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Synthesis and anticancer activity of novel 2-(4-amino-5-isocyano-methyl-2,3-dihydro-thiophen-2-yl)-7-[hydroxy-3-methyl-6,7-dihydrothiazolo[3,2-a]pyrimidin-5-one derivatives

Naema A. Mahmoud

Chemistry Department, Faculty of Science(Girls),Al-Azhar university, Nasr City, Egypt

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ABSTRACT

Base catalyzed reaction of 2-acetyl-3,5-dimethyl-isothiazolo[2,3-a]pyrimidin-7-one (1) with malononitrile yielded the corresponding compound (2) which is cyclized with sulphur to give (3), also, compound (1) reacted with 2-cyanoacetohydrazide in acetic acid to give (4). On the other hand, by treatment of compound (1) with 2-amino-2-(hydroxy methyl) propane-1,3 diol gives (5) which reacted with phosphorous oxychloride to give (6).Finally, chalcone (7) is formed by the reaction of compound (1) with trimethoxy benzaldehyde (scheme.1). Compound (3) is reacted with ρ.nitrobenzaldehyde to give compound (8) which is reacted with thioglycolic acid in the presence of anhydrous Na_2SO_4 to give compound (9). Compound (3) is cyclized by ethylenediamine to give (10), also compound (3) reacted with chloroacetylchloride to give chloroderivative (11) which under heating with potassium thiocyanate yielded (12) (scheme 2). Compound (3) under heating with formic acid, formamide, malononitrile and ethylcyanoacetate yielded the compounds (13-16) respectively (scheme 3). Diazotization of the compound (3) with concentrated HCl and sodium nitrite at 0-5 ℃ yielded azodye derivative (17). On the other hand, Coupling of (3) with diazotized p.nitroaniline gave (18).Finally (3) is condensed with acetic anhydride and phthalic anhydride to give (19,20) respectively(Scheme 4).

Key words: Thiophene, thiophene derivatives

INTRODUCTION

As the world's population increases and health problems expand accordingly, need to discover new the rapeutics will become even more diring. The design of drug molecules arguably offers some of the greatest hopes for success in present and future era. Heterocyclic compounds are widely distributed in nature and are essential for life. There are vast numbers of pharmacologically active heterocyclic compounds many of which are in regular clinical use **[1]**. The investigational approaches towards Structure- Activity Relationship focusing the search of optimized candidates have become immensely important. Thiophene belongs to a class of heterocyclic compounds containing a five membered ring made up of one sulphur as heteroatom with the formula C4H4S. Thiophene and its derivatives exist in petroleum or coal. Thiophene is taken from the word theion, the Greek word for sulfur, and another Greek word phaino which means shinning. Thiophene structure can be found in certain natural products and is also incorporated in several pharmacologically active compounds.

In medicinal chemistry, thiophene derivatives have been very well known for their therapeutic applications. The simple thiophenes are stable liquids which closely resemble the corresponding benzene compounds in boiling point and even in smell**[2]**.

Substituted thiophenes are among the most important aromatic heterocyclic derivatives. Many molecules incorporating the thiophene nucleus have, in fact, shown important pharmacological activities **[3–6]**. Moreover,

thiophene derivatives find large application in material science **[7–17]** and in coordination chemistry **[18,19]**, and as intermediate in organic synthesis **[20,21]**. The classical approaches to substituted thiophenes are mainly based on condensation-like reactions or on subsequent functionalization of the thiophene ring **[22–32]**. However, during the last years, innovative approaches to the regioselective synthesis of substituted thiophenes starting from acyclic precursors have been developed, mainly based on heterocyclization of functionalized alkynes **[33]**.

In this article we are using 2-amino thiophene as a starting materials for building of fused heterocyclic systems with an electron-withdrawing group such as cyano, ethoxy carbonyl or aminocarbonyl in the 3-position and alkyl,aryl and hetaryl groups in the 4 and 5 position[**34-40]** are prepared utilizing the Gewald reaction to obtain new compounds with higher biological activity than the parent compounds**[41]**.

MATERIALS AND METHODS

Melting points were determined in open capillary tubes on an electrothermal 9100 digital melting point apparatus (Buchi, stritzerland) Elmer 2400 analyzer (USA).

IR spectra were recorded on a Perkin-Elmer 160 FTIR (USA) as KBr. The ^{1}H-NMR and ^{13}C-NMR spectra were measured on Brüker DRX 500 and 125 MHz at Max-Plank Institute, Germany in DMSO-d_6.

The elemental analysis were carried out at microanalytical, faculty of science, Cairo university by using Perkin-Elmer 2400 C,H,N elemental analyzer.

Synthesis of 2-acetyl -3,5-dimethyl-isothiazol[2,3-a] pyrimidin-7-one (1)
A solution of thiobarbituric (0.01 mol), ethylacetoacetate (0.01 mol) in acetic acid (20 ml) containing few drop of conc H_2SO_4 was refluxed for 2h.

The solid obtained after cooling and neutralized of the solution with ammonium hydroxide was filtered off, washed with water ,dried and crystalized from benzene. Yield 45%, M.P 200°C, FT-IR (KBr, υ,cm-1), 3353(OH),1692(C=O ketone),1608(C=N),1680(C=O amide)., Anal.Calcd for $C_9H_{10}N_2O_3S$(226). C;47.78,H;4.42, N;12.38,S:14.15., Found, C,48.00, H,4.40, N:12.40,S:14.00%.

