



Synthesis and Antioxidant Ability of New 5-amino-1,2,4-Triazole Derivatives Containing 2,6-dimethoxyphenol

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ABSTRACT

4-amino-3-(4-(((4-hydroxy-3,5-dimethoxybenzyl)oxy)methyl)phenyl)-1,2,4-triazole-5-thione was synthesized by to method the first one from melt reaction of 4-(((4-hydroxy-3,5-dimethoxybenzyl)oxy)methyl)benzoic acid with Thiocarbonyldihydrazide, the second method from convert the corresponded acid hydrazide to potassium 2-(4-(((4-hydroxy-3,5-dimethoxybenzyl)oxy)methyl)benzoyl)hydrazinecarbodithioate salt then react with hydrazine hydrate. Newly Schiff base (7a-7f) were synthesized from reaction the 4-amino-1,2,4-triazol with substituted hydroxybenzaldehyde. The resulting compounds were characterized by IR, ¹H-NMR, ¹³C-NMR, and HRMS data. 2,2-Diphenyl-1-picrylhydrazide (DPPH) and ferric reducing antioxidant power (FRAP) assays were used to screened the antioxidant properties of the synthesized compounds. Compounds 7d , 7e and 7f exhibited significant free-radical scavenging ability in both assays.

Key words: 2,6-dimethoxyphenol ,4-amino-1,2,4-triazole, Schiff base, Antioxidant, DPPH, FRAP.

INTRODUCTION

The reactive oxygen species (ROS) and other related free radicals species are witty to react either directly or indirectly, to damage all biomolecules. This damage can cause many diseases ¹, such as inflammatory², cancers³, degenerative⁴ and chronic diseases⁵. The antioxidant compounds are the most important species which can inhibit the oxidative stress in biological system and prevent any free radicals damage. The phenolic

compounds are one of the significant antioxidant type, as well reported it possess broad biological activity such as anti inflammatory⁶, anticancer⁷ and gastroprotective⁸.

Generally, antioxidants compounds donate protons to become stable free radicals. This stability increases with the extent of delocalization and enhances the antioxidant ability^{9, 10}. As such, many synthesized compounds containing long chain resonance exhibited significant antioxidant

activity. Furthermore, the compounds which can be considered a strong antioxidant usually possess common structural features. They often own multiple phenolic hydroxyl groups like flavonoids¹¹ or which have full conjugation δ system like carotenoids¹². Moreover, exhibited substituted groups might influence on the scavenging ability. This indicates the existence of a close relationship between the chemical structure and the ability to scavenge free radicals.

Derivatives of 1,2,4-triazole exhibited various types of biological activities e.g. Antimicrobial¹³, antiviral,^{14,15} inhibitors of Methionine Aminopeptidase-2,¹⁶ Anhydrase inhibitors,¹⁷ anti-cancer¹⁸, anti-inflammatory,¹⁹ inhibitors of the HIV-1,²⁰ analgesic²¹ and antioxidant²². As well the compounds of Schiff base derivatives displayed broader properties and applications such as nonlinear optical devices²³ liquid crystalline^{24,25}, dyes²⁶, biological²⁷⁻²⁹ and pharmaceutical³⁰ applications.

The 2,6-dimethoxyphenol derivatives won wide attention in recent years as antioxidants^{31,32}. In this work we presented the synthesis of new 4-((arylidine)amino)-3-(4-(((4-hydroxy-3,5-dimethoxybenzyl)oxy)methyl)phenyl)-1,2,4-triazole-5-thione (7a-7f) as new antioxidant material.

MATERIAL AND METHOD

Chemistry

The IR spectra were obtained with a Perkin Elmer 400 Fourier Transform Infrared (FTIR) Spectrometer. ¹H and ¹³C-NMR spectra were recorded at Joel Lambda spectrometers at 400 MHz (UM, Malaysia). DMSO-*d*₆ were used as solvents with TMS as the internal standard. The mass spectra were recorded using an Agilent 5975 system for EI/MS and a Finnigan TSQ7000 for HREIMs (NUS, Singapore). For UV spectroscopy, a Power Wave X340 (BIOTEK Instruments, Inc., Winooski, VT, USA) was used to record the FRAP and DPPH. Melting points were measured on a Sturt-SMP10 melting point apparatus in open-end capillary tubes. Flash column chromatography on silica gel 60 (230–400 mesh, E. Merck) was employed. General grade solvents and reagents were purchased from commercial suppliers and used without further purification.

