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Research Article

Synthesis and Antifungal Studies of (2*E*)-*N*-Benzyl-*N*′-phenylbut-2-enediamide and (2*E*)-*N*,*N*′-Dibenzylbut-2-enediamide Analogues

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A series of eleven butanediamine analogues, of which nine were new, were synthesized by the nucleophilic substitution of aromatic amines and benzylamines with maleic anhydride and tested on four yeast strains of *Candida* species using the broth microdilution method. Compounds **3a** and **3c** with an unsubstituted phenyl ring and a 3-methoxyphenyl ring, respectively, are the most active against the fungal species with MIC values ranging from 20.2 to 80.6 μM for *C. albicans* and *C. parapsilosis* and 178.5 and 161.2 μM for *C. krusei*, respectively.

1. Introduction

Molecules having an amide functionality have played a significant role in medicine and pharmacology, being used as anticancer agents [1–4], antiproliferative agents [5], for inflammation [6], Parkinson's disease [7], and as antimicrobial agents [8, 9], antioxidants [10], and anti-HIV agents [11, 12]. The HIV epidemic has led to an increase in the number of immunocompromised patients, which in turn has resulted in an increase in the number of systemic fungal infections. Due to this, recent research has started to focus on new potential antifungal agents [13–20]. Clinical therapy has relied mainly on the use of either amphotericin B (ergosterol-binding polyene) or azole (lanosterol demethylase inhibitors) antifungal agents [21]. Resistance to these drugs necessitates the need for the synthesis of new antifungal drugs. This prompted us to investigate the potential of the butanediamines to act as possible antifungal agents.

Some of the synthetic approaches to synthesising these amides include the Aza-Michael addition of an amine to *N*-phenylmaleimide using *trans-N,N,N′,N′*-tetramethylcyclohexanediamine (TMCDA) as a receptor [22], cross-metathesis of acrylic amide with NHC Grubbs ruthenium catalyst [23], and the use of DCC, either with 1-hydroxybenzotriazole (HOBT) or but-2-en-1,4-dioic acid and benzylamine [24], which we have chosen to adopt with some modification along with the modified method of esterification of carboxylic acids, in which we have formed amides rather than the esters by using amines instead of alcohols [25].

To the best of our knowledge, no biological studies of these compounds have been reported and only the syntheses of **3a** [22] and **3i** [23] have been reported. We present here the synthesis of several butanediamine analogues with different electron donating and withdrawing groups (OCH_3, NO_2, Cl, F) and explored their potential to act as antifungal agents.

2. Materials and Methods

2.1. General Experimental Details. All chemicals were reagent grade and purchased from Sigma-Aldrich (Germany) through Capital Laboratories (South Africa). Analytical thin layer chromatography (TLC) was performed using precoated silica gel 60 (F_{254}) from MERCK (South Africa). Visualization was achieved by UV light (254 nm) and by heating with 20% H_2SO_4 acid in MeOH. NMR spectra were recorded

using a Bruker Avance^{III} 400 MHz spectrometer at room temperature in DMSO-d_6 with chemical shifts (δ) recorded against the internal standard, tetramethylsilane (TMS). The infrared spectra of the solid samples were recorded on a Perkin Elmer Spectrum 100 FTIR spectrometer with universal ATR sampling accessory. The gas chromatography mass spectrometry (GC-MS) was carried out by the electron impact ionization method, using an Agilent GC-MSD apparatus equipped with a DB-5SIL MS fused-silica capillary column. UV/Vis analysis was carried out on a Perkin Elmer Lambda 35 UV/VIS spectrometer with a scan range of 200–400 nm. Melting point determination was carried out on an Ernst Leitz Wetzlar number 461086 microhot stage melting point apparatus. High resolution mass spectrometry (HRMS) was recorded on a waters Q-TOF Micromass spectrometer with a lock spray source.

2.2. Synthesis of the Intermediate Carboxylic Acids ***(2a–k)***. Maleic anhydride (2.8 g, 28 mmol) was weighed into a round bottom flask containing 20 mL of toluene. The substituted anilines (**1a–g**), 3-aminopyridine (**1h**) or substituted benzylamines (**1i–k**) (25 mmol) were then added to the mixture, which was then stirred under reflux for 2 h. The heat was then removed and the reaction stirred for a further 30 min. The solid carboxylic acid intermediates **2a** (48% yield), **2b** (36%), **2c** (74%), **2d** (42%), **2e** (51%), **2f** (42%), **2g** (34%), **2h** (47%), **2i** (45%), **2j** (64%), and **2k** (65%) were obtained after being filtered under suction and washed with toluene. Compounds **2a–k** were all known, and their structures were confirmed by comparing their spectroscopic and physical data with that in the literature [26–30].

