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Synthesis and Anticandidosic Activities of Imidazo[1,2-a]pyridinehydrazone Derivatives

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Abstract

The synthesis and antifungal activity study of new hydrazide hydrazones derivatives (5a-r) containing the imidazo[1,2-a]pyridine backbone are presented in this paper. They were obtained by condensation reaction between 2-hydrazino-3-nitroimidazo[1,2-a]pyridine 3 and various aromatic aldehydes (4a-r) in the presence of acetic acid under reflux of methanol. Synthesized compounds were characterized by ^{1}H, ^{13}C Nuclear Magnetic Resonance (NMR), and High-Resolution Mass Spectrometry (HRMS) analyses. Among these compounds, twelve (12) were evaluated for their potential antifungal activity on *Candida albicans* n°396 from the CeDReS collection. The results demonstrate that antifungal activity varies according to the substituent present on the phenyl ring of each derivative. The weakly electron-donating or electron-withdrawing compounds seem to be the most active. Thus, methylated (5d) and brominated (5i) derivatives were the most efficient with respectively minimum inhibition concentrations (MICs) of 4.06 and 8.61 µmol/L.

Keywords:* *Hydrazones, Imidazo[1,2-a]pyridine, Antifungal, Candida albicans

Introduction

Candida albicans (C. albicans) is a fungus species that is commensal to humans. Generally harmless, it can under certain circumstances become pathogenic, causing candidiasis [1]. These infections can be superficial, but on fragile subjects, particularly those who are immunocompromised, they could become invasive or even generalized, and ultimately life-threatening [2]. It is considered that 75% of women have experienced at least one episode of vaginal candidiasis in their life [3]. C. albicans is the first fungemia cause in the world and is also considered the first opportunistic infection caused during HIV infection [4]. However, there are treatments for these candida infections [5–7]. Antifungal azoles, as well as newer echinocandins, are the most prominent treatments. Other drugs such as amphotericin B, may also be considered. They offer prospects for treatment and improve the prognosis of fungal infections, with good tolerance in general. However, in this field, as with all microorganisms, the drug resistance phenomena are developing and spreading [8,9]. This complicates care, forcing the use of higher doses or less well-

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tolerated drugs. It should also be noted that the selection pressure exerted by antifungal agents is leading to a change in the epidemiology of fungal infections, leading to more frequent infections to other Candida species, such as *C. tropicalis, C. glabrata* and of new multi-resistant species emergence such as *C. auris* [10].

The needs for innovative molecules, active on *C. albicans*, to circumvent drug resistance problems while having good tolerance is still relevant. New potential targets discovery allows design of new alternatives [11]. Use of chemical motifs with anti-infectious potential could be an interesting avenue. The phenylhydrazone scaffold has demonstrated anti-infective and antifungal potential and is found in zinoconazole and various other compounds under research [12,13]. Based on the juxtaposition of bioactive entities concept, the use of this motif combined with a carrier also known for its antifungal activity could lead to these new anticandidal targets. Bicyclic heterocycles to pentagonal heterocycles attached to benzene ring, such as benzimidazole and its isosteric imidazopyridine have these characteristics. Indeed, several studies have demonstrated their interest in support for anti-infectious activities, particularly antifungal [14,15]. These were carried out with Michael acceptors, of the acrylonitrile and arylpropenone type, linked to the heterocycle. Other work has also shown that coupling of benzimidazole ring with phenylhydrazone linkage leads to antifungal compounds that are active against plant pathogenic fungi [16]. Following on from this, the question is whether such a combination, this time with imidazopyridine as support, would lead significant antifungal activity. Present work thus aims to design, synthesize and evaluate the activity of new imidazopyridine-supported phenylhydrazone against *C. albicans.*

Experimental part

Materials and methods of Chemistry

All reagents and solvents were purchased from Sigma Aldrich and used without further purification unless otherwise noted. All anhydrous solvents, reagent grade solvents for chromatography and starting materials were purchased at the highest commercial quality from either Aldrich Chemical or Fisher Scientific. The reactions were monitored by TLC on precoated Merck 60 F254 silica gel plates and visualized using UV-Lamp (6 W, 254 nm and/or 365 nm) or KMnO4 solution followed by heating. Unless otherwise indicated, 1H and 13C NMR spectra were recorded either on a Bruker Advance at 300, 400, 500 and 75, 101, 126 MHz. The spectra were internally referenced to the residual proton solvent signal. Residual solvent peaks were taken as reference (CDCl3: 7.26 ppm, Acetone-d6: 2.05 ppm, DMSO-d6: 2.50 ppm) at room temperature. For 1H NMR assignments, the chemical shifts are given in ppm on the δ scale. Multiplicities are described as s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet), m (multiplet) and further qualified as app (apparent), br (broad signal) coupling constants, J are reported in Hz. HRMS were measured in the electrospray (ESI) mode on an LC-MSD TOF mass analyzer. Solid compound melting points were measured using a Köfler bench.

