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Synthesis and anti-*Candidal* activity of *N*-(4-aryl/cyclohexyl)-2-(pyridine-4-yl carbonyl) hydrazinecarbothioamide

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ABSTRACT

Eighteen *N*-(4-aryl/cyclohexyl)-2-(pyridine-4-yl carbonyl) hydrazinecarbothioamide derivatives were synthesized, evaluated against ten clinical isolates of *Candida* spp. and compared with itraconazole. Introduction of *p*-chloro (**2c**), *p*-iodo (**2q**), *m*-chloro (**2l**) and *o*-nitro (**2r**) substitution at phenyl ring of thiosemicarbazide enhanced the anti-*Candida* activity. Compound (**2c**) bearing *p*-cholorophenyl ring was found to be the most effective against *Candida albicans* ATCC 66027, *Candida* spp. 12810 (blood) and *Candida* spp. 178 (HVS) with MIC value of 0.09–0.78 μg/mL, whereas itraconazole exhibits the inhibitory activity with MIC value of 0.04–1.56 μg/mL against all tested strains. There is a correlation between anti-*Candidal* activity and *p*-chloro substitution at phenyl ring of thiosemicarbazide. All synthesized compounds were investigated for their potential cytotoxicity against non cancer cell line MCF-10A. The active compounds **2c**, **2r** and **2a** were further investigated for their cytotoxic effects on three cancer cell lines; HT1080 (skin), HepG2 (liver) and A549 (lung). The active compounds showed minimal cytotoxic activity against non cancer cell line and all three cancer cell lines. Moreover, compound **2c** displaying better activity against *C. albicans* ATCC66027 and *Candida* spp. [blood] compared to reference drug (itraconazole), represents a good lead for the development of newer, potent and broad spectrum anti-*Candidal* agents.

Invasive fungal infections (IFIs) are life-threatening opportunistic infections that are an increasingly important cause of morbidity and mortality in patients, especially those with compromised immune function and those hospitalized with serious underlying diseases.[1] The majority of these infections are caused by *Candida* spp., with over 50% due to *Candida albicans*.[2] These fungi are responsible for various forms of disease, ranging from superficial infections of the mucosal surfaces or skin to systemic infections, which in most cases is life threatening.[3] In general, for treatment of an infection with *Candida* spp., amphotericin and azole drugs are used, but these agents are not considered to satisfy medical needs due to their toxicity, side effects, drug interactions, limited routes, emergence of drug-resistant and drug-low-susceptible strains.[4] The most frequently implicated risk factors include treatment with broad-spectrum antibiotics, use of central venous catheters and implantable prosthetic devices, parenteral nutrition, prolonged intensive care unit stay, hemodialysis and immunosuppression (including HIV infection, neutropenia, use of glucocorticosteroids, chemotherapeutic agents, and immunomodulators).[5]

Based on docking and molecular modeling studies it was proposed that the possible target for antifungal activity of 4-arylthiosemicarbazides is the enzyme *N*-myristoyltransferase (NMT). The ligand recognition process is connected with high-electron density around the sulfur atom and geometry of NH—NH—C(=S)—NH core.[6] In the course of our search for prototype antifungal agents from the class of thiosemicarbazide derivatives, we have preliminary screened a series of compounds against clinical isolates of *Candida* spp. and ATCC strains of *Candida* species. Among tested compounds are 4-aryl/cyclohexyl thiosemicarbazides. Few other aryl thiosemicarbazides that exhibit significant anti-*Candidal* activity have been reported in the literature.[7–15] Those bearing isoquinoline ring are acting against several *Candida* species.[16] Thus such template could be seen as starting point for further optimization of novel antifungal agents. In order to develop new potent antifungal compounds, a new set of thiosemicarbazides (**2a**–**r**) based on compound A (Fig. 1) were synthesized and in vitro anti-*Candida* activity was studied. Pyridyl ring, a prominent scaffold present in

