Bull. Chem. Soc. Ethiop. **2024**, 38(3), 739-750.
DOI: https://dx.doi.org/10.4314/bcse.v38i3.15

ISSN 1011-3924
Printed in Ethiopia
Online ISSN 1726-801X

MULTICOMPONENT REACTIONS OF CYANOACETANILIDE DERIVATIVES: SYNTHESIS OF COUMARIN AND QUINOLINE DERIVATIVE AND EVALUATIONS OF THEIR CYTOTOXICITY

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(Received November 13, 2023; Revised January 9, 2024; Accepted January 12, 2024)

ABSTRACT. In this study, several fused heterocyclic systems involving tetrahydrobenzopyrans **7a-i** and tetrahydrobenzopyridines **9a-i** were synthesized. The reactions proceed via a multicomponent reaction of different aromatic aldehydes, cyanoacetanilide derivatives, and cyclohexane-1,3-dione. All the produced compounds were tested for their anticancer activity by using six cancer cell lines such as A549, HT-29, MKN-45, U87MG, SMMC-7721 and H460 utilizing foretinib as the positive control and the standard MTT assay *in vitro*. The results indicated that many synthesized derivatives were the most potent towards all the cancer cell lines used, six compounds **7e**, **7f**, **9b**, **9d**, **9e**, and **9g** showed the highest potency. The results obtained in this work enhance further investigations in the future.

KEY WORDS: Multi-component reactions, Cyanoacetanilide, Pyran, Pyridine, Ctotoxicity

INTRODUCTION

In recent years, multicomponent reactions (MCRs) have become essential, efficient, bond-forming methods for expedient synthesis of a wide range of active organic compounds and natural products without separation and purification of intermediates. The MCRs, which are important classes of chemical transformations, have recently attracted much attention owing to their high efficacy, shorter reaction times, mild conditions, simplicity, and environmental friendliness [1-4]. These effective and attractive processes also eliminate waste production and costly purification processes. They are also simple strategies for synthesis of heterocyclic structures in a single vessel from three or more components with high atom and structural economy [5]. One of the most important tartget molecules that can be produced through MCR's are the pyran derivatives. Such group of compounds were characterized by their wide range of pharamaceutical applications. Pyran derivatives occupy an important place in the realm of natural and synthetic organic chemistry, because of their biological and pharmacological properties as anti-sterility and anti-cancer agents [6]. During the last several years, the diverse applications of such 2-amino-4*H*-pyran heterocyclic scaffolds in medicinal chemistry have drawn appreciable attention among synthetic chemists to explore useful synthetic routes to these heterocycles of potential interest with anti-microbial and anti-tumor activities [7.8]. The fused pyran ring skeleton is a well-known heterocycle and important core unit in several natural products [9-11]. Natural products bearing a fused pyran ring system possess distinct properties of general interest and have a variety of valuable biological activities and potential medicinal applications [12-16]. It is worthwhile to note that many of these natural products exhibit cancer cell growth inhibitory activity and have been further investigated as potential anti-cancer agents [17, 18]. As more valuable properties emerge, these pyran-containing structures begin to inspire chemists to develop efficient synthetic approaches and identify novel biologically active compounds [19, 20]. On the other hand, pyridine nucleus a well-studied six-membered heterocyclic moiety also displayed various

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biological activities and was found in a variety of drugs such as isoniazid, ethionamide, amrinone, bupicomide, sulphapyridine [21]. It was reported in the literature that molecular hybridization of two or more biologically active pharmacophore into a single chemical structure showed significant synergistic effects [22]. The combination of thiophene with other heterocyclic rings showed wide range of biological activities [23]. Recently, our research group was involved through comprehensive program involving the use of cyclohexan-1,3-dione in many heterocyclic transformations [24-27]. Many of these reactions were multi-component reactions leading to the formation of fused pyran and pyridine derivatives [28]. As a continuation of this program, we report, in the present work, on the synthesis and the spectroscopic, structural, and physicochemical characterization of new heterocyclic derivatives incorporating fused pyran or pyridine moiety starting from the cyanoacetanilide derivatives. The anti-proliferative activity of the synthesized compounds toward different cancer cell lines was also explored. The rule of such agents in the future is to improve the survival rates and quality of life of patients with tumors.

