RSC Medicinal Chemistry

ROYAL SOCIETY OF CHEMISTRY

RESEARCH ARTICLE

View Article Online
View Journal

Cite this: DOI: 10.1039/d0md00175a

Rational design, synthesis and testing of novel tricyclic topoisomerase inhibitors for the treatment of bacterial infections part 2†

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Received 25th May 2020,
Accepted 7th August 2020

DOI: 10.1039/d0md00175a

rsc.li/medchem

Building on our previously-reported novel tricyclic topoisomerase inhibitors (NTTIs), we disclose the discovery of REDX07965, which has an MIC_{90} of 0.5 μg mL^{-1} against *Staphylococcus aureus*, favourable *in vitro* pharmacokinetic properties, selectivity *versus* human topoisomerase II and an acceptable toxicity profile. The results herein validate a rational design approach to address the urgent unmet medical need for novel antibiotics.

1. Introduction

The structure activity relationship (SAR) of a number of potent, novel tricyclic topoisomerase inhibitor (NTTI) derivatives *e.g.* REDX05931 (Fig. 2C) has been described in part 1 of this work.[1] Despite displaying favourable MICs against a range of Gram-positive and fastidious Gram-negative bacterial strains, including ≤4 μg mL^{-1} against the clinically relevant FQ resistant strain of *N. gonorrhoeae* (WHO L),[2] REDX05931 and closely related analogues inhibited multiple forms of CYP enzymes. Our SAR analysis indicated that the 2′-methylene amino moiety was responsible for this profile, which precluded this series from further study. As such, a new medicinal chemistry strategy was necessary.

Multiple generations of fluoroquinolones have depended on saturated heterocycles in the C-7 position. Optimisation of the saturated amine heterocycle at this position of fluoroquinolones has led to improvements in the antibacterial spectrum of activity; covering Gram-positive, Gram-negative and anaerobic bacteria, whilst also improving *in vitro* pharmacokinetic and physicochemical properties.[3] To address the low solubility of the aryl C-7 NTTIs, and to potentially broaden the spectrum of activity to include a range of Gram-negative organisms, we explored the addition of saturated heterocycles in the C-7 position (R_1; Fig. 1a). Structural superposition of REDX05931 (Fig. 2B and C) onto the X-ray crystal structure of moxifloxacin (Fig. 1b and 2A) bound to topoisomerase IV (PDB 2XKK) indicates that the tricyclic core provides a good overlap with the moxifloxacin quinolone core. Therefore, we reasoned that saturated heterocycles in the C-7 position of the NTTI series could provide similar binding interactions to our original work and maintain activity against FQ resistant organisms.[1]

2. Results and discussion

2.1 SAR of R_1 saturated heterocyclic amine substitution

Matched pair analysis of all saturated heterocycles in the R_1 position where X = C–Cl *vs.* X = C–H (Table 1, REDX06598 *vs.* REDX05762, REDX05855 *vs.* REDX07181, REDX05840 *vs.* REDX07815) clearly suggests that improved potency against *S. aureus* ATCC 29213, *S. pneumoniae* ATCC 49619 and *E. coli* ATCC 25922 (ref. 7) is observed with X = C–Cl, confirming that the nature of the X group plays an important role in potency. The results are consistent with the premise that the steric bulk of the Cl, and REDX05931 consequent out of plane distortion of the core encourages DNA breakage once intercalated.[4]

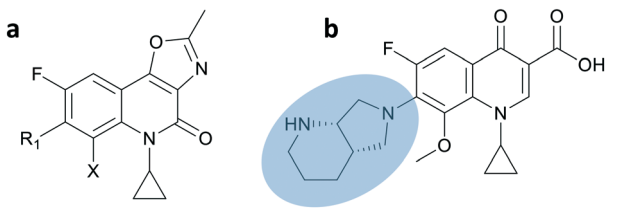

Fig. 1 a: Points of derivatisation of our NTTI series. b: Comparative fluoroquinolone moxifloxacin, highlighting the saturated heterocycle in the C-7 position.