Synthesis methods of 2-chloro-3-nitroimidazo[1,2-a]pyridine 2: A round bottom flask containing 15 mL of H_2SO_4 and 1 eq (1.5 g, 9.83 mmol) of 2-chloro-H-imidazo[1,2-a]pyridine 1 was immersed in an ice bath, and then 3.5 eq (1.6 mL, 34.40 mmol) of HNO_3 were added to the solution. The reaction mixture was stirred at room temperature for 3 h and followed by TLC analysis. The reaction mixture was extracted with DCM and the organic layer was dried over Na_2SO_4. The organic phase was evaporated under vacuum and dried to yield 1.76 g (91%) compound 5 as yellow crystals, m.p: 166-168°C. 1H NMR (400 MHz, Acetone-d6) δ 9.42 (dt, J=7.0, 1.1 Hz, 1H; HAr), 7.92–7.79 (m, 2H; HAr), 7.52 (td, J=7.0, 1.5 Hz, 1H; HAr). ^{13}C NMR (400 MHz, Acetone-d6) δ 132.08, 117.33. HRMS (ESI): Calc. for $C_7H_5ClN_3O_2$ $[M+H]^+$=198.8974 Found=198.8977.

Synthesis methods of 2-hydrazino-3-nitroimidazo[1,2-a]pyridine 3: To a flask containing 5 mL of ethanol, 1 eq (1 mmol) of compound 2 was added and hydrated hydrazide (20 eq, 20 mmol) was added dropwise. The mixture was stirred at 60°C-70°C and then monitored by TLC for 30 minutes. The precipitate was filtered, washed with 2 mL of ethanol and recrystallized in ethanol to yield 78% 2-hydrazino-3-nitroimidazo[1,2-a]pyridine. Yellow powder, m.p=198°C -200°C. 1H NMR (300 MHz, CDCl3) δ 9.42 (d, J=6.8 Hz, 1H, HAr), 8.23 (s, 1H, NH), 7.65 (dd, J=11.7, 4.4 Hz, 1H, HAr), 7.52 (d, J=8.8 Hz, 1H, HAr), 7.13 (t, J=6.5 Hz, 1H, HAr), 4.25 (s, 2H, NH2). 13C NMR (75 MHz, CDCl3) δ 133.48, 128.62, 117.29, 115.44, 114.39. HRMS (ESI): Calc for $C_7H_5ClN_2O_2$ $[M+H]^+$=194.0832 Found=194.0834.

General procedure for the synthesis of 1-(3-nitroimidazo[1,2-a]pyridinyl)-3-phenylhydrazone derivatives 5a-r: The compound 3 (1 eq, 1 mmol) and aromatic aldehydes 4 (1 eq, 1 mmol) were dissolved in 5 mL of methanol. Then two drops of acetic acid were added to the mixture medium. The reaction mixture was refluxed for 30 min to 1 h. After cooling to room temperature, the precipitate was filtered, dried and then purified by recrystallization in ethanol to give compounds 5a-r with yields between 49 and 95%.

1-(3-nitroimidazo[1,2-a]pyridinyl)-3-phenylhydrazone 5a: Yellow powder, m.p=258-260°C; yield=80%. 1H NMR (300 MHz, DMSO-d6) δ 11.25 (s, 1H, NH), 9.34 (d, J=6.8 Hz, 1H, HAr), 8.67 (s, 1H, CH=N), 7.89–7.65 (m, 4H, HAr), 7.56–7.41 (m, 3H, HAr), 7.29 (td, J=7.0, 1.2 Hz, 1H, HAr). 13C NMR (75 MHz, DMSO-d6) δ 150.57, 148.37, 146.75, 134.85, 134.55, 130.40, 129.34, 129.03, 127.47, 116.01, 115.37. HRMS (ESI) Calc. for C14H12N5O2 $[M+H]^+$=282.1881 Found=282.1883.

1-(3-nitroimidazo[1,2-a]pyridinyl)-3-(4-methoxyphenyl)hydrazone 5b: Yellow powder, m.p=251-253°C, yield=89%. 1H NMR (300 MHz, DMSO-d6) δ 11.12 (s, 1H, NH), 9.32 (d, J=6.8 Hz, 1H, HAr), 8.58 (s, 1H, CH=N), 7.87–7.74 (m, 1H, HAr), 7.67 (d, J=8.8 Hz, 3H, HAr), 7.27 (td, J=7.0, 1.0 Hz, 1H, HAr), 7.03 (d, J=8.8 Hz, 2H, HAr), 3.82 (s, 3H, OCH3). ^{13}C NMR (75 MHz, DMSO-d6) δ 161.15, 150.60, 148.39, 146.90, 134.59, 129.11, 129.04, 127.37, 117.59, 115.83, 115.22, 114.83, 55.79. HRMS (ESI) $C_{15}H_{14}N_5O_3$ $[M+H]^+$=312.2571 Found=312.2573.