RESULTS AND DISCUSSION

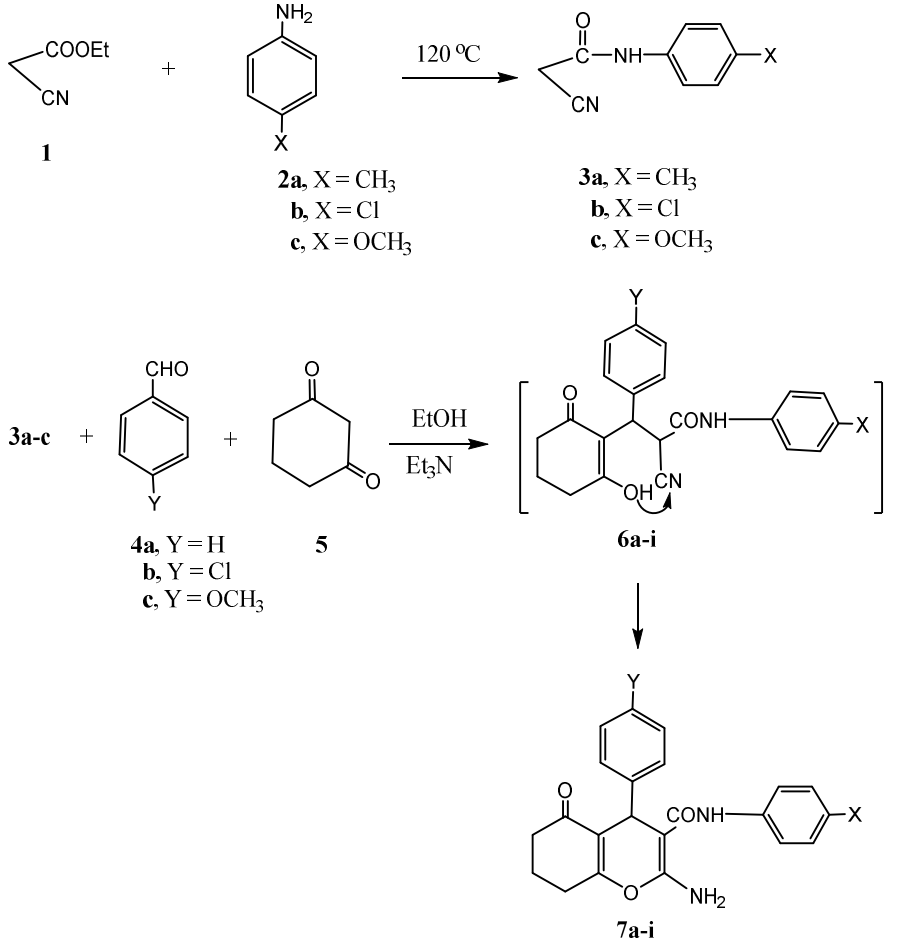
Through this work the cyanoacetanilide derivatives **3a-c** that were synthesized from the reaction of ethyl cyanoacetate (**1**) with the aniline derivatives **2a-c** in an oil bath at 120 ºC were used as the key starting materials for the synthesis of the new heterocyclic compounds. The reaction sequences were demonstrated through Schemes 1-4. The multicomponent reactions of **3a-c** with the aromatic aldehydes namely benzaldehyde (**4a**), 4-chlorobenzaldehyde (**4b**) or 4-methoxy-benzaladehyde (**4c**) and cyclohean-1,3-dione (**5**) in ethanol containing triethylamine produced the tetrahydro-4*H*-chromene-3-carboxamide derivatives **7a-i**, respectively (Scheme 1). The structures of the latter products were confirmed based on analytical and spectral data. Thus, the ^{1}H NMR spectrum of **7a** showed three signals at the δ 1.60, 1.90 and 2.90 ppm for the three CH_2 groups. A singlet at δ 2.22 ppm for the CH_3 group and a singlet at δ 3.85 ppm for CH-pyran ring, a multiplet at δ 7.06-7.43 ppm for the phenyl moieties. Finally, the presence of the two signals at δ 8.30 and 10.18 ppm confirmed the appearance of the two NH groups.

On the other hand, the multicomponent reactions of **3a-c** with benzaldehyde (**4a**), 4-chlorobenzaldehyde (**4b**) or 4-methoxybenzaladehyde (**4c**) and cyclohexan-1,3-dione (**5**) in ethanol containing ammonium acetate produced the 1,4,5,6,7,8-hexahydroquinoline-3-carboxamide derivatives **9a-i**, respectively (Scheme 2). The structures of the latter products were based on their respective analytical and spectral data (see experimental section).

Structure activity relationship

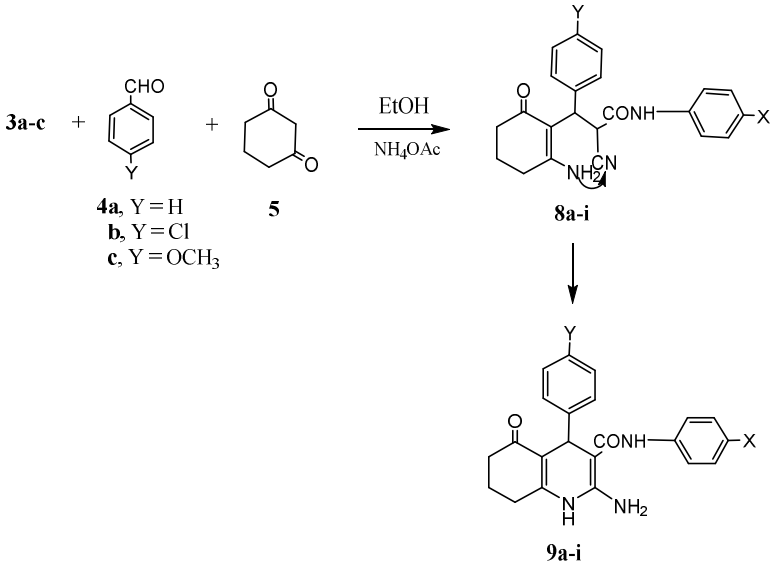
Table 1 summarized the anti-cancer effects for all the prepared compounds which tested against six cancer cell lines namely, A549, HT-29, MKN-45, U87MG, and SMMC-7721 and using the standard MTT assay *in vitro*, with foretinib as the positive control. About six compounds were exhibited potent IC_{50} values compared with the standatrd reference drug which was used. The first series which were the tetrahydrobenzopyran derivatives **7a-i**, indicated that compounds **7e** (X = Cl, Y = Cl) and **7f** (X = Cl, Y = OCH_3) were the most active compounds among all the other compounds in the same series. Their high activity were attributed to the presence of the Cl and the OCH_3 moieties in their structures. In addition, the other group of tetrahydrobenzopyridine derivatives **9a-i**, represents the apperence of the activity for four compounds **9b** (X = CH_3, Y = Cl), **9d** (X = Cl, Y= H), **9e** (X = Cl, Y = Cl) and **9g** (X = OCH_3, Y = H). The presence of the Cl within the molecular structure of compounds **9b**, **9d** and **9e** enhanced the high inhibitions of such compounds. Moreover, the presence of the two Cl groups in compound **9e** make it the most active one among all the other derivatives in such series of compounds. From the above results, we can conclude that the presence of the electronegative Cl group with the structure of the molecule was

responsible for its high inhibitions. The results obtained in this work will encourage us to make further studies in another heterocyclic ring systems to form new anti-cancer agents.