2.3. Synthesis of the But-2-enediamides ***(3a–k)***. To a solution of the substituted acids **(2a–k)** (2.3 mmol) and diisopropylcarbodiimide (DIC, 0.32 g; 2.5 mmol) in DMF (7 mL), dimethylaminopyridine (DMAP, 0.08 g; 0.65 mmol) was added and the mixture stirred at room temperature for at least 30 min. Benzylamine (0.27 g; 2.5 mmol) was then added and the reaction mixture stirred at room temperature under nitrogen for 12 hrs. A volume of 10 mL of dichloromethane (DCM) was then introduced and the contents stirred for a further 5 min and then washed with brine (3 × 10 mL), separated, and dried on $MgSO_4$. A precipitate was observed upon cooling the organic layer on ice, which was filtered under suction and washed with DCM, producing the products (**3a–k**) in pure form.

Compounds **3a** and **3i** were synthesised previously [22, 23]; however NMR data for **3a** are not reported in Bi et al. [22] and are therefore presented below. The ^{1}H and ^{13}C NMR data for **3i** (32% yield) was consistent with that reported in Streuff and Muñiz [23].

(2E)-N-Benzyl-N′-phenylbut-2-enediamide ***(3a)***. White powder (35% yield); mp 265–267°C; UV λ_{max} (CH_3OH) nm (log ε) 290 (4.31); IR (KBr) υ_{max} (cm^{-1}) 3266 (NH), 3061, 1633 (C=O), 1598, 1542; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 10.47 (s, 1H, NH-5), 9.01 (bt, 1H, J = 5.9 Hz, NH-6), 7.68 (d, 2H, J = 8.1 Hz, H-2′/6′), 7.34–7.36 (m, 4H, H-3′/5′, H-3″/5″), 7.28–7.33 (m, 3H, H-2″/6″, H-4″), 7.10–7.12 (m, 1H, H-4′), 7.11 (d, 1H, J = 15.1 Hz, H-3), 7.04 (d, 1H, J = 15.1 Hz, H-2), 4.41 (d, 2H, J = 5.9 Hz, H-7″); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 164.0 (C-1), 162.7 (C-4), 139.4 (C-1″), 139.3 (C-1′), 134.1 (C-2), 133.7 (C-3), 129.3 (C-3′/5′), 128.9 (C-3″/5″), 127.8 (C-2″/6″), 127.4 (C-4″), 124.3 (C-4′), 119.8 (C-2′/6′), 42.9 (C-7″).

(2E)-N-Benzyl-N′-(2-methoxyphenyl)but-2-enediamide ***(3b)***. White powder (18% yield); mp 260–262°C; UV λ_{max} (CH_3OH) nm (log ε) 310 (4.76), 275 (4.92), 240 (5.23); IR (KBr) υ_{max} (cm^{-1}) 3271 (NH), 3034, 1626 (C=O), 1537, 1492; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.73 (s, 1H, NH-5), 8.95 (bt, 1H, J = 5.6 Hz, NH-6), 8.01 (d, 1H, J = 7.7 Hz, H-6′), 7.35 (d, 1H, J = 15.1 Hz, H-3), 7.33–7.35 (m, 2H, H-3″/5″), 7.28–7.30 (m, 3H, H-2″/6″, H-4″), 7.11 (m, 1H, H-5), 7.01 (d, 1H, J = 15.1 Hz, H-2), 7.00 (d, 1H, J = 7.7 Hz, H-3′), 6.92 (t, 1H, J = 7.7 Hz, H-4′), 4.40 (d, 2H, J = 5.6 Hz, H-7″), 3.83 (s, 3H, 2′-OCH_3); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 164.2 (C-1), 163.0 (C-4), 150.5 (C-2′), 139.4 (C-1″), 134.1 (C-2), 134.0 (C-3), 128.9 (C-3″/5″), 127.8 (C-2″/6″), 127.4 (C-4″), 125.5 (C-5′), 122.8 (C-6′), 120.67 (C-4′), 111.8 (C-3′), 56.15 (2′-O$\underline{C}H_3$), 42.85 (C-7″); EIMS (m/z) 310 [M$^+$], 281, 249, 207, 188, 160, 106, 91; HRMS (m/z): 310.1318 (calculated for $C_{18}H_{18}N_2O_3$, 310.1315).

