Scientific paper

Heteroannelation of Cyclic Ketones: Synthesis, Characterization and Antitumor Evaluation of Some Condensed Azine Derivatives

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Abstract

A series of pyrimidine and thiazine derivatives was synthesized by one-pot reaction of cyclopentanone with a mixture of an aromatic aldehyde, namely *o*-anisaldehyde, and different ureas, namely urea, guanidine and thiourea, respectively. Furthermore, cycloaddition reaction of active methylene reagents, namely acetyl acetone, malononitrile, ethyl cyanoacetate, cyanoacetamide and *N*-phenyl cyanoacetamide with 2,6-bis(2-methoxybenzylidene)cyclohexanone afforded chromene and quinoline derivatives in basic medium. The antitumor evaluation of some new compounds against three human cell lines, namely MCF-7, NCI-H460 and SF-268 showed significant and moderate activity compared with the positive control doxorubicin.

Keywords: Cyclopentapyrimidine, Thiazolopyrimidine, Quinazoline, Chromene, Antitumor activity

1. Introduction

The azines have been reported to have antibacterial,[1,2] analgesic,[3] antitubercular,[4,5] anti-inflammatory,[6,7] antioxidant,[8,9] and antiviral activities.[11–14] 2-Oxo-1,2-dihydropyridine-3-carbonitrile derivatives were reported as inhibitors of the oncogenic serine/threonine kinase[15,16] and for the treatment of the congestive heart failure.[17,18]

Cycloalkanones, such as cyclopentanone and cyclohexanone, react cleanly with urea or thiourea and aromatic aldehydes to give three families of fused heterobicyclic, benzylidene heterobicyclic, and spiro heterotricyclic pyrimidines as key intermediates for the preparations of many biologically active compounds.[19–28] The modification, however, is still able to maintain the active moiety of the compound.

In view of these observations and due to our recent interest in developing novel multicomponent reactions (MCRs) for heterocyclic synthesis via dipolar intermediates,[29–39] we report herein the synthesis of some new derivatives of condensed pyrimidines of cycloalkanone and aldehyde bearing ortho effect with nitrogen nucleophiles and preliminarily evaluate their anticancer properties.

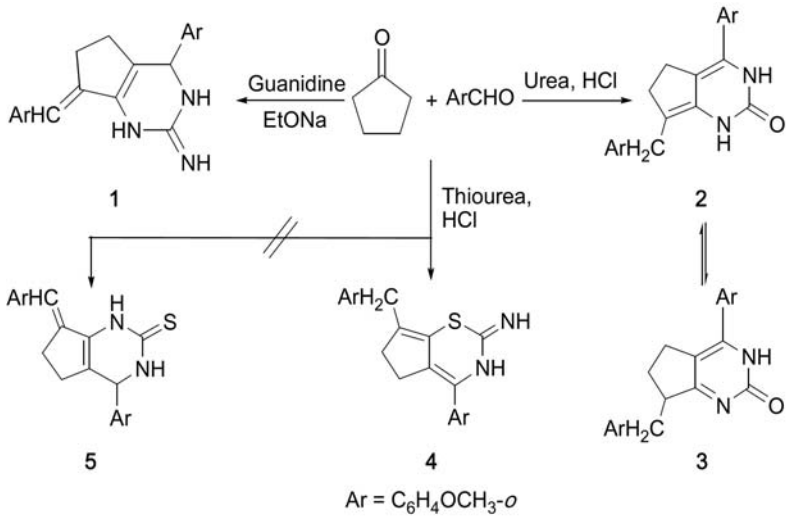
Furthermore, reaction of 2,6-bis(2-methoxybenzylidene)cyclohexanone (**6**) with different cyano nucleophiles yielded chromene and quinoline derivatives of promising antitumor activity.

2. Results and Discussion

2. l. Chemistry

The goal of this work was to study the possibility of azine synthesis by [3+3] cycloaddition of α,β-unsaturated systems to diverse nucleophiles, to afford condensed pyrimidine and pyridine ring systems. These compounds are readily available in high yields under the conditions of both acidic and basic catalysis. Thus, one-pot three component reaction of *o*-anisaldehyde, guanidine sulphate and cyclopentanone in a basic medium resulted in a Michael-type adduct that was identified as the cyclic product **1** (Scheme 1).

The ^{1}H NMR spectrum of **1** exhibited three singlets at δ 10.25–8.60 (D_2O exchangeable) corresponding to the guanidine protons and a singlet at 5.65 ppm belonging to the CH methylenic group. ^{13}C NMR of **1** was in agreement with the expected structure that can exist in equilibrium with its non isolable tautomers. On the other hand, acid induced [3+3] cycloaddition of cyclopentanone, anisaldehyde and urea afforded cyclopentapyrimidine derivati-



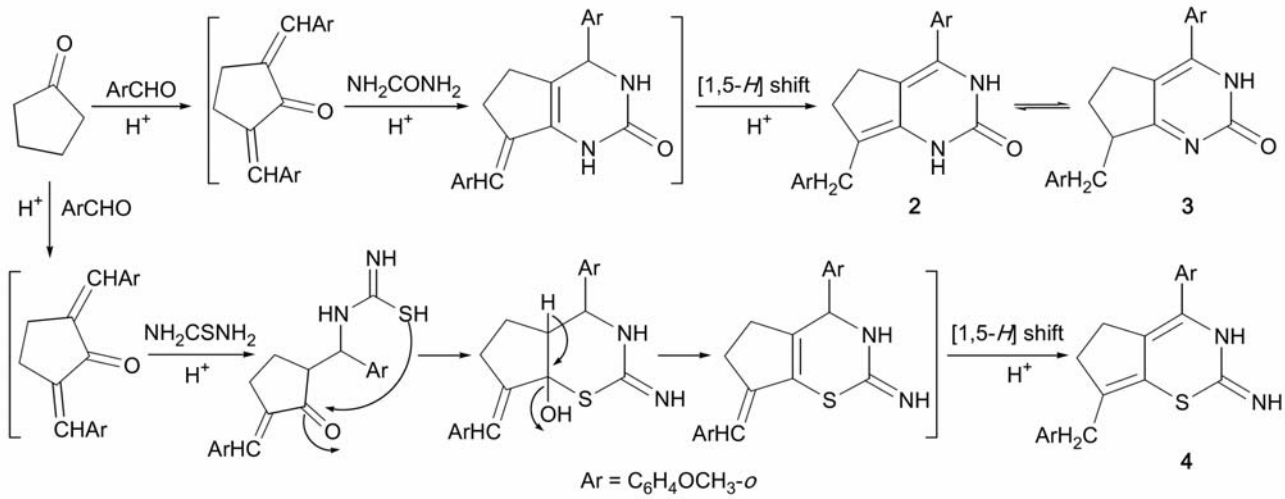
Scheme 1. One pot synthesis of cyclopenta[*d*]pyrimidines **1**, **2**, **3** and cyclopenta[*e*][1,3]thiazine **4** derivatives.

ves **2** and **3** (in ratio 1:1) as shown in Scheme 1. The structures of the latter products were established on the basis of analytical and spectral data. Thus, the ^{1}H NMR spectrum of **2** showed two singlets at δ 11.81 and 9.98 (D_2O exchangeable) corresponding to the two NH groups and a singlet at δ 3.80 ppm indicating CH_2 benzylic group. The ^{1}H NMR spectrum of **3** showed a multiplet at δ 5.40, a triplet at 3.75 and a multiplet at δ 3.64–2.65 ppm corresponding to CH methylenic, CH_2 benzylic groups and CH_2 of cyclopentane, respectively.

