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Design, synthesis and antifungal activity of pterolactam-inspired amide Mannich bases

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ABSTRACT

Pterolactam (5-methoxypyrrolidin-2-one) is a heterocycle naturally occurring in plants. In an attempt to identify antifungal agents, a series of novel Mannich bases of amide derivated from Pterolactam have been designed, synthesized and their antifungal activities were evaluated on a panel of nine fungal strains and three *non albicans candida* yeasts species which have demonstrated reduced susceptibility to commonly used antifungal drugs. A third of the target compounds exhibited good to high antifungal activities on at least one strain with EC_{50} lower than the control antifungal agent. *N,N′*-aminals derivated from Pterolactam proved to be good candidates for the development of biosourced fungicides, with compound **3o** being the most broader-spectrum agent, active against five strains and devoted of any cytotoxicity.

1. Introduction

Statistics show that nearly two million people die each year from invasive fungal infections, and that, for instance, this mortality is three-fold higher than for malaria [1]. Synthetic drugs have proved to be highly successful in the past, but the fungal resistance poses a real risk, which comes naturally from the use of antifungal drugs, and many novel resistance patterns have been already observed [2]. Despite the numerous available antimicrobial agents the treatment of pathogenic microbial contaminations remains a central challenge in the global healthcare [3] and in particular the increase of fungal resistance led to make mycoses a major cause of morbidity and mortality in immunocompromised patients [4]. Fungi also trigger most severe plant pathologies and are one of the principal sources of agricultural losses [5]. The identification of new antifungal schedule is thus imbedded in drug discovery among the different scientific disciplines [6]. Plants afford a valuable resource platform for such endeavors owing the chemical diversity of the many scaffolds they provide, contributing to their presence in traditional approaches towards fungal diseases [7]. It is well known that many plant-derived metabolites exhibit antifungal properties [8–10]. Pterolactam **A** was extracted from bracken (*Pteridium aquilinum* Kuhn var. *latiusculum* Underwood) [11], from the leaves of *Phyteuma japonicum* [12], from *Villasenoria orcuttii*, the only species of the genus *Villasenoria* (*Asteraceae, Senecioneae*), which is a shrub that grows in the Mexican rainforest at 100 to 2000 *m* of elevation [13], or from the aerial parts of *Chrysanthemum coronarium L.* [14] (Fig. 1).

This natural occurring 5-membered lactam ring belongs to the pyrrolidine-2-one derivatives. It is notable that pyrrolidone derivatives are well described for their antifungal properties [15–17]. Moreover, *N,N′*-aminals and *N,O*-acetals originated from the substitution of the position 5 of this heterocycle are largely distributed among the plant metabolites.

From a chemical point of view, these scaffolds are chemically equivalent to constrained Mannich bases of amides, many of them known for their antifungal properties [23,24]. However, to the best of our knowledge, no systematic study of the antifungal properties of

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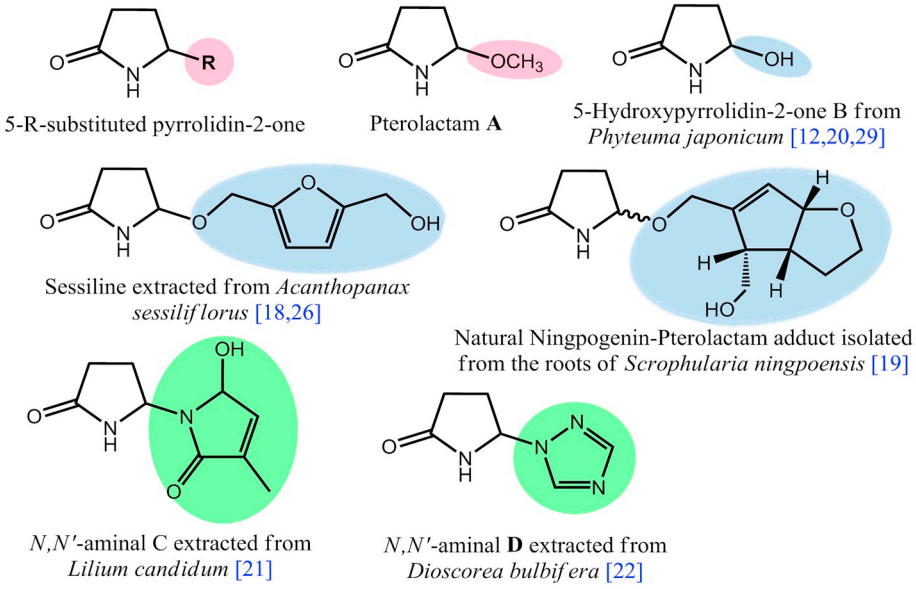


Fig. 1. Natural compounds derivated from pyrrolidin-2-one in position 5 [18-22, 29].

entities issued from natural Pterolactam **A** was reported, and only 5-hydroxypyrrolidone **B** was described as antibacterial (Fig. 1). Thus, we decided to examine a diversity of representative derivatives of these natural building blocks, and four series of related 5-substituted 2-pyrrolidinones were synthesized, covering the range of aliphatic ethers and thioethers (**Series a**), aliphatic amines (**Series b**), aromatic amines (**Series c**) and heterocyclic amines (**Series d**) (Scheme 1).

2. Material and methods

2.1. Chemistry

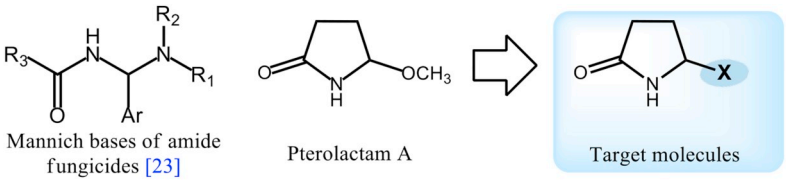
Solvents of analytical reagent grade were used without further purification. Analytical thin-layer chromatographies (TLC) were performed on E. Merck 60 F254 silica gel plates. ^{1}H NMR and ^{13}C NMR spectra were obtained at 25 °C on a Varian 400-MR spectrometer (400 MHz for ^{1}H, 100 MHz for ^{13}C, respectively). Chemical shifts (δ) are quoted in parts per million (ppm) and are referenced to TMS as an internal standard. Coupling constants (J) are quoted in hertz. Column chromatographies were performed with a CombiFlash Rf Companion (Teledyne-Isco System) using RediSep packed columns. IR spectra were recorded on a Varian 640-IR FT-IR Spectrometer. Melting points were measured on a MPA 100 OptiMelt® apparatus. Elemental analyses (C, H, N, S) of new compounds were determined on a Thermo Electron apparatus by 'Pôle Chimie Moléculaire-Welience', Faculté de Sciences Mirande, Université de Bourgogne, Dijon, France. Yield refers to the isolated analytically pure material. Starting materials were purchased from Solabia group (France), Sigma Aldrich, Alfa Aesar and TCI, and were used without further purification.

2.2. General procedure for the preparation of target compounds

The target compounds were synthesized according to a clean solvent-free protocol recently reported by the lab (Scheme 2) [25]. Pterolactam A (17.4 mmol, 1 eq) and CsF (0.086 mmol, 5 mol%)were added to the nucleophile (17.4 mmol, 1 eq), without any solvent, and the mixture was stirred under moderate vacuum (30 mmHg) at 80 °C, until the ^{1}H NMR conversion showed no more progression, or after caking of the medium. After cooling at room temperature, diethyl ether (20 mL) was added to the crude mixture. The precipitate was filtered off and the solid obtained was recrystallized from EtOH or purified by flash chromatography (silica gel, gradient of ethyl acetate in *n*-heptane), to afford the target compounds. In these conditions, *N,O* and *N,S*-acetals **1a-j**, and *N,N′*-aminals **2a-g, 3a-t** and **4a-f** were obtained in moderate to excellent yields (Table 1).

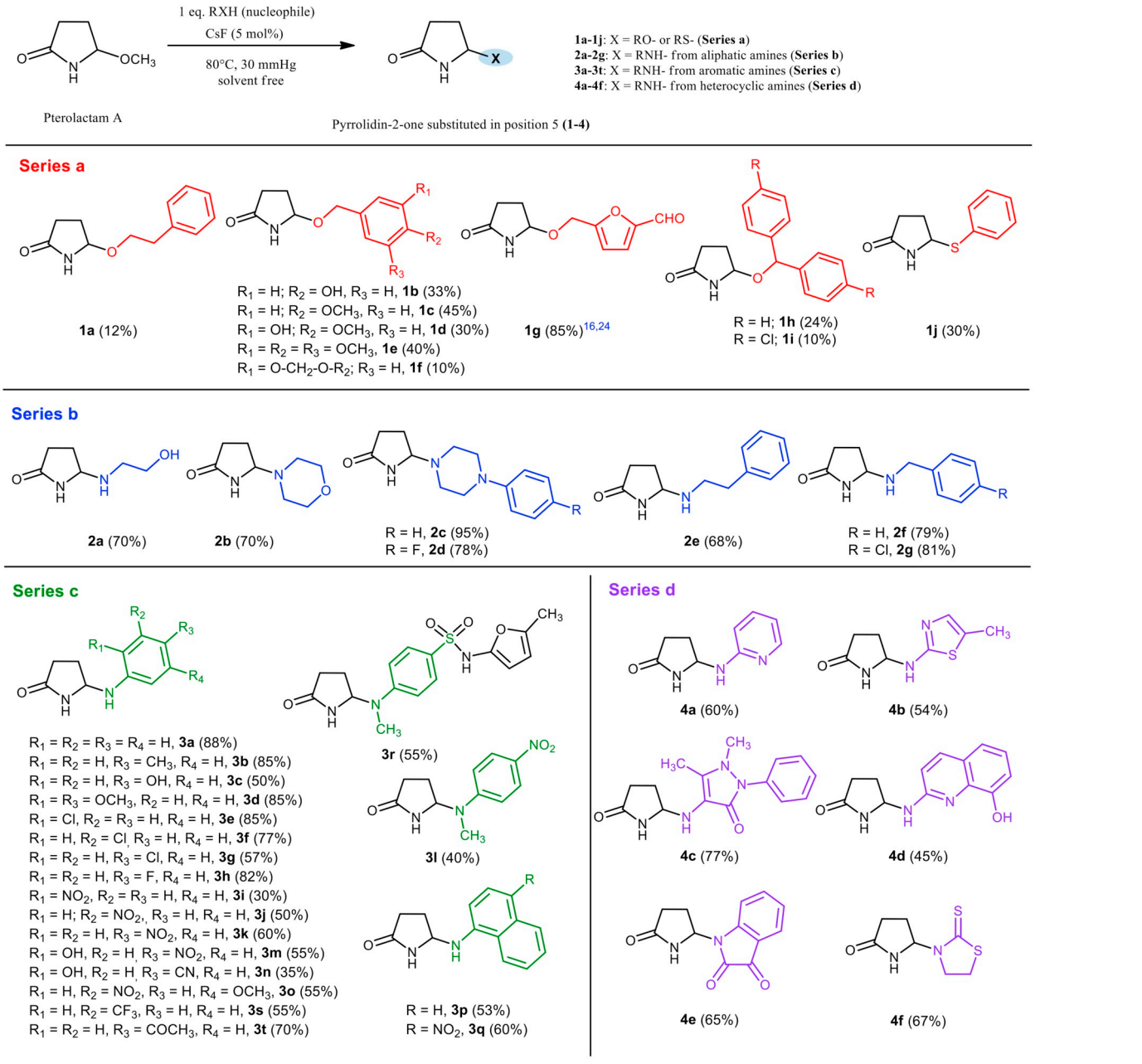
2.2.1. 5-Phenethoxypyrrolidin-2-one (1a)

Following the general procedure, the crude product was precipitated in diethyl ether (20 mL), filtered off and washed with ethanol (2 × 10 mL), affording compound **1a** as a white solid (0.25 g, 12% yield); m.p. 144–145 °C; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.66 (s, 1H, NH), 7.30–7.19 (m, 5H, CHAr), 4.89 (d, J = 6.5 Hz, 1H), 3.65 (q, J = 7.0 Hz, 1H, CH_2-CH_2-O), 3.49 (q, J = 7.0 Hz, 1H, CH_2-CH_2-O), 2.79 (t, J = 7.9 Hz, 2H, CH_2-CH_2-O), 2.28–2.12 (m, 2H, CH_2-CH_2-CH), 2.06–1.96 (m, 1H, CH_2-CH_2-CH), 1.87–1.78 (m, 1H, CH_2-CH_2-CH); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 177.9 (C=O), 139.2 (C), 129.3 (2 CHAr), 128.6 (2 CHAr), 126.5 (CHAr), 85.6 (CH), 67.7 (CH_2), 35.9 (CH_2), 28.5 (CH_2), 28.0 (CH_2). IR ν (cm^{-1}): 3178, 3106, 2913, 1683, 1278, 1067, 1027. Anal. Calcd for $C_{12}H_{15}NO_2$: C, 70.22; H, 7.37; N, 6.82; found: C, 69.84; H, 7.51; N, 7.03%.



1a-1j: X = RO- or RS- (**Series a**)
2a-2g: X = RNH- from aliphatic amines (**Series b**)
3a-3t: X = RNH- from aromatic amines (**Series c**)
4a-4f: X = RNH- from heterocyclic amines (**Series d**)

Scheme 1. Fungicidal Mannich bases of amides (**1–4**) from Pterolactam A.