Synthesis of 7-hydroxy-3-methyl-5-oxo-6,7-dihydro-5-H-thiazolo-[3,2-a]pyrimidin-2-yl)-ethylidin)-malononitrile (2)
A mixture of 1 (0.01 mol) and malononitrile (0.01 mol) in absolute alcohol (20 ml) containing few drops of pipredine was refluxed for 6-8 h., after cooling the reaction mixture was poured onto ice-water (50 ml), the solid that formed was filtered off , air dried , and recrystallization from pet. Ether (60-80), yield 70 %, M.P. 100°C. FT-IR (KBr, υ,cm-1) , 1683(C=O),1619(C=N),1527(C=C), 2188-2164 (2CN), 3312(OH), 1386-1311(CH_3), ^{1}H-NMR spectrum (DMSO-d_6), 11.2(s,H,OH),4.7-4.5 (s,6H,2CH_3),7.7(m,3H,CH_2-CH-pyrimidine),Anal.Calcd, for $C_{12}H_{10}N_4O_2S$(274) for C, 52.5 H,3.6 N, 20.04,S:11.67 Found: C,52.40 H, 3.50, N: 19.8,S:11.70 %.

Synthesis of 2-(4-Amino-5-isocyano-2-methyl-2,3-dihydrothiophene-2-yl)-7-hydroxy-3-methyl-6,7-dihydrothiazolo[3,2-a]pyrimidin-5-one (3)
To a solution of compound 2 (0.05 mol) and sulphur (1.6 gm,0.05 mol) in absolute ethanol (50 ml),diethyl amine (3.65 gm,0.05 mol) was added dropwise at 15°C, the reaction mixture was stirred for 2h.at 65°C after evaporation of all solvents,the residue was dissolved in absolute ethanol (50 ml),followed by further stirring for 30 min. in an ice bath. The solid that formed filtered off,washed with water ,air dried and recrystallized from absolute ethanol to give the title compound. Yield 75 %,M.P 230°C.

FT-IR (KBr, υ,cm^{-1}) ,3417 (OH),1650(C=O), 1558(C=C),1602 (C=N),1340(CH_3),3302 (NH_2),2112(CN), ^{1}H-NMR spectrum (DMSO-d_6), 12.2(S,H,OH), 7.5(m,3H,CH_2-CH-Pyrimidine), 8.5(S,2H,NH_2), 3.6(S,3H,CH_3), 8.1(S,H,thiophene), ^{13}C-NMR 116.71(C≡N), 23.30(CH_3), 63.75(C=N), 61.65(CH_2), 157.67(C=O). Anal Calcd. For $C_{12}H_{10}N_4O_2S_2$(306), C,47;05, H; 3.26, N; 18.30,S;20.91 . Found: C; 47.00, H; 3.00, N;18.00,S;21.00 %.

Synthesis of cyano-acetic acid [1-(7-hydroxy-3-methyl-3-methyl-5-oxo-6,7-dihydro-5H-thiazolo[3,2-a]pyrimidin-2-yl)-ethylidene]-hydrazide (4).
To a solution of compound 1 (0.01 mol) in acetic acid 95 %) (10 ml) was added 2-cyanoacetohydrazide (0.01 mol). The reaction mixture was stirred at room temperature for 30 min. the solid formed was filtered off, washed with water and recrystallized from absolute ethanol.yield 75%,M.P 180°C., FT-IR (KBR, υ,cm^{-1}) 1620 (C=O), 1527(C=N), 1384-1346(CH_3), 3414(OH), 3028(NH), 2121(CN). ^{1}H- NMR 3.8-3.5 (S, 6H, 2CH_3), 11.2 (S, H, OH),

4.8-4.6 (s, 2H, CH_2), 7.3(d,t,3H,CH_2-CH-pyrimidine ring), 10.3 (s, H, NH). Anl cald for $C_{12}H_{13}N_5O_3S$(307), C, 46.9, H, 4.2, N, 22.8, S;10.42Found C46.4, H 4.1, N 22.5,S;10.37 %.

Synthesis of 5-H-hydroxy-2-[1-(3-hydroxy-propylimino)-ethyl-3-methyl-5,6-dihydro-isothiazolo[2,3-a]pyrimidin-7-one (5).

To a solution of 1 (0.01 mol) in acetic acid (96%,10 ml) was added 2-amino-2-(hydroxymethyl) propane 1,3 diol (0.01 mol) , the solid formed was filtered off,washed with water , air dried and recrystallized from benzene, yield 65% m.p. 120°C FT-IR (KBr, υ,cm^{-1}) (3749-3321(4OH) 1604(C=O), 1527 (C=C), 1602 (C=N), 1384 (CH_3), ^{1}H-NMR 13.8-10.4 (s, 4H, 4OH) 3.8-3.5 (s, 6H, $2CH_3$), 2.4 (dd, 2H, CH_2-pyrimidine), 7.7 (t, H, CH–pyrimidine),3.5(s,6H,3CH_2) Anal. Calcd for $C_{13}H_{19}O_5N_3S$(329): C, 47.41, H, 5.77, N, 12.76,S;9.72 found: C, 47.10, H, 6.00, N, 13.00,S;10.00%.

Synthesis of 2-(1-(3-chloro-propy, limino)-ethyl)-5-hydroxy-methyl-5,6-dihydro-thiazolo[2,3-a] pyrimidin-7-one (6)

A solution of compound 6 (0.01 mol) in phosphorus oxy chloride (50 ml) was heated under reflux for 3h.the reaction mixture was cooled and then poured into ice water , while stirring the solid that formed was filtered off,washed with water , air dried and recrystallized from ethanol,yield 65%,M.P over 300°C. FT-IR 3417(OH), 1616 (C=O), 1534(C=N), 1384(CH_3) 783(Cl), Anl. Calcd for $C_{13}H_{16}$ $N_3O_2Cl_3S$(384.5): C, 40.57, H, 4.16, N, 10.92,S;8.32,Cl;27.69, Found: C, 40.1, H, 4.5, N, 10.7,S;8.36,Cl;28.00%.