The three-components Biginelli-like reaction of *o*-anisaldehyde, cyclopentanone and thiourea in an acidic medium resulted in heterocyclization potentiated by the more reactive SH than NH group (*i.e.* kinetic product)[40] affording thiazine derivative **4** and none of the pyrimidine derivative **5** was obtained (Scheme 1). The structure of **4** was established from its analytical and spectral data. Thus, the ^{1}H NMR spectrum of **4** showed two singlets at δ 10.00 and 9.94 (D_2O exchangeable) corresponding to two NH groups and a singlet at 3.91 ppm indicating CH_2 of benzyl group.

Formation of the pyrimidinones **2**, **3** and thiazinimine **4** from cyclopentanone, *o*-anisaldehyde, urea and/or thiourea presumably proceeds via the formation of acyclic Michael-type adducts of 2,5-bis(2-methoxybenzylidene)cyclopentanone, followed by the heterocyclization and a series of hydrogen shifts with the subsequent isomerization in the case of urea cycloaddition as shown in Scheme 2.

Furthermore, synthesis of pyrimidine thione **7** was achieved via a base induced [3+3] cycloaddition of thiourea and α,β-unsaturated system **6** as shown in Scheme 3. ^{1}H NMR spectrum of **7** showed two singlets at δ 9.13 and 8.69 corresponding to NH groups and a singlet at δ 5.18 ppm corresponding to the CH methylenic proton. Com-



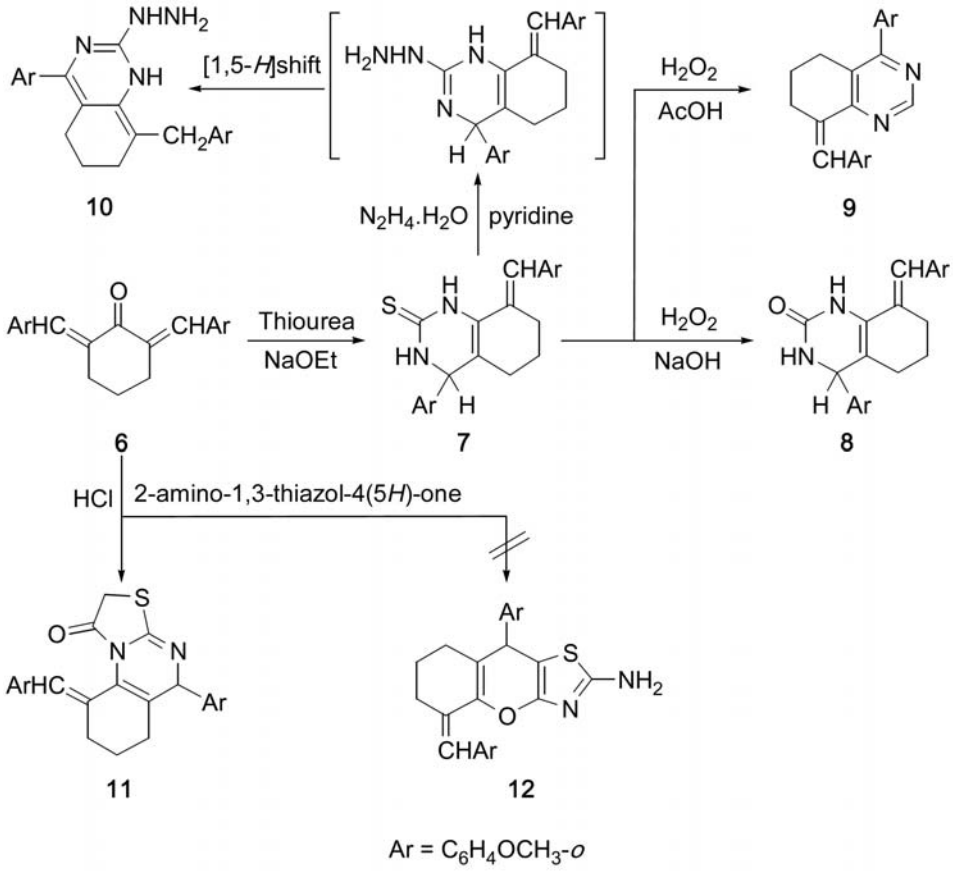
Scheme 2. Postulated mechanism for the formation of cyclopenta[*d*]pyrimidin-2-ones **2**, **3** and cyclopenta[*e*][1,3]thiazin-2(3*H*)-imine **4** derivatives.

pound **7** was reacted with H_2O_2 in the presence of NaOH to produce the oxidized product that was identified as the pyrimidinone **8**. Whereas using H_2O_2 in acetic acid as the oxidizing agent resulted dehydrogenation, in addition to the desulfurization, afforded the quinazoline derivative of type **9**. Also, the pyrimidine thione **7** was allowed to react with hydrazine hydrate in dry pyridine resulting in the hydrazinolysis in addition to the basic isomerization producing the final product **10** (Scheme 3).

The structures of the latter products were established on the basis of analytical and spectral data. The IR spectrum of **8** revealed a peak at 1671 cm^{-1} of the carbonyl group and ^{1}H NMR spectrum showed a singlet at δ 8.07 ppm corresponding to the NH group. ^{1}H NMR spectrum of **9** showed a multiplet at δ 8.20–6.96 ppm corresponding to the aromatic and ethylenic protons. The ^{1}H NMR of the hydrazino derivative **10** showed two singlets at δ 9.13 and 8.68 (D_2O exchangeable) corresponding to NH groups, a singlet at 5.18 (D_2O exchangeable) belonging to the NH_2 group and a singlet at 3.84 ppm indicating CH_2 benzylic protons.

Curiously, α,β-unsaturated system of the type **6** underwent intermolecular cycloaddition with 2-amino-1,3-thiazol-4(5*H*)-one to produce thiazolopyrimidine derivative **11** potentiated by the high nucleophilicity of the ring nitrogen than the enolic tautomer of thiazolone, therefore none of the chromenothiazole **12** was obtained (Scheme 3). The analytical and spectral data were consistent with the proposed structure. Thus, the IR spectrum of **11** revealed a peak at 1696 cm^{-1} of the carbonyl group and the ^{1}H NMR spectrum showed double doublet at δ 4.14 corresponding to the CH_2 group of thiazole, a singlet at δ 4.50 indicating CH methylenic and a multiplet at δ 7.95–6.93 ppm corresponding to Ar-H and CH ethylenic group.

Upon the reaction of *o*-anisalcyclohexanone **6** with acetyl acetone (AcAc) a cycloaddition took place forming chromene derivative, which in turn underwent a hydrogen shift giving the final product **13**. None of the naphthalene derivative **14** was obtained due to the enolic tautomer of the intermediate adduct facilitating the attack of the enolic OH to the acetyl carbonyl under the reaction conditions to produce the desired chromene **13** (Scheme 4). The analytical and spectral data were consistent with the proposed structure. Thus, the IR spectrum of **13** revealed a peak at 1660 cm^{-1} of the carbonyl group and the ^{1}H NMR spectrum showed a singlet at δ 3.88 indicating the CH_2 benzylic group, a singlet at δ 2.49 corresponding to the acetyl protons and a singlet at δ 2.46 ppm belonging to methyl protons.



