Synthesis of Gallic-Acid-1-Phenyl-*1H*-[1,2,3]Triazol-4-yl Methyl Esters as Effective Antioxidants

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Key words
- gallic acid
- ascorbic acid
- triazoles
- antioxidants
- food-industry

Abstract

Using a click chemistry approach, a series of gallic-acid-1-phenyl-*1H*-[1,2,3]triazol-4-ylmethyl esters was synthesized to develop more effective antioxidants. The results of DPPH screening indicate that few of the synthesized analogs display better antioxidant effect compared to the standards. Among all, compounds, **9** and **20** displayed highest DPPH radical scavenging effect with IC_{50} values as low as 6.4±0.2 and 7.9±0.4 µM respectively, compared to the standard ascorbic acid (IC_{50}=12±0.8 µM) and gallic acid (IC_{50}=9.0±0.6 µM). Compound **10** also displayed a potent antioxidant effect with IC_{50} of 10.80±0.4 µM. This study provides an important aspect with regard to the use of these gallic-acid based synthetic antioxidants in food industry as dietary supplements.

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Introduction

During the past few years there has been an upsurge in the newer developments in the areas of disease prevention, especially in the role of free radicals in causing the disease and antioxidants in the prevention of disease [1,2]. In normal human body the occurrence of prooxidants particularly in the form of reactive oxygen species and reactive nitrogen species are effectively kept under check by some degree of antioxidant defense. Nature has rendered each cell with its own protective mechanism against any kind of harmful effects whatsoever, be it free radicals, sodium glutathione peroxide, glutathione reductase or thiols [3]. However exposure to adverse physiological, environmental, or pathological agents such as pollutants, cigarette smoking, UV-rays, toxic chemicals, over-nutrition etc. results in the shift of this delicately maintained balance leading to the lipid peroxidation, oxidative damage to DNA, proteins etc. and under such conditions the dietary intake of antioxidants is warranted to exert promising therapeutic potential to combat the radicals and prevent them from causing the oxidative stress which otherwise may lead to chronic disorders like cardiovascular diseases, cancers and neurodegeneration [4]. Plant natural-products constitute an abundant source of antioxidants and include anthocyanins, aprons, chalcones, flavanones (naringenin), flavanols (procyanidin), flavan-3-ol (epicatechin, catechin), flavones (apigenin, luteolin etc.), flavonols (kaempferol, quercetin, rutin etc.) and isoflavonoids (genistein, daidzein etc.), hydroxybenzoic acid (gallic acid), hydroxycinnamic acid (caffeic acid, ferulic acid etc.), proanthocyanidins, vitamin C and vitamin E (α-Tocopherols, Tocotreinols) [5]. Gallic acid (GA, 3,4,5-trihydroxybenzoic acid) along with its derivatives which occur widely within the plant kingdom represent a large family of plant secondary polyphenolic metabolites with known antioxidant properties. GA along with its derivatives are considered the main polyphenolic compounds in grapes, different berries, mango, areca nut, walnut, green tea and other fruits as well as in wine [6]. Gallic acid shows different biological activities such as induction of apoptosis in tumour cells in higher proportions than normal cells and is an excellent free radical scavenger [7]. Due to this antioxidant effect, GA-containing plants have shown various possible bioactivities which mainly include antiangiogenic, antidiabetic and antimelanogenic effects along with reduced heart infarction incidence and oxidative kidney and liver damage [8–10]. GA has also been used in cosmetics, food industry and in pharmaceuticals as an effective antioxidant [11]. It is non-toxic to mammalian organisms at pharma-

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cological doses. LD_{50} dose for GA has been found to be 5 g/kg body weight in rats [12]. Gallic acid has been subjected to a number of structural modifications to access its structure-activity relationships. Alkyl esters of gallic acid have been found to exhibit potential antioxidant as well as anticancer properties [13]. Novel galloyl-pyrrolidine derivatives have been used as potential anti-tumor agents [14]. Till date there has not been any attempt towards the synthesis of azole containing analogs of gallic acid and evaluate their antioxidant potential. Triazoles, a versatile bunch of heterocycles are known chemotherapeutic agents. 1,2,3-triazoles, as clinical drug candidates have been frequently employed for the treatment of various diseases, which have shown their large developmental value and wide potential as medicinal agents exhibiting diverse array of biological functions which include anticancer, antifungal, antitubercular, anti-inflammatory, antiviral, antibacterial and anticonvulsant, analgesic, antidiabetic, antiparasitic, antihistaminic, obesitic, antihypertensive, antineuropathic, as well as other biological activities [15–27]. But a very little or no study documents the antioxidant potential of the triazolyl compounds. Since gallic acid (**1**) is reported to possess effective antioxidant potential and triazoles are also reported to possess versatile biological properties with the triazole ring being used as an attractive linker to combine different pharmacophore fragments to produce innovative bifunctional drug molecules, in a convenient and efficient pathway. Therefore it is expected from combination principle that if we link triazoles with gallic acid, the resulting compounds with triazole scaffold should be better antioxidants. Keeping in view this fact that the whole is always greater than the sum as well as our previous work to carry out structural diversification of natural products for synthesizing medicinally important leads [28–30], we designed a click chemistry based approach to synthesize the triazolyl gallic acid analogs for better antioxidant activity.

