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RESEARCH ARTICLE

Synthesis, Characterization and Anti-Breast Cancer Activity of some new pyrazole, thiazole, chromene and pyridine derivatives

Sawsan Ahmed Fouad

Chemistry Department, Faculty of Science, Al-Azhar University (for Girls), Nasr City, Cairo, Egypt.

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Corresponding Author

Sawsan Ahmed Fouad

Abstract

As a part of ongoing studies in developing new potent antitumor agents. A novel synthesis of 2-cyano-N-(cyclohexyl) acetamide (2) has been reported. 2-Cyano-N-(cyclohexyl) acetamide (2) was utilized as key intermediate for the synthesis of some new pyrazole, thiazole, chromene, thiophene, and pyridine derivatives. The structures of the newly synthesized compounds were confirmed by elemental analysis, IR, ^{1}H NMR, and mass spectral data. Representative compounds of the synthesized products were tested and evaluated as Anti-breast Cancer gents.

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Introduction

Cyanoacetamides and their related heterocyclic derivatives have generated a great deal of attention, due to their interesting biological and therapeutic value. Their pharmaceutical activities include: antimicrobial [1,2], antifungal [3], insulin releasing [4], carbonic anhydrase inhibitory [5], anti-inflammatory [6], and anti-tumor properties [7]. Among these heterocyclic, pyrazoles, thiophene and thiazoles are an interesting group of compounds, many of which possess wide spread pharmacological properties such as analgesic, antipyretic and anti-androgenic activities [8-11]. Pyrazoles also possess antidepressant, anti-inflammatory and anti-rheumatic activities [12–14]. Over the past few decades, the literature has been enriched with progressive findings about the anticonvulsant activities of various substituted thiazole derivatives [15–19] that are of interest as potential neuro protective agents [20,21] and anti-tumor agents [22, 23]. Some 2-pyridones are also reported to possess antitumor [24], antibacterial [25] and other biological activities [26, 27]. Also, a large number of thiophene derivatives have found to exhibit pharmacological activity [28-30]. Recently, natural and synthetic chromene attracted great attention due to their wide range of biological properties, including anticancer [31], anti-HIV [32], anti-inflammatory [33] and antibacterial [34] activities. Moreover, the coumarin nucleus is prevalent in numerous natural products and is extremely important in the chemistry of biological activities [35] which have found applications in treatment of antibacterial, antitumor, anti-inflammatory, antithrombotic, cardio protectors or enzymatic inhibitors antimicrobial and antifungal [36-40]. In view of these findings and in continuation of our work on the synthesis of novel heterocyclic systems exhibiting biological activity, we undertook the synthesis of a new series of compounds and evaluate their anti-breast cancer activity.

EXPERIMENTAL SECTION

General

All melting points were determined on a Stuart melting point apparatus. IR spectra were recorded on a Shimadzu-440 IR spectrophotometer using the KBr disk technique (Shimadzu, Japan). ^{1}H NMR spectra were measured on a Varian Mercury VX-300 NMR spectrometer in DMSO-d6 as a solvent and were run at 300 MHz, using tetramethylsilane (TMS) as an internal standard. The mass spectra were recorded on Shimadzu GCMS-QP-1000EX mass spectrometers at 70 eV. The purity of the synthesized compounds was monitored by TLC. Elemental

analyses were carried out by the Microanalytical Research Centre- Faculty of Science- Cairo University. Analytical results for C, H, N and S were within ±0.4 of the calculated values.

Chemistry

2-Cyano-N-(cyclohexyl) acetamide (2).

Equimolar amounts (0.01 mol) of both ethyl cyanoacetate and cyclohexylamine in ethanol (20 mL). The reaction mixture was heated for 3 h, and then allowed to cool, then poured into ice /water and the formed solid product was collected by filtration and recrystallized from ethanol to give white powder **2**.Yield 95%; mp 60 °C; IR (KBr) νcm^{-1}: 3300 (NH), 2260 (C≡N), and 1651 (C=O). ^{1}H-NMR ((300 MHz, DMSO-d6) δ: 0.83-1.41(m, 10H, CH_2 cyclohexyl), 3.01-3.31(m, 1H, CH-N cyclohexyl), 3.27 (s, 2H, CH_2–CN), and 8.12 (s, 1H, NH). MS m/z (%):1.66 (27. 89), 56 (100).Anal. Calcd. For $C_9H_{14}N_2O$ (166): C, 65.06; H, 8.43; N, 16.86%; found: C, 64.92 %; H, 8.41 %; N, 16.83%.

4-Amino-N-cyclohexyl-2, 3-dihydro-3-phenyl-2-thioxothiazole-5-carboxamide (3).

To a solution of compound **2** (0.01mol) in ethanol (20 mL) containing triethylamine (0.5 mL), elemental sulfur (0.01mol) and phenyl isothiocyanate (0.01mol) were added. The reaction mixture was heated for 3 h, with continuous stirring and then poured into a beaker containing an ice/water mixture containing few drops of hydrochloric acid. The formed solid product was collected by filtration, dried, and recrystallized from ethanol to give yellow powder **3**. Yield 65%; m. p. 140 ^{0}C; IR (KBr) ν cm^{-1}: 3432 (NH_2), 3268 (NH), 1693, (C=O) and 1288 (C=S). ^{1}H-NMR ((300 MHz, DMSO-d6) δ: 0.83-1.44 (m, 10H, CH_2 cyclohexyl), 3.11-3.32 (m, 1H, CH-N cyclohexyl), 6.66 (s, 2H, NH_2, D2O-exchangable), 7.32-7.60 (m, 8H, Ar +NH)). MS m/z (%): 333 (4.19), 56 (100). Anal. Calcd. For $C_{16}H_{19}N_3S_2O$ (333): C, 57.65%; H, 5.70%; N, 12.60 %; found: C, 57.54%; H, 5.69%; N, 12.58%.

3, 5-Diamino-4-cyano-N-cyclohexylthiophene-2-carboxamide (4).

