Heterocycl. Commun., Vol. 17(3-4), pp. 155–160, 2011 • Copyright © by Walter de Gruyter • Berlin • Boston. DOI 10.1515/HC.2011.033

Utility of 2-(4,5-dihydro-4-oxothiazol-2-yl)acetonitrile in the synthesis of fused heterocyclic derivatives with anti-tumor activities

Wagnat W. Wardakhan*, Diaa A. Ibrahim and Mayssoune Y. Zaki

National Organization for Drug Control and Research, P.O. Box 29, Cairo, A.R., Egypt

*Corresponding author
e-mail: wagnatward@hotmail.com

Abstract

2-(4,5-Dihydro-4-oxothiazol-2-yl)acetonitrile (**1**) was reacted with acetophenone (**2**) to give 2-(4,5-dihydro-4-oxothiazol-2-yl)-3-phenylbut-2-enenitrile (**3**). The reactivity of product **3** towards aromatic aldehydes **4a–d**, cyanomethylene reagents **6a,b**, aryl diazonium salts **10a–d**, phenylisothiocyanate and elemental sulfur was studied. Some of the newly synthesized compounds were used to synthesize fused derivatives. The anti-tumor evaluation of selected compounds against three cancer cells namely breast adenocarcinoma (MCF-7), non-small cell lung cancer (NCI-H460) and CNS cancer (SF-268) showed that some of them have a high inhibitory effect and compound **19** was found to be more active than the standard on NCI-H460 and SF-268.

Keywords: anti-tumor; pyrazole; pyridazine; thiazole.

Introduction

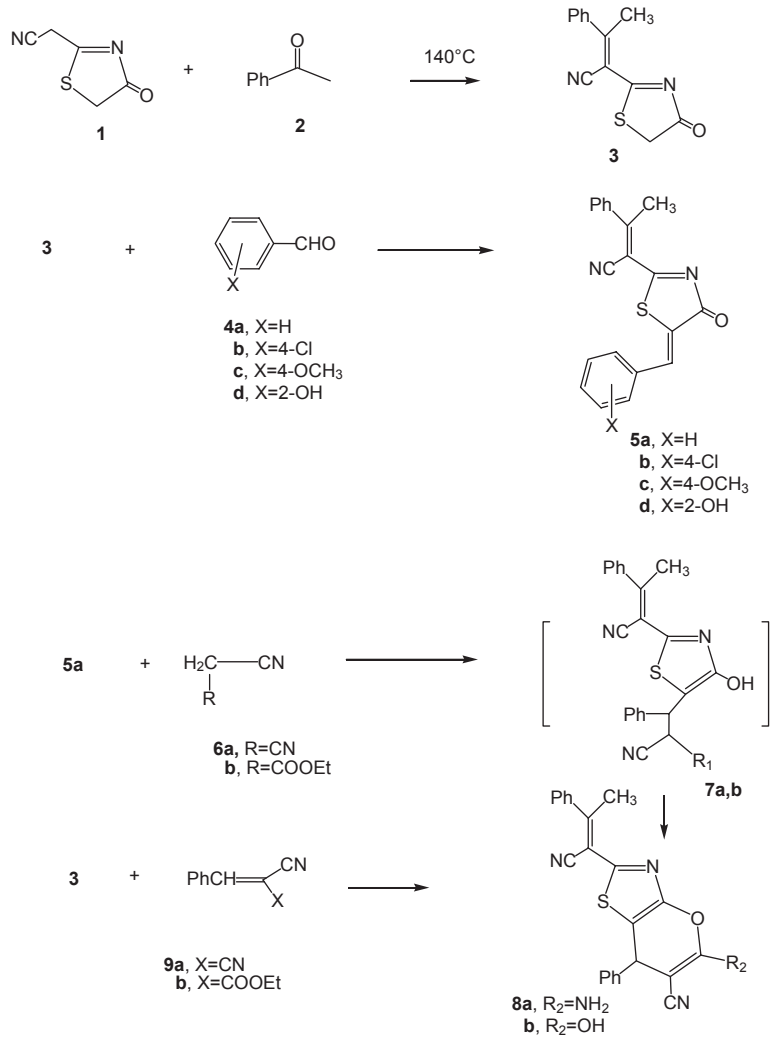
Thiazoles play a prominent role in nature. For example, the thiazolium ring present in vitamin B1 serves as an electron sink and its coenzyme form is important for the decarboxylation of α-keto acids (Sondhi et al., 2005). Various pesticides possessing a thiazole nucleus are well known in agriculture. Large numbers of thiazole derivatives have emerged as active pharmaceutical ingredients in several drugs for their potential anti-inflammatory (Yang et al., 2005), anti-tumor (Turan-Zitouni et al., 2005), anti-hyperlipidemic (Li et al., 2005), anti-hypertensive (Narayana et al., 2004), and several other biological properties (Vicini et al., 2003). In addition, thiazoles are also synthetic intermediates and common substructures in numerous biologically active compounds (Bhattacharya et al., 2005; Nettekoven et al., 2005; Bondock et al., 2007; Yu et al., 2007; Al-Sarawy et al., 2008; Peng-Cheng et al., 2009; Tomasz and Boduszek, 2010; Mansour et al., 2011).

