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SYNTHESIS, ANTIMICROBIAL AND ANTITUMOR STUDY OF NEW PYRIDO[2,1-*a*]ISOQUINOLINES *VIA* ISOQUINOLINE-1-ACETONITRILE

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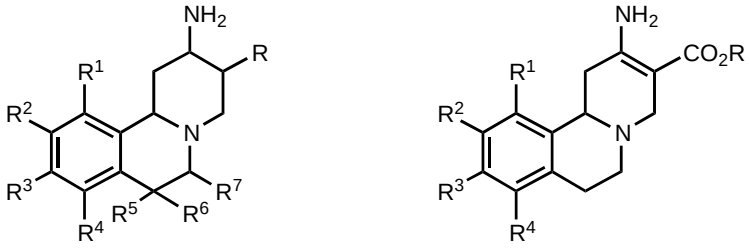
Abstract – Refluxing of enaminonitrile **3** with arylacetonitriles **2** in ethanol in the presence of piperidine afforded 4*H*-pyrido[2,1-*a*]isoquinoline-1-carbonitriles **6**. Refluxing of 2-(6,7-dimethoxy-3,4-dihydroisoquinolin-1-yl)acetonitrile **1** with ethyl 3-aryl-2-cyanoacrylates **9** in acetonitrile in the presence of piperidine gave the corresponding 4*H*-pyrido[2,1-*a*]isoquinoline-1,3-dicarbonitriles **13**. All the new synthesized compounds were identified by elemental analysis and spectral data. Cytotoxic assay was investigated for *in vitro* antitumor screening against MCF7, HepG2 and HCT-116 cell lines. Molecular docking using Mcule.com. software was carried out for the most potent compound **6b**. The results are compared with doxorubicin standard anticancer drug. Antimicrobial activities were investigated, and all compounds revealed no antimicrobial activities against all tested strains except compounds **13b** and **13e**.

INTRODUCTION

Bridge headed nitrogen heterocyclic compounds showed a significant role as natural and synthetic products.[1-3] Many compounds bearing such moiety exhibit beneficial biological activities, in addition to acting as agrochemical or pharmaceutical agents.[4-11] For example, azaphenanthrene of type (**I**) and (**II**) (Figure 1) containing pyrido[2,1-*a*]isoquinoline moiety are useful for treatment and/or prophylaxis of diabetes associated with enzyme DPP IV (dipeptidyl peptidase IV), such as diabetes mellitus and firstly non-insuline dependent diabetes mellitus and disturbed tolerance of glucose.[12-14] To our knowledge, only a few methods are available in literature for synthesis of pyrido[2,1-*a*]isoquinolines.[15,16] Most of reported protocols for their synthesis need transition metals, multisynthetic steps and low yields.[15,16] In

continuation to our work on utility of isoquinolines in the synthesis of such heterocyclic compounds,[17-29] we reported herein efficient, metal free and one-pot synthesis of new 4*H*-pyrido[2,1-*a*]isoquinoline derivatives using 2-(6,7-dimethoxy-3,4-dihydroisoquinolin-1-yl)acetonitrile. Also, we studied the biological activities of the target compounds.