To a solution of compound **2** (0.01 mol) in absolute ethanol (25 mL) contained triethylamine (0.5 mL), malononitrile (0.01 mol) and elemental sulfur (0.01 mol) were added. The reaction mixture was heated under reflux for 3 h, and then allowed to cool, then poured into ice/water and the formed solid product was collected by filtration and recrystallized from ethanol to give green powder **4**. Yield 90%; m. p. 100-110 ^{0}C; IR (KBr) ν cm-1: 3418, 3327 (NH_2+NH), 2214 (C≡N), 1680 (C=O). ^{1}H-NMR ((300 MHz, DMSO-d6) δ: 0.84-1.72 (m, 10H, CH_2 cyclohexyl), 3.19-3.31(m, 1H, CH-N cyclohexyl), 6.21, 7.25 (2s, 4H, $2NH_2$, D2O-exchangable), 11.60 (s, 1H, NH,). MS m/z (%): 264 (5.24), 133 (100). Anal. Calcd. For $C_{12}H_{16}N_4OS$ (264): C, C, 54.54%; H, 6.06 %; N, 21.21%. Found: C, 54.42%; H 6.04%; N, 21.16%.

6-Amino-4-(4-chlorophenyl)-1-cyclohexyl-1, 2, 3, 4-tetrahydro-2-oxopyridine-3, 5-dicarbonitrile (6).

A mixture of compound **2** (0.01 mol) and the appropriate α-cyano-4-chlorophenyl cinnamonitrile **5** (0.01 mol) in ethanol (30 mL) containing few drops of piperidine (5 drops) were stirred at reflux for 3 h, then the reaction mixture was cooled at room temperature. The solid which formed was collected by filtration, washed with hot ethanol, and recrystallized from ethanol to give pale brown crystals **6**. Yield 85%; m. p. 140 ^{0}C; IR (KBr) ν cm^{-1}: 3412, 3335 (NH_2), 1675 (C=O) and 2213(C≡N). ^{1}H-NMR ((300 MHz, DMSO-d6) δ: 0.83-1.51(m, 10H, CH2 cyclohexyl), 3.29-3.36 (m, 1H, CH-N cyclohexyl), 3.97 (d, d, 2H, CH pyridine), 7.49-7.63 (m, 4H, Ar-H) and 8.39 (s, 2H, NH_2). MS m/z (%):355 (16.39), 70 (100), Anal. Calcd. For $C_{19}H_{19}N_4OCl$ (355): C, 64.22%; H, 5.35%; N, 15.77%, Found: C, 64.23%; H, 5.38%; N, 15.78%.

4, 6-Diamino-1-cyclohexyl-1,2-dihydro-2-oxopyridine-3-carbonitrile (7).

A mixture of compound **2** (0.01 mol) and malononitrile (0.01 mol) with a few drops of piperidine in an oil bath was heated for 1 h at 160 ^{0}C, then allowed to cool. The solid product was collected and recrystallized from ethanol to give brown crystals **7**.Yield 65%, m. p. 120^{0}C; IR (KBr) ν cm^{-1}: 3316, 3292 (NH_2), 2195 (C≡N), 1658 (C=O). ^{1}H-NMR ((300 MHz, DMSO-d6) δ: 0.83-1.30(m, 10H, CH_2 cyclohexyl), 2.89-3.31(m, 1H, CH-N cyclohexyl), 3.60 (s, 1H, CH pyridine), 5.42 (s, 2H, NH_2), 8.15(s, 2H, NH2). MS m/z (%): 233 [M+1] (8.29), 65 (100). Anal. Calcd. For $C_{12}H_{16}N_4O$ (232): C, 62.05%; H, 6.89%; N, 24.12%. Found: C, 62.92%; H, 6.88%; N, 24.08%.

2-Cyano -N-cyclohexyl- 3-(4- hydroxy-3-methoxyphenyl) acryl amide (8).

To a solution of compound **2** (0.01 mol) in absolute ethanol (15 mL) containing 3 drops of sodium hydroxide (10%), vanillin (0.01 mol) was added .The reaction mixture was heated under reflux or 3 h, and then allowed to cool. The precipitate that formed was collected by filtration, washed with ethanol, dried and recrystallized from a mixture of EtOH /DMF (1:1) to give yellow powders **8**. Yield 80%; m. p. 98 ^{0}C; IR (KBr) ν cm^{-1}: 3355 (OH), 3279 (NH), 2210 (C≡N), 1648 (C=O).^{1}H-NMR ((300 MHz, DMSO-d6) δ: 0.82-1.48 (m, 10H, CH_2 cyclohexyl), 3.14-3.31(m, 1H, CH-N cyclohexyl), 3.87(s, 3H, OCH_3), 6.94-7.64 (m, 4H, ArH), 8.22 (s, 1H, NH), 10.2(s, 1H, OH) .MS m/z (%): 299 [M-1] (5.8), 217 (100) . Anal. Calcd. For $C_{17}H_{20}N_2O_3$ (300): C, 67.98%; H, 6.66%; N, 9.33%; found: C, 67.88%; H, 6.69%; N, 9.30%.

6-amino-1-cyclohexyl-1,2,3,4-tetrahydro-4-(4-hydroxy-3-methoxyphenyl)-2-oxopyridine-3,5-dicarbonitrile (9).

To a solution of compound **8** (0.01mol) in absolute ethanol (30 mL) containing piperidine (0.5 mL) malononitrile (0.01 mol) was added. The whole reaction mixture was heated in a boiling water bath for 8 h then left to cool. The formed solid product was triturated with ethanol to give pale yellow crystals **9**. Yield 65%, m. p. 225-230^{0}C; IR (KBr) ν cm^{-1}: 3301 (OH), 3213 (NH), 2214 (C≡N), 1650 (C=O). MS m/z (%): 368 [M+2] (34.16), 316 (100). Anal. Calcd. For $C_{20}H_{22}N_4O_3$(366): C, 65.57%; H, 6.01%; N, 15.30%; Found C, 65.45%; H, 5.99%; N, 15.27%.

5- Amino-N- cyclohexyl -3- (4-Hydroxy -3- methoxyphenyl) -1H -pyrazole-4-carboxamid (10).