Results and discussion

Chemistry

In this work, we describe the uses of 2-(4,5-dihydro-4-oxothiazol-2-yl)acetonitrile (**1**), which was obtained earlier (Elnagdi et al., 1979, 1981) in heterocyclic synthesis (Scheme 1). Compound **1** undergoes a reaction with acetophenone (**2**) in the presence of ammonium acetate at 140°C to give the Knoevenagel condensation product **3**. The structure of compound **3** is based on analytical and spectral data. Thus, the ^{1}H NMR spectrum shows a singlet at δ 2.89 corresponding to the methyl group, a singlet at δ 5.65 corresponding to the methylene group, and a multiplet at δ 7.30–7.39 for phenyl protons.

The reaction of compound **3** with aromatic aldehydes gives arylidene derivatives. Thus, compound **3** undergoes a reaction with either benzaldehyde (**4a**), 4-chlorobenzaldehyde (**4b**), 4-methoxybenzaldehyde (**4c**) or salicylaldehyde (**4d**), to give in each case a single product for which the structures **5a–d** were assigned. These structures are based on analytical and spectral data (see Experimental section). The reaction of compound **5a** with either malononitrile (**6a**) or ethyl cyanoacetate (**6b**) gives the substituted pyrano[2,3-*d*]thiazole derivatives **8a,b**. The reaction took place via the intermediate formation of **7a,b** (Scheme 1). Structures of compounds **8a** and **8b** were established on the basis of analytical and spectral data (see Experimental section). Further confirmation for structures **8a,b** was obtained through the synthesis of these compounds using an independent synthetic route. Thus, the reaction of compound **3** with either α-cyanocinnamonitrile **(9a)** or ethyl α-cyanocinnamate **(9b)** (Scheme 1) in 1,4-dioxane containing a catalytic amount of triethylamine gave the same products **8a** and **8b**, respectively. The reaction of compound **3** with benzenediazonium chloride **(10a)**, 4-chlorobenzenediazonium chloride **(10b)**, 4-methybenzenediazonium chloride **(10c)** or 4-methoxybenzenediazonium chloride (**10d**) gave the corresponding arylhydrazone derivatives **11a–d**. The analytical and spectral data of the latter products are consistent with the proposed structures (see Experimental section). Compounds **11a–d** undergo cyclization when heated under reflux in ethanolic/NaOH solution to give the 4-thiazol-2-yl-pyridazine derivatives **12a–d**. The reaction of compound **5** with phenylisothiocyanate (**13**) in 1,4-dioxane containing triethylamine gave the 3-thiazol-2-yl-pyridine derivative **14**, the structure of which is based on analytical and spectral data. Thus, the ^{1}H NMR spectrum shows a singlet at δ 4.89 corresponding to pyridine CH_2, a singlet at δ 5.87 for the thiazole



Scheme 1 Synthesis of compounds **3–8a,b.**

CH_2 and a multiplet at δ 7.26–7.39 corresponding to the phenyl protons. The reaction of compound **3** with malononitrile (**6a**) or ethyl cyanoacetate (**6b**) gave the respective substituted pyrano[2,3-*d*]thiazole derivatives **15a** and **15b**. The analytical and spectral data of these products are in agreement with the proposed structures. The reaction of compound **3** with elemental sulfur in 1,4-dioxane containing triethylamine gave thiophene derivative **16** (Scheme 2).

Antitumor activity

The effect of the newly synthesized products **3–16** 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 48 h. The results are summarized in Table 1.

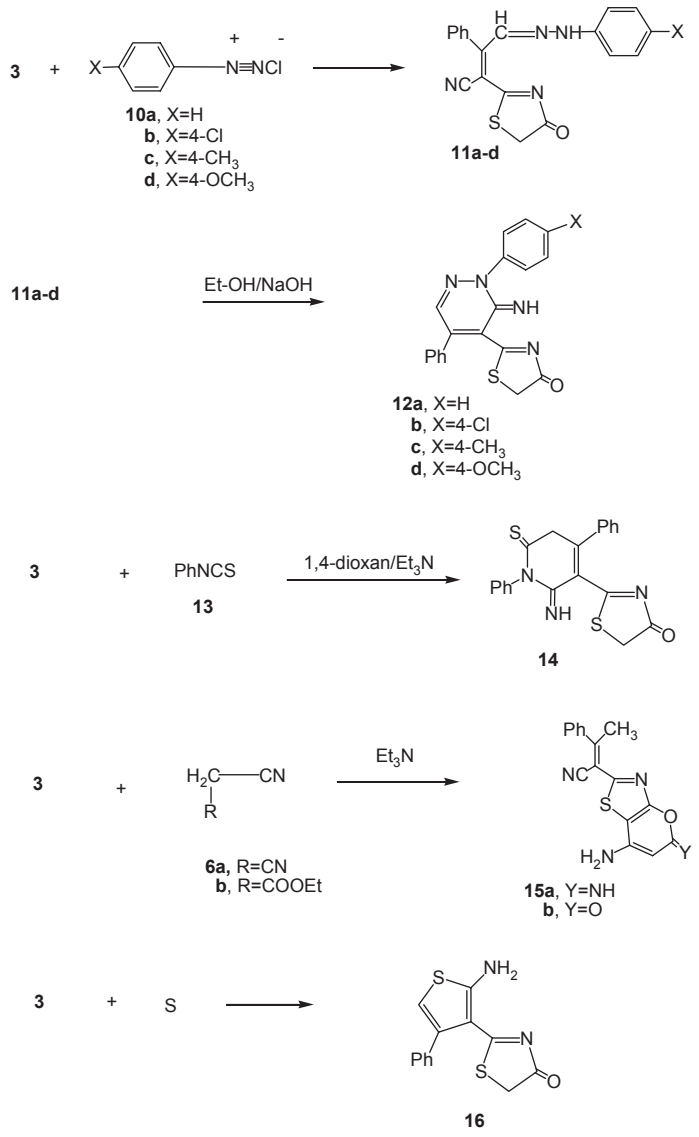
All compounds inhibit the growth of the human tumor cell lines in a dose-dependent manner (data not shown). The 2-pyridylthiazole derivative **14** and the 2-thiophenylthiazole derivatives **16** show the best results. By contrast, compounds **5a**, **8a**, **11b**, **11d** and **12a** show a moderate growth inhibitory effect.