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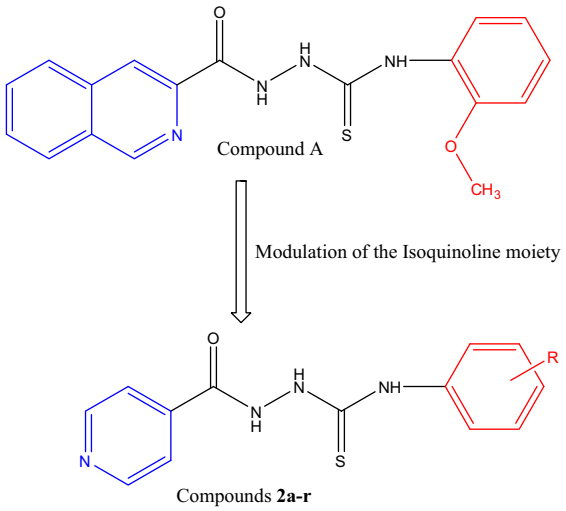
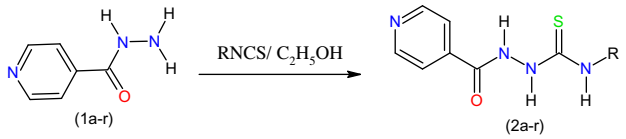


Figure 1. Structure of the lead compound A and newly synthesized compounds (**2a**–**r**).



Scheme 1. Synthetic protocol of the compounds (**2a**–**r**).

various bioactive molecules, has played a vital role in the development of different medicinal agents.[17–19] The anti-*Candidal* activity of pyridine has also been reported.[20] The pharmacophore was preserved and isoquinoline moiety was replaced by pyridine moiety of the isoniazid.

The synthesis of isoniazid (INH) derivatives was carried out in single step shown in (Scheme 1). Isoniazid was reacted with appropriate substituted phenyl/cyclohexyl isothiocyanate in presence of absolute ethanol to yield *N*-(4-aryl/cyclohexyl)-2-(pyridin-4-yl-carbonyl) hydrazinecarbothioamide (**2a**–**r**).[21–24] The purity of the compounds was checked by TLC and elemental analysis. The compounds of this series were identified by spectral data. In the ^{1}H NMR spectra, the signals of the respective protons of the derivatives were verified on the basis of chemical shift, multiplicity and coupling constant. The spectra of all compounds showed D_2O exchangeable singlet at 7.9–10.1 ppm and 10.5–14.5 ppm corresponding to NH protons and CONH protons.[25] Analytical and spectral data of the synthesized compounds were in good agreement with composition of the synthesized compounds. The data of the physiochemical properties of all the compounds is given in (Table 1). Single crystal X-ray crystallography was obtained for compound **2b** (Fig. 2). All the compounds were screened for their in vitro anti-*Candidal* activity against ten strains of *Candida* species (Table 2).[26] All the synthesized compounds were investigated for their potential cytotoxicity against non cancer cell line MCF-10A. The active compounds **2c**, **2r** and **2a** were further investigated for their cytotoxic effects on three cancer cell lines; HT1080 (skin), HepG2 (liver) and A549 (lung).[27,28]

In an initial screening program to determine potential anti-*Candida* activities of thiosemicarbazides, compound A (MIC,

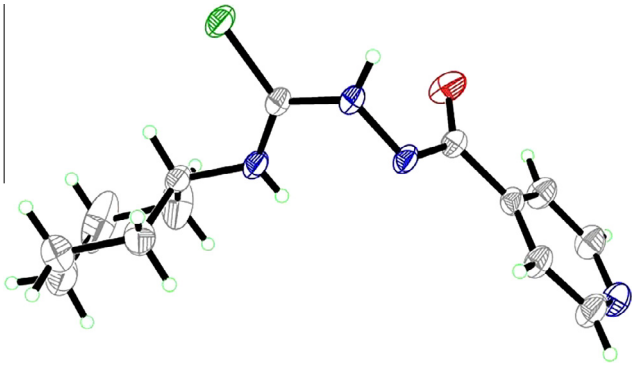
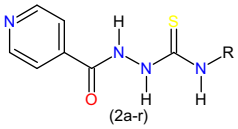


Figure 2. ORTEP diagram of **2b** at 50% probability.

