A Novel Route to Isoquinoline[2,1-g][1,6]naphthyridine, Pyrazolo[5,1-a]

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(E)-2-Chloro-3-(2-cyanovinyl)-9,10-dimethoxy-4-oxo-6,7-dihydro-4\textsuperscript{H}-pyrido[2,1-a]isoquinoline-1-carbonitrile (5) was obtained by treatment of the 2-chloro-3-formylpyrido[2,1-a]isoquinoline derivative 3 with 2-(triphenylphosphoranylidene)acetonitrile (4). Treatment of 5 with sodium azide afforded the corresponding azido compound 6 which could be reduced by sodium dithionite to compound 7. A novel isoquinolino[2,1-g][1,6]naphthyridine derivative 11 was obtained by the reaction of phenyl isothiocyanate with the phosphorane compound 8, which was prepared by the reaction of compound 6 with triphenylphosphine. Treatment of 5 with amines 12a–c and thiophenols 14a–c in refluxing ethanol afforded the corresponding substitution products 13a–c and 15a–c, respectively. Also, the reaction of 1 with \(\alpha\)-oxo hydroxamoyl chlorides 16 was reinvestigated, and the synthesized pyrazoloprisquinolines 19a–f and pyridazinopyrazoloisquinolines 20a, e were screened for their \textit{in vitro} antitumor activities.

\textbf{Key words:} Isoquinoline-1-acetonitrile, Naphthyridines, Pyrazoloisoquinolines, Pyridazinopyrazoloisoquinolines, Hydroxamoyl Chlorides, Antitumor Activity

\section*{Introduction}

Isoquinolines and their derivatives are important constituents of pharmacologically active compounds, as these systems have shown a broad spectrum of biological activities such as cardiovascular [1], anti-inflammatory [2], anti-depressant [3] and anticancer [4 – 6]. Among them, pyrido[2,1-a]isoquinolines are well known, and several methods for their preparation have been reported [7 – 12]. The present study is a part of our program directed towards the synthesis of fused isoquinoline derivatives [13 – 24]. The aim of this study, on one hand is to introduce a simple and convenient method for the synthesis of the title compounds in good yields and on the other hand to reinvestigate the proposed structures resulting from the reaction of isoquinoline-1-acetonitrile 1 with \(\alpha\)-oxo hydroxamoyl chlorides 18a–f. Some of the synthesized compounds (19a–f and 20a, e) were tested for antitumor activity against hepatocellular carcinoma (HepG2), breast carcinoma (MCF-7) and colon carcinoma (HCT).

\section*{Results and Discussion}

\textbf{Chemistry}

2-Chloro-3-formyl-9,10-dimethoxy-4-oxo-6,7-dihydro-4\textsuperscript{H}-pyrido[2,1-a]isoquinoline-1-carbonitrile 3 was prepared according to a literature procedure (Scheme 1) [25]. The target compound 5, which has not been reported hitherto, was prepared in this study by stirring of 3 with 2-(triphenylphosphoranylidene)acetonitrile 4 in chloroform at room temperature (Scheme 2). The structure of 5 was based on its elemental and spectral analyses. The \(^1\text{H}\) NMR spectrum showed two doublets at \(\delta = 6.85\) and 7.75 ppm with a coupling constant \(J = 16\) Hz that indicated a \textit{trans} configuration for the vinylic protons, in addition to signals of a pyrido[2,1-a]isoquinoline moiety. The mass spectrum of 5 showed the molecular ion peak at \(m/z = 367\), and its IR spectrum revealed bands at \(\nu = 2245, 2214\) and 1658 cm\(^{-1}\) assignable to cyano and amide carbonyl groups, respectively.

Azido derivative 6 was prepared by stirring of 5 with sodium azide in a dioxane/water mixture for 3 h at