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† Electronic supplementary information (ESI) available. See DOI: 10.1039/d0md00175a

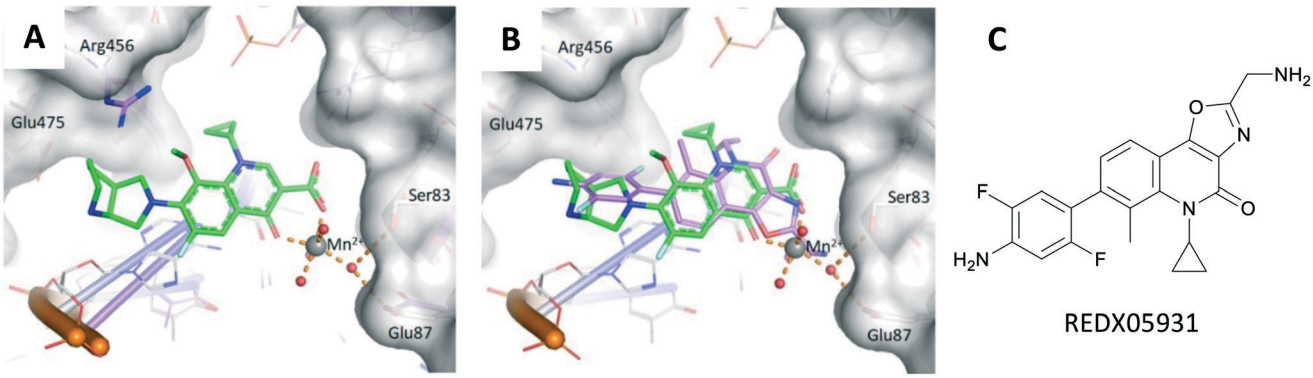


Fig. 2 A) Moxifloxacin bound to topoisomerase IV taken from PDB 2XKK (lime green). B) Overlaid superposition of REDX05931 (magenta) over moxifloxacin (lime green). C) Chemical structure of REDX05931.

Substitution of a methylene amino group from a primary amine to a secondary amine (REDX07948 and REDX07046) did not improve the microbiological profiles, nor did introduction of steric bulk around the methylene region (REDX05848 and REDX05960; Table 1). Additionally, the *N*,*N*-dimethyamino group (REDX07156 and REDX06885) displayed a markedly reduced microbiological profile, indicating the necessity of an accessible H-bond donor. Microbiological data for the 3-amino pyrrolidine derivative (REDX05761) compared with the methylene amino pyrrolidine derivatives suggest that the extended amine has a beneficial effect on activity. This trend is also seen with the six-membered counterparts. The minimum inhibitory concentration (MIC) of the piperazine analogue (REDX06937) was 2 μg mL^{-1} against methicillin-sensitive *S. aureus* ATCC 29213 (MSSA); its 4-aminopiperidine analogue (REDX07181) demonstrated an MIC of 0.25 μg mL^{-1}. Interestingly these six-membered saturated heterocycles consistently demonstrated a loss of activity against resistant strains (data not shown). Replacing the terminal amino group with a hydroxy group in REDX05944 (3-hydroxypyrrolidine) resulted in a loss of activity, with an MIC against MSSA of 32 μg mL^{-1}. Bicyclic systems such as REDX06876 and REDX06921 did not improve the microbiological profile, indicating that the orientation and lipophilicity surrounding the terminal nitrogen is important. The latter point being accentuated with the comparative analogues REDX06937 (piperazine) and REDX06803 (2,6-dimethyl piperazine) with MICs of 0.25 and 8 μg mL^{-1}, respectively, *versus* MSSA. The fused core derivatives showed a strong preference for the (*S*)-stereoisomer (REDX06215 *vs.* REDX06415), consistent with the relative activities of levofloxacin and dextrofloxacin.[5] However, due to an eight-fold increase in MIC against methicillin-resistant *S. aureus* strains (namely MRSA NRS74), the morpholino derivative core was not pursued as a priority.

A thorough investigation of related topoisomerase inhibitors revealed several studies of an quinazolinedione series,[6,7] cumulating in the discovery of PD0305970 (Fig. 3a). A published SAR examination of aminoalkyls at the C-7 revealed the absolute stereochemistry of the (*S*)-1-((*R*)-pyrrolidin-3-yl)ethan-1-amine at the C-7 position impacts the microbiological activity.[8] A crystal structure of PD0305970 in topoisomerase IV from *S. pneumoniae* reveals that the 3-(aminomethyl)pyrrolidinyl is surrounded by Arg 456, Glu 474, Glu 475 and Asp 435 residues (ParE).[7] Based on this result and our computational modelling of our NTTI series; we hypothesised that incorporation of the most active stereoisomer ((*S*)-1-((*R*)-pyrrolidin-3-yl)ethan-1-amine) at the C-7 position of our NTTI series would orientate the primary amine towards GyrB-Glu466 (ref. 9) (*S. aureus* nomenclature). Using this rationale in conjunction with the learnings from part 1 (ref. 1) and the SAR described above we synthesised REDX07965 (Fig. 3b).