To a solution of compound **8** (0.01 mol) in ethanol (30 mL) hydrazine hydrate (0.01 mol) was added. The reaction mixture was heated under reflux for 6 h, and then poured into ice/water containing few drops of hydrochloric acid. The formed solid product was collected by filtration and crystallized from dioxane to give yellow crystals **10**.Yield 77%, m. p. 132–136 ^{0}C; IR (KBr) ν cm^{-1}: 342 (NH), 3301(NH_2), 1687(C=O). ^{1}H-NMR ((300 MHz, DMSO-d6) δ: 0.83-1.39(m, 10H, CH_2 cyclohexyl), 3.02-3.26 (m, 1H, CH-N cyclohexyl), 3.83 (s, 3H, OCH_3), 5.85 (s, 1H, NH_2), 7.23-7.45 (m, 3H, ArH), 8.11(s, 1H, NH-pyrazole), 8.55(s, 1H, NHCO), 9.61 (s, 1H, OH). MS m/z (%): 329 [M-1] (25), 97 (100). Anal. Calcd. For $C_{17}H_{22}N_4O_3$ (330): C, 61.80%; H, 6.71%; N, 16.96%. Found C, 61.73%; H, 6.80%; N, 16.83%.

1-Cyclohexyl-1, 2-dihydro-4, 6-dimethyl-2-oxopyridine-3-carbonitrile (11).

Equimolar amounts of **2** (0.01 mol) and acetyl acetone (0.01 mol) with a few drops of piperidine in an oil bath were heated for 1 h at 160 ^{0}C, then allowed to cool. The solid product was collected and recrystallized from dioxane to give yellowish White crystals **11.** Yield 55%, mp 70 ^{0}C; IR (KBr) ν cm^{-1}: 3078 (CH-aroma.), 2924 (CH-aliph.), 2220 (C≡N), 1654 (C=O). ^{1}H-NMR ((300 MHz, DMSO-d6) δ: 0.83-1.54(m, 10H, CH_2 cyclohexyl), 3.24-3.31(m, 1H, CH-N cyclohexyl), 3.37 (s, 3H, CH_3), 3.88 (s, 3H, CH_3), 6.28 (s, 1H, CH pyridine). MS m/z (%): 230 (4.88), 148 (100). Anal. Calcd. For $C_{14}H_{18}N_2O$ (230): C, 73.04%; H, 7.82%; N, 12.16 %. Found: C, 72.90%; H, 7. 81%; N 12.15%.

N-Cyclohexyl-2-imino-2H-chromene-3-carboxamide (12).

To a solution of compound **2** (0.01 mol) in absolute ethanol (20 mL) containing piperidine (0.5 mL), salicylaldehyde (0.01 mol) was added. The reaction mixture was heated under reflux for 3 h, and then allowed to cool. The precipitate formed was filtered off, washed with ethanol, dried and recrystallized from ethanol to give Pale yellow crystal **12**. Yield 90%; m. p. 80 ^{0}C; IR (KBr) ν cm^{-1}: 3453, 3230 (2NH), 1680 (C=O). ^{1}H-NMR ((300 MHz, DMSO-d6) δ: 0.85-1.64(m, 10H, CH_2 cyclohexyl), 3-3.29 (m, 1H, CH-N cyclohexyl), 7.54-7.77 (m, 5H, ArH+CH), 8.96 (s, 1H, NH D2O-exchangeable), 11.79 (s, 1H, NHCO D2O-exchangeable). MS m/z (%):271 [M+1] (11.65), 88 (100). Anal. Calcd. For $C_{16}H_{18}N_2O_2$ (270): C, 71.11%; H, 6.66%; N, 10.36%; found: C, 70.99%; H, 6.65%; N, 10.35%.

N-Cyclohexyl-2-(hydroxymethylamino)-2H-chromene-3-carboxamide (13).

A mixture of compound **12** (0.01 mol) and formalin (37%, 0.01 mol) was refluxed in ethanol (15 mL) for 4 h. The reaction mixture after cooling at room temperature was poured into ice/water. The separated solid was filtered and recrystallized from ethanol to give Pale brown crystal **13**. Yield 66%; m. p. 90 ^{0}C; IR (KBr) ν cm^{-1}:3433 (OH), 3319, 3218 (2NH), 1654 (C=O). ^{1}H-NMR ((300 MHz, DMSO-d6) δ: 0.81-1.51(m, 10H, CH_2 cyclohexyl), 3.23-3.34 (m, 1H, CH-N cyclohexyl), 4.79 (s, 2H, CH_2) 7.40-7.98 (m, 5H, ArH+CH), 8.65 (s, 1H, NH), 8.84 (s, 1H, NHCO), 11.21(s, 1H, OH). MS m/z (%): 303 [M+1] (11.65), 88 (100). Anal. Calcd. For $C_{17}H_{22}N_2O_3$ (302): C, 67.53%; H, 7.28%; N, 9.26%; found: C, 67.43%; H, 7.27%; N, 9.25%.

2-Amino-3-cyclohexyl-4, 5-dihydro-5-imino-4-oxo-3H-chromeno [3, 4-c] pyridine-1-carbonitrile (14).
A mixture of compound **12** (0.01 mol) and malononitrile (0.01 mol) was refluxed in ethanol (15 mL) containing 3 drops piperidine for 4 h. The reaction mixture after cooling at room temperature was poured into ice/water. The separated solid was filtered and recrystallized from ethanol to give pure pale brown crystal **14**.Yield 86%; m. p. 90 ^{0}C; IR (KBr) ν cm$^{-1;}$3440, 3349 (NH_2), 3237 (NH) 2209 (CN), 1654 (C=O). ^{1}H-NMR ((300 MHz, DMSO-d6) δ: 0.81-1.51(m, 10H, CH_2 cyclohexyl), 3.24-3.37 (m, 1H, CH-N cyclohexyl), 7.40-7.98 (m, 6H, ArH+NH_2), 8.84 (s, 1H, NH D2O-exchangeable). MS m/z (%): 335 [M+1] (11.65), 300 (100). Anal. Calcd. For $C_{19}H_{18}N_4O_2$ (334): C, 68.25%; H, 5.39%; N, 16.76%; found: C, 68.18%; H, 5.37%; N, 16.74%.