(2E)-N-Benzyl-N′-(3-methoxyphenyl)but-2-enediamide ***(3c)***. White powder (65% yield); mp 240-241°C; UV λ_{max} (CH_3OH) nm (log ε) 310 (3.88), 280 (3.88), 240 (4.04); IR (KBr) υ_{max} (cm^{-1}) 3266 (NH), 3070, 1633 (C=O), 1602 (NH), 1544, 1492; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 10.45 (s, 1H, NH-5), 9.02 (bt, 1H, J = 5.8 Hz, NH-6), 7.38 (s, 1H, H-2′), 7.33–7.35 (m, 2H, H-3″/H-5″), 7.25–7.29 (m, 3H, H-2″/6″, H-4″), 7.19–7.23 (m, 2H, H-5′,6′), 7.08 (d, 1H, J = 15.1 Hz, H-2), 7.02 (d, 1H, J = 15.1 Hz, H-3), 6.69 (d, 1H, J = 7.8 Hz, H-4′), 4.40 (d, 2H, J = 5.8 Hz, H-7″), 3.73 (s, 3H, 3′-OCH_3); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 164.0 (C-1), 162.7 (C-4), 160.0 (C-3′), 140.4 (C-1′), 139.3 (C-1″), 134.2 (C-2), 133.7 (C-3), 130.1 (C-5′), 128.9 (C-3″/5″), 127.8 (C-2″/6″), 127.5 (C-4″), 112.2 (C-6′), 109.7 (C-4′), 105.7 (C-2′), 55.5 (3′-OCH_3), 42.9 (C-7″). EIMS (m/z): 310 [M$^+$], 281, 207, 187, 176, 149, 106, 91; HRMS (m/z): 310.1315 (calculated for $C_{18}H_{18}N_2O_3$, 310.1315).

(2E)-N-Benzyl-N′-(2-chlorophenyl)but-2-enediamide ***(3d)***. Pale brown powder (24% yield); mp 244-245°C; UV λ_{max} (CH_3OH) nm (log ε) 275 (4.36); IR (KBr) υ_{max} (cm^{-1}) 3267 (NH), 3032, 1626 (C=O), 1531, 1494; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 10.08 (s, 1H, NH-5), 9.00 (bt, 1H, J = 5.9 Hz, NH-6), 7.79 (d, 1H, J = 7.9 Hz, H-6′), 7.53 (d, 1H, J = 7.9 Hz, H-3′), 7.32–7.35 (m, 2H, H-3″/5″), 7.25–7.29 (m, 3H, H-2″/6″, H-4″), 7.07 (d, 2H, J = 15.2 Hz, H-2/3), 4.42 (d, 2H, J = 5.9 Hz, H-7″); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 164.0 (C-1), 163.1 (C-4), 139.3 (C-1″), 134.9 (C-2), 134.8 (C-3), 133.1 (C-4′), 130.1 (C-3′), 128.9 (C-3″/5″), 127.9 (C-4″), 127.8 (C-2″/6″), 127.4 (C-5′), 126.9 (C-6′), 42.9 (C-7″); EIMS (m/z): 314 [M$^+$], 281, 207, 187, 138, 106; HRMS (m/z): 314.0820 (calculated for $C_{17}H_{15}ClN_2O_2$, 314.0820).

(2E)-N-Benzyl-N′-(3-chlorophenyl)but-2-enediamide ***(3e)***. Pink powder (30% yield); mp 272–274°C; UV λ_{max} (CH_3OH) nm (log ε) 290 (4.66), 240 (4.60); IR (KBr) υ_{max} (cm^{-1}) 3265

(NH), 3066, 1633 (C=O), 1592, 1541, 1482; ^{1}H NMR (DMSO-d$_6$, 400 MHz) δ 10.63 (s, 1H, NH-5), 9.03 (bt, 1H, J = 5.9 Hz, NH-6), 7.92 (s, 1H, H-2′), 7.51 (d, 1H, J = 8.5 Hz, H-4′), 7.36–7.38 (m, 1H, H-5), 7.32–7.34 (m, 2H, H-3″/5″), 7.25–7.29 (m, 3H, H-2″/6″, H-4″), 7.17 (d, 1H, J = 8.5 Hz, H-6′), 7.06 (s, 2H, H-2/3), 4.41 (d, 2H, J = 5.9 Hz, H-7″); ^{13}C NMR (DMSO-d$_6$, 100 MHz) δ 163.4 (C-1), 162.5 (C-4), 140.2 (C-1′), 138.8 (C-1″), 134.2 (C-2), 133.1 (C-3), 132.7 (C-3′), 130.5 (C-5′), 128.4 (C-3″/5″), 127.3 (C-2″/6″), 126.9 (C-4″), 123.5 (C-6′), 118.8 (C-2′), 117.8 (C-4′), 42.4 (C-7″). EIMS (m/z) 314 [M$^+$], 281, 207, 180, 153, 138, 117, 106, 91; HRMS (m/z): 314.0826 (calculated for $C_{17}H_{15}ClN_2O_2$, 314.0820).

