Synthesis of Fused Quinoline Derivatives With Antiproliferative Activities and Tyroine Kinases, Pim-1 Kinase Inhibitions

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Abstract

Cyclohexan-1,3-dione (**1**) reacted with either 2-aminoprop-1-ene-1,1,3-tricarbonitrile (**2a**) or diethyl 3-amino-2-cyanopent-2-enedioate (**2b**) to give the 5,6,7,8-tetrahydronaphthalene derivatives **3a** and **3b**, respectively. The latter compounds underwent further heterocyclization reactions to give thieno[2',3':5,6]-benzo[1,2-*e*][1,3]oxazine derivatives. On the other hand, the reaction of compound **1** with trichloroacetonitrile afforded the 2,2,2-trichloroethylidene)-cyclohexane derivative **14**. The latter underwent a series of reactions to produce 2,3,6,7-tetrahydroquinazoline, dihydrothieno[2,3-*h*]isoquinoline, octahydrobenzo-[*h*]quinazoline and dihydrothieno[2,3-*h*]isoquinoline derivatives. The synthesized compounds were tested toward six cancer cell lines where most of them gave high inhibitions together with c-Met enzymatic activity together with tyrosine kinases and Pim-1 inhibitions. The results obtained will encourage further work through such compounds to produce optimized anticancer agents.

Keywords: cyclohexan-1,3-dione, trichloroacetonitrile, quinoline, isoquinoline, cytotoxicity

1. Introduction

With its origins rooted in organic synthesis and medicinal chemistry, heterocyclic compounds present themselves as a fundamental division of organic chemistry. Defined by IUPAC as "cyclic compounds having as ring members atoms of at least two different elements" (IUPAC Gold Book 2015),[1]heterocycles' ring structures are in essence composed by elements other than carbon, where the most frequent substituents are oxygen, nitrogen and sulfur.[2,3] According to the

heteroatom(s) present in the ring structures, heterocycles can be classified as oxygen, nitrogen or sulfur based and, within each class, compounds are organized based on the size of the ring structure size determined by the total number of atoms.[4] The type and size of ring structures, together with the substituent groups of the core scaffold, impact strongly on the physicochemical properties.[2,5] Among the various clinical applications, heterocyclic compounds have a considerable active role as anti-bacterial,[6,7] anti-viral,[8] anti-fungal,[9] anti-inflmmatory[10] and anti-tumor drugs.[11-13] The engineering and rationale behind drug design are closely related to the strategic incorporation of heterocyclic like fragments with specific physicochemical properties. Potency and selectivity through bioisosteric replacements, lipophilicity, polarity, and aqueous solubility can ultimately be fine-tuned to the point of altering and conditioning the possible mechanisms of action of pharmaceutical drugs in an attempt to obtain molecularly targeted agents.[14] Despite their versatility and potential, as for any other pharmaceutical, there are several issues hindering wider application and further development of such compounds into market drugs. Oncology is one of the areas where this is perhaps most noticeable, partially due to the intrinsic limitations regarding main therapeutic routes of chemotherapy, concomitant side effects and toxicity to healthy tissues. Such deleterious effects may be circumvented via selective targeting of delivery, passively or actively into cancerous cells.[15] It should be referred that for some playmakers within the chemotherapy field, the success of "molecularly targeted agents", such as imatinib are merely fortunate exceptions and that the number of success in this area is considerably low.[16] Recent advances in interdisciplinary field of nanobiotechnology have led to the development of newly inventive therapeutic strategies and drug delivery alternatives taking advantage of the architectural geniality of systems based on nanoscale devices particularly tailored to deliver drugs to a selected tissue.[17-19] Recently our research group reported several reactions of cyclic β-diketones to produce thaizoles and thiophene derivatives. The produced compounds showed high anti-proliferative activities against cancer cell lines together with high inhibitions toward tyrosine kinases.[20-22] This encouraged us to continue this goal through the reaction of

cyclohexan-1,3-dione with dimeric cyanomethylene and trichloroacetonitrile reagents together with using the produced molecule as a suitable starting material for subsequent heterocyclization to produce a variety of fused derivatives. The antiproliferative activities of the synthesized compounds and their inhibitions toward tyrosine kinases were determined.

2. Experimental

2.1. General

All melting points were uncorrected and were recorded using an Electrothermal digital melting point apparatus. IR spectra (KBr discs) were measured using a FTIR plus 460 or PyeUnicam SP-1000 spectrophotometer. ^{1}HNMR spectra were measured using a Varian Gemini-300 (300 MHz) and Jeol AS 500 MHz instruments spectra were performed in DMSO-d_6 as solvent using TMS as internal standard and chemical shifts are expressed as δ ppm. MS (EI) spectra were measured using Hewlett Packard 5988 A GC/MS system and GCMS-QP 1000 Ex Shimadzu instruments. Analytical data were obtained from the Micro-analytical Data Unit at Cairo University and were performed on Vario EL III Elemental analyzer. The Anti-tumor evaluation has been carried out through the National Cancer Research Center at Cairo, Egypt where the IC_{50} values were calculated.

2.1.1. General procedure for the synthesis of the 5,6,7,8-tetrahydronaphthalene 3a,b

Equimolar amounts of dry solids of compound **1** (1.12 g, 0.01 mol) and either of **2a** (1.32 g, 0.01 mol) or **2b** (2.14 g, 0.01 mol) and ammonium acetate (1.50 g) were heated in an oil bath at 120 ^{o}C for 1 h then were left to cool. The remaining product was triturated with diethylether and the formed solid product, in each case, was collected by filtration.

2.1.1.1. 2,4-Diamino-5-oxo-5,6,7,8-tetrahydronaphthalene-1,3-dicarbonitrile (3a)

Yellow crystals from 1,4-dioxane, yield (1.58 g, 70 %), m.p 256-258 °C. IR (KBr) υ max cm^{-1}: 3488-3352 (NH_2), 3055 (CH, aromatic), 2223, 2220 (2CN), 1703 (CO), 1632 (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 4.93, 4.53 (s, 4H, D_2O exchangeable, 2NH_2), 2.93-2.80 (m, 4H, 2CH_2), 1.98-1.28 (m, 2H, CH_2); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 174.2 (C-5), 127.9, 125.6, 124.9, 123.5, 121.8, 120.4 (C-1, C-2, C-3, C-4, C-5, C-6),

116.8, 116.3 (2CN), 40.6, 38.9, 17.4 (C-6, C-7, C-8). Anal. Calculated for $C_{12}H_{10}N_4O$: C, 63.71; H, 4.46; N, 24.76. Found: C, 63.92; H, 4.79; N, 24.80. MS: m/e 226 (M^+, 36 %).

2.1.1.2. Ethyl 2-amino-3-cyano-4-hydroxy-5-oxo-5,6,7,8-tetrahydronaphthalene-1-carboxylate (3b)

Orange crystals from ethanol, yield (1.89 g, 69 %), m.p 180-183 °C. IR (KBr) υ max cm^{-1}: 3554-3338 (OH, NH_2), 3055 (CH, aromatic), 2220 (CN), 1708, 1689 (2CO), 1636 (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 10.26 (s, 1H, D_2Oexchangeable, OH), 4.92 (s, 2H, D_2O exchangeable, NH_2), 4.22 (q, 2H, J = 7.31 Hz, O$\underline{CH_2}CH_3$), 2.80-2.96 (m, 4H, $2CH_2$), 1.98-1.28 (m, 2H, CH_2), 1.12 (t, 3H, J = 7.31 Hz, $OCH_2\underline{CH_3}$), ^{13}C NMR (DMSO-d_6, 75 MHz): δ 165.2, 164.3 (C-5, ester CO), 125.4, 123.0, 122.8, 122.5, 121.9, 120.5, 119.2 (C-1, C-2, C-3, C-4, C-5, C-6), 116.9 (CN), 50.3 (O$\underline{CH_2}CH_3$), 40.1, 38.5, 17.1 (C-6, C-7, C-8), 16.2 ($OCH_2\underline{CH_3}$), Anal. Calculated for $C_{14}H_{14}N_2O_4$: C, 61.31; H, 5.14; N, 10.21. Found: C, 61.26; H, 5.39; N, 10.36. MS: m/e 274 (M^+, 28 %).

2.1.2. General procedure for the synthesis of the 5,6,7,8-tetrahydronaphthalene derivatives 4a,b

A solution of either compound **3a** (2.26 g, 0.01 mol) or **3b** (2.74 g, 0.01 mol) in acetic acid (40 mL) and acetic anhydride (15 mL) was heated under reflux for 3 h then left to cool. The reaction mixture, in each case was evaporated under vacuum and the remaining product was triturated with ethanol and the formed solid product was collected by filtration.

2.1.2.1. *N*-(3-Amino-2,4-dicyano-8-oxo-5,6,7,8-tetrahydronaphthalen-1-yl)acetamide (4a)

Pale yellow crystals from 1,4-dioxane, yield (1.82 g, 68 %), m.p 236-239 °C. IR (KBr) υ max cm^{-1}: 3464-3342 (NH_2, NH), 3055 (CH, aromatic), 2223, 2220 (2CN), 1702, 1688 (2CO), 1630 (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 8.26 (s, 1H, D_2O exchangeable, NH), 4.56 (s, 2H, D_2O exchangeable, CH_2), 3.02 (s, 3H CH_3), 2.93- 2.85 (m, 4H, $2CH_2$), 1.96-1.84 (m, 2H, CH_2); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 174.3, 166.2 (C-8, CO amide), 125.9, 123.9, 123.7, 122.5, 122.0, 121.6, 119.8 (C-1, C-2, C-3, C-4, C-5, C-6), 116.5, 116.4 (2CN), 40.6, 38.5, 17.6 (C-6, C-7, C-8), 24.8 (CH_3); Anal. Calculated for $C_{14}H_{12}N_4O_2$: C, 62.68; H, 4.51; N, 20.88. Found: C, 62.93; H, 4.63; N, 20.68. MS: m/e 268 (M^+, 44 %).

2.1.2.2. Ethyl 4-acetoxy-2-amino-3-cyano-5-oxo-5,6,7,8-tetrahydronaphthalene-1-carboxylate (4b)

Pale brown crystals from ethanol, yield (2.17 g, 60 %), m.p 158-161 °C. IR (KBr) υ max cm^{-1}: 3473-3328 (NH), 3055 (CH, aromatic), 2220 (CN), 1705, 1688 (2CO), 1630 (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 4.72 (s, 2H, D_2O exchangeable, NH_2), 4.23 (q, 2H, J = 6.56, O$\underline{CH_2}CH_3$), 3.01 (s, 3H CH_3), 2.96-2.82 (m, 4H, $2CH_2$), 1.96- 1.81 (m, 2H, CH_2), 1.12 (t, 3H, J = 6.56, $OCH_2\underline{CH_3}$); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 174.3, 166.1 (C-8, CO ester), 120.3, 121.8, 122.6, 123.2, 124.1, 125.1, 125.2 (C-1, C-2, C-3, C-4, C-5, C-6), 117.0 (CN), 50.2 (O$\underline{CH_2}CH_3$), 40.1, 38.5, 17.3 (C-6, C-7, C-8), 24.8 (CH_3), 16.6, 16.3 (two $OCH_2\underline{CH_3}$), Anal. Calculated for $C_{16}H_{16}N_2O_5$: C, 60.75; H, 5.10; N, 8.86. Found: C, 60.43; H, 5.28; N, 8.90. MS: m/e 316 (M^+, 30 %).

2.1.3. General procedure for the synthesis of the 3,4,7,8,9,10-hexahydro-2*H*-naphtho[2,1-*e*][1,3]azine derivatives 6a,b

To a solution of either of compound **4a** (2.68 g, 0.01 mol) or **4b** (3.16 g, 0.01 mol) in ethanol (40 mL) containing triethylamine (1.0 mL) phenylisothiocyanate (1.30 g, 0.01 mol) was heated under reflux for 3h then left to cool. The formed solid crystals, in each case, were collected by filtration.

2.1.3.1. 5-Amino-4-imino-10-oxo-3-phenyl-2-thioxo-1,2,3,4,7,8,9,10-octahydrobenzo[*h*]-quinazoline-6-carbonitrile (6a)

Yellowish white crystals from 1,4-dioxane, yield (2.64 g, 73 %), m.p 212-215 °C. IR (KBr) υ max cm^{-1}: 3480-3329 (NH), 3055 (CH, aromatic), 2220 (CN), 1689 (CO), 1630 (C=C), 1209 (C=S); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 8.35, 8.28 (2s, 2H, D_2O exchangeable, 2NH), 7.42-7.26 (m, 5H, C_6H_5), 4.52 (s, 2H, D_2O exchangeable, NH_2), 2.96-2.83 (m, 4H, $2CH_2$), 1.96-1.82 (m, 2H, CH_2), ^{13}C NMR (DMSO-d_6, 75 MHz): δ 179.8 (C-2), 173.6 (C-10), 126.8, 126.1, 125.2, 125.1, 124.6, 124.1, 123.8, 123.2, 122.6, 121.8, 120.3 (C-1, C-2, C-3, C-4, C-5, C-6, C_6H_5), 117.0, 116.3, 116.1 (3CN), 46.8, 40.2, 38.5 (C-7, C-8, C-9). Anal. Calculated for $C_{19}H_{15}N_5OS$: C, 63.14; H, 4.18; N, 19.38; S, 8.87. Found: C, 63.28; H, 4.25; N, 19.26; S, 8.69. MS: m/e 361 (M^+, 28 %).

