Organic & Biomolecular Chemistry

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Organic and biomolecular chemistry

Communication

An improved synthesis of pyrido[2,3-*d*]pyrimidin-4(1*H*)-ones and their antimicrobial activity

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The screening of a small library of diverse chemical structures resulted in the identification of 2-thioxodihydropyrido[2,3-*d*]pyrimidine **10a** as having broad spectrum antibacterial activity (MIC 0.49 - 3.9 µg mL^{-1}), and reasonable antifungal activity (MIC 0.49 - 3.9 µg mL^{-1}). An expeditious synthesis of **10a** was optimized by varying solvents, catalysts and the use of microwave irradiation with the best conditions using DMF as a solvent, I_2 (10 mol%) and a 30 minute reaction time compared to 15 h for classic conventional heating. The pharmacokinetic properties and calculation of drug likeness of **10a** suggested good traditional drug-like properties and led to the synthesis of a small library with seven compounds **10a** and **10d-i** showing broad antimicrobial activity (MIC = 0.49 – 7.81 µg mL^{-1}) and selectivity indices of more than 5.6 against the normal colon cell line (CCD-33Co). The antifungal activity of compounds **10d-i** was moderate to strong with MIC values of 1.95 – 7.81 µg mL^{-1}.

Introduction

The paucity of novel antimicrobial agents in contemporary science is highly challenging with only 1.6% of new antibiotic drugs in clinical trials, a situation arising from the reduction in relevant drug discovery research, which leads to fewer novel registered therapeutics.[1-6] Since the dawn and overuse of antibiotics, bacteria have exploited windows to readily develop resistance to the established anti-infective agents.[3-12] *In vitro* target-based approaches have been used widely to discover potent enzyme inhibitors, but many are devoid of whole cell antibacterial activity, presumably due either to the inability to reach the intracellular targets, or as a result of the process of bacterial active efflux.[13, 14] More promising is whole-cell screening, where antimicrobial potencies have been identified *a priori* followed by determination of the modes of action.[15, 16] Most current antibiotics approved by the FDA for clinical applications are derivatives of a limited number of chemical classes or scaffolds, with many discovered in the mid-1980s.[17, 18] Strikingly, most candidates in recent phase 3 clinical trials were based on known scaffolds,[19] including the tetracycline derivative omadacycline **I**, the macrolide derivative solithromycin **II**, trimethoprim analogue iclaprim **III** and the quinolones derivatives zabofloxacin **IV** and delafloxacin **V** (Figure 1).[19]

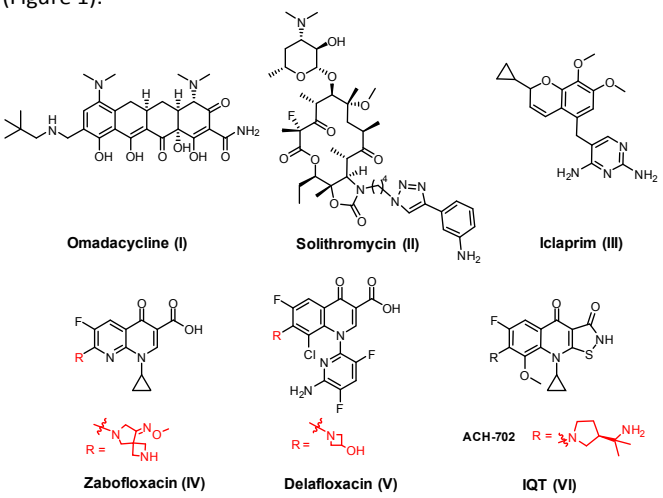

Figure 1. Examples of antibacterial agents in phase 3 clinical trials (**I-V**), isothiazoloquinoline derivatives (**VI**).

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Attempts to discover novel antimicrobial scaffolds related to known classes include the isothiazoloquinolones IQT **VI** (Figure 1), which are related to the known quinolones.[20] These derivatives showed activity against methicillin sensitive *Staphylococcus aureus* (MSSA) (MIC = 0.13 - 0.0020 µg/mL) against methicillin resistant *Staphylococcus aureus* (MRSA) (MIC = 4.0 - 0.060 µg/mL) and against *Escherichia coli* (*E. coli*) (MIC = 0.50 - 0.0040 µg/mL). Examples of ITQ derivatives were found to have 8- to 16- fold greater activity than the standard drug against MRSA, and ACH-702 is in pre-clinical development.[20]

The continued need for new anti-infective agents is well documented, especially those based on new chemical classes. As part of an ongoing program searching for new leads, we report here 2-thioxodihydropyrido[2,3-*d*]pyrimidine **10a** which revealed broad spectrum antibacterial activity against three Gram -ve and two Gram +ve bacteria with MIC values ranging from 0.98 to 3.9 µg/mL, (control drug 0.49 - 0.98 µg/mL), and interestingly it showed MIC = 31.3 µg/mL against MRSA (control drug 3.9 µg/mL) and two fungi (control drug 0.49 - 0.98 µg/mL).

