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RESEARCH ARTICLE

Design and synthesis of thiazol derivatives with biological evaluations as antitumor agents

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ABSTRACT:

The synthesis of 2-cyanomethyl-thiazole-4-one **(1)** was obtained via the reaction of malononitrile with thioglycolic acid, then the formed product was directed toward the reaction with either acetoacetanlide or 4-chloro acetoacetanlide to produce compounds **2, 3**. The latter compounds were reacted with either aromatic aldehyde or diazonium chloride derivatives to produce compounds **10, 11, 16-21** and **28-33**. Finally compound **14** and **15** were obtained through the reaction of compound **10** with either malononitrile or ethylcyanoacetate. The newly synthesized compounds were evaluated for antitumor activity.

KEYWORDS: Thiazol, thiophene, pyridazine, antitumor activity.

1. INTRODUCTION:

Thiazoles and thiazolidones[1] derivatives exhibit an interesting numbers of biological properties such as antimicrobial[2-4], antiprotozoal[5,6], anti-inflammatory, antiallergic[7,8], anticonvulsant[9], cardiotonic[10], analgesic and antithermic[11] and anticancer[12], among others. In this article we have synthesized new thiazol-4-one derivatives purposely to try to improve the antitumor activity against three different cell lines. The structures of the newly synthesized compounds were established using IR, NMR and Mass spectrometry techniques.

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2. MATERIAL AND METHODS:

2.1. Experimental

All melting points were determined in open capillaries and are uncorrected. Elemental analyses were performed on a Yanaco CHNS Corder elemental analyzer (Japan). IR spectra were measured using KBr discs on a Pye Unicam SP-1000 spectrophotometer. ^{1}H NMR spectra were measured on a Varian EM 390-200 M*Hz* instrument in CD_3SOCD_3 as solvent using TMS as internal standard and chemical shifts were expressed as δ ppm. Mass spectra were recorded on Kratos (75 eV) MS equipment (Germany).

2.1.1. General procedures for the synthesis of 2-cyanomethylenothiazol-4-one (1):

The titled compound **1** obtained via the reaction of malononitrile with thioglycolic acid in the presence of glacial acetic acid according to procedures described before[13].

2.1.2. General procedures for the synthesis of:- 3-Methyl-4-phenylformamido-α-yl-2-(thiazolo-4'-one-2'-yl) crotononitrile (2):

Equimolecular amounts of compound **1** (1.4 g, 0.01 mol) and acetoacetanilide (1.7 g, 0.01 mol), in presence of catalytic amount of ammonium acetate were heated under reflux at 140˚C for 1h. The reaction mixture was then evaporated in vacuum. The remaining product was triturated with ethanol and the solid product formed was collected by filtration.

Compound **2:** Pale yellow crystals from 1,4-dioxane, yield: 83.4 % (2.5 g); mp: 60-62ºC. IR (KBr): υ/cm^{-1} = 3460-3380 (NH), 3057 (CH-aromatic), 2988 (CH_3), 2875 (CH_2), 2222 (CN), 1715, 1680 (2CO), 1658 (C=N). ^{1}H NMR (DMSO-d_6): δ = 2.94 (s, 3H, CH_3), 5.34 (s, 2H, CH_2), 6.82 (s, 2H, thiazol CH_2), 7.32-7.37 (m, 5H, C_6H_5), 8.94 (s, 1H, NH, D_2O-exchangeable). MS (relative intensity) m/z: 299 (M^+, 34%). Analysis for $C_{15}H_{13}N_3O_2S$ Calcd: C, 60.14; H, 4.73; N, 14.08, S,10.7 Found: C, 60.3; H, 4.57; N, 14.03, S, 10.88 %.

3-Methyl-4-(4'-Cl-phenyl) formamido-α-yl-2-(thiazole - 4'-one-2'-yl) crotononitrile (3):

Equimolar amounts of compound **1** (1.4 g, 0.01 mol) and 4-chloroacetoacetanilide (2.12 g, 0.01 mol), in the presence of catalytic amount of ammonium acetate were heated under reflux at 140˚C for 1.5 hr. The solid product formed upon neutralization with ice/water mixture containing few drops of hydrochloric acid was collected by filtration.

Compound **3:** Brown crystals from 1,4-dioxane , yield: 83.3 % (2.78 g); mp: 160-162 ºC. IR (KBr): υ/cm^{-1} = 3456-3374 (NH), 3060 (CH-aromatic), 2980 (CH_3), 2865 (CH_2), 2220 (CN), 1710, 1684 (2CO), 1640 (C=C). ^{1}H NMR (DMSO-d_6): δ = 2.93 (s, 3H, CH_3), 5.3 (s, 2H, CH_2), 6.8 (s, 2H, thiazol CH_2), 7.33-7.36 (m, 4H, C_6H_4), 9.24 (s, 1H, NH, D_2O-exchangeable). MS (relative intensity) m/z: 334 (M^+, 21%). Analysis for $C_{15}H_{12}N_3O_2SCl$ Calcd: C, 53.93; H, 3.62; N, 12.63, S, 9.59 Found: C, 53.7; H, 3.8; N, 12.81, S, 9.72 %.

2.1.3. General procedure for the synthesis of compounds:- 3-Methyl-4-phenylformamido-α-yl-2-(thiazole-4'-one-2'-yl-)-5-phenyl-2,4-dienovaleronitrile (10) and 3-Methyl-4-phenyl- formamido-α-yl-2-(thiazole-4'-one-2'-yl)-5-(2'-hydroxy phenyl)-2,4-dienovaleronitrile (11):

A mixture of compounds **2** (2.9 g, 0.01 mol) and either benzaldehyde (1.08 g, 0.01 mol) or salicylaldehyde (1.2 g, 0.01 mol), in dimethylformamide (40 ml) containing a catalytic amount of piperidine (0.5 ml) was heated under reflux for 2hrs. The reaction mixture was cooled at room temperature and poured onto ice/water mixture containing few drops of hydrochloric acid the solid products, so formed, were filtered off and dried.

