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Synthesis of novel potent anti-cancer agent derived from heterocyclization of cyclohexanone

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ABSTRACT

The 2-benzylidenecyclohexanone derivatives have been used as starting material to form a series of novel pyran, pyridine, pyrazole and thiophene derivatives. New approaches based on the reactivity of the starting materials towards different chemical reagents were developed and evaluated. Thirty one compounds of all the synthesized structures were selected and evaluated as significant anti-cancer agents. The results showed that some compounds with high activities towards the six cancer cell lines, NUGC (gastric cancer), DLDI (colon cancer), HA22T (liver cancer), HEPG2 (liver cancer), HONEI (nasopharyngeal carcinoma), HR (gastric cancer), MCF (breast cancer), WI38 (normal fibroblast cells) and comparing the results to inhibitory compound reference, CHS828.

Keywords: *thiophene, pyran, pyrazole, pyridine, pyrimidine.*

1. INTRODUCTION

In recent decades, multicomponent reactions (MCR's) have gained wide applicability in the field of synthetic organic chemistry as they increase the efficiency of the reaction and decrease the number of laboratory operations along with quantities of solvent and chemicals used. These methods also considerably reduce the reaction time and facilitate the yield of products than the normal multiple step methods. One-pot, four-component synthesis of symmetrically substituted 1,4-dihydropyridines were first reported[1]. Hantzsch 1,4-dihydropyridines (1,4-DHPs) and their derivatives are an important class of bioactive molecules in the pharmaceutical field[2]. They possess anti-inflammatory, anti-microbial,[3] anti-oxidant, antiulcer activities[4]. Several calcium channel blockers such as DHPs are used for the treatment of cardiovascular diseases, including hypertension[5]. Recently, the synthesis of DHPs with respect to multi drug Resistance (MDR) reversal in tumor cell gave a new dimension to their applications[6,7]. In addition, 1,4-DHP class of compounds are excellent starting synthons for development of antituber-cularagents[8,9]. Oxidative aromatization reactions of DHPs are taking place in biological systems in presence of certain enzymes. The nitrogen heterocyclic thus prepared by Hantzsch method is of great importance because of their role in biological systems. They have been served as model compounds for the NAD-NAPH biological redox systems[10-12]. Recently, antibiotic-resistant microbes are making their inexorablemarch and medicinal chemists have now realized that the discovery of more powerful antibiotics is not the only answer to this threat. But, a real need exists in searching a novel antimicrobial that expresses antimicrobial properties, possibly acting through mechanisms different from those of existing drugs. In this context, it is very essential to successfully develop novel, efficient antimicrobial agents with clinically unexploited mode of action. Further, pyrazole derivatives have showed significant biological activities, such as anti-microbial[13] analgesic[14] anti-inflammatory[15] and anticancer[16]. This gave a great impetus to the search for potential pharmacologically active drugs carrying pyrazole substituent. Keeping in view of this and in continuation of our search on biologically potent molecules[17-21], we here reported the synthesis of pyran, pyridine, pyrimidine, thiophene, pyrazole and thiazole derivatives using cyclohexanone as well as their cytotoxicity study against six cancer and one human normal cell lines.

2. EXPERIMENTAL SECTION

Electrothermal digital melting point apparatus was used to determin all melting points which are uncorrected. IR spectra (KBr discs) were recorded on a FTIR plus 460 or Pyeunicam SP-1000 spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra with chemical shifts expressed as δ ppm were recorded using Mercury-300BB (300 MHz) instrument in DMSO-*d*6 as solvent using TMS as internal standard in Cairo University. Analytical data were performed on Vario El III Elemental CHNS analyzer and obtained from the Microanalytical Data Unit, Cairo University, Egypt. Compounds 3a and 3b were synthesized according to the reported literature [22].

Synthesis of 2-benzylidenecyclohexanone derivatives (3a-c). A mixture of 1 (5 mL, 0.05 mol) and benzaldehyde (5 mL, 0.05 mol), p-chlorobenzaldehyde (6.7 g, 0.05 mol) or p-methoxybenzaldehyde (6.5 mL, 0.05 mol) with a catalytic amount of piperidine (0.5 mL) was heated under reflux at 120^{o}C in an oil bath for 2 hours. After cooling to room temperature, the reaction mixture was poured onto ice/water. After neutralization by HCl, the solid product formed was collected by filtration and crystallized from ethanol.

2-(4-Methoxybenzylidene)cyclohexanone (3c). Yellow crystals, m.p. 70^{o}C, yield 6.9 g (64%). IR (υ-cm^{-1}): 3104, 3062 (CH aromatic), 3018 (CH_2), 1662 (C=O), 1536 (C=C). ^{1}H NMR (δ-

ppm): 1.52-1.99 (m, 4H, $2CH_2$), 2.27-2.50 (m, 4H, $2CH_2$), 3.80 (s, 3H, OCH_3), 7.00-7.50 (m, 4H, C_6H_4), 7.58 (s, 1H, CH=C). ^{13}C NMR (δ-ppm): 35.7, 37.5, 38.7, 40.5 ($4CH_2$), 52.3 (OCH_3), 120.51, 121.03, 123.12, 129.4, (C_6H_4), 147.5, 150.0 (C=CH), 160.5 (CO). Analysis Calcd for: $C_{14}H_{16}O_2$ (216.28): C, 77.75; H, 7.46. Found: C, 77.57; H, 7.66.

Synthesis of 2-amino-5,6,7,8-tetrahydro-4-phenyl-4*H*-chromene-3-carbonitrile derivatives (5a-c). A mixture of malononitrile (0.66 g, 0.01 mol) and compound 3a (1.86 g, 0.01 mol), 3b (2.2 g, 0.01 mol) or 3c (2.16 g, 0.01 mol) were dissolved in ethanol (25 mL) containing a catalytic amount of triethylamine (0.50 mL). After heating for 3 h under reflux., the reaction mixture was allowed to cool to room temperature and then poured onto ice/water mixture. The mixture was neutralized by adding a few drops of concentrated HCl. The solid product formed was collected by filtration and crystallized from ethanol.

2 - Amino-5, 6, 7, 8 - tetrahydro -4- phenyl - 4*H* – chromene – 3 -carbonitrile (5a). Yellow crystals, m.p. 85-87^oC, yield 1.9 g (75%). IR (υ-cm^{-1}): 3406-3313 (NH_2), 3205, 3106 (CH aromatic), 2931 (CH_2), 2210 (CN). ^1H NMR (δ-ppm): 1.15-1.18 (m, 4H, $2CH_2$), 1.54-1.81 (m, 4H, $2CH_2$), 3.96 (s, 2H, NH_2), 6.96 (s, 1H, pyranH-4), 7.13-7.42 (m, 5H, C_6H_5). ^{13}C NMR (δ-ppm): 35.2, 36.6, 39.2, 40.8 ($4CH_2$), 115.0 (CN), 120.2, 121.1, 121.5, 123.0, 124.7, 127.2, 129.6, 130.8, 142.4 (C_6H_5, pyran C). Analysis Calcd for: $C_{16}H_{16}N_2O$ (252.31): C, 76.16; H, 6.39; N, 11.10. Found: C, 76.29; H, 6.66; N, 10.93.

2-Amino-5,6,7,8-tetrahydro-4-(4-chlorophenyl)-4*H*-chromene-3-carbonitrile (5b). Brown crystals, m.p. 95-97^oC, yield 2.4 g (85%) IR (υ-cm^{-1}): 3323-3418 (NH_2), 3064 (CH aromatic), 2921 (CH_2), 2216 (CN), 1536 (C=C). ^1H NMR (δ-ppm): 1.17-1.18 (m, 4H, CH_2), 1.53-2.18 (m, 4H, CH_2), 3.63 (s, 2H, NH_2), 6.93 (s, 1H, pyran H-4), 7.23-7.44 (m, 4H, C_6H_4). ^{13}C NMR (δ-ppm): 36.2, 38.8, 39.4, 40.3 ($4CH_2$), 113.4 (CN), 120.5, 121.01, 121.3, 123.3, 124.9, 127.7, 129.4, 130.5, 148.9 (C_6H_4, pyran C). Analysis Calcd for: $C_{16}H_{15}ClN_2O$ (286.76): Calcd: C, 67.02; H, 5.27; N, 9.77. Found: C. 67.19; H, 5.25; N, 9.75.

2 - Amino - 5, 6, 7, 8 - tetrahydro - 4 - (4-methoxyphenyl) - 4*H* - chromene – 3 - carbonitrile (5c). Brown crystals, m.p. 100-103^oC, yield 2.4 g (87%). IR (υ-cm-1): 3326-3426 (NH_2), 3055 (CH aromatic), 2215 (CN), 1563-1512 (C=C). ^1H NMR (δ-ppm): 1.12-1.16 (m, 4H, $2CH_2$), 2.49-2.50 (m, 4H, $2CH_2$), 3.77 (s, 2H, NH_2), 3.86 (s, 3H, OCH_3), 7.01 (s, 1H, pyranH-4), 7.18-7.40 (m, 4H, C_6H_4). ^{13}C NMR (δ-ppm): 36.7, 38.6, 39.9, 40.0 ($4CH_2$), 49.33 (OCH_3), 117.2 (CN), 120.51, 121.03, 123.12, 129.4, 128.7, 129.4, 130.5, 133.0, 148.8 (C_6H_4, pyran C). Analysis Calcd for: $C_{17}H_{18}N_2O_2$ (282.34): C, 72.32; H, 6.43; N, 9.92. Found: C, 72.48; H, 6.33; N, 10.09.