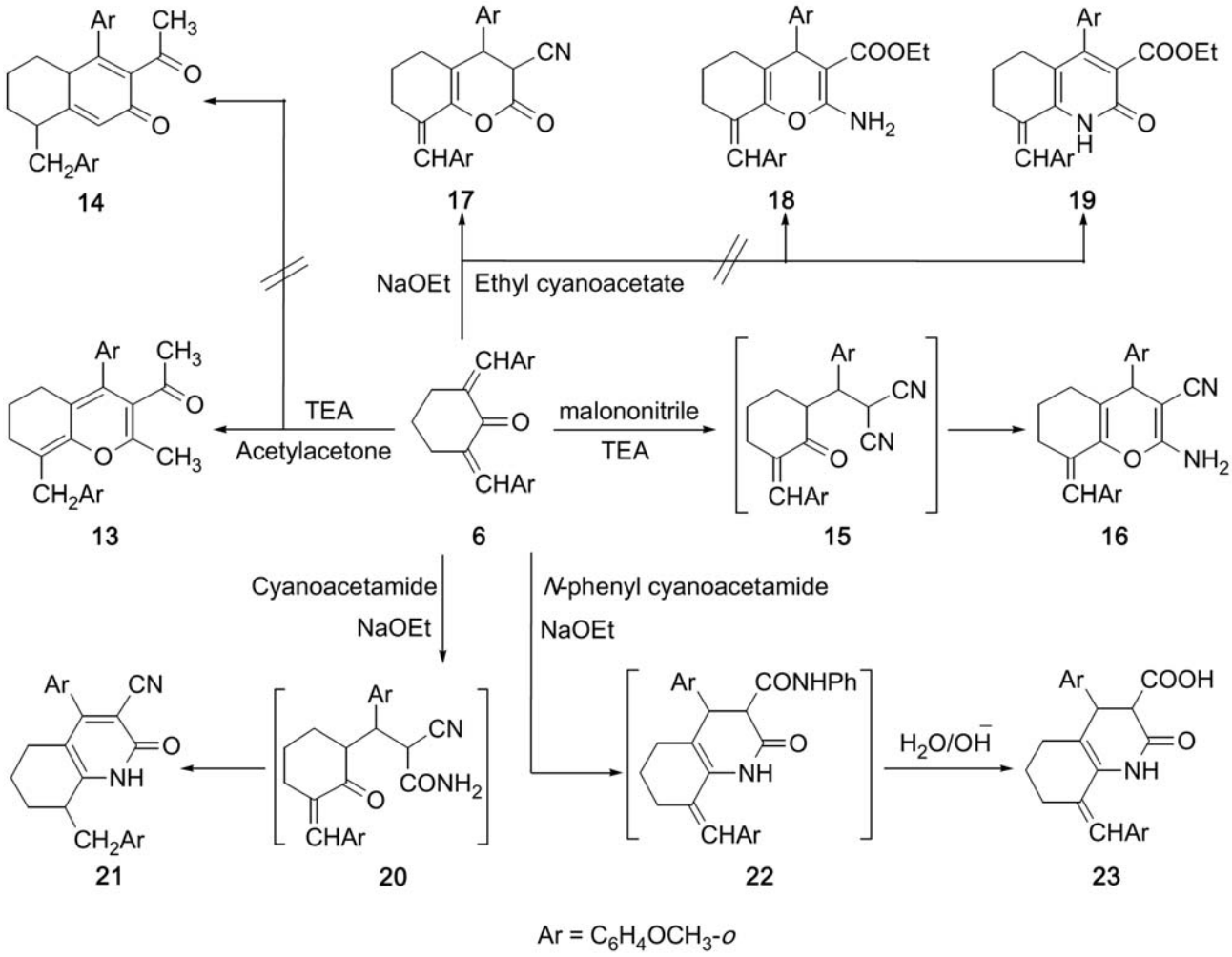
Scheme 3. The synthetic route for cycloaddition of α,β-unsaturated cyclic ketone.

The high yield of α,β-unsaturated system of the type **6** encouraged us to study their further reactivity towards cyanomethylene reagents. Thus, malononitrile added its nucleophilic carbon to the electrophilic carbon of **6** producing acyclic Michael-type adduct **15** that intramolecularly cyclizes producing chromene-3-carbonitrile of the type **16**. While, α,β-unsaturated system **6** when allowed to react with ethyl cyanoacetate afforded chromene-3-carbonitrile of the type **17**. None of the products **18** and **19** were obtained. Concerning the proposed mechanism, we expected that attack of the enolic OH to the ester carbonyl, which is more electrophilic than the cyano carbon, leads to the formation of chromene-3-carbonitrile **17** (Scheme 4). The analytical and spectral data of the obtained products were in agreement with the assigned structures. Thus, the ^{1}H NMR spectrum of **17** (as an example) showed beside the expected signals of the cyclohexane moiety, two singlets at δ 3.83 and 3.78 ppm corresponding to the two CH groups, a multiplet at δ 7.80–6.97 ppm including the aromatic protons with CH ethylenic groups and the IR spectrum exhibited peaks at 2197 and 1674 cm^{-1} of the cyano and carbonyl groups, respectively.

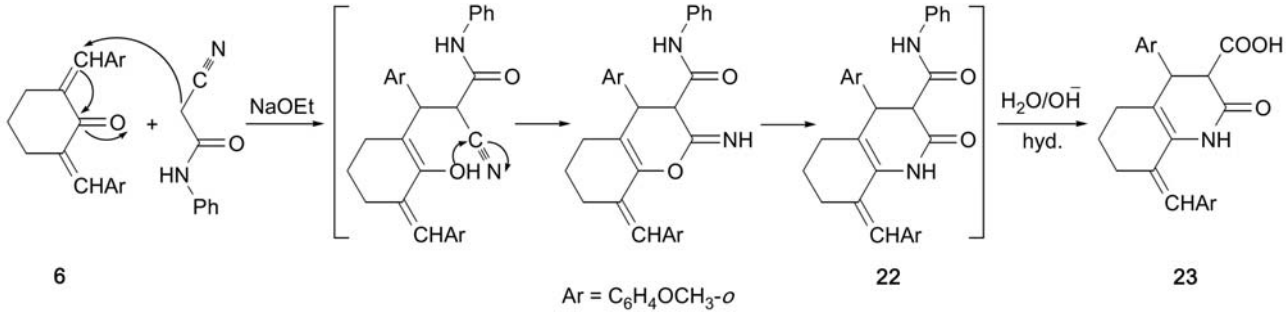
Also, cyanoacetamide produced the Michael-type adduct **20** upon its reaction with ketonic compound **6** followed by basic isomerization giving the final quinoline product **21**. The IR spectrum of **21** revealed a peak at 2223 cm^{-1} of the CN group and the ^{1}H NMR spectrum showed a singlet at δ 12.05 according to NH group and doublet at δ 3.71 ppm indicating the Ar-C$\underline{H}_2$ protons.

Finally, reaction of 2-cyano-*N*-phenylacetamide with the chalcone **6** in a basic medium afforded the intermediate product **22** which in turn underwent basic hydrolysis producing quinoline derivative **23** (Scheme 4). This reaction presumably proceeds via Michael addition followed by an intramolecular cyclization and subsequent Dimroth rearrangement affording **22** which in turn underwent basic hydrolysis producing quinoline derivative **23** (Scheme 5). The analytical and spectral data were consistent with the proposed structure.