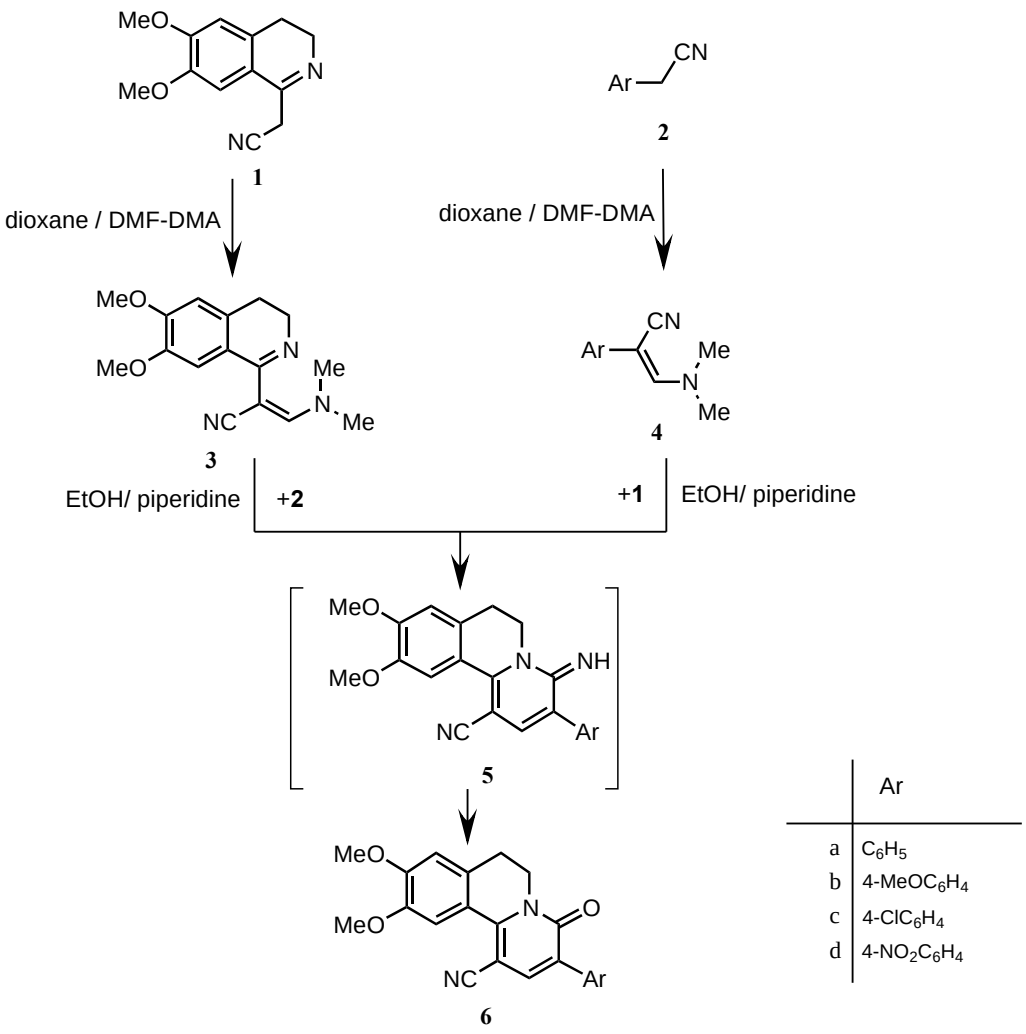
I **II**

Figure 1. Structure of azaphenanthrene

RESULTS AND DISCUSSION

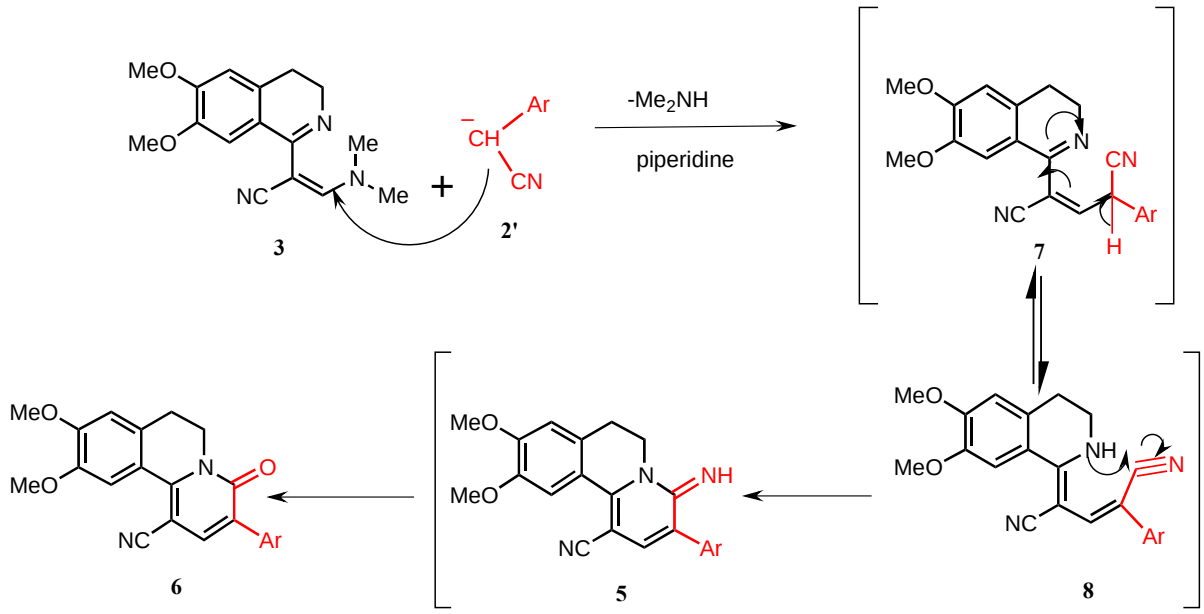
Treatment of 2-(6,7-dimethoxy-3,4-dihydroisoquinolin-1-yl)acetonitrile **1** with *N*,*N*-dimethylformamide dimethyl acetal (DMF-DMA) in refluxing dioxane afforded enaminonitrile **3**.[26] Refluxing of the latter enaminonitrile **3** with 2-arylacetonitriles **2** in ethanol in the presence of piperidine yielded 4*H*-pyrido[2,1-*a*]isoquinoline-1-carbonitrile derivatives **6a-d** (Scheme 1). The latter products **6a-d** were also prepared by treatment of enaminonitriles **4a-d**[30,31] [prepared by refluxing of 2-arylacetonitriles **2** with *N*,*N*-dimethylformamide dimethyl acetal in dioxane] with **1** in refluxing ethanol in the presence of piperidine (Scheme 1).

The proposed mechanism was the reacting of **3**, being the Michael acceptor, with 2-arylacetonitriles **2**, being the Michael donor, to afford the corresponding Michael adduct which underwent *in situ* cyclization to give intermediate **5** followed by hydrolysis during work up to yield the final product **6** (Scheme 2). The structures of products **6a-d** were identified by their elemental analysis and spectral data. For example, the IR spectrum of compound **6d** showed bands at ν 1620 and 2199 cm^{-1} assignable to CO and CN groups, respectively. Its ^{1}H NMR spectrum revealed five singlet signals at δ 3.82, 3.88, 7.10, 7.27 and 7.81 corresponding to two MeO groups, isoquinoline-H, the proton of the pyrido ring and another isoquinoline-H, respectively, a pair of doublet at δ 7.73 and 8.28 corresponding to a *para*-substituted benzene ring, in addition to two multiplet signals at δ 2.94-2.96 (CH_2 group of isoquinoline) and 4.13-4.15 (CH_2 group of isoquinoline). Its ^{13}C NMR revealed 20 signals for asymmetric carbon atoms. The mass spectrum showed a molecular ion beak at *m/z* = 403.

