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Nitrophenyl-Group-Containing Heterocycles. I. Synthesis, Characterization, Crystal Structure, Anticancer Activity, and Antioxidant Properties of Some New 5,6,7,8-Tetrahydroisoquinolines Bearing 3(4)-Nitrophenyl Group

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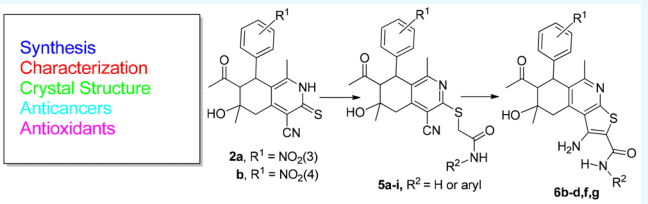
ABSTRACT: Regioselective cyclocondensation of 2,4-diacetyl-5-hydroxy-5-methyl-3-(3-nitrophenyl/4-nitrophenyl)cyclohexanones **1a,b** with cyanothioacetamide afforded the corresponding 7-acetyl-4-cyano-1,6-dimethyl-6-hydroxy-8-(3- and -4-nitrophenyl)-5,6,7,8-tetrahydrosoquinoline-3(2*H*)-thiones **2a,b**. Reaction of compounds **2a,b** with ethyl iodide, 2-chloroacetamide (**4a**), or its *N*-aryl derivatives **4b−e** in the presence of sodium acetate trihydrate gave 3-ethylthio-5,6,7,8-tetrahydroisoquinoline **3** and (5,6,7,8-tetrahydroisoquinolin-3-ylthio)acetamides **5a−i**, respectively. Cyclization of compounds **5b−d,f,g** into their isomeric 1-amino-6,7,8,9-tetrahydrothieno[2,3-*c*]isoquinoline-2-carboxamides **6b−d,f,g** was achieved by heating in ethanol containing a catalytic amount of sodium carbonate. Structures of all synthesized compounds were characterized on the basis of their elemental analyses and spectroscopic data. The crystal structure of 5,6,7,8-tetrahydroisoquinoline **5d** was determined by X-ray diffraction analysis. In addition, the biological evaluation of some synthesized compounds as anticancer agents was performed, and only six compounds showed moderate to strong activity against PACA2 (pancreatic cancer cell line) and A549 (lung carcinoma cell line). Moreover, the antioxidant properties of most synthesized compounds were examined. The results revealed high antioxidant activity for the most tested compounds.



1. INTRODUCTION

The 5,6,7,8-tetrahydroisoquinoline ring system is a structural fragment of many alkaloids that are next to indole alkaloids in 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 have also been 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-tetrahyroisoquinolines and related 6,7,8,9-tetrahyrothieno[2,3-*c*]isoquinolines bearing a 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. The crystal structure of 2-[(7-acetyl-4-cyano-1,6-dimethyl-6-hydroxy-8-(3-nitrophenyl)-5,6,7,8-tetrahydroisoquinolin-3-yl)thio]-*N*-(4-chlorophenyl)acetamide (**5d**) was determined by X-ray diffraction analysis. In addition, the applications of the synthesized compounds as anticancer and/or as antioxidant agents have been carried out, and the obtained results are reported herein.

2. RESULTS AND DISCUSSION

2.1. 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 a regioselective cyclocondensation reaction affording the corresponding 7-acetyl-8-(3- and -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). The pathway of this reaction is similar to that reported before.[24−27]

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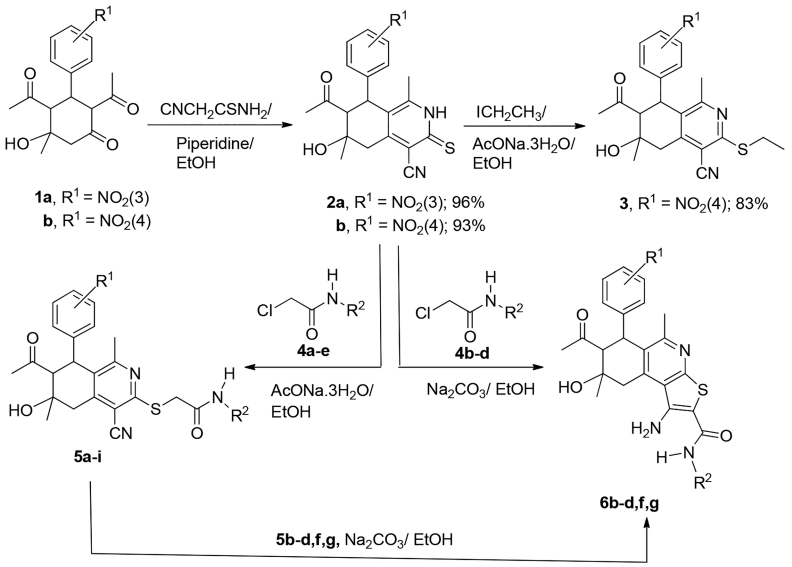
Scheme 1. Synthesis of Compounds 2a,b, 3, 5a−i, and 6b−d,f,g