Table 1
Physical data of the synthesized compounds (**2a–r**)



Compound	R	Molecular formula	Yield %	mp (°C)	*C*Log*P*[a]
2a	Phenyl	$C_{13}H_{12}N_4OS$	70	200–202	1.37
2b	Cyclohexyl	$C_{13}H_{18}N_4OS$	75	223–225	1.68
2c	4-Chloro phenyl	$C_{13}H_{11}ClN_4OS$	70	210–212	2.36
2d	4-Methoxy phenyl	$C_{14}H_{14}N_4O_2S$	72	235–237	1.32
2e	2,6-Dimethyl phenyl	$C_{15}H_{16}N_4OS$	80	290–292	2.29
2f	3-Ethyl phenyl	$C_{15}H_{16}N_4OS$	75	220–222	2.37
2g	4-Nitro phenyl	$C_{13}H_{11}N_5O_3S$	70	238–240	1.83
2h	4-Sulfapyrimidine phenyl	$C_{17}H_{15}N_7O_3S_2$	65	256–258	0.70
2i	4-Methyl phenyl	$C_{14}H_{14}N_4OS$	80	260–262	1.83
2j	3-Methyl phenyl	$C_{14}H_{14}N_4OS$	75	278–280	1.83
2k	2-Methyl phenyl	$C_{14}H_{14}N_4OS$	70	268–270	1.83
2l	3-Chloro phenyl	$C_{13}H_{11}ClN_4OS$	65	290–292	2.36
2m	2-Methoxy phenyl	$C_{14}H_{14}N_4O_2S$	70	270–272	1.32
2n	3-Methoxy phenyl	$C_{14}H_{14}N_4O_2S$	75	245–247	1.32
2o	4-Acetamido phenyl	$C_{15}H_{15}N_5O_2S$	60	290–292	0.79
2p	4-Ethoxy phenyl	$C_{15}H_{16}N_4O_2S$	65	170–172	1.86
2q	4-Iodo phenyl	$C_{13}H_{11}IN_4OS$	60	210–212	2.80
2r	2-Nitro phenyl	$C_{13}H_{11}N_5O_3S$	70	248–250	1.83

[a] *C*Log*P* calculated by ACD/Chem Sketch.

Table 2
Anti-*Candidal* activity of the compounds (**2a**–**r**) as MIC values (μg/mL)

Compound	*C. tropicalis* ATCC 66029	*C. parapsilosis* ATCC 22019	*C. albicans* ATCC 66027	*Candida* sp. [HVS*] 13184	*Candida* sp. [HVS] 11972	*Candida* sp. [HVS] 178	*Candida* sp. [urine] 300	*Candida* sp. [urine] 12341	*Candida* sp. [urine] 12485	*Candida* sp. [blood] 12810
2a	1.6	0.39	0.19	12.5	6.25	6.25	0.19	1.6	1.6	0.78
2b	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
2c	0.19	0.19	0.09	0.78	0.19	0.19	0.39	0.19	0.39	0.09
2d	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
2e	0.78	0.78	0.78	6.25	12.5	6.25	0.78	6.25	6.25	1.6
2f	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
2g	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
2h	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
2i	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
2j	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
2k	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
2l	1.6	0.78	0.19	6.25	0.39	6.25	0.78	0.78	6.25	0.09
2m	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
2n	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
2o	0.19	0.19	0.39	1.6	0.78	0.19	1.6	1.6	1.6	1.6
2p	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
2q	0.19	0.19	0.39	1.6	0.19	0.09	1.6	0.19	0.78	0.39
2r	0.19	6.25	1.6	0.78	6.25	0.39	1.6	6.25	0.39	0.39
IT#	0.19	0.04	1.56	0.39	0.02	0.19	0.09	0.09	0.04	0.25

* HVS: High Vaginal Swab.
\# IT: itraconazole.