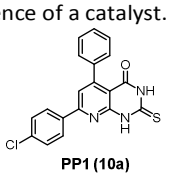
Here we describe the development of this scaffold with the synthesis of a small library of compounds and their screening against Gram +ve, Gram -ve and MRSA bacteria as well as antifungal agents. The calculation of the pharmacokinetic properties of all compounds and the assessment of the cytotoxicity of the most active compounds was also performed.

Results and discussions

Synthesis of pyrido[2,3-*d*]pyrimidine derivatives 10a-j:

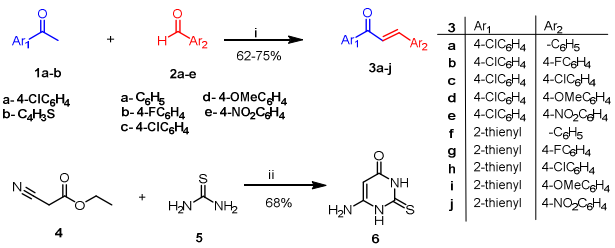
The required chalcone starting materials **3a-j** were prepared *via* a typical Claisen-Schmidt condensation of either *p*-chloroacetophenone **1a** or 2-acetyl thiophene **1b** with aromatic aldehydes **2a-e** in alcoholic alkaline solution in yields of 62-75% (Scheme 1).[21] The synthesis of 6-amino-2-thiouracil **6** was accomplished by heating thiourea **5** at reflux with ethyl cyanoacetate **4** in the presence of sodium ethoxide in 68% yield (Scheme 1).

The classic Skraup synthesis of quinoline uses hot aniline, nitrobenzene and glycerol in the presence of catalytic sulfuric acid and produces low yields of product (Figure 3).[22, 23] Doebner and von Miller replaced the glycerol with α,β-unsaturated carbonyl compounds, with heating under acidic conditions or in the presence of iodine as a catalyst to obtain quinolines in good yields (Figure 3).[23] A further development replaced the aniline with 6-amino-2-thiouracil to synthesize the pyrido[2,3-*d*]pyrimidine ring instead of quinoline (Figure 3).[24] We report here the continued evolution of this synthesis, to synthesize the versatile pyrido[2,3-*d*]pyrimidine scaffold in DMF at reflux in the absence of a catalyst.



PP1 (10a)

Figure 2. Chemical structure of compound PP1 (**10a**).



Scheme 1: Reagents and conditions: (i) KOH, methanol, rt, (ii) NaOEt/ethanol, reflux, 4 h.

Therefore, 5-aryl-7-(thiophen-2-yl/4-chlorophenyl)-2-thioxo-2,3-dihydropyrido[2,3-*d*]pyrimidin-4(1*H*)-ones **10a-j** were prepared by heating at reflux equimolar quantities of 6-amino-2-thiouracil **6** and the chalcones **3a-j** in dry DMF for 15-20 h in yields of 44-65% (Scheme 2). In a typical example, analysis of the ^{1}H NMR spectra of **10b** revealed a resonance at 7.22 ppm assigned to C5-Ar H3 and H5 with the doublet at 7.49 ppm assigned to C5-Ar H2 and H6. The pyridyl H6 was assigned to the singlet at δ 7.67 in the ^{1}H NMR spectra. To the corresponding C7 *para* substituted aryl substituent, Ar H3 and H5 was assigned the resonance at 7.58 ppm, while the resonance at 8.25 ppm was assigned to Ar H2 and H6. Two resonances at δ 12.38 and 13.08 were assigned to the two pyrimidyl NHs. The ^{13}C NMR spectrum of the fluorine containing compound **10b** showed the splitting pattern of the fluorine and carbon with coupling constants $^1J_{F\text{-}C}$ = 243.1 Hz at resonance 161.3 ppm, $^2J_{F\text{-}C}$ = 20.9 Hz at resonance 114.2 ppm and $^3J_{F\text{-}C}$ = 8.6 Hz at resonance 131.0 ppm.