Synthesis of 5-hydroxy-3-methyl-2-(3-[3,4,5-trimethoxy-phenyl)-acroloyl]-5,6-dihydro-isithiazolo[2,3-a]pyrimidin-7-one(7)

To a solution of 1 (0.01 mol) in ethanol (10 ml)containing potassium hydroxide solution (5 ml,KOH (2.5 gm) ,H_2O (100 ml)) ., trimethoxy benzaldehyde(0.01 mol,0.1 ml) was added , the reaction mixture was stirred 2h. at room temperature, and then left overnight at refrigerator , the reaction mixture was poured onto ice-water (50 ml)and neutralized with dilute hydrochloric acid (1:1) ., the solid that formed was filtered off , washed with water,air dried and recrystallized from pet. ether (60-80°C) yield 85% m.p. 150°C FT-IR (KBr, υ,cm^{-1})1660(C=O), 1578(C=N) 1318(CH_3) 1170-1107 (3OCH_3), ^{1}H NMR 8.9(s, H, OH), 7.2-7.4(m, 4H, CH=CH, H–Ar), 4.1-4.4 (s, 9H, 3OCH_3), 3.89 (s, 3H, CH_3), 3.81-3.8 (m, 2H, CH_2), ^{13}C NMR 129.4-129.3 (Ar–C), 40.59-40.4 (CH), 192.4 (C=O), 26.8 (CH_3), 54.8-54.6 (3OCH_3), 167.4 (C=N) Anal.$C_{19}H_{20}N_2O_6S$(404) For C, 56.43, H, 4.95, N, 6.93,S;7.92 Found: C, 56.10, H, 4.82, N, 6.70,S;8.00%.

Synthesis of 5-(5-hydroxy-3- methyl-7-oxo-6,7-dihydro-5H-isothiazolo [2,3-a]pyrimidin-2-yl)-3-[4-Nitro-benzylidin)-amino]-thiophene-2-carbonitrile (8).

To a solution of compound 3 (0.01 mol) and p. nitrobenzaldehyde (0.01 mol) in methanol (50 ml) with 4 drops of glacial acetic acid was refluxed for 6 h., the solid obtained was filtered off ,dried and recrystallized from benzene , yield 45%,M.P. 190°C. FT-IR,(KBr, υ,cm^{-1}), 3455(OH), 2122(CN), 1616(C=O), 1611(C=N), 1570 (C=C), 1315 (CH_3), ^{1}H NMR 11.8 (s, H, OH), 10.1 (s, H, CH=N), 8.2 (s, H, thiophene), 7.4-7.1 (m, 7H, CH_2–CH–pyrimidine, Ar–H), Anal.$C_{19}H_{13}N_5O_2S$(407) Calcd. For : C, 56.01, H, 3.19 N, 17.19,S;15.72 Found : C, 56.00, H, 3.00, N, 17.00,S;16.00%.

Synthesis of 5-(5-hydroxy-3-methyl-7-oxo-6,7-dihydro-5H-isothiazolo [2,3-a]pyrimidin-2-yl)-3-[2-(4-nitro-phenyl)-4-oxo-thiazolidin-3-yl]-thiophene-2-carbonitrile (9)

When the equimolar solution of compound 9 and mercaptoacetic acid (0.01 mol) with a pinch of anhydrous $ZnCl_2$ in methanol (30 ml) was refluxed for 6 h., the solid obtained was filtered and recrystalized from benzene ,yield 55%,M.P. 160 °C. FT IR, (KBr, υ,cm^{-1}) 1620 (C=O, 3450 (OH) 1630 (C=N), 1310(CH_3), 2130 (CN). ^{1}H NMR 12.1 (s, H, OH), 3.6 (s, 3H, CH_3) 7.4-7.1 (m, 9H, CH_2–CH-pyrimidine, CH-thiophene, Ar–H) ^{13}C NMR, 40.59(CH) 23.3 (CH_3) 117.1 (CN), 63.2 (CH_2), 165.1 (C=O) Anal.$C_{21}H_{15}N_5O_5S_3$(513) Calcd. For : C, 49.12 H, 2.92, N, 13.64,S;18.71 Found: C, 49.0, H, 3.00, N, 13.60,S;19.00%.

Synthesis of 2-[5-Amino-4-(4,5-dihydro-1H-imidazol-2-yl]thiophen-2-yl]-5-hydroxy-3-methyl-5,6-dihydro-isothiazolo[2,3-a]pyrimidin-7-one (10).

A mixture of 3 (0.01 mol),ethylene diamine (5 ml) in ethanol(10 ml)was refluxed for 6 h. the solid that formed on hot was filtered off, dried, recrystalized from chloroform yield 55 %,M.P. 180°C. FT-IR (KBr, υ, cm^{-1}), 1660 (C=O), 3401 (OH), 1320(CH_3), 3330 (NH_2), 3320(NH). Mass spectra m/z = 349, base peak = 80, Anal.$C_{14}H_{15}N_5O_2S_2$(349) Calcd. For $C_{14}H_{15}N_5O_2S_2$ C, 48.13, H, 4.29, N, 20.05.S;18.33 Found, C, 48.10, H, 4.30, N, 20.00,S;18.37%.

Synthesis of 2-chloro-N-[5-(5-hydroxy-3-methyl-oxo-6,7-dihdro-5H-isothiazolo[2,3-a]pyrimidin-2-yl)-2-isocyano-thiophen-3-yl]-acetamide (11).

