European Journal of Chemistry

Journal webpage: www.eurjchem.com

Synthesis, reactions and antimicrobial activity of benzothiazoles

Eman Mostafa Abbas [1,*], Marwa Saied Salem [2], Asmaa Fathy Kassem [1], Sherein Ismail Abd El-Moez [3] and Mohamed Youssef El-Kady [2]

[1] *Department of Chemistry, Natural and Microbial Products, National Research Center, Dokki, Giza, 12622, Egypt*
[2] *Synthetic Organic Chemistry Laboratory, Chemistry Department, Faculty of Science, Ain Shams University, Abbasiya, Cairo, 11566, Egypt*
[3] *Microbiology and Immunology Department, Veterinary Division, National Research Center, Dokki, Giza, 12622, Egypt*

** Corresponding author at: Department of Chemistry, Natural and Microbial Products, National Research Center, Dokki, Giza, 12622, Egypt. Tel.: +2.012.23502762. Fax: +2.37161666. E-mail address: eman_m69@yahoo.com (E.M. Abbas).*

ARTICLE INFORMATION

DOI: 10.5155/eurjchem.6.2.98-106.1161

Received: 23 September 2014
Received in revised form: 16 December 2014
Accepted: 11 January 2015
Published online: 30 June 2015
Printed: 30 June 2015

KEYWORDS

Pyrrole
Schiff's base
Benzothiazole
Thioglycolic acid
Mercaptotriazole
Antimicrobial activity

ABSTRACT

Benzothiazoles have been proven to be potent antimicrobial agents. In this study, 3-(5,6-dimethoxy-2-oxo-1,3-benzothiazol-3(2*H*)-yl)propanohydrazide has been utilized as a scaffold for synthesis of pyrrole, indolylidene, pyrazoles, mercaptotriazole, oxadiazole, triazole and oxothiazolidine derivatives. Structures of the synthesized compounds were elucidated on the basis of elemental analyses and spectral data. All the synthesized compounds were screened for their antimicrobial activity.

Cite this: *Eur. J. Chem.* **2015**, *6(2)*, 98-106

1. Introduction

Despite numerous attempts to develop new structural prototype in the search for more effective antimicrobials, benzothiazoles still remain as one of the most versatile class of compounds against microbes [1-7] and therefore, they are useful substructures for further molecular exploration. Benzothiazole derivatives have attracted continuing interest because of their various biological activities *viz* antitumor [8-13], antitubercular [14], antimalarial [15], anticonvulsant [16], anthelmintic [17], analgesic [18], anti-inflammatory [19] and antidiabetic [20]. Recently benzothiazole derivatives have been evaluated as potential amyloidal-binding diagnostic agents in neurodegenerative disease [21,22] and as selective fatty acid amide hydrolase inhibitors [23]. The above observations encouraged us to synthesize a novel series of benzothiazole derivatives and evaluated their antimicrobial activity.

2. Experimental

2.1. Instrumentation

All melting points were determined on an electrothermal Gallenkamp apparatus. The IR spectra were measured on a Pye-Unicam SP300 instrument in potassium bromide discs. The ^{1}H NMR spectra were recorded on Varian Mercury VXR-300 MHz spectrometer (300 or 400 MHz) and the chemical shifts δ (ppm) down field from tetramethylsilane (TMS) as an internal standard. The mass spectra were recorded on a GCMS-Q1000-EX Shimadzu and GCMS 5988-A HP spectrometers, the ionizing voltage was 70 eV. Elemental analyses were carried out by the Microanalytical Center of Cairo University, Giza, Egypt. TLC was run on silica gel G coated plates and iodine vapor as visualizing agent.

2.2. Synthesis

Solvents were generally distilled and dried by standard literature procedures prior to use.

2.2.1. Synthesis of 3-(5,6-dimethoxy-2-oxobenzothiozol-3-yl)-propionic acid ethyl ester (2)

A mixture of compound **1** (2.1 g, 0.01 mol), ethyl bromo propanoate (1.6 mL, 0.01 mol) and anhydrous potassium carbonate (0.76 g, 0.01 mol) in 50 mL dry acetone was refluxed for 10 h. The reaction mixture was cooled and poured onto ice/cold water; the solid that separated out was filtered,

ISSN 2153-2249 (Print) / ISSN 2153-2257 (Online)
http://dx.doi.org/10.5155/eurjchem.6.2.98-106.1161

dried and recrystallized from ethanol to give compound **2** (Scheme 1). Color: Pale yellow crystals. Yield: 80%. M.p.: 83-85 °C. FT-IR (KBr, ν, cm^{-1}): 1719, 1671 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.19 (t, 3H, CH_2CH_3), 2.76 (t, 2H, CH_2), 3.86 (s, 3H, OCH_3), 3.92 (s, 3H, OCH_3), 4.11 (q, 2H, CH_2CH_3), 4.21 (t, 2H, -N-CH_2), 6.74 (s, 1H, Ar-H), 6.77(s, 1H, Ar-H). MS (*m/z* (%)): 311 ($M^{+\cdot}$, 100), 296 (40), 266 (5), 211 (5), 196 (15), 180 (25), 101 (10), 85 (10), 55 (20). Anal. calcd. for $C_{14}H_{17}NO_5S$: C, 54.01; H, 5.50; N, 4.50; S, 10.30. Found: C, 54.08; H, 5.57; N, 4.49; S, 10.35%.

2.2.2. Synthesis of 3-(5,6-dimethoxy-2-oxobenzo[d]thiazol-3(2H)-yl) propanohydrazide (3)

A mixture of compound **2** (3.11 g, 0.01 mol) hydrazine hydrate (98%) (2 mL, 0.04 mol) and 30 mL absolute ethanol was refluxed for 6 h. The reaction mixture was cooled; the formed precipitate was filtered, dried and recrystallized from acetic acid to give compound **3** (Scheme 1). Color: White solid. Yield: 80%. M.p.: 192-194 °C. FT-IR (KBr, ν, cm^{-1}): 3444, 3346, 3215(NH, NH_2), 1674, 1612 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 2.38 (t, 2H, CH_2), 3.78, (s, 3H, OCH_3), 3.79 (s, 3H, OCH_3), 3.80 (t, 2H, -N-CH_2), 4.09 (s, 2H, NH_2, exchangeable by D_2O), 7.01 (s, 1H, Ar-H), 7.29 (s, 1H, Ar-H), 9.09 (s, 1H, NH, exchangeable by D_2O). MS (*m/z* (%)): 297 (M^+, 100), 266 (65), 224 (60), 196 (50), 180 (15), 87 (10), 55 (50). Anal. calcd. for $C_{12}H_{15}N_3O_4S$: C, 48.47; H, 5.08; N, 14.13; S, 10.78. Found: C, 48.33; H, 5.37; N, 14.19; S, 10.80%.

2.2.3. Synthesis of N-(3,4-dichloro-2,5-dioxo-2H-pyrrol-1 (5H)-yl)-3-(5,6-dimethoxy-2-oxobenzo[d]thiazol-3-(2H)-yl)propanamide (4)

A mixture of compound **3** (0.6 g, 0.002 mol) and dichloromaleic anhydride (0.33 g, 0.002 mol) in 5 mL acetic acid was refluxed for 8 h. The formed precipitate was filtered, dried and recrystallized from acetic acid to give compound **4** (Scheme 1). Color: Orange crystals. Yield: 60%. M.p.: 226-228 °C. FT-IR (KBr, ν, cm^{-1}): 3187 (NH), 1749, 1708, 1661, 1612 (C=O). MS (*m/z* (%)): 447 ($[M+2]^+$, 17), 446 ($[M+1]^+$, 5), 445 ($[M]^+$, 19), 430 (6), 283 (7), 266 (9), 196 (31), 211 (22), 87 (35). Anal. calcd. for $C_{16}H_{13}Cl_2N_3O_6S$: C, 43.06; H, 2.94; N, 9.42; S, 7.19. Found: C, 43.33; H, 2.89; N, 9.40; S, 7.20 %.

2.2.4. Synthesis of 3-(5,6-dimethoxy-2-oxobenzothiazol-3-yl)-propionic acid (2-oxo-1,2-dihydro-indol-3-ylidene) hydrazide (5)

To a solution of compound **3** (0.6 g, 0.002 mol) in DMF (10 mL) was added isatin(0.3 g, 0.002 mol) refluxed for 4 h, left to cool then poured onto crushed ice. The product formed was filtered off and recrystallized from ethanol to give compound **5** (Scheme 1). Color: Pale yellow crystals. Yield: 80%. M.p.: 198-200 °C. FT-IR (KBr, ν, cm^{-1}): 3450, 3214 (NH), 1669, 1618, 1590 (CO). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 3.29 (t, 2H, CH_2), 3.68, (s, 3H, OCH_3), 3.75 (s, 3H, OCH_3), 4.23(t, 2H, N-CH_2), 6.86 (d, 1H, Ar-H), 6.88 (t, 1H, Ar-H), 7.11 (s, 2H, Ar-H), 7.21(d, 1H, Ar-H), 7.30 (t, 1H, Ar-H), 11.22(s, 1H, NH exchangeable by D_2O), 12.49 (s, 1H, NH, exchangeable by D_2O). MS (*m/z* (%)): 427($[M^++1]$, 10), 426 ($[M^+]$, 34), 417 (4), 377 (6), 356 (7), 300 (5), 266 (12), 224 (35), 210 (13) 196 (34), 132 (35), 104 (51), 77 (53), 55 (100). Anal. calcd. for $C_{20}H_{18}N_4O_5S$: C, 56.33; H, 4.25; N, 13.14; S, 7.52. Found: C, 56.39; H, 4.08; N, 13.23; S, 7.46%.