4-(((4-hydroxy-3,5-dimethoxybenzyl)oxy)methyl)benzoic acid 4

This compound was synthesized according to the procedure described by K. F. Ali³³. Recrystallized with ethanol to obtain white microcrystals. Yield 60 %, Mp 150-152 °C [lit. 152-154 °C³³], IR (KBr) ν_{\max} 3354 (OH), 3046 (CH_{Ar}), 2972, 2893 (CH_{aliphatic}), 1676 (C=O) 1585 (C=C), 1180 (Ar-O-C), cm⁻¹. ¹H-NMR (400MHz, CDCl₃): 3.87(6H, s, 2xOCH₃), 4.33 (2H, s, OCH₂), 4.38 (2H, s, OCH₂), 6.66 (s, 2H, H-3), 7.65 (2H, d, J 8.2, H-8), 8.12 (2H, d, J 8.0, H-9), 9.15 (1H, bs, OH), ¹³C-NMR (100 MHz, CDCl₃): 56.11(2C, OCH₃), 71.22(1C, CH₂OCH₂), 72.77 (1C, CH₂OCH₂), 109.05(2C, CH), 128.84(2C, CH), 129.37(1C), 131.21(2C, CH), 134.23 (1C), 139.65 (1C), 142.13(1C) 152.01 (2C), 169.58 (1C, C=O) HREIMs m/z = 318.1100 [M⁺] (calc. for C₁₇H₁₈O₆, 318.1103).

4-(((4-hydroxy-3,5-dimethoxybenzyl)oxy)methyl)benzohydrazide 5

This compound was synthesized according to the procedure described by K. F. Ali³³. The crude was recrystallized from ethanol to give white solid. Yield 87%, Mp 94-96 °C [lit. 92-94 °C³³], IR (KBr) ν_{\max} 3422 (OH_{phenol}), 3308, 3218 (NH, NH₂), 3037 (CHAr), 2975-2885 (CHaliphatic), 1661 (C=O), 1585 (C=C), 1151 (Ar-O-C), cm⁻¹. ¹H-NMR (400MHz, DMSO-*d*₆): 3.71 (6H, s, 2x OCH₃), 3.93 (2H, s, OCH₂), 4.09 (2H, s, OCH₂), 4.63 (bs, 2H, NH₂), 6.52 (s, 2H, H-3), 7.67 (2H, d, J 8.1, H-8), 8.22 (2H, d, J 7.92, H-9), 8.863 (1H, bs, CONH), 9.21 (1H, bs, OH), ¹³C-NMR (100 MHz, DMSO-*d*₆): 56.3 (2C, OCH₃), 71.14 (1C, CH₂OCH₂), 72.60 (1C, CH₂OCH₂), 106.88(2C, CH), 129.11(2C, CH), 128.92(1C), 130.01(2C, CH), 133.42(1C), 139.04(1C), 141.55(1C) 152.01 (2C), 166.15(C=O). HREIMs m/z = 332.1369 [M⁺] (calc. for C₁₇H₂₀N₂O₅, 332.1372)

General synthesis of 4-amino-3-(4-(((4-hydroxy-3,5-dimethoxybenzyl)oxy)methyl)phenyl)-1,2,4-triazole-5-thione. 6

Method A

4-(((4-hydroxy-3,5-dimethoxybenzyl)oxy)methyl)benzoic acid (3.2gm 10.05,mmol) and Thiocar-bonyldihydrazide (1.17 gm, 11 mmol) was mixed then grinded after that the mixture was transferred to conical flask and heated until melt at 160-165 °C for three hour. The mixture left to cool

to ambient temperature. The crude was boiled with (2x20 mL) dichloromethane then filtrated. The organic combined evaporated under reduced pressure. The crude precipitated was recrystallized from ethanol to afford white crystals. Yield 51% Mp 68-70 °C,

Method B

To a solution of 4-(((4-hydroxy-3,5-dimethoxybenzyl)oxy)methyl)benzohydrazide (3.33 g, 10 mmol) in xx mL absolute ethanol was added. Carbon disulphide (0.92 g, 12 mmol) was added and potassium hydroxide (0.56 g, 10 mmol) at room temperature. The mixture was stirred for 24 h, then 25 mL dry ether was added and allow to stir for another 2 h. The precipitated was filtrated and washed with dry ether. The product was dried at 80 °C to give white solid of potassium 2-(4-(((4-hydroxy-3,5-dimethoxybenzyl)oxy)methyl)benzoyl)hydrazinecarbodithioate salt (4 g, 9 mmol). The resulting product was dissolved in excess of hydrazine hydrate 80%. The mixture was heated and refluxed for 7 h then cooled and poured into ice water. The pH was adjusted to 5 by using 10% HCl. The precipitate was filtrated, washed with water, dried and recrystallized from ethanol to obtain yield 68% of white crystals. Mp 68-70.

