A Novel Route to Isoquinoline[2,1-*g*][1,6]naphthyridine, Pyrazolo[5,1-*a*] isoquinoline and Pyridazino[4′,5′:3,4]pyrazolo[5,1-*a*]isoquinoline Derivatives With Evaluation of Antitumor Activities

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(*E*)-2-Chloro-3-(2-cyanovinyl)-9,10-dimethoxy-4-oxo-6,7-dihydro-4*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 α-oxo hydroxamoyl chlorides **16** was reinvestigated, and the synthesized pyrazoloisoquinolines **19a–f** and pyridazinopyrazoloisoquinolines **20a**, **e** were screened for their *in vitro* antitumor activities.

Key words: Isoquinoline-1-acetonitrile, Naphthyridines, Pyrazoloisoquinolines, Pyridazinopyrazoloisoquinolines, Hydroxamoyl Chlorides, Antitumor Activity

Introduction

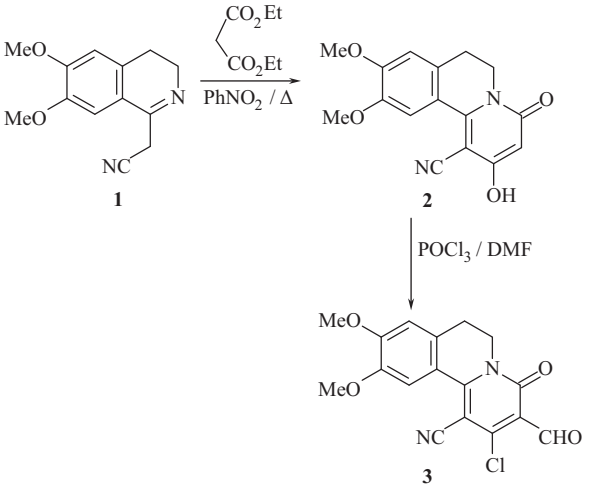
Isoquinolines and their derivatives are important constituents of pharmacologically active compounds, as these systems have shown a broad spectrum of biological activities such as cardiovascular [1], anti-inflammatory [2], anti-depressant [3] and anti-cancer [4 – 6]. Among them, pyrido[*a*]isoquinolines 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-acetonitirile **1** with α-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).

Results and Discussion

Chemistry

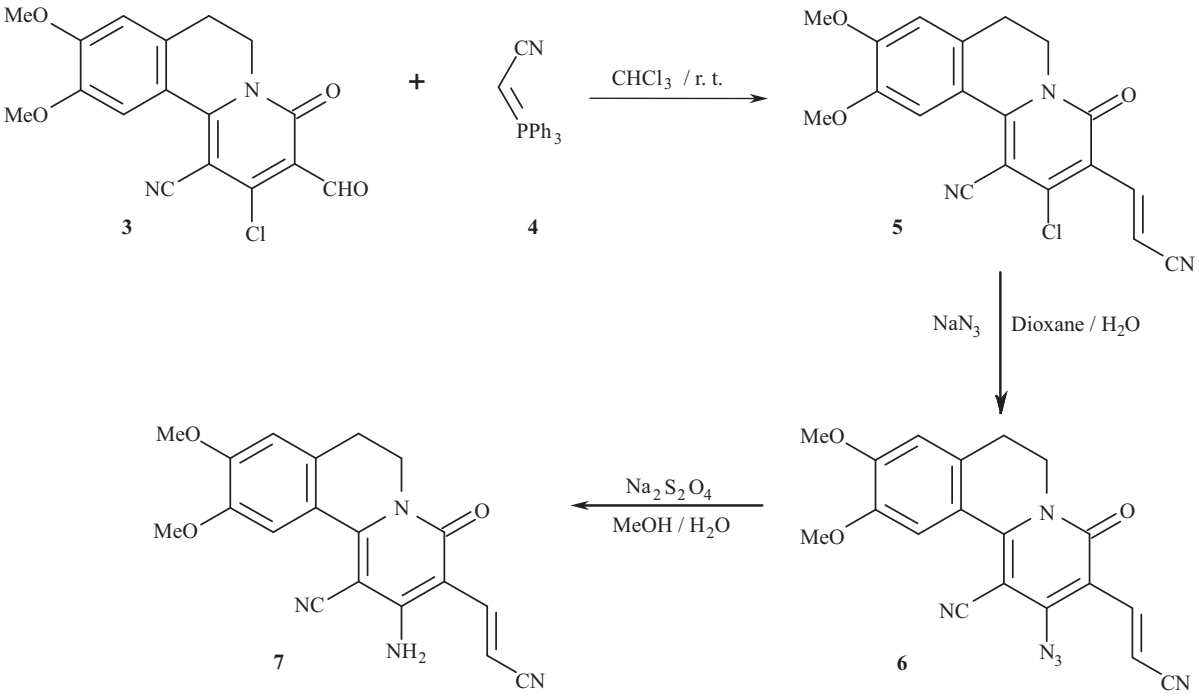
2-Chloro-3-formyl-9,10-dimethoxy-4-oxo-6,7-dihydro-4*H*-pyrido[2,1-*a*]isoquinoline-1-carbonitril **3** was prepared according to a literature procedure (Scheme 1) [25]. The target compund **5**, which has not been reported hitherto, was prepared in this study by stirring of **3** with 2-(triphenylphosphoranylidene)acetonitrile **4** in chloroform at room temperature (Scheme 2). The structure of **5** was based on its elemental and spectral analyses. The ^{1}H NMR spectrum showed two doublets at $\delta = 6.85$ and 7.75 ppm with a coupling constant $J = 16$ Hz that indicated a *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