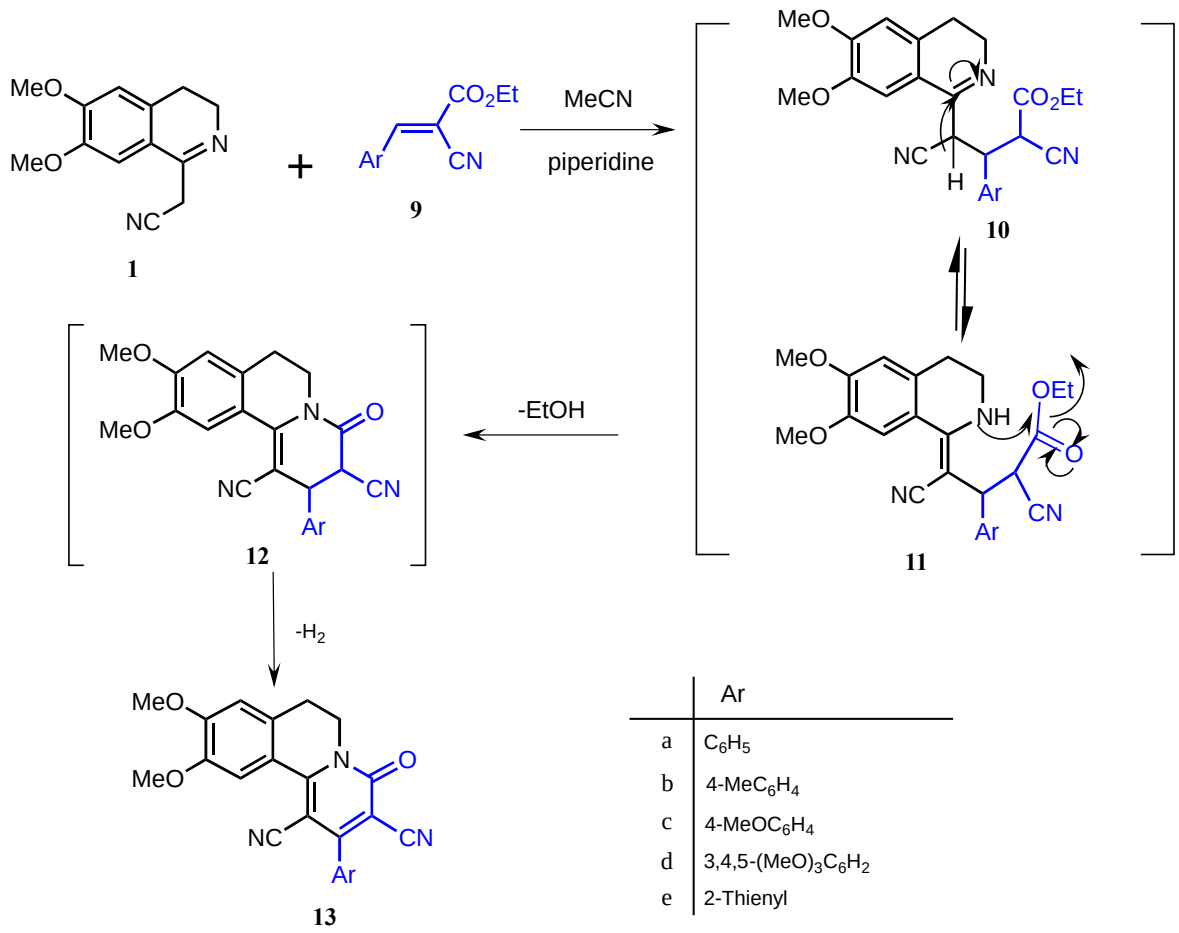


Scheme 1. Synthesis of 4*H*-pyrido[2,1-*a*]isoquinoline-1-carbonitrile derivatives **6a-d**

Reaction of 2-(6,7-dimethoxy-3,4-dihydroisoquinolin-1-yl)acetonitrile **1** with ethyl 3-aryl-2-cyanoacrylates **9a-e**[32,33] in refluxing acetonitrile and in the presence of piperidine yielded the corresponding 4*H*-pyrido[2,1-*a*]isoquinoline-1,3-dicarbonitrile derivatives **13a-e** (Scheme 3). The formation of **13** is supposed to be carried out by addition reaction of 2-(6,7-dimethoxy-3,4-dihydroisoquinolin-1-yl)acetonitrile **1** to ethyl 3-aryl-2-cyanoacrylates **9** to give the corresponding Michael adduct followed by cyclization *via in situ* elimination of ethanol to afford intermediate **12**. The latter underwent aromatization *via in situ* oxidation to give **13**. The structures of the isolated products **13** were identified by their elemental analysis and spectral data. For example, the IR spectrum of **13b** revealed three characteristic bands at ν 1651, 2188 and 2214 cm^{-1} assignable to CO group and two CN groups, respectively. Its ^{1}H NMR showed a singlet signal at δ 2.44 assignable to 4-MeC_6H_4, a pair of doublet at δ 7.35 and 7.45 corresponding to a *para*-substituted benzene ring, in addition to the signals of isoquinoline moiety. Its ^{13}C NMR showed 22 signals for asymmetric carbon atoms. The mass spectrum showed a molecular ion peak at *m/z* = 397.



