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Original article

2-Acylhydrazino-5-arylpyrrole derivatives: Synthesis and antifungal activity evaluation

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ABSTRACT

The synthesis and antifungal activity of 2-acylhydrazino-5-arylpyrroles **21–62** are described. Pyrrole derivatives **21–62** were evaluated for their antifungal activity towards *Candida albicans* ATCC 10231 and three *Candida* non-*albicans* isolated from clinical specimens. Most of them showed very good antifungal activities against *Candidae*, having MIC values in the 0.39–3.12 μg/mL range and enhanced inhibition potency as compared to that of fluconazole. In addition, some of the most active compounds were tested for cytotoxic activities against breast (MCF-7), lung (H-460), and central nervous system (SF-268) human cancer cell lines with the NCI anticancer drug screen. The activity of pyrroles described in this paper, along with the low toxicity, shows promise for the future development of non-toxic new antimycotic agents. The relationship between functional group variation and biological activity of the evaluated compounds is also discussed.

1. Introduction

The incidence of fungal infections has increased significantly in the past two decades mainly due to the growing number of immunocompromised patients, such as cancer patients, patients who have undergone organ transplantation, and patients with AIDS, and the frequent use of cytotoxic and/or antibacterial drugs [1–6]. Clinically, candidiasis and aspergillosis account for between 80% and 90% of systemic fungal infections in immunocompromised patients. *Candida* species have been found to be the fourth most prevalent group of pathogens and have been isolated from 8% of patients with nosocomial bloodstream infections [7]. They are also identified as critical pathogens in infections of wounds and other body fluids [8]. The change in mortality rate associated with disseminated candidiasis has been insignificant even after treatment with effective antifungal drugs [9]. Until recently, amphotericin B was the standard therapy for many fungal infections, but a high frequency of renal toxicity has limited its use [10,11]. In recent times, new antifungal agents, such as azoles (fluconazole and voriconazole), and echinocandins (caspofungin), have increased the ability to treat fungal infections [10,12]. However, all of them have a narrow spectrum of activity, the development of high resistance has been documented, especially, for fluconazole, and the mortality due to fungal infections even with the novel antifungal agents is still unacceptably high [10,13–15]. Currently, a small number of agents are available, and all have some drawbacks regarding their spectrum, toxicity, tissue distribution, central nervous system (CNS) penetration, and high cost [13–17]. In view of the increasing resistance to existing azole antifungals [18,19] and the lack of sufficient chemical diversity in the existing classes of antifungals, the need for new antifungals remains high. As part of our ongoing development of efficient protocols for the preparation of biologically active heterocycles from common intermediates and keeping in view the biological activity of the pyrrole derivatives, we have published the synthesis and preliminary evaluation of in vitro antifungal activity of a series of 2-acetyl (or ethoxycarbonyl)-hydrazinopyrrole derivatives [20]. Our results indicated that some of the derivatives displayed appreciable fungistatic effect, especially against a strain of *Candida albicans* isolated from clinical specimens, making this class of compounds suitable for further development as potential antimycotic agents. Therefore we present the design, synthesis, and biological evaluation of a novel group of potent antifungal pyrroles derived from the substances described in our previous study.

2. Results and discussion

2.1. Synthesis

The synthetic approach to obtain 2-acylhydrazino-5-arylpyrrole derivatives **21–62** followed the reactions shown in Scheme 1. The

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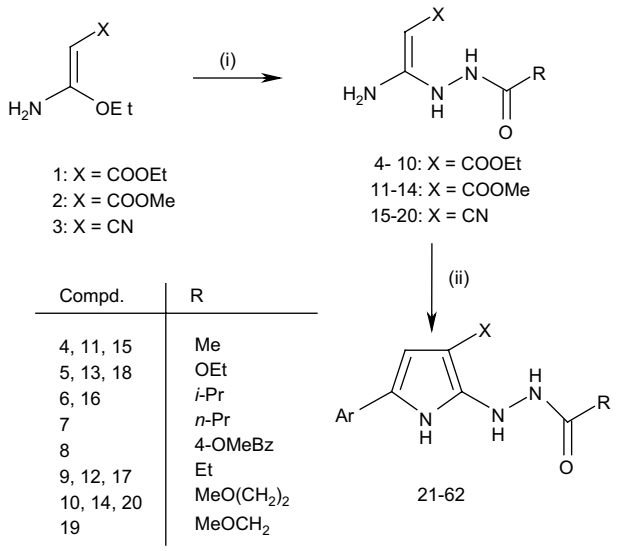


Scheme 1. Synthetic pathway to pyrroles **21**–**62**. Reagents and conditions: (i) $RCONHNH_2$, EtOH, r.t.; (ii) $ArCOCH_2Br$, $NaHCO_3$, MeCN, reflux.

required 3-(2-acylhydrazino)-3-aminopropenoates **4**–**14** and 3-(2-acylhydrazino)-3-aminopropenenitriles **15**–**20** were prepared by treatment of imidates **1**–**3** with acylhydrazines using the method described in our previous papers [20–23]. Intermediates **4**–**20** were converted to pyrroles **21**–**62** (Tables 1 and 2) by treatment with the appropriate α-bromoketone in 1:1 molar ratio, in the presence of sodium hydrogencarbonate, in anhydrous MeCN solution. All the newly synthesized compounds gave corrected analytical data. The IR and NMR spectral data were consistent with the assigned structure.

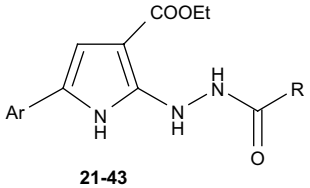
2.2. Antifungal activity

The antifungal properties of compounds **21**–**62** were evaluated by the broth microdilution method according to the NCCLS reference document M27-A [24] against a panel of four *Candidae*: *C. albicans* ATCC 10231, and *Candida glabrata*, *Candida parapsilosis* and *Candida krusei* isolated from clinical specimens. Fluconazole and amphotericin B were used as positive controls.

2.3. Discussion

The antifungal effect of compounds **21**–**43** is reported in Table 1. From the analysis of data we can note that compounds **24**, **25**, **27**, **28**, **31**, **32**, and **40**–**43** display minimum inhibitory concentration (MIC) values against *C. albicans* in the 3.12–6.25 μg/mL range. Furthermore MIC values of compounds **24**, **25**, **27**, **28**, **29**, and **40**–**42** against *C. glabrata* are 2- to 4-fold better than that of the reference drug fluconazole. The same **24**, **25**, **28**, **32**, as well as **40** showed

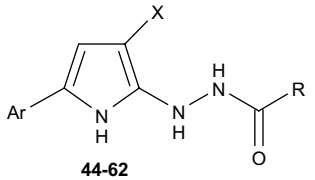
Table 1
Antifungal activity of pyrroles **21**–**43**[a]



Compound	Ar	R	*c* log *P*[b]	MIC (μg/mL)				MFC[c] (μg/mL)			
				C. albicans ATCC 10231	*C. glabrata*	*C. parapsilosis*	*C. krusei*	*C. albicans* ATCC 10231	*C. glabrata*	*C. parapsilosis*	*C. krusei*
21	Ph	Me	1.02	12.5	12.5	50	50	12.5	>100	100	50
22	4-ClPh	Me	1.58	>100	>100	12.5	25	>100	>100	25	50
23	4-MePh	Me	1.51	>100	>100	50	100	>100	>100	50	>100
24	4-OMePh	Me	0.9	3.12	6.25	50	12.5	3.12	12.5	50	25
25	Ph	OEt	1.95	3.12	6.25	6.25	12.5	3.12	12.5	12.5	25
26	4-ClPh	OEt	2.51	25	100	12.5	50	>100	>100	12.5	>100
27	4-MePh	OEt	2.43	6.25	3.12	100	100	6.25	25	>100	>100
28	4-OMePh	OEt	1.82	3.12	6.25	6.25	12.5	3.12	25	25	100
29	Ph	*i*-Pr	2.24	>100	6.25	25	>100	>100	12.5	100	>100
30	4-ClPh	*i*-Pr	2.8	>100	>100	>100	>100	>100	>100	>100	>100
31	4-MePh	*i*-Pr	2.73	6.25	>100	25	25	12.5	>100	>100	100
32	4-OMePh	*i*-Pr	2.12	6.25	>100	6.25	12.5	12.5	>100	>100	>100
33	Ph	*n*-Pr	2.09	12.5	>100	100	>100	12.5	>100	>100	>100
34	4-ClPh	*n*-Pr	2.65	>100	>100	>100	>100	>100	>100	>100	>100
35	4-MePh	*n*-Pr	2.58	>100	>100	25	>100	>100	>100	>100	>100
36	4-OMePh	*n*-Pr	1.97	12.5	>100	12.5	50	12.5	>100	>100	100
37	Ph	4-OMeBz	2.61	>100	>100	50	50	>100	>100	>100	>100
38	4-ClPh	4-OMeBz	3.3	>100	>100	>100	>100	>100	>100	>100	>100
39	4-MePh	4-OMeBz	3.23	>100	>100	100	100	>100	>100	>100	>100
40	Ph	Et	1.68	3.12	6.25	6.25	12.5	12.5	12.5	25	25
41	4-OMePh	Et	1.55	3.12	6.25	12.5	25	12.5	12.5	25	100
42	Ph	$MeO(CH_2)_2$	0.98	3.12	6.25	6.25	12.5	6.25	25	25	50
43	4-OMePh	$MeO(CH_2)_2$	0.85	6.25	12.5	25	50	6.25	100	100	100
AmB[d]				0.39	0.78	0.78	0.78	0.78	1.56	1.56	1.56
FLC[e]				0.78	12.5	0.39	25	>100	>100	>100	50

[a] Antifungal activity was determined with the microbroth dilution assay following the NCCLS guidelines.
[b] Calculated using ChemDraw Ultra software version 8.0.3.
[c] Defined as the minimum concentration of compound which resulted in the killing of >99% of the original inoculum.
[d] Amphotericin B.
[e] Fluconazole.