Compound **10:** Pale brown crystals from 1,4-dioxane , yield: 77.3 % (2.99 g); mp: 98-100 ºC. IR (KBr): υ/cm^{-1} = 3455-3375 (NH), 3055 (CH-aromatic), 2975(CH_3), 2863 (CH_2), 2220 (CN), 1693, 1683 (2CO), 1658 (C=N), 1636 (C=C). ^{1}H NMR (DMSO-d_6): δ = 2.91 (s, 3H, CH_3), 6.83 (s, 2H, thiazol CH_2), 7.01 (s,1H, CH=C), 7.32-7.39 (m, 10H, $2C_6H_5$), 8.91 (s, 1H, NH, D_2O-exchangeable). MS (relative intensity) m/z: 387 (M^+, 27%). Analysis for $C_{22}H_{17}N_3O_2S$ Calcd: C, 68.16; H, 4.42; N, 10.88, S, 8.27 Found: C, 68.2; H, 4.6; N, 10.9, S, 8.41 %.

Compound **11:** Brown crystals from 1,4-dioxane , yield: 74.8 % (3.02 g); mp: 70-73 ºC. IR (KBr): υ/cm^{-1} = 3520-3360 (OH, NH), 3050 (CH-aromatic), 2966 (CH_3), 2861 (CH_2), 2222 (CN), 1700, 1680 (2CO), 1660 (C=N), 1634 (C=C). ^{1}H NMR (DMSO-d_6): δ = 2.95 (s, 3H, CH_3), 6.68 (s, 2H, thiazol CH_2), 6.98 (s, 1H, CH=C), 7.36-7.42 (m, 9H, C_6H_5, C_6H_4), 8.89 (s, 1H, NH, D_2O-exchangeable), 10.36 (s, 1H, OH). MS (relative intensity) m/z: 403 (M^+, 20%). Analysis for $C_{22}H_{17}N_3O_3S$ Calcd: C, 65.46; H, 4.24; N, 10.45, S, 7.94 Found: C, 65.6; H, 4.42; N, 10.59, S, 7.99 %.

2.1.4. General procedure for the synthesis of:- 1-Amino-6-cyano-3-methyl-5-phenyl-4-phenylformamido-α-yl-2-(thiazole-4'-one-2'-yl-) benzene (14) and Ethyl-1-amino-6-cyano -3-methyl-5-phenyl-4-phenylformamido-α-yl-2-(thiazole-4'-one-2'-yl) benzoate (15):

To a solution of compound **10** (3.8 g, 0.01 mol) in dimethylformamide (50 ml) containing a catalytic amount of triethylamine (0.5 ml), either malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.13 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 2hrs. Then it was poured onto ice/water mixture containing few drops of hydrochloric acid. The formed solid product was collected by filtration.

Compound **14:** Yellow crystals from 1,4-dioxane , yield: 71.6 % (3.055 g); mp: 210-212 ºC. IR (KBr): υ/cm^{-1} = 3460-3345 (NH_2, NH), 3058 (CH-aromatic), 2983 (CH_3), 2869 (CH_2), 2225 (CN), 1664 (C=N), 1638 (C=C). ^{1}H NMR (DMSO-d_6): δ = 3.11 (s, 3H, CH_3), 5.48 (s, 2H, NH_2, D_2O-exchangeable), 6.83 (s, 2H, thiazol CH_2), 7.32-7.37 (m, 10H, $2C_6H_5$), 8.81 (s, 1H, NH, D_2O-exchangeable). MS (relative intensity) m/z: 426 (M^+, 16%). Analysis for $C_{24}H_{18}N_4O_2S$ Calcd: C, 67.55; H, 4.25; N, 13.18, S, 7.51 Found: C, 67.76; H, 4.41; N, 13.29, S, 7.38 %.

Compound **15:** Yellowish brown crystals from 1,4-dioxane , yield: 67.5 % (3.197 g); mp: 140-142 ºC. IR (KBr): υ/cm^{-1} = 3470-3325 (NH_2, NH), 3060 (CH-aromatic), 2979 (CH_3), 2883 (CH_2), 1705, 1690-1683 (3C=O), 1655 (C=N), 1637 (C=C). ^{1}H NMR (DMSO-d_6): δ = 1.51 (t, 3H, CH_3), 3.19 (s, 3H, CH_3), 4.32 (q, 2H,

CH_2), 5.36 (s, 2H, NH_2, D_2O-exchangeable), 6.81 (s, 2H, thiazol CH_2), 7.31-7.44 (m, 10H, $2C_6H_5$), 8.89 (s, 1H, NH, D_2O-exchangeable). MS (relative intensity) m/z: 473 (M^+, 23%). Analysis for $C_{26}H_{23}N_3O_4S$ Calcd: C, 65.92; H, 4.89; N, 8.90, S, 6.76 Found: C, 65.81; H, 4.66; N, 8.72, S, 6.58 %.

2.1.5. General procedure for the synthesis of compounds:- 3-Methyl-4-phenylformamido-α-yl-4-phenylhydrazono-2-(thiazole-4'-one-2'-yl) crotono-nitrile (16), 3-Methyl-4-(hydrazo-3-cyano-4',5',6',7'-tetrahydrobenzo[*b*] thiophenophenylformamido-α-yl-)-2-(thiozole-4'-one-2'-yl) crotononitrile (17) and 4-[Ethyl 2–hydrazo (4',5',6',7'-tetrahydrobenzo[*b*] thiopheno-3-carboxylate)phenylforma-mido-α-yl] - 3-methyl-2-(thiozole-4'-one-2'-yl) crotononitrile (18):

To a cold solution of compound **2** (2.9 g, 0.01 mol), in ethanol (50 ml) containing sodium acetate (0.82 g, 0.01 mol), either benzene diazonium chroride (0.9 g, 0.01 mol), or 1-cyano-2-diazo-4,5,6,7- tetrahydrobenzo [*b*]thiophene (1.7 g, 0.01 mol), or ethyl 2-diozo- 4,5,6,7-tetrahydrobenzo [*b*]thiophencarboxylate (2.25 g, 0.01 mol) was added respectively with continuous stirring. The reaction mixture was stirred at room temperature for 2hrs and the formed solid product was collected by filtration.