4	R^2
a	H
b	Ph
c	$C_6H_4Me(4)$
d	$C_6H_4Cl(4)$
e	$C_6H_4COMe(4)$

5	R^1	R^2
a	$NO_2(3)$	H; 91%
b	$NO_2(3)$	Ph; 93%
c	$NO_2(3)$	$C_6H_4Me(4)$; 95%
d	$NO_2(3)$	$C_6H_4Cl(4)$; 84%
e	$NO_2(4)$	H; 88%
f	$NO_2(4)$	Ph; 92%
g	$NO_2(4)$	$C_6H_4Me(4)$; 90%
h	$NO_2(4)$	$C_6H_4Cl(4)$; 94%
i	$NO_2(4)$	$C_6H_4COMe(4)$; 86%

6	R^1	R^2
b	$NO_2(3)$	Ph; 87%
c	$NO_2(3)$	$C_6H_4Me(4)$; 92%
d	$NO_2(3)$	$C_6H_4Cl(4)$; 94%
f	$NO_2(4)$	Ph; 91%
g	$NO_2(4)$	$C_6H_4Me(4)$; 92%

Reaction of compounds **2a,b** with some halo compounds, namely ethyl iodide, 2-chloroacetamide (**4a**), or *N*-aryl-2-chloroacetamide (**4b−e**), in refluxing in ethanol in the presence of slightly excess molar amounts of sodium acetate trihydrate for 1 h 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).

Cyclization of compounds **5b−d,f,g** into the corresponding 7-acetyl-1-amino-*N*-aryl-5,8-dimethyl-8-hydroxy-6-(3- and -4-nitrophenyl)-6,7,8,9-tetrahydrothieno[2,3-*c*]isoquinoline-2-carboxamides **6b−d,f,g** was achieved by heating with catalytic amounts of anhydrous sodium carbonate in absolute ethanol for 3 h. Compounds **6b−d,f,g** were also synthesized by heating compounds **2a,b** with the respective *N*-aryl-2-chloroacetamides **4b−d** in absolute ethanol in the presence of slightly excess molar amounts of sodium carbonate (Scheme 1). Conversion of **5b−d,f,g** into the corresponding **6b−d,f,g** may obey intramolecular Thorpe−Ziegler cyclization, whose mechanism is outlined before in our publication.[28]

2.2. Characterization. All newly synthesized compounds were characterized on the basis of their elemental analyses and spectroscopic data (cf. Experimental Section). Thus, the 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 previously.[27] The IR spectrum of **3** revealed the disappearance of ν_{NH}, whereas its ^1H NMR spectrum showed the presence of an ethyl group. The IR spectra of **5a,e** showed absorption bands in the regions 3481−3355 cm^{-1} for (OH and NH_2), 2222−2215 cm^{-1} for (C≡N), 1709−1701 cm^{-1} for (C═O, acetyl), and 1662−1660 cm^{-1} for (C═O, amide). The ^1H NMR spectra of **5a,e** showed the presence of a double doublet signal corresponding to an SCH_2 group with a δ value around 3.85 and two singlet signals overlapped with those of aromatic protons corresponding to the $CONH_2$ group.[27] The 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). The ^1H NMR spectra of **5b−d,f−i** showed the presence of a double doublet signal corresponding to the SCH_2 group at a δ value around 4.00 and a singlet signal at a δ range from 10.12 to 10.57 equivalent to an NH group. IR spectra of **6b−d,f,g** revealed the disappearance of the carbonitrile band and the presence of four absorption bands in the region 3517−3314 cm^{-1} characteristic for OH, NH_2, and NH groups in addition to two other bands in the regions 1705−1698 and 1651−1624 cm^{-1} corresponding to an acetyl group and an amidic carbonyl group, respectively. ^1H NMR spectra of **6b−d,f,g** showed a singlet signal at δ values ranging from 9.33 to 9.56 for the NH group and a broad singlet signal for the amino group at δ value ranging from 7.05 to 7.13 instead of the signal of the SCH_2 group, which exists in the ^1H NMR spectra of **5b−d,f,g**. The

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presence of a tertiary alcoholic group in all compounds was ascertained from their ^{1}H NMR spectra which possess a singlet signal at δ values ranging from 4.84 to 5.05 for one proton of the OH group. The ^{1}H NMR spectra of all compounds displayed characteristic signals at certain δ values tha tare equivalent to the protons of cyclohexene ring and in accordance with those reported before for their analogues.[27] ^{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 (cf. Experimental Section).

From a stereochemistry point of view, the structure of starting compounds **2a,b** and all products generated thereof contains three consecutive stereogenic centers, and hence, four diastereoisomers are possible for each compound. Additionally the α-carbonyl stereogenic center is base-labile. From the single-crystal X-ray data of compound **5d** in the current paper and those of other reported related compounds,[24−26,29−34] it is apparent that the *cis,trans−cis* isomer crystallized: aryl, acetyl, and hydroxy are *cis*/*trans*/*cis* with a hydrogen bonding between acetyl and hydroxy. Only one diastereoisomer is isolated as a reaction product during the course of the current investigation and previously reported ones.[24−26,29−34] All reactions of starting compounds **2a,b** which take place far away form their three consecutive stereogenic centers resulted in no epimerization processes.[24−26,29−34]

