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ORIENTAL JOURNAL OF CHEMISTRY
An International Open Free Access, Peer Reviewed Research Journal

www.orientjchem.org

ISSN: 0970-020 X
CODEN: OJCHEG
2017, Vol. 33, No.(5):
Pg. 2492-2500

Synthesis and Antioxidant Ability of Some New 6-amino-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-3-yl) Derivatives Bearing 2,6-Dimethoxy-4-(methoxymethyl)Phenol Moiety

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http://dx.doi.org/10.13005/ojc/330543

(Received: August 09, 2017; Accepted: August 30, 2017)

ABSTRACT

Compound 4-(((6-amino-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-3-yl)methoxy)methyl)-2,6-dimethoxyphenol (6) was synthesized by multi steps. The corresponding acetonitrile thioalkyl (7) was cyclized by refluxing with acetic acid to afford 4-(((6-amino-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-3-yl)methoxy)methyl)-2,6-dimethoxyphenol (8). Two new series of 4-(((6-(3-(4-aryl)thioureido)-7H-[1,2,4]triazolo[3,4-b][1,3,4] thiadiazin-3-yl)methoxy)methyl)-2,6-dimethoxyphenol (9a-c) and of 4-(((6-(substitutedbenzamido)7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-3-yl)methoxy)methyl)-2,6-dimethoxyphenol (10a-c) were synthesized as new derivatives for fused 1,2,4-trizaole-thiadiazine(8). The antioxidants of newly compounds were evaluated by DPPH and FRAP assays. Compound 9b showed significant antioxidant ability in both assays(higher than ascorbic acid) as well compound 6,8 and 10a-c showed antioxidant higher than BHT.

Keywords: 2,6-dimethoxyphenol, 6-aminothiadiazine, Fused 1,2,4-triazole, Antioxidant.

INTRODUCTION

The free radicals are one of the most important factors that cusses critical disadvantagetobiomolecules, for instance, proteins, carbohydrates,lipids, and DNA[1]. Such this disadvantage considered as source of many infirmity such as inflammatory[2] and cancers infirmity [3], degenerative infirmity [4] and Chronic infirmity. For that, theantioxidants compounds considered one of thesignificant materials due to their capacity to terminate the free radicals ordiminish the oxidation effect. Furthermore, many anti-inflammatory and antinecrotic medications reported it possess antioxidant ability besides their therapeutic properties[5]. Commonly, the free radical scavengingcompounds endue protons and transform toextra stable free radicals. This stability rises with the presence of delocalization and rises the antioxidant ability[6,7]. Otherwise,the presence of multiple hydroxyl groups or presence full conjugation (π system) in structure and steric hindrance these factors has positive influence on the antioxidant ability[8-10].

Fused rings with 1,2,4-triazole exhibited intensive interests for their wide biological activity. For instance, antimicrobials activity[11], antitumor[12,13], herbicidal activity[14], anti-inflammatory[15], antifungal activity[16], anti-HIV-1 activity[17] and antioxidant activity[18]. Moreover, fused 1,2,4-triazole –thiadiazine exhibited wide biological activity[19-21]. In the other hand, the 2,6-dimethoxyphenol derivatives, has been reported exhibited interesting antioxidant properties[22,23]. In this work we presented synthesis some new 6-amino-7h-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-3-yl) derivatives bearing 2,6-dimethoxy-4-(methoxymethyl)phenol moiety. And evaluated their antioxidant ability by DPPH and FRAP assays.

MATERIAL AND METHOD

Chemistry

The melting point was detected by open capillary tube method utilizingOMEGA MPS10, apparatus and it is uncorrected. The purities of synthesized compounds were checked with a thin layer chromatography (Silica gel TLC) plates and the spots were visualized either with UV lights or iodine vapors. The FTIR spectra were obtained with Perkin Elmer 400 Fourier Transform Infrared (FTIR) Spectrometer. 1D NMR spectra were recorded by Bruker AVN 400MHz. $CDCl_3$ and DMSO-d_6 wereutilized as a solvent with TMS as internal standard, measurements.The accurate mass spectra were recorded by utilizing a Finnigan TSQ7000 for HREIMs (NUS, Singapore). The FRAP Assay and DPPH assay (as antioxidant assay) were recording byusing ultravioletspectroscopy Power Wave X340,BIO-TEK instrument.