2.2.5. Synthesis of 3-(3-(3,5-dimethyl-1H-pyrazol-1-yl)-3-oxopropyl)-5,6-dimethoxybenzo[d]thiazol-2(3H)-one (6)

Refluxing a mixture of compound **3** (0.6 g, 0.002 mol), with acetyl acetone (2 mL, 0.002 mol) in 15 mL acetic acid for 8 h. The formed precipitate after cooling was filtered, dried and recrystallized from methanol:ethyl acetate (1:1, *v*:*v*) to give compound **6** (Scheme 1). Color: White crystals. Yield: 65 %. M.p.: 189-191 °C. FT-IR (KBr, ν, cm^{-1}): 1684, 1610 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 2.21 (s, 3H, CH_3), 2.33 (s, 3H, CH_3), 3.51 (t, 2H, CH_2), 3.86, (s, 3H, OCH_3),3.90 (s, 3H, OCH_3), 4.36 (t, 2H, CH_2), 5.97 (s, 1H, CH), 6.77 (s, 1H, Ar-H), 6.96 (s, 1H, Ar-H). ^{13}C NMR (400 MHz, DMSO-d_6, δ, ppm): 14.18, 14.43 (2 CH_3), 33.17, 39.87 (2 CH_2), 56.54 (2 OCH_3), 97.33 (=CH), 98.42, 108.16, 112.29, 130.31, 143.52, 146.87 (Ar-C), 169.77 (C=O), 171.59 (C=N), 172.43 (C=O). MS (*m/z* (%)): 363 ($[M+2]^+$, 4), 361($[M^+]$, 20), 360($[M^+-1]$, 10), 236 (12), 211 (29), 151 (43), 97 (69), 55 (100). Anal. calcd. for $C_{17}H_{19}N_3O_4S$: C, 56.50; H, 5.30, N, 11.63; S, 8.87. Found: C, 56.71; H, 5.28, N, 11.68; S, 8.90%.

2.2.6. Synthesis of ethyl 5-amino-1-(3-(5,6-dimethoxy-2-oxobenzo[d]thiazol-3(2H)-yl)propanoyl)-1H-pyrazole-4-carboxylate (7)

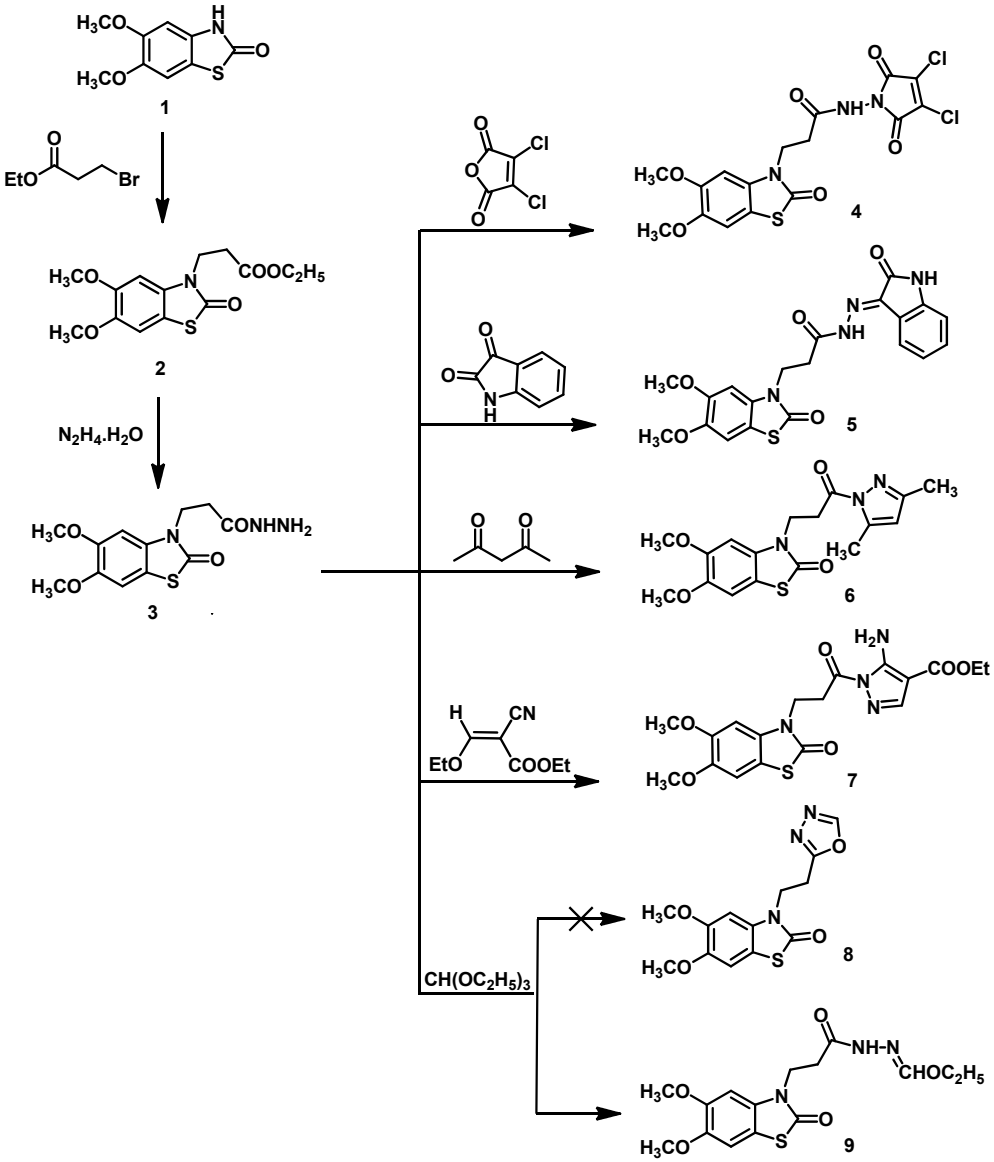
A mixture of compound **3** (0.6 g, 0.002 mol) and ethyl (ethoxymethylene) cyano-acetate (0.34 g, 0.002 mol) in absolute ethanol (20 mL) was heated under reflux for 8h. After cooling, the product was collected by filtration then recrystallized from ethanol to give compound **7** (Scheme 1). Color: White crystals. Yield: 74%. M.p.: 172-174 °C. FT-IR (KBr, ν, cm^{-1}): 3469, 3357 (NH_2), 1734, 1679, 1613 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.21(t, 3H, CH_2-CH_3), 2.60 (t, 2H, CH_2), 3.70, (s, 3H, OCH_3), 3.77 (s, 3H, OCH_3), 3.78 (q, 2H, CH_2-CH_3), 4.17 (t, 2H, CH_2), 7.00 (s, 1H, Ar-H), 7.29 (s, 1H, Ar-H), 7.71 (s, 1H, N=CH), 9.97 (s, 2H, NH_2, exchangeable by D_2O). MS (*m/z* (%)): 420 ($[M^+]$, 40), 375 (5), 353 (15), 307 (10), 266 (60), 237 (35), 224 (50), 211 (45), 196 (40), 109 (25), 55 (100). Anal. calcd. for $C_{18}H_{20}N_4O_6S$: C, 51.42; H, 4.79; N, 13.33; S, 7.63. Found: C, 51.47; H, 4.68; N, 13.45; S, 7.42%.

2.2.7. Synthesis of ethyl N'-(3-(5,6-dimethoxy-2-oxobenzo[d] thiazol-3(2H)-yl)propanoyl)formohydrazonate (9)

A mixture of compound **3** (0.6 g, 0.002 mol) and triethylorthoformate (5 mL) was heated under reflux for 12 h. After cooling, the solvent was evaporated under reduced pressure and the solid product obtained was filtered off and recrystallized from ethanol to give compound **9** (Scheme 1). Color: White crystals. Yield: 55%. M.p.: 135-137 °C. FT-IR (KBr, ν, cm^{-1}): 3210 (NH), 1682, 1612 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.29 (t, 3H, CH_2-CH_3), 2.81(t, 2H, CH_2), 3.77(s, 3H, OCH_3),3.78 (s, 3H, OCH_3), 4.12 (t, 2H, N-CH_2), 4.15 (q, 2H, CH_2-CH_3), 7.01 (s, 1H, Ar-H), 7.27 (s, 1H, Ar-H), 8.09 (s, 1H, N=CH), 10.59 (s, 1H, NH, exchangeable by D_2O). MS (*m/z* (%)): 353($[M^+]$, 100), 307 (45), 292 (15), 266 (15), 211 (30), 196 (35), 97 (25), 55 (50). Anal. calcd. for $C_{15}H_{19}N_3O_5S$: C, 50.98; H, 5.42; N, 11.89; S, 9.07. Found: C, 50.88; H, 5.49; N, 11.83; S, 9.23%.