Results and Discussion

Taking cue from above literature that more potent antioxidants can be synthesized, while modifying the carboxylic acid moiety of gallic acid, we designed a click approach to carry out the synthetic modications at this moiety to obtain more potent and effective antioxidant derivatives of gallic acid. Gallic acid (**1**) was thus subjected to propargylation in dry DMF using NaH as base. The propargylation reaction delivered the required alkyne at the desired position which could easily be confirmed from its ^{1}H and ^{13}C NMR spectra (**Fig. 1a, b**). However a very little amount of side-product with one alkyne moiety tethered to the acid group and the other alkyne moiety to one of the 3 hydroxyl functionalities of the benzene nucleus was also formed (**2**). To localize as which of the 3 hydroxyl moieties underwent propargylation, we resorted to close analysis of its ^{1}H and ^{13}C NMR spectra (**Fig. 1c, d**). As can be seen in the ^{1}H NMR of compound **2**, a singlet integrating for 2 equivalent aromatic protons corresponding to positions 2 and 6 in the aromatic ring is a clear indication of the 2 OH groups (position 3 and 5) in the aromatic ring being intact and that the *para*-hydroxyl might have been propargylated. This was further supplemented by the ^{13}C NMR of compound **2**. Presence of only 11 signals for 13 carbon atoms suggested a symmetrical nature of aromatic ring and hence evidencing the propargylation at para-hydroxyl moiety. After propargylation various aromatic azides were prepared from their respective aromatic amines by diazotization with sodium nitrite in acidic conditions followed by displacement with sodium azide in quantitative yields. 1,3-dipolar cycloaddition reaction of **3** with aromatic azides in presence of $CuSO_4.5H_2O$ and sodium ascorbate in *t*-BuOH:H_2O (2:1) resulted in regioselective formation of 1,4-disubstituted-triazolyl derivatives (**4–20**) in excellent yields (**Fig. 2**). All the reactions were carried out at room temperature under ultra-sonication and completed within 4 h. The products were confirmed by ^{1}H and ^{13}C NMR spectral data analysis. In ^{1}H NMR cyclization of azides to form triazoles was confirmed by *H*-5 resonance of triazole ring in aromatic region as well as by other proton absorptions in the same region (**Fig. 1e**). The structure was further supported by ^{13}C NMR and DEPT, which showed all the expected carbon signals corresponding to acid triazolyl derivatives. ESI-MS of all the derivatives was in good agreement with the desired structures. By employing the above reaction conditions, a series of such triazolyl gallic acid-derivatives that vary at substitutions on aromatic ring were synthesized from a range of aromatic azides. It was observed that all the reactions worked smooth under ultra-sonication conditions (**Table 1**). Gallic acid along with its triazolyl analogs were then studied for the possible antioxidant activity using a well-known DPPH scavenging assay system. Preliminary screening of all the analogs was done at 10 and 5 µM concentrations. Almost all the analogs displayed an appreciable scavenging effect. The analogs were then screened at different concentrations to evaluate their DPPH scavenging effect in terms of the IC_{50} values depicted in **Table 2**. Both parent gallic acid (**1**) as well as ascorbic acid (**21**) served as positive controls in this assay. In general a dose-dependent antioxidant potential of the analogs was observed. Among all the synthesized analogs, compound **2**, prop-2-ynyl-3,5-dihydroxy-4-(prop-2-ynyloxy) benzoate formed as a side product in the propargylation reaction of gallic acid and propargyl bromide, was found to be least active of all. Since in compound **2** the free *para*-OH moiety as well as the OH of acid moiety are in esterified state, it lead to a loss in antioxidant action, suggesting that the OH groups in gallic acid molecule are very important for exhibiting the DPPH scavenging effect. This fact is in strong conformity with the previously documented fact that OH-group para to the acid group in gallic acid [31], its derivatives is necessary for exhibiting a potential DPPH scavenging effect. Therefore no efforts to modify compound **2** were invested. It was also noted that to produce analogs with better activity than the parent gallic acid molecule only acid group accepts any sort of modification. Since the desired product, prop-2-ynyl-3, 4, 5-trihydroxy benzoate (**3**), with all the 3 phenolic OH groups in free state and that of the carboxylic acid moiety in esterified state, was formed in excellent yields and exhibited an appreciable scavenging effect displaying IC_{50} value of 25.67 ± 0.5 µM, less than that of standard gallic acid and ascorbic acid with IC_{50} of 9.01 ± 0.6 and 12.07 ± 0.8 µM respectively. This observation was in strong concurrence with the previous reports that the various long chain esters of gallic acid possess a lower scavenging effect compared to gallic acid and follow the order as gallic acid (IC_{50} = 6.0 ± 0.1 µM), methyl gallate (IC_{50} = 7.2 ± 0.1 µM), > *n*-propyl gallate (IC_{50} = 8.2 ± 0.1 µM), > *n*-octyl gallate (IC_{50} = 11.8 ± 0.2 µM) > *n*-dodecyl gallate (IC_{50} = 13.2 ± 0.2 µM) against DPPH (190 µM) in ethanolic solution [31]. Compound **3** was further subjected to Huisgen's cycloaddition reaction at its well poised alkyne moiety to afford a range of triazolyl analogs (**4–20**). Among all the synthesized analogs, a notable difference in the antioxidant potential was observed which may be attributed to the position of substituents depending upon the type of