Thus, the IR spectrum of **23** revealed peaks at 3432 for the acidic OH (broad) and 1707–1628 cm^{-1} characteri-



Scheme 4. Condensation reactions of α,β-unsaturated cyclic ketones with active methylene reagents.



Scheme 5. Mechanism for the formation of product **23**.

stic for the carbonyl groups. The ^{1}H NMR spectrum showed a multiplet at δ 7.56–6.88 corresponding to the Ar-H and CH ethylenic, a singlet at δ 9.52 (D_2O exchangeable) indicating the NH group and a singlet at δ 12.11 ppm belonging to the carboxylic proton, in addition to the expected signals of the cyclohexane moiety.

3. 2. Antitumor Activity

2. 2. 1. Tumor Cell Growth Assay

The effects of compounds **1**, **13**, **16**, **17** and/or **21** on the *in vitro* growth of human tumor cell lines were evaluated according to the procedure adopted by the National Cancer Institute (NCI, USA) in the '*In vitro* Anticancer Drug Discovery Screen' that uses the protein-binding dye sulforhodamine B to assess cell growth.[41,42] Briefly, exponentially, cells growing in 96-well plates were then exposed for 48 h to five serial concentrations of each compound, starting from a maximum concentration of 150 μM. Following this exposure period adherent cells were fixed, washed, and stained. The bound stain was solublized and the absorbance was measured at 492 nm in a plate reader (Bio-Tek Instruments Inc., Power wave XS, Winooski, USA). For each test compound and cell line, a dose–response curve was obtained and the growth inhibition of 50% (GI_{50}), corresponding to the concentration of the compounds that inhibited 50% of the net cell growth was calculated as described elsewhere.[43] Doxorubicin was used as a positive control and tested in the same manner. For our newly synthesized products we selected the three cancer cell lines: the breast adenocarcinoma (MCF-7), non-small cell lung cancer (NCI-H460) and CNS cancer (SF-268) as our compounds are electron rich systems substituted with electronegative groups and many reports from previous work used such cell lines together with the use of doxorubicin which was showed to be the best positive control against the three cell lines (Table 1).

Results are given in concentrations that were able to cause 50% of cell growth inhibition (GI_{50}) after a continuous exposure of 48 h and show means ± SEM of three independent experiments performed in duplicate.

2. 2. 2. Structure Activity Relationship (SAR)

The compound **16** with –CN substitution at C-3 position of chromene ring and $–NH_2$ substitution at C-2 position exhibited potent antitumor activity in MCF-7, NCI-H460 and significant effect in SF-268. Also, compound **17** with –CN substitution at C-3 position of chromen-2-one ring exhibited potent antitumor activity in SF-268, NCI-H460 and significant effect in MCF-7. However, compound **13** with $–COCH_3$ substitution at C-3 position of chromene moiety as well as $–CH_3$ substitution at C-2 position showed significant effect in MCF-7 and moderate activity in both NCI-H460 and SF-268. On the other hand, 2-oxoquinolinecarbonitrile **21** with –CN substitution at C-3 position was the lowest in both. Comparing the antitumor activity of the tested compounds and their analogous described in the literature,[37–38] it is obvious that the highest cytotoxicity might be attributed to the presence of

Table 1. Effect of compounds **1, 13, 16, 17** and **21** on the growth of three human tumor cell lines

Compound	GI_{50} (μM) (% growth)		
	MCF-7	NCI-H460	SF-268
1	20.23 ± 4.50	18.28 ± 4.21	42.62 ± 4.80
13	14.27 ± 6.07	18.15 ± 4.05	20.27 ± 2.40
16	4.16 ± 1.09	7.25 ± 1.30	12.80 ± 3.90
17	13.48 ± 4.22	6.09 ± 1.88	4.62 ± 1.12
21	22.31 ± 3.40	18.29 ±2.40	28.11 ± 10.30
Doxorubicin	0.04 ± 0.008	0.09 ± 0.008	0.09 ± 0.007

the cyanoaminochromene and cyanochromen-2-one moiety bearing 2-$CH_3OC_6H_4$ group.

3. Experimental

3. 1. Chemistry

All melting points were determined using a Stuart melting point apparatus by the open capillary tube method and are uncorrected. IR spectra were recorded on a Perkin–Elmer model 1600 FT-IR instrument as KBr pellets. ^{1}H and ^{13}C NMR spectra were recorded on a Varian 300 MHz in DMSO-d_6 as solvent, using TMS as internal standard and chemical shifts are expressed as δ ppm. Antitumor activity and elemental analyses were performed by the Micro Analytical Center, Cairo University, Egypt. The starting material **6** was prepared as described in the literature.[44] The progress of the reaction and the purity of the compounds were routinely monitored on TLC by pre-coated aluminum silica gel $60F_{254}$ thin layer plates obtained from Merck (Germany) eluting with petroleum ether/ethyl acetate. The yields of all products were not optimized. All reagents used were obtained from commercial sources. All solvents were of analytical grade and used without further purification.

7-(2-Methoxybenzylidene)-4-(2-methoxyphenyl)-1,3,4,5,6,7-hexahydro-2*H*-cyclopenta[*d*]pyrimidin-2-imine (1)

A mixture of *o*-anisaldehyde (2.72 g, 0.02 mol), cyclopentanone (0.8 g, 0.01 mol) and guanidine sulphate (1.57 g, 0.01 mol) in 50 mL ethoxide solution [prepared by dissolving Na (0.92 g, 0.04 mol) in 50 mL absolute ethanol] was heated under reflux for 5 h. The reaction mixture was cooled, poured onto crushed ice and neutralized with acetic acid. The separated solid was filtered off, dried and recrystallized from acetic acid.

Yield: 78%; m.p.: 258–260 °C; IR (KBr, cm^{-1}): 3434 (NH), 2925, 2856 (CH aliphatic), 1635 (C=N); ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.25, 9.85, 8.60 (s, 3H, 3NH), 7.82–6.90 (m, 9H, Ar-H + CH ethylenic), 5.65 (s, 1H, Ar-CH), 3.85, 3.78 (s, 6H, $2OCH_3$), 3.17–2.85 (m, 4H, CH_2 cyclopentane); ^{13}C NMR (100 MHz, DMSO-d_6): δ 26.59, 28.28, 28.92, 29.32, 51.49, 55.78, 55.86, 55.98, 56.21, 111.44, 111.61, 111.84, 112.06, 115.96, 116.23, 118.35, 118.80, 119.35, 119.55, 119.63, 119.66, 120.09, 120.36, 120.57, 120.74, 120.84, 121.00, 121.36, 122.92, 124.07, 125.52, 125.87, 127.42, 128.12, 128.27, 128.45, 128.61, 128.97, 129.09, 129.64, 129.91, 129.97, 130.25, 130.45, 131.43, 132.37, 133.19, 136.92, 138.24, 138.90, 140.29, 152.77, 156.66, 156.72, 156.84, 156.95, 157.87, 157.95, 160.79, 160.83, 161.00, 161.28, 171.40, 195.79. Anal. Calcd. for $C_{22}H_{23}N_3O_2$ (361.43): C, 73.11; H, 6.41; N, 11.63. Found: C, 73.05; H, 6.17; N, 11.56.