To a solution of 3 (0.02 mol) in dry benzene (60 ml) , a solution of chloroacetylchloride (5 ml, 0.04 mol)in dry benzene (20 ml) was added dropwise under vigorous stirring at 0-5°C., after all addition the reaction mixture was refluxed for 3h., the solvent was evaporated under vacuum and the solid $NaHCO_3$ and then with water ,air dried and recrystallized from chloroform ,yield 80%,M.P. 120°C. FT-IR, (KBr, υ, cm^{-1}) 3435, 1615 (C=O), 1340 (CH_3), 3320(NH), C, 796(Cl), 2163(CN), 1530(C=N). Mass spectra m/z = 382.5, Base peak (M^+, 416) and 158 (100%). Anal. Calc. $C_{14}H_{11}N_4O_3S_2Cl$(382.5)Cacd.for, C, 43.92, H, 2.87, N, 14.65, S;16.73,Cl;9.28 .Found C, 44.00, H, 3.00, N, 15.00,S;16.00,Cl;9.30%.

Synthesis of 5-hydroxy-2-(4-(2-imino-4-oxo-thiozolidin-3-yl)-5-isocyano-thiophen2-yl]-3-methle -5,6-dihydro-isothiazolo[2,3-a]pyrimidin-7-one (12).

A mixture of compound 12 (0.03 mol),potassium thiocyanate (0.06 mol) in dry acetone (100 ml) was refluxed for 3h.

The solid that formed on hot was filtered off,dried and recrystallized from chloroform, yield 45 %,M.P. 175°C. FT-IR (KBr, υ,cm^{-1}) ,1627(C=O),1600(C=N),1384(CH_3),3421(OH), 3236(NH),2129(CN), ^{1}H NMR spectrum (DMSO-d_6), reveals signals (δ, ppm) at 11.9 (s,H,OH)8.9 (S,H,NH),3.6 (s,3H,CH_3),5.2(s,2H,CH_2),1.2(s,H,NH-imino group),7.1(S,2H,CH_2),1.2(S,H,CH-thiophene),7.4 (m,3H,CH_2-CH pyrimidine),^{13}C-NMR,159.4(C=N),23.5 (CH_3),117.1(CN),192.6 (C=O).Anal Calcd for $C_{15}H_{11}N_5O_3S_3$(405): C, 44.44; H, 2.71; N, 17.28,S;23.70. Found C, 44.10; H, 2.5;N, 17.10,S;23.78 %.

Synthesis of 6-(5-hydroxy-3-methyl-7-oxo-6,7-dihydro-5H-isothiazolo [2,3-a]pyrimidin-2-yl)-3H-thieno[3,2-d]pyrimidin-4-one (13)

A solution of 3 (0.005 mol)in formic acid (85 %) (10 ml) was refluxed for 3h.., the solid was formed on hot ,was refluxed ,dried and recrystallized from ethanol ,yield 65%, m.p.190°C. FT-IR (KBr, υ,cm^{-1}), 3460(OH), 1660(C=O),1620(C=N), 2180(CN),1330(CH_3) and its mass spectrum m/z = 334 and base peak at 255. Anal Calcd for $C_{13}H_{10}N_4O_3S_2$(334): C,46.70; H,2.99;N,16.76,S;19.16 Found, C,46.10;H,2.77;N,16.15,S;19.00 %.

Synthesis of 2-(4-Amino-theino [3, 2-d] pyrimidin-6-yl)-5-hydroxy-3-methl-5,6-dihydro-isothiazolo[3,2-a] pyrimidin-7-one (14).

A mixture of 3 (0.005 mol) in formamide (20 ml) was refluxed for 5h. the solid was formed on hot was filtered off, dried and recrystallized from ethanol, yield 60%,M.P. 120°C FT-IR (KBr, υ,cm^{-1}), 1660(C=O), 3450(OH), 1340(CH_3),3320(NH_2),1620(C=N). ^{1}H NMR spectrum (DMSO-d6), reveals signals (δ, ppm) at 8.6(S,H,OH),8.4-8.3(m,4H,CH-thiophene), CH_2,CH-pyrimidine), ^{13}C-NMR, 17.7(CH_3), 160.0(C=N), 65.5(CH_2), 195.5(C=O). Anal Calcd for $C_{13}H_{11}N_5O_2S_2$(333): C, 46.84,H; 3.30,N; 21.02,S;19.21 Found: C, 47.00, H, 3.60, N, 21.00,S;19.00%.

Synthesis of 2-(5,7-Diamino-6-isocyano-6,7-dihydro-thieno[3,2-b] pyridine-2-yl)-5-hydroxy-3-methyl-5,6-dihydro-isothiazolo[2,3-a]pyridine-7-one (15).

A mixture of 3 (0.01 mol), malononitrile (0.01mol) in absolute ethanol (10 ml) containing few drops of pipridine was refluxed for 10 h. after cooling , the reaction mixture was poured onto ice-water (50 ml), the solid that formed ,filtered off, dried and recrystallized from ethanol ,yield 70 %, M.P. 210°C, FT-IR (KBr, υ,cm-1),3420(OH), 1660(C=O), 1630(C=N),1320(CH_3), 3330(NH_2), 2195(CN). ^{1}H-NMR spectrum (DMSO-d6), reveals signals (δ, ppm) at 11.2(s,H,OH), 8.9-8.2(s,4H,2NH_2), 6.5(m,4H,CH_2-CH-pyrimidine, CH-thiophene), Anal Calcd for $C_{15}H_{12}N_6O_2S_2$(372), C, 48.38, H, 3.22, N, 22.58,S;17.20. Found , C, 48.40, H, 3.50, N, 22.10,S;17.00%.