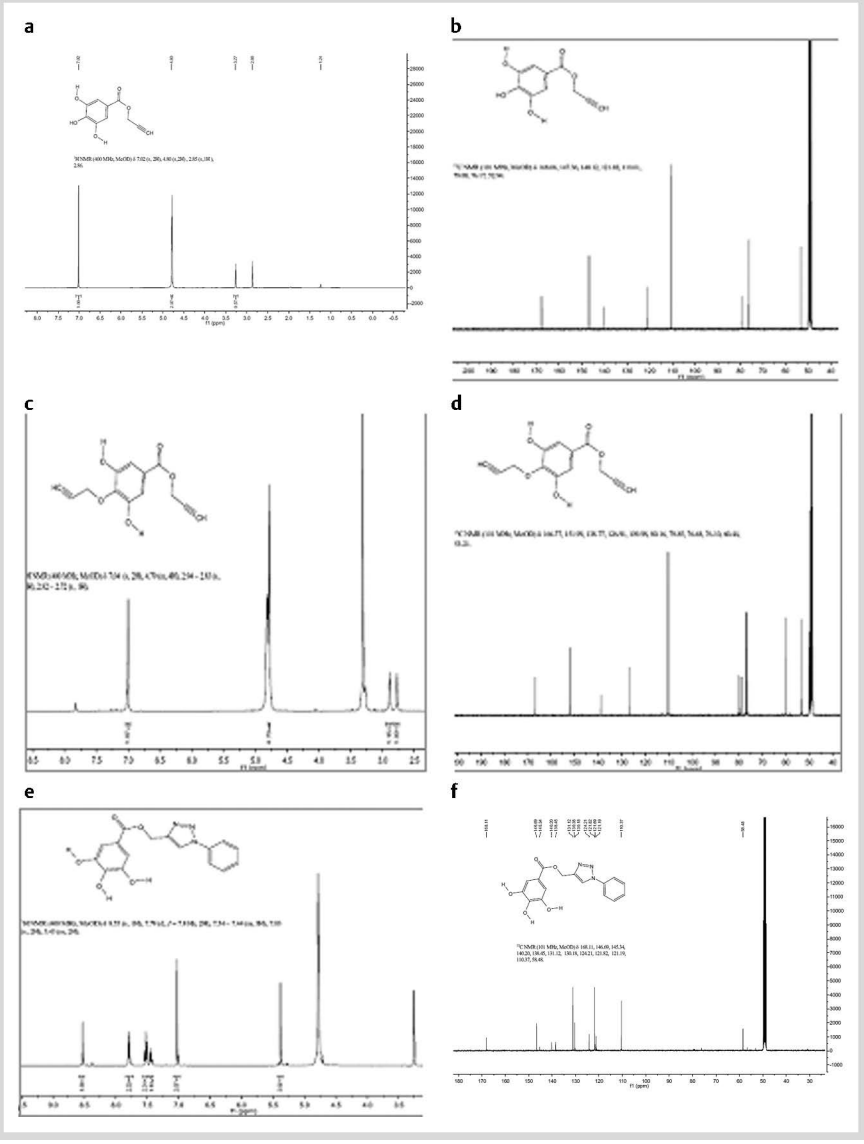


Fig. 1 (**a** and **b**) 1H and 13C NMR spectra of compound 3. (**c** and **d**) 1H and 13C NMR spectra of compound 2. (**e** and **f**) 1H and 13C NMR spectra of compound 4.