Compound **16:** Brown crystals from ethanol, yield: 81.7 % (3.299 g); mp: 175-177 ºC. IR (KBr): υ/cm^{-1} = 3480-3410 (2NH), 3060 (CH-aromatic), 2977 (CH_3), 2880 (CH_2), 2227(CN), 1690, 1684 (2C=O), 1658 (C=N), 1634 (C=C). ^{1}H NMR (DMSO-d_6): δ = 2.94 (s, 3H, CH_3), 6.84 (s, 2H, thiazol CH_2), 7.29-7.36 (m, 10H, $2C_6H_5$), 8.44, 8.78 (2s, 2H, 2NH, D_2O-exchangeable). MS (relative intensity) m/z: 403 (M^+, 24%). Analysis for $C_{21}H_{17}N_5O_2S$ Calcd: C, 62.47; H, 4.24; N, 17.41, S, 7.94 Found: C, 62.71; H, 4.16, N, 17.67, S, 7.68 %.

Compound **17:** Dark brown crystals from 1,4-dioxane , yield: 71.5 % (3.496 g); mp: 233-235 ºC. IR (KBr): υ/cm^{-1} = 3474-3370 (2NH), 3051 (CH-aromatic), 2984 (CH_3), 2890 (CH_2), 2226, 2223 (2CN), 1694, 1685 (2C=O), 1665 (C=N), 1643 (C=C). ^{1}H NMR (DMSO-d_6): δ = 2.25-2.28 (m, 4H, $2CH_2$), 2.31-2.35 (m, 4H, $2CH_2$), 2.94 (s, 3H, CH_3), 6.78 (s, 2H, thiazol CH_2), 7.28-7.35 (m, 5H, C_6H_5), 8.41, 8.79 (2s, 2H, 2NH, D_2O-exchangeable). MS (relative intensity) m/z: 489 (M^+, 31%). Analysis for $C_{24}H_{20}N_6O_2S_2$ Calcd: C, 58.95; H, 4.12; N, 17.26; S, 13.11 Found: C, 58.82; H, 4.06; N, 17.44; S, 13.34 %.

Compound **18:** Brown crystals from 1,4-dioxane , yield: 59.3 % (3.176 g); mp: 170-172 ºC. IR (KBr): υ/cm^{-1} = 3478-3324 (2NH), 3048 (CH-aromatic), 2987 (CH_3), 2890 (CH_2), 2221 (CN), 1693, 1688, 1680 (3C=O), 1670 (C=N), 1642 (C=C). ^{1}H NMR (DMSO-d_6): δ = 1.14 (t, 3H, CH_3), 2,24-2.27 (m, 4H, $2CH_2$), 2.34-2.37 (m, 4H, $2CH_2$), 2.90 (s, 3H, CH_3), 4.25 (q, 2H, CH_2), 6.45 (s, 2H, thiazol CH_2), 7.32-7.39 (m, 5H, C_6H_5), 8.51, 8.76 (2s, 2H, 2NH, D_2O-exchangeable). MS (relative intensity) m/z: 535 (M^+, 21%). Analysis for $C_{26}H_{25}N_5O_4S_2$ Calcd: C, 58.30; H, 4.66; N, 13.06; S, 11.94 Found: C, 58.03; H, 4.70; N, 13.24; S, 11.74 %.

2.1.6. General procedure for the synthesis of compounds:- 3-Methyl-4-(hydrazophenyl–4'–chlorophenyl- formamido-α-yl)-2-(thiazole-4'-one-2'-yl)- crotononitrile (19), 3-Methyl–4–[hydrazo–3'–cyano–4-(4',5',6',7'-tetrahydro- benzo[*b*] thiophene–4-chloro phenyl formamido–α-yl]–2-(thiazole–4'-one–2'–yl) crotononitrile (20) and 4-[Ethyl -2–hydrazo(4',5',6',7'-tetrahydrobenzo [*b*]- thiopheno-3-carboxylate) 4-chlorophenyl- formamido-α-yl]-3-methyl-2-(thiozole-4'-one-2'-yl) crotononitrile (21):

To a cold solution of compound **3** (3.3 g, 0.01 mol) in ethanol (50 ml) containing sodium acetate (0.82 g, 0.01 mol), either benzene diazonium chloride (0.9 g, 0.01 mol), or 1-cyano-2-diazo– 4,5,6,7–tetrahydrobenzo[*b*]thiophene (1.7 g, 0.01 mol), or ethyl 2-diazo–4,5,6,7–terahydrobenzo[*b*]thiophene carboxylate (2.25 g, 0.01 mol) was added respectively with continuous stirring. The reaction mixture was stirred at room temperature for 3hrs the formed solid product in each case was collected by filtration.

Compound **19:** Deep brown crystals from 1,4-dioxane , yield: 66.1 % (2.897 g); mp: 222-225 ºC. IR (KBr): υ/cm^{-1} = 3465-3340 (2NH), 3060 (CH-aromatic), 2980 (CH_3), 2877 (CH_2), 2224 (CN), 1691, 1683 (2C=O), 1660 (C=N), 1644 (C=C). ^{1}H NMR (DMSO-d_6): δ = 2.89 (s, 3H, CH_3), 6.83 (s, 2H, thiazol CH_2), 7.27-7.38 (m, 9H, C_6H_5, C_6H_4), 8.48, 8.82 (2s, 2H, 2NH, D_2O-exchangeable). MS (relative intensity) m/z: 438 (M^+, 17%). Analysis for $C_{21}H_{16}N_5O_2SCl$ Calcd: C, 57.55; H, 3.68; N, 16.04; S, 7.31 Found: C, 57.76; H, 3.97; N, 16.23; S, 7.12 %.