(2E)-N-Benzyl-N′-(3-nitrophenyl)but-2-enediamide ***(3f)***. Cream powder (29% yield); mp 259–261°C; UV λ_{max} (CH_3OH) nm (log ε) 275 (5.21); IR (KBr) υ_{max} (cm^{-1}) 3271 (NH), 3038, 1643 (C=O), 1621, 1529; ^{1}H NMR (DMSO-d$_6$, 400 MHz) δ 10.95 (s, 1H, NH-5), 9.07 (bt, 1H, J = 5.3 Hz, NH-6), 8.74 (s, 1H, H-2′), 7.97 (d, 2H, J = 7.8 Hz, H-4′/6′), 7.67 (t, 1H, J = 7.8 Hz, H-5′), 7.33–7.35 (m, 2H, H-3″/5″), 7.26–7.29 (m, 3H, H-2″/6″, H-4″), 7.10 (s, 2H, H-2/3), 4.43 (d, 2H, J = 5.3 Hz, H-7″); ^{13}C NMR (DMSO-d$_6$, 100 MHz) δ 163.8 (C-1), 163.4 (C-4), 148.5 (C-3′), 140.4 (C-1′), 139.3 (C-1″), 135.0 (C-2), 133.0 (C-3), 130.8 (C-5′), 128.9 (C-3″/5″), 127.9 (C-2″/6″), 127.5 (C-4″), 125.8 (C-6′), 118.9 (C-4′), 113.9 (C-2′), 42.9 (C-7″). EIMS (m/z): 325 [M$^+$], 280, 234, 191, 164, 149, 117, 91; HRMS (m/z): 325.1057 (calculated for $C_{18}H_{18}N_2O_3$, 325.1060).

(2E)-N-Benzyl-N′-(4-nitrophenyl)but-2-enediamide ***(3g)***. Pale brown powder (12% yield); mp 274–276°C; UV λ_{max} (CH_3OH) nm (log ε) 325 (4.90); IR (KBr) υ_{max} (cm^{-1}) 3267 (NH), 3062, 1626 (C=O), 1534, 1492; ^{1}H NMR (DMSO-d$_6$, 400 MHz) δ 11.05 (s, 1H, NH-5), 9.07 (bt, 1H, J = 5.9 Hz, NH-6), 8.26 (d, 2H, J = 9.1 Hz, H-3″/5″), 7.92 (d, 2H, J = 9.1 Hz, H-2″/6″), 7.29–7.35 (m, 2H, H-3″/5″), 7.25–7.27 (m, 3H, H-2″/6″, H-4″), 7.11 (s, 2H, H-2,3), 4.42 (d, 2H, J = 5.9 Hz, H-7″); ^{13}C NMR (DMSO-d$_6$, 100 MHz) δ 163.6 (C-1), 163.3 (C-4), 145.2 (C-1′), 142.9 (C-4′), 139.0 (C-1″), 135.2 (C-2), 132.7 (C-3), 128.6 (C-3″/5″), 127.7 (C-2″/6″), 127.3 (C-4″), 125.3 (C-3′/5′), 119.5 (C-2′/6′), 42.8 (C-7″); EIMS (m/z): 325, 281, 249, 207, 187, 167, 149, 106; HRMS (m/z): 325.1059 (calculated for $C_{17}H_{15}N_3O_4$, 325.1060).