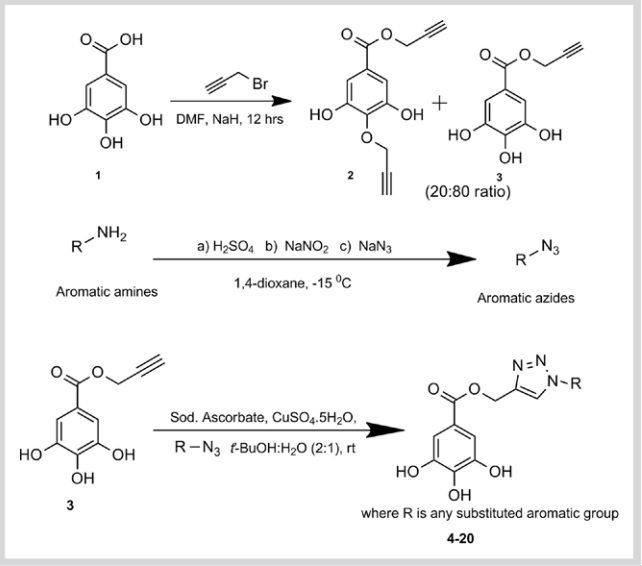
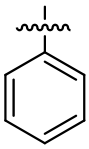
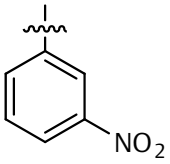
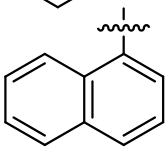
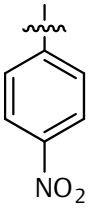
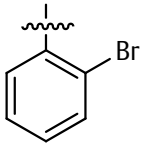
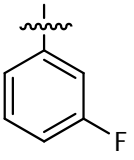
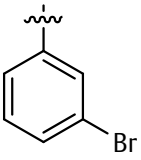
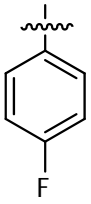
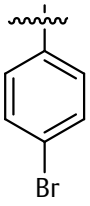
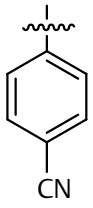
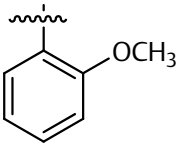
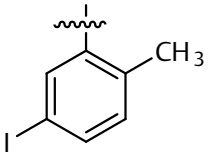
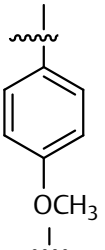
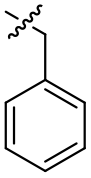
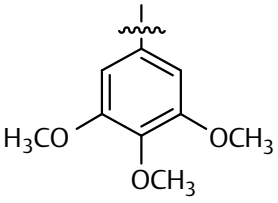
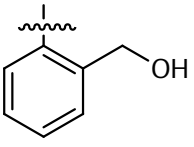
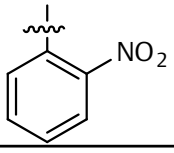


Fig. 2 Synthesis of triazole analogs of Gallic acid.

aromatic azide used. The highest DPPH scavenging effect was observed in case of [1-(2-methoxyphenyl)-1H-1,2,3-triazol-4-yl]methyl-3,4,5–trihydroxy benzoate (**9**) displaying IC_{50} of 6.41 ± 0.2 µM compared to standard gallic acid and ascorbic acid with IC_{50} of 9.01 ± 0.6 and 12.07 ± 0.8 µM respectively. However its congener, [1-(4-methoxyphenyl)-1H-1,2,3-triazol-4-yl] methyl-3,4,5-trihydroxybenzoate (**10**) with OCH_3 at *para*-position was less active than standard gallic acid but more active than ascorbic acid displaying IC_{50} of 12.07 ± 0.8 µM. The trimethoxy analog (**11**) displayed a lower antioxidant effect with IC_{50} of 20.8 ± 1.1 µM, depicting the effect of number and position of methoxy groups in exhibiting the desired scavenging effect. Compound **20** with *ortho*-hydroxymethyl moiety also exhibited potent DPPH scavenging effect only after compound **9** and displayed IC_{50} of 7.9 ± 0.4 µM. However compounds with simple phenyl (**4**) and napthyl (**5**) moieties were found to be moderately active displaying IC_{50} of 17.12 ± 1.3 and 14.20 ± 0.9 µM respectively. Similarly compound **18** having 5-Iodo-2-methyl phenyl moiety exhibited a moderate DPPH scavenging effect with IC_{50} of 19.90 ± 0.8 µM. All the remaining compounds exhibited lower DPPH scavenging effect as compared to both the standard: gallic (**1**) and ascorbic acid (**21**). All the results have been summed up in **Table 2**.

Table 1 Preparation of various analogs of gallic acid.

S. No.	R	Product	[a]Yield%	S.No.	R	Product	[a]Yield%
1		**4**	69	10		**13**	70
2		**5**	71	11		**14**	86
3		**6**	65	12		**15**	68
4		**7**	62	13		**16**	79
5		**8**	88	14		**17**	83
6		**9**	71	15		**18**	72
7		**10**	90	16		**19**	80
8		**11**	84	17		**20**	75
9		**12**	63				

'R' refers to any substituted aryl moiety

'a' refers to the product yield after isolation/purification process

Table 2 DPPH scavenging potential of various analogs of gallic acid.