Table 2
Antifungal activity of pyrroles **44**–**62**[a]



44-62

Compound	X	Ar	R	*c* log *P*[b]	MIC (μg/mL)				MFC[c] (μg/mL)			
					C. albicans ATCC 10231	*C. glabrata*	*C. parapsilosis*	*C. krusei*	*C. albicans* ATCC 10231	*C. glabrata*	*C. parapsilosis*	*C. krusei*
44	CN	Ph	Me	0.9	3.12	6.25	3.12	3.12	3.12	12.5	12.5	6.25
45	CN	4-OMePh	Me	0.77	1.56	3.12	3.12	1.56	6.25	12.5	6.25	3.12
46	CN	Ph	Et	1.55	3.12	3.12	3.12	1.56	6.25	12.5	12.5	12.5
47	CN	4-OMePh	Et	1.43	0.39	0.78	0.78	0.78	6.25	12.5	12.5	12.5
48	CN	4-OMePh	*i*-Pr	1.99	3.12	50	25	50	3.12	>100	>100	>100
49	CN	Ph	OEt	1.82	3.12	3.12	6.25	3.12	3.12	6.25	12.5	6.25
50	CN	4-OMePh	OEt	1.70	3.12	12.5	25	25	3.12	50	100	50
51	CN	Ph	$MeOCH_2$	0.56	6.25	25	6.25	6.25	6.25	50	6.25	25
52	CN	4-OMePh	$MeOCH_2$	0.43	6.25	25	6.25	6.25	6.25	50	12.5	12.5
53	CN	Ph	$MeO(CH_2)_2$	0.85	6.25	3.25	6.25	6.25	6.25	12.5	25	12.5
54	CN	4-OMePh	$MeO(CH_2)_2$	0.73	3.12	6.25	6.25	3.12	6.25	12.5	12.5	6.25
55	COOMe	Ph	Me	0.69	3.12	6.25	6.25	12.5	6.25	25	25	12.5
56	COOMe	4-OMePh	Me	0.56	3.12	3.12	3.12	3.12	3.12	12.5	6.25	6.25
57	COOMe	Ph	Et	1.34	3.12	6.25	6.25	12.5	6.25	25	12.5	25
58	COOMe	4-OMePh	Et	1.21	3.12	6.25	3.12	6.25	3.12	12.5	12.5	6.25
59	COOMe	Ph	OEt	1.61	3.12	3.12	3.12	6.25	6.25	12.5	12.5	25
60	COOMe	4-OMePh	OEt	1.48	3.12	12.5	25	50	6.25	50	>100	>100
61	COOMe	Ph	$MeO(CH_2)_2$	0.64	6.25	6.25	12.5	12.5	12.5	25	100	25
62	COOMe	4-OMePh	$MeO(CH_2)_2$	0.51	6.25	6.25	6.25	6.25	6.25	25	12.5	25
AmB[d]					0.39	0.78	0.78	0.78	0.78	1.56	1.56	1.56
FLC[e]					0.78	12.5	0.39	25	>100	>100	>100	50

[a] Antifungal activity was determined with the microbroth dilution assay following the NCCLS guidelines.
[b] Calculated using ChemDraw Ultra software version 8.0.3.
[c] Defined as the minimum concentration of compound which resulted in the killing of >99% of the original inoculum.
[d] Amphotericin B.
[e] Fluconazole.

a 2-fold inhibition potency against *C. krusei* as compared to fluconazole. Also it is interesting to note that pyrroles **21**, **24**, **25**, **27**, **28**, and **43** exhibit fungicidal effect against *C. albicans* ATCC 10231 at concentrations of the corresponding MICs.

As regards structure–activity relationships (SARs), we found that activity of this series of compounds was dependent on the substituent present at 5-position of the pyrrole ring. These compounds with a methoxy substituent in the *para* position or with an unsubstituted phenyl showed, in general, a more potent antifungal activity than other compounds synthesized. Replacement of the methoxy group with chlorine or methyl resulted in decreased or impaired activity. Furthermore the presence at 2-position of the pyrrole ring of butyrylhydrazino, isobutyrylhydrazino, or (4-methoxyphenyl)acetylhydrazino moieties led to compounds endowed with poor activity. With the mechanism of the antifungal effect being unknown, we assumed that while a certain degree of lipophilicity is necessary for the compound to penetrate the fungal cell wall, the presence of a hydrophilic moiety can play a most vital role in binding to its biological target molecule. As matter of fact, the active compounds are characterized by *c* log *P* values minor than 2.4. In view of the results obtained and bearing in mind that the desired biological activity is supported by the presence of a phenyl ring or 4-methoxyphenyl in 5-position, we shifted attention to the substitution at C(3) of the pyrrole ring. We settled on to determine the effect of replacing the COOEt moiety with CN or COOMe groups. Thus, we designed a new series of pyrroles (compounds **44**–**62**) bearing CN or COOMe groups at C-3 of pyrrole ring as well as a 2-acylhydrazino moiety that give to the molecule *c* log *P* values minor than 2.

The results of antifungal activity of pyrroles **44**–**62** are displayed in Table 2. Pyrroles **44**–**62** show a general enhancement in antifungal activity with respect to compounds **21**–**43**. MIC values of these compounds are in the 0.39–6.25 and 0.78–50 μg/mL range against *C. albicans* ATCC 10231, and the three *Candida* non-*albicans* isolated from clinical specimens, respectively. Compound **47** showed the highest activity of the whole series. In particular, the analysis of pyrrole **47** MIC data reveals a 2- to 32-fold inhibition potency against *C. albicans*, *C. glabrata* and *C. krusei* as compared to fluconazole. On the contrary the same compound displays antifungal activity minor than fluconazole against *C. parapsilosis*. Furthermore **47** is endowed of the same antifungal potency of amphotericin B against *C. albicans* ATCC 10231 and against clinically isolated *Candida* species. Compound **47** was shown to be fungicidal against all the strains tested at concentrations 8 doubling dilutions greater than the corresponding MICs (MCF 6.25–12.5 μg/mL). Replacing the 3-COOEt (pyrrole **41**) or 3-COOMe (pyrrole **58**) with a 3-cyano group, to produce pyrrole **47**, led to an approximate 8- to 32-fold increase in activity. Replacement of the ethylcarbonylhydrazino moiety of pyrrole **47** by an ethoxycarbonylhydrazino (compound **50**), methoxymethylcarbonylhydrazino (compound **52**) or methoxyethylcarbonylhydrazino (compound **54**) resulted in reduced activity (4- to 32-fold).

In addition to the antifungal evaluation, the most active compounds **45**, **47**, **50**, and **56** were evaluated by the National Cancer Institute (NCI, Bethesda, MD) for cytotoxic activities against breast (MCF-7), lung (H-460), and central nervous system (SF-268) human cancer cell lines [25], in order to determine the selectivity of pyrroles towards fungi. Results showed (Table 3) that all the tested

Table 3
Cytotoxic activity[a] of compounds **45**, **47**, **50** and **56**

Compound	Growth inhibition percentage[b] at 10^{-5} M concentration		
	MCF-7	H-460	SF-268
45	0	0	0
47	4.6	0	2.1
50	0.9	31	0
56	0	0	0

[a] Data obtained from the NCI's in vitro disease-oriented human tumor cells screen (see Refs. [25–27] for details).
[b] Calculated with respect to untreated cells.

pyrroles displayed poor or no cytotoxic activity at 10^{-5} M concentration. Thus compounds **45**, **47**, **50**, and **56** showed high selectivity towards *Candidae*.

3. Conclusion

We synthesized a novel series of 2-acylhydrazinopyrrole derivatives. Most of the synthesized compounds exhibited potent antifungal activity against *C. albicans* ATCC 10231 and clinically isolated *Candida* species. These novel compounds may serve as lead for the development of drugs with different structural feature from those of the currently utilized antifungal agents. The structure–activity relationships discussed above can provide useful information for further design and synthesis of compounds with optimized bioactivity profile. Additional studies to optimize the structural feature as well as to explore the mechanism of action of this class of compounds is planned to start in our group in near future.

