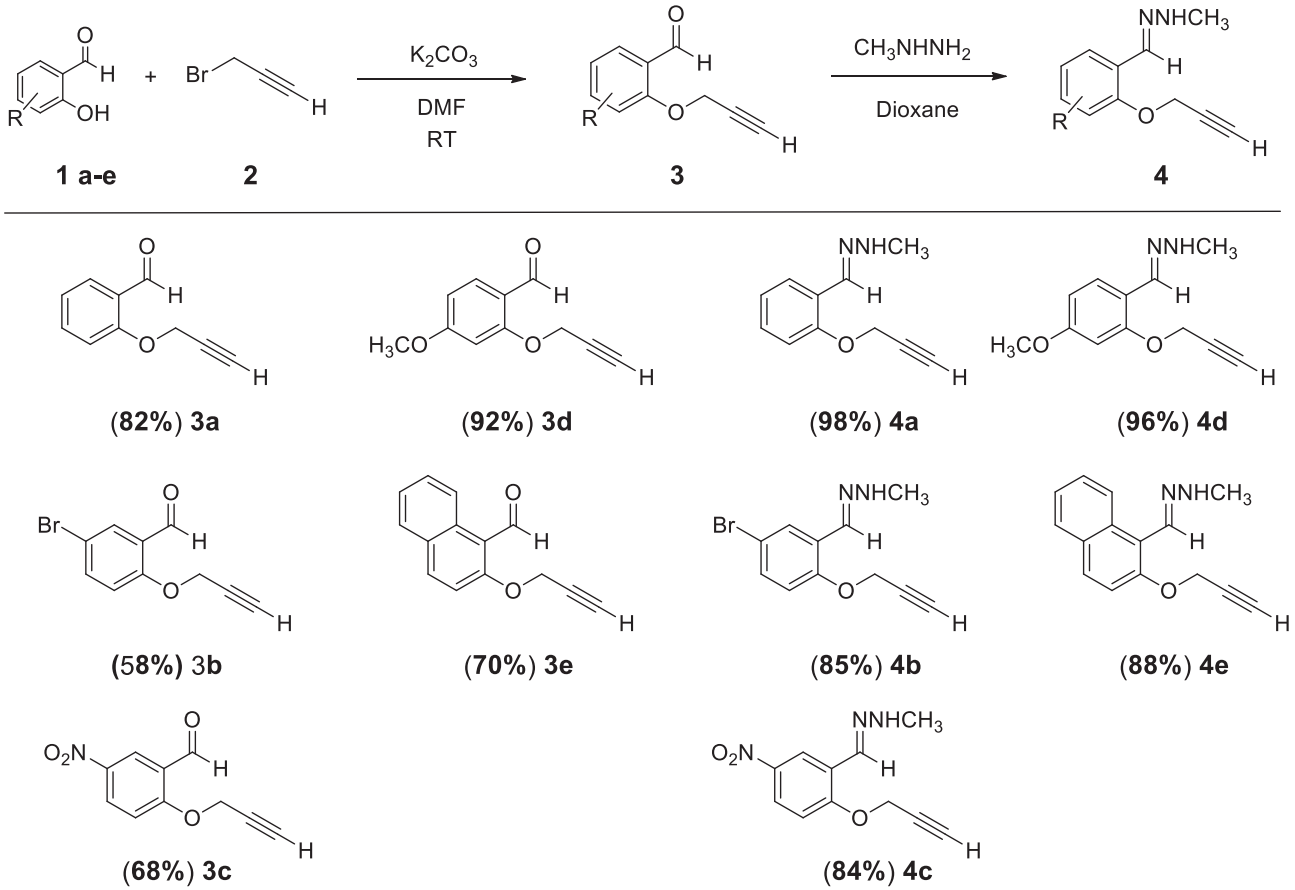
In the present study, novel hydrazone derivatives were designed and synthesized via condensation reactions between methyl hydrazine and 2-(prop-2-yn-1-yloxy)benzaldehyde derivatives. Their antioxidant potentials were also tested using the ABTS assay. In this assay, 2,2'-azino-bis(3-ethylbenzthiazoline-6-acid) (ABTS) is turned into its blue/green radical cation (ABTS $^{\bullet}+$) with the action of potassium persulfate, which is a strong oxidizing agent. The ABTS $^{\bullet}+$ cation is highly reactive towards antioxidant compounds and soluble in both aqueous and organic solvents. This makes it very useful for the determination of the antioxidant capacity of both lipophilic and hydrophilic antioxidants.[40] In addition, this assay can be applied over a wide pH range without affecting the ionic strength of medium.[41]