ethyl2-((3,5-dimethoxy-4-((trimethylsilyl) oxy)benzyl)oxy)acetate3

Ethyl bromoacetate (5.1g, 30 mmol) was added drop wise with in 1 h. to stirring suspension of(3,5-dimethoxy-4-((trimethylsilyl)oxy)phenyl)methanol (7.69 g, 30 mmol) in 50 ml DCM and 20 ml of 15% sodium hydroxide in the presence of tetrahexy lammonium bromide (THAB) (0.65 g, 5 % mmol). After complete the addition the mixture was left under stirring for 18 h at room temperature. The mixture transferred to separation funnel to extract the organic layer. The organic layer was extracted with distilled water (10 ml × 2). The organic layer was evaporated under reduced pressure after it dried under sodium sulfate. The product was purified by column chromatography utility hexane: ethyl acetate (6:1) to afford yellow oil which is solidified at 0°C to give white solid. Yield 74%, Mp 7-9°C. FTIR (liquid film) ν_{max}; 3046 (CH_{Ar}), 2983, 2867($CH_{aliphatic}$), 1724 (C=O), 1601,1584 (C=C), 1208 (Ar-O-C), 870(Si-CH_3) cm^{-1}.^{1}H NMR 400 MHz, $CDCl_3$): δ 0.16 (9H, s, Si(CH_3)3,1.34 (3H,t,*J* 7.2,CH_3),.3.85 (6H,s, 2×OCH_3), 4.24(2H, q,*J* 7.3,OCH_2CH_3),4.40 (2H,s, OCH_2CO_2Et), 4.78(2H,s, CH_2OCH_2),6.61(2H,s,H-3).^{13}C NMR (100MHz. CDCl3) δ; -0.057(3C,Si(CH_3)$_3$), 15.22 (1C,CH_3), 57.09 (2C,2× OCH_3), 59.35(1C, OCH_2CH_3) ,65.18(1C,OCH_2CO_2Et), 71.07 (1C,CH_2OCH_2), 109.11(2C,C_3).127.94 (1C,C1), 132.54 (1C, C-4),150.91 (1C,C-2), 171.04(1C,C=O). HREIMs m/z = 342.1499 [M^+] (calc. for $C_{16}H_{26}O_6Si$, 342.1499).

2-((4-hydroxy-3,5-dimethoxybenzyl)oxy)acetic acid. 4

A suspension of ethyl 2-((3,5-dimethoxy-4-((trimethylsilyl)oxy)benzyl)oxy)acetate. (3) (7.5 g, 21mmol) in 25 ml of 50 % acetic acid: THF (2:1) was refluxed overnight. After cooling the PH of the mixture was adjusted to 8-9 by saturated solution of sodium hydrogen carbonate, and then extracted by chloroform. The organic layer was neglected. The aqueous layer was acidified using 5% hydrochloric acid. The precipitated was filtrated, washed with distilled water and dried to obtain white amorphous solid. Yield 69.2%.Mp 128-130°C.FTIR (KBr) ν_{max}; 3455 (OH), 3033 (CH_{Ar}), 2977, 2854($CH_{aliphatic}$), 1687 (C=O), 1595, 1483 (C=C), 1198 (Ar-O-C) cm^{-1}.^{1}H NMR 400 MHz, DMSO-d_6): δ 3.87 (6H,s, 2×OCH_3),4.34(2H,s,OCH_2COOH), 4.81(2H,s,CH_2OCH_2),6.65(2H,s,H-3),9.24 (bs,1H,OH), 12.76(1H, bs, COOH).^{13}C NMR (100MHz, DMSO-d_6) δ; 56.83 (2C,2× OCH_3),64.77 (1C,OCH_2COEt),70.88(1C,CH_2OCH_2), 108.94(2C,C-3), 132.60 (1C, C-4),137.94 (1C,C-1), 151.03 (1C,C-2), 178.25(1C,C=O). HREIMs m/z = 242.0786[M^+] (calc. for$C_{11}H_{14}O_6$, 242.0790).

2-((4-hydroxy-3,5-dimethoxybenzyl)oxy)acetohydrazide 5

Thionylchloride (5ml) was added droop wise to 2-((4-hydroxy-3,5-dimethoxybenzyl)oxy)acetic acid. (4) (3.6 g, 14.86mmol). The stirring mixture was refluxed for 3h.The remains of thionyl chloride was evaporated under reduce pressure. The resulting acid chloride (without further purification) was transferred to an addition funnel with 10 ml dry benzene. 5 ml of hydrazine hydrate

(98 %) in 10 ml dried benzene was added into a two neck flask that equipped with a condenser. After that the addition funnel fixed onto the flask and the solution of acid chloride was added dropwise at 0 °C. Aftercompletion the addition, the mixture incubated for 1 h with stirring at an ambient temperature, then for further it was refluxed 3 h .The solvent was removed under reduce pressure. The crude product collected and washed with water then recrystallized from aqueous ethanol to afford white needle crystal. Yield 82%.Mp103-106 °C.FTIR (KBr) ν_{max}; 3464 (OH), 3318, 3205, 3197 ($NHNH_2$(, 3042 (CH_{Ar}), 2984, 2870($CH_{aliphatic}$),1661 (C=O), 1603, 1485 (C=C), 1206(Ar-O-C) cm^{-1}.^{1}H NMR (400 MHz, DMSO-d_6): δ 3.89 (6H,s, 2×OCH_3), 4.37 (2H,s, OCH_2CO), 4.79(2H,s,CH_2OCH_2), 4.54(1H, bs,NH_2), 6.66(2H,s,H-3), 8.76(1H, bs.CONH), 9.32 (1H, bs,OH).^{13}C NMR (100MHz, DMSO-d_6) δ ; 56.91 (2C,2× OCH_3), 68.22(1C, OCH_2CONH), 71.27(1C,CH_2OCH_2), 109.09 (2C,C-3). 132.67 (1C, C-4),137.83 (1C,C-1), 150.95(1C,C-2), 166.05(1C,C=O).HREIMs m/z = 256.1054[M^+](calc. for$C_{11}H_{16}N_2O_5$, 256.1059).