4. Experimental protocols

4.1. Synthesis

4.1.1. Materials and general methods

Melting points were determined on a Stuart Scientific Melting point SMP1 and are uncorrected. Proton NMR spectra were recorded on a Varian Unity 300 spectrometer. The chemical shift are reported in parts per million (δ, ppm) downfield from tetramethylsilane (TMS), which was used as internal standard. Infrared spectra were obtained with a Bruker Vector 22 spectrophotometer. Elemental analyses were carried out with a Carlo Erba model 1106 Elemental Analyzer and the values found were within 0.4% of theoretical values. Ethyl 3-(2-acetylhydrazino)-3-amino-2-propenoate (**4**) [20], ethyl 3-amino-3-(2-ethoxycarbonylhydrazino)-2-propenoate (**5**) [20], ethyl 3-amino-3-(2-isobutyrylhydrazino)-2-propenoate (**6**) [21], ethyl 3-amino-3-(2-(4-methoxyphenyl)acetyl)hydrazino-2-propenoate (**8**) [22], 3-amino-3-(2-acetylhydrazino)-2-propenenitrile (**15**) [23], 3-amino-3-(2-isobutyrylhydrazino)-2-propenitrile (**16**) [23] and pyrroles **21–28** [20] were obtained with previously described procedures.

4.1.2. General procedure for the synthesis of compounds ***4–20***

A solution of ethyl 3-amino-3-ethoxypropenoate (**1**), methyl 3-amino-3-ethoxypropenoate (**2**), or 3-amino-3-ethoxypropenenitrile (**3**) (0.01 mol) and the appropriate acylhydrazine (0.01 mol) in dry EtOH (50 mL) was heated at 70 °C for 5 min and then allowed to stand overnight at r.t. The formed precipitate was filtered off, washed with diethyl ether and recrystallized from the solvent indicated.

*4.1.2.1. Ethyl 3-amino-3-(2-butyrylhydrazino)-2-propenoate (**7**).* Yield 86%. Mp 177–180 °C (cyclohexane). ^{1}H NMR (DMSO-d_6): δ 0.81 (t, 3H, $J = 5.1$, CH_3), 1.57 (m, 2H, CH_2), 2.29 (m, 2H, CH_2), 4.03 (q, 2H, $J = 5.1$ Hz, CH_2), 5.04 (s, 1H, CH), 6.09 (s, 2H, NH_2), 9.24, 10.02 (s, 2H, NH). IR (Nujol) 3417 (NH), 3234 (NH), 3059 (NH), 1716 (C=O), 1657, 1607 cm^{-1}. Anal. Calcd for $C_9H_{17}N_3O_3$: C, 50.22; H, 7.96; N, 19.52. Found: C, 50.18; H, 7.98; N, 19.54.

*4.1.2.2. Ethyl 3-amino-3-(2-propanoylhydrazino)-2-propenoate (**9**).* Yield 86%. Mp 119–120 °C (benzene). ^{1}H NMR ($CDCl_3$): δ 1.05 (t, 3H, $J = 7.6$ Hz, CH_3), 1.18 (t, 3H, $J = 7.3$ Hz, CH_3), 4.10 (m, 4H, CH_2), 5.57 (s, 1H, CH), 6.21 (s, 2H, NH_2), 10.22, 10.39 (s, 2H, NH). IR (Nujol) 3400 (NH), 3210 (NH), 3047 (NH), 1742 (C=O), 1653, 1618, 1595 cm^{-1}. Anal. Calcd for $C_8H_{15}N_3O_3$: C, 47.75; H, 7.51; N, 20.88. Found: C, 47.71; H, 7.49; N, 20.92.

*4.1.2.3. Ethyl 3-amino-3-(2-(3-methoxypropanoyl)hydrazino)-2-propenoate (**10**).* Yield 82%. Mp 64–65 °C (*n*-hexane). ^{1}H NMR ($CDCl_3$): δ 1.20 (t, 3H, $J = 7.3$ Hz, CH_3), 2.43 (m, 2H, CH_2), 3.63 (m, 2H, CH_2), 4.13 (q, 2H, $J = 7.3$ Hz, CH_2), 5.61 (s, 1H, CH), 5.97 (s, 2H, NH_2), 10.02, 10.42 (s, 2H, NH). IR (Nujol) 3432 (NH), 3180 (NH), 1723 (C=O), 1687 (C=O), 1600, 1582 cm^{-1}. Anal. Calcd for $C_9H_{17}N_3O_4$: C, 46.74; H, 7.41; N, 18.17. Found: C, 46.71; H, 7.43; N, 18.13.

*4.1.2.4. Methyl 3-(2-acetylhydrazino)-3-amino-2-propenoate (**11**).* Yield 90%. Mp 149–150 °C (toluene). ^{1}H NMR (DMSO-d_6): δ 1.89 (s, 3H, CH_3), 3.60 (s, 3H, CH_3), 5.06 (s, 1H, CH), 6.12 (s, 2H, NH_2), 9.32 (s, 2H, NH). IR (Nujol) 3498 (NH), 3414 (NH), 3184 (NH), 1724 (C=O), 1684 (C=O), 1614 cm^{-1}. Anal. Calcd for $C_6H_{11}N_3O_3$: C, 41.61; H, 6.40; N, 24.27. Found: C, 41.67; H, 6.39; N, 24.24.

*4.1.2.5. Methyl 3-amino-3-(2-propanoylhydrazino)-2-propenoate (**12**).* Yield 88%. Mp 139–140 °C (2-PrOH). ^{1}H NMR (DMSO-d_6): δ 0.94 (t, 3H, $J = 5.3$ Hz, CH_3), 2.04 (q, 2H, $J = 5.3$ Hz, CH_2), 3.56 (s, 3H, CH_3), 5.07 (s, 1H, CH), 6.09 (s, 2H, NH_2), 9.24 (s, 2H, NH). IR (Nujol) 3420 (NH), 3178 (NH), 1727 (C=O), 1682 (C=O), 1657, 1624 cm^{-1}. Anal. Calcd for $C_7H_{13}N_3O_3$: C, 44.91; H, 7.00; N, 22.45. Found: C, 44.95; H, 7.02; N, 22.42.

*4.1.2.6. Methyl 3-amino-3-(2-ethoxycarbonylhydrazino)-2-propenoate (**13**).* Yield 94%. Mp 137–138 °C (2-PrOH). ^{1}H NMR (DMSO-d_6): δ 1.10 (t, 3H, $J = 6.9$ Hz, CH_3), 3.56 (s, 3H, CH_3), 4.00 (q, 2H, $J = 6.9$ Hz, CH_2), 5.04 (s, 1H, CH), 5.99 (s, 2H, NH_2), 8.88, 9.21 (s, 2H, NH). IR (Nujol) 3416 (NH), 3232 (NH), 3043 (NH), 1720 (C=O), 1694 (C=O), 1639, 1584 cm^{-1}. Anal. Calcd for $C_7H_{13}N_3O_4$: C, 41.38; H, 6.45; N, 20.68. Found: C, 41.35; H, 6.44; N, 20.72.

*4.1.2.7. Methyl 3-amino-3-(2-(3-methoxypropanoyl)hydrazino)-2-propenoate (**14**).* Yield 84%. Mp 65–66 °C (*n*-hexane). ^{1}H NMR ($CDCl_3$): δ 2.25 (m, 2H, CH_2), 3.06 (s, 3H, CH_3), 3.45 (s, 3H, CH_3), 3.54 (m, 2H, CH_2), 5.47 (s, 1H, CH), 5.90 (s, 2H, NH_2), 9.91, 10.02 (s, 2H, NH). IR (Nujol) 3410 (NH), 3320 (NH), 3186 (NH), 1741 (C=O), 1722, 1648 cm^{-1}. Anal. Calcd for $C_8H_{15}N_3O_4$: C, 44.23; H, 6.96; N, 19.34. Found: C, 44.29; H, 6.97; N, 19.30.

*4.1.2.8. 3-Amino-3-(2-propanoylhydrazino)-2-propenenitrile (**17**).* Yield 98%. Mp 154–155 °C (toluene). ^{1}H NMR (DMSO-d_6): δ 0.93 (t, 3H, $J = 7.7$ Hz, CH_3), 2.37 (q, 2H, $J = 7.7$ Hz, CH_2), 5.39 (s, 1H, CH), 6.30 (s, 2H, NH_2), 9.42, 9.98 (s, 2H, NH). IR (Nujol) 3451 (NH), 3408 (NH), 3205 (NH), 2257 (CN), 1612, 1599 cm^{-1}. Anal. Calcd for $C_6H_{10}N_4O$: C, 46.74; H, 6.54; N, 36.34. Found: C, 46.77; H, 6.56; N, 36.36.