Synthesis of 2-(6-Amino-5-isocyano-7-oxo-4, 7-dihydro-thieno[3,2-b]pyridine-2-yl)-5-hydroxy-3-methyl-5,6-dihydro-isothiazolo[2,3-a]pyrimidine-7-one(16)

A mixture of 3 (0.01 mol) and ethylcyanoacetate (0.01 mol) was fused at 180-200 C for 2h. , the reaction mixture was allowed to cool and the solid product was collected and recrystallized from benzene. Yield 55%, M.P. 180°C. FT-IR (KBr, υ,cm-1), 3450(OH), 3320-3310 (NH_2,NH), 1660(C=O)1370 (CH_3),2199(CN),1610 (C=N),AnalCalcd for $C_{15}H_{13}N_5O_3S_2$(375): C,48.00; H,3.46; N,18.66,S;17.06. Found, C,48.10; H, 3.50; N,19.00,S;17.00 %.

Synthesis of 5-hydroxy-2-[5-(4-hydroxy-naphthalene-7-yl-azo)-4-isocyano-thiophen-2-yl]-3-methyl-5,6-dihydro-isothiazolo[2,3-a]pyrimidin-7-one (17)

To a solution of 3 (0.01 mol) in a mixture of concentrated hydrochloric acid (10 ml), cooled aqueous solution of $NaNO_2$ (0.96 gm),0.01 mol) in ice water (10 ml), was added dropwise under stirring at 0-5°C , the diazonium salt solution, thus prepared was added dropwise to a solution of B-naphthol (1.44 gm, 0.01 mol) and sodium hydroxide (0.8 gm, 0.02 mol) in ethanol and water (1:1) (50 ml) under stirring from 0-5°C. the solid that formed was filtered off ,dried and recrystallized from ethanol, yield 79 %, M.p 160°C. FT-IR (KBr, υ,cm-1),3430(OH),

1665(C=O),1607(CH_3), 2201(CN) Anal Calcd for $C_{22}H_{15}N_5O_3S_2$(461): C,57.26;H,3.25;N,15.18,S;13.88. Found, C,57.12;H,3.28;N,15.00,S14.00 %.

Synthesis of 6-(5-Hydroxy-3-methyl-7-oxo-6,7-dihydro-5H-isothiazolo [2,3-a]pyridine-2-yl)-2-methyl-1H-thieno[3,2-d] pyrimidin-4-one (18)

To a solution of p.nitroaniline (1.38 gm,0.01 mol) in a mixture of concentrated hydrochloric acid (10 ml) and ice water (10 ml) ,cooled aqueous solution NaNO2 (0.96 gm,0.01 mol) in ice water (10 ml) was added dropwise under stirring 0-5°C.The diazonium salt solution thus prepared was added dropwise to a solution of 3 (0.01 mol) in ethanol and water (1:1) (50 ml) under stirring at 0-5°C.The solid that formed was filtered off, crystallized from ethanol ,yield 76%,M.P. 190 C. FT-IR (KBr, υ,cm-1), 1620(C=O),3405(OH),1360(CH_3),1607(C=N),^{1}H -NMR spectrum (DMSO-d_6), reveals signals (δ, ppm) at 3.3(s,3H,CH_3,8.3 (s,H,OH),7.9-7.5(m, 4H,CH_2-CH pyrimidine; CH-thiazol),1.2 (s,3H,CH_3), ^{13}C-NMR 23.2(CH_3), 165.1(C=N), 180.2(C=O), 55.5(CH_3), Anal Calcd for $C_{18}H_{12}N_6O_4S_2$(440): C, 49.09; H, 2.72;N,19.09,S;14.54. Found C, 49.00; H, 3.00; N, 19.00,S;14.50%.

Synthesis of 2-[5-hydroxy-3-methyl-7-oxo-6,7-dihydro-5H-isothiazolo [2,3-a]pyrimidin-2-yl)-3-isocyano-thiophene-2-yl]-isoindle-1,3-dione (19).

Treatment of 3 (0.01 mol) with acetic anhydride solution was refluxed for 2h.and the crude product was separated ,filtered and recrystallized from ethanol , yield 70 %, M.P. 200°C. FT-IR (KBr, υ,cm^{-1}) 1650-1600(C=O), 3410(OH), 1352(CH_3), 2192(CN), 1610(C=N) Anal Calcd for $C_{20}H_{12}N_4O_4S_2$(436): C,55.04; H, 2.75; N,12.84,S;14.67 Found C, 54.80; H, 2.50; N, 12.60,S;15.00 %.

Synthesis of 2-[3-Amino-4-isocyano-5-(4-nitro-phenylazo)-thiophene -2-yl]-5-hydroxy-3-methyl-5,6-dihydro-isothiazolo[2,3-a]pyrimidin-7-one (20).

A mixture of 3 and phthalic anhydride was dissolved in glacial acetic acid , the solution was refluxed for 6 h. the crude product was separated, filtered off and recrystallized from benzene ,.yield 65%, M.P. 220°C. FT-IR (KBr, υ, cm-1), 3450(OH), 1630(C=O), 3303(NH_2), 1309(CH_3), 2133(CN), and its mass spectrum (EL/MS) shows molecular ion peak at m/z = 455 and base peak at m/z = 74 (100%). Anal Calcd for $C_{14}H_{12}N_4O_3S_2$(348): C,48.27; H, 3.44; N, 16.09,S;18.39. Found, C;48.35; H, 3.00;N, 16.00,S;18.52%.