2.2.8. Synthesis of 3-(2-(5-mercapto-1H-1,2,4-triazol-3-yl) ethyl)-5,6-dimethoxybenzo[d]thiazol-2(3H)-one (10)

A mixture of compound **3** (0.6 g, 0.002 mol) and ammonium thiocyanate (0.15 g, 0.002 mol) was fused at 200 °C for 30 min. The solid mass was triturated with hot water, left to cool and acidified with concentrated hydrochloric acid. The formed precipitate was filtered and recrystallized from ethanol to give compound **10** (Scheme 2). Color: Pale yellow crystals. Yield: 77%. M.p.: 208-210 °C. FT-IR (KBr, ν, cm^{-1}): 3171, (NH), 1646 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 2.87 (t, 2H, CH_2), 3.73 (s, 3H, OCH_3), 3.79 (s, 3H, OCH_3), 4.18 (t, 2H, N-CH_2), 6.91 (s, 1H, Ar-H), 7.26 (s, 1H, Ar-H), 7.28 (s, 1H, NH, exchangeable by D_2O), 11.19 (s, 1H, SH, exchangeable by D_2O). MS (*m/z* (%)): 339 ($[M^++1]$, 21), 338 ($[M^+]$, 58), 323(13), 311(15), 291(16), 146 (14), 224 (15), 211 (71), 196 (100), 180 (51), 128 (74), 55 (72). Anal. calcd. for $C_{13}H_{14}N_4O_3S_2$: C, 46.14; H, 4.17; N, 16.56; S, 18.95. Found: C, 46.18; H, 4.09; N, 16.39; S, 18.82%.



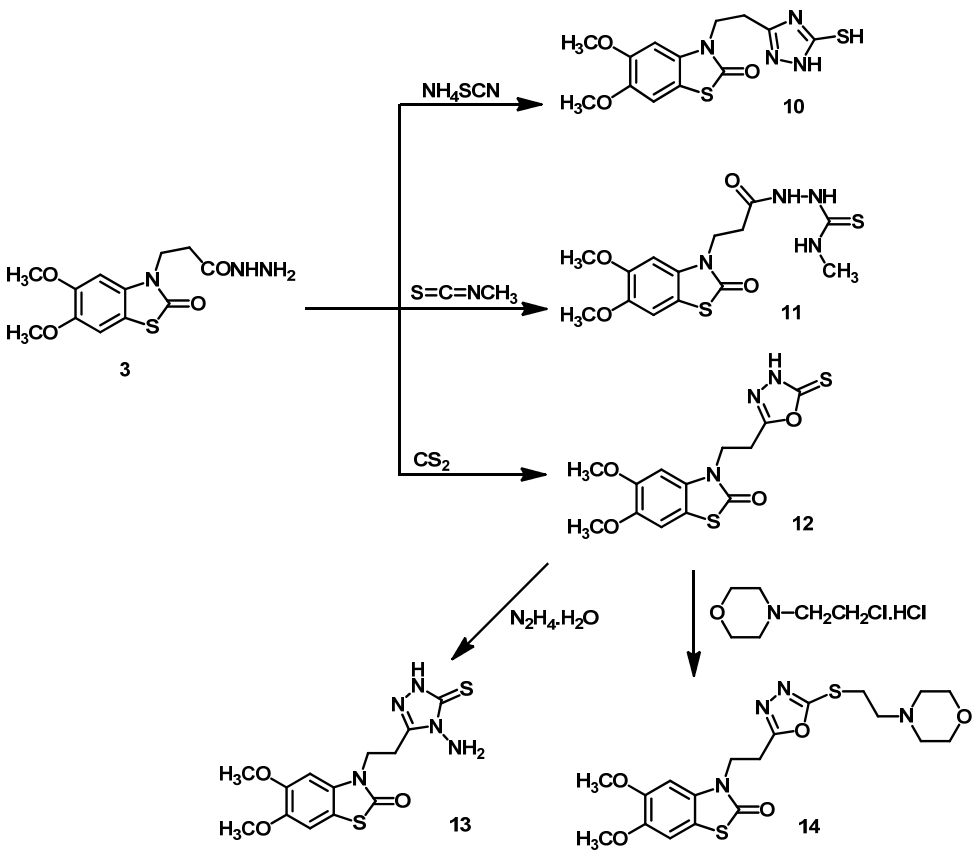
Scheme 1

2.2.9. Synthesis of 1-(3-(5,6-dimethoxy-2-oxobenzo[d]thiazol-3-(2H)-yl)propanoyl)-4-methylthio-semicarbazide (11)

A mixture of compound **3** (0.6 g, 0.002 mol) and methyl isothiocyanate (0.15 g, 0.002 mol) in 10 mL DMF was refluxed for 6 h. After cooling pouring onto ice/cold water, acidified by hydrochloric acid, the formed precipitate was filtered dried and recrystallized from methanol to give compound **11** (Scheme 2). Color: White crystals. Yield: 70 %. M.p.: 192-194 °C. FT-IR (KBr, ν, cm^{-1}): 3395, 3329, 3199 (NH), 1650, 1610 (C=O), 1249 (C=S). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 2.47 (t, 2H, CH_2), 2.82 (d, 3H, CH_3), 3.71, (s, 3H, OCH_3), 3.79 (s, 3H, OCH_3), 4.13 (t, 2H, N-CH_2), 7.02 (s, 1H, Ar-H), 7.31 (s, 1H, Ar-H), 7.78 (s, 1H, NH, exchangeable by D_2O), 9.17 (s, 1H, NH, exchangeable by D_2O), 9.80 (s, 1H, NH, exchangeable by D_2O). MS (*m/z* (%)): 371 [(M^++1], 61), 370 ([M^+], 80), 351 (74), 338 (61), 318 (95), 296 (77), 279 (70), 257 (78), 232 (78), 198 (74), 167 (86), 73 (100), 51 (70). Anal. calcd. for $C_{14}H_{18}N_4O_4S_2$: C, 45.39; H, 4.90; N, 15.12; S, 17.31. Found: C, 45.42; H, 4.87; N, 15.18; S, 17.16%.

2.2.10. Synthesis of 5,6-dimethoxy-3-(2-(5-thioxo-4,5-dihydro-1,3,4-oxadiazol-2-yl)ethyl)benzo[d]thiazol-2(3H)-one (12)

A mixture of compound **3** (0.6 g, 0.002 mol) and carbon disulfide (6 mL) in pyridine (10 mL) and DMF (5 mL) was heated under reflux on water bath for 8 h. After cooling, the solvent was evaporated under reduced pressure and residue was triturated with an ice- water mixture and neutralized with diluted HCl. The solid precipitate formed was filtered off and recrystallized from ethanol to afford compound **12** (Scheme 2). Color: Pale yellow crystals. Yield: 99%. M.p.: 110-112 °C. FT-IR (KBr, ν, cm^{-1}): 3152 (NH), 1658 (C=O), 1208 (C=S). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 3.34 (t, 2H, CH_2), 3.77 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 4.27 (t, 2H, N-CH_2), 6.97 (s, 1H, Ar-H), 7.29 (s, 1H, Ar-H), 14.30 (s, 1H, NH. exchangeable by D_2O). ^{13}C NMR (400 MHz, DMSO-d_6, δ, ppm): 16.84, 23.80 (2 CH_2), 56.65 (2 OCH_3), 97.31, 107.37, 111.65, 127.91, 130.23, 142.25 (Ar-C), 155.07 (C=N), 164.18 (C=S), 169.83(C=O).



Scheme 2

MS (*m/z* (%)): 339 ([M⁺], 100), 324 (5), 283 (15), 264 (10), 224 (20), 211 (40), 196 (65), 180 (25), 143 (15), 108 (10), 55 (15). Anal. calcd. for $C_{13}H_{13}N_3O_4S_2$: C, 46.01; H, 3.86; N, 12.38; S, 18.90. Found: C, 45.08; H, 3.62; N, 12.29; S, 18.93%.

2.2.11. Synthesis of 3-(2-(4-amino-5-thioxo-4,5-dihydro-1H-1,2,4-triazol-3-yl)ethyl)-5,6-dimethoxybenzo[d]thiazol-2 (3H)-one (13)

A mixture of the oxadiazolinethione **12** (0.34 g, 0.001 mol) and hydrazine hydrate (3 mL) in absolute ethanol (20 mL) was heated under refluxed 6 h. After cooling, the solvent was removed in vacuum and the residue obtained was triturated with water. The solid product formed was filtered off and recrystallized from ethanol to afford compound **13** (Scheme 2). Color: White crystals. Yield: 80%. M.p.: 150-152 °C. FT-IR (KBr, ν, cm^{-1}): 3194, 2952 (NH, NH_2), 1646 (C=O), 1203 (C=S). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm):2.97 (t, 2H, CH_2), 3.77 (s, 3H, OCH_3), 3.79 (s, 3H, OCH_3), 4.18 (t, 2H, N-CH_2), 5.15 (s, 1H, NH exchangeable by D_2O), 6.00 (s, 2H, NH_2, exchangeable by D_2O), 6.98 (s, 1H, Ar-H), 7.30 (s, 1H, Ar-H). MS (*m/z* (%)): 353 ([M⁺], 10), 297 (25), 282 (5), 266 (25), 224 (30), 211 (100), 196 (70), 168 (20), 140 (20), 108 (25), 57 (55). Anal. calcd. for $C_{13}H_{15}N_5O_3S_2$: C, 44.18; H, 4.28; N, 19.82; S, 18.15. Found: C, 44.30; H, 4.19; N, 19.86; S, 18.09%.