*4.1.2.9. 3-Amino-3-(2-ethoxycarbonylhydrazino)-2-propenenitrile (**18**).* Yield 97%. Mp 169–170 °C (MeCN). ^{1}H NMR (DMSO-d_6): δ 1.11 (t, 3H, $J = 6.9$ Hz, CH_3), 3.99 (q, 2H, $J = 6.9$ Hz, CH_2), 5.35 (s, 1H, CH), 6.21 (s, 2H, NH_2), 9.01, 9.62 (s, 2H, NH). IR (Nujol) 3451 (NH), 3408 (NH), 3205 (NH), 2257 (CN), 1612, 1599 cm^{-1}. Anal. Calcd for $C_6H_{10}N_4O_2$: C, 42.35; H, 5.92; N, 32.92. Found: C, 42.40; H, 5.90; N, 32.90.

*4.1.2.10. 3-Amino-3-(2-(2-methoxyacetyl)hydrazino)-2-propenenitrile (**19**).* Yield 86%. Mp 159–160 °C (MeCN). ^{1}H NMR (DMSO-d_6): δ 3.29 (s, 3H, CH_3), 4.13 (s, 2H, CH_2), 5.24 (s, 1H, CH), 6.33 (s, 2H, NH_2), 9.36, 10.00 (s, 2H, NH). IR (Nujol) 3451 (NH), 3408 (NH), 3205 (NH), 2257 (CN), 1612, 1599 cm^{-1}. Anal. Calcd for $C_6H_{10}N_4O_2$: C, 42.35; H, 5.92; N, 32.92. Found: C, 42.40; H, 5.90; N, 32.90.

*4.1.2.11. 3-Amino-3-(2-(3-methoxypropanoyl)hydrazino)-2-propenitrile (**20**).* Yield 87%. Mp 124–125 °C (*n*-hexane). ^{1}H NMR (DMSO-d_6): δ 2.26 (t, 2H, J = 6.5 Hz, CH_2), 3.40 (s, 3H, CH_3), 3.67 (t, 2H, J = 6.5 Hz, CH_2), 5.16 (s, 1H, CH), 6.31 (s, 2H, NH_2), 9.42, 9.73 (s, 2H, NH). IR (Nujol) 3410 (NH), 3320 (NH), 3186 (NH), 1741 (C=O), 1722, 1648 cm^{-1}. Anal. Calcd for $C_7H_{12}N_4O_2$: C, 45.64; H, 6.57; N, 30.42. Found: C, 45.69; H, 6.56; N, 30.46.

*4.1.3. General procedure for the synthesis of pyrrole derivatives (**21**–**62**)*

A mixture of compounds **4**–**20** (10 mmol), $NaHCO_3$ (0.92 g, 11 mmol), and the appropriate phenacyl bromide (10 mmol) in dry MeCN (10 mL) was refluxed for 20 min and then stirred at room temperature for 2 h. The formed precipitate was filtered off and purified by crystallization from the adequate solvent to give the pyrrole derivatives **21**–**62**.

*4.1.3.1. Ethyl 2-(2-isobutyrylhydrazinyl)-5-phenyl-1H-pyrrole-3-carboxylate (**29**).* Yield 83%. Mp 168–170 °C (MeCN). ^{1}H NMR (DMSO-d_6): δ 1.00 (d, 6H, J = 6.9 Hz, CH_3), 1.20 (t, 3H, J = 7.2 Hz, CH_3), 2.45 (m, 1H, CH), 4.18 (q, 2H, J = 7.2 Hz, CH_2), 6.53 (s, 1H, H-4), 7.25–7.60 (m, 5H, Ph), 7.87, 9.63 (s, 2H, NH), 10.81 (s, 1H, NH). IR (Nujol) 3311 (NH), 3278 (NH), 1677 (C=O), 1657, 1607 cm^{-1}. Anal. Calcd for $C_{17}H_{21}N_3O_3$: C, 64.74; H, 6.71; N, 13.32. Found: C, 64.77; H, 6.70; N, 13.36.

*4.1.3.2. Ethyl 5-(4-chlorophenyl)-2-(2-isobutyrylhydrazinyl)-1H-pyrrole-3-carboxylate (**30**).* Yield 70%. Mp 173–174 °C (MeCN). ^{1}H NMR (DMSO-d_6): δ 1.03 (d, 6H, J = 6.9 Hz, CH_3), 1.21 (t, 3H, J = 7.3 Hz, CH_3), 2.42 (m, 1H, CH), 4.13 (q, 2H, J = 7.3 Hz, CH_2), 6.55 (s, 1H, H-4), 7.30 (d, 2H, J = 8.1 Hz, Ph), 7.56 (d, 2H, J = 8.1 Hz, Ph), 7.83, 9.62 (s, 2H, NH), 10.82 (s, 1H, NH). IR (Nujol) 3311 (NH), 3278 (NH), 1677 (C=O), 1657, 1607 cm^{-1}. Anal. Calcd for $C_{17}H_{20}ClN_3O_3$: C, 58.37; H, 5.76; N, 12.01. Found: C, 58.32; H, 5.77; N, 12.04.

*4.1.3.3. Ethyl 5-(4-methylphenyl)-2-(2-isobutyrylhydrazinyl)-1H-pyrrole-3-carboxylate (**31**).* Yield 80%. Mp 190–192 °C (MeCN). ^{1}H NMR (DMSO-d_6): δ 1.03 (d, 6H, J = 6.5 Hz, CH_3), 1.21 (t, 3H, J = 7.7 Hz, CH_3), 2.23 (s, 3H, CH_3), 2.41 (m, 1H, CH), 4.11 (q, 2H, J = 7.7 Hz, CH_2), 6.42 (s, 1H, H-4), 7.08 (d, 2H, J = 7.7 Hz, Ph), 7.42 (d, 2H, J = 7.7 Hz, Ph), 7.80, 9.59 (s, 2H, NH), 10.72 (s, 1H, NH). IR (Nujol) 3350 (NH), 3312 (NH), 3208 (NH), 1745 (C=O), 1657, 1599 cm^{-1}. Anal. Calcd for $C_{18}H_{23}N_3O_3$: C, 65.63; H, 7.04; N, 12.76. Found: C, 65.67; H, 7.02; N, 12.79.

*4.1.3.4. Ethyl 5-(4-methoxyphenyl)-2-(2-isobutyrylhydrazinyl)-1H-pyrrole-3-carboxylate (**32**).* Yield 62%. Mp 187–188 °C (MeCN). ^{1}H NMR (DMSO-d_6): δ 1.04 (d, 6H, J = 6.9 Hz, CH_3), 1.22 (t, 3H, J = 6.9 Hz, CH_3), 2.40 (m, 1H, CH), 3.71 (s, 3H, CH_3), 4.13 (q, 2H, J = 6.9 Hz, CH_3), 6.34 (s, 1H, H-4), 6.86 (d, 2H, J = 8.8 Hz, Ph), 7.46 (d, 2H, J = 8.8 Hz, Ph), 7.79, 9.59 (s, 2H, NH), 10.70 (s, 1H, NH). IR (Nujol) 3286 (NH), 1686 (C=O), 1650, 1596 cm^{-1}. Anal. Calcd for $C_{18}H_{23}N_3O_4$: C, 62.59; H, 6.71; N, 12.17. Found: C, 64.57; H, 6.70; N, 12.20.

*4.1.3.5. Ethyl 2-(2-butyrylhydrazinyl)-5-phenyl-1H-pyrrole-3-carboxylate (**33**).* Yield 71%. Mp 194–195 °C (MeCN). ^{1}H NMR (DMSO-d_6): δ 0.86 (t, 3H, J = 7.3 Hz, CH_3), 1.22 (t, 3H, J = 7.3 Hz, CH_3), 1.54 (m, 2H, CH_2), 2.13 (m, 2H, CH_2), 4.13 (q, 2H, J = 7.3 Hz, CH_2), 6.48 (s, 1H, H-4), 7.08–7.53 (m, 5H, Ph), 7.82, 9.80 (s, 2H, NH), 10.73 (s, 1H, NH). IR (Nujol) 3320 (NH), 3269 (NH), 1666 (C=O), 1611, 1597 cm^{-1}. Anal. Calcd for $C_{17}H_{21}N_3O_3$: C, 64.74; H, 6.71; N, 13.32. Found: C, 64.77; H, 6.70; N, 13.36.