IR (KBr) ν_{\max} 3507 (OH_{phenol}), 3410, 3330 and 3170 (NH, NH₂), 3013 (CH_{Ar}), 2995-2887 (CH_{aliphatic}), 1628 (C=N), 1585 (C=C), 1362 (C-N), 1245 (C=S), 1201 (Ar-O-C), cm⁻¹. ¹H-NMR (400 MHz, DMSO-d₆): 3.82 (6H, s, 2x OCH₃), 3.89 (2H, s, OCH₂), 3.98 (2H, s, OCH₂), 6.13 (bs, 2H, NH₂), 6.58 (s, 2H, H-3), 7.69 (2H, d, J 8.0, H-8), 8.25 (2H, d, J 7.82, H-9), 9.27 (1H, bs, OH), 14.03 (1H, bs, NH). ¹³C-NMR (100 MHz, DMSO-d₆): 57.21, (2C, OCH₃), 72.04 (1C, CH₂OCH₂), 72.69 (1C, CH₂OCH₂), 107.17 (2C, CH), 128.78 (2C, CH), 129.90 (1C), 131.10 (2C, CH), 133.72 (1C), 138.86 (1C), 142.53 (1C), 149.54 (1C, C=N), 152.01 (2C), 166.61 (C=S). HREIMS m/z = 388.1202 [M⁺] (calc. for C₁₈H₂₀N₄O₄S, 388.1205)

General synthesis of 4-((arylidene)amino)-3-(4-(((4-hydroxy-3,5-dimethoxybenzyl)oxy)methyl)phenyl)-1,2,4-triazole-5-thione 7a-7f

aryl aldehyde (0.51 mmol) was added to a stirred solution of 4-amino-3-(4-(((4-hydroxy-3,5-dimethoxybenzyl)oxy)methyl)phenyl)-1,2,4-

triazole-5-thione (0.2 gm, 0.51 mmol) in 5 mL glacial acetic acid and the mixture was refluxed for 9h. After cooling the mixture was poured into 50 mL crushed ice. the precipitate collected, washed with cold distilled water and recrystallized from suitable solvent.

4-(((4-hydroxybenzylidene)amino)-3-(4-(((4-hydroxy-3,5-dimethoxybenzyl)oxy)methyl)phenyl)-1,2,4-triazole-5-thione 7a

Recrystallized from methanol to give pale yellow precipitate. Yield 83%, Mp 115-117 °C IR (KBr) ν_{\max} 3498 (OH_{phenol}), 3152 (NH), 3062 (CH_{Aromatic}), 1623 (CH=N), 1607 (C=N_{triazole}), 1995, 1985 (C=C), 1247 (C=S), 1201 (C-O), cm⁻¹. ¹H-NMR (DMSO-d₆, δ): 3.62 (6H, s, 2x OCH₃), 4.34 (2H, s, OCH₂), 4.52 (2H, s, OCH₂), 6.67 (s, 2H, H-3), 6.84 (2H, d, J 8.2), 7.86 (4H, d, J 8.4), 7.93 (2H, d, J 8.2), 8.24 (2H, d, J 8.21), 8.46 (1H, s, CH=N), 9.22 (1H, bs, OH), 9.95 (bs, 2H, OH), 14.21 (1H, bs, NH). ¹³C-NMR (100 MHz, DMSO-d₆): 56.09 (2C, OCH₃), 70.88 (1C, CH₂OCH₂), 69.90 (1C, CH₂OCH₂), 107.85 (2C, CH), 116.11 (2C), 125.56 (1C), 129.07 (2C, CH), 129.25 (1C), 130.47 (2C), 131.23 (2C, CH), 133.09 (1C), 138.85 (1C), 142.17 (1C), 151.91 (2C), 157 (1C), 160.31 (CH=N), 160.93 (C=N_{triazole}), 182.43 (1C, C=S). HREIMS m/z = 492.1461 [M⁺] (calc. for C₂₅H₂₄N₄O₅S, 492.1467).

4-(((4-hydroxy-3-methoxybenzylidene)amino)-3-(4-(((4-hydroxy-3,5-dimethoxybenzyl)oxy)methyl)phenyl)-1,2,4-triazole-5-thione 7b

Recrystallized from ethanol to afford yellow precipitate. Yield 78% Mp 81-83 °C IR (KBr) ν_{\max} 3568 (OH_{phenol}), 3169 (NH), 3053 (CH_{Ar}), 1621 (CH=N), 1605 (C=N_{triazole}), 1595, 1983 (C=C), 1232 (C=S), 1195 (C-O); cm⁻¹. ¹H NMR (DMSO-d₆, δ): 3.57 (6H, s, 2x OCH₃), 3.89 (s, 3H, OCH₃), 4.41 (2H, s, OCH₂), 4.59 (2H, s, OCH₂), 6.62 (s, 2H, H-3), 6.83 (d, 1H, J 7.79), 6.86 (2H, d, J 8.0), 7.26 (dd, 2H, J 8.22; 1.8), 7.43 (s, 1H) 7.91 (2H, d, J 8.22), 8.22 (2H, d, J 8.2), 8.59 (s, 1H, CH=N), 9.23 (1H, bs, OH), 10.06 (1H bs, OH), 14.01 (1H, bs, NH). ¹³C-NMR (100 MHz, DMSO-d₆): 56.09 (2C, OCH₃), 56.13 (1C, OCH₃), 70.91 (1C, CH₂OCH₂), 69.78 (1C, CH₂OCH₂), 108.04 (2C, CH), 110.31 (1C), 116.1 (1C), 116.37 (2C), 124.25 (1C), 125.63 (1C), 126.08 (1C), 129.11 (2C, CH), 129.19 (1C), 130.52 (2C), 131.53 (2C, CH), 133.29 (1C), 138.65 (1C), 142.17 (1C), 148.45 (1C), 151.92 (2C), 153.39 (1C), 157.1 (1C), 161.10 (CH=N),

