Nitrophenyl-group-containing Heterocycles. Part I. Synthesis, Characterization, Anticancer Activity and Antioxidant Properties of Some New 5,6,7,8-tetrahydro-isoquinolines Bearing 3(4)-nitrophenyl Group

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Abstract

Regioselective cyclocondensation of 2,4-diacetyl-5-hydroxy-5-methyl-3-(3-nitrophenyl/4-nitrophenyl)cyclohexanones **1a,b** with cyanothioacetamide afforded the corresponding 7-acetyl-4-cyano-1,6-dimethyl-6-hydroxy-8-(3-nitrophenyl/4-nitrophenyl)-5,6,7,8-tetrahydroisoquinoline-3(2*H*)-thiones **2a,b** in 93-96% yield. Reaction of compounds **2a,b** with some ethyl iodide, 2-chloroacetamide (**4a**) or its *N*-aryl derivatives **4b-e** by refluxing in ethanol, in the presence of slightly excess molar amounts of sodium acetate trihydrate, for one hour gave 3-ethylthio-5,6,7,8-tetrahydroisoquinoline **3** and (5,6,7,8-tetrahydroisoquinolin-3-ylthio)acetamides **5a-i**, respectively. On heating compounds **5b-d,f,g** in ethanol containing a catalytic amount of sodium carbonate, they converted into their isomeric 1-amino-6,7,8,9-tetrahydrothieno[2,3-*d*]isoquinoline-2-carboxamides **6b-d,f,g**. Structural formulae of all synthesized compounds were characterized on the basis of their elemental analyses and spectroscopic data. Also, the biological evaluation of the synthesized isoquinolines as anticancer and antioxidant agents have been carried out and the obtained results are reported herein.

Introduction

5,6,7,8-Tetrahydroisoquinoline ring system is a structural fragment of many alkaloids that are next to indole alkaloids in their abundance [1–4]. Compounds containing a 5,6,7,8-tetrahydroisoquinoline fragment are used as intermediate products in the synthesis of alkaloids [5–7], precursors to enzyme inhibitors [8, 9], fungicides [10, 11], potassium receptor antagonists [12], and drugs for the treatment of cardiovascular diseases, bronchial asthma, tumors, and viral infections [4, 13]. 5,6,7,8-tetrahydroisoquinoline derivatives were also shown to exhibit anticonvulsant [14–16], antibacterial [17], neurotropic [18] and antimicrobial activities [19].

On the other hand, many nitro-group-containing compounds are reported to possess versatile applications in the fields of biochemistry and medicine [20–23].

In view of the above observations, the current work was planned to synthesize and characterize of some new 5,6,7,8-tetrahydroisoquinolines and related 6,7,8,9-tetrahydrothieno[2,3-*d*]isoquinolines bearing 3-nitrophenyl or 4-nitrophenyl moiety with the hope that these new compounds will find good applications in both biological and medicinal fields owing to their incorporation of various pharmacophores. Also, the applications of some synthesized compounds as anticancer and as antioxidant agents have been carried out and the obtained results are reported herein.

Results And Discussions

Synthesis

Treatment of 1,3-dicarbonyl compounds **1a,b** with cyanothioacetamide in refluxing ethanol in the presence of piperidine as a basic catalyst resulted in regioselective cyclocondensation reaction affording

the corresponding, 7-acetyl-8-(3-nitrophenyl or 4-nitrophenyl)-4-cyano-1,6-dimethyl-6-hydroxy-5,6,7,8-tetrahydroisoquinoline-3(2*H*)-thiones **2a,b** in 93-96% yield (Scheme 1).

Reaction of compounds **2a,b** with some halocompounds namely; ethyl iodide, 2-chloroacetamide (**4a**) or *N*-aryl-2-chloroacetamide (**4b-e**) by refluxing in ethanol, in the presence of slightly excess molar amounts of sodium acetate trihydrate, for one hour gave 3-ethylthio-5,6,7,8-tetrahydroisoquinoline **3**, (5,6,7,8-tetrahydroisoquinolin-3-ylthio)acetamides **5a,e** and *N*-aryl-(5,6,7,8-tetrahydroisoquinolin-3-ylthio)acetamides **5b-d,f-i** respectively (Scheme 1).

On refluxing of compounds **5b-d,f,g** with catalytic amounts of anhydrous sodium carbonate in abs. ethanol for 2 hours, they converted into 7-acetyl-1-amino-*N*-aryl-5,8-dimethyl-8-hydroxy-6-(3-nitrophenyl/4-nitrophenyl)-6,7,8,9-tetrahydrothieno[2,3-*c*] isoquinoline-2-carboxamides **6b-d,f,g**. Compounds **6b-d,f,g** were also synthesized *via* heating compound **2a,b** with the respective *N*-aryl-2-chloroacetamides **4b-e** in abs. ethanol in the presence of slightly excess molar amounts of sodium carbonate (Scheme 1).

Cyclization of compounds **5b-d,f,g** into the corresponding 6,7,8,9-tetrahydrothieno[2,3-*c*]isoquinolines **6b-d,f,g** may obey intramolecular Thorpe-Ziegler cyclization which its mechanism is outlined before in our publication [24].

Characterization

All newly synthesized compounds were characterized on the basis of their elemental analyses spectroscopic data (*cf.* Experimental part). Thus, IR spectra of **2a,b** showed characteristic absorption bands in the regions 3482-3429 cm⁻¹ for (O-H), 3235- 3230 cm⁻¹ for (NH), 2221-2220 cm⁻¹ for (C≡N), and 1710-1708 cm⁻¹ for (C=O, acetyl). ¹H NMR spectra of **2a,b** are in agreement with those of their analogues which were reported before [25]. IR spectrum of **3** revealed the disappearance of NH whereas its ¹H NMR spectrum showed the presence of ethyl group. IR spectra of **5a,e** showed absorption bands in the regions 3481-3355 cm⁻¹ for (OH and NH₂), 2222-2215 cm⁻¹ for (C≡N), 1709-1701 cm⁻¹ for (C=O, acetyl) and 1662-1660 cm⁻¹ for (C=O, amide). ¹H NMR spectra of **5a,e** showed the presence of a double doublet signal corresponding to SCH₂ group at δ value around 3.85 and two singlet signals at δ value overlapped with that of aromatic protons corresponding to CONH₂ group [25]. IR spectra of **5b-d,f-i** showed absorption bands in the regions 3563-3456 cm⁻¹ for (OH), 3401-3289 cm⁻¹ for (NH), 2221-2213 cm⁻¹ for (C≡N), 1705-1683 cm⁻¹ for (C=O, acetyl) and 1687-1666 cm⁻¹ for (C=O, amide). ¹H NMR spectra of **5b-d,f-i** showed the presence of a double doublet signal corresponding to SCH₂ group at δ value around 4.00 and a singlet signal at δ value ranged from 10.12 to 10.57 equivalent to NH group. IR spectra of **6b-d,f,g** revealed the disappearance of the carbonitrile band and presence of four absorption bands in the region 3517-3314 cm⁻¹ characteristic for OH, NH₂ and NH groups beside other two bands in the regions 1705-1698 cm⁻¹ and 1651-1624 cm⁻¹ corresponding to acetyl group and amidic carbonyl group, respectively. ¹H NMR spectra of **6b-d,f,g** showed singlet signal at δ value ranged from 9.33-9.56 equivalent to NH group and a broad singlet signal referred to the amino group at δ value ranged from 7.05 to 7.13 instead of the signal of SCH₂ group which exists in the ¹H NMR spectra of **5b-d,f,g**. The presence of tertiary

alcoholic group in all compounds was ascertained from their ^1H NMR spectra which possess a singlet signal at δ value ranged from 4.84 to 5.05 equivalent to one proton of (OH) group. ^1H NMR spectra of all compounds displayed characteristic signals at certain δ values which are equivalent to the protons of cyclohexene ring and in accordance with those reported before for their analogues [25]. ^{13}C NMR spectra of compounds **5a,c,d,f,h** and **6b-d,f,g** displayed characteristic peaks at certain δ values which are in agreement with their structures.