Room temperature (Scheme 2). Elemental analyses and spectral data were used to elucidate the structure of the product 6. Thus, the IR spectrum gave a band at $\nu = 2125 \text{ cm}^{-1}$ assignable to an azide group. Reduction of the azido derivative 6 to the corresponding amino compound 7 was accomplished by stirring with sodium dithionite in methanol/water mixture at room temperature for 24 h (Scheme 2). The structure of compound 7 was confirmed by elemental and spectral analyses. Its IR spectrum revealed the absence of the azide stretching band, instead two new bands for the amino group were clearly visible at 3367 and 3267 cm$^{-1}$. Treatment of 6 with triphenylphosphine in boiling ether afforded the iminophosphorane 8 in good yield. The IR spectrum of 8 revealed the absence of the azide group, and its mass spectrum showed the molecular ion peak at $m/z = 608$. Refluxing of iminophosphorane 8 with phenyl isothiocyanate in 1,2-dichlorobenzene for 6 h afforded the novel 2,3-dimethoxy-8-oxo-11-(phenylamino)-6,8-dihydro-5H-isoquinolino[2,1-g][1,6]naphthyridine-10,13-dicarbonitrile (11) in 76% yield (Scheme 3). It is proposed that the pathway of the formation of the novel tetracyclic system 11 involves an initial Aza-Wittig-type reaction between the iminophosphorane group and phenyl isothiocyanate to give the reactive intermediate carbodiimide 9, which gives the intermediate 10 via intramolecular cyclization by nucleophilic attack of the $\beta$-carbon atom of the vinyl moiety. The latter yielded the final product 11.


via a proton shift (Scheme 3) [26]. Combustion analysis and mass spectrum confirmed the molecular formula C_{28}H_{19}N_{5}O_{3}. The IR spectrum of compound 11 revealed a band at 3255 cm\(^{-1}\) corresponding to anilino NH. Its \(^1\)H NMR spectrum showed a singlet signal at \(\delta = 8.58\) corresponding to the proton of the pyridine ring at position 9.

The chlorine atom in compound 5 showed high reactivity towards nitrogen and sulfur nucleophiles. Thus refluxing 5 with amine derivatives 12a–c in ethanol for 6 h afforded the substitution products (E)-2-(aralkylamino)-3-(2-cyanovinyl)-9,10-dimethoxy-4-oxo-6,7-dihydro-4H-pyrido[2,1-a]isoquinoline-1-carbonitrile 13a–c (Scheme 4). Similarly, the substitution products 15a–c were obtained by refluxing of 5 with sulfur nucleophiles 14a–c in ethanol in the presence of triethylamine (Scheme 4). The structures of the products 13a–c and 15a–c were confirmed on the basis of elemental and spectral analyses (see Experimental Section).

It was claimed that 3-aroyl-3-hydroxyimino-2-(6,7-dimethoxy-1,2,3,4-tetra-hydroisoquinolin-1-ylidene)propanenitrile (18) can be obtained in good yield from the reaction of hydroxamoyl chlorides 16a–f with 1 (Scheme 5) [27]. In our hands, stirring of 1 with 16a in acetonitrile at room temperature afforded a product which gave analytical data consistent with its formulation as 2-benzoyl-8,9-dimethoxy-5,6-dihydropyrazolo[5,1-a]isoquinoline-1-carbonitrile 19a. Mass spectral and combustion analysis data indicated its molecular formula as C_{21}H_{17}N_{3}O_{3}. Similarly, 1 reacts with 16b–f to give 19b–f. The IR spectra of compounds 19a–f were free of OH and NH bands. The reaction pathway that seems to account for the formation of 19a–f from reaction of 1 with 16a–f is outlined in Scheme 5. It is proposed that the reaction involves nucleophilic substitution to give 17a–f. The latter intermediates tautomerize to give 18a–f, which cyclize via elimination of water to give 19a–f. The structures
of 19a–f were further confirmed by their reaction with hydrazine hydrate. For example, refluxing of 19a, e with hydrazine hydrate in ethanol afforded the 9-aryl-2,3-dimethoxy-5,6-dihydro-pyrazolo[4′,5′:3,4]pyrazolo[5,1-a]isoquinolin-12-amines 20a, e. The IR spectra of compounds 20 revealed two bands at ca. 3340 and 3244 cm\(^{-1}\) assignable to asymmetric and symmetric stretch of an amino group.