Compound **20:** Brown crystals from acetic acid, yield: 63 % (3.298 g); mp: 158-159 ºC. IR (KBr): υ/cm^{-1} = 3458-3347 (2NH), 3050 (CH-aromatic), 2993 (CH_3), 2866 (CH_2), 2225, 2222 (2CN), 1692, 1677 (2C=O), 1660 (C=N), 1645 (C=C). ^{1}H NMR (DMSO-d_6): δ = 2.12-2.19 (m, 4H, $2CH_2$), 2.25-2.38 (m, 4H, $2CH_2$), 2.88 (s, 3H, CH_3), 6.81 (s, 2H, thiazol CH_2), 7.34-7.45 (d.d, 4H, C_6H_4), 8.39, 8.68 (2s, 2H, 2NH, D_2O-exchangeable). MS (relative intensity) m/z: 523 (M^+, 27%). Analysis for $C_{24}H_{19}N_6O_2SCl$ Calcd: C, 55.07; H, 3.65; N, 16.12; S, 12.25 Found: C, 55.28; H, 3.91; N, 16.34; S, 12.09 %.

Compound **21:** Brown crystals from acetic acid, yield: 64.8 % (3.696 g); mp: 140-142 ºC. IR (KBr): υ/cm^{-1} = 3460-3330 (2NH), 3061 (CH-aromatic), 2984 (CH_3),

2870 (CH_2), 2220 (CN), 1705, 1691, 1684, 1680 (3C=O), 1656 (C=N), 1641 (C=C). ^{1}H NMR (DMSO-d_6): δ = 1.16 (t, 3H, CH_3), 1.89-1.95 (m, 4H, $2CH_2$), 2.11-2.22 (m, 4H, $2CH_2$), 2.95 (s, 3H, CH_3), 4.21 (q, 2H, CH_2), 6.73 (s, 2H, thiazol CH_2), 7.37-7.54 (m, 5H, C_6H_4), 8.33, 8.79 (2s, 2H, 2NH, D_2O-exchangeable). MS (relative intensity) m/z: 570 (M^+, 29%). Analysis for $C_{26}H_{24}N_5O_4S_2Cl$ Calcd: C, 54.74; H, 4.24; N, 12.33; S, 11.24 Found: C, 54.91; H, 4.37; N, 12.12; S, 11.03 %.

2.1.7. General procedure for the synthesis of compounds:- 3-Phenylformamido-α-yl-6-oxo-4-methyl-5-(thiazole-4'-one-2'-yl)-1-phenylpyridazine (28), 3-Phenylforma-mido-α-yl-6-oxo-4-methyl-5-(thiazole-4'-one-2'-yl)-1-(3'-cyano-4'-[4',5',6',7'-tetra-hydrobenzo[*b*]- thiopheno)]pyridazine (29), 1-(Ethyl 4'-(4, 5,6,7– tetrahydrobenzo [*b*] thiopheno) –3'– carboxylate–3– phenylformamido–α-yl–6–oxo–4-methyl–5-(thiazole–4'-one-2'-yl -pyridazine (30).
A suspension of each compound of **16–18** (0.01 mol) in sodium ethoxide solution (40 ml), was heated in a boiling water bath for 2hrs, and then left to cool. The solid product formed upon pouring onto ice/water mixture containing few drops of hydrochloric acid was collected by filtration.

Compound **28:** Yellowish brown crystals from dimethylformamide, yield: 69.1 % (2.796 g); mp: 216-218 ºC. IR (KBr): υ/cm^{-1} = 3360-3315 (NH), 3054 (CH-aromatic), 2978 (CH_3), 2885 (CH_2), 1705, 1695, 1682, (3C=O), 1656 (C=N), 1638 (C=C). ^{1}H NMR (DMSO-d_6): δ = 2.99 (s, 3H, CH_3), 6.82 (s, 2H, thiazol CH_2), 7.21-7.45 (m, 10H, $2C_6H_5$), 8.49 (s, 1H, NH, D_2O-exchangeable). MS (relative intensity) m/z: 404 (M^+, 29%). Analysis for $C_{21}H_{17}N_5O_2S$ Calcd: C, 62.32; H, 3.98; N, 13.90, S, 7.92 Found: C, 62.61; H, 4.16, N, 13.73, S, 7.65 %.

Compound **29:** Brown crystals from acetic acid, yield: 71.4 % (3.498 g); mp: 180-182 ºC. IR (KBr): υ/cm^{-1} = 3415-3384 (NH), 3053 (CH-aromatic), 2983 (CH_3), 2897 (CH_2), 2220 (CN), 1700, 1684 (2C=O), 1660 (C=N), 1648 (C=C). ^{1}H NMR (DMSO-d_6): δ = 2.05-2.14 (m, 4H, $2CH_2$), 2.26-2.37 (m, 4H, $2CH_2$), 2.88 (s, 3H, CH_3), 6.82 (s, 2H, thiazol CH_2), 7.37-7.48 (m, 5H, C_6H_5), 8.73 (s, 1H, NH, D_2O-exchangeable). MS (relative intensity) m/z: 489 (M^+, 22%). Analysis for $C_{24}H_{19}N_5O_3S_2$ Calcd: C, 58.84; H, 3.90; N, 14.35; S, 13.09 Found: C, 58.65; H, 4.08; N, 14.14; S, 13.32 %.

