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Synthesis and biological evaluation of new pyridines containing imidazole moiety as antimicrobial and anticancer agents

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Abstract: The synthesis of a novel series of pyridine and bipyridine derivatives is described via one-pot multicomponent reaction of 5-acetylimidazole, malonitrile (or ethylcyanoacetate or diethylmalonate), substituted benzaldehyde (or terephthaldehyde), and ammonium acetate in good yields. The structures of all the new compounds were elucidated on the basis of elemental analysis and spectral data. The antimicrobial activities of the synthesized compounds were screened and the results showed that most of such compounds exhibit considerable activities.

Furthermore, some of the newly synthesized compounds were screened for their anticancer activity against human breast cell line (MCF-7) and liver carcinoma cell line (HEPG2) in comparison to doxorubicin. Most of the tested compounds exhibited promising activity.

Key words: 5-Acetylimidazole, cyanopyridone, bipyridine, multicomponent reactions, anticancer activity

1. Introduction

Cancer is the second leading cause of death in both developed and developing countries.[1,2] Chemotherapy has become one of the methods adopted to treat cancer. Many compounds have been synthesized with this aim, but their clinical use has been limited by their relatively high risk of toxicity, because they lack specificity and produce adverse effects related to the impact on rapidly dividing noncancerous cells.[2,3] Therefore, to improve efficacy and decrease the adverse effect potential is one of the goals in developing new anticancer drugs. Another major goal for developing new anticancer agents is to overcome cancer resistance to drug treatment, which has made many of the currently available chemotherapeutic agents ineffective.[4]

Novel 2-oxo-1,2-dihydropyridine-3-carbonitrile derivatives were reported as inhibitors of the oncogenic serine/threonine kinase PIM-1, which plays a role in cancer cell survival, differentiation, and proliferation (Figure 1a).[5] Moreover, several cyanopyridines with higher lipophilic properties (Figure 1b) can inhibit survivin, which is a member of the inhibitors of apoptosis (IAP) family.[6] Survivin is highly expressed in most human tumors and fetal tissue but undetectable in most terminally differentiated adult tissues. This fact makes survivin an ideal target for cancer therapy.[7,8] Milrinone (Figure 1c) is a 3-cyanopyridine derivative that has been used for the treatment of congestive heart failure via PDE3 inhibition. Recent studies showed that PDE3, PDE4, and PDE5 are overexpressed in cancerous cells compared with in normal cells. In addition, inhibition of tumor cell growth and angiogenesis may be due to cross inhibition of PDE3 together with other PDEs.[9,10]

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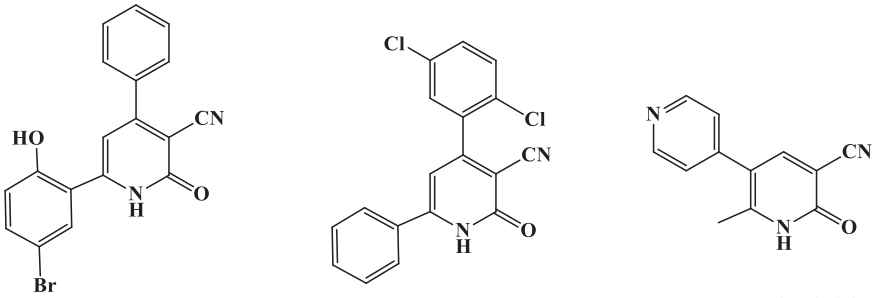


a. PIM-1 kinase inhibition **b. Survivin inhibition** **c. PDE3 inhibition**

Figure 1. Various 3-cyano-2-oxopyridine derivatives with potential growth inhibitory and/or antiangiogenic actions through PIM-1 kinase inhibition (a), survivin inhibition (b) or PDE3 inhibition (c).

Pyridines are a class of both synthetically and naturally occurring heterocyclic compounds with a wide range of biological applications.[11–13] Moreover, the current interest in the development of new antimicrobial and anticancer agents can be partially ascribed to both the increasing emerging resistance among new pathogens and the appearance of multidrug resistance, and adverse side effects are a serious threat to public health. Therefore, the development of new and efficacious drugs is a very important goal, and most of the research efforts in this field are directed towards the design of new agents.[4,14,15] It is reported that some important anticancer drugs possess a pyridine nucleus.[16–18] Thus, this study gives promising compounds possessing a pyridine nucleus that can be investigated for future in vivo and clinically oriented studies. It is suggested that the linkage between alpha carbons of pyridine is important for cytotoxic effects regardless of 4-substituents. From the structure–activity relationships, it is revealed that the terpyridine skeleton is important for cytotoxicity against several human cancer cell lines, which supports the previous results.[19–21]

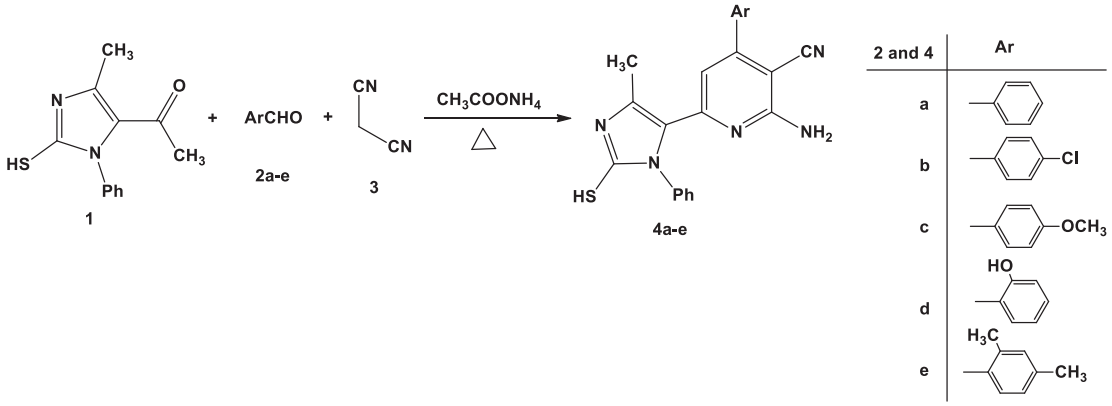
Multicomponent reactions (MCRs) are powerful tools in modern medicinal chemistry because such reactions have constituted an increasingly valuable approach to drug discovery efforts in recent years.[22–24]

In view of these observations and in continuation of our previous work,[25–34] we report herein the synthesis of some new derivatives of pyridines in MCRs and preliminarily evaluate their anticancer properties with the aim of obtaining better antimicrobial and anticancer drugs without side effects.