7	a	b	c	d	e	f	g	h	i
X	CH_3	CH_3	CH_3	Cl	Cl	Cl	OCH_3	OCH_3	OCH_3
Y	H	Cl	OCH_3	H	Cl	OCH_3	H	Cl	OCH_3

Scheme 1. Synthesis of compounds **3a-c** and **7a-i**.



9	a	b	c	d	e	f	g	h	i
X	CH_3	CH_3	CH_3	Cl	Cl	Cl	OCH_3	OCH_3	OCH_3
Y	H	Cl	OCH_3	H	Cl	OCH_3	H	ClH	OCH_3

Scheme 2. Synthesis of compounds **9a-i**.

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
7a	4.82± 1.81	5.72 ± 1.93	5.62 ± 1.94	3.80 ± 1.80	4.27 ±122	6.32 ± 2.46
7b	0.48± 0.29	2.52 ± 1.26	3.55 ± 1.65	4.52 ±2.53	2.72 ± 1.09	1.94 ± 0.73
7c	8.23± 2.72	8.26 ± 2.42	8.53 ± 2.80	6.59 ± 2.25	8.52 ±2.31	7.61 ± 2.29
7d	2.80 ± 1.50	3.82 ± 1.31	2.31± 0.89	4.69 ± 1.32	4.52 ±1.32	5.62 ± 2.13
7e	0.28± 0.18	0.31 ± 0.08	0.52 ± 0.18	0.28 ± 0.17	0.29 ± 0.08	0.38 ± 0.20
7f	0.38± 0.15	0.29 ± 0.07	0.42 ± 0.16	0.39 ± 0.16	0.27 ± 0.17	0.32 ± 0.15
7g	1.68± 0.79	2.52 ± 1.26	3.55 ± 1.65	4.52 ±2.53	2.72 ± 1.09	1.94 ± 0.73
7h	1.68± 0.93	2.53 ± 1.69	3.62 ± 1.53	4.93 ± 1.90	3.62 ±1.152	6.52 ± 2.40
7i	3.62± 1.53	4.80 ± 1.17	6.52 ± 2.41	5.96 ± 2.72	6.88 ±2.32	5.20 ± 2.26
9a	2.57 ± 1.31	3.72 ± 1.26	2.53 ± 0.91	3.75 ± 1.26	2.63 ± 1.79	2.69 ±1.27
9b	0.32±0.14	0.28±0.13	0.29± 0.17	0.36 ±0.18	0.32±0.15	0.26 ± 0.18
9c	8.28 ±2.26	8.61 ±2.46	5.30 ± 1.18	6.24 ± 2.85	7.38 ± 2.25	6.58 ±1.68
9d	0.23 ± 0.19	0.39 ± 0.21	0.43 ±0.20	0.54 ± 0.21	0.38 ±0.16	0.42 ± 0.28
9e	0.16 ± 0.04	0.28 ± 0.17	0.18 ± 0.04	0.28 ± 0.14	0.36 ± 0.21	0.25 ±0.14
9f	6.94 ± 1.38	8.24 ± 2.19	7.58±1.36	5.34± 1.58	8.61 ± 2.28	6.28 ± 1.62
9g	0.26 ± 0.12	0.32 ±0.13	0.48 ± 0.31	0.29 ± 0.16	0.24 ±1.78	0.41 ± 0.23
9h	2.61±0.86	3.67 ± 1.58	4.86 ± 1.72	5.18 ± 1.68	4.38 1.25	2.45 ± 1.63
9i	8.25 ±2.58	8.41 ±2.57	7.47 ± 1.82	7.33 ±2.56	8.61 ± 2.46	8.33 ± 2.24
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

Determination of morphological changes of A549cell line

The lung tissue was found to be regulated and refined by the effect of anticancer agents, for that reason in the present work we investigated the morphological changes of compound **9d** and **9e** toward A549 cell line [29, 30]. There are many reports concerned with morphological changes of other cell lines [31, 32]. The ability of **9d** and **9e** in apoptosis induction that enhance morphological changes on the cancer cell line A549 was studied. Different concentrations of **9d** and **9e** were used and images were expressed in Figure 1A that were obtained after 72 hours and showed the apoptotic changes resulting from shrinking of the cell line. In the present study, after treatment with compounds **9d** and **9e** for 72 hours on A549 cell line, the appearance of the membrane and the number of cells with the concentration of the used compound was shown in Figure 1B. Where the red rectangle area expressed through Figure 1B (indicated by an arrow) showed the effect of compounds **9d** and **9e** on A549 cell line. The morphological changes were detected via a fluorescent microscope using acridine orange staining. The use of DAPI visualized the chromatin condensation, pyknotic (inset of 1.25 µM), and condensed (bright colored: inset of 2.5 µM) nuclei formation as indicated in Figure 1C. The red arrows indicated the effect of compounds **9d** and **9e** on A549 cell line and these were directed through the area of such effect. Figure 1 indicated the morphological changes of compound **9d** while Figure 2 for the morphological changes of A549 cell line by compound **9e**. It was obvious that compound **9e** showed more shrinking of the cell line than compound **9d**.