Compound	[a]IC_{50} (μM)	Compound	[a]IC_{50} (μM)
2	nd	**13**	25.67 ± 2.0
3	25.67 ± 0.5	**14**	28.24 ± 1.6
4	17.12 ± 1.3	**15**	nd
5	14.20 ± 0.9	**16**	61.66 ± 2.2
6	77.00 ± 2.0	**17**	24.24 ± 1.0
7	nd	**18**	19.19 ± 0.8
8	41.40 ± 1.0	**19**	29.88 ± 1.5
9	6.41 ± 0.2	**20**	07.92 ± 0.4
10	10.80 ± 0.4	**21 (AA)**	12.07 ± 0.8
11	20.87 ± 1.1	**1 (GA)**	09.01 ± **0**.6
12	nd		

[a] refers to the mean value of 3 independent readings ± SD
GA and AA refer to standard gallic and ascorbic acids

Conclusion

In conclusion, a range of galloyl triazole analogs were synthesized using click chemistry approach and studied for the possible antioxidant potential. Compound **9, 10**, **20** displayed potent scavenging effect with IC_{50} values of 6.41 ± 0.2, 10.80 ± 0.4 and 07.92 ± 0.4, μM respectively as compared to the standard gallic acid and ascorbic acid with IC_{50} of 12.07 ± 0.8 and 9.01 ± 0.6 μM respectively. Since gallic acid derivatives find their use as food antioxidant additives, this study provides an important aspect with regard to the use of these gallic acid based synthetic antioxidants in food industry as dietary supplements and are more potent than their natural precursor-gallic acid.

Experimental

General

^{1}H and ^{13}C NMR spectra were recorded on Bruker 400, 100 MHz Bruker spectrometers using TMS as internal standard. Chemical shifts (δ) are expressed as ppm. Mass spectra were recorded on Shimadzo lab solutions, Chromatography was carried out using ordinary phase column chromatography silica gel 60–120 mesh (Merck grade) and precoated TLC plates with silica gel 60 F254 (Merck, 0.25 mm). Detection was done by using cerric-sulfate solution and *para*-anisaldehyde followed by heating.

Synthesis

Synthesis of *prop-2-ynyl-3, 4, 5-trihydroxybenzoate (3)*

A solution of compound **1** (1 000 mg, 5.88 mmol) in dimethyl formamide (DMF) (5 ml) and propargyl bromide (0.151 mmol) was heated under reflux for 12 h in presence of base DBU. After cooling, the reaction mixture was evaporated *under vaccuo* on a rotary evaporator and the residue obtained was subjected to normal silica-gel column chromatography using Hexane-EtOAc (60:40) as eluent to furnsh 2 products **2** and **3**.

General procedure for synthesis of azides

To a solution of particular aromatic amine in 1,4-dioxane at −15 °C, 5 equivalents of 2 M Sulphuric acid was added in small installments while stirring. After 5 min 2 equivalents of 3 M sodium nitrite was added drop wise and after 30 min 3 equivalents of 3 M sodium azide was added drop wise carefully. Reaction was brought to room temperature and extracted with diethyl ether for at least 3 times. Organic layers were washed with saturated sodium bicarbonate solution twice, dried over anhydrous sodium sulphate and concentrated to a minimum volume under reduced pressure on rotary evaporator without making use of heating from water bath.

General procedure for the synthesis of triazole analogs of compound 3 (4–20)

To a solution of compound **3** (25 mg, 0.083 mmol) in *t*-BuOH:H_2O (2:1, 3 ml), sodium ascorbate (2.0 mg, 0.012 mmol) and $CuSO_4.5H_2O$ (2 mg, 0.0075 mmol) were added at room temperature. To this mixture, aryl azide (0.12 mmol) was added and the reaction mixture sonicated till its completion. The crude mixture was extracted with ethylacetate (3 × 20 ml) and the combined organic layers dried over sodium sulphate and purified through column chromatography to give pure **4–20** in 62-90% yield.

Spectral analysis of the representative compounds

Prop-2-ynyl-3,5-dihydroxy-4-(prop-2-ynyloxy)benzoate (2)

^{1}H NMR (400 MHz, MeOD) δ 7.00 (s, 2H), 4.78–4.82 (m, 4H), 2.93 (s, 1H), 2.77 (s, 1H). ^{13}C NMR (101 MHz, MeOD) δ 167.08, 152.31, 138.7, 126.6, 11.23, 80.15, 78.98, 76.85, 76.21, 61.69, 53.35. ESI-MS at *m/z* = 247 for $[M+H]^+$ calculated for $C_{13}H_{10}O_5$.