2. Results and discussion

2.1. Chemistry

A variety of novel methyl hydrazones, namely 1-(2-(but-3-ynyl)benzylidene)-2-methylhydrazine (**4a**), 1-(5-bromo-2-(but-3-ynyl)benzylidene)-2-methylhydrazine (**4b**), 1-(2-(but-3-ynyl)-5-nitrobenzylidene)-2-methylhydrazine (**4c**), 1-(2-(but-3-ynyl)-4-methoxybenzylidene)-2-methylhydrazine (**4d**), and 1-((2-(ethynyloxy)naphthalen-1-yl)methylene)-2-methylhydrazine (**4e**), were synthesized. Initially, varieties of 2-(prop-2-yn-1-yloxy)benzaldehyde **3** were prepared from the corresponding 2-hydroxybenzaldehyde derivatives (**1**) by using literature procedures. A simple method for the preparation of 2-(prop-2-yn-1-yloxy)benzaldehyde **3** involves the S_N2 nucleophilic substitution reactions with propargyl bromide **2** under basic conditions. Using this approach, a variety of 2-(prop-2-yn-1-yloxy)benzaldehydes (**3a**, **3b**, **3c**, **3d**, and **3e**) were synthesized as starting compounds (Scheme). When 2-hydroxybenzaldehyde was allowed to react with propargyl bromide **2** in the presence of potassium carbonate, corresponding **3a** was formed in 82% yields. While the highest yield (92%) was obtained from the reaction between 2-hydroxyl-4-methoxybenzaldehyde **1d** and propargyl bromide **2**, the bromo-substituted aldehyde **1b** gave a very low yield of the expected intermediate **3b**. Moreover, **3a**, **3c**, and **3e** were also isolated in good yields (Scheme).

After preparation and characterization of 2-(prop-2-yn-1-yloxy)benzaldehyde **3**, the condensation reactions between **3a**–**3d** and methyl hydrazine were used for the formation of corresponding hydrazone derivatives (**4a**–**4e**). When **3a** underwent a condensation reaction with methyl hydrazine in dioxane at room temperature under inert atmosphere, desired product **4a** was obtained in 98% yield. The scope and limitations were tested for a variety of groups including electron-withdrawing groups (NO_2), electron-donating groups (MeO-), halogen (Br), and poly-aromatics (naphthyl). When **3b** was allowed to react with methyl hydrazine for the formation



Scheme. Synthesis of 1-(2-(but-3-ynyl)benzylidene)-2-methylhydrazine **4**.

of **4b**, the yield was found to be 85%. When a strong electron-withdrawing nitro group present on phenyl **3c** reacted with the same hydrazine to form the corresponding hydrazone, the yield (84%) of **4c** was found relatively lower than that of electron-donating methoxy-substituted aromatic **4d**(96%). Moreover, poly-aromatic compound **3e** was applied for the synthesis of corresponding hydrazone **4e** and the yield obtained was 88%. These results showed that the reactions between **3** and methyl hydrazine can be easily used in synthesis of novel hydrazone derivatives **4**. In addition, these hydrazones may be readily elaborated for more complex products from propargyl groups by using known chemistry.[42–44]

^{1}H NMR and ^{13}C NMR spectra of **4a**–**4e** were recorded in $CDCl_3$ solvent. Aromatic peaks were observed between 7 and 9 ppm. Moreover, acetylenic hydrogens (≡—) gave rise to shielded hydrogens or relatively high-field chemical shifts for ^{1}H NMR. This could be explained by the cylindrical $\pi - \pi$ cloud around the carbon-carbon triple bond. The ^{1}H NMR spectra of **3** and **4** show a high-field signal due to the acetylenic hydrogen on the terminal alkyne. The acetylenic protons appear as a singlet at 2.5 ppm. Propargyl groups had also a CH_2 and it was found around 4.8 ppm. Interestingly, CH_2 was also affected by the acetylenic proton and gave a doublet on ^{1}H NMR due to long-range proton-proton couplings (Figure 1).[45] The ^{13}C NMR signals for propargyl groups of **3** and **4** were shifted upfield between 60 ppm and 80 ppm. After isolation of the desired compounds, it was observed that there was a singlet at 3 ppm for methyl's protons ($NHCH_3$) and a singlet at 7.8 ppm for hydrazone's proton (CH = N). Moreover, not only the aldehyde peaks on ^{1}H NMR but also

the carbonyl peaks on ^{13}C NMR disappeared after condensation reactions between **3** and methyl hydrazine (Figure 2).