REDX07965 (Fig. 3b) displayed superior activity within the series, with a *S. aureus* MIC_{90} (ref. 14) of 0.5 μg mL^{-1} against clinical isolates (see Table S1†). Assessment of target bacterial enzyme inhibition by REDX07965 showed it to be a potent inhibitor of DNA gyrase and topoisomerase IV displaying an IC_{50} of 1.82 and 6.10 μM respectively (see Table S2†).

2.2 Profiling of REDX07965

REDX07965 demonstrated excellent potency against a range of *S. aureus* strains, including fluoroquinolone and multidrug-resistant (MDR) strains. Despite reduced activity *versus Enterococcus faecium*, REDX07965 displayed good activity against the Gram-negative organisms *E. coli*, *H. influenzae* and *N. gonorrhoeae*.[7] A microbiological profile of REDX07965 is shown in Table 2.

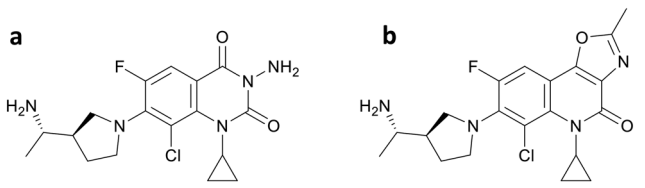
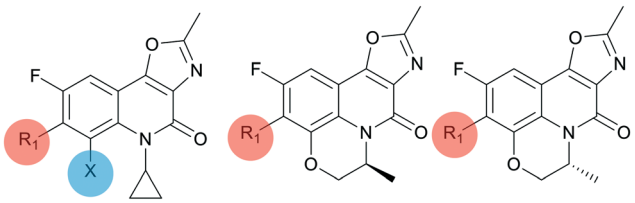


Fig. 3 a: Chemical structure of PD0305970. b: Chemical structure of REDX07965.

Table 1 Selected microbiological and solubility data of saturated R_1 analogues. MICs data are based on at least two experimental replicates; data in μg mL^{-1}



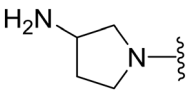
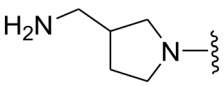
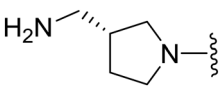
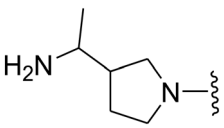
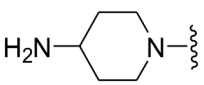
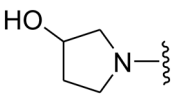
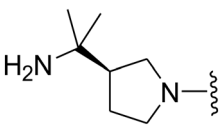
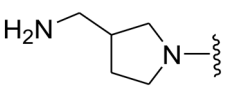
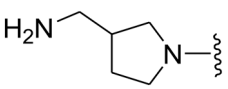
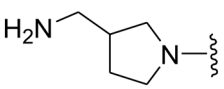
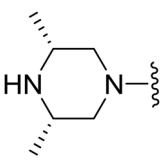
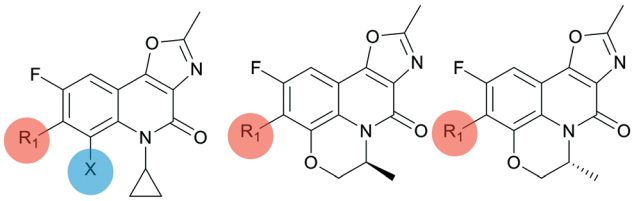
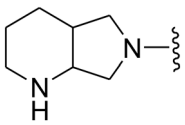
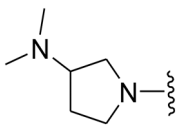
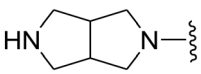
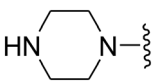
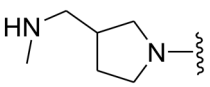
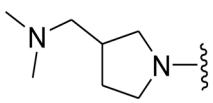
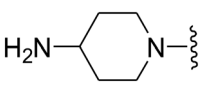
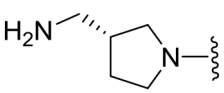
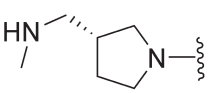
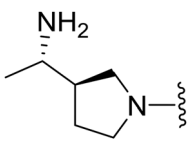
#	R_1	X	MIC *S. aureus* ATCC 29213[a]	MIC *S. pneumoniae* ATCC 49619[a]	MIC *E. coli* ATCC 25922[a]	MIC *H. influenzae* ATCC 49247[a]	TD sol[b] (mg L^{-1})
REDX05761		H	2	8	1	1	n.d.
REDX05762		H	0.5	0.5	2	8	n.d.
REDX05840		H	1	0.5	2	8	1403
REDX05848		H	1	1	4	4	1350
REDX05855		H	1	1	2	1	1403
REDX05944		H	32	64	64	4	n.d.
REDX05960		H	2	2	8	16	1353
REDX06215		FC1	0.5	0.5	2	4	ND
REDX06415		FC2	64	128	64	128	ND
REDX06598		Cl	0.12	0.12	1	n.d.	950
REDX06803		Cl	8	16	4	8	543