Prop-2-ynyl-3,4,5-trihydroxybenzoate (3)

^{1}H NMR (400 MHz, MeOD) δ 7.06 (s, 2H), 4.84 (s, 2H), 2.92 (s, 1H). ^{13}C NMR (101 MHz, MeOD) δ 167.60, 146.71, 140.25, 121.02, 110.35, 79.21, 76.17, 53.05. ESI-MS at *m/z* = 209 for $[M+H]^+$.

[1-(Phenyl)-1H-1,2,3-triazol-4-yl]methyl-3,4,5-trihydroxybenzoate (4)

^{1}H NMR (400 MHz, MeOD) δ 8.53 (s, 1H), 7.80 (m, 2H), 7.4–7.6 (m, 3H), 7.02 (s, 2H), 5.41 (s, 2H).^{13}C NMR (101 MHz, MeOD) δ 168.11, 146.69, 145.34, 140.19, 138.44, 131.11, 130.35, 124.21, 121.82, 121.18, 110.37, 58.48. ESI-MS at *m/z* = 328 $[M+H]^+$ (calculated for $C_{16}H_{13}N_3O_5$, 327).

[1-(Napthalen-1-yl)-1H-1,2,3-triazol-4-yl]methyl-3,4,5-trihydroxybenzoate (5)

^{1}H NMR (400 MHz, MeOD) δ 8.40 (s, 1H), 7.9–8.1 (s, 2H), 7.45–7.62 (m, 5H), 7.05 (s, 2H), 5.46 (s, 2H). ^{13}C NMR (101 MHz, MeOD) δ 168.17, 163.97, 146.74, 144.89, 141.10, 135.84, 132.02, 130.03, 129.65, 129.29, 128.70, 128.44, 126.40, 125.18, 123.14, 121.24, 110.39, 58.57. ESI-MS at *m/z* = 378 $[M+H]^+$ (calculated for $C_{20}H_{15}N_3O_5$, 377).

[1-(2-bromophenyl)-1H-1,2,3-triazol-4-yl]methyl-3,4,5-trihydroxybenzoate (6)

^{1}H NMR (400 MHz, MeOD) δ 8.32 (s, 1H), 7.83 (m, 1H), 7.4–7.6 (m, 3H), 7.0 (s, 2H), 5.40 (s, 2H).^{13}C NMR (101 MHz, MeOD) δ 168.09, 146.70, 144.48, 140.19, 137.91, 135.19, 133.18, 130.05, 129.75, 128.24, 121.20, 120.38, 110.36, 58.43. ESI-MS at *m/z* = 406 $[M+2]^+$ (calculated for $C_{16}H_{12}BrN_3O_5$, 404).

[1-(3-bromophenyl)-1H-1,2,3-triazol-4-yl]methyl-3,4,5-trihydroxybenzoate (7)

^{1}H NMR (400 MHz, MeOD) δ 8.30 (s, 1H), 7.70 (s, 1H), 7.75–7.33 (m, 3H), 7.01 (s, 2H), 5.47 (s, 2H). ^{13}C NMR (101 MHz, MeOD) δ 168.26, 145.99, 144.31, 140.01, 135.13, 132.16, 130.05, 129.75, 128.24, 124.31, 121.20, 119.98, 109.36, 58.43. ESI-MS at *m/z* = 406 $[M+2]^+$ (calculated for $C_{16}H_{12}BrN_3O_5$, 404).

[1-(4-bromophenyl)-1H-1,2,3-triazol-4-yl]methyl-3,4,5-trihydroxybenzoate (8)

^{1}H NMR (400 MHz, MeOD) δ 8.23 (s, 1H), 7.65 (d, J = 7.8 Hz, 1H), 7.53 (d, J = 8.0 Hz, 2H), 7.11 (s, 2H), 5.42 (s, 2H). ^{13}C NMR (101 MHz, MeOD) δ 168.23, 146.12, 144.21, 140.24, 136.76, 133.07, 129.15, 124.34, 121.24, 120.30, 109.00, 58.01. ESI-MS at m/z = 406 $[M+2]^+$ (calculated for $C_{16}H_{12}BrN_3O_5$, 404).

[1-(2-methoxyphenyl)-1H-1,2,3-triazol-4-yl]methyl-3,4,5-trihydroxybenzoate (9)

^{1}H NMR (400 MHz, MeOD) δ 8.34 (s, 1H), 7.61 (d, J = 8.6 Hz, 1H), 7.46–7.48 (m, 1H), 7.21 (d, J = 8.2 Hz, 1H), 7–7.07 (m, 1H), 6.99 (s, 2H), 5.39 (s, 2H), 3.85 (s, 3H). ^{13}C NMR (101 MHz, MeOD) δ 168.15, 153.45, 146.70, 144.07, 140.17, 132.22, 128.10, 127.35, 126.89, 122.24, 121.26, 115.95, 110.35, 58.52, 56.78. ESI-MS at m/z = 358 $[M+H]^+$ (calculated for $C_{17}H_{15}N_3O_6$, 357).