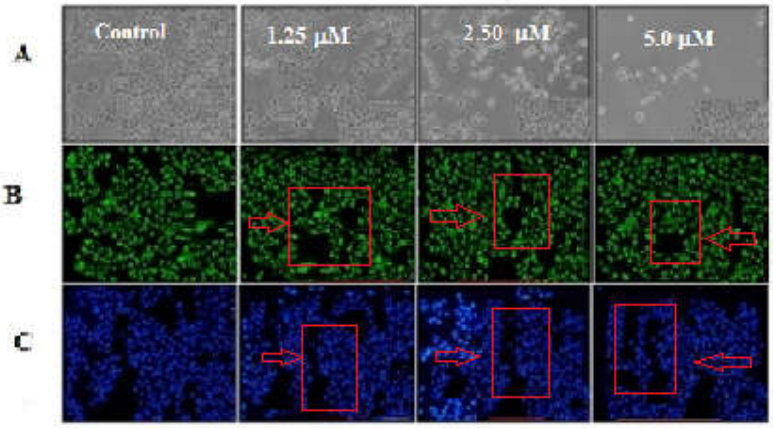


Figure 1. The microscopic indication of changes due to the use of different concentrations of compound **9d**. Area (A) the use of phase-contrast microscopy. Area (B) the use of fluorescent microscope. Area (C) showed nuclear changes by DAPI staining by a fluorescent microscope.

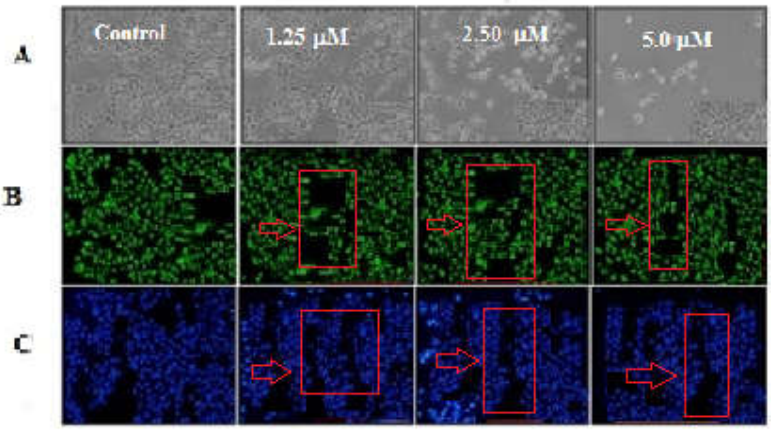


Figure 2. The microscopic indication of changes due to the use of different concentrations of compound **9e**. Area (A) the use of phase-contrast microscopy. Area (B) the use of fluorescent microscope. Area (C) showed nuclear changes by DAPI staining by a fluorescent microscope.

EXPERIMENTAL

All melting points were determined on an Electro-thermal digital meting point apparatus and are uncorrected. IR Spectra (KBr discs) were recorded on a FITR plus 460 or Pye Unicam SP-1000 spectrophotometer. ^{1}H NMR spectra were recorded with Varian Gemini-200 (200 MHZ) (Cairo University) and Jeol AS 500 MHz (National Research Center) instruments in DMSO-d6 as solvent using TMS as internal standard and chemical shifts are expressed as δ ppm. The mass spectra were recorded with Hewlett Packard 5988 AGC/MS system and GCMS-QP1000 Ex shimadzu instruments. Analytical data were obtained from the Micro analytical data unit at Cairo University and were performed on Vario El III Elemental CHNS analyzer.

General procedure for the synthesis of 2-amino-5-oxo-N,4-diphenyl-5,6,7,8-tetrahydro-4H-chromene-3-carboxamide derivatives ***(7a-i)***

To a solution of compound 2-cyano-*N*-(*p*-tolyl)acetamide **3a** (1.74 g, 0.01 mol), *N*-(4-chlorophenyl)-2-cyanoacetamide **3b** (1.94 g, 0.01 mol) or 2-cyano-*N*-(4-methoxyphenyl) acetamide **3c** (1.90 g, 0.01 mol), in ethanol (20 mL) containing triethylamine (0.50 mL), either benzaldehyde **4a** (1.06 g, 0.01 mol), 4-chlorobenzaldehyde **4b** (1.40 g, 0.01 mol) or 4-methoxy-benzaldehyde **4c** (1.36 g, 0.01 mol) in the presence of cyclohexane-1,3-dione **5** (1.12 g, 0.01 mol) were added. The solid products formed upon cooling in an ice-bath were collected by filtration, washed with water and crystallized from absolute alcohol.

2-Amino-5-oxo-4-phenyl-N-(p-tolyl)-5,6,7,8-tetrahydro-4H-chromene-3-carboxamide ***(7a)***. Yellow crystals, m.p. 163-165 °C, yield: 2.81 g (75%). Elemental analysis for $C_{23}H_{22}N_2O_3$ (374.43), (% calcd./found): 73.78/73.99 (C), 5.92/5.75 (H), 7.48/7.70 (N). IR (ν, cm^{-1}): 3426, 3296 (NH_2), 3207 (NH), 3136 (CH-aromatic), 2923 (CH_2, CH_3), 1664, 1613 (2C=O), 1552, 1457 (C=C). ^{1}H NMR (*δ*, ppm): 1.60, 1.90, 2.90 (m, 6H, $3CH_2$), 2.22 (s, 3H, CH_3), 3.85 (s, 1H, CH-pyran), 7.06-7.43 (m, 9H, C_6H_5, C_6H_4), 8.30 (s, 2H, NH_2), 10.18 (s, 1H, NH). MS (EI): *m/z* (%) 374 [M^+] (28.37), 373 [M^+-1] (34.54), and 194 (100.00).