**Antitumor activity**

The cytotoxic potencies of the synthesized pyrazoloisoquinolines 19a–f and pyridazinopyrazoloisoquinolines 20a, e against a panel of human tumor cell lines were investigated and compared with the reference drug Doxorubicin. The human tumor cell line panel consisted of hepatocellular carcinoma (HepG2), breast carcinoma (MCF-7) and colon carcinoma (HCT). The results are summarized in Table 1. The cytotoxic potency of pyrazoloisoquinoline derivatives substituted with 4-nitrobenzoyl (19d), thiophene-2-carbonyl (19e) and 2-naphthoyl (19f) at position 2 showed a weak, but distinct difference, and the IC\(_{50}\) values of these compounds ranged from 30 to 50 µg. Exceptionally, compound 19d was selectively active against colon carcinoma cell lines (IC\(_{50}\) = 11.7 µg). Compounds bearing a phenyl, a 4-methoxybenzoyl and a 4-bromobenzoyl group at position 2 (compounds 19a–c) and 20a, e displayed a broad spectrum of cytotoxic activities with IC\(_{50}\) value lower
than 25 µg against the three tumor cell lines. Interestingly, compound 19c exhibited a strong cytotoxic effect against the HCT cell line with IC50 = 5.2 µg. An overview of the cytotoxic activities data of all examined compounds clearly confirmed that the 4-bromobenzoyl-substituted pyrazolo-isoquinoline 19c was most active against all tumor cell lines tested.

**Experimental Section**

Melting points were determined on a Stuart melting point apparatus and are uncorrected. IR spectra were measured as KBr pellets on a FTIR Bruker-Vector 22 spectrophotometer. The 1H NMR and 13C NMR spectra were recorded in CDCl3 or [D6]DMSO on a Varian Mercury VX R 300 spectrometer (300 MHz for 1H NMR and 75 MHz for 13C NMR) using TMS as internal standard. Chemical shifts are reported in δ units (ppm). Mass spectra were measured on a Shimadzu GCMS-Q-1000 EX mass spectrometer at 70 eV. Elemental analyses were performed at the Microanalytical Center, Cairo University. Isoquinoline-1-acetonitrile 1 [28], 2-hydroxy-9,10-dimethoxy-4-oxo-6,7-dihydro-4H-pyrido[2,1-a]isoquinoline-1-carbonitrile 2 [29], 2-chloro-3-formyl-9,10-dimethoxy-4-oxo-6,7-dihydro-4H-pyrido[2,1-a]isoquinoline-1-carbonitrile 3 [25], 2-(triphenylphosphoranylidene)acetonitrile 4 [30], hydroxamoyl chlorides 18a-f [31, 32] were prepared according to the procedures in the literature.

**Synthesis of (E)-2-chloro-3-(2-cyanovinyl)-9,10-dimethoxy-4-oxo-6,7-dihydro-4H-pyrido[2,1-a]isoquinoline-1-carbonitrile (5)**

To a solution of 2-(triphenylphosphoranylidene)acetonitrile (4, 5.64 g, 20.0 mmol) in chloroform (50 mL), 2-chloro-3-formyl-9,10-dimethoxy-4-oxo-6,7-dihydro-4H-pyrido[2,1-a]isoquinoline-1-carbonitrile (3, 6.88 g, 20.0 mmol) was added. The reaction mixture was stirred for 3 h at room temperature then evaporated to dryness under reduced pressure. Ethanol (30 mL) was added to the residue, and the solid formed was filtered, washed with ethanol and crystallized from DMF to give compound 5. Yellow crystals; m. p. 246–248 °C; yield: 6.24 g (85%). – IR (KBr): ν = 2245 (CN), 2214 (CN), 1658 (C=O) cm⁻¹. – 1H NMR (300 MHz, DMSO): δ = 2.91 (t, J = 7 Hz,
A solution of 5 (1.84 g, 5.0 mmol) in a dioxane-water mixture (4:1 (v/v)) 60 mL was treated with a solution of sodium azide (0.65 g, 10.0 mmol) in the same solvent mixture. The reaction mixture was vigorously stirred for 3 h at room temperature, then diluted with water (100 mL). The solution was collected and crystallized from DMF to afford compound 6 at room temperature, then diluted with water (100 mL). The reaction mixture was vigorously stirred for 3 h. The solid that precipitated was filtered, washed with ethanol and crystallized from DMF to give compound 6. Canary-yellow crystals; m. p. 270 – 272 °C; yield: 2.31 g (76%). – IR (KBr): v = 3255 (NH), 2227 (CN), 2204 (C=O) cm⁻¹. – 1H NMR (300 MHz, CDCl₃): δ = 7.02, 7.10 (m, 2H), 7.32, 7.86 (d, J = 16 Hz, 1H); 8.59 (s, 1H). – 13C NMR (75 MHz, CDCl₃): δ = 27.09, 41.25, 55.70, 56.02, 80.41, 92.25, 96.74, 110.61, 112.35, 117.43, 118.13, 120.89, 133.26, 141.75, 146.78, 150.89, 152.36, 154.51, 159.49. – MS (EL, 70 eV): m/z (%) = 346 (86) [M+Cl]⁺, 262 (100). – C₁₉H₁₇ClN₄O₂S (348.3): calcd. C 59.41, H 4.91, N 12.53; found C 59.53, H 4.92, N 12.55.