[1-(4-methoxyphenyl)-1H-1,2,3-triazol-4-yl]methyl-3,4,5-trihydroxybenzoate (10)

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.20 (s, 1H), 7.76 (d, J = 8.0 Hz, 2H), 7.00 (d, J = 8.0 Hz, 2H), 6.95 (s, 2H), 5.41 (s, 2H), 3.89 (s, 3H). ^{13}C NMR (101 MHz, MeOD) δ 168.25, 154.47, 146.43, 144.27, 140.17, 131.20, 124.12, 122.32, 119.70, 115.81, 110.08, 58.85, 57.12. ESI-MS at m/z = 358 $[M+H]^+$ (calculated for $C_{17}H_{15}N_3O_6$, 357).

[1-(3,4,5-trimethoxyphenyl)-1H-1,2,3-triazol-4-yl]methyl-3,4,5-trihydroxybenzoate (11)

^{1}H NMR (400 MHz, MeOD) δ 8.68 (s, 1H), 7.24 (s, 1H), 7.19 (s, 2H), 5.51 (s, 2H), 3.98 (s, 6H), 3.89 (s, 3H). ^{13}C NMR (101 MHz, MeOD) δ 168.12, 155.51, 146.70, 145.22, 140.21, 139.78, 134.38, 124.52, 121.19, 110.37, 99.83, 61.39, 58.48, 57.13. ESI-MS at m/z = 418 $[M+H]^+$ (calculated for $C_{19}H_{19}N_3O_8$, 419).

[1-(2-nitrophenyl)-1H-1,2,3-triazol-4-yl]methyl-3,4,5-trihydroxybenzoate (12)

^{1}H NMR (400 MHz, MeOD) δ 8.57 (s, 1H), 8.31 (d, J = 8.4 Hz, 1H), 8.01–7.75 (m, 3H), 7.02 (s, 2H), 5.40 (s, 2H). ^{13}C NMR (101 MHz, MeOD) δ 167.54, 150.43, 146.80, 144.31, 139.17, 132.59, 127.13, 126.36, 123.45, 121.20, 118.96, 110.39, 57.50. ESI-MS at m/z = 373 $[M+H]^+$ (calculated for $C_{16}H_{12}N_4O_7$, 372).

[1-(3-nitrophenyl)-1H-1,2,3-triazol-4-yl]methyl-3,4,5-trihydroxybenzoate (13)

^{1}H NMR (400 MHz, MeOD) δ 8.70 (s, 1H), 7.8–8.5 (m, 4H), 7.12 (s, 2H), 5.51 (s, 2H). ^{13}C NMR (101 MHz, MeOD) δ 167.89, 150.81, 150.58, 146.80, 139.17, 139.16, 132.59, 127.23, 124.56, 122.67, 121.19, 116.66, 110.39, 56.59. ESI-MS at m/z = 373 $[M+H]^+$ (calculated for $C_{16}H_{12}N_4O_7$, 372).

[1-(4-nitrophenyl)-1H-1,2,3-triazol-4-yl]methyl-3,4,5-trihydroxybenzoate (14)

^{1}H NMR (400 MHz, MeOD) δ 8.72 (s, 1H), 8.40 (d, J = 9.2 Hz, 2H), 8.10 (d, J = 8.8 Hz, 2H), 7.02 (s, 2H), 5.43 (s, 2H). ^{13}C NMR (101 MHz, MeOD) δ 168.07, 149.06, 146.72, 146.09, 142.66, 140.25, 126.66, 124.43, 122.20, 121.14, 110.39, 58.39. ESI-MS at m/z = 373 $[M+H]^+$ (calculated for $C_{16}H_{12}N_4O_7$, 372).

[1-(3-flurophenyl)-1H-1,2,3-triazol-4-yl]methyl-3,4,5-trihydroxybenzoate (15)

^{1}H NMR (400 MHz, MeOD) δ 8.55 (s, 1H), 7.62 (d, J = 8.5 Hz, 2H), 7.48–7.53 (m, 1H), 7.14–7.18 (m, 1H), 7.01 (s, 2H), 5.38 (s, 2H). ^{13}C NMR (101 MHz, MeOD) δ 168.06, 165.84, 163.39, 146.66, 145.51, 140.17, 132.80, 124.23, 121.15, 117.34, 117.01, 116.80, 110.38, 58.42. ESI-MS at m/z = 346. $[M+H]^+$ (calculated for $C_{16}H_{12}FN_3O_5$, 345).