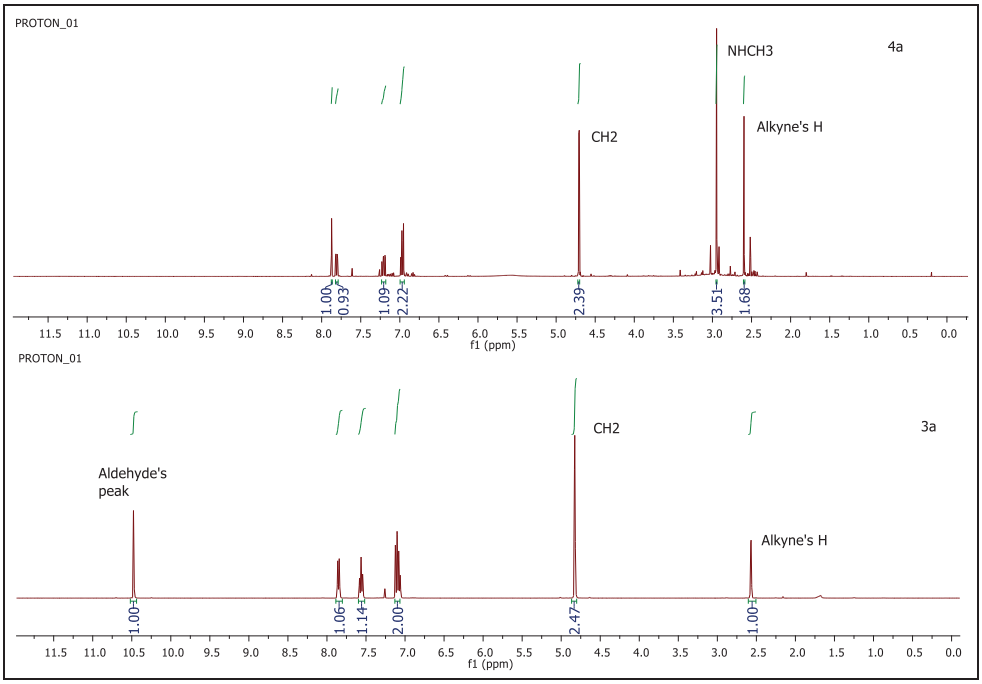


Figure 1. The comparison of ^{1}H NMR spectra for 2-(prop-2-ynyloxy)benzaldehyde **3a** and 1-(2-(but-3-ynyl)benzylidene)-2-methylhydrazine **4a**.

2.2. Antioxidant capacities

When the synthesized structures were analyzed for their electron density, it was observed that the strong electron-withdrawing groups, such as nitro groups, draw electrons away from the aromatic ring. This is known as the polar effect or electronic effect in chemistry. Therefore, electrons of hydrazone's nitrogen atoms were forced to move to the aromatic ring. As a result of the polar effect, the N-H bond in the structure of hydrazone is weakened, which increases the tendency of reacting with the ABTS molecule. It was observed that more polar hydrazones were more amenable to react with ABTS compared to less polar ones.

A possible reaction mechanism between ABTS and **4** was proposed. First, ABTS was allowed to react with the strong oxidant potassium persulfate and its cationic radical intermediates were formed. During this reaction, the colorless neutral form of ABTS is converted into its blue/green ABTS radical cation. The radical is then shifted onto the nitrogen atom between the two aromatic rings of ABTS (**7**). In the next step of the mechanism, there exists a radical substitution reaction by the interaction of the formed intermediate **7** with hydrazone **4** and colorless ABTS^{+} carbocation form **8** is generated. Meanwhile, hydrazone radical **9** is obtained mechanistically (Figure 3). The same mechanism pattern was applied for the other derivatives.