(2E)-N-Benzyl-N′-(pyridin-3-yl)but-2-enediamide ***(3h)***. Brown powder (27% yield); mp 198–200°C; UV λ_{max} (CH_3OH) nm (log ε) 270 (4.64), 240 (4.75); IR (KBr) υ_{max} (cm^{-1}) 3268 (NH), 3061, 1625 (C=O), 1532 (NH), 1493; ^{1}H NMR (DMSO-d$_6$, 600 MHz) δ 10.69 (s, 1H, NH-5), 9.04 (bt, 1H, J = 5.6 Hz, NH-6), 8.81 (s, 1H, H-2′), 8.31 (d, 1H, J = 8.2 Hz, H-6′), 8.14 (d, 1H, J = 8.2 Hz, H-4′), 7.38-7.39 (m, H-5′), 7.34–7.36 (m, 2H, H-3″/5″), 7.26–7.30 (m, 3H, H-2″/6″, H-4″), 7.10 (d, 1H, J = 15.1 Hz, H-3), 7.07 (d, 1H, J = 15.1 Hz, H-2), 4.42 (d, 2H, J = 5.6 Hz, H-7″); ^{13}C NMR (DMSO-d$_6$, 150 MHz) δ 163.9 (C-1), 163.2 (C-4), 145.2 (C-6′), 141.4 (C-2′), 139.3 (C-3′), 136.0 (C-1″), 134.7 (C-2), 133.0 (C-3), 128.9 (C-3″/5″), 127.8 (C-2″/6″), 127.5 (C-4″), 126.8 (C-4′), 124.2 (C-5′), 42.9 (C-7″). EIMS (m/z) 281 [M$^+$], 207, 187, 147, 119, 106, 91; a high resolution mass could not be detected for this compound despite several attempts.

(2E)-N-Benzyl-N′-(3-fluorobenzyl)but-2-enediamide ***(3j)***. White powder (45% yield); mp 298–300°C; UV λ_{max} (CH_3OH) nm (log ε) 250 (4.08); IR (KBr) υ_{max} (cm^{-1}) 3275 (NH), 3085, 1626 (C=O), 1591, 1555; ^{1}H NMR (DMSO-d$_6$, 400 MHz) δ 8.94 (t, 1H, J = 5.9 Hz, NH-5), 8.93 (t, 1H, J = 5.9 Hz, NH-6), 7.30–7.36 (m, 3H, H-5′, H-3″/5″), 7.25–7.27 (m, 3H, H-2″/6″, H-4″), 7.05–7.11 (m, 3H, H-2′,4′,6′), 6.93 (s, 2H, H-2/3), 4.39 (d, 2H, J = 5.9 Hz, H-7′), 4.37 (d, 2H, J = 5.9 Hz, H-7″); ^{13}C NMR (DMSO-d$_6$, 100 MHz) δ 163.8 (C-4), 163.6 (C-1), 162.2 (d, J_{CF} = 242.0 Hz, C-3′), 141.9 (d, J_{CF} = 7.2 Hz, C-1′), 138.9 (C-1″), 132.9 (C-2), 132.6 (C-3), 130.3 (d, J_{CF} = 8.4 Hz, C-5′), 128.3 (C-3″/5″), 127.3 (C-2″/6″), 126.9 (C-4″), 123.2 (d, J_{CF} = 2.7 Hz, C-6′), 113.9 (d, J_{CF} = 21.3 Hz, C-2′), 113.6 (d, J_{CF} = 20.9 Hz, C-4′), 42.3 (C-7′), 41.8 (C-7″); ^{19}F-NMR (DMSO-d$_6$, 376.5 MHz) δ −113.46, HRMS (m/z): 312.1270 (calculated for $C_{18}H_{17}N_2O_2F$, 312.1272).