Synthesis of 2-amino-1,4,5,6,7,8-hexahydro-4-phenylquinoline-3-carbonitrile derivatives (6a-c). To a solution of compound 3a (1.85 g, 0.01 mol), 3b (2.2 g, 0.01 mol) or 3c (2.16 g, 0.01 mol) in ethanol (20 mL) containing ammonium acetate (0.77 g, 0.01 mol), malononitrile (0.66 g, 0.01 mol) was added. After heating for 3 h under reflux., the reaction mixture was allowed to cool to room temperature and poured onto ice/water mixture. The solid product formed after neutralization by hydrochloric acid was collected by filtration and crystallized from ethanol.

2-Amino-1, 4, 5, 6, 7, 8 – hexahydro – 4 – phenylquinoline – 3 - carbonitrile (6a). Reddish brown crystals, m.p. 72-75^oC, yield 1.6 g (65%). IR (υ-cm^{-1}): 3423, 3272 (NH, NH_2), 3143 (CH aromatic), 2919 (CH_2), 2216 (CN), 1589-1536 (C=C). ^1H NMR (δ-ppm): 1.22-1.35 (m, 4H, $2CH_2$), 1.90-1.94 (m, 4H, $2CH_2$), 3.56 (s, 2H, NH_2), 7.04 (s, 1H, pyridine H-4), 7.16-7.52 (m, 5H, C_6H_5), 10.02 (s, 1H, NH). ^{13}C NMR (δ-ppm): 30.2, 38.9, 39.2, 40.0 ($4CH_2$), 116.6 (CN), 120.5, 120.6, 121.4, 123.4, 124.6, 129.2, 133.1, 134.4, 140.4 (C_6H_5, pyridine C). Analysis Calcd for: $C_{16}H_{17}N_3$ (251.33): C, 76.46; H, 6.82; N, 16.72. Found: C, 76.27; H, 6.88; N, 16.54.

2-Amino-1,4,5,6,7,8-hexahydro-4-(4-chlorophenyl)quinoline-3-carbonitrile (6b). Yellow crystals, m.p 39^oC, yield 2.4 g (85%). IR (υ-cm^{-1}): 3383-3210 (NH_2), 3065 (CH aromatic), 2931 (CH_2), 2192 (CN), 1554 (C=C). ^1H NMR (δ-ppm): 1.60-1.63 (m, 4H, $2CH_2$), 2.48-2.51 (m, 4H, $2CH_2$), 3.01 (s, 2H, NH_2), 7.17 (s, 1H, pyridine H-4), 7.20-7.59 (m, 4H, C_6H_4), 9.63 (s, 1H, NH). ^{13}C NMR (δ-ppm): 30.2, 45.8, 49.4, 50.2 ($4CH_2$), 115.2 (CN), 120.4, 121.1, 122.8, 123.2, 124.3, 128.2, 128.3, 128.8, 129.4 (C_6H_4, pyridine C). Analysis Calcd for: $C_{16}H_{16}ClN_3$ (285.77): C, 67.25; H, 5.46; N, 14.70. Found: C, 67.39; H, 5.25; N, 14.18.

2-Amino-1,4,5,6,7,8-hexahydro-4-(4-methoxyphenyl)quinoline-3-carbonitrile (6c). Yellow crystals, m.p 45-48^oC, yield 2.1 g (77%). IR (υ-cm^{-1}): 3351-3201 (NH_2), 3222-3000 (CH aromatic), 2930, 2843 (CH_2), 2197 (CN), 1564 (C=C). ^1H NMR (δ-ppm): 1.59-2.87 (m, 8H, $4CH_2$), 3.22 (s, 2H, NH_2), 3.80 (s, 3H, OCH_3), 7.07 (s, 1H, pyridine H-4), 6.86-7.49 (m, 5H, C_6H_5), 9.40 (s, 1H, NH). ^{13}C NMR (δ-ppm): 30.2, 40.8, 44.3, 44.6 ($4CH_2$), 50.5 (OCH_3), 116.0 (CN), 120.2, 121.5, 122.9, 123.1, 124.1, 128.5, 128.8, 129.0, 149.5 (C_6H_4, pyridine C). Analysis Calcd for: $C_{17}H_{19}N_3O$ (281.35): C, 72.57; H, 6.81; N, 14.94. Found: C, 72.29; H, 5.44; N, 14.79.

Synthesis of 2-amino-4-benzylidene-4,5,6,7-tetrahydrobenzo-[*b*]thiophene-3-carbonitrile derivatives (7a-c). A mixture of malononitrile (0.66 g, 0.01 mol), elementals sulfur (0.3 g, 0.01 mol) and compound 3a (1.8 g, 0.01 mol), 3b (2.2 g, 0.01 mol), or 3c (2.16 g, 0.01 mol) in 1,4-dioxane containing triethylamine (0.50 mL) was heated at 120^oC for 45 min. After cooling to room temperature, the reaction mixture was poured onto ice/water then neutralized by adding a few drops of concentrated hydrochloric acid. The formed solid product was collected by filtration and crystallized from 1,4-dioxane.

2-Amino-4-benzylidene-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3-carbonitrile (7a). Brown crystals, m.p 113-114^oC, yield 1.8 g (80%). IR (υ-cm^{-1}): 3418-3320 (NH_2), 3063 (CH aromatic), 2958-2918 (CH, CH_2), 2215 (CN), 1583, 1536 (C=C). ^1H NMR (δ-ppm): 1.54-2.18 (m, 6H, $3CH_2$), 3.96 (s, 2H, NH_2), 7.16-7.37 (m, 5H, C_6H_5), 7.40 (s, 1H, CH=C). ^{13}C NMR (δ-ppm): 32.2, 44.6, 55.3 ($3CH_2$), 116.2 (CN), 120.3, 120.9, 121.6, 124.0, 124.4, 127.4, 129.0, 130.2 (C_6H_5, thiophene), 147.9, 150.0 (CH=C). Analysis Calcd for $C_{16}H_{14}N_2S$ (266.36): C, 72.15; H, 5.30; N, 10.52; S, 12.04. Found: C, 71.48; H, 5.33; N, 10.82; S, 12.93.

2 - Amino – 4 - (4-chlorobenzylidene) - 4, 5, 6, 7 - tetrahydrobenzo[*b*]thiophene - 3 - carbonitrile (7b). Brown crystals, m.p. 50-53°C, yield 2.5 g (85%). IR (υ-cm^{-1}): 3325-3180 (NH_2), 3056 (CH aromatic), 2931, 2856 (CH, CH_2), 2200 (CN), 1604, 1520 (C=C). ^{1}H NMR (δ-ppm): 1.63-2.57 (m, 6H, CH_2), 3.56 (s, 2H, NH_2), 7.48-7.57 (m, 4H, C_6H_4), 7.58 (s, 1H, C=CH). ^{13}C NMR (δ-ppm): 30.2, 45.1, 65.3 ($3CH_2$), 116.0 (CN), 120.5, 121.0, 121.4, 123.8, 124.9, 127.7, 129.4, 130.6 (C_6H_4, thiophene), 148.9, 150.3 (CH=C). Analysis Calcd for: $C_{16}H_{13}ClN_2S$ (300.81): C, 63.89; H, 4.36; N, 9.31; S, 10.66. Found: C, 63.71; H, 4.22; N, 9.12; S, 10.44.

2 - Amino - 4 - (4 - methoxybenzylidene) - 4, 5, 6, 7-tetrahydrobenzo[*b*]thiophene - 3 - carbonitrile (7c). Brown crystals, m.p. 85°C, yield 2.3 g (80%). IR (υ-cm^{-1}): 3380 (NH_2), 3056 (CH aromatic), 2932, 2836 (CH, CH_2), 2199 (CN), 1593 (C=C). ^{1}H NMR (δ-ppm): 1.68-2.87 (m, 6H, $3CH_2$), 3.74 (s, 2H, NH_2), 3.80 (s, 3H, OCH_3), 7.00-7.52 (m, 4H, C_6H_4), 7.58 (s, 1H, C=CH). ^{13}C NMR (δ-ppm): 30.7, 44.3, 55.1 ($3CH_2$), 52.5 (OCH_3), 116.4 (CN), 121.1, 121.6, 123.7, 126.5, 127.9, 129.9, 130.6, 148.9 (C_6H_4, thiophene), 149.9, 150.5 (CH=C). Analysis Calcd for: $C_{17}H_{16}N_2OS$ (296.39): C, 68.89; H, 5.44; N, 9.45; S, 10.82. Found: C, 68.59; H, 5.66; N, 9.55; S, 10.63.

Synthesis of 2-benzylidenecyclohexylidenethiourea derivatives (9a-c). Equimolar amount of compound 3a (1.86 g, 0.01 mol), 3b (2.2 g, 0.01 mol), or 3c (2.16 g, 0.01 mol) and thiourea (0.76 g, 0.01 mol) in ethanol (25 mL) containing triethylamine (0.50 mL) was heated under reflux for 3 h and then poured onto ice/water mixture. The solid product formed after neutralization by hydrochloric acid was collected by filtration and crystallized from ethanol.