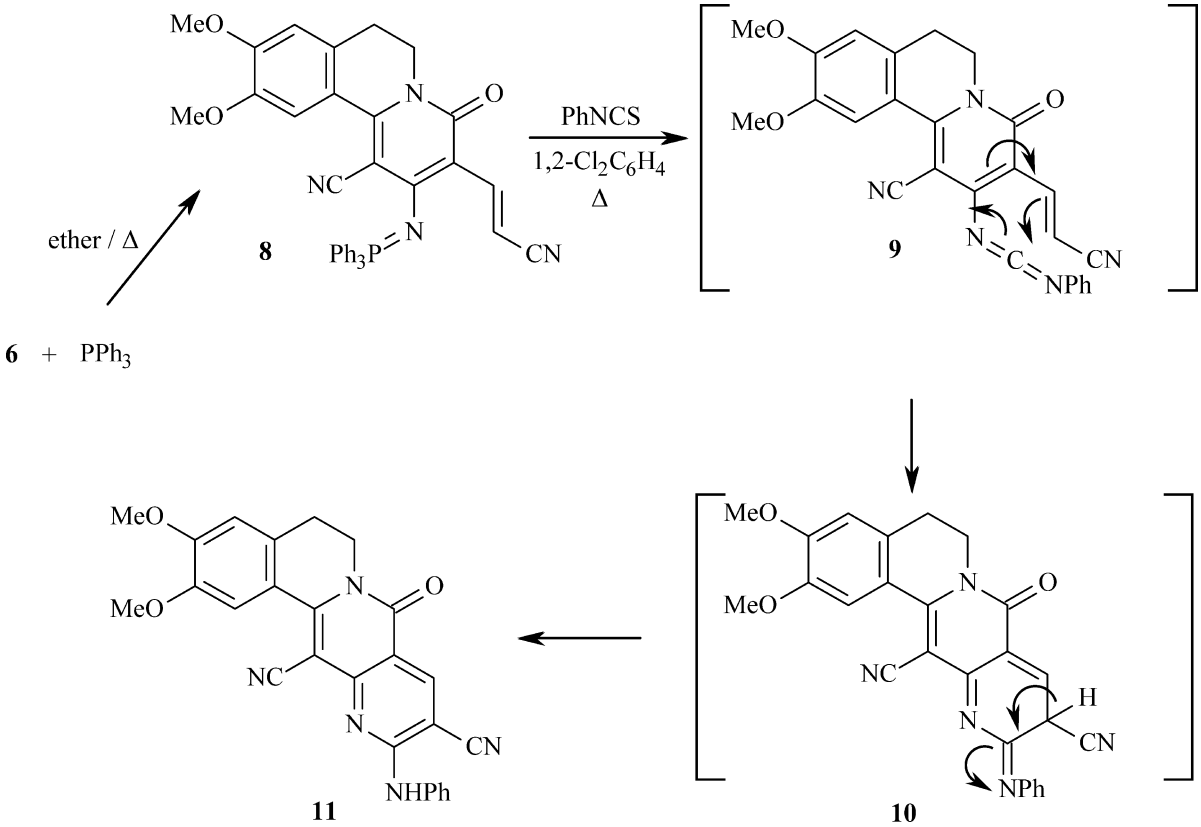


Scheme 1. Synthesis of 2-chloro-3-formylpyrido[2,1-*a*]isoquinoline **3**.

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 $\nu = 2125\,\mathrm{cm}^{-1}$ 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-dimeth-oxy-8-oxo-11-(phenylamino)-6,8-dihydro-5*H*- 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 β-carbon atom of the vinyl moiety. The latter yielded the final product **11**



Scheme 2. Synthesis of (*E*)-3-(2-cyanovinyl)pyrido[2,1-*a*]isoquinoline derivatives.