25 μg/mL against a fluconazole) was chosen as the first hit for further derivatization.[6] Replacement of the isoquinoline nucleus by pyridine and preserving the pharmacophore [—(C═O)—NH—NH—(C═S)—NH—R] was studied. Surprisingly when the R was changed by electron donating groups (compounds **2d**, **2f**, **2i**, **2j**, **2k**, **2m** and **2n**), the anti-*Candidal* activity was lost. Insertion of cyclohexy group, **2b** also led to the loss of activity. These disappointing results led us to switch to the electron withdrawing groups. Compounds **2c**, **2q**, **2l**, **2o** and **2r** achieved the strongest anti-*Candida* activity. The best activity was observed when phenyl was replaced by *p*-chlorophenyl group, compound **2c** presents a good anti-*Candida* activity with a selectivity oriented towards *C. albicans* ATCC 66027 and *Candida* spp. 12810 [blood] (MIC, 0.09 μg/mL). In particular, this compound showed similar to better activity than reference drug for several *Candida* strains. The compound with *p*-iodo substitution on the phenyl group was found to be more active against *Candida* spp. 178 [HVS] with (MIC, 0.09 μg/mL) and compound with *m*-chloro substitution on phenyl group was found to be active against *Candida* spp. 12810 [blood] with (MIC, 0.09 μg/mL) whereas itraconazole exhibits inhibitory activity with (MIC, 0.04–1.56 μg/mL). The compound **2c** bearing *p*-chloro substitution on phenyl group was found to be most potent derivative of the series against all strains of *Candida* spp. This could result from increased lipophilicity associated with chloro phenyl group. The biological results indicate that *C. albicans* ATCC 66027 was more susceptible to compounds **2a**, **2c**, **2e**, **2l**, **2o** and **2q** (MIC, 0.09–0.78 μg/mL) as compared to itraconazole (MIC, 1.56 μg/mL). *Candida tropicalis* ATCC 66029 was equally susceptible to compounds **2c**, **2o**, **2q** and **2r** (MIC, 0.19 μg/mL) as to itraconazole (0.19 μg/mL). *Candida* spp. 178 [blood] was more susceptible to compounds **2c**, **2o** and **2q** (MIC, 0.09–0.19 μg/mL) as compared to itraconazole (MIC, 0.19 μg/mL). It is apparent that there is a positive correlation between anti-*Candidal* activity and electronegative functional groups like chloro, iodo and nitro. The hydrophobicity balance was already proved to be important in such series to obtains anti *Candida* activity.[29] From a pharmacological point of view it is important for the studied compounds to exhibit high bioactivity and at the same time show no or low cytotoxicity effects, otherwise the activity might just be due to general toxicity, which disqualifies the compound as a drug or lead molecule candidate. All the compounds (**2a**–**r**) were evaluated against non cancer cell line; MCF-10A (non-tumorigenic epithelial cell line) for their cytotoxic properties using WST-1 assay. None of the active compounds showed any cytotoxicity to non cancer cell line up to highest concentration of (IC_{50} >300 μM), indicating a high selectivity of anti-*Candidal* activity. The compounds **2c**, **2r** and **2a** were evaluated against three cancer celines; HT1080 (skin), HepG2 (Liver), A549 (Lung) using MTT assay (Table 3). The biological study indicated that screened compounds showed minimal cytotoxicity (IC_{50} >400 μg/mL), against all three cancer cell lines.

Table 3
In vitro cytotoxicity of the compounds against cancer cell lines

Compound	IC_{50} (μg/mL)		
	HT 1080 (Skin)	HepG2 (Liver)	A549 (Lung)
2c	540.12	1200.5	409.55
2r	833.4	409.55	813
2a	684.14	715.83	2042.5
Itraconazole	201.63	142	182

In conclusion we focused on the synthesis of thiosemicarbazide derivatives of isoniazid (**2a**–**r**) which were screened in vitro against ten strains of *Candida* spp. The compounds bearing *p*-chlorophenyl, *p*-iodophenyl, *m*-chlorophenyl, *o*-nitrophenyl and *p*-acetamido substitution were more active than the compounds bearing methylphenyl, methoxyphenyl, cyclohexyl, sulfonamidophenyl and ethoxyphenyl against all *Candida* spp. Compound **2c** was the most effective compound against *C. albicans ATCC66027* and *Candida* spp. [blood] (MIC, 0.09 μg/mL). This outcome confirms that *p*-chlorophenyl substitution have a considerable influence on anti-*Candidal* activity. The active compounds showed lowest cytotoxicity against tested three cancer cell lines and non cancer cell line. Based on the reported results, compounds **2c**, **2q**, **2l**, **2r** and **2o** could also be used in association with azole derivatives to evaluate their antifungal activity. Moreover, compound **2c** displaying better activity against *C. albicans* ATCC66027 and *Candida* spp. [blood] compared to reference drug (itraconazole), represents a good lead for the development of newer, potent and broad spectrum anti-*Candidal* agents.

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Supplementary data

Supplementary data (Crystallographic Data Center (CCDC) number 959803 contains crystallographic data for the structure **2b**. Copies of the data can be obtained, free of charge, on application to CCDC 12 Union Road, Cambridge CB2 1EZ, UK (Fax: +44 1223 336033; e-mail: deposit@ccdc.cam.ac.uk/http://www.ccdc.cam.ac.uk). associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.01.060.

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