*4.1.3.6. Ethyl 5-(4-chlorophenyl)-2-(2-butyrylhydrazinyl)-1H-pyrrole-3-carboxylate (**34**).* Yield 76%. Mp 149–150 °C (MeCN). ^{1}H NMR (DMSO-d_6): δ 0.88 (t, 3H, J = 6.9 Hz, CH_3), 1.22 (t, 3H, J = 7.3 Hz, CH_3), 1.56 (m, 2H, CH_2), 2.20 (m, 2H, CH_2), 4.13 (q, 2H, J = 6.9 Hz, CH_2), 6.20 (s, 1H, H-4), 7.28 (d, 2H, J = 8.0 Hz, Ph), 7.58 (d, 2H, J = 8.0 Hz, Ph), 7.83, 9.63 (s, 2H, NH), 10.67 (s, 1H, NH). IR (Nujol) 3374 (NH), 3337 (NH), 3299 (NH), 1684 (C=O), 1656, 1616, 1595 cm^{-1}. Anal. Calcd for $C_{17}H_{20}ClN_3O_3$: C, 58.37; H, 5.76; N, 12.01. Found: C, 58.34; H, 5.75; N, 12.03.

*4.1.3.7. Ethyl 5-(4-methylphenyl)-2-(2-butyrylhydrazinyl)-1H-pyrrole-3-carboxylate (**35**).* Yield 82%. Mp 194–195 °C (MeCN). ^{1}H NMR (DMSO-d_6): δ 0.85 (t, 3H, J = 7.7 Hz, CH_3), 1.21 (t, 3H, J = 7.3 Hz, CH_3), 1.52 (m, 2H, CH_2), 2.13 (m, 2H, CH_2), 2.22 (s, 3H, CH_3), 4.30 (q, 2H, J = 7.3 Hz, CH_2), 6.41 (s, 1H, H-4), 7.10 (d, 2H, J = 7.7 Hz, Ph, H-3 and H-5), 7.60 (d, 2H, J = 7.7 Hz, Ph, H-2 and H-6), 7.80, 9.59 (s, 2H, NH), 10.69 (s, 1H, NH). IR (Nujol) 3324 (NH), 1659 (C=O), 1602 cm^{-1}. Anal. Calcd for $C_{18}H_{23}N_3O_3$: C, 65.63; H, 7.04; N, 12.76. Found: C, 65.68; H, 7.05; N, 12.74.

*4.1.3.8. Ethyl 5-(4-methoxyphenyl)-2-(2-butyrylhydrazinyl)-1H-pyrrole-3-carboxylate (**36**).* Yield 92%. Mp 180–182 °C (MeCN). ^{1}H NMR (DMSO-d_6): δ 0.86 (t, 3H, J = 7.3 Hz, CH_3), 1.21 (t, 3H, J = 6.9 Hz, CH_3), 1.52 (m, 2H, CH_2), 2.13 (m, 2H, CH_2), 3.79 (s, 3H, CH_3), 4.13 (q, 2H, J = 6.9 Hz, CH_2), 6.33 (s, 1H, H-4), 6.85 (d, 2H, J = 8.8 Hz, Ph, H-3 and H-5), 7.45 (d, 2H, J = 8.8 Hz, Ph, H-2 and H-6), 7.77, 9.58 (s, 2H, NH), 10.65 (s, 1H, NH). IR (Nujol) 3268 (NH), 1669 (C=O), 1633, 1596 cm^{-1}. Anal. Calcd for $C_{18}H_{23}N_3O_4$: C, 62.59; H, 6.71; N, 12.17. Found: C, 62.64; H, 6.70; N, 12.14.

*4.1.3.9. Ethyl 2-(2-(2-(4-methoxyphenyl)acetyl)hydrazinyl)-5-phenyl-1H-pyrrole-3-carboxylate (**37**).* Yield 86%. Mp 144–145 °C (MeCN). ^{1}H NMR (DMSO-d_6): δ 1.20 (t, 3H, J = 6.9 Hz, CH_3), 3.42 (s, 2H, CH_2), 3.67 (s, 3H, CH_3), 4.13 (q, 2H, J = 6.9 Hz, CH_2), 6.49 (s, 1H, H-4), 6.82 (d, 2H, J = 7.7 Hz, Ph, H-3 and H-5), 7.10–7.28 (m, 5H, Ph), 7.52 (d, 2H, J = 7.7 Hz, Ph, H-2 and H-6), 7.85, 9.88 (s, 2H, NH), 10.76 (s, 1H, NH). IR (Nujol) 3444 (NH), 3291 (NH), 1670 (C=O), 1608, 1593 cm^{-1}. Anal. Calcd for $C_{22}H_{23}N_3O_4$: C, 67.16; H, 5.89; N, 10.68. Found: C, 67.21; H, 5.90; N, 10.66.

*4.1.3.10. Ethyl 5-(4-chlorophenyl)-2-(2-(2-(4-methoxyphenyl)acetyl)hydrazinyl)-1H-pyrrole-3-carboxylate (**38**).* Yield 39%. Mp 179–180 °C (MeCN). ^{1}H NMR (DMSO-d_6): δ 1.21 (t, 3H, J = 7.0 Hz, CH_3), 3.42 (s, 2H, CH_2), 3.67 (s, 3H, CH_3), 4.13 (q, 2H, J = 7.0 Hz, CH_2), 6.45 (s, 1H, H-4), 6.84 (d, 2H, J = 8.0 Hz, 4-OMePh), 7.09 (d, 2H, J = 7.8 Hz, 4-OMePh), 7.25 (d, 2H, J = 8.0 Hz, 4-ClPh), 7.44 (d, 2H, J = 7.8 Hz, 4-ClPh), 7.86, 9.83 (s, 2H, NH), 10.85 (s, 1H, NH). IR (Nujol) 3380 (NH), 3233 (NH), 1669 (C=O), 1611 cm^{-1}. Anal. Calcd for $C_{22}H_{22}ClN_3O_4$: C, 61.75; H, 5.18; N, 9.82. Found: C, 61.69; H, 5.19; N, 9.80.

*4.1.3.11. Ethyl 5-(4-methylphenyl)-2-(2-(2-(4-methoxyphenyl)acetyl)hydrazinyl)-1H-pyrrole-3-carboxylate (**39**).* Yield 66%. Mp 169–170 °C (MeCN). ^{1}H NMR (DMSO-d_6): δ 1.20 (t, 3H, J = 6.9 Hz, CH_3), 2.23 (s, 3H, CH_3), 3.41 (s, 2H, CH_2), 3.67 (s, 3H, CH_3), 4.11 (q, 2H, J = 6.9 Hz, CH_2), 6.42 (s, 1H, H-4), 6.82 (d, 2H, J = 8.1 Hz, 4-OMePh), 7.10 (d, 2H, J = 7.7 Hz, 4-OMePh), 7.20 (d, 2H, J = 8.1 Hz, 4-MePh), 7.42 (d, 2H, J = 7.7 Hz, 4-MePh), 7.83, 9.87 (s, 2H, NH), 10.71 (s, 1H, NH). IR (Nujol) 3444 (NH), 3286 (NH), 1669 (C=O), 1597 cm^{-1}. Anal. Calcd for $C_{23}H_{25}N_3O_4$: C, 67.80; H, 6.18; N, 10.31. Found: C, 67.77; H, 6.20; N, 10.28.

*4.1.3.12. Ethyl 5-phenyl-2-(2-propanoylhydrazinyl)-1H-pyrrole-3-carboxylate (**40**).* Yield 27%. Mp 159–160 °C (MeCN). ^{1}H NMR (DMSO-d_6): δ 1.00 (t, 3H, J = 7.3 Hz, CH_3), 1.22 (t, 3H, J = 7.3 Hz, CH_3), 2.17 (q, 2H, J = 7.3 Hz, CH_2), 4.13 (q, 2H, J = 7.3 Hz, CH_2), 6.49 (s, 1H, H-4), 7.08–7.54 (m, 5H, Ph), 7.80, 9.61 (s, 2H, NH), 10.75 (s, 1H, NH). IR (Nujol) 3319 (NH), 1661 (C=O), 1597 cm^{-1}. Anal. Calcd for $C_{16}H_{19}N_3O_3$: C, 63.77; H, 6.36; N, 13.94. Found: C, 63.70; H, 6.37; N, 13.96.

*4.1.3.13. Ethyl 5-(4-methoxyphenyl)-2-(2-propanoylhydrazinyl)-1H-pyrrole-3-carboxylate (**41**).* Yield 35%. Mp 187–188 °C (MeCN). ^{1}H NMR (DMSO-d_6): δ 0.99 (t, 3H, J = 7.3 Hz, CH_3), 1.21 (t, 3H, J = 6.9 Hz, CH_3), 2.15 (q, 2H, J = 7.3 Hz, CH_2), 3.60 (s, 3H, CH_3), 4.12 (q, 2H, J = 6.9 Hz, CH_2), 6.32 (s, 1H, H-4), 6.85 (d, 2H, J = 8.6 Hz, Ph, H-3 and H-5), 7.49 (d, 2H, J = 8.6 Hz, Ph, H-2 and H-6), 7.75, 9.59 (s, 2H, NH), 10.66 (s, 1H, NH). IR (Nujol) 3519 (NH), 3320 (NH), 1668 (C=O), 1642, 1604, 1585 cm^{-1}. Anal. Calcd for $C_{17}H_{21}N_3O_4$: C, 61.62; H, 6.39; N, 12.68. Found: C, 61.67; H, 6.38; N, 12.65.