164.22(C=N_{triazole}), 179.93(1C, C=S). HREIMs m/z = 522.1568 [M⁺] (calc. for C₂₆H₂₆N₄O₆S, 522.1573).

4-((4-hydroxy-3-ethoxybenzylidene)amino)-3-(((4-hydroxy-3,5-dimethoxybenzyl)oxy)methyl)phenyl)-1,2,4-triazole-5-thione 7c

The crude product was recrystallized from acetonitrile to afford yellow crystals. Yield 71% Mp 104-106 °C. IR (KBr) ν_{\max} , 3584(OH_{phenol}), 3170(NH), 3037(CH_{Ar}), 2987, 2865 (CH_{aliphatic}) 1618 (CH=N), 1607 (C=N_{triazole}), 1595, 1983(C=C), 1238(C=S). 1201(C-O), cm⁻¹. ¹H-NMR (400MHz, DMSO-d₆) 1.43 (t, 3H, J 6.9, O-CH₂CH₃), 3.72(6H, s, 2x OCH₃), 3.92 (q, 2H, J 7.1, O-CH₂), 4.39 (2H, s, OCH₂), 4.74 (2H, s, OCH₂), 6.54 (s, 2H, H-3), 6.85 (d, 2H, J 8.0, 7.24 (1H, dd, J 8.1; 1.68) 7.71 (2H, d, J 8.22, H-8), 8.26 (2H, d, J 8.2), 8.59 (1H, s, CH=N), 9.16(1H, bs, OH), 9.78 (1H, bs, OH), 14.14(1H, bs, NH), ¹³C-NMR (100 MHz, DMSO-d₆): 14.94 (1C, O-CH₂CH₃), 55.21(2C, OCH₃), 64.32 (1C, OCH₂), 70.96(1C, CH₂OCH₂), 69.87(1C, CH₂OCH₂), 108.05(2C, CH), 111.32(1C), 117.13 (1C), 124.77 (1C), 127.51(1C), 129.39(2C, CH), 129.65(1C), 132.36(2C, CH), 134.16(1C), 138.82(1C), 142.72 (1C), 147.86 (1C), 150.61 (1C) 151.89 (2C), 161.01(1C, CH=N), 163.12 (C=N_{triazole}), 181.34(1C, C=S). HREIMs m/z = 5536.1725 [M⁺] (calc. for C₂₇H₂₈N₄O₆S, 536.1730).

4-((4-hydroxy-3,5-dimethoxybenzylidene)amino)-3-(((4-hydroxy-3,5-dimethoxybenzyl)oxy)methyl)phenyl)-1,2,4-triazole-5-thione 7d

Crude product recrystallized from chloroform-ethanol to afford pale yellow precipitate. Yield 73%. Mp 136-138 °C. IR (KBr) ν_{\max} , 3605(OH_{phenol}), 3172(NH), 3062(CH_{Ar}), 2984, 2890 (CH_{aliphatic}) 1614 (CH=N), 1605 (C=N_{triazole}), 1595, 1980(C=C), 1221(C=S), 1198(C-O), cm⁻¹. ¹H-NMR (400MHz, DMSO-d₆) 3.73(6H, s, 2x OCH₃), 3.85 (s, 6H, 2xOCH₃) 4.42 (2H, s, OCH₂), 4.56 (2H, s, OCH₂), 6.59 (s, 2H, H-3), 7.24 (s, 2H) 7.71 (2H, d, J 8.2, H-8), 8.28 (2H, d, J 8.0), 8.51(1H, s, CH=N), 9.08(2H, bs, OH), 9.65(1H, bs, OH), 13.97(1H, bs, NH) ¹³C-NMR (100 MHz, DMSO-d₆): 56.28(2C, OCH₃), 56.31(2C, OCH₃) 70.17(1C, CH₂OCH₂), 68.99(1C, CH₂OCH₂), 106.07 (2C, CH), 107.96(2C, CH), 129.27(2C, CH), 124.78(1C) 129.28(1C), 131.23(2C, CH), 133.43(1C), 138.66(1C), 142.41 (2C) 151.85 (4C), 160.35(1C, CH=N), 162.13 (1C,

C=N_{triazole}), 181.22(1C, C=S). HREIMs m/z = 552.1673 [M⁺] (calc. for C₂₇H₂₈N₄O₇S, 552.1679).