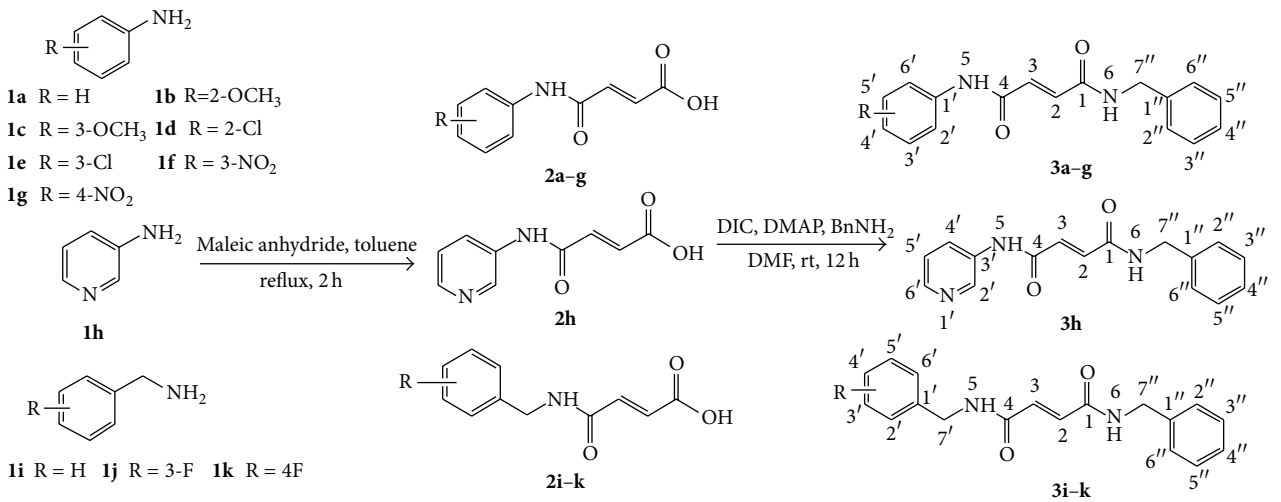
(2E)-N-Benzyl-N′-(4-fluorobenzyl)but-2-enediamide ***(3k)***. Pink powder (50% yield); mp 299–301°C; UV λ_{max} (CH_3OH) nm (log ε) 250 (4.50); IR (KBr) υ_{max} (cm^{-1}) 3275 (NH), 3084, 1624 (C=O), 1539, 1487; ^{1}H NMR (DMSO-d$_6$, 400 MHz) δ 8.97 (bs, 1H, NH-5), 8.93 (bs, 1H, NH-6), 7.36–7.38 (m, 2H, H-3″/5″), 7.31-7.32 (m, 2H, H-2′/6′), 7.26-7.27 (m, 3H, H-2″/6″, H-4″), 7.09–7.11 (m, 2H, H-3′/5′), 6.93 (s, 2H, H-2/3), 4.38 (bs, 4H, H-7′/7″); ^{13}C NMR (DMSO-d$_6$, 100 MHz) δ 164.2 (C-1), 164.1 (C-4), 161.6 (d, J_{CF} = 252.5 Hz, C-4′), 139.4 (C-1′), 135.7 (C-1″), 133.3 (C-2), 133.2 (C-3), 129.8 (d, J_{CF} = 8.0 Hz, C-2′/6′), 128.8 (C-3″/5″), 127.8 (C-2″/6″), 127.4 (C-4″), 115.6 (d, J_{CF} = 20.9 Hz, C-3′/5′), 42.8 (C-7′), 42.1 (C-7″); ^{19}F-NMR (DMSO-d$_6$, 376.5 MHz) δ −116.0, HRMS (m/z): 312.1271 (calculated for $C_{18}H_{17}FN_2O_2$, 312.1272).

2.4. Biological Assay

Microbial Strains. The test compounds were evaluated on American Type Culture Collection (ATCC) strains of four *Candida* yeasts, *Candida albicans* (90028), *Candida albicans* (10231), *Candida krusei* (6258), and *Candida parapsilosis* (22019). Yeast strains were grown on sabouraud dextrose agar (SDA) and incubated at 35°C for 24 h.

Antifungal Agents. The test compounds were dissolved in DMSO and made to a final concentration of 2% DMSO in each well. All dilutions were done just prior to carrying out the assays. The final concentration of the test compounds ranged from 3.8 nM to 715.0 μM. All drug dilutions were carried out in 96-well flat bottom microtitre plates. Amphotericin-B from Sigma Aldrich was used as a reference drug for the antifungal assay.

Antifungal Susceptibility Test. Evaluation of the susceptibility of *Candida albicans* and non-*Candida albicans* species as performed using the broth microdilution method according to M27-A2 for yeast guidelines [31]. Yeast strains were grown aerobically overnight at 35°C on SDA plates. Yeasts were



Scheme 1: Preparation of butenediamide analogues.

harvested and suspended in 1% sterile saline, and the turbidity of the supernatants measured spectrophotometrically at 625 nm with an absorbance of 0.08–0.1 is equivalent to a 0.5 McFarland standard following the NCCLS M27-A2 guidelines. The working suspension was diluted 20 times in a mixture containing RPMI 1640 medium and 0.165 M morpholinepropanesulfonic acid buffered to a pH of 7.0. The working suspension was further diluted 50 times to obtain the final test inoculums ($1 - 5 \times 10^3$ CFU mL^{-1}). The microtitre plates that contained different concentrations of the test compounds were allowed to thaw and equilibrate to room temperature under aseptic conditions. Aliquots of working inoculum suspensions were dispensed into each well, and the plates incubated in an aerobic environment at 35°C for 24 h. After incubation, 20 μL of 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium salt (MTS, Promega Corporation, Madison, USA) was added directly to each well, incubated at 37°C for 4 h and the absorbance recorded at 490 nm on a 96-well plate reader (VACUTEC). All analyses were performed in triplicate, and data are reported as the mean ± standard error of the mean of ≤5.

3. Results and Discussion

3.1. Chemistry. Our synthetic route involved two steps (Scheme 1). The first was the formation of the acid by nucleophilic addition of the amine to maleic anhydride. The reaction takes place in toluene under reflux conditions, where the amine attacks the carbonyl carbon of maleic anhydride, which breaks open forming the first amide bond and the carboxylic acids (intermediates **2a–k**). The second step occurs via a Steglich esterification, in which diisopropylcarbodiimide (DIC) is used as a coupling reagent and dimethylaminopyridine (DMAP) as a catalyst [24].

The reaction involves abstraction of a proton from the acid by DMAP. The carboxylate ion that forms attacks the DIC to form the *O*-acylisourea which is more reactive toward nucleophilic substitution than the acid. The amine now attacks this *O*-acylisourea to form the diamide with diisopropylurea (DIU) as an unwanted by-product, which complicates the purification and diminishes the yield of the final product.

The reaction takes place at room temperature for at least 12 h under nitrogen gas. With dichloromethane (DCM) alone, an insoluble DIU was formed as a solid by-product. The product yield obtained after filtration of the DIU for this step of the reaction was unsatisfactory at between 2–20%. Changing the solvent to DMF in subsequent reactions improved the yields for this step considerably by approximately 10% on average, and in some cases stepwise yields of between 50% and 85% were achieved. The overall yields are given in the experimental section and ranged between 12% and 65%. For the phenylamino derivatives **3a–3g**, electron donating substituents at the 2′-position resulted in low overall yields, 18% (**3b**) and 24% (**3d**) compared to the same substituents at the 3′-position, 65% (**3c**) and 30% (**3e**). Electron withdrawing substituents, such as the nitro group, at the 3′-position are better for benzylamine coupling with the carboxyl group than the same substitution at the 4′-position, 29% for **3f** compared to 12% (**3g**). Substituting electron donating substituents at the 2′-position makes the carboxyl carbon less electrophilic to substitution with the benzylamine as does electron withdrawing substituents at the 4′-position. The pyridin-3-yl and the fluorobenzylamino derivatives had acceptable overall yields of 27% (**3h**), 32% (**3i**), 45% (**3j**), and 50% (**3k**). Since the DIU by-product was completely soluble in DMF, DCM is needed to extract the organic product. The remaining DIU is removed by washing with water, and the pure products (**3a–k**) precipitated out of the DCM fraction upon cooling.

The structures of the molecules were confirmed by ^{1}H and ^{13}C NMR spectroscopy and by 2D correlation (HSQC and HMBC). For example, the ^{1}H NMR spectrum of **3a** shows a pair of doublets at δ_H 7.11 (H-3, J = 15.1 Hz) and δ_H 7.04 (H-2, J = 15.1 Hz), the large coupling constant

Table 1: Antifungal activity of the synthesised butenediamides (MIC) μM.

Compound	*C. albicans* (ATCC90028)	*C. albicans* (ATCC10231)	*C. krusei* (ATCC6258)	*C. parapsilosis* (ATCC22019)
3a	**44.6 ± 0.57**	**44.6 ± 1.21**	178.5 ± 0.36	**44.6 ± 0.46**
3b	—	—	—	—
3c	**40.3 ± 0.48**	**80.6 ± 0.42**	161.2 ± 1.61	**20.2 ± 0.39**
3d	159.2 ± 0.45	159.2 ± 0.60	159.2 ± 0.51	159.2 ± 0.41
3e	636.8 ± 0.64	636.8 ± 2.55	159.2 ± 0.80	159.2 ± 1.43
3f	—	—	615.2 ± 0.65	615.2 ± 1.54
3g	307.6 ± 0.31	307.6 ± 0.77	307.6 ± 0.40	153.8 ± 2.21
3h	355.7 ± 1.78	355.7 ± 0.75	177.9 ± 0.60	355.7 ± 0.89
3i	170.0 ± 0.44	170.0 ± 0.41	170.0 ± 1.16	170.0 ± 0.61
3j	320.4 ± 0.54	320.4 ± 0.51	640.8 ± 1.60	640.8 ± 1.60
3k	640.8 ± 1.12	640.8 ± 0.80	640.8 ± 0.74	640.8 ± 0.51
Amphotericin-B	1.3 ± 0.60	1.3 ± 0.49	5.4 ± 0.54	1.3 ± 0.27

All experiments were carried out in triplicate. Data reported as the mean of three experiments.
Key: —: No activity.