1-(3-nitroimidazo[1,2-a]pyridinyl)-3-(4-fluorophenyl)hydrazone 5c: Yellow powder, m.p=260-262° C, yield=88%. 1H NMR (300 MHz, DMSO-d6) δ 11.27 (s, 1H, NH), 9.42–9.30 (m, 1H, HAr), 8.66 (s, 1H, CH=N), 7.85–7.76 (m, 3H, HAr), 7.70 (d, J=8.8 Hz, 1H, HAr), 7.37–7.25 (m, 3H, HAr). 13C NMR (75 MHz, DMSO-d6) δ 150.56, 147.18, 146.73, 134.54, 131.49, 131.45, 129.65, 129.53, 129.03, 116.58, 116.29, 116.00, 115.39. HRMS (ESI) Calc. for $C_{14}H_{11}FN_5O_2$ $[M+H]^+$=300.1752 Found=300.1756.

1-(3-nitroimidazo[1,2-a]pyridinyl)-3-(2-methylphenyl)hydrazone 5d: Yellow powder, m.p=234-236°C, yield=94%. 1H NMR (300 MHz, CDCl3) δ 10.53 (s, 1H, NH), 9.41 (dt, J=6.8, 1.1 Hz, 1H, HAr), 8.44 (s, 1H, CH=N), 8.11 (dd, J=7.6, 1.5 Hz, 1H, HAr), 7.69–7.63 (m, 2H, HAr), 7.33–7.27 (m, 1H, HAr), 7.25–7.10 (m, 3H, HAr), 2.51 (s, 3H, CH_3). 13C NMR (75 MHz, $CDCl_3$) δ 150.47, 147.11, 146.24, 137.09, 133.69, 131.41, 130.78, 130.37, 128.52, 127.09, 126.31, 116.32, 114.74, 19.45. HRMS (ESI) Calc. for $C_{15}H_{14}N_5O_2$ $[M+H]^+$=296.1072 Found=296.1074.

1-(3-nitroimidazo[1,2-a]pyridinyl)-3-(4-methylphenyl)hydrazone 5e: Yellow powder, m.p=n.d (>266°C), yield=71%. 1H NMR (300 MHz, DMSO-d6) δ 11.20 (s, 1H, NH), 9.34 (d, J=6.8 Hz, 1H, HAr), 8.63 (s, 1H, N=CH), 7.82 (dd, J=8.5, 7.1, 1.3 Hz, 1H, HAr), 7.67 (dd, J=18.0, 8.4 Hz, 3H, HAr), 7.28 (dd, J=9.8, 4.3 Hz, 3H, HAr), 2.36 (s, 3H, CH3). 13C NMR (75 MHz, DMSO-d6) δ 150.61, 148.47, 146.82, 140.26, 134.59, 132.15, 129.96, 129.06, 127.49, 115.97, 115.32, 21.54. HRMS (ESI) Calc. for $C_{15}H_{13}N_5O_2Na$ $[M+Na]^+$ =318.1835 Found=318.1837.

1-(3-nitroimidazo[1,2-a]pyridinyl)-3-(4-chlorophenyl)hydrazone 5f: Yellow powder, m.p=264-266°C, yield=90%. 1H NMR (300 MHz, DMSO-d6) δ 11.31 (s, 1H, NH), 9.34 (d, J=6.8 Hz, 1H, HAr), 8.65 (s, 1H, CH=N), 7.87–7.65 (m, 4H, HAr), 7.54 (d, J=8.5 Hz, 2H, HAr), 7.29 (td, J=7.0, 1.2 Hz, 1H, HAr). 13C NMR (75 MHz, DMSO-d6) δ 150.45, 146.87, 146.65, 134.75, 134.51, 133.80, 129.44, 129.02, 116.04, 115.44. HRMS (ESI) Calc. for $C_{14}H_{10}ClN_5O_2Na$ $[M+Na]^+$ =338.0381 Found=338.0384.

1-(3-nitroimidazo[1,2-a]pyridinyl)-3-(2-hydroxylphenyl)hydrazone 5g: Yellow powder, m.p=n.d (>266°C), yield=95%. 1H NMR (300 MHz, DMSO-d6) δ 11.55 (s, 1H, OH), 11.29 (s, 1H, NH), 9.33 (d, J=6.8 Hz, 1H, HAr), 8.85 (s, 1H, CH=N), 7.81 (ddd, J=8.5, 7.1, 1.2 Hz, 1H, HAr), 7.68 (d, J=8.8 Hz, 1H, HAr), 7.47 (dd, J=8.0, 1.6 Hz, 1H, HAr), 7.36 – 7.23 (m, 2H, HAr), 6.92 (dd, J=10.3, 4.5 Hz, 2H, HAr). 13C NMR (75 MHz, DMSO-d6) δ 157.80, 150.02, 148.89, 146.51, 134.48, 131.62, 130.05, 128.99, 119.82, 119.24, 116.92, 116.01, 115.47. HRMS (ESI) Calc. for $C_{14}H_{11}N_5O_3Na$ $[M+Na]^+$ =320.0538 Found=320.0543.