Biological Activity

Antiproliferative activity:

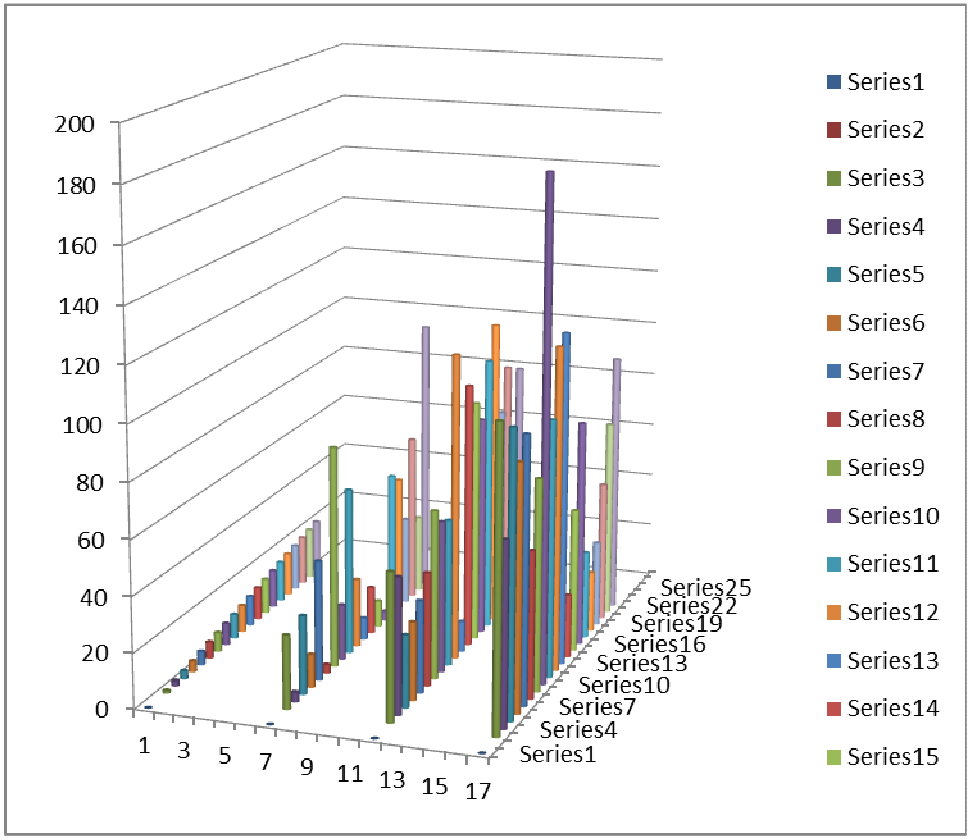
MTT ASSAY

MTT assay is a sensitive, quantitative and reliable colorimetric method that measures viability of cells[**42]** Cytotoxicity assay was determined by MTT stain (3-[4,5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide) as a water soluble tetrazolium salt, which is converted to an insoluble purple MTT -formazan Complex by cleavage of the tetrazolium ring by succinate dehydrogenase within the mitochondria. The formazan product is impermeable to the cell membranes and therefore it accumulates in viable cells.

Table (1): IC_{50} % (µg/ml) values of new compounds towards (HEPG-2), (MCF-7), (HCT-116) cell lines, and diagrammed in Fig.(1)

Inhibition concentration (50%) (IC_{50}) µg/ml			
Compound	**HEPG-2**	**MCF-7**	**HCT-116**
1	26.6	53.3	108.2
2	3.6	49.1	66.3
3	28.6	26.2	102.3
4	12.03	28.3	88.5
5	43.5	33.5	96.1
6	3.1	41.2	53.3
7	80.2	61.2	76.5
8	20.3	55.1	180.1
9	60.6	53.4	93.2
10	25.1	111.1	117.1
11	8.1	11.2	120.2
12	17.3	96.3	23.1
13	9.7	88.1	52.2
14	3.3	180.1	82.3
15	53.2	99.9	32.1
16	49.7	111.5	22.2
17	32.3	77.1	31.2
18	61.3	92.2	51.3
19	28.6	67.1	72.2
20	101.1	88.2	95.1

Antiproliferative activity of the newly synthesized compounds against human carcinoma cell lines:
IC_{50}% (µg/ml) of newly synthesized compounds on human hepatocyte carcinoma cell line (HEPG-2), human breast cancer cell line (MCF-7) and human colon cancer cell line (HCT-116).



RESULTS AND DISCUSSION

The new derivatives were prepared according to the reaction sequences depicted in schemes (1-4).

Reaction of 2- acetyl- 3,5- dimethyl-isothiazolo(2,3-a)-pyrimidin-7- one (1) with malononitrile under reflux in ethanol yielded the corresponding 7-hydroxy-3-methyl-5-oxo-6,7-dihydro-5-H-thiazolo[3,2-a]pyrimidin-2-yl)-ethyledine-malononitrile (2)which is cyclized with sulphur in ethanol and in the presence of diethylamine as acatalyst (Gewald reaction) to give the corresponding (3).

Reaction of compound (1) with 2-cyanoacetohydrazide in acetic anhydride gives compound (4).