Cytotoxic activity

The cytotoxic activity of compounds coded with **2a, 3, 5a, 5c, 5d, 5g, 5h** and **5i** against PACA2 (Pancreatic cancer cell line) and that of compounds coded with **5e, 5f, 5g, 6b, 6d, 6f, 6g** against A549 (Lung carcinoma cell line) has evaluated *in vitro* at different concentrations ranged from 0.78 to 100 μM using the MTT assay method. In this work, doxorubicin was used as a positive control drug for comparison purposes with the drug candidates **2a, 3, 5a, 5c, 5d, 5e, 5f, 5g, 5h, 5i, 6b, 6d, 6f** and **6g** under the same experimental conditions. Different concentrations of these compounds were tested to reach the concentration which could cause death for 50% of the cancer cells; IC_{50} value and the IC_{50} range of each compound was estimated and the relation between log concentration and the probit were plot as given in Figures 1 and 2. After the cells were exposed to the solutions of the compounds under test for an incubation time of 72 h, cytotoxic activity was determined and expressed as IC_{50} and IC_{50} ranges.

The results obtained (Tables 1 and 2) revealed that among all tested compounds: (i) four compounds **3, 5c, 5h** and **5i** showed mild to strong cytotoxic activity against PACA2 (Pancreatic cancer cell line) with IC_{50} of 53.5, 60.1, 25.9 and 73.4 μM respectively, (ii) only three compounds **6b, 6d** and **6g** which showed considerable cytotoxic activity against A549 (Lung carcinoma cell line) with IC_{50} of 34.9, 57.6 and 46.3 μM respectively, (iii) compounds **5h** and **6b** exhibit the highest activity and (iv) rest of the tested compounds being inactive against the two cell lines under investigation.

Table 1

Cytotoxic activity of compounds **2a, 3, 5a, 5c, 5d, 5g, 5h** and **5i** against PACA2 (Pancreatic cancer cell line) at concentration of 100 μM and their IC_{50} values.

95% Confidence Limits for conc.				95% Confidence Limits for log (conc.) ^a		
Compd. No.	Estimate $\text{IC}_{50}(\mu\text{M})$	Lower bound	Upper bound	Log conc(μM) at probability 0.5	Lower bound	Upper bound
3	53.5	48.349	59.677	1.728	1.684	1.776
5c	60.1	43.310	96.761	1.779	1.637	1.986
5h	25.9	21.724	31.121	1.414	1.337	1.493
5i	73.4	62.900	88.630	1.865	1.799	1.948

Table 2

Cytotoxic activity of compounds **5e**, **5f**, **5g**, **6b**, **6d**, **6f** and **6g** against A549 (Lung carcinoma cell line) at concentration of 100 μ M and their IC₅₀ values.

Compd. No.	95% Confidence Limits for conc.			95% Confidence Limits for log (conc.) ^a		
	Estimate IC ₅₀ (μ M)	Lower bound	Upper bound	Log conc(μ M) at probability 0.5	Lower bound	Upper bound
6b	34.9	30.782	39.855	1.543	1.488	1.600
6d	57.6	49.404	69.055	1.761	1.694	1.839
6g	46.3	40.490	53.765	1.666	1.607	1.731

Antioxidant activity

Fourteen compounds were evaluated for DPPH scavenging activity as a measurement of their antioxidant activity. Data are represented by Mean $\pm$ SD of 3 replicates. DPPH scavenging activity are represented as %. Table 3 declared variable percentage of inhibition of DPPH scavenging activity of the tested compounds in a dose-dependent relationship compared with vitamin C as a standard. The highest dose of synthesized compounds that is 0.10 μ g/mL represents the highest antioxidant activity of all compounds relative to vitamin C. The synthesized compounds **2a**, **2b**, **5a** and **6b** showed the highest antioxidant activity at concentration of 0.1 μ g/mL (dose-dependent manner) .

Table 3
DPPH Scavenging activity of isoquinioline derivatives.*

Compd. No.	Conc.	R1	R2	R1 Inhibition	R1 Inhibition	Mean	St.De (%)
2a	0.10	0.016	0.019	96.72	96.10	96.41	0.44 ^a
2a	0.05	0.264	0.269	45.82	44.80	45.31	0.73 ^b
2a	0.01	0.342	0.344	29.82	29.41	29.61	0.29 ^c
2b	0.10	0.018	0.017	96.31	96.51	96.41	0.15 ^a
2b	0.05	0.019	0.020	96.10	95.90	96.00	0.15 ^a
2b	0.01	0.028	0.027	94.25	94.46	94.36	0.15 ^a
3	0.10	0.163	0.166	66.55	65.93	66.24	0.44 ^d
3	0.05	0.198	0.202	59.37	58.55	58.96	0.58 ^e
3	0.01	0.249	0.253	48.90	48.08	48.49	0.58 ^b
5a	0.10	0.021	0.024	95.69	95.07	95.38	0.44 ^a
5a	0.05	0.023	0.021	95.28	95.69	95.49	0.29 ^a
5a	0.01	0.052	0.055	89.33	88.71	89.02	0.44 ^f
5c	0.10	0.127	0.130	73.94	73.32	73.63	0.44 ^g
5c	0.05	0.210	0.216	56.91	55.67	56.29	0.87 ^e
5c	0.01	0.280	0.297	42.54	39.05	40.80	2.47 ^b
5d	0.10	0.178	0.175	63.47	64.09	63.78	0.44 ^d
5d	0.05	0.269	0.272	44.80	44.18	44.49	0.44 ^b
5d	0.01	0.280	0.289	42.54	40.69	41.62	1.31 ^b
5e	0.10	0.078	0.080	83.99	83.58	83.79	0.29 ^f
5e	0.05	0.170	0.176	65.11	63.88	64.50	0.87 ^d
5e	0.01	0.266	0.269	45.41	44.80	45.11	0.44 ^b
5f	0.10	0.171	0.175	64.91	64.09	64.50	0.58 ^d

Table 3
Continued.

Compd. No.	Conc.	R1	R2	R1 Inhibition	R1 Inhibition	Mean	St.De (%)
5f	0.05	0.200	0.205	58.96	57.93	58.44	0.73 ^e
5f	0.01	0.241	0.245	50.54	49.72	50.13	0.58 ^b
5g	0.10	0.101	0.106	79.27	78.25	78.76	0.73 ^g
5g	0.05	0.173	0.177	64.50	63.68	64.09	0.58 ^d
5g	0.01	0.288	0.293	40.90	39.87	40.39	0.73 ^b
5h	0.10	0.187	0.191	61.63	60.80	61.21	0.58 ^d
5h	0.05	0.212	0.215	56.49	55.88	56.19	0.44 ^e
5h	0.01	0.226	0.229	53.62	53.01	53.31	0.44 ^e
6b	0.10	0.037	0.039	92.41	92.00	92.20	0.29 ^a
6b	0.05	0.042	0.045	91.38	90.77	91.07	0.44 ^a
6b	0.01	0.175	0.179	64.09	63.27	63.68	0.58 ^d
6f	0.10	0.172	0.174	64.70	64.29	64.50	0.29 ^d
6f	0.05	0.257	0.251	47.26	48.49	47.88	0.87 ^b
6f	0.01	0.298	0.292	38.85	40.08	39.46	0.87 ^b
6g	0.10	0.152	0.155	68.81	68.19	68.50	0.44 ^d
6g	0.05	0.188	0.191	61.42	60.80	61.11	0.44 ^d
6g	0.01	0.233	0.236	52.19	51.57	51.88	0.44 ^b

* Data are represented by Mean±SD of 3 replicats. DPPH scavenging activity represented as %. Statistical analysis is carried out using two way ANOVA coupled with CO-state computer program where similar letters are insignificant and different letters are significant at $P \leq 0.05$. Vitamin C standard, was used as positive control. DPPH scavenging activity was calculated as following: % Inhibition = $100 - [\text{Absorbance of the test compound} / \text{Absorbance of the control}] \times 100$

The DPPH scavenging activity of the most potent compounds **2a**, **2b**, **5a**, **6b** compared with that of Vitamine C in a dose dependent manner is given in Table 4. The DPPH scavenging activity of the latter compounds obeys the order **2b** > **5a** > **6b** > **2a** (Figure 3).