Experimental

Synthesis

Melting points are uncorrected. IR spectra were recorded in KBr discs on a Pye Unicam SP-1000 Spectrophotometer. ^{1}H NMR spectra were measured on a Varian EM-390-200 MHz and Bruker AVANCE DRX-500-300 MHz in DMSO-d_6 as solvent using TMS as internal standard. ^{13}C NMR spectra were measured on the Bruker AVANCE DRX-500 instrument. Analytical data were obtained from the Micro Analytical Data Unit at Cairo University, Giza, Egypt.

2-(4,5-Dihydro-4-oxothiazol-2-yl)-3-phenylbut-2-enenitrile (3) A mixture of compound **1** (0.01 mol, 1.87 g), acetophenone (1.20 g, 0.01 mol) and ammonium acetate (2.0 g) was heated to 140°C in an oil bath for 1 h, then left to cool. The solid product formed after treatment with hot ethanol was collected by filtration



Scheme 2 Synthesis of compounds **11a–d–16**.

and crystallized from ethanol: yellow crystals; yield 1.84 g (76%); m.p. 145–148°C; IR: υ 3367–3141, 2905, 1705, 1672, 1638 cm^{-1}; MS: *m/z* 242 (100), 127 (40), 101 (14.2); ^{1}H NMR: δ 2.89 (s, 3H), 5.65 (s, 2H), 7.30–7.39 (m, 5H); ^{13}C NMR: δ 20.4, 59.0, 116.9, 120.7, 125.8, 126.9, 128.3, 142.4, 146.4, 166.0, 168.1. Analysis: calcd. for $C_{13}H_{10}N_2OS$ (242.30): C, 64.44; H, 4.16; N, 11.56; S, 13.23. Found: C, 64.32; H, 4.37; N, 11.71; S, 13.40.

Compounds 5a-d

A solution of compound **3** (0.01 mol, 2.89 g) and piperidine (0.5 ml) in ethanol (30 ml) was treated with benzaldehyde (0.01 mol, 1.06 ml), *p*-chlorobenzaldehyde (1.40 g, 0.01 mol), *p*-methoxybenzaldehyde (1.36 g, 0.01 mol) or salicylaldehyde (1.22 g, 0.01 mol). The mixture was heated under reflux for 3 h, and then poured into an ice/water mixture containing a few drops of hydrochloric acid. The solid product formed was collected by filtration.

2-(5-Benzylidene-4,5-dihydro-4-oxothiazol-2-yl)-3-phenylbut-2-enenitrile (5a) Yellow crystals from ethanol; yield 2.01 g (61%); m.p. 158–161°C; IR: υ 3060, 2976–2869, 1693, 1665, 1638 cm^{-1}; ^{1}H NMR: δ 2.86 (s, 3H), 7.26–7.38 (m, 11H); ^{13}C NMR: δ 19.6, 89.1, 97.5, 116.9, 120.6, 122.8, 125.3, 126.8, 127.0, 135.6, 137.8, 140.4, 143, 166.6, 167.9. Analysis: calcd. for $C_{20}H_{14}N_2OS$ (330.08): C, 72.70; H, 4.27; N, 8.48; S, 9.70. Found: C, 72.95; H, 4.37; N, 8.57; S, 9.33.

2-(5-Benzylidene-4,5-dihydro-4-oxothiazol-2-yl)-3-(4-chlorophenyl)but-2-enenitrile (5b) Yellow crystals from ethanol; yield 2.44 g (67%); m.p. 221–224°C; IR: υ 3055, 2949, 1687, 1655, 1598 cm^{-1}; ^{1}H NMR: δ 2.83 (s, 3H,), 7.23–7.42 (m, 10H); ^{13}C NMR: δ 19.5, 116.8, 120.6, 122.6, 123.4, 125.4, 126.4, 127.5, 134.8, 144.6, 156.5, 157.2, 159.9, 166.8, 169.4. Analysis: calcd. for $C_{20}H_{13}ClN_2OS$ (364.85): C, 65.84; H, 3.59; N, 3.59; S, 8.79. Found: C, 66.15; H, 4.54; N, 3.73; S, 8.82.