Scheme 2. Reagents and conditions: 1 eq of H_2N-NH-R, CsF (5 mol%), solvent-less, 6–20 h, 80 °C [25]

2.2.2. 5-((4-Hydroxybenzyl)oxy)pyrrolidin-2-one (1b)

Following the general procedure, the crude product was purified through flash liquid chromatography being eluted with a gradient of *n*-heptane/ethyl acetate, the wanted compound being eluted in 70% ethyl acetate, as a white solid (1.2 g, 33% yield); m.p. 142–143 °C; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.38 (s, 1H, OH), 8.76 (s, 1H, NH), 7.12 (d, *J* = 8.5 Hz, 2H, CHAr), 6.73 (d, *J* = 8.5 Hz, 2H, CHAr), 4.93 (d, *J* = 6.2 Hz, 1H, CH), 4.39 (d, *J* = 11.4 Hz, 1H, CH_2-O), 4.25 (d, *J* = 11.4 Hz, 1H, CH_2-O), 2.29–2.13 (m, 2H, CH_2-CH_2-CH), 2.06–1.98 (m, 1H, CH_2-CH_2-CH), 1.87–1.82 (m, 1H, CH_2-CH_2-CH); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 178.0 (C=O), 157.24 (C), 130.0 (2 CHAr), 128.6 (C), 115.4 (2 CHAr), 84.8 (CH), 68.3 (CH_2), 28.5 (CH_2), 28.0 (CH_2). IR ν (cm^{-1}): 3203, 2935, 1668, 1516, 1454, 1227, 1056, 1037. Anal. Calcd for $C_{11}H_{13}NO_3$: C, 63.76; H, 6.32; N, 6.76; found: C, 63.36; H, 6.40; N, 6.91%.

2.2.3. 5-((4-Methoxybenzyl)oxy)pyrrolidin-2-one (1c)

Following the general procedure, the crude product was purified through flash liquid chromatography being eluted with a gradient of *n*-heptane/ethyl acetate, the wanted compound being eluted in 70% ethyl acetate, as a of a white solid (1.2 g, 46% yield); m.p. 71–72 °C; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.78 (s, 1H, NH), 7.25 (d, *J* = 6.9 Hz, 2H, CHAr), 6.90 (d, *J* = 6.9 Hz, 2H, CHAr), 4.94 (d, *J* = 6.5 Hz, 1H), 4.45 (d, *J* = 11.6 Hz, 1H, CH_2), 4.30 (d, *J* = 11.6 Hz, 1H, CH_2), 3.73 (s, 3H, OCH_3), 2.32–2.14 (m, 2H, CH_2-CH_2-CH), 2.03–2.01 (m, 1H, CH_2-CH_2-CH), 1.88–1.84 (m, 1H, CH_2-CH_2-CH); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 178.0 (C=O), 159.1 (C), 130.4 (C), 129.8 (2 CHAr), 114.1 (2 CHAr), 84.9 (CH), 68.0 (CH_2), 55.5 (CH_3), 28.5 (CH_2), 28.0 (CH_2). IR ν (cm^{-1}): 3413, 3194, 2914, 1668, 1256, 1172, 1027. Anal. Calcd for $C_{12}H_{15}NO_3$: C, 65.14; H, 6.83; N, 6.33; found: C, 64.95; H, 6.91; N, 6.07%.

Table 1
Antifungal activity of target compounds at 100 μg/mL concentration.[a,b]

			Average inhibition rate (n = 3)											
Entry		Compound	SS	BC	AO	PV	PO	CC	AA	FS	GC	CK	CP	CT
1		**1a**	23	0	9	7	0	11	0	10	0	0	32	0
2		**1b**	0	0	0	26	0	0	0	0	0	0	12	0
3		**1c**	0	0	0	12	29	18	0	0	0	50	0	5
4		**1d**	0	0	9	14	30	26	18	0	0	42	12	0
5	**Series a**	**1e**	0	28	10	33	41	33	22	10	48	0	13	0
6		**1f**	0	0	12	30	**80**	0	2	0	0	2	0	0
7		**1g**	0	11	0	31	37	36	8	0	27	0	33	0
8		**1h**	0	0	0	43	34	0	8	30	64	0	23	8
9		**1i**	10	6	64	**78**	0	60	8	73	**79**	**92**	8	38
10		**1j**	0	0	25	57	27	0	10	**91**	7	17	**81**	46
1112		**2a**	30	0	15	12	19	5	0	65	53	0	0	5
13		**2b**	0	14	19	47	17	0	0	36	0	9	23	34
14		**2c**	6	36	12	19	30	21	0	18	31	0	0	5
15	**Series b**	**2d**	0	0	9	15	0	31	15	**100**	3	0	20	22
16		**2e**	15	0	24	12	26	0	0	**95**	0	32	0	5
17		**2f**	0	0	8	0	0	0	0	33	20	12	35	0
18		**2g**	28	0	25	36	0	19	5	**100**	0	29	0	26
19		**3a**	28	0	37	2	31	26	0	10	0	29	0	17
20		**3b**	2	0	6	51	1	15	0	36	0	25	0	35
21		**3c**	29	0	0	30	11	0	0	**100**	10	5	51	31
22		**3d**	23	0	0	0	22	34	0	0	0	0	30	28
23		**3e**	11	0	0	13	32	24	0	29	21	0	25	33
24		**3f**	10	0	0	17	21	0	43	2	18	28	40	32
25		**3g**	0	10	10	23	0	15	0	45	20	19	8	31
26		**3h**	32	0	0	15	39	8	0	70	0	35	32	22
27		**3i**	0	0	22	53	7	0	0	49	0	25	5	31
28	**Series c**	**3j**	6	0	12	3	0	28	25	43	0	0	12	21
29		**3k**	0	26	0	17	16	62	40	**100**	1	42	10	25
30		**3l**	11	0	34	0	0	2	22	**84**	36	30	18	51
31		**3m**	11	0	44	46	33	36	37	0	8	39	43	40
32		**3n**	8	0	10	0	0	40	0	52	0	32	33	34
33		**3o**	0	18	39	**96**	**98**	15	**75**	57	24	38	**97**	**75**
34		**3p**	6	43	**84**	54	**83**	29	0	**98**	43	**75**	21	48
35		**3q**	66	0	0	16	0	0	34	0	0	0	2	0
36		**3r**	12	26	0	52	**76**	26	**86**	64	41	70	58	38
37		**3s**	0	4	14	15	0	5	0	28	0	8	0	23
38		**3t**	0	0	16	31	2	0	20	35	0	2	28	32
39		**4a**	71	0	0	32	17	0	39	2	0	0	48	33
40		**4b**	12	0	14	9	19	0	0	3	31	0	0	0
41	**Series d**	**4c**	0	0	9	0	0	18	10	**98**	0	27	20	15
42		**4d**	72	0	0	40	47	0	3	42	62	43	17	0
43		**4e**	9	0	22	36	0	0	0	62	0	18	47	32
44		**4f**	17	0	10	26	32	16	0	48	31	28	0	45

SS - *Sclerotinia sclerotiorum;* BC - *Botrytis cinerea*; AO - *Aspergillus oryzae;* PV - *Paecilomyces variotii;* PO - *Penicillium ochrochloron;* CC - *Cladosporium cladosporioides;* AA - *Alternaria alternata;* FS - *Fusarium solani;* GC - *Geotrichum candidum;* CK - *Candida krusei;* CP - *Candida pseudotropicalis;* CT - *Candida tropicalis.*

[a] All the data are the average value of three replications.

[b] The colored bold values represent data above or equal to 75% inhibition of the corresponding strain.

*2.2.4. 5-((3-Hydroxy-4-methoxybenzyl)oxy)pyrrolidin-2-one (**1d**)*

Following the general procedure, the crude product was purified through flash liquid chromatography being eluted with a gradient of *n*-heptane/ethyl acetate, the wanted compound being eluted in 70% ethyl acetate, as a white solid (1.2 g, 30% yield); m.p. 121–122 °C; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.93 (s, 1H, OH), 8.73 (bs, 1H, NH), 6.85 (d, J = 8.4 Hz, 1H, CHAr), 6.75 (d, J = 1.8 Hz, 1H, CHAr), 6.71 (dd, J = 8.4 Hz, J = 1.8 Hz, 1H, CHAr), 4.94 (d, J = 6.2 Hz, 1H, CH), 4.39 (d, J = 11.7 Hz, 1H, CH_2), 4.24 (d, J = 11.7 Hz, 1H, CH_2), 3.74 (s, 3H, CH_3), 2.33–2.13 (m, 2H, CH_2-CH_2-CH), 2.08–1.98 (m, 1H, CH_2-CH_2-

CH), 1.92–1.83 (m, 1H, CH_2-CH_2-CH); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 178.0 (C=O), 147.5 (C), 146.8 (C), 131.0 (C), 119.1 (CHAr), 115.7 (CHAr), 112.3 (CHAr), 84.9 (CH), 68.3 (CH_2), 56.1 (CH_3), 28.5 (CH_2), 28.0 (CH_2). IR ν (cm^{-1}): 3212, 3194, 1674, 1679, 1254, 1172, 1027. Anal. Calcd for $C_{12}H_{15}NO_4$: C, 60.75; H, 6.37; N, 5.90; found: C, 60.36; H, 6.40; N, 6.12%.

2.2.5. 5-((3,4,5-Trimethoxybenzyl)oxy)pyrrolidin-2-one (**1e**)

Following the general procedure, the crude product was purified through flash liquid chromatography being eluted with a gradient of *n*-heptane/ethyl acetate, the wanted compound being eluted in 70% ethyl acetate, being obtained as a white solid (1.8 g, 40% yield); m.p. 91–92 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.76 (s, 1H, NH), 6.63 (s, 2H, CHAr), 4.96 (d, J = 5.2 Hz, 1H, CH), 4.46 (d, J = 9.6 Hz, 1H, CH_2), 4.32 (d, J = 9.6 Hz, 1H, CH_2), 3.77 (s, 6H, OCH_3), 3.64 (s, 3H, OCH_3), 2.32–2.28 (m, 2H, CH_2CH_2CH), 2.10–2.03 (m, 1H, CH_2CH_2CH), 1.98–1.92 (m, 1H, CH_2CH_2CH); ^{13}C{^{1}H}NMR (DMSO-d_6, 100 MHz): δ 178.0 (C=O), 153.2 (2 C), 137.2 (C), 134.1 (C), 105.4 (2 CHAr), 85.0 (CH), 68.5 (CH_2), 60.4 (OCH_3), 56.2 (2 OCH_3), 28.5 (CH_2), 28.0 (CH_2). IR ν (cm^{-1}): 3184, 3092, 1682, 1590, 1462, 1230, 1132. Anal. Calcd for $C_{14}H_{19}NO_5$: C, 59.78; H, 6.81; N, 4.98; found: C, 60.01; H, 6.99; N, 5.03%.

2.2.6. 5-(Benzo[d][1,3]dioxol − 5-ylmethoxy)pyrrolidin-2-one (**1f**)

Following the general procedure, the crude product was purified through flash liquid chromatography being eluted with a gradient of DCM/methanol, the wanted compound being eluted in 20% methanol, affording the compound as a white solid (0.4 g, 10% yield); m.p. 134–135 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.0 (s, 1H, NH), 6.83 (s, 1H, CHAr), 6.78 (s, 2H, CHAr), 5.95–5.91 (m, 2H, CH_2), 5.05 (d, J = 6.0 Hz, 1H, CH), 4.50 (d, J = 11.2 Hz, 1H, CH_2), 4.38 (d, J = 11.2 Hz, 1H, CH_2), 2.57–2.32 (m, 1H, CH_2CH_2CH), 2.30–2.22 (m, 2H, CH_2CH_2CH), 2.21–2.08 (m, 1H, CH_2CH_2CH); ^{13}C{^{1}H}NMR (100 MHz, $CDCl_3$): δ 179.6 (C=O), 147.8 (C), 147.3 (C_{IV}), 131.1 (C), 121.5 (CHAr), 108.6 (CHAr), 108.2 (CHAr), 101.0 (CH_2), 85.2 (CH), 69.4 (CH_2), 28.4 (CH_2), 28.3 (CH_2). IR ν (cm^{-1}): 3205, 3156, 1684, 1252, 1101, 1078. Anal. Calcd for $C_{12}H_{13}NO_4$: C, 61.27; H, 5.57; N, 5.95; found: C, 61.42, H, 5.59; N, 5.97%.

2.2.7. 5-(Benzhydryloxy)pyrrolidin-2-one (**1 h**)

Following the general procedure, the crude product was purified through flash liquid chromatography being eluted with a gradient of *n*-heptane/ethyl acetate, the wanted compound being eluted in 90% ethyl acetate, as a white solid (1.1 g, 24% yield); m.p. 98–99 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.79 (s, 1H, N*H*) 7.29–7.36 (m, 10H, Ar*H*), 5.63 (s, 1H, CH), 4.85 (d, J = 5.8 Hz, 1H, CH), 2.35–2.25 (m, 1H, CH_2CH$_2$CH), 2.17–2.13 (m, 1H, CH$_2$CH_2CH), 2.10–1.85 (m, 2H, CH$_2$CH_2CH); ^{13}C{^{1}H}NMR (DMSO-d_6, 100 MHz): δ 178.0 (C=O), 143.0 (C), 142.3 (C), 128.9 (2 CHAr), 128.7 (2 CHAr), 127.9 (CHAr), 127.6 (CHAr), 127.5 (2 CHAr), 126.8 (2 CHAr), 85.7 (CH), 78.7 (CH), 28.5 (CH_2), 28.2 (CH_2). IR ν (cm^{-1}): 3183, 3104, 2935, 1697, 1283, 1059. Anal. Calcd for $C_{17}H_{17}NO_2$: C, 76.38; H, 6.41; N, 5.24; found: C, 76.12; H, 6.26; N, 5.08%.