1-(3-nitroimidazo[1,2-a]pyridinyl)-3-(3-cyanophenyl)hydrazone 5h: Yellow powder, m.p=n.d (>266°C), yield=91%. 1H NMR (300 MHz, DMSO-d6) δ 11.44 (s, 1H, HAr), 9.33 (d, J=6.7 Hz, 1H, HAr), 8.68 (s, 1H, CH=N), 8.09 (s, 1H, HAr), 8.05 (d, J=8.0 Hz, 1H, HAr), 7.84 (dd, J=2.0, 7.6 Hz, 2H, HAr), 7.69 (dd, J=15.1, 7.9 Hz, 2H, HAr), 7.30 (t, J=6.9 Hz, 1H, HAr). 13C NMR (75 MHz, DMSO-d6) δ 150.36, 146.52, 145.74, 136.22, 134.50, 133.45, 131.52, 130.70, 129.02, 116.12, 115.58, 112.52. HRMS (ESI) Calc. for $C_{15}H_{10}N_6O_2Na$ $[M+Na]^+$ =329.0487 Found=329.0489.

1-(3-nitroimidazo[1,2-a]pyridinyl)-3-(3-bromophenyl)hydrazone 5i: Yellow powder, m.p =260-262°C, yield=65%. 1H NMR (300 MHz, DMSO-d6) δ 11.37 (s, 1H, NH), 9.34 (d, J=6.8 Hz, 1H, HAr), 8.63 (s, 1H, CH=N), 7.93 (t, J=1.6 Hz, 1H, HAr), 7.88–7.79 (m, 1H, HAr), 7.71 (dd, J=8.2, 7.2 Hz, 2H, HAr), 7.63 (ddd, J=7.9, 1.9, 0.9 Hz, 1H, HAr), 7.44 (t, J=7.8 Hz, 1H, HAr), 7.31 (td, J=6.9, 1.3 Hz, 1H, HAr). 13C NMR (75 MHz, DMSO-d6) δ 150.43, 146.60, 146.36, 137.33, 134.54, 132.85, 131.58, 129.18, 129.03, 126.77, 122.68, 116.09, 115.53. HRMS (ESI) Calc. for $C_{14}H_{11}BrN_5O_2$ $[M+H]^+$ =361.0991 Found=361.0995.

1-(3-nitroimidazo[1,2-a]pyridinyl)-3-(2-nitrophenyl)hydrazone 5j: Yellow powder, m.p =260-262°C, yield=73%. 1H NMR (300 MHz, DMSO-d6) δ 11.71 (s, 1H, NH), 9.37–9.31 (m, 1H, HAr), 9.09 (s, 1H, CH=N), 8.17 (dd, J=7.9, 1.2 Hz, 1H, HAr), 8.07 (dd, J=8.2, 1.1 Hz, 1H, HAr), 7.82 (ddd, J=8.4, 6.9, 2.7 Hz, 2H, HAr), 7.74–7.62 (m, 2H, HAr), 7.31 (td, J=7.0, 1.3 Hz, 1H, HAr). 13C NMR (75 MHz, DMSO-d6) δ 150.27, 148.63, 146.35, 142.68, 134.35, 134.07, 130.88, 129.18, 128.98, 128.19, 125.08, 116.15, 115.60. HRMS (ESI) Calc. for $C_{14}H_{11}N_6O_4$ $[M+H]^+$=327.0664 Found=327.0668.

1-(3-nitroimidazo[1,2-a]pyridinyl)-3-furanylhydrazone 5k: Yellow powder, m.p =258-260°C, yield=49%. 1H NMR (300 MHz, DMSO-d6) δ 11.29 (s, 1H, NH), 9.33 (d, J=6.7 Hz, 1H, HAr), 8.58 (s, 1H, CH=N), 7.82 (dd, J=17.5, 9.5 Hz, 2H, HAr), 7.67 (d, J=8.7 Hz, 1H, HAr), 7.28 (t, J=6.8 Hz, 1H, HAr), 6.89 (d, J=3.2 Hz, 1H, HAr), 6.65 (d, J=1.2 Hz, 1H, HAr). 13C NMR (75 MHz, DMSO-d6) δ 150.45, 150.07, 146.69, 145.59, 138.01, 134.53, 129.02, 117.65, 115.97, 115.34, 113.61, 112.75. HRMS (ESI) Calc. for $C_{12}H_{10}N_5O_3$ $[M+H]^+$ =272.0921 Found=272.0926