2. Results and discussion

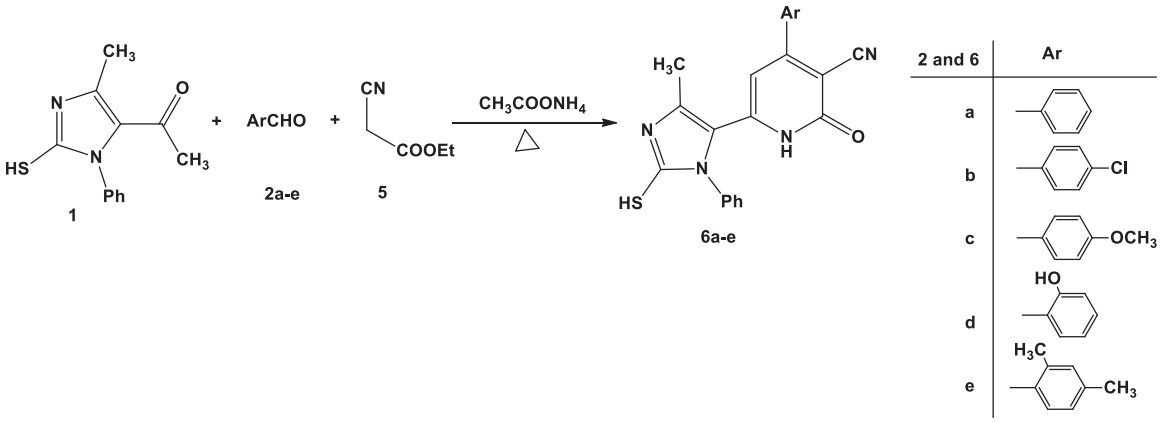
2.1. Chemistry

The required 5-acetyl-2-mercapto-4-methyl-1-phenyl-1*H*-imidazole **1** was prepared according to the literature method.[35] A series of 3-cyanopyridine derivatives **4a**–**e** were prepared by one-pot condensation of acetylimidazole **1**, an aldehyde **2a**–**e**, malononitrile **3**, and ammonium acetate in refluxing acetic acid (Scheme 1). The structures of compounds **4a**–**e** were confirmed by their spectral data. The IR spectra of compound **4a** showed CN and NH_2 groups in their expected locations at v_{max} = 2212, 3254, and 3431 cm^{-1}, respectively. The ^1H NMR of compound **4a** showed a singlet (1H) at δ = 8.03 ppm attributable to pyridine H-5, along with the expected D_2O exchangeable protons at δ =7.83 ppm assignable for NH_2 protons. Moreover, an EI mass spectroscopic technique gave its correct molecular ion peak at m/z = 383 (see Experimental section). The reaction goes in parallel to the literature.[36–38]



Scheme 1. Synthesis of pyridine derivatives **4a**–**e**.

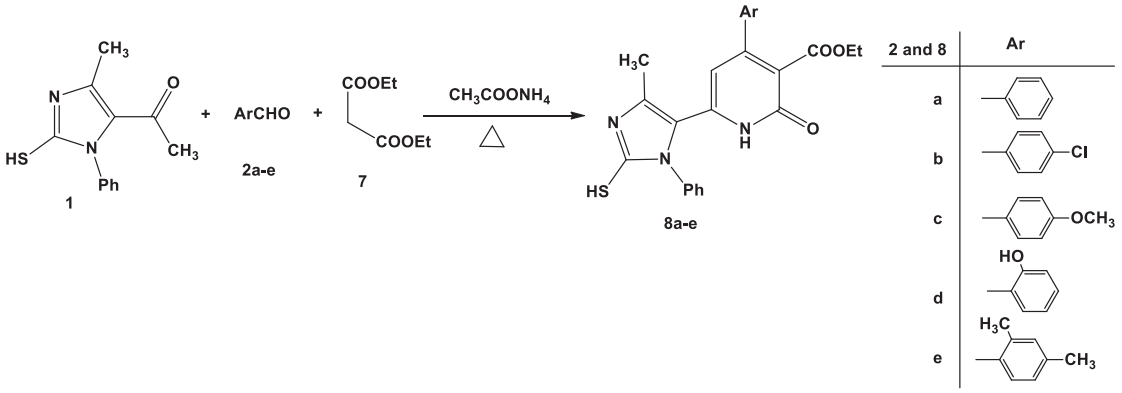
In a similar manner, acetyl compound **1** was condensed with the appropriate aromatic aldehydes and ethyl cyanoacetate in the presence of excess ammonium acetate in acetic acid to give the corresponding cyanopyridones **6a**–**e** in a one-pot reaction (Scheme 2).



Scheme 2. Synthesis of pyridine derivatives **6a**–**e**.

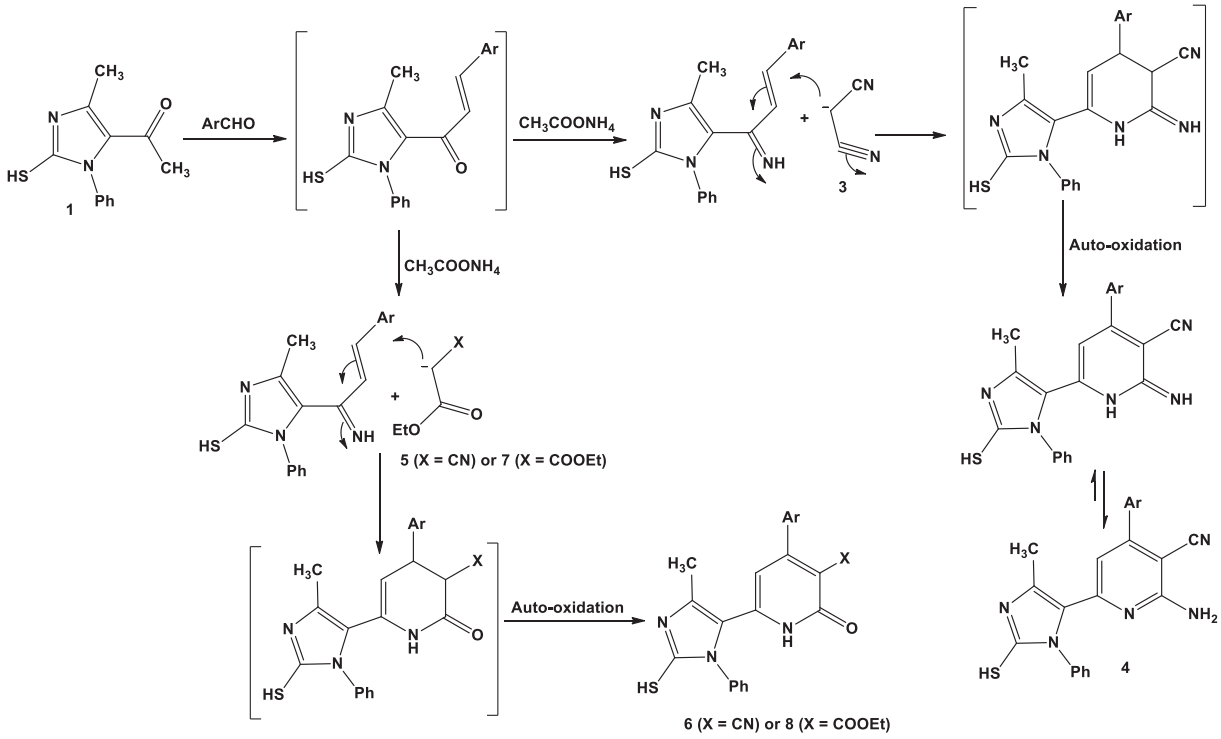
The structure of the isolated products was confirmed on the basis of their elemental analysis and spectral data. For example, taking compound **6a** as a typical example, its IR spectrum exhibited absorption bands at υ_{max} = 1668, 2221, and 3431 cm^{-1} due to CO, CN, and NH groups, respectively. Its ^{1}H NMR spectrum showed singlet signals (D_2O exchangeable) at δ = 11.32 ppm, due to NH proton, in addition to an aromatic multiplet in the region δ = 7.02–7.65 ppm, whereas the mass spectrum showed a peak corresponding to its molecular ion at m/z 384 (see Experimental section).