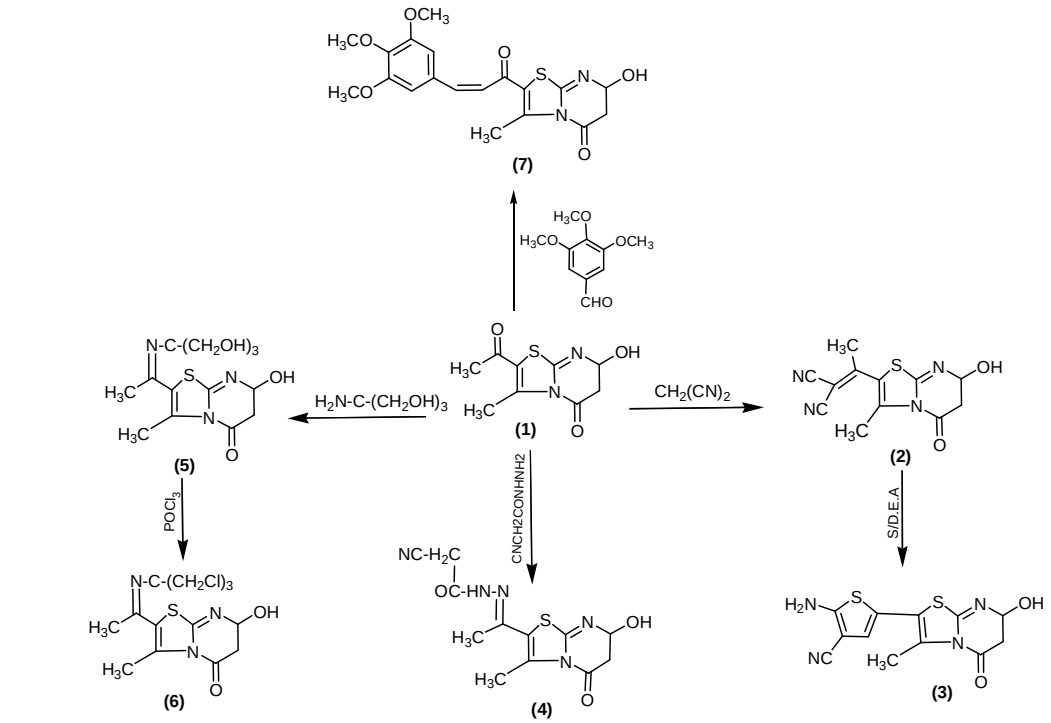
Also, compound (1) is reacted with 2-amino-2-(hydroxy methyl)-propane -1,3 diol in acetic acid to give (5) which is reacted with phosphorous oxy chloride to form (6).
On the other hand, compound (1) is reacted with trimethoxy benzaldehyde in ethanol in the presence of potassium hydroxide (25 %) to give α,β unsaturated ketone (7). (Scheme 1).

The compound (3) reacted with p.nitrobenzaldehyde in glacial acetic acid give the corresponding schiff"s base (8) which is corresponding to thiazolidinone derivative (9).

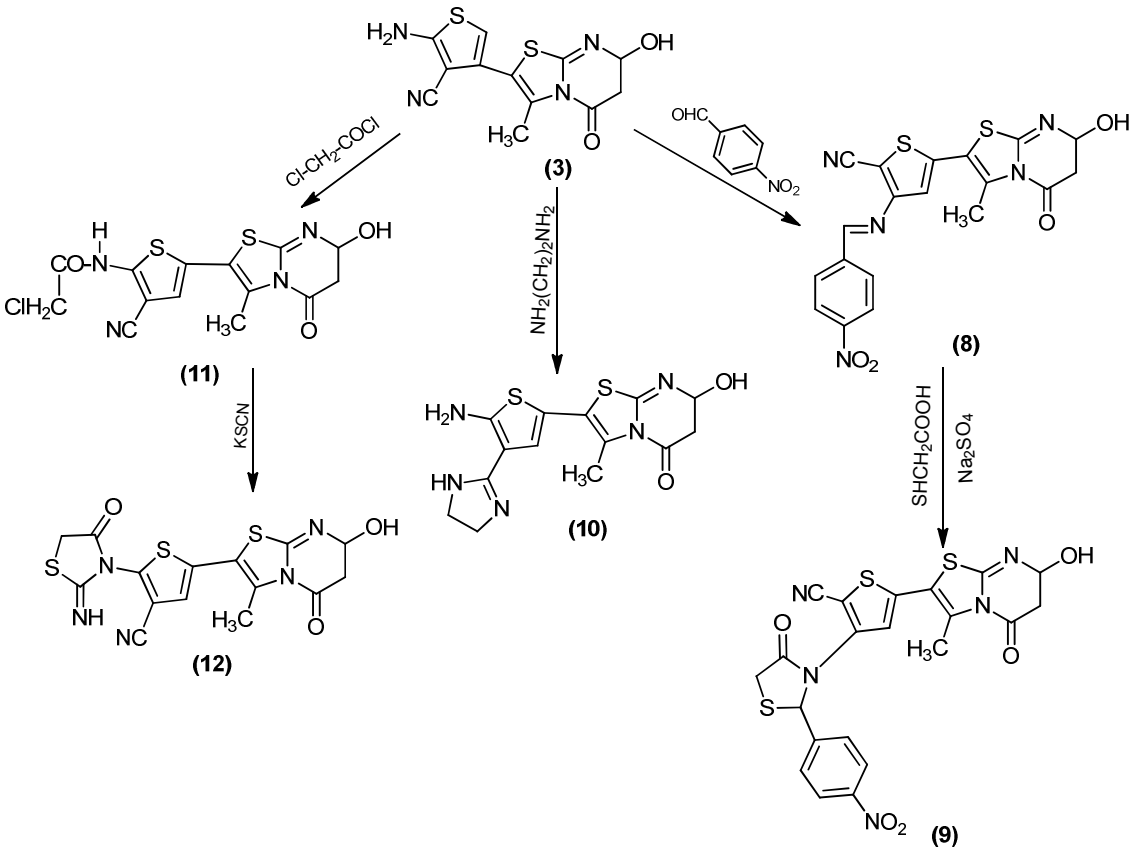
Also, condensation of cyanogroup of (3) with ethylene diamine in ethanol leads to formation of imidazole derivative (10).

Moreover , reaction of compound (3) with chloro acetylchloride in dry benzene gives the chloroacetamido derivative (11) which under heating with potassium thiocyanate in dry acetone yielded 2-imino-thiazolidinone derivative (12) (scheme 2).