Table 4

DPPH scavenging activity of the potent isoquinoline compounds compared with ascorbic acid.*

Compound No.	% of inhibition at dose 10 micro of 0.1gm	% of inhibition at dose 10 micro of 0.05gm	% of inhibition at dose 10 micro of 0.01gm
Ascorbic acid	99.20±4.22 ^a	66.70±5.32 ^d	48.78±2.22 ^b
2a	96.41±0.44 ^a	45.31±0.73 ^b	29.31±0.29 ^c
2b	96.41±0.15 ^a	96.00±0.15 ^a	94.36±0.15 ^a
5a	95.38±0.44 ^a	95.49±0.29 ^a	89.02±0.44 ^a
6b	92.20±0.29 ^a %	91.07±0.44 ^a %	63.68±0.58 ^d %

* Data are represented by Mean±SD of 3 replicats. DPPH scavenging activity represented as %. Statistical analysis is carried out using two way ANOVA coupled with CO-state computer program where similar letters are insignificant and different letters are significant at $P \leq 0.05$. Vitamin C standard, was used as positive control. DPPH scavenging activity was calculated as following: % Inhibition = $100 - [\text{Absorbance of the test compound} / \text{Absorbance of the control}] \times 100$

Conclusions

In this paper, we have successfully synthesized 7-acetyl-4-cyano-1,6-dimethyl-6-hydroxy-8-(3-nitrophenyl/4-nitrophenyl)-5,6,7,8-tetrahydroisoquinoline-3(2*H*)-thiones **2a,b** in excellent yields *via* cyclocondensation reaction of 2,4-diacetyl-5-hydroxy-5-methyl-3-(3-nitrophenyl/4-nitrophenyl)cyclohexanones **1a,b** with cyanothioacetamide. Compounds **2a,b** were used as starting materials for synthesizing two new series of isoquinoline derivatives; 3-substituted thio-5,6,7,8-tetrahydroisoquinoline-4-carbonitriles **3** and **5a-i**, and related 1-amino-*N*-aryl-6,7,8,9-tetrahydrothieno[2,3-*c*]isoquinoline-2-carboxamides **6b-d,f,g**. Structural formulae of all new compounds were characterized on the basis of their elemental analyses and spectroscopic data. Some of the synthesized compounds showed good activity as anticancer agents and excellent activity as antioxidants.

Experimental Section

General

Melting points were determined on a Gallan-Kamp apparatus and are uncorrected. The IR spectra were recorded on a Shimadzu 470 IR-spectrophotometer (KBr; ν_{max} in cm^{-1}). The ^1H and ^{13}C NMR spectra were recorded on a Varian A5 500 MHz spectrometer using DMSO- d_6 (except for compounds **3** and **5a** in CDCl_3) as a solvent and tetramethylsilane (TMS) as internal reference. Coupling constants (J values) are given in Hertz (Hz). The purity of the obtained products is checked by TLC.

Reaction of 2-acetylcyclohexanones **1a,b** with cyanothioacetamide; Synthesis compounds **2a,b**; general method.

A mixture of compound **1a,b** (10 mmol), cyanothioacetamide (10 mmol) and piperidine (0.8 mL, 10 mmol) in ethanol (100 mL) was refluxed for 2 h. The yellow crystals that formed on hot were collected, washed with methanol, dried in air to give compounds **2a,b**. The purity of these products is 100% and needs no any purification.

7-Acetyl-4-cyano-1,6-dimethyl-6-hydroxy-8-(3-nitrophenyl)-5,6,7,8-tetra-hydroisoquinoline-3(2 H)-thione (2a). It was synthesized by reaction of **1a** with cyanothioacetamide. Yield: 96%; m. p: 279-280°C. IR: 3429 (O-H), 3235 (N-H); 3139 (C-H, sp^2); 2971 (C-H, sp^3); 2221 (C $\equiv$ N); 1710 (C=O). 1H NMR: δ 13.68 (s, 1H, NH); 7.95-8.05 (m, 2H, ArH); 7.51-7.58 (m, 2H, ArH); 5.05 (s, 1H, OH); 4.61-4.63 (d, $J=10$, 1H, C 8 H); 3.23-3.26 (d, $J=15$, 1H, C 5 H), 2.88-2.90 (d, $J=10$, 1H, C 7 H), 2.83-2.87 (d, $J=20$, 1H, C 5 H); 2.12 (s, 3H, COCH $_3$); 1.86 (s, 3H, CH $_3$); 1.23 (s, 3H, CH $_3$). Anal. calcd for C $_{20}$ H $_{19}$ N $_3$ O $_4$ S (397.11): C, 60.44; H, 4.82; N, 10.57%. Found: C, 60.67; H, 5.11; N, 10.28%.

7-Acetyl-4-cyano-1,6-dimethyl-6-hydroxy-8-(4-nitrophenyl)-5,6,7,8-tetra-hydroisoquinoline-3(2 H)-thione (2b). It was synthesized by reaction of **1b** with cyanothioacetamide. Yield: 93%; m. p 290-291°C. IR: 3482 (O-H); 3235 (NH); 3106 (C-H, sp^2); 2971, 2872 (C-H, sp^3); 2220 (C $\equiv$ N); 1708 (C=O). 1H NMR: δ 13.83 (s, H, NH), 7.84-7.86 (d, $J=10$, H, ArH); 7.62-7.64 (d, $J=10$, H, ArH); 7.51-7.53 (d, $J=10$, H, ArH); 7.33-7.34 (d, $J=5$, H, Ar), 5.04 (s, 1H, OH); 4.97-4.99 (d, $J=10$, 1H, C 8 H); 3.33-3.16 (d, $J=20$, 1H, C 5 H), 3.16-3.10 (d, $J=5$, 1H, C 7 H), 2.86-2.90 (d, $J=20$, 1H, C 5 H); 2.02 (s, 3H, COCH $_3$); 1.93 (s, 3H, CH $_3$); 1.29 (s, 3H, CH $_3$). Anal. calcd for C $_{20}$ H $_{19}$ N $_3$ O $_4$ S (397.11): C, 60.44; H, 4.82; N, 10.57%. Found: C, 60.32; H, 5.04; N, 10.33%.

Reaction of compounds **2a,b** with ethyl iodide, 2-chloroacetamide (**4a**) or its N-aryl-2-chloroacetamides **4b-f**; Synthesis of compounds **3** and **5a-j**; general method.

A mixture of **2a,b** (10 mmol), ethyl iodide, 2-chloroacetamide (**4a**) or N-aryl-2-chloroacetamides **4b-f** (10 mmol) and sodium acetate trihydrate (1.50 g, 11 mmol) in ethanol (100 mL) was refluxed for one hour. The solid that formed after cooling (50 mL) was collected and then recrystallized from ethanol to give white crystals of compounds **3** and **5a-i**.