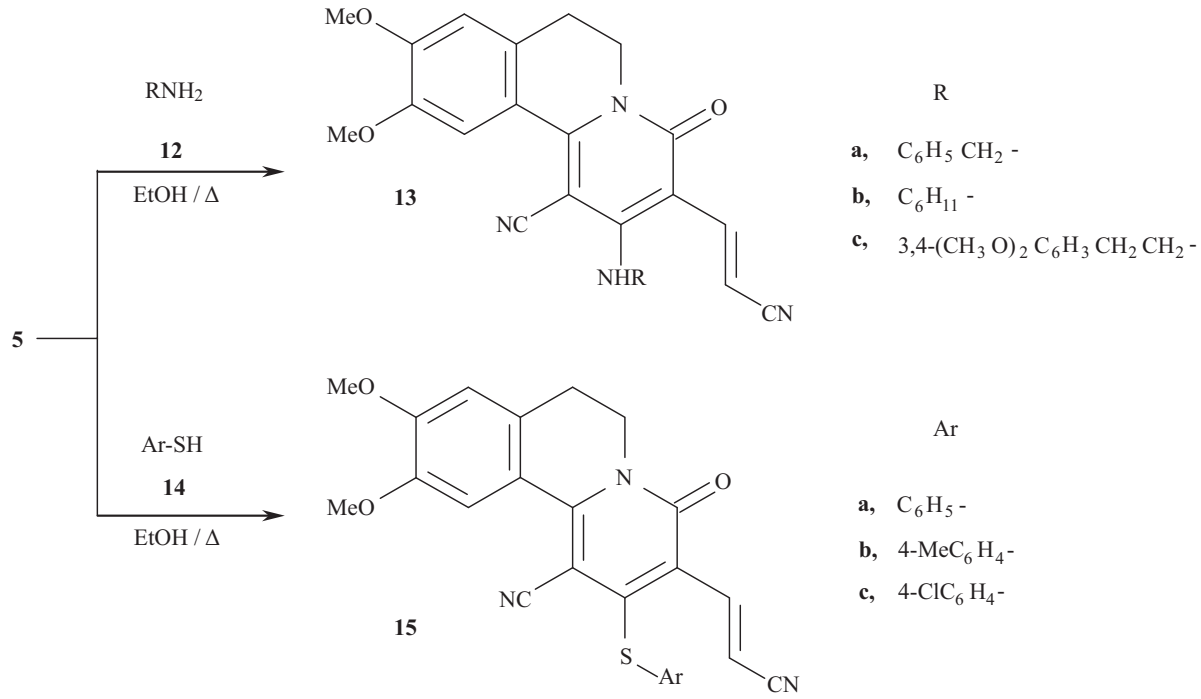


Scheme 3. Synthesis of isoquinolino[2,1-*g*][1,6]naphthyridine derivative **11**.

via a proton shift (Scheme 3) [26]. Combustion analysis and mass spectrum confirmed the molecular formula $C_{28}H_{19}N_5O_3$. The IR spectrum of compound **11** revealed a band at 3255 cm^{-1} corresponding to anilino NH. Its ^1H 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-4*H*-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 $C_{21}H_{17}N_3O_3$. 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



Scheme 4. Synthesis of (*E*)-3-(2-cyanovinyl)pyrido[2,1-*a*]isoquinoline derivatives **13** and **15**.

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-pyridazino[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.