1-(2-Benzylidenecyclohexylidene)thiourea (9a). Yellow crystals, m.p. 67-68°C, yield 1.9 g (80%). IR (υ-cm^{-1}): 3413-3320 (NH_2), 3102 (CH aromatic), 2921 (CH, CH_2), 1582 (C=C). ^{1}H NMR (δ-ppm): 1.69-1.73 (m, 4H, $2CH_2$), 2.48-2.50 (m, 4H, $2CH_2$), 3.52 (s, 2H, NH_2), 7.27-7.55 (m, 5H, C_6H_5), 7.63 (s, 1H, C=CH). ^{13}C NMR (δ-ppm): 30.2, 45.8, 49.6, 55.2 ($4CH_2$), 120.6, 121.0, 129.2, 129.4 (C_6H_5), 133.0, 133.2 (CH=C), 165.0 (C=S), 170.0 (C=N). Analysis Calcd for: $C_{14}H_{16}N_2S$ (244.36): C, 68.81; H, 6.60; N, 11.46; S, 13.12. Found: C, 68.71; H, 6.79; N, 11.56; S, 13.29.

1-(2-(4-Chlorobenzylidene)cyclohexylidene)thiourea (9b). Yellow crystals, m.p. 105-107°C, yield 2.2 g (80%). IR (υ-cm^{-1}): 3425-3422 (NH_2), 3062 (CH aromatic), 2914 (CH, CH_2), 1440 (C=C). ^{1}H NMR (δ-ppm): 1.69-1.71 (m, 4H, $2CH_2$), 2.50-2.54 (m, 4H, $2CH_2$), 3.66 (s, 2H, NH_2) 7.46-7.57 (m, 4H, C_6H_4), 7.58 (s, 1H, CH=C). ^{13}C NMR (δ-ppm): 31.2, 45.7, 49.2, 54.1 ($4CH_2$), 120.2, 121.9, 129.0, 129.8 (C_6H_5), 135.4, 136.2 (CH=C), 165.1 (C=S), 170.0 (C=N). Analysis Calcd for: $C_{14}H_{15}ClN_2S$, (278.80): C, 60.31; H, 5.42; N, 10.05; S, 11.50. Found: C, 60.46; H, 5.39; N, 10.03; S, 11.73.

2-(4-Methoxybenzylidene)cyclohexylidenethiourea (9c). Brown crystals, m.p. 125-128°C, yield 2.2 g (80%). IR (υ-cm^{-1}): 3339-3321 (NH_2), 3066 (CH Aromatic), 1582 (C=C). ^{1}H NMR (δ-ppm): 1.69-1.79 (m, 4H, $2CH_2$), 2.53-2.88 (m, 4H, $2CH_2$), 3.52 (s, 2H, NH_2), 3.80 (s, 3H, OCH_3), 6.99-7.52 (m, 4H, C_6H_4), 7.58 (s, 1H, CH=C). ^{13}C NMR (δ-ppm): 30.2, 45.8, 49.6, 65.2 ($4CH_2$), 56.2 (OCH_3), 120.1, 122.5, 130.9, 142.6 (C_6H_5), 136.0, 138.2 (CH=C), 164.9 (C=S), 170.4 (C=N). Analysis Calcd for: $C_{15}H_{18}N_2OS$ (274.38): C, 65.66; H, 6.61; N, 10.21; S, 11.69. Found: C, 65.71; H, 6.99; N, 11.36; S, 11.36.

Synthesis of 3-(5,6,7,8-tetrahydro-2-thioxoquinazoline-3(2*H*)-yl)-3-oxopropanenitrile derivatives (11a-c). Equimolar amount of ethyl 2-cyanoacetate 10 (1.13 mL, 0.01 mol), thiourea 8 (0.76 g, 0.01 mol) and compound 3a (1.86 g, 0.01 mol), 3b (2.2 g, 0.01 mol) or 3c (2.16 g, 0.01 mol) were dissolved in ethanol (25 mL) containing triethylamine (0.50 mL). After heating for 3 h under reflux, the solid product formed after neutralization by hydrochloric acid was collected by filtration and crystallized from ethanol.

3-(4-Phenyl-5,6,7,8-tetrahydro-2-thioxoquinazolin-3(2*H*)-yl)-3-oxopropanenitrile (11a). Reddish brown crystals, m.p. 82-87°C; yield 2.6 g (85%). IR (υ-cm-1): 3055 (CH aromatic), 2932 (CH_2), 2187 (CN), 1675 (C=O). ^{1}H NMR (δ-ppm): 1.14-1.18 (m, 4H, $2CH_2$), 1.59-1.80 (m, 4H, $2CH_2$), 2.50 (s, 2H, CH_2), 7.05-7.54 (m, 5H, C_6H_5). Analysis Calcd for: $C_{17}H_{15}N_3OS$ (309.39): C, 66.00; H, 4.89; N, 13.58; S, 10.36. Found: C, 66.39; H, 4.73; N, 13.58; S, 10.53.

3-(4-(4-Chlorophenyl)-5,6,7,8-tetrahydro-2-thioxoquinazoline-3(2*H*)-yl)-3-oxopropane-nitrile (11b). Pale orange crystals, m.p. 63-65°C; yield 2.9 g (85%). IR (υ-cm^{-1}): 3057 (CH aromatic), 2953-2862 (CH_2), 2223 (CN), 1737 (C=O). ^{1}H NMR (δ-ppm): 1.01-1.05 (m, 4H, $2CH_2$), 1.56-1.68 (m, 4H, $2CH_2$), 2.50 (s, 2H, CH_2), 7.15-7.26 (m, 4H, C_6H_4). Analysis Calcd for: $C_{17}H_{14}ClN_3OS$ (343.83): C, 59.38; H, 4.10; N, 12.22; S, 9.33. Found: C, 59.22; H, 4.38; N, 12.30; S, 9.52.

3 - (4-(4-methoxyphenyl) - 5, 6, 7, 8 – tetrahydro – 2 – thioxoquinazoline - 3(2*H*)-yl)-3-oxopropanenitrile (11c). Yellow crystals, m.p. 130-133°C; yield 2.9 g (85%). IR (υ-cm^{-1}): 3006 (CH aromatic), 2973-2831 (CH_2), 2013 (CN), 1737 (C=O). ^{1}H NMR (δ-ppm): 1.03-1.08 (m, 4H, $2CH_2$), 1.07-1.74 (m, 4H, $2CH_2$), 2.50 (s, 2H, CH_2), 3.80 (s, 3H, OCH_3), 7.03-7.56 (m, 4H, C_6H_4). Analysis Calcd for: $C_{18}H_{17}N_3O_2S$ (339.41): C, 63.70; H, 5.65; N, 12.38; S, 9.45. Found: C, 63.55; H, 5.72; N, 12.49; S, 9.42.

Synthesis of 4-phenyl-5,6,7,8-tetrahydro-2-hdroxy-4*H*-chromene-3-carbonitrile derivatives (12a,b). A mixture of ethyl 2-cyanoacetate (1.13 mL, 0.01 mol) and either 3b (2.2 g, 0.01 mol) or 3c (2.16 g, 0.01 mol) in ethanol (25 mL) containing triethylamine was heated under reflux for 3 h and then poured onto ice/water mixture containing few drops of hydrochloric acid. The formed solid product was collected by filtration.

4 - (4-Chlorophenyl) - 5, 6, 7, 8 – tetrahydro – 2 – hdroxy - 4*H* - chromene-3-carbonitrile (12a). Reddish brown crystals, m.p. 38-40°C, yield 2.4 g (85%). IR (υ-cm^{-1}): 3442 (OH), 3048 (CH aromatic), 2935-2861 (CH, CH_2), 2189 (CN). ^{1}H NMR (δ-ppm): 1.23-1.57 (m, 4H, $2CH_2$), 2.48-2.51 (m, 4H, $2CH_2$), 7.16 (s, 1H, pyran H-4), 7.19-7.51 (m, 4H, C_6H_4), 8.24 (s, 1H, D_2O exchangeable, NH). ^{13}C NMR (δ-ppm): 30.1, 45.6, 49.5, 50.5 ($4CH_2$), 117.6 (CN), 120.5, 120.6, 124.6, 129.2, 129.8, 133.1, 134.5, 140.3, 145.2 (C_6H_4, pyran C). Analysis Calcd for:

$C_{16}H_{14}ClNO_2$ (287.74): C, 66.79; H; 4.90; N, 4.87. Found: C, 66.73; H, 4.99; N, 4.93.