2.3. Crystal Structure of 5d. The details of data collection, structure solution, and refinement are given in Table S1, while metrical parameters are listed in Tables S2 and S3. The molecule adopts an approximate chair conformation in which the tetrahydroisoquinoline moiety forms the seat, the 4-nitrophenyl and 4-chlorophenylacetamide substituents are the back legs, and the acetyl and hydroxyl groups are the front legs (Figure 1). The orientation of the acetyl group is determined by the intramolecular O2−H2···O1 hydrogen bond (Figure 1). The conformation of the tetrahydroisoquinoline moiety is such that the heterocyclic ring is not planar, and a puckering analysis[35−38] of this ring gave the parameters Q = 0.0911(11) Å, θ = 82.6(7)°, and φ = 104.6(7)°. The analysis of the C2···C7 ring gave the parameters Q = 0.5289(12) Å, θ = 54.50(13)°, and φ = 161.49(16)°. The nitro group is essentially coplanar with the C11···C16 ring as indicated by the O4−N4−C15−C14 torsion angle of 179.27(14)°. In the crystal, the *c*-glide plane generates chains of molecules parallel to the *c*-axis direction through N3−H3···O3 hydrogen bonds (Table S3) which are linked in pairs through C21−H21B···O2 hydrogen bonds (Figure 2). The double chains are connected by C27−H27···O4 hydrogen bonds into layers parallel to the *ac* plane (Figure 3).

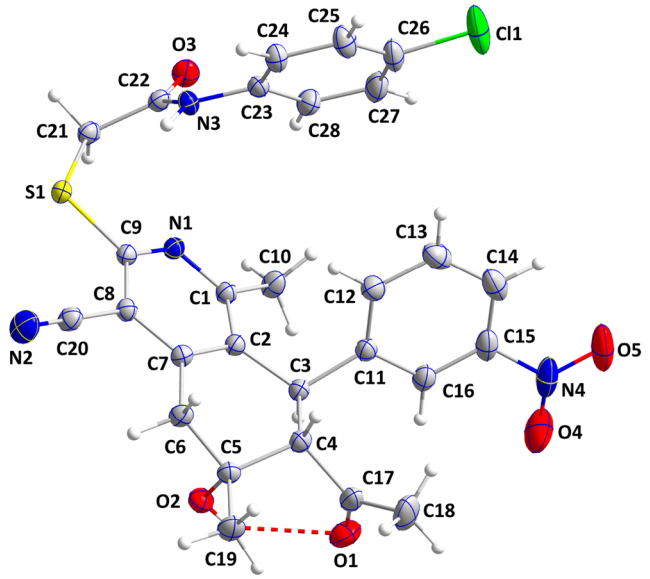

Figure 1. Perspective view of **5d** with labeling scheme and 50% probability ellipsoids. The intramolecular O2−H2···O1 hydrogen bond is depicted by a dashed line.

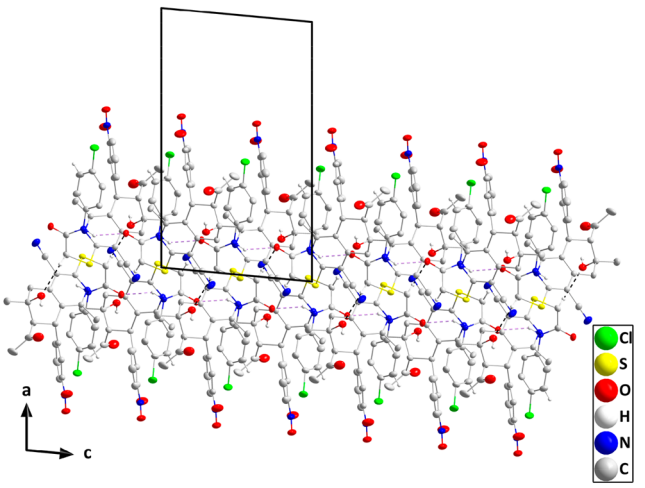

Figure 2. Portion of one double chain in **5d** viewed along the *b*-axis direction with N−H···O and C−H···O hydrogen bonds depicted, respectively, by violet and black dashed lines. Noninteracting hydrogen atoms are omitted for clarity.

2.4. Cytotoxic Activity. The cytotoxic activity of compounds **2a**, **3**, and **5a−d,g,h,I** against PACA2 (pancreatic cancer cell line) and that of compounds **5e−g**, **6b,d,f,g** against A549 (lung carcinoma cell line) has been evaluated *in vitro* at different concentrations ranging from 0.78 to 100 μM using the MTT assay method. In this work, doxorubicin was used as

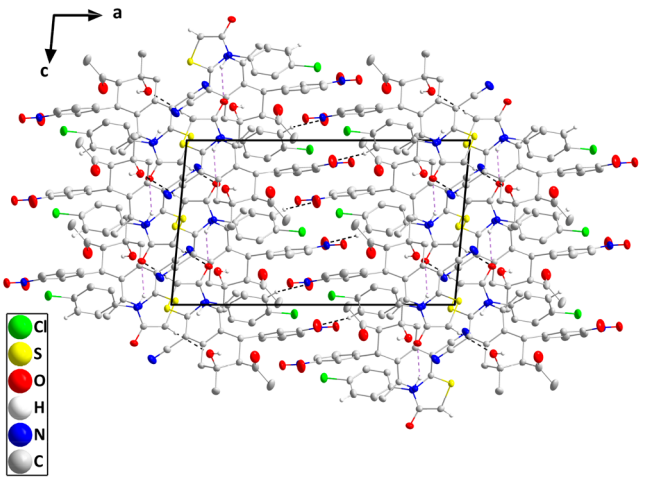

Figure 3. Packing of **5d** viewed along the *b*-axis direction with N−H···O and C−H···O hydrogen bonds depicted, respectively, by violet and black dashed lines. Noninteracting hydrogen atoms are omitted for clarity.