All synthesized hydrazone derivatives **4a**–**4e** were tested to find the antioxidant capacities. According to the experimental results of the ABTS assay, calculated EC_{50} values reveal an order of **4b** > **4d** > **4e** > **4a** > **4c** for all analyzed derivatives. In addition, their antioxidant capacities were found as **4c** > **4a** > **4e** > **4d** > **4b**. The highest antioxidant capacity was measured for structure **4c** including a strong electron-

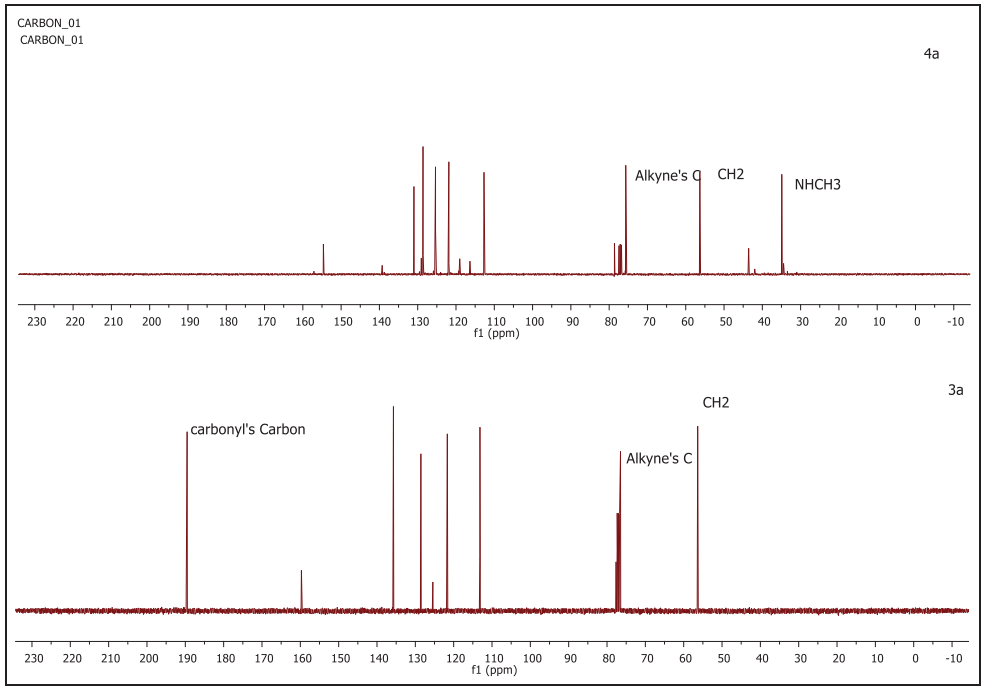


Figure 2. The comparison of ^{13}C NMR spectra for 2-(prop-2-ynyloxy)benzaldehyde **3a** and 1-(2-(but-3-ynyl)benzylidene)-2-methylhydrazine **4a**.