2.1.3.2. Diethyl 2-cyano-4-(3-oxocyclohexylidene)-3-(3-phenylthioureido)pent-2-enedioate (6b)

Orange crystals from ethanol, yield (3.09 g, 67 %), m.p 211-214 °C. IR (KBr) υ max cm^{-1}: 3468-3347 (NH), 3055 (CH, aromatic), 1689, 1687 (2CO), 1630 (C=C), 1209 (C=S); ^{1}H NMR (DMSO-*d*$_6$, 200 MHz): δ = 8.32 (s, 1H, D_2O exchangeable, NH), 7.40-7.23 (m, 5H, C_6H_5), 4.53 (s, 2H, D_2O exchangeable, NH_2), 4.22 (2q, 2H, J= 7.03, O$\underline{CH_2}CH_3$), 2.96-2.83 (m, 4H, $2CH_2$), 1.96-1.82 (m, 2H, CH_2), 1.12 (t, 3H, J= 7.03 $OCH_2\underline{CH_3}$); ^{13}C NMR (DMSO-*d*$_6$, 75 MHz): δ 179.7 (C-2), 174.6, 166.1 (C-3, ester CO), 126.9, 126.5, 125.3, 125.0, 124.9, 124.6, 123.4, 123.1, 122.9, 122.3, 120.1 (C-1, C-2, C-3, C-4, C-5, C-6, C_6H_5), 50.1 (O$\underline{CH_2}CH_3$), 40.5, 38.5, 17.1 (C-7, C-8, C-9), 16.3 ($OCH_2\underline{CH_3}$). Anal. Calculated for $C_{21}H_{19}N_3O_4S$: C, 61.60; H, 4.68; N, 10.26; S, 7.83. Found: C, 61.39; H, 4.78; N, 10.58; S, 7.57. MS: m/e 409 (M^+, 38 %).

2.1.4. General procedure for the synthesis of the 3,4,7,8,9,10-hexahydro-2H-naphtho[2,1-*e*][1,3]azinone derivatives 7a,b

A suspension of either compound **6a** (3.61 g, 0.01 mol) or **6b** (4.09 g, 0.01 mol) in sodium ethoxide [prepared through dissolving metallic sodium (0.46 g, 0.02 mol) in absolute ethanol (50 mL)] was heated in a boiling water bath for 6h. The reaction mixture was poured onto ice/water then triturated with hydrochloric acid (till pH 7) and the formed solid product was collected by filtration.

2.1.4.1. 5-Amino-4,10-dioxo-3-phenyl-2-thioxo-1,2,3,4,7,8,9,10-octahydrobenzo[*h*]-quinazoline-6-carbonitrile (7a)

Yellow crystals from ethanol, yield (1.99 g, 55 %), m.p 210-212 °C., IR (KBr) υ max cm^{-1}: 3472-3346 (NH_2), 3055 (CH, aromatic), 2220 (CN), 1688 (CO), 1630 (C=C); ^{1}H NMR (DMSO-*d*$_6$, 200 MHz): δ = 8.31 (s, 1H, D_2O exchangeable, NH), 7.24-7.48 (m, 5H, C_6H_5), 4.80 (s, 2H, D_2O exchangeable, NH_2), 2.98-2.81 (m, 4H, $2CH_2$), 1.94-1.80 (m, 2H, CH_2); ^{13}C NMR (DMSO-*d*$_6$, 75 MHz): δ 179.5 (C-2), 173.4, 168.2 (C-4, C-10), 126.9, 126.1, 125.7, 125.2, 124.4, 124.0, 123.8, 123.4, 122.3, 122.6, 120.0 (C-1, C-2, C-3, C-4, C-5, C-6, C_6H_5), 116.6 (CN), 40.6, 38.2, 17.3 (C-7, C-8, C-9), Anal. Calculated for $C_{19}H_{14}N_4O_2S$: C, 62.97; H, 3.89; N, 15.46; S, 8.85. Found: C, 62.77; H, 4.19; N, 15.52; S, 8.59. MS: m/e 362 (M^+, 38 %).

2.1.4.2. Ethyl 5-amino-4,10-dioxo-3-phenyl-2-thioxo-3,4,7,8,9,10-hexahydro-2*H*-naphtho-[2,1-*e*][1,3]oxazine-6-carboxylate (7b)

Pale brown crystals from ethanol, yield (2.70 g, 66 %), m.p 177-179 °C. IR (KBr) υ max cm^{-1}: 3462, 3330 (NH_2), 3055 (CH, aromatic), 1689-1687 (3CO), 1630 (C=C), 1209 (C=S); ^1H NMR (DMSO-d_6, 300 MHz): δ = 7.25-7.42 (m, 5H, C_6H_5), 4.83 (s, 2H, D_2O exchangeable, NH_2), 4.23 (q, 2H, J= 7.43, $O\underline{CH_2}CH_3$), 2.98-2.83 (m, 4H, $2CH_2$), 1.93-1.80 (m, 2H, CH_2), 1.12 (t, 3H, J= 7.43, $OCH_2\underline{CH_3}$), ^{13}C NMR (DMSO-d_6, 75 MHz): δ 179.8 (C-2), 174.2, 166.5 (C-4, C-10), 127.1, 126.4, 125.9, 125.0, 124.6, 124.3, 123.5, 123.1, 122.5, 122.6, 120.3 (C-1, C-2, C-3, C-4, C-5, C-6, C_6H_5), 50.3 ($O\underline{CH_2}CH_3$), 40.8, 38.5, 17.0 (C-7, C-8, C-9), 16.1 ($OCH_2\underline{CH_3}$). Anal. Calculated for $C_{21}H_{18}N_2O_5S$: C, 61.45; H, 4.42; N, 6.83; S, 7.81. Found: C, 61.50; H, 4.38; N, 4.40; S, 7.14. MS: m/e 410 (M^+, 18 %).

2.1.5. 2-Phenyl-4-thioxo-7,8-dihydro-4*H*-benzo[*e*][1,3]oxazin-5(6*H*)-one (10)

To a solution of compound **1** (1.12 g, 0.01 mol) in 1,4-dioxane (40 mL) benzoylisothiocyanate (1.63 g, 0.01 mol) [prepared by addition benzoyl chloride (1.40 g, 0.01 mol) to ammonium thiocyanate (0.76 g, 0.01 mol) in 1,4-dioxane (20 ml) with gentile heating for 5 min followed by filtration of the produced ammonium chloride] was heated under reflux for 3h then left to cool. The formed solid crystals, was collected by filtration.

White crystals from ethanol, yield (1.74 g, 67 %), m.p 188-191 °C. IR (KBr) υ max cm^{-1}: 3055 (CH, aromatic), 1689 (CO), 1630 (C=C), 1208 (C=S); ^1H NMR (DMSO- d_6, 300 MHz): δ = 7.43-7.22 (m, 5H, C_6H_5), 2.78-2.68 (m, 2H, CH_2), 1.93- 1.67 (m, 4H, $2CH_2$); ^{13}C NMR (DMSO- d_6, 75 MHz): δ 180.4 (C-4), 174.2 (C-2), 168.2 (C-5), 142.7, 133.3, 126.3, 125.2, 123.6, 121.1 (C_6H_5, C-2, C-4a, C-8a), 39.8, 36.8, 16.0 (C-6, C-7, C-8). Anal. Calculated for $C_{14}H_{11}NO_2S$: C, 65.35; H, 4.31; N, 5.44; S, 12.46. Found: C, 65.26; H, 4.28; N, 5.60; S, 12.36. MS: m/e 257 (M^+, 36 %).

2.1.6. General procedure for the synthesis of the thieno[2',3':5,6]benzo[1,2-*e*][1,3]oxazine derivatives 12a,b

To a solution of compound **10** (2.57 g, 0.01 mol), in ethanol (40 mL) containing triethylamine (0.50 mL) either malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.07 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 3 h then poured onto ice/water mixture containing a few drops of hydrochloric acid and the formed solid product was collected by filtration.

2.1.6.1. 8-Amino-3-phenyl-1-thioxo-5,6-dihydro-1*H*-thieno[2',3':5,6]benzo[1,2-*e*][1,3]oxazine-9-carbonitrile (12a)

Orange crystals from ethanol, yield (2.35 g, 70 %), m.p 180-183 °C. IR (KBr) υ max cm^{-1}: 3472-3353 (NH_2), 3055 (CH, aromatic), 2220 (CN), 1630 (C=C), 1208 (C=S); ^1H NMR (DMSO-d_6, 300 MHz): δ = 7.42-7.26 (m, 5H, C_6H_5), 4.78 (s, 2H, D_2O exchangeable, NH_2), 2.82-2.60 (2t, 4H, $2CH_2$); ^{13}C NMR (DMSO- d_6, 75 MHz): δ 179.6 (C-1), 176.3 (C-3), 142.6, 140.7, 134.2, 132.6, 132.7, 132.3, 127.2, 124.8, 122.4, 121.6 (C_6H_5, C-5, C-6, C-6a, C-9a, C-4a, C-9b), 116.8 (CN), 39.8, 36.3 (C-5, C-6). Anal. Calculated for $C_{17}H_{11}N_3OS_2$: C, 60.51; H, 3.29; N, 12.45; S, 19.01. Found: C, 60.37; H, 3.63; N, 12.72; S, 18.93. MS: m/e 337 (M^+, 28 %).

2.1.6.2. Ethyl 8-amino-3-phenyl-1-thioxo-5,6-dihydro-1*H*-thieno[2',3':5,6]benzo[1,2-*e*][1,3]oxazine-9-carboxylate (12b)

Grey crystals from acetic acid, yield (2.84 g, 74 %), m.p 177-180 °C. IR (KBr) υ max cm^{-1}: 3459-3337 (NH_2), 3055 (CH, aromatic), 2930, 2970 (CH_2, CH_3), 1689 (CO), 1630 (C=C); ^1H NMR (DMSO-d_6, 300 MHz): δ = 1.12 (t, 3H, J = 6.59 Hz, CH_3), 2.78-2.65 (2t, 4H, $2CH_2$), 4.26 (q, 2H, J = 6.59 Hz, CH_2), 4.80 (s, 2H, D_2O exchangeable, NH_2), 7.45-7.21 (m, 5H, C_6H_6); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 180.4 (C-1), 176.2 (C-3), 168.4 (ester CO), 142.3, 141.3, 134.1, 132.6, 132.9, 132.3, 128.3, 124.9, 123.3, 120.6, (C_6H_5, C-5, C-6, C-6a, C-9a, C-4a, C-9b), 16.1 ($OCH_2\underline{CH_3}$), 52.3 ($O\underline{CH_2}CH_3$), 39.7, 36.1 (C-5, C-6), Anal. Calculated for $C_{19}H_{16}N_2O_3S_2$: C, 59.35; H, 4.19; N, 7.29; S, 16.68. Found: C, 59.52; H, 4.27; N, 7.37; S, 16.39. MS: m/e 384 (M^+, 28 %).

2.1.7. 2-(1-Amino-2,2,2-trichloroethylidene)cyclohexane-1,3-dione (14)

Equimolar amounts of cyclohexan-1,3-dione (1.12 g, 0.01 mol) and trichloroacetonitrile (1.42 g, 0.01 mol) in absolute ethanol (40 mL) containing triethylamine (0.50 mL) was heated under reflux for 3 h. The solid product formed upon evaporated the excess alcohol was collected by filtration.

Yellow crystals from ethanol, yield (1.99 g, 78 %), m.p 204-207 °C. IR (KBr) υ max cm^{-1}: 3472-3346 (NH_2), 1702, 1688 (2CO), 1630 (C=C); ^1H NMR (DMSO-d_6, 300 MHz): δ = 4.85 (s, 2H, D_2O exchangeable, NH_2), 1.96-1.82 (m, 2H, CH_2), 2.95- 2.80 (m, 4H, $2CH_2$); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 173.4, 168.0 (C-1, C-3), 112.3, 90.8 (C-2, C-1 ethylidene), 94.8 (CCl_3), 40.8, 38.2, 17.1 (C-4, C-5, C-6), Anal. Calculated for

$C_8H_8ClNO_2$: C, 37.46; H, 3.14; N, 5.46. Found: C, 37.80; H, 3.39; N, 5.52. MS: m/e 256 (M^+, 28 %).

2.1.8. 1-Phenyl-2-thioxo-4-(trichloromethyl)-2,3,6,7-tetrahydroquinazolin-5(1*H*)-one (16)

Equimolar amounts of compound **14** (2.56 g, 0.01 mol) and phenylisothiocyanate (1.30 g, 0.01 mol) in 1,4-dioxane (40 mL) containing triethylamine (0.50 mL) was heated under reflux for 2 h. The solid product formed upon pouring onto ice/water mxture was collected by filtration.