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a positive control drug for comparison purposes with the drug candidates **2a**, **3**, **5a**,**c**−**i**, and **6b**,**d**,**f**,**g** 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; the 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 4 and 5.

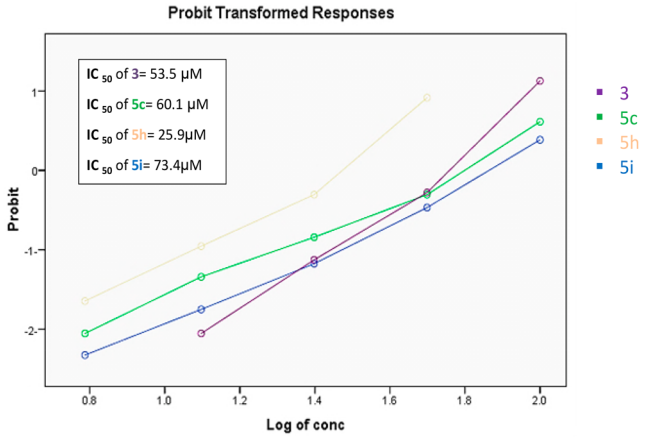

Figure 4. Cytotoxic activity of different concentrations of compounds **3**, **5c**, **5h**, and **5i** against PACA2.

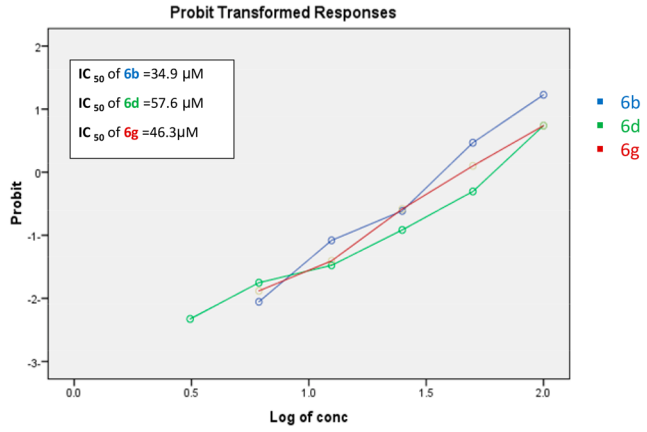

Figure 5. Cytotoxic activity of different concentrations of compounds **6b**, **6d**, and **6g** against A549.

The results obtained (Tables 1 and 2 and Figures 4 and 5) 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** were more active than doxorubicin against PACA2 and A549, respectively; (iv) the cytotoxic activity against PACA2 (pancreatic cancer cell line) obeys the order **5h** > **3** > **5c** > **5i**, (v) the cytotoxic activity against A549 (lung carcinoma cell line) obeys the order **6b** > **6g** > **6d**, and (vi) rest of the tested compounds being inactive against the two cell lines under investigation.

2.5. Antioxidant Activity. Fourteen compounds were evaluated for DPPH scavenging activity as a measurement of their antioxidant activity. Data are represented by mean ± SD of three replicates. DPPH scavenging activity is represented as percent Table 3 declared a variable percentage of inhibition of DPPH scavenging activity of the tested compounds in a dose-dependent relationship compared with ascorbic acid as a standard. The highest dose of synthesized compounds that is 0.10 μg/mL represents the highest antioxidant activity of all compounds relative to ascorbic acid. The synthesized compounds **2a**, **2b**, **5a**, and **6b** showed the highest antioxidant activity at a concentration of 0.1 μg/mL (dose-dependent manner). The DPPH-scavenging activity of the latter compounds at different concentrations compared with that of ascorbic acid obeys the order: ascorbic acid > **2b** > **5a** > **6b** > **2a** (Figure 6).

3. CONCLUSIONS

In this paper, we have successfully synthesized 7-acetyl-4-cyano-1,6-dimethyl-6-hydroxy-8-(3- and -4-nitrophenyl)-5,6,7,8-tetrahydrosoquinoline-3(2*H*)-thiones **2a**,**b** in excellent yields via cyclocondensation reaction of 2,4-diacetyl-5-hydroxy-5-methyl-3-(3- and -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**. Structures of all new compounds were characterized on the basis of their elemental analyses and spectroscopic data. The crystal structure of compound **5d** was determined by X−ray diffraction analysis. Some of the synthesized compounds showed good activity as anticancer agents, and most of them showed excellent activity as antioxidants.