**Synthesis of (E)-2-azido-3-(2-cyanovinyl)-9,10-dimethoxy-4-oxo-6,7-dihydro-4H-pyrido[2,1-a]isoquinoline-1-carbonitrile (7)**

To a stirred suspension of 6 (1.87 g, 5.0 mmol) in 4:1 MeOH:H₂O (40 mL) mixture, sodium dithionite (4.0 g, 20.0 mol) was added portionwise. The reaction mixture was stirred for 24 h, then poured into H₂O (20 mL). The resulting solid product was filtered, washed with water and crystallized from DMF-EtOH to give compound 7. Yellow crystals; m. p. 282 – 284 °C; yield: 1.37 g (79%). – IR (KBr): v = 3367, 3267 (NH₂), 2225 (CN), 2198 (CN), 1639 (C=O) cm⁻¹. – 1H NMR (300 MHz, DMSO): δ = 2.88 (t, J = 7 Hz, 2H, CH₂), 3.51 (s, 3H, OMe), 3.88 (s, 3H, OMe), 4.00 (t, J = 7 Hz, 2H, CH₂), 6.86 (d, J = 16 Hz, 1H), 7.07 (s, 1H), 7.21 (s, 2H, NH₂), 7.75 (d, J = 16 Hz, 1H), 7.81 (s, 1H). – 13C NMR (75 MHz, DMSO): δ = 26.85, 40.35, 55.67, 55.89, 79.17, 92.25, 96.85, 110.85, 111.93, 117.34, 117.97, 120.68, 133.38, 141.61, 146.73, 150.70, 152.33, 154.38, 159.13. – MS (EL, 70 eV): m/z (%) = 348 (100) [M⁺], 260, 126. – C₁₀H₁₆N₂O₃ (348.3): calcd. C 65.51, H 4.63, N 16.08; found C 65.32, H 4.75, N 16.31.

**Synthesis of (E)-3-(2-cyanovinyl)-9,10-dimethoxy-4-oxo-6,7-dihydro-4H-pyrido[2,1-a]isoquinoline-1-carbonitrile (8)**

A solution of 6 (1.87 g, 5.0 mmol) and triphenylphosphine (1.3 g, 5.0 mmol) in dry ether (25 mL) was refluxed for 1 h and then cooled. The solid that precipitated was filtered, washed with ethanol and crystallized from DMF to give compound 8. Phenyl isothiocyanate (0.12 g, 1.0 mmol) was added to a solution of 8 (0.61 g, 1.0 mmol) in 1,2-dichlorobenzene (10 mL). The reaction mixture was refluxed for 6 h, and then the solvent was removed under reduced pressure. The solid was collected and crystallized from DMF to give compound 11. Yellow crystals; m. p. 308 – 310 °C; yield: 0.34 g (76%). – IR (KBr): v = 3255 (NH), 2227 (CN), 2204 (CN), 1655 (C=O) cm⁻¹. – 1H NMR (300 MHz, CDCl₃): δ = 2.90 (t, J = 7 Hz, 2H, CH₂), 3.84 (s, 3H, OMe), 3.88 (s, 3H, OMe), 4.05 (t, J = 7 Hz, 2H, CH₂), 7.05 (s, 1H), 7.10 – 7.99 (m, 6H), 8.58 (s, 1H), 9.52 (s, 1H, NH). – 13C NMR (75 MHz, CDCl₃): δ = 26.80, 40.37, 55.66, 55.90, 86.73, 93.73, 110.23, 110.81, 112.09, 115.44, 117.41, 118.14, 121.55, 123.70, 128.74, 130.60, 133.45, 133.83, 141.81, 146.87, 152.43, 155.27, 155.57, 158.49. – MS (EI, 70 eV): m/z (%) = 449 (100) [M⁺], 448 (54). – C₂₆H₂₄N₄O₃ (449.4): calcd. C 69.48, H 4.26, N 15.88; found C 69.71, H 4.22, N 15.63.
(50 mL) was refluxed for 6 h in the presence of triethylamine (0.4 mL). The solvent was evaporated and the residue cooled. The resulting solid product was collected, washed with ethanol and crystallized from CH$_2$CN to give compounds 13a-c.