4-((4-hydroxy-3,5-di-tert-butylbenzylidene)amino)-3-(((4-hydroxy-3,5-dimethoxybenzyl)oxy)methyl)phenyl)-1,2,4-triazole-5-thione 7e

The crude products recrystallized from Chloroform afforded off white precipitate. Yield 60%, mp 158-160, IR (KBr) ν_{\max} , 3599.7 (OH_{phenol}), 3177(NH), 3068 (CH_{Ar}), 2990-2885 (CH_{aliph}), 1622(CH=N), 1608(C=N_{triazole}), 1595-1589 (C=C) and 1262(C=S). ¹H NMR, δ 3.333334.65 (2H, s, OCH₂), 5.62 (1H, bs, OH), 6.64 (2H, s, H-3), 7.62 (2H, d, J 8.22, H-8), 7.91(s, 2H), 8.18(2H, d, J 8.1), 8.60(1H, s, CH=N), 9.14(1H, bs, OH), (1H, bs, NH) ¹³C-NMR (100 MHz, DMSO-d₆): 30.37(6C, 4(C(CH₃)₃), 34.46(2C, C(CH₃)₃) 56.11(2C, OCH₃), 71.24(1C, CH₂OCH₂), 69.93(1C, CH₂OCH₂), 107.65(2C, CH), 116.86 (1C), 124.80 (1C), 129.07(2C, CH), 129.35(1C), 131.38(2C, CH), 133.14(1C), 138.85(1C), 142.17 (1C) 151.79 (2C), 157.15 (1C), 161.77 (1C, CH=N), 165.11 (C=N_{triazole}), 182.71(1C, C=S). HREIMs m/z = 604.2715 [M⁺] (calc. for C₃₃H₄₀N₄O₅S, 604.2719).

4-((2-hydroxy-3,5-di-tert-butylbenzylidene)amino)-3-(((4-hydroxy-3,5-dimethoxybenzyl)oxy)methyl)phenyl)-1,2,4-triazole-5-thione 7f

The crude products recrystallized from Chloroform-ethanol afforded pale yellow precipitate. Yield 73 %, mp 122-124 °C, IR (KBr) ν_{\max} , 3603 (OH_{phenol}), 3173(NH), 3068 (CH_{Ar}), 2980-2885 (CH_{aliph}), 1621(CH=N), 1604(C=N_{triazole}), 1597-1589 (C=C) and 1262(C=S) ¹H NMR, δ (ppm, CDCl₃, 400 MHz): 1.32(9H, s, o-(t-Bu), 1.45(9H, s, p-(t-Bu), 3.71(6H, s, 2x OCH₃), 4.42 (2H, s, OCH₂), 4.45 (2H, s, OCH₂), 6.57 (s, 2H, H-3), 7.47(1H, d, J 2.4) 7.52(1H, d, J 2.2) 7.74 (2H, d, J 8.22, H-8), 8.18 (2H, d, J 8.2), 8.56(1H, s, CH=N) 9.14(1H, bs, OH), 10.21 1H, bs, OH), 14.29(1H, bs, NH), ¹³C-NMR (100 MHz, DMSO-d₆): 29.32(3C, p-(C(CH₃)₃), 31.52(3C, o-(C(CH₃)₃), 34.56(1C, o-C(CH₃)₃), 35.41(1C, p-C(CH₃)₃) 56.31(2C, OCH₃), 71.02 (1C, CH₂OCH₂), 72.57 (1C, CH₂OCH₂), 106.35(1C), 108.95(2C, CH), 120.41 (1C), 128.29(1C), 128.81(2C, CH), 129.32(1C), 131.24(2C, CH), 134.13 (1C), 137.61(1C), 139.55 (1C), 141.7(1C), 142.17(1C) 151.91 (2C), 154(1C), 162.07 (1C, CH=N), 164.08 (C=N_{triazole}), 182.88(1C, C=S) HREIMs m/z = 604.2716 [M⁺] (calc. for C₃₃H₄₀N₄O₅S, 604.2719)

Antioxidant**DPPH assay**

The assay was performed as reported by Gerhauser *et al.* ³⁴. Five microliters of the sample (dissolved in ethanol) was added into 195 μ L of 100 μ M DPPH reagent in ethanol (96%) and mixed in a 96-well plate. The intensity of the colour was measured for 3 h at an interval of 20 min at 515 nm. Ascorbic acid and BHT were used as reference.