4-amino-3-(((4-hydroxy-3,5-dimethoxybenzyl)oxy)methyl)-1H-1,2,4-triazole-5(4H)-thione 6

To stirred a solution of 2-((4-hydroxy-3,5-dimethoxybenzyl)oxy)acetohydrazide(3.0g, 11.70 mmol) in 12 ml absolute ethanolCarbon disulphide (1.35 g, 17.5 mmol) and potassium hydroxide (065 g, 11.7mmol) were added at ambient temperature. The mixture allowed to stirred for 24 h, and then 20 ml dry diethylether was added and thenstirring for further 2 h. The precipitated was collected by filtration thenwashed with dry diethyl ether. The product was dried at 70 °C to give white solid potassium 2-(2-((4-hydroxy-3,5-dimethoxybenzyl)oxy)acetyl)hydrazine carbodithioatesalt (3.69 g, 11.15 mmol). The product was dissolved in 10 mL of hydrazine hydrate 80 %. And heated under refluxed for 7 h. after coolingit was poured into crushed ice. The pH of solution was adjusted to 5-6 by utilizing 5 % HCl. The precipitated was collected and washed with water, dried then recrystallized from methanol to obtain 2.25 g (65 %) of white precipitate, m.p.142-144°C.FTIR (KBr) ν_{max}3511 (OH_{phenol}), 3411, 3304and 3169 (NH_2, NH), 3025 (CHAr), 2986 -2877 (CHaliphatic), 1630 (C=N), 1594-1477 (C=C), 1358(C-N), 1231(C=S), 1202 (Ar-O-C), cm^{-1}.^{1}H-NMR (400MHz, DMSO-d_6) δ;3.63(2H,s, CH_2OCH_2),3.84 (6H,s,2×OCH_3), 4.55 (2H,s,CH_2OCH_2), 5.98 (2H, bs, NH_2), 6.69 (2H, s, H-3), 9.30 (1H,bs, OH), 11.07 (1H, bs, NH). ^{13}C-NMR (100 MHz, DMSO-d_6) δ ; 57.13 (2C, 2×OCH_3), 67.83 (1C,CH_2OCH_2) ,71.95(1C, CH_2OCH_2), 110.73(2C,C-3),132.85 (1C,C-4), 138.54(1C,C-1),149.59(1C,C=N), 152.11(2C,C-2), 169.08 (1C, C=S). HREIMsm/z=312.0888[M^+](calc. for$C_{12}H_{16}N_4O_4S$,312.0892).

2-((4-amino-5-(((4-hydroxy-3,5-dimethoxybenzyl)oxy)methyl)-4H-1,2,4-triazol-3-yl)thio)acetonitrile 7

Chloroacetonitrile(0.5 g, 6.62 mmol) was added in small portions to a stirred suspension of compound 6 (2.06 g, 6.62 mmol)in anhydrous acetone and anhydrous potassium carbonate (0.91g, 6.62 mmol). The mixture was left to stand overnight with stirring at ambient temperature. The solvent was evaporated and the residue extracted with 30 ml chloroform. It was dried under anhydrous sodiumsulfate and evaporated under reduced pressure. The crud product was recrystallized from acetonitrile to afford off white precipitate. Yield 84%, Mp 68-70 °C.FTIR (KBr) ν_{max}3556 (OH_{phenol}), 3411, 3304(NH_2), 3031(CHAr), 2985-2865 (CHaliphatic), 2249(CN), 1626(C=N), 1598,1475(C=C), 1347(C-N), 1192(Ar-O-C), cm^{-1}.^{1}H-NMR (400MHz, DMSO-d_6) δ; 3.84(6H, s, 2× OCH_3), 4.30(2H, s, CH_2CN), 4.55 (2H,s,CH_2OCH_2), 4.60 (2H, s, CH_2OCH_2), 6.02 (2H, bs, NH_2), 6.65 (2H, s, H-3), 9.30 (1H,bs, OH).^{13}C-NMR (100 MHz, DMSO-d_6)δ; 18.33(1C, CH_2CN)56.81(2C, 2×OCH_3), 65.44 (1C, CH_2OCH_2), 70.58 (1C, CH_2OCH_2), 110.73 (2C, C-3), 115.0(1C, CN), 132.49 (1C,C-4), 137.98 (1C,C-1), 151.89 (2C, C-2), 152.02 (1C, C=N), 157.18 (1C, C=N). HREIMs m/z= 351.0998[M^+](calc. for $C_{14}H_{17}N_5O_4S$, 351 . 1 001).

4-(((6-amino-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-3-yl)methoxy)methyl)-2,6-dimethoxyphenol8

2-((4-amino-5-(((4-hydroxy-3,5-dimethoxybenzyl)oxy)methyl)-4H-1,2,4-triazol-3-yl)thio) acetonitrile (1.80g, 5.12 mmol) in 10 ml acetic acid was heated under reflux for 24 h after cooling the precipitated was collected and washed with 5 % sodium hydrogen carbonate solution then distilled water . Thecrude product was recrystallized from aqueous DMF to obtain pale yellow precipitated. Yield 59%. Mp 114-116°C.FTIR (KBr) ν_{max};3542 (OH_{phenol}), 3343, 3219(NH_2), 3028 (CHAr), 2988, 2873 (CHaliphatic), 1618 (C=N), 1595, 1477 (C=C), 1342(C-N), 1197(Ar-O-C), cm^{-1}. .^{1}H-NMR (400MHz, DMSO-d_6) δ; 3.85(2H, s, 2× OCH_3), 4.41(2H, s, H-9),

