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Rational design, synthesis and testing of novel tricyclic topoisomerase inhibitors for the treatment of bacterial infections part 1†

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The alarming reduction in drug effectiveness against bacterial infections has created an urgent need for the development of new antibacterial agents that circumvent bacterial resistance mechanisms. We report here a series of DNA gyrase and topoisomerase IV inhibitors that demonstrate potent activity against a range of Gram-positive and selected Gram-negative organisms, including clinically-relevant and drug-resistant strains. In part 1, we present a detailed structure activity relationship (SAR) analysis that led to the discovery of our previously disclosed compound, REDX05931, which has a minimum inhibitory concentration (MIC) of 0.06 $\mu\text{g mL}^{-1}$ against fluoroquinolone-resistant *Staphylococcus aureus*. Although *in vitro* hERG and CYP inhibition precluded further development, it validates a rational design approach to address this urgent unmet medical need and provides a scaffold for further optimisation, which is presented in part 2.

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1. Introduction

As reported by the World Health Organisation (WHO) and the Centers for Disease Control and Prevention (CDC), there is a clear and growing unmet need for novel antibacterial agents to combat infections.^{1,2} The rapid spread of multi-drug resistant (MDR) bacterial strains, coupled with the lack of new antibiotics in clinical development, could soon result in society entering a ‘post-antibiotic era’.^{3–5} For example, in a recent hospital admission a patient presented with an acute bacterial infection resistant to 26 different antibiotics, including all aminoglycosides and polymyxins.⁶ Left unaddressed, it is predicted that by 2050 deaths attributed to bacterial infections could rise to 10 million per year, with an estimated cost to the global economy of \$100 trillion.⁷

Staphylococcus aureus (*S. aureus*) causes a range of illnesses, from skin and soft tissue infections, to pneumonia, endocarditis and sepsis. The CDC estimates that there were around 80 000 *S. aureus* infections leading to 11 000 deaths in the US in 2011 alone. Despite a near decade-long decline as a result of improved antibiotic stewardship and hospital hygiene,⁸ Public Health England (PHE) has recently (2016/2017) reported an 8.9% rise in incidence of *S. aureus* bacteraemia of 8.9%.⁹

Fluoroquinolones are a first-line antibacterial therapy used to treat both Gram-negative and Gram-positive bacterial infections. Fluoroquinolones act by inhibiting bacterial type II topoisomerases, DNA gyrase and topoisomerase IV, whose function are to maintain DNA topology during replication.¹⁰ DNA gyrase/topoisomerase IV are tetrameric enzymes that consist of two GyrA (DNA gyrase)/ParC (topoisomerase IV) subunits and two GyrB (DNA gyrase)/ParE (topoisomerase IV) subunits. Fluoroquinolones inhibit the enzymes by creating a cleaved complex between these bacterial enzymes and DNA. From a combination of mechanistic studies, and crystal structures of enzyme–fluoroquinolone complexes, a model for fluoroquinolone binding has been established.^{11–14} A primary interaction involves chelation by the β -keto acid moiety on the fluoroquinolone to a non-catalytic Mg^{2+} ion, which is anchored to the active site within GyrA *via* a water-mediated metal ion bridge with serine and an acidic residue^{15,16} (Fig. 1A). Human type II topoisomerases lack these key residues, resulting in fluoroquinolone selectivity for bacterial enzymes. The fluoroquinolone core also intercalates with the bacterial DNA (Fig. 1B). This π -stacking interaction plays a key role in determining fluoroquinolone potency¹³ which is likely to be influenced by the distribution of electron density within the fluoroquinolone core. The amine functionality at C7 (Fig. 1D) has no obvious contacts with the enzymes across multiple species but may interact with Arg458 (*S. aureus* GyrA nomenclature, Fig. 1C).

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Fig. 1 Moxifloxacin bound to *S. aureus* DNA gyrase, taken from PDB: 5CDQ. A) Moxifloxacin chelation to a non-catalytic magnesium ion, which in turn has polar interactions with Ser84 and Glu88 through an expected water network, as observed in homologous crystal structures 5BTI through to 5BTN. B) Intercalation of moxifloxacin with DNA. C) Nearby amino acid Arg458 at the C-7 position. D) 2D representation of moxifloxacin, highlighting the C-7 position.

Fluoroquinolone resistance (FQR) is frequently driven by point mutations in the quinolone resistance-determining regions (QRDRs) of the DNA gyrase (encoded by GyrA and GyrB) and topoisomerase IV (encoded by ParC and ParE; known as

GrlA and GrlB for *S. aureus*),^{17–20} resulting in target-based resistance. One of the most widely-studied resistance mechanisms is the mutation of the key serine and/or acidic residues within the active site of GyrA, which disrupts the

Fig. 2 Chemical structures of A) zoliflodacin, B) gepotidacin, and C) EV-035. D) EV-035 orthogonal view, exemplifying the torsion angle between the methyl/cyclopropyl group.