4-(4-Methoxyphenyl) - 5, 6, 7, 8 – tetrahydro – 2 – hdroxy - 4*H* - chromene-3-carbonitrile (12b). Yellow light crystals, m.p.160-163^{o}C, yield 2.4 g (85%). IR (υ-cm^{-1}): 3442 (OH), 3003 (CH aromatic), 2937-2830 (CH_2), 2210 (CN). ^{1}H NMR (δ-ppm): 1.23-1.57 (m, 4H, $2CH_2$), 2.48-2.51 (m, 4H, $2CH_2$), 3.81 (s, 3H, OCH_3), 7.00 (s, 1H, pyran H-4), 7.00-7.58 (m, 4H, C_6H_4) 8.23 (s, 1H, D_2O exchangeable, NH). ^{13}C NMR (δ-ppm): 30.2, 45.4, 49.3, 50.2 ($4CH_2$), 56.7 (OCH_3), 115.2 (CN), 120.4, 121.1, 122.8, 124.3, 124.5, 128.2, 129.4, 155.5, 165.7 (C_6H_4, pyran). Analysis Calcd for: $C_{17}H_{17}NO_3$ (283.32): C, 72.07; H, 6.05; N, 4.94. Found: C, 71.95; H, 6.25; N, 5.03.

Synthesis of 4-phenyl-1,4,5,6,7,8-hexahydroquinoline-3-carbonitrile derivatives (13a,b). To a solution of ethyl 2-cyanoacetate (1.13 mL, 0.01 mol) in ethanol (25 mL) containing ammonium acetate (0.77 g, 0.01 mol), 2-(4-chlorobenzylidene)cyclohexanone 3b (2.2 g, 0.01 mol) or 2-(4-methoxybenzylidene)cyclohexanone 3c (2.16 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 3h then poured onto ice/water mixture containing few drops of hydrochloric acid. The formed solid product was collected by filtration and crystallized from ethanol.

4-(4-Chlorophenyl)-1, 4, 5, 6, 7, 8-hexahydroquinoline-3-carbonitrile (13a). Yellow crystals, m.p. 57-60^{o}C, yield 2.3 g (80%). IR (υ-cm^{-1}): 3416 (OH), 3260 (NH), 3060 (CH aromatic), 2932-2859 (CH_2), 2221 (CN), 1632 (C=C). ^{1}H NMR (δ-ppm): 1.61-2.87 (m, 8H, $4CH_2$), 7.18 (s, 1H, pyridine H-4), 7.20-7.57 (m, 4H, C_6H_4), 8.60 (s, 1H, NH), 10.21 (s, 1H, D_2O exchangeable, OH). ^{13}C NMR (δ-ppm): 30.2, 45.0, 49.0, 49.2 ($4CH_2$), 115.9 (CN), 120.4, 120.6, 125.6, 129.2, 129.8, 133.4, 134.7, 140.3, 145.8 (C_6H_4, pyridine C). Analysis Calcd for: $C_{16}H_{15}ClN_2O$ (286.76): C, 67.02; H, 5.27; N, 9.77. Found: C, 67.19; H, 4.93; N, 9.67.

2 - Hydroxy - 4 - (4-methoxyphenyl) - 1, 4, 5, 6, 7, 8-hexahydroquinoline – 3 - carbonitrile (13b). Brown crystals, m.p 60-62^{o}C, yield 2.4 g (85%). IR (υ-cm^{-1}): 3431 (OH), 3364 (NH), 3066 (CH aromatic), 2932-2859 (CH_3), 2217 (CN). ^{1}H NMR (δ-ppm): 1.03-1.08 (m, 4H, $2CH_2$), 1.22-1.26 (m, 4H, $2CH_2$), 3.78 (s, 3H, OCH_3), 7.07 (s, 1H, pyridine H-4), 7.10-7.14 (m, 4H, C_6H_4), 9.55 (s, 1H, NH), 10.23 (s, 1H, D_2O exchangeable, OH). ^{13}C NMR (δ-ppm): 30.6, 45.3, 49.5, 50.0 ($4CH_2$), 56.9 (OCH_3), 116.0 (CN), 120.6, 121.1, 122.9, 124.5, 126.5, 129.0, 130.5, 154.9, 167.2 (C_6H_4, pyridine C). Analysis Calcd for: $C_{17}H_{18}N_2O_2$ (282.34): C, 72.32; H, 6.43; N, 9.92. Found: C, 72.08; H, 6.57; N, 9.89.

Ethyl 4 - (4-methoxybenzylidene) – 2 – amino – octa - hydrobenzo[*b*]thiophene – 3 - carboxylate (14). A mixture of compound 3c (2.16 g, 0.01 mol), elementals sulfur (0.32 g, 0.01 mol) and ethyl 2-cyanoacetate (1.06 mL, 0.01 mol) were dissolved in ethanol (20 mL) containing triethylamine (0.50 mL). After heating under reflux for 3 h, the reaction mixture was allowed to cool to room temperature and then poured onto ice/water mixture. The solid product formed after neutralization by hydrochloric acid was collected by filtration and crystallized from ethanol.

Pale yellow crystals, m.p. 120-123^{o}C, yield 2.9 g (85%). IR (υ-cm^{-1}): 3432-3332 (NH_2), 3003 (CH aromatic), 2936-2830 (CH_2), 1725 (C=O), 1592 (C=C). ^{1}H NMR (δ–ppm): 1.72 (t, 3H, CH_3), 2.49-2.50 (m, 6H, $3CH_2$), 3.60 (q, 3H, CH_3), 3.79 (s, 3H, CH_3), 3.82 (s, 2H, D_2O exchangeable, NH_2), 7.00-7.58 (m, 4H, C_6H_4). ^{13}C NMR (δ-ppm): 25.5 (CH_3), 30.7, 44.3, 55.1 ($3CH_2$), 52.5 (OCH_3), 62.5 (OCH_2), 121.0, 121.4, 123.8, 126.1, 127.9, 129.8, 130.6, 148.9 (C_6H_4, thiophene), 149.7, 150.5 (CH=C), 156.5 (CO). Analysis Calcd for: $C_{19}H_{21}NO_3S$ (343.44): C, 66.54; H, 6.16; N, 4.08. Found: C, 66.39; H, 6.07; N, 3.89.

4 - (4 - Methoxybenzylidene) – hexahydro – 3 - phenylbenzo(*d*)thiazole - 2(3*H*) – thione (16). A mixture of 2-(4-methoxybenzylidene)cyclohexanone 3c (2.16 g, 0.01 mol), elementals sulfur (0.32 g, 0.01 mol) and phenylisothiocyanate 15 (1.2 mL, 0.01 mol) was dissolved in ethanol (20 mL) containing triethylamine (0.50 mL) and heated under reflux for 3h. After cooling to room temperature, the reaction mixture was poured onto ice/water containing few drops of hydrochloric acid. The solid product formed was collected by filtration and crystallized from ethanol.

Brown crystals, m.p. 105-107^{o}C, yield 3.3 g (90%). IR (υ–cm^{-1}): 3004 (CH aromatic), 2937-2830 (CH_2), 1593 (C=C). ^{1}H NMR (δ–ppm): 1.70-2.89 (m, 6H, $3CH_2$), 3.73 (s, 3H, OCH_3), 7.01-7.52 (m, 9H, C_6H_5, C_6H_4), 7.58 (s, 1H, C=CH). ^{13}C NMR (δ-ppm): 31.7, 42.5, 53.5 ($3CH_2$), 55.8 (OCH_3), 119.8, 120.2, 121.5, 121.8, 122.5, 126.1, 127.9, 129.8, 130.6, 148.9 (C_6H_5, C_6H_4, thiophene), 147.7, 151.6 (CH=C), 175.7 (C=S). Analysis Calcd for: $C_{21}H_{19}NOS_2$ (365.51): C, 69.81; H, 5.24; N, 3.83; S, 17.55. Found: C, 69.94; H, 5.38; N, 4.05; S, 17.36.

4 – Amino – 9 - (4-methoxybenzylidene) – 3 – phenyl – 6 , 7, 8 , 9-tetrahydro-benzo[4,5]-thieno[3,2-*d*]pyrimidine-2(3*H*)-thione (17). A mixture of 7c (3.2 g, 0.01 mol) and phenylisothiocyanate (1.3 mL, 0.01 mol) were dissolved in ethanol (20 mL) containing triethylamine (0.05 mL). The reaction mixture was then heated under reflux for 4 h. After cooling to room temperature, the reaction mixture was poured onto ice/water containing hydrochloric acid. The solid product formed was collected by filtration and crystallized from ethanol.

Yellow crystals, m.p. 103-105^{o}C, yield 3.7 g (86%). IR (υ-cm^{-1}): 3429-3326 (NH_2), 3004 (CH aromatic), 2937-2829 (CH_2). ^{1}H NMR (δ–ppm): 1.72 (s, 2H, D_2O exchangeable, NH_2), 2.49-2.89 (m, 6H, $3CH_2$), 3.80 (s, 3H, OCH_3), 7.00-7.78 (m, 9H, C_6H_5, C_6H_4), 7.69 (s, 1H, C=CH). ^{13}C NMR (δ-ppm): 30.7, 44.3, 55.1 ($3CH_2$), 54.5 (OCH_3) 118.4, 121.1, 121.6, 123.7, 124.3, 126.5, 127.9, 128.8, 129.9, 130.6, 148.9, 155.5 (C_6H_5, C_6H_4, pyrimidine, thiophene), 149.9, 150.5 (CH=C), 176.5 (C=S). Analysis Calcd for: $C_{24}H_{21}N_3OS_2$ (431.57): C, 66.79; H, 4.90; N, 9.74; S, 14.86. Found: C, 66.84; H, 5.17; N, 9.79; S, 14.69

Synthesis of 4,5,6,7-tetrahydro-2*H*-indazole derivatives 19a, b. To a solution of either compound 3b (2.2 g, 0.01 mol) or 3c (2.1 g, 0.01 mol) in ethanol (25 mL) containing triethylamine (0.50 mL), hydrazine hydrate (0.50 mL, 0.01 mol) was added. The solution

was then heated under reflux for 2 hours. The reaction mixture was allowed to cool to room temperature and then poured onto ice/water. The solid product formed after neutralization by hydrochloric acid was collected by filtration and crystallized from ethanol.