Table 1 *(continued)*



#	R_1	X	MIC *S. aureus* ATCC 29213[a]	MIC *S. pneumoniae* ATCC 49619[a]	MIC *E. coli* ATCC 25922[a]	MIC *H. influenzae* ATCC 49247[a]	TD sol[b] (mg L^{-1})
REDX06876		Cl	2	4	2	8	1061
REDX06885		Cl	4	4	4	8	n.d.
REDX06921		H	2	4	4	2	1195
REDX06937		Cl	2	4	0.5	0.5	885
REDX07046		Cl	0.25	0.25	1	1	1181
REDX07156		Cl	1	2	4	16	1194
REDX07181		Cl	0.25	0.5	0.5	1	922
REDX07815		Cl	0.12	0.12	0.5	1	601
REDX07948		Cl	0.25	0.25	2	1	—
REDX07965		Cl	0.06	0.015	0.5	1	n.d.

[a] Strain description – see ref. 13. [b] TD sol = thermodynamic solubility.

REDX07965 demonstrated an acceptable *in vitro* ADMET profile (Table 3). However, *in vitro* investigation of cardiac ion channel activity revealed inhibition of hERG, Ca^{2+} and Na^{+} channels (IC_{50} 28.7, 21.5 and 93.6 μM, respectively).

Table 2 Microbiological profile of REDX07965

Strain[a]	MIC (μg mL^{-1})	Strain	MIC (μg mL^{-1})
S. aureus ATCC 29213	0.06	*S. aureus* NRS271	0.5
S. aureus NRS70	0.06	*S. aureus* VRS8	0.5
S. aureus NRS100	0.12	*E. faecalis* ATCC 29212	0.12
S. aureus NRS106	0.12	*S. epidermidis* ATCC 12228	0.06
S. aureus NRS107	0.12	*S. epidermidis* NRS101	1
S. aureus NRS108	0.12	*E. faecium* ATCC 19434	8
S. aureus NRS384	0.12	*E. faecium* ATCC 700221	8
S. aureus ATCC 43300	0.12	*S. pneumoniae* ATCC 49619	0.015
S. aureus NRS74	0.12	*S. pyogenes* ATCC 19615	0.015
S. aureus NRS1	0.25	*E. coli* ATCC 25922	0.5
S. aureus NRS127	0.5	*H. influenzae* ATCC 49247	1
S. aureus VRS1	0.5	*N. gonorrhoeae* ATCC 49226	0.12
S. aureus NRS482	0.12	*N. gonorrhoeae* ATCC 700825	0.03

[a] Strain description – see note 13.

Table 3 Selected ADMET properties of REDX07965

In vitro assay (units)	Result
HepG2 cytotoxicity CC_{50} (μg mL^{-1})	32–64
Human PPB (% free)	24.4
% LBF (predicted from Hheps)	27.1
Hhep predicted half life (min)	461.9
Hhep clint (μL min^{-1} per 10^6 cells)	3.0
Caco2 efflux ratio	2.2
Caco 2 Papp (10^{-6} cm s^{-1})	20.7
CYP1A2 IC_{50} (μM)	30
CYP2C9 IC_{50} (μM)	16.4
CYP2C19 IC_{50} (μM)	30
CYP2D6 IC_{50} (μM)	30
CYP3A4 IC_{50} (μM)	17.5
CYP3A4T IC_{50} (μM)	30
Kinetic solubility pH 7.4 (μM)	>100
hERG patch IC_{50} (μM)	28.7
NaV1.5 patch IC_{50} (μM)	21.5
CaV1.2 patch IC_{50} (μM)	93.6
Genotox BlueScreen (−S9) at 200 μM	Non-toxic
Genotox BlueScreen (+S9) at 200 μM	Toxic