Yellow crystals from ethanol, yield (2.61 g, 70 %), m.p 168-17 °C. IR (KBr) υ max cm^{-1}: 3470-3380 (NH), 3050 (CH aromatic), 1689 (CO), 1630 (C=C), 1208 (C=S); ^1H NMR (DMSO-d_6, 300 MHz): δ = 8.28 (s, 1H, D_2O exchangeable, NH), 7.42-7.29 (m, 5H, C_6H_5), 5.21 (t, 1H, CH), 2.95-2.80 (m, 4H, $2CH_2$), ^{13}C NMR (DMSO-d_6, 75 MHz): δ 178.8 (C-2), 168.2 (C-5), 135.2, 133.6, 130.3, 129.0, 123.9, 123.6, 121.5, 120.5 (C_6H_5, C-8, C-9, C-3, C-4), 94.4 (CCl_3), 40.8, 38.2 (C-6, C-7), Anal. Calculated for $C_{15}H_{11}ClN_2OS$: C, 48.21; H, 2.97; N, 7.50. Found: C, 48.45; H, 3.19; N, 7.28. MS: m/e 373 (M^+, 42 %).

2.1.9. General procedure for the synthesis of the 3,5,6,7-tetrahydroquinazoline derivatives 18a,b

To a solution of compound **16** (3.73 g, 0.01 mol) in absoute ethanol (60 mL) either hydrazine hydrate (1.0 mL, 0.02 mol) or phenylhydrazine (2.16 g, 0.02 mol) was added. The reaction mixture, in each case, was heated under reflux for 2 h then poured onto ice/water containing a few drops of hydrochloric acid and the formed solid product was collected by filtration.

2.1.9.1. 4-Hydrazinyl-5-hydrazono-1-phenyl-3,5,6,7-tetrahydroquinazoline-2(1*H*)-thione (18a)

Orange crystals from ethanol, yield (2.04 g, 68 %), m.p 210-212 °C. IR (KBr) υ max cm^{-1}: 3489-3329 (NH_2), 3054 (CH, aromatic), 1630 (C=C), 1210 (C=S); ^1H NMR (DMSO-d_6, 300 MHz): δ = 8.41, 8.29 (2s, 2H, D_2O exchangeable, 2NH), 7.45- 7.28 (m, 5H, C_6H_5), 5.62 (t, 1H, CH), 2.89-2.64 (m, 4H, $2CH_2$), 4.90, 4.78 (2s, 4H, D_2O exchangeable, $2NH_2$), ^{13}C NMR (DMSO-d_6, 75 MHz): δ 179.3 (C-2), 168.6 (C-5), 142.6, 140.7, 134.2, 132.6, 132.7, 132.3, 127.2, 124.8, 122.4, 121.6 (C_6H_5, C-4, C-4a, C-8, C-8),

39.8, 36.7 (C-6, C-7). Anal. Calculated for $C_{14}H_{16}N_6S$: C, 55.98; H, 5.37; N, 27.98; S, 10.67. Found: C, 56.26; H, 5.49; N, 27.73; S, 10.88. MS: m/e 300 (M^+, 40 %).

2.1.9.2. 1-Phenyl-4-(2-phenylhydrazinyl)-5-(2-phenylhydrazono)-3,5,6,7-tetrahydroquinazoline-2(1*H*)-thione (18b)

Orange crystals from ethanol, yield (2.71 g, 60 %), m.p 177-180 °C. IR (KBr) υ max cm^{-1}: 3449-3352 (NH), 3055 (CH, aromatic), 1630 (C=C), 1208 (C=S); ^1H NMR (DMSO-d_6, 300 MHz): δ = 8.44-8.29 (4s, 4H, D_2O exchangeable, 4NH), 7.49-7.29 (m, 15H, $3C_6H_5$), 5.60 (t, 1H, CH), 2.93-2.64 (m, 4H, $2CH_2$); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 179.6 (C-2), 168.4 (C-5), 141.8, 140.7, 133.0, 132.7, 132.1, 131.8, 127.2, 126.7, 126.5, 124.8, 123.8, 123.6, 123.3, 122.4, 120.9, 120.6, 120.3 ($3C_6H_5$, C-4, C-4a, C-8, C-8), 39.9, 36.5 (C-6, C-7). Anal. Calculated for $C_{26}H_{24}N_6S$: C, 69.00; H, 5.35; N, 18.57; S, 7.09. Found: C, 69.21; H, 5.58; N, 18.80; S, 7.26. MS: m/e 452 (M^+, 36 %).

2.1.10. General procedure for the synthesis of the 6,7-dihydroisoquinoline derivatives 20a,b

To a solution of compound **14** (2.65 g, 0.01 mol) in 1,4-dioxane containing ammonium acetate (2.00 g) either malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.13 g, 0.01 mol) was added. The whole reaction mixture was heated under reflux for 3 h and the solid product formed, in each case, upon poring onto ice/water containing a few drops of hydrochloric acid was collected by filtration.

2.1.10.1. 3-Amino-8-oxo-1-(trichloromethyl)-5,6,7,8-tetrahydro-isoquinoline-4-carbonitrile (20a)

Orange crystals from 1,4-dioxane, yield (1.97 g, 65 %), m.p 211-214 °C. IR (KBr) υ max cm^{-1}: 3458, 3332 (NH_2), 3050 (CH aromatic), 2220 (CN), 1689 (CO), 1630 (C=C); ^1H NMR (DMSO-d_6, 300 MHz): δ = 4.80 (s, 2H, D_2O exchangeable, NH_2), 2.93-2.82 (m, 4H, $2CH_2$), 1.86-1.62 (m, 2H, CH_2). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 168.2 (C-8), 164.2 (C-3), 124.2, 123.8, 121.5, 120.3, 119.6 (C-1, C-4, C-4a, C-8a), 117.2 (CN), 94.6 (CCl_3), 40.8, 38.2, 24.8 (C-5, C-6, C-7). Anal. Calculated for $C_{11}H_8Cl_3N_3O$: C, 43.38; H, 2.65; N, 13.80. Found: C, 43.52; H, 2.80; N, 13.68. MS: m/e 304 (M^+, 28 %).

2.1.10.2. Ethyl 3-amino-8-oxo-1-(trichloromethyl)-5,6,7,8-tetrahydroiso-quinoline-4-carboxylate (20b)

Pale brown crystals from 1,4-dioxane, yield (2.52 g, 72 %), m.p 180-180 °C. IR (KBr) υ max cm^{-1}: 3468, 3329 (NH_2), 3045 (CH aromatic), 1702, 1689 (2CO), 1630 (C=C); ^1H NMR (DMSO-d_6, 300 MHz): δ = 4.85 (s, 2H, D_2O exchangeable, NH_2), 4.23 (q, 2H, *J* = 6.80 Hz, O$\underline{CH_2}CH_3$), 2.96-2.80 (m, 4H, $2CH_2$), 1.86-1.61 (m, 2H, CH_2), 1.12 (t, 3H, *J* = 6.80 Hz, $OCH_2\underline{CH_3}$), ^{13}C NMR (DMSO-d_6, 75 MHz): δ 168.2 (C-8), 164.8 (C=N), 124.9, 123.5, 122.8, 120.1, 119.8 (C-1, C-4, C-4a, C-8a), 94.5 (CCl_3), 50.3 (O$\underline{CH_2}CH_3$), 40.8, 38.7, 24.3 (C-5, C-6, C-7), 16.5 ($OCH_2\underline{CH_3}$). Anal. Calculated for $C_{13}H_{13}Cl_3N_2O_3$: C, 44.41; H, 3.73; N, 7.97. Found: C, 44.60; H, 3.84; N, 18.26. MS: m/e 350 (M^+, 28 %).

2.1.11. General procedure for the synthesis of the 5,6-dihydrothieno[2,3-*h*]isoquinoline derivatives 21a-d

To a solution of either compound **20a** (3.04 g, 0.01 mol) or **20b** (3.50 g, 0.01 mol) in 1,4-dioxane (40 mL) containing triethylamine (1.00 mL) either malononitrile (0.66 g, 0.01 mol) or ethyl cyanoacetate (1.07 g, 0.01 mol) was added. The reaction mixture, in each case, was heated under reflux for 1 h then poured onto ice/water containing a few drops of hydrochloric acid and the formed solid product was collected by filtration.

2.1.11.1. 3,8-Diamino-1-(trichloromethyl)-5,6-dihydrothieno[2,3-*h*]isoquinoline-4,9-dicarbonitrile (21a)

Pale brown crystals from 1,4-dioxane, yield (2.95 g, 77 %), m.p > 300 °C. IR (KBr) υ max cm^{-1}: 3493-3362 (NH_2), 3050 (CH aromatic), 2223, 2220 (2CN), 1630 (C=C); ^1H NMR (DMSO-d_6, 300 MHz): δ = 2.98-2.86 (m, 4H, $2CH_2$), 4.87, 4.84 (2s, 4H, D_2O exchangeable, $2NH_2$); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 164.7 (C-3), 134.5, 132.4, 130.2, 129.8, 124.9, 122.6, 120.8, 120.6, 119.8 (C-1, C-4, C-4a, C-9, C-8, C-8a, C-6a, C-9a),117.1, 116.8 (2CN), 94.8 (CCl_3), 40.9, 38.6 (C-5, C-6). Anal. Calculated for $C_{14}H_8Cl_3N_5S$: C, 43.71; H, 2.10; N, 18.21; S, 8.34. Found: C, 43.52; H, 1.89; N, 17.82; S, 8.08. MS: m/e 384 (M^+, 62 %).

2.1.11.2. Ethyl 3,8-diamino-9-cyano-1-(trichloromethyl)-5,6-dihydro-thieno[2,3-*h*]iso-quinoline-4-carboxylate (21b)

Pale brown crystals from 1,4-dioxane, yield (3.17 g, 73 %), m.p 284-287 °C. IR (KBr) υ max cm^{-1}: 3482-3339 (NH_2), 3050 (CH aromatic), 2220 (CN), 1688 (CO), 1630 (C=C); ^1H NMR (DMSO-d_6, 300 MHz): δ = 1.12 (t, 3H, *J* = 6.47 Hz, $OCH_2\underline{CH_3}$), 2.84-2.96 (m,

4H, $2CH_2$), 4.22 (q, 2H, J = 6.47 Hz, $O\underline{CH_2}CH_3$), 4.86, 4.86 (2s, 4H, D_2O exchangeable, $2NH_2$); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 168.2 (CO ester), 164.8 (C-3), 133.6, 130.3, 128.0, 127.2, 124.7, 123.7, 122.7, 120.8, 119.6 (C-1, C-4, C-4a, C-9, C-8, C-8a, C-6a, C-9a), 116.9 (CN), 94.5 (CCl_3), 50.6 ($O\underline{CH_2}CH_3$), 40.6, 38.8 (C-5, C-6), 16.8 ($OCH_2\underline{CH_3}$). Anal. Calculated for $C_{16}H_{13}Cl_3N_4O_2S$: C, 44.51; H, 3.04; N, 12.98; S, 7.43. Found: C, 44.72; H, 3.29; N, 13.18; S, 7.72. MS: m/e 431 (M^+, 54 %).

2.1.11.3. Ethyl 3,8-diamino-4-cyano-1-(trichloromethyl)-5,6-dihydro-thieno[2,3-*h*]iso-quinoline-9-carboxylate (21c)

Pale brown crystals from 1,4-dioxane, yield (2.58 g, 60 %), m.p 179-182 °C. IR (KBr) υ max cm^{-1}: 3459-3321 (NH_2), 3050 (CH aromatic), 2220 (CN), 1689 (CO), 1630 (C=C); ^1H NMR (DMSO-d_6, 300 MHz): δ = 4.88, 4.84 (2s, 4H, D_2O exchangeable, $2NH_2$), 4.21 (q, 2H, J = 7.25 Hz, $O\underline{CH_2}CH_3$), 2.98-2.82 (m, 4H, $2CH_2$), 1.13 (t, 3H, J = 7.25 Hz, $OCH_2\underline{CH_3}$); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 168.6 (CO ester), 164.5 (C-3), 116.8 (CN), 133.9, 131.2, 128.5, 127.6, 125.2, 123.9, 122.8, 120.6, 120.3 (C-1, C-4, C-4a, C-9, C-8, C-8a, C-6a, C-9a), 94.7 (CCl_3), 50.3 ($O\underline{CH_2}CH_3$), 40.8, 38.6 (C-5, C-6), 16.9 ($OCH_2\underline{CH_3}$). Anal. Calculated for $C_{16}H_{13}Cl_3N_4O_2S$: C, 44.51; H, 3.04; N, 12.98; S, 7.43. Found: C, 44.72; H, 3.29; N, 13.18; S, 7.72. MS: m/e 431 (M^+, 54 %).