N-Cyclohexyl-2-N-4-(N-quinoxalin-2-yl-sulfamoyl)-2H-chromene-3-carboxamide (15).
To a stirred solution of chromene **12** (0.01 mol) in acetic acid (2 mL) sulfaquinoxaline (0.01 mol) was added and the resulting mixture was stirred at reflux for 12 h. The solvent was removed under reduced pressure, and the reaction mixture leaves to overnight then ppt. dissolved in ethanol and poured into crushed ice .the solid product was filtered off and recrystallized from ethanol to give pale yellow crystal **15**.Yield 70%; mp 100 ^{0}C; IR (KBr) ν cm^{-1}: 3327, 3220 (2NH), 1645 (CONH), 1339, 1145 (SO_2). ^{1}H-NMR ((300 MHz, DMSO-d6) δ: 0.84-1.97 (m, 10H, CH_2 of cyclohexyl), 3.28-3.32 (m, 1H, CH-N cyclohexyl), 7.41-7.96 (m, 14H, ArH+CH quinoxaline, CH pyrane), 8.83 (s, 1H, NHCO), 10.26 (s, 1H, $NHSO_2$). MS m/z (%):556 [M+3] (9.88), 58 (100). Anal. Calcd. For $C_{30}H_{27}N_5O_4S$ (553): C, 65.08%; H, 4.88%; N, 12. 65%; found: C, 64.98%; H, 4.87%; N, 12.61%.

2-Cyano-N-cyclohexyl-2-(4-oxo-3-phenylthiazolidin-2-ylidene) acetamide (17) and 2-Cyano-N-cyclohexyl-2-(4-(N-quinoxalin-2-yl-sulfamoyl)-3-phenyl-thiazolidin-2-ylidene) acetamide (18).
General procedure:
To a cold suspension of finally divided KOH (0.01mol) in dry DMF (25 mL) compound **2** (0.01 mol) was added followed by phenyl isothiocyanate (0.01 mol). The mixture was stirred at room temperature for 6 h, then cooled again to 0 ^{0}C, treated with chloroacetyle chloride and or 2-Chloro-N-(4-(N-quinoxalin-2-ylsulfamoyl) phenyl) acetamide (0.01mol) the stirring was continued at room temperature for further 5 h. The reaction mixture was poured into 50 mL of cold water. The resultant solid products were collected by filtration and recrystallized from a mixture of EtOH / DMF (1:1) to give compounds **17** and **18**.

2-Cyano-N-cyclohexyl-2-(4-oxo-3-phenylthiazolidin-2-ylidene) acetamide (17).
Yellow powder; Yield 84%; m. p. 150 ^{0}C; IR (KBr) ν cm^{-1}: 3431 (NH), 2205 (C≡N), 1738, 1643 (2C=O). ^{1}H-NMR ((300 MHz, DMSO-d6) δ: 0.82-1.37(m, 10H, CH_2 cyclohexyl), 3.03-3.30 (m, 1H, CH-N cyclohexyl), 3.92(s, 2H, CH_2 thiazole), 7.36-7.53 (m, 6H, Ar-H+NH). MS m/z (%): 341 (11.65), 286.05 (100). Anal. Calcd. For $C_{18}H_{19}N_3O_2S$ (341): C, 63.32%; H, 5.57%; N, 12.31 %. Found: C, 63.22%; H, 5.56%; N, 12.29%.

2-Cyano-N-cyclohexyl-2-(4-(N-quinoxalin-2-yl-sulfamoyl)-3-phenylthiazolidin-2-ylidene) acetamide (18).
Brownish yellow powder; Yield 90%; mp 110 ^{0}C; IR (KBr) ν cm^{-1}: 3431 (NH), 2208 (C≡N), 1736 (C=O). ^{1}H-NMR ((300 MHz, DMSO-d6) δ: 0.81-2.08(m, 10H, CH2 cyclohexyl), 3.19-3.42 (m, 1H, CH-N cyclohexyl), 4.26 (s, 1H, CH thiazole), 6.56–7.74 (m, 9H, Ar-H + NH), 8.61 (s, 1H, CH quinoxaline), 10.72 (s, 1H, NHCO), 11.9 (s, 1H, $NHSO_2$).MS m/z (%): 623 (5.51), 63 (100). Anal. Calcd. For $C_{32}H_{29}N_7O_3S_2$ (623): C, 61.62%; H, 4.65%; N, 15.72%. Found: C, 61.51%; H, 4.64%; N, 15.70%.

N-Cyclohexyl-2-(4, 5-dihydro-4-oxothiazol-2-yl) acetamide (19).
A mixture of compound **2** (0.01 mol) and thioglycolic acid(0.01 mol) in dry pyridine (20 mL) was heated under reflux for 8 h, allowed to cool, and poured into cold water (50 mL). The solid product obtained was collected by filtration, dried and recrystallized from ethanol to give yellow crystals **19**. Yield 62%; m. p. 120 ^{0}C; IR (KBr) ν cm^{-1}: 3394 (OH), 3284 (NH), 1638 (C=O). MS m/z (%): 240 (3.15), 56 (100). Anal. Calcd. For $C_{11}H_{16}N_2O_2S$ (240): C, 55.05%; H, 6.71%; N, 11.72 %, Found: C, 54.97%; H, 6.65%; N, 11.70%.

2-Amino-N-cyclohexyl-4, 5, 6, 7-tetrahydrobenzo[b]thiophene-3-carboxamide (20).
A mixture of compound **2** (0.01 mol), cyclohexanone (0.01 mol), and elemental sulfur (0.015 mol) in ethanol (20 mL) containing morpholine (0.01 mol) was heated at 60 ^{0}C for 2 h with stirring. After the reaction mixture was allowed to cool, the precipitate that formed was collected by filtration, dried and recrystallized from chloroform to give pale yellow crystals **20**. Yield 76%; m.p.110 ^{0}C; IR (KBr) ν cm^{-1}: 3440 (NH_2), 3210 (NH), 1658 (C=O). ^{1}H-NMR ((300 MHz, DMSO-d6) δ: 0.85-1.24 (m, 10H, CH_2 cyclohexyl), 2.43-2.56 (m, 4H, CH_2 cyclohexyl

thiophene), 3.33-3.45 (m, 1H, CH-N cyclohexyl), 4.58 (s, 2H, NH_2), 8.97 (s, 1H, NH). MS m/z (%): 278 (2.12), 172 (100), Anal. Calcd. For $C_{15}H_{22}N_2OS$ (278): C, 64.74%; H, 7.91%; N, 10.06%, Found: C, 64.62%; H, 7.89%; N, 10.01%.