4.58 (2H,s,CH_2OCH_2), 4.68 (2H, s, CH_2OCH_2), 6.65 (6H, s, H-3), 7.34 (2H, bs, NH_2), 9.07 (1H,bs, OH).^{13}C-NMR (100 MHz, DMSO-d_6) δ; 37.57(1C, C-9) 57.21 (2C, 2×OCH_3), 60.62 (1C, CH_2OCH_2), 70.49 (1C, CH_2OCH_2), 110.24(2C, C-3), 131.87 (1C,C-4), 138.25 (1C,C-1), 151.73 (2C, C-2), 154.48 (1C, C=N), 157.16 (1C, C=N),157.70(1C, C=N). HREIMs m/z = 351.0995 [M^+] (calc. for $C_{14}H_{17}N_5O_4S$, 351.1001).

General synthesis of 4-(((6-(3-(4-aryl)thioureido)-7H-[1,2,4]triazolo[3,4-b][1,3,4] thiadiazin-3-yl)methoxy)methyl)-2,6-dimethoxyphenol 9a-c

aryl isothiocyanate (0.6 mmole) was added with small portion to hot solution of 4-(((6-amino-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-3-yl)methoxy)methyl)-2,6-dimethoxyphenol (0.21g, 0.6 mmol) in absolute ethanol (10 ml). The mixture heated at 50 C for 2 h. Upon cooling the precipitate filtrated and washed with cold ethanol. The crud material was recrystallized from suitable solvent.

4-(((6-(3-(4-chlorophenyl)thioureido)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-3-yl)methoxy)methyl)-2,6-dimethoxyphenol 9a

The crude solid recrystallized from aqueous methanol to obtain white precipitate 83% Yield. Mp 167-169°C.FTIR (KBr) ν_{max}3496 (OH_{phenol}), 3230 (NH), 3032 (CHAr), 2990 ,2868 (CHaliphatic), 1622 (C=N), 1596,1481 (C=C), 1340 (C-N), 1257 (C=S), 1197 (Ar-O-C) cm^{-1}.^{1}H-NMR (400MHz, DMSO-d_6) δ; 3.83(6H, s, 2× OCH_3), 4.38 (2H, s, H-9), 4.61 (2H,s,CH_2OCH_2), 4.71 (2H, s, CH_2OCH_2), 6.64 (2H, s, H-3), 6.81(2H,d,*J* 8.21,H-13),7.34(2H,d, *J* 8.20, H-14), 8.84 (1H, bs, NH), 8.90(1H, bs, NH), 9.11 (1H,bs, OH). ^{13}C-NMR (100 MHz, DMSO-d_6) δ; 37.45(1C, C-9) 58.01 (2C, 2×OCH_3), 60.67 (1C, CH_2OCH_2), 70.55 (1C, CH_2OCH_2), 109.94(2C, C-3), 128.83(2C, C-14), 130.52(2C, C-13), 132.08 (1C, C-4), 134.42(1C, C-15), 137.15 (1C, C-12), 138.77 (1C,C-1), 151.68 (2C, C-2), 154.53 (1C, C=N), 157.20 (1C, C=N),157.751(1C, C=N). 181.92 (1C, C=S) HREIMs m/z= 520.0752[M^+] (calc. for $C_{21}H_{21}ClN_6O_4S_2$,520.0754).

4-(((6-(3-(4-methylphenyl)thioureido)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-3-yl)methoxy)methyl)-2,6-dimethoxyphenol 9b

The crude product recrystallized from methanol to obtain white precipitate 85% Yield. Mp 134-136°C.FTIR (KBr) ν_{max}3528(OH_{phenol}),3235 (NH), 3019 (CHAr), 2996, 2872 (CHaliphatic), 1629 (C=N), 1595, 1488 (C=C), 1341 (C-N), 1250 (C=S), 1210 (Ar-O-C) cm^{-1}.^{1}H-NMR (400MHz, DMSO-d_6)δ; 2.21(3H,s, CH_3), 3.86(6H, s, 2× OCH_3), 4.41 (2H, s, H-9), 4.64 (2H,s,CH_2OCH_2), 4.70 (2H, s, CH_2OCH_2), 6.56 (2H,d, *J* 7.78,H-13), 6.68 (2H, s, H-3), 7.23 (2H,d, *J* 8.02, H-14), 8.86 (1H, bs, NH), 8.93 (1H, bs, NH), 9.35 (1H, bs, OH). ^{13}C-NMR (100 MHz, DMSO-d_6) δ; 19.80 (1C, CH_3), 37.59 (1C, C-9) 57.89 (2C, 2×OCH_3), 60.83 (1C, CH_2OCH_2), 71.05 (1C, CH_2OCH_2), 111.13(2C, C-3), 127.92 (2C, C-13), 129.74 (2C, C-14), 132.33 (1C, C-4), 134.88 (1C, C-12), 137.15 (1C, C-15), 139.08. (1C,C-1), 150.67 (2C, C-2), 155.51 (1C, C=N), 157.35 (1C, C=N),157.76(1C, C=N). 181.69 (1C, C=S) HREIMs m/z = 500.1294[M^+] (calc. for $C_{22}H_{24}N_6O_4S_2$, 500.1300).