Table 1 Effect of selected compounds on the growth of three human tumor cell lines.

Compound	GI_{50} (μmol/l)		
	MCF-7	NCI-H460	SF-268
3	20±0.8	14.3±1.6	20.3±1.5
5a	16±0.4	20.3±0.8	31±0.6
5d	35.4±10.2	24.1±0.8	18.9±6.8
8a	10.8±2.4	12.5±2.4	18.7±1.8
11a	50.1±0.7	23.2±4.8	18.4±1.8
11b	20.0±0.2	22.6±1.4	38.2±0.9
11c	33.0±1.8	41.0±0.8	22.5±2.5
11d	20.0±0.6	20.0±0.4	30.5±8.0
12a	11.9±0.6	14.1±0.6	20.3±0.5
12d	35.4±10.2	24.1±0.8	18.9±6.8
14	0.8±0.06	0.5±0.03	0.7±0.01
15b	32.4±8.2	20.1±0.4	16.6±3.8
16	0.06±0.004	0.05±0.002	0.07±0.01
Doxorubicin	0.04±0.008	0.09±0.008	0.09±0.007
Doxorubicin	0.04±0.008	0.09±0.008	0.09±0.007

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. Compounds **5b**, **5c**, **8b**, **12b**, **12c** and **15a** gave low inhibitory effect <30 μм/l against the three cell lines.

2-(5-Benzylidene-4,5-dihydro-4-oxothiazol-2-yl)-3-(4-methoxyphenyl)but-2-enenitrile (5c) Yellow crystals from ethanol; yield 3.09 g (86%); m.p. 130–133°C; IR: υ 3059, 2960, 1689, 1663, 1590 cm^{-1}; ^{1}H NMR: δ 2.99, 3.34 (2s, 6H), 7.28–7.42 (m, 10H); ^{13}C NMR: δ 20.6, 55.0, 89.8, 114.9, 116.8, 118.5, 120.8, 120.8, 122.7, 125.3, 126.7, 128.4, 130.9, 164.6, 166.4. Analysis: calcd. for $C_{21}H_{16}N_2O_2S$ (360.43): C, 69.98; H, 4.47; N, 7.77; S, 8.88. Found: C, 69.81; H, 4.43; N, 7.84; S, 8.62.

2-(5-Benzylidene-4,5-dihydro-4-oxothiazol-2-yl)-3-(2-hydroxyphenyl)but-2-enenitrile (5d) Yellow crystals from ethanol; yield 1.85 g (53.6%); m.p. 244–246°C; IR: υ 3560–3243, 3060, 2930, 1689, 1666, 1597 cm^{-1}; ^{1}H NMR: δ 2.84 (s, 3H), 7.25–7.39 (m, 10H), 10.22 (s, 1H); ^{13}C NMR: δ 19.9, 55.8, 89.9, 114.5, 226.5, 118.4, 120.8, 120.8, 122.7, 126.5, 129.8, 130.9, 133.6, 138.6, 140.7, 166.5, 168.7. Analysis: calcd. for $C_{20}H_{14}N_2O_2S$ (346.08): C, 69.35; H, 4.07; N, 8.09; S, 9.26. Found: C, 69.53; H, 4.11; N, 7.99; S, 9.32.

Compounds 8a,b

Method A To a solution of compound **5a** (0.01 mol, 3.96 g) in ethanol (30 ml) containing triethylamine (0.5 ml), malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.13 g, 0.01 mol) was added, and the mixture was heated under reflux for 4 h. The solid product formed upon pouring onto an ice/water mixture was collected by filtration.

Method B To a solution of compound **3** (0.01 mol, 2.42 g) in ethanol (30 ml) containing triethylamine (0.5 ml), α-cyanocinnamonitrile (1.70 g, 0.01 mol) or ethyl α-cyanocinnamate (1.97 g, 0.01 mol) was added. The mixture was heated under reflux for 5 h, then poured onto an ice/water mixture containing a few drops of hydrochloric acid, and the formed solid product was collected by filtration.

5-Amino1-cyano-2-phenylprop-1-enyl)-7-phenyl-7*H*-pyrano[2,3-*d*]thiazole-6-carbonitrile (8a) Orange crystals from ethanol; yield 2.22 g (56%); m.p. 184–186°C; IR: υ 3464–3323, 3052, 2936, 2220, 1638, 1564 cm^{-1}; ^{1}H NMR: δ 2.84 (s, 3H), 5.52 (s, 2H), 7.31–7.52 (m, 11H); ^{13}C NMR: δ 19.4, 58.2, 84.3, 116.6, 118.6, 120.1, 119.0, 120.2, 121.9, 122.4, 123.6, 123.9, 124.9, 124.2, 126.3, 128.8, 129.6, 131.5, 136.9, 140.2, 144.6, 166.8, 168.6. Analysis: calcd. for $C_{23}H_{16}N_4OS$ (396.46): C, 69.68; H, 4.07; N, 14.13; S, 8.09. Found: C, 69.94; H, 4.27; N, 14.05; S, 8.16.