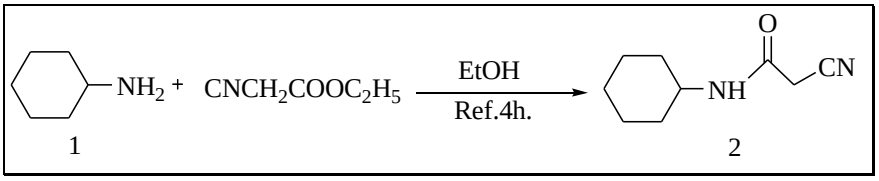
1- Cyclohexyl -2, 3-dihydro-2-oxo-5-phenyl-1H-pyrrole-3-carbonitrile (21).

A mixture of compound **2** (0.01mol) and phenacyl bromide (0.01mol) in ethanol (30 mL) containing triethylamine (0.5 mL) was refluxed for 5 h. The formed solid product was filtered off, dried and recrystallized from ethanol to give pale yellow powder **21**. Yield 45%; m. p. 100 ^{0}C; IR (KBr) ν cm^{-1}: 2195 (C≡N), 1640, (C=O). ^{1}H-NMR ((300 MHz, DMSO-d6) δ: 0.85-1.66 (m, 10H, CH_2 cyclohexyl), 3.19-3.42 (m, 1H, CH-N of cyclohexyl), 4.54 (d, 1H, H-3 pyrrole), 5.65 (d, 1H, H-4 pyrrole), 7.27 (m, 5H, ArH). MS m/z (%): 266 (15.65), 56 (100). Anal. Calcd. For: $C_{17}H_{18}N_2O$ (266): C, 76.69%; H, 6.76%; N, 10.52%. Found: C, 76.55%; H, 6.65%; N, 10.49%.

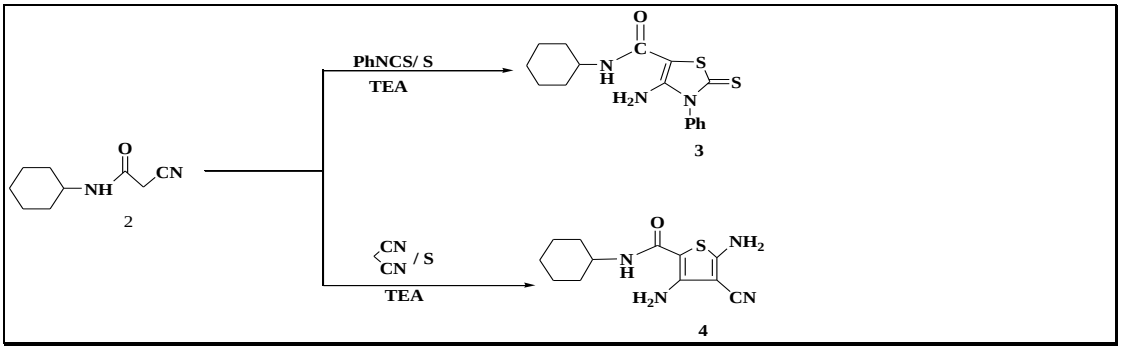
RESULTS AND DISCUSSION

Chemistry

One reason of our interest enamines is related to the conversion possibility of their NH_2 group to for $NHCOCH_2CN$ group, which leads to cyano acetamide with further useful functionalization at this position [41, 42]. N-Cyclohexyl cyano acetamide **2** was synthesized by cyano acetylating of **1** with ethyl cyanoacetate [43] as previously described.



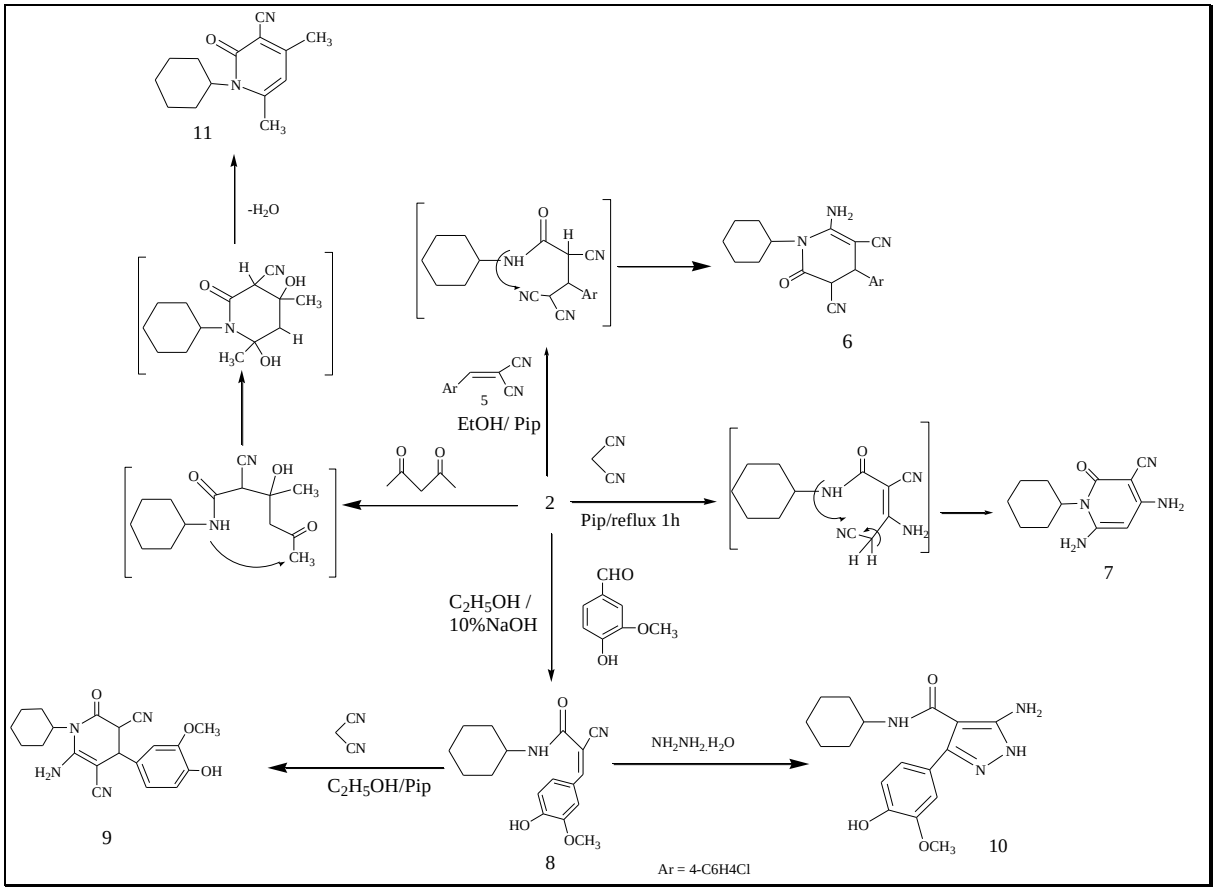
In view of the growing biological importance of thiazole derivatives, it was considered of interest to synthesize some new derivative of thiazole. Thus, the reaction of compound **2** with phenyl isothiocyanate and elemental sulfur gave the thiazole-2-thione derivative **3**. Also, the reaction of cyano acetamide **2** with elemental sulfur and malononitrile gave the thiophene derivative **4** as previously reported in the literature [44] (Scheme 1).Analytical and spectral data of the products are in agreement with the proposed structure (see Experimental section).