water-Mg²⁺ ion bridge, thereby preventing fluoroquinolone chelation.^{21–23} Recently, two new classes of bacterial topoisomerase II inhibitors, which do not rely on this interaction, have completed phase II clinical trials. The spiropyrimidinetrione, zoliflodacin (Fig. 2A), interacts with DNA gyrase and topoisomerase IV in a similar pocket to fluoroquinolones, and demonstrates activity against Gram-positive and fastidious Gram-negative organisms.^{16,24–28} The crystal structure of a closely-related compound, quinolone pyrimidone trione-1 (QPT-1), provides compelling evidence that this series binds in a unique fashion compared to fluoroquinolones, relying upon interactions with DNA gyrase Asp437 (*S. aureus* nomenclature) and DNA. A number of non-fluoroquinolone ‘novel bacterial type II topoisomerase inhibitors’ (NBTIs), (such as gepotidacin, Fig. 2B), have also been reported in recent years, and are under active development. The NBTI class works by binding to DNA gyrase in a pocket close to, but distinct from, the fluoroquinolone binding site,^{15,16,29} and intercalating into the DNA. As a result, this class does not display cross-resistance with fluoroquinolones.^{30,31} Another series of experimental bacterial topoisomerase II inhibitors are the 2-pyridones (isomeric quinolones), exemplified by EV-035 (Fig. 2C). This series of compounds binds to the same pocket as the fluoroquinolones with similar interactions, owing to their β -keto acid functionality and 2-pyridone core. EV-035 displays broad-spectrum *in vitro* activity against both wild type (WT) and fluoroquinolone-resistant strains. This is ascribed to the apparent increased wedge angle between the methyl/cyclopropyl motifs³² (Fig. 2D) which is thought to be complementary to the slightly distorted bound DNA in this region.³³

An early-stage drug discovery project was undertaken to identify novel antibacterials unaffected by FQR mechanisms, leading to the discovery of REDX04139 (Fig. 3), which lacks the β -keto-acid function essential for fluoroquinolone activity and incorporates the aromatic C-7 substituent found in EV-035. REDX04139 demonstrated excellent *in vitro* microbiology against all tested variants of *S. aureus*, including fluoroquinolone and multidrug-resistant strains, with a MIC₉₀ of 0.25 $\mu\text{g mL}^{-1}$. It also demonstrated an excellent microbiological profile against a range of resistant *Neisseria gonorrhoeae* strains with MICs ranging from 0.06 to 1 $\mu\text{g mL}^{-1}$.^{34,35} It is noteworthy to add, however, that there was no measured activity against *Escherichia coli* (*E. coli*), possibly due to the relatively high lipophilicity (measured $\log D = 2.96$) observed for REDX04139, which is known to disfavor permeability into Gram-negative bacteria.^{36,37} REDX04139

Fig. 3 Structure of REDX04139.

Fig. 4 Areas of derivatisation.

had low thermodynamic solubility at physiological pH (3 μM). Without an obvious solubilising group, this property alone would require extensive optimisation for progression. For these reasons, we decided to conduct a medicinal chemistry optimisation program to explore the SAR of this series (Fig. 4), focusing on modifications of the core (X and R₁), R₄ substitution (aromatic and saturated heterocycles), as well as simple substitution at the oxazole 2-position (R₃). Since our initial publications,^{34,35} two papers on an imidazopyrazinones (IPYs) series have been published which have a similar scientific rationale to our novel tricyclic topoisomerase inhibitor (NTTI) series. Although similar, the IPY series did not demonstrate superior potency to the NTTI series and displayed genotoxic liabilities.^{38,39} Herein, we describe the results of our initial work, presenting the chemical, *in vitro* biological and pharmacokinetic profiles of our optimisation program.

2. Results and discussion

2.1 SAR of tricyclic core

Given the predicted mechanism-of-action, initial efforts focused on simple modifications of the core to establish the similarity of the series with the fluoroquinolones. It was quickly identified that R₁ = cyclopropane proved optimal for antibacterial activity (Table 1). Microbiological activity was $\leq 0.25 \mu\text{M}$ when X = C-Me or C-Cl (REDX04139 and REDX07368, respectively), but when X = C-H (REDX05166), the microbiological activity increased to 2 μM (Table 1). Incorporation of F at the R₂ position is a known strategy to improve traditional fluoroquinolone potency^{40,41} and whilst there was a modest improvement in the X = C-H series (REDX05166 vs. REDX05557), this was not seen in the X = Me, R₁ = Et series (REDX05237 vs. REDX05028). Additionally, unlike its fluoroquinolone counterpart, enoxacin, X = N (REDX05634) was inactive. Replacement of the H substituent at the R₃ position of the oxazole moiety with CH₃ (REDX05282) was tolerated without significant impact on potency versus the Gram-positive strains tested (Table 1). Finally, the fused core (REDX06345, levofloxacin analogue) had an MIC of 0.5 $\mu\text{g mL}^{-1}$.

The apparent beneficial effect of R₁ = cyclopropane and X = C-Me led us to evaluate the conformation of a difluoro intermediate (Fig. 5A) via small molecule X-ray crystallography (Fig. 5B). The results indicate that the R₁ cyclopropyl substituent is significantly displaced from the plane of the core, with increased trigonal character of the amide nitrogen.

Table 1 SAR of tricyclic core, minimum inhibitory concentrations (MIC) versus *S. aureus* ATCC 29213 are given in $\mu\text{g mL}^{-1}$

The chemical structure shows a tricyclic core consisting of a benzene ring fused to a pyridine ring, which is further fused to an oxazole ring. Substituents are indicated as R₁, R₂, R₃, and X. The inset shows a fused core (Fc) with X = C and a methyl group.

Compound	X	R ₁	R ₂	R ₃	MIC
Ciprofloxacin	—	—	—	—	0.25
REDX04139	C-Me	Cprop	H	H	0.03
REDX05028	C-Me	Et	H	H	2
REDX05166	C-H	Cprop	H	H	2
REDX05237	C-Me	Et	F	H	1
REDX05282	C-Me	Cprop	H	Me	0.03
REDX05557	C-H	Cprop	F	H	0.25
REDX05634	N	Cprop	F	H	128
REDX06345	Fc	Fc	H	H	0.5
REDX07368	C-Cl	Cprop	H	H	0.25

The torsion angle between the C-Me and N-cyclