SYNTHESIS AND ANTIFUNGAL ACTIVITY OF DIARYL HYDRAZONES FROM 2,4-DINITROPHENYLHYDRAZINE

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ABSTRACT

A new series of diarylhydrazones derived from 2,4-dinitrophenylhydrazine were synthesized via condensation with aromatic aldehydes whose structures have been determined by mass spectrometry, infrared spectroscopy, and nuclear magnetic resonance spectra. Yields were 50–99%. All compounds were screened *in vitro* for their antifungal activity. Preliminary results indicated that compounds **3g** and **3h** exhibited promising antifungal potency. Understanding of the structure of these compounds establishes a preliminary structure-activity relationship to form the basis of further investigation.

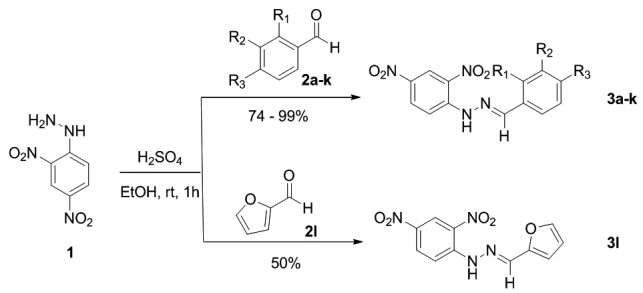
Keywords: Antifungal Activity, Diarylhydrazones, Schiff bases.

INTRODUCTION

Aryl hydrazones are key compounds for the synthesis of heterocyclic rings such as indoles and pyrazoles.[1–3] In recent decades the use and interest in Schiff bases has increased because of their wide range of applications, including biological, organic, inorganic and analytical chemical uses. They are used as pigments, dyes, catalysts, ligands in organometallic complexes and polymer stabilisers.[4–9] The biological activity of these compounds results in a wide variety of effects, including antidepressant, analgesic, anti-inflammatory, antiplatelet, antimalarial, antimicrobial, antimycobacterial, antitumor and antioxidant.[9–11] However, the antifungal activity of hydrazones is known only from reports on acylhydrazones.[12–14]

RESULT AND DISCUSSION

A new series of diarylhydrazones **3a-l** was obtained from 2,4-dinitrophenylhydrazine **1** and aromatic aldehydes **2a-l** (Scheme 1). The new series was synthesized through formation of a hemiaminal, between an amine and the corresponding aldehyde, with subsequent dehydration. The acidic medium was provided by the addition of sulfuric acid to facilitate the dehydration process, and ethanol was used as the solvent.



Scheme 1. Synthesis of diarylhydrazones **3a-l**.

The spectroscopic data (^{1}H NMR, ^{13}C NMR and IR) were consistent with the proposed structures for compounds **3a-l** (Table 1). The hydrazone bond was characterised by the presence of a band at 1614–1621 cm^{-1} corresponding to C=N stretching and a band at 3270–3290 cm^{-1} corresponding to N–H stretching in FT-IR and two singlets at δ_H 8.61–9.10 ppm (N=C–H) and 11.57–11.96 ppm (N–H) in the ^{1}H NMR spectrum. In the ^{13}C NMR spectra, a chemical shift was seen at δ_C 138.9–150.7 ppm corresponding to the imine bond (C=N).

Table 1. Spectroscopy of newly synthesised compounds **3a-l**.

Compound	R_1	R_2	R_3	IR (cm^{-1})			^{1}H NMR δ (ppm)			^{13}C NMR δ (ppm)
				C=N	N-H		N=C-H	N-H		C=N
3a	H	H	H	1620	3286		8.71	11.67		149.4
3b	OH	H	H	1620	3273		8.95	11.70		144.9
3c	OMe	H	H	1616	3270		8.98	11.74		144.9
3d	H	OMe	OH	1621	3278		8.56	11.57		150.7
3e	H	H	OMe	1621	3290		8.64	11.59		149.5
3f	Cl	H	H	1616	3288		9.10	11.93		144.8
3g	H	H	Cl	1615	3284		8.69	11.69		148.5
3h	Cl	H	Cl	1614	3286		9.06	11.96		143.7
3i	H	H	Br	1619	3297		8.63	11.72		-
3j	H	H	F	1614	3286		8.70	11.66		148.8
3k	H	H	NO_2	1616	3289		8.79	11.83		146.5
3l	Furan-2-yl			1618	3280		8.61	11.64		138.9