In addition, compound **1** was reacted with diethyl malonate, aldehyde, and ammonium acetate to give the corresponding ethyl 6-(imidazol-5-yl)-2-oxo-1,2-dihydropyridine-3-carboxylate derivatives **8a**–**e** (Scheme 3) based on elemental and spectral data. IR spectra for compound **8a** showed the stretching vibrations of 2CO and NH groups at 1667, 1725, and 3280 cm^{-1}, respectively. In addition, mass spectra of all derivatives displayed all correct molecular ion peaks. The ^{1}H NMR spectrum displayed characteristic signals at δ =1.22(t), 4.26(q), and 11.96 ppm related to the ethyl group and NH protons, respectively (see Experimental section).



Scheme 3. Synthesis of pyridine derivatives **8a**–**e**.

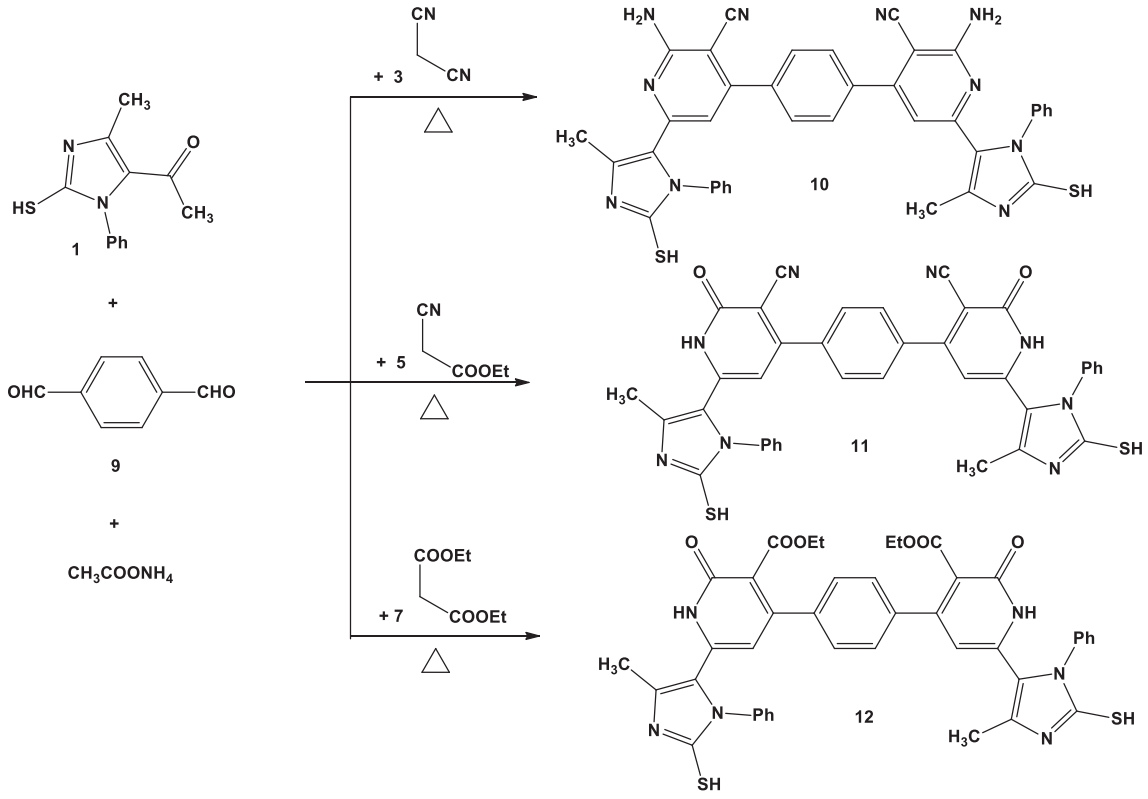
The mechanism of the one-pot synthesis of pyridine derivatives **4a**–**e**, **6a**–**e**, and **8a**–**e** is known to be through the formation of α, β-unsaturated ketones intermediate via the Claisen-Schmidt reaction between the ketone and aromatic aldehydes. This reaction is followed by condensation with active methylene compounds (e.g., malononitrile or ethyl cyanoacetate or diethyl malonate) through the Michael addition reaction in the presence of ammonium acetate, cyclization, and aromatization to afford the corresponding pyridine derivatives **4a**–**e**, **6a**–**e**, and **8a**–**e** (Scheme 4).



Scheme 4. Mechanism of the synthesis of pyridine derivatives **6a**–**e** and **8a**–**e**.

We extended our protocol to the synthesis of bipyridine derivatives (**10**–**12**) via reacting 5-acetylimidazole **1** (1.0 mmol) with terephthaldehyde (0.5 mmol), malononitrile (or ethyl cyanoacetate or diethyl malonate) (1.0 mmol) under optimized conditions to give the corresponding bipyridine derivatives (**10**–**12**) (Scheme 5). Structure confirmation of compounds **10**–**12** was assisted by their analytical and spectral data. For instance,

the IR spectrum of compound **10** displayed characteristic absorption bands at 3430 and 3140 cm^{-1} related to the NH_2 group as well as a cyano stretching vibration at 2211 cm^{-1}. Its ^1H NMR spectrum showed a singlet signal integrating for four protons ($2NH_2$) at 2.58 ppm, and a singlet signal at 7.82 ppm, which was assigned to the 5-H pyridine proton. The mass spectrum of **10** showed a peak in accordance with the proposed structure at m/z (%) = 688 (see Experimental section).



Scheme 5. Synthesis of bipyridine derivatives **10**–**12**.

2.2. Biology

2.2.1. Antimicrobial activity

The in vitro antibacterial activity of the newly synthesized compounds was evaluated against two gram-positive bacteria, namely *Staphylococcus pneumoniae* (SP) and *Bacillus subtilis* (BS), and two gram-negative bacteria, namely *Pseudomonas aeruginosa* (PA) and *Escherichia coli* (EC). They were also tested for their in vitro antifungal activity against three fungi species, namely *Aspergillus fumigatus* (AF), *Geotrichum candidum* (GC), *Candida albicans* (CA), and *Syncephalastrum racemosum* (SR). The organisms were tested against the activity of solutions of concentration (5 μg/mL) of each compound and using inhibition zone diameter (IZD) in mm as the criterion for antimicrobial activity (agar diffusion well method). The bactericides ampicillin and gentamicin and the fungicide amphotericin B were used as references to evaluate the potency of the tested compounds under the same conditions.