4-(((6-(3-(4-methoxylphenyl)thioureido)-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-3-yl)methoxy)methyl)-2,6-dimethoxyphenol 9c

The crude product recrystallized from ethanol to obtain white precipitate 78 % Yield. Mp 128-130 °C.FTIR (KBr) ν_{max}3547 (OH_{phenol}), 3231 (NH), 3049 (CHAr), 2994, 2875 (CHaliphatic), 1630 (C=N), 1601, 1486(C=C), 1338 (C-N), 1247 (C=S), 1212 (Ar-O-C) cm^{-1}.^{1}H-NMR (400MHz, DMSO-d_6) δ; 3.84(9H, s, 3× OCH_3), 4.39 (2H, s, H-9), 4.62 (2H,s, CH_2OCH_2), 4.72 (2H, s, CH_2OCH_2), 6.37 (2H,d, *J*8.2,H-13), 6.68 (2H, s, H-3), 6.98 (2H,d, *J*8.12, H-14), 8.85 (1H, bs, NH), 8.91 (1H, bs, NH), 9.14 (1H, bs, OH). ^{13}C-NMR (100 MHz, DMSO-d_6) δ; 37.59 (1C, C-9), 56.92 (1C, OCH_3), 57.83 (2C, 2×OCH_3), 61.54 (1C, CH_2OCH_2), 70.95 (1C, CH_2OCH_2), 111.13 (2C, C-3), 121.32 (2C, C-14), 127.44 (2C, C-13), 132.51 (1C, C-4), 133.66 (1C, C12), 138.58 (1C, C1), 150.67 (2C, C-2),151.04(1C, C-15), 155.69 (1C, C=N), 156.75 (1C, C=N), 158.22 (1C, C=N). 181.09 (1C, C=S) HREIMs m/z = 516.1247[M^+] (calc. for $C_{22}H_{24}N_6O_5S_2$, 516.1250).

General synthesis of 4-(((6-(substitutedbenzamido) 7H-[1,2,4] triazolo[3,4-b][1,3,4]thiadiazin-3-yl) methoxy)methyl)-2,6-dimethoxyphenol 10a-c

To a stirring suspension 4-(((6-amino-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-3-yl)methoxy)methyl)-2,6-dimethoxyphenol (0.21g, 0.6 mmol) in 15 ml dry pyridine at 0C, aryloxy acid chloride (0.65 mmole) was added dropwise in period 30 mint through additional funnel. After completion the addition the mixture left to stirring at

ambient temperature overnight. The mixture poured in to 50 ml crushed ice then extracted from DCM. The organic layer washed by 2% hydrochloric acid then distilled water after that dried over sodium sulfate. The crude product was purified by column chromatography.

4-(((6-(4-hydroxybenzamido)7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-3-yl)methoxy)methyl)-2,6-dimethoxyphenol 10a

The crude product was purified by column chromatography utilizing ethyl acetate: hexane (3:1) to offered pale yellow precipitate. Yield 61%.Mp 102-104 °C.FTIR (KBr) ν_{max}3610 (OH $_{phenol}$), 3345 (NH), 3060 (CHAr), 2990, 2879 (CHaliphatic), 1669(C=O), 1625 (C=N), 1597, 1484(C=C), 1342 (C-N), 1267 (Ar-O-C) cm^{-1}.^{1}H-NMR (400MHz, DMSO-d_6) δ; 3.85(6H, s, 2× OCH_3), 4.40 (2H, s, H-9), 4.68 (2H,s, CH_2OCH_2), 4.75 (2H, s, CH_2OCH_2), 6.62 (2H, s, H-3), 7.18 (2H,d, *J* 8.24,H-14), 7.69 (2H,d, *J* 8.2, H-13), 9.14 (1H, bs, OH). 9.87 (1H, bs, OH), 11.57 (1H, bs, NH).^{13}C-NMR (100 MHz, DMSO-d_6) δ; 37.43 (1C, C-9), 58.03 (2C, 2×OCH_3), 62.14 (1C, CH_2OCH_2), 70.55 (1C, CH_2OCH_2), 110.24 (2C, C-3), 119.72 (2C, C-13), 125.05(1C-C-12), 129.81 (2C, C-14), 132.78 (1C, C-4), 139.29 (1C, C-1), 150.82 (2C, C-2),151.84 (1C, C-15), 156.19 (1C, C=N), 157.63 (1C, C=N), 158.15 (1C, C=N). 173.46 (1C, C=O) HREIMs m/z = 471.1208 [M$^+$] (calc. for $C_{21}H_{21}N_5O_6S$,471.1213).