2.1.11.4. Diethyl 3,8-diamino-1-(trichloromethyl)-5,6-dihydrothieno[2,3-h]isoquinoline-4,9-dicarboxylate (21d)

Pale brown crystals from 1,4-dioxane, yield (2.58 g, 60 %), m.p 179-182 °C. IR (KBr) υ max cm^{-1}: 3459-3321 (NH_2), 3050 (CH aromatic), 2220 (CN), 1689 (CO), 1630 (C=C); ^1H NMR (DMSO-d_6, 300 MHz): δ = 4.88, 4.84, (2s, 4H, D_2O exchangeable, $2NH_2$), 4.23, 4.21 (2q, 4H, J = 5.80, 7.25 Hz, two $O\underline{CH_2}CH_3$), 2.98- 2.82 (m, 4H, $2CH_2$), 1.13, 1.12 (2t, 6H, J = 5.80, 7.25 Hz, two $OCH_2\underline{CH_3}$); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 168.6, 167.2 (2CO ester), 164.5 (C-3), 133.9, 131.2, 128.5, 127.6, 125.2, 123.9, 122.8, 120.6, 120.3 (C-1, C-4, C-4a, C-9, C-8, C-8a, C-6a, C-9a), 116.8 (CN), 94.7 (CCl_3), 50.6, 50.3 (two $O\underline{CH_2}CH_3$), 40.8, 38.6 (C-5, C-6), 16.9, 16.7 (two $OCH_2\underline{CH_3}$), Anal. Calculated for $C_{18}H_{18}Cl_3N_8O_4S$: C, 45.16; H, 3.79; N, 8.78; S, 6.70. Found: C, 44.92; H, 3.59; N, 8.92; S, 6.82. MS: m/e 478 (M^+, 54 %).

2.1.15. General procedure for the synthesis of the 1-hydroxy-5,6-dihydro-thieno[2,3-*h*]isoquinoline derivatives 22a-d

A solution of either **21a** (3.86 g, 0.01 mol), **21b** (4.29 g, 0.01 mol), **21c** (4.31 g, 0.01 mol) or **21d** (4.31 g, 0.01 mol) in ethanol (60 mL) containing sodium hydroxide solution (10 %, 5 mL) was heated under reflux for 4 h till ammonia gas evaluation cease. The solid product formed, in each case; upon pouring onto ice/water containing a few drops of hydrochloric acid (till pH 6) was collected by filtration.

2.1.15.1. 3,8-Diamino-1-hydroxy-5,6-dihydrothieno[2,3-h]isoquinoline-4,9-dicarbonitrile (22a)

Pale yellow crystals from 1,4-dioxane, yield (1.98 g, 66 %), m.p 220-223 °C. IR (KBr) υ max cm^{-1}: 3563-3362 (OH, NH_2), 3050 (CH aromatic), 2224, 2220 (2CN), 1630 (C=C); ^{1}H NMR (DMSO-*d*$_6$, 300 MHz): δ = 10.27 (s, 1H, D_2O exchangeable, OH), 4.89, 4.81 (2s, 4H, D_2O exchangeable, $2NH_2$), 2.96-2.83 (m, 4H, $2CH_2$); ^{13}C NMR (DMSO-*d*$_6$, 75 MHz): δ 164.8 (C-2), 133.8, 131.4, 130.6, 129.3, 125.3, 124.6, 123.8, 121.6, 120.7, 120.2 (C-1, C-4, C-4a, C-9, C-8, C-8a, C-6a, C-9a), 117.0, 116.5 (2CN), 40.7, 38.4 (C-5, C-6). Anal. Calculated for $C_{13}H_9N_5OS$: C, 55.11; H, 3.20; N, 24.72; S, 11.32. Found: C, 54.85; H, 3.59; N, 24.83; S, 11.48. MS: m/e 283 (M^+, 55 %).

2.1.15.2. Ethyl 3,8-diamino-9-cyano-1-hydroxy-5,6-dihydrothieno[2,3-*h*]isoquinoline-4-carboxylate (22b)

Pale brown crystals from 1,4-dioxane, yield (2.17 g, 66 %), m.p 189-192 °C. IR (KBr) υ max cm^{-1}: 3542-3359 (OH, NH_2), 3050 (CH aromatic), 2220 (CN), 1689 (CO), 1630 (C=C); ^{1}H NMR (DMSO-*d*$_6$, 300 MHz): δ = 10.22 (s, 1H, D_2O exchangeable, OH), 4.89, 4.84 (2s, 4H, D_2O exchangeable, $2NH_2$), 4.24 (q, 2H, *J* = 7.02 Hz, $O\underline{CH_2}CH_3$), 2.98-2.85 (m, 4H, $2CH_2$), 1.12 (t, 3H, *J* = 7.02 Hz, $OCH_2\underline{CH_3}$), ^{13}C NMR (DMSO-*d*$_6$, 75 MHz): δ 168.6 (CO ester), 164.3 (C-3), 133.8, 130.1, 128.2, 126.5, 124.1, 122.8, 122.2, 120.9, 120.6 (C-1, C-4, C-4a, C-9, C-8, C-8a, C-6a, C-9a), 116.7 (CN), 50.3 ($O\underline{CH_2}CH_3$), 40.8, 38.5 (C-5, C-6), 16.9 ($OCH_2\underline{CH_3}$). Anal. Calculated for $C_{15}H_{14}N_4O_3S$: C, 54.53; H, 4.27; N, 16.96; S, 9.71. Found: C, 54.66; H, 4.30; N, 17.16; S, 9.89. MS: m/e 330 (M^+, 28 %).

2.1.15.3. Ethyl 3,8-diamino-4-cyano-1-hydroxy-5,6-dihydrothieno[2,3-*h*]isoquinoline-9-carboxylate (22c)

Pale brown crystals from 1,4-dioxane, yield (1.98 g, 60 %), m.p 201-204 °C. IR (KBr) υ max cm^{-1}: 3539-3345 (OH, NH_2), 3050 (CH aromatic), 2220 (CN), 1689 (CO), 1630

(C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 10.22 (s, 1H, D_2O exchangeable, OH), 5.01, 4.86 (2s, 4H, D_2O exchangeable, $2NH_2$), 4.23 (q, 2H, J = 6.85 Hz, $O\underline{CH_2}CH_3$), 2.96-2.80 (m, 4H, $2CH_2$), 1.12 (t, 3H, J = 6.85 Hz, $OCH_2\underline{CH_3}$); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 168.4 (CO), 164.6 (C=N), 133.7, 132.5, 129.7, 127.9, 124.8, 123.3, 122.5, 120.4, 120.1 (C-1, C-4, C-4a, C-9, C-8, C-8a, C-6a, C-9a), 116.8 (CN), 50.5 ($O\underline{CH_2}CH_3$), 40.9, 38.2 (C-5, C-6), 16.7 ($OCH_2\underline{CH_3}$), Anal. Calculated for $C_{15}H_{14}N_4O_3S$: C, 54.53; H, 4.27; N, 16.96; S, 9.71. Found: C, 54.82; H, 4.08; N, 17.26; S, 9.87. MS: m/e 330 (M^+, 36 %).

2.1.15.4. Diethyl 3,8-diamino-1-hydroxy-5,6-dihydrothieno[2,3-h]isoquinoline-4,9-dicarboxylate (22d)

Pale brown crystals from 1,4-dioxane, yield (2.58 g, 60 %), m.p 179-182 °C. IR (KBr) υ max cm^{-1}: 3559-3321 (NH_2), 3050 (CH aromatic), 2220 (CN), 1689 (CO), 1630 (C=C); ^{1}H NMR (DMSO-d_6, 300 MHz): δ = 10.21 (s, 1H, D_2O exchangeable, OH), 4.84, 4.80 (2s, 4H, D_2O exchangeable, $2NH_2$), 4.24, 4.21 (q, 2H, J = 6.39, 7.25 Hz, two $O\underline{CH_2}CH_3$), 2.98-2.82 (m, 4H, $2CH_2$), 1.13, 1.12 (2t, 6H, J = 6.39, 7.25 Hz, two $OCH_2\underline{CH_3}$); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 169.0, 168.6 (two CO ester) 164.5 (C-3), 133.9, 131.2, 128.5, 127.6, 125.2, 123.9, 122.8, 120.6, 120.3 (C-1, C-4, C-4a, C-9, C-8, C-8a, C-6a, C-9a), 116.8 (CN), 50.3, 50.1 (two $O\underline{CH_2}CH_3$), 40.8, 38.6 (C-5, C-6), 16.9, 16.6 (two $OCH_2\underline{CH_3}$). Anal. Calculated for $C_{17}H_{19}N_3O_5S$: C, 54.10; H, 5.07; N, 11.13; S, 8.50. Found: C, 54.26; H, 4.85; N, 11.26; S, 8.79. MS: m/e 377 (M^+, 68 %).

2.1.16. General procedure for the synthesis of the 5,6-dihydrothieno[2,3-*h*]isoquinoline derivatives 24a-h

To a solution of either **21a** (3.86 g, 0.01 mol), **21b** (4.29 g, 0.01 mol), **21c** (4.31 g, 0.01 mol) or **21d** (4.31 g, 0.01 mol) in ethanol (60 mL) either potassium cyanide (1.28 g, 0.02 mol) or potassium thiocyanide (1.94 g, 0.01 mol) dissolved in water (10 ML) was added drop-wise. After complete addition, the whole mixture, in each case, was heated in a water bath at 60 ºC for 2 h then was poured onto ice/water mixture containing a few drops of hydrochloric acid and the formed solid product was collected by filtration.

2.1.16.1. 3,8-Diamino-5,6-dihydrothieno[2,3-*h*]isoquinoline-1,4,9-tricarbonitrile (24a)

Pale brown crystals from 1,4-dioxane, yield (1.69 g, 58 %), m.p 266-268 °C. IR (KBr) υ max cm^{-1}: 3469-3341 (NH_2), 3045 (CH aromatic), 2223-2220 (3CN), 1630 (C=C); ^{1}H

NMR (DMSO-d_6, 300 MHz): δ = 2.86-2.98 (m, 4H, $2CH_2$), 4.84, 4.87 (2s, 4H, D_2O exchangeable, $2NH_2$); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 164.7 (C-3), 133.5, 132.6, 129.4, 127.8, 124.8, 122.7, 121.5, 120.9, 120.6 (C-1, C-4, C-4a, C-9, C-8, C-8a, C-6a, C-9a), 117.2, 117.1, 116.5 (3CN), 40.8, 38.4 (C-5, C-6), Anal. Calculated for $C_{14}H_8N_6S$: C, 57.52; H, 2.76; N, 28.75; S, 10.97. Found: C, 57.69; H, 2.80; N, 28.66; S, 10.57. MS: m/e 292 (M^+, 58 %).

2.1.16.2. Ethyl 3,8-diamino-1,9-dicyano-5,6-dihydrothieno[2,3-*h*]isoquinoline-4-carboxylate (24b)

Pale yellow crystals from 1,4-dioxane, yield (2.10 g, 62 %), m.p 180-184 °C. IR (KBr) υ max cm^{-1}: 3488-3331 (NH_2), 3050 (CH aromatic), 2224, 2220 (2CN), 1689 (CO), 1630 (C=C); ^1H NMR (DMSO-d_6, 300 MHz): δ = 4.97, 4.84 (2s, 4H, D_2O exchangeable, $2NH_2$), 4.22 (q, 2H, J = 6.41 Hz, $O\underline{CH_2}CH_3$), 2.98-2.83 (m, 4H, $2CH_2$), 1.14 (t, 3H, J = 6.41 Hz, $OCH_2\underline{CH_3}$); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 168.8 (CO ester), 164.8 (C-3), 133.9, 132.1, 128.3, 126.8, 124.3, 123.6, 121.9, 120.8, 120.4 (C-1, C-4, C-4a, C-9, C-8, C-8a, C-6a, C-9a), 117.0, 116.3 (2CN), 50.3 ($O\underline{CH_2}CH_3$), 40.7, 38.4 (C-5, C-6), 16.8 ($OCH_2\underline{CH_3}$). Anal. Calculated for $C_{16}H_{13}N_5O_2S$: C, 56.63; H, 3.86; N, 20.64; S, 9.45. Found: C, 56.80; H, 3.96; N, 20.80; S, 9.62. MS: m/e 339 (M^+, 63 %).

2.1.16.3. Ethyl 3,8-diamino-1,4-dicyano-5,6-dihydrothieno[2,3-*h*]isoquinoline-9-carboxylate (24c)

Pale yellow crystals from 1,4-dioxane, yield (2.03 g, 60 %), m.p 222-225 °C. IR (KBr) υ max cm^{-1}: 3488-3331 (NH_2), 3050 (CH aromatic), 2223, 2220 (2CN), 1689 (CO), 1630 (C=C); ^1H NMR (DMSO-d_6, 300 MHz): δ = 4.99, 4.86 (2s, 4H, D_2O exchangeable, $2NH_2$), 4.23 (q, 2H, J = 7.22 Hz, $O\underline{CH_2}CH_3$), 2.96-2.81 (m, 4H, $2CH_2$), 1.13 (t, 3H, J = 7.22 Hz, $OCH_2\underline{CH_3}$); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 168.8 (CO ester), 164.9 (C-3), 133.9, 132.1, 128.1, 126.2, 124.1, 123.8, 121.9, 120.8, 120.1 (C-1, C-4, C-4a, C-9, C-8, C-8a, C-6a, C-9a), 117.0, 116.3 (2CN), 50.4 ($O\underline{CH_2}CH_3$), 40.8, 38.4 (C-5, C-6), 16.2 ($OCH_2\underline{CH_3}$), Anal. Calculated for $C_{16}H_{13}N_5O_2S$: C, 56.63; H, 3.86; N, 20.64; S, 9.45. Found: C, 56.49; H, 3.77; N, 20.41; S, 9.53. MS: m/e 339 (M^+, 44 %).