The results are summarized in Tables 1 and 2. They indicate the following:

1. Compounds **4c**, **4d**, and **8d** exhibit high inhibitory effects against *Staphylococcus pneumoniae*, while compounds **4a**, **4b**, **4e**, **8a**, and **10** exhibit moderate inhibitory effects.

2. Compounds **4c**, **6a**, **6e**, **8b**, **8d**, **10**, **11**, and **12** exhibit high inhibitory effects against *Bacillus subtilis*, while compounds **4a**, **4b**, **4e**, **6b**, and **8c** exhibit moderate inhibitory effects and no inhibitory effect towards *Pseudomonas aeruginosa*.

3. Compounds **4c**, **4d**, **6a**, **6e**, **8d**, and **12** exhibit high inhibitory effects against *Escherichia coli*.

4. Compounds **4c**, **4d**, **6a**, **8d**, **10**, **11**, and **12** exhibit high inhibitory activities against *Aspergillus fumigatus*, *Syncephalastrum racemosum*, and *Geotrichum candidum*, while compounds **4a** an**d 8a** have moderate inhibitory activity and all compounds have no activity against *Candida albicans*.

Table 1. Antibacterial activity of the synthesized compounds.

Compound	Inhibitory activity against the tested bacteria (zone of inhibition in mm)			
	Gram-positive bacteria		Gram-negative bacteria	
	Staphylococcus pneumoniae	*Bacillus subtilis*	*Pseudomonas aeruginosa*	*Escherichia coli*
4a	16.9 ± 0.37	15.7 ± 0.44	NA	12.9 ± 0.25
4b	15.9 ± 0.44	14.5 ± 0.37	NA	12.1 ± 0.58
4c	18.6 ± 0.44	20.8 ± 0.58	NA	18.6 ± 0.25
4d	19.4 ± 0.17	20.7 ± 0.29	NA	19.9 ± 0.42
4e	16.3 ± 0.44	15.5 ± 0.44	NA	12.4 ± 0.25
6a	16.6 ± 0.44	21.2 ± 0.37	NA	18.3 ± 0.44
6b	13.8 ± 0.44	15.2 ± 0.37	NA	10.3 ± 0.44
6c	9.7 ± 0.37	12.1 ± 0.19	NA	8.5 ± 0.37
6d	13.9 ± 0.44	17.5 ± 0.25	NA	10.7 ± 0.25
6e	16.8 ± 0.44	21.4 ± 0.37	NA	19.7 ± 0.44
8a	15.0 ± 0.44	18.3 ± 0.37	NA	11.1 ± 0.25
8b	16.3 ± 0.44	20.9 ± 0.37	NA	17.6 ± 0.44
8c	12.4 ± 0.58	16.3 ± 0.37	NA	10.4 ± 0.25
8d	21.6 ± 0.43	22.4 ± 0.25	NA	20.3 ± 0.44
8e	11.7 ± 0.58	12.0 ± 0.58	NA	9.8 ± 0.44
10	14.2 ± 0.44	19.4 ± 0.25	NA	12.8 ± 0.44
11	15.3 ± 0.44	21.0 ± 0.25	NA	13.9 ± 0.44
12	16.4 ± 0,25	22.6 ± 0.30	NA	19.6 ± 0.14
Ampicillin	23.8 ± 0.2	32.4 ± 0.3	-	-
Gentamicin	-	-	17.3 ± 0.1	19.9 ± 0.3

NA: No activity, data are expressed in the form of mean of inhibition zone diameter for test compound performed in triplicate ± SD.

2.2.2. Anticancer activity

The cytotoxicity of synthesized products **4b**, **4c**, **4d**, **6b**, **6d**, **8b**, **8c**, **10**, **11**, and **12** was evaluated against human breast cell line (MCF-7) and liver carcinoma cell line (HEPG-2) using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay Doxorubicin and vinblastine sulfate were used as reference drugs (IC_{50} value of 0.42 ± 0.03 and 5.7 ± 0.60 μg/mL against MCF-7 as well as 0.46 ± 0.04 and 4.6 ± 0.5 μg/mL, against HepG2, respectively). Data generated were used to plot a dose response curve, from which the concentration of test compounds required to kill 50% of the cell population (IC_{50}) was determined. Cytotoxic activity was expressed as the mean IC_{50} of three independent experiments. The results are represented in Tables 3–6. They indicated that:

Table 2. Antifungal activity of the synthesized compounds.

Compound	Inhibitory activity against the tested fungi (zone of inhibition in mm)			
	Aspergillus fumigatus	*Syncephalastrum racemosum*	*Geotrichum candidum*	*Candida albicans*
4a	15.0 ± 0.44	17.0 ± 0.25	13.3 ± 0.32	NA
4b	14.4 ± 0.44	15.6 ± 0.58	12.8 ± 0.4	NA
4c	17.7 ± 0.22	19.8 ± 0.44	16.7 ± 0.44	NA
4d	18.8 ± 0.22	20.4 ± 0.25	16.9 ± 0.44	NA
4e	14.8 ± 0.58	16.7 ± 0.19	14.9 ± 0.25	NA
6a	18.2 ± 0.44	19.3 ± 0.58	18.2 ± 0.19	NA
6b	13.7 ± 0.25	12.9 ± 0.44	13.8 ± 0.44	NA
6c	9.8 ± 0.15	8.7 ± 0.19	13.5 ± 0.38	NA
6d	13.5 ± 0.58	12.7 ± 0.37	14.8 ± 0.58	NA
6e	19.7 ± 0.44	20.2 ± 0.58	18.4 ± 0.19	NA
8a	15.7 ± 0.37	16.1 ± 0.27	13.3 ± 0.44	NA
8b	18.2 ± 0.44	19.3 ± 0.58	17.8 ± 0.19	NA
8c	13.6 ± 0.40	11.0 ± 0.30	13.40 ± 0.58	NA
8d	22.3 ± 0.37	19.3 ± 0.44	20.5 ± 0.58	NA
8e	12.7 ± 0.37	13.1 ± 0.44	14.0 ± 0.19	NA
10	17.3 ± 0.58	19.4 ± 0.44	15.3 ± 0.25	NA
11	19.9 ± 0.58	20.6 ± 0.44	17.1 ± 0.25	NA
12	20.4 ± 0.13	20.9 ± 0.44	18.9 ± 0.25	NA
Amphotericin B	23.7 ± 0.1	19.7 ± 0.2	28.7 ± 0.2	25.4 ± 0.1

NA: No activity, data are expressed in the form of mean of inhibition zone diameter for test compound performed in triplicate ± SD

Table 3. Viability values of tested compounds against breast carcinoma cells (*MCF-7*) using MTT assay.