Cyclization of (3) upon heating with formic acid, formamide, malononitrile and ethylcyanoacetate gives pyrimidine and pyridine derivatives (13-16) respectively (scheme 3).



Scheme (1)

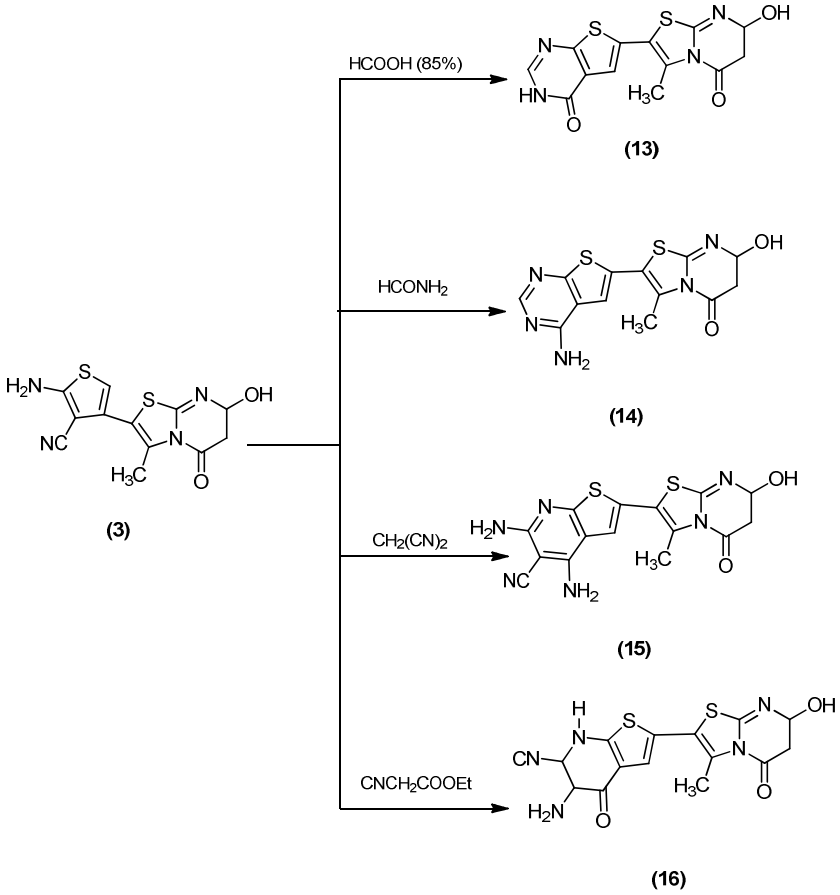


Scheme (2)

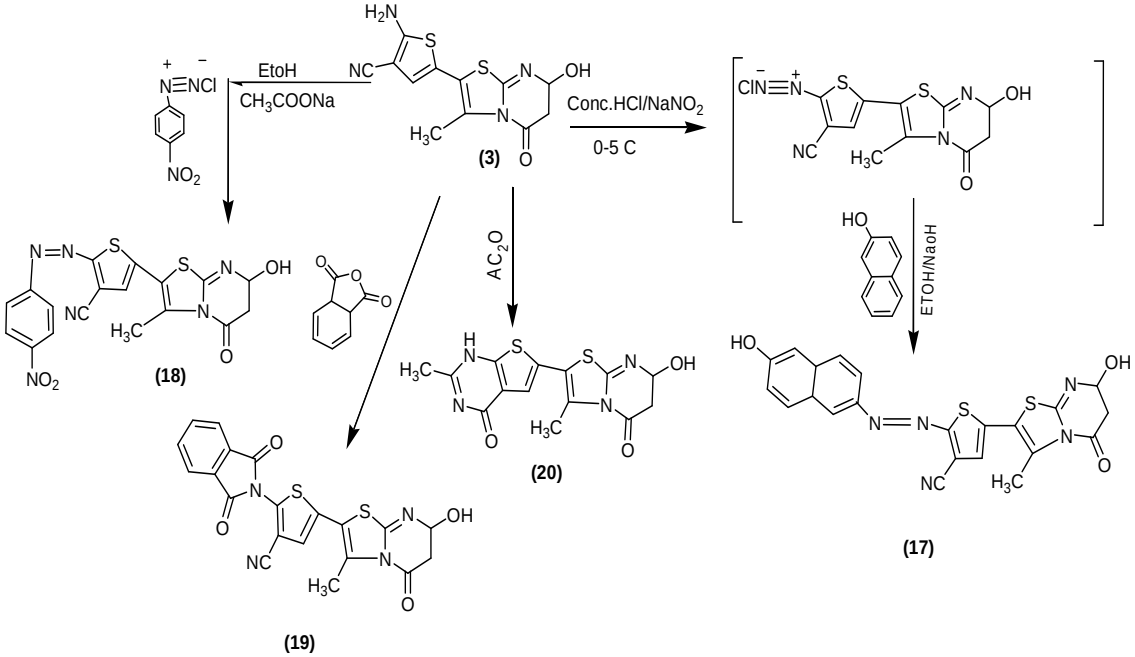
Diazodization of compound (3) with concentrated hydrochloric acid and sodium nitrite at 0-5 C yielded the corresponding diazonium salt which coupling with β-naphthol gave the corresponding azodye derivative (17).

On the other hand, coupling of (3) with diazotized p.nitroaniline gave pyrimidine derivative (18).

Finally, condensation of (3) with acetic anhydride and phthalic anhydride to give isoindole and 4-nitrophenylazothiophene derivatives (19,20) (scheme 4).



(Scheme 3)



(Scheme 4)

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