Compound **30:** Brown crystals from acetic acid, yield: 78.2 % (4.198 g); mp: 165-167 ºC. IR (KBr): υ/cm^{-1} = 3438-3369 (NH), 3056 (CH-aromatic), 2974 (CH_3), 2886 (CH_2), 1712, 1693, 1681, 1674 (4C=O), 1655 (C=N), 1646 (C=C). ^{1}H NMR (DMSO-d_6): δ = 1.21 (t, 3H, CH_3), 2,23-2.29 (m, 4H, $2CH_2$), 2.32-2.38 (m, 4H, $2CH_2$), 3.08 (s, 3H, CH_3), 4.22 (q, 2H, CH_2), 6.74 (s, 2H, thiazol CH_2), 7.36-7.44 (m, 5H, C_6H_5), 8.42 (s, 1H, NH, D_2O-exchangeable). MS (relative intensity) m/z: 536 (M^+, 17%). Analysis for $C_{26}H_{24}N_4O_5S_2$ Calcd: C, 58.16; H, 4.50; N, 10.48; S, 11.94 Found: C, 58.42; H, 4.71; N, 10.21; S, 11.76 %.

2.1.8. General procedure for the synthesis of compounds:-3-(4'-Chlorophenyl- formamido -α-yl)-6-oxo-4-methyl -5-(thiazole-4'-one-2'-yl)pyridazine (31), 1-[3'-Cyano-4'(4,5,6,7-tetra-hydrobenzo[*b*]thiopheno)] -3-(4'-chloro phenylformamido-α-yl)-4-methyl-6-oxo-5-(thiazole-4'-one-2'-yl)-pyridazine (32) and 1-[Ethyl 4-(4',5',6',7'- tetrahydro-benzo[*b*] thiopheno)-3-carboxylate]-3(4'-chlorophenyl-formamido-α-yl)-4-methyl-6-oxo-5-(thiazol -4'-one-2'– yl)pyridazine (33).
A suspension of each compound of **19–21** (0.01 mol) in sodium ethoxide solution (40 ml), was heated in a boiling water bath for 3hrs, and then left to cool. The solid product formed upon pouring onto ice/water mixture containing few drops of hydrochloric acid was collected by filtration.

Compound **31:** Brown crystals from dimethylformamide, yield: 75.1 % (3.298 g); mp: 266-268 ºC. IR (KBr): υ/cm^{-1} = 3463-3398 (NH), 3064 (CH-aromatic), 2975 (CH_3), 2863 (CH_2), 1703, 1688, 1677 (3C=O), 1655 (C=N), 1641 (C=C). ^{1}H NMR (DMSO-d_6): δ = 2.98 (s, 3H, CH_3), 6.78 (s, 2H, thiazol CH_2), 7.35-7.42 (m, 9H, C_6H_5, C_6H_4), 8.81 (s, 1H, NH, D_2O-exchangeable). MS (relative intensity) m/z: 439 (M^+, 14%). Analysis for $C_{21}H_{15}N_4O_3SCl$ Calcd: C, 57.43; H, 3.44; N, 12.81; S, 7.30 Found: C, 57.64; H, 3.22; N, 12.62; S, 7.13 %.

Compound **32:** Brown crystals from acetic acid, yield: 66.3 % (3.477 g); mp: 221-223 ºC. IR (KBr): υ/cm^{-1} = 3455-3390 (NH), 3056 (CH-aromatic), 2990 (CH_3), 2863 (CH_2), 2225 (CN), 1698, 1681, 1673 (3C=O), 1654 (C=N), 1648 (C=C). ^{1}H NMR (DMSO-d_6): δ = 1.86-1.97 (m, 4H, $2CH_2$), 2.08-2.15 (m, 4H, $2CH_2$), 2.77 (s, 3H, CH_3), 6.89 (s, 2H, thiazol CH_2), 7.29-7.41 (d.d, 4H, C_6H_4), 8.91, (s, 1H, NH, D_2O-exchangeable). MS (relative intensity) m/z: 524 (M^+, 33%). Analysis for $C_{24}H_{18}N_5O_3S_2Cl$ Calcd: C, 54.97; H, 3.46; N, 13.41; S, 12.22 Found: C, 55.08; H, 3.64; N, 13.34; S, 12.42 %.

Compound **33:** Brown crystals from dimethyformamide, yield: 64.8 % (3.702 g); mp: 232-235 ºC. IR (KBr): υ/cm^{-1} = 3451-3386 (NH), 3046 (CH-aromatic), 2994 (CH_3), 2883 (CH_2), 1710, 1693, 1685, 1678 (4C=O), 1660 (C=N), 1643 (C=C). ^{1}H NMR (DMSO-d_6): δ = 1.19 (t, 3H, CH_3), 1.84-1.93 (m, 4H, $2CH_2$), 2.11-2.19 (m, 4H, $2CH_2$), 2.83 (s, 3H, CH_3), 4.31 (q, 2H, CH_2), 6.89 (s, 2H, thiazol CH_2), 7.31-7.52 (d.d, 4H, C_6H_4), 8.95 (s, 1H, NH, D_2O-exchangeable). MS (relative intensity) m/z: 571 (M^+, 16%). Analysis for $C_{26}H_{23}N_4O_5S_2Cl$ Calcd: C, 54.65; H, 4.05; N, 9.84; S, 11.22 Found: C, 54.84; H, 4.31; N, 9.76; S, 11.01 %.

