Evaluation of arylpiperazines from an internal compound library: broad-spectrum antimicrobial activity and favorable pharmacokinetic profiles

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ABSTRACT

The rise of multidrug-resistant pathogens underscores the critical need for novel antimicrobials. We screened 58 synthetic compounds from four chemical classes against 15 bacterial and 5 fungal clinically relevant species, identifying arylpiperazines **18**, **20**, **26**, **27** and **29** as potent broad-spectrum. These compounds exhibited low cytotoxicity and favorable pharmacokinetic profiles: optimal lipophilicity (LogP), high gastrointestinal permeability, metabolic stability (human/mouse liver microsomes), and aqueous solubility. Notably active against *Staphylococcus epidermidis*, *S. aureus*, *Lactobacillus paracasei*, and *Candida orthopsilosis*, they represent promising hit-to-lead candidates for antibacterial/antifungal development.

Keywords: Broad-spectrum antimicrobials, Hit discovery, Drug discovery, ADMET, Arylpiperazines

Introduction

Infections caused by resistant microorganisms represent a severe global health crisis. Epidemiological data reveal that hospital-acquired infections affect 5.7-19% of patients in developing countries, with pathogens such as *Staphylococcus aureus* and *Escherichia coli* being among the main culprits. Concurrently, fungal infections caused by *Candida* spp. show alarming mortality rates, particularly among immunocompromised patients, exacerbated by the emergence of strains resistant to conventional treatments. (1)

Some FDA-approved drugs, such as antifungals (posaconazole) and antibiotics (norfloxacin), share the arylpiperazine moiety in their structures. However, with the rapid rise of microbial resistance (projected to cause 20 million deaths annually by 2050) and the scarcity of new antimicrobials on the market, the development of new therapeutic agents has become urgent. In this context, the present work aimed to discover potential hits against 15 species of bacteria and 5 species of fungi from an internal library of synthetic compounds for lead optimization. (1)

We synthesized 58 compounds containing arylpiperazines and another structural moiety, characterized by $^{1}H/^{13}C$ NMR and HRMS. Comprehensive *in vitro* cytotoxicity and pharmacokinetic profiling established the safety and efficacy profile of promising candidates for future *in vivo* studies. Notable compounds **18**, **20**, **26**, **27** and **29** demonstrated: (1) broad-spectrum activity (MICs 12.5-15.6 µg/mL); (2) low cytotoxicity; and (3) favorable pharmacokinetic profiles (permeability, metabolic stability, and solubility). These results identify promising candidates to initiate a hit-to-lead optimization process for developing novel antimicrobial agents. (1)

Experimental

Synthesis

To synthesize the most compound, we employed two synthetic strategies principal. The first involves a nucleophilic aromatic substitution (SNAr) reaction between aryl chloride **S2** and piperazine derivative **S3**. Compound **S2** can be prepared via amide formation of *p*-chloronicotinic acid **S4**, while the piperazine derivative **S3** can be synthesized through various routes, including amide formation, SNAr, SN1, SN2, sulfonamide formation, or Chan-Lam coupling, starting from piperazine **S5**. The second strategy can utilize amide formation, SNAr, sulfonamide formation, or Chan-Lam coupling with amine **S6**, which can be synthesized via amide formation of *p*-chloronicotinic acid **S4** followed by SNAr with piperazine **S5** (Figure 1).

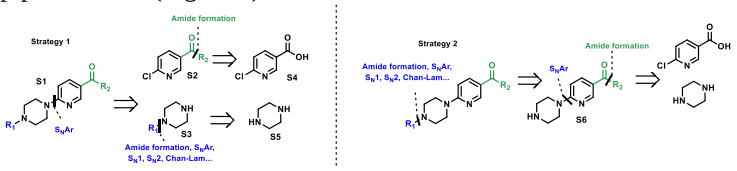

Figure 1. Retrosynthetic scheme of some arylpiperazines analogs.

Antimicrobial Activity

Minimum Inhibitory Concentrations (MICs) were determined via broth microdilution following CLSI guidelines with modifications. Bacterial strains were tested in Mueller-Hinton broth using resazurin (0.02%) as growth indicator, while fungal assays employed RPMI-1640 medium standardized to 1.2×10^{3} CFU/mL (McFarland 0.5). Plates were incubated at 37°C (24h bacteria/48h fungi) with amphotericin B and solvent controls. Test concentrations ranged 1.46-1000 µg/mL (5% DMSO).

Cytotoxicity Assays

THP-1 and L929 cells were cultured in RPMI-1640 (10% FBS) and differentiated with PMA (20 ng/mL). Compounds were evaluated at increasing concentrations (72-96h incubation), with viability assessed via AlamarBlue™ reduction. CC_{50} values were calculated using GraphPad Prism (nonlinear regression). HepG2 cytotoxicity data were obtained from previous studies.

ADME Profile

The distribution coefficient was determined by reverse-phase HPLC using an Ascentis RP-amide column. We established a calibration curve with eight reference compounds spanning a wide lipophilicity range (acyclovir, LogD 1.86 to amiodarone, LogD 6.10) to accurately calculate LogD values based on compound retention times. Gastrointestinal absorption potential was evaluated using the parallel artificial membrane permeability assay. Test compounds in PBS pH 6.5 (simulating intestinal conditions) were placed in donor chambers, separated by an artificial membrane from acceptor chambers containing PBS pH 7.4 (simulating blood pH). After 5 hours at 37°C (mimicking body temperature), compound transfer was quantified by LC-MS/MS to calculate permeability coefficients. Compounds were incubated with human and mouse liver microsomes (0.25 mg/mL) in the presence of NADPH cofactor to simulate liver metabolism. Aliquots were taken at 11 time points (0-60 minutes) and analyzed by LC-MS/MS to determine half-life and intrinsic clearance. Kinetic solubility was assessed in physiologically relevant buffers (PBS pH 2.0 for stomach, pH 7.4 for intestine) at 250 µM concentration. After 24 hours of shaking at 25°C (simulating gastrointestinal transit time), samples were centrifuged and supernatants analyzed by LC-MS/MS against a 5-point calibration curve for accurate quantification.