General Procedure for the Synthesis of Compounds 2, 3 and 4

A mixture of *o*-anisaldehyde (2.72 g, 0.02 mol), cyclopentanone (0.8 g, 0.01 mol) with 0.60 g urea and/or 0.76 g thiourea (0.01 mol), and conc. HCl (0.03 mol) in ethanol (30 mL) was heated under reflux for 5 h. The reaction mixture was cooled and poured into ice cold water. The precipitated solid was filtered off, dried and recrystallized from the proper solvent to give the products **2**, **3** and **4**, respectively.

7-(2-Methoxybenzyl)-4-(2-methoxyphenyl)-1,3,5,6-tetrahydro-2*H*-cyclopenta[*d*]pyrimidin-2-one (2). Yield: 40% from benzene; m.p.: 240–242 °C; IR (KBr, cm^{-1}): 3414 (NH), 2924, 2854 (CH aliphatic), 1626 (C=O); ^{1}H NMR (300 MHz, DMSO-d_6): δ 11.81, 9.98 (s, 2H, 2NH), 7.86–6.92 (m, 8H, Ar-H), 3.87, 3.82 (s, 6H, $2OCH_3$), 3.80 (s, 2H, Ar-CH_2), 3.04, 2.62 (m, 4H, $2CH_2$ cyclopentane). Anal. Calcd. for $C_{22}H_{22}N_2O_3$ (362.42); C, 72.91; H, 6.12; N, 7.73. Found: C, 73.22; H, 5.82; N, 7.33.

7-(2-Methoxybenzyl)-4-(2-methoxyphenyl)-1,5,6,7-tetrahydro-2*H*-cyclopenta[*d*]pyrimidin-2-one (3). Yield: 45% from methanol; m.p.: 200–202 °C; IR (KBr, cm^{-1}): 3408 (NH), 3076 (CH aromatic), 2930, 2854 (CH aliphatic) 1646 (C=O amide); ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.95 (s, 1H, NH), 7.87–6.80 (m, 8H, Ar-H), 5.40 (m, 1H, Ar-CH), 3.87, 3.82 (s, 6H, $2OCH_3$), 3.75 (t, 2H, J = 10.2 Hz, Ar-CH_2), 3.64–2.65 (m, 4H, $2CH_2$ cyclopentane). Anal. Calcd. for $C_{22}H_{22}N_2O_3$ (362.42): C, 72.91; H, 6.12; N, 7.73. Found: C, 72.63; H, 6.00; N, 7.45.

7-(2-Methoxybenzyl)-4-(2-methoxyphenyl)-5,6-dihydrocyclopenta[*e*][1,3]-thiazin-2(3*H*)-imine (4). Yield: 79% from aqueous methanol; m.p.: 220–222 °C; IR (KBr, cm^{-1}): 3383, 3205 (NH), 3066 (CH aromatic), 2925, 2857 (CH aliphatic), 1592 (C=N); ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.00, 9.94 (s, 2H, 2NH), 7.54–6.46 (m, 8H, Ar-H), 3.91 (s, 2H, Ar-CH_2), 3.87, 3.74 (s, 6H, $2OCH_3$), 3.17–2.73 (m, 4H, $2CH_2$ cyclopentane). Anal. Calcd. for $C_{22}H_{22}N_2O_2S$ (378.48): C, 69.81; H, 5.86; N, 7.40. Found: C, 70.12; H, 5.78; N, 7.14.

Synthesis of 4-(2-Methoxyphenyl)-8-[(2-methoxyphenyl)methylidene]-3,4,5,6,7,8-hexahydro-2(1*H*)-quinazolinethione (7)

A mixture of compound **6** (3.34 g, 0.01 mol), thiourea (0.76 g, 0.01 mol) and sodium ethoxide (0.02 mol) [prepared of sodium (0.46 g) dissolved in absolute ethanol (20 mL)] in absolute ethanol (30 mL) was heated under reflux for 4 h. The solid product obtained upon cooling was poured onto crushed ice and acidified with acetic acid, filtered off, dried and recrystallized from acetic acid.

Yield: 85%; m.p.: 165–167 °C; IR (KBr, cm^{-1}): 3404, 3247 (NH), 3063 (CH aromatic), 2933, 2832 (CH aliphatic), 1655 (C=N); 1594 (C=C), 1243 (C=S); ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.14, 8.70 (s, 2H, 2NH), 7.31–6.89 (m, 9H, Ar-H + CH ethylenic), 5.19 (s, 1H,

Ar-CH), 3.81, 3.79 (s, 6H, $2OCH_3$), 2.50–1.46 (m, 6H, CH_2 cyclohexane). ^{13}C-NMR (75 MHz, DMSO-d_6): δ 22.25 (CH_2), 26.11 (CH_2), 26.63 (CH_2), 52.46 (N-C-C), 55.17 (OCH_3), 55.56 (OCH_3), 110.81, 111.24, 113.72, 119.00, 119.78, 120.81, 125.51, 127.53, 127.56, 128.32, 128.98, 130.14, 130.67 (N-C=C), 156.00 (O-C=C), 156.88 (O-C=C), 174.48 (C=S). Anal. Calcd. for $C_{23}H_{24}N_2O_2S$ (392.51): C, 70.38; H, 6.16; N, 7.14. Found: C, 70.03; H, 5.86; N, 6.83.

Synthesis of 4-(2-Methoxyphenyl)-8-[(2-methoxyphenyl)methylidene]-3,4,5,6,7,8-hexahydro-2(1*H*)-quinazolinone (8)

A mixture of **7** (3.92 g, 0.01 mol) and sodium hydroxide (0.40 g, 0.01 mol) was dissolved in DMF (30 mL). To this solution, H_2O_2 (0.02 mol) was added drop wise with stirring at r.t. for 2 h. The reaction mixture was neutralized by HCl, and the precipitated solid was filtered off, dried and recrystallized from methanol.

Yield: 89%; m.p.: 180–182 °C; IR (KBr, cm^{-1}): 3407 (OH enolic); 3336, 3235 (NH), 3111, 3067 (CH aromatic), 2947, 2878 (CH aliphatic), 1671 (C=O), 1594 (C=N); ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.07 (s, 2H, 2NH), 7.28–6.82 (m, 9H, Ar-H + CH ethylenic), 5.19 (s, 1H, Ar-CH), 3.80, 3.78 (s, 6H, $2OCH_3$), 2.49–1.49 (m, 6H, CH_2 cyclohexane). ^{13}C NMR (75 MHz, DMSO-d_6): δ 22.45 (CH_2), 25.93 (CH_2), 26.64 (CH_2), 52.35 (N-C-C), 55.71 (OCH_3), 55.56 (OCH_3), 110.83, 110.98, 111.24, 118.99, 119.75, 120.74, 125.87, 127.36, 127.56, 128.13, 128.62, 128.97, 130.68, 131.58 (N-C=C), 153.73 (C=O), 156.20 (O-C=C), 156.90 (O-C=C). Anal. Calcd. for $C_{23}H_{24}N_2O_3$ (376.44): C, 73.38; H, 6.43; N, 7.44. Found: C, 73.03; H, 6.53; N, 7.63.

Synthesis of 8-(2-Methoxybenzylidene)-4-(2-methoxyphenyl)-5,6,7,8-tetrahydroquinazoline (9)

To a solution of **7** (3.92 g, 0.01 mol) in acetic acid (20 mL), H_2O_2 (0.02 mol) was added drop wise at r.t. with stirring. Furthermore, the reaction mixture was stirred at r.t. for 3 h. The separated solid was collected by filtration, washed with water, dried and recrystallized from methanol.