Scheme 2. Proposed pathway for synthesis of 4*H*-pyrido[2,1-*a*]isoquinoline-1-carbonitrile derivatives **6a-d**



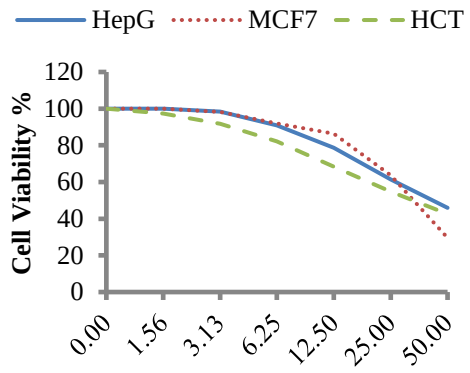
Scheme 3. Synthesis of 4*H*-pyrido[2,1-*a*]isoquinoline-1,3-dicarbonitrile derivatives **13a-e**

Antitumor Screening

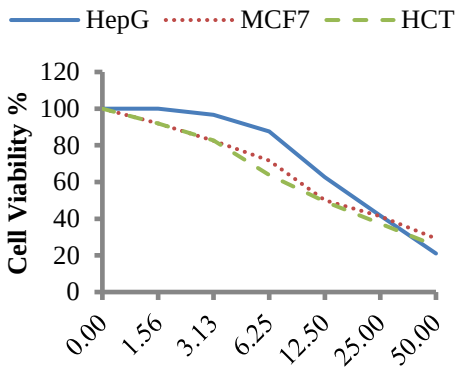
The new synthesized compounds **6a-d** were subjected to *in vitro* antitumor screening against different human cancer cell lines namely: HepG2, MCF7 and HCT-116. It is clear from IC_{50} values in Table 1, according to Shier scale all the tested compounds showed moderate to weak antitumor activity against HepG2, MCF7 and HCT-116. Compound **6b** revealed moderate activities against all tested cell lines followed by compound **6d** compared to the positive doxorubicin drug. On the other hand, compounds **6a** and **6c** showed very weak activities against all tested cell lines compared to the positive doxorubicin drug.

Table 1. IC_{50} of the tested compounds **6a-d** for their *in vitro* antitumor activity against (HepG2), (MCF7) and (HCT-116).

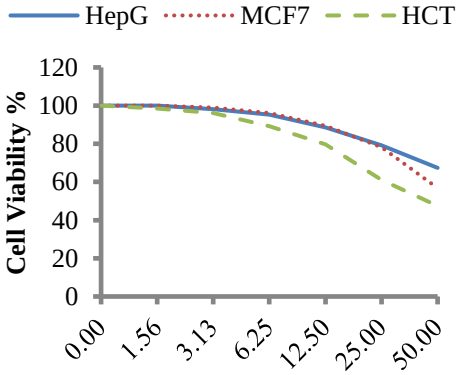
Compd.	IC_{50} (µg)		
	HepG2	MCF7	HCT-116
Doxorubicin	1.20	2.38	0.469
6a	43.4	35.0	34.7
6b	20.0	12.6	12.2
6c	>50	>50	45.3
6d	25.6	15.3	19.9



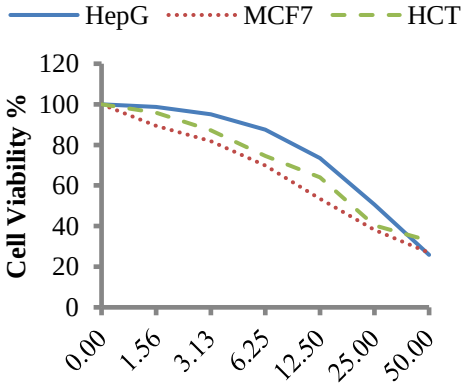
Antitumor cytotoxicity of different concentrations of compound **6a**



Antitumor cytotoxicity of different concentrations of compound **6b**



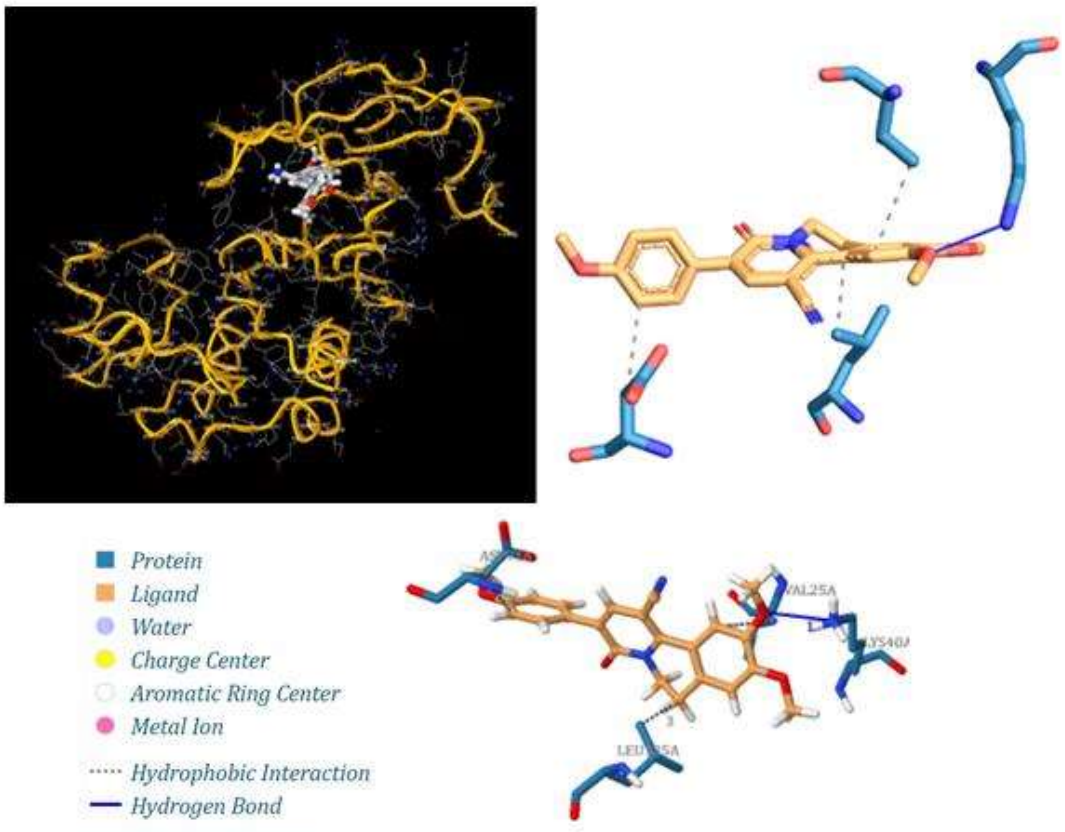
Antitumor cytotoxicity of different concentrations of compound **6c**