7-Acetyl-4-cyano-1,6-dimethyl-3-ethylthio-6-hydroxy-8-(4-nitrophenyl)-5,6,7,8-tetrahydroisoquinoline (3): It was synthesized by reaction of **2b** with ethyl iodide. Yield: 83%; m.p.: 144-145°C. IR: 3509 (O-H); 3098 (C-H, sp^2); 2974, 2919 (C-H, sp^3); 2213 (C $\equiv$ N); 1698 (C=O), 1603 (C=N). 1H NMR: δ 8.13-8.15 (d, $J=10$, 2H, ArH), 7.35-7.37 (d, $J=10$, 2H, ArH), 4.99 (s, 1H, OH), 4.75-4.78 (d, $J=15$, 1H, C 8 H), 3.15-3.31 (m, 3H: C 5 H and SCH $_2$), 2.87-2.95 (m, 2H: C 7 H and C 5 H), 2.18 (s, 3H, COCH $_3$), 1.98 (s, 3H, CH $_3$), 1.31 (s, 3H, CH $_3$), 1.29 (t, 3H, CH $_3$). Anal. Calcd for C $_{22}$ H $_{23}$ N $_3$ O $_4$ S (425.14): C, 62.10; H, 5.45; N, 9.88%. Found: C, 62.37; H, 5.18; N, 10.01%.

2-[(7-Acetyl-4-cyano-1,6-dimethyl-6-hydroxy-8-(3-nitrophenyl)-5,6,7,8-tetrahydro-isoquinolin-3-yl)thio]acetamide (5a): It was synthesized by reaction of **2a** with 2-chloroacetamide (**4a**). Yield: 91%; m.p.: 174-175°C. IR: 3481, 3373 (O-H, NH₂); 2991, 2930 (C-H, sp³); 2215 (C≡N); 1701 (C=O, acetyl); 1660 (C=O, amide). ¹H NMR: δ 7.26-8.18 (m, 6H: NH₂ and ArH), 4.53-4.55 (d, *J* = 10.0, 1H, OH), 3.82- 3.97 (dd, *J* = 15.0, 2H: C⁷H and C⁸H), 3.02-3.21(m, 4H: SCH₂ and C⁵H₂), 1.96 (s, 3H, COCH₃), 1.87 (s, 3H, CH₃), 1.42 (s, 3H, CH₃). ¹³C NMR: δ 214.79, 175.43, 161.92, 160.77, 160.44, 160.11, 159.78, 158.14, 149.88, 149.66, 145.64, 134.84, 131.34, 129.23, 123.41, 122.78, 118.74, 116.47, 114.65, 114.20, 111.93, 106.45, 69.90, 64.12, 45.89, 42.55, 35.59, 33.60, 28.30, 25.83. Anal. Calcd. for C₂₂H₂₂N₄O₅S (454.13): C, 58.14; H, 4.88; N, 12.33%. Found: C, 58.00; H, 5.03; N, 11.98%.

2-[(7-Acetyl-4-cyano-1,6-dimethyl-6-hydroxy-8-(3-nitrophenyl)-5,6,7,8-tetrahydro-isoquinolin-3-yl)thio]- N -phenylacetamide (5b). It was synthesized by reaction of **2a** with *N*-phenyl-2-chloroacetamide (**4b**).Yield: 93%; m.p.: 191-192°C. IR: 3467 (O-H); 3335(N-H); 3063 (C-H, sp²); 2999, 2914 (C-H, sp³); 2214 (C≡N); 1702 (C=O, acetyl); 1687 (C=O, amide). ¹H NMR: δ 10.25 (s, 1H, NH), 8.06-8.08 (d, 1H, ArH), 7.94-7.95 (d, 1H, ArH), 7.51-7.56 (m, 4H, ArH), 7.24-7.28 (m, 2H, ArH), 7.00-7.04 (m, 1H, ArH), 5.00 (s, 1H, OH), 4.76-4.79 (d, *J* = 15, 1H, C⁸H), 4.08-4.18 (dd, *J* = 15, 2H, SCH₂), 3.45 (m, 1H, C⁵H), 2.93-2.97 (m, 2H: C⁷H and C⁵H), 2.19 (s, 3H, COCH₃), 1.91 (s, 3H, CH₃), 1.28 (s, 3H, CH₃). Anal. Calcd. for C₂₈H₂₆N₄O₅S (530.16): C, 63.38; H, 4.94; N, 10.56%. Found: C, 62.99; H, 5.12; N, 10.33%.

2-[(7-Acetyl-4-cyano-1,6-dimethyl-6-hydroxy-8-(3-nitrophenyl)-5,6,7,8-tetrahydro-isoquinolin-3-yl)thio]- N -(4-tolyl)acetamide (5c). It was synthesized by reaction of **2a** with *N*-(4-tolyl)-2-chloroacetamide (**4c**).Yield: 95%; m.p.: 187-188°C. IR: 3559 (O-H); 3317 (N-H); 3034 (C-H, sp²); 2973, 2924 (C-H, sp³); 2213 (C≡N); 1701 (C=O, acetyl); 1675 (C=O, amide). ¹H NMR: δ 10.12 (s, 1H, NH), 8.06-8.08 (d, 1H, ArH), 7.94-7.95 (m, 1H, ArH), 7.53-7.55 (m, 2H, Ar-H), 7.38-7.40 (d, 2H, ArH), 7.04-7.06 (d, 2H, ArH), 4.99 (s, 1H, OH), 4.76-4.78 (d, *J* = 10.0, 1H, C⁸H), 4.06-4.15 (dd, *J* = 15, 2H, SCH₂), 2.89-3.32 (m, 3H: C⁷H and C⁵H₂), 2.21 (s, 3H, CH₃ of 4-tolyl residue), 2.17 (s, 3H, COCH₃), 1.99 (s, 3H, CH₃), 1.28 (s, 3H, CH₃). ¹³C NMR: δ 208.74, 200.27, 181.20, 165.58, 160.36, 157.54, 150.02, 147.75, 145.84, 136.23, 134.97, 132.03, 130.00, 128.88, 128.47, 122.54, 121.56, 118.87, 114.90, 103.87, 67.23, 65.74, 55.86, 43.11, 42.28, 34.55, 30.84, 27.33, 24.51, 20.21, 18.36. Anal. Calcd. Anal. calcd for C₂₉H₂₈N₄O₅S (544.18): C, 63.95; H, 5.18; N, 10.29%. Found: C, 64.04; H, 4.92; N, 9.91%.

2-[(7-Acetyl-4-cyano-1,6-dimethyl-6-hydroxy-8-(3-nitrophenyl)-5,6,7,8-tetrahydro-isoquinolin-3-yl)thio]- N -(4-chlorophenyl)acetamide (5d): It was synthesized by reaction of **2a** with *N*-(chlorophenyl)-2-chloroacetamide (**4d**). Yield: 84%; m.p.: 205-206°C. IR: 3536 (O-H); 3289 (N-H); 3074 (C-H, sp²); 2973, 2924 (C-H, sp³); 2216 (C≡N); 1694 (C=O, acetyl); 1666 (C=O, amide). ¹H NMR: δ 10.37 (s, 1H, NH), 8.06 (d, 1H, ArH), 7.94 (s, 1H, ArH), 7.54-7.56 (m, 4H, ArH), 7.29-7.31 (d, *J* = 10, 2H, ArH), 4.99 (s, 1H, OH), 4.76-4.78 (d, *J* = 10, 1H C⁸H), 4.14-4.17 (dd, 2H, SCH₂), 3.30-3.32 (d, *J* = 10, 1H, C⁵H), 2.93-2.95 (m, 2H: C⁷H and C⁵H), 2.17 (s, 3H, COCH₃), 1.89 (s, 3H, CH₃), 1.28 (s, 3H, CH₃). ¹³C NMR: δ 204.15, 161.49, 155.77, 152.85, 145.47, 143.16, 141.23, 133.10, 130.38, 125.41, 123.93, 123.84, 122.10, 117.96, 116.97, 115.78, 110.30,

99.30, 62.66, 61.15, 38.53, 37.70, 30.01, 26.27, 22.75, 19.90. Anal. Calcd. for $C_{28}H_{25}ClN_4O_5S$ (564.12): C, 59.52; H, 4.46; N, 9.92%. Found: C, 59.31; H, 4.50; N, 10.13%.