2.2.8. 5-(Bis(4-chlorophenyl)methoxy)pyrrolidin-2-one (**1i**)

Following the general procedure, the crude product was purified through flash liquid chromatography being eluted with a gradient of *n*-heptane/ethyl acetate, the wanted compound being eluted in 80% ethyl acetate, as a white solid (0.6 g, 10% yield); m.p. 172–173 °C; ^{1}H-NMR (DMSO-d_6, 400 MHz): δ ppm 8.79 (s, 1H, NH), 7.44–7.30 (m, 8H, CHAr), 5.65 (s, 1H, NH), 4.85 (d, 1H, J = 6.4 Hz, CH), 2.41–2.27 (m, 1H, CH_2CH_2CH), 2.21–2.18 (m, 1H, CH_2CH_2CH), 2.05–1.95 (m, 2H, CH_2CH_2CH); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 178.0 (C), 141.6 (C), 140.9 (C), 132.7 (C), 132.3 (C), 129.4 (2 CHAr), 129.0 (2 CHAr), 128.8 (2 CHAr), 128.7 (2 CHAr), 83.9 (C), 77.2 (CH), 28.4 (CH_2), 28.2 (CH_2). IR ν (cm^{-1}): 3196, 3160, 2915, 1695, 1488, 1278, 1045. Anal. Calcd for $C_{17}H_{15}Cl_2NO_2$: C, 60.73; H, 4.50; N, 4.17; found: C, 60.53; H, 4.59; N, 4.23%.

2.2.9. 5-(Phenylthio)pyrrolidin-2-one (**1j**) [27]

Following the general procedure, the crude product was precipitated in diethyl ether (20 mL), filtered off and washed with ethanol (2 × 10 mL), affording the compound as a white solid (1.0 g, 30% yield); m.p.75–76 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.48 (s, 1H, NH), 7.47 (dd, J = 7.9 Hz, J = 2.1 Hz, 2H, CHAr), 7.38–7.33 (m, 1H, CHAr), 7.35 (dd, J = 7.9 Hz, J = 2.1 Hz, 2H, CHAr), 5.16 (d, J = 7.4 Hz, 1H, CH), 2.46–2.43 (m, 1H, CH_2CH_2CH), 2.03–1.80 (m, 3H, CH_2CH_2CH); ^{13}C{^{1}H}NMR (DMSO-d_6, 100 MHz): δ 176.8 (C=O), 133.3 (2 CHAr), 132.9 (C), 129.6 (2 CHAr), 128.2 (CHAr), 62.2 (CH), 29.2 (CH_2), 28.9 (CH_2). IR ν (cm^{-1}): 3389, 3158, 1657, 1453, 1258.

2.2.10. 5-((2-Hydroxyethyl)amino)pyrrolidin-2-one (**2a**)

Following the general procedure, the crude product was precipitated in diethyl ether (20 mL), filtered off and washed with ethanol (2 × 10 mL), affording compound **2a** as a white solid (1.75 g, 70% yield); m.p. 136–137 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ ppm 8.09 (bs, 1H, NH), 4.51 (bs, 1H, CH), 4.34 (bs, 1H, NH), 3.41 (bs, 2H, CH_2), 2.67 (bs, 1H, OH), 2.15–2.01 (m, 5H, CH_2CH_2NH, CH_2CH_2CH), 1.65–1.60 (m, 1H, CH_2CH_2CH); ^{13}C{^{1}H}NMR (DMSO-d_6, 100 MHz): δ ppm 176.8 (C=O), 69.7 (CH), 61.3 (CH_2), 48.0 (CH_2), 47.5 (CH_2), 29.7 (CH_2), 28.3 (CH_2). IR ν (cm^{-1}): 3223, 3079, 2850, 1668, 1455, 1261, 1060.

2.2.11. 5-Morpholinopyrrolidin-2-one (**2b**) [28]

Following the general procedure, the crude product was precipitated in diethyl ether (20 mL), filtered off and washed with ethanol (2 × 10 mL), affording the compound as a white-off solid (2.0 g, 70% yield); m.p. 141–142 °C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.29 (bs, 1H, NH), 4.48 (d, J = 7.4 Hz, 1H, CH), 3.73 (d, J = 5.2 Hz, 4H, CH_2), 2.38 (d, J = 5.2 Hz, 4H, CH_2), 2.30–2.26 (m, 3H, CH_2CH_2CH), 2.08–2.03 (m, 1H, CH_2CH_2CH); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 178.4 (C=O), 75.1 (CH), 66.8 (2 CH_2), 47.3 (2 CH_2), 29.4 (CH_2), 23.1 (CH_2). IR ν (cm^{-1}): 3153, 2966, 2854, 1673, 1263, 1107, 1068.

2.2.12. 5-(4-Phenylpiperazin-1-yl)pyrrolidin-2-one (**2c**)[27]

Following the general procedure, the crude product was precipitated in diethyl ether (20 mL), filtered off and washed with ethanol (2 × 10 mL), affording compound **2c** as a white solid (4.0 g, 95% yield); m.p. 174–175 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.22 (d, J = 8.0 Hz, 2H, CHAr), 6.92 (d, J = 7.8 Hz, 2H, CHAr), 6.76 (t, J = 7.6 Hz, 1H, CHAr), 4.41 (dd, J = 7.9 Hz, J = 2.25 Hz, 1H, CH), 3.11 (t, J = 5.0 Hz, 4H, NCH_2CH_2N), 2.70–2.60 (m, 4H, NCH_2CH_2N), 2.22–2.08 (m, 3H, CH_2CH_2CH), 1.86–1.82 (m, 1H, CH_2CH_2CH); ^{13}C{^{1}H}NMR (DMSO-d_6, 100 MHz): δ 177.2 (C=O), 151.4 (C), 129.3 (2 CHAr), 119.2 (CHAr), 115.8 (2 CHAr), 74.4 (CH), 48.6 (2 CH_2), 46.8 (2 CH_2), 26.6 (CH_2), 23.7 (CH_2). IR ν (cm^{-1}): 3214, 2825, 1686, 1651, 1241.

2.2.13. 5-(4-(4-Fluorophenyl)piperazin-1-yl)pyrrolidin-2-one (**2d**)

Following the general procedure, the crude product was precipitated in diethyl ether (50 mL), filtered off and washed with ethanol (2 × 10 mL), affording the compound as a white solid (3.6 g, 78% yield); m.p. 172–173 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.23 (bs, 1H, NH), 7.04 (t, J = 9.2 Hz, 2H, Ar*H*), 6.95 (dd, J = 8.8 Hz, J = 4.8 Hz, 2H, CHAr), 4.41 (d, J = 5.2 Hz, 1H, CH), 3.06 (bs, 4H, NCH_2CH_2-N), 2.68–2.65 (m, 2H, NCH_2CH_2N), 2.50–2.46 (m, 2H, NCH_2CH_2N), 2.20–2.08 (m, 3H, CH_2CH_2CH), 1.89–1.87 (m, 1H, CH_2CH_2CH); ^{13}C{^{1}H}NMR (DMSO-d_6, 100 MHz): δ 177.3 (C=O), 157.6 (C, $J_{C\text{-}F}$ = 233.9 Hz), 148.3 (C, $J_{C\text{-}F}$ = 1.5 Hz), 117.5 (2 CHAr, $J_{C\text{-}F}$ = 7.6 Hz), 115.6 (2 CHAr, $J_{C\text{-}F}$ = 21.3 Hz), 74.4 (CH), 49.4 (2 CH_2), 46.8 (2 CH_2), 29.7 (CH_2), 23.8 (CH_2). IR ν (cm^{-1}): 3214, 2825, 1686, 1651, 1241. Anal. Calcd for $C_{14}H_{18}FN_3O$: C, 63.86; H, 6.89; N, 15.96; found: C, 64.01; H, 6.59; N, 15.73%.

*2.2.14. 5-(Phenethylamino)pyrrolidin-2-one (**2e**)*

Following the general procedure, the crude product was precipitated in diethyl ether (20 mL), filtered off and washed with ethanol (2 × 10 mL), affording compound **2e** as a white solid (2.4 g, 68% yield); m.p. 71–72 °C; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 8.15 (bs, 1H, NH), 7.26 (d, *J* = 7.9 Hz, 2H, CHAr), 7.19 (d, *J* = 7.9 Hz, 2H, CHAr), 7.16 (d, *J* = 7.9 Hz, 1H, CHAr), 4.34 (s, 1H, CH), 2.86–2.84 (m, 1H, NH), 2.68–2.64 (m, 3H, CH_2-CH_2), 2.19–2.14 (m, 3H, CH_2-CH_2), 2.03–1.90 (m, 1H, CH_2-CH_2), 1.66–1.58 (m, 1H, CH_2-$\underline{CH_2}$-CH); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 176.74 (C=O), 140.84 (C), 129.04 (2 CHAr), 128.63 (2 CHAr), 126.27 (CHAr), 69.50 (CH), 47.45 (CH_2), 36.58 (CH_2), 29.67 (CH_2), 28.3 (CH_2). IR ν (cm^{-1}): 3301, 3286, 3173, 2943, 1681, 1497. Anal. Calcd for $C_{12}H_{16}N_2O$: C, 70.56; H, 7.90; N, 13.71; found: C, 70.53; H, 7.59; N, 13.73%.

*2.2.15. 5-(Benzylamino)pyrrolidin-2-one (**2f**) [28]*

Following the general procedure, the crude product was precipitated in diethyl ether (20 mL), filtered off and washed with ethanol (2 × 10 mL), affording compound **2f** as a white solid (2.6 g, 79% yield); m.p. 106–107 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.34–7.27 (m, 5H, CHAr), 4.62 (d, *J* = 6.3 Hz, 1H, CH), 3.87 (d, *J* = 12.7 Hz, 1H, CH_2-NH), 3.82 (d, *J* = 12.7 Hz, 1H, CH_2-NH), 2.38–2.46 (m, 2H, CH_2CH_2CH), 1.85–1.80 (m, 1H, CH_2CH_2CH); ^{13}C{^{1}H}NMR (DMSO-d_6, 100 MHz): δ 177.8 (C=O), 139.5 (C), 128.6 (2 CHAr), 128.1 (2 CHAr), 121.3 (CHAr), 69.5 (CH), 50.0 (CH_2), 29.5 (CH_2), 28.7 (CH_2). IR ν (cm^{-1}): 3264, 3029, 1677, 1487, 1262.

*2.2.16. 5-((4-Chlorobenzyl)amino)pyrrolidin-2-one (**2 g**)*

Following the general procedure, the crude product was precipitated in diethyl ether (20 mL), filtered off and washed with ethanol (2 × 10 mL), affording compound **2 g** as a white solid (3.2 g, 81% yield); m.p. 145–146 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.23 (bs, 1H, NH), 7.33 (s, 4H, CHAr), 4.29 (bs, 1H, CH), 3.75 (d, *J* = 14.2 Hz, 1H, CH_2), 3.59 (d, *J* = 14.2 Hz, 1H, CH_2), 2.75 (bs, 1H, NH), 2.25–2.10 (m, 2H, CH_2CH_2CH), 2.03–1.96 (m, 1H, CH_2CH_2CH), 1.69–1.62 (m, 1H, CH_2CH_2CH); ^{13}C{^{1}H}NMR (DMSO-d_6, 100 MHz): δ 176.8 (C=O), 140.2 (C), 131.4 (C), 130.2 (2 CHAr), 128.4 (2 CHAr), 68.9 (CH), 48.4 (CH_2), 29.7 (CH_2), 28.2 (CH_2). Anal. Calcd for $C_{10}H_{12}N_2O$: C, 58.80; H, 5.83; N, 12.47; found: C, 58.76; H, 5.76; N, 12.63%.

*2.2.17. 5-(Phenylamino)pyrrolidin-2-one (**3a**) [30]*

Following the general procedure, the crude product was precipitated in diethyl ether (20 mL), filtered off and washed with ethanol (2 × 10 mL), affording compound **3a** as a white solid (2.7 g, 88% yield); m.p. 135–136 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.25 (bs, 1H, NH), 7.09 (t, *J* = 6 Hz, 2H, CHAr), 6.62 (d, *J* = 9.6 Hz, 2H, CHAr), 6.57 (d, *J* = 6.6 Hz, 1H, NH), 6.11 (d, *J* = 9.6 Hz, 1H, CHAr), 5.10 (bs, 1H, CH), 2.46–2.20 (m, 2H, CH_2CH_2CH), 2.16–2.05 (m, 1H, CH_2CH_2CH), 1.95–1.73 (m, 1H, CH_2CH_2CH); ^{13}C{^{1}H}NMR (DMSO-d_6, 100 MHz): δ 176.8 (C=O), 147.2 (C), 129.4 (2 CHAr), 117.1 (CHAr), 114.4 (2 CHAr), 64.2 (CH), 29.5 (CH_2), 28.5 (CH_2). IR ν (cm^{-1}): 3338, 3165, 1669, 1598, 1253.