FRAP assay

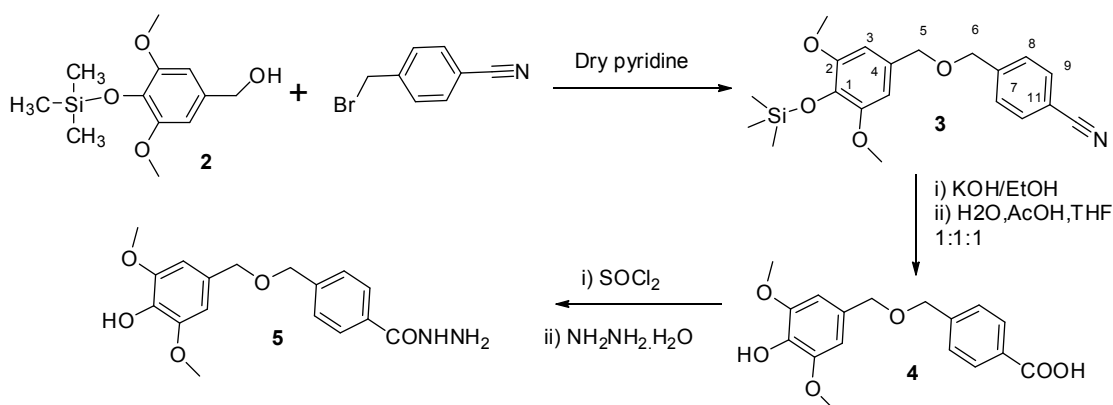
The FRAP assay was performed according to the Benzie and Strain ³⁵ method. The FRAP reagent was prepared by combining 300 mM acetate buffer and 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) solution in 40 mM HCl and 20 mM FeCl₃·6H₂O, in a ratio of 10:1:1. The FRAP reagent was incubated at 37 °C prior to use. Ten microliters of the sample was reconstituted in the carrier (solvent or ultrapure water) and mixed with 300 μ L of FRAP reagent. The mixture was incubated at 37 °C for 4 min in a microplate reader. The absorbance of the complex was 593 nm. The FRAP value can be calculated using the following equation³⁶ :

$$\text{FRAP} = \frac{[(0-4 \text{ min } \Delta A_{593 \text{ nm}} \text{ of test sample}) / (0-4 \text{ min } \Delta A_{593 \text{ nm}} \text{ of standard})] \times [\text{standard}] (\mu\text{M}) \times Y}{\times 1000}$$

Where; Y is absorbance of the spectrophotometer.

RESULTS AND DISCUSSION**Chemistry**

The 4-(((4-hydroxy-3,5-dimethoxybenzyl)oxy)methyl) benzoic acid **4** and their corresponded



Scheme 1: Synthetic route of 4-(((4-hydroxy-3,5-dimethoxybenzyl)oxy)methyl) benzoic acid **4 and their corresponded acid hydrazide **5****

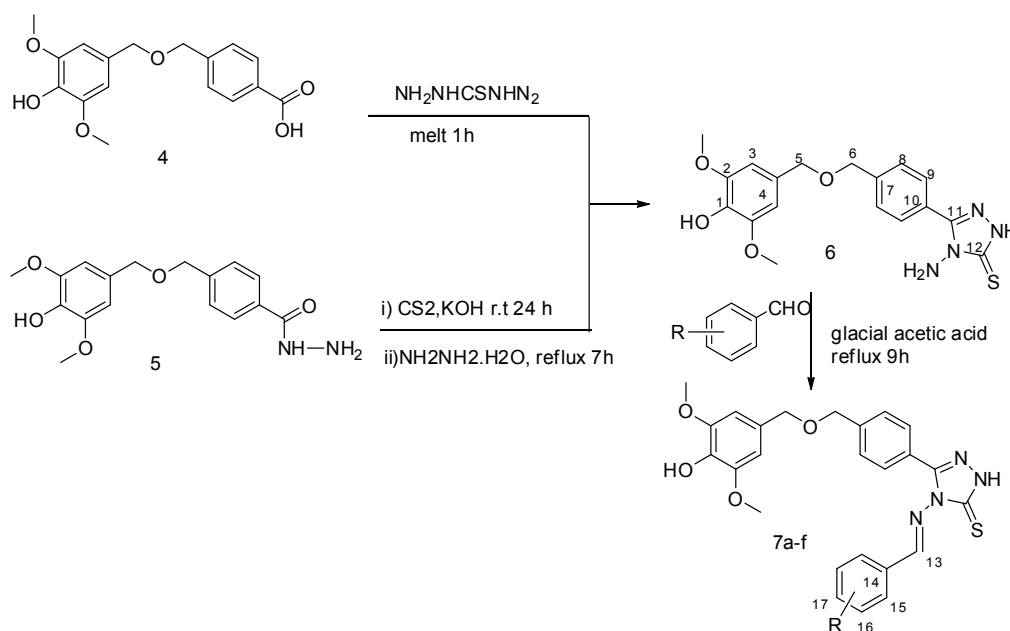
acid hydrazide **5** were synthesized from according to the procedure described by K. F. Ali ³³ as depicted in Scheme 1