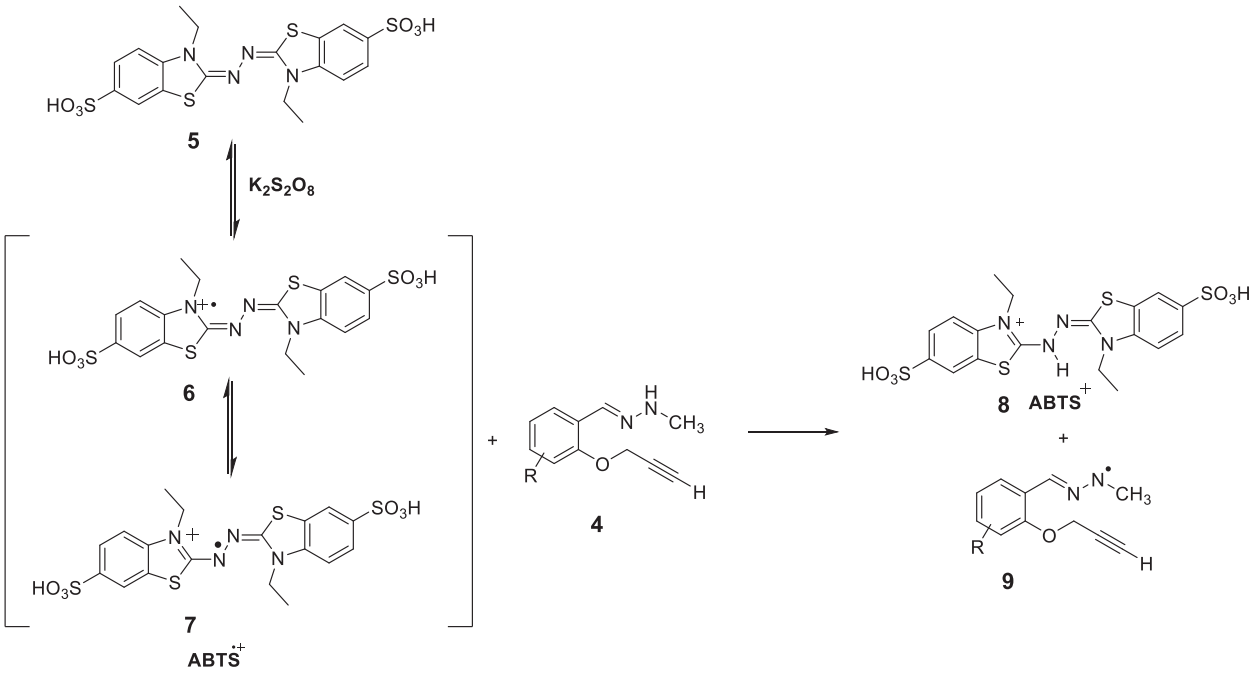


Figure 3. Proposed ABTS cation radical scavenging mechanism of hydrazone derivatives.

withdrawing nitro (NO_2) group on the aromatic ring. On the other hand, bromo-substituted **4b** displayed the least antioxidant capacity (Figure 4).

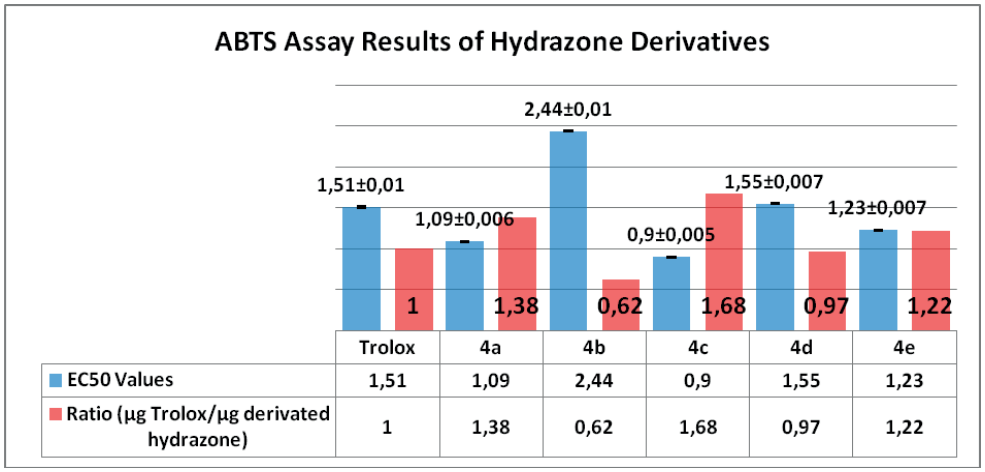


Figure 4. Antioxidant capacities of synthesized hydrazine derivatives. EC_{50} values of derivatives were calculated as the concentration (μg/mL) exhibiting 50% inhibition of the ABTS radical. Each value represents mean $\pm$ standard deviation. All the measurements were done in triplicate.

In the ABTS assay, the measured antioxidant capacities of hydrazone derivatives were compared with Trolox and they were predicated as the ratio of μg trolox/μg derivated hydrazone. Referring to Figure 4, while derivatives **4b** and **4d** could not reach the antioxidant capacity of the general standard of Trolox, **4c**, **4a**, and **4e** surpassed this level by different percentages ranging from 22% to 68%. By exceeding the antioxidant capacity of Trolox, those derivatives have considerable potential to be used in the design and further derivatization of more biologically active agents.

2.3. Conclusions

In the present study, a novel synthetic route to a variety of methyl-2-(2-(prop-2-yn-1-yloxy)benzylidene)hydrazines **4a**–**4e** by the condensation reaction of methyl hydrazine and 2-(prop-2-ynyloxy)benzaldehydes has been developed. The reaction tolerates a variety of 2-(prop-2-yn-1-yloxy)benzaldehyde and affords the corresponding hydrazones in good to excellent yields. This methodology provides a useful new route for the synthesis of substituted methyl-2-(2-(prop-2-yn-1-yloxy)benzylidene)hydrazine, which should display promising antioxidant properties. The EC_{50} values of the synthesized compounds were found to increase in the order of **4b** > **4d** > **4e** > **4a** > **4c**. **4a** and **4c** were determined as the most potent scavengers of the ABTS•+ cation radical and compound **4c** showed a considerable degree of antioxidant capacity as it exceeded the level of Trolox by about 1.7 times.

These results might help in the development of new antioxidative drugs with important pharmaceutical functions by giving the advantage of the design and further derivatization of more biologically active agents.