The reaction is an apparent heterocyclic condensation of the uracil amino group with the propenone carbonyl **3a-j** to afford the Schiff bases **7a-j** (Scheme 2). This mechanism has been investigated[23] indicating an initial conjugate addition, followed by fragmentation with subsequent multistep reactions to produce the '1,2-addition' product. This then undergo intramolecular cyclization providing the 5,6-dihydropyrido[2,3-*d*]pyrimidines **8a-j** which tautomerizes to the 5,8-dihydropyrido[2,3-*d*]pyrimidines **9a-j** which then oxidizes to **10a-j**.[24-26] It has been reported that condensation of 6-amino-2-thiouracil **6** with chalcone analogues containing Ar_1 or Ar_2 *para*-substitution that are electron withdrawing groups (eg, NO_2, Cl), the oxidized form **10** was directly obtained. Cases in which this position is unsubstituted or is *para*-methoxy substituted, the 5,8-dihydropyrido[2,3-*d*]pyrimidines **9** predominated.[24] In our study, all examples were isolated as the oxidized **10a-j**, including the *para*-methoxy substituent **10i**, presumably due to the extended reaction time (20 h) in air.

Microwave-assisted synthesis of the pyrido[2,3-*d*]pyrimidine derivative 10a:

The Doebner von Miller cyclocondensation reaction of 6-amino-2-thiouracil **6** with chalcone **3a** was also investigated under microwave conditions. Conventional heating of starting materials **6** and **3a** at reflux in DMF gave 44% yield of **10a** (table 1, entry 1). Replacing the solvent with trifluoracetic acid (TFA) and maintaining heating for 12 h decreased the yield

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(28%) (table 1, entry 2), with further heating resulting in no significant change. The use of microwave irradiation under catalyst free

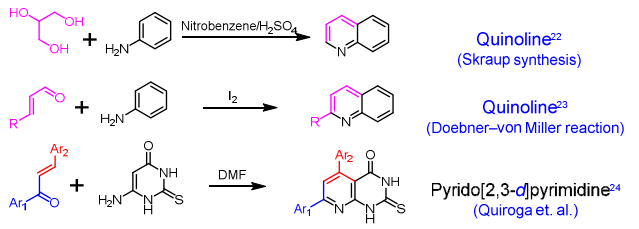
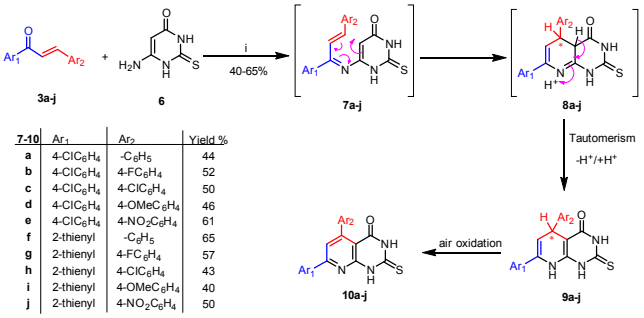


Figure 3. Skraup and Doebner-Miller synthesis of quinolone and Quiroga *et. al* synthesis of pyrido[2,3-*d*]pyrimidine.



7-10	Ar_1	Ar_2	Yield %
a	4-ClC_6H_4	-C_6H_5	44
b	4-ClC_6H_4	4-FC_6H_4	52
c	4-ClC_6H_4	4-ClC_6H_4	50
d	4-ClC_6H_4	4-$OMeC_6H_4$	46
e	4-ClC_6H_4	4-$NO_2C_6H_4$	61
f	2-thienyl	-C_6H_5	65
g	2-thienyl	4-FC_6H_4	57
h	2-thienyl	4-ClC_6H_4	43
i	2-thienyl	4-$OMeC_6H_4$	40
j	2-thienyl	4-$NO_2C_6H_4$	50

Scheme 2: Reagents and conditions: (i) Dry DMF, reflux 15-20 h.

conditions for 1 h in DMF at 160 °C gave **10a** in low yield (18%) (table 1, entry 3), which improved marginally with increased temperature and time (table 1, entry 4). Reaction monitoring by mass spectrometry analysis indicated the reduced form **9a** was synthesized, presumably as the reaction was performed in a sealed tube with a limited oxygen supply. Upon workup, the reaction was left overnight to crystallize resulting in an efficient oxidation of **9a** to **10a**. Application of higher temperature (240 °C) for longer time periods (4 h) in catalyst free conditions only gave traces of **10a** (table 1, entry 5).