1-(3-nitroimidazo[1,2-a]pyridinyl)-3-(4-dimethylaminophenyl)hydrazone 5l: Orange powder, m.p=n.d (>266°C), yield=68%. 1H NMR (500 MHz, DMSO-d6) δ 10.98 (s, 1H, NH), 9.40 – 9.26 (m, 1H, HAr), 8.48 (s, 1H, CH=N), 7.80 (ddd, J=8.6, 7.1, 1.3 Hz, 1H, HAr), 7.66 (d, J=8.8 Hz, 1H, HAr), 7.56 (d, J=8.9 Hz, 2H, HAr), 7.25 (td, J=7.0, 1.2 Hz, 1H, HAr), 6.78 (d, J=8.9 Hz, 2H, HAr), 2.99 (s, 6H, N(CH3)2). 13C NMR (126 MHz, DMSO-d6) δ 151.48, 150.09, 149.03, 146.64, 134.17, 128.58, 128.46, 121.54, 115.29, 114.52, 111.80, 39,75. HRMS (ESI) Calc. for $C_{16}H_{16}N_6O_2Na$ $[M+Na]^+$=347.1556 Found=347.1559

1-(3-nitroimidazo[1,2-a]pyridinyl)-3-(3-nitrophenyl)hydrazone 5m: Yellow powder, m.p=n.d (>266°C), yield=91%. 1H NMR (500 MHz, DMSO-d6) δ 11.45 (s, 1H, NH), 9.34 (dt, J=6.8, 1.1 Hz, 1H, HAr), 8.78 (s, 1H, CH=N), 8.55–8.51 (m, 1H, HAr), 8.26 (ddd, J=8.2, 2.4, 1.0 Hz, 1H, HAr), 8.14–8.09 (m, 1H, HAr), 7.85–7.81 (m, 1H, HAr), 7.79–7.74 (m, 2H, HAr), 7.31 (td, J=6.9, 1.3 Hz, 1H, HAr). 13C NMR (126 MHz, DMSO-d6) δ 149.82, 148.25, 146.02, 145.20, 136.20, 133.99, 133.31, 130.52, 128.50, 123.99, 120.44, 115.69, 115.12. HRMS (ESI) Calc. for $C_{14}H_{11}N_6O_4$ $[M+H]^+$=327.0562 Found=327.0567.

1-(3-nitroimidazo[1,2-a]pyridinyl)-3-(4-hydroxy-3-methoxyphenyl)hydrazone 5n: Orange powder, m.p=n.d (>266°C), yield=79%. 1H NMR (300 MHz, DMSO- d6) δ 11.07 (s, 1H, NH), 9.59 (s, 1H, OH), 9.34 (d, J=6.7 Hz, 1H, HAr), 8.53 (s, 1H, CH=N), 7.81 (ddd, J=8.5, 7.1, 1.2 Hz, 1H, HAr), 7.67 (d, J=8.8 Hz, 1H, HAr), 7.28 (ddd, J=13.9, 6.4, 1.4 Hz, 3H, HAr), 7.11 (dd, J=8.2, 1.8 Hz, 1H, HAr), 6.87 (d, J=8.1 Hz, 1H, HAr), 3.86 (s, 3H, OCH3). 13C NMR (75 MHz, DMSO-d6) δ 150.57, 149.50, 149.25, 148.52, 146.97, 134.66, 129.08, 126.17, 122.59, 117.55, 116.02, 115.87, 115.18, 109.62, 56.11. HRMS (ESI) Calc. for $C_{15}H_{14}N_5O_4$ $[M+H]^+$=328.1522 Found=328.1525.

1-(3-nitroimidazo[1,2-a]pyridinyl)-3-(2,4-dichlorophenyl)hydrazone 5o: Yellow powder, m.p=n.d (>266°C), yield=66%. 1H NMR (300 MHz, DMSO- d6) δ 11.70 (s, 1H, NH), 9.34 (d, J=6.8 Hz, 1H, HAr), 9.06 (s, 1H, CH=N), 8.06 (d, J=8.6 Hz, 1H, HAr), 7.89–7.65 (m, 4H, HAr), 7.55 (dd, J=8.6, 2.0 Hz, 1H, HAr), 7.31 (td, J=7.0, 1.2 Hz, 1H, HAr). 13C NMR (75 MHz, DMSO-d6) δ 150.28, 146.42, 142.83, 135.19, 134.39, 134.15, 131.54, 129.86, 128.99, 128.44, 116.12, 115.56. HRMS (ESI) Calc. for $C_{14}H_{10}Cl_2N_5O_2$ $[M+H]^+$ =351.1725 Found=351.1727.

1-(3-nitroimidazo[1,2-a]pyridinyl)-3-pyridin-4-ylhydrazone 5p: Yellow powder, m.p=n.d (>266°C), yield=81%. 1H NMR (300 MHz, DMSO- d6) δ 11.52 (s, 1H, NH), 9.35 (d, J=6.8 Hz, 1H, HAr), 8.67 (s, 3H, HAr, CH=N), 7.88 – 7.78 (m, 1H, HAr), 7.74 (d, J=8.7 Hz, 1H, HAr), 7.66 (d, J=5.9 Hz, 2H, HAr), 7.33 (td, J=6.9, 1.3 Hz, 1H, HAr). 13C NMR (75 MHz, DMSO-d6) δ 150.66, 150.23, 146.41, 145.51, 141.99, 134.48, 129.02, 121.30, 117.89, 116.22, 115.70. HRMS (ESI) Calc. for $C_{13}H_{11}N_6O_2$ $[M+H]^+$ =283.0745 Found=283.0747.