3. Experimental

The design, synthesis, and biological properties of novel hydrazone derivatives were studied. Synthesized molecules were determined by ^{1}H and ^{13}C NMR. ^{1}H and ^{13}C NMR spectra were recorded on an Agilent NMR (400 MHz) spectrometer. Chemical shifts were reported in parts per million (ppm) downfield from an internal trimethylsilane reference. Coupling constants (J) were reported in Hz. In addition, spin multiplicities were presented by the following symbols: s (singlet), brs (broad singlet), d (doublet), t (triplet), q (quartet), and m

(multiplet). ^{13}C NMR information was given in parentheses as C, CH, CH_2, and CH_3. Flash chromatography was performed using thick-walled glass columns and flash-grade silica (Merck 230-400 mesh). Thin-layer chromatography was performed using commercially prepared 0.25-mm silica gel plates and visualization was done with a short-wavelength UV lamp. The relative proportions of solvents in chromatography solvent mixtures referred to the volume-to-volume ratio. Absorption spectra were measured on a Thermo Scientific Multiskan GO UV-VIS spectrophotometer. All commercially available reagents were used directly without purification unless otherwise stated. All the solvents used in reaction experiments were distilled for purity. Inert atmosphere was created by slight positive pressure (ca. 0.1 psi) of argon. All glassware was dried in an oven prior to use.

3.1. Synthesis of compounds

3.1.1. General procedure for the synthesis of 2-(prop-2-ynyloxy) benzaldehydes

The corresponding 2-hydroxybenzaldehyde (10 mmol) was dissolved in DMF. Then 3-bromoprop-1-yne (1.5 equiv.) and potassium carbonate (1 equiv.) were added at room temperature. The resulting mixture was flushed with argon and stirred at room temperature for 4 h. After the reaction was over, the reaction mixture was cooled at 0 °C, filtered, and washed with distilled water to afford the desired corresponding product.

3.1.1.1. 2-(Prop-2-ynyloxy)benzaldehydes (3a)

Purification by filtration afforded the product as a light yellow solid (yield: 82%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.49 (s, 1H, aldehyde), 7.87 (d, J = 7.7 Hz; 1H), 7.58–7.56 (m, 1H), 7.12–7.10 (m, 2H), 4.83 (s, 2H, CH_2), 2.58 (s, 1H, alkynes H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 189.5, 159.7, 135.7, 128.6, 125.4, 121.7, 113.2, 77.6, 76.5, 56.3. IR (ATR) 3268 (acetylenic H), 2873, 2116 (triple bond), 1679 (carbonyl), 1581, 1480, 1456, 1329, 1286, 1220, 1006, 755, 675. The spectral data were in agreement with those reported previously for this compound.[46]

3.1.1.2. 5-Bromo-2-(prop-2-ynyloxy)benzaldehydes (3b)

Purification by filtration afforded the product as a white solid (yield: 58%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.37 (s, 1H, aldehyde), 7.93 (d, J = 2.4 Hz, 1H), 7.63 (dd, J = 8.8 and 2.5 Hz, 1H), 7.02 (d, J = 8.8 Hz, 1H), 4.81 (s, 2H, CH_2), 2.58 (t, J = 2.4 Hz, 1H, alkyne's H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 188.1, 158.6, 138.1, 131.1, 126.7, 115.3, 114.6, 77.3, 76.9, 56.6. IR (ATR) 3234 (acetylenic H), 2883, 2118 (triple bond), 1681 (carbonyl), 1589, 1479, 1406, 1329, 1286, 1219, 1018, 880, 691. The spectral data were in agreement with those reported previously for this compound.[46]

3.1.1.3. 5-Nitro-2-(prop-2-ynyloxy)benzaldehydes (3c)

Purification by filtration afforded the product as a brown solid (yield: 68%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.43 (s, 1H, aldehyde), 8.70–8.67 (m, 1H), 8.43 (dt, J = 9.2 and 2.5 Hz, 1H), 7.28 (d, J = 9.2 Hz, 1H), 4.97 (s, 2H, CH_2), 2.66 (t, J = 2.4 Hz, 1H, alkyne); ^{13}C NMR (100 MHz, $CDCl_3$) δ 187.2, 163.3, 142.1, 130.3, 125.1, 124.6, 113.7, 77.9, 76.2, 57.1. IR (ATR) 3240 (acetylenic H), 2887, 2114 (triple bond), 1661 (carbonyl), 1575, 1480, 1412, 1315, 1286, 1219, 1018, 885, 705. The spectral data were in agreement with those reported previously for this compound.[46]