The Doebner-Von Miller synthesis of quinoline has been reported to be catalysed by iodine, Lewis acids ($InCl_3$, $Yb(OTf)_3$, $Sc-(OTf)_3$, $ZnCl_2$) and Brønsted acids (TsOH, $HClO_4$, Amberlite).[23] Therefore, further optimization proceeded using catalytic ytterbium(III) trifluoromethanesulfonate $Yb(OTf)_3$ or indium chloride ($InCl_3$), which resulted in traces of the desired compound, however, the use of iodine (40 mol%) gave a 15% yield in a 1 h reaction (table 1, entry 8). Reducing the reaction time enabled the catalytic loading to be reduced to 10 mole%, providing **10a** in 59% isolated yield (table 1, entry 10).

Higher catalytic loadings of I_2 and $InCl_3$ (entry 8 and entry 7, respectively) resulted in the synthesis of **11** as a by-product in yields of 45% and 38%, respectively. The ^{1}H NMR data of **11** (see Supplementary Information) indicated a resonance for only one NH of the pyrimidine at 11.20 ppm and a resonance at 3.14 ppm was assigned to the two CH_3 of the *N*,*N*-dimethyl, which suggests a nucleophilic substitution reaction of the thiol group in **10a** yielding compound **11**. The proposed mechanism starts with the generation of dimethylamine, which undergoes nucleophilic addition to C2 of **10a** with the thiol group acting as the leaving group. The replacement of DMF with formamide, while maintaining catalytic I_2 at 40 mol% (entry 11) provides 2-aminopyrido[2,3-*d*]pyrimidine **12** (see Supplementary Information), which supports the proposed mechanism using either I_2 (entry 8) or the Lewis acid indium chloride (entry 7) as catalysts.

Table 1. Formation of the pyridopyrimidine **10a** under conventional heating or microwave irradiation in the presence or absence of catalyst

Entry	Solvent	Catalyst	Method	Time	Yield%
1	DMF	Free	Reflux	15 h	44
2	TFA	Free	Reflux	12 h	28
3	DMF	Free	MW (160 °C, 150 W)	1 h	18
4	DMF	Free	MW (240 °C, 150 W)	2 h	26
5	DMF	Free	MW (240 °C, 150 W)	4 h	Traces
6	DMF	$Yb(OTf)_3$ (40 mol%)	MW (160 °C, 150 W)	1 h	Traces
7	DMF	$InCl_3$ (50 mol%)	MW (160 °C, 150 W)	1 h	Traces
8	DMF	I_2 (40 mol%)	MW (160 °C, 150 W)	1 h	15
9	DMF	I_2 (30 mol%)	MW (160 °C, 150 W)	40 min	32
10	DMF	I_2 (10 mol%)	MW (160 °C, 150 W)	30 min	59
11	Formamide	I_2 (40 mol%)	MW (160 °C, 150 W)	1h	Traces

Pharmacokinetic properties and drug likeness calculations

Drug likeness estimations have been used as tools to minimize attrition in the process of drug discovery and refers to the similarity of compound properties to existing oral drugs.[27] Strategies to assess drug likeness include the Lipinsiki guidelines.[28] Interestingly, all compounds fulfil the Lipinsiki criteria, which indicate the possibility of oral bioavailability (Table 2). All compounds **10a-j** have 2-3 rotatable bonds (Table 2). Reduced molecular flexibility is measured with the number of rotatable bonds and is greatly linked to the oral bioavailability[29] with the number of rotatable bonds less or equal to ten potentially increases the oral bioavailability.[30] Percentage of the absorbed drugs (%ABS) is inversely proportional to the molecular volume and topological polar surface area (TPSA) and is calculated using the formula %ABS = 109 ± 0.345 x TPSA (Table 2).[31] Compounds **10a-j** possess %ABS ranging from 71.96 to 87.77. The number of rotatable bonds (*nrot*), TPSA, molecular volume and %ABS provide indications to sustain good oral bioavailability of drug candidates.