(Scheme 1)

The reactivity of **2** toward some active methylene reagents was investigated. Thus, compound **2** reacted with malononitrile (1:1 M ratio) in an oil bath at 160 ^{0}C to yield a single product that was identified as 4, 6-diamino-1-cyclohexyl-1, 2-dihydro-2-oxopyridine-3-carbonitrile**7**, on the basis of elemental analysis and spectral data. Its mass spectrum showed a molecular ion peak at m/z 233 together with base peak at m/z 65. The formation of **7** is assumed to proceed via the Michael addition of **2** to cyano group of malononitrile to form the acyclic Michael adduct followed by the situ cyclization to the pyridine skeleton, (Scheme 2). Also, The active methylene in the cyano acetamide **2** underwent nucleophilic addition reaction to the double bond of α-cyano-4-chlorophenyl cinnamonitrile

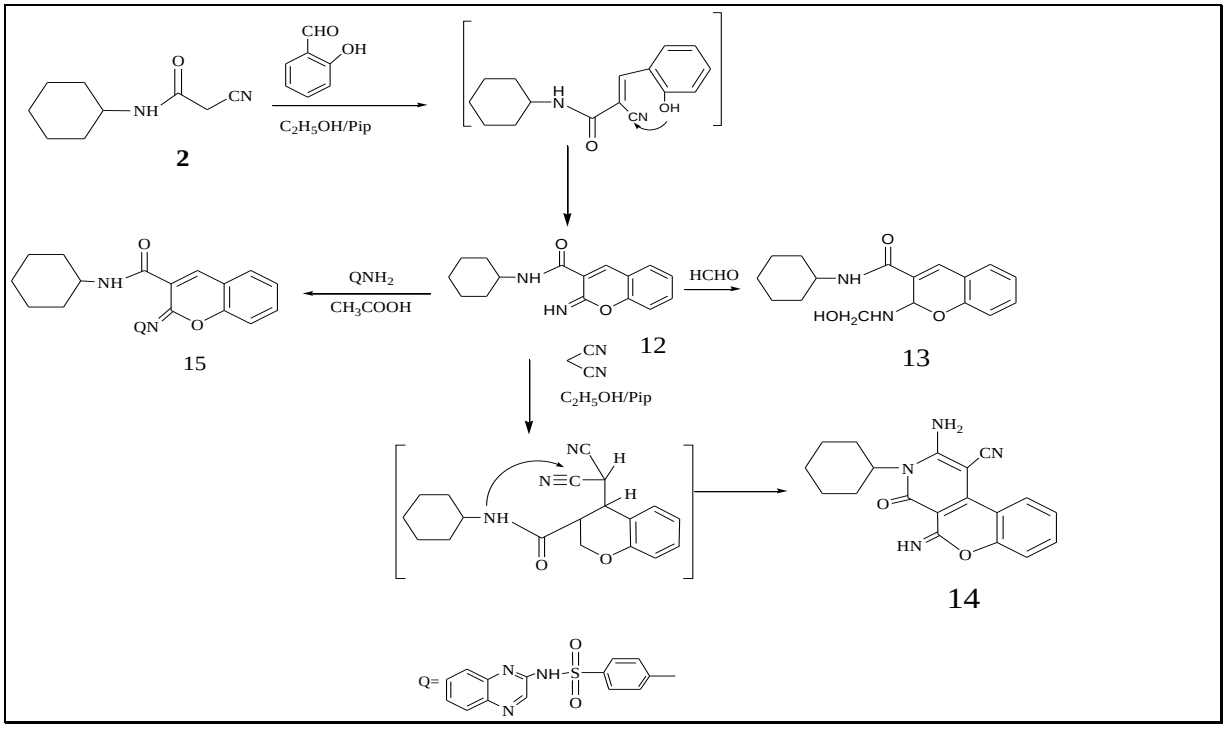
5 via a Michael addition reaction, by refluxing in ethanol containing few drops of piperidine afford the 2-pyridone derivative **6** .To further explore the synthetic amide **2** was suspected to the Knoevenagel condensation by heating with 4-hydroxy-3-methoxy benzaldehyde in ethanolic sodium hydroxide (10%) afford 2-cyano-N-cyclohexyl-3-(4-hydroxy-3-methoxy-pheny) acryl amide (**8**).Ternary condensation of compound **8** with malononitrile in ethanol in the presence of piperidine afford 2-pyridone derivative **9** (Scheme 2). The structure of compound **6, 7** and **9** was elucidated on the basis of its analytical and spectral data. IR spectrum of compound **6** taken as a typical example of series prepared, revealed absorption bands at 1675, 2213, 3355, 3412 cm−1 corresponding to carbonyl, nitrile and NH_2 functions, respectively. Its ^{1}H-NMR spectrum showed signals at δ 8.39 ppm due to NH_2 protons in addition to aromatic protons at δ 7.49-7.63 and 3.97 ppm due to CH pyridine. Its mass spectrum showed a molecular ion peak at m/z 355 corresponding to a molecular formula $C_{19}H_{19}N_4OCl$. The addition of hydrazine hydrate to the activated double bond of compound **8** in boiling ethanol afforded 5-amino-N-cyclohexyl-3-(4-Hydroxy-3-methoxyphenyl)-1H-pyrazole-4-carboxamid **10** elemental analysis, IR, NMR and MS are in agreement with the proposed structure. The reaction of **2** with 1, 3-dicarbonyl compound was studied in the aim of formation of pyridine derivatives with potential biological activities [44, 45]. Thus, when the cyano acetamide **2** was reacted with acetyl acetone in the presence of a catalytic amount of piperidine, the cyclo condensation reaction occurred and the 2-pyridinone derivatives **11** were smoothly afforded. It can be postulated that the reaction initially proceeds via a nucleophilic attack to form the Michael adduct which in turn cyclized and elimination of two water molecules, affording the final product **11** (Scheme 2).