(E)-2-(Benzylamino)-3-(2-cyanovinyl)-9,10-dimethoxy-4-oxo-6,7-dihydro-4H-pyrido[2,1-a]isoquinoline-1-carbonitrile (13a)

Yellow crystals; m. p. 160 – 161 °C; yield: 1.02 g (78%).

- IR (KBr): $\nu$ 3284 (NH), 2225 (CN), 2210 (CN), 1669 (C=O) cm$^{-1}$. – $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ = 2.91 (t, $J$ = 7 Hz, 2H, CH$_2$), 3.82 (s, 3H, OMe), 3.83 (s, 3H, OMe), 4.04 (t, $J$ = 7 Hz, 2H, CH$_2$), 4.74 (s, 2H, CH$_2$), 6.71 (d, $J$ = 16 Hz, 1H), 6.78 – 6.83 (m, 6H), 6.86 (s, 1H), 7.26 (d, $J$ = 16 Hz, 1H), 7.75 (s, 1H). – $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta$ = 26.71, 40.42, 49.65, 55.76, 55.98, 83.35, 101.02, 110.96, 112.35, 115.36, 116.45, 118.13, 119.27, 127.50, 127.96, 128.33, 129.02, 134.52, 136.89, 138.92, 142.24, 152.10, 152.68, 156.02. – MS (EI, 70 eV): $m/z$ (%) = 438 (9) [M$^+$], 398 (85), 91 (100). – C$_{26}$H$_{22}$N$_2$O$_3$ (438.4): calcd. C 69.75, H 6.09, N 13.01; found C 69.79, H 5.82, N 9.93.

Synthesis of (E)-2-(arylamino)-3-(2-cyanovinyl)-9,10-dimethoxy-4-oxo-6,7-dihydro-4H-pyrido[2,1-a]isoquinoline-1-carbonitriles 15a-c

General procedure: These compounds were prepared as previously described for the synthesis of 13 using arylthiols 14 in the presence of triethylamine (0.4 mL) instead of amines 12. The resulting solid products were collected, washed with ethanol and crystallized from CH$_2$CN to give compounds 15a-c.

(E)-3-(2-Cyanovinyl)-9,10-dimethoxy-4-oxo-6,7-dihydro-4H-pyrido[2,1-a]isoquinoline-1-carbonitrile (15a)

Yellow crystals; m. p. 166 – 168 °C; yield: 1.03 g (78%).

- IR (KBr): $\nu$ = 2214 (CN), 2209 (CN), 1645 (C=O) cm$^{-1}$. – $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ = 2.90 (t, $J$ = 7 Hz, 2H, CH$_2$), 3.82 (s, 3H, OMe), 3.92 (s, 3H, OMe), 4.02 (t, $J$ = 7 Hz, 2H, CH$_2$), 6.77 (s, 1H), 6.95 – 7.21 (m, 6H), 7.81 (s, 1H), 7.85 (d, $J$ = 16 Hz, 1H). – $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta$ = 24.81, 24.99, 26.75, 33.75, 40.40, 55.85, 55.97, 56.46, 82.13, 102.45, 111.25, 112.64, 115.23, 115.61, 117.86, 118.71, 135.59, 137.24, 146.70, 150.64, 151.22, 152.49, 156.33. – MS (EI, 70 eV): $m/z$ (%) = 441 (100) [M$^+$], 440 (92), 364 (74), – C$_{25}$H$_{19}$N$_2$O$_3$S (441.4): calcd. C 68.02, H 4.34, N 9.52, S 7.25; found C 67.86, H 4.52, N 9.34, S 7.19.

(E)-3-(2-Cyanovinyl)-9,10-dimethoxy-4-oxo-2-(p-tolylthio)-6,7-dihydro-4H-pyrido[2,1-a]isoquinoline-1-carbonitrile (15b)

Yellow crystals; m. p. 196 – 197 °C; yield: 1.06 g (78%).