2-Amino-4-(4-chlorophenyl)-5-oxo-N-(p-tolyl)-5,6,7,8-tetrahydro-4H-chromene-3-carboxamide ***(7b)***. Yellow crystals, m.p. 173-175 °C, yield: 3.27 g (80%). Elemental analysis for $C_{23}H_{21}ClN_2O_3$ (408.88), (% calcd./found): 67.56/67.79 (C), 5.18/5.40 (H), 6.85/6.50 (N). IR (ν, cm^{-1}): 3321, 3196 (NH_2, NH), 3032 (CH-aromatic), 2923 (CH_2, CH_3), 1675, 1609 (2C=O), 1538, 1465 (C=C). ^{1}H NMR (δ, ppm): 1.76, 2.17, 2.99 (m, 6H, $3CH_2$), 2.29 (s, 3H, CH_3), 3.86 (s, 1H, CH-pyran), 7.04-7.70 (m, 8H, $2C_6H_4$), 8.26 (s, 2H, NH_2), 10.40 (s, 1H, NH).

2-Amino-4-(4-methoxyphenyl)-5-oxo-N-(p-tolyl)-5,6,7,8-tetrahydro-4H-chromene-3-carboxamide ***(7c)***. Yellow crystals, m.p. 147-149 °C, yield: 3.20 g (79%). Elemental analysis for $C_{24}H_{24}N_2O_4$ (404.46), (% calcd./found): 71.27/71.49 (C), 5.98/5.70 (H), 6.93/7.20 (N). IR (ν, cm^{-1}): 3335, 3196 (NH_2), 3120 (NH), 3032 (CH-aromatic), 2923 (CH_2, OCH_3), 1673, 1605 (2C=O), 1513, 1459 (C=C). ^{1}H NMR (δ, ppm): 2.24 (s, 3H, CH_3), 2.28- 2.51 (m, 6H, $3CH_2$), 3.71 (s, 3H, OCH_3), 3.87 (s, 1H, CH-pyran), 7.07-8.18 (m, 10H, $2C_6H_4$, NH_2), 10.17 (s, 1H, NH). ^{13}C NMR (δ, ppm): 20.9, 21.0, 39.4, 39.6, 55.4, 104.2, 113.6, 115.3, 115.7, 119.7, 119.9, 129.6, 129.7, 133.0, 133.7, 136.3, 150.7, 161.2.

2-Amino-N-(4-chlorophenyl)-5-oxo-4-phenyl-5,6,7,8-tetrahydro-4H-chromene-3-carboxamide ***(7d)***. Yellow crystals, m.p. 139-141 °C, yield: 3.20 g (81%). Elemental analysis for $C_{22}H_{19}ClN_2O_3$ (394.85), (% calcd./found): 66.92/67.20 (C), 4.85/5.10 (H), 7.09/6.80 (N). IR (ν, cm^{-1}): 3408, 3304 (NH, NH_2), 3028 (CH-aromatic), 2931 (CH_2), 1685, 1656 (2C=O), 1601, 1487 (C=C). ^{1}H NMR (δ, ppm): 2.18-2.31, 2.99 (m, 6H, $3CH_2$), 3.97 (s, 1H, CH-pyran), 7.06-7.70 (m, 9H, C_6H_4, C_6H_5), 8.40 (s, 2H, NH_2), 10.60 (s, 1H, NH). MS (EI): *m*/*z* (%) 394 [M^+] (20.73), 393 [M^+-1] (28.93), 392 [M^+-2] (12.92), 112 (100.00).

2-Amino-N,4-bis(4-chlorophenyl)-5-oxo-5,6,7,8-tetrahydro-4H-chromene-3-carboxamide ***(7e)***. Browinsh yellow crystals, m.p. 116-118 °C, yield: 3.18 g (74 %). Elemental analysis for $C_{22}H_{18}Cl_2N_2O_3$ (429.30), (% calcd./found): 61.55/61.70 (C), 4.23/4.43 (H), 6.53/6.30 (N). IR (ν, cm^{-1}): 3258, 3191 (NH, NH_2), 3057 (CH-aromatic), 2943 (CH_2), 1687, 1602 (2C=O), 1536, 1487 (C=C). ^{1}H NMR (δ, ppm): 1.74-2.51 (m, 6H, $3CH_2$), 3.94 (s, 1H, CH-pyran), 6.96-7.62 (m, 10H, $2C_6H_4$, NH_2), 10.40 (s, 1H, NH). ^{13}C NMR (δ, ppm): 21.2, 29.8, 36.7, 38.5, 77.8, 112.6, 121.1, 121.2, 128.1, 128.9, 129.9, 129.9, 130.1, 130.4, 130.7, 140.9, 146.8, 152.0, 195.3.