Antitumor cytotoxicity of different concentrations of compound **6d**

Modeling Studies

Docking studies visualization was performed using PLIP (Protein-Ligand Interaction Profiler).[34] We performed these studies to anticipate the binding mode between the ligand and the binding site of target proteins which is EGFR (epidermal growth factor receptor), one of the human proteins which are highly expressed in most tumor cells to validate and specify the mechanism of action in the context of anticancer activity. Protein was selected and downloaded from the Protein Data Bank (PDB ID: 4i23). From the results, four binding sites have been detected, three hydrophobic interactions and one hydrogen bond for this complex. The hydrophobic interactions with Val229, Asp819 and Leu 1282 with bond distances 3.75, and 3.82 and 3.54 A° respectively. The fourth interaction is hydrogen bonding between Lys373 and oxygen atom with bond distance 2.95 A° and angle 136.65 (Figure 2). All these binding modes confirmed



Hydrophobic Interactions ····

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1	25A	VAL	3.75	2728	229
2	91A	ASP	3.82	2746	819
3	135A	LEU	3.54	2738	1282

Hydrogen Bonds —

Index	Residue	AA	Distance H-A	Distance D-A	Donor Angle	Protein donor?	Side chain	Donor Atom	Acceptor Atom
1	40A	LYS	2.13	2.95	136.65	✓	✓	373 [N3+]	2752 [O2]

Figure 2. 3D and 2D dimensional modeling representation of compound **6b** with EGFR

our suggestion that 9,10-dimethoxy-3-(4-methoxyphenyl)-4-oxo-6,7-dihydro-4*H*-pyrido[2,1-*a*]-isoquinoline-1-carbonitrile **6b** may have weak anticancer activity and play a minor role in inhibiting the cancer progression.

Antimicrobial Screening

The antibacterial and antifungal activities of new synthesized compounds were studied by the disc diffusion method. The antibacterial activities were studied gainst the following pathogenic organisms; the gram-positive bacteria *Bacillus subtilis* and *Staphylococcus aureus*, the gram negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa*. Moreover, antifungal activities against *Candida albicans*

Table 2. *In vitro* antibacterial and antifungal activity of some new synthesized compounds (Inhibition zone in mm). (G^- = Gram negative; G^+ = gram positive; R. A. = relative activity)

Compounds	**Antimicrobial activity**									
	Bacterial species (G^+)				Bacterial species (G^-)				**Fungi**	
	Bacillus subtilis		*Staphylococcus aureus*		*Escherichia coli*		*Pseudomonas aeruginosa*		*Candida albicans*	
	IZ	RA%	IZ	RA%	IZ	RA%	IZ	RA%	IZ	RA%
Control: DMSO	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ampicillin	26	100	21	100	25	100	26	100		
Amphotericin									21	100
13b	15	57.7	12	57.1	13	52	9	34.6	16	76.2
13e	10	38.5	0.0	0.0	9.0	52.9	0.0	0.0	0.0	0.0

were studied. The synthesized compounds were used at the concentration of 20 mg/mL using DMSO as a solvent. The Ampicillin and the Gentamicin 10 μL/disc were used as a standard antibacterial agents and the Nystatin 20 μg/mL as standard antifungal agent. All the tested compounds showed no antimicrobial activities except for compounds **13b** and **13e**.

EXPERIMENTAL

Melting points were measured with a Stuart melting point apparatus and are uncorrected. The IR spectra were recorded using a FTIR Bruker–vector 22 spectrophotometer as KBr pellets. The ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ or DMSO–d_6 as solvent on Varian Gemini NMR spectrometer at 300 MHz and 75 MHz, respectively, using TMS as internal standard. Chemical shifts are reported as δ values in ppm. Mass spectra were recorded with a Shimadzu GCMS–QP–1000 EX mass spectrometer in EI (70

eV) model. The elemental analyses were performed at the Micro analytical center, Cairo University. The 2-(6,7-dimethoxy-3,4-dihydroisoquinolin-1-yl)acetonitrile **1**,[35] enaminonitrile **3**,[26] enaminonitrile **4a-d**[30,31] and ethyl 3-aryl-2-cyanoacrylate **9a-e**[32,33] were prepared using the reported procedures. *In vitro* antitumor activity was conducted at The Regional Center for Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt. The *in vitro* antimicrobial testing was performed at Microanalytical Center, Cairo University. The agar disc diffusion method and a panel of standard strains (*S. aureus* ATCC 6588, *B. subtilis* CMGB 215, *E. coli* ATCC 11775, *P. aeruginosa* ATCC 15442, and *C. albicans* ATCC 7102) were employed.