The drug likeness score (DLS) of all the compounds **10a-g** were calculated using MolSoft software and[32] received a positive drug likeness score from 0.05 to 0.66 (Table 2). Compounds eliciting positive values should be considered as drug candidates, while those with zero or negative values might not be considered as drug-like candidates.[31] The 2-thioxo-2,3-dihydropyrido[2,3-*d*]pyrimidinone **10h** counterpart emerged as the most similar to drugs (DLS = 0.66) followed by 2-thioxo-2,3-dihydropyrido[2,3-*d*]pyrimidinone **10a** and **10d** (Table 2).

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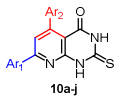
Our physicochemical calculation indicated that the compounds **10a-j** fulfilled the Lipinski guidelines and have positive drug-likeness model scores, which enables them to be potential oral bioavailable leads.

Table 2. Drug likeness calculations and Lipinski parameters of the compounds **10a**-**10j**



10a-j

Com	Ar_1	Ar_2	M. Wt[a]	LogP[b]	HBA[c]	HBD[d]	*n* violations[e]	Rule of 5	*n*rot[f]	TPSA[g]	Volume[h]	%ABS[i]	Drug-likeness model score
Rule			≤500	≤5.0	≤10	≤5	≤1						
10a	4-ClC_6H_4	-C_6H_5	365.04	4.39	3	2	0	Pass	2	61.55	297.05	87.77	0.61
10b	4-ClC_6H_4	4-FC_6H_4	383.03	4.51	3	2	0	Pass	2	61.55	301.98	87.77	0.36
10c	4-ClC_6H_4	4-ClC_6H_4	399.00	4.98	3	2	0	Pass	2	61.55	310.58	87.77	0.39
10d	4-ClC_6H_4	4-$OMeC_6H_4$	395.05	4.46	4	2	0	Pass	3	70.78	322.59	84.58	0.61
10e	4-ClC_6H_4	4-$NO_2C_6H_4$	411.03	4.46	5	3	0	Pass	3	107.37	320.38	71.96	0.22
10f	2-thienyl	-C_6H_5	337.03	3.81	4	2	0	Pass	2	61.55	274.22	87.77	0.05
10g	2-thienyl	4-FC_6H_4	355.02	3.94	4	2	0	Pass	2	61.55	279.15	87.77	0.57
10h	2-thienyl	4-ClC_6H_4	371.00	4.37	4	2	0	Pass	2	61.55	287.76	87.77	0.66
10i	2-thienyl	4-$OMeC_6H_4$	367.04	3.79	5	5	0	Pass	3	70.78	299.77	84.58	0.45
10j	2-thienyl	4-$NO_2C_6H_4$	383.03	3.96	6	3	0	Pass	3	107.37	297.56	71.96	0.22

[a] Molecular weight; [b] Lipophilicity; [c] Number of hydrogen bond acceptors; [d] Number of hydrogen bond donors, [e] Number of violations, [f] Number of rotatable bonds, [g] Topological polar surface area; [h] Molecular volume; [i] Percentage absorption.

Anti-microbial and Cytotoxicity Evaluation

Antibacterial activities and selectivity indices

Compounds **10a**, **10d-10i** showed promising inhibition zone values in comparison to the standard drugs ampicillin (Am), for Gram +ve bacteria, and gentamycin (Gm), for Gram -ve bacteria. These compounds were further tested for their minimum inhibition concentration (MIC) in μg mL^{-1} and showed excellent to good activities across the tested Gram +ve and Gram –ve bacteria (Table 3).

The activity of 2-thioxo-2,3-dihydropyrido[2,3-*d*]pyrimidinone **10a** was excellent against *S. pneumonia* and *E.coli* (MIC = 0.98 μg mL^{-1}) with a therapeutic index SI = 22.1 (Table 3). It also displayed good activity against *B. subtilis*, *S. aureus* and *P. aeruginosa* (MIC = 1.95, 3.9 and 3.9 μg mL^{-1}, respectively). Grafting fluorine or chlorine in the Ar2 *para*-position (**10b** and **10c** respectively) decreased activity against all strains. The addition of the *para*-methoxy group (**10d**) increased activity against *S. pneumonia* (MIC = 0.49 μg mL^{-1}), while maintaining the antibacterial activity of the standard drug against the Gram +ve bacteria *B. subtilis* and the Gram -ve bacteria *P. aeruginosa* and *E. coli* (MIC = 0.49, 0.98 and 0.49 μg mL^{-1}, respectively). Compound **10d** is the least cytotoxic compound with a SI against all the tested strains >46.8. Compound **10e** bearing an Ar2 4-nitro substituent retained the antibacterial activity of **10a** and showed excellent potency against *S. pneumonia* and *E. coli* (MIC = 0.98 μg mL^{-1}) and an acceptable selectivity index (35.2) and elicited good activities against the other three bacteria (MIC = 0.98 μg mL^{-1}) with SI = 17.7. The effect of substitution of the 7-*para*-chloropyrido[2,3-*d*]pyrimidines **10a-e**, grafting a methoxy or a nitro group onto the Ar2 *para*-position is beneficial for the activity, whereas, substitution with halogens decreased the activity dramatically.