(Scheme 2)

Cyclo condensation of compound **2** with salicylaldehyde in boiling ethanol containing a catalytic amount of piperidine afforded 2-iminochomarin **12** in high yield [46]. IR spectrum of the reaction product **12** revealed the disappearance of cyano absorption band and showed absorption bands at 1680, 3453 and 3230 cm^{-1} corresponding to carbonyl and two NH functions, respectively. Its ^{1}H-NMR spectrum showed two D2O-exchangeable signal at δ 8.96 and 11.79 ppm due to two NH protons, in addition to an aromatic multiple in the region 7.54-7.77 ppm . Its

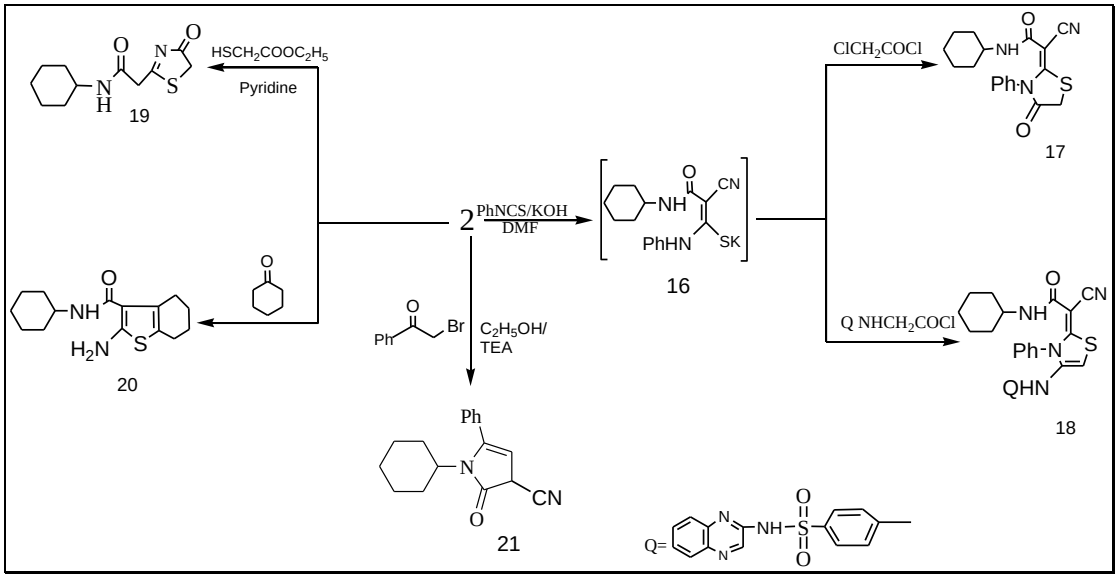
mass spectrum showed a molecular ion peak at (M^+) m/z 271 corresponding to a molecular formula $C_{16}H_{18}N_2O_2$. Compounds **12** on heating with formalin in ethanol afforded the corresponding N-cyclohexyl-2-(hydroxyl methyl amino)-2H-chromene-3-carboxamide **13** (Scheme 3). The structure of compound **13** was confirmed by their spectroscopic IR, ^{1}HNMR and MS and analytical data. Moreover, the resulting chromene derivatives have latent functional constituents, which have the potential for further chemical transformations that give new routes for the preparation of substituted chromene derivatives. Reaction of chromene **12** with malononitrile in refluxing ethanol containing a catalytic amount of piperidine afford the novel chromeno [3, 4-c] pyridine derivative **14** in high chemical yield. The molecular structure of **14** was established through analytical and spectral data. Its infrared spectrum showed absorption bands at 3440, 3349, 3237 and 2209 cm^{-1} due to amino, imino and cyano function groups, respectively. Also, its mass spectrum revealed a molecular ion peak at (M^+) m/z=335 (11.65%) corresponding to a molecular formula $C_{19}H_{18}N_4O_2$ .The formation of **14** was assumed to proceed via the Michael addition of the active methylene function of malononitrile to the activated double bond center in **12** to yield the acyclic Michael adduct which cyclize and aromatize through auto-oxidation under the reaction conditions. Reaction of **12** with sulfaquinoxaline furnished the desired (quinoxalin-2-yl) iminochromenes **15** (Scheme 3).The molecular structure of **15** was established through analytical and spectral data.