4. EXPERIMENTAL SECTION

4.1. Instrumentation. 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 ^{1}H and ^{13}C NMR spectra were

Table 1. Cytotoxic Activity of Compounds 3 and 5c,h,I against PACA2 (Pancreatic Cancer Cell Line) at a Concentration of 100 μM and Their IC_{50} Values

	95% confidence limits for conc			95% confidence limits for log (conc)		
compd no.	estimated IC_{50}(μM)	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
doxorubicin	69.2	56.800	88.300	1.840	1.750	1.940

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Table 2. Cytotoxic Activity of Compounds 6b, 6d, and 6g against A549 (Lung Carcinoma Cell Line) at a Concentration of 100 μM and Their IC_{50}values

	95% confidence limits for conc			95% confidence limits for log (conc)		
compd no.	estimate IC_{50}(μM)	lower bound	upper bound	log conc (μM) 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
doxorubicin	54.8	41.600	77.100	1.730	1.610	1.880

Table 3. DPPH Scavenging Activity of Isoqunioline Derivatives[a]

compd. no.	conc (μg/mL)	mean ± SD (%)	compd no.	conc (μg/mL)	mean ± SD (%)
2a	0.10	96.41 ± 0.44	**5f**	0.10	64.50 ± 0.58
2a	0.05	45.31 ± 0.73	**5f**	0.05	58.44 ± 0.73
2a	0.01	29.61 ± 0.29	**5f**	0.01	50.13 ± 0.58
2b	0.10	96.41 ± 0.15	**5g**	0.10	78.76 ± 0.73
2b	0.05	96.00 ± 0.15	**5g**	0.05	64.09 ± 0.58
2b	0.01	94.36 ± 0.15	**5g**	0.01	40.39 ± 0.73
3	0.10	66.24 ± 0.44	**5h**	0.10	61.21 ± 0.58
3	0.05	58.96 ± 0.58	**5h**	0.05	56.19 ± 0.44
3	0.01	48.49 ± 0.58	**5h**	0.01	53.31 ± 0.44
5a	0.10	95.38 ± 0.44	**6b**	0.10	92.20 ± 0.29
5a	0.05	95.49 ± 0.29	**6b**	0.05	91.07 ± 0.44
5a	0.01	89.02 ± 0.44	**6b**	0.01	63.68 ± 0.58
5c	0.10	73.63 ± 0.44	**6f**	0.10	64.50 ± 0.29
5c	0.05	56.29 ± 0.87	**6f**	0.05	47.88 ± 0.87
5c	0.01	40.80 ± 2.47	**6f**	0.01	39.46 ± 0.87
5d	0.10	63.78 ± 0.44	**6g**	0.10	68.50 ± 0.44
5d	0.05	44.49 ± 0.44	**6g**	0.05	61.11 ± 0.44
5d	0.01	41.62 ± 1.31	**6g**	0.01	51.88 ± 0.44
5e	0.10	83.79 ± 0.29	ascorbic acid	0.10	99.20 ± 4.22
5e	0.05	64.50 ± 0.87	ascorbic acid	0.05	66.70 ± 5.32
5e	0.01	45.11 ± 0.44	ascorbic acid	0.01	48.78 ± 2.22

[a]Data are represented by Mean ± St.De (%) of 3 replicats. DPPH scavenging activity (%) = 100−[Absorbance of the test compound/Absorbance of the control] × 100. Statistical analysis is carried out using two way ANOVA coupled with CO-state computer program.

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.

4.2. Reaction of 2-Acetylcyclohexanones 1a,b with Cyanothioacetamide: Synthesis of 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 were collected, washed with methanol, and dried in air to give compounds **2a,b**. The purity of these products is 100% and needs no any purification.

*4.2.1. 7-Acetyl-4-cyano-1,6-dimethyl-6-hydroxy-8-(3-nitrophenyl)-5,6,7,8-tetrahydroisoquinoline-3(2H)-thione (**2a**).* Compound **2a** was synthesized by reaction of **1a** with cyanothioacetamide. Yield: 96%. Mp: 279−280 °C. IR: 3429 (O−H); 3235 (N−H); 3139 (C−H, sp^2); 2971 (C−H, sp^3); 2221 (C≡N); 1710 (C═O). ^{1}H 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^8H); 3.23−3.26 (d, J = 15, 1H, C^5H), 2.88−2.90 (d, J = 10, 1H, C^7H), 2.83−2.87 (d, J = 20, 1H, C^5H); 2.12 (s, 3H, $COCH_3$); 1.86 (s, 3H, CH_3); 1.23 (s, 3H, CH_3). Anal. Calcd for $C_{20}H_{19}N_3O_4S$ (397.11): C, 60.44; H, 4.82; N, 10.57. Found: C, 60.67; H, 5.11; N, 10.28.

*4.2.2. 7-Acetyl-4-cyano-1,6-dimethyl-6-hydroxy-8-(4-nitrophenyl)-5,6,7,8-tetrahydroisoquinoline-3(2H)-thione (**2b**).* Compound **2b** was synthesized by reaction of **1b** with cyanothioacetamide. Yield: 93%. Mp: 290−291 °C. IR: 3482 (O−H); 3235 (NH); 3106 (C−H, sp^2); 2971, 2872 (C−H, sp^3); 2220 (C≡N); 1708 (C═O). ^{1}H 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^8H); 3.10−3.16 (dd, 2H: C^7H and C^5H), 2.86−2.90 (d, J = 20, 1H, C^5H); 2.02 (s, 3H, $COCH_3$); 1.93 (s, 3H, CH_3); 1.29 (s, 3H, CH_3). Anal. Calcd for $C_{20}H_{19}N_3O_4S$ (397.11): C, 60.44; H, 4.82; N, 10.57. Found: C, 60.32; H, 5.04; N, 10.33.