3.1.1.4. 4-Methoxy-2-(prop-2-ynyloxy)benzaldehydes (3d)

Purification by filtration afforded the product as a light yellow (yield: 92%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.49 (s, 1H, aldehyde), 7.47–7.45 (m, 1H), 7.17–7.15 (m, 2H), 4.87 (s, 2H, CH_2), 3.88 (s, 3H, OMe), 2.47 (s, 1H, alkyne); ^{13}C NMR (100 MHz, $CDCl_3$) δ 190.6, 152.8, 152.6, 131.2, 124.9, 118.8, 117.6, 78.2, 76.9, 60.8, 56.0. IR (ATR) 3281 (acetylenic H), 2936, 2119 (triple bond), 1688 (carbonyl), 1588, 1472, 1412, 1345, 1273, 1062, 1065, 990, 785. The spectral data were in agreement with those reported previously for this compound.[47]

3.1.1.5. 2-(Ethynyloxy)-1-naphthaldehyde (3e)

Purification by filtration afforded the product as a light yellow (yield: 70%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.9 (s, 1H, aldehyde), 9.27 (d, J = 8.7 Hz, 1H), 8.09–8.06 (m, 1H), 7.81–7.79 (m, 1H), 7.65–7.63 (m, 1H), 7.45–7.43 (m, 1H), 7.39–7.36 (m, 1H), 4.94 (s, 2H, CH_2), 2.77 (t, J = 2.3 Hz, 1H, alkyne's H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.7, 161.9, 137.3, 131.4, 129.9, 129.1, 128.2, 125.2, 125.1, 118.0, 113.9, 77.6, 76.7, 57.4. IR (ATR) 3258 (acetylenic H), 2966, 2123 (triple bond), 1696 (carbonyl), 1495, 1363, 1177, 1227, 1062, 1001, 985, 740. The spectral data were in agreement with those reported previously for this compound.[46]

3.1.2. General procedure for the synthesis of 1-(2-(but-3-ynyl)benzylidene)-2-methylhydrazines

The corresponding 2-(prop-2-ynyloxy)benzaldehydes **3** (0.5 mmol) were dissolved in dioxane (2 mL). Then methylhydrazine (2 mL) was added at room temperature. The resulting mixture was flushed with argon and stirred at room temperature for 1 h. After the reaction was over, the solvent was removed under vacuum and the residue was purified by column chromatography over silica gel with hexane-EtOAc (9:1) to afforded the desired hydrazones.

3.1.2.1. 1-(2-(But-3-ynyl)benzylidene)-2-methylhydrazine (4a)

The product was isolated as a brown oil (yield: 98%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87 (s, 1H, CH = N), 7.81 (dd, J = 8.0 and 1.9 Hz, 1H), 7.22–7.19 (m, 1H), 6.98–6.97 (m, 2H), 5.60 (brs, 1H, NH), 4.70 (d, J = 2.4 Hz, 2H, CH_2), 2.94 (s, 3H, $NHCH_3$), 2.59 (s, 1H, alkyne's H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.6, 139.2, 130.9, 128.6, 125.3, 121.8, 112.7, 78.6, 75.7, 56.3, 34.9. IR (ATR) 3284 (acetylenic H), 2960, 2866, 2793, 2116 (triple bond), 1600, 1483, 1463, 1223, 1098, 1021, 762.

3.1.2.2. 1-(5-Bromo-2-(but-3-ynyl)benzylidene)-2-methylhydrazine (4b)

The product was isolated as a brown oil (yield: 85%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94 (d, J = 2.5 Hz, 1H), 7.72 (s, 1H, CH = N), 7.28 (dd, J = 8.7 and 2.5 Hz, 1H), 6.86 (d, J = 8.8 Hz, 1H), 4.70 (d, J = 2.4, 2H, CH_2), 2.96 (s, 3H, $NHCH_3$), 2.52 (t, J = 2.4 Hz, 1H, alkyne's H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 153.4, 136.9, 130.8, 128.6, 127.8, 118.2, 114.5, 78.1, 76.0, 56.5, 34.7. IR (ATR) 3290 (acetylenic H), 2980, 2920, 2797, 2120 (triple bond), 1594, 1475, 1406, 1224, 1115, 1020, 799, 633.