indicating *trans* olefinic methine groups. The H-3 resonance was assigned further downfield than the H-2 resonance, since this resonance is seen to shift downfield when a methoxy group is present at the 2′-position. For example, H-3 in **3a** is at δ_H 7.11, while H-3 in **3b** is at δ_H 7.35. The H-2 resonance does not shift and appears at approximately δ_H 7.01. These resonances also show HMBC correlations to both the carbonyl amide resonances at δ_C 164.0 (C-1) and δ_C 162.7 (C-4). The C-1 carbonyl resonance occurs more downfield than the C-4 carbonyl resonance. In **3a**, there is no HMBC correlation to prove this; however, in the 2′-methoxy derivative (**3b**), a distinct correlation can be seen between the NH-6 triplet and the C-1 carbonyl resonance. The NH-6 triplet in **3a** appears at δ_H 9.01, and the NH-5 resonance appears at δ_H 10.47 as a singlet resonance. These resonances and 2D correlations confirm the but-2-enediamide moiety.

The H-7″ benzyl methylene resonance was critical in assigning the aromatic proton resonances and distinguishing the resonances of the two aromatic rings. The H-7″ resonance at δ_H 4.41 showed HMBC correlations to both the C-1″ and C-2″/6″ resonances at δ_C 139.4 and δ_C 127.8. The other singlet aromatic carbon resonance at δ_C 139.3 was then assigned to C-1′ on the other aromatic ring, which allowed H-3′/5′ to be assigned since a correlation between C-1′ and H-3′/5′ was seen in the HMBC spectrum. The H-2′/6′ proton resonance was distinctly shifted more downfield by the anisotropic effect of the amide group and appeared at δ_H 7.68 as a doublet with $J = 8.1$ Hz. This resonance showed an HMBC correlation to C-4′ at δ_C 124.3. The H-3″/5″ resonance overlapped with the H-3′/5′ resonance at δ_H 7.34–7.36 as a multiplet, and the remaining H-4″ resonance was then assigned together with the H-2″/6″ resonance as the multiplet at δ_H 7.28–7.33. Using the HSQC spectrum to identify the corresponding proton and carbon resonances, all the resonances could be identified. The resonances of the other compounds were determined in a similar fashion.

3.2. Biological Results. The synthesised compounds were tested against two strains of *Candida albicans* (ATCC90028 and ATCC10231) as well as *Candida krusei* (ATCC6258) and *Candida parapsilosis* (ATCC22019). Compounds **3a** and **3c** had the best activity from all the compounds tested against (Table 1). These were the compounds with an unsubstituted phenyl ring (**3a**) and the 3′-methoxysubstituted derivative (**3c**). Compound **3d** with a 2′-chlorosubstituent and **3i** with two benzyl groups attached to the butanediamine also had moderate activity against the four strains of fungi. Moderate strain specific activity against *C. krusei* and *C. parapsilosis* was also seen by **3e** with a 3′-chloro group; however, *C. albicans* was totally resistant to this compound. Of the four strains, *C. krusei* was the most resistant to compounds **3a** and **3c**, the most active compounds. The benzyl pyridin-3-ylbut-2-enediamide (**3h**), although susceptible to *C. albicans* and *C. parapsilosis*, showed moderate activity against *C. krusei* and **3g**, the 4′-nitro compound showed moderate activity against *C. parapsilosis*.

In general, the positions of the methoxy- and chlorosubstituents on the phenyl ring were important as the activity of the methoxygroup was associated with the 3′-position only, and the 2′-chlorocompound (**3d**) showed better activity than the 3′-chlorocompound against *C. albicans*. Compound **3b**, the 3-methoxy derivative, was completely susceptible to all the fungal strains as were the fluorosubstituted benzyl derivatives (**3j** and **3k**) and the 3-nitrophenyl derivative (**3f**).

4. Conclusion

The substituted aromatic but-2-enediamides were successfully synthesised and their structures confirmed by NMR spectroscopy. In general, the (2*E*)-*N*-benzyl-*N*′-phenylbut-2-enediamide class showed better antifungal ability than the (2*E*)-*N*-*N*′-dibenzylbut-2-enediamide series which showed lower antifungal activity. Even though these compounds were not as active as the standard drug Amphotericin B, the compounds may be useful in situations where resistance is built up to currently used antifungal drugs. Compounds **3a** and **3c** can definitely be explored for their potential to act as antifungal agents.

Conflict of Interests

The authors declare that they do not have any financial relations with any of the commercial entities mentioned in the paper that could lead to a conflict of interests.

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