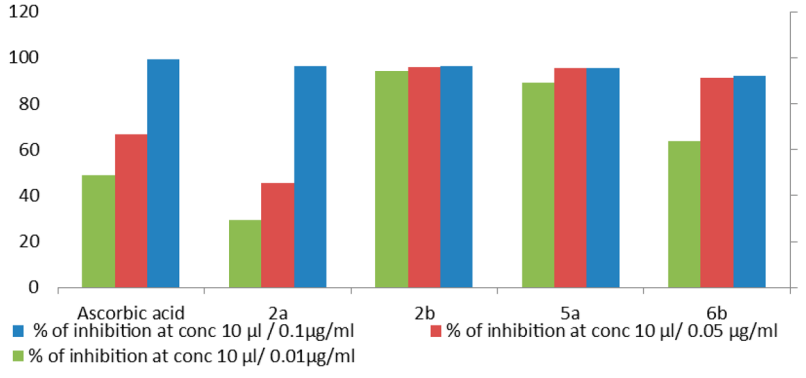

Figure 6. Antioxident activity of compounds **2a**, **2b**, **5a**, and **6b** and ascorbic acid as a standard.

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4.3. Reaction of Compounds 2a,b with Ethyl Iodide, 2-Chloroacetamide (4a), or Its *N*-Aryl-2-chloroacetamides 4b−e: 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−e** (10 mmol) and sodium acetate trihydrate (1.50 g, 11 mmol) in ethanol (100 mL) was refluxed for 1 h. The solid that formed after cooling was collected and then recrystallized from ethanol to give yellowish white crystals of compounds **3** and **5a−i**.

*4.3.1. 7-Acetyl-4-cyano-1,6-dimethyl-3-ethylthio-6-hydroxy-8-(4-nitrophenyl)-5,6,7,8-tetrahydroisoquinoline (**3**).* Compound **3** was synthesized by reaction of **2b** with ethyl iodide. Yield: 83%. Mp: 144−145 °C. IR: 3509 (O−H); 3098 (C−H, sp^2); 2974, 2919 (C−H, sp^3); 2213 (C≡N); 1698 (C═O), 1603(C = N). ^{1}H 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^8H), 3.15−3.31 (m, 3H: C^5H and SCH_2), 2.87−2.95 (m, 2H: C^7H and C^5H), 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 $C_{22}H_{23}N_3O_4S$ (425.14): C, 62.10; H, 5.45; N, 9.88. Found: C, 62.37; H, 5.18; N, 10.01.

*4.3.2. 2-[(7-Acetyl-4-cyano-1,6-dimethyl-6-hydroxy-8-(3-nitrophenyl)-5,6,7,8-tetrahydroisoquinolin-3-yl)thio]-acetamide (**5a**).* Compound **5a** was synthesized by reaction of **2a** with 2-chloroacetamide (**4a**). Yield: 91%. Mp: 174−175 °C. IR: 3481, 3373 (O−H, NH_2); 2991, 2930 (C−H, sp^3); 2215 (C≡N); 1701 (C═O, acetyl); 1660 (C═O, amide). ^{1}H NMR: δ 8.16−8.18 (d, 1H, ArH), 7.79 (s, 1H, ArH), 7.56−7.58 (d, 3H: NH and ArH), 7.10 (s, 1H, NH), 4.53−4.55 (d, J = 10, 1H, OH), 3.82- 3.97 (dd, J = 15, 2H: C^8H and C^5H), 3.02−3.21(m, 4H: SCH_2 and C^7H and C^5H), 1.96 (s, 3H, $COCH_3$), 1.87 (s, 3H, CH_3), 1.42 (s, 3H, CH_3). ^{13}C NMR: δ 214.79, 175.43, 161.92, 160.11, 158.14, 149.66, 145.64, 134.84, 131.34, 129.23, 123.41, 122.78, 118.74, 116.47, 114.65, 106.45, 69.90, 64.12, 45.89, 42.55, 35.59, 33.60, 28.30, 25.83. Anal. Calcd for $C_{22}H_{22}N_4O_5S$ (454.13): C, 58.14; H, 4.88; N, 12.33. Found: C, 58.00; H, 5.03; N, 11.98.

*4.3.3. 2-[(7-Acetyl-4-cyano-1,6-dimethyl-6-hydroxy-8-(3-nitrophenyl)-5,6,7,8-tetrahydroisoquinolin-3-yl)thio]-N-phenylacetamide (**5b**).* Compound **5b** was synthesized by reaction of **2a** with *N*-phenyl-2-chloroacetamide (**4b**).Yield: 93%. Mp: 191−192 °C. IR: 3467 (O−H); 3335 (N−H); 3063 (C−H, sp^2); 2999, 2914 (C−H, sp^3); 2214 (C≡N); 1702 (C═O, acetyl); 1687 (C═O, amide). ^{1}H NMR: δ 10.25 (s, 1H, NH), 8.06−8.08 (d, J = 10, 1H, ArH), 7.94−7.95 (d, J = 5, 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, C^8H), 4.08−4.18 (dd, J = 15, 2H, SCH_2), 3.45 (m, 1H, C^5H), 2.93−2.97 (m, 2H: C^7H and C^5H), 2.19 (s, 3H, $COCH_3$), 1.91 (s, 3H, CH_3), 1.28 (s, 3H, CH_3). 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, 10.33%.