*2.2.18. 5-(p-Tolylamino)pyrrolidin-2-one (**3b**) [30]*

Following the general procedure, the crude product was precipitated in diethyl ether (30 mL), filtered off and washed with ethanol (2 × 10 mL), affording compound **3b** as a white solid (2.8 g, 85% yield); m.p. 139–140 °C; ^{1}H-NMR (DMSO-d_6, 400 MHz): δ ppm 6.90 (d, 2H, *J* = 8.2 Hz, CHAr) 6.54 (d, 2H, *J* = 8.2 Hz, CHAr), 5.93 (d, 1H, *J* = 8.2 Hz, NH), 5.05 (td, 1H, *J* = 8.2 Hz, *J* = 4.1 Hz, CH), 2.46–2.29 (m, 2H, CH_2CH_2CH), 2.15 (s, 3H, CH_3), 2.09–2.13 (m, 1H, CH_2CH_2CH), 1.85–1.78 (m, 1H, CH_2CH_2CH); ^{13}C-NMR (100 MHz, DMSO-d_6): δ ppm 176.8 (C), 144.9 (C), 129.8 (2 CHAr), 125.5 (C), 113.6 (2 CHAr), 64.5 (CH), 29.5 (CH_2), 28.4 (CH_2), 20.5 (CH_3). IR ν (cm^{-1}): 3283, 3161, 1680, 1583, 1485, 1246, 1176.

*2.2.19. 5-((4-Hydroxyphenyl)amino)pyrrolidin-2-one (**3c**)*

Following the general procedure, the crude product was precipitated in diethyl ether (20 mL), filtered off and washed with ethanol (2 × 10 mL), affording compound **3c** as a white solid (1.8 g, 50% yield); m.p. 190–191 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.49 (s, 1H, NH), 8.16 (s, 1H, NH), 6.48 (d, *J* = 9.1 Hz, *J* = 2.46 Hz, 2H, CHAr), 6.55 (d, *J* = 9.1 Hz, *J* = 2.46 Hz, 2H, CHAr), 5.47 (d, *J* = 9.5 Hz, 1H, CH), 4.99–4.97 (m, 1H, OH), 2.31–2.26 (m, 2H, CH_2CH_2CH), 2.12–2.04 (m, 1H, CH_2CH_2CH), 1.82–1.74 (m, 1H, CH_2CH_2CH); ^{13}C{^{1}H}NMR (DMSO-d_6, 100 MHz): δ 176.74 (C=O), 149.50 (C), 139.76 (C), 116.11 (2 CHAr), 115.12 (2 CHAr), 65.45 (CH), 29.56 (CH_2), 28.47 (CH_2). IR ν (cm^{-1}): 3361, 3141, 1658, 1511, 1244, 1231. Anal. Calcd for $C_{10}H_{12}N_2O_2$: C, 62.49; H, 6.29; N, 14.57; found: C, 62.49; H, 6.14; N, 14.20%.

*2.2.20. 5-((2,4-Dimethoxyphenyl)amino)pyrrolidin-2-one (**3d**)*

Following the general procedure, the crude product was precipitated in diethyl ether (20 mL), filtered off and washed with ethanol (2 × 10 mL), affording compound **3d** as a white solid (3.5 g, 85% yield); m.p. 135–136 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 6.59 (d, *J* = 8.2 Hz, 1H, CHAr), 6.47 (d, *J* = 2.4 Hz, 1H, CHAr), 6.44 (dd, *J* = 8.2 Hz, *J* = 2.4 Hz, 1H, CHAr), 6.32 (bs, 1H, NH), 5.22 (dd, *J* = 6.7 Hz, *J* = 4.6 Hz, 1H, CH), 4.16 (bs, 1H, NH), 3.82 (s, 3H, CH_3), 3.76 (s, 3H, CH_3), 2.65–2.35 (m, 3H, CH_2CH_2CH), 2.02–1.93 (m, 1H, CH_2CH_2CH); ^{13}C{^{1}H}NMR (DMSO-d_6, 100 MHz): δ 176.8 (C=O), 153.6 (C), 148.9 (C), 128.9 (C), 112.7 (CHAr), 103.9 (CHAr), 99.5 (CHAr), 65.7 (CH), 55.7 (CH_3), 55.5 (CH_3), 29.2 (CH_2), 28.8 (CH_2).

*2.2.21. 5-((2-Chlorophenyl)amino)pyrrolidin-2-one (**3e**)*

Following the general procedure, the crude product was precipitated in diethyl ether (20 mL), filtered off and washed with ethanol (2 × 10 mL), affording the wanted compound as a white solid (3.1 g, 85% yield); m.p. 125–126 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.32 (s, 1H, NH), 7.28 (dd, *J* = 8.0 Hz, *J* = 1.6 Hz, 1H, CHAr), 7.14 (td, *J* = 8.8 Hz, *J* = 2.0 Hz, 1H, CHAr), 6.84 (d, *J* = 7.2 Hz, 1H, CHAr), 6.66 (td, *J* = 8.0 Hz, *J* = 1.6 Hz, 1H, CHAr), 5.61 (d, *J* = 8.8 Hz, 1H, NH), 5.22 (t, *J* = 4.0 Hz, 1H, CH), 2.40–2.25 (m, 2H, CH_2CH_2CH), 2.13–2.06 (m, 2H, CH_2CH_2CH); ^{13}C{^{1}H}NMR (DMSO-d_6, 100 MHz): δ 176.9 (C=O), 142.7 (C), 129.6 (C), 128.4 (CHAr), 118.9 (C), 118.3 (CH), 113.3 (CHAr), 64.2 (CH), 29.5 (CH_2), 28.1 (CH_2); IR ν (cm^{-1}): 3398, 3169, 1692, 1597, 1497, 1265, 1181; Anal. Calcd for $C_{10}H_{11}ClN_2O$: C, 57.01; H, 5.26; N, 13.30%.

*2.2.22. 5-((3-Chlorophenyl)amino)pyrrolidin-2-one (**3f**) [30]*

Following the general procedure, the crude product was precipitated in diethyl ether (20 mL), filtered off and washed with ethanol (2 × 10 mL), affording the wanted compound as a white solid (2.8 g, 77% yield); m.p. 139–140 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.10 (t, *J* = 7.9 Hz, 1H, CHAr), 6.66 (t, *J* = 2.2 Hz, 1H, CHAr), 6.57 (td, *J* = 7.9 Hz, *J* = 2.2 Hz, 2H, CHAr), 5.10 (t, *J* = 5.2 Hz, 1H, CH), 2.37–2.25 (m, 2H, CH_2CH_2CH), 2.12–2.06 (m, 1H, CH_2CH_2CH), 1.82–1.79 (m, 1H, CH_2CH_2CH); ^{13}C{^{1}H}NMR (DMSO-d_6, 100 MHz): δ 176.8 (C=O), 148.8 (C), 134.1 (C), 130.8 (CHAr), 116.4 (CHAr), 112.5 (CHAr), 112.0 (CHAr), 64.0 (CH), 29.5 (CH_2), 28.4 (CH_2);

IR ν (cm^{-1}): 3328, 3168, 1682, 1595, 1482, 1242, 1089.

*2.2.23. 5-((4-Chlorophenyl)amino)pyrrolidin-2-one (**3 g**)*

Following the general procedure, the crude product was precipitated in diethyl ether (20 mL), filtered off and washed with ethanol (2 × 10 mL), affording the wanted compound as a grey solid (2.1 g, 57% yield); m.p. 169–170 °C; ^{1}H-NMR (DMSO-d_6, 400 MHz): δ ppm 7.12 (d, *J* = 9.2 Hz, 2H, CHAr) 6.63 (d, *J* = 9.2 Hz, 2H, CHAr), 6.39 (bs, 1H, NH), 5.08 (s, 1H, CH), 2.25–2.31 (m, 2H, CH_2CH_2CH), 2.07–2.12 (m, 1H, CH_2CH_2CH), 1.84–1.79 (m, 1H, CH_2CH_2CH); ^{13}C-NMR (100 MHz, DMSO-d_6) δ ppm 176.8 (C) 146.2 (C), 129.0 (2 CHAr), 120.3 (C), 114.8 (2 CHAr), 64.2 (CH), 29.5 (CH_2), 28.4 (CH_2). IR ν

(cm^{-1}): 3281, 3159, 3088, 1681, 1590, 1487, 1247, 1176. Anal. Calcd for $C_{10}H_{11}ClN_2O$: C, 57.01; H, 5.26; N, 13.30; found: C, 61.55; H, 5.61; N, 14.22%.

*2.2.24. 5-((4-Fluorophenyl)amino)pyrrolidin-2-one (**3 h**)*

Following the general procedure, the crude product was precipitated in diethyl ether (20 mL), filtered off and washed with ethanol (2 × 10 mL), affording compound **3 h** as a white solid (2.8 g, 82% yield); m.p. 146–147 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.15 (bs, 1H, NH), 6.93 (t, J = 8.8 Hz, 2H, CHAr), 6.63 (dd, J = 8.8 Hz, J = 4.4 Hz, 2H, CHAr), 6.11 (d, J = 7.8 Hz, 1H, NH), 5.05 (bs, 1H, CH), 2.36–2.26 (m, 2H, CH_2CH_2CH), 2.14–2.05 (m, 1H, CH_2CH_2CH), 1.85–1.80 (m, 1H, CH_2CH_2CH); ^{13}C{^{1}H}NMR (DMSO-d_6, 100 MHz): δ 176.85 (C=O), 155.2 (C, C-F, J = 232 Hz), 143.85 (C), 115.7 (2 CHAr, J = 21.2 Hz), 114.3 (2 CHAr, J = 7.1 Hz), 64.7 (CH), 29.5 (CH_2), 28.4 (CH_2). IR ν (cm^{-1}): 3284, 3177, 1683, 1505, 1245. Anal. Calcd for $C_{10}H_{11}FN_2O$: C, 61.85; H, 5.71; N, 14.42; found: C, 61.55; H, 5.61; N, 14.22%.

*2.2.25. 5-((2-Nitrophenyl)amino)pyrrolidin-2-one (**3i**)*

Following the general procedure, the crude product was precipitated in diethyl ether (20 mL), filtered off and washed with ethanol (2 × 10 mL), affording the compound as a yellow solid (1.2 g, 30% yield); m.p. 182–183 °C; ^{1}H-NMR (DMSO-d_6, 400 MHz): δ ppm 8.52 (s, 1H, NH), 8.09 (dd, J = 9.2, 1.6 Hz, 1H, CHAr), 7.97 (d, J = 7.5 Hz, 1H, NH), 7.57 (t, J = 9.2 Hz, 1H, CHAr), 7.12 (d, J = 9.2 Hz, 1H, CHAr), 6.80 (d, J = 7.5 Hz, 1H, NH), 5.43 (td, J = 7.5, 3.1 Hz, 1H, CH), 2.42–2.30 (m, 2H, CH_2CH_2CH), 2.20–1.25 (m, 1H, CH_2CH_2CH), 1.90–1.86 (m, 1H, CH_2CH_2CH); ^{13}C-NMR (DMSO-d_6, 100 MHz): δ ppm 177.0 (C=O), 143.4 (C), 137.0 (CHAr), 132.3 (C), 126.7 (CHAr), 117.0 (CHAr), 115.8 (CHAr), 64.0 (CH), 29.2 (CH_2), 28.5 (CH_2). IR ν (cm^{-1}): 3345, 3156, 1686, 1342, 1228, 1178.

*2.2.26. 5-((3-Nitrophenyl)amino)pyrrolidin-2-one (**3j**)*

Following the general procedure, the crude product was precipitated in diethyl ether (20 mL), filtered off and washed with ethanol (2 × 10 mL), affording the compound as a yellow solid (1.9 g, 50% yield); m.p. 170–171 °C; ^{1}H-NMR (DMSO-d_6, 400 MHz): δ ppm 8.39 (s, 1H, NH), 7.43 (d, J = 8.9 Hz, 2H, CHAr), 7.38 (t, J = 8.9 Hz, 1H, CHAr), 7.05 (d, J = 8.9 Hz, 1H, CHAr), 6.88 (d, J = 8.4 Hz, 1H, NH), 5.23 (td, J = 7.7, 3.7 Hz, 1H, CH), 2.49–2.31 (m, 2H, CH_2CH_2CH), 2.17–2.10 (m, 1H, CH_2CH_2CH), 1.88–1.82 (m, 1H, CH_2CH_2CH); ^{13}C-NMR (100 MHz, DMSO-d_6) δ ppm 176.2 (C=O), 148.7 (C), 147.8 (C), 130.0 (CHAr), 119.0 (CHAr), 110.8 (CHAr), 106.9 (CHAr), 63.4 (CH), 28.9 (CH_2), 27.8 (CH_2). IR ν (cm^{-1}): 3349, 3259, 1681, 1594, 1477, 1326, 1254, 1176, 1121.

*2.2.27. 5-((4-Nitrophenyl)amino)pyrrolidin-2-one (**3 k**) [30]*

Following the general procedure, the crude product was precipitated in diethyl ether (20 mL), filtered off and washed with ethanol (2 × 10 mL), affording the compound as a yellow solid (2.3 g, 60% yield); m.p. 200–201 °C; ^{1}H-NMR (DMSO-d_6, 400 MHz): δ ppm 8.03 (bs, 1H, NH), 8.02 (d, J = 8.8 Hz, 2H, CHAr), 6.74 (d, J = 8.8 Hz, 2H, CHAr), 5.28 (q, J = 3.4 Hz, 1H, CH), 2.41–2.23 (m, 2H, CH_2CH_2CH), 2.18–2.13 (m, 1H, CH_2CH_2CH), 1.89–1.85 (m, 1H, CH_2CH_2CH); ^{13}C-NMR (DMSO-d_6, 100 MHz): δ ppm 176.9 (C=O), 153.2 (C), 137.0 (C), 126.5 (3 CHAr), 112.1 (CHAr), 63.6 (CH), 29.2 (CH_2), 28.2 (CH_2). IR ν (cm^{-1}): 3349, 3259, 1681, 1594, 1477, 1326, 1254, 1176, 1121.