1-(3-nitroimidazo [1,2-a]pyridinyl)-3-(4-hydroxylphenyl) hydrazone 5q: Orange powder, m.p=n.d (> 266°C), yield=90%, 1H NMR (500 MHz, DMSO-d6) δ 11.07 (s, 1H, NH), 9.93 (s, 1H, OH), 9.42 – 9.26 (m, 1H, HAr), 8.54 (s, 1H, CH=N), 7.80 (ddd, J=8.6, 7.1, 1.3 Hz, 1H, HAr), 7.67 (d, J=8.8 Hz, 1H, HAr), 7.60–7.56 (m, 2H, HAr), 7.27 (td, J=7.0, 1.2 Hz, 1H, HAr), 6.88–6.83 (m, 2H, HAr). 13C NMR (126 MHz, DMSO-d6) δ 159.37, 150.16, 148.40, 146.49, 134.11, 128.81, 128.56, 125.33, 115.74, 115.37, 114.65. HRMS (ESI) Calc. for $C_{14}H_{12}N_5O_3$ $[M+H]^+$ =298.0733 Found=298.0736.

1-(3-nitroimidazo[1,2-a]pyridinyl)-3-(2,4-dimethoxyphenyl)hydrazone 5r: Orange powder, m.p=262-264°C, yield=76%. 1H NMR (300 MHz, DMSO-d6) 11.23 (s, 1H, NH), 9.33 (d, J=6.8 Hz, 1H, HAr), 8.85 (s, 1H, CH=N), 7.93–7.78 (m, 2H, HAr), 7.66 (d, J=8.8 Hz, 1H, HAr), 7.26 (td, J=7.0, 1.2 Hz, 1H, HAr), 6.74–6.59 (m, 2H, HAr), 3.87 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3) ; 13C NMR (75 MHz, DMSO-d6) δ 162.86, 159.62, 150.61, 146.90, 144.11, 134.52, 129.04, 127.25, 117.45, 115.83, 115.10, 106.89, 98.75, 56.28, 55.93. HRMS (ESI) Calc. for $C_{16}H_{16}N_5O$ $[M+H]^+$ =342.2956 Found=342.2960

Materials and methods of Biology

The antifungal activity evaluation was carried out at the Parasitology and Mycology Laboratory of the Centre for Diagnosis and Research on AIDS and other infectious diseases (CeDReS) in Côte d'Ivoire. The fungal carrier is a Fluconazole-resistant strain of Candida albicans. This clinical strain of C. albicans n°396 comes from CeDReS collection. The strain was grown on Sabouraud glucose agar (Sabouraud 4% glucose agar, Fluka). The synthesis products are composed of eighteen (18) 1-(3-nitroimidazo[1,2-a]pyridinyl)-3-arylhydrazone (5a-r) derivatives. The solvents used to solubilize the chemical products were dimethyl sulfoxide (DMSO) and distilled water.

The anticandidosic activity was determined by measuring the compounds' minimum inhibitory concentration (MIC). The strain used for the tests was the C. albicans strain n°396, from the CeDReS collection, which is resistant to fluconazole. The microdilution method was used to evaluate the MIC of the different extracts. This method consists of putting Candida inoculum in contact with an increasing dilution of the antifungal agent in microplates of 96-wells (12 rows of 8 wells). The observed detection of purple discolouration evaluated the inhibition of fungal growth. This purple colouration is due to the dehydrogenase activity of the mitochondria of living cells. The MIC is given by the lowest concentration which does not result in a colour change of the Methyl chloride Thiazolyl tetrazolium (MTT). Cultures of Candida were prepared on agar Sabouraud glucose (Sabouraud 4% glucose agar, Fluka) in a Petri dish, and incubated at 30°C for 48 hours. One to three colonies were seeded in 50 mL of sterile Brain Heart Broth (BHB), then left agitating overnight at room temperature. The next day, 10 mL of the broth was removed and transferred to a new BHB and left under agitation for 6 hours (time needed to achieve exponential growth of Candida). At the time of the test, 5 mL of approximately 6 h old BHB is added to 50 mL of sterile BHB to obtain an inoculum containing approximately 105 cells/mL (cell density check by hematometra cell). The test was performed in 96 well microplates.