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All compounds were tested *in vitro* for antifungal activity at a concentration of 400 µg/mL in 2% DMSO in Sabouraud-dextrose broth by counting colony-forming units. Standardised strains of *C. dubliniensis* CBS 7987, *C. glabrata* ATCC 2001, *C. tropicalis* ATCC 13803 and *C. krusei* ATCC 6258 and fluconazole, as the reference drug, were used to test the antifungal activity of the compounds. Preliminary *in vitro* screening of newly synthesised compounds (Table 2) showed compounds **3g** and **3h** were active against three of the *Candida* strains: *C. krusei*, *C. tropicalis* and *C. glabrata*. The compounds showed no antifungal activity against *C. dubliniensis*.

Table 2: Percent inhibition of *Candida* species by newly synthesised compounds

Compound[a]	Inhibition (%)			
	C. dubliniensis	*C. glabrata*	*C. tropicalis*	*C. krusei*
3a	0	0	0	0
3b	0	0	0	0
3c	0	0	0	0
3d	0	0	0	0
3e	0	0	0	0
3f	0	0	0	0
3g	0	**26**	**52**	**30**
3h	0	**20**	**18**	**15**
3i	0	0	0	0
3j	0	0	0	0
3k	0	0	0	0
3l	0	0	0	0
DMSO 2%[b]	0	0	0	0
Flu.[c]	98	95	95	96

[a] concentration 400 µg/mL
[b] solvent control
[c] reference drug, fluconazole

In a second study, the active compounds were tested on *Candida* strains at decreased concentrations: 200 µg/mL and 100 µg/mL (Table 3). Compound **3g** was the most active compound, showing up to 41% of inhibition of *C. tropicalis* at a concentration of 100 µg/mL. Compound **3h** showed a decrease in activity with decreasing concentration.

Table 3. Percent inhibition of *Candida* species at different concentrations by compounds **3g**, **3h** and fluconazole (Flu).

Comp.	Concentration	Inhibition (%)		
		C. glabrata	*C. tropicalis*	*C. krusei*
3g	400 µg/mL	26	52	30
	200 µg/mL	23	47	21
	100 µg/mL	19	41	14
3h	400 µg/mL	20	18	15
	200 µg/mL	15	14	13
	100 µg/mL	13	9	7
Flu.	400 µg/mL	95	95	96
	200 µg/mL	93	92	89
	100 µg/mL	91	90	87

In general, all compounds nonhalogenated not exhibit antifungal activity. This could be due to the lipophilicity of the compounds since halogens improve this property.[15] Both active compounds (**3g** and **3h**) have chlorine atoms in the aromatic ring. Compound **3h** has two chlorine atoms in the *ortho* position of the ring, while compound **3g** has a *para* monosubstitution of chlorine. Compound **3g** showed greater inhibition of three *Candida* strains than compound **3h**. This may be due to the existence of a chlorine atom in the *ortho* position in compound **3h**. Notably, compound **3f** presents monosubstitution in the *ortho* position by a chlorine atom, but no antifungal properties. We infer that a substitution of chlorine in the *para* position is favorable for antifungal activity. However, the presence of a second atom of chlorine apparently decreases the activity of the compound; likewise the absence of chlorine generates a completely inactive compound. Other halogens in the *para* position were tested, but do not show antifungal activity. Other authors have reported a high resistance of *Candida spp*. to nitro hydrazine derivatives.[16] *C. dubliniensis* is known for its resistance to azoles, and in this case we observed that compounds are inactive against this strain.[17]

EXPERIMENTAL

The reagents and solvents used in this work were obtained from Fluka, Aldrich or Merck and were used without further purification. Melting points were determined on a Kofler-type apparatus and are uncorrected. The infrared spectroscopy (IR) was done on a Perkin-Elmer 200 spectrophotometer with KBr. Nuclear magnetic resonance (NMR) spectra were collected in DMSO-d_6 or $CDCl_3$ on a Varian Unity Inova 500 MHz spectrometer equipped with a microflow probe from Protasis. Mass spectra (MS) were recorded on a Micromass-LCT Premier Time-of-Flight electrospray (ESI) spectrometer with Acquity UPLC (Ultra Performance Liquid Chromatography) interface system**.** Thin-layer chromatography (TLC) was performed on silica gel plates Merck 60 F_{254} and components were visualised by spraying with phosphomolybdic acid reagent, followed by heating**.**

General synthesis procedure

To a solution of 2,4-dinitrophenylhydrazine **1** (300.0 mg, 1.51 mmol) and the corresponding aldehyde **2a-l** (1.51 mmol) in 50.0 mL of ethanol was added 0.5 mL of concentrated sulfuric acid. The mixture was stirred for one hour at room temperature, then cooled in an ice bath and the precipitate was collected by filtration. The precipitate was dried for four hours at 60°C and crystallized from acetone, ethanol or isopropanol at room temperature.

(E)-1-benzylidene-2-(2,4-dinitrophenyl)hydrazine (**3a**)

Compound **3a** was obtained as orange crystals (acetone) at 74% yield, mp: 207–208°C, R_f: 0.62 (hexane:EtOAc, 7:3). IR (cm^{-1}) ν: 3286 (N–H), 3104 ($C_{sp}{}^2$–H), 1620 (N=C), 1588 (C_{Ar}–C_{Ar}), 1509 (NO_2). ^{1}H-NMR (DMSO-d_6, 500 MHz) δ (ppm): 7.50 (m, 3H), 7.82 (dd, *J* = 2.0, 7.6 Hz, 2H), 8.13 (d, *J* = 9.6 Hz, 1H), 8.39 (dd, *J* = 2.7, 9.6 Hz, 1H), 8.72 (s, 1H, N=CH), 8.88 (d, *J* = 2.7 Hz, 1H), 11.68 (s, 1H, N–H). ^{13}C-NMR (DMSO-d_6, 125 MHz) δ (ppm): 116.8 (CH), 123.0 (CH), 127.4 (CH), 128.9 (CH), 129.5 (C), 129.9 (CH), 130.5 (CH), 133.8 (C), 137.0 (C), 144.6 (C), 149.4 (CH, N=C). HRESIMS(+): calculated for $C_{13}H_{11}N_4O_4$ $[M+H]^+$: 287.07802; found 287.08102.

(E)-2-((2-(2,4-dinitrophenyl)hydrazono)methyl)phenol (**3b**)

Compound **3b** was obtained as orange crystals (EtOH) at 99% yield, mp: 260–261°C, R_f: 0.55 (hexane:EtOAc, 7:3). IR (cm^{-1}) ν: 3365 (O–H), 3273 (N–H), 3099 ($C_{sp}{}^2$–H), 1620 (N=C), 1591 (C_{Ar}–C_{Ar}), 1510 (NO_2). ^{1}H-NMR (DMSO-d_6, 500 MHz) δ (ppm): 6.92 (dd, *J* = 1.1, 8.3 Hz, 2H), 7.30 (t, *J* = 7.2 Hz, 1H), 7.85 (d, *J*= 7.7 Hz, 1H), 8.04 (d, *J* = 9.6 Hz, 1H), 8.37 (d, *J* = 9.7 Hz, 1H), 8.87 (d, *J* = 2.4 Hz, 1H), 8.96 (s, 1H, N=CH), 10.22 (s, 1H, OH), 11.72 (s, 1H, N-H). ^{13}C-NMR (DMSO-d_6, 125 MHz) δ (ppm): 116.3 (CH), 116.6 (CH), 119.5 (CH), 120.1 (C), 123.1 (CH), 126.6 (CH), 129.5 (C), 129.7 (CH), 131.9 (CH), 137.0 (C), 144.6 (C), 146.7 (CH, N=C), 156.9 (C). HRESIMS(+): calculated for $C_{13}H_{11}N_4O_5$ $[M+H]^+$: 303.07293; found 303.07416.

(E)-1-(2,4-dinitrophenyl)-2-(2-methoxybenzylidene)hydrazine (**3c**)

Compound **3c** was obtained as red crystals (acetone) at 97% yield, mp: 196–197°C, R_f: 0.60 (hexane:EtOAc, 7:3). IR (cm^{-1}) ν: 3270 (N–H), 3110 ($C_{sp}{}^2$–H), 2935 ($C_{sp}{}^3$–H), 1616 (N=C), 1585 (C_{Ar}-C_{Ar}), 1502 (NO_2), 1255 (C-O). ^{1}H-NMR (DMSO-d_6, 500 MHz) δ (ppm): 4.02 (s, 3H, OCH_3), 7.05 (t, *J* = 7.5 Hz, 1H), 7.14 (d, *J* = 7.6 Hz, 1H,), 7.46 (t, *J* = 7.7 Hz, 1H,), 7.97 (d, *J* = 7.2, 1H), 8.10 (d, *J* = 9.6 Hz, 1H), 8.36 (d, *J* = 10.0 Hz, 1H), 8.86 (s, 1H), 8.99 (s, 1H, N=CH), 11.76 (s, 1H, N-H). ^{13}C-NMR (DMSO-d_6, 125 MHz) δ (ppm): 56.5 (CH_3), 112.1 (CH), 116.8 (CH), 120.8 (CH), 122.0 (C), 123.0 (CH), 125.8 (CH), 129.4 (C), 129.6 (CH), 132.1 (CH), 136.8 (C), 144.5 (C), 144.9 (CH, N=C), 158.0 (C). HRESIMS(+): calculated for $C_{14}H_{13}N_4O_5$ $[M+H]^+$: 317.08858; found 317.09125.

(E)-4-((2-(2,4-dinitrophenyl)hydrazono)methyl)-2-methoxyphenol (**3d**)

Compound **3d** was obtained as red crystals (EtOH) at 92% yield, mp: 272–273°C, R_f: 0.30 (hexane:EtOAc, 7:3). IR (cm^{-1}) ν: 3385(O–H), 3278 (N–H), 3108 ($C_{sp}{}^2$–H), 2942 ($C_{sp}{}^3$–H), 1621 (N=C), 1589 (C_{Ar}–C_{Ar}), 1515 (NO_2), 1269

(C–O). ^{1}H-NMR (DMSO-d_6, 500 MHz) δ (ppm): 3.87 (s, 3H, OCH_3), 6.88 (d, J = 8.1 Hz, 1H), 7.18 (dd, J = 1.9, 8.2 Hz, 1H), 7.40 (d, J = 1.9 Hz, 1H), 8.09 (d, J = 9.66 Hz, 1H), 8.34 (dd, J = 2.7, 9.6 Hz, 1H), 8.57 (s, 1H, N=CH), 8.87 (d, J = 2.6 Hz, 1H), 9.68 (s, 1H, OH), 11.59 (s, 1H, N-H). ^{13}C-NMR (DMSO-d_6, 125 MHz) δ (ppm): 56.2 (CH_3), 110.2 (CH), 116.1 (CH), 117.2 (CH), 123.0 (CH), 123.6 (CH), 129.6 (C), 125.6 (C), 130.0 (CH), 136.7 (C), 145.0 (C), 148.6 (C), 149.9 (C), 150.7 (CH, N=C). HRESIMS(+): calculated for $C_{14}H_{13}N_4O_6$ $[M+H]^+$: 333.08350; found 333.08572.

(E)-1-(2,4-dinitrophenyl)-2-(4-methoxybenzylidene)hydrazine (**3e**)

Compound **3e** was obtained as red crystals (acetone) at 84% yield, mp: 253–254°C, R_f: 0.54 (hexane:EtOAc, 7:3). IR (cm^{-1}) ν: 3290 (N–H), 3112 (C_{sp^2}–H), 2944 (C_{sp^3}–H), 1621 (N=C), 1585 (C_{Ar}–C_{Ar}), 1515 (NO_2), 1255 (C–O). ^{1}H-NMR (DMSO-d_6, 500 MHz) δ (ppm): 3.83 (s, 3H, OCH_3), 7.06 (d, J = 8.30 Hz, 2H), 7.76 (d, J = 8.28 Hz, 2H), 8.09 (d, J = 9.60 Hz, 1H), 8.37 (dd, J = 2.72, 9.70 Hz, 1H), 8.65 (s, 1H, N=CH), 8.88 (d, J = 2.63 Hz, 1H), 11.60 (s, 1H, N-H). ^{13}C-NMR (DMSO-d_6, 125 MHz) δ (ppm): 55.4 (CH_3), 114.5 (CH), 116.7 (CH), 123.0 (CH), 126.3 (C), 128.7 (C), 129.1 (CH), 129.7 (CH), 136.7 (C), 144.5 (C), 149.5 (CH, N=C), 161.3 (C). HRESIMS(+): calculated for $C_{14}H_{13}N_4O_5$ $[M+H]^+$: 317.08858; found 317.09015.

(E)-1-(2-chlorobenzylidene)-2-(2,4-dinitrophenyl)hydrazine (**3f**)

Compound **3f** was obtained as orange crystals (EtOH) at 89% yield, mp: 216–217°C, R_f: 0.60 (hexane:EtOAc, 7:3). IR (cm^{-1}) ν: 3288 (N–H), 3104 (C_{sp^2}–H), 1616 (N=C), 1582 (C_{Ar}–C_{Ar}), 1508 (NO_2). ^{1}H-NMR (DMSO-d_6, 500 MHz) δ (ppm): 7.46 (td, J = 1.4, 7.4 Hz, 1H), 7.49 (td, J = 2.3, 7.4 Hz, 1H), 7.56 (dd, J = 1.6, 7.8 Hz, 1H), 8.14 (dd J= 1.7, 9.5 Hz, 1H), 8.39 (dd, J = 2.7, 9.6 Hz, 1H), 8.87 (d, J = 2.7 Hz, 1H), 9.11 (s, 1H, N=CH), 11.95 (s, 1H, N-H). ^{13}C-NMR (DMSO-d_6, 125 MHz) δ (ppm): 116.7 (CH), 122.6 (CH), 127.0 (CH), 127.4 (CH), 129.4 CH), 129.5 (C), 129.8 (CH), 131.2 (C), 131.5 (CH), 133.4 (C), 137.2 (C), 144.2 (C), 144.8 (CH, N=C). HRESIMS(+): calculated for $C_{13}H_{10}ClN_4O_4$ $[M+H]^+$: 321.03904; found 321.03925.

(E)-1-(4-chlorobenzylidene)-2-(2,4-dinitrophenyl)hydrazine (**3g**)

Compound **3g** was obtained as orange crystals (^{i}PrOH) at 90% yield, mp: 272–273°C, R_f: 0.63 (hexane:EtOAc, 7:3). IR (cm^{-1}) ν: 3284 (N–H), 3090 (C_{sp^2}–H), 1615 (N=C), 1586 (C_{Ar}–C_{Ar}), 1511 (NO_2). ^{1}H-NMR (DMSO-d_6, 500 MHz) δ (ppm): 7.57 (d, J = 8.5 Hz, 2H), 7.84 (d, J = 8.6 Hz, 2H), 8.11 (d, J = 9.6 Hz, 1H), 8.37 (d, J = 9.7 Hz, 1H), 8.70 (s, 1H, N=CH), 8.87 (d, J = 2.7 Hz, 1H,), 11.71 (s, 1H, N-H). ^{13}C-NMR (DMSO-d_6, 125 MHz) δ (ppm): 117.2 (CH), 123.5 (CH), 129.4 (CH), 129.5 (CH), 130.0 (CH), 130.4 (C), 133.3 (C), 135.4 (C), 137.4 (C), 144.4 (C), 148.5 (CH, N=C). HRESIMS(-): calculated for $C_{13}H_8ClN_4O_4$ $[M-H]^-$: 3191.02339; found 319.02060.

(E)-1-(2,4-dichlorobenzylidene)-2-(2,4-dinitrophenyl)hydrazine (**3h**)

Compound **3h** was obtained as yellow crystals (EtOH) at 89% yield, mp: 222–223°C, R_f: 0.70 (hexanes:EtOAc, 7:3). IR (cm^{-1}) ν: 3286 (N–H), 3096 (C_{sp^2}–H), 1614 (N=C), 1589 (C_{Ar}–C_{Ar}), 1512 (NO_2). ^{1}H-NMR (DMSO-d_6, 500 MHz) δ (ppm): 7.55 (dd, J = 1.6, 8.4 Hz, 1H), 7.75 (d, J = 2.1 Hz, 1H), 8.14 (d, J = 9.5 Hz, 1H), 8.15 (d, J = 8.7 Hz, 1H), 8.38 (dd, J = 2.7, 9.7 Hz, 1H), 8.87 (d, J = 2.7 Hz, 1H), 9.07 (s, 1H, N=CH), 11.97 (s, 1H, N-H). ^{13}C-NMR (DMSO-d_6, 125 MHz) δ (ppm): 116.8 (CH), 122.6 (CH), 127.7 (CH), 128.2 (CH), 129.3 (CH), 129.3 (CH), 129.9 (C), 130.2 (C), 133.8 (C), 134.9 (C), 137.2 (C), 143.7 (CH, N=C), 144.1 (C). HRESIMS(+): calculated for $C_{13}H_9Cl_2N_4O_4$ $[M+H]^+$: 355.00007; found 355.00113.

(E)-1-(4-bromobenzylidene)-2-(2,4-dinitrophenyl)hydrazine (**3i**)

Compound **3i** was obtained as orange crystals (^{i}PrOH) at 95% yield, mp: 263–264°C, R_f: 0.70 (hexanes:EtOAc, 7:3). IR (cm^{-1}) ν: 3297 (N–H), 3090 (C_{sp^2}–H), 1619 (N=C), 1587 (C_{Ar}–C_{Ar}), 1510 (NO_2). ^{1}H-NMR (DMSO-d_6, 500 MHz) δ (ppm): 7.69 (d, J = 8.4 Hz, 2H), 7.76 (d, J = 7.8 Hz, 2H), 8.05 (d, J = 8.9 Hz, 1H), 8.27 (s, 1H), 8.64 (s, 1H, N=CH), 8.82 (s, 1H), 11.73 (s, 1H, N-H). ^{13}C-NMR (DMSO-d_6, 125 MHz) δ (ppm): 116.5 (CH, d, J = 20.0 Hz), 117.2 (CH), 123.5 (CH), 130.1 (CH), 130.0 (CH, d, J = 8.6 Hz), 130.1 (C), 131.0 (C), 137.4 (C), 145.0 (C), 148.8 (CH, N=C), 163.5 (C-F, d, J = 251.8 Hz). HRESIMS(-): calculated for $C_{13}H_8BrN_4O_4$ $[M-H]^-$: 362.97288; found 362.97180.

(E)-1-(2,4-dinitrophenyl)-2-(4-fluorobenzylidene)hydrazine (**3j**)

Compound **3j** was obtained as orange crystals (^{i}PrOH) at 83% yield, mp: 280–281°C, R_f: 0.61 (hexanes:EtOAc, 7:3). IR (cm^{-1}) ν: 3286 (N–H), 3091 (C_{sp^2}–H), 1614 (N=C), 1589 (C_{Ar}–C_{Ar}), 1504 (NO_2). ^{1}H-NMR (DMSO-d_6, 500 MHz) δ (ppm): 7.35 (t, J = 8.8 Hz, 2H), 7.88 (dd, J = 5.7, 8.6 Hz, 2H), 8.11 (d, J = 9.6 Hz, 1H), 8.37 (dd, J = 2.7, 9.7 Hz, 1H), 8.71 (s, 1H, N=CH), 8.88 (d, J = 2.7 Hz, 1H), 11.67 (s, 1H, N-H). ^{13}C-NMR (DMSO-d_6, 125 MHz) δ (ppm): 116.5 (CH, d, J = 20.0 Hz), 117.2 (CH), 123.5 (CH), 130.1 (CH), 130.0 (CH, d, J = 8.6 Hz), 130.1 (C), 131.0 (C), 137.4 (C), 145.0 (C), 148.8 (CH, N=C), 163.5 (C-F, d, J = 251.8 Hz). HRESIMS(-): calculated for $C_{13}H_8FN_4O_4$ $[M-H]^-$: 303.05294; found 303.05228.

(E)-1-(2,4-dinitrophenyl)-2-(4-nitrobenzylidene)hydrazine (**3k**)

Compound **3k** was obtained as orange crystals (acetone) at 92% yield, mp: 349–350°C, R_f: 0.48 (hexanes:EtOAc, 7:3). IR (cm^{-1}) ν: 3289 (N–H), 3091 (C_{sp^2}–H); 1616 (N=C), 1577 (C_{Ar}–C_{Ar}), 1508 (NO_2). ^{1}H-NMR (DMSO-d_6, 500 MHz) δ (ppm): 8.07 (d, J = 8.53 Hz, 2H), 8.16 (d, J = 9.58 Hz, 1H), 8.34 (d, J = 8.59 Hz, 2H), 8.40 (d, J = 9.52 Hz, 1H), 8.81 (s, 1H, N=CH), 8.88 (d, J = 2.69 Hz, 1H), 11.85 (s, 1H, N-H). ^{13}C-NMR (DMSO-d_6, 125 MHz) δ (ppm): 117.0 (CH), 122.9 (CH), 124.2 (CH), 128.1 (CH), 129.6 (CH), 130.6 (C), 137.1 (C), 140.3 (C), 144.4 (C), 146.5 (CH, N=C), 147.9 (C). HRESIMS(-): calculated for $C_{13}H_8N_5O_6$ $[M-H]^-$: 330.04744; found 330.04697.

(E)-1-(2,4-dinitrophenyl)-2-(furan-2-ylmethylene)hydrazine (**3l**)

Compound **3l** was obtained as red precipitate at 50% yield, mp: 149–150°C, R_f: 0.52 (hexanes:EtOAc, 7:3). IR (cm^{-1}) ν: 3280 (N–H), 3118 (C_{sp^2}–H), 1618 (N=C), 1581 (C_{Ar}–C_{Ar}), 1511 (NO_2). ^{1}H-NMR (DMSO-d_6, 500 MHz) δ (ppm): 6.68 (dd, J = 1.8, 3.4 Hz, 1H), 6.99 (d, J = 3.4 Hz, 1H), 7.90, (d, J = 1.7 Hz, 1H), 7.95 (d, J = 9.6 Hz, 1H), 8.38 (dd, J = 2.7, 9.7 Hz, 1H), 8.62 (s, 1H, N=CH), 8.85 (d, J = 2.7 Hz, 1H), 11.66 (s, 1H, N-H). ^{13}C-NMR (DMSO-d_6, 125 MHz) δ (ppm): 112.5 (CH), 114.9 (CH), 116.6 (CH), 122.9 (CH), 129.4 (C), 129.8 (CH), 137.0 (C), 138.9 (CH, N=C), 144.3 (C), 145.8 (CH), 149.0 (C). HRESIMS(-): calculated for $C_{11}H_7N_4O_5$ $[M-H]^-$: 275.04163; found 275.03949.

Antifungal test

The compounds were tested for their *in vitro* antifungal activity against *Candida dubliniensis* CBS 7987, *C. glabrata* ATCC 2001, *C. tropicalis* ATCC 13803 and *C. krusei* ATCC 6258 strains by counting colonies with serial dilutions in liquid broth, for determination of MIC90. Fluconazole was used as the reference drug. All compounds and the reference drug were dissolved in dimethylsulfoxide (DMSO) at a concentration of 1000 µg/mL with subsequent dilution in Sabouraud-dextrose broth (400 µg/mL) to obtain a 2% solution. An initial inoculum was created using 4.8 mL Sabouraud-dextrose broth plus 0.1 mL of solution of each compound (400 µg/mL) and 0.1 mL of yeast strain matched to a 0.5% McFarland turbidity standard. This initial inoculum was incubated at 36°C for 48 hours. After this time, 100 mL of each inoculum was placed on Petri dishes with Sabouraud-dextrose agar, seeded with a seed rake and incubated at 36°C for 48 hours. Finally, we counted the colonies formed to calculate percent inhibition of each strain by each compound against the reference drug, as shown in Formula 1. Each assay was performed in triplicate. The same methodology was used for the tests of compounds at 200 µg/mL and 100 µg/mL.

$$\%\ Inhibition = 100 - \frac{Growth\ control\ (UFC)\ \times 100}{Growth\ with\ compounds}$$

Formula 1: Preliminary calculation of percent inhibition

CONCLUSION

In this work, we report screening of a series of diarylhydrazones synthesised from commercially available substances using a simple method with yields of 50–99%. Two of the compounds showed promising antifungal activity, establishing the first foundations for understanding of the correlation between structure and antifungal activity in this family of compounds that constitutes the basis for further structural modifications.

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REFERENCES

1. Regina G. L., Coluccia A., Piscitelli F., Bergamini A., Sinistro A., Cavazza A., Maga G., Samuele A., Zanoli S., Novellino E., Artico M., Silvestri R. *J. Med. Chem.* 2007, **50**, 5034.
2. Regina G.L., Sarkar T., Bai R., Edler M. C., Saletti R., Coluccia A., Piscitelli F., Minelli L., Gatti V., Mazzoccoli C., Palermo V., Mazzoni C., Falcone C., Scovassi A. I., Giansanti V., Campiglia P., Porta A., Maresca B., Hamel E., Brancale A.. Novellino E., Silvestri R. *J. Med. Chem*. 2009, **52**, 7512.
3. Silvestri R., Cascio M. G., La Regina G., Piscitelli F., Lavecchia A., Brizzi A., Pasquini S., Botta M., Novellino E., Di Marzo V., Corelli F. *J. Med. Chem*. 2008, **51**, 1560.
4. Colak M., Demirel N. *Tetrahedron: Asymmetry* 2008, **19**, 635.
5. Yoshikawa N., Shibasaki M. *Tetrahedron* 2002, **58**, 8289.

6. Radecka-Paryzek W., Patroniak V., Lisowski J. *Coord. Chem. Rev*. 2005, **249**, 2156.
7. Gupta K. C., Sutar A. K., Lin C-C. *Coord. Chem. Rev*. 2009, **253**, 1926.
8. Mobinikhaledi A., Jabbarpour M., Hamta A. *J. Chil. Chem. Soc.* 2011, **56**, 812.
9. Rollas S., Küçükgüzel S. G. *Molecules* 2007, **12**, 1910.
10. Cheng L., Tang J-J., Luo H., Jin X-L., Dai F., Yang J., Qian Y-P., Li X-Z., Zhou B. *Bioorg. Med. Chem. Lett.* 2010, **20**, 2417.
11. Török, B., Sood, A., Bag S., Tulsan R., Ghosh S., Borkin D., Kennedy A. R., Melanson M., Madden R., Zhou W., LeVine H., Török M. *Biochemistry*. 2013, **52**, 1137.
12. Vicini, P., Zani, F., Cozzini, P., Doytchinova, I. *Eur. J. Med. Chem*. 2002, **37**, 553.
13. Küçükgüzel Ş. G,, Mazi A., Şahin F., Öztürk S., Stables J. P. *Eur. J. Med. Chem*. 2003, **38**, 1005.
14. Loncle C., Brunel J., Vidal N., Dherbomez M., Letourneux Y. *Eur. J. Med. Chem*. 2004, **39**, 1067.
15. Fortún J., Medicine, 1998, **91**, 4231.
16. Altıntop M. D., Özdemir A., Turan-Zitouni G., Ilgın S., Atlı Ö., Demirci F., Kaplancıklı Z. A. *Molecules* 2014, **19**, 14809.
17. Moran G. P., Sullivan D. J., Henman M. C., Creary C. E. M. C., Harrington B. J., Shanley D. B., Coleman D. C. Antimicrob Agents Chemother. 1997, **41**, 617.