Sample conc.	Viability %											
(μg/mL)	Vinb-S	Dox	**4b**	**4c**	**4d**	**6b**	**6d**	**8b**	**8c**	**10**	**11**	**12**
50	7.82	4.91	23.24	6.36	6.26	14.42	4.14	64.28	10.91	6.13	4.38	36.77
25	15.18	8.32	39.82	11.58	11.38	27.69	6.87	79.43	25.28	10.58	6.92	75.42
12.5	29.6	11.73	74.13	22.92	20.46	36.18	12.98	87.52	38.46	17.43	12.77	84.35
6.25	48.75	18.04	89.59	45.64	32.88	46.23	24.21	96.45	53.22	28.51	24.52	92.48
3.125	60.35	25.79	94.76	69.38	43.07	58.54	39.96	99.08	62.94	37.25	42.66	98.81
1.56	76.24	36.41	97.55	82.52	64.58	69.18	53.47	100	74.18	51.38	59.62	100
0.78	84.02	46.12	100	91.08	79.22	78.92	68.42	100	83.75	68.74	70.43	100
0.39	89.13	51.43	100	97.13	85.35	85.46	79.57	100	90.89	76.22	78.74	100
0	100	100	100	100	100	100	100	100	100	100	100	100

Where Vinb-S and Dox were standard drugs vinblastine sulfate and doxorubicin, respectively

Table 4. IC_{50} values of tested compounds ± standard deviation against (*MCF-7*).

Compound	IC_{50}	Compound	IC_{50}
Doxorubicin	0.46	**6d**	2.0
Vinblastine sulfate	5.7	**8b**	Above 50
4b	21.3	**8c**	7.6
4c	5.7	**10**	1.7
4d	2.6	**11**	2.5
6b	5.3	**12**	41.4

Table 5. Viability values of tested compounds against hepatocellular carcinoma cells (*HepG-2*) using MTT assay.

Viability %												Sample conc.
12	**11**	**10**	**8c**	**8b**	**6d**	**6b**	**4d**	**4c**	**4b**	Dox	Vinb-S	(μg/mL)
45.82	6.17	7.42	12.78	58.76	6.32	12.74	7.38	9.87	21.97	3.24	8.38	50
74.91	13.96	14.54	31.49	76.94	10.76	25.92	18.94	18.36	36.86	6.55	16.13	25
87.38	30.72	32.91	47.52	85.68	21.89	41.76	34.57	34.91	68.94	11.74	24.25	12.5
94.52	41.94	48.67	72.31	93.31	37.56	52.38	48.62	49.82	82.71	17.22	45.13	6.25
98.74	56.36	69.82	84.58	98.74	48.72	64.96	61.88	73.54	89.82	21.18	55.00	3.125
100	73.63	82.84	91.32	100	65.94	81.53	78.63	86.28	94.78	30.86	72.13	1.56
100	81.74	90.75	96.24	100	71.82	90.48	89.21	93.12	98.36	42.96	80.24	0.78
100	90.92	94.36	98.97	100	80.61	95.12	93.82	98.53	100	50.72	86.17	0.39
100	100	100	100	100	100	100	100	100	100	100	100	0

Where Vinb-S and Dox were standard drugs vinblastine sulfate and doxorubicin, respectively.

Table 6. IC_{50} values of tested compounds ± standard deviation against (*HepG-2*).

Compound	IC_{50}	Compound	IC_{50}
Doxorubicin	0.42	**6d**	3.0
Vinblastine sulfate	4.6	**8b**	Above 50
4b	19.9	**8c**	11.9
4c	6.2	**10**	6
4d	5.9	**11**	4.5
6b	7.7	**12**	46.4

- The order of activity was **10** > **6d** > **11** > **4d** > **6b** > **4c** > **8c** > **4b** > **12** > **8b**, which is in accordance with the order of breast carcinoma cells inhibitory activity (Table 4).
- The order of activity was **6d** > **11** > **4d** > **10** > **4c** > **6b** > **8c** > **4b** > **12** > **8b**, which is in accordance with the order of hepatocellular carcinoma cells inhibitory activity (Table 6).

3. Experimental section

3.1. General

Melting points were measured on Electrothermal IA 9000 series digital melting point apparatus. The IR spectra were recorded in potassium bromide discs on a Pye Unicam SP 3300 and Shimadzu FT IR 8101 PC infrared spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were recorded in deuterated dimethyl sulfoxide (DMSO-$d6$) using a Varian Gemini 300 NMR spectrometer (300 MHz for ^{1}H NMR and 75 MHz for ^{13}C NMR). Mass spectra were recorded on a Shimadzu GCMS-QP1000 EX mass spectrometer at 70 eV. Elemental analysis was carried out at the Microanalytical Center of Cairo University, Giza, Egypt. All reactions were followed by TLC (silica gel, Merck). Antitumor activity was evaluated by the Regional Center for Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt.

3.1.1. General procedure for synthesis of pyridine derivatives 4a–e, 6a–e, and 8a–e

A mixture of 5-acetyl-2-mercapto-4-methyl-1-phenyl-1*H*-imidazole **1** (0.232 g, 1 mmol), malononitrile **3** or ethyl cyanoacetate **5** or diethylmalonate **7** (1 mmol), the appropriate aldehyde **2a**–**e** (1 mmol), and ammonium

acetate (0.616 g, 8 mmol) in glacial acetic acid (20 mL) was refluxed for 6–8 h (monitored by TLC). The mixture was cooled to room temperature and the precipitated products were separated by filtration, washed successively with water, dried, and crystallized from ethanol. The synthesized compounds together with their physical and spectral data are listed below.

2-Amino-6-(2-mercapto-4-methyl-1-phenyl-1*H*-imidazol-5-yl)-4-phenylnicotinonitrile (4a). Yield 70%; yellow solid; mp 81–83 °C; IR (KBr): v_{max} 1602 (C=N), 2212 (CN), 3254, 3431 (NH_2) cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.41 (s, 3H, CH_3), 7.02–7.59 (m, 10H, ArH), 7.83 (s, 2H, D_2O exchangeable, NH_2), 8.03 (s, 1H, pyridine-H5), 10.48 (s, 1H, SH); MS m/z (%): 383 (M^+, 46), 275 (45), 217 (45), 148 (47), 104 (64), 77 (100). Anal. Calcd for $C_{22}H_{17}N_5S$ (383.47): C, 68.91; H, 4.47; N, 18.26. Found C, 68.68; H, 4.33; N, 18.04%.