2-Amino-N-(4-chlorophenyl)-4-(4-methoxyphenyl)-5-oxo-5,6,7,8-tetrahydro-4H-chromene-3-carboxamide ***(7f)***. Yellow crystals, m.p. 168-170 °C, yield: 3.31 g (78%). Elemental analysis for $C_{23}H_{21}ClN_2O_4$ (424.88), (% calcd./found): 65.02/65.30 (C), 4.98/5.23 (H), 6.59/6.35 (N). IR (ν, cm^{-1}): 3389, 3319 (NH_2), 3108 (NH), 3017 (CH-aromatic), 2957, 2835 (CH_2, CH_3), 1676, 1601 (2C=O), 1533, 1458 (C=C). ^{1}H NMR (δ, ppm): 1.30, 1.90-2.29 (m, 6H, $3CH_2$), 3.73 (s, 3H, OCH_3), 3.87 (s, 1H, CH-pyran), 6.53-8.04 (m, 8H, $2C_6H_4$), 8.21 (s, 2H, NH_2), 10.39 (s, 1H, NH).

2-Amino-N-(4-methoxyphenyl)-5-oxo-4-phenyl-5,6,7,8-tetrahydro-4H-chromene-3-carboxamide ***(7g)*** . Grey crystals, m.p. 167-169 °C, yield: 3.20 g (82%). Elemental analysis for $C_{23}H_{22}N_2O_4$ (390.43), (% calcd./found): 70.75/70.90 (C), 5.68/5.80 (H), 7.17/6.85 (N). IR (ν, cm^{-1}): Broad band 3308 (NH, NH_2), 3054, 3025 (CH-aromatic), 2956, 2834 (CH_2, CH_3), 1671, 1637 (2C=O), 1602, 1455 (C=C). ^{1}H NMR (δ, ppm): 1.82-1.93, 2.10-2.25 (m, 6H, $3CH_2$), 3.75 (s, 3H, OCH_3), 3.96 (s, 1H, CH-pyran), 6.81-7.98 (m, 9H, C_6H_4, C_6H_5), 8.27 (s, 2H, NH_2), 10.34 (s, 1H, NH). MS (EI): *m*/*z* (%) 390 [M^+] (36.42), and 121 (100.00).

2-Amino-4-(4-chlorophenyl)-N-(4-methoxyphenyl)-5-oxo-5,6,7,8-tetrahydro-4H-chromene-3-carboxamide ***(7h)***. Grey crystals, m.p. 156-158 °C, yield: 4.12 g (97%). Elemental analysis for $C_{23}H_{21}ClN_2O_4$ (424.88), (% calcd./found): 65.02/65.30 (C), 4.98/5.20 (H), 6.59/6.29 (N). IR (ν, cm^{-1}): Broad band 3317 (NH, NH_2), 3066 (CH-aromatic), 2947 (CH_2, CH_3), 1667, 1604 (2C=O),

1538, 1510 (C=C). ^{1}H NMR (δ, ppm): 1.79-1.91, 2.10-2.19, 2.22-2.33 (m, 6H, $3CH_2$), 3.74 (s, 3H, OCH_3), 3.84 (s, 1H, CH-pyran), 6.87-8.01 (m, 9H, $2C_6H_4$), 8.26 (s, 2H, NH_2), 10.28 (s, 1H, NH).

2-Amino-N,4-bis(4-methoxyphenyl)-5-oxo-5,6,7,8-tetrahydro-4H-chromene-3-carboxamide ***(7i)***. Grey crystals, m.p. 133-135 ºC, yield: 3.20 g (76%). Elemental analysis for $C_{24}H_{24}N_2O_5$ (420.46), (% calcd./found): 68.56/68.70 (C), 5.75/5.90 (H), 6.66/6.30 (N). IR (ν, cm^{-1}): 3388, 3315 (NH_2), 3136 (NH), 3063 (CH-aromatic), 2953, 2835 (CH_2, CH_3), 1664, 1606 (2C=O), 1547, 1459 (C=C). ^{1}H NMR (δ, ppm): 1.77-1.86, 2.18-2.28 (m, 6H, $3CH_2$), 3.75, 3.79 (s, 6H, $2OCH_3$), 3.87 (s, 1H, CH-pyran), 6.71-8.03 (m, 8H, $2C_6H_4$), 8.19 (s, 2H, NH_2), 10.13 (s, 1H, NH). ^{13}C NMR (δ, ppm): 21.2, 26.9, 36.9, 39.3, 55.3, 55.5, 104.1, 113.8, 114.3, 114.4, 114.6, 114.8, 121.5, 122.8, 129.7, 131.8, 131.9, 134.0, 151.5, 156.1, 156.5, 160.9, 163.1, 196.8.

General procedure for the synthesis of 2-amino-5-oxo-N,4-diphenyl-1,4,5,6,7,8-hexahydroquinoline-3-carboxamide derivatives ***(9a-i)***

To a solution of either **3a** (1.74 g, 0.01 mol), **3b** (1.94 g, 0.01 mol) or **3c** (1.90 g, 0.01 mol), in ethanol (20 mL) containing a catalytic amount of ammonium acetate, each of benzaldehyde **4a** (1.06 g, 0.01 mol), 4-chlorobenzaldehyde **4b** (1.40 g, 0.01 mol) or 4-methoxybenzaldehyde **4c** (1.36 g, 0.01 mol) and cyclohexane-1,3-dione (1.12 g, 0.01 mol) were added. The reaction mixture was heated under the reflux conditions for 3h and the produced solid product upon cooling in an ice bath was collected by filtration and crystallized from ethanol.