*2.2.28. 5-(Methyl(4-nitrophenyl)amino)pyrrolidin-2-one (**3 l**)*

Following the general procedure, the crude product was purified through flash liquid chromatography being eluted with a gradient of *n*-heptane/ethyl acetate, the wanted compound being eluted in 90% ethyl acetate, as 1.6 g of a yellow solid, in 40% yield; m.p. 152–153 °C; ^{1}H-NMR (DMSO-d_6, 400 MHz): δ ppm 8.22 (s, 1H, NH), 8.08 (d, J = 9.5 Hz, 2H, CHAr), 6.98 (d, J = 9.5 Hz, 2H, CHAr), 5.83 (q, J = 3.4 Hz, 1H, CH), 2.82 (s, 3H, CH_3), 2.45–2.37 (m, 2H, CH_2CH_2CH), 2.17–2.23 (m, 1H, CH_2CH_2CH), 1.90–1.84 (m, 1H, CH_2CH_2CH); ^{13}C-NMR (DMSO-d_6, 100 MHz): δ ppm 176.8 (C=O), 154.0 (C), 126.1 (2 CHAr), 112.6 (2 CHAr), 68.3 (CH), 30.5 (CH_2), 29.6 (CH_2), 25.1 (CH_3). IR ν (cm^{-1}): 3151, 3066, 2892, 1693, 1583, 1462, 1311, 1238, 1081.

*2.2.29. 5-((2-Hydroxy-4-nitrophenyl)amino)pyrrolidin-2-one (**3 m**)*

Following the general procedure, the crude product was purified through flash liquid chromatography being eluted with a gradient of *n*-heptane/ethyl acetate, the wanted compound being eluted in 100% ethyl acetate, as 4.0 g of a yellow solid, in 55% yield; m.p. 169–170 °C; ^{1}H-NMR (DMSO-d_6, 400 MHz): δ ppm 10.42 (bs, 1H, OH), 8.34 (s, 1H, NH), 7.68 (d, J = 9.0 Hz, 1H, CHAr), 7.51 (d, J = 2.73 Hz, 1H, CHAr), 6.74 (d, J = 9.0 Hz, 1H, CHAr), 6.51 (d, J = 9.0 Hz, 1H, NH), 5.32 (td, J = 8.5, 3.8 Hz, 1H, CH), 2.35–2.39 (m, 2H, CH_2CH_2CH), 2.04–2.11 (m, 2H, CH_2CH_2CH); ^{13}C-NMR (DMSO-d_6, 100 MHz): δ ppm 177.0 (C=O), 143.7 (C), 143.0 (C), 136.9 (C), 118.2 (CHAr), 109.0 (CHAr), 108.3 (CHAr), 63.6 (CH), 29.4 (CH_2), 27.9 (CH_2). IR ν (cm^{-1}): 3404, 3332, 1658, 1589, 1475, 1255, 1221.

2.2.30. 5-Nitro-2-((5-oxopyrrolidin-2-yl)amino)benzonitrile (3n) [30]

Following the general procedure, the crude product was purified through flash liquid chromatography being eluted with a gradient of *n*-heptane/ethyl acetate, the wanted compound being eluted in 100% ethyl acetate, as a yellow solid (1.5 g, 35% yield); m.p. 199–200 °C; ^{1}H-NMR (DMSO-d_6, 400 MHz): δ ppm 8.47 (d, J = 2.8 Hz, 1H, NH), 8.39 (d, J = 2.8 Hz, 1H, NH), 8.22 (dd, J = 10.0 Hz, J = 2.8 Hz, 1H, CHAr), 7.89 (d, J = 8.4 Hz, 1H, CHAr), 7.04 (d, J = 10 Hz, 1H, CHAr), 5.45 (td, J = 7.6, 2.5 Hz, 1H, CH), 2.43–2.40 (m, 2H, CH_2CH_2CH), 2.12–1.98 (m, 2H, CH_2CH_2CH); ^{13}C-NMR (DMSO-d_6, 100 MHz): δ ppm 177.2 (C=O), 153.1 (C), 137.1 (C), 131.4 (CHAr), 130.0 (CHAr), 116.4 (C), 113.0 (CHAr), 95.3 (C), 64.4 (CH), 29.2 (CH_2), 27.6 (CH_2). IR ν (cm^{-1}): 3332, 3167, 3094, 2230, 1697, 1587, 1503, 1305, 1270, 1171.

*2.2.31. 5-((3-Methoxy-5-nitrophenyl)amino)pyrrolidin-2-one (**3o**)*

Following the general procedure, the crude product was precipitated in diethyl ether (20 mL), filtered off and washed with ethanol (2 × 10 mL), affording the wanted compound as an orange solid (2.4 g, 55% yield); m.p. 194–195 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.53 (s, 1H, NH), 7.87 (d, J = 7.5 Hz, 1H, NH), 7.53 (s, 1H, CHAr), 7.31 (dd, J = 9.5 Hz, J = 2.5 Hz, 1H, CHAr), 7.12 (d, J = 7.5 Hz, 1H, CHAr), 5.42 (bs, 1H, CH), 3.76 (s, 3H, CH_3), 2.43–2.32 (m, 2H, CH_2CH_2CH), 2.22–2.11 (m, 1H, CH_2CH_2CH), 2.02–1.90 (m, 1H, CH_2CH_2CH) ppm; ^{13}C{^{1}H}NMR (DMSO-d_6, 100 MHz): δ 176.97 (C=O), 150.27 (C), 138.99 (C), 131.58 (C), 127.16 (CHAr), 117.46 (CHAr), 107.25 (CHAr), 64.16 (CH), 56.08 (CH_3), 29.22 (CH_2), 28.63 (CH_2). IR ν (cm^{-1}): 3359, 3169, 1695, 1511, 1345, 1232, 1178. Anal. Calcd for $C_{11}H_{13}N_3O_4$: C, 52.59; H, 5.22; N, 16.73; found: C, 52.24; H, 4.89; N, 16.36%.

*2.2.32. 5-((4-Nitronaphthalen-1-yl)amino)pyrrolidin-2-one (**3p**)*

Following the general procedure, the crude product was precipitated in diethyl ether (20 mL), filtered off and washed with ethanol (2 × 10 mL), affording the wanted compound as a white solid (2.5 g, 53% yield); m.p. 204–205 °C; ^{1}H-NMR (DMSO-d_6, 400 MHz): δ ppm 8.83 (dd, J = 9.2 Hz, J = 1.0 Hz, 1H, NH), 8.48 (d, J = 8.0 Hz, 2H, CHAr), 8.40 (d, J = 8.0 Hz, 1H, CHAr), 7.90 (d, J = 8.0 Hz, 1H, CHAr), 7.77 (d, J = 8.0 Hz, 1H, CHAr), 7.61 (t, J = 8.0 Hz, 1H, CHAr), 6.77 (d, J = 9.2 Hz, 1H, NH), 5.52 (t, J = 5.1 Hz, 1H, CH), 2.45–2.35 (m, 2H, CH_2CH_2CH), 2.19–2.15 (m, 2H, CH_2CH_2CH); ^{13}C-NMR (DMSO-d_6, 100 MHz): δ ppm 176.7 (C=O), 149.2 (C), 133.5 (C), 130.0 (CHAr), 129.5 (CHAr), 127.0 (C), 125.4 (CHAr), 123.3 (CHAr), 123.0 (CHAr), 121.7 (C), 102.5 (CHAr), 63.8 (CH), 28.8 (CH_2), 27.3 (CH_2). IR ν (cm^{-1}): 3402, 3164, 3079, 1692, 1573, 1482, 1253, 1182.

*2.2.33. 5-(Naphthalen-1-ylamino)pyrrolidin-2-one (**3q**)*

Following the general procedure, the crude product was precipitated in diethyl ether (20 mL), filtered off and washed with ethanol

(2 × 10 mL), affording the wanted compound as a white solid (2.4 g, 60% yield); m.p. 137–138 °C; ^{1}H-NMR (DMSO-d_6, 400 MHz): δ ppm 8.39 (bs, 1H, N*H*), 8.21 (d, *J* = 8.0 Hz, 1H, CHAr), 7.77 (d, *J* = 8.0 Hz, 1H, CHAr), 7.46–7.39 (m, 2H, CHAr), 7.27 (t, *J* = 7.6 Hz, 1H, CHAr), 7.19 (d, *J* = 7.6 Hz, 1H, CHAr), 6.65 (d, *J* = 7.6 Hz, 1H, CHAr), 6.46 (d, *J* = 8.4 Hz, 1H, NH), 5.31 (d, *J* = 3.4 Hz, 1H, CH), 2.45–2.32 (m, 2H, CH_2CH_2CH), 2.11–2.09 (m, 2H, CH_2CH_2CH); ^{13}C-NMR (DMSO-d_6, 100 MHz): δ ppm 176.5 (C=O), 141.9 (C), 133.9 (C), 127.8 (CHAr), 126.5 (CHAr), 125.6 (CHAr), 124.0 (CHAr), 123.2 (CHAr), 121.9 (CHAr), 116.7 (C), 105.0 (CHAr), 64.1 (CH), 29.1 (CH_2), 27.7 (CH_2). IR ν (cm^{-1}): 3353, 3048, 1702, 1579, 1530, 1409, 1258.

*2.2.34. 5-((4-(((5-Methylisoxazol-3-yl)methyl)sulfonyl)phenyl) amino) pyrrolidin-2-one (**3r**)*

Following the general procedure, the crude product was precipitated in diethyl ether (30 mL), filtered off and washed with ethanol (3 × 10 mL), affording the compound as a white solid (3.2 g, 55% yield); m.p. 231–232 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 11.00 (bs, 1H, NH), 8.36 (s, 1H, NH), 7.56 (d, *J* = 8.0 Hz, 2H, CHAr), 7.12 (d, *J* = 8.0 Hz, 1H, CHAr), 6.71 (d, *J* = 8.0 Hz, 2H, CHAr), 6.11 (s, 1H, NH), 5.18 (bs, 1H, CH), 2.50–2.34 (m, 2H, CH_2CH_2CH), 2.28 (s, 3H, CH_3), 2.1–2.12 (m, 2H, CH_2CH_2CH), 1.84–1.81 (m, 2H, CH_2CH_2CH); ^{13}C{^{1}H}NMR (DMSO-d_6, 100 MHz): δ 176.8 (C), 170.4 (C), 158.3 (C), 151.1 (C), 129.1 (2 CHAr), 126.1 (C), 112.3 (2 CHAr), 95.7 (CHAr), 63.5 (CH), 29.4 (CH_2), 28.3 (CH_2), 12.5 (CH_3). IR ν (cm^{-1}): 3387, 3220, 1681, 1596, 1325, 1157, 1095.

*2.2.35. 5-((3-(Trifluoromethyl)phenyl)amino)pyrrolidin-2-one (**3 s**)*

Following the general procedure, the crude product was precipitated in diethyl ether (20 mL), filtered off and washed with ethanol (2 × 10 mL), affording the wanted compound as an orange solid (1.4 g, 33% yield); m.p. 149–150 °C. ^{1}H NMR (DMSO-d6, 400 MHz): δ 8.40 (s, 1H, NH), 7.32 (t, *J* = 8.2 Hz, 1H, CHAr), 6.91 (d, *J* = 8.2 Hz, 2H, CHAr), 6.87 (s, 1H, NH), 6.65 (d, *J* = 8.2 Hz, CHAr), 5.19 (td, *J* = 8.0 Hz, *J* = 3.5 Hz, 1H, CH), 2.41–2.20 (m, 2H, CH_2CH_2CH), 2.10–2.03 (m, 1H, CH_2CH_2CH), 1.80–1.75 (m, 1H, CH_2CH_2CH); ^{13}C{1H}NMR (DMSO-d6, 100 MHz): δ 176.8 (C=O), 147.7 (C), 130 (CHAr), 129.0 (q, $J_{C\text{-}F}$ = 31.2 Hz, C), 124.8 (q, $J_{C\text{-}F}$ = 272.0 Hz, C), 116.7 (CHAr), 113.0 (d, $J_{C\text{-}F}$ = 3.8 Hz, CHAr), 109.3 (d, $J_{C\text{-}F}$ = 3.8 Hz, CHAr), 63.9 (CH), 29.5 (CH_2), 28.4 (CH_2). IR ν (cm^{-1}): 3314, 1695, 1612, 1451, 1286, 1107, 1068.

*2.2.36. 5-((4-Acetylphenyl)amino)pyrrolidin-2-one (**3 t**)*

Following the general procedure, the crude product was precipitated in diethyl ether (20 mL), filtered off and washed with ethanol (2 × 10 mL), affording compound **3 t** as a white solid (2.6 g, 70% yield); m.p. 171–172 °C; ^{1}H-NMR (DMSO-d_6, 400 MHz): δ ppm 7.74 (d, *J* = 8.4 Hz, 2H, Ar*H*), 6.68 (d, *J* = 8.4 Hz, 2H, Ar*H*), 5.22 (td, *J* = 6.8 Hz, *J* = 3.2 Hz, 1H, C*H*), 2.36–2.29 (m, 2H, C*H*$_2$CH$_2$CH), 2.35 (s, 3H, C*H*$_3$), 2.16–2.11 (m, 1H, CH$_2$C*H*$_2$CH), 1.87–1.83 (m, 1H, CH$_2$C*H*$_2$CH); ^{13}C-NMR (DMSO-d_6, 100 MHz): δ ppm 195.7 (C=O), 176.8 (C=O), 151.4 (C), 130.8 (2CHAr), 126.2 (C), 112.1 (2CHAr), 63.6 (CH), 29.4 (CH_2), 28.3 (CH_2), 26.4 (CH_3). IR ν (cm^{-1}): 3280, 1686, 1576, 1424, 1240, 1162. Anal. Calcd for $C_{12}H_{14}N_2O_2$: C, 66.04; H, 6.47; N, 12.84, found: C, 66.35; H, 6.31; N, 12.44%.