2.1.16.4. Diethyl 3,8-diamino-1-cyano-5,6-dihydrothieno[2,3-h]isoquinoline-4,9-dicarboxylate (24d)

Yeloow crystals from 1,4-dioxane, yield (2.70 g, 70 %), m.p 177-180 °C. IR (KBr) υ max cm^{-1}: 3493-3352 (NH_2), 3050 (CH aromatic), 2220 (CN), 1689 (CO), 1630 (C=C); ^1H NMR (DMSO-d_6, 300 MHz): δ = 4.97, 4.84 (2s, 4H, D_2O exchangeable, $2NH_2$), 4.24, 4.22 (2q, 4H, J = 6.80, 7.51 Hz, two O$\underline{CH_2}CH_3$), 2.97-2.83 (m, 4H, $2CH_2$), 1.14, 1.12 (2t, 6H, J = 6.80, 7.51 Hz, two $OCH_2\underline{CH_3}$); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 170.1, 168.9 (two CO ester), 164.7 (C-3), 134.2, 131.7, 128.5, 125.8, 124.2, 123.4, 121.6, 120.6, 120.3 (C-1, C-4, C-4a, C-9, C-8, C-8a, C-6a, C-9a), 117.1 (CN), 50.4, 50.1 (two O$\underline{CH_2}CH_3$), 40.9, 38.2 (C-5, C-6), 16.5, 16.2 (two $OCH_2\underline{CH_3}$). Anal. Calculated for $C_{18}H_{18}N_4O_4S$: C, 55.95; H, 4.70; N, 14.50; S, 8.30. Found: C, 56.25; H, 4.59; N, 14.73; S, 8.62. MS: m/e 386 (M^+, 36 %).

2.1.16.5. 3,8-Diamino-1-thiocyanato-5,6-dihydrothieno[2,3-*h*]isoquinoline-4,9-dicarbonitrile (24e)

Pale brown crystals from 1,4-dioxane, yield (2.52 g, 78 %), m.p 243-247 °C. IR (KBr) υ max cm^{-1}: 3482-3326 (NH_2), 3045 (CH aromatic), 2224-2220 (3CN), 1630 (C=C); ^1H NMR (DMSO-d_6, 300 MHz): δ = 4.86, 4.82 (2s, 4H, D_2O exchangeable, $2NH_2$), 2.96-2.84 (m, 4H, $2CH_2$); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 164.8 (C-3), 133.9, 132.8, 129.6, 127.3, 123.2, 122.1, 121.8, 120.7, 120.4 (C-1, C-4, C-4a, C-9, C-8, C-8a, C-6a, C-9a), 40.6, 38.1 (C-5, C-6), 117.1, 116.4 110.8 (3CN), Anal. Calculated for $C_{14}H_8N_6S_2$: C, 51.84; H, 2.49; N, 25.91; S, 19.77. Found: C, 51.69; H, 2.63; N, 26.25; S, 19.80. MS: m/e 324 (M^+, 40 %).

2.1.16.6. Ethyl 3,8-diamino-9-cyano-1-thiocyanato-5,6-dihydrothieno[2,3-*h*]isoquinoline-4-carboxylate (24f)

Yellow crystals from 1,4-dioxane, yield (2.74 g, 74 %), m.p 170-172 °C. IR (KBr) υ max cm^{-1}: 3479-3343 (NH_2), 3050 (CH aromatic), 2221, 2220 (2CN), 1689 (CO), 1630 (C=C); ^1H NMR (DMSO-d_6, 300 MHz): δ = 4.97, 4.83 (2s, 4H, D_2O exchangeable, $2NH_2$), 4.22 (q, 2H, J = 6.84 Hz, O$\underline{CH_2}CH_3$), 2.98-2.81 (m, 4H, $2CH_2$), 1.13 (t, 3H, J = 6.84 Hz, $OCH_2\underline{CH_3}$); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 168.6 (CO ester), 164.8 (C-3), 116.8, 112.6 (2CN), 133.5, 132.4, 128.8, 126.2, 124.4, 123.9, 122.3, 120.6, 120.0 (C-1, C-4, C-4a, C-9, C-8, C-8a, C-6a, C-9a), 50.2 (O$\underline{CH_2}CH_3$), 40.6, 38.1 (C-5, C-6), 16.5 ($OCH_2\underline{CH_3}$), Anal. Calculated for $C_{16}H_{13}N_5O_2S_2$: C, 51.74; H, 3.53; N, 18.85; S, 17.27. Found: C, 52.01; H, 3.49; N, 18.63; S, 17.08. MS: m/e 371 (M^+, 32 %).

2.1.16.7. Ethyl 3,8-diamino-4-cyano-1-thiocyanato-5,6-dihydrothieno[2,3-*h*]iso-quinoline-9-carboxylate (24g)

Yellow crystals from 1,4-dioxane, yield (2.04 g, 55 %), m.p 193-196 °C. IR (KBr) υ max cm^{-1}: 3493-3329 (NH_2), 3050 (CH aromatic), 2224, 2220 (2CN), 1688 (CO), 1630 (C=C); ^1H NMR (DMSO-d_6, 300 MHz): δ = 4.97, 4.82 (2s, 4H, D_2O exchangeable, $2NH_2$), 4.23 (q, 2H, *J* = 7.12 Hz, $O\underline{CH_2}CH_3$), 2.96-2.80 (m, 4H, $2CH_2$), 1.12 (t, 3H, *J* = 7.12 Hz, $OCH_2\underline{CH_3}$); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 168.4 (CO ester), 164.7 (C-3), 132.8, 131.6, 120.3, 129.2, 126.2, 124.1, 123.9, 122.6, 121.8 (C-1, C-4, C-4a, C-9, C-8, C-8a, C-6a, C-9a), 116.6, 111.5 (2CN), 50.2 ($O\underline{CH_2}CH_3$), 40.6, 38.4 (C-5, C-6), 16.3 ($OCH_2\underline{CH_3}$). Anal. Calculated for $C_{16}H_{13}N_5O_2S_2$: C, 51.74; H, 3.53; N, 18.85; S, 17.27. Found: C, 51.91; H, 3.42; N, 18.43; S, 17.33. MS: m/e 371 (M^+, 36 %).

2.1.16.8. Diethyl 3,8-diamino-1-thiocyanato-5,6-dihydrothieno[2,3-*h*]isoquinoline-4,9-dicarboxylate (24h)

Pale yellow crystals from 1,4-dioxane, yield (2.71 g, 52 %), m.p 166-169 °C. IR (KBr) υ max cm^{-1}: 3470-3332 (NH_2), 3050 (CH aromatic), 2221, 2220 (CN), 1689 (CO), 1630 (C=C); ^1H NMR (DMSO-d_6, 300 MHz): δ = 4.97, 4.84 (2s, 4H, D_2O exchangeable, $2NH_2$), 4.23, 4.22 (2q, 4H, *J* = 6.49, 6.21 Hz, two $O\underline{CH_2}CH_3$), 2.98-2.81 (m, 4H, $2CH_2$), 1.13, 1.12 (2t, 6H, *J* = 6.49, 6.21 Hz, two $OCH_2\underline{CH_3}$); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 168.8 (CO ester), 164.5 (C-3), 133.8, 131.3, 128.2, 124.9, 124.7, 122.6, 121.9, 120.8, 120.1, (C-1, C-4, C-4a, C-9, C-8, C-8a, C-6a, C-9a), 112.3, 117.0 (2CN), 50.3, 50.6 (two $O\underline{CH_2}CH_3$), 40.8, 38.3 (C-5, C-6), 16.1, 16.4 (two $OCH_2\underline{CH_3}$), Anal. Calculated for $C_{18}H_{18}N_4O_4S_2$: C, 51.66; H, 4.34; N, 13.39; S, 15.32. Found: C, 51.49; H, 4.60; N, 13.26; S, 15.53. MS: m/e 418 (M^+, 24 %).

2.2. Biology section

2.2.1. Cell proliferation assay

The anti-proliferative activities of the newly synthesized compounds (Table 1) were evaluated against the six cancer cell lines A549, HT-29, MKN-45, U87MG, and SMMC-7721 and H460 using the standard MTT assay *in vitro*, with foretinib as the positive control.[23] The cancer cell lines were cultured in minimum essential medium (MEM) supplemented with 10% fetal bovine serum (FBS).

Approximate 4 x 10^3 cells, suspended in MEM medium, were plated onto each well of a 96-well plate and incubated in 5% CO_2 at 37 ^oC for 24 h. The compounds tested at the indicated final concentrations were added to the culture medium and the cell cultures were continued for 72 h. Fresh MTT was added to each well at a terminal concentration of 5 μg/mL, and incubated with cells at 37 ^oC for 4 h. The formazan crystals were dissolved in 100 μL of DMSO each well, and the absorbency at 492 nM (for absorbance of MTT formazan) and 630 nM (for the reference wavelength) was measured with an ELISA reader. All of the compounds were tested three times in each cell line. The results expressed as IC_{50} (inhibitory concentration 50%) were the averages of three determinations and calculated by using the Bacus Laboratories Incorporated Slide Scanner (Bliss) software.

The mean values of three independent experiments, expressed as IC_{50} values, were presented in Table 1. Most of the synthesized compounds exhibited potent anti-proliferative activity with IC_{50} values less than 30 μM. Generally, the variations of substituent's within the aryl moiety together with the hetero cycle ring being attached have a notable influence on the anti-proliferative activity.

Table 1. In vitro growth inhibitory effects IC_{50} ± SEM (μM) of the newly synthesized compounds against cancer cell lines

Compound No	IC_{50} ± SEM (μM)					
	A549	H460	HT29	MKN-45	U87MG	SMMC-7721
3a	8.72 ± 2.62	6.25 ± 3.06	7.83 ± 2.54	8.01 ± 2.41	8.72 ± 2.63	8.08 ± 3.19
3b	3.46 ± 1.29	4.53 ± 1.44	3.65 ± 1.64	2.43 ± 0.86	3.82 ± 1.06	2.63 ± 1.16
4a	5.83±1.43	6.73±2.54	3.29 ±1.13	2.62 ± 0.74	4.80 ±2.43	3.78 ± 0.62
4b	7.72 ± 2.63	8.69± 2.36	6.73± 2.33	8.62 ± 1.43	7.25 ± 2.49	8.30± 3.59
6a	9.29±2.59	8.17±2.89	5.08± 1.69	4.32± 2.41	6.50± 1.52	6.30±2.83
6b	0.32 ± 0.20	0.34 ±0.13	0.52 ± 0.24	0.45 ± 0.12	0.53 ± 0.25	0.39 ± 0.14
7a	5.63 ± 1.28	3.49 ± 1.28	5.46 ± 2.36	6.05 ± 2.47	4.29± 1.59	6.07 ± 2.62
7b	1.26 ± 0.85	0.99 ± 0.63	0.86 ± 0.49	0.32± 0.19	0.68 ± 0.19	0.80 ± 0.38
10	8.24 ± 3.68	6.26 ± 2.34	5.29 ± 2.89	6.27 ± 1.29	3.83 ± 1.53	5.59 ± 2.32
12a	6.28 ±1.78	4.83 ± 1.23	4.70 ± 1.20	6.73 ± 2.30	5.82 ± 2.69	6.49 ± 2.28
12b	0.34 ± 0.21	0.69 ± 0.30	0.53 ± 0.32	0.28 ± 2.39	0.42 ± 0.29	0.52 ± 0.26
14	0.52 ± 0.13	0.83 ± 0.20	0.71 ± 1.82	0.26 ± 0.12	0.60 ± 0.21	0.15 ± 0.02