2-Amino-5-oxo-4-phenyl-N-(p-tolyl)-1,4,5,6,7,8-hexahydroquinoline-3-carboxamide ***(9a)***. Yellow crystals, m.p. 128-130 ºC, yield: 3.18 g (85%). Elemental analysis for $C_{23}H_{23}N_3O_2$ (373.45), (% calcd./found): 73.97/74.20 (C), 6.21/6.40 (H), 11.25/11.40 (N). IR (ν, cm^{-1}): 3345, 3193 (2NH, NH_2), 3033 (CH-aromatic), 2925 (CH_2, CH_3), 1681, 1601 (2C=O), 1533, 1452 (C=C). ^{1}H NMR (δ, ppm): 1.08-1.10 (s, 3H, CH_3), 1.29-1.34, 2.18-2.29 (m, 6H, $3CH_2$), 4.91 (s, 1H, CH-pyridine), 7.01-8.00 (m, 9H, C_6H_4, C_6H_5), 8.28 (s, 2H, NH_2), 10.32, 10.40 (2s, 2H, 2NH). ^{13}C NMR (δ, ppm): 21.0, 21.3, 26.8, 37.3, 39.6, 107.9, 112.9, 119.5, 119.7, 125.9, 127.9, 127.9, 128.1, 128.2, 129.6, 129.7, 133.9, 136.2, 147.9, 151.1, 155.6, 160.8, 195.3.

2-Amino-4-(4-chlorophenyl)-5-oxo-N-(p-tolyl)-1,4,5,6,7,8-hexahydroquinoline-3-carboxamide ***(9b)***. Yellow crystals, m.p. 165-167 ºC, yield: 3.18 g (78%). Elemental analysis for $C_{23}H_{22}ClN_3O_2$ (407.89), (% calcd./found): 67.73/67.90 (C), 5.44/5.66 (H), 10.30/10.50 (N). IR (ν, cm^{-1}): 3321 (2NH, NH_2), 3045 (CH-aromatic), 2942 (CH_2, CH_3), 1674, 1600 (2C=O), 1533, 1490 (C=C). ^{1}H NMR (δ, ppm): 1.09-1.10 (s, 3H, CH_3), 1.84, 2.10-2.50 (m, 6H, $3CH_2$), 3.86 (s, 1H, CH-pyridine), 6.91-8.01 (m, 8H, $2C_6H_4$), 8.26 (s, 2H, NH_2), 10.33, 10.40 (2s, 2H, 2NH).

2-Amino-4-(4-methoxyphenyl)-5-oxo-N-(p-tolyl)-1,4,5,6,7,8-hexahydroquinoline-3-carboxamide ***(9c)***. Dark yellow crystals, m.p. 165-167 ºC, yield: 3.19 g (79%). Elemental analysis for $C_{24}H_{25}N_3O_3$ (403.47), (% calcd./found): 71.44/71.60 (C), 6.25/6.46 (H), 10.41/10.60 (N). IR (ν, cm^{-1}): 3400, 3335 (2NH, NH_2), 3021 (CH-aromatic), 2929, 2842 (CH_2, CH_3), 1674, 1604 (2C=O), 1591, 1457 (C=C). ^{1}H NMR (δ, ppm): 1.29 (s, 3H, CH_3), 2.19-2.51 (m, 6H, $3CH_2$), 3.87 (s, 3H, OCH_3), 4.29 (s, 1H, CH-pyridine), 6.70-8.10 (m, 8H, $2C_6H_4$), 8.21 (s, 2H, NH_2), 8.30, 10.19 (2s, 2H, 2NH).

2-Amino-N-(4-chlorophenyl)-5-oxo-4-phenyl-1,4,5,6,7,8-hexahydroquinoline-3-carboxamide ***(9d)***. Yellow crystals, m.p. 263-265 ºC, yield: 3.23 g (82%). Elemental analysis for $C_{22}H_{20}ClN_3O_2$ (393.87), (% calcd./found): 67.09/67.30 (C), 5.12/5.40 (H), 10.67/10.80 (N). IR (ν, cm^{-1}): 3269, 3178 (2NH, NH_2), 3052 (CH-aromatic), 2939 (CH_2), 1684, 1602 (2C=O), 1533, 1484 (C=C). ^{1}H

NMR (δ, ppm): 1.78-1.93, 2.10-2.50 (m, 6H, $3CH_2$), 4.92 (s, 1H, CH-pyridine), 6.54-7.74 (m, 9H, C_6H_4, C_6H_5), 8.31 (s, 2H, NH_2), 9.42, 10.20 (2s, 2H, 2NH).

2-Amino-N,4-bis(4-chlorophenyl)-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxamide ***(9e)***. Faint yellow crystals, m.p. 136-138 ºC, yield: 3.21 g (75%). Elemental analysis for $C_{22}H_{19}Cl_2N_3O_2$ (428.31), (% calcd./found): 61.69/61.80 (C), 4.47/4.60 (H), 9.81/9.99 (N). IR (ν, cm^{-1}): 3400, 3322 (2NH, NH_2), 3054 (CH-aromatic), 2924 (CH_2), 1683, 1602 (2C=O), 1594, 1485 (C=C). ^{1}H NMR (δ, ppm): 1.78-1.93, 2.10-2.50 (m, 6H, $3CH_2$), 4.88 (s, 1H, CH-pyridine), 6.53-8.01 (m, 8H, $2C_6H_4$), 8.30 (s, 2H, NH_2), 9.46, 10.54 (2s, 2H, 2NH).