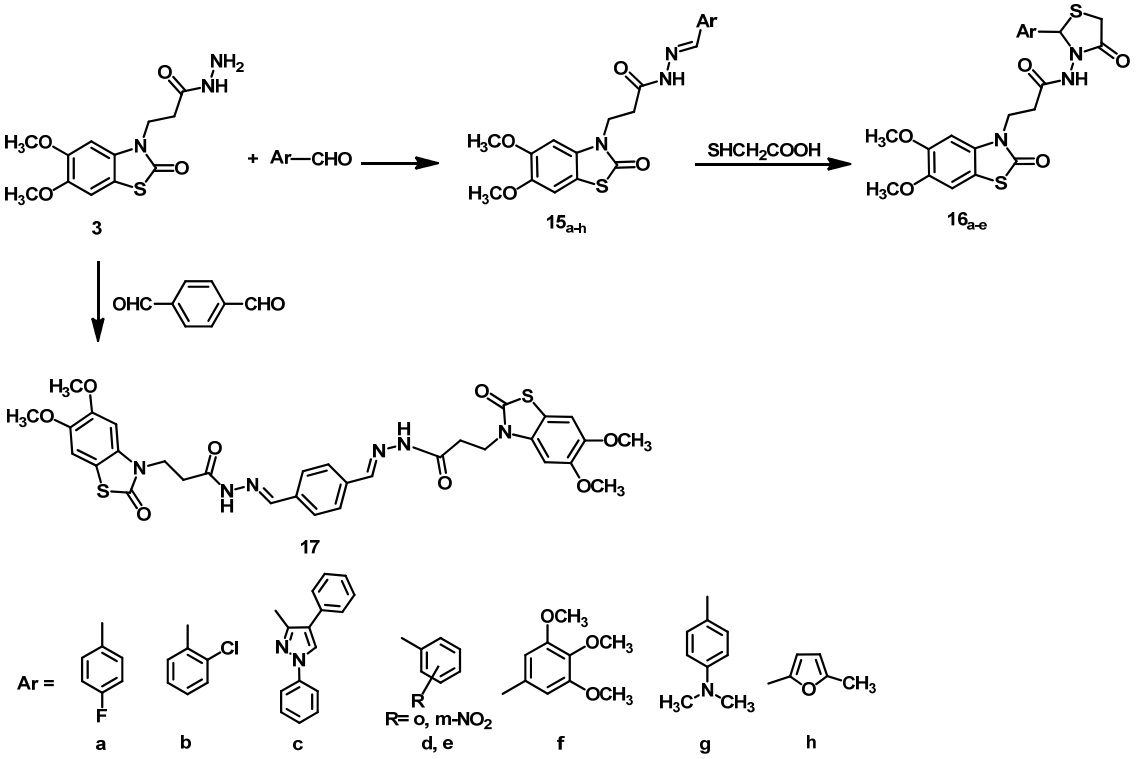
2.2.12. Synthesis of 5,6-dimethoxy-3-(2-(5-((2-morpholino ethyl)thio)-1,3,4-oxadiazol-2-yl)ethyl)benzo[d]thiazol-2(3H)-one (14)

A mixture of the oxadiazolethione **12** (0.68 g, 0.002 mol), sodium acetate (0.38 g, 0.002 mol) and 4-(2-chloroethyl) morpholine hydrochloride (0.37 g, 0.002 mol) in ethanol (30 mL) was heated under reflux for 6 h. The solvent was evaporated and the residue was triturated with water. The solid product formed was collected by filtration and recrystallized from ethanol to give afford compound **14** (Scheme 2). Color: Bright gray crystals. Yield: 80%. M.p.: 85-87 °C. FT-IR (KBr, ν, cm^{-1}): 1662 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.66 (t, 4H, OCH_2), 2.54 (t, 4H, N-CH_2), 2.56 (t, 2H, CH_2), 3.19 (t, 2H, S-C*H*$_2$-CH_2-N), 3.23 (t, 2H, S-CH_2-C*H*$_2$-N), 3.70 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3), 4.28 (t, 2H, N-CH_2), 6.96 (s, 1H, Ar-H), 7.29 (s, 1H, Ar-H). MS (*m/z* (%)): 453([M+1]⁺, 4), 452 ([M]⁺, 5), 450 (3), 430 (3), 414 (4), 392 (6), 375 (5), 351 (4), 339 (12), 288 (5), 255 (5), 211 (6), 196 (8), 113 (36), 100 (100), 85 (16), 70 (12), 55 (16). Anal. calcd. for $C_{19}H_{24}N_4O_5S_2$: C, 50.43; H, 5.35; N, 12.38; S, 14.17. Found: C, 50.30; H, 5.27; N, 12.43; S, 14.22%.

2.2.13. General procedure for preparation of Schiff's base compounds 15a-h

To a solution of compound **3** (0.6 g, 0.002 mol) in absolute ethanol (20 mL) containing glacial acetic acid (5 mL) was added different aromatic aldehydes (0.01 mol) the reaction mixture was refluxed for 5 h. Then cooled, poured onto crushed ice. The product was filtered off and recrystallized from acetic acid to give **15a-h** (Scheme 3).

3-(5,6-Dimethoxy-2-oxo-benzothiazol-3-yl)-propionic acid (4-fluoro-benzylidene)-hydrazide (**15a**): Color: Pale white crystals. Yield: 85%. M.p.: 180-183 °C. FT-IR (KBr, ν, cm^{-1}): 3141 (NH), 1606 (C=N), 1711, 1672 (C=O).



Scheme 3

^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 3.31 (t, 2H, CH_2), 3.68, (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3), 4.23 (t, 2H, CH_2), 7.05 (d, 1H, Ar-H), 7.06 (d, 1H, Ar-H), 7.13 (s, 1H, Ar-H), 7.20 (d, 1H, Ar-H), 7.27 (d, 1H, Ar-H), 7.52 (s, 1H, Ar-H), 7.87 (s, 1H, N=CH), 11.38(s, 1H, NH, exchangeable by D_2O.). MS (*m/z* (%)): 404 ([M$^+$+1], 15), 403 ([M$^+$], 69), 266 (13), 244 (27), 211 (84), 196 (57), 193 (49), 168 (14), 150 (8), 55 (100). Anal. calcd. for $C_{19}H_{18}FN_3O_4S$: C, 56.57; H, 4.50; N, 10.42; S, 7.95. Found; C, 56.49; H, 4.31; N, 10.49; S, 7.36%.

3-(5,6-Dimethoxy-2-oxo-benzothiazol-3-yl)-propionic acid (2-chloro-benzylidene)-hydrazide (**15b**): Color: White crystals. Yield: 80 %. M.p.: 120-122 °C. FT-IR (KBr, ν, cm^{-1}): 3184 (NH), 1598 (C=N), 1669 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm):2.97 (t, 2H, CH_2), 3.77 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 4.22 (t, 2H, N-CH_2),6.99 (d, 1H, Ar-H), 7.01 (s, 1H, Ar-H), 7.08 (m, 1H, Ar-H), 7.22 (m, 1H, Ar-H), 7.39 (d, 1H, Ar-H), 8.21 (s, 1H, Ar-H), 8.92 (s, 1H, N=CH), 11.47 (s, 1H, NH, exchangeable by D_2O). MS (*m/z* (%)): 421 ([M+1]$^+$, 21), 419 ([M]$^+$, 55), 266 (18), 224 (30), 211 (97), 196 (62), 168 (15), 137 (28), 89 (30), 55 (100). Anal. calcd. for $C_{19}H_{18}ClN_3O_4S$: C, 54.35; H, 4.32; N, 10.01; S, 7.64. Found: C, 54.40; H, 4.29; N, 10.13; S, 7.36%.

3-(5,6-Dimehoxy-2-oxo-benzothiazole-3-yl)-propionic acid (1,4-diphenyl-1H-pyrazol-3-ylmethylene)-hydrazide (**15c**): Color: Pale white crystals. Yield: 80 %. M.p.: 205-207 °C. FT-IR (KBr, ν, cm^{-1}): 3127 (NH), 1599(C=N), 1680 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm):2.86 (t, 2H, CH_2), 3.76 (s, 3H, OCH_3),3.79 (s, 3H, OCH_3), 4.18 (t, 2H, N-CH_2), 7.03 (s, 1H, Ar-H), 7.25 (s, 1H, Ar-H), 7.32 (m, 3H, Ar-H), 7.47 (m, 3H, Ar-H), 7.59 (d, 2H, Ar-H), 7.86 (d, 2H, Ar-H), 8.01 (s, 1H, Ar-H), 8.90 (s, 1H, N=CH), 11.21 (s, 1H, NH, exchangeable by D_2O). MS (*m/z* (%)): 527 ([M]$^+$, 15), 526 ([M-1]$^+$, 15), 428 (15), 317 (11), 282 (28), 245 (55), 196 (47), 147 (18), 77 (100), 55 (81). Anal. calcd. for $C_{28}H_{25}N_5O_4S$: C, 63.74; H, 4.78; N, 13.27; S, 6.08. Found:C, 63.80; H, 4.61; N, 13.42; S, 6.10%.

3-(5,6-Dimethoxy-2-oxo-benzothiazol-3-yl)-propionic acid (2-nitro-benzylidene)-hydrazide (**15d**): Color: Yellow crystals. Yield: 85 %. M.p.: 237-239 °C. FT-IR (KBr, ν, cm^{-1}): 3274 (NH), 1651 (C=N), 1697(C=O). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 3.26 (t, 2H, CH_2), 3.69 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3), 4.23(t, 2H, N-CH_2), 7.06 (d, 1H, Ar-H), 7.07(s, 1H, Ar-H), 7.12 (s, 1H, Ar-H), 7.57 (t, 1H, Ar-H),7.89 (d, 1H, Ar-H), 7.98 (s, 1H, Ar-H), 8.25 (s, 1H, N=CH), 11.62 (s, 1H, NH, exchangeable by D_2O.). MS (*m/z* (%)): 432 ([M$^+$+2], 5), 431 ([M$^+$+1], 8), 430 ([M$^+$], 27), 211 (23), 196 (30), 77 (8), 55 (100). Anal. calcd. for $C_{19}H_{18}N_4O_6S$: C, 53.02; H, 4.22; N, 13.02; S, 7.45. Found: C, 53.31; H, 4.40; N, 13.11; S, 7.23%.