16	0.31 ± 0.22	0.13 ± 0.07	0.22 ± 0.16	0.32 ± 0.17	0.42 ± 0.19	0.36 ± 0.15
18a	1.63 ± 0.23	1.63 ± 0.34	1.08 ± 0.81	0.92 ± 0.63	0.90 ± 0.71	1.31 ± 0.80
18b	0.62 ± 0.39	0.72 ± 0.53	0.39± 0.26	0.49 ± 0.26	0.58 ± 0.19	0.64 ± 0.28
20a	1.26 ± 0.69	1.38 ± 0.99	1.79 ± 0.82	0.96 ± 0.42	0.86 ± 0.26	0.57 ± 0.30
20b	0.30 ± 0.19	0.24 ±0.10	0.43 ± 0.27	0.52 ± 0.18	0.23 ± 0.0.8	0.32 ± 0.17
21a	1.16 ±0.75	1.80 ± 0.69	1.25 ± 0.48	2.04 ± 0.38	1.90 ± 0.58	1.49 ± 0.78
21b	0.31 ± 0.20	0.39 ± 0.12	0.23 ± 0.06	0.23 ± 0.06	0.28 ± 0.16	0.56 ± 0.23
21c	0.58 ± 0.17	0.52 ± 0.23	0.62 ± 0.22	0.42 ± 0.19	0.53 ± 0.25	0.72 ± 0.19
21d	0.49 ± 0.21	0.52 ± 0.15	0.46 ± 0.19	0.50 ± 0.27	0.70 ± 0.25	0.38 ± 0.18
22a	1.02 ± 0.72	1.26 ± 0.59	1.42 ± 0.69	1.26 ± 0.82	0.86 ± 0.31	1.63 ± 0.82
22b	1.12 ± 0.69	1.04 ± 0.80	1.36± 0.88	1.26 ± 0.73	2.13 ± 1.79	0.85 ± 0.41
22c	0.26 ± 0.19	0.35 ± 0.16	0.42 ± 0.27	0.19 ± 0.02	0.36 ± 0.18	0.28 ± 0.06
22d	0.32 ± 0.18	0.26 ± 0.13	0.51 ± 0.29	0.35 ± 0.29	0.28 ± 0.06	0.18 ± 0.07
24a	1.44± 0.86	1.22 ± 0.76	0.89 ± 0.54	0.95 ± 0.63	0.86 ± 0.39	1.39 ± 2.28
24b	1.05 ±0.61	1.70 ±0.72	0.96 ± 0.26	0.83 ± 0.39	1.46 ± 0.79	1.36 ± 0.87
24c	2.82 ± 0.93	1.69 ± 0.93	1.38 ± 0.62	0.79 ± 0.42	0.89 ± 0.32	1.67 ± 0.58
24d	0.27 ± 0.12	0.38 ± 0.09	0.52 ± 0.28	0.38 ± 0.19	0.46 ± 0.21	0.39 ± 0.18
24e	1.66 ±0.26	1.42 ± 0.73	1.39 ± 0.86	0.72 ± 0.63	0.68 ± 0.31	1.41 ± 0.89
24f	0.87 ± 0.32	0.69 ± 0.28	0.39± 0.21	0.62 ± 0.28	0.72 ± 0.39	0.83 ± 0.36
24g	0.77 ± 0.26	0.82 ± 0.31	0.59 ± 0.82	0.32 ± 0.17	0.32 ± 0.18	0.21 ± 0.05
24h	0.53 ± 0.14	0.61 ±0.28	0.42 ± 0.21	0.62 ± 0.18	0.60 ± 0.32	0.59 ± 0.15
Foretinib	0.08 ± 0.01	0.18 ± 0.03	0.15 ± 0.023	0.03±0.0055	0.90 ± 0.13	0.44 ± 0.062

2.2.3. Structure activity relationship

It is clear from Table 1 that most of the tested compounds have high inhibitions toward the six cancer cell lines. Considering the 5,6,7,8-tetrahydronaphthalene derivatives **3a** and **3b**, it is clear that compound **3b** (X = OH, R = COOEt) that is an oxygen rich compound has higher inhibition than **3a** (X = NH_2, R = CN). Reaction of either compound **3a** or **3b** with acetic acid and acetic anhydride gave the acetylated derivatives **4a** and **4b**, respectively where both of two compounds showed moderate inhibition, surprisingly, compound **3a** exhibited higher inhibition than **4b**. For the 1,2,3,4,7,8,9,10-octahydrobenzo[*h*]quinazoline **6a** and the 3,4,7,8,9,10-hexahydro-2H-naphtho[2,1-*e*][1,3]oxazine **6b**, it is obvious that compound **6b** (Y = O, R = COOEt) showed higher inhibitions toward the six cancer cell lines than that **6a** (Y = NH, R = CN). The same

findings was noticed after hydrolysis of the exocyclic C=NH group present in **6a** and **6b** into C=O where compound **7b** exhibited more inhibitions than **7a**. The 7,8-dihydro-4H-benzo[*e*][1,3]oxazine derivative **10** exhibited low inhibitions. The reaction of compound **10** with either malononitrile or ethyl cynoacetate and elemental sulfur produces the thieno[2',3':5,6]benzo[1,2-*e*][1,3]oxazine derivatives **12a** and 1**2b**, respectively. It is obvious from Table 1 that compound **12b** (R = COOEt) with higher inhibitions than compound **12a** (R = CN). The reaction of cyclohexan-1,3-dione with trichloroacetonitrile gave the 2,2,2-trichloroethylidene)-cyclohexane-1,3-dione derivative **14** which exhibited high inhibitions toward the six cancer cell lines. Its conversion into the 2,3,6,7-tetrahydroquinazoline derivative **16** through its reaction with phenylisothiocyanate support the inhibition of the molecule where compound **16** showed high inhibitions. Increasing the nitrogen content of 16 through its reaction with either hydrazine hydrate or phenylhydrazine to give either **18a** or **18b**, respectively resulting also high cytotoxicities. Moreover, compound **18b** (R = Ph) was more cytotoxic than **18a** (R = H). The same argument appeared in case of **20a** (R = CN) and **20b** (R = COOEt) where the latter showed higher cytotoxicities than the first. Considering the 5,6-dihydrothieno[2,3-*h*]isoquinoline derivatives **21a-d**, for which**21b** (R_1 = CN, R_2 = COOEt), **21c** (R_1 = COOEt, R_2 = CN) and **21d** (R_1 = R_2 = COOEt) were of high inhibitions toward the six cancer cell lines. However, in case of the hydroxyl derivatives **22a-d** only compounds **22c** and **22d** were the most cytotoxic compounds. Finally, for the nucleophilic substituted compounds with the CN or the SCN moieties to give the eight compounds **24a-h**, all of them exihibited high inhibitions. However, compounds **24a**, **24b**, **24c** and **24e** showed from moderate to high inhibitionstogether with compounds **24d**, **24f**, **24g** and **24h** exhibited the high inhibitions. It is of great value to mention that compounds **6b**, **7b**, **12b**, **14**, **16**, **18b**, **20b**, **21b**, **21c**, **21d**, **22c**, **22d**, **24d**, **24f**, **24g** and **24h** were the most cytotoxic compounds among the tested compounds. On the other hand, compounds **3b**, **7a**, **18a**, **20a**, **21a**, **22a**, **22b**, **24a**, **24b**, **24c** and **24e** have moderate inhibitions.

2.2.4. HTRF kinase assay

c-Met (mesenchymal epithelial transition factor) is a multifunctional transmembrane tyrosine kinase and acts as a receptor for hepatocyte growth

factor/Scatter factor (HGF/SF).[24]It is expressed during embryogenesis in multiple epithelial tissues (liver, pancreas, prostate, kidney, muscle, bone-marrow) and is also discovered in numerous tumor cell communities on the cell surface.[25] Multiple oncogenetic characteristics of c-Met were outlined shortly after its discovery, including cell dissociation stimulation, migration, motility, and extracellular matrix invasion.[26,27] Moreover, c-Met kinase activity has been revealed to be correlated with prostate cancer where c-Met played a key role in the conversion of prostate cancer from the primary androgen-sensitive to androgen-insensitive status along with the increase in radioresistance. Firstly, an inverse relationship between the expression of androgen receptor (AR) and c-Met has been observed in prostate epithelium and prostate cancer cells[28] Secondly, AR signaling suppressed c-Met transcription while androgen removal improved the expression of c-Met.[29] Thirdly, it is observed that c-Met expression is high in late stage bone metastatic prostate cancer.30Furthermore, a latest research has shown that c-Met expression is closely related to cellular radiosensitivity.[30]

Based on these reported observations, the c-Met kinase activity of all compounds was evaluated using homogeneous time-resolved fluorescence (HTRF) assay as previously reported.[31] Taking foretinib as the positive control, the results expressed as IC_{50} were summarized in Table 2. The anti-proliferative activity of all target compounds against the human prostatic cancer PC-3 cell line was measured by MTT assay using anibamine as the reference drug. The mean values of three independent experiments, expressed as IC_{50} values, were presented in Table 1. Generally, the variations of substituents within the aryl moiety together with the heterocyclic ring being attached have a notable influence on the anti-proliferative activity.

HTRF assay utilizes the signal generated by the fluorescence resonance energy transfer between donor and acceptor molecules in close proximity. Dual-wavelength detection helps to eliminate media interference, and the final signal is proportional to the extent of product formation. Thus far, the reported applications of this technology for in vitro kinase assays have mainly focused on high-throughput screening. The MTT assay is a colorimetric assay for assessing cell

metabolic activity. NAD(P)H-dependent cellular oxidoreductase enzymes may, under defined conditions, reflect the number of viable cells present. These enzymes are capable of reducing the tetrazolium dye MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide to its insoluble formazan, which has a purple colour. Other closely related tetrazolium dyes including XTT, MTS and the WSTs, are used in conjunction with the intermediate electron acceptor, 1-methoxy phenazine methosulfate (PMS). With WST-1, which is cell-impermeable, reduction occurs outside the cell via plasma membrane electron transport. However, this traditionally assumed explanation is currently contended as proof has also been found of MTT reduction to formazan in lipidic cellular structures without apparent involvement of oxidoreductases. Tetrazolium dye assays can also be used to measure cytotoxicity (loss of viable cells) or cytostatic activity (shift from proliferation to quiescence) of potential medicinal agents and toxic materials. MTT assays are done in the dark since the MTT reagent is sensitive to light. Within this protocol, replace serum-containing media with serum-free media and MTT reagent in cell cultures incubate for 3 hr at 37 ºC add MTT solvent and incubate for 15 min analyze with microplate reader.

As illustrated in Table 2, all the tested compounds displayed potent c-Met enzymatic activity with IC_{50} values ranging from 0.21 to 8.41 nM and potent prostate PC-3 cell line inhibition with IC_{50} values ranging from 0.26 to 8.46 μM. Compared with foretinib (IC_{50} = 1.16 nM), the ten compounds (**3b**, **4a**, **4b**, **14**, **16**, **18b**, **20b**, **21c**, **22b**, **22d** and **24c**) exhibited higher potency with IC_{50} values less than 1.00 nM. Remarkably, among the synthesized compounds, **3a**, **3b**, **4a**, **4b**, **6a**, **6b**, **10**, **2a**, **12b**, **14**, **16**, **18b**, **20a**, **20b**, **21c**, **21d**, **22a**, **22b**, **22c**, **24c**, **24d**, **24f** and **24h** displayed much higher anti-proliferation activities against PC-3 cell line than the standard anibamine (IC_{50} = 3.26 μM).

All synthesized compounds were tested against the VERO, Monkey Kidney normal cell line, where they showed low activity against the normal cell line. Interestingly, from Table 2 compounds **3a**, **22c**, **22d** and **24b** showed SI > 30 while the fourteen compounds **3b**, **4b**, **6a**, **6b**, **12b**, **14**, **16**, **18b**, **20b**, **21d**, **22b**, **24c**, **24f** and **24h** exhibited SI > 100 with the rest of compounds showed SI < 30.

Table 2. c-Met enzymatic activity and PC-3 inhibition of the newly synthesized compounds.

Compound No	IC_{50} (nM) c-Met	IC_{50}(μM) PC-3	VERO[a] (μM)	SI PC-3[b]
3a	1.42 ± 0.80	1.73 ± 0.73	58.41±6.32	33.76
3b	0.31 ± 0.16	0.25 ± 0.17	55.61±6.24	> 100
4a	0.54 ± 0.16	2.31 ± 0.92	36.22±6.27	15.68
4b	0.32 ± 0.21	0.28 ± 0.23	50.68 ± 6.14	> 100
6a	4.16±1.83	0.26 ±0.10	39.56 ± 6.31	> 100
6b	4.28 ± 1.80	0.30 ± 2.53	58.23 ±5.16	> 100
7a	6.27 ±2.19	8.46 ±2.24	36.69 ± 8.12	4.37
7b	2.47 ± 0.88	4.05 ± 1.82	58.36 ±6.27	14.41
10	4.72±1.83	1.26 ±0.97	32.28 ±5.71	25.62
12a	8.41 ± 2.53	2.82 ± 1.03	58.27 ±5.80	20.66
12b	1.33 ± 0.78	0.29± 0.06	60.81 ± 7.26	> 100
14	0.29 ±0.09	0.26±0.18	58.32 ±6.93	> 100
16	0.63 ±0.42	0.29 ±0.13	60.35 ± 6.56	> 100
18a	4.51 ±1.86	6.41±2.20	63.40 ± 8.27	9.89
18b	0.82± 0.32	0.42 ± 0.23	60.22 ±7.32	> 100
20a	2.46 ±1.30	2.80 ±1.01	56.32 ±6.57	20.11
20b	0.49 ± 0.21	0.53 ± 0.12	65.43±6.81	> 100
21a	3.65 ± 1.83	4.82 ± 1.26	40.41±8.32	8.38
21b	4.82 ± 1.16	3.20 ± 1.68	30.23±7.19	9.44
21c	0.22 ± 0.13	0.38 ± 0.16	42.53±6.63	> 100
21d	1.18±0.92	0.24 ±0.07	40.53 ± 5.63	> 100
22a	5.28± 1.47	2.79 ± 1.01	60.29 ±8.20	21.61
22b	0.36 ±0.14	0.42±0.09	42.49±6.53	> 100
22c	1.81 ± 0.96	1.48 ± 0.79	56.27 ±8.93	38.02
22d	0.36±0.18	0.63 ±0.17	36.58 ±5.30	58.06
24a	6.36 ± 2.31	5.57 ± 1.29	60.47 ±6.93	10.86
24b	1.42 ± 0.80	1.73 ± 0.73	58.41±6.32	33.76
24c	0.21 ± 0.05	0.39 ± 0.15	58.37±6.19	> 100
24d	2.58 ± 0.80	4.18 ± 1.48	48.26±5.39	11.54

24e	2.68 ± 1.72	3.80 ± 1.49	58.01 ± 5.77	15.26
24f	1.18±0.89	0.24 ±0.11	54.52 ± 6.70	> 100
24g	2.37 ± 1.16	4.93 ± 1.77	38.73 ± 4.83	7.85
24h	1.32±0.93	0.28 ±0.17	60.72 ± 8.19	> 100
	Foretinib 1.16 ± 0.17	**Anibamine** 3.26 ± 0.35	**-**	**-**

[a] VERO, Monkey Kidney cell line (Cat No-11095–080).
[b] Selectivity index (SI) were calculated by IC_{50} values in normal cell line divided by IC_{50} values in PC-3 cancer cell line.