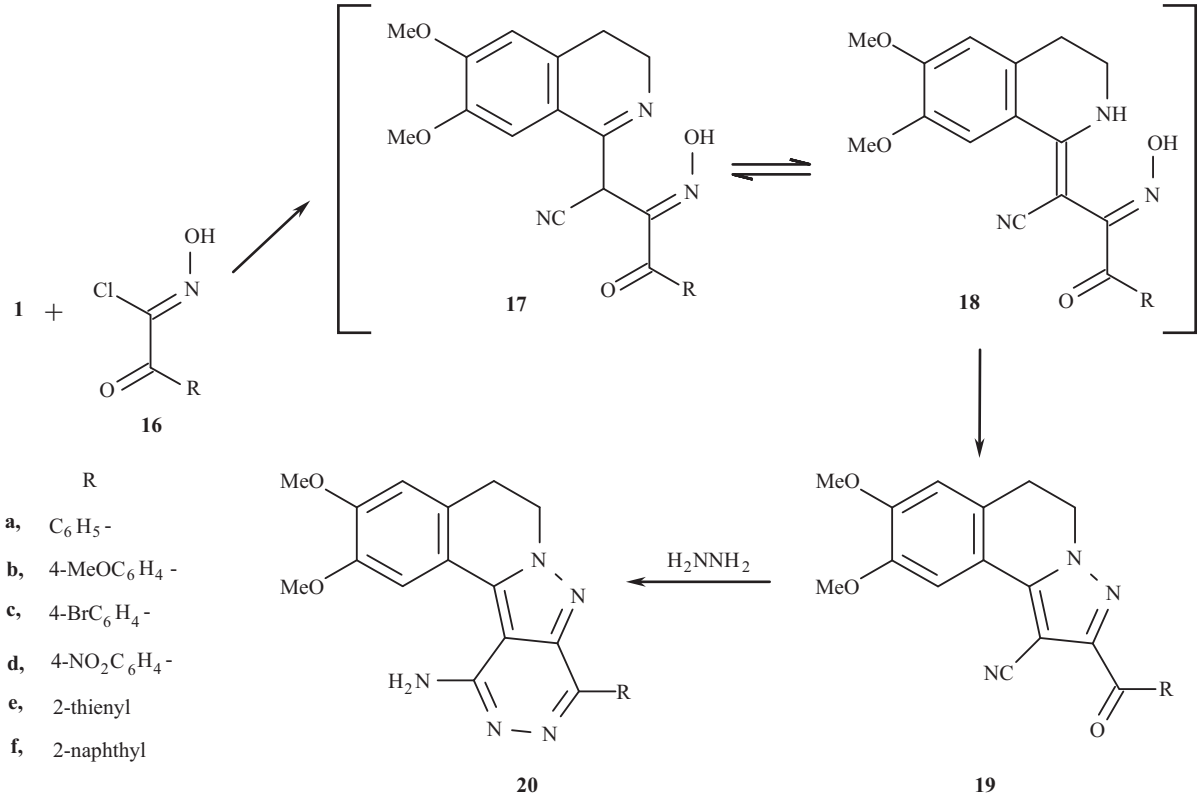
Antitumor activity

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 ($IC_{50} = 11.7\ \mu$g). 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

Table 1. Evaluation of *in vitro* antitumor activities of pyrazoloisoquinolines **19a–f** and pyridazinopyrazoloisoquinolines **20a**, **e**.

	IC_{50} (μg)[a]		
Compound	HepG2[b]	MCF-7	HCT
19a	20.0	12.6	12.2
19b	25.6	15.3	19.9
19c	18.8	11.1	5.2
19d	48.6	33.4	11.7
19e	> 50	> 50	45.3
19f	43.4	35.0	34.7
20a	19.7	18.0	25.0
20e	25.2	23.7	22.1
Doxorubicin	1.2	2.38	0.469

[a] Cytotoxicity as IC_{50} for each cell line is the concentration of compound which reduced the optical density of treated cells by 50% with respect to untreated cells; [b] cell lines include hepatocellular carcinoma (HepG2), breast carcinoma (MCF-7) and colon carcinoma (HCT).



Scheme 5. Synthesis of 2-aroylpyrazolo[5,1-*a*]isoquinoline derivatives.

than 25 μg against the three tumor cell lines. Interestingly, compound **19c** exhibited a strong cytotoxic effect against the HCT cell line with $IC_{50} = 5.2\ \mu g$. 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 ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ or [D_6]DMSO on a Varian Mercury VXR 300 spectrometer (300 MHz for ^{1}H NMR and 75 MHz for ^{13}C 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-4*H*-pyrido[2,1-*a*]isoquinoline-1-carbonitrile **2** [29], 2-chloro-3-formyl-9,10-dimethoxy-4-oxo-6,7-dihydro-4*H*-pyrido-[2,1-*a*]isoquinoline-1-carbonitilie **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-4*H*-pyrido[2,1-*a*]isoquinoline-1-carbonitilie (**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}. – ^{1}H NMR (300 MHz, DMSO): δ = 2.91 (t, J = 7 Hz,

2H, CH_2), 3.78 (s, 3H, OMe), 3.85 (s, 3H, OMe), 4.03 (t, $J = 7$ Hz, 2H, CH_2), 6.85 (d, $J = 16$ Hz, 1H), 7.08 (s, 1H), 7.75 (d, $J = 16$ Hz, 1H), 7.86 (s, 1H). – ^{13}C NMR (75 MHz, DMSO): δ = 27.19, 40.21, 55.72, 55.97, 79.24, 92.25, 96.86, 110.89, 111.93, 117.44, 118.20, 120.87, 133.49, 141.75, 146.77, 150.85, 152.36, 154.52, 159.24. – MS (EI, 70 eV): $m/z(\%) = 369$ (34) $[M+2]^+$, 367 (100) $[M]^+$, 366 (66). – $C_{19}H_{14}ClN_3O_3$ (367.8): calcd. C 62.05, H 3.84, Cl 9.64, N 11.43; found C 61.89, H 3.92, Cl 9.61, N 11.20.

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

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 solid that precipitated was collected and crystallized from DMF to afford compound **6**. Yellow crystals; m. p. 318 – 320 °C; yield: 1.48 g (79 %). – IR (KBr): ν = 2230 (CN), 2214 (CN), 2125 (N_3), 1651 (C=O) cm^{-1}. – ^{1}H NMR (300 MHz, DMSO): $\delta = 2.91$ (t, $J = 7$ Hz, 2H, CH_2), 3.99 (s, 3H, OMe), 4.02 (s, 3H, OMe), 4.15 (t, $J = 7$ Hz, 2H, CH_2), 7.00 (d, $J = 16$ Hz, 1H), 7.19 (s, 1H), 7.78 (d, $J = 16$ Hz, 1H), 7.86 (s, 1H). – ^{13}C NMR (75 MHz, DMSO): 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 (EI, 70 eV): $m/z(\%) = 346$ (86) $[M–N_2]^+$, 331 (100). – $C_{19}H_{14}N_6O_3$ (374.3): calcd. C 60.96, H 3.77, N 22.45; found C 60.74, H 3.85, N 22.19.