3-(4-Chlorophenyl)-4,5,6,7-tetrahydro-2*H*-indazole (19a). Orange crystals, m.p. 139-141°C, yield 1.9 g (85%). IR (υ-cm^{-1}): 3425 (NH), 3036 (CH aromatic), 2969 (CH_2), 1584 (C=C). ^{1}H NMR (δ–ppm): 1.34-1.50 (m, 4H, $2CH_2$), 2.49-2.51 (m, 4H, $2CH_2$), 7.40-7.95 (m, 4H, C_6H_4), 8.37 (s, 1H, D_2O, NH), ^{13}C NMR (δ-ppm): 38.6, 39.4, 44.6, 50.7 (CH_2), 115.2, 120.2, 121.3, 130.4, 134.1, 138.9, 140.6 (C_6H_4, pyrazole). Analysis Calcd for: $C_{13}H_{13}ClN_2$ (232.71): C, 67.10; H, 5.63; N, 12.04. Found: C, 67.25; H, 5.73; N, 12.19.

3-(4-Methoxyphenyl)-4,5,6,7-tetrahydro-2*H*-indazole (19b). Yellow crystals, m.p. 80-85°C, yield 1.8 g (82%). IR (υ-cm^{-1}): 3437 (NH), 3021 (CH aromatic), 2843 (CH_2), 1594 (C=C). ^{1}H NMR (δ–ppm): 1.70-1.73 (m, 4H, $2CH_2$), 2.49-2.50 (m, 4H, $2CH_2$), 2.71 (s, 3H, OCH_3), 7.01-7.52 (m, 4H, C_6H_4) 7.58 (s, 1H, NH). ^{13}C NMR (δ-ppm): 37.7, 39.0, 45.2, 50.9 (CH_2), 55.7 (OCH_3), 115.6, 120.6, 123.5, 134.5, 138.5, 140.6, 145.6 (C_6H_4, pyrazole C). Analysis Calcd for: $C_{14}H_{16}N_2O$ (228.29): C, 73.66; H, 7.06; N, 12.27. Found: 73.74; H, 6.95; N, 12.39.

Synthesis of 4,5,6,7-tetrahydroindazol2-yl)-3-oxopropane-nitrile derivatives (21a,b). To a solution of ethyl cyanoacetate (1.13 g, 0.01 mol) in dimethylformamide (20 mL), compound 3b (2.2 g, 0.01 mol) or 3c (2.1 g, 0.01 mol) was added. The reaction mixture was heated under reflux for 2 h. After cooling to room temperature, the reaction mixture was poured onto ice/water and the formed solid product was collected by filtration.

3-(3-(4-Chlorophenyl) - 4, 5, 6, 7 - tetrahydroindazol-2-yl)-3-oxopropane-nitrile (21a). Orange crystals, m.p. 122-125°C, yield 2.5 g (85%). IR (υ-cm^{-1}): 3061 (CH aromatic), 2932-2863 (CH_2), 2261 (CN), 1676 (C=O). ^{1}H NMR (δ–ppm): 1.56-2.49 (m, 8H, $4CH_2$), 2.56 (s, 2H, CH_2), 7.25-7.58 (m, 4H, C_6H_4). ^{13}C NMR (δ-ppm): 24.2, 37.5, 38.2, 43.2, 50.1 (CH_2), 115.5 (CN), 115.9, 121.1, 121.5, 131.6, 135.4, 137.8, 141.9 (C_6H_4, pyrazole C), 164.3 (C=O). Analysis Calcd for: $C_{16}H_{14}ClN_3O$ (299.75): C, 64.11; H, 4.71; N, 14.02. Found: C, 64.33; H, 4.59; N, 13.93.

3(3-(4-Methoxyphenyl)-4,5,6,7-tetrahydroindazol2-yl)-3-oxopropanenitrile (21b). Yellow crystals, m.p. 125-228°C, yield 2.5 g (87%). IR (υ-cm^{-1}): 3073-3003 (CH aromatic), 2937-2830 (CH_2), 2261 (CN), 1673 (C=O). ^{1}H NMR (δ–ppm): 1.72-2.75 (m, 8H, $4CH_2$), 2.49 (s, 2H, CH_2), 3.80 (s, 3H, OCH_3), 7.02-7.52 (m, 4H, C_6H_4). 24.5, 38.2, 38.7, 44.3, 50.0 (CH_2), 57.0 (OCH_3), 115.2 (CN), 116.1, 121.1, 122.8, 131.6, 136.5, 138.3, 148.8 (C_6H_4, pyrazole), 164.0 (C=O). Analysis Calcd for: $C_{17}H_{17}N_3O_2$ (295.34): C, 69.14; H, 5.80; N, 14.23. Found: C, 69.08; H, 6.11; N, 14.52.

7 – Amino - 9, 10, 11, 12 - tetrahydrochromeno[3,4-*c*]-chromen-6(12b*H*)-one (23). A mixture of compound 1 (0.89 mL, 0.01 mol), salicylaldehyde (1.10 mL, 0.01 mol) and malononitrile (0.66 g, 0.01 mol) in absolute ethanol (20 mL) containing triethylamine (0.50 mL) was heated under reflux for 1 h. The reaction mixture allowed to cool to room temperature and then poured onto ice/water. The solid product formed after neutralization by hydrochloric acid was collected by filtration and crystallized from ethanol.

Yellow crystals, m.p. 66-69°C, yield 2.4 g (88%). IR (υ-cm^{-1}): 3540-3326 (NH_2), 3063 (CH aromatic), 2937 (CH_2), 1661 (C=O). ^{1}H NMR (δ–ppm): 1.59-1.93 (m, 8H, $4CH_2$), 2.96 (s, 2H, NH_2), 6.90 (s, 1H, pyran 4H), 7.10-7.43 (m, 4H, C_6H_4). ^{13}C NMR (δ–ppm): 40.3, 45.5, 49.1, 65.4, ($4CH_2$), 120.4, 120.5, 121.1, 122.2, 124.2, 128.4, 128.9, 129.4, 129.4, 139.1, 140.5 (C_6H_4, pyran C), 162.6 (C=O). Analysis Calcd for: $C_{16}H_{15}NO_3$ (269.30): C, 71.36; H, 5.61; N, 5.20. Found: C, 71.28; H, 5.70; N, 5.19.

4,5,6,7-Tetrahydrobenzo(d)thiazol-2-amine (25). To a solution of compound 1 (6 mL, 0.06 mol) in acetic acid (30 mL), bromine (4.8 mL, 0.06 mol) was added drop-wise. After stirring for 40 min at room temperature, thiourea (4.7 g, 0.06 mol) in absolute ethanol (25 mL) was added and the reaction mixture was heated under reflux for 1 h. The reaction mixture was allowed to cool to room temperature and then poured onto ice/water mixture. The solid product formed was collected by filtration and crystallized from ethanol.

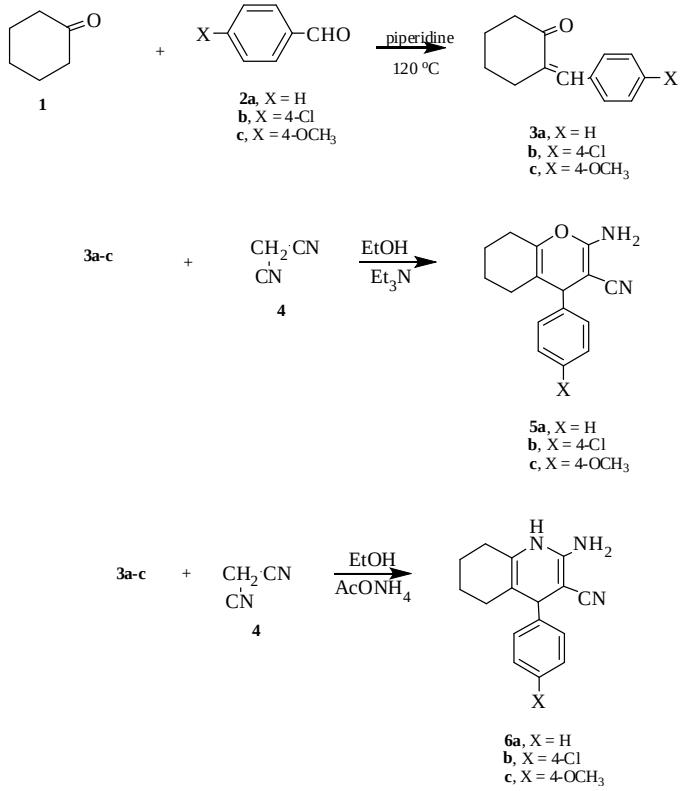
Yellow crystals, m.p. 85°C, yield 1.3 g (86%). IR (υ-cm-1): 3326-3753 (NH_2), 3053 (CH aromatic), 2927-2850 (CH_2). ^{1}H NMR (δ–ppm): 1.14-1.59 (m, 4H, $2CH_2$), 1.75-1.84 (m, 4H, $2CH_2$), 3.56 (s, 2H, NH_2). ^{13}C NMR (δ–ppm): 40.06, 40.3, 45.5, 49.8 (CH_2), 115.6, 120.4, 120.8 (thiazole C). Analysis Calcd for: $C_7H_{10}N_2S$ (154.23): C, 54.51; H, 6.54; N, 18.16; S, 20.79. Found: C, 54.44; H, 6.39; N, 18.29; S, 20.66.