3-(5,6-dimethoxy-2-oxo-benzothiazol-3-yl)-propionic acid (3-nitro-benzylidene)-hydrazide (**15e**): Color: White solid. Yield: 85 %. M.p.: 238-240 °C. FT-IR (KBr, ν, cm^{-1}): 3222 (NH), 1640 (C=N), 1710 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 3.22 (t, 2H, CH_2), 3.69 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 4.23 (t, 2H, N-CH_2), 7.16 (s, 1H, Ar-H), 7.89 (d, 2H, Ar-H), 7.96 (s, 1H, Ar-H), 8.11 (d, 2H, Ar-H), 8.23 (s, 1H, N=CH), 11.69 (s, 1H, NH, exchangeable by D_2O). MS (*m/z* (%)): 430 ([M$^+$], 0.4), 419 (7), 266 (5), 224 (3), 211 (11), 196 (9), 180 (8), 118 (20), 55 (100). Anal. calcd. for $C_{19}H_{18}N_4O_6S$: C, 53.02; H, 4.22; N, 13.02; S, 7.45. Found: C 53.12; H, 4.41; N, 13.11; S, 7.30%.

3-(5,6-dimethoxy-2-oxobenzothiazol-3-yl) propionic acid (3,4,5-trimethoxy-benzylidene)-hydrazide (**15f**): Color: White crystals. Yield: 85 %. M.p.: 189-191 °C. FT-IR (KBr, ν, cm^{-1}):3175 (NH), 1595(C=N), 1682 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 3.19 (t, 2H, CH_2), 3.82 (s, 6H, 2OCH_3), 3.92 (s, 9H, 3OCH_3), 4.34 (t, 2H, N-CH_2), 6.83-7.64 (m, 4H, Ar-H), 8.03 (s, 1H, N=CH), 9.53 (s, 1H, NH, exchangeable by D_2O.). MS (*m/z* (%)): 476 ([M+1]$^+$, 17), 474 ([M]$^+$, 35), 429 (5), 302 (15), 266 (32), 196 (51), 94 (18), 77 (11), 55 (100). Anal. calcd. for $C_{22}H_{25}N_3O_7S$:C, 55.57; H, 5.30; N, 8.84; S, 6.74. Found: C, 55.47; H, 5.29; N, 8.86; S, 6.83%.

3-(5,6-dimethoxy-2-oxobenzothiazol-3-yl) propionic acid (4-dimethylamino-benzylidene)-hydrazide (**15g**): Color: White crystals. Yield: 80 %. M.p.: 178-180 °C. FT-IR (KBr, ν, cm^{-1}): 3203 (NH), 1599 (C=N), 1670 (C=O). ^{1}H NMR (400 MHz,

DMSO-d_6, δ, ppm): 2.89 (s, 6H, 2CH_3), 2.91 (t, 2H, CH_2), 3.77 (s, 3H, OCH_3), 3.79 (s, 3H, OCH_3), 4.20 (t, 2H, N-CH_2), 6.59 (d, 1H, Ar-H), 6.61 (d, 1H, Ar-H),7.05 (s, 1H, Ar-H), 7.22 (d, 1H, Ar-H), 7.26 (d, 1H, Ar-H), 7.74 (s, 1H, Ar-H), 7.90 (s, 1H, N=CH), 11.08 (s, 1H, NH, exchangeable by D_2O). MS (*m/z* (%)): 429 ([M+1]$^+$, 15), 428 ([M]$^+$, 54), 427 ([M-1]$^+$, 17), 266 (15), 218 (56), 146 (100), 132 (29), 55 (94). Anal. calcd. for $C_{21}H_{24}N_4O_4S$: C, 58.86; H, 5.65; N, 13.07; S, 7.48. Found: C, 58.63; H, 5.72; N, 13.13; S, 7.39%.

3-(5, 6-Dimehoxy-2-oxo-benzothiazol-3-yl)-propionic acid (5-methyl-furan-2-ylmethylene)-hydrazide (**15h**): Color: White crystals. Yield: 85%. M.p.: 110-112 °C. FT-IR (KBr, ν, cm^{-1}): 3127 (NH), 1596 (C=N), 1672 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 1.23 (s, 3H, CH_3), 3.15 (t, 2H, CH_2), 3.86 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3), 4.31 (t, 2H, -N-CH_2), 6.16 (d, 1H, = CH. furan), 6.59 (s, 1H, Ar-H), 6.89 (d, 1H, =CH. furan), 7.52 (s, 1H, Ar-H), 8.47 (s, 1H, N=CH), 10.24(s, 1H, NH, exchangeable by D_2O.). MS (*m/z* (%)): 389 ([M]$^+$, 80), 311 (15), 296 (5), 266 (35), 224 (25), 196 (55), 179 (75), 108 (35), 79 (25), 55 (100). Anal. calcd. for $C_{18}H_{19}N_3O_5S$ (389.1): C, 55.52; H, 4.92; N, 10.79; S, 8.23. Found: C, 55.60; H, 4.98; N, 10.62; S, 8.33%.

2.2.14. General method for preparation of oxathiazolidin compound 16a-e

Thioglycolic acid (0.01 mol) was added to a well stirred solution of Schiff's bases (0.01 mol) **15a-d** in dry benzene (50 mL). Then refluxed for 5 h and excess of solvent was evaporated under reduced pressure and the residue was washed by 2% $NaHCO_3$, then treated with petroleum ether. The solid product was filtered off, washed with petroleum ether, and then recrystallized from methanol to give compound **16a-e**, respectively (Scheme 3).

3-(5, 6-Dimethoxy-2-oxo-benzothiazol-3-yl)-N-(2-(4-fluro-phenyl)-4-oxo-thiazolidin-3-yl)-propanamide (**16a**): Color: Pale white crystals. Yield: 65 %. M.p.: 230-232 °C. FT-IR (KBr, ν, cm^{-1}): 3315 (NH), 1725, 1657, 1611 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm):3.67 (s, 3H, OCH_3), 3.71 (s, 3H, OCH_3), 3.81 (t, 2H, CH_2), 3.92 (t, 2H, -N-CH_2), 4.00 (m, 2H, -S-CH_2), 5.50 (s, 1H, -N-CH), 6.96 (s, 1H, Ar-H), 7.12 (d, 2H, Ar-H), 7.14 (d, 2H, Ar-H), 7.32 (s, 1H, Ar-H), 10.20 (s, 1H, NH, exchangeable by D_2O). MS (*m/z* (%)): 478 ([M+1]$^+$, 4), 477 ([M]$^+$, 11), 467 ([M-1]$^+$, 6), 282 (8), 266 (9), 224 (12), 212 (9), 196 (56), 122 (31), 55 (100). Anal. calcd. for $C_{21}H_{20}FN_3O_5S_2$: C, 52.82; H, 4.22; N, 8.80; S, 13.43. Found: C, 52.70; H, 4.32; N, 8.73; S, 13.50%.

N-[2-(4-Chloro-phenyl)-4-oxo-thiazolidin-3-yl)-3-(5, 6-dimethoxy-2-oxo-benzothiazol-3-yl)-propionamide (**16b**): Color: Pale white crystals. Yield: 65 %. M.p.: 140-142 °C. FT-IR (KBr, ν, cm^{-1}): 3237 (NH), 1726, 1671, 1595 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 2.52 (t, 2H,CH_2), 3.78,(s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 3.83 (q, 2H, -S-CH_2), 4.07 (t, 2H, -N-CH_2), 5.90 (s, 1H, -N-CH), 6.94 (s, 1H, Ar-H), 7.28 (s, 1H, Ar-H), 7.32-7.41(m, 4H, Ar-H), 10.42 (s, 1H, NH, exchangeable by D_2O). ^{13}C NMR (400 MHz, DMSO-d_6, δ, ppm): 29.12, 31.84, 39.82(3 CH_2), 56.59, 56.70 (2 OCH_3), 58.88 (-N-CH), 97.79, 107.26, 111.71, 128.26, 130.32, 130.51, 130.67, 132.57, 136.00, 146.24, 149.08 (Ar-C), 169.62, 169.72, 171.07 (3C=O). MS (*m/z* (%)): 493 ([M]$^+$, 1), 411 (10), 266 (13), 211 (100), 196 (48), 168 (17), 55 (61). Anal. calcd. for $C_{21}H_{20}ClN_3O_5S$: C, 51.06; H, 4.08; N, 8.51; S, 12.98. Found: C, 51.32; H, 4.21; N, 8.10; S, 12.61%.