*Synthesis of (E)-2-amino-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 a 4 : 1 MeOH:H_2O (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 H_2O (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): ν = 3367, 3267 (NH_2), 2225 (CN), 2198 (CN), 1639 (C=O) cm^{-1}. – ^{1}H NMR (300 MHz, DMSO): $\delta = 2.88$ (t, $J = 7$ Hz, 2H, CH_2), 3.51 (s, 3H, OMe), 3.88 (s, 3H, OMe), 4.00 (t, $J = 7$ Hz, 2H, CH_2), 6.86 (d, $J = 16$ Hz, 1H), 7.07 (s, 1H), 7.21 (s, 2H, NH_2), 7.75 (d, $J = 16$ Hz, 1H), 7.81 (s, 1H). – ^{13}C 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 (EI, 70 eV): $m/z(\%) = 348$ (100) $[M]^+$. – $C_{19}H_{16}N_4O_3$ (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-2-((triphenylphosphoranylidene)-amino)-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**. Canary-yellow crystals; m. p. 270 – 272 °C; yield: 2.31 g (76 %). – IR (KBr): ν = 2225 (CN), 2203 (CN), 1641 (C=O) cm^{-1}. – ^{1}H NMR (300 MHz, DMSO): $\delta = 2.85$ (t, $J = 7$ Hz, 2H, CH_2), 3.70 (s, 3H, OMe), 3.84 (s, 3H, OMe), 4.00 (t, $J = 7$ Hz, 2H, CH_2), 6.58 (d, $J = 16$ Hz, 1H), 7.04 (s, 1H), 7.43 (d, $J = 16$ Hz, 1H), 7.47 (s, 1H), 7.58 – 7.71 (m, 15H). – ^{13}C NMR (75 MHz, DMSO): δ = 26.82, 40.32, 55.55, 55.78, 90.16, 90.30, 91.89, 109.02, 109.10, 110.68, 111.85, 118.14, 119.63, 120.19, 128.34, 128.96, 129.45, 129.13, 129.73, 132.12, 132.26, 132.66, 132.69, 133.06, 144.38, 146.54, 149.88, 152.00, 159.97, 160.65, 162.15. – MS (EI, 70 eV): $m/z(\%) = 608$ (15) $[M]^+$, 262 (100). – $C_{37}H_{29}N_4O_3P$ (608.6): calcd. C 73.02, H 4.80, N 9.21, P 5.09; found C 72.84, H 4.65, N 9.06, P 5.31.

*Synthesis of 2,3-dimethoxy-8-oxo-11-(phenylamino)-6,8-dihydro-5H-isoquinolino[2,1-g]-[1,6]naphthyridine-10,13-dicarbonitrile (**11**)*

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): ν = 3255 (NH), 2227 (CN), 2204 (CN), 1665 (C=O) cm^{-1}. – ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 2.90$ (t, $J = 7$ Hz, 2H, CH_2), 3.84 (s, 3H, OMe), 3.88 (s, 3H, OMe), 4.05 (t, $J = 7$ Hz, 2H, CH_2), 7.05 (s, 1H), 7.10 – 7.99 (m, 6H), 8.58 (s, 1H), 9.52 (s, 1H, NH). – ^{13}C NMR (75 MHz, $CDCl_3$): 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, 130.62, 133.45, 138.53, 143.81, 146.87, 152.43, 154.27, 155.57, 158.49. – MS (EI, 70 eV): $m/z(\%) = 449$ (100) $[M]^+$, 448 (54). – $C_{26}H_{19}N_5O_3$ (449.4): calcd. C 69.48, H 4.26, N 15.58; found C 69.71, H 4.22, N 15.63.

*Synthesis of (E)-2-amino-3-(2-cyanovinyl)-9,10-dimethoxy-4-oxo-6,7-dihydro-4H-pyrido[2,1-a]isoquinoline-1-carbonitriles **13a–c***

General procedure: A mixture of compound **5** (1.10 g, 3.0 mmol) and an amine **12a–c** (3.0 mmol) in absolute EtOH

(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_3CN 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): $\nu = 3249$ (NH), 2225 (CN), 2210 (CN), 1669 (C=O) cm^{-1}. – ^1H 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, $CDCl_3$): $\delta = 26.71, 41.02, 49.63, 55.76, 55.98, 83.35, 101.02, 110.96, 112.35, 115.36, 116.45, 118.13, 119.27, 127.50, 127.96, 128.33, 129.02, 134.52, 136.89, 138.92, 146.24, 152.10, 152.68, 156.02$. – MS (EI, 70 eV): $m/z(\%) = 438$ (9) $[M]^+$, 398 (85), 91 (100). – $C_{26}H_{22}N_4O_3$ (438.4): calcd. C 71.22, H 5.06, N 12.78; found C 71.12, H 4.94, N 12.93.