*4.1.3.14. Ethyl 2-(2-(3-methoxypropanoyl)hydrazinyl)-5-phenyl-1H-pyrrole-3-carboxylate (**42**).* Yield 22%. Mp 141–142 °C (MeCN). ^{1}H NMR (DMSO-d_6): δ 1.23 (t, 3H, J = 6.9 Hz, CH_3), 2.41 (m, 2H, CH_2), 3.21 (s, 3H, CH_3), 3.54 (m, 2H, CH_2), 4.13 (q, 2H, J = 6.9 Hz, CH_2), 6.50 (s, 1H, H-4), 7.09–7.50 (m, 5H, Ph), 7.85, 9.75 (s, 2H, NH), 10.64 (s, 1H, NH). IR (Nujol) 3274 (NH), 3257 (NH), 1672 (C=O), 1641, 1605, 1592 cm^{-1}. Anal. Calcd for $C_{17}H_{21}N_3O_4$: C, 61.62; H, 6.39; N, 12.68. Found: C, 61.66; H, 6.38; N, 12.72.

*4.1.3.15. Ethyl 5-(4-methoxyphenyl)-2-(2-(3-methoxypropanoyl) hydrazinyl)-1H-pyrrole-3-carboxylate (**43**).* Yield 68%. Mp 157–158 °C (MeCN). ^{1}H NMR (DMSO-d_6): δ 1.21 (t, 3H, J = 7.0 Hz, CH_3), 2.39 (m, 2H, CH_2), 3.19 (s, 3H, CH_3), 2.54 (m, 2H, CH_2), 3.70 (s, 3H, CH_3), 4.12 (q, 2H, J = 7.0 Hz, CH_2), 6.33 (s, 1H, H-4), 6.85 (d, 2H, J = 7.3 Hz, Ph, H-3 and H-5), 7.44 (d, 2H, J = 7.3 Hz, Ph, H-2 and H-6), 7.79, 9.72 (s, 2H, NH), 10.54 (s, 1H, NH). IR (Nujol) 3281 (NH), 3261 (NH), 1674 (C=O), 1639, 1596 cm^{-1}. Anal. Calcd for $C_{18}H_{23}N_3O_5$: C, 59.82; H, 6.41; N, 11.63. Found: C, 59.77; H, 6.40; N, 11.66.

*4.1.3.16. 2-(2-Acetylhydrazinyl)-5-phenyl-1H-pyrrole-3-carbonitrile (**44**).* Yield 98%. Mp 198–200 °C (MeCN). ^{1}H NMR (DMSO-d_6): δ 1.83 (s, 3H, CH_3), 6.50 (s, 1H, H-4), 7.07–7.50 (m, 5H, Ph), 8.09, 9.81 (s, 2H, NH), 11.05 (s, 1H, NH). IR (Nujol) 3364 (NH), 3260 (NH), 2215 (CN), 1679 (C=O), 1618 cm^{-1}. Anal. Calcd for $C_{13}H_{12}N_4O$: C, 64.99; H, 5.03; N, 23.32. Found: C, 65.03; H, 5.02; N, 23.36.

*4.1.3.17. 2-(2-Acetylhydrazinyl)-5-(4-methoxyphenyl)-1H-pyrrole-3-carbonitrile (**45**).* Yield 40%. Mp 194–195 °C (MeCN). ^{1}H NMR (DMSO-d_6): δ 1.83 (s, 3H, CH_3), 3.80 (s, 3H, CH_3), 6.32 (s, 1H, H-4), 6.33 (d, 2H, J = 8.4 Hz, Ph, H-3 and H-5), 7.41 (d, 2H, J = 8.4 Hz, Ph, H-2 and H-6), 7.98, 9.78 (s, 2H, NH), 10.93 (s, 1H, NH). IR (Nujol) 3279 (NH), 2197 (CN), 1697 (C=O), 1673, 1602 cm^{-1}. Anal. Calcd for $C_{14}H_{14}N_4O_2$: C, 62.21; H, 5.22; N, 20.73. Found: C, 62.27; H, 5.21; N, 20.77.

*4.1.3.18. 5-Phenyl-2-(2-propanoylhydrazinyl)-1H-pyrrole-3-carbonitrile (**46**).* Yield 27%. Mp 189–190 °C (MeCN). ^{1}H NMR (DMSO-d_6): δ 1.33 (t, 3H, J = 5.1 Hz, CH_3), 4.03 (q, 2H, J = 5.1 Hz, CH_2), 6.50 (s, 1H, H-4), 7.10–7.50 (m, 5H, Ph), 8.11, 9.17 (s, 2H, NH), 11.08 (s, 1H, NH). IR (Nujol) 3280 (NH), 2205 (CN), 1731 (C=O), 1707, 1613 cm^{-1}. Anal. Calcd for $C_{14}H_{14}N_4O$: C, 66.13; H, 5.55; N, 22.03. Found: C, 66.17; H, 5.56; N, 22.06.

*4.1.3.19. 5-(4-Methoxyphenyl)-2-(2-propanoylhydrazinyl)-1H-pyrrole-3-carbonitrile (**47**).* Yield 39%. Mp 179–180 °C (MeCN). ^{1}H NMR (DMSO-d_6): δ 1.00 (m, 3H, CH_3), 2.13 (m, 2H, CH_2), 3.70 (s, 3H, CH_3), 6.32 (s, 1H, H-4), 6.86 (d, 2H, J = 7.0 Hz, Ph, H-3 and H-5), 7.42 (d, 2H, J = 7.0 Hz, Ph, H-2 and H-6), 7.96, 9.76 (s, 2H, NH), 10.96 (s, 1H, NH). IR (Nujol) 3332 (NH), 3321 (NH), 2201 (CN), 1735 (C=O), 1708, 1617 cm^{-1}. Anal. Calcd for $C_{15}H_{16}N_4O_2$: C, 63.37; H, 5.67; N, 19.71. Found: C, 63.42; H, 5.66; N, 19.74.

*4.1.3.20. 5-(4-Methoxyphenyl)-2-(2-isobutyrylhydrazinyl)-1H-pyrrole-3-carbonitrile (**48**).* Yield 24%. Mp 208–210 °C (MeCN). ^{1}H NMR (DMSO-d_6): δ 0.98 (d, 6H, J = 6.9 Hz, CH_3), 2.39 (m, 1H, CH), 3.68 (s, 3H, CH_3), 6.54 (s, 1H, H-4), 7.18 (d, 2H, J = 7.8 Hz, Ph, H-3 and H-5), 7.50 (d, 2H, J = 7.8 Hz, Ph, H-2 and H-6), 7.94, 9.86 (s, 2H, NH), 11.09 (s, 1H, NH). IR (Nujol) 3259 (NH), 2204 (CN), 1696 (CO), 1672, 1599 cm^{-1}. Anal. Calcd for $C_{16}H_{18}N_4O_2$: C, 64.41; H, 6.08; N, 18.78. Found: C, 64.47; H, 6.07; N, 18.76.

*4.1.3.21. 2-(2-Ethoxycarbonylhydrazinyl)-5-phenyl-1H-pyrrole-3-carbonitrile (**49**).* Yield 34%. Mp 204–205 °C (MeCN). ^{1}H NMR (DMSO-d_6): δ 1.16 (t, 3H, J = 6.9 Hz, CH_3), 4.03 (q, 2H, J = 6.9 Hz, CH_2), 6.51 (s, 1H, H-4), 7.05–7.53 (m, 5H, Ph), 8.13, 9.17 (s, 2H, NH), 11.09 (s, 1H, NH). IR (Nujol) 3286 (NH), 2200 (CN), 1699 (C=O), 1675, 1612 cm^{-1}. Anal. Calcd for $C_{14}H_{14}N_4O_2$: C, 62.21; H, 5.22; N, 20.73. Found: C, 62.16; H, 5.20; N, 20.78.

*4.1.3.22. 2-(2-Ethoxycarbonylhydrazinyl)-5-(4-methoxyphenyl)-1H-pyrrole-3-carbonitrile (**50**).* Yield 38%. Mp 205–206 °C (MeCN). ^{1}H NMR (DMSO-d_6): δ 1.00 (t, 3H, J = 7.7 Hz, CH_3), 3.86 (s, 3H, CH_3), 4.02 (q, 2H, J = 7.7 Hz, CH_2), 6.32 (s, 1H, H-4), 6.86 (d, 2H, J = 8.4 Hz, Ph, H-3 and H-5), 7.41 (d, 2H, J = 8.4 Hz, Ph, H-2 and H-6), 7.94, 9.86 (s, 2H, NH), 10.95 (s, 1H, NH). IR (Nujol) 3301, 2198 (CN), 1695 (C=O), 1673, 1619 cm^{-1}. Anal. Calcd for $C_{15}H_{16}N_4O_3$: C, 59.99; H, 5.37; N, 18.66. Found: C, 60.06; H, 5.38; N, 18.63.