- IR (KBr): $\nu$ = 2214 (CN), 2207 (CN), 1644 (C=O) cm$^{-1}$. – $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ = 2.82 (s, 3H, Me), 2.91 (t, $J$ = 7 Hz, 2H, CH$_2$), 3.87 (s, 3H, OMe), 3.92 (s, 3H, OMe), 4.22 (t, $J$ = 7 Hz, 2H, CH$_2$), 6.76 (s, 1H), 7.06 – 7.36 (m, 5H), 7.80 (s, 1H), 7.98 (d, $J$ = 16 Hz, 1H). – $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta$ = 20.88, 27.27, 40.22, 55.96, 56.09, 93.70, 102.39, 110.01, 111.90, 116.95, 117.77, 117.89, 122.94, 129.34, 129.83, 130.15, 130.24, 143.12, 147.59, 150.02, 150.44, 153.00, 158.41. – MS (EI, 70 eV): $m/z$ (%) = 441 (76) [M$^+$], 415 (66), 91 (100). – C$_{26}$H$_{23}$N$_2$O$_3$S (455.4): calcd. C 68.56, H 4.65, N 9.23, S 7.03; found C 68.48, H 4.70, N 9.21, S 6.87.
Synthesis of 2-aryl-8,9-dimethoxy-5,6-dihydropyrazolo-[5,1-a]isoquinoline-1-carbonitriles 19a–f

General procedure: A mixture of equimolar amounts of isoquinoline-1-acetonitrile 1 and hydroxylammon chloride 16 (5 mmol each) was stirred for 2 h in acetonitrile (30 mL) at room temperature, during which time the compounds dissolved, and the product 19 precipitated. The solids were collected and crystallized from CH3CN to give compounds 19a–f.

2-Benzoyl-8,9-dimethoxy-5,6-dihydropyrazolo[5,1-a]isoquinoline-1-carbonitrile (19d)

Dark-green crystals; m. p. 256–258 °C; yield: 1.36 g (76%). – IR (KBr): ν = 2202 (CN) cm⁻¹. – ¹H NMR (300 MHz, CDCl₃): δ = 3.10 (t, J = 7 Hz, 2H, CH₂), 3.79 (s, 3H, OMe), 3.85 (s, 3H, OMe), 4.26 (t, J = 7 Hz, 2H, CH₂), 7.11 (s, 1H), 7.65–7.96 (m, 6H). – MS (EI, 70 eV); m/z (%) = 359 (100) [M⁺], 358 (59). – C₁₅H₁₁N₃O₅ (359.3): calcd. C 57.40, H 3.54, Br 18.16, N 10.77, yield: 1 g (72%).

8,9-Dimethoxy-2-(4-methoxybenzoyl)-5,6-dihydropyrazolo[5,1-a]isoquinoline-1-carbonitrile (19b)

Green crystals; m. p. 262–264 °C; yield: 1.50 g (77%). – IR (KBr): ν = 2205 (CN) cm⁻¹. – ¹H NMR (300 MHz, DMSO): δ = 3.07 (t, J = 7 Hz, 2H, CH₂), 3.76 (s, 3H, OMe), 3.82 (s, 3H, OMe), 3.86 (s, 3H, OMe), 4.25 (t, J = 7 Hz, 2H, CH₂), 7.09 (s, 1H), 7.18 (d, J = 7.5 Hz, 2H), 7.58 (s, 1H), 7.88 (d, J = 7.5 Hz, 2H). – ¹³C NMR (75 MHz, DMSO): δ = 27.21, 42.51, 55.53, 55.64, 55.71, 69.83, 107.49, 112.06, 114.14, 116.31, 116.65, 118.40, 128.88, 133.47, 138.78, 147.93, 148.84, 150.83, 159.71, 161.33. – MS (EI, 70 eV); m/z (%) = 389 (100) [M⁺], 328 (86). – C₂₁H₁₉N₃O₅ (389.4): calcd. C 67.86, H 4.92, N 10.79; found C 67.62, H 5.12, N 10.96.