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

Yellow crystals; m. p. 212 – 214 °C; yield: 1.01 g (78 %). – IR (KBr): $\nu = 3299$ (NH), 2228 (CN), 2209 (CN), 1665 (C=O) cm^{-1}. – ^1H NMR (300 MHz, $CDCl_3$): $\delta = 1.24$ (m, 10H), 2.91 (t, $J = 7$ Hz, 2H, CH_2), 3.80 (s, 3H, OMe), 3.86 (s, 3H, OMe), 4.01 (t, $J = 7$ Hz, 2H, CH_2), 4.20 (m, 1H), 5.90 (d, 1H, NH), 6.82 (s, 1H), 6.85 (d, $J = 16$ Hz, 1H), 7.47 (d, $J = 16$ Hz, 1H), 7.75 (s, 1H). – ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 24.81, 24.99, 26.75, 33.75, 40.40, 55.85, 55.97, 56.46, 82.13, 102.45, 111.25, 112.64, 115.23, 115.61, 117.86, 118.71, 133.59, 137.24, 146.70, 150.64, 151.22, 152.49, 156.33$. – MS (EI, 70 eV): $m/z(\%) = 430$ (36) $[M]^+$, 390 (100), 333 (78). – $C_{25}H_{26}N_4O_3$ (430.5): calcd. C 69.75, H 6.09, N 13.01; found C 69.79, H 5.82, N 13.14.

*(E)-3-(2-Cyanovinyl)-2-((3,4-dimethoxyphenethyl)amino)-9,10-dimethoxy-4-oxo-6,7-dihydro-4H-pyrido[2,1-a]isoquinoline-1-carbonitrile (**13c**)*

Yellow crystals; m. p. 92 °C; yield: 1.18 g (77 %). – IR (KBr): $\nu = 3260$ (NH), 2225 (CN), 2207 (CN), 1657 (C=O) cm^{-1}. – ^1H NMR (300 MHz, $CDCl_3$): $\delta = 2.87$ (t, $J = 7$ Hz, 2H, CH_2), 2.91 (t, $J = 7$ Hz, 2H, CH_2), 3.76 (t, $J = 7$ Hz, 2H, CH_2), 3.81 (s, 3H, OMe), 3.83 (s, 3H, OMe), 3.94 (s, 3H, OMe), 3.97 (s, 3H, OMe), 4.12 (t, $J = 7$ Hz, 2H, CH_2), 4.98 (s, 1H, NH), 6.70 (d, $J = 16$ Hz, 1H), 6.74 – 6.77 (m, 3H), 6.81 (s, 1H), 7.23 (d, $J = 16$ Hz, 1H), 7.73 (s, 1H). – ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 27.59, 36.92, 39.32, 51.00, 55.74, 55.80, 56.12, 56.23, 82.16, 95.54, 100.87, 110.17, 111.55, 111.81, 112.00, 117.63, 118.23, 120.19, 120.99, 129.32, 132.53, 142.00, 147.69, 148.02, 149.11, 150.35, 152.79, 155.81, 159.98$. – MS (EI, 70 eV): $m/z(\%) = 512$ (14) $[M]^+$, 348 (100), 163 (91). – $C_{29}H_{28}N_4O_5$ (512.5): calcd. C 67.96, H 5.51, N 10.93; found C 67.69, H 5.48, N 11.02.

*Synthesis of (E)-2-(arylthio)-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_3CN to give compounds **15a–c**.

*(E)-3-(2-Cyanovinyl)-9,10-dimethoxy-4-oxo-2-(phenylthio)-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}. – ^1H NMR (300 MHz, $CDCl_3$): $\delta = 2.90$ (t, $J = 7$ Hz, 2H, CH_2), 3.86 (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, $CDCl_3$): $\delta = 27.27, 40.21, 55.96, 56.09, 93.70, 102.39, 110.01, 111.90, 116.95, 117.77, 118.79, 122.94, 129.34, 129.83, 130.15, 132.04, 138.22, 143.12, 147.59, 150.02, 150.44, 153.00, 158.41$. – MS (EI, 70 eV): $m/z(\%) = 441$ (100) $[M]^+$, 440 (92), 364 (74). – $C_{25}H_{19}N_3O_3S$ (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}. – ^1H NMR (300 MHz, $CDCl_3$): $\delta = 2.28$ (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, $CDCl_3$): $\delta = 20.88, 27.27, 40.22, 55.96, 56.09, 93.70, 102.39, 110.01, 111.90, 116.95, 117.77, 118.79, 122.94, 129.34, 129.83, 130.15, 132.04, 138.22, 143.12, 147.59, 150.02, 150.44, 153.00, 158.41$. – MS (EI, 70 eV): $m/z(\%) = 455$ (76) $[M]^+$, 415 (66), 91 (100). – $C_{26}H_{21}N_3O_3S$ (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.