1-Cyano-2-phenylprop-1-enyl)-5-hydroxy-7-phenyl-7*H*-pyrano[2,3-*d*]thiazole-6-carbonitrile (8b) Yellow crystals from ethanol; yield 2.22 g (56%); m.p. 160–163°C; IR: υ 3586–3142, 3055, 2967, 2224, 1663, 1637 cm^{-1}; ^{1}H NMR: δ 2.85 (s, 3H), 7.27–7.44 (m, 11H), 9.01 (s, 1H); ^{13}C NMR: δ 19.9, 64.3, 88.4, 11.8, 120.7, 116.9, 119.8, 120.6, 121.8, 122.5, 123.6, 124.8, 125.1, 125.5, 126.9, 127.8, 129.0, 130.6, 131.8, 136.8, 140.3, 148.7, 166.0, 169.5. Analysis: calcd. for $C_{23}H_{15}N_3O_2S$ (397.45): C, 69.50; H, 3.80; N, 10.57; S, 8.07. Found: C, 69.34; H, 4.11; N, 10.82; S, 8.31.

Compounds 11a–d

To a cold mixture (0–5°C) of compound **3** (2.89 g, 0.01 mol g), acetic acid/ethanol (1:4, 50 ml) and an aqueous solution of sodium hydroxide (5 ml, 10%), benzenediazonium chloride (0.01 mol), 4-chlorobenzenediazonium chloride (0.01 mol), 4-methylbenzenediazonium chloride (0.01 mol) or 4-methoxybenzenediazonium chloride (0.01 mol) was added with stirring. The mixture was left at 0–5°C for 4 h and the formed solid product was collected by filtration.

4-(2-(4-Phenyl)-hydrazono)-2-(4,5-dihydro-4-oxothiazol-2-yl)-3-phenylbut-2-enenitrile (11a) Red crystals from ethanol; yield 2.90 g (84%); m.p. 132–136°C; IR: υ 3463–3320, 3054, 1687, 1657, 1638 cm^{-1}; ^{1}H NMR: δ 5.37 (s, 2H), 6.09 (s, 1H), 7.26–7.47 (m, 10H), 8.36 (s, 1H); ^{13}C NMR: δ 19.8, 44.6, 116.9, 112.3, 120.4, 119.9, 121.6, 122.9, 123.9, 124.0, 124.2, 125.3, 126.9, 128.0, 128.7, 134.9, 137.9, 140.0, 142.6, 164.3, 168.1, 169.8. Analysis: calcd. for $C_{19}H_{14}N_4OS$ (346.41): C, 65.88; H, 4.07; N, 16.17; S, 9.26. Found: C, 66.14; H, 4.16; N, 16.32; S, 9.24.

4-(2-(4-Chlorophenyl)-hydrazono)-2-(4,5-dihydro-4-oxothiazol-2-yl)-3-phenylbut-2-enenitrile (11b) Dark brown powder from ethanol; yield 2.66 g (70%); m.p. 230–233°C; IR: υ 3455–3338, 3055, 1689, 1658, 1638 cm^{-1}; ^{1}H NMR: δ 5.66 (s, 2H), 6.20 (s, 1H), 7.26–7.39 (m, 9H), 9.33 (s, 1H); ^{13}C NMR: δ 55.6, 116.5, 111.8, 120.8, 119.6, 120.8, 121.3, 123.3, 124.6, 124.9, 126.2, 128.1, 129.6, 137.3, 142.4, 148.7, 163.4, 166.8, 169.8. Analysis: calcd. for $C_{19}H_{13}ClN_4OS$ (380.85): C, 59.92; H, 3.44; N, 14.71; S, 8.42. Found: C, 59.85; H, 3.74; N, 14.95; S, 8.64.

4-(2-(4-Methylphenyl)-hydrazono)-2-(4,5-dihydro-4-oxothiazol-2-yl)-3-phenylbut-2-enenitrile (11c) Brown crystals form ethanol; yield 3.10 g (76%); m.p. 210–214°C; IR: υ 3448–3305, 3060, 2969, 2908, 1686, 1660, 1634 cm^{-1}; ^{1}H NMR: δ 3.21 (s, 3H, CH_3), 5.48 (s, 2H), 6.23 (s, 1H), 7.28–7.38 (m, 9H), 8.36 (s, 1H). Analysis: calcd. for $C_{20}H_{16}N_4OS$ (360.43): C, 66.65; H, 4.47; N, 15.54; S, 8.90. Found: C, 66.69; H, 4.75; N, 15.55; S, 8.78.