*2.2.37. 5-(Pyridin-2-ylamino)pyrrolidin-2-one (**4a**)*

Following the general procedure, the crude product was precipitated in diethyl ether (20 mL), filtered off and washed with ethanol (2 × 10 mL), affording compound **4a** as a white solid (4.6 g, 60% yield); m.p. 158–159 °C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.10 (d, *J* = 5.8 Hz, 1H, CHAr), 7.45 (td, *J* = 8.1 Hz, *J* = 1.6 Hz, 1H, CHAr), 6.68 (t, *J* = 8.1 Hz, 1H, CHAr), 6.59 (bs, 1H, NH), 6.45 (d, *J* = 8.1 Hz, 1H, CHAr), 5.59 (td, *J* = 7.4 Hz, *J* = 4.9 Hz, 1H, CH), 4.74 (d, *J* = 7.0 Hz, 1H, NH), 2.62–2.41 (m, 2H, CH_2CH_2CH), 2.40–2.35 (m, 1H, CH_2CH_2CH), 1.90–1.61 (m, 1H, CH_2CH_2CH); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 176.2 (C=O), 157.1 (C), 148.0 (CHAr), 137.4 (CHAr), 114.4 (CHAr), 109.0 (CHAr), 62.9 (CH), 29.0 (CH_2), 28.1 (CH_2). IR ν (cm^{-1}): 3416, 3211, 1679, 1599, 1525, 1484, 1284, 1266. Anal. Calcd for $C_9H_{11}N_3O$: C, 61.00; H, 6.26; N, 23.71; found: C, 60.86; H, 6.36; N, 23.43%.

*2.2.38. 5-((5-Methylthiazol-2-yl)amino)pyrrolidin-2-one (**4b**)*

Following the general procedure, the precipitated product was washed with ether several times in order to afford the wanted compound, as a white solid (1.85 g, 54% yield); m.p. 166–167 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.82 (s, 1H, NH), 7.63 (s, 1H, NH), 6.69 (s, 1H, CH), 5.29 (s, 1H, CH), 2.50 (bs, 2H, CH_2CH_2CH), 2.30 (s, 3H, CH_3), 2.10 (bs, 1H, CH_2CH_2CH), 1.88 (bs, 1H, CH_2CH_2CH); ^{13}C{^{1}H}NMR (DMSO-d_6, 100 MHz): δ 176.5 (C=O), 166.2 (C), 136.0 (CH), 120.8 (C), 65.4 (CH), 29.2 (CH_2), 28.1 (CH_2). IR ν (cm^{-1}): 3280, 1670, 1450, 1280, 1109.

*2.2.39. 2,5-Dimethyl-4-((5-oxopyrrolidin-2-yl)amino)-1-phenyl-1H-pyrazol-3(2H)-one (**4c**)*

Following the general procedure, the crude product was precipitated in diethyl ether (20 mL), filtered off and washed with ethanol (2 × 10 mL), affording compound **4c** as a light-yellow solid (3.8 g, 77% yield); m.p. 149–150 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.00 (bs, 1H, NH), 7.43 (t, *J* = 8.0 Hz, 2H, CHAr), 7.34 (d, *J* = 8.0 Hz, 2H, CHAr), 7.23 (t, *J* = 8.0 Hz, 1H, CHAr), 4.90 (bs, 1H, CH), 4.25 (d, *J* = 8.8 Hz, 1H, NH), 2.84 (s, 3H, CH_3), 2.26–2.18 (m, 2H, CH_2CH_2CH), 2.12 (s, 3H, CH_3), 2.02–1.99 (m, 1H, CH_2CH_2CH), 1.82–1.80 (m, 1H, CH_2CH_2CH); ^{13}C{^{1}H}NMR (DMSO-d_6, 100 MHz): δ 176.4 (C=O), 163.0 (C=O), 146.2 (C), 135.9 (C), 129.4 (2 CHAr), 126.0 (2 CHAr), 123.1 (CHAr), 117.6 (C), 67.7 (CH), 37.5 (CH_3), 29.7 (CH_2), 28.3 (CH_2), 10.7 (CH_3).

*2.2.40. 5-((8-Hydroxynaphthalen-2-yl)amino)pyrrolidin-2-one (**4d**)*

Following the general procedure, the crude product was precipitated in diethyl ether (20 mL), filtered off and washed with ethanol (2 × 10 mL), affording the compound as a white solid (1.9 g, 45% yield); m.p. 122–123 °C; ^{1}H-NMR (DMSO-d_6, 400 MHz): δ ppm 8.95 (bs, 1H, NH), 7.84 (d, *J* = 8.8 Hz, 1H, CHAr), 7.55 (bs, 1H, CHAr), 7.04–6.98 (m, 2H, CHAr), 6.84 (dd, *J* = 6.9 Hz, *J* = 1.9 Hz, 1H, CHAr), 6.73 (d, *J* = 8.8 Hz, 1H, CHAr), 5.63 (bs, 1H, CH), 3.45 (bs, 1H, OH), 2.32–2.40 (m, 2H, CH_2CH_2CH), 2.17–2.08 (m, 1H, CH_2CH_2CH), 1.96–1.90 (m, 1H, CH_2CH_2CH); ^{13}C-NMR (DMSO-d_6, 100 MHz): δ ppm 176.3 (C=O), 154.6 (C), 137.7 (C), 136.6 (C), 136.6 (CHAr), 123.5 (CHAr), 122.2 (CHAr), 116.5 (C), 112.8 (CHAr), 111.8 (CHAr), 61.8 (CH), 29.1 (CH_2), 27.4 (CH_2). IR ν (cm^{-1}): 3364, 3161, 1694, 1612, 1529, 1266, 1241.

*2.2.41. 1-(5-Oxopyrrolidin-2-yl)indoline-2,3-dione (**4e**)*

Following the general procedure, the crude product was precipitated in diethyl ether (20 mL), filtered off and washed with ethanol (2 × 10 mL), affording compound **4e** as an orange solid (2.6 g, 65% yield); m.p. 187–188 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.14 (s, 1H, NH), 7.68 (td, *J* = 7.64 Hz, *J* = 1.30 Hz, 1H, CHAr), 7.61 (dd, *J* = 7.64 Hz, *J* = 1.30 Hz, 1H, CHAr), 7.17 (t, *J* = 7.6 Hz, 1H, CHAr), 7.15 (d, *J* = 7.6 Hz, 1H, CHAr), 5.91 (dd, *J* = 9.0 Hz, *J* = 3.2 Hz, 1H, CH), 2.65–2.53 (m, 2H, CH_2CH_2CH), 2.34–2.20 (m, 2H, $CH_2C\underline{H_2}CH$); ^{13}C{^{1}H}NMR (DMSO-d_6, 100 MHz): δ 183.5 (C=O), 177.2 (C=O), 158.23 (C=O), 149.59 (C), 138.62 (CHAr), 125.27 (CHAr), 123.78 (CHAr), 118.23 (C), 111.96 (CHAr), 62.6 (CH), 29.7 (CH_2), 24.1 (CH_2). IR ν (cm^{-1}): 3216, 1742, 1679, 1606, 1468, 1254. Anal. Calcd for $C_{12}H_{10}N_2O_3$: C, 62.60; H, 4.38; N, 12.17; found: C, 62.26; H, 4.17; N, 11.82%.

*2.2.42. 3-(5-Oxopyrrolidin-2-yl)thiazolidine-2,4-dione (**4f**)*

Following the general procedure, the crude product was precipitated in diethyl ether (20 mL), filtered off and washed with ethanol (2 × 10 mL), affording the wanted compound as a white solid (2.3 g,

67% yield); m.p. 186–187 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.05 (s, 1H, NH), 5.68 (d, J = 8.9 Hz, 1H, CH), 2.60–2.39 (m, 4H, CH_2CH_2CH, O = CCH_2CH_2C = O), 2.20–2.00 (m, 2H, CH_2CH_2CH); ^{13}C{^{1}H}NMR (DMSO-d_6, 100 MHz): δ 177.7 (C = O), 172.0 (C = O), 171.6 (C = O), 63.2 (CH), 33.5 (CH_2), 29.4 (CH_2), 24.1 (CH_2). IR ν (cm^{-1}): 3037, 2943, 1747, 1662, 1277, 1149. Anal. Calcd for $C_7H_8N_2O_3S$, 0.5H_2O: C, 40.18; H, 4.34; N, 13.39; S, 15.33; found: C, 40.57; H, 3.93; N, 13.52; S, 15.28%

2.3. Strains and culture conditions

The antifungal activities of the synthesized compounds were tested *in vitro* against a total of twelve species. Eight phytopathogenic fungal strains and four yeast strains were selected from the collection of our mycology laboratory. The strains *Aspergillus oryzae* (MUCL19009), *Alternaria alternata* (MUCL53651), *Paecilomyces variotii* (MUCL 39890), *Penicillium ochrochloron* (MUCL 38775), *Sclerotinia sclerotiorum* (MUCL 011553), *Fusarium solani* (MUCL 035016), *Cladosporium cladosporioides* (Laboratory's isolate), *Botrytis cinerea* (Laboratory's isolate), and yeasts *Geotrichum candidum* (Laboratory's isolate), *Candida* boratory's isolate), *Candida pseudotropicalis* (MUCL46196), *Candida tropicalis* (MUCL29893), packed in tubes on PDA medium, were further plated on Petri dishes containing potato dextrose agar, and incubated at 22 °C for 5 to 7 days so that pure young cultures could be obtained, with periodic subculturing.

2.4. Culture procedures

The mineral salts medium consisted of (g/L): KCl, 0.5; NaH_2PO4, 1.544; Na_2HPO4, 0.008; $MgSO_4$, 0.244; NH_4NO_3, 1; and trace-element solution consisting of (mg/L): $ZnSO_4.7H_2O$, 1; $MnCl_2.4H_2O$, 0.1; $FeSO_4.7H_2O$, 1; $CuSO_4.5H_2O$, 0.5; $CaCl_2.2H_2O$, 0.1; MoO_3, 0.2. The carbon source was glucose at 10 g/L. Each fungal isolate was singly grown in Erlenmeyer flasks (500 mL) for one week at 22 °C at 140 rpm under a 12 h/12 h photoperiod in a Binder incubator including an orbital shaker. Fungi broth microdilutions were performed in 96-well microtiter plates. For the assay, compound test wells (CTWs) were prepared with stock solutions of each compound in DMSO. The compounds possessing good activity (inhibitory rate above 75% at 100 μg/mL) were further evaluated using different concentrations by diluting the above stock solution in the range 1 to 100 μg/mL. An inoculum suspension (100 μL) was added to each well (final volume in the well = 200 μL). A growth control well (GCW) (containing medium, inoculum and the same amount of DMSO used in a CTW) was included for each fungus tested. Microtiter trays were incubated in a moist chamber at 22 °C for 5 to 7 days for all yeasts. DMSO served as the negative control, having no observed influence on the tested panel. The cultures were incubated for 7 days, and turbidity was measured through the optical density using a microplate reader (Multiskan Go, Thermo), at 600 nm. Tests were performed in triplicates.

2.5. Cell culture and cytotoxic assay

The human embryonic kidney 293 cell line (HEK293) was cultured in Dulbecco's modified Eagle medium (DMEM) (Gibco, Waltham, MA) supplemented with 2 mM L-glutamine, 100 IU/mL penicillin/streptomycin, non-essential amino acid solution (1/100) and 5% (*v*/v) heat-inactivated fetal bovine serum (Sigma-Aldrich, Saint-Louis, MO), and grown at 37 °C in a humidified incubator with 5% CO_2. Cells were seeded at 3000 cells per well onto 96-well plates in DMEM medium. Cells were incubated in a culture medium that contained 100 μM of the different test compounds and 2 μM of the references, each dissolved in less than 1% DMSO. After 72 h of incubation, cell viability (in proliferation and cytotoxicity) was estimated by the colorimetric MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium) assay.

3. Results and discussion

3.1. Synthetic chemistry

This study needs the introduction of a heteroatom linked to an alkyl or aryl group to the position 5 of pyrrolidin-2-one. That can be realized in the case of few amines by reacting them directly with the lactam in oxidative and metal catalyzed conditions [31]. A more general reaction started from pterolactam **A** relying on the use of a triflic acid catalyzed intermolecular α-amination of pterolactam *via N*-acyliminium species to produce 5-arylamino pyrrolidinones [32]. However the scope of this reaction is limited. In fact, this acidic protocol gave good yields with anilines, but failed when using aliphatic amines or benzyl alcohol derivatives for instance. We recently described a new procedure for the reaction of pterolactam with nucleophiles in non-acidic conditions [25]. Using this method, the starting nucleophile and a catalytic amount of cesium fluoride were reacted under a slight vacuum, with lactam **A** at 80 °C. With this very efficient method, all target compounds were obtained in moderate to good yields and gave analytical and spectroscopic data in full accordance with their structures. Four series of *N,O*-, *N,S*-acetals and *N,N′*-aminals derivated from natural pterolactam **A**, structurally similar to some plant metabolites, were obtained and tested against a panel of fungal strains and yeasts.