4-(((6-(4-methylbenzamido)7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-3-yl)methoxy)methyl)-2,6-dimethoxyphenol 10b

The crude product was purified by column chromatography utilizing ethyl acetate: hexane (3:2) to offered pale yellow precipitate. Yield 64%.Mp 89-91 °C.FTIR (KBr) ν_{max}3604 (OH $_{phenol}$), 3338 (NH), 3051 (CHAr), 2996, 2873 (CHaliphatic), 1671(C=O), 1631 (C=N), 1595, 1485(C=C), 1343 (C-N), 1255 (Ar-O-C) cm^{-1}.^{1}H-NMR (400MHz, DMSO-d_6) δ; 2.29 (3H, CH_3), 3.83(6H, s, 2× OCH_3), 4.38 (2H, s, H-9), 4.76 (2H,s, CH_2OCH_2), 4.77 (2H, s, CH_2OCH_2), 6.59 (2H, s, H-3), 7.31 (2H,d, *J* 8.22,H-14), 7.60 (2H,d, *J* 8.4, H-13), 9.35 (1H, bs, OH), 11.39 (1H, bs, NH).^{13}C-NMR (100 MHz, DMSO-d_6) δ; 21.24 (1C, CH_3), 38.81 (1C, C-9), 57.74 (2C, 2×OCH3), 61.87 (1C, CH_2OCH_2), 71.03 (1C, CH_2OCH_2), 110.24 (2C, C-3), 126.55(1C, C-12), 128.78 (2C, C-14), 130.01 (2C, C-13), 133.15 (1C, C-4), 139.29,. (1C, C-1), 140.80 (1C, C-15), 151.02 (2C, C-2), 157.16 (1C, C=N), 157.85 (1C, C=N), 158.92 (1C, C=N). 1732.29 (1C, C=O) HREIMs m/z = 469.1417 [M$^+$] (calc. for $C_{22}H_{23}N_5O_5S$, 469.1420).

4-(((6-(4-chlorobenzamido)7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazin-3-yl)methoxy)methyl)-2,6-dimethoxyphenol 10c

The crude product was purified by column chromatography utilizing ethyl acetate: hexane (3:2) to afford off white precipitate. Yield 68%.Mp 120-122 °C.FTIR (KBr) ν_{max}3557 (OH $_{phenol}$), 3320 (NH), 3034 (CHAr), 2926, 2863 (CHaliphatic), 1668(C=O), 1627 (C=N), 1598, 1480(C=C), 1322 (C-N), 1247 (Ar-O-C) cm^{-1}.^{1}H-NMR (400MHz, DMSO-d_6) δ; 3.85 (6H, s, 2× OCH3), 4.43 (2H, s, H-9), 4.75 (2H,s, CH_2OCH_2), 4.792H, s, CH_2OCH_2), 6.61 (2H, s, H-3), 7.42 (2H,d, *J* 8.18,H-14), 7.76 (2H,d, *J* 8.20, H-13), 9.52 (1H, bs, OH), 11.21 (1H, bs, NH).^{13}C-NMR (100 MHz, DMSO-d_6) δ; 39.05 (1C, C-9), 56.98 (2C, 2×OCH_3), 62.37 (1C, CH_2OCH_2), 70.93 (1C, CH_2OCH), 110.13 (2C, C-3), 127.19 (1C, C-12), 128.65 (2C, C-14), 130.42 (2C, C-13), 133.15 (1C, C-4), 137.90 (1C, C15), 138.21 (1C, C-1), 151.44 (2C, C-2), 158.06 (1C, C=N), 158.25 (1C, C=N), 158.99 (1C, C=N). 172.71 (1C, C=O). HREIMs m/z = 489.0870 [M$^+$] (calc. for $C_{21}H_{20}ClN_5O_5S$, 489.0874).

Antioxidant

The DPPH assay was carried out as reported by Gerhauser *et al.* [24]and the FRAP assay was carried out according to the Benzie and Strain[25] method as described in previous publications[26,27]. 2,6-dimethoxyphenol (2,6-DMP), BHT and ascorbic acid were used as standard references.