Bioisosteric replacement of the 7-(*para*-chlorophenyl) motif in **10a-e** with a 2-thienyl gave compounds **10f-j** (Table 3). The unsubstituted **10f** reduced activity against *B. subtilis* and *E. coli* by a factor of four with an MIC value of 1.95 μg mL^{-1}. Insertion of fluorine or chlorine in the Ar2 *para*-position resulted in **10g** and **10h**, with the former maintaining activity against *S. pneumonia* (MIC = 0.98 μg mL^{-1}, SI of 30.9) and *B. subtilis* (MIC = 0.98 μg mL^{-1} and SI = 30.9), with acceptable activity against *S. aureus*, *P. aeruginosa* and *E. coli* (MIC = 1.95, 3.90 and 1.95 μg mL^{-1}, respectively). The chloro derivative **10h** was equipotent to the controls against *B. subtilis*, *P. aeruginosa* and *E. coli* (MIC = 0.49, 0.98 and 0.49 μg mL^{-1}, respectively) and possessed half the activity against *S. aureus* (MIC = 0.98 μg mL^{-1}) with a selectivity index of >13.7. Generally, the chloro counterpart **10h** showed the best activity against all the tested strains except for *S. pneumonia*. Substituting a OMe at the Ar_2 C4 of derivative **10f** resulted in a halving of activity against *S. pneumonia* and *B. subtilis* (MIC = 1.95 and 0.98 μg mL^{-1}, respectively), and a SI >51.3. The observed different activities of **10i** towards the rest of the strains were good to fair (MIC = 3.9 - 7.81 μg mL^{-1}, SI > 12.8).

The *para*-methoxy derivative **10i** is the least cytotoxic among all the tested compounds (IC_{50} > 100 μM). The presence of the methoxy group increased the activity of **10i** relative to the unsubstituted **10f** towards *S. pneumonia and B. subtilis*, while maintaining the activity against *S. aureus and p. aeruginosa*. Insertion of a nitro group in C4 of Ar_2 (**10j**), showed only small inhibition zones and was not measured for MIC determination due to reduced antibacterial activity. Generally, the thiophene containing analogues **10f-j** activities showed excellent to moderate activities, but with different trend to the 7-*p*-chlorophenyl analogues **10a-e.** The activity decreased in the order of 4-Cl>4-F>4-OMe>unsubstituted>nitro. Therefore, this might unravel the particularity of the incorporation of a small halogen or an electron donating group in the *para* position of the phenyl motif of the 5-phenyl-7-(thiophen-2-yl)-2-thioxo-2,3-dihydropyrido[2,3-*d*]pyrimidin-4(1*H*)-one **10f** (Table 3).

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Table 3. *In vitro* antibacterial activity screening of the synthesized compounds **10a-j** as inhibition Zones (IZ) in millimetres and minimum inhibitory concentrations (MICs) in μg mL^{-1}