At the same, the stock solutions of the different extracts were prepared with DMSO at a concentration of 1 mg/mL. Then a dilution was carried out with the Tryptone Soy broth (TBS) containing the yeast to obtain solutions concentrated to 100 µg/mL (one volume of the extract was mixed with 9 volumes of the BHB containing the yeast). 100 µL of this dilution is deposited in the wells of the first column. Then 50 µL of BHB broth containing the yeast in the following wells (wells 2 to 10), and 50 µL of the first well solution are used to obtain the dilution range from 100 to 0.2 µg/mL. The filled plates were incubated at 30°C for 48 hours. For the revelation of the microplates thus prepared, 40 µL of a solution of MTT chloride prepared in DMSO at the concentration of 2.5 mg/mL were distributed in the wells and incubated for another 30 min at room temperature. The MTT solution is yellow. Wells containing cells that are still active turn purple as a result of the dehydrogenase activity of the mitochondria. Reading is done with the naked eye. The MIC was defined as the lowest concentration for which no colour change in MTT was observed. All samples were duplicated and the tests were repeated and improved twice.

Results and Discussion

Chemistry

The evaluated compounds were obtained by multi synthesis steps. As regards the design of structures, the choice was made on a phenylhydrazone pattern fixed at position-2 of imidazo[1,2-a]pyridine. Previously published work focused on a Michael acceptor fixed in position-3 [17]. For this series of compounds, position-3 was substituted by a nitro group, an activity modulator regularly found in anti-infectious compounds. Apart from these two positions, the imidazo[1,2-a]pyridine ring was not substituted. Structural variations focused on substitution at the phenyl ring. A reference compound with an unsubstituted ring, was first synthesized, followed by different derivatives with different substituents: electron-donating groups (EDG), halogens and electron-withdrawing groups (EWG) (**Figure 1**).

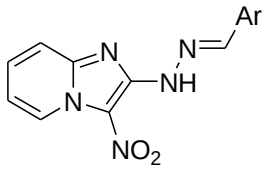


FIG. 1. **General structure of imidazo[1,2-a]pyridine supported phenylhydrazones**

The synthesis of compounds **5a-r** was carried out in three steps starting with the intermediate 2-chloroimidazo[1,2-a]pyridine **1.** This intermediate **1** was obtained in two steps according to the method described by Brad et *al* [18]. The synthesis of the new 1-(3-nitroimidazo[1,2-a]pyridinyl)-3-phenylhydrazone derivatives **(5a-r)** was performed by condensation between 2-hydrazino-3-nitroimidazo[1,2-a]pyridine 3 with **4a-r** aromatic aldehydes (**Figure 2**).

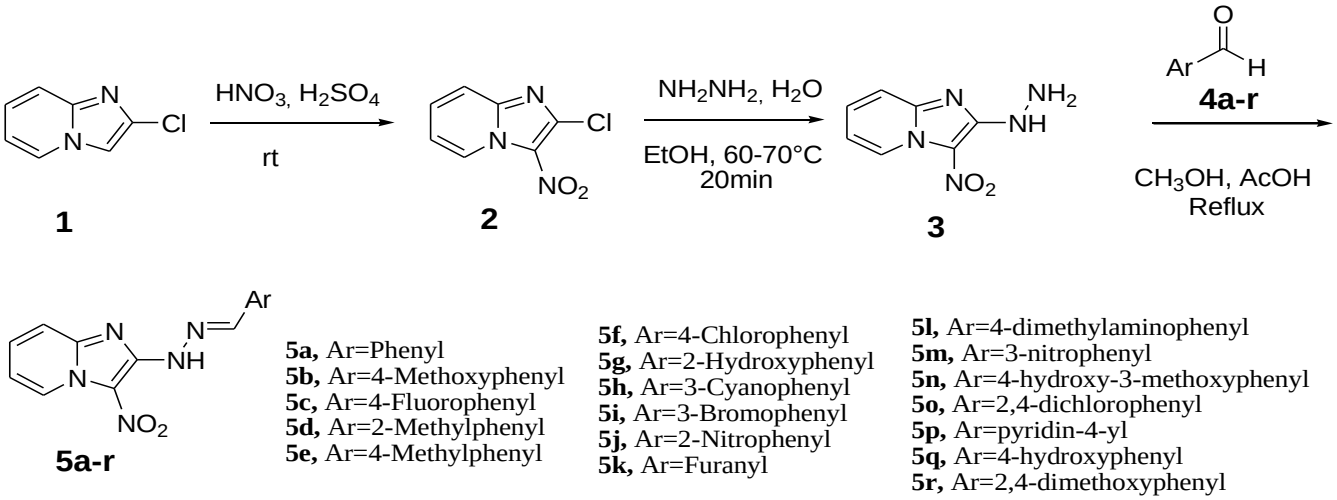


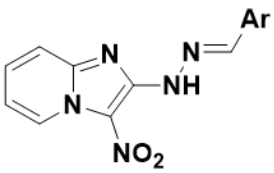
FIG. 2**. Synthesis route of compounds 5a-r**