3-(5, 6-Dimethoxy-2-oxo-benzothiazol-3-yl)-N-[2-(1, 4-di phenyl-1H-pyrazol-3-yl)-4-oxo-thiazolidin-3-yl]-propionamide (**16c**): Color: Pale white crystals. Yield: 60 %. M.p.: 128-130 °C. FT-IR (KBr, ν, cm^{-1}): 3281 (NH), 1720, 1661, 1597 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 2.46 (t, 2H, CH_2), 3.62 (t, 2H, -N-CH_2), 3.68 (s, 3H, OCH_3), 3.69 (s, 3H, OCH_3), 3.72 (q, 2H, -S-CH_2), 5.86 (s, 1H, -N-CH), 7.15-8.55 (m, 13H, Ar-H), 10.37 (s, 1H, NH, exchangeable by D_2O). MS (*m/z* (%)): 602 ([M+1]$^+$, 3), 600 ([M-1]$^+$, 2), 585 (3), 524 (2), 431 (3), 318 (27), 277 (6), 263 (17), 246 (21), 211 (27), 196 (16), 168 (10), 108 (20), 77 (95), 55 (100). Anal. calcd. for $C_{30}H_{27}N_5O_5S_2$: C, 59.88; H, 4.52; N, 11.64; S, 10.66 .Found: C, 59.79; H, 4.60; N, 11.36; S, 10.81%.

3-(5, 6-Dimethoxy-2-oxo-benzothiazol-3-yl)-N-[2-(2-nitro-phenyl)-4-oxo-thiazolidin-3-yl]-propionamide (**16d**): Color: Pale white crystals. Yield: 65 %. M.p.: 237-239 °C. FT-IR (KBr, ν, cm^{-1}):3120 (NH), 1722, 1670, 1625 (3 C=O). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 3.13 (t, 2H, CH_2), 3.74(s, 3H, OCH_3), 3.76 (s, 3H, OCH_3), 3.92 (q, 2H, -S-CH_2), 4.03 (t, 2H, -N-CH_2), 5.72 (s, 1H, -N-CH), 6.92 (s, 1H, Ar-H), 7.24 (s, 1H, Ar-H), 7.63 (m, 3H, Ar-H), 8.14 (s, 1H, Ar-H), 10.30 (s, 1H, NH, exchangeable by D_2O). MS (*m/z* (%)): 505 ([M+1]$^+$, 2), 504 ([M]$^+$, 5), 503 ([M-1]$^+$, 3), 282 (3), 223 (24), 211 (22), 196 (13), 55 (100). Anal. calcd. for $C_{21}H_{20}N_4O_7S_2$: C, 49.99; H, 4.00; N, 11.10; S, 12.71. Found: C, 49.71; H, 4.32; N, 11.36; S, 12.52%.

3-(5, 6-Dimethoxy-2-oxo-benzothiazol-3-yl)-N-[2-(3-nitro-phenyl)-4-oxo-thiazolidin-3-yl]-propionamide (**16e**): Color: Pale white crystals. Yield: 65 %. M.p.: 198-200 °C. FT-IR (KBr, ν, cm^{-1}):3332 (NH), 1733, 1674, 1600 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 3.29 (t, 2H, CH_2), 3.68 (s, 3H, OCH_3), 3.71 (s, 3H, OCH_3), 4.03 (t, 2H, -NCH_2), 4.06 (q, 2H, SCH_2), 5.65 (s, 1H, -NCH), 6.14 (s, 1H, Ar-H), 7.24 (s, 1H, Ar-H), 7.29 (d, 2H, Ar-H), 8.12 (d, 2H, Ar-H), 10.30 (s, 1H, NH, exchangeable by D_2O). MS (*m/z* (%)): 505 ([M+1]$^+$, 16), 504 ([M]$^+$, 24), 498 (18), 468 (13), 461 (22), 435 (20), 420 (29), 408 (34), 303 (31), 276 (25), 212 (26), 195 (23), 132 (59), 91(100), 77 (54), 55 (22). Anal. calcd. for $C_{21}H_{20}N_4O_7S_2$: C, 49.99; H, 4.00; N, 11.10; S, 12.71. Found: C, 49.75; H, 4.09; N, 11.23; S, 12.59%.

2.2.15. Synthesis of 3-(5,6-dimethoxy-2-oxo-benzothiazol-3-yl)-propionicacid(4-{[3-5,6-dimethoxy-2-oxobenzothiazol-3-yl)-propionyl]-hydrazonomethyl}-benzlidene)-hydrazide (17)

To a solution of compound **3** (0.6 g, 0.002 mol) in absolute ethanol (20 mL) containing glacial acetic acid (5 mL) was added benzene 1,4 dicarbaldehyde (0.54 g, 0.004 mol). The reaction mixture was refluxed for 5 h then cooled, poured onto crushed ice. The product was filtered off and crystallized from acetic acid to give compound **17** (Scheme 3). Color: Yellow crystals. Yield: 85 %. M.p.: 249-251 °C. FT-IR (KBr, ν, cm^{-1}): 3465, 3181 (NH), 1610, 1660 (C=O). ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm): 2.97 (t, 4H, 2CH_2), 3.69 (s, 3H, OCH_3), 3.77, (s, 3H, OCH_3), 3.79 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 4.22 (t, 4H, 2NCH_2), 7.06-7.46 (m, 8H, Ar-H), 7.56 (s, 1H,N=CH), 7.85 (s, 1H, N=CH), 11.49 (s, 1H, NH, exchangeable by D_2O), 11.93 (s, 1H, NH, exchangeable by D_2O). MS (*m/z* (%)): 693 ([M$^+$+1], 61), 692 ([M$^+$], 66), 691 ([M$^+$-1], 59), 618 (45), 597 (62), 586 (62), 519 (79), 413 (100), 189 (83), 96 (60). Anal. calcd. for $C_{32}H_{32}N_6O_8S_2$: C, 55.48; H, 4.66; N, 12.13; S, 9.26Found: C, 55.51; H, 4.60; N, 12.25; S, 9.32%.

2.3. Biological evaluations

2.3.1. Preparation of microbial suspensions

Antimicrobial activities were tested out against highly pathogenic reference strains accused of causing food poisoning from food of animal origin. Two Gram positive bacteria *(Methicillin resistance Staphylococcus aureus* (MRSA), *Staphylococcus aureus* NCINB 50080), two Gram negative bacteria (*Escherichia coli* O157ATCC 700728*, Escherichia coli* ATCC 11775) and one mycotic strain (*Candida albicans* ATCC10231) were selected as model organisms. Agar well diffusion (qualitative method) and minimum inhibitory concentration (MIC) (quantitative method) were used in this study. Wherein a suspension ofbacterial and mycotic strains were freshly prepared by inoculating fresh stock culture from each strain into separate broth tubes, each containing 7 mL of Muller Hinton Broth for bacterial strains and Sabaroud Dextrose broth for mycotic strain. The inoculated tubes were incubated at 37 and 28 °C for 24 h, respectively.

Table 1. Agar well diffusion method showing antimicrobial activities of the tested compounds compared with reference drugs, results given in mm *.

Chemical/Compound	*E. coli* O157, ATCC 700728	*E. coli*, ATCC 11775	MRSA	*S. aureus*, NCINB 50080	*C. albicans*, ATCC10231
3	-ve	13	-ve	12	11
4	11	12	-ve	12	10s
5	-ve	10	-ve	10	-ve
6	-ve	10	-ve	11	-ve
7	10-12s	10	-ve	10	-ve
9	12	10	12	10	-ve
10	13	11	12	12	10
11	10	10	-ve	10	-ve
12	12	12	-ve	11	-ve
13	11	10	-ve	10	-ve
14	11	12	-ve	10	-ve
15a	12s	15	-ve	14	-ve
15b	-ve	11	-ve	9	10
15c	-ve	11	-ve	11	-ve
15d	11s	9	-ve	8	9s
15e	11s	-ve	-ve	-ve	9s
15f	12s	12	-ve	12	-ve
15g	-ve	9	-ve	9	9s
15h	11s	12	-ve	12	-ve
16a	12	11	12	9	10
16b	10s	10	-ve	10	11s
16c	10s	8	-ve	9	-ve
16d	11s	7	-ve	7	-ve
16e	-ve	9	-ve	9	-ve
17	11	10	-ve	-ve	-ve
Ciprofloxacin, 100 µg/mL	39	42	-ve	39	ND
Fluconazole, 100 µg/mL	ND	ND	ND	ND	32
Control negative, DMSO	-ve	-ve	-ve	-ve	-ve

* ND = not defined, -ve = indicates that the tested compound did not show any hindrance activity against the tested isolate.

Serial dilutions were carried out for each strain, dilution matching with 0.5 Mc-Farland (about 1×10^8 cells/mL), was selected for screening of antimicrobial activities. Ciprofloxacin 100 µg/mL and fluconazole 100 µg/mL were used as reference drugs (Oxoid), DMSO was used as control negative.

2.3.2. Determination of antimicrobial activity by agar well diffusion method

Muller Hinton and Sabaroud Dextrose agar plates were prepared [24,25]. Bacterial and fungal strains matching with 0.5 Mc-Farland were spread onto the surface of the agar plates using sterile cotton swabs. For evaluation of antibacterial activities, wells were formed in the agar plates using pasture pipette and each well was filled with 50 µL of the compound dissolved in DMSO (300 µg of the tested compound dissolved in 1 mL DMSO), others were saturated with 50 µL ciprofloxacin (100 µg/mL) and others 50 µL DMSO as control negative. The same method was used for evaluation of antimycotic activities using fluconazole (100 µg/mL). Then inoculated agar plates and left for 1 h at 25 °C to allow a period of pre-incubation diffusion in order to minimize the effects of variation in time between the applications of different solutions. The plates were re-incubated at 37 °C and 28 °C for 24 h for bacterial and mycotic isolates, respectively. After incubation, plates were observed for antimicrobial activities, zone of inhibition were measured in mm using a ruler. The experiment was carried out in duplicate and the mean of the zone of inhibition was tabulated in Table 1.