8,9-Dimethoxy-2-(4-nitrobenzoyl)-5,6-dihydropyrazolo[5,1-a]isoquinoline-1-carbonitrile (19d)

Yellowish-green crystals; m. p. 258–260 °C; yield: 1.58 g (78%). – IR (KBr): ν = 2182 (CN) cm⁻¹. – ¹H NMR (300 MHz, DMSO): δ = 3.09 (t, J = 7 Hz, 2H, CH₂), 3.75 (s, 3H, OMe), 3.83 (s, 3H, OMe), 4.29 (t, J = 7 Hz, 2H, CH₂), 7.11 (s, 1H), 7.57 (s, 1H), 8.01 (d, J = 7.5 Hz, 2H), 8.13 (d, J = 7.5 Hz, 2H). – ¹³C NMR (75 MHz, DMSO): δ = 27.09, 42.71, 55.49, 55.70, 69.35, 107.39, 111.75, 116.10, 116.72, 124.86, 125.65, 128.97, 131.46, 133.74, 139.23, 148.55, 149.82, 152.10, 160.04. – MS (EI, 70 eV); m/z (%) = 404 (38) [M⁺], 150 (100). – C₁₂H₁₀N₂O₄ (404.3): calcd. C 62.37, H 3.99, N 13.86; found C 62.15, H 4.12, N 13.74.

8,9-Dimethoxy-2-(thiophene-2-carbonyl)-5,6-dihydropyrazolo[5,1-a]isoquinoline-1-carbonitrile (19c)

Yellowish-brown crystals; m. p. 264–266 °C; yield: 1.41 g (77%). – IR (KBr): ν = 2210 (CN) cm⁻¹. – ¹H NMR (300 MHz, DMSO): δ = 3.12 (t, J = 7 Hz, 2H, CH₂), 3.77 (s, 3H, OMe), 3.84 (s, 3H, OMe), 4.41 (t, J = 7 Hz, 2H, CH₂), 7.12 (s, 1H), 7.42 (t, J = 7.5 Hz, 1H), 7.62 (s, 1H), 7.86 (d, J = 7.5 Hz, 1H), 8.11 (d, J = 7.5 Hz, 1H). – MS (EI, 70 eV); m/z (%) = 365 (100) [M⁺], 111 (52). – C₁₀H₁₀N₂O₃S (365.3): calcd. C 62.46, H 4.14, N 11.50, S 8.76; found C 62.35, H 4.20, N 11.59, S 8.58.

8,9-Dimethoxy-2-(naphthyl)-5,6-dihydropyrazolo[5,1-a]isoquinoline-1-carbonitrile (19f)

Pale-green crystals; m. p. 172–173 °C; yield: 1.60 g (78%). – IR (KBr): ν = 2209 (CN) cm⁻¹. – ¹H NMR (300 MHz, DMSO): δ = 3.12 (t, J = 7 Hz, 2H, CH₂), 3.81 (s, 3H, OMe), 3.86 (s, 3H, OMe), 4.34 (t, J = 7 Hz, 2H, CH₂), 7.15 (s, 1H), 7.50 (d, J = 7.5 Hz, 2H), 7.75 (d, J = 7.5 Hz, 2H). – MS (EI, 70 eV); m/z (%) = 389 (100) [M⁺], 328 (86). – C₂₁H₁₉N₃O₅ (389.4): calcd. C 67.86, H 4.92, N 10.79; found C 67.62, H 5.12, N 10.96.
2,3-Dimethoxy-9-(thiophen-2-yl)-5,6-dihydropyrazido-
[4′,5′,6]pyrazolo[5,1-a]isquinolin-12-amine (20e)

Yellow crystals; m. p. 268 – 270 °C; yield: 1.36 g (72%).

In vitro cytotoxicity assays

The cells were propagated in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% heat-inactivated fetal calf serum, 1% L-glutamine, HEPES buffer, and 50 µg mL⁻¹ gentamycin. The cells were maintained at 37 °C in a humidified atmosphere with 5% CO₂. The cytotoxicity assay was carried out using 100 µL of cell suspension, containing 10,000 cells seeded in each well of a 96-well microtiter plate (Falcon, NJ, USA). Fresh medium containing different dilutions of the test sample was added after 24 h of seeding. Control cells were incubated without test sample. The microtiter plates were incubated at 37 °C in a humidified incubator with 5% CO₂ for a period of 48 h. Three wells were used for each concentration of the test sample. The cell cytotoxic effect of each tested compound was determined using MTT assay [33].

References