*4.3.4. 2-[(7-Acetyl-4-cyano-1,6-dimethyl-6-hydroxy-8-(3-nitrophenyl)-5,6,7,8-tetrahydroisoquinolin-3-yl)thio]-N-(4-tolyl)acetamide (**5c**).* Compound **5c** was synthesized by reaction of **2a** with *N*-(4-tolyl)-2-chloroacetamide (**4c**).Yield: 95%. Mp: 187−188 °C. IR: 3559 (O−H); 3317 (N−H); 3034 (C−H, sp^2); 2973, 2924 (C−H, sp^3); 2213 (C≡N); 1701 (C═O, acetyl); 1675 (C═O, amide). ^{1}H NMR: δ 10.12 (s, 1H, NH), 8.06−8.08 (d, J = 10, 1H, ArH), 7.94−7.95 (m, 1H, ArH), 7.53−7.55 (m, 2H, Ar−H), 7.38−7.40 (d, J = 10, 2H, ArH), 7.04−7.06 (d, J = 10, 2H, ArH), 4.99 (s, 1H, OH), 4.76−4.78 (d, J = 10, 1H, C^8H), 4.06−4.15 (dd, J = 15, 2H, SCH_2), 2.89−3.32 (m, 3H: C^7H and C^5H_2), 2.21 (s, 3H, CH_3 of 4-tolyl residue), 2.17 (s, 3H, $COCH_3$), 1.99 (s, 3H, CH_3), 1.28 (s, 3H, CH_3). ^{13}C 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, 122.54, 121.56, 118.87, 114.90, 103.87, 67.23, 65.74, 43.11, 42.28, 34.55, 30.84, 27.33, 24.51, 20.21. Anal. Calcd Anal. Calcd for $C_{29}H_{28}N_4O_5S$ (544.18): C, 63.95; H, 5.18; N, 10.29. Found: C, 64.04; H, 4.92; N, 9.91.

*4.3.5. 2-[(7-Acetyl-4-cyano-1,6-dimethyl-6-hydroxy-8-(3-nitrophenyl)-5,6,7,8-tetrahydroisoquinolin-3-yl)thio]-N-(4-chlorophenyl)acetamide (**5d**).* Compound **5d** was synthesized by reaction of **2a** with *N*-(chlorophenyl)-2-chloroacetamide (**4d**). Yield: 84%. Mp: 205−206 °C. IR: 3536 (O−H); 3289 (N−H); 3074 (C−H, sp^2); 2973, 2924 (C−H, sp^3); 2216 (C≡N); 1694 (C═O, acetyl); 1666 (C═O, amide). ^{1}H 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 C^8H), 4.14−4.17 (dd, 2H, SCH_2), 3.30−3.32 (d, J = 10, 1H, C^5H), 2.93−2.95 (m, 2H: C^7H and C^5H), 2.17 (s, 3H, $COCH_3$), 1.89 (s, 3H, CH_3), 1.28 (s, 3H, CH_3). ^{13}C 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.

*4.3.6. 2-[(7-Acetyl-4-cyano-1,6-dimethyl-6-hydroxy-8-(4-nitrophenyl)-5,6,7,8-tetrahydroisoquinolin-3-yl)thio]-acetamide (**5e**).* Compound **5e** was synthesized by reaction of **2b** with 2-chloroacetamide (**4a**).Yield: 88%. Mp: 178−179 °C. IR: 3466, 3355 (O−H, NH_2); 2968, 2919 (C−H, sp^3); 2222 (C≡N); 1709 (C═O, acetyl); 1662 (C═O, amide).^{1}H NMR: δ 8.09−8.11 (d, J = 10.0, 2H, ArH), 7.54 (s, 1H, NH), 7.30−7.32 (dd, J = 5, 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, 2H, SCH_2), 3.25−3.28 (d, J = 15, 1H, C^5H), 2.88−2.90 (d, J = 10, 1H, C^7H), 2.83−2.87 (d, J = 20, 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.

*4.3.7. 2-[(7-Acetyl-4-cyano-1,6-dimethyl-6-hydroxy-8-(4-nitrophenyl)-5,6,7,8-tetrahydroisoquinolin-3-yl)thio]-N-phenylacetamide (**5f**).* Compound **5f** was synthesized by reaction of **2b** with *N*-phenyl-2-chloroacetamide (**4b**). Yield: 92%. Mp: 137−138 °C. IR: 3525 (O−H); 3322 (N−H); 3061 (C−H, sp^2); 2994, 2935 (C−H, sp^3); 2217 (C≡N); 1702 (C═O, acetyl); 1687 (C═O, amide).^{1}H NMR: δ 10.21 (s, 1H, NH); 8.09−8.11 (d, J = 10, 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.