3.1.2.3. 1-(2-(But-3-ynyl)-5-nitrobenzylidene)-2-methylhydrazine (4c)

The product was isolated as a brown oil (yield: 84%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.70–8.67 (m, 1H), 8.09–8.07 (m, 1H), 7.68 (s, 1H, CH = N), 7.06–7.04 (m, 1H), 5.93 (brs, 1H, NH), 4.83 (s, 2H, CH_2), 3.00 (s,

3H, $NHCH_3$), 2.58 (s, 1H, alkyne); ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.3, 142.5, 126.8, 123.4, 123.0, 120.9, 111.9, 77.2, 76.8, 56.5, 34.4. IR (ATR) 3280 (acetylenic H), 2940, 2915, 2788, 2117 (triple bond), 1601, 1455, 1450, 1232, 1145, 1060, 787, 702.

3.1.2.4. 1-(2-(But-3-ynyl)-4-methoxybenzylidene)-2-methylhydrazine (4d)

The product was isolated as a brown oil (yield: 96%): ^1H NMR (400 MHz, $CDCl_3$) δ 7.96 (s, 1H, CH = N), 7.42 (dd, J = 7.9 and 1.4 Hz, 1H), 7.04 (td, J = 8.0 and 0.5 Hz, 1H), 6.80 (dd, J = 8.02 and 1.4 Hz, 1H), 4.71 (d, J = 2.5 Hz, 2H, CH_2), 3.84 (s, 3H), 2.98 (s, 3H, $NHCH_3$), 2.97 (s, 1H, alkyne's H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 152.5, 131.5, 130.9, 124.7, 116.8, 111.1, 79.4, 75.5, 60.3, 55.7, 34.7. IR (ATR) 3400, 3281 (acetylenic H), 2936, 2838, 2796, 2119 (triple bond), 1588, 1472, 1437, 1302, 1273, 1065, 990, 743.

3.1.2.5. 1-((2-(Ethynyloxy)naphthalen-1-yl)methylene)-2-methylhydrazine (4e)

The product was isolated as a brown oil (yield: 88%): ^1H NMR (400 MHz, $CDCl_3$) δ 9.06 (d, J = 8.7 Hz, 1H), 8.28 (s, 1H, CH = N), 7.79–7.77 (m, 1H), 7.76–7.75 (m, 1H), 7.53–7.50 (m, 1H), 7.39–7.36 (m, 1H), 7.32 (d, J = 9 Hz, 1H), 4.83 (d, J = 2.4 Hz, 2H, CH_2), 3.09 (s, 3H, $NHCH_3$), 2.52 (t, J = 2.4 Hz, 1H, alkyne's H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 153.7, 136.5, 133.5, 129.8, 128.1, 127.2, 126.7, 126.2, 124.3, 122.9, 114.8, 78.7, 75.8, 57.7, 35.4. IR (ATR) 3291 (acetylenic H), 2956, 2865, 2775, 2114 (triple bond), 1577, 1475, 1445, 1377, 1272, 1066, 995, 756.

3.2. Trolox equivalent antioxidant capacity (ABTS assay)

All chemicals used in the ABTS assay, including 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), potassium persulfate ($K_2S_2O_8$), and methanol (CH_3OH), were purchased from Sigma-Aldrich. Antioxidant capacities of synthesized hydrazone derivatives were determined according to the modified method of Re et al.[48] First the ABTS stock solution was prepared in dH_2O, including ABTS (7 mM) and potassium persulfate (2.45 mM), by incubating at room temperature for 12–16 h. Then, in order to obtain a working ABTS solution, the ABTS stock solution was diluted with methanol to achieve an absorbance of 0.700 $\pm$ 0.02 at 734 nm. Hydrazone derivatives and Trolox (standard) were dissolved in methanol and diluted with methanol. Finally, a standard or synthesized hydrazone compound was mixed with the ABTS working solution (1:1) and incubated in the dark at 25 °C for 30 min. Absorbances of standard/synthesized compounds and a control tube, containing only methanol and ABTS, were measured at a wavelength of 734 nm. All measurements were done in triplicate under dim light. Trolox was used as an antioxidant standard.

The percentage of radical scavenging capability was calculated using the following equation:

$$\text{RadicalScavengingCapability}(\%) = \frac{(\text{ControlAbsorbance-SampleAbsorbance})}{(\text{ControlAbsorbance})} \times 100$$

The EC_{50} value is the concentration of a compound that is able to reduce the absorbance value of the ABTS•+ radical cation solution to half of its original value. This value was obtained from the linear curve of radical scavenging capability versus different concentrations of tested compound.

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