Com	Gram +ve Bacteria								Gram -ve Bacteria				IC_{50} (μM) (CCD-33Co)
	Sp		Bs		Sa		MRSA		Pa		Ec		
	I.Z	MIC	I.Z	MIC	I.Z	MIC	I.Z	MIC	I.Z	MIC	I.Z	MIC	
10a	21.9±0.58	0.98 (22.1)	21.2±0.67	1.95 (11.1)	20.1±2.1	3.90 (5.6)	16.3±0.67	31.25 (0.7)	20.4±0.46	3.90 (5.6)	21.6±1.2	0.98 (22.1)	21.65
10b	12.3±0.58	NT	14.6±0.67	NT	13.5±1.2	NT	NT	NT	14.2±0.58	NT	16.3±0.46	NT	NT
10c	16.3±0.44	NT	17.2±1.2	NT	15.4±0.63	NT	NT	NT	16.7±0.58	NT	18.2 1.2	NT	NT
10d	24.2±0.44	0.49 >100	23.6±1.2	0.49 >100	21.3±0.63	1.95 (46.8)	19.6±0.58	3.90 (23.4)	22.4±0.58	0.98 (93.2)	24.6±1.2	0.49 >100	91.30
10e	22.4±1.2	0.98 (35.2)	21.3±0.25	1.95 (17.7)	20.6±1.2	1.95 (17.7)	18.3±0.25	7.81 (4.4)	21.3±0.25	1.95 (17.7)	22.4±0.63	0.98 (35.2)	34.50
10f	18.7±0.44	3.90	21.3± 0.25	1.95	20.2±0.25	3.9	NA	NA	18.3±0.44	7.81	21.2±0.44	1.95	NT
10g	22.3±0.63	0.98 (30.9)	23.4±1.5	0.98 (30.9)	20.9±0.58	1.95 (15.5)	NA	NA	19.2±1.2	3.90 (7.8)	21.4±1.2	1.95 (15.5)	30.26
10h	20.4±0.44	3.90 (13.7)	24.3±1.2	0.49 >100	23.5±0.23	0.98 (54.7)	21.3±1.2	1.95 (27.4)	23.4±0.63	0.98 (54.7)	24.9±0.58	0.49 >100	53.50
10i	21.3±0.44	1.95 >51.3	22.1±1.5	0.98 >100	20.3±0.67	3.90 >25.6	NA	NA	18.4±0.63	7.81 >12.8	20.3±1.2	3.90 >25.6	>100.00
10j	19.4±0.67	NT	18.1±0.67	NT	16.9±1.5	NT	NT	NT	16.3±1.5	NT	18.2 ±0.46	NT	NT
Am	23.8±0.63	0.98	32.4± 0.72	0.49	26.2±1.2	0.49							
Gm									22.3±0.58	0.98	25.4±1.2	0.49	
VM							20.3±0.63	3.90					

NT: Not tested: Selectivity Indices presented in brackets below the MIC values. **The screening organisms**, Gram +ve bacteria: *Streptococus pneumoniae* (RCMB 010010, Sp), *Bacillus subtilis* (RCMB 010067, Bs) and *Staphylococcus aureus* (RCMB) 010028, Sa), MRSA: Methicillin Resistant Staphylococcus aureus. Gram -ve bacteria: *Pseudomonas aeruginosa* (RCMB 010044, Pa) and *Escherichia coli* (RCMB 010053, Ec), **Am: Ampicillin, Gm: Gentamicin, Vm: Vancomycin**

The encouraging antimicrobial results with compounds **10a**, **10d-i** inspired the further testing of these compounds against MRSA (Table 3). The 2-thioxo-2,3-dihydropyrido[2,3-*d*]pyrimidinone **10h** provided a two-fold activity increase (MIC = 1.95 μg mL^{-1}, SI = 27.4) relative to that of vancomycin (MIC = 3.90 μg mL^{-1}). The 4-OMe derivative **10d** maintained the activity of the vancomycin (MIC = 3.90 μg mL^{-1}, with SI = 23.4) (Table 3), while, compounds **10a** (MIC = 31.25 μg mL^{-1} and SI = 0.7) and **10e** (MIC = 7.81 μg mL^{-1} and SI = 4.4) showed modest activity (Table 3). The 2-thioxo-2,3-dihydropyrido[2,3-*d*]pyrimidinones **10a**, **10d, 10e** and **10h** inhibited the growth of MRSA, however, the remaining compounds were inactive. Among the tested compounds **10a**, **10d-i**, the 2-thioxo-2,3-dihydropyrido[2,3-*d*]pyrimidinone **10h** (MIC = 1.95 μg mL^{-1}) emerged as the most active, eliciting double the activity of vancomycin.

Anti-fungal activity

The antifungal activity of compounds **10a**, **10d-i** (Table 4) showed that 2-thioxo-2,3-dihydropyrido[2,3-*d*]pyrimidinones **10d**, **10g** and **10i** as the most active agents against *A. fumigatus* with MIC values = 1.95 μg mL^{-1} followed by **10e**, **10f** and **10h** with MIC values = 3.90 μg mL^{-1}. Selectivity indices of the tested compounds ranged from 13.7 to >51.3 except for compound **10a** (0.7) and **10e** (8.9). The 2-thioxo **10d** analogue (MIC = 1.95 μg mL^{-1}) was the most active analogue followed by **10g** and **10h** (MIC = 3.90 μg mL^{-1}). The 2-thioxo-2,3-dihydropyrido[2,3-*d*]pyrimidinones **10f** and **10i** showed fair activity against *C. albicans* (MIC = 7.81 μg mL^{-1}) and compound **10e** demonstrated weak activity (MIC = 15.63 μg mL^{-1}). The selectivity indices of **10d**, **10h** and **10i** were within the acceptable range. The compounds showed better activities and selectivity indices against *A. fumigatus* than *C. albicans*.