2-[(7-Acetyl-4-cyano-1,6-dimethyl-6-hydroxy-8-(4-nitrophenyl)-5,6,7,8-tetrahydro-isoquinolin-3-yl)thio]acetamide (5e): It was synthesized by reaction of **2b** with 2-chloroacetamide (**4a**). Yield: 88%; m.p.: 178-179°C. IR: 3466, 3355 (O-H, NH_2); 2968, 2919 (C-H, sp^3); 2222 ($C\equiv N$); 1709 (C=O, acetyl); 1662 (C=O, amide). 1H NMR: δ 8.09-8.11 (d, J = 10.0, 2H, ArH), 7.54 (s, 1H, NH), 7.30-7.32 (dd, J = 5.0, 2H, ArH), 7.09 (s, 1H, NH), 5.00 (s, 1H, OH), 4.70-4.72 (d, J = 10.0, 1H, C^8H), 3.81-3.89 (dd, J = 15.0, 2H, SCH_2), 3.25-3.28 (d, J = 15.0, 1H, C^5H), 2.88-2.90 (d, J = 10.0, 1H, C^7H), 2.83-2.87 (d, J = 20.0, 1H, C^5H), 2.23 (s, 3H, $COCH_3$), 1.91 (s, 3H, CH_3), 1.24 (s, 3H, CH_3). Anal. calcd for $C_{22}H_{22}N_4O_5S$ (454.13): C, 58.14; H, 4.88; N, 12.33%. Found: C, 57.92; H, 4.59; N, 12.52%.

2-[(7-Acetyl-4-cyano-1,6-dimethyl-6-hydroxy-8-(4-nitrophenyl)-5,6,7,8-tetrahydro-isoquinolin-3-yl)thio]-N-phenylacetamide (5f): It was synthesized by reaction of **2b** with *N*-phenyl-2-chloroacetamide (**4b**). Yield: 92%; m.p.: 137-138°C. IR: 3525 (O-H); 3322 (N-H); 3061 (C-H, sp^2); 2994, 2935 (C-H, sp^3); 2217 ($C\equiv N$); 1702 (C=O, acetyl); 1687 (C=O, amide). 1H NMR: δ 10.21 (s, 1H, NH); 8.09-8.11 (d, J = 10.0, 2H, ArH); 7.50-7.52 (d, J = 10, 2H, ArH); 7.32-7.33 (d, J = 5, 2H, ArH); 7.23-7.24 (d, J = 5, 2H, ArH); 6.98-7.01 (t, J = 5, 1H, ArH); 4.98 (s, 1H, OH); 4.73-4.75 (d, J = 10, 1H, C^8H); 4.08-4.16 (dd, 2H, SCH_2); 3.28-3.30 (d, J = 10, 1H, C^5H), 2.89-2.94 (m, 2H: C^7H and C^5H); 2.16 (s, 3H, $COCH_3$); 1.89 (s, 3H, CH_3); 1.28 (s, 3H, CH_3). ^{13}C NMR: δ 208.52, 166.01, 160.51, 157.73, 151.77, 150.03, 146.07, 138.89, 129.52, 128.67, 123.78, 123.26, 119.05, 115.01, 104.03, 78.72, 67.41, 65.71, 56.02, 42.70, 34.74, 31.07, 27.48, 24.50, 18.50. Anal. Calcd. for $C_{28}H_{26}N_4O_5S$ (530.16): C, 63.38; H, 4.94; N, 10.56%. Found: C, 62.99; H, 5.09; N, 10.53%.

2-[(7-Acetyl-4-cyano-1,6-dimethyl-6-hydroxy-8-(4-nitrophenyl)-5,6,7,8-tetrahydro-isoquinolin-3-yl)thio]-N-(4-tolyl)acetamide (5g): It was synthesized by reaction of **2b** with *N*-(4-tolyl)-2-chloroacetamide (**4c**). Yield: 90%; m.p.: 234-235°C. IR: 3456 (O-H); 3297 (N-H); 3107 (C-H, sp^2); 2970 (C-H, sp^3); 2218 ($C\equiv N$); 1702 (C=O, acetyl); 1682 (C=O, amide). 1H NMR: δ 10.12 (s, 1H, NH), 8.07-8.09 (d, J = 10, 2H, ArH), 7.28-7.36 (m, 4H, ArH), 7.01-7.04 (d, 2H, ArH), 4.99 (s, 1H, OH), 4.68-4.71 (d, 1H, C^8H), 4.05-4.07 (m, 2H, SCH_2), 3.25-3.32 (d, 1H, C^5H), 2.86-2.88 (m, 2H: C^7H and C^5H), 2.18 (s, 3H, CH_3 of 4-tolyl residue), 2.13 (s, 3H, $COCH_3$), 1.84 (s, 3H, CH_3), 1.24 (s, 3H, CH_3). Anal. Calcd. for $C_{29}H_{28}N_4O_5S$ (544.62): C, 63.95; H, 5.18; N, 10.29%. Found: C, 64.08; H, 4.91; N, 9.93%.

2-[(7-Acetyl-4-cyano-1,6-dimethyl-6-hydroxy-8-(4-nitrophenyl)-5,6,7,8-tetrahydro-isoquinolin-3-yl)thio]-N-(4-chlorophenyl)acetamide (5h): It was synthesized by reaction of **2b** with *N*-(4-chlorophenyl)-2-chloroacetamide (**4d**). Yield: 94%; m.p.: 144-145°C. IR: 3563 (O-H), 3344 (N-H); 3134 (C-H, sp^2); 2971, 2937 (C-H, sp^3); 2221 ($C\equiv N$); 1705 (C=O, acetyl); 1681 (C=O, amide). 1H NMR: δ 10.35 (s, 1H, NH), 8.08-8.11 (m, 2H, ArH), 7.60-7.62 (d, 2H, ArH), 7.29-7.54 (m, 4H, ArH), 4.98 (s, 1H, OH), 4.71-4.73 (d, J = 10, 1H, CH at C-8), 4.06-4.14 (dd, J = 15.0, 2H, SCH_2), 3.42-3.44 (d, J = 7.0, 1H, C^5H), 2.90-2.92 (m, 2H: C^7H and C^5H), 2.15 (s, 3H, $COCH_3$), 1.85 (s, 3H, CH_3), 1.27 (s, 3H, CH_3). ^{13}C NMR: δ 208.53, 166.23, 164.75, 160.47,

157.63, 151.75, 150.04, 146.07, 137.85, 129.52, 128.73, 128.60, 126.83, 123.77, 120.90, 120.53, 114.98, 103.98, 67.39, 65.71, 55.99, 43.21, 42.65, 34.72, 31.02, 27.46, 24.45, 18.50. Anal. Calcd. for $C_{28}H_{25}ClN_4O_5S$ (564.12): C, 59.52; H, 4.46; N, 9.92%. Found: C, 59.20; H, 4.67; N, 10.07%.

2-[(7-Acetyl-4-cyano-1,6-dimethyl-6-hydroxy-8-(4-nitrophenyl)-5,6,7,8-tetrahydro-isoquinolin-3-yl)thio]-N-(4-acetylphenyl)acetamide (5i): It was synthesized by reaction of **2a** with *N*-(4-acetylphenyl)-2-chloroacetamide (**4e**). Yield: 86%; m.p.: 193-194°C. IR: 3540 (O-H); 3337(N-H); 3109 (C-H, sp^2); 2968 (C-H, sp^3); 2220 ($C\equiv N$); 1683 (3 C=O); 1595 (C=N). 1H NMR: δ 10.57 (s, 1H, NH), 8.06-8.11 (d, 2H, ArH), 7.84-7.86 (d, 2H, ArH), 7.62-7.65 (d, 2H, ArH), 7.28-7.31 (d, 2H, ArH), 5.02 (s, 1H, OH), 4.76-4.78 (d, 1H, C^8H), 4.36-4.38 (d, 1H, C^5H), 4.11-4.13 (dd, 2H, SCH_2), 2.88-2.91 (m, 2H: C^7H and C^5H), 2.12 (s, 3H, $COCH_3$), 1.80 (s, 3H, $COCH_3$), 1.23 (s, 3H, CH_3 attached to pyridine ring), 1.03 (s, 3H, CH_3). Anal. Calcd. for $C_{30}H_{28}N_4O_6S$ (572.17): C, 62.92; H, 4.93; N, 9.78%. Found: C, 63.00; H, 4.85; N, 10.06%.