4-(2-(4-Methoxyphenyl)-hydrazono)-2-(4,5-dihydro-4-oxothiazol-2-yl)-3-phenylbut-2-enenitrile (11d) Brown crystals from ethanol; yield 3.85 g (89%); m.p. 148°C; IR: υ 3456–3328, 3055, 2987, 2908, 1689, 1621, 1593 cm^{-1}; ^{1}H NMR: δ 3.38 (s, 3H, CH_3),

5.69 (s, 2H, CH_2), 6.30 (s, 1H, CH=N), 7.28–7.48 (m, 9H, C_6H_4, C_6H_5), 8.93 (s, 1H, NH). Analysis: calcd. for $C_{20}H_{16}N_4O_2S$ (376.43): C, 63.81; H, 4.28; N, 14.88; S, 8.52. Found: C, 64.08; H, 4.51; N, 15.09; S, 8.47.

Compounds 12a–d

A solution of either **11a** (3.46 g, 0.01 mol), **11b** (3.80 g, 0.01 mol), **11c** (4.60 g, 0.01 mol) or **11d** (4.76 g, 0.01 mol) in ethanol (50 ml) containing sodium hydroxide (0.50 g) was heated under reflux for 4 h. The formed solid product, upon pouring onto an ice/water mixture containing hydrochloric acid (pH 6), was collected by filtration.

2-(2,3-Dihydro-3-imino-2,5-diphenylpyridazin-4-yl)thiazol-4-(5*H*)-one (12a) Pale yellow crystals from 1,4-dioxane; yield 2.29 g (66%); m.p. 180–183°C; IR: υ 3050, 2888, 1689, 1669, 1637 cm^{-1}; ^1H NMR: δ 5.62 (s, 2H), 6.29 (s, 1H), 7.28–7.44 (m, 10H), 8.91 (s, 1H); ^{13}C NMR: δ 54.6, 120.6, 122.6, 121.6, 122.9, 123.4, 124.9, 126.8, 127.9, 134.6, 140.7, 146.1, 163.5, 167.9, 164.8. Analysis: calcd. for $C_{19}H_{14}N_4OS$ (346.41): C, 65.88; H, 4.07; N, 16.17; S, 9.26. Found: C, 65.62; H, 4.09; N, 15,92; S, 9.01.

2-(2-(4-Chlorophenyl)-2,3-dihydro-3-imino-5-phenylpyridazin-4-yl)thiazol-4(5*H*)-one (12b) Yellowish brown crystals from ethanol; yield 2.51 g (66%); m.p. 145–149°C; IR: υ 3050, 2887, 1690, 1652, 1636 cm^{-1}; ^1H NMR: δ 5.85 (s, 2H), 6.62 (s, 1H), 7.25–7.38 (m, 9H), 9.01 (s, 1H); ^{13}C NMR: δ 56.8, 119.8, 121.8, 122.0, 123.4, 123.9, 124.5, 126.3, 132.8, 134.3, 140.2, 145.8. 164.6, 167.4 163.9. Analysis: calcd. for $C_{19}H_{13}ClN_4OS$ (380.85): C, 59.92; H, 3.44; N, 14.71; S, 8.42. Found: C, 60.13; H, 3.79; N, 14.83; S, 8.37.

2-(2-(4-Methylphenyl)-2,3-dihydro-3-imino-5-phenylpyridazin-4-yl)thiazol-4(5*H*)-one (12c) Brown crystals form 1,4-dioxane; yield 2.85 g (79%); m.p. 196–198°C; IR: υ 3050, 2941, 1689, 1658, 1634 cm^{-1}; ^1H NMR: δ 5.69 (s, 2H), 6.69 (s, 1H), 7.24–7.37 (m, 9H), 8.93 (s, 1H). Analysis: calcd. for $C_{20}H_{16}N_4OS$ (360.43): C, 66.64; H, 4.47; N, 15.54; S, 8.90. Found: C, 66.75; H, 4.37; N, 15.84; S, 8.69.

2-(2-(4-Methoxyphenyl)-2,3-dihydro-3-imino-5-phenylpyridazin-4-yl)thiazol-4(5*H*)-one (12d) Yellow crystals from acetic acid; yield 2.59 g (69%); m.p. 274–277°C; IR: υ 3061, 2893, 1686, 1654, 1638 cm^{-1}; ^1H NMR: δ 3.22 (s, 3H), 5.62 (s, 2H), 6.43 (s, 1H), 7.25–7.39 (m, 9H), 9.04 (s, 1H); ^{13}C NMR: δ 24.9, 53.8, 119.9, 120.8, 121.3, 121.9, 122.6, 123.8, 124.9, 126.7, 136.6, 143.5, 148.3, 165.8, 167.2, 169.0. Analysis: calcd. for $C_{20}H_{16}N_4O_2S$ (376.43): C, 63.81; H, 4.28; N, 14.88; S, 8.52. Found: C, 63.86; H, 4.35; N, 14.97; S, 8.46.