The low IC_{50} for the hERG ion channel suggested a risk of QT prolongation at a therapeutic dose. Additionally, despite an acceptable cytotoxicity profile (CC_{50} 32–64 μg mL^{-1} in a HepG2 cytotoxicity assay), the compound proved to be genotoxic with the addition of S9 at 200 μM (BlueScreen assay[10]). For these reasons, further development of this compound was terminated (Table 3).

3. Conclusion

Using a rational, structure-guided drug design strategy, we have identified a promising lead compound (REDX07965), that inhibits bacterial DNA gyrase and topoisomerase IV with broad-spectrum antibacterial potency and has favourable *in vitro* pharmacokinetic properties. However, the risk of cardiac and genetic toxicology, revealed by the hERG and BlueScreen assays respectively, precludes the further development of REDX07965.

4. Chemistry

4.1 Chemistry of saturated LHS analogues

The synthesis of the saturated C-7 derivative series began with the synthesis of a late stage versatile intermediate (**6**). Trifluorobenzoic acid (**1**) was activated with oxalyl chloride followed by addition and ring closure with ethyl isocyanoacetate under basic conditions to give a C–H oxazole (**2**).[11] This can be hydrolytically cleaved with aqueous HCl to give the hydroxylamine, followed by ring closure with triethylorthoacetate to give the methyl oxazole (**3**). The ester was hydrolysed under basic conditions and activated/functionalised with oxalyl chloride-cyclopropylamine to give amide (**5**). Intramolecular SnAr gave the versatile tricyclic intermediate (**6**). This advanced intermediate allowed for derivatisation at the C-7 position *via* microwave-assisted intermolecular SnAr to give intermediate (**7**). At this point, the tricyclic core can be further functionalised by the introduction of a chlorine in the C-8 position, by addition of 1 equivalent of 1,3-dichloro-5,5-dimethyl-imidazolidine-2,4-dione (DCDMH) in DCM at room temperature.[12] The saturated heterocycles were then deprotected with TFA in DCM (if the saturated heterocycle contains a Boc protected amine) to give compounds **9a**/**b**.

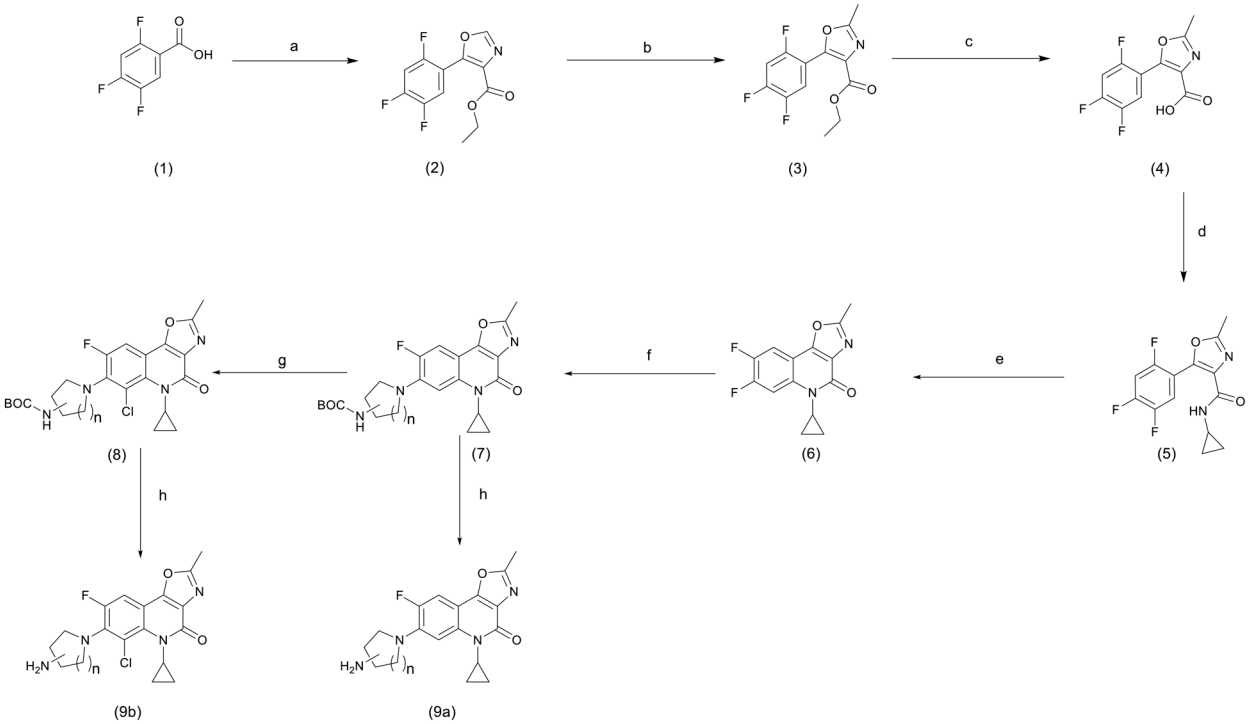
The fused core intermediate was synthesised in an analogous procedure to that previously described[1] using tetrafluorobenzoic acid (**10**). The tetracyclic core (**11**) was then derivatised using standard SnAr conditions described in Scheme 1, procedure f (Scheme 2).