3. RESULTS AND DISCUSSION:

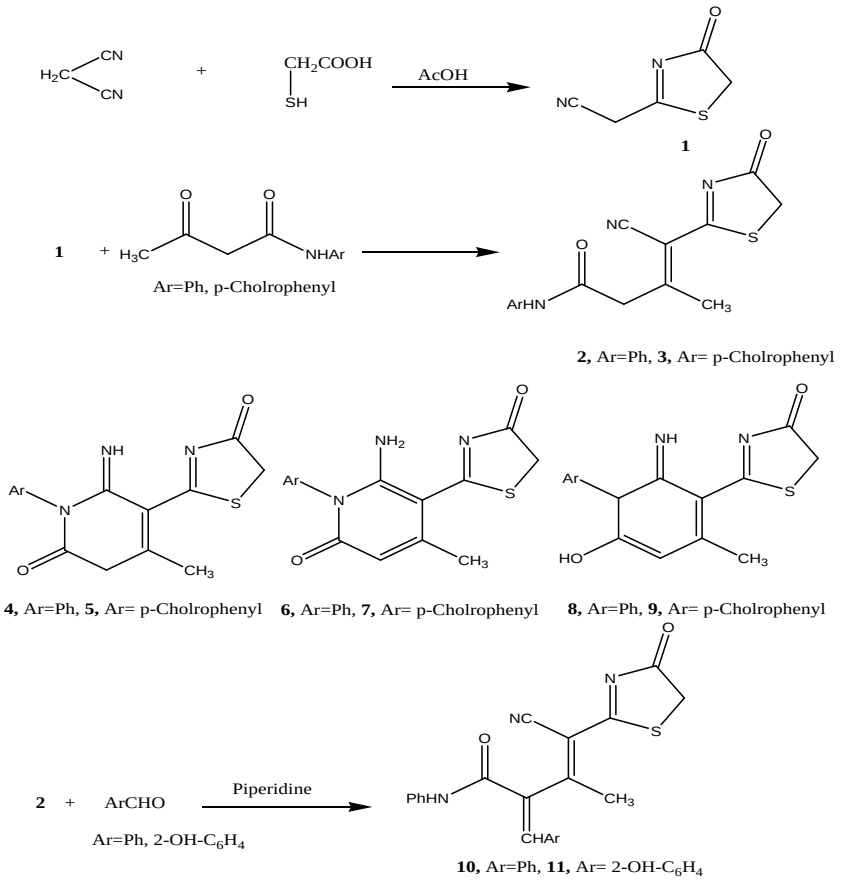
2-Cyanomethylenothiazol-4-one **(1)** obtained via the reaction of malononitrile with thioglycolic acid in presence of glacial acetic acid[13] compound **1** containing two methylene groups capable for many chemical transformations. It has been reported that when it coupled with either acetoacetanilide and p-chloroacetoacetanilide in the presence of ammonium acetate at 140 ^{o}C gave a single product with molecular formula $C_{15}H_{13}N_3SO_2$.

Four different pairs isomeric structures were considered **2–9**, structures **4**, **5**, **6**, **7** and **8**, **9** were ruled out on the basis of IR spectrum of the reaction product which showed the presence of one CN group stretching at ν^- 2222 cm^{-1} and absence of any NH_2 group stretching which might be expected to appear if structure **6**, **7** is considered and one OH group stretching if structure **8**, **9** is considered. Further the confirmations for structures **2**, **3** are obtained through studying their reactivity towards chemical reagents. Thus, compound **2** (as an example) reacts with aryl aldehydes namely benzaldehyde or salicyladehyde to give the arylidene derivatives **10** and **11**. **(Scheme.1)**

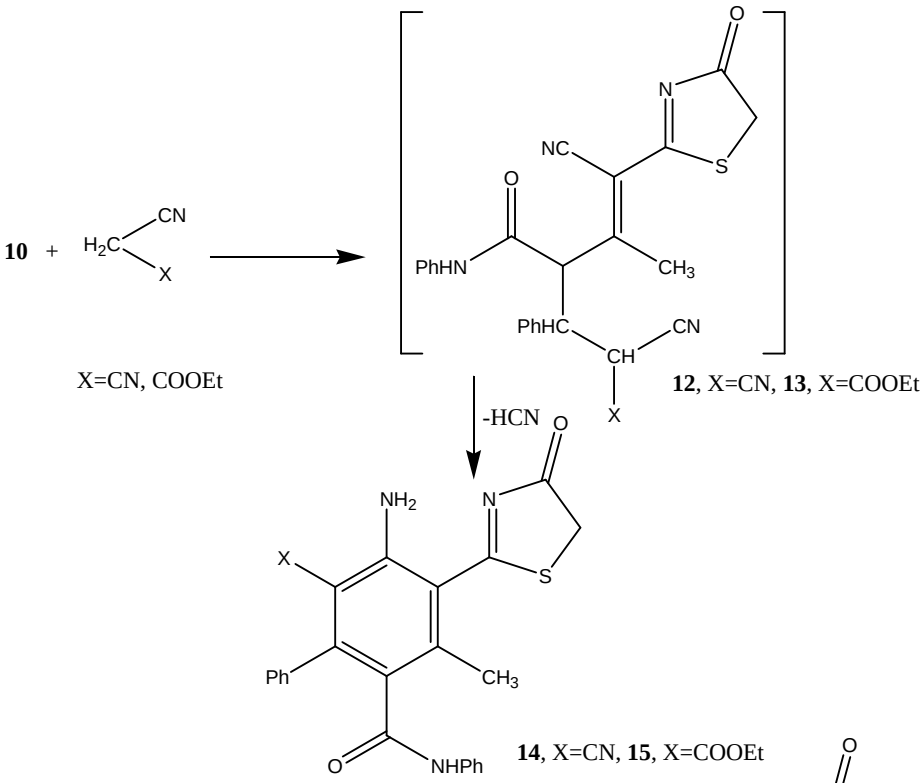
The reaction of **10** with either malononitrile or ethyl cyanoacetate gave the polyfunctionally substituted benzene derivatives **14** and **15** respectively through the intermediate formation of **12**, **13** followed by cyclization and HCN elimination.