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

Method A:

To the appropriate solution of 2-(6,7-dimethoxy-3,4-dihydroisoquinolin-1-yl)-3-(dimethylamino)-acrylonitrile **3** (0.57 g, 2.0 mmol) and 2-arylacetonitriles **2** (2.0 mmol) in EtOH (20 mL), piperidine (0.50 mL) was added and the reaction mixture was refluxed for 6 h. the solvent was evaporated under reduced pressure and the residue was treated with MeOH (50 mL) where it solidified. The crude product was collected and crystallized from suitable solvent to give **6** in yield (60-52%).

Method B:

To the appropriate solution of 2-aryl-3-(dimethylamino)acrylonitriles **4a-d** (2.0 mmol) and 2-(6,7-dimethoxy-3,4-dihydroisoquinolin-1-yl)acetonitrile **1** (0.46 g, 2.0 mmol) in EtOH (20 mL), piperidine (0.5 mL) was added and the reaction mixture was refluxed for 6 h. The solvent was evaporated under reduced pressure and the residue was treated with MeOH (50 mL) where it solidified. The crude product was collected and crystallized from suitable solvent to give **6**. The compounds prepared with their physical data are listed below in yield (81-79%).

***9,10-Dimethoxy-4-oxo-3-phenyl-6,7-dihydro-4H-pyrido[2,1-a]isoquinoline-1-carbonitrile* (6a).** Yellow crystals; mp 200-202 °C (MeCN); yield (79%); IR (ν_{max}, cm^{-1}) ν 1631 (CO), 2204 (CN); ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.94-2.99 (m, 2H, CH_2), 3.81 (s, 3H, CH_3O), 3.87 (s, 3H, CH_3O), 4.15-4.20 (m, 2H, CH_2), 7.10 (s, 1H, isoquinoline-H), 7.12 (s, 1H, Ar-H), 7.30-7.41 (m, 5H, Ar-H), 7.79 (s, 1H, isoquinoline-H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 26.49, 40.18, 55.35, 55.63, 87.03, 110.82, 113.75, 119.43, 127.55, 128.36, 128.47, 128.95, 128.98, 131.84, 132.72, 137.22, 146.96, 148.63, 151.75, 159.34; MS (EI, 70 eV) *m/z* (%): 358 (M^+, 34.41), 356 (100), 178 (13.88), 102 (22.83), 51 (66.64). Anal. Calcd. for $C_{22}H_{18}N_2O_3$ (358.40): C, 73.73; H, 5.06; N, 7.82. Found: C, 73.83; H, 4.93; N, 7.71.

***9,10-Dimethoxy-3-(4-methoxyphenyl)-4-oxo-6,7-dihydro-4H-pyrido[2,1-a]isoquinoline-1-carbonitrile* (6b).** Yellow crystals; mp 244-246 °C (MeCN); yield (81%); IR (ν_{max}, cm^{-1}) ν 1643 (CO), 2206 (CN); ^{1}H

NMR (300 MHz, DMSO-d_6) δ 2.92-2.96 (m, 2H, CH_2), 3.78 (s, 3H, CH_3O), 3.82 (s, 3H, CH_3O), 3.86 (s, 3H, CH_3O), 4.13-4.16 (m, 2H, CH_2), 6.93 (d, J = 9 Hz, 2H, Ar-H), 7.08 (s, 1H, isoquinoline-H), 7.68 (d, J = 9 Hz, 2H, Ar-H), 7.88 (s, 1H, Ar-H), 7.92 (s, 1H, isoquinoline-H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 26.81, 40.13, 55.20, 55.69, 55.92, 86.39, 110.93, 113.53, 118.46, 119.55, 124.33, 127.50, 129.77, 131.24, 132.45, 137.09, 147.06, 148.41, 151.90, 159.11, 159.60; MS (EI, 70 eV) *m/z* (%): 388 (M^+, 100), 373 (44.03), 194 (14.91), 75 (9.48), 51 (9.020). Anal. Calcd. for $C_{23}H_{20}N_2O_4$ (388.42): C, 71.12; H, 5.19; N, 7.21. Found: C, 71.05; H, 5.31; N, 7.35.

***3-(4-Chlorophenyl)-9,10-dimethoxy-4-oxo-6,7-dihydro-4H-pyrido[2,1-a]isoquinoline-1-carbonitrile* (6c).** Yellow crystals; mp 249-251 °C (MeCN); yield (80%); IR (ν_{max}, cm^{-1}) ν 1630 (CO), 2201 (CN); ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.94-2.96 (m, 2H, CH_2), 3.82 (s, 3H, CH_3O), 3.88 (s, 3H, CH_3O), 4.15-4.17 (m, 2H, CH_2), 7.10-7.56 (m, 6H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 26.63, 40.23, 55.20, 55.67, 86.53, 110.40, 113.93, 119.69, 127.67, 129.00, 129.53, 129.89, 130.12, 132.52, 134.40, 137.10, 147.15, 148.43, 151.82, 159.01; MS (EI, 70 eV) *m/z* (%): 392 (M^+, 74.85), 391 (100), 178 (28.73), 140 (22.55), 75 (22.36). Anal. Calcd. for $C_{22}H_{17}ClN_2O_3$ (392.84): C, 67.26; H, 4.36; Cl, 9.02; N, 7.13. Found: C, 67.39; H, 4.24; Cl, 9.11; N, 7.23.