RESULTS AND DISCUSSION

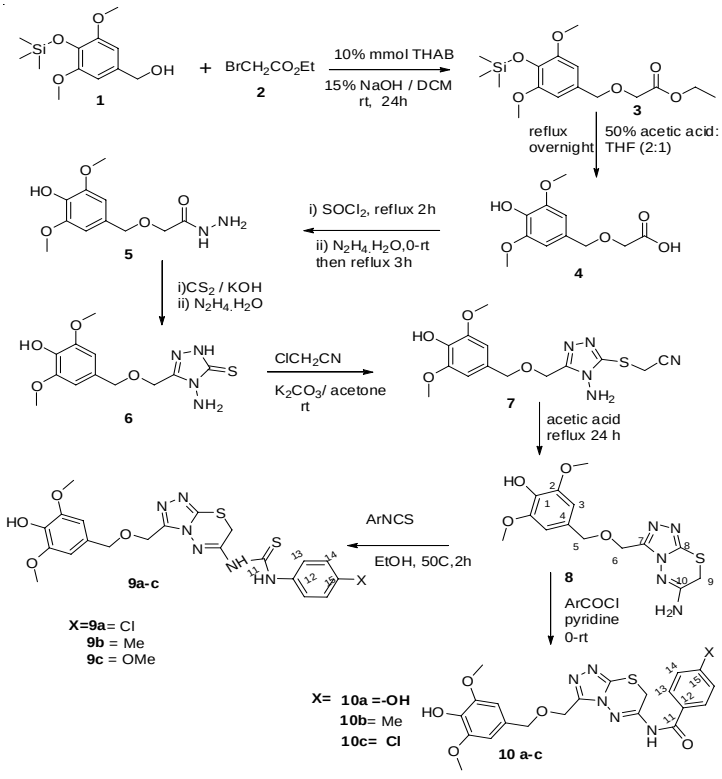
Chemistry

Newly compound 3 was synthesized from reaction equal equivalent of (3,5-dimethoxy-4-((trimethylsilyl)oxy)phenyl)methanol (1) with ethyl bromoacetate(2). Hydrolysis of compound 3 in 50% acetic acid afforded compound (4) which is converted to their corresponding acid hydrazide (5). The acid hydrazide cyclized to their corresponding new 1,2,4-triazole-5-thione (6) as depicted in Scheme 1. Alkylation of compound 6 with chloroacetonitrilein the presence of potassium carbonate in acetone at room temperature afforded compound (7). Alkylation this compound

temperature e.g up to 50°C will permit alkylation at phenolic hydroxyl group.

Refluxing compound (7) in acetic acid afforded corresponding fused 1,24-triazole-thiadiazine-6-amine ring (8). Compound 8 react with aryl isothiocyanate to afford the corresponding thioureaderivatives (9a-c), while it react with aryl acid chloride afford corresponding amide derivatives (10a-c). The para substituent and some physical properties were tabulated in Table 1. Thestructures of all synthesized compounds were confirmed from their

FTIR, ^{1}H NMR,^{13}C NMR besides to HREIMs spectra. The FTIR of compound 3 exhibitedband at 1725 cm^{-1} attributed to carbonyl group which also appeared in ^{13}C NMR spectrum at 171.04. The ^{1}H NMR spectrum of this compound displayed new two singlet peak for CH_2OCH_2 as well the ethoxy group of ester as triplet for CH_3 and quartet for CO_2CH_2. The FTIR of compounds4-7 were convenient with proposed structure as well the ^{1}H NMR and ^{13}C NMR. Furthermore, the HREIMs spectra of these compounds were compatible with calculated mass. The FTIR spectrum of compound 8 was exhibited disappearanceof the CN band at 2249 cm^{-1}. Moreover it shows the band of OH at 3542 cm^{-1}, while the band of NH_2 located at3343 and 3219 cm^{-1}. The band of C=N was appeared at 1618 cm^{-1}.



Scheme. 1. Synthetic route ofsynthesis compound 3-8 besides to 9a-c and 10a-c

The 1H NMR of this compound showed the two protons of H-9 at 4.41ppm and the two protons of NH_2 at 7.34 as broad singlet peak besides to all expected protons were appeared at their expected regions . The ^{13}C NMR also showed disappearance carbon signal of CN group as well it exhibited new peak at37.57 ppm attributed to C-9.three carbons signals at were located at 154.48, 157.16 and 157.70 ppm respectively attributed to three C=N for fused heterocyclic. The FTIR spectra of compounds 9a-c displayed new band at 3230-3235 cm^{-1} attributed to NH of thiourea part as well the band of C=S was located at 1247-1257 cm^{-1} besides to the popular band in their structure such as OH, CH aromatic & aliphatic and C=N. The 1HNMR spectra showed the new two doublet peaks each on with

integration for two protons belonging toarylthiourea part, moreover the 1HNMR displayed peak at 2.21ppm for three protons of *para* CH_3 of compound 9b and peak at 3.84 ppm for nine protons for three set of OCH_3 for compound 9c. Furthermore, two broad singlets were located at 8.84-8.86 and 8.90-8.93 ppm attributed to two NH of thiourea part. 13C NMR spectra of these compounds were exhibited four new carbons attributed to arylthiourea part and interesting peak for C=S was located at 181.09-181.92 ppm besides to the expected carbons were located in their expected region. The accurate mass spectra (HREIMs) were in agreement with the structure and the molecular formula for synthesized compound 9a-c (Table 1).

The FTIR spectra of compounds 10a-c showed interested two band, first one at 3320-3345 cm^{-1} of NH and the second one at 1668-1671 to C=O which are indicated to successfully formation of amide. The ^1H NMR spectra exhibited the peak of NH amide at 11, 21-11.57 ppm and new peaks for aromatic protons of aryl partof the amide as two doublets for four protons, each one integral for two protons. Furthermore, new broad singlet peak was located with compound 10a attributed to para OH at 9.87 ppm. The para CH_3 of compound 10b was located at 2.29 ppm. The ^{13}C NMR spectra of these compounds showed characteristic peak for carbonyl of amide at 172.71-173.46 ppm. Furthermore, the spectra exhibited all carbons of aryl amide group at their expected regions. The HREMs spectra were compatible with the calculated molecular mass.