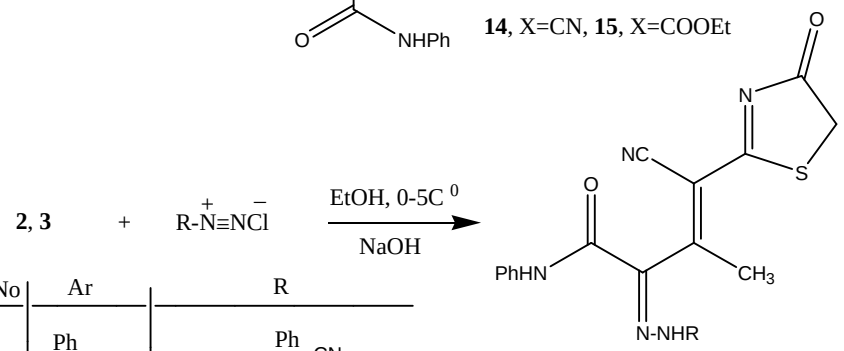
The reactivity of either **2** or **3** towards aryl and heterocyclic diazonium salts was studied to form hydrazones capable for cyclization. Thus, the reaction of either compound **2** or **3** with benzenediazonium chloride or 2-diazo-4, 5, 6, 7-tetrahydrobenzo[*b*]thiophene derivatives gave the corresponding hydrazone derivatives **16-21**. The structures of the latter products were based on analytical and spectral data. **(Scheme.2)**

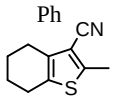
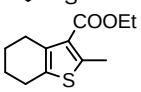
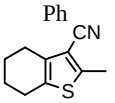
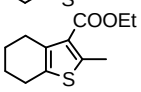
Compounds **16-21** underwent ready cyclization when heated in sodium ethoxide solution to give the corresponding 6-oxo-pyridazine derivatives **28-33**. Formation of the latter products is assumed to take place via the intermediate formation of the 6-iminopyridazine derivatives **22-27** followed by hydrolysis of the imino group into the oxo group through ammonia liberation[14]. The IR and ^{1}H NMR spectra of the products are in-consistent with the assigned structures. **(Scheme.3)**



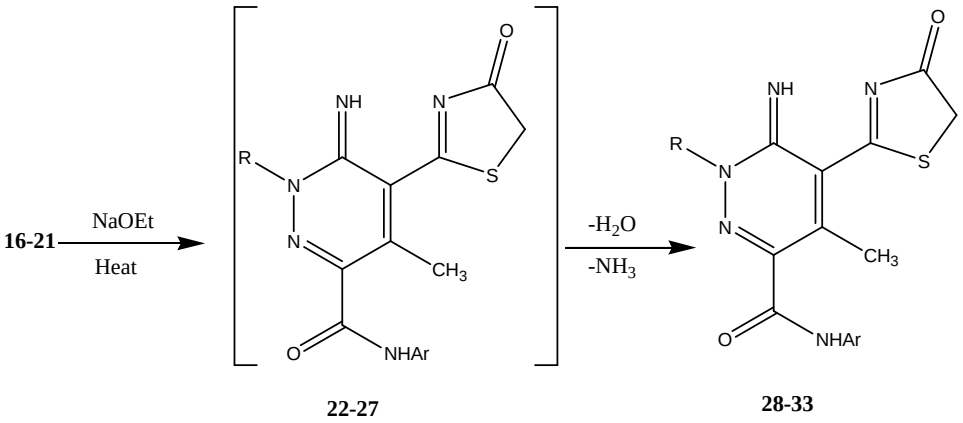
Scheme 1. Synthesis of compounds **1--3, 10, 11**

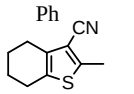
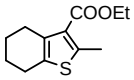
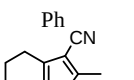
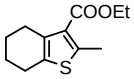




Comp.No	Ar	R
16	Ph	
17	Ph	
18	Ph	
19	4-Cl-C_6H_4	
20	4-Cl-C_6H_4	
21	4-Cl-C_6H_4	

Scheme 2. Synthesis of compounds **14-21**.



Comp.No	Ar	R
28	Ph	
29	Ph	
30	Ph	
31	4-Cl-C_6H_4	
32	4-Cl-C_6H_4	
33	4-Cl-C_6H_4	

Scheme 3. Synthesis of compounds **28-33**.

3.1 Antitumor activity tests:

Reagents: Fetal bovine serum (FBS) and L-glutamine from Gibco Invitrogen Co. (Scotland, UK). RPMI-1640 medium from Cambrex (New Jersey, USA). Dimethyl sulfoxide (DMSO), doxorubicin, penicillin, streptomycin and sulforhodamine B (SRB) were from Sigma Chemical Co. (Saint Louis, USA).

Cell cultures: Three human tumor cell lines, MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer), and SF-268 (CNS cancer) were used. MCF-7 was obtained from the European Collection of Cell Cultures (ECACC, Salisbury, UK) and NCI-H460, SF-268 and normal fibroblast cells (WI 38) were kindly provided by the National Cancer Institute (NCI, Cairo, Egypt). They grow as monolayer and routinely maintained in RPMI-1640 medium supplemented with 5% heat inactivated FBS, 2 mM glutamine and antibiotics (penicillin 100 U/mL, streptomycin 100 µg/mL), at 37^{o}C in a humidified atmosphere containing 5% CO_2. Exponentially growing cells were obtained by plating 1.5 X 10^5 cells/mL for MCF-7 and SF-268 and 0.75 X 10^4 cells/mL for NCI-H460, followed by 24 h of incubation. The effect of the vehicle solvent (DMSO) on the growth of these cell lines was evaluated in all the experiments by exposing untreated control cells to the maximum concentration (0.5%) of DMSO used in each assay.

Tumor cell growth assay: The effects of **2–33** on the in vitro growth of human tumor cell lines were evaluated according to the procedure adopted by the National Cancer Institute (NCI, USA) in the '*In vitro* Anticancer Drug Discovery Screen' that uses the protein-binding dye sulforhodamine B to assess cell growth. Briefly, exponentially, cells growing in 96-wellplates were then exposed for 48 h to five serial concentrations of each compound[15], starting from a maximum concentration of 150 µM. Following this exposure period adherent cells were fixed, washed, and stained. The bound stain was solubilized and the absorbance was measured at 492 nm in a plate reader (Bio-Tek Instruments Inc., Power wave XS, Wincoski, USA). For each test compound and cell line, a dose–response curve was obtained and the growth inhibition of 50% (GI_{50}), corresponding to the concentration of the compounds that inhibited 50% of the net cell growth, was calculated as described elsewhere[16]. Doxorubicin was used as a positive control and it was tested in the same manner.