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

Yellow crystals; m. p. 174 – 176 °C; yield: 1.08 g (76 %). – IR (KBr): $\nu = 2215$ (CN), 2209 (CN), 1652 (C=O) cm^{-1}. – ^1H NMR (300 MHz, $CDCl_3$): $\delta = 2.91$ (t, $J = 7$ Hz, 2H, CH_2), 3.86 (s, 3H, OMe), 3.90 (s, 3H, OMe), 4.28 (t, $J = 7$ Hz, 2H, CH_2), 6.79 (s, 1H), 6.99 (d, $J = 16$ Hz, 1H), 7.21 – 7.46 (m, 4H), 7.82 (s, 1H), 7.88 (d, $J = 16$ Hz, 1H). – ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 27.29$, 40.20, 55.85, 56.12, 93.36, 101.52, 109.78, 112.57, 117.00, 117.94, 119.21, 123.16, 129.24, 130.01, 130.25, 132.31, 139.16, 143.31, 147.88, 150.31, 150.45, 153.09, 158.70. – MS (EI, 70 eV): $m/z(\%) = 477$ (43) $[M+2]^+$, 475 (100) $[M]^+$, 364 (96). – $C_{25}H_{18}ClN_3O_3S$ (475.8): calcd. C 63.10, H 3.81, Cl 7.45, N 8.83, S 6.72; found C 63.25, H 3.99, Cl 7.36, N 8.61, S 6.84.

*Synthesis of 2-aroyl-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 hydroxamoyl 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 CH_3CN to give compounds **19a–f**.

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

Dark-green crystals; m. p. 256 – 258 °C; yield: 1.36 g (76 %). – IR (KBr): $\nu = 2202$ (CN) cm^{-1}. – ^1H NMR (300 MHz, DMSO): $\delta = 3.10$ (t, $J = 7$ Hz, 2H, CH_2), 3.79 (s, 3H, OMe), 3.85 (s, 3H, OMe), 4.26 (t, $J = 7$ Hz, 2H, CH_2), 7.11 (s, 1H), 7.65 – 7.96 (m, 6H). – MS (EI, 70 eV): $m/z(\%) = 359$ (100) $[M]^+$, 358 (59). – $C_{21}H_{17}N_3O_3$ (359.3): calcd. C 70.18, H 4.77, N 11.69; found C 70.05, H 4.70, N 11.83.

*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): $\nu = 2205$ (CN) cm^{-1}. – ^1H NMR (300 MHz, DMSO): $\delta = 3.07$ (t, $J = 7$ Hz, 2H, CH_2), 3.76 (s, 3H, OMe), 3.82 (s, 3H, OMe), 3.86 (s, 3H, OMe), 4.25 (t, $J = 7$ Hz, 2H, CH_2), 7.09 (s, 1H), 7.18 (d, $J = 7.5$ Hz, 2H), 7.58 (s, 1H), 7.88 (d, $J = 7.5$ Hz, 2H). – ^{13}C NMR (75 MHz, DMSO): $\delta = 27.21$, 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). – $C_{22}H_{19}N_3O_4$ (389.4): calcd. C 67.86, H 4.92, N 10.79; found C 67.62, H 5.12, N 10.96.

*2-(4-Bromobenzoyl)-8,9-dimethoxy-5,6-dihydropyrazolo-[5,1-a]isoquinoline-1-carbonitrile (**19c**)*

Reddish-brown crystals; m. p. 260 – 262 °C; yield: 1.73 g (79 %). – IR (KBr): $\nu = 2211$ (CN) cm^{-1}. – ^1H NMR (300 MHz, DMSO): $\delta = 3.08$ (t, $J = 7$ Hz, 2H, CH_2), 3.76 (s, 3H, OMe), 3.86 (s, 3H, OMe), 4.24 (t, $J = 7$ Hz, 2H, CH_2), 7.10 (s, 1H), 7.60 (s, 1H), 7.85 (d, $J = 7.5$ Hz, 2H), 7.88 (d, $J = 7.5$ Hz, 2H) ppm. – ^{13}C NMR (75 MHz, DMSO): $\delta = 27.09$, 42.69, 55.58, 55.79, 69.46, 107.36, 111.88, 116.11, 116.41, 124.86, 125.62, 128.88, 131.59, 133.73, 138.88, 147.29, 147.93, 150.90, 159.55. – MS (EI, 70 eV): $m/z(\%) = 439$ (100) $[M+2]^+$, 437 (97) $[M]^+$. – $C_{21}H_{16}BrN_3O_3$ (438.2): calcd. C 57.55, H 3.68, Br 18.23, N 9.59; found C 57.51, H 3.54, Br 18.16, N 9.72.