The 4-amino-3-(4-(((4-hydroxy-3,5-dimethoxybenzyl)oxy)methyl)phenyl)-1,2,4-triazole-5-thione. **6** were synthesized from two different methods. The first one, from melt reaction with Thiocarbonyldihydrazide for three hour at 160-165 °C to obtained 51% yield. The second methods, was from converted the corresponded acid hydrazide **5** to their corresponded potassium hydrazinecarbodithioate salt as the first step. The next step was reacted this salt with hydrazine hydrate under reflux for 7h. this method exhibited yield higher than method one. The compound **7a-7f** were synthesized from reaction compound **6** with hydroxyl substituted benzaldehyde in the presence of glacial acetic acid. As depicted in Scheme 2

All synthesized compounds were characterized by IR, ¹H-NMR, ¹³C-NMR spectrum beside the EIMS and HRMIS. The IR spectrum of compound **4** displayed rise a new band of C=O at 1676 cm⁻¹ and disappeared the band of C≡N at 2243 cm⁻¹. compound **5** showed shifting at carbonyl group which is appeared at 1661 cm⁻¹ of acid to hydrazide as well new bands of NH and NH₂ were appeared at 3308-3218 cm⁻¹. The IR spectrum of compound **6** exhibited disappeared of carbonyl group and new interesting bands were appeared at 1628 cm⁻¹ corresponded the C=N group of the triazole ring as and at 1245 cm⁻¹ of C=S beside the value of NH and NH₂ bands were shifted to 3410,3330 and 3170 cm⁻¹ respectively. The spectra of **7a-7f**

displayed disappeared the band of NH₂ and a new interesting band for the Schiff base was appeared at 1623-1614 cm⁻¹ besides the C=N of the triazole ring which appeared at 1608-1604 cm⁻¹. The ¹H-NMR spectrum of compound **4** showed disappearing of tri methylsilyl group at 0.17 and appears of the broad singlet of OH for the converting the nitrile group to carboxylic acid. The ¹H-NMR spectrum of compound **5** showed the disappearing hydroxyl group of the carboxylic as well a new two board singlet of NH₂ and NH were appeared at 4.63 δ and 8.86 δ

respectively. The ¹H-NMR of compound **6** displayed all expected peaks in triazole structure. The ¹H-NMR spectra of compounds **7a-7f** showed the appearance of new peaks represented the Schiff base (imin group) and the expected proton of the substituted hydroxybenzylidene (see the experimental suction) also appeared in their expected rang. The ¹³C-NMR spectrum of compound **4** exhibited two carbons of CH₂OCH₂ at 3.87 & 433 δ , beside disappearing the carbon of Ca''N and raised a new carbon of C=O. That enhances the evidence of successful convert