Results and Discussion

The antimicrobial evaluation of 58 synthetic compounds from four structural classes revealed several promising candidates with distinct activity profiles. Against Gram-positive bacteria, compounds **18**, **26**, **27**, and **43** demonstrated notable efficacy, particularly against *S. aureus* and *S. epidermidis* (MICs between 12.5-25 µg/mL, Figure 2). Compound **27** emerged as the most promising candidate, demonstrating consistent potency against *S. aureus*/*S. epidermidis* (MIC = 12.5 µg/mL) and maintaining better activity against cariogenic bacteria (*S. sobrinus*, *S. mitis*, *S. mutans*, *L. paracasei*) compared to other tested compounds, confirming its superior antibacterial profile (Figure 2). The structure-activity relationship analysis highlighted the critical role of the piperazine linker, with *N,N'*-disubstituted derivatives containing nicotinic ring exhibiting enhanced potency.

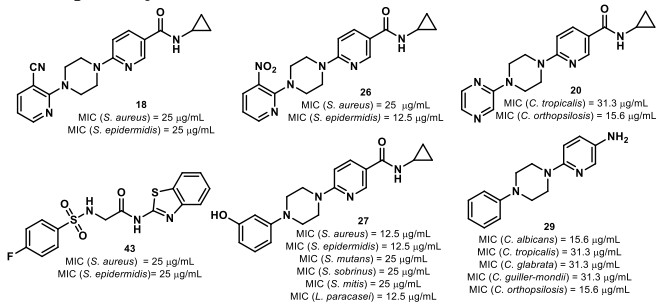

Figure 2. Structure of compounds 18, **20**, **26**, **27**, **29** and their antimicrobial activity.

In antifungal screening, eight compounds showed activity against *Candida* species, derivatives **18** and **29** displaying particularly broad-spectrum efficacy. Compound **29** inhibited all five tested *Candida* species (MICs between 15.6-31.3 µg/mL), including emerging clinically relevant strains like *C. orthopsilosis* and *C. guillermondii* (Figure 2). The maintained potency against *non-albicans* species is especially significant given the rising incidence of infections caused by these treatment-resistant fungi. The structural similarity between active antibacterial and antifungal compounds suggests these arylpiperazine derivatives may act through conserved microbial targets or membrane disruption mechanisms. (1)

Comprehensive ADME profiling of the most active compounds revealed excellent drug-like properties. All tested derivatives showed balanced lipophilicity (logD 2.35-3.07), high gastrointestinal permeability (PAMPA >7.99×10^{-6} cm/s), and good metabolic stability in both human and mouse liver microsomes ($t_{1/2}$ up to 10.5 hours) (Table 1). Particularly noteworthy was the aqueous solubility profile (>83 µM across pH 2.0 and pH 7.4), suggesting favorable absorption characteristics. Cytotoxicity assessments revealed generally low citotoxicity (CC_{50} >50 µM in most cell lines), with compound **20** showing exceptional safety across all tested cell types (CC_{50} >100 µM). These combined pharmacokinetic properties and antimicrobial activity position these compounds, particularly **18**, **20**, **26**, **27** and **29**, as promising hits and leads candidates for further optimization toward broad-spectrum antimicrobial agents with potential for oral administration. (1)

Table 1. ADMET profile of compounds **18**, **20**, **26**, **27** and **29**.

Comp.	log D	PAMPA GI	KS	MLM		HLM		CC_{50} HepG2	CC_{50} THP-1	CC_{50} L929
				$T_{1/2}$	CL	$T_{1/2}$	CL			
18	2.88	14.73	>100	3.1	14.8	1.9	24.4	NT	264	28
20	2.35	8.88	>83	10.5	<12	1.8	25.2	>100	780	435
26	3.07	22.03	>100	2.3	20.0	2.0	22.8	51	183	68
27	2.75	7.99	>100	1.9	24.0	1.7	26.8	36	120	105
29	2.45	17.87	>100	1.2	38.4	2.3	20.0	NT	1228	71

logD: lipophilicity at pH 7.4; PAMPA GI: gastrointestinal permeability in 10^{-6}cm s^{-1}; KS: kinetic solubility at pH 2.0 and 7.4 (µM); $T_{1/2}$: microsomal half-life (h); CL (mic): Intrinsic clearance in microsome (µL/min/ mg); CC_{50}: *in vitro* cytotoxicity (µM); NT: Not tested.

Conclusions

This study identified potent antimicrobial candidates among 58 synthetic compounds screened against 15 bacterial and 5 fungal species. The pyridylpiperazine derivatives **26** and **27** showed exceptional broad-spectrum antibacterial activity, while compounds **18**, **20**, **29** exhibited strong antifungal effects. All active compounds demonstrated low cytotoxicity in HepG2, THP-1, and L929 cell lines. Notably, five of the six most promising hits were pyridylpiperazine derivatives, which also displayed favorable ADME profiles for oral administration. These results position this class compounds, particularly **26**, **27**, and **29**, as prime candidates for hit-to-lead optimization and future *in vivo* studies to develop novel broad-spectrum antimicrobial agents.

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Reference

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