2-Amino-4-(4-chlorophenyl)-6-(2-mercapto-4-methyl-1-phenyl-1*H*-imidazol-5-yl)nicotinonitrile (4b). Yield 66%; yellow solid; mp 93–95 °C; IR (KBr): v_{max} 1604 (C=N), 2209 (CN), 3195, 3405 (NH_2) cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.35 (s, 3H, CH_3), 6.99–7.78 (m, 9H, ArH), 7.92 (s, 2H, D_2O exchangeable, NH_2), 8.12 (s, 1H, pyridine-H5), 10.49 (s, 1H, SH); MS m/z (%): 419 (M^+ +2, 21), 417 (M^+, 68), 307 (100), 286 (29), 170 (77), 145 (65), 82 (79). Anal. Calcd for $C_{22}H_{16}ClN_5S$ (417.91): C, 63.23; H, 3.86; N, 16.76. Found C, 63.29; H, 3.79; N, 16.45%.

2-Amino-6-(2-mercapto-4-methyl-1-phenyl-1*H*-imidazol-5-yl)-4-(4-methoxyphenyl) nicotinonitrile (4c). Yield 69%; yellow solid; mp 88–90 °C; IR (KBr): v_{max} 1603 (C=N), 2213 (CN), 3190, 3396 (NH_2) cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.42 (s, 3H, CH_3), 3.83 (s, 3H, OCH_3), 6.58–7.60 (m, 9H, ArH), 7.93 (s, 2H, D_2O exchangeable, NH_2), 8.19 (s, 1H, pyridine-H5), 10.29 (s, 1H, SH); ^{13}C NMR (75 MHz, DMSO-d_6): δ = 18.4, 55.2 (2CH3), 89.6 (CN), 113.9, 114.3, 117.5, 118.0, 120.5, 121.4, 127.6, 128.0, 128.5, 129.3, 131.3, 135.4, 138.7, 163.8, 169.8 (Ar-C) ppm; MS m/z (%): 413 (M^+, 44), 307 (27), 267 (27), 149 (76), 58 (100). Anal. Calcd for $C_{23}H_{19}N_5OS$ (413.49): C, 66.81; H, 4.63; N, 16.94. Found C, 66.59; H, 4.60; N, 16.76%.

2-Amino-4-(2-hydroxyphenyl)-6-(2-mercapto-4-methyl-1-phenyl-1*H*-imidazol-5-yl)nicotinonitrile (4d). Yield 66%; yellow solid; mp 92–94 °C; IR (KBr): v_{max} 1603 (C=N), 2232 (CN), 3254, 3431 (NH_2 and OH) cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.41 (s, 3H, CH_3), 5.10 (s, 1H, OH), 7.02–7.80 (m, 9H, ArH), 7.98 (s, 2H, D_2O exchangeable, NH_2), 8.10 (s, 1H, pyridine-H5), 10.34 (s, 1H, SH); MS m/z (%): 399 (M^+, 9), 239 (12), 172 (37), 150 (92), 127 (95), 65 (93), 51 (100). Anal. Calcd for $C_{22}H_{17}N_5OS$ (399.47): C, 66.15; H, 4.29; N, 17.53. Found C, 66.15; H, 4.29; N, 17.53%.

2-Amino-4-(2,4-dimethylphenyl)-6-(2-mercapto-4-methyl-1-phenyl-1*H*-imidazol-5-yl)nicotinonitrile (4e). Yield 72%; yellow solid; mp 125–127 °C; IR (KBr): v_{max} 1613 (C=N), 2198 (CN), 3272, 3409 (NH_2) cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.41 (s, 3H, CH_3), 2.63 (s, 3H, CH_3), 2.79 (s, 3H, CH_3), 6.68–7.80 (m, 8H, ArH), 7.88 (s, 2H, D_2O exchangeable, NH_2), 7.96 (s, 1H, pyridine-H5), 10.66 (s, 1H, SH); MS m/z (%): 411 (M^+, 69), 307 (15), 203 (12), 176 (98), 112 (71), 75 (100). Anal. Calcd for $C_{24}H_{21}N_5S$ (411.52): C, 70.05; H, 5.14; N, 17.02. Found C, 70.23; H, 5.11; N, 16.89%.

6-(2-Mercapto-4-methyl-1-phenyl-1*H*-imidazol-5-yl)-2-oxo-4-phenyl-1,2-dihydropyridine-3-carbonitrile (6a). Yield 68%; yellow solid; mp 103–105 °C; IR (KBr): v_{max} 1611 (C=N), 1668 (C=O), 2221 (CN), 3431 (NH) cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.42 (s, 3H, CH_3), 7.02–7.65 (m, 10H, ArH), 8.07 (s, 1H, pyridine-H5), 10.82 (s, 1H, SH), 11.23 (s, 1H, D_2O exchangeable, NH); MS m/z (%): 384 (M^+, 45), 316 (64), 184 (49), 232 (100), 107 (64), 77 (99). Anal. Calcd for $C_{22}H_{16}N_4OS$ (384.45): C, 68.73; H, 4.19; N, 14.57. Found C, 68.45; H, 4.05; N, 14.39%.

4-(4-Chlorophenyl)-6-(2-mercapto-4-methyl-1-phenyl-1*H*-imidazol-5-yl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (6b). Yield 76%; yellow solid; mp 123–124 °C; IR (KBr): υ_{max} 1615 (C=N), 1659 (C=O), 2219 (CN), 3429 (NH) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 2.41 (s, 3H, CH_3), 7.02–7.72 (m, 9H, ArH), 8.14 (s, 1H, pyridine-H5), 10.71 (s, 1H, SH), 11.48 (s, 1H, D_2O exchangeable, NH); MS m/z (%): 420 (M^+ +2, 8), 418 (M^+, 18), 340 (48), 232 (87), 104 (46), 77 (84), 69 (100). Anal. Calcd for $C_{22}H_{15}ClN_4OS$ (418.90): C, 63.08; H, 3.61; N, 13.37. Found C, 63.02; H, 3.60; N, 13.23%.

6-(2-Mercapto-4-methyl-1-phenyl-1*H*-imidazol-5-yl)-4-(4-methoxyphenyl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (6c). Yield 70%; yellow solid; mp 85–87 °C; IR (KBr): υ_{max} 1610 (C=N), 1666 (C=O), 2211 (CN), 3444 (NH) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 2.41 (s, 3H, CH_3), 3.85 (s, 3H, OCH_3), 6.99–7.74 (m, 9H, ArH), 8.09 (s, 1H, pyridine-H5), 10.70 (s, 1H, SH), 11.30 (s, 1H, D_2O exchangeable, NH); MS m/z (%): 415 (M^+ +1, 25), 414 (M^+, 51), 329 (35), 230 (43), 184 (100), 61 (68). Anal. Calcd for $C_{23}H_{18}N_4O_2S$ (414.48): C, 66.65; H, 4.38; N, 13.52. Found C, 66.42; H, 4.27; N, 13.40%.