Table. 1: Substituent group and some physical properties of synthesized compounds

No.	X	Yield %	Mp C	Molecular Formula	HREIMs Found	HREIMs Calc
8	-	59	114-116	$C_{14}H_{17}N_5O_4S$	351.0995	351.1001
9a	Cl	83	167-169	$C_{21}H_{21}ClN_6O_4S_2$	520.0752	520.0754
9b	CH_3	85	134-136	$C_{22}H_{24}N_6O_4S_2$	500.1294	50.1300
9c	OCH_3	78	128-130	$C_{22}H_{24}N_6O_5S_2$	516.1247	516.1250
10a	OH	61	102-104	$C_{21}H_{21}N_5O_6S$	471.1208	471.1213
10b	CH_3	64	89-91	$C_{22}H_{23}N_5O_5S$	469.1417	469.1420
10c	Cl	68	120-122	$C_{22}H_{20}ClN_5O_5S$	489.0870	489.0874

Antioxidant Activity

The antioxidant ability of newly synthesized compounds 6-8, 9a-c and 10a-c were evaluated with DPPH assay which prefer atom transfer mechanism (HAT)[28]. Furthermore, the antioxidant ability of these compounds were evaluated utilizing FRAP assay which is undergoes single electron transfer mechanism(SET)[29]. The DPPHinhibition % and theIC_{50}valuefor these compounds tabulated in Table 2. Compound 6 exhibited antioxidant ability higher than BHT and less than ascorbic acid. And their IC_{50} value was less BHT. The antioxidant of compound 7 (alkyl derivative of compound 6) showed eminent deficiency in antioxidant ability. This deficiencycould be point to fade the thioamide group which werereported as free radical scavengers.[30] Thioamide isconsidered as a part of thiourea system. Compound 8 showed DPPH inhibition slightly less than BHT as well their IC_{50} is higher than BHT. The antioxidant capacity of compounds 9a-c showed significant antioxidant capacity. Compound 9b exhibited DPPH inhibitions higher than ascorbic acid, although their IC50 was higher than ascorbic acid. Compound 9c exhibited antioxidant less than ascorbic acid and 9ashowed DPPH inhibition less than compound 9b and 9c. Increase the antioxidant ability of compounds 9a-c when compared to antioxidant ability of compound 8 could be attributed to thiourea system which is known as effective antioxidant[31]. Furthermore, the difference in DPPH results between 9a, 9b and 9capprove that the inductive effectof electron-donating groups (EDG) of substituent group at para position play vitalrole to enhance the antioxidant ability, while the as electron-withdrawing groups

(EWG) demotethe antioxidant ability. Compounds 10a-c exhibited moderated antioxidant ability. Their antioxidant was higher than compound 8, but less than 9a-c. Compound 10a showed higher antioxidant than10b and 10c and that could due the existence of another phenolic hydroxyl group which can enhances the antioxidant properties.

Table. 2: DPPH inhibition % and IC_{50} for synthesized compounds 6-8,9a-cand 10a-c

CompoundNo.	x	DPPH Inhibition % ± SD [a]	IC_{50} ±SEM [b](100µg/mL)
6	-	69.07 ±0.034	81.77 ±0.044
7	-	58.26 ±0.058	> 100
8	-	63.91 ±0.011	> 100
9a	Cl	79.71 ±0.024	30.8±0.01
9b	CH_3	92.81 ±0.035	56.43±0.016
9c	OCH_3	83.67 ±0.021	61.18±0.035
10a	OH	75.37 ±0.028	65.4±0.071
10b	CH_3	70.51 ±0.020	> 100
10c	Cl	68.09 ±0.047	> 100
2,6-DMP	-	42.16 ±0.078	> 100
BHT	-	66.23 ±0.025	78.64±0.015
Ascorbic acid	-	89.34 ±0.025	21.50±0.020

[a]Standard deviation (SD) value in FRAP was between 0.01–0.2; [b]SEM standard error of mean and IC_{50}: 50 % effective concentration.

The FRAP value of these compounds Fig.1. were harmonious with the DPPH results. These results indicate that these compounds capable to undergoes with HAT and SET mechanisms.

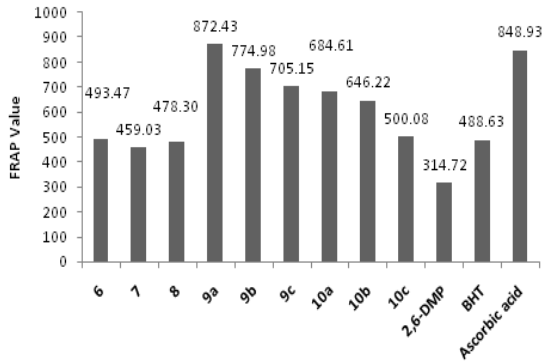


Fig.1. FRAP value of compounds 6-8,9a-cand 10a-c

CONCLUSIONS

4-amino-3-(((4-hydroxy-3,5-dimethoxy benzyl)oxy)methyl)-1H-1,2,4-triazole-5(4H)-thione6 successfully converted to their corresponding thioacetonitrilederivatives7 which cyclized to obtain 4-(((6-amino-7H-[1,2,4]triazolo [3,4-b][1,3,4]thiadiazin-3-yl)methoxy) methyl)-2,6-dimethoxyphenol 8 two series were synthesized from compound 8. First onewas as the corresponding thiourea derivative 9a-c.The second one was as the corresponding amid derivatives10a-c. All compounds were characterized successfully and screened their antioxidant ability. Compound 9a showed significant antioxidant ability, moreover 9a-c exhibited antioxidant higher than 10a-c and that could be attributed to existent of thiourea part which enhances the antioxidant ability.

ACKNOWLEDGEMENTS

The authors would like acknowledge University of Baghdad for their supporting and funding of this research. As well we would like to present our thanks to University of Malaya for their cooperation.

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