7-Acetyl-1-amino-2-(N-arylcarbamoyl)-5,8-dimethyl-8-hydroxy-6-(3-nitrophenyl or 4-nitrophenyl)-6,7,8,9-tetrahydrothieno[2,3-c]isoquinolines 6b-d,f,g; general methods.

Method A)

To a suspension of **5b-d,f,g** (10 mmol) in abs. ethanol (60 mL), anhydrous sodium carbonate (0.30 g) was added. The reaction mixture was refluxed for 3 hours. The yellow crystals that formed while hot were collected, washed with water, dried in air and then recrystallized from dioxane to give **6b-d,f,g**, respectively.

7-Acetyl-1-amino-5,8-dimethyl-8-hydroxy-6-(3-nitrophenyl)-N-phenyl-6,7,8,9-tetrahydrothieno[2,3-c]isoquinoline-2-carboxamide (6b).

Yield: 87%; m.p.: 287-288°C. IR: 3415, 3388, 3314 (O-H, NH_2 , N-H); 2914 (C-H, sp^3); 1703 (C=O, acetyl); 1622 (C=O, amide). 1H NMR: δ 9.43 (s, 1H, NH); 7.31-7.84 (m, 9H, ArH); 7.09 (s, 2H, NH_2); 4.86-4.88 (d, $J=10$, 1H, C^6H); 4.84 (s, 1H, OH); 3.64-3.67 (d, $J=15$, 1H, C^9H), 3.41-3.44 (d, $J=15$, 1H, C^7H); 2.92-2.94 (d, $J=10$, 1H, C^9H); 2.21 (s, 3H, $COCH_3$); 2.03 (s, 3H, CH_3); 1.33 (s, 3H, CH_3). ^{13}C NMR: δ 209.44, 164.31, 158.22, 156.58, 149.38, 147.92, 147.07, 142.88, 138.83, 135.08, 130.11, 128.36, 128.24, 123.45, 123.02, 122.40, 121.51, 121.26, 97.03, 67.14, 65.90, 42.90, 41.98, 31.17, 27.94, 24.74. Anal. Calcd. for $C_{28}H_{26}N_4O_5S$ (530.16): C, 63.38; H, 4.94; N, 10.56%. Found: C, 62.99; H, 5.12; N, 10.46%.

7-Acetyl-1-amino-5,8-dimethyl-8-hydroxy-6-(3-nitrophenyl)-N-(4-tolyl)-6,7,8,9-tetrahydrothieno[2,3-c]isoquinoline-2-carboxamide (6c). Yield: 92%; m.p.: 291-292°C. IR: 3418, 3386, 3313 (O-H, NH_2 , N-H); 3075 (C-H, sp^2); 2914 (C-H, sp^3); 1706 (C=O, acetyl); 1624 (C=O, amide). 1H NMR: δ 9.35 (s, 1H, NH); 7.06-8.08 (d, $J=10$, 1H, ArH); 7.84 (s, 1H, ArH); 7.53-7.58 (m, 4H, ArH); 7.12-7.14 (d, 2H, $J=10$, ArH); 7.07 (s, 2H, NH_2); 4.86-4.88 (d, $J=10$, 1H, C^6H); 4.84 (s, 1H, OH); 3.64-3.67 (d, $J=15$, 1H, C^9H), 3.41-3.45 (d, $J=20$, 1H, C^7H); 2.93-2.95 (d, $J=10$, 1H, C^9H); 2.28 (s, 3H, CH_3 of 4-tolyl residue); 2.21 (s, 3H, $COCH_3$); 2.03 (s, 3H, CH_3); 1.33 (s, 3H, CH_3). ^{13}C NMR: δ 209.44, 164.19, 158.12, 156.53, 149.19, 147.92, 147.08, 142.83, 136.25, 135.07, 132.42, 130.11, 128.77, 128.21, 123.08, 122.40, 121.51, 121.31, 97.20, 67.15, 65.90, 42.91, 41.97,

31.18, 27.95, 24.73, 20.46. Anal. Calcd. for $C_{29}H_{28}N_4O_5S$ (544.18): C, 63.95; H, 5.18; N, 10.29%. Found: C, 64.14; H, 4.92; N, 9.93%.

7-Acetyl-1-amino- N -(4-chlorophenyl)-5,8-dimethyl-8-hydroxy-6-(3-nitrophenyl)-6,7,8,9-tetrahydrothieno[2,3- c]isoquinoline-2-carboxamide (6d). It was obtained by cyclization of compound **8c**. Yield: 94%; m.p.: 293-294°C. IR: 3417, 3383, 3314 (O-H, NH_2 , N-H); 3095 (C-H, sp^2); 2967, 2916 (C-H, sp^3); 1706 (C=O, acetyl); 1622 (C=O, amide). 1H NMR: δ 9.56 (s, 1H, NH); 7.36-8.08 (m, 8H, ArH); 7.13 (s, 2H, NH_2); 4.86-4.88 (d, J =10, 1H, C^6H); 4.85 (s, 1H, OH); 3.64-3.67 (d, J =15, 1H, C^9H), 3.40-3.44 (d, J =20, 1H, C^7H); 2.93-2.95 (d, J =10, 1H, C^9H); 2.21 (s, 3H, $COCH_3$); 2.04 (s, 3H, CH_3); 1.33 (s, 3H, CH_3). ^{13}C NMR: δ 209.42, 164.35, 158.33, 156.65, 149.62, 147.92, 147.04, 142.94, 135.07, 130.10, 128.27, 128.23, 126.96, 122.95, 122.65, 122.41, 121.51, 96.81, 67.14, 65.88, 42.8, 41.99, 31.17, 27.94, 24.74. Anal. Calcd. for $C_{28}H_{25}ClN_4O_5S$ (564.12): C, 59.52; H, 4.46; N, 9.92%. Found: C, 59.69; H, 4.41; N, 10.16%.

7-Acetyl-1-amino-5,8-dimethyl-8-hydroxy-6-(4-nitrophenyl)-N-phenyl-6,7,8,9-tetrahydrothieno[2,3- c]isoquinoline-2-carboxamide (6f). Yield: 91%; m.p.: 285-286°C. IR: 3406, 3320 (O-H, NH_2 , N-H); 2921 (C-H, sp^3); 1703 (C=O, acetyl); 1622 (C=O, amide). 1H NMR: δ 9.41 (s, 1H, NH); 8.11-8.13 (d, J =10, 2H, ArH); 7.67-7.69 (d, J =10, 2H, ArH); 7.28-7.33 (m, 5H, ArH); 7.08 (s, 2H, NH_2); 4.84 (s, 1H, OH); 4.82-4.84 (d, J =10, 1H, C^6H); 3.59-3.63 (d, J =20, 1H, C^9H), 3.40-3.43 (d, J =15, 1H, C^7H); 2.87-2.89 (d, J =10, 1H, C^9H); 2.19 (s, 3H, $COCH_3$); 2.00 (s, 3H, CH_3); 1.32 (s, 3H, CH_3). ^{13}C NMR: δ 209.25, 164.33, 158.17, 156.61, 152.92, 149.35, 145.94, 142.71, 138.84, 129.40, 128.37, 128.22, 123.80, 123.46, 123.02, 121.26, 97.03, 67.14, 65.73, 43.19, 41.96, 31.19, 27.92, 24.61. Anal. Calcd. for $C_{28}H_{26}N_4O_5S$ (530.16): C, 63.38; H, 4.94; N, 10.56%. Found: C, 62.98; H, 5.01; N, 10.62%.