2-(1,2,5,6-Tetrahydro-2-imino-1,4-diphenyl-6-thioxopyridin-3-yl)thiazol-4(5*H*)-one (14) A mixture of compound **5** (2.42 g, 0.01 mol) and phenylisothiocyanate (1.35 g, 0.01 mol) in 1,4-dioxane (30 ml) containing triethylamine (0.50 ml) was heated under reflux for 3 h, then poured onto an ice/water mixture containing a few drops of hydrochloric acid. The resultant solid product was collected by filtration: orange crystals from ethanol; yield 2.94 g (78%); m.p. 130–132°C; IR: υ 2884, 1688, 1659, 1638 cm^{-1}; ^1H NMR: δ 4.89 (s, 2H, pyridine CH_2), 5.87 (s, 2H, thiazole CH_2), 7.26–7.39 (m, 10H, $2C_6H_5$), 9.63 (9s, 1H, NH); ^{13}C NMR: δ 56.6, 120.8, 121.9, 122.6, 123.9, 123.8, 125.3, 126.0, 134.9, 136.7, 140.0, 143.9, 166.8, 166.6. Analysis: calcd. for $C_{20}H_{15}N_3OS_2$ (377.48): C, 63.64; H, 4.01; N, 11.13; S, 16.99. Found: C, 63.96; H, 3.99; N, 11.06; S, 17.25.

Compounds 15a,b

A solution of compound **3** (2.42 g, 0.01 mol) and triethylamine (0.5 ml) in ethanol (20 ml) and either malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.13 g, 0.01 mol) was added. The mixture was heated under reflux for 6 h, and then poured onto an ice/water mixture containing a few drops of hydrochloric acid. The solid product formed was collected by filtration.

2-(7-Amino-5-oxo-5H-pyrano[2,3-*d*]thiazole-2-yl)-3-phenylbut-2-enenitrile (15a) Yellow crystals from methanol; yield 2.00 g (65%); m.p. 210–214°C; IR: υ 3475–3354, 3050, 2993, 1668, 1632; ^1H NMR: δ 2.63 (s, 3H), 4.95 (s, 2H), 5.86 (s, 1H), 7.24–7.43 (m, 5H), 8.86 (s, 1H); ^{13}C NMR: δ 24.3, 86.4, 116.6, 119.6, 120.6, 123.0, 125.8, 126.1, 128.3, 140.8, 146.3, 164.8, 168.3. Analysis: calcd. for $C_{16}H_{12}N_4OS$ (308.36): C, 62.32; H, 3.92; N, 18.17; S, 10.40. Found: C, 62.41; H, 4.31; N, 18.04; S, 10.52.

2-(7-Amino-5-imino-5-H-pyrano[2,3-*d*]thiazol-2-yl)-3-phenylbut-2-enenitrile (15b) Yellow crystals from ethanol; yield 1.63 g (53%); m.p. 190–193°C; IR: υ 3060, 2978, 1679, 1646, 1638 cm^{-1}; ^1H NMR: δ 2.68 (s, 3H), 4.95 (s, 2H), 5.93 (s, 1H), 7.25–7.38 (m, 5H); ^{13}C NMR: δ 24.8, 116.9, 118.9, 119.4, 120.8, 121.8, 122.6, 123.0, 123.7, 124.8, 127.0, 133.8, 134.9, 143.8, 168.0. Analysis: calcd. for $C_{16}H_{11}N_3O_2S$ (309.34): C, 62.12; H, 3.58; N, 13.58; S, 10.37. Found: C, 62.31; H, 3.68; N, 13.84; S, 10.52.

2-(2-Amino-4-phenylthiophen-3-yl)thiazol-4(5H)-one (16) A mixture of compound **3** (2.42 g, 0.01 mol), triethylamine (0.5 ml), elemental sulfur (0.32 g, 0.01 mol) and 1,4-dioxane (30 ml) was heated under reflux for 3 h, then poured onto an ice/water mixture containing a few drops of hydrochloric acid. The solid product formed was collected by filtration and crystallized from ethanol: yield 1.80 g (56%); m.p. 180–183°C; IR: υ 3055, 1687, 1637, 1578 cm^{-1}; ^1H NMR: δ 4.96, 5.36 (s, 2H), 5.22 (s, 2H), 7.26–7.38 (m, 5H); ^{13}C NMR: δ 58.8, 87.4, 111.6, 117.8, 120.3, 123.6, 124.9, 127.0, 128.0, 128.6, 129.7, 133.0, 166.2. Analysis: calcd. for $C_{13}H_{10}N_2OS_2$ (274.36): C, 56.91; H, 3.67; N, 10.21; S, 23.37. Found: C, 56.73; H, 3.83; N, 10.42; S, 23.29.

Antitumor activity

Fetal bovine serum (FBS) and L-glutamine were from Gibco Invitrogen Co. (Scotland, UK). RPMI-1640 medium was from Cambrex (NJ, USA). Dimethyl sulfoxide (DMSO), doxorubicin, penicillin, streptomycin and sulforhodamine B were from Sigma Chemical Co. (Saint Louis, MO, USA). Stock solutions of compounds **3–16** were prepared in DMSO and kept at -20°C. Appropriate dilutions of the compounds were freshly prepared just prior to the assays.