Yield: 65%; m.p.: 136–138 °C; IR (KBr, cm^{-1}): 2924, 2856 (aliphatic CH), 1600 (C=N); ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.20–6.96 (m, 10H, Ar-H + CH ethylenic), 3.81, 3.72 (s, 6H, $2OCH_3$), 2.72–0.74 (m, 6H, CH_2 cyclohexane). Anal. Calcd for $C_{23}H_{22}N_2O_2$ (358.43): C, 77.07; H, 6.19; N, 7.82. Found C, 76.79; H, 5.98; N, 7.59.

Synthesis of 2-Hydrazino-8-(2-methoxybenzyl)-4-(2-methoxyphenyl)-1,5,6,7-tetrahydroquinazoline (10)

A mixture of **7** (3.92 g, 0.01 mol) and hydrazine hydrate (0.015 mol) in pyridine (20 mL) was refluxed for 5 h. The reaction mixture was cooled and neutralized with dilute HCl. The separated solid was filtered off, dried and recrystallized from methanol.

Yield: 54%; m.p.: 130–132 °C; IR (KBr, cm^{-1}): 3400–3264 (NH, NH_2), 2926–2856 (CH aliphatic); ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.13, 8.68 (s, 2H, 2NH, D_2O exchangeable), 7.32–6.89 (m, 8H, Ar-H), 5.18 (s, 2H, NH_2, D_2O exchangeable), 3.84 (s, 2H, Ar-CH_2), 3.81, 3.78 (s, 6H, $2OCH_3$), 2.45–1.05 (m, 6H, CH_2 cyclohexane); ^{13}C NMR (100 MHz, DMSO-d_6): δ 22.77 (CH_2), 26.64 (CH_2), 27.11 (CH_2), 42.64 (Ar-CH_2), 52.91, 55.57 (OCH_3), 56.07 (OCH_3), 111.31, 111.75, 114.31, 119.55, 120.29, 121.33, 126.00, 128.02, 128.05, 128.85, 129.46, 129.52, 130.66, 131.20 (C-$NHNH_2$), 156.47 (Ar-C), 157.38 (Ar-C), 174.96 (C=N). Anal. Calcd. for $C_{23}H_{26}N_4O_2$ (390.48): C, 70.75; H, 6.71; N, 14.35. Found: C, 70.51; H, 6.91; N, 14.63.

Synthesis of 5-(2-Methoxyphenyl)-9-[(2-methoxyphenyl)methylidene]-6,7,8,9-tetrahydro-5*H*-[1,3]thiazolo[3,2-a]quinazolin-1(2*H*)-one (11)

A mixture of chalcone **6** (3.34 g, 0.01 mol), 2-amino-1,3-thiazol-4(5*H*)-one (1.16 g, 0.01 mol) and conc. HCl (1.5 mL) in ethanol (30 mL) was refluxed for 5 h. The reaction mixture was left to cool at room temperature. The precipitated solid was filtered off, dried and recrystallized from acetic acid.

Yield 63%; m.p.: > 360 °C; IR (KBr, cm^{-1}): 3411 (OH enolic), 2927–2859 (CH aliphatic), 1696 (C=O), 1618 (C=N); ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.95–6.93 (m, 9H, Ar-H + CH ethylenic), 4.50 (s, 1H, Ar-CH), 4.14 (d, 2H, J = 0.6 Hz, CH_2 of thiazole), 3.83, 3.80 (s, 6H, $2OCH_3$), 2.86–1.70 (m, 6H, CH_2 cyclohexane). Anal. Calcd. for $C_{25}H_{24}N_2O_3S$ (432.53): C, 69.42; H, 5.59; N, 6.48. Found: C, 69.12; H, 5.45; N, 6.64.

General Procedure for the Synthesis of Chromene Derivatives 13 and 16

A mixture of **6** (3.34 g, 0.01 mol), acetyl acetone and/or malononitrile (0.01 mol) and a few drops of TEA in dimethyl formamide (30 mL) was heated under reflux for 20 h. The solid product obtained upon cooling, poured into ice cold water and acidified by acetic acid, filtered off, dried, and recrystallized from the proper solvent gave compounds **13** and **16**, respectively.

1-[8-(2-Methoxybenzyl)-4-(2-methoxyphenyl)-2-methyl-6,7-dihydro-5*H*-chromen-3-yl]-1-ethanone (13). Yield: 69% from aqueous methanol; m.p.: 170–173 °C; IR (KBr, cm^{-1}): 3064 (CH aromatic), 2925, 2851 (CH aliphatic), 1660 (C=O), 1600 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.38–6.64 (m, 8H, Ar-H), 3.88 (s, 2H, CH_2 benzylic), 3.84, 3.71 (s, 6H, $2OCH_3$), 2.49 (s, 3H, $COCH_3$), 2.46 (s, 3H, CH_3), 2.79–1.23 (m, 6H, CH_2 cyclohexane). Anal. Calcd. for $C_{27}H_{28}O_4$ (416.50): C, 77.86; H, 6.78. Found: C, 77.58; H, 6.67.

2-Amino-4-(2-methoxyphenyl)-8-[(2-methoxyphenyl) methylidene]-5,6,7,8-tetrahydro-4*H*-chromene-3-car-

bonitrile (16). Yield: 73% from methanol; m.p.: 280–282 °C; IR (KBr, cm^{-1}): 3340–3223 (NH_2), 3089 (CH aromatic), 2935 (CH aliphatic), 2205 (CN), 1664 (C=N), 1593 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.00 (s, 2H, NH_2), 7.43–6.20 (m, 9H, Ar-H + CH ethylenic), 4.08 (s, 1H, Ar-CH), 3.78, 3.76 (s, 6H, $2OCH_3$), 2.82–1.50 (m, 6H, CH_2 cyclohexane); ^{13}C NMR (100 MHz, DMSO-d_6): δ 28.38 (CH_2), 28.99 (CH_2), 32.96 (CH_2), 33.95 (Ar-CH), 34.54, 55.79, 77.18, 85.81, 111.64, 113.72, 114.40, 118.33, 120.91, 124.90, 126.21, 126.49, 128.56, 128.78, 131.04, 156.31 (Ar-C), 158.27 (C-NH_2), 164.49 (Ar-C). Anal. Calcd. for $C_{25}H_{24}N_2O_3$ (400.46): C, 74.98; H, 6.04; N, 7.00. Found: C, 74.69; H, 5.95; N, 6.74.

General Procedure for the Synthesis of Compounds 17, 21 and 23

A mixture of chalcone **6** (3.34 g, 0.01 mol), ethyl cyanoacetate, cyanoacetamide and/or *N*-phenyl cyanoacetamide (0.01 mol) and sodium ethoxide (0.02 mol) [prepared of 0.46 g sodium dissolved in ethanol absolute (20 mL)] in ethanol (30 mL) was refluxed for 3 h. The reaction mixture was cooled, poured into ice cold water and neutralized with acetic acid. The precipitated solid was filtered off, dried to give crude material of **17**, **21** and **22**, respectively. The crude product **22** in 20 mL aqueous NaOH (10%) was heated under reflux for 1 h. The resultant solution was cooled, diluted with ice cold water and acidified with HCl. The precipitated solid was filtered off, dried to give compound **23**.