2.3.3. Determination of Minimum Inhibitory concentration (MIC)

Microtiter dilution plate quantitative method, i.e. the minimum inhibitory concentration (MIC) was used for evaluation of the antimicrobial activity of tested compounds. Determination of MIC of extract against tested strains was achieved using 96-well sterile micro plates. The first well contain the concentrated form of the tested compound used in the agar disk diffusion method (300 µg of the tested compound dissolved in 1 mL DMSO), then two fold serial dilutions was carried out for the tested compounds, reference drugs (ciprofloxacin and fluconazole) and DMSO, Then wells were inoculated with 100 µL of tested isolates (0.5 Mc-Farland, about 1×10^8 cells/mL) and incubated at 37-28 °C for 24 h for bacterial and fungal strains respectively. After incubation, plates were examined visually for bacterial or fungal growth precipitation. The experiment was repeated three times. The lowest concentration that showed complete hindrance of growth was taken as MIC.

3. Results and discussion

3.1. Chemistry

The key intermediate **3** was prepared by condensation of 5,6-dimethoxy-3*H*-benzothiazol-2-one (**1**) with ethylbromo propanoate to give ethyl 3-(5,6-dimethoxy-2-oxobenzo[*d*] thiazol-3(2*H*)-yl)propanoate (**2**) which was condensed with hydrazine hydrate (98%) in ethanol to give 3-(5,6-dimethoxy-2-oxobenzo[*d*]thiazol-3(2*H*)-yl) propanehydrazide (**3**) which is very useful starting material for the synthesis of all target compounds. The structures of compounds **2** and **3** were established on the basis of elemental analyses and spectral data. The reaction of compound **3** with dichloromaleic anhydride in acetic acid gave the amide compound **4** [26]. The mass spectrum of compound **4** showed the molecular ion peak M^+ at *m/e* 445. Simple reaction of hydrazide (**3**) with isatin in absolute ethanol containing a catalytic amount of glacial acetic acid gave compound **5** [27]. ^{1}H NMR spectrum of compound **5** showed the appearance of two singlet signals representing the proton of two NH groups near δ 11.22 and 12.49 ppm. Also, cyclization of compound **3** with acetylacetone afforded the corresponding pyrazole derivative **6** [28]. Structure of compound **6** was assigned to the reaction product on the basis of ^{1}H NMR spectrum, which revealed the absence of amino group and exhibited signals corresponding to two methyl groups. Furthermore, hydrazide **3** reacted with ethyl (ethoxymethylene)cyanoacetate in boiling ethanol to give the corresponding pyrazole derivative **7**. The ^{1}H NMR spectrum of compound **7** displayed the characteristic triplet and quartet signals at δ 1.21 and 3.78 ppm, respectively assigned to the ethyl protons and D_2O exchangeable singlet signal at δ 9.97 ppm due to NH_2 protons.

Table 2. Minimum inhibitory concentration showing antimicrobial activities of the tested compounds compared with reference drugs, results given in mm *.

Chemical/Compound	*E. coli* O157, ATCC 700728	*E. coli*, ATCC 11775	*S. aureus*, NCINB 50080	*C. albicans*, ATCC10231
3	ND	50	100	100
4	100	100	100	ND
6	ND	ND	100	ND
9	100	100	ND	ND
10	50	100	100	ND
12	100	100	100	ND
13	100	100	ND	ND
14	100	100	ND	ND
15a	ND	25	25	ND
15b	ND	100	ND	ND
15c	ND	100	ND	ND
15f	ND	100	100	ND
15h	ND	100	100	ND
16a	100	100	ND	ND
17	100	ND	ND	ND
Ciprofloxacin, 100 µg/mL	1.56	0.78	1.56	ND
Fluconazole, 100 µg/mL	ND	ND	ND	3.125
Control negative, DMSO	-ve	-ve	-ve	-ve

* ND = not defined; -ve = indicates that the tested compound did not show any hindrance activity against the tested isolate.

When compound **3** was allowed to react with triethyl orthoformate, the product was not the expected oxadiazole derivative **8** but it was identified as the methylenepropano hydrazide derivative **9** (Scheme 1) [29].

Moreover, fusion of hydrazide **3** with ammonium thiocyanate gave the corresponding mercaptotriazole derivative **10**. Furthermore, treatment of hydrazide **3** with methyl isothiocyanate gave 4-methylthiosemicarbazide derivative **11**. The structure of compound **11** was confirmed by its IR spectrum, which displayed absorption bands at 3329 cm^{-1} for NH, 1650 cm^{-1} due to C=O and 1249 cm^{-1} corresponding to C=S stretch vibrations. The reaction of compound **3** with carbon disulfide in boiling pyridine and DMF gave the corresponding oxadiazole derivative **12** which was converted to the aminotriazole derivative **13** through condensation with hydrazine hydrate. Also alkylation of compound **12** with 4-(2-chloroethyl) morpholine hydrochloride in boiling ethanol in the presence of fused sodium acetate gave the corresponding morpholine derivative **14** (Scheme 2).

To get a new series of expected biologically active Schiff's bases **15a-h**, it was of interest to condense compound **3** with different aromatic and/or heterocyclic aldehydes in ethanol containing few drops of glacial acetic acid (Scheme 3).

The structures of compounds **15a-h** were established on the basis of elemental analyses and spectral data. The IR spectrum of compound **15a** showed an absorption peak at 1606 cm^{-1} due to C=N stretching vibrations, its ^{1}HNMR spectrum displayed a singlet signal at δ 7.87 ppm attributed to -N=CH proton and a multiple at δ 7.05-7.54 ppm for Ar-H protons and its mass spectra showed the molecular ion peak M^+ at *m/e* 403 corresponding to the molecular formula $C_{19}H_{18}FN_3O_4S$. Also the reactivity of compounds **15a-e** towards other reagents has been investigated to obtain new biologically active heterocyclic systems. Thus, the reaction of Schiff's bases **15a-e** with thioglycolic acid in dry benzene gave the thiazolidinones **16a-e** (Scheme 3) [30]. The cyclic structures were readily determined on the basis of ^{1}H NMR spectra, which clearly indicated the absence of the ylidenic proton and the appearance of new singlet signals at δ 5.60-5.90 ppm assigned to azomethine proton. It was of interest to react hydrazide **3** with benzene-1,4-dicarbaldehyde to afford the *bis*-benzothiazole **17**. ^{1}H NMR spectrum of the compound confirmed the proposed structure by revealing an increase in the integration of the aromatic protons relative to hydrazide **3**.

3.2. Biological activities

All the newly synthesized compounds **3-15h** were screened for their antimicrobial activity determined by agar diffusion method [24,25] for determination of the preliminary antibacterial and antifungal activity and the results were recorded for each tested compound as the average diameter of inhibition zones (IZ) of bacterial or fungal growth around the discs in mm (Table 1). In addition, the minimum inhibitory concentrations (MIC) were recorded for compounds that showed promising growth inhibition using the two-fold serial dilution method [31,32]. The MIC (µg/mL) values against the tested bacterial and fungal isolates were recorded in Table 2.

The potentiality of the synthesized compounds as antimicrobials was appraised for their antimicrobial studies against two strains of Gram positive bacteria *(S. aureus* NCINB 50080, MRSA), and two strains of Gram negative bacteria (*E. coli* ATCC 11775, *E. coli* O157ATCC 700728) using ciprofloxacin as a standard drug (100 µg/mL). They were also evaluated for their *in vitro* antifungal activity against the mycotic strain (*C. albicans* ATCC10231) using fluconazole as a standard antifungal drug (100 µg/mL).

According to the data of Table 1 and 2, it is clear that six compounds **15a**, **3**, **4**, **10**, **15f** and **15h** were found the most potent, they showed moderate activity against *E. coli* ATCC 11775 and *S. Aureus* NCINB 50080 strains compared to the reference standard ciprofloxacin (Figure 1). DMSO was also taken in a control experiment which showed no effect in the experiment.

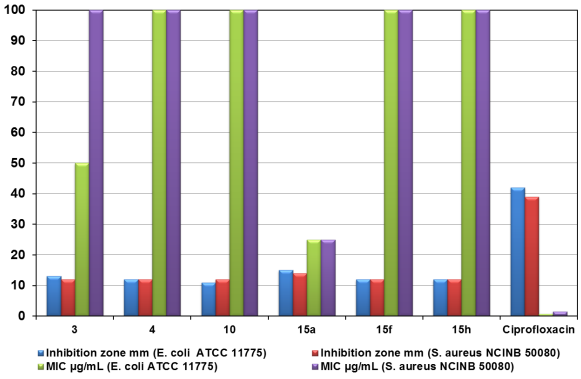


Figure 1. Antibacterial activity of the bioactive compounds against *E. coli* ATCC 11775 and *S. Aureus* NCINB 50080 strains determined by zone of inhibition and MIC.

The Schiff base **15a** containing 4-fluro substituted phenyl ring was found the most potent against *E. coli* ATCC 11775 and *S. Aureus* NCINB 50080 strains with zone of inhibition 15 and 14 mm, respectively, and MIC equals 25 µg/mL. It was more potent than the parent hydrazide **3** (zone of inhibition 13 and 12 mm and MIC equals 50 and 100 µg/mL, respectively) and the two Schiff's bases **15f** and **15h** containing trimethoxy substituted phenyl and 5-meethylfuryl functionalities, respect-

tively (zone of inhibition 12 mm and MIC equals 100 µg/mL). Furthermore, the dichloropyrrol dione compound **4** and the mercaptotriazole derivative **10** displayed equal antibacterial activity (zone of inhibition 12 mm, 11 and 12 mm, respectively and MIC equals 100 µg/mL).