Scheme 2: Synthetic route of compound **6** and **7a-7f**

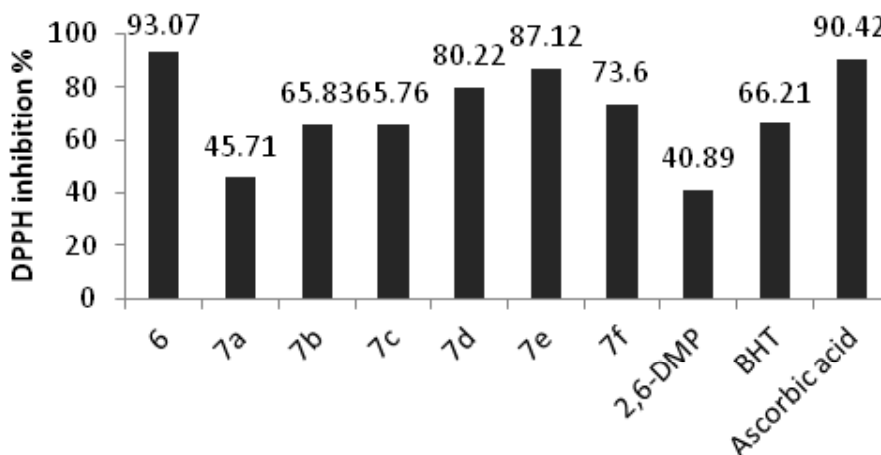


Fig. 1: DPPH inhibitions % of **7a-7f**

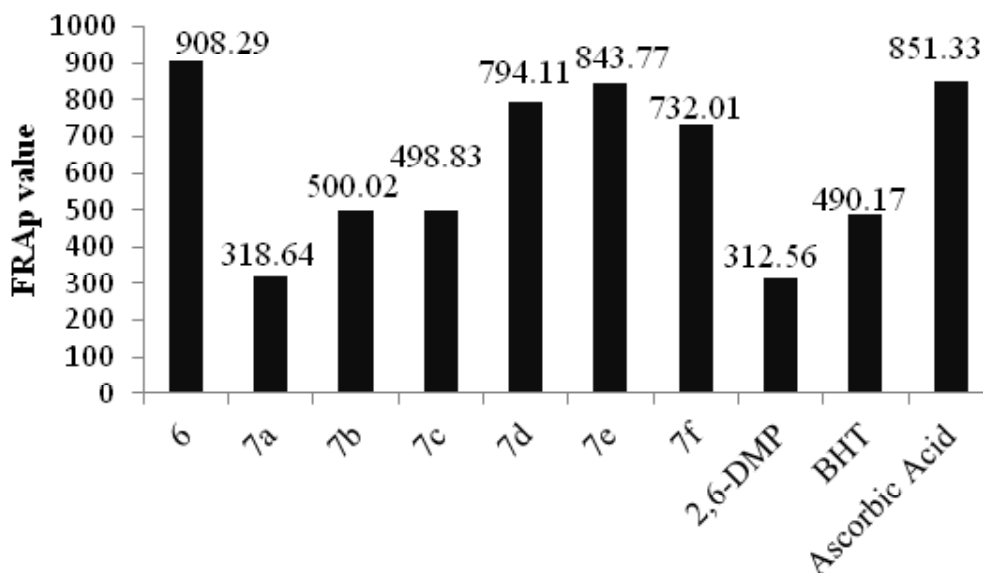


Fig. 2: FRAP value of 7a-7f

compound 3 to 4 besides the spectra of IR and $^1\text{H-NMR}$. The $^{13}\text{C-NMR}$ showed change in value of carbonyl of compound 5 due to convert the acid to acid hydrazide. The spectrum of compound 6 showed two new carbons first on the C=N at 149.54 \AA of triazole ring and the second one at 166.61 \AA represented the C=S. The ^{13}C spectra of compounds 7a-7f were showed an interested peak of CH=N beside the carbons of the hydroxybenzylidene. The substituent group for 7b-7f were appeared in the expected range. The EIMS spectra showed the molecular ion M^+ for all compounds and the base peak (100%) as well the HREIMS value was confirmed the accurate mass and the molecular formula .as depicted in Table 1.

Antioxidant activity

The synthesized compound 6 and 7a-7f were showed high antioxidant ability in both assays (DPPH and FRAP). Compound 6 showed antioxidant ability higher than 7a-7b in both assays and that could attributed to exhibited a pro-oxidative effect³⁷. Even though compounds 7a-7f exhibited significant antioxidant ability. The type of substituted at hydroxybenzylidene play an important role to enhance the antioxidant ability. Compound 7e exhibited higher antioxidant ability (slightly less than ascorbic acid) in both assays (DPPH and FRAP) as shown in Figure 1 and Figure 2.

Compound 7d showed free radical scavenging ability more than compound 7f and that could attributed to the *t*-Bu group at position *para* reduce the antioxidant ability³⁸, the compound 7b and 7c exhibited nearly same antioxidant ability and that in agreement with most literatures. Finally compound 7a without any substituted group around the hydroxyl group of phenol show the less antioxidant ability.


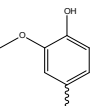
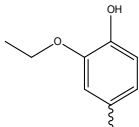
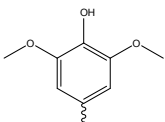
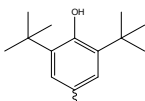
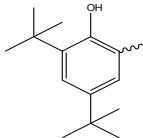
CONCLUSIONS

A series of newly Schiff base compounds from 4-amino-1,3,4-triazole incorporating hindered phenol moieties were successfully synthesized and characterized. All of the newly compounds were tested for antioxidant activity by DPPH and FRAP assays. The antioxidant ability of these compound increases with increasing the hindered phenol.

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Table 1: The hydroxyl substituted and selected properties of synthesized compound 7a-7f

No	aldehyde	Yield %	M.P. C	Molecular Formula	HREIMS calc.	HREIMS Found
7a		83	115-117	C ₂₅ H ₂₄ N ₄ O ₅ S	492.1467	492.1461
7b		78	81-83	C ₂₆ H ₂₆ N ₄ O ₆ S	522.1573	522.1568
7c		71	104-106	C ₂₇ H ₂₈ N ₄ O ₆ S	5536.1730	5536.1725
7d		73	136-138	C ₂₇ H ₂₈ N ₄ O ₇ S	552.1679	552.1673
7e		60	158-160	C ₃₃ H ₄₀ N ₄ O ₅ S	604.2719	604.2715
7f		73	122-124	C ₃₃ H ₄₀ N ₄ O ₅ S	604.2719	604.2716

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