*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): $\nu = 2182$ (CN), cm^{-1}. – ^1H NMR (300 MHz, DMSO): $\delta = 3.09$ (t, $J = 7$ Hz, 2H, CH_2), 3.75 (s, 3H, OMe), 3.83 (s, 3H, OMe), 4.29 (t, $J = 7$ Hz, 2H, CH_2), 7.11 (s, 1H), 7.57 (s, 1H), 8.01 (d, $J = 7.5$ Hz, 2H), 8.13 (d, $J = 7.5$ Hz, 2H). – ^{13}C NMR (75 MHz, DMSO): $\delta =$ 27.09, 42.71, 55.49, 55.70, 69.35, 107.39, 111.75, 116.10, 116.42, 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). – $C_{21}H_{16}N_4O_5$ (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 (**19e**)*

Yellowish-brown crystals; m. p. 264 – 266 °C; yield: 1.41 g (77 %). – IR (KBr): $\nu = 2210$ (CN) cm^{-1}. – ^1H NMR (300 MHz, DMSO): $\delta = 3.12$ (t, $J = 7$ Hz, 2H, CH_2), 3.77 (s, 3H, OMe), 3.84 (s, 3H, OMe), 4.41 (t, $J = 7$ Hz, 2H, CH_2), 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). – $C_{19}H_{15}N_3O_3S$ (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-(2-naphthoyl)-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): $\nu = 2209$ (CN) cm^{-1}. – ^1H NMR (300 MHz, DMSO): $\delta = 3.12$ (t, $J = 7$ Hz, 2H, CH_2), 3.81 (s, 3H, OMe), 3.86 (s, 3H, OMe), 4.34 (t, $J = 7$ Hz, 2H,

CH_2), 7.13 (s, 1H), 7.57 – 8.55 (m, 8H). – MS (EI, 70 eV): $m/z(\%) = 409$ (4) $[M]^+$, 154 (100). Anal. for $C_{25}H_{19}N_3O_3$ (409.4): calcd. C 73.34, H 4.68, N 10.26; found C 73.39, H 4.79, N 9.98.

Synthesis of 9-aryl-2,3-dimethoxy-5,6-dihydropyridazino-[4′,5′:3,4]pyrazolo[5,1-a]isoquinolin-12-amines ***20***

General procedure: A mixture of a 2-aroyl-8,9-dimethoxy-5,6-dihydropyrazolo[5,1-*a*]isoquinoline- 1 -carbonitrile **19a**, **e** (5 mmol) in ethanol (30 mL) and hydrazine hydrate 99 % (0.7 g, 14 mmol) was refluxed for 6 h, during which the corresponding pyridazino-[4′,5′:3,4]pyrazoloisoquinoline **20a**, **e** precipitated. The resulting solid products were collected, washed with ethanol and crystallized from DMF to give compounds **20**.

*2,3-Dimethoxy-9-phenyl-5,6-dihydropyridazino-[4′,5′:3,4]pyrazolo[5,1-a]isoquinolin-12-amine (**20a**)*

Yellow crystals; m. p. 280 – 282 °C; yield: 1.31 g (70 %). – IR (KBr): ν = 3340, 3244 (NH_2) cm^{-1}. – ^1H NMR (300 MHz, DMSO): $\delta = 2.81$ (t, $J = 7$ Hz, 2H, CH_2), 3.16 (t, $J = 7$ Hz, 2H, CH_2), 3.77 (s, 3H, OMe), 3.86 (s, 3H, OMe), 5.62 (s, 2H, NH_2), 7.06 (s, 1H), 7.19 (s, 1H), 7.45 – 8.62 (m, 5H). – MS (EI, 70 eV): $m/z(\%) = 373$ (7) $[M]^+$, 149 (100). – $C_{21}H_{19}N_5O_2$ (373.4): calcd. C 67.55, H 5.13, N 18.76; found C 67.51, H 4.92, N 18.83.

*2,3-Dimethoxy-9-(thiophen-2-yl)-5,6-dihydropyridazino-[4′,5′:3,4]pyrazolo[5,1-a]isoquinolin-12-amine (**20e**)*

Yellow crystals; m. p. 268 – 270 °C; yield: 1.36 g (72 %). – IR (KBr): ν = 3475, 3348 (NH_2) cm^{-1}. – ^1H NMR (300 MHz, DMSO): $\delta = 2.82$ (t, $J = 7$ Hz, 2H, CH_2), 3.17 (t, $J = 7$ Hz, 2H, CH_2), 3.76 (s, 3H, OMe), 3.85 (s, 3H, OMe), 5.62 (s, 2H, NH_2), 7.04 (s, 1H), 7.16 (s, 1H), 7.23 (t, J = 7.5 Hz, 1H), 7.64 (d, $J = 7.5$ Hz, 1H), 8.45 (d, $J = 7.5$ Hz, 1H). – MS (EI, 70 eV): $m/z(\%) = 379$ (4) $[M]^+$, 365 (100). – $C_{19}H_{17}N_5O_2S$ (379.3): calcd. C 60.15, H 4.52, N 18.46, S 8.43; found C 60.01, H 4.70, N 18.55, S 8.67.

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 % CO_2. 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 % CO_2 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].

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