***9,10-Dimethoxy-3-(4-nitrophenyl)-4-oxo-6,7-dihydro-4H-pyrido[2,1-a]isoquinoline-1-carbonitrile* (6d)**. Red crystals; mp 260-262 °C (DMF); yield (81%); IR (ν_{max}, cm^{-1}) ν 1620 (CO), 2199 (CN); ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.94-2.96 (m, 2H, CH_2), 3.82 (s, 3H, CH_3O), 3.88 (s, 3H, CH_3O), 4.13-4.15 (m, 2H, CH_2), 7.10 (s, 1H, isoquinoline-H), 7.27 (s, 1H, Ar-H), 7.73 (d, 2H, Ar-H), 7.81 (s, 1H, isoquinoline-H), 8.28 (d, 2H, Ar-H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 26.38, 40.09, 55.46, 55.83, 86.67, 110.54, 113.57, 119.67, 122.73, 127.72, 129.81, 130.19, 132.58, 137.05, 139.30, 146.99, 148.22, 148.53, 152.16, 159.27; MS (EI, 70 eV) *m/z* (%): 403 (M^+, 46.21), 238 (26.89), 156 (42.33), 91 (76.94), 51 (100). Anal. Calcd. for $C_{22}H_{17}N_3O_5$ (403.39): C, 65.50; H, 4.25; N, 10.42. Found: C, 65.58; H, 4.32; N, 10.30.

***Synthesis of 2-aryl-9,10-dimethoxy-4-oxo-6,7-dihydro-4H-pyrido[2,1-a]isoquinoline-1,3-dicarbonitrile* (13a-e):**

To a solution of the appropriate ethyl 3-aryl-2-cyanoacrylates **9a-e** (10 mmol) and 2-(6,7-dimethoxy-3,4-dihydroisoquinolin-1-yl)acetonitrile **1** (2.3 g, 10 mmol) in MeCN (10 mL) was added piperidine (0.08 mL, 10 mmol) at room temperature. The reaction mixture was refluxed for 6 h, the solvent was evaporated under reduced pressure and the residue was treated with MeOH (5 mL) where it solidified. The crude products was collected and crystallized from suitable solvent to give **13**.

***9,10-Dimethoxy-4-oxo-2-phenyl-6,7-dihydro-4H-pyrido[2,1-a]isoquinoline-1,3-dicarbonitrile* (13a).** Yellow crystals; mp 226-228 °C (MeCN); yield (81%); IR (ν_{max}, cm^{-1}) ν 1649 (CO), 2187 (CN), 2213 (CN); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.97-3.01 (m, 2H, CH_2), 3.95 (s, 3H, CH_3O), 3.99 (s, 3H, CH_3O),

4.28-4.32 (m, 2H, CH_2), 6.84 (s, 1H, isoquinoline-H), 7.51-7.55 (m, 5H, Ar-H), 7.96 (s, 1H, isoquinoline-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 26.73, 40.98, 56.36, 56.52, 89.12, 100.83, 109.86, 110.89, 113.64, 117.26, 117.52, 127.69, 128.73, 128.99, 133.29, 133.57, 148.02, 153.35, 153.52, 158.63, 160.86; MS (EI, 70 eV) *m/z* (%): 383 (M^+, 100), 382 (61.48), 368 (35.20), 352 (7.77), 296 (5.74). Anal. Calcd. for $C_{23}H_{17}N_3O_3$ (383.41): C, 72.05; H, 4.47; N, 10.96. Found: C, 72.11; H, 4.59; N, 10.89.

9,10-Dimethoxy-4-oxo-2-(p-tolyl)-6,7-dihydro-4H-pyrido[2,1-a]isoquinoline-1,3-dicarbonitrile **(13b).** Yellow crystals; mp 236-238 °C (MeCN); yield (80%); IR (ν_{max}, cm^{-1}) ν 1651 (CO), 2188 (CN), 2214 (CN); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.44 (s, 3H, 4-C$\underline{H}_3C_6H_4$), 2.96-3.01 (m, 2H, CH_2), 3.95 (s, 3H, CH_3O), 3.99 (s, 3H, CH_3O), 4.28-4.32 (m, 2H, CH_2), 6.84 (s, 1H, isoquinoline-H), 7.35 (d, 2H, Ar-H), 7.45 (d, 2H, Ar-H), 7.96 (s, 1H, isoquinoline-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.48, 27.36, 40.58, 56.30, 56.62, 89.43, 101.08, 110.21, 111.91, 114.54, 117.37, 117.83, 128.22, 129.54, 130.51, 133.15, 141.46, 147.85, 153.43, 153.68, 158.59, 161.14; MS (EI, 70 eV) *m/z* (%): 397 (M^+, 100), 396 (32.06), 383 (18.36), 382 (64.47), 366 (9.45). Anal. Calcd. for $C_{24}H_{19}N_3O_3$ (397.43): C, 72.53; H, 4.82; N, 10.57. Found: C, 72.64; H, 4.68; N, 10.64.

9,10-Dimethoxy-2-(4-methoxyphenyl)-4-oxo-6,7-dihydro-4H-pyrido[2,1-a]isoquinoline-1,3-dicarbonitrile **(13c).** Yellow crystals; mp 272-274 °C (DMF); yield (85%); IR (ν_{max}, cm^{-1}) ν 1652 (CO), 2191 (CN), 2214 (CN); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.96-2.99 (m, 2H, CH_2), 3.92, (s, 3H, CH_3O), 3.95 (s, 3H, CH_3O), 4.00 (s, 3H, CH_3O), 4.26-4.30 (m, 2H, CH_2), 6.83 (s, 1H, isoquinoline-H), 7.39 (d, J = 9 Hz, 2H, Ar-H), 7.51 (d, J = 9 Hz, 2H, Ar-H), 7.98 (s, 1H, isoquinoline-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 27.26, 41.35, 56.11, 56.26, 56.45, 89.32, 101.22, 109.54, 111.67, 114.35, 116.19, 117.38, 117.61, 124.83, 129.19, 133.33, 147.98, 153.44, 153.82, 158.61, 160.40, 160.93; MS (EI, 70 eV) *m/z* (%): 413 (M^+, 100), 412 (27.11), 399 (11.70), 398 (41.08), 382 (18.27). Anal. Calcd. for $C_{24}H_{19}N_3O_4$ (413.43): C, 69.72; H, 4.63; N, 10.16. Found: C, 69.81; H, 4.72; N, 10.27.