4-(2-Hydroxyphenyl)-6-(2-mercapto-4-methyl-1-phenyl-1*H*-imidazol-5-yl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (6d). Yield 66%; yellow solid; mp 92–94 °C; IR (KBr): υ_{max} 1606 (C=N), 1688 (C=O), 2218 (CN), 3280 (NH), 4211 (OH) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 2.41 (s, 3H, CH_3), 5.74 (s, 1H, OH), 6.99–7.76 (m, 9H, ArH), 7.92 (s, 1H, pyridine-H5), 10.61 (s, 1H, SH), 11.29 (s, 1H, D_2O exchangeable, NH); MS m/z (%): 400 (M^+, 38), 372 (69), 332 (62), 217 (65), 146 (37), 69 (100), 55 (92). Anal. Calcd for $C_{22}H_{16}N_4O_2S$ (400.45): C, 65.98; H, 4.03; N, 13.99. Found C, 65.81; H, 4.12; N, 13.68%.

4-(2,4-Dimethylphenyl)-6-(2-mercapto-4-methyl-1-phenyl-1*H*-imidazol-5-yl)-2-oxo-1,2-dihydropyridine-3-carbonitrile (6e). Yield 72%; yellow solid; mp 123–125 °C; IR (KBr): υ_{max} 1623 (C=N), 1668 (C=O), 2206 (CN), 3270 (NH) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 2.33 (s, 3H, CH_3), 2.41 (s, 3H, CH_3), 2.99 (s, 3H, CH_3), 6.65–7.61 (m, 8H, ArH), 7.91 (s, 1H, pyridine-H5), 10.87 (s, 1H, SH), 11.10 (s, 1H, D_2O exchangeable, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ = 14.8, 18.4, 21.0 (3CH3), 91.9 (CN), 111.5, 117.5, 118.0, 118.2, 122.1, 122.6, 129.1, 133.7, 139.9, 153.6, 154.1, 156.5, 163.4, 165.0, 172.0 (Ar-C), 188.9 (C=O) ppm; MS m/z (%): 412 (M^+, 47), 354 (38), 232 (42), 217 (39), 80 (98), 64 (100). Anal. Calcd for $C_{24}H_{20}N_4OS$ (412.51): C, 69.88; H, 4.89; N, 13.58. Found C, 69.70; H, 4.76; N, 13.47%.

Ethyl 6-(2-mercapto-4-methyl-1-phenyl-1*H*-imidazol-5-yl)-2-oxo-4-phenyl-1,2-dihydropyridine-3-carboxylate (8a). Yield 68%; yellow solid; mp 98–100 °C; IR (KBr): υ_{max} 1615 (C=N), 1667, 1725 (2C=O), 3280 (NH) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 1.22 (t, 3H, CH_3, J = 7.4 Hz), 2.41 (s, 3H, CH_3), 4.26 (q, 2H, CH_2, J= 7.4 Hz), 7.03–7.63 (m, 10H, ArH), 7.76 (s, 1H, pyridine-H5), 10.74 (s, 1H, SH), 11.96 (s, H, D_2O exchangeable, NH); ^{13}C NMR (75 MHz, DMSO-d_6): δ = 14.1, 18.8 (CH3), 61.3 (CH2), 118.1, 118.2, 118.0, 120.6, 122.1, 122.6, 122.8, 128.4, 129.0, 139.9, 141.7, 156.5, 157.7, 165.1, 165.0 (Ar-C), 180.7, 188.9 (C=O) ppm; MS m/z (%): 432 (M^+, 23), 339 (40), 232 (27), 217 (100), 104 (69), 77 (99). Anal. Calcd for $C_{24}H_{21}N_3O_3S$ (431.51): C, 66.80; H, 4.91; N, 9.74. Found C, 66.71; H, 4.73; N, 9.67%.

Ethyl 4-(4-chlorophenyl)-6-(2-mercapto-4-methyl-1-phenyl-1*H*-imidazol-5-yl)-2-oxo-1,2-dihydropyridine-3-carboxylate (8b). Yield 66%; yellow solid; mp 138–140 °C; IR (KBr): υ_{max} 1609 (C=N), 1660, 1729 (2C=O), 3280 (NH) cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 1.22 (t, 3H, CH_3, J = 7.4 Hz), 2.42 (s, 3H, CH_3), 4.25 (q, 2H, CH_2, J= 7.4 Hz), 7.02–7.60 (m, 9H, ArH), 7.74 (s, 1H, pyridine-H5), 10.65 (s, 1H, SH), 11.99 (s, H, D_2O exchangeable, NH); MS m/z (%): 467 (M^++2, 11), 465 (M^+, 29), 318 (59), 222 (56),

172 (54), 114 (76), 69 (100). Anal. Calcd for $C_{24}H_{20}ClN_3O_3S$ (465.95): C, 61.86; H, 4.33; N, 9.02. Found C, 61.58; H, 4.19; N, 8.71%.

Ethyl 6-(2-mercapto-4-methyl-1-phenyl-1*H*-imidazol-5-yl)-4-(4-methoxyphenyl)-2-oxo-1,2-dihydropyridine-3-carboxylate (8c). Yield 67%; yellow solid; mp 185–187 °C; IR (KBr): v_{max} 1602 (C=N), 1640, 1716 (2C=O), 3280 (NH) cm^{-1}; ^1H NMR (DMSO-d_6): δ 1.23 (t, 3H, CH_3, J = 7.4 Hz), 2.41 (s, 3H, CH_3), 3.82 (s, 3H, OCH_3), 4.23 (q, 2H, CH_2, J= 7.4 Hz), 6.99–7.60 (m, 9H, ArH), 7.74 (s, 1H, pyridine-H5), 10.73 (s, 1H, SH), 11.96 (s, H, D_2O exchangeable, NH); MS m/z (%): 462 (M^+ +1, 18), 461 (M^+, 24), 381 (48), 305 (64), 215 (37), 155 (43), 55 (100). Anal. Calcd for $C_{25}H_{23}N_3O_4S$ (461.53): C, 65.06; H, 5.02; N, 9.10. Found C, 64.85; H, 4.87; N, 9.01%.

Ethyl 4-(2-hydroxyphenyl)-6-(2-mercapto-4-methyl-1-phenyl-1*H*-imidazol-5-yl)-2-oxo-1,2-dihydropyridine-3-carboxylate (8d). Yield 76%; yellow solid; mp 95–97 °C; IR (KBr): v_{max} 1617 (C=N), 1685, 1754 (2C=O), 3278 (NH), 3401 (OH) cm^{-1}; ^1H NMR (DMSO-d_6): δ 1.31 (t, 3H, CH_3, J = 7.4 Hz), 2.41 (s, 3H, CH_3), 4.28 (q, 2H, CH_2, J= 7.4 Hz), 5.10 (s, 1H, OH), 6.92–7.64 (m, 9H, ArH), 7.75 (s, 1H, pyridine-H5), 11.03 (s, 1H, SH), 11.88 (s, H, D_2O exchangeable, NH); MS m/z (%): 448 (M^+ +1, 17), 447 (M^+, 32), 232 (96), 217 (100), 104 (69), 77 (92). Anal. Calcd for $C_{24}H_{21}N_3O_4S$ (447.51): C, 64.41; H, 4.73; N, 9.39. Found C, 64.32; H, 4.49; N, 9.18%.