2.2.5. Inhibitory effect of the most active compounds towards tyrosine kinases

The most active compounds that showed the highest inhibitions toward the six cancer cell lines were further evaluated against other five tyrosine kinases (c-Kit, Flt-3, VEGFR-2, EGFR, and PDGFR) using the same screening method (Table 3). These receptor tyrosine kinases (RTKs) have been implicated in vascular development by affecting the proliferation and migration of endothelial cells or pericytes. It is clear from Table 3 that compounds **7b**, **12b**, **16**, **20b**, **21b**, **22c**, **22d**, **24d**, **24f** and **24h** were the most potent towards the five tyrosine kinases. Compound **25g** showed high inhibitions towards the four kinases Flt-3 and VEGFR-2 with IC_{50}'s 0.32 and 0.29 nM, respectively while it showed moderate inhibition towards c-Kit and EGFR with IC_{50} 1.52 and 1.93 nM, respectively. Compound **24d** was the most active compound against Flt-3 kinase with IC_{50} 0.17 nM, respectively. Compounds **12b**, **16** and **24f** were the most active toward PDGFR with IC_{50}'s 0.28, 0.26 and 0.27 nM, respectively. Compounds **6b**, **18b** and **21c** showed the lowest potency among the tested compounds.

Table 3. Inhibition of tyrosine kinases [Enzyme IC_{50} (nM)] by compounds **6b**, **7b**, **12b**, **14**, **16**, **18b**, **20b**, **21b**, **21c**, **21d**, **22c**, **22d**, **24d**, **24f**, **24g** and **24h**

Compound	c-Kit	Flt-3	VEGFR-2	EGFR	PDGFR
6b	1.80	2.43	1.72	2.93	1.05
7b	0.21	0.17	0.23	0.26	0.42
12b	0.30	0.51	0.29	0.33	0.28

14	1.08	2.62	1.17	2.39	1.52
16	0.28	0.16	0.52	0.74	0.26
18b	1.16	2.39	1.12	2.83	1.29
20b	0.31	0.46	0.35	0.29	0.33
21b	0.41	0.28	0.26	0.42	0.50
21c	1.22	2.96	1.53	2.72	1.38
22c	0.38	0.29	0.52	0.41	0.70
22d	0.27	0.25	0.41	0.66	0.37
24d	0.17	0.26	0.50	0.61	0.39
24f	0.25	0.36	0.42	0.36	0.27
24g	1.52	0.32	0.29	1.93	2.53
24h	0.22	0.26	0.36	0.28	0.37

2.2.6. Inhibition of selected compounds towards Pim-1 kinase

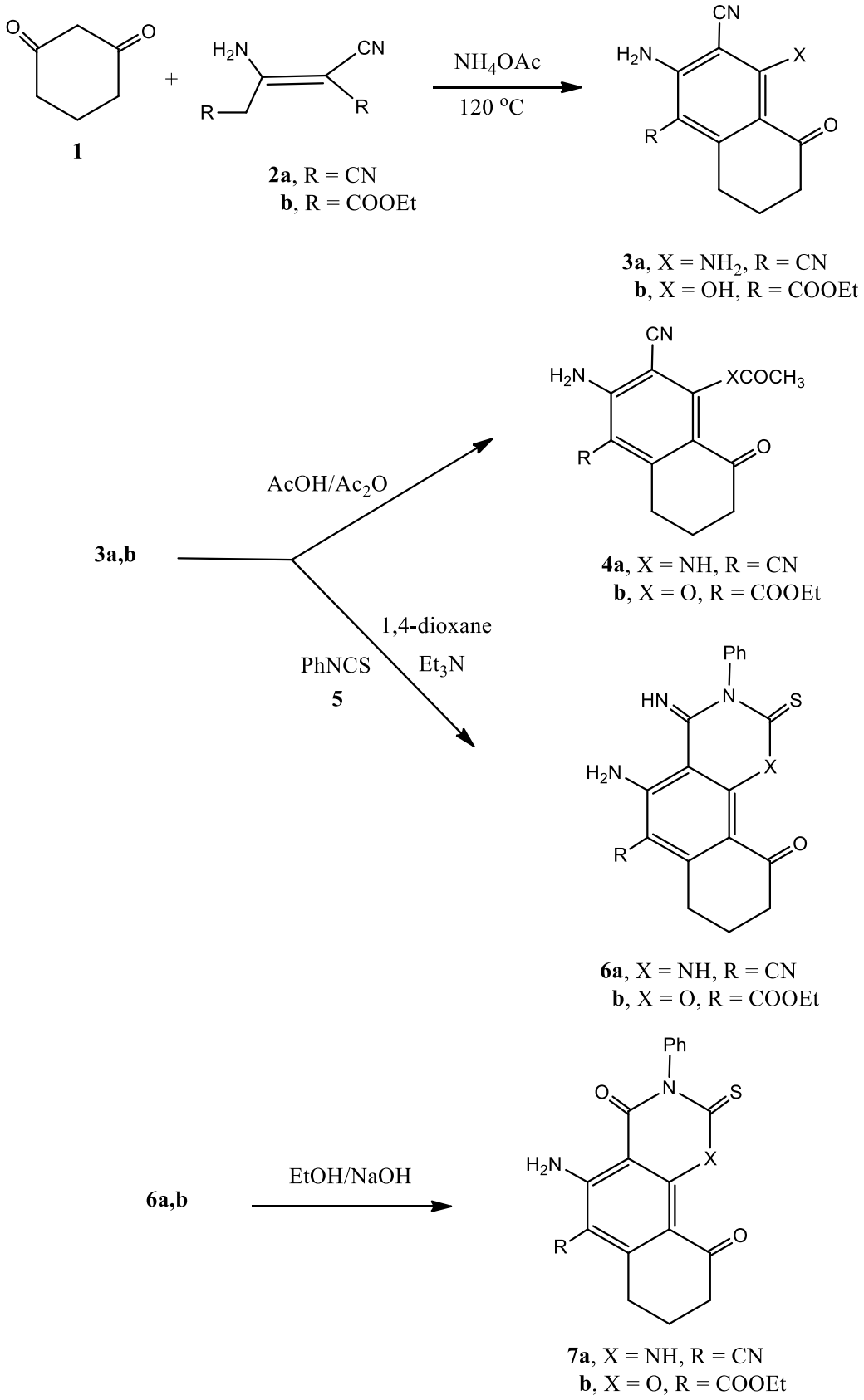
Compounds **7b**, **12b**, **16**, **20b**, **21b**, **22c**, **22d**, **24d**, **24f** and **24h** were selected to examine their Pim-1 kinase inhibition activity (Table 4) as these compounds showed high inhibition towards the tested cancer cell lines at a range of 10 concentrations and the IC_{50} values were calculated.Compounds **7b**, **12b**, **16**, **22c** and **24f** were the most potent to inhibit Pim-1 kinase with IC_{50} value of 0.22, 0.28, 0.24, 0.30 and 0.26 μM, respectively. On the other hand, compounds **20b**, **21b, 22d, 24d** and **24h** were less effective (IC_{50}> 10 μM). These profiles in combination with cell growth inhibition data of compounds **7b**, **12b**, **16**, **20b**, **21b**, **22c**, **22d**, **24d**, **24f** and **24h** were listed in Table 3 indicated that Pim-1 was a potential target of these compounds where SGI-1776 was used as positive control with IC_{50} 0.048 μM in the assay.

Table 4. The inhibitor activity of compounds **7b**, **12b**, **16**, **20b**, **21b**, **22c**, **22d**, **24d**, **24f** and **24h** toward Pim-1 Kinase.

Compound	Inhibition ratio At 10 μM	IC_{50} (μM)
7b	96	0.22
12b	90	0.28
16	94	0.24
20b	22	> 10
21b	32	>10
22c	89	0.30
22d	24	> 10
24d	20	> 10
24f	92	0.26
24h	30	> 10
SGI-1776	-	0.048

3. Results and discussion

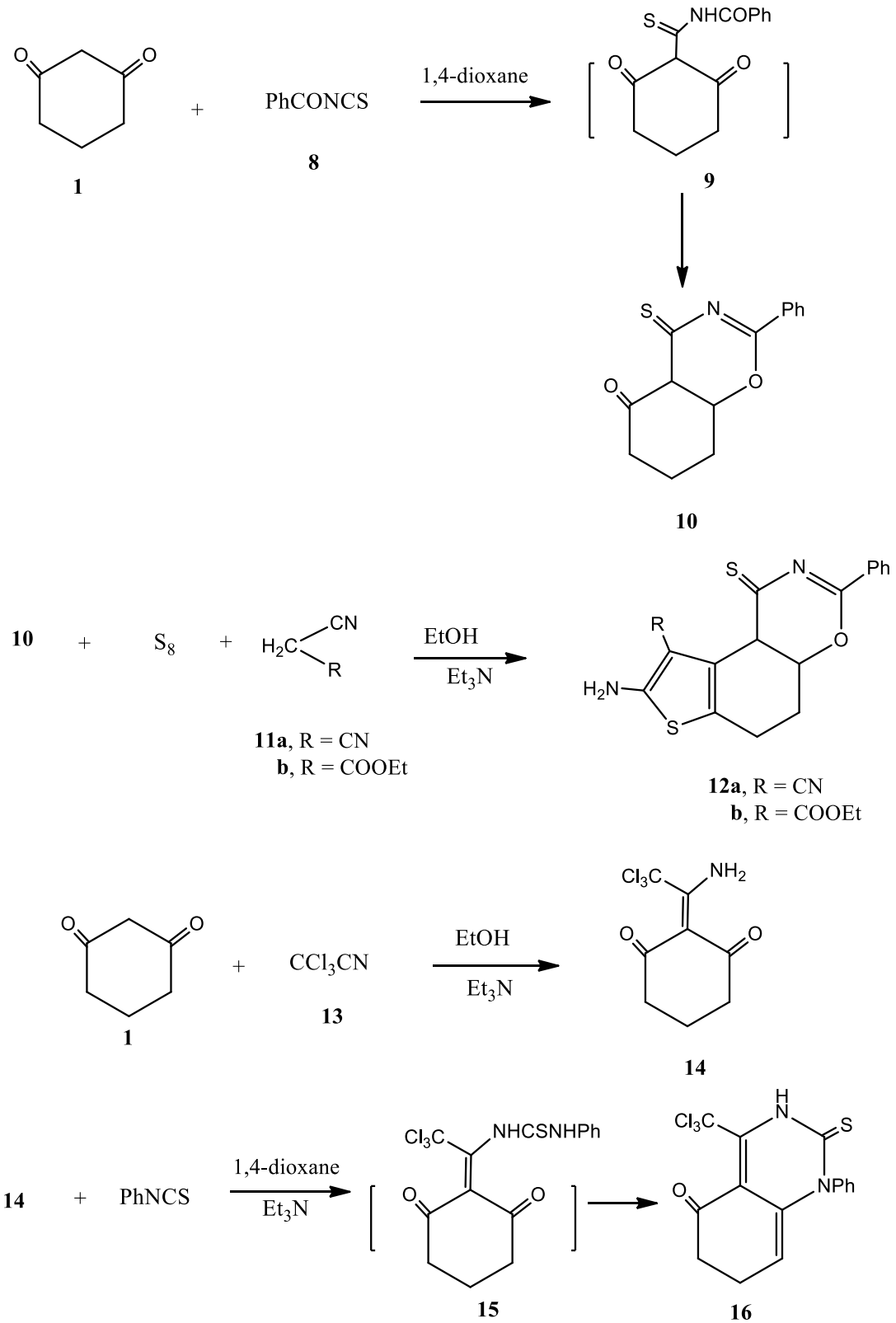
Initially cyclohexan-1,3-dione was chosen as the model substrate for the synthesis of fused heterocyclic compounds through studying its reactivity toward some dimeric compounds and nitrile reagents to produce biologically active products. Thus, we studied the condensation of cyclohexane-1,3-dione (**1**) with some dimeric compounds. Thus, the reaction of cyclohexane-1,3-dione (**1**) with either of 2-aminoprop-1-ene-1,1,3-tricarbonitrile (**2a**) or diethyl 3-amino-2-cyanopent-2-enedioate (**2b**) in the presence of a catalytic amount of ammonium acetate in an oil bath at 120 ^{o}C gave the 5,6,7,8-tetrahydronaphthalene derivatives **3a** and **3b**, respectively. The structures of the latter products were confirmed through their respective analytical and spectral data. Thus, the ^{1}H NMR spectrum of compound **3a** revealed beside the expected signals, two singlet's, D_2O exchangeable, indicating two NH_2 groups and the ^{13}C NMR spectrum showed the presence of a signal at δ 174.2 due to the C=N bonding, signals at δ 127.9, 125.6, 124.9, 123.5, 121.8, 120.4 indicating the aromatic carbons and two signals at δ116.8, 116.3 confirming the presence of the two CN groups. The latter compounds when heated in acetic acid/acetic anhydride solution gave the N-acetamido derivative**4a** and the acetate ester derivative **4b**, respectively. On the other hand the reaction of either compound **4a** or **4b** with phenylisothiocyanate (**5**) in 1,4-dioxane solution containing a catalytic amount of triethylamine gave the 1,2,3,4,7,8,9,10-octahydrobenzo[*h*]quinazoline**6a** and the 3,4,7,8,9,10-hexahydro-2*H*-naphtho[2,1-*e*][1,3]oxazine **6b**, respectively. Compounds **6a** and **6b** underwent ready hydrolysis for the exocyclic C=N when heated ethanol containing hydrochloric acid to afford the corresponding 4,10-dione derivatives **7a** and **7b**, respectively via ammonia liberation (Scheme 1). The chemical structures of new compounds were assured by spectral data (IR, ^{1}H, ^{13}C-NMR, MS).