9,10-Dimethoxy-4-oxo-2-(3,4,5-trimethoxyphenyl)-6,7-dihydro-4H-pyrido[2,1-a]isoquinoline-1,3-dicarbonitrile **(13d).** Yellow crystals; mp 252-254 °C (MeCN); yield (82%); IR (ν_{max}, cm^{-1}) ν 1653 (CO), 2188 (CN), 2214 (CN); ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.96-3.00 (m, 2H, CH_2), 3.92 (s, 3H, CH_3O), 3.93 (s, 6H, $2CH_3O$), 3.96 (s, 3H, CH_3O), 4.00 (s, 3H, CH_3O), 4.28-4.32 (m, 2H, CH_2), 6.78 (s, 2H, Ar-H), 6.82 (s, 1H, isoquinoline-H), 7.98 (s, 1H, isoquinoline-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 27.39, 40.66, 56.30, 56.33, 56.51, 60.92, 89.35, 101.09, 105.99, 110.20, 112.02, 114.49, 117.21, 117.78, 128.26, 133.19, 140.37, 147.88, 153.25, 153.43, 153.75, 158.53, 160.69; MS (EI, 70 eV) *m/z* (%): 473 (M^+, 100), 458 (22.72), 430 (17.84), 398 (11.32), 344 (10.66). Anal. Calcd. for $C_{26}H_{23}N_3O_6$ (473.49): C, 65.95; H, 4.90; N, 8.87. Found: C, 65.84; H, 4.84; N, 8.98.

9,10-Dimethoxy-4-oxo-2-(thiophen-2-yl)-6,7-dihydro-4H-pyrido[2,1-a]isoquinoline-1,3-dicarbonitrile **(13e).** Red crystals; mp 238-240 °C (MeCN); yield (81%); IR (ν_{max}, cm^{-1}) ν 1652 (CO), 2188 (CN), 2214

(CN); ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.98-3.02 (m, 2H, CH_2), 3.83 (s, 3H, CH_3O), 3.91 (s, 3H, CH_3O), 4.13-4.17 (m, 2H, CH_2), 7.16-8.02 (m, 5H, Ar-H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 26.12, 40.40, 55.80, 56.08, 89.17, 99.80, 110.91, 112.48, 115.19, 117.46, 117.57, 127.91, 131.29, 131.82, 132.73, 134.43, 146.88, 152.98, 153.48, 153.94, 158.27; MS (EI, 70 eV) *m/z* (%): 389 (M^+, 100), 388 (55.03), 375 (10.37), 374 (37.74), 358 (9.49). Anal. Calcd. for $C_{21}H_{15}N_3O_3S$ (389.43): C, 64.77; H, 3.88; N, 10.79; S, 8.23. Found: C, 64.87; H, 3.82; N, 10.87; S, 8.36.

Cytotoxicity Assay

The cells were propagated in DMEM supplemented with 10% heat-inactivated FBS, 1% L-glutamine, HEPES buffer and 50 µg/mL gentamycin. All cells were maintained at 37 °C in humidified atmosphere with 5% CO_2 and were subcultured two times a week. Cell toxicity was monitored by determining the effect of the test samples on cell morphology and cell viability. The cells were seeded in 96-well plate at a cell concentration of 1×10^4 cells per well in 100 µL of growth medium. Fresh medium containing different concentration of the test sample was added after 24 h of seeding. Serial two-fold dilutions of the tested chemical compound were added to confluent cell monolayers dispensed into 96-well, flat-bottomed microtiter plates (Flacon, NJ, USA) using a multichannel pipette. The microtiter plates were incubated at 37 °C in a humiditifed incubator with 5% CO_2 for a period of 48 h. Three wells were used for each concentration of the test sample. Control cells were incubated without test sample and with or without DMSO. The little percentage of DMSO present in the wells (maximal 0.1%) was found not to affect the experiment. After incubation of the cells for 24 h at 37 °C, various concentrations of sample (50, 25, 12.5, 6.25, 3.125 and 1.56 µg) were added, and the incubation was continued for 48 h and viable cells yield was determined by a colorimetric method.[36]

Molecular Modeling

Modelling studies were done for the most active compound **6b**, to predict the protein-ligand interactions at the active site. Protein structure of EGFR (epidermal growth factor receptor) were downloaded from the Protein Data Bank (PDB ID: 4i23). Proteins were prepared for docking, and other instructions were described according to the previous literatures[37] for the modeling investigation using Mcule.com. By the end, the final data was visualized using PLIP (Protein-Ligand Interaction Profiler).[34]

Antimicrobial Activity Evaluation

Antimicrobial activity of the tested compounds was determined using a modified Kirby-Bauer disc diffusion method.[38] Briefly, 100 µL of the test bacteria/fungi were grown in 10 mL of fresh media until they reached a count of approximately 10^8 cells/mL for bacteria or 10^5 cells/mL for fungi[39] 100 µL of

microbial suspension was spread onto agar plates corresponding to the broth in which they were maintained. Of the many media available, NCCLS recommends Mueller-Hinton agar due to: it results in good batch-to-batch reproducibility. Disc diffusion method for yeasts developed by using approved standard method (M44-P) by the (NCCLS, 2009).[40]

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