Compound	*Aspergillus fumigatus*		*Candida albicans*	
	IZ	MIC	IZ	MIC
10a	19.2 ± 0.6	31.25 (0.7)	16.4 ± 1.2	31.25 (0.7)
10b	15.2 ± 1.2	NT	14.1 ± 0.3	NT
10c	15.2 ± 1.2	NT	13.4 ± 1.5	NT
10d	21.3 ± 1.5	1.95 (46.8)	20.8 ± 1.2	1.95 (46.8)
10e	20.1 ± 0.6	3.90 (8.9)	17.3 ± 0.6	15.63 (2.2)
10f	18.6 ± 1.2	3.90	18.3 ± 0.6	7.81
10g	21.3 ± 1.5	1.95 (15.5)	19.2 ± 0.6	3.90 (7.8)
10h	20.3 ± 1.2	3.90 (13.7)	19.3 ± 1.5	3.90 (13.7)
10i	20.6 ± 1.2	1.95 (>51.3)	18.3 ± 0.6	7.81 (>12.8)
10j	16.3 ± 0.6	NT	12.6 ± 2.1	NT
AB	23.7± 1.2	0.98	25.4± 0.6	0.49

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Table 4. *In vitro* antifungal activity screening of the synthesized compounds **10a-j** as inhibition Zones (IZ) in millimetres and minimum inhibitory concentrations (MICs) in µg mL^{-1}.

NT: Not tested, Selectivity indices between brackets, **I.Z:** inhibition diameter zones expressed in millimetres**, MIC:** Minimum inhibitory concentration expressed in µg/mL. **The screening organisms**: Mould: *Aspergillus fumigatus* (RCMB 02568), Yeast: *Candida albicans* (RCMB 05036).

Conclusions

The synthesis and antimicrobial evaluation of the functionalized 5,7-disubstituted pyrido[2,3-*d*]pyrimidine derivatives were performed with a microwave assisted synthesis of **10a** optimized to produce better yields in shorter times. Using DMF as the solvent and with 10 mol% of I_2 under microwave radiation for 30 minutes gave the best yield 59% yield. The compounds were tested *in vitro* for their antibacterial activity and moderate to excellent activity was observed for compounds **10a** and **10d-i** with MIC = 0.49–7.81 µg mL^{-1} (Figure 4). Compounds **10d-e** and **10h** displayed potent inhibition of MRSA (MIC =1.95–3.90 µg mL^{-1}). Among the tested compounds, derivatives **10d**, **10g** and **10i** exhibited the best anti-*aspergillus* activity (MIC = 1.95 µg mL^{-1}) and compound **10d** represent the best obtained against *Candia albicans* (MIC = 1.95 µg mL^{-1}). All the compounds showed excellent *in silico* pharmacokinetic properties calculation. The best compounds **10d** and **10h** will be subjected to a further cycle of derivatization to explore more about the structure antimicrobial relationship of this scaffold and to eventually develop the best derivatives into potentially novel and safe antimicrobial agents.

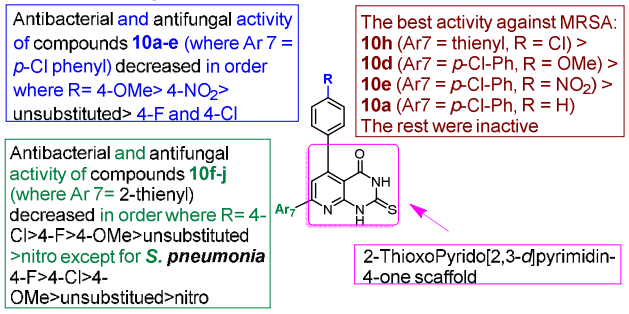

Figure 4. SAR of all the compounds against the tested bacterial and fungi.

Conflicts of interest

There are no conflicts to declare.

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An improved synthesis of pyrido[2,3-*d*]pyrimidin-4(1*H*)-ones and their antimicrobial activity