3. RESULTS SECTION

Chemistry. Reaction of cyclohexanone with aromatic aldehyde such as benzaldehyde, 4-chlorobenzaldehyde or 4-methoxybenzaldehyde in the presence of few drops of piperidine produced the 2-arylidenecyclohexanone derivatives **3a-c**, respectively (Scheme1). The structures of the latter products were confirmed based on their analytical and spectral data. Thus, the ^{1}H NMR spectrum of **3c** showed two multiplets at δ 1.52-1.99 and 2.27-2.50 ppm equivalent to the four CH_2 groups a singlet at δ 3.80 indicating the CH_3 group, a multiplet at δ 7.00-7.50 ppm for the phenyl protons and a singlet at δ 7.58 ppm for the CH group. Pyran derivatives **5a-c** were synthesized by the reaction of 2-arylidenecyclohexanone derivatives **3a-c** with malononitrile **4** in absolute ethanol in presence of catalytic amount of triethylamine (Scheme 1). On the other hand, carrying the same reaction but using ammonium acetate instead of triethylamine produced the pyridine derivatives **6a-c** (Scheme 1).

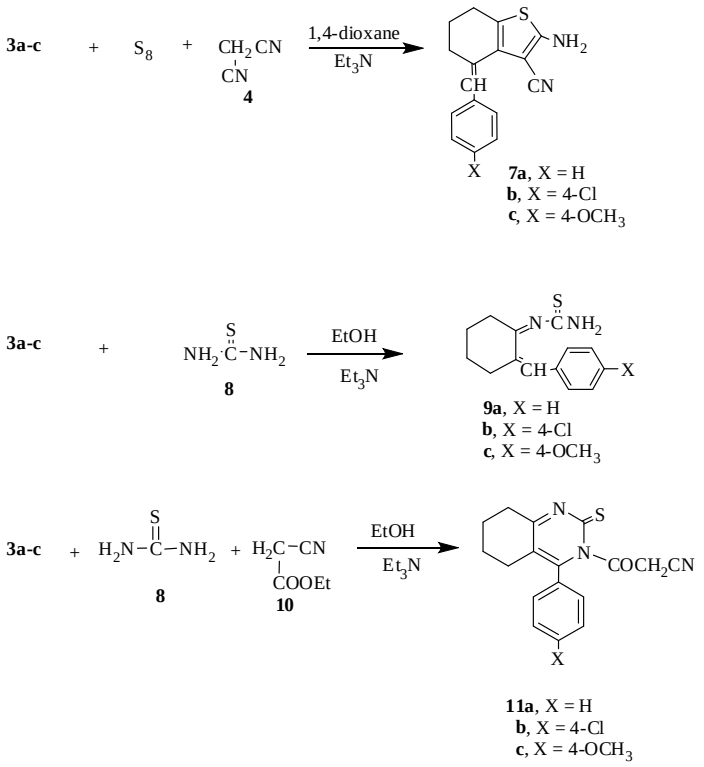
Formation of 2-arylidenecyclohexanone compounds **3a-c** in a good yield promoted us to study their reactivity towards thiophene synthesis using the well-known Gewald's thiophene synthesis[23,24]. Therefore, the reaction of 2-arylidenecyclohexanone derivatives **3a-c** with elemental sulfur and malononitrile **4** formed the thiophene derivatives **7a-**

c,(Scheme 2). The analytical and spectral data of the latter products are consistent with their respective structures. Thus, the ^{1}H NMR spectrum of compound **7a** showed a multiplet at δ1.54-2.18 ppm equivalent to the three CH_2 groups, a singlet at δ 3.96 ppm for the NH_2 group, a multiplet at δ 7.16-7.37 ppm for the C_6H_5 and a singlet at δ 7.40 for the CH group. On the other hand, the (2-arylidenecyclohexylidene)thiourea derivatives **9a-c** were successfully prepared by the reaction of 2-arylidenecyclohexanone derivatives **3a-c** with thiourea at 150^{o}C (Scheme 2). Moreover, the three-component reaction of thiourea **8**, ethyl cyanoacetate **10b** and either compound **3a**, **3b** or **3c** in ethanol containing triethylamine formed the pyrimidine derivatives **11a-c** (Scheme 2). The structures of later compounds **11a-c** were established on the basis of their analytical and spectral data as indicated in the experimental part.



Scheme 1. Synthesis of compounds 3a-c, 5a-c and 6a-c.

Furthermore, the reaction of ethyl cyanoacetate **10** with either of compound **3b** and **3c** in ethanol containing a catalytic amount of trimethylamine led to the formation of pyran derivatives **12a** and **12b**, respectively. On the other hand, using ammonium acetate instead of triethylamine led to the formation of pyridine derivatives **13a** and **13b**, respectively. In addition, ethyl4-(methoxybenzylidene)-2-aminoctahydrobenzo[*b*]thiophene-3-carbo-xylate **14** was formed by the reaction of compound **3c** with elemental sulfur and ethyl cyanoacetate **10** in ethanol containing a catalytic amount of trimethylamine. In contrast, the reaction of compound **3c** with elemental sulfur and phenylisothiocyanat **15** formed the thiazole derivative **16.** Its structure was based on analytical and spectral data. Thus, the ^{1}H NMR spectrum showed a multiplet at δ 1.70-2.89 ppm equivalent to the three CH_2 groups a singlet at δ 3.73 ppm for the OCH_3 group a multiplet at δ 7.01-7.52 ppm for the C_6H_5 and C_6H_4 and a singlet at δ 7.58 ppm for the CH group. The reaction of compound **7c** with phenylisothiocyanate **15** in ethanol containing a catalytic amount of trimethylamine produced the 4-amino-9-(4-methoxybenzylidene)-3-phenyl-6,7,8,9-tetrahydrobenzo[4,5]thieno[3,2-*d*]pyrimidine-2(3*H*)-thione **17** (Scheme 3).



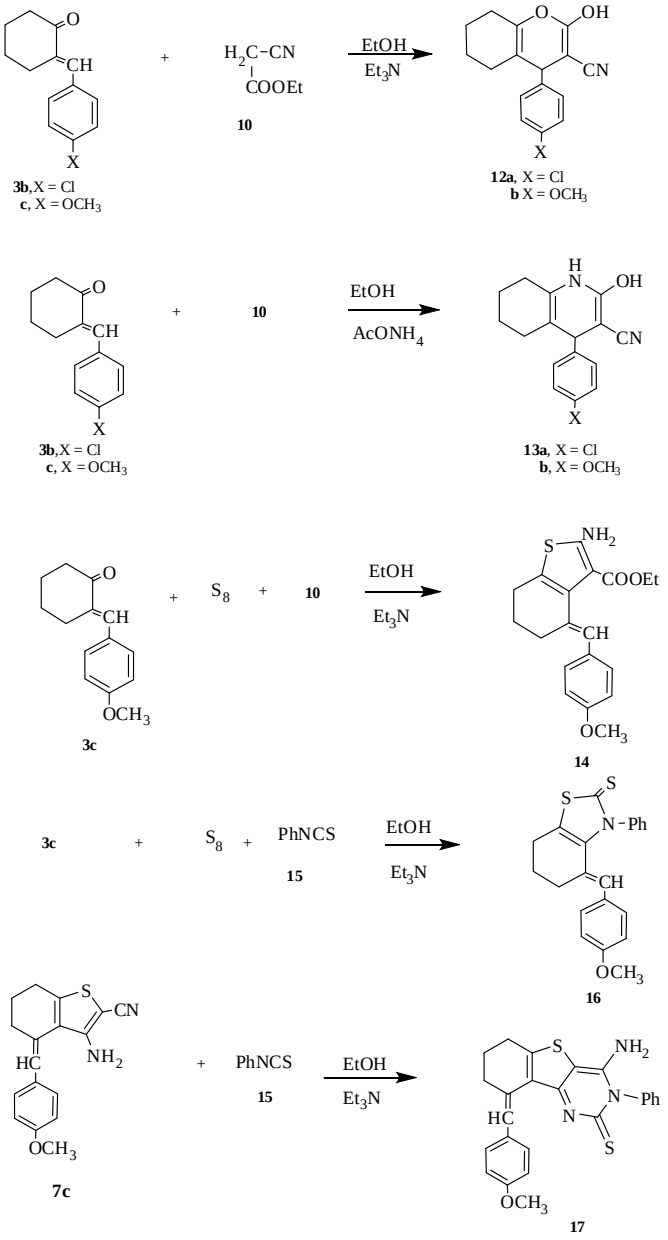
Scheme 2. Synthesis of compounds 7a-c, 9a-c and 11a-c.