These new compounds **5a-r** were obtained using the method described by Cledualdo *et al*. [19]. It consists of heating the mixture of compound **3** and aromatic aldehydes **4a-r** in the presence of two drops of acetic acid under reflux of methanol for one hour. A precipitate was formed, isolated by hot filtration, and then washed with methanol. The compounds **5a-r** were isolated and purified by recrystallization in ethanol. The synthesis of compound **3**, is a nucleophilic substitution reaction between 2-chloro-3-nitroimidazo[1,2-a]pyridine **2** and hydrazine hydrate following works done by Mostafa et *al*. [20] and Wafaa et *al*. [21]. The 2-Chloro-3-nitro imidazo[1,2-a]pyridine **2** reacts with hydrazine hydrate under reflux in ethanol for 10 minutes. By doing so, the product was isolated with a yield of 20%. The low efficiency obtained led us to carry out optimization tests by varying the temperature. The results show that the best yield was obtained at temperatures between 60 and 70°C. At these temperatures, the product was formed after twenty (20) minutes as a yellowish solid with a better yield of 78%. When the boiling temperature of ethanol was reached, we observed product degradation. 2-Chloro-3-nitro imidazo[1,2-a]pyridine **2** was obtained by the nitration of the position-3 of compound **1** in sulfuric acid in the presence of nitric acid at room temperature. The analysis of ^{1}H NMR spectrum of compound **3** (see Supporting information) revealed the presence of peaks corresponding to the protons of the different nitrogen. We note the presence of two new singlets, one at 4.25 ppm integrating for two protons corresponding to the NH_2 proton and the other at 8.23 ppm integrating for one proton corresponding to the NH proton. The NMR spectra of the compounds **5a-r** (see Supporting information) obtained show, besides the presence of new peaks in the aromatic zone, the disappearance of the singlet at 4.25 ppm corresponding to the protons of the NH_2 group of compound **3** and the appearance of a singlet in the zone of 8.4 to 9 ppm characteristic of the imine function proton (N=CH). We also note a strong deshielding of the NH proton of 8.23 ppm at around 11 ppm. This deshielding may explained by the fact that this proton is conjugated with the double bond of the imine function.

Biology

The synthesized compounds **5a-l** were evaluated for their antifungal activity against *C. albicans*. The minimum inhibitory concentrations (MICs) of these compounds (**TABLE 1**).

TABLE 1: **Antifungal activity of compounds 5a-l**

Compounds	General structure	Ar	MIC (µmol/L)
5a		Phenyl	22.4
5b		4-Methoxyphenyl	20.24
5c		4-Fluorophenyl	>300
5d		2-Methylphenyl	4.06
5e		4-Methylphenyl	42.33
5f		4-Chlorophenyl	19.96
5g		2-Hydroxyphenyl	>300
5h		3-Cyanophenyl	20.57
5i		3-Bromophenyl	8.61
5j		2-Nitrophenyl	>300
5k		Furanyl	>300
5l		4-Dimethylaminophenyl	>300

Analysis of the results provides insight towards validating the design as potential antifungals. Indeed, seven (7) of the twelve (12) compounds assessed have antifungal activity. However, the functional group on the phenyl ring strongly influences the antifungal activity. The absence of substituent (compound **5a)** on the phenyl ring allows an activity with an MIC of 22.4 µM. Substitution of position-2 methyl group on the phenyl ring (compound **5d**) allows a strong increase in activity, marked by a reduced MIC of the order of 4 µM. The substitution of methyl by another EDG of the hydroxy type results in a loss of activity (compound **5g**). This activity is restored when a substituent is a methoxy group (compound **5b**). When the substituent is a halogenide, the activity also varied. The MIC seems to increase with the electronegativity of the compounds, the most active being the brominated derivative (compound **5i**) with a MIC of 8.61 µM while the fluorinated derivative is inactive **(**compound **5c)**. Finally, with the EWG, it appears that only the cyano-function compound (compound **5h**) is active with a MIC close to that of compound **5a.** The duplication of nitro function and the introduction of a dimethylamino group did not result in a gain in activity, but rather a loss (compounds **5j, 5l**). Replacement of the phenyl ring with a furan-like heterocycle (compound **5k**) did not improve the antifungal activity.

Conclusion

This work led to the development of eighteen (18) 1-(3-nitroimidazo[1,2-a]pyridinyl)-3-arylhydrazone. All these compounds were characterized by 1H and 13C NMR spectroscopy and HRMS mass spectrometry. The antifungal activity of twelve (12) of them was studied on a C. albicans strain resistant to Fluconazole. The obtained results show that the antifungal activity varied according to the substituent linked or fixed on the phenyl ring. The weakly electron-donating or electron-withdrawing compounds are the most potent. Thus, the methylated (compound 5d) and brominated (compound 5i) derivatives were the most active against C. albicans, opening the way to new perspectives on Quantitative Structure-Activity Relationship (QSAR) studies.

Author Contributions

K.F.A performed the syntheses. S.C. and D.S participated in the design and direction of the project. K.A and M.O conceptualized the biological study and methodology. C.T.E, S.C., and KFA wrote the paper. C.S, A.A, D.S, supervised the project. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Supporting Information: Full experimental details for the synthesis methods as well as copies of relevant NMR spectra can be found via the "Supplementary Information" section of this article's webpage.

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