2-Amino-N-(4-chlorophenyl)-4-(4-methoxyphenyl)-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxamide ***(9f)***. Off white crystals, m.p. 167-169 ºC, yield: 3.18 g (75%). Elemental analysis for $C_{23}H_{22}ClN_3O_3$ (423.89), (% calcd./found): 65.17/65.30 (C), 5.23/5.40 (H), 9.91/9.69 (N). IR (ν, cm^{-1}): 3427, 3318 (2NH, NH_2), 3012 (CH-aromatic), 2935 (CH_2, CH_3), 1675, 1603 (2C=O), 1593, 1509 (C=C). ^{1}H NMR (δ, ppm): 1.79-1.93, 2.17-2.50 (m, 6H, $3CH_2$), 3.88 (s, 3H, OCH_3), 4.85 (s, 1H, CH-pyridine), 6.70-8.10 (m, 8H, $2C_6H_4$), 8.23 (s, 2H, NH_2), 9.39, 10.42 (2s, 2H, 2NH). ^{13}C NMR (δ, ppm): 21.3, 26.9, 36.9, 39.8, 55.3, 103.8, 113.2, 115.3, 115.4, 122.6, 124.8, 129.1, 129.4, 133.1, 133.9, 137.9, 151.1, 157.6, 161.5, 163.3, 196.8.

2-Amino-N-(4-methoxyphenyl)-5-oxo-4-phenyl-1,4,5,6,7,8-hexahydroquinoline-3-carboxamide ***(9g)***. Grey crystals, m.p. 134-136 ºC, yield: 3.31 g (85%). Elemental analysis for $C_{23}H_{23}N_3O_3$ (389.45), (% calcd./found): 70.93/71.20 (C), 5.95/6.10 (H), 10.79/10.49 (N). IR (ν, cm^{-1}): Broad band 3316 (2NH, NH_2), 3053 (CH-aromatic), 2940 (CH_2, CH_3), 1674, 1608 (2C=O), 1510, 1371 (C=C). ^{1}H NMR (δ, ppm): 2.18-2.28, 2.49-2.51 (m, 6H, $3CH_2$), 3.84 (s, 3H, OCH_3), 4.91 (s, 1H, CH-pyridine), 6.89-8.00 (m, 9H, C_6H_4, C_6H_5), 8.27 (s, 2H, NH_2), 10.10, 10.27 (2s, 2H, 2NH).

2-Amino-4-(4-chlorophenyl)-N-(4-methoxyphenyl)-5-oxo-1,4,5,6,7,8-hexahydro-quinoline-3-carboxamide ***(9h)***. Grey crystals, m.p. 184-186 ºC, yield: 3.18 g (75%). Elemental analysis for $C_{23}H_{22}ClN_3O_3$ (423.89), (% calcd./found): 65.17/65.30 (C), 5.23/5.40 (H), 9.91/9.69 (N). IR (ν, cm^{-1}): 3317, 3193 (2NH, NH_2), 3052, 3012 (CH-aromatic), 2949, 2834 (CH_2, CH_3), 1672, 1607 (2C=O), 1597, 1458 (C=C). ^{1}H NMR (δ, ppm): 1.75-2.10, 2.20-2.40 (m, 6H, $3CH_2$), 3.75 (s, 3H, OCH_3), 4.90 (s, 1H, CH-pyridine), 6.93-8.01 (m, 8H, $2C_6H_4$), 8.26 (s, 2H, NH_2), 10.28, 10.40 (2s, 2H, 2NH). ^{13}C NMR (δ, ppm): 39.4, 55.7, 108.4, 114.4 (2), 122.7 (2), 128.2 (2), 129.9 (2), 131.3, 131.6, 132.2, 137.4, 149.6, 156.6, 160.4.

2-Amino-N,4-bis(4-methoxyphenyl)-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxamide ***(9i)***. Grey crystals, m.p. 164-166 ºC, yield: 3.50 g (83%). Elemental analysis for $C_{24}H_{25}N_3O_4$ (419.47), (% calcd./found): 68.72/68.99 (C), 6.01/6.30 (H), 10.02/9.79 (N). IR (ν, cm^{-1}): 3399, 3321 (2NH, NH_2), 3056, 3007 (CH-aromatic), 2951, 2834 (CH_2, CH_3), 1671, 1606 (2C=O), 1598, 1459 (C=C). ^{1}H NMR (δ, ppm): 1.80-2.10, 2.26, 2.49-2.51 (m, 6H, $3CH_2$), 3.75, 3.87 (2s, 6H, $2OCH_3$), 4.31 (s, 1H, CH-pyridine), 6.74-8.19 (m, 8H, $2C_6H_4$), 8.31 (s, 2H, NH_2), 10.13, 10.20 (2s, 2H, 2NH). ^{13}C NMR (δ, ppm): 39.4, 55.7, 56.1, 62.6, 104.1, 114.3, 115.3, 115.4, 117.4, 121.3, 122.7, 124.9, 129.4, 131.8, 133.0, 131.0, 150.6, 156.4, 161.0, 163.1.

Biological activity applications

Cell proliferation assay of 18 compounds

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 [33-36]. 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 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 substituents within the heterocyclic moiety together with the hetero cycle ring being attached have a notable influence on the anti-proliferative activity.

CONCLUSION

Through this work the multicomponent reactions of cyanoacetanilide derivatives **3a-c** which were used to produce novel tetrahydrobenzopyrans, tetrahydrobenzopyridines thiophene derivatives **7a-i**, and **9a-i**, respectively. The antiproliferative activity for all the synthesized compounds was tested against six cancer cell lines namely, A549, HT-29, MKN-45, U87MG, SMMC-7721 and, H460. The results were promising and showed that many prepared compounds were the most active products for all the tested cancer cell lines. The presence of the Cl group together with the nature of the heterocyclic ring have a strong impact through the activity of the compound. This appeared through the high potency of compounds **7e**, **7f**, **9b**, **9d**, **9e** and **9g**. The results obtained in this work encourage further work to be done in the future aiming to produce new anti-cancer agents.

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