(Scheme 3)

The reactivity of cyano acetamide **2** towards isothiocyanate was also investigated. Thus, when **2** was left to react with phenyl isothiocyanate in dimethylformamid, in presence of potassium hydroxide, at room temperature, the corresponding potassium salt **16** was obtained. Hetero cyclization of the intermediate **16** with chloroacetyle chloride or 2-Chloro-N-(4-(N-quinoxalin-2-ylsulfamoyl) phenyl) acetamide furnished in each case, one isolable product. Probably the reaction proceeds via nucleophilic displacement of the halogen atom to give an S-alkylated intermediate followed by loss of water of the latter intermediate to give thiazole derivatives **17** and **18** as the final products. The structure of the synthesized product was established on the basis of their elemental analysis and spectral data (see Experimental section). On the other hand, thiazolidin-4-one **19** was achieved via the reaction of amide **2** with thioglycolic acid in boiling pyridine [47] (Scheme4). 2-Amino-N-cyclohexyl-4,5,6,7-tetra-hydrobenzo [b] thiophene-3-carboxamide **20** could be achieved according to the method described by Gewald, by reacting amide **2** with sulfur and cyclohexanone in the presence of morpholine as a basic catalyst. The assignment of the structure of compound **20** was based on analytical and spectroscopic data. Thus, its IR spectrum displayed absorption bands at 3440 and 3210 cm^{-1} assignable to NH_2 and NH groups. The mass spectrum showed molecular ion peak at m/z 278

corresponding to a molecular formula $C_{15}H_{22}N_2OS$. Recently, we have reported the reaction of cyano acetamide moiety with α-halo carbonyl compounds which represents a new, simple and efficient synthetic route for the synthesis of pyrrole derivatives. Therefore, it was interesting to study the reaction of **2** with phenacyl bromide. Cyclo condensation of **2** with phenacyl bromide in boiling ethanol using triethylamine as a basic catalyst furnished the pyrrole derivative **21** (scheme 4).



(Scheme 4)

In vitro antitumor activity

Chemicals Used, dimethyl sulfoxide (DMSO), crystal violet and trypan blue dye were purchased from Sigma (St. Louis, Mo., USA) [48, 49]. Fetal bovine serum, DMEM, RPMI-1640, HEPES buffer solution, L-glutamine, gentamycin and 0.25% trypsin-EDTA were purchased from Lonza.

Crystal violet stain (1%): It composed of 0.5% (w/v) crystal violet and 50% methanol then made up to volume with de ionized water and filtered through a Whatmann No.1 filter paper. The cells were propagated in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat in activated fetal bovine serum, 1% L-glutamine, HEPES buffer and 50 µg/mL gentamycin. All cells were maintained at 37 ºC in humidified atmosphere with 5% $CO_2$and was sub cultured two times a week. Cell toxicity was monitored by determining the effect of the test samples on cell morphology and cell viability.

Cytotoxicity evaluation using viability assay: For cytotoxicity assay, the cells were seeded in 96-well plate at a cell concentration of 1×10^4cells per well in 100µl of growth medium. Fresh medium containing different concentrations of the test sample was added after 24 h of seeding. Serial two-fold dilutions of the tested chemical compound were added to confluent cell mono layers dispensed into 96-well, flat-bottomed micro titer plates (Falcon, NJ, USA) using a multichannel pipette. The micro titer plates were incubated at 37 ºC in a humidified incubator with 5% CO_2 for a period of 48 h. Three Cells were used for each concentration of the test sample. Control cells were incubated without test sample and with or without DMSO. The little percentage of DMSO present in the wells (maximal 0.1%) was found not to affect the experiment. After incubation of the cells for 24 h at 37 °C, various concentrations of sample (50, 25, 12.5, 6.25, 3.125 & 1.56 µg) were added, and the incubation was continued for 48 h and viable cells yield was determined by a colorimetric method. In brief, after the end of the incubation period, media were aspirated and the crystal violet solution (1%) was added to each well for at least 30 minutes. The stain was removed and the plates were rinsed using tap water until all excess stain was removed. Glacial acetic acid (30%) was then added to all wells and mixed thoroughly, and then the absorbance of the plates were measured after gently shaken on Micro plate reader (TECAN, Inc.), using a test wavelength of 490 nm. All results were corrected for background absorbance detected in wells without added stain. Treated samples were compared with the cell

control in the absence of the tested compounds. All experiments were carried out in triplicate. The cell cytotoxic effect of each tested compound was calculated [48, 49].

In vitro anticancer screening

The newly synthesized compounds were evaluated for their in vitro cytotoxic activity against human breast cancer cell line, MCF7. Doxorubicin, which is one of the most effective anticancer agents, was used as the reference drug in this study. The relationship between surviving fraction and drug concentration was plotted to obtain the survival curve of breast cancer cell line (MCF7). The response parameter calculated was the IC50 value, which corresponds to the concentration required for 50% inhibition of cell viability. Table 1 shows the in vitro cytotoxic activity of the synthesized compounds **2, 3, 4, 6, 7, 8, 9, 10, 11, 12, 14, 15, 17, 18**, **20** and **21**. Most of the synthesized compounds exhibited significant activity compared to the reference drug, Doxorubicin. From the analysis of Table 1, it has been shown that **6, 7, 9** and **17** are the compounds of lowest IC50 which means that they are the most effective cytotoxic drugs, accordingly compounds **6, 7, 9** and **17** can be used as very potent cytotoxic drug for breast carcinoma cell, while compounds **4, 15 , 18** and **20** have moderate IC50 which means that they are lower effective cytotoxic drug for breast carcinoma cell, on the other hand the remaining compounds **2, 3, 8, 10, 11, 12, 14** and **21** are very weak cytotoxic drug compared with the reference drug, Doxorubicin. The results are summarized in Table 1.

Table 1: In-vitro anticancer screening of compounds2-21 human breast cell line (MCF7)

Compound	Cytotoxicity (IC50) (µg / mL)
	MCF7
2	›50
3	45.1
4	24.2
6	12.3
7	9.21
8	38.6
9	15.7
10	›50
11	›50
12	39.3
14	›50
15	26.7
17	5.82
18	30.1
20	31.5
21	39.7
Doxorubicin	0.426

IC50 compounds concentration required to inhibit tumor cell proliferation by 50%

CONCLUSION

The objective of the present study was to synthesize and investigate the anticancer activity of new thiazole, imidazole, thiophene, chromene and pyridinone derivatives containing biologically active moiety. Some of the new synthesized compounds **6, 7, 9** and **17** are found as active as doxorubicin control. Compound **4, 15, 18** and **20** exhibited a moderate activity and compounds **3, 8, 12** and **21** showed a weak activity, while compounds **2, 10, 11** and **14** revealed no activity. Further investigations on different probable mechanisms of action and dose response studies should be helpful in identifying the specific site(s) of action and optimum doses of the synthesized cyano acetamide derivatives. These investigations would be crucial in discovering more potent and more selective anti-breast cancer agents.

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