7-Acetyl-1-amino-5,8-dimethyl-8-hydroxy-6-(4-nitrophenyl)- N -(4-tolyl)-6,7,8,9-tetrahydrothieno[2,3- c]isoquinoline-2-carboxamide (6g). Yield: 92%; m.p.: 292- 293°C. IR: 3400, 3322 (O-H, NH_2 , N-H); 2919 (C-H, sp^3); 1701 (C=O, acetyl); 1623 (C=O, amide). 1H NMR: δ 9.33 (s, 1H, NH); 8.11-8.13 (d, J =10, 2H, Ar-H); 7.55-7.57 (d, J =10, 2H, ArH); 7.27-7.29 (d, J =10, 2H, ArH); 7.11-7.13 (d, J =10, 2H, ArH); 7.05 (s, 2H, NH_2); 4.84 (br s, 1H, OH); 4.82-4.84 (d, J =10, 1H, C^6H); 3.59-3.62 (d, J =15, 1H, C^9H), 3.40-3.44 (d, J = 20, 1H, C^7H); 2.86-2.89 (d, J =15, 1H, C^9H); 2.27 (s, 3H, CH_3 of 4-tolyl residue); 2.19 (s, 3H, $COCH_3$); 2.01 (s, 3H, CH_3); 1.32 (s, 3H, CH_3). ^{13}C NMR: δ 209.25, 164.19, 158.06, 156.55, 152.92, 149.14, 145.76, 142.65, 136.26, 129.38, 128.77, 128.17, 123.79, 123.06, 121.30, 97.19, 67.13, 65.74, 43.18, 41.94, 31.18, 27.92, 24.59, 20.44. Anal. Calcd. for $C_{29}H_{28}N_4O_5S$ (544.18): C, 63.95; H, 5.18; N, 10.29%. Found: C, 64.13; H, 4.92; N, 9.99%.

Method B).

To mixture of **2a,b** (10 mmol) and respective *N*-aryl-2-chloroacetamide **4b-d** (10 mmol) in ethanol (60 mL), anhydrous sodium carbonate (1.30 g) was added. The resulting mixture was refluxed for 3 hours. The

solid that formed while hot was collected, washed with water, dried in air and then recrystallized from dioxane to give compounds **6b-d,f,g**; yield: 80-86%.

Cytotoxic activity

The cytotoxicity activity of the some synthesized compounds was determined according to the MTT method [26–28].

The pancreatic (PACA2) and human cancer lung (A549) cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and 1% GlutaMAX. Then the cells were seeded into sterile 96-well plates at a density of 10×10^3 cells/well and maintained at 37°C for 24 h. Cancerous cells were exposed to compounds at concentrations of 0.75, 1.75, 3.125, 6.250, 12.500, 25, 50, and 100 μ M for 72 h. The media was removing and add 40 μ L MTT stock solution to each well. The resulting solutions were incubated for more than 4 h. Subsequently, then add 120 μ L of 10% SDS as solubilising reagent. SPSS Software program was used to calculate the IC₅₀ and IC₅₀ ranges.

Antioxidant activity

DPPH has been used for measurement of free radical scavenging ability of antioxidants. Reduction of an alcoholic DPPH solution [29–31] in the presence of a hydrogen-donating antioxidant is the mainly step of this method. Hydrogen atom or electron-donation ability of the tested compounds were measured spectrophotometrically from the bleaching of the purple-colored ethanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH). In this study, antioxidant activity of the tested compounds was measured using the stable radical 2,2- diphenyl-1-picrylhydrazyl (DPPH). The free radical scavenging capacity of the tested compounds was determined using DPPH. A solution 1: prepared by dissolving DPPH (0.002 gm) in ethanol (50 mL ethanol). Solution 2: prepared by dissolving different weights 0.1, 0.05, 0.01 grams of each sample in 1mL of DMSO then take 10 μ L of each sample solution with 1mL ethanol. Then mix 1mL of solution 1 with 1mL of solution 2 and the resulting mixture was vortexed thoroughly and left in the dark for about 30 min. The absorbance of the mixture was spectrophotometrically measured at $\lambda_{\text{max}} = 517$ nm against blank 1mL absolute ethanol and compared to the ascorbic acid (Vitamin C). DPPH radical scavenging activity (% RSA) of compounds was calculated from the absorbance at the start (0) and after some reaction time (T) according to the equation (1).

$$(\% \text{ RSA}) = (\text{ABS}-\text{ATS}) / \text{ABS} \times 100 \text{ (1)}$$

Where ABS is the absorbance of blank sample (DPPH) solution without the compound to be tested and ATS is the absorbance of tested sample.

Declarations

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References

1. Kisel VM, Kostyrko E O, Kovtunenkov VA. Synthesis and biological properties of isoquinolines spirofused with carbocycles and heterocycles at position 4. *Chem. Heterocycl. Compd.*, 2002; 38: 295-1318.
2. Potikha LM, Kovtunenkov VA. Synthesis and properties of 3-aminodihydro-isoquinolines. *Russ. Chem. Rev.* 2009; 78: 513-533. <http://dx.doi.org/10.1070/RC2009v078n06ABEH004041>
3. Buske A, Busemann S, Muhlbacher J, Schmidt J, Porzel A, Bringmann G, Adam G. Antidesmone, a novel type isoquinoline alkaloid from *Antidesma membranaceum* (Euphorbiaceae). *Tetrahedron*, 1999; 55: 1079-1086. [https://doi.org/10.1016/S0040-4020\(98\)01107-7](https://doi.org/10.1016/S0040-4020(98)01107-7)
4. Dyachenko I V, Dyachenko VD. Cycloalka[c]pyridine Derivatives. *Methods of Synthesis and Chemical Properties*. *Russ. J. Org. Chem.* 2017; 53: 1769-1787.
5. Liou J-P, Cheng C.-Y. Total synthesis of (±)-desoxycodine-D: A novel route to the morphine skeleton. *Tetrahedron Lett.* 2000; 41: 915-918. [https://doi.org/10.1016/S0040-4039\(99\)02188-7](https://doi.org/10.1016/S0040-4039(99)02188-7)
6. Lipinska T. Microwave-induced solid-supported Fischer indolization, a key step in the total synthesis of the sempervirine type methoxy analogues. *Tetrahedron Lett.* 2004; 45: 8831-8834.
7. Vernier J-M, Holsenback H, Cosford N D P, Whitten J P, Menzaghi F, Reid R, Rao, T S, Sacca, A I, Lloyd G K, Suto S M, Chavez-Noriega L E, Washburn M S, Urrutia A and McDonald I A, Conformationally restricted analogues of nicotine and anabasine. *Bioorg. Med. Chem. Lett.*, 1998; 8: 2173-2178.
8. Peukert S, Schwahn U, Gusstegen S, Schreuder H and Hofmeister A. Poly(ADP-Ribose) Polymerase-1 (PARP-1) Inhibitors Based on a Tetrahydro-1(2H)-isoquinolinone Scaffold: Synthesis, Biological Evaluation and X-ray Crystal Structure. *Synthesis*, 2005; 1550-1554.
9. Wu, S C, Yoon, D, Chin J, van Kirk K, Seethala R, Golla R, He B, Harrity T, Kunselman L K, Morgan N N, Ponticello R P, Taylor J R, Zebo R, Harper TW, Li W, Wang M, Zhang L, Sleczonek BG, Nayeem A, Sheriff S, Camac DM, Mozin PE, Everlof JG, Li, Y-X, Ferraro CA, Kieltyka, K, Shon W, Vath M B, Zvyaga T A, Gordon DA and Robl JA. Discovery of 3-hydroxy-4-cyanoisoquinolines as novel, potent, and selective inhibitors of human 11b-hydroxydehydrogenase 1 (11b-HSD1). *Bioorg. Med. Chem. Lett.* 2011; 21: 6693-6698.
10. Al-Omran F, Elassar A-Z A and El-Khair A A. Synthesis of condensed heteroaromatics: novel synthesis of aminoquinolinone derivatives as anti-HIV agents. *Tetrahedron* 2001; 57: 10163-10170. [https://doi.org/10.1016/S0040-4020\(01\)01039-0](https://doi.org/10.1016/S0040-4020(01)01039-0)
11. Hunt J C A, Briggs E, Clarke E D and Whittingham W G. Synthesis and SAR studies of novel antifungal 1,2,3-triazines. *Bioorg. Med. Chem. Lett.* 2007; 17: 5222-5226. <https://doi.org/10.1016/j.bmcl.2007.06.076>