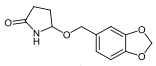
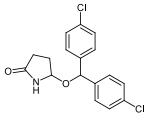
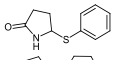
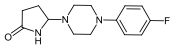
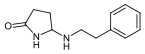
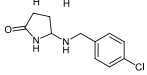
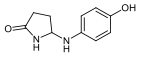
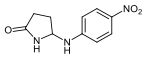
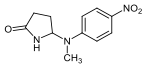
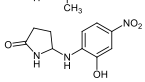
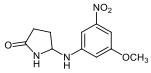
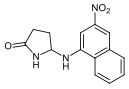
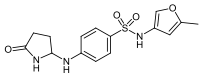
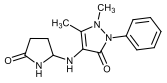
3.2. Antifungal activity and structure-activity relationships (SAR)

The *in vitro* antifungal activities of these original compounds were evaluated using the micro-dilution method, by using hymexazol - a systemic soil and seed broad spectrum fungicide -, fluconazole and ketoconazole as the positive controls. A panel of twelve fungi (*Fusarium solani, Paecilomyces variotii, Penicillium ochrochloron*, *Aspergillus oryzae, Alternaria alternata*, *Cladosporium cladosporioides, Geotrichum candidum, Sclerotinia sclerotiorum* and *Botrytis cinerea*) known for causing allergies, asthma, mycosis or plant pathologies, and also *non albicans candida* yeasts species (*Candida pseudotropicalis*, *Candida tropicalis* and *Candida krusei*) which have demonstrated reduced susceptibility to commonly used antifungal drugs was selected. The results of the first screening at a concentration of 100 μg/mL were listed in Table 1. To further evaluate the inhibitory potencies of the most promising synthesized compounds, the half maximal inhibitory concentration (EC_{50}) values of products with high inhibition rate (> 75%) were determined (Table 2).

Data presented in Table 1 indicate that the *Sclerotiniaceae* family *(S. sclerotiorum* and *B. cinerea)* and *C. cladosporiodoides* are not sensitive to the new Mannich base of amides inspired by pterolactam. However, in term of antifungal activity, a third of the newly synthesized compounds displayed antifungal activities against at least one strain (Tables 1–2). The most sensitive strains to the tested compounds were *P. ochrochloron* and *F. solani*, with five and four compounds displaying EC_{50} in the same range as for the control (EC_{50}: 0.077–0.236 *vs* 0.628 μmol/mL and 0.050–0.357 *vs* 0.157 μmol/mL respectively) (Table 2, lines PO and FS). This result is of particular interest for *F. solani* considering the described problems of drug resistance in this species for both human health and agricultural purposes [33]. It is also notable than some compounds can be considered as broad-spectrum antifungal agents (*ie* compounds **1i**, **3o** and **3p**) whereas others targeted selectively one pathogenic strain (*ie* compounds **1f**, **3 l**, **4c**) (Table 2, entries 2–4, 14–18, 19–22, 1, 12, 25, respectively).

In the **Series a** of ethers and thioethers it is interesting to note that only a third of the compounds exhibited some activity against at least one strain at 100 μg/mL (Table 1, entries 1–10). Sessiline **1 g** [26] as well as the other benzyl ethers **1b-e** substituted by oxygenated groups demonstrated no activity. Compound **1f**, bearing a benzodioxole ring appeared three times more potent than the control against *P. ochrochloron* (EC_{50}: 0.136 *vs* 0.628 μmol/mL) (Table 2, entry 1). This compound is structurally similar to sesamol, known for its antimycotic properties [34]. Concerning the benzhydryl ether derivatives **1 h** and **1i**

Table 2
EC_{50} (μmol/mL) of selected compounds against the sensitive fungi and yeasts.[d]

Entry	Compound	Structure	M (g/mol)	Strain	Toxic regression (y = ax + b)	R^2	EC_{50} (μmol/mL)	Reference EC_{50} (μmol/mL)	Spectrum[e]
1	**1f**		235.24	PO	y = 291.48× + 10.102	0.9840	0.136	0.628 [a]	1
2	**1i**		336.21	PV	y = 1195× + 32.26	0.9708	0.014	0.577 [a]	3
3				GC	y = 623.33× + 46.927	0.9216	0.005	0.003 [c]	
4				CK	y = 225.74× + 41.12	0.9525	0.039	0.045 [b]	
5	**1j**		196.03	FS	y = 412.51×-8.1445	0.9772	0.139	0.157 [a]	2
6				CP	y = 683.24×-1.5085	0.9772	0.076	0.019 [c]	
7	**2d**		263.31	FS	11% inhibition rate at 50 μg/mL	–	> 0.3	0.157 [a]	1
8	**2e**		204.27	FS	No inhibition rate at 50 μg/mL	–	> 0.3	0.157 [a]	1
9	**2 g**		224.69	FS	20% inhibition rate at 50 μg/mL	–	> 0.3	0.157 [a]	1
10	**3c**		192.21	FS	y = 307.54×-60	1	0.357	0.157 [a]	1
11	**3 k**		221.21	FS	15% inhibition rate at 50 μg/mL	–	> 0.3	0.157 [a]	1
12	**3 l**		235.24	FS	y = 134.53× + 28.047	0.9901	0.164	0.157 [a]	1
13	**3 m**		237.21	FS	No inhibition rate at 50 μg/mL	–	> 0.3	0.157 [a]	1
14	**3o**		251.24	PV	y = 3140.5× + 37.5	1	0.004	0.577 [a]	5
15				PO	y = 301.49× + 15.75	0.9985	0.114	0.628 [a]	
16				AA	y = 2263.2×-4.377	0.9693	0.024	0.387 [a]	
17				CP	y = 250.96×-0.4469	0.9989	0.201	0.019 [c]	
18				CT	y = 319.85×-30.718	0.9489	0.252	0.030 [c]	
19	**3p**		271.27	AO	y = 219.87× + 6.9737	0.9990	0.195	0.461 [a]	4
20				PO	y = 254.99× + 30.4	0.8982	0.077	0.628 [a]	
21				FS	y = 907.81× + 3.8812	0.9466	0.050	0.157 [a]	
22				CK	y = 334.05× + 32.880	0.8937	0.051	0.045 [b]	
23	**3r**		335.38	PO	y = 415.87–48	1	0.236	0.628 [a]	2
24				AA	y = 2202× + 37.984	0.9455	0.005	0.387 [a]	
25	**4c**		286.33	FS	y = 248.3 × 24.055	0.9419	0.104	0.157 [a]	1

SS - *Sclerotinia sclerotiorum;* BC - *Botrytis cinerea*; AO - *Aspergillus oryzae;* PV - *Paecilomyces variotii;* PO - *Penicillium ochrochloron;* CC - *Cladosporium cladosporioides;* AA - *Alternaria alternata;* FS - *Fusarium solani;* GC - *Geotrichum candidum;* CK - *Candida krusei;* CP - *Candida pseudotropicalis;* CT - *Candida tropicalis.*

[a] Hymexazol, used as positive control for bioassay.
[b] Fluconazole, used as positive control for bioassay.
[c] Ketoconazole, used as positive control for bioassay.
[d] Sequential dilutions from 100 μg/mL to 1 μg/mL were performed for bioassays.
[e] Spectrum refers to the number of strains killed by the compound.

it is notable that the dichloro compound **1i** is highly active whereas **1 h** is not (Table 1, entries 8 and 9). The dichlorobenzhydryl product **1i** proved to be as active as the control against *G. candidum* and *C. krusei* but above all fifty time more potent as the control against *P. variotii* (EC_{50}: 0.014 *vs* 0.577 μmol/mL, Table 2, entry 2). Turning to the thiophenyl ether, introduction of the sulfur linkage furnished **1j** with the same activity as the control against *F. solani* but four time less against *C. pseudotropicalis* (EC_{50} 0.076 μmol/mL) (Table 2, entry 3).

Concerning **Series b** of aliphatic amino-5-pyrrolidone derivatives, even if some inhibition rates were observed for compounds **2d**, **2e** and **2 g** at 100 μg/mL against *F. solani*, their EC_{50} were above 0.3 μmol/mL and, consequently, they were considered as inactive (Table 1, entries 14, 15 and 17 and Table 2, entries 7–9). Almost the same considerations can be provided for the heterocycles of the **Series d**, for which only the Mannich base **4c** combining a pyrrolidin-2-one and phenyl dimethyl pyrazole moieties proved to be slightly more active as the control against *F. solani* (EC_{50}: 0.104 *vis* 0.157 μmol/mL).

The **Series c** of aromatic amines Mannich bases furnished the more promising compounds (Table 2, entries 10–24). Indeed, while the product **3a** issued from aniline proved to be inactive, addition of an OH group in *para* position led to **3c**, two times less potent against *F. solani* than hymexazol (EC_{50}: 0.357 *vs* 0.157 μmol/mL). Exchanging the OH for the nitro group of **3 k** did not help increasing the activity (Table 2, entry 11).

The *N*-methylation of the linker, leading to compound **3 l** slightly increased the activity (EC_{50}: 0.164 μmol/mL) compared to **3 k** (Table 2, entry 12). Now, replacing the nitro-phenyl group of **3 k** by the bulkier nitro-naphthalene yielded **3p**. Not only this compound is three time more potent as the control against *F. solani* (EC_{50}: 0.050 μmol/mL), but it is also equal or eight time more active as the control against *A. oryzae*, *P. ochrochloron* and *C. krusei* (EC_{50}: 0.195 vs 0.461 μmol/mL, 0.077 vis 0.628 μmol/mL and 0.051 vis 0.045 μmol/mL respectively) (Table 2, entries 19–22). This increase of activity comes from the nitro group because the naphtyl analog **3q** displayed no biological properties

(Table 1, entry 35). Noteworthy also, the *para*-sulfamide withdrawing group led to **3r** which displayed two and ten times more activity against *P. ochrochloron and A. alternata* (EC_{50}: 0.236 and 0.005 μmol/mL respectively). The modification of the substitution on the aromatic ring is not well tolerated. Exchanging the NO_2 group for a methyl or a halogen substituent abolished the activity (Table 1, entries 2, 25–26).

The same observation was done when varying the position of the NO_2 group from *para* to *ortho* or *meta* (Table 1, entries 28–30). Moreover, keeping the *para*-nitro group and adding a second substituent on the aromatic ring – cyano or hydroxy - led to no activity (Table 1, entries 31–32). However, Mannich base **3o**, formed from pyrrolidin-2-one and 3-nitro-5-methoxy aniline become inactive against *F. solani*, and ten times less potent as the control against *C. pseudotropicalis* and *C. tropicalis* while becoming five and fifty times more active as the control against *P. ochrochloron* and *A. alternata* and even provide a very impressive 0.004 μmol/mL EC_{50} value against *P. variotii* (control: 0.58 μmol/mL) (Table 2, entry 11). Thus, compound **3o** prove to be a very interesting platform molecule because his broad antimicrobial spectrum (five sensitive strains among twelve tested).

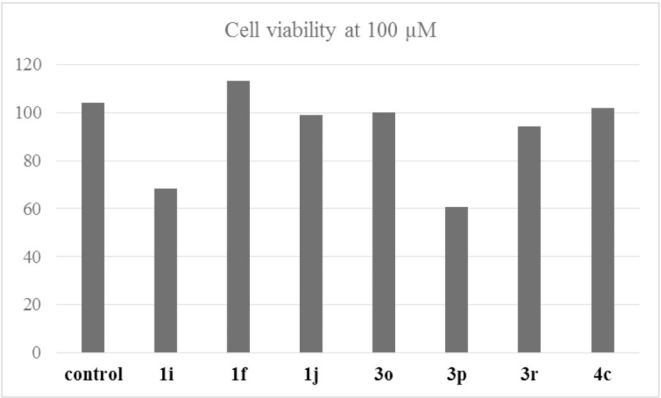


Fig. 2. HEK293 cell line (TPP culture plates) cell viability of selected molecules tested at 100 μM concentration.

3.3. Molecular physicochemical properties of the selected antimicrobial agents (Table 3)

To further evaluate their potential for candidate lead fungicide, some of the molecular physicochemical properties [octanol/ water partition coefficient (LogP), hydrophilic–lipophilic balance (HLB), topology polar surface area (TPSA), number of hydrogen bond donor sites (NDS), number of hydrogen bond acceptors sites (NAS) and molecular weight (MW)] were calculated (Table 3) [35]. Except **1i**, all these compounds meet the Lipinski "Rule of five" criteria [36] with Log $P < 1.8$, NDS < 4, NAS < 9 and MW < 350, and the Briggs "Ground rules of three" suggesting that for a fungicidal to be available, it should have a Log $P < 3$ [37]. It can also be observed that most of the active compounds are lipophilic. However, no obvious correlation can be found between the antifungal activity over a particular fungal species and only one of these parameters.

3.4. Toxicity evaluation

In order to check the mammalian cell toxicity of the tested compounds presented herein, the most active products were screened against human embryonic kidney cells (HEK293) at a very high concentration of 100 μM (Fig. 2) [38]. Compound **3o**, the broader-spectrum antimicrobial discovered agent exhibited no cytotoxicity. However, all compounds showed no to a slight toxicity in viable kidney cells, the viability of cells dropping down around 40% for the most toxic compounds at a concentration of 100 μM. On the other side, fifteen compounds (**1b**, **1d**, **1j**, **2a**, **2f**, **2a**, **3c**, **3f**, **3 k**, **3o**, **4b**, **4c**, **4d**, **4e**, **4f**) were selected by the National Cancer Institute (NCI) for screening on a large panel of cancer cells, and they exhibited slight to no cytotoxicity (see data in ESI).