Pyrazole derivatives **19a** and **19b** were synthesized by the reaction of 2-arylidenecyclohexanone derivatives **3b** or **3c** with hydrazine hydrate **18**, while *N*-acetylpyrazole derivatives **21a** and **21b** were synthesized by the reaction of 2-arylidenecyclohexanone derivatives **3b** or **3c** with cyanoacetylhydrazine **20**, (Scheme 4). the three-component reaction of cyclohexanone **1** with salicylaldehyde **22** and malononitrile **4** in ethanol containing a catalytic amount of triethylamine formed the 7-amino-9,10,11,12-tetrahydrochromeno[3,4-*c*]chromen-6(12b*H*)-one **23**. The analytical and spectral data of compound **23** were in agreement with its structure as indicated in the experimental part. Finally, the reaction of cyclohexanone **1** with bromine in presence of acetic acid gives 2-bromocyclohexanone **24** which reacts further with thiourea to produce 4,5,6,7-tetrahydrobenzo(*d*)thiazol-2-amine **25** (Scheme 4). Structure of compound **25** was confirmed based on its analytical and spectral data as indicated in the experimental part.

In Vitro Cytotoxicity. Both L-glutamine and fetal bovine serum (FBS) were purchased from Gibco Invitrogen Co. (Scotland, UK). RPMI-1640 medium was purchased from Cambrex (New Jersey, USA). Dimethyl sulfoxide (DMSO), doxorubicin, penicillin, streptomycin and sulforhodamine B (SRB) were purchased from Sigma Chemical Co. (Saint Louis, USA).

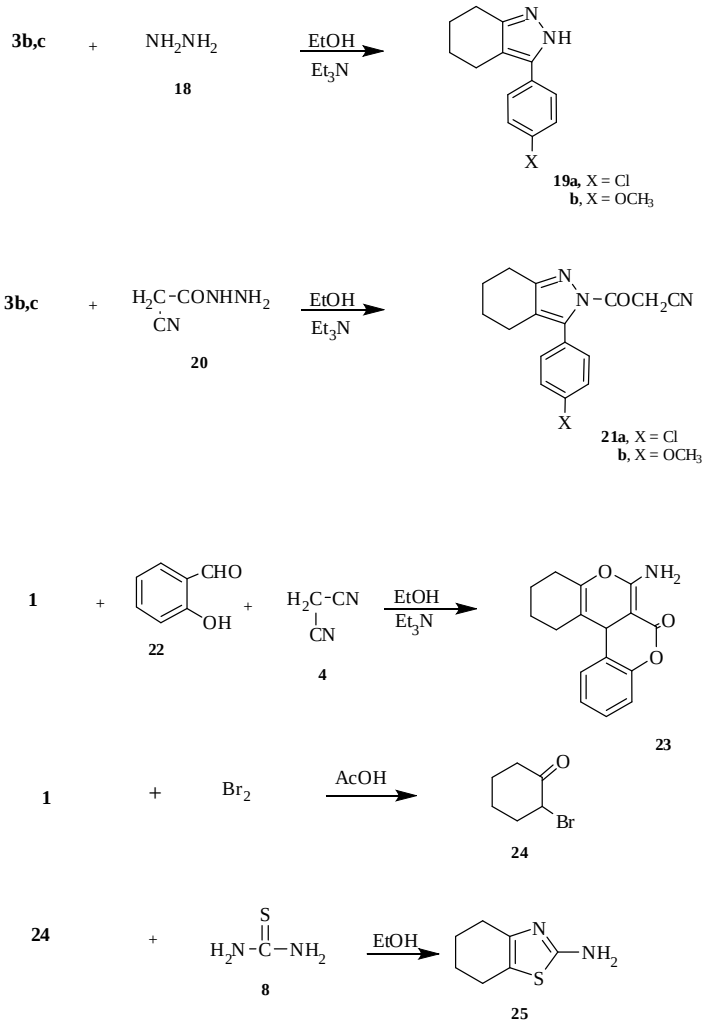
Cell cultures were obtained from the European Collection of cell Cultures (ECACC, Salisbury, UK) and human gastric cancer (NUGC and HR), human colon cancer (DLD1), human liver cancer (HA22T and HEPG2), human breast cancer (MCF), nasopharyngeal carcinoma (HONE1) and normal fibroblast cells (WI38) were kindly provided by the National Cancer Institute (NCI, Cairo, Egypt). They grow as monolayer and routinely maintained in RPMI-1640 medium supplemented with 5% heat inactivated FBS, 2 mM glutamine and antibiotics (penicillin 100 U/mL, streptomycin 100 lg/mL), at 37^{o}C in a humidified

atmosphere containing 5% CO_2. Exponentially growing cells were obtained by plating 1.5 x 10^5 cells/mL for the seven human cancer cell lines including cells derived from 0.75 x 10^4 cells/mL followed by 24 h of incubation. The effect of the vehicle solvent (DMSO) on the growth of these cell lines was evaluated in all the experiments by exposing untreated control cells to the maximum concentration (0.5%) of DMSO used in each assay.



Scheme 3. Synthesis of compounds 3a, b; 14; 16 and 17.

The newly synthesized heterocyclic compound, which prepared in this study, were evaluated according to standard protocols for their *in-vitro* cytotoxicity against seven human cancer cell lines, human gastric cancer (NUGC and HR), human colon cancer (DLD1), human liver cancer (HA22T and HEPG2), human breast cancer (MCF), nasopharyngeal carcinoma (HONE1) and normal fibroblast cells (WI38). A pyridyl cyanoguanidine, CHS 828, was used as a standard antitumor drug. All the synthesized compounds were tested for their cytotoxicity against normal fibroblast cells. All of the IC_{50} values (concentration that produces 50% reduction in cell growth) are listed in Table 1 in nanomolars (nM). Some heterocyclic compounds was observed with significant cytotoxicity against most of the cancer cell lines tested (IC_{50}=10–1000 nM). The results obtained showed that normal fibroblasts cells (WI38) were affected to a much lesser extent (IC50>10,000 nM).



Scheme 4. Synthesis of compounds 19a, b; 21a, b; 23; 24 and 25.

From Table 1, it is clear that the cyclohexene moiety was found to be crucial for the cytotoxic effect of the cyclic compounds **3c-25**. Compounds **5c, 7c, 16**, **19b** and **23** exhibited optimal cytotoxic effect against cancer cell lines, with IC_{50} in the nM range. Comparing the cytotoxicity of the pyran derivatives **5a**, **5b** and **5c**, it is obvious that the cytotoxicity of **5c** is higher than that of **5a** and **5b**. Moreover, the latter compound, **5c**, exhibited high cytotoxicity effect (IC50=32 nM) against gastric cancer (NUGC) compared to the standard CHS 828 (IC50=25 nM). The remarkable activity of **5c** was due to the presence of the 4-OCH_3 aryl moiety. In addition, the cytotoxicity of **5b** was higher than **5a** due to the presence of 4-chloro aryl moiety. The high cytotoxicity of compound **6c** relative to compound **6a** and **6b** is also explained in terms of the presence of the 4-OCH_3 aryl moiety. On the other hand, by considering the 2-amino-4-benzylidene-4,5,6,7-tetrahydrobenzothiophene-3-carbonitrile derivative **7a-7c,** it is clear that the presence of the 4-OCH_3 group present in **7c** is responsible for its high potency than **7b** and **7a**. The 2-cyclohexylidenethiourea derivatives **9a-c**, **9a** and **9b** showed low cytotoxicity effect towards the six cancer cell lines, but **9c** showed high cytotoxicity effect towards HA22T cancer cell line. In 3-(5,6,7,8-tetrahydro-2-thioxoquinazoline-3(2*H*)-yl)-3-oxopropanenitrile derivatives **11a-c**, the high cytotoxicity of compound **11c** relative to compound **11a** and **11b** is also explained in terms of the presence of the 4-OCH_3 aryl moiety. The cytotoxicity of **11b** is higher than that of **11a** due to presence of 4-

chloro aryl moiety. Comparing the activities of compounds **5b** and **5c** with **12a** and **12b**, it is observed that compounds **5b** and **5c** showed higher cytotoxicity compared to compounds **12a** and **12b**. Higher cytotoxicity of **5b** and **5c** is due to the presence of NH_2 group attached to the pyrane ring while in case of compounds **12a** and **12b**, the OH group lowered their cytotoxicity. The high cytotoxicity of **12b** relative to **12a** and high cytotoxicity of **13b** relative to **13a** is also explained in terms of the presence of the 4-OCH_3 aryl moieties. Considering the pyrazol derivatives **19a**,**b** and **21a,b**, compounds **21a,b** showed lower growth inhibitory effects. This may be attributed to the presence of *N*-acetyl group bonded to pyrazol ring. From Table 1, it is noticed that all tested compounds showed no cytotoxicity effect towards the normal cell line WI38 except compounds **11b** and **13a** indicated low potent effect.

Table 1. Cytotoxicity of synthesized compounds against a variety of six human cancer cell lines [IC_{50}[b] (nM)] and normal human cell line.