12. Hsin L-W, Chang L-T, Rothman RB, Dersch, CM, Fishback JA and Matsumoto RR. Synthesis and Opioid Activity of Enantiomeric N-Substituted 2,3,4,4a,5,6,7, 7a-Octahydro-1H-benzofuro[3,2-e]isoquinolines. *J. Med. Chem.* 2010; 53: 1392-1396. <https://doi.org/10.1021/jm901503e>
13. Chen L, Stefanac T, Turcotte N, Hu Z, Chen Y, Bedard J, May S and Jin H. Design and evaluation of dihydroisoquinolines as potent and orally bioavailable human cytomegalovirus inhibitors. *Med. Chem. Lett.* 2000; 10: 1477-1480. [https://doi.org/10.1016/S0960-894X\(00\)00265-1](https://doi.org/10.1016/S0960-894X(00)00265-1)
14. Sirakanyan SN, Akopyan EK, Paronikyan RG, Akopyan AG and Ovakimyan A A. Synthesis and anticonvulsant activity of 7(8)-amino derivatives of condensed thieno[3,2-d]pyrimidines. *Pharm. Chem. J.* 2016; 50: 296-300. <https://doi.org/10.1007/s11094-016-1439-5>
15. Paronikyan EG, Noravyan AS, Akopyan Sh F, Dzhagatspanyan IA, Nazaryan IM, and Paronikyan RG. Synthesis and anticonvulsant activity of pyrano[4,3:4,5]pyrido[2,3-b]thieno[3,2-d]pyrimidine derivatives and pyrimido[5,4:2,3]thieno[2,3-c]isoquinoline derivatives. *Pharm. Chem. J.* 2007; 41: 466-469.
16. Paronikyan, EG, Sirakanyan SN, Noravyan AS, Paronikyan RG and Dzhagatspanyan IA. Synthesis and anticonvulsant activity of pyrazolo[3,4-b]pyrano (thiopyrano)[4,3-d]pyridine and pyrazolo[3,4-c]isoquinoline derivatives. *Pharm. Chem. J.* 2001; 35: 8-10.
17. Paronikyan EG, Akopyan Sh F, Noravyan A S, Gaioosh G, Dashyan ShSh, Paronikyan RV, and Stepanyan GM. Synthesis and antibacterial activity of N- amino-derivatives of condensed pyridines. *Pharm. Chem. J.* 2013; 47: 257-260.
18. Paronikyan EG, Dashyan Sh Sh, Noravyan A S, Dzhagatspanyan IA, Paronikyan RG , Nazaryan I M and Akopyan, AG Synthesis and neurotropic activity of amino derivatives of cyclopenta[4,5]pyrido[3,2:4,5]thieno[3,2-d] pyrimidines and pyrimido [4,5:4,5]thieno[2,3-c]isoquinolines. *Pharm. Chem. J.* 2016; 50:301-305. <https://doi.org/10.1007/s11094-016-1440-z>
19. Kamal AM , Radwan SM and Zaki RM. Synthesis and biological activity of pyrazolothienotetrahydroisoquinoline and [1,2,4]triazolo[3,4-a]thienotetrahydro-isoquinoline derivatives. *Eur. J. Med. Chem.* 2011;46:567-578. <https://doi.org/10.1016/j.ejmech.2010.11.036>
20. Ju, KS and Parales RE. Nitroaromatic compounds, from synthesis to biodegradation. *Microbiol. Mol. Biol. Rev.* 2010; 74: 250-272.
21. Noboru O. The Nitro Group in Organic Synthesis; Wiley VCH: Weinheim, Germany, 2001; pp 1-363.
22. Strauss, M. The nitroaromatic group in drug design. *Pharmacology (for nonpharmacologists). Ind. Eng. Chem. Prod. Res. Dev.* 1979; 18: 158-166.
23. Nepali K, Lee H-Y, Liou J-P. Nitro-Group-Containing Drugs. *J. Med. Chem.* 2019; 62: 2851-2893. <https://doi.org/10.1021/acs.jmedchem.8b00147>
24. El-Ossaily Y A , Al-Taifi, EA, Bakhite, EA, Marae IS and Zaki R M. Synthesis and characterization of new quinazolinylmethylsulfanylpyridines, quinazolinylthieno[2,3-b]pyridines and pyrido[3",2":4',5']thieno[3',2':4,5]pyrimido [6,1-b]quinazolines. *Arkivoc* 2019; part vi: 446-458.

25. Ozols AI, Pelcher YE, Kalme ZA, Popelis YY, Turovskis IV and Duburs GY. Synthesis and chemical properties of 8-aryl-7-acyl-1,6-dimethyl-6-hydroxy-4-cyano-5,6,7,8-tetrahydro-3(2H)-isoquinolinones and isoquinolinethiones. *Chem. Heterocycl. Compd.* 1996; 32: 52–58.
26. Akkoç S, Kayser V, İlhan I O. Synthesis and In Vitro Anticancer Evaluation of Some Benzimidazolium Salts. *J. heterocycl. Chem.* 2019; 56: 2934-2944.
27. Akkoç S, İlhan IO, Gök Y, Upadhyay PJ, Kayser VJ. *In vitro* cytotoxic activities of new silver and PEPPSI palladium *N*-heterocyclic carbene complexes derived from benzimidazolium salts. *Inorg. Chim. Acta.* 2016; 449: 75-81
28. Akkoç S, Kayser V, İlhan IO, Hibbs D E, Gök Y, Williams PA, Hawkins B and Lai FJ. New compounds based on a benzimidazole nucleus: synthesis, characterization and cytotoxic activity against breast and colon cancer cell lines. *J. Organomet. Chem.* 2017; 839: 98-107.
29. M. Fatiha, T. Abdelkader, Study of antioxidant activity of pyrimidinium betaines by DPPH radical scavenging method, *Journal of Analytical & Pharmaceutical Research*, 2019; 8(2): 33–36.
30. Salem M S, Guirguis D B, El-Helw EAE, Ghareeb MA, Derbala, HAY. Antioxidant Activity of Heterocyclic Compounds Derived from 4-(4-Acetamidophenyl)-4-oxobut-2-enoic Acid. *Intern. J. Sci. & Res.* 2014; 3: 1274-1282.
31. Wilhelm EA, Ferreira ATA, Pinz MP, dosReis AAS, Vogt AG, Stein , Zeni G and Luchese C, Antioxidant effect of quinoline derivatives containing or not selenium: Relationship with antinociceptive action quinolines are antioxidant and antinociceptive, *J. Anais da Academia Brasileira de Ciências.* 2017; 89:457-467.

Scheme

Scheme 1 is available in the Supplemental Files section.

Figures

Figure 1

Cytotoxic activity of different concentrations of compounds **3**, **5c**, **5h** and **5i** against PACA2

Figure 2

Cytotoxic activity of different concentrations of compounds **6b**, **6d** and **6g** against A549.

Figure 3

Antioxident activity of compounds **2a**, **2b**, **5a**, **6b** and ascorbic acid as a standard.

Supplementary Files

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