*4.3.8. 2-[(7-Acetyl-4-cyano-1,6-dimethyl-6-hydroxy-8-(4-nitrophenyl)-5,6,7,8-tetrahydroisoquinolin-3-yl)thio]-N-(4-tolyl)acetamide (**5g**).* Compound **5g** was synthesized by reaction of **2b** with *N*-(4-tolyl)-2-chloroacetamide (**4c**).Yield: 90%. Mp: 234−235 °C. IR: 3456 (O−H); 3297 (N−H); 3107

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(C−H, sp^2); 2970 (C−H, sp^3); 2218 (C≡N); 1702 (C═O, acetyl); 1682 (C═O, amide). ^{1}H 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), 3.25−33.28(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.

*4.3.9. 2-[(7-Acetyl-4-cyano-1,6-dimethyl-6-hydroxy-8-(4-nitrophenyl)-5,6,7,8-tetrahydroisoquinolin-3-yl)thio]-N-(4-chlorophenyl)acetamide (**5h**).* Compound **5h** was synthesized by reaction of **2b** with *N*-(4-chlorophenyl)-2-chloroacetamide (**4d**).Yield: 94%. Mp: 144−145 °C. IR: 3563 (O−H), 3344 (N−H); 3134 (C−H, sp^2); 2971, 2937 (C−H, sp^3); 2221 (C≡N); 1705 (C═O, acetyl); 1681 (C═O, amide). ^{1}H 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, C^8H), 4.06−4.14 (dd, *J* = 15, 2H, SCH_2), 3.42−3.44 (d, *J* = 10, 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.

*4.3.10. 2-[(7-Acetyl-4-cyano-1,6-dimethyl-6-hydroxy-8-(4-nitrophenyl)-5,6,7,8-tetrahydroisoquinolin-3-yl)thio]-N-(4-acetylphenyl)acetamide (**5i**).* Compound **5i** was synthesized by reaction of **2b** with *N*-(4-acetylphenyl)-2-chloroacetamide (**4e**). Yield: 86%. Mp: 193−194 °C. IR: 3540 (O−H); 3337(N−H); 3109 (C−H, sp^2); 2968 (C−H, sp^3); 2220 (C≡N); 1683 (3 C═O); 1595 (C = N). ^{1}H 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, *J* = 10, 1H, C^8H), 4.36−4.38 (d, *J* = 10, 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.

4.4. 7-Acetyl-1-amino-2-(*N*-arylcarbamoyl)-5,8-dimethyl-8-hydroxy-6-(3- and 4-nitrophenyl)-6,7,8,9-tetrahydrothieno[2,3-*c*]isoquinolines 6b−d,f,g. General Methods. *4.4.1. Method A.* To a suspension of **5b−d,f,g** (10 mmol) in absolute ethanol (60 mL) was added anhydrous sodium carbonate (0.30 g). The reaction mixture was refluxed for 3 h. 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**, repectively.

*4.4.1.1. 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**).* Compound **6b** was obtained by cyclization of compound **5b**. Yield: 87%. Mp: 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). ^{1}H 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.

*4.4.1.2. 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**).* Compound **6c** was obtained by cyclization of compound **5c**. Yield: 92%. Mp: 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). ^{1}H 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, *J* = 10, 2H, 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.

*4.4.1.3. 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**).* Compound **6d** was obtained by cyclization of compound **5d**. Yield: 94%. Mp: 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). ^{1}H 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.

*4.4.1.4. 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**).* Compound **6f** was obtained by cyclization of compound **5f**. Yield: 91%. Mp: 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). ^{1}H 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.

*4.4.1.5. 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**).* Compound **6g** was obtained by cyclization of compound **5g**. Yield: 92%. Mp: 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). ^{1}H NMR: δ 9.33 (s, 1H, NH); 8.11−8.13 (d, *J* = 10, 2H, Ar−H); 7.55−

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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.

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

4.5. Cytotoxic Activity. The cytotoxic activity of the some synthesized compounds was determined according to the MTT method.[39−41] 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% Gluta MAX. 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, 6.25, 12.50, 25, 50, and 100 μM for an incubation time of 72 h. The media was removed, and 40 μL of MTT stock solution was added to each well. The resulting solutions were incubated for more than 4 h. Subsequently, 120 μL of 10% SDS was added as a solubilizing reagent. The SPSS Software program was used to calculate the IC_{50} and IC_{50} ranges.

4.6. Antioxidant Activity. DPPH has been used for measurement of the free-radical scavenging ability of antioxidants. Reduction of an alcoholic DPPH solutio[42−44] 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-picrylhydraziyl (DPPH). Solution 1 was prepared by dissolving DPPH (0.002 g) in ethanol (50 mL). Solution 2 was prepared by dissolving different weights 0.1, 0.05, and 0.01 g of each sample in 1 mL of DMSO then mixing 10 μL of each sample solution with 1 mL ethanol. Then 1 mL of solution 1 was mixed with 1 mL 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 λ_{max} = 517 nm against blank 1 mL absolute ethanol and compared to ascorbic acid (vitamin C). The DPPH radical scavenging activity (% RSA) of compounds was calculated from the absorbance at the start (0) and after some reaction time (T) according to eq 1

$$(\%RSA) = (ABS - ATS)/ABS \times 100 \quad (1)$$

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

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.1c06994.

IR, ^1H NMR, and ^{13}C NMR spectral data as well as the raw data for biological activity (PDF)

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Notes

The authors declare no competing financial interest.

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