4-(2-Methoxyphenyl)-8-[(2-methoxyphenyl)methylidene]-2-oxo-3,4,5,6,7,8-hexahydro-2*H*-chromene-3-carbonitrile (17). Yield: 78% from methanol; m.p.: 148–150 °C; IR (KBr, cm^{-1}): 3432 (OH enolic), 3055 (CH aromatic), 2927–2846 (CH aliphatic), 2197 (CN), 1674 (C=O), 1594 (C=C); ^{1}H NMR (300 MHz, DMSO-d_6): δ 7.80–6.97 (m, 9H, Ar-H + CH ethylenic), 3.83, 3.78 (dd, 2H, J = 9.0; 6.6 Hz, 2CH), 3.77, 3.72 (s, 6H, $2OCH_3$), 2.79–1.56 (m, 6H, CH_2 cyclohexane). Anal. Calcd. for $C_{25}H_{23}NO_4$ (401.54): C, 74.79; H, 5.77; N, 3.49. Found: C, 74.47; H, 5.47; N, 3.14.

8-(2-Methoxybenzyl)-4-(2-methoxyphenyl)-2-oxo-1,2,5,6,7,8-hexahydro-3-quinolinecarbonitrile (21). Yield: 75% from acetic acid; m.p.: 265–267 °C; IR (KBr, cm^{-1}): 3468 ((NH), 3011 (CH aromatic), 2932, 2837 (CH aliphatic), 2223 (CN), 1635 (C=O); ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.82 (s, 1H, NH), 7.59–6.83 (m, 8H, Ar-H), 4.31 (d, 2H, J = 4.2 Hz, Ar-CH_2), 3.82, 3.72 (s, 6H, $2OCH_3$), 2.45–1.55 (m, 7H, CH cyclohexane). ^{13}C NMR (75 MHz, DMSO-d_6): δ 22.13 (CH_2), 25.06 (CH_2), 26.48 (CH_2), 55.40 (OCH_3), 55.65 (OCH_3), 111.13, 111.72, 115.91, 119.97, 120.76, 124.19, 124.69, 127.40, 128.80, 129.68, 130.10, 130.87, 155.20 (O-C=C)), 157.33 (O-C=C)), 160.18 (C=O). Anal. Calcd. for $C_{25}H_{24}N_2O_3$ (400.47): C, 74.98; H, 6.04; N, 7.00. Found: C, 75.33; H, 5.95; N, 6.78.

4-(2-Methoxyphenyl)-8-[(2-methoxyphenyl)methylidene]-2-oxo-1,2,3,4,5,6,7,8-octahydro-3-quinolinecarboxylic acid (23). Yield: 67% from benzene; m.p.: 238–240 °C; IR (KBr, cm^{-1}): 3432 (OH broad), 2924, 2854 (CH aliphatic), 1707, 1628 (C=O); ^{1}H NMR (300 MHz, DMSO-d_6): δ 12.11 (s, 1H, OH), 9.52 (s, 1H, NH), 7.56–6.88 (m, 9H, Ar-H + CH ethylenic), 4.92 (d, 1H, J = 3 Hz, CH-CO), 3.77 (d, 1H, J = 4 Hz, Ar-CH), 3.74, 3.70 (s, 6H, $2OCH_3$), 2.73–1.23 (m, 6H, CH_2 cyclohexane). Anal. Calcd. for $C_{25}H_{25}NO_5$ (419.47): C, 71.58; H, 6.01; N, 3.34. Found: C, 71.93; H, 5.86; N, 3.66.

3. 2. Antitumor Activity Tests

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

Cell cultures: Three human tumor cell lines, MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer), and SF-268 (CNS cancer) were used. MCF-7, XF498, colon; A549, ovarian; HCT15, stomach; was obtained from the European Collection of Cell Cultures (ECACC, Salisbury, UK), NCI-H460, SF-268 and normal fibroblast cells (WI 38) were kindly provided by the National Cancer Institute (NCI, Cairo, Egypt). They grow as a 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 μM), at 37 °C in a humidified atmosphere containing 5% CO_2. Exponentially growing cells were obtained by plating 1.5×10^5 cells/mL for MCF-7, NCI-H460 and SF-268 and 0.75×10^4 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.

4. Conclusion

A series of novel condensed pyrimidine, pyran and pyridine derivatives were synthesized and assayed for their antitumor activity against three human cell lines namely MCF-7, NCI-H460 and SF-268. The activity comparison and the structure correlation of the tested compounds had shown that these potencies paralleled the electron withdrawing powers of the substituent groups. Hence, the higher cytotoxcity of compounds **14** and **15** was attributed to the presence of the electronegative cyano group.

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6. Supplementary Material

Copies of the IR, ^{1}H, ^{13}C NMR spectra and antitumor evaluations of the new compounds are available on the Journal's website.