Compound	Cytotoxicity (IC_{50} in nM)						
	NUGC	DLDI	HA22T	HEPG2	HONE1	MCF	WI38
3c	1084	1890	3166	399	2234	3365	na
5a	288	308	2983	3520	2850	3265	na
5b	38	160	1880	1160	1265	2875	na
5c	32	130	1170	680	220	na	na
6a	1220	3241	3296	4355	688	428	na
6b	1195	440	3266	1277	1670	1262	na
6c	124	280	120	415	4527	2176	na
7a	3470	1893	2196	3346	2877	4036	na
7b	640	190	2155	420	1188	1652	na
7c	182	50	720	320	442	164	na
9a	1926	4420	2061	4118	2186	2444	na
9b	1335	3460	1310	2766	1820	1293	na
9c	1001	1080	58	1320	1180	342	na
11a	3124	2539	2343	4730	2850	1286	na
11b	2784	2280	1165	4321	2166	1080	244
11c	1288	1940	380	166	180	189	na
12a	1277	2237	2655	3320	1770	2876	na
12b	1080	1287	2237	428	1168	580	na
13a	231	1893	2187	2175	6273	4940	144
13b	535	480	160	1180	1128	346	na
16	310	55	120	36	49	2254	na
17	2860	1259	2694	2287	1659	2869	na
19a	1120	2263	2143	312	2466	39	na
19b	42	50	2163	38	1480	830	na
21a	1184	2549	3265	1265	4423	2533	na
21b	180	60	2533	365	2154	2840	na
23	48	66	884	560	2160	40	na
25	680	2655	1580	3121	2316	1463	na
CHS 828	25	2315	2067	1245	15	18	378

[a]NUGC, gastric cancer, DLDI, colon cancer, HA22T, liver cancer, HEPG2, liver cancer; HONEI, nasopharyngeal carcinoma; HR, gastric cancer; MCF, breast cancer; WI38, normal fibroblast cells.

[b]The sample concentration produces a 50% reduction in cell growth

4. CONCLUSIONS

Thirty one of the synthesized compounds were assessed for their anti-proliferative activities on six human cancer cell lines as well as normal human cell line. Most of the compounds were found to be promising anti-proliferative agents. Moreover, the results showed that compounds **5c, 7c, 16, 19b** and **23** are the most active compounds towards the six tumor cell lines namely NUGC (gastric cancer), DLDI (colon cancer), HA22T (liver cancer), HONEI (nasopharngeal carcinoma), MCF (breast cancer) and WI38 (normal fibroblasts human cell).

5. REFERENCES

[1] Hantzsch A., Ueber die synthese pyridinartiger verbindungen aus acetessigäther und aldehydammoniak, *Justus Liebigs Ann. Chem*., 215, 1, 1-82, **1882**.

[2] Stout D. M., Meyers A. I., *Recent advances in the chemistry of dihydropyridines*, *Chem. Rev.*, 82, 2, 223-243, **1982**.

[3] Kumar R. S., Idhayadhulla A., Nasser A. J. A., Selvin, J., Synthesis and antimicrobial activity of a new series of 1,4-dihydropyridine derivatives, *J. Serb. Chem. Soc.*, 76, 1, 1-11, **2011**.

[4] Vijesh A. M., Isloo A. M., Peethamba S. K., Shivanand K. N. Arulmoli, T., Isloor N. A., Hantzsch reaction: synthesis and characterization of some new 1,4-dihydropyridine derivatives as potent antimicrobial and antioxidant agents, *Eur. J. Med. Chem.*, 46, 11, 5591-5597, **2011**.

[5] Swarnalatha G., Prasanthi G., Sirisha N., Chetty C. M., 1,4-Dihydropyridines: A multtifunctional molecule- a review, *Int. J. Chem. Tech Res*., 3, 1, 75-89, **2011**.

[6] Gaudio A. C., Korolkovas A., Takahata Y., Quantitative structure-activity relationships for 1,4-dihydropyridine calcium channel antagonists (nifedipine analogues): A quantum chemical/classical approach, *J. Pharm. Sci.*, 83, 8, 1110-1115, **1994**.

[7] Costantino G., Maltoni K., Marinozzi M., Camaioni E., Prezeau L., Pin J. P., Pellicciari R., Synthesis and biological evaluation of 2-(3′-(1*H*-tetrazol-5-yl)bicyclo[1.1.1]pent-1-yl)glycine (*S*-TBPG), a novel mGlu1 receptor antagonist, *Bioorg. Med. Chem.*, 9, 2, 221-227, **2001**.

[8] Tasaka S., Ohmori H., Gomi N., Iino M., Machida T., Kiue A., Naito S., Kuwano M., Synthesis and structure–activity analysis of novel dihydropyridine derivatives to overcome multidrug resistance, *Bioorg. Med. Chem. Lett*., 11, 2, 275-277, **2001**.

[9] Kharkar P. S., Desai B., Gaveria H., Varu B., Loriya R., Naliapara Y., Shah A., Kulkarni V. M., Three-dimensional quantitative structure−activity relationship of 1,4-dihydropyridines as antitubercular agents, *J. Med. Chem.*, 45, 22, 4858–4867, **2002**.

[10] Khojastehnezhad A., Maleki B., Mofrad A. V., Silica-supported molybdenum oxide (MoO_3-SiO_2): As a heterogeneous and recyclable catalyst for the synthesis of polyhydroquinoline derivatives**,** *Biointerface Res. Appl. Chem*., 7, 3, 2030 - 2034, **2017**.

[11] Schellenberg K. A., Westheimer F. H., A free-radical oxidation of a dihydropyridine, *J. Org. Chem.*, 30, 6, 1859–1862, **1965**.

[12] Berben L. A., Craig D. C., Suriñach C. G., Robinson A., Sugiyarto K. H., Colbran S. B., Palladium(II) complexes of imidazolin-2-ylidene *N*-heterocyclic carbene ligands with redox-active dimethoxyphenyl or (hydro)quinonyl substituents, *Inorg. Chim. Acta*, 370, 1, 374-381, **2011**.

[13] Isloor A. M.; Kalluraya B., Shetty P., Regioselective reaction: Synthesis, characterization and pharmacological studies of some new Mannich bases derived from 1,2,4-triazoles, *Eur. J. Med. Chem.*, 44, 9, 3784-3787, **2009**.

[14] Isloor A. M., Kalluraya B., Pai K. S., Synthesis, characterization and biological activities of some new benzo[b]thiophene derivatives, *Eur. J. Med. Chem.*, 45, 2, 825-830, **2010**.

[15] Achutha D. K., Kameshwar V. H., Ningappa M. B., Kariyappa A. K., Synthesis and in vitro biological evaluation for antioxidant, anti-inflammatory activities and molecular docking studies of novel pyrazole derivatives., *Biointerface Res. Appl. Chem.*, 7, 3, 2040 - 2047, **2017**.

[16] Grosse S., Mathieu V., Pillard C., Massip S., Marchivie M., Jarry C., Bernard P., Kiss R., Guillaumet G., New imidazo[1,2-*b*]pyrazoles as anticancer agents: Synthesis, biological evaluation and structure activity relationship analysis, *Eur. J. Med. Chem.*, 84, 718-730, **2014**.

[17] Mohareb R. M., Al-Omran F., Azzam R. A., Heterocyclic ring extension of estrone: Synthesis and cytotoxicity of fused pyran, pyrimidine and thiazole derivatives, *Steroids*, 84, 46–56, **2014**.

[18] Dhorajiya B. D., Dholakiya B. Z., Mohareb R. M., Hybrid probes of aromatic amine and barbituric acid: highly promising leads for anti-bacterial, anti-fungal and anti-cancer activities, *Med. Chem. Res.*, 23, 9, 3941–3952, **2014**.

[19] Mohareb R. M., Al Farouk F. O., Sherif S. M., Karaghiosoff K., Hybrid probes of aromatic amine and barbituric acid: highly promising leads for anti-bacterial, anti-fungal and anti-cancer activities, *Med. Chem. Res.*, 23, 9, 3165–3177, **2014**.

[20] Mohareb R. M., Abdallah A. E. M., New approaches for the synthesis of pyrazole, thiophene, thieno[2,3-*b*]pyridine, and thiazole derivatives together with their anti-tumor evaluations, *Med. Chem. Res.*, 23, 2, 564–579, **2014**.

[21] Mohareb R. M., El-Sayed N. N. E., Abdelaziz M. A., The Knoevenagel reactions of pregnenolone with cyanomethylene reagents: Synthesis of thiophene, thieno[2,3-*b*]pyridine, thieno[3,2-*d*]isoxazole derivatives of pregnenolone and their in vitro cytotoxicity towards tumor and normal cell lines, 12-13, *Steroids*, 78, 1209–1219, **2013**.

[22] Koch K., Smitrovich J. H., Hydroxy-directed hydroaluminations: A stereoselective approach to cycloalkanols from β-aryl enones, *Tetrahed. Lett.*, 35, 8, 1137-1140, **1994**.

[23] Wang D. L., Wu J. Y., Cui Q. T., An efficient one-pot synthesis of thiophene-fused pyrido[3,2-a]azulenes *via* Gewald reaction, *Chin. Chem. Lett.*, 25, 12, 1591-1594, **2014**.

[24] Zhao D., Li L., Xu F., Wu Q., Lin X., Bovine serum albumin-catalyzed one-pot synthesis of 2-aminothiophenes via Gewald reaction, *J. Molec. Catal. B: Enzy.*, 95, 29-35, **2013**.