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 and SF-268 were kindly provided by the National Cancer Institute (NCI, Cairo, Egypt). They were grown as a monolayer and routinely maintained in RPMI-1640 medium supplemented with 5% heat inactivated FBS, 2 mM glutamine and antibiotics (penicillin 100 μg/ml, streptomycin 100 μg/ml), at 37°C in a humidified atmosphere containing 5% CO_2. Exponentially growing cells were obtained by plating 1.5×10^5 cells/ml for MCF-7 and SF-268 and 0.75×10^4 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.

References

Al-Sarawy, A. A.; Fouda, A. S.; Shehab El-Dein, W. A. Some thiazole derivatives as corrosion inhibitors for carbon steel in acidic medium. *Desalination* **2008**, *229*, 279–293.

Bhattacharya, P.; Leonard, J. T.; Roy, K. Exploring QSAR of thiazole and thiadiazole derivatives as potent and selective human adenosine A3 receptor antagonists using FA and GFA techniques. *Bioorg. Med. Chem.* **2005**, *13*, 1159–1165.

Bondock, S.; Khalifa, W.; Fadda, A. A. Synthesis and antimicrobial evaluation of some new thiazole, thiazolidinone and thiazoline derivatives starting from 1-chloro-3,4-dihydronaphthalene-2-carboxaldehyde. *Eur. J. Med. Chem.* **2007**, *42*, 948–954.

Elnagdi, M. H.; Elmoghayer, M. -R.; Hammam, A. G.; Khallaf, S. A. The reaction of malononitrile with thioglycolic acid. A novel procedure for the synthesis of thiazolone derivatives. *J. Heterocycl. Chem.* **1979**, *16*, 1541–1546.

Elnagdi, M. H.; Khalifa, M. A.; Ibrahim, M. K.; Elmoghayer, M. R. Synthesis of new azaindenes and pyrazole derivatives. *J. Heterocycl. Chem.* **1981**, *8*, 877–889.

Li, Z.; Yang, Q.; Qian, X. Novel heterocyclic family of phenyl naphthothiazole carboxamides derived from naphthalimides: synthesis, antitumor evaluation, and DNA photocleavage. *Bioorg. Med. Chem.* **2005**, *13*, 3149–3155.

Mansour, S. A.; Mahmoud, S. B.; Al-qasoumi, S. I.; Horab, M. M. Anti-breast cancer activity of some novel 1,2-dihydropyridine, thiophene and thiazole derivatives. *Eur. J. Med. Chem.* **2011**, *46*, 137–141.

Narayana, B.; Vijaya Raj, K. K.; Ashalatha, B. V.; Kumari, N. S.; Sarojini, B. K. Synthesis of some new 5-(2-substituted-1,3-thiazol-5-yl)-2-hydroxy benzamides and their 2-alkoxy derivatives as possible antifungal agents. *Eur. J. Med. Chem.* **2004**, *39*, 867–872.

Nettekoven, M.; Guba, W.; Neidhart, W.; Mattei, P.; Pflieger, P.; Roche, O.; Taylor, S. Isomeric thiazole derivatives as ligands for the neuropeptide Y5 receptor. *Bioorg. Med. Chem. Lett*. **2005**, *15*, 3446–3449.

Peng-Cheng, L. V.; Kai-Rui Wang, K. R.; Yang, Y.; Mao, W. J.; Jin Chen, J.; Jing Xiong, J.; Zhu, H. L. Design, synthesis and biological evaluation of novel thiazole derivatives as potent FabH inhibitors. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6750–6754.

Sondhi, M. S.; Singh, N.; Johar, M.; Kumar, A. Synthesis, anti-inflammatory and analgesic activities and evaluation of some mono, bi and tricyclic pyrimidine derivatives. *Bioorg. Med. Chem.* **2005**, *13*, 6158–6166.

Tomasz, K. O.; Boduszek, B. A brief perspective on catalysis from its origins and at the threshold of the 21st century. *Tetrahedron* **2010**, *66*, 8661–8666.

Turan-Zitouni, G. Z. A.; Kaplancikli, M. T.; Chevallet, Y. P.; Kaya, D. Synthesis and antimicrobial activity of 4-phenyl/cyclohexyl-5-(1-phenoxyethyl)-3-[*N*-(2-thiazolyl)acetamido]thio-4H-1,2,4-triazole derivatives. *Eur. J. Med. Chem.* **2005**, *40*, 607–613.

Vicini, P. L.; Geronikaki, A.; Incerti, M.; Busonera, B.; Poni, G.; Cabras, C. A.; Colla, P. Synthesis and biological evaluation of benzo[d]isothiazole, benzothiazole and thiazole Schiff bases. *Bioorg. Med. Chem.* **2003**, *11*, 4785–4789.

Yang, Z.; Li, Q.; Qian, X. Novel thiazonaphthalimides as efficient antitumor and DNA photocleaving agents: effects of intercalation, side chains and substituents groups. *Bioorg. Med. Chem.* **2005**, *13*, 4864–4870.

Yu, H.; Shao, L. H.; Fang, J. Synthesis and biological activity research of novel ferrocenyl-containing thiazole imine derivatives. *J. Organomet. Chem*. **2007**, *692*, 991–996.

Received June 29, 2011; accepted August 8, 2011