Associated content

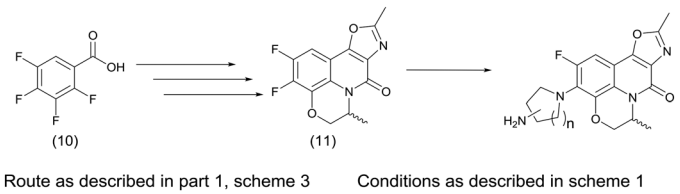
Table S1 and experimental data are available in ESI.†

Funding

This study was funded by Redx Pharma Plc in collaboration with the Royal Liverpool and Broadgreen University Hospitals NHS Trust.



Scheme 1 Reagents and conditions: (a) (**1**) oxalyl chloride, DCM, 0 °C-rt, 2 h, quant; (**2**) ethyl isocyanoacetate, THF, NEt_3, 0 °C-rt 16 h, 48%. (b) (**1**) 1 M HCl, 1,4 dioxane, rt, 72 h, quant; (**4**) triethylortho acetate, 110 °C, 2 h, quant. (c) LiOH, 1,4-dioxane, rt, 16 h, 98%; (d) (**1**) oxalyl chloride, DMF, DCM, rt, 1 h, quant; (**2**) NEt_3, cyclopropylamine, DCM, rt, 18 h, 79%. (e) K_2CO_3, 18-crown-6, DMSO, 140 °C, 50 min, 64%. (f) Amine, DIPEA, MW,140 °C, 80 min, 71–91% (g) DCDM, DCM, rt, 20 min, 43–59%. (h) TFA, DCM, rt, 18 h, 85–99%.



Scheme 2 General synthesis of tetracyclic core.

Conflicts of interest

All authors were or are employees of Redx Pharma and may hold shares or share options in the company.

Acknowledgements

We are grateful for the provision of bacterial strains by the Network on Antimicrobial Resistance in *Staphylococcus aureus* (now part of BEI Resources, Manassas, USA), to IHMA Europe Sarl (Epalinges, Switzerland) for performing the clinical isolate MIC_{90} studies, to Inspiralis Ltd (Norwich, UK) for performing the DNA supercoiling, decatenation and cleavage assays, to Apconix for performing the multi-ion channel assessment (K^+, Ca^{2+}, Na^+) and to Gentronix for performing the genotoxicity assessments.

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14 Abbreviations used: TMP – trimethoprim, LZD – linezolid, FQ – fluoroquinolones, TET – tetracyclines, VAN – vancomycin, MUP – mupirocin, ERY – erythromycin, AMG – aminoglycoside. WHO – World Health Organisation, CDC – Centers for Disease Control and Prevention, MDR – multidrug resistant, MSSA – methicillin-susceptible *S. aureus*, MRSA – methicillin-resistant *S. aureus* ADMET – adsorption, distribution, metabolism, excretion, toxicity, Physchem – physical chemical, TD – thermodynamic, PPB – plasma protein binding, Cli – intrinsic clearance, HEP – hepatocytes, SOL – solubility, MIC_{90} = minimum inhibitory concentration required to inhibit the growth of 90% of organisms.