[1-(4-flurophenyl)-1H-1,2,3-triazol-4-yl]methyl-3,4,5-trihydroxybenzoate (16)

^{1}H NMR (400 MHz, MeOD + DMSO) δ 8.721 (s, 1H), 8.03 (d, J = 8 Hz, 2H), 7.46 (d, J = 8 Hz, 2H), 7.18 (s, 2H), 5.546 (s, 2H). ^{13}C NMR (101 MHz, MeOD + DMSO) δ 167.78, 165.27, 146.80, 140.11, 134.85, 124.19, 121.21, 118.04, 117.80, 110.37, 58.22. ESI-MS at m/z = 346 $[M+H]^+$ (calculated for $C_{16}H_{12}FN_3O_5$, 345).

[1-(4-cyanophenyl)-1H-1,2,3-triazol-4-yl]methyl-3,4,5-trihydroxybenzoate (17)

^{1}H NMR (400 MHz, MeOD) δ 8.68 (s, 1H), 8.06 (d, J = 8.8 Hz, 2H), 7.91 (d, J = 8.6 Hz, 2H), 7.00 (s, 2H), 5.43 (s, 2H). ^{13}C NMR (101 MHz, MeOD) δ 168.07, 146.72, 145.97, 141.41, 135.38, 124.25, 122.19, 121.14, 118.94, 113.79, 110.37, 58.38. ESI-MS at m/z = 353 $[M+H]^+$ (calculated for $C_{17}H_{12}N_4O_5$, 352).

[1-(5-Iodo-2-methyl-phenyl)-1H-1,2,3-triazol-4-yl]methyl-3,4,5-trihydroxybenzoate (18)

^{1}H NMR (400 MHz, MeOD) δ 8.32 (s, 1H), 7.78–77.82 (m, 2H), 7.23 (d, J = 8 Hz, 1H), 7.08 (s, 2H), 5.45 (s, 2H), 2.20 (s, 3H). ^{13}C NMR (101 MHz, MeOD) δ 168.10, 146.69, 144.69, 140.50, 140.19, 138.78, 135.90, 135.11, 134.39, 127.63, 121.18, 110.36, 90.94, 58.46, 17.68. ESI-MS at m/z = 468 $[M+H]^+$ (calculated for $C_{17}H_{14}IN_3O_5$, 467).

[1-benzyl]-1H-1,2,3-triazol-4-yl]methyl-3,4,5-trihydroxybenzoate (19)

^{1}H NMR (400 MHz, MeOD) δ 7.96 (s, 1H), 7.28 (s, 5H), 6.95 (s. 2H), 5.53 (s, 2H), 5.26 (s, 2H). ^{13}C NMR (101 MHz, MeOD) δ 168.59, 146.66, 144.48, 139.74, 130.33, 129.73, 129.43, 125.66, 120.90, 110.23, 58.19, 55.32. ESI-MS at m/z = 342 $[M+H]^+$ (calculated for $C_{17}H_{15}N_3O_5$, 341).

[1-[2-(hydroxymethyl)phenyl]-1H-1,2,3-triazol-4-yl] methyl-3,4,5-trihydroxybenzoate (20)

^{1}H NMR (400 MHz, MeOD) δ 8.33 (s, 1H), 7.67 (d, J = 8.1 Hz, 1H), 7.5–7.65 (m, 1H), 7.4–7.48 (m, 2H), 7.02 (s, 2H), 5.41 (s, 2H), 4.4 (s, 2H). ^{13}C NMR (101 MHz, MeOD) δ 168.11, 146.69, 144.62, 140.18, 137.97, 136.67, 131.50, 130.72, 129.78, 127.84, 126.94, 121.21, 110.36, 61.03, 58.48. ESI-MS at m/z = 358 $[M+H]^+$ (calculated for $C_{17}H_{15}N_3O_6$, 357).

DPPH scavenging effect

DPPH free radical scavenging activity was evaluated by measuring the scavenging activity of the samples on stable 2.2-diphenyl-1-picryl hydrazyl radical (DPPH) [32]. A 0.5 mM solution of DPPH in methanol was prepared. Different concentrations of each sample (5–50 µM) were added to 1.0 ml (0.5 mM DPPH) and final volume made up to 3.0 ml with methanol. The mixture was shaken vigorously and kept standing at room temperature for 15 min. Then the absorbance of the mixture was measured at 517 nm on UV spectrophotometer. The decrease in the absorbance indicates an increase in DPPH-radical scavenging activity. The percentage inhibition was calculated by the following equation:

$$\text{DPPH radical scavenging } (\%) = A_C - A_S / A_C \times 100$$

where A_C is the absorbance of control and A_S is absorbance of sample. Ascorbic acid and gallic acid both were used as positive control. The experiment was done in triplicate and mean values were calculated. Standard deviation for the triplicate analysis was also calculated. IC_{50} value was calculated as the concentration of sample required to scavenge 50 % of DPPH free radicals.

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Conflict of Interest

The authors declare that there is no conflict of interest.

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