Ethyl 4-(2,4-dimethylphenyl)-6-(2-mercapto-4-methyl-1-phenyl-1*H*-imidazol-5-yl)-2-oxo-1,2-dihydropyridine-3-carboxylate (8e). Yield 72%; yellow solid; mp 89–91 °C; IR (KBr): v_{max} 1626 (C=N), 1685, 1729 (2C=O), 3272 (NH) cm^{-1}; ^1H NMR (DMSO-d_6): δ 1.24 (t, 3H, CH_3, J = 7.4 Hz), 2.33 (s, 3H, CH_3), 2.41 (s, 3H, CH_3), 2.96 (s, 3H, CH_3), 4.20 (q, 2H, CH_2, J= 7.4 Hz), 6.72–7.60 (m, 8H, ArH), 7.69 (s, 1H, pyridine-H5), 10.97 (s, 1H, SH), 11.89 (s, H, D_2O exchangeable, NH); MS m/z (%): 459 (M^+, 28), 392 (55), 272 (42), 217 (34), 137 (50), 69 (100). Anal. Calcd for $C_{26}H_{25}N_3O_3S$ (459.56): C, 67.95; H, 5.48; N, 9.14. Found C, 67.78; H, 5.38; N, 9.03%.

3.1.2. General procedure for synthesis of bipyridine derivatives 10–12

A mixture of 5-acetylimidazole **1** (0.464 g, 2 mmol), malononitrile **3** or ethyl cyanoacetate **5** or diethyl malonate **7** (2 mmol), the terephthalaldehyde **9** (0.134 g, 1 mmol), and ammonium acetate (1.232 g, 16 mmol) in acetic acid (30 mL) was refluxed for 6–8 h (monitored by TLC). The reaction mixture was cooled and poured into cold water; the resulting precipitate was filtered off, washed with water, and recrystallized from dioxane to give the corresponding bipyridine products **10**–**12**. The synthesized compounds together with their physical and spectral data are listed below.

4,4'-(1,4-Phenylene)bis(2-amino-6-(2-mercapto-4-methyl-1-phenyl-1*H*-imidazol-5-yl)nicotinonitrile) (10). Yield 68%; yellow solid; mp 126–128 °C; IR (KBr): v_{max} 1609 (C=N), 2211 (CN), 3430, 3140 (NH_2) cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.42 (s, 6H, $2CH_3$), 2.58 (s, 4H, D_2O exchangeable, $2NH_2$), 7.04–7.61 (m, 14H, ArH), 7.82 (s, 2H, pyridine-H5), 10.57 (s, 2H, 2SH); MS m/z (%): 688 (M^+, 45), 340 (39), 232 (83), 104 (46), 77 (84), 69 (100). Anal. Calcd for $C_{38}H_{28}N_{10}S_2$ (688.83): C, 66.26; H, 4.10; N, 20.33. Found C, 66.08; H, 4.14; N, 20.12%.

4,4'-(1,4-Phenylene)bis(6-(2-mercapto-4-methyl-1-phenyl-1*H*-imidazol-5-yl)-2-oxo-1,2-dihydropyridine-3-carbonitrile) (11). Yield 66%; yellow solid; mp 120–122 °C; IR (KBr): v_{max} 1628 (C=N), 1686 (C=O), 2218 (CN), 3256 (NH) cm^{-1}; ^1H NMR (DMSO-d_6): δ 2.42 (s, 6H, $2CH_3$), 7.04–7.61

(m, 14H, ArH), 7.89 (s, 2H, pyridine-H5), 10.70 (s, 2H, 2SH), 11.30 (s, 2H, D_2O exchangeable, 2NH); MS m/z (%): 690 (M^+, 34), 251 (49), 153 (65), 127 (78), 77 (100). Anal. Calcd for $C_{38}H_{26}N_8O_2S_2$ (690.80): C, 66.07; H, 3.79; N, 16.22. Found C, 65.89; H, 3.70; N, 16.13%.

Diethyl 4,4'-(1,4-phenylene)bis(6-(2-mercapto-4-methyl-1-phenyl-1*H*-imidazol-5-yl)-2-oxo-1,2-dihydropyridine-3-carboxylate) (12). Yield 67%; yellow solid; mp 143–145 °C; IR (KBr): v_{max} 1628 (C=N), 1688, 1720 (2C=O), 3256 (NH) cm^{-1}; ^1H NMR (DMSO-d_6): δ 1.24 (t, 6H, $2CH_3$, J = 7.4 Hz), 2.42 (s, 6H, $2CH_3$), 4.28 (q, 2H, $2CH_2$, J = 7.4 Hz), 7.02–7.61 (m, 14H, ArH), 7.85 (s, 2H, pyridine-H5), 11.07 (s, 2H, 2SH), 11.41 (s, 2H, D_2O exchangeable, 2NH); MS m/z (%): 784 (M^+, 17), 339 (34), 232 (73), 217 (100), 104 (45), 77 (95). Anal. Calcd for $C_{42}H_{36}N_6O_6S_2$ (784.90): C, 64.27; H, 4.62; N, 10.71. Found C, 64.19; H, 4.43; N, 10.59%.

3.2. Biological part

3.2.1. Antimicrobial activity test

Agar diffusion is the method adopted for such tests. The microorganism inocula were uniformly spread using a sterile cotton swab on a sterile petri dish of malt extract agar (for fungi) and nutrient agar (for bacteria). Then 100 μL of each sample was added to each well (10 mm diameter holes cut in the agar gel, 20 mm apart from one another). The systems were incubated for 24–48 h at 37 °C (for bacteria) and at 28 °C (for fungi). After incubation, the microorganism's growth was observed. Inhibition of the bacterial and fungal growth was measured as IZD in mm. The tests were performed in triplicate.[39]

3.2.2. Cytotoxic activity

The method of Skehan et al.[40] was used for potential cytotoxicity measurements of the synthesized compounds using Sulfo-Rhodamine-B (SRB) stain. Cells were plated in 96-multiwell plates (10^4 cells/well) for 24 h before treatment with the tested compound to allow attachment of cells to the wall of the plate. Next, 0, 1.56, 3.125, 6.25, 12.5, 25, and 50 μg/mL of the testing compound were added to the cell monolayer in triplicate wells individual dose, and monolayer cells were incubated with the compounds for 48 h at 37 °C and in atmosphere of 5% CO_2. After 48 h, the cells were fixed, washed, and stained with SRB stain. Excess stain was washed with acetic acid and attached stain was recovered with tris-EDTA buffer. Color intensity was measured using an ELISA reader. The relation between surviving fraction and drug concentration was plotted. The response parameter calculated was the IC_{50} value, which corresponds to the compound concentration causing 50% mortality in net cells.

4. Conclusions

The synthesis of some new pyridine and bipyridine derivatives from 5-acetylimidazole in a MCR was established. Moreover, some of the newly synthesized products were tested for antimicrobial and anticancer activities and the results obtained were promising.

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