*4.1.3.23. 2-(2-(2-Methoxyacetyl)hydrazinyl)-5-phenyl-1H-pyrrole-3-carbonitrile (**51**).* Yield 24%. Mp 181–182 °C (MeCN). ^{1}H NMR (DMSO-d_6): δ 2.40 (s, 2H, CH_2), 3.90 (s, 3H, CH_3), 6.50 (s, 1H, H-4), 7.07–7.51 (m, 5H, Ph), 8.16, 9.87 (s, 2H, NH), 11.07 (s, 1H, NH). IR (Nujol) 3296 (NH), 3232 (NH), 2204 (CN), 1702 (C=O), 1678, 1613, 1595 cm^{-1}. Anal. Calcd for $C_{14}H_{14}N_4O_2$: C, 62.21; H, 5.22; N, 20.73. Found: C, 62.27; H, 5.23; N, 20.68.

*4.1.3.24. 2-(2-(2-Methoxyacetyl)hydrazinyl)-5-(4-methoxyphenyl)-1H-pyrrole-3-carbonitrile (**52**).* Yield 35%. Mp 189–190 °C (MeCN). ^{1}H NMR (DMSO-d_6): δ 2.48 (s, 2H, CH_2), 3.36 (s, 3H, CH_3), 3.70 (s, 3H, CH_3), 6.33 (s, 1H, H-4), 6.86 (d, 2H, J = 8.4 Hz, Ph, H-3 and H-5), 7.41 (d, 2H, J = 8.4 Hz, Ph, H-2 and H-6), 8.07, 9.85 (s, 2H, NH), 10.97 (s, 1H, NH). IR (Nujol) 3312 (NH), 2201 (CN), 1682 (C=O), 1663, 1619 cm^{-1}. Anal. Calcd for $C_{15}H_{16}N_4O_3$: C, 59.99; H, 5.37; N, 18.66. Found: C, 59.92; H, 5.38; N, 18.70.

*4.1.3.25. 2-(2-(3-Methoxypropanoyl)hydrazinyl)-5-phenyl-1H-pyrrole-3-carbonitrile (**53**).* Yield 29%. Mp 187–188 °C (MeCN). ^{1}H NMR (DMSO-d_6): δ 2.38 (t, 2H, J = 6.1 Hz, CH_2), 3.17 (s, 3H, CH_3), 3.54 (t, 2H, J = 6.1 Hz, CH_2), 6.50 (s, 1H, H-4), 7.10–7.48 (m, 5H, Ph), 8.17, 9.87 (s, 2H, NH), 11.01 (s, 1H, NH). IR (Nujol) 3298 (NH), 2205 (CN), 1696 (C=O), 1672, 1611 cm^{-1}. Anal. Calcd for $C_{15}H_{16}N_4O_2$: C, 63.37; H, 5.67; N, 19.71. Found: C, 63.41; H, 5.69; N, 19.68.

*4.1.3.26. 5-(4-Methoxyphenyl)-2-(2-(3-methoxypropanoyl)hydrazinyl)-1H-pyrrole-3-carbonitrile (**54**).* Yield 37%. Mp 174–175 °C (MeCN). ^{1}H NMR (DMSO-d_6): δ 2.38 (t, 2H, J = 6.6 Hz, CH_2), 3.18 (s, 3H, CH_3), 3.54 (t, 2H, J = 6.6 Hz, CH_2), 3.70 (s, 3H, CH_3), 6.32 (s, 1H, H-4), 6.86 (d, 2H, J = 8.6 Hz, Ph, H-3 and H-5), 7.41 (d, 2H, J = 8.6 Hz, Ph, H-2 and H-6), 8.07, 9.84 (s, 2H, NH), 10.89 (s, 1H, NH). IR (Nujol) 3278 (NH), 1668 (C=O), 1644, 1596 cm^{-1}. Anal. Calcd for $C_{16}H_{18}N_4O_3$: C, 61.13; H, 5.77; N, 17.82. Found: C, 61.17; H, 5.76; N, 17.86.

*4.1.3.27. Methyl 2-(2-acetylhydrazinyl)-5-phenyl-1H-pyrrole-3-carboxylate (**55**).* Yield 34%. Mp 174–175 °C (MeCN). ^{1}H NMR (DMSO-d_6): δ 1.87 (s, 3H, CH_3), 3.64 (s, 3H, CH_3), 6.50 (s, 1H, H-4), 7.08–7.53 (m, 5H, Ph), 7.81, 9.67 (s, 2H, NH), 10.74 (s, 1H, NH). IR (Nujol) 3224 (NH), 1686 (C=O), 1647, 1611 cm^{-1}. Anal. Calcd for $C_{14}H_{15}N_3O_3$: C, 61.53; H, 5.53; N, 15.38. Found: C, 61.49; H, 5.54; N, 15.36.

*4.1.3.28. Methyl 2-(2-acetylhydrazinyl)-5-(4-methoxyphenyl)-1H-pyrrole-3-carboxylate (**56**).* Yield 31%. Mp 179–180 °C (MeCN). ^{1}H NMR (DMSO-d_6): δ 1.86 (s, 3H, CH_3), 3.63 (s, 3H, CH_3), 3.70 (s, 3H, CH_3), 6.33 (s, 1H, H-4), 6.85 (d, 2H, $J = 8.8$ Hz, Ph, H-3 and H-5), 7.46 (d, 2H, $J = 8.8$ Hz, Ph, H-2 and H-6), 7.76, 9.65 (s, 2H, NH), 10.65 (s, 1H, NH). IR (Nujol) 3327 (NH), 3340 (NH), 1686 (C=O), 1642, 1603 cm^{-1}. Anal. Calcd for $C_{15}H_{17}N_3O_4$: C, 59.40; H, 5.65; N, 13.85. Found: C, 59.35; H, 5.66; N, 13.89.

*4.1.3.29. Methyl 5-phenyl-2-(2-propanoylhydrazinyl)-1H-pyrrole-3-carboxylate (**57**).* Yield 34%. Mp 149–150 °C (MeCN). ^{1}H NMR (DMSO-d_6): δ 1.00 (t, 3H, $J = 7.7$ Hz, CH_3), 2.16 (q, 2H, $J = 7.7$ Hz, CH_2), 3.64 (s, 3H, CH_3), 6.50 (s, 1H, H-4), 7.05–7.53 (m, 5H, Ph), 7.61, 9.61 (s, 2H, NH), 10.74 (s, 1H, NH). IR (Nujol) 3311 (NH), 3272 (NH), 1685 (C=O), 1647, 1609 cm^{-1}. Anal. Calcd for $C_{15}H_{17}N_3O_3$: C, 62.71; H, 5.96; N, 14.63. Found: C, 62.77; H, 5.95; N, 14.60.

*4.1.3.30. Methyl 5-(4-methoxyphenyl)-2-(2-propanoylhydrazinyl)-1H-pyrrole-3-carboxylate (**58**).* Yield 44%. Mp 185–186 °C (MeCN). ^{1}H NMR (DMSO-d_6): δ 1.00 (t, 3H, $J = 8.4$ Hz, CH_3), 2.16 (q, 2H, $J = 8.4$ Hz, CH_2), 3.64 (s, 3H, CH_3), 3.70 (s, 3H, CH_3), 6.34 (s, 1H, H-4), 6.85 (d, 2H, $J = 8.1$ Hz, Ph, H-3 and H-5), 7.46 (d, 2H, $J = 8.1$ Hz, Ph, H-2 and H-6), 7.76, 9.59 (s, 2H, NH), 10.66 (s, 1H, NH). IR (Nujol) 3518 (NH), 3307 (NH), 1675 (C=O), 1643, 1601 cm^{-1}. Anal. Calcd for $C_{16}H_{19}N_3O_4$: C, 60.56; H, 6.03; N, 13.24. Found: C, 60.63; H, 6.02; N, 13.26.

*4.1.3.31. Methyl 2-(2-ethoxycarbonylhydrazinyl)-5-phenyl-1H-pyrrole-3-carboxylate (**59**).* Yield 21%. Mp 151–152 °C (MeCN). ^{1}H NMR (DMSO-d_6): δ 1.16 (t, 3H, $J = 6.8$ Hz, CH_3), 3.64 (s, 3H, CH_3), 4.03 (q, 2H, $J = 6.8$ Hz, CH_2), 6.51 (s, 1H, H-4), 7.07–7.49 (m, 5H, Ph), 7.84, 9.02 (s, 2H, NH), 10.88 (s, 1H, NH). IR (Nujol) 3554 (NH), 3309 (NH), 1703 (C=O), 1650 (C=O), 1614 cm^{-1}. Anal. Calcd for $C_{15}H_{17}N_3O_4$: C, 59.40; H, 5.65; N, 13.85. Found: C, 59.46; H, 5.64; N, 13.86.