4. Conclusions

The inhibition activity of 43 hemisynthetic compounds inspired from natural pterolactam and its metabolites were evaluated against a large panel of fungal strains and yeasts, some of them being particularly sensitive to this class of compounds. According to the structure-activity relationships, *N,N′*-aminals from pterolactam were identified as more potent antimicrobial agents than *N,O*- or *N,S*-acetals. Moreover, aromatics bearing a nitro-group were of particular interest, especially to fight against *F. solani*. This screening underlined that compound **3o**, combining a pyrrolidin-2-one and a 3-methoxy-5-nitroaniline moieties, is a broad-spectrum antifungal agent with adequate "fungicide-like" properties and no visible cytotoxicity.

Table 3
Predicted molecular properties of the new compounds.

Compound	Log P[a]	HLB[b]	TPSA[c]	NDS[d]	NAS[e]	MW[f]	Sensible strains	Spectrum
1f	0.82	11.4	56.79	1	4	235.24	PO	1
1i	4.19	5.42	38.33	1	2	336.21	PV – GC - CK	3
1j	1.56	8.39	29.10	1	1	193.26	FS - CP	2
2d	1.42	14.38	35.58	1	3	263.32	FS	1
2e	1.17	11.87	41.11	2	2	204.27	FS	1
2 g	1.48	12.56	41.13	2	2	224.69	FS	1
3c	0.49	9.06	61.36	3	3	192.22	FS	1
3 k	0.73	10.11	86.95	2	4	221.21	FS	1
3 l	1.37	10.29	78.16	1	4	235.24	FS	1
3 m	0.43	11.77	107.18	3	5	237.21	FS	1
3o	0.57	11.94	96.18	2	5	251.24	PV – PO – AA – CT - CP	5
3p	1.72	8.03	86.95	2	4	271.28	AO – PO – FS – CK	4
3r	0.62	8.98	100.44	3	4	335.38	PO – AA	2
4c	0.11	19.44	64.68	2	4	286.33	FS	1

[a] Log P = octanol/water partition coefficient.
[b] Hydrophilic–lipophilic balance (HLB) is the balance of the size and strength of the hydrophilic and lipophilic moieties of a compound.
[c] TPSA = topology polar surface area.
[d] NDS = number of hydrogen bond donor sites.
[e] NAS = number of hydrogen bond acceptors sites.
[f] MW = molecular weight (g/mol).

Declaration of Competing Interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fitote.2020.104581.

References

[1] F. Bongomin, S. Gago, R.O. Oladele, D.W. Denning, Global and multi-national prevalence of fungal diseases-estimate precision, J. Fungi 3 (2017) 1–29, https://doi.org/10.3390/jof3040057.
[2] D. Sanglard, Emerging threats in antifungal-resistant fungal pathogens, Front. Med. 3 (2016) 11, https://doi.org/10.3389/fmed.2016.00011.
[3] U. Theuretzbacher, Accelerating resistance, inadequate antibacterial drug pipelines and international responses, Int. J. Antimicrob. Agents 39 (2012) 295–299, https://doi.org/10.1016/j.ijantimicag.2011.12.006.
[4] A. Srinivasan, J.L. Lopez-Ribot, A.K. Ramasubramanian, Overcoming antifungal resistance, Drug discovery today. Technologies 11 (2014) 65–71, https://doi.org/10.1016/j.ddtec.2014.02.005.
[5] F. Almeida, M.L. Rodrigues, C. Coelho, The still underestimated problem of fungal diseases worldwide, Front. Microbiol. 10 (2019) 214, https://doi.org/10.3389/fmicb.2019.00214.
[6] K.V. Ramana, S. Kandi, P. Venkata-Bharatkumar, C.H.V. Sharada, R. Rao, R. Mani, S.D. Rao, Invasive fungal infections: a comprehensive review, Am. J. Infect. Dis. Microbiol. 1 (2013) 64–69, https://doi.org/10.12691/ajidm-1-4-2.
[7] M.J. Abad, M. Ansuategui, P. Bermejo, Active antifungal substances from natural sources, ARKIVOC 7 (2007) 116–145.
[8] M.-Y. Yoon, B. Cha, J.-C. Kim, Recent trends in studies on botanical fungicides in agriculture, Plant Pathol. J. 29 (2013) 1–9, https://doi.org/10.5423/PPJ.RW.05.2012.0072.
[9] R.J. Grayer, J.B. Harborne, A survey of antifungal compounds from higher plants, 1982-1993, Phytochemistry 37 (1994) 19–42, https://doi.org/10.1016/0031-9422(94)85005-4.
[10] K. Hostettmann, O. Potterat, J.-L. Wolfender, The potential of higher plants as a source of new drugs, Chimia 52 (1998) 10–17.
[11] K. Takatori, S. Nakano, S. Nagata, K. Okumura, I. Hirono, M. Shimizu, Pterolactam, a new compound isolated from bracken, Chem. Pharm. Bull. 20 (1972) 1087.
[12] Chung Sub Kim, Oh. Wook Kwon, Sun Yeou Kim, Ki Hyun Kim, Kang Ro Lee, A new cyclic triterpene saponin from *Phyteuma Japonicum*, Heterocycles 89 (2014) 1913–1922, https://doi.org/10.3987/COM-14-13035.
[13] A. Arciniegas, A.L. Pérez-Castorena, K. González, M. Reyes-Lezama, J.L. Villaseñorc, A. Romo de Vivar, Pyrrolizidine Alkaloids and Diterpenes from *Villasenoria orcuttii*, J. Braz. Chem. Soc. 24 (2013) 1167–1171 https://doi.org/10.5935/0103-5053.20130149.
[14] M.-C. Song, H.-J. Yang, T.-S. Jeong, K.-T. Kim, N.-I. Baek, Heterocyclic compounds from *Chrysanthemum coronarium L.* and their inhibitory activity on hACAT-1, hACAT-2, and LDL-oxidation, Archives Pharmacal. Research 31 (2008) 573–578, https://doi.org/10.1007/s12272-001-1195-4.
[15] R. Amara, G. Bentabed-Ababsa, M. Hedidi, J. Khoury, H. Awad, E. Nassar, T. Roisnel, V. Dorcet, F. Chevallier, Z. Fajloun, F. Mongin, Synthesis of *N*-Aryl and *N*-heteroaryl γ-, δ-, and ε-Lactams using deprotometalation–iodination and *N*-arylation, and properties thereof, Synthesis 49 (2017) 4500–4516, https://doi.org/10.1055/s-0036-1590798.
[16] K. Saurav, K. Kannabiran, Interaction of 5-(2,4-dimethylbenzyl)pyrrolidin-2-one with selected antifungal drug target enzymes by *in silico* molecular docking studies, Interdisciplinary Sciences: Computational Life Sciences 3 (2011) 198–203, https://doi.org/10.1007/s12539-011-0098-1.
[17] P. Moya, A. Cantin, M.A. Miranda, J. Primo, E. Primo-Yufera, Synthesis and biological evaluation of new analogues of the active fungal metabolites N-(2-Methyl-3-oxodecanoyl)-2-pyrroline and N-(2-Methyl-3-oxodec-8-enoyl)-2-pyrroline, J. Agric. Food Chem. 48 (2000) 3682–3688.
[18] S. Lee, J. Ji, K.H. Shin, B.K. Kim, Sessiline, a new nitrogenous compound from the fruits of *Acanthopanax sessiliflorus*, Planta Med. 68 (2002) 939–941, https://doi.org/10.1055/s-2002-34925.
[19] W. Zhang, P. Liu, X.-Y. Ji, X.-Y. Chen, Y.-Y. Jiang, B. Liu, A pair of new non-glycosidic iridoid epimers from *Scrophularia ningpoensis*, Heterocycles 96 (2018) 1991–1998, https://doi.org/10.3987/COM-18-13991.
[20] M. Kuhnt, A. Probstle, H. Rimpler, R. Bauer, M. Heinrich, Biological and pharmacological activities and further constituents of Hyptis verticillata, Planta Med. 61 (1995) 227–232, https://doi.org/10.1055/s-2006-958061.
[21] E. Eisenreichova, M. Haladova, A. Buckova, J. Tomko, D. Uhrin, K. Ubik, *A pyrroline-pyrrolidine alkaloid from Lilium candidum* bulbs, Phytochemistry. 31 (1992) 1084–1085, https://doi.org/10.1016/0031-9422(92)80088-V.
[22] S. Ghosh, A. Derle, M. Ahire, P. More, S. Jagtap, S. Phadatare, A.B. Patil, A.M. Jabgunde, G.K. Sharma, V.S. Shinde, K. Pardesi, D.D. Dhavale, B.A. Chopade, Phytochemical analysis and free radical scavenging activity of medicinal plants *Gnidia glauca* and *Dioscorea bulbifera*, PLoS One 8 (2013), https://doi.org/10.1371/journal.pone.0082529 e82529/1-e82529/18.
[23] K.C. Chaluvaraju, K. Ishwar Bhat, Aminobenzylated Mannich bases of amides, J. Chem. Pharm. Sciences 3 (2010) 138–140.
[24] G. Roman, Mannich bases in medicinal chemistry and drug design Eur, J. Med. Chem. 89 (2015) 743–816, https://doi.org/10.1016/j.ejmech.2014.10.076.
[25] A.E. Dascalu, A. Ghinet, E. Lipka, M. Collinet, B. Rigo, M. Billamboz, Cesium salts as superior catalysts for solvent-free modifications of biosourced pterolactam, Mol. Cat. 470 (2019) 32–39, https://doi.org/10.1016/j.mcat.2019.03.003.
[26] V. Ilkei, K. Farago, Z. Santa, M. Dekany, L. Hazai, C. Jr, Szantay, C. Szantay, G. Kalaus. The first synthesis of sessiline. Int. J. Org. Chem. 4 (2014) 309–313, https://doi.org/10.4236/ijoc.2014.45033.
[27] M. Eguchi, Q. Zen, A. Korda, I. Ojima, Synthesis of pyrrolizidine alkaloids *via* rhodium-catalyzed silylformylation and amidocarbonylation, Tetrahedron Lett. 34 (1993) 915–918, https://doi.org/10.1016/S0040-4039(00)77453-3.
[28] Y. Kosugi, H. Hamaguchi, T. Nagasaka, N. Ozawa, S. Ohki, Synthesis of 5-amino-2-pyrrolidinone and its derivatives, Heterocycles 14 (1980) 1245–1249 http://pascal-francis.inist.fr/vibad/index.php?action=getRecordDetail&idt=PASCAL8130207169.
[29] E. Toja, C. Gorini, C. Zirotti, F. Barzaghi, G. Galliani, Amnesia-reversal activity of a series of 5-alkoxy-1-arylsulfonyl-2-pyrrolidinones, Eur. J. Med. Chem. 26 (1991) 403–413, https://doi.org/10.1016/0223-5234(91)90101-R.
[30] S. Priyadarshini, P.J. Amal Joseph, M. Lakshmi Kantam, Copper catalyzed oxidative cross-coupling of aromatic amines with 2-pyrrolidinone: a facile synthesis of *N*-aryl-γ-amino-γ-lactams, Tetrahedron 70 (2014) 6068–6074, https://doi.org/10.1016/j.tet.2014.04.070.
[31] M. Sun, T. Zhang, W. Bao, $FeCl_3$-catalyzed sp3 C-H amination. Synthesis of aminals with aryl amines and amides, etrahedron Letters 55 (2014) 893–896 https://doi.ord/10.1016/j.tetlet.2013.12.043.
[32] G.-M. Dumitriu, E. Bîcu, U. Eryuruk, D. Belei, B. Rigo, A. Daïch, A. Ghinet, Triflic acid catalyzed intermolecular α-amination of pterolactams to give 5-arylaminopyrrolidinones *via N*-acyliminium species, Synlett. 27 (2016) 934–940, https://doi.org/10.1055/s-0035-1561333.
[33] K. O'Donnell, D.A. Sutton, A. Fothergill, D. McCarthy, M.G. Rinaldi, M.E. Brandt, N. Zhang, D.M. Geiser, Molecular phylogenetic diversity, multilocus haplotype nomenclature, and in vitro antifungal resistance within the *Fusarium solani* species complex, J. Clin. Microbiol. 46 (2008) 2477–2490, https://doi.org/10.1128/JCM.02371-07.
[34] M.A. Ansari, F. Zeeshan, S. Hameed, Mechanistic insights into the mode of action of anticandidal sesamol, Microb. Pathog. 98 (2016) 140–148, https://doi.org/10.1016/j.micpath.2016.07.004.
[35] The herein calculations were performed with MarvinSketch 18.24, a ChemAxon software.
[36] C.A. Lipinski, F. Lombardo, B.W. Dominy, P.J. Feeney, Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings, Adv. Drug Deliv. Rev. 23 (1997) 3–25, https://doi.org/10.1016/S0169-409X(96)00423-1.
[37] G.G. Briggs, "Predicting Uptake and Movement of Agrochemicals from Physical Properties" Talk Provided at the SCI Meeting on Uptake of Agrochemicals and Pharmaceuticals, London, UK, December (1997).
[38] The human embryonic kidney 293 cell line (HEK293) was cultured in Dulbecco"s modified Eagle medium (DMEM) (Gibco, Waltham, MA) supplemented with 2 mM L-glutamine, 100 IU/mL penicillin/streptomycin, non-essential amino acid solution (1/100) and 5% (v/v) heat-inactivated foetal bovine serum (Sigma-Aldrich, Saint-Louis, MO), and grown at 37 °C in a humidified incubator with 5% CO2. Cells were seeded at 3000 cells per well onto 96-well plates in DMEM medium. Cells were incubated in a culture medium that contained 100 μM of the different test compounds and 2 μM of the references, each dissolved in less than 1% DMSO. After 72 h of incubation, cell viability (in proliferation and cytotoxicity) was estimated by the colorimetric MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) assay.