Table 1. Effect of compounds **2-33** on the growth of three human tumor cell lines

Compound	GI_{50} («mol L^{-1})			
	MCF-7	**NCI-H460**	**SF-268**	**WI38**
2	21.7 ± 6.9	19.9 ± 4.7	22.5 ± 5.3	na
3	21.2 ± 5.6	18.0 ± 3.9	17.7 ± 4.7	na
10	20.6 ± 3.8	17.1 ± 2.9	21.3 ±2.5	75.2±13.5
11	41.7 ± 7.9	32.2 ± 6.8	24 ± 7.8	na
14	39.2 ± 6.8	37.3 ± 6.4	35.9 ± 6.9	>100
15	46.2± 7.8	33.1 ± 8.4	28.4 ± 5.8	>100
16	38.0 ± 7.5	33.0 ± 8.9	32.5 ± 7.3	>100
17	11.8 ± 1.6	10.5 ± 2.2	11.4 ± 2.1	58.4 ± 9.6
18	22.0 ± 7.7	20.6 ± 6.3	22.4 ± 8.2	885.1 ± 12.3
19	44.4 ± 6.4	42.1 ± 8.7	38.3 ± 6.3	na
20	2.6 ± 0.07	2.4± 0.06	2.1 ± 0.08	53.7± 9.3
21	12.3 ±1.2	11.6 ± 0.9	9.7 ± 0.7	69.2 ± 11.5
28	21.3 ± 2.4	14.9 ± 2.8	12.6 ± 3.6	78.9 ± 12.9
29	13.1± 1.7	14.5 ±1.9	12.3 ± 2.2	80.2 ± 14.6
30	13.0 ± 0.9	12.0 ± 1.4	12.5 ± 1.8	77.6 ± 13.5
31	31.5 ± 7.5	29.2 ± 8.2	27.0 ± 6.1	83.8 ± 14.8
32	1.4 ± 0.03	1.6 ± 0.04	0.09 ± 0.02	42.7± 8.8
33	24.0 ± 1.8	24.0 ± 0.8	10.5 ± 1.1	49.7 ± 10.1
Doxorubicin	0.04 ±0.008	0.09±0.008	0.09±0.007	>100

Results are given in concentrations that were able to cause 50 % of cell growth inhibition (GI_{50}) after a continuous exposure of 48 h and show means ± SEM of three-independent experiments performed in duplicate.

The inhibitory effect of compounds **2-33** was evaluated on the *in vitro* growth of three human tumor cell lines representing different tumor types, namely, breast adenocarcinoma (MCF-7), non-small cell lung cancer (NCI-H460) and CNS cancer (SF-268) after a continuous exposure for 48h. All of the tested compounds were able to inhibit the growth of the tested human tumor cell lines in a dose-dependent manner (data not shown). The results indicated through **Table 1** represented that hydrazothieno-thiazol derivatives **20** and thiazolopyridazine derivatives **32** showed the highest inhibitory effect against all the three tumor cell lines corresponding to reference standard material (Doxorubicin), also compounds **17, 21, 29** and **30** showed the highest inhibitory effect against all the three tumor cell lines corresponding to the remaining synthesized compounds. On the other hand for compounds **2, 3, 10, 18, 28** and **33** showed moderate inhibitory effects against the three cancer cell lines. The rest of the compounds **11, 14, 15, 16, 19** and **31** showed a low growth inhibitory effect.

Comparing phenylformamido thiazole derivatives derivatives **2** and **3** it was found that the two compounds may be nearly the same effect regarding the presence of p-chlorophenyl group in compound **3** instead of phenyl group in compound **2,** on the other hand the dieno-thiazole derivatives **10** and **11** it was found that compound **10** with phenyl group it has higher effect than that of compound **11** which it is containing p-chlorophenyl group, comparing anilinothiazole derivatives derivatives **14** and **15** it was clear that the two compounds may be nearly the same effect although they are containing two different groups, compound **14** containing CN group and compound **15** containing COOEt.

Also comparing the hydrazothieno-thiazol derivatives **16-21** it was found that compound **20** with the Ar = 4-Cl-C_6H_4, R= 3-Cyanotetrahydrobenzo[*b*]thieno group showed the highest inhibitory effect among the six compounds and then compound **17** which it has Ar = Ph instead of 4-Cl-C_6H_4 as in compound **20** has highest inhibitory effect against all the three tumor cell lines corresponding to the remaining synthesized group. Finally comparing compounds **28-33** it is obvious that compound **32** with the Ar = 4-Cl-C_6H_4, R= 3-Cyanotetrahydrobenzo[*b*]thieno group showed the highest inhibitory effect among the six compounds and then compound **30** has highest inhibitory effect against all the three tumor cell lines corresponding to the remaining synthesized group which it has Ar = Ph instead of 4-Cl-C_6H_4 and R= 3- Ethylcaboxylate-tetrahydrobenzo[*b*]thieno group instead of 3-Cyanotetrahydrobenzo[*b*]thieno group as in compound **32.**

4. CONCLUSIONS:

In this article the newly synthesized compounds thiazol-4-one derivatives were investigated to detect their antitumor activity against three different cell lines corresponding to reference standard "doxorubicin". Among the newly synthesized products hydrazothieno-thiazol derivatives **20** and thiazolopyridazine derivatives **32** showed the highest inhibitory effect against all the three tumor cell lines, also compounds **17, 21, 29** and **30** showed the highest inhibitory effect against all the three tumor cell lines corresponding to the remaining synthesized compounds.

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