7. References

1. S. Chitra, D. Devanathan, K. Pandiarajan, *Eur. J. Med. Chem.* **2009**, *45*, 1–5.
2. N. M. Hamada, N. Y. Megally, *Molecules* **2015**, *20*, 10468–10486. http://dx.doi.org/10.3390/molecules200610468
3. P. G. Baraldi, S. Manfredini, R. Romagnoli, L. Stevanato, A. N. Zaid, R. Manservigi, *Nucleos. Nucleot. Nucl.* **1998**, *17*, 2165–2173. http://dx.doi.org/10.1080/07328319808004307
4. R. Sawant, V. Sarode, *Iran. J. pharma. Res.* **2011**, *10(4)*, 733–739.
5. M. M. Kamel, N. Y. Megally, *Eur. J. Med. Chem.* **2014**, *86*, 75–80. http://dx.doi.org/10.1016/j.ejmech.2014.08.047
6. V. K. Narayanaswamy, S. K. Nayak, M. Pillay, R. Prasanna, Y. M. Coovadia, B. Odhav, *Chem. Biol. Drug Des.* **2013**, *81*, 219–227. http://dx.doi.org/10.1111/cbdd.12065
7. O. I. El-Sabbagh, S. Mostafa, H. A. Abdel-Aziz, H. S. Ibrahim, M. M. Elaasser, *Arch. Pharm. Chem. Life Sci.* **2013**, *346*, 688–698. http://dx.doi.org/10.1002/ardp.201300193
8. M. M. Elaasser, M. M. Abdel-Aziz, R. A. El-Kassas, *J. Microbiol. Biotech. Res.* **2011**, *1(4)*, 5–17.
9. N. J. P. Subhashini, J. Amanaganti, L. Boddu, P. A. Nagarjuna, *J. Chem. Pharm. Res.* **2013**, *5(1)*, 140–146.
10. R. Al-Salahi, I. Alswaidan, H. A. Ghabbour, E. Ezzeldin, M. Elaasser, M. Marzouk, *Molecules* **2015**, *20*, 5099–5111. http://dx.doi.org/10.3390/molecules20035099
11. M. M. Youssef, M. A. Amin, *Molecules* **2012**, *17*, 9652–9667. http://dx.doi.org/10.3390/molecules17089652
12. M. M. Ramiz, W. A. El-Sayed, E. Hagag, A. A. Abdel-Rahman, *J. Heterocycl. Chem.* **2011**, *48*, 1028–1038. http://dx.doi.org/10.1002/jhet.686
13. E. R. Kotb, N. M. Yousif, M. A. M. Salama, M. M. Ali, H. S. Khalaf, *RJPBCS* **2015**, *6(1)*, 516–528.
14. A. A. Fayed, A. F. El-Farargy, N. M. Yousif, F. A. Gad, A. Kalmouch, *Der Pharma Chemica* **2015**, *7(2)*, 316–322.
15. A. E. Rashad, A. H. Shamroukh, M. A. El-Hashash, A. F. El-Farargy, N. M. Yousif, M. A. Salama, A. Mostafa, M. El-Shahat, *J. Heterocycl. Chem.* **2012**, *49*, 1130–1135. http://dx.doi.org/10.1002/jhet.966
16. I. W. Cheney, S. Yan, T. Appleby, H. Walker, T. Vo, N. Yao, R. Hamatake, Z. Hong, J. Z. Wu, *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1679–1683. http://dx.doi.org/10.1016/j.bmcl.2006.12.086
17. P. Gary, J.-W. Soh, Y. Mao, M.-G. Kim, R. Pamukcu, H. Li, W. J. Thompson, I. B. Weinstein, *Clin. Cancer Res.* **2000**, *6*, 4136–4141.
18. J. Cheng, J. P. Grande, *Exp. Biol. Med.* **2007**, *232*, 38–51.
19. Y.-L. Zhu, S.-L. Huang, Y.-J. Pan, *Eur. J. Org. Chem.* **2005**, 2354–2367. http://dx.doi.org/10.1002/ejoc.200400845
20. S. Rahmani, A. Amoozadeh, E. Kolvari, *Catalysis Communications* **2014**,, 184–188. http://dx.doi.org/10.1016/j.catcom.2014.07.002
21. A. Amoozadeh, S. Rahmani, F. Nemati, *Heterocycl. Commun.* **2013**, *19(1)*, 69–73. http://dx.doi.org/10.1515/hc-2012-0157
22. C. Mukhopadhyay, A. Datta, *)*, 438–449. http://dx.doi.org/10.1080/00397911.2011.602497
23. M. D. Wendt, C. Sun, A. Kunzer, D. Sauer, K. Sarris, E. Hoff, L. D. Yu, G. Nettesheim, J. Chen, S. Jin, *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3122–3129. http://dx.doi.org/10.1016/j.bmcl.2007.03.042
24. N. A. Aqui, R. H. Vonderheide, *Cancer Biol. Ther.* **2008**, *7*, 1888–1889. http://dx.doi.org/10.4161/cbt.7.12.7219
25. G. Ambrosini, C. Adida, D. C. Altieri, *Nat. Med.* **1997**, *3*, 917–921. http://dx.doi.org/10.1038/nm0897-917
26. S. M. Riyadh, *Molecules* **2011**, *16*, 1834–1853. http://dx.doi.org/10.3390/molecules16021834
27. M. Abbas, S. M. Riyadh, M. A. Abdallah, S. M. Gomha, *J. Heterocycl. Chem.,* **2006**, *43*, 935–942. http://dx.doi.org/10.1002/jhet.5570430419
28. S. M. Gomha, S. M. Riyadh, *Molecules* **2011**, *16*, 8244–8256. http://dx.doi.org/10.3390/molecules16108244
29. A. E. Rashad, A. H. Shamroukh, N. M. Yousif, M. A. Salama, H. S. Ali, M. M. Ali, A. E. Mahmoud, M. El-Shahat, *Arch. Pharm. Chem. Life Sci.* **2012**, *345*, 729–738. http://dx.doi.org/10.1002/ardp.201200119
30. E. Abdelghani, *Heterocycles* **2001**, *55(12)*, 2413–2421. http://dx.doi.org/10.3987/COM-01-9348
31. E. Abdelghani, *J. Chem. Res.* **1999**, *(S)*, 174-175; *(M)*, 1135–1150.
32. M. G. Assy, E. Abdelghani, *Pol. J. Chem.* **1995**, *69*, 685–687.
33. M. G. Assy, H. H. Sayed, A. H. Moustafa, M. N. Yousif, M. A. El-Hallim, *Phosphorus Sulfur* **2008**, *183*, 2318–2329. http://dx.doi.org/10.1080/10426500801963590
34. E. Abdelghani, S. A. Said, M. G. Assy, A. M. Abdel Hamid, *J. Iran. Chem. Soc.* **2015**, *12*, 1809–1817.
35. F. J. Smit, R. A. van Biljon, L.-M. Birkholtz, D. D. N'Da, *Eur. J. Med. Chem.* **2015**, 90, 33–44. http://dx.doi.org/10.1016/j.ejmech.2014.11.016
36. P. Kaswan, K. Pericherla, D. Purohit, A. Kumar, *Tetrahedron Lett*. **2015**, *56,* 549–553. http://dx.doi.org/10.1016/j.tetlet.2014.11.121
37. N. Y. Megally Abdo, *Acta Chim. Slov.* **2015**, *62,* 168–180. http://dx.doi.org/10.17344/acsi.2014.867
38. M. M. Kamel, *Acta Chim. Slov.* **2015**, *62,* 136–151.

http://dx.doi.org/10.17344/acsi.2014.828
39. L. Wang, G. Chen, X. Lu, S. Wang, S. Han, Y. Li, G. Ping, X. Jiang, H. Li, J. Yang, C. Wu, *Eur. J. Med. Chem.* **2015**, *89*, 88–97. http://dx.doi.org/10.1016/j.ejmech.2014.10.036
40. V. Kanagarajan, M. Gopalakrishnan, *Pharm. Chem. J.* **2011**, *45*(4), 248–256. http://dx.doi.org/10.1007/s11094-011-0611-1
41. E. Campaigne, *Comprehen. Het*er. *Chem.* **1984**, *4*, 863–984.
42. P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J. T. Warren, H. Bokesch, S. Kenny, M. R. Boyd, *J. Natl. Cancer Inst.* **1990**, *82*, 1107–1112. http://dx.doi.org/10.1093/jnci/82.13.1107
43. A. Monks, D. Scudiero, P. Skehan, R. Shoemaker, R. Storeng, K. Paul, D. Vistica, C. Hose, J. Langley, P. Cronise, A. Vaigro-Wolff, M. Gray-Goodr, H. Campbell, J. Mayo, J. M. Boyd, *J. Natl. Cancer Inst.* **1991**, *83*, 757–766. http://dx.doi.org/10.1093/jnci/83.11.757
44. M. M. Al-Arab, F. S. Al-Saleh, S. M. Mayoof, *J. Heterocycl. Chem.* **1998**, *35*, 1473–1476. http://dx.doi.org/10.1002/jhet.5570350643

Povzetek

Z »one-pot« reakcijo med ciklopentanonom, ustreznim aromatskimi aldehidom (*o*-anisaldehid) in različnimi sečninami (sečnina, gvanidin, tiosečnina) smo sintetizirali serijo pirimidinskih in tiazinskih derivatov. Pri cikloadiciji reagentov, ki vsebujejo aktivno metilensko skupino (acetil aceton, malononitril, etil cianoacetat, cianoacetamid in *N*-fenil cianoacetamid), z 2,6-bis(2-metoksibenziliden)cikloheksanonom pod bazičnimi pogoji nastanejo kromenski in kinolinski derivati. Za nekatere nove spojine smo preučili tudi njihove antitumorne lastnosti proti trem človeškim rakastim celičnim linijam, in sicer MCF-7, NCI-H460 in SF-268, ter ugotovili zmerno dobre aktivnosti glede na pozitivno kontrolo doksorubicin.