*4.1.3.32. Methyl 2-(2-ethoxycarbonylhydrazinyl)-5-(4-methoxy phenyl)-1H-pyrrole-3-carboxylate (**60**).* Yield 11%. Mp 141–142 °C (MeCN). ^{1}H NMR (DMSO-d_6): δ 1.14 (t, 3H, $J = 6.8$ Hz, CH_3), 3.64 (s, 3H, CH_3), 3.70 (s, 3H, CH_3), 4.02 (q, 2H, $J = 6.8$ Hz, CH_2), 6.49 (s, 1H, H-4), 6.85 (d, 2H, $J = 8.1$ Hz, Ph, H-3 and H-5), 7.52 (d, 2H, $J = 8.1$ Hz, Ph, H-2 and H-6), 7.58, 9.60 (s, 2H, NH), 10.43 (s, 1H, NH). IR (Nujol) 3518 (NH), 3307 (NH), 1675 (C=O), 1643, 1601 cm^{-1}. Anal. Calcd for $C_{16}H_{19}N_3O_5$: C, 57.65; H, 5.75; N, 12.61. Found: C, 57.60; H, 5.74; N, 12.63.

*4.1.3.33. Methyl 2-(2-(3-methoxypropanoyl)hydrazinyl)-5-phenyl-1H-pyrrole-3-carboxylate (**61**).* Yield 59%. Mp 172–173 °C (MeCN). ^{1}H NMR (DMSO-d_6): δ 2.40 (m, 2H, CH_2), 3.20 (s, 3H, CH_3), 3.54 (m, 2H, CH_2), 3.65 (s, 3H, CH_3), 6.50 (s, 1H, H-4), 7.06–7.53 (m, 5H, Ph), 7.85, 9.73 (s, 2H, NH), 10.61 (s, 1H, NH). IR (Nujol) 3287 (NH), 3257 (NH), 1668 (C=O), 1646, 1606 cm^{-1}. Anal. Calcd for $C_{16}H_{19}N_3O_4$: C, 60.56; H, 6.03; N, 13.24. Found: C, 60.65; H, 6.01; N, 13.26.

*4.1.3.34. Methyl 5-(4-methoxyphenyl)-2-(2-(3-methoxypropanoyl)hydrazinyl)-1H-pyrrole-3-carboxylate (**62**).* Yield 54%. Mp 174–175 °C (MeCN). ^{1}H NMR (DMSO-d_6): δ 2.40 (m, 2H, CH_2), 3.19 (s, 3H, CH_3), 3.53 (m, 2H, CH_2), 3.64 (s, 3H, CH_3), 3.70 (s, 3H, CH_3), 6.34 (s, 1H, H-4), 6.85 (d, 2H, $J = 6.5$ Hz, Ph, H-3 and H-5), 7.44 (d, 2H, $J = 6.5$ Hz, Ph, H-2 and H-6), 7.80, 9.72 (s, 2H, NH), 10.54 (s, 1H, NH). IR (Nujol) 3278 (NH), 1668 (C=O), 1644, 1596 cm^{-1}. Anal. Calcd for $C_{17}H_{21}N_3O_5$: C, 58.78; H, 6.09; N, 12.10. Found: C, 58.72; H, 6.07; N, 12.12.

4.2. Antifungal susceptibility assays

MICs were determined by the broth microdilution method according to the NCCLS reference document M27-A [24]. RPMI 1640 medium (Sigma Chemicals) without $NaHCO_3$, supplemented with L-glutamine (Gibco, Invitrogen) and buffered with 0.165 M morpholinepropanesulfonic acid (MOPS, Sigma Chemicals) at pH 7.0 was used as test medium. Twofold dilutions of the drugs with concentrations ranging between 0.008 and 200 mg/L were obtained in RPMI 1640 and dispensed into the wells of plastic microdilution trays. Starting inoculum suspensions were obtained by the spectrophotometric method of inoculum preparation, adjusted to 10^6 CFU/mL and then diluted in test medium to 2×10^4 cells/mL. A 100-μl yeast inoculum was added to each well of the microdilution trays to obtain final concentrations of the drugs ranging between 0.004 and 100 mg/L and final inocula of 10^4 cells/mL. The inoculated plates were incubated overnight at 35 °C in a humid atmosphere. After agitation, plates were visually read with the aid of a reading mirror and spectrophotometrically with an automatic plate reader (Sunrise Tecan, Grödig/Salzburg, Austria) set at 450 nm. MICs were determined either visually or by spectrophotometric evaluation showed excellent agreement.

After the MIC determination, a 20-μl sample from each well was seeded in plates of Sabouraud Dextrose agar. Plates were incubated for 72 h at 35 °C. The minimum fungicidal concentration (MFC) was defined as the minimum concentration of compound which resulted in the growth of less than two colonies representing the killing of >99% of the original inoculum.

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References

[1] J.M. Brown, Curr. Opin. Infect. Dis. 17 (2004) 347–352.
[2] G. Garber, Drugs 61 (2001) 1–12.
[3] T.J. Walsh, A.H. Groll, Transpl. Infect. Dis. 1 (1999) 247–261.
[4] E. Anaissie, Clin. Infect. Dis. 14 (1992) S43–S53.
[5] P. Eggimann, J. Garbino, D. Pittet, Lancet Infect. Dis. 3 (2003) 685–702.
[6] N.M. Ampel, Emerg. Infect. Dis. 2 (1996) 109–116.
[7] M.A. Pfaller, Clin. Infect. Dis. 19 (Suppl. 1) (1994) S8–S13.
[8] W.G. Powderly, G.S. Kobayashi, G.P. Herzig, G. Medoff, Am. J. Med. 84 (1988) 826.
[9] M.A. Pfaller, S.A. Messer, R.J. Hollis, R.N. Jones, G.V. Doern, M.E. Brandt, R.A. Hajjeh, Diagn. Microbiol. Infect. Dis. 33 (1999) 217–222.
[10] V. Fanos, L. Cataldi, J. Chemother. 12 (2000) 463–470.
[11] D.J. Sheehan, C.A. Hitchcock, C.M. Sibley, Clin. Microbiol. Rev. 12 (1999) 40–79.
[12] G. Deray, J. Antimicrob. Chemother. 49 (2002) 37–41.
[13] B. Dupont, J. Antimicrob. Chemother. 49 (2002) 31–36.
[14] J.W. Baddley, S.A. Moser, Clin. Lab. Med. 24 (2004) 721–735.
[15] M.A. Ghannoum, L.B. Rice, Clin. Microbiol. Rev. 12 (1999) 501–517.
[16] J.R. Perfect, Oncology (Williston Park) 18 (2004) 15–22.
[17] V.C. Krcmery Jr., Med. Princ. Pract. 14 (2005) 125–135.
[18] D. Sanglard, F.C. Odds, Lancet Infect. Dis. 2 (2002) 73–85.
[19] (a) P. Marichal, Curr. Opin. Anti-Infect. Invest. Drugs 1 (1999) 318; (b) C. Jana, S. Julius, Int. J. Antimicrob. Agents 27 (2006) 403.
[20] M.T. Cocco, C. Congiu, A. Maccioni, M.L. Schivo, G. Palmieri, Farmaco XLIII (1988) 319–331.
[21] M.T. Cocco, C. Congiu, V. Onnis, A. Maccioni, J. Heterocycl. Chem. 28 (1991) 797–800.
[22] M.T. Cocco, C. Congiu, V. Onnis, Trends of Heterocyclic Chemistry, Research Trends, Council of Scientific Informations, 1995, pp. 13–23.

[23] A.M. Bernard, M.T. Cocco, C. Congiu, V. Onnis, P.P. Piras, J. Heterocycl. Chem. 33 (1996) 1771–1773.
[24] National Committee for Clinical Laboratory Standards (NCCLS), Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts: Approved Standard M27-A, NCCLS, Villanova, PA, 1997.
[25] A. Monks, D.A. Scudiero, G.S. Johnson, K.D. Paull, E.A. Sausville, Anticancer Drug Des. 12 (1997) 533.
[26] A. Monks, D. Scudiero, P. Skehan, R. Shoemaker, K.D. Paull, D. Vistica, C. Hose, J. Langley, P. Cronise, A. Vaigro-Wolff, M. Gray-Goodrich, H. Campbell, J. Mayo, M. Boyd, J. Natl. Cancer Inst. 83 (1991) 757–766.
[27] J.N. Weinstein, T.G. Myers, P.M. O'Connor, S.H. Friend, A.J. Fornance Jr., K.W. Kohn, T. Fojo, S.E. Bates, L.V. Rubinstein, N.L. Anderson, J.K. Buolamwini, W.W. van Osdol, A.P. Monks, D.A. Scudiero, E.A. Sausville, D.W. Zaharevitz, B. Bunow, V.N. Viswanadhan, G.S. Johnson, R.E. Wittes, K.D. Paull, Science 275 (1997) 343–349.