Moreover, four compounds; mercaptotriazole **10** and its oxadiazole bioisoster **12** (zone of inhibition 13 mm, 11mm and 12 mm, and MIC equals 50, 100 µg/mL and 100 µg/mL, respectively) as well as the hydrazone **9** (zone of inhibition 12 mm,10 mm and MIC equals 100 µg/mL) and the 4-fluoro substituted thiazolidinone **16a** (zone of inhibition 12 mm, 11 mm and MIC equals 100 µg/mL) were the most potent antibacterial compounds tested against Gram negative bacterial strains; *E. coli* ATCC 11775 and *E. coli* O157 ATCC 700728 (Figure 2).

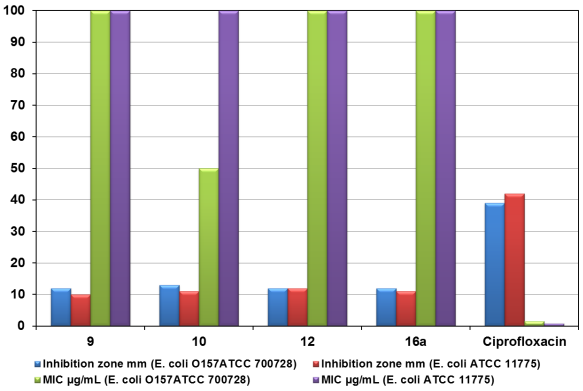


Figure 2. Antibacterial activity of the bioactive compounds against *E. coli* ATCC 11775 and *E. coli* O157 ATCC 700728 strains determined by zone of inhibition and MIC.

It is obvious from the analysis activity results that the 4-flourophenyl substituted compounds have considerable activity against both tested Gram positive (*S. Aureus* NCINB 50080) and Gram negative (*E. coli* ATCC 11775 and *E. coli* O157 ATCC 700728) bacterial strains. This observation is supported by the antibacterial activity shown by compounds **15a** and **16a**. On the other hand, all the tested compounds showed negative hindrance activities against tested *Methicillin Resistance Staph aureus*.

Further, these compounds **3**-**15h** were also screened for their antifungal activity against *C. Albicans* ATCC1023. Fluconazole was taken as a standard drug throughout the experiment. Only compound **3** showed hindrance effect against *C. Albicans* ATCC10231 with zone of inhibition 11 mm and MIC equals 100 µg/mL.

4. Conclusion

In conclusion, we report the synthesis of new derivatives of pyrrole, indolylidene, pyrazoles, mercaptotriazole, oxadiazole, triazole and oxothiazolidine incorporated with the benzothiazole unit via reaction of 3-(5,6-dimethoxy-2-oxobenzo[*d*] thiazol-3(2*H*)-yl)propanohydrazide (**3**) with different reagents. The structures of the newly synthesized compounds were established on the basis of spectral data (IR, ^{1}H NMR, ^{13}C NMR, Mass) and elemental analyses. Also some of the newly synthesized compounds were evaluated for antimicrobial activity and the results showed that some compounds showed good activity against the tested microorganisms.

Acknowledgements

The authors thank ALLAH for giving them the potential to achieve this work. Also, we would like to express our thanks to Microbiology and Immunology Department, Veterinary Division, National Research Center and Microanalytical Center of Cairo University.

References

[1]. Yamazaki, K.; Kaneko, Y.; Suwa, K.; Ebara, S.; Nakazawa, K.; Yasuno, K. *Bioorg. Med. Chem.* **2005**, *13*, 2509-2522.
[2]. Latrofa, A.; Franco, M.; Lopedota, A.; Rosato, A.; Carone, D.; Vitali, C. *IL Farmaco* **2005**, *60*, 291-297.
[3]. Yalcin, I.; Oren, I.; Sener, E.; Akin, A.; Ucarturk, N. *Eur. J. Med. Chem.* **1992**, *27*, 401-406.
[4]. Bujdakova, H.; Muckova, M. *Int. J. Antimicrob. Agents* **1994**, *4*, 303-308.
[5]. Rajeeva, B.; Srinivasulu, N.; Shantakumar, S. M. *Eur. J. Med. Chem.* **2009**, *6*, 775-779.
[6]. Soni, B.; Ranawat, M. S.; Shrma, R.; Bhandari, A.; Sharma, S. *Eur. J. Med. Chem.* **2010**, *45(7)*, 2938-2942.
[7]. El-Emary; T. I.; El-Mohsen, S. A. A. *Molecules* **2012**, *17*, 14464-14483.
[8]. Weekes, A. A.; Westwell, A. D. *Curr. Med. Chem.* **2009**, *16*, 2430-2440.
[9]. Kini, S.; Swain, S. P.; Gandhi, A. M. *Indian J. Pharm. Sci.* **2007**, *69*, 46-50.
[10]. Lion, C. J.; Matthews, C. S.; Wells, G.; Bradshaw, T. D.; Stevens, M. F. G.; Westwell, A. D. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5005-5008.
[11]. Mortimer, C. S.; Wells, G.; Crochard, P. J.; Stone, E. L.; Bradshaw, T. D.; Stevens, M. F. G.; Westwell, A. D. *J. Med. Chem.* **2006**, *49*, 179-185.
[12]. Wells, G.; Berry, J. M.; Bradshaw, T. D.; Burger, A. M.; Seaton, A.; Wang, B.; Westwell, A. D.; Stevens, M. F. G. *J. Med. Chem.* **2003**, *46*, 532-541.
[13]. Hutchinson, I.; Jennings, S. A.; Vishnuvajjala, B. R.; Westwell, A. D.; Stevens, M. F. G. *J. Med. Chem.* **2002**, *45*, 744-747.
[14]. Palmer, F. J.; Trigg, R. B.; Warrington, J. V. *J. Med. Chem.* **1971**, *14*, 248-251.
[15]. Burger, A.; Sawhey, S. N. *J. Med. Chem.* **1968**, *11*, 270-273.
[16]. Chakole, R. D.; Amnerkar, N. D.; Khedekar, P. B.; Bhusari, K. P. *Indian J. Heterocycl. Chem.* **2005**, *15*, 27-30.
[17]. Jayachandran, E.; Bhatia, K.; Naragud, L. V. G.; Roy, A. *Indian Drugs* **2003**, *40*, 408-411.
[18]. Siddiqui, N.; Alam, M.; Siddiqui, A. A. *Asian J. Chem.* **2004**, *16*, 1005-1008.
[19]. Gurupadayya, B. M.; Gopal, M.; Padmashali, B.; Vaidya, V. P. *Int. J. Heterocycl. Chem.* **2005**, *15*, 169-172.
[20]. Diaz, H. M.; Molina, R. V.; Andrade, R. O.; Coutino, D. D ; Franco, L. M.; Webster, S. P.; Binnie, M.; Soto, S. E.; Barajas, M. I.; Rivera, I. L ; Vazquez, G. N. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 2871-2877.
[21]. Henriksen, G.; Hauser, A. I.; Westwell, A. D.; Yousefi, B. H.; Schwaiger, M.; Drzega, A.; Wester, H. J. *J. Med. Chem.* **2007**, *50*, 1087-1089.
[22]. Mathis, C. A.; Wang, Y.; Holt, D. P.; Haung, G. F.; Debnath, M. L.; Klunk, W. E. *J. Med. Chem.* **2003**, *46*, 2740-2754.
[23]. Wang, X.;Sarris, K.; Kage, K.; Zhang, D.;Brown, S. P.; Kolasa, T.; Surowy, C.; ElKouhen, O. F.; Muchmore, S. W.; Brioni, J. D.; Stewart, A. O. *J. Med. Chem.* **2009**, *52*, 170-180.
[24]. Sgouras, D.; Maragkoudakis, P.; Petraki, K.; Martinez-Gonzalez, B.; Eriotou, E.; Michopoulos, S.; Kalantzopoulos, G.; Tsakalidou, E.; Mentis, A. *Appl. Environ. Microbiol*. **2004**, *70*, 518-526.
[25]. Wiart, C. *Oxford. C. J. Med.* **2007**, *4*, 299-311.
[26]. El-Zahar, M. I.; Abd EL-Karim, S. S.; Haiba, M. E. *World J. Chem.* **2009**, *4,* 182-194.
[27]. Hall, M. D.; Salam, N. K.; Hellawell, J. L.; Fales, H. M.; Kensler, C. B.; Ludwig, J. A.; Szakacs, G.; Hibbs, D. E.; Gottesman, M. M. *J. Med. Chem.* **2009**, *52*, 3191-3204.
[28]. Amin, K. M.; El-Zahar, M. I.; Anwar, M. M.; Kamel, M. M.; Mohamed, M. H. *Acta Poloniae Pharm.* **2009**, *66*, 279-291.
[29]. Farghaly, A.; El-Kashef, H. *Arkivoc* **2006**, *11*, 76-90.
[30]. Bashwan, S. A.; Fayed, A. A.; Amer, A. A. *Nat. Sci.* **2010**, *8*, 86-91.
[31]. Andrews, J. M. *J. Antimicrob. Chemother.* **2001**, *48*, 5-16.
[32]. Khalifa, N. M.; Adel, A. H. A.; Sherein, I. A. E.; Omar, A. F.; Amina, A. A. E. *Res. Chem. Intermed.* **2015**, *41*, 2295-2305.