Scheme 1. Synthesis of compounds **3a,b**; **4a,b**; **6a,b** and **7a,b**.

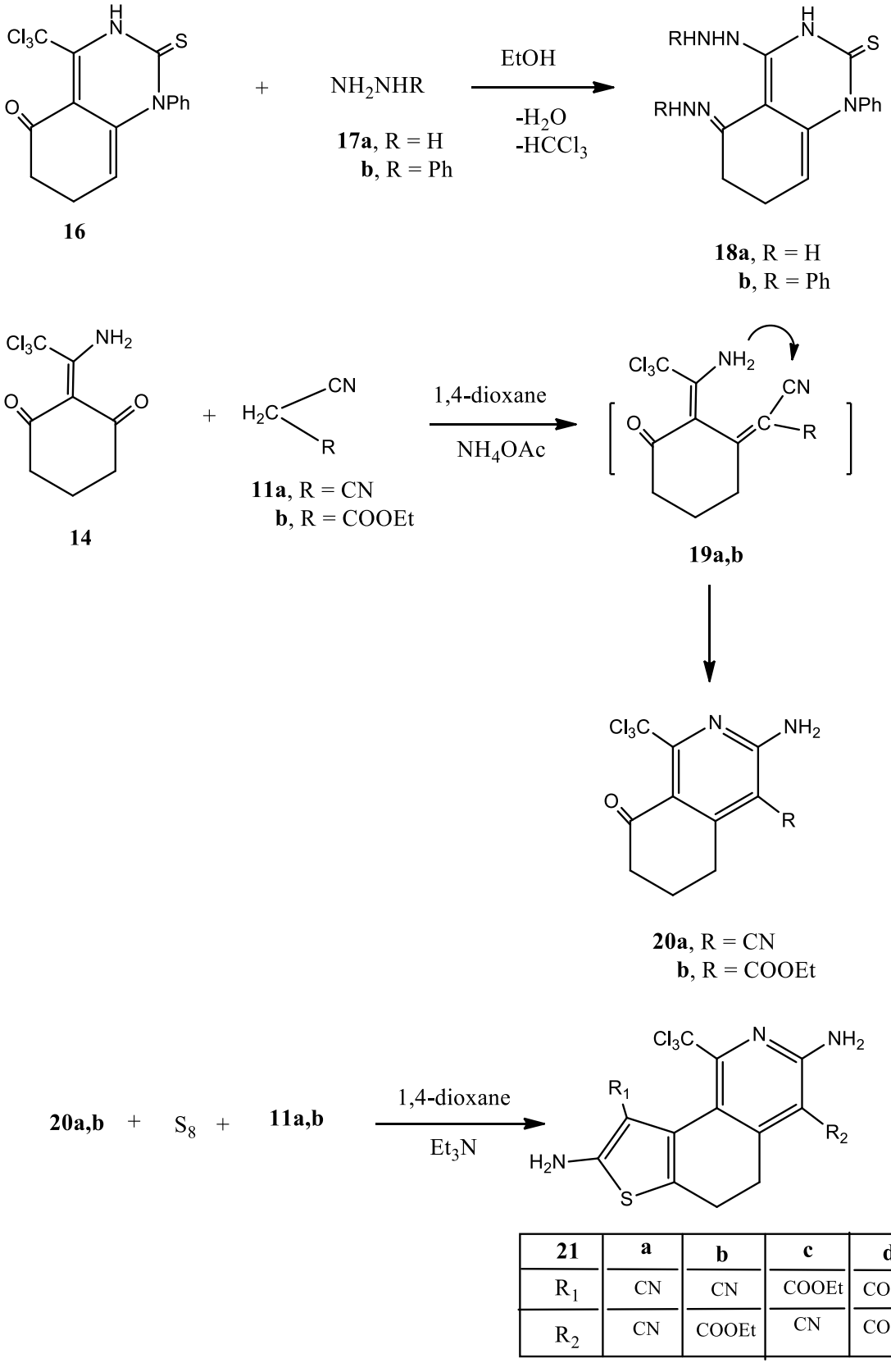
The reaction of cyclohexane-1,3-dione (**1**) with benzoyl isothiocyanate (**8**) in 1,4-dioxane gave the benzo[*e*][1,3]oxazin-5(6*H*)-one derivative**10**. Formation of the latter product was explained through the first addition the methyleno group of compound **1** to the isothiocyanate moiety of **8** to give the intermediate **9** followed by elimination of one molecule of water. The structure of compound **10** was based on the obtained analytical and spectral data. Thus, its mass spectrum revealed m/e 257 corresponding to its molecular mass. The ^{1}H NMR spectrum showed the presence of a multiplet at δ7.43-7.22 due to the C_6H_5 group and the ^{13}C NMR spectrum showed three signals at δ180.4, 174.2, 168.2 due to the presence of C-4, C-2 and C-5, respectively and signals at δ142.7, 133.3, 126.3, 125.2, 123.6, 121.1 indicating the C_6H_5, C-2, C-4a, C-8a carbons.

Compound **10** is capable for forming fused thiophene derivatives through Gewald's thiophene reaction.[32,33] Thus, the reaction of compound **10** with elemental sulfur and either of malononitrile (**11a**) or ethyl cyanoacetate (**11b**)in ethanol containing triethylamine gave the thieno[2',3':5,6]benzo[1,2-*e*][1,3]oxazine derivatives **12a** and 1**2b**, respectively. Next we studied the reaction of cyclohexan-1,3-dione with trichloroacetonitrile followed by heterocyclization of the product in the aim of producing halogen rich compounds that characterized by high inhibitions toward cancer cell lines. Therefore, the reaction of cyclohexan-1,3-dione (**1**) with trichloroacetonitrile (**13**) in ethanol solution containing triethylamine gave the 2,2,2-trichloroethylidene)cyclohexane derivative **14**. The latter compound showed interesting reactivity's toward variety of chemical reagents. Thus, the reaction of compound **14** with phenyisothiocyanate (5) in 1,4-dioxane solution containing a catalytic amount of triethylamine gave the 2,3,6,7-tetrahydroquinazoline derivatives **16** via the intermediate formation of Michael addition adduct **15** followed by cyclization through water elimination (Scheme 2). The analytical and spectral data of compound **16** were in agreement with its proposed structure. Thus, the ^{1}H NMR spectrum showed the presence of a multiplet at δ7.29-7.42 due to the presence of C_6H_5 group and a singlet at δ 8.28, D_2O exchangeable, due to the NH group and the ^{13}C NMR spectrum showed a signal at 94.4 indicating the CCl_3 group, signals at 120.5, 121.5, 123.6, 123.9, 19.0, 130.3, 133.6, 135.2 for the C_6H_5, C-8, C-9, C-3 and C-4 carbons and two signals at 168.2,178.8 for the C-5 and C-2 carbons.



Scheme 2. Synthesis of compounds **10**, **12a,b**; **14** and **16**.

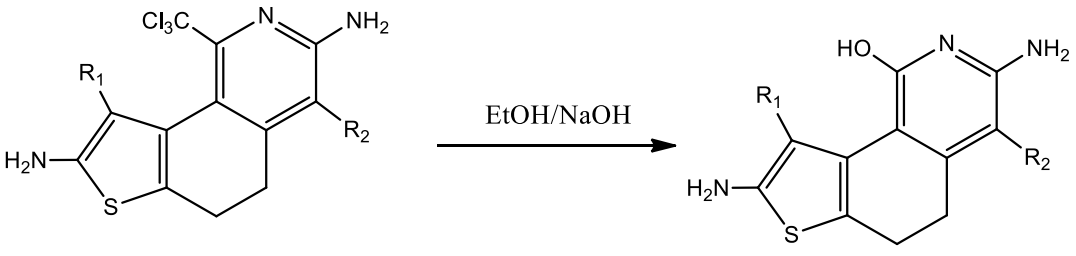
The reaction of compound **16** with two fold of either hydrazine hydrate (**17a**) or phenylhydrazine (**17b**) gave the 5-hydrazono-1-phenyl-3,5,6,7-tetrahydroquinazoline derivatives **18a** and **18b**, respectively. On the other hand, the reaction of compound **14** with either malononitrile (**11a**) or ethyl cyanoacetate (**11b**) in 1,4-dioxane solution containing a catalytic amount of triethylamine gave the 6,7-dihydroisoquinoline derivatives **20a** and **20b**, respectively. Formation of the latter products was assumed to took place via the first Knoevenagel condensation of the cyanomethylene reagent to give the intermediates **19a,b** followed by Michael addition to produce **21a,b**. The Gewald's thiophene reactions of either **20a** or **20b** with elemental sulfur and either malononitrile (**11a**) or ethyl cyanoacetate (**11b**) gave the 5,6-dihydrothieno[2,3-*h*]isoquinoline derivatives **21a-d**, respectively (Scheme 3).



21	a	b	c	d
R_1	CN	CN	COOEt	COOEt
R_2	CN	COOEt	CN	COOEt

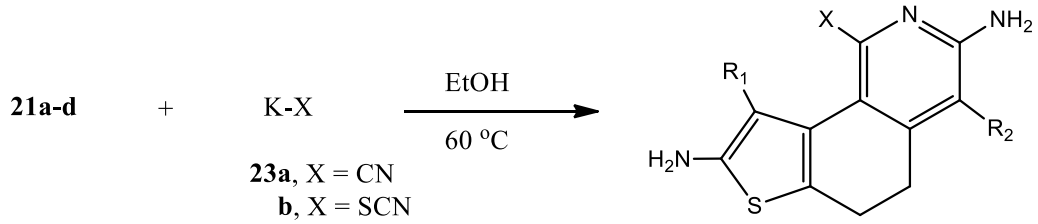
Scheme 3. Synthesis of compounds **18a,b**; **20a,b** and **21a-d**.

The trichloromethyl moiety present in compounds **21a-d** showed interesting reactivity's toward nucleophilic displacement reactions. Thus, the heating of either**21a**, **21b**, **21c** or **21d** in ethanolic sodium hydroxide solution gave the 1-hydroxy-5,6-dihydrothieno[2,3-*h*]isoquinoline derivatives **22a-d**, respectively. On the other hand, the reaction of either **21a**, **21b**, **21c** or **21d** with either potassiumcyanide (**23a**) or potassium thiocyanate (**23b**) gave the corresponding nucleophlic displacement products **24a-h**, respectively (Scheme 4). All new compounds were confirmed by their correct compatible spectrum data and spectrum data and elemental analyses values (see the Experimental section).



21a-d

22	**a**	**b**	**c**	**d**
R_1	CN	CN	COOEt	COOEt
R_2	CN	COOEt	CN	COOEt



24a-h

24	**a**	**b**	**c**	**d**	**e**	**f**	**g**	**h**
R_1	CN	CN	COOEt	COOEt	CN	CN	COOEt	COOEt
R_2	CN	COOEt	CN	COOEt	CN	COOEt	CN	COOEt
X	CN	CN	CN	CN	SCN	SCN	SCN	SCN

Scheme 4. Synthesis of compounds **22a-d** and **24a-h**.

4. Conclusion

The main findings of these studies is the synthesis of a series of novel heterocyclic derivatives synthesized from cyclohexan-1,3-dione followed by screening of the newly synthesized compounds towards six cancer cell lines. Sixteen compounds exhibited high inhibitions toward the cancer cell lines and the c-Met enzymatic activity showed that compounds revealed that eleven

compounds were more active than the reference foretinib. In addition, compounds twenty three compounds displayed much higher anti-proliferation activities against PC-3 cell line than the standard anibamine. Further tests toward the five tyrosine kinases c-Kit, Flt-3, VEGFR-2, EGFR, and PDGFR and Pim-1 kinase showed that compounds **7b**, **12b**, **16**, **20b**, **21b**, **22c**, **22d**, **24d**, **24f** and **24h** were the most potent of the tested compounds toward the five tyrosine kinases and compounds **7b**, **12b**, **16**, **22c** and **24f** were of the highest inhibitions toward Pim-1 kinase. The results obtained will encourage further work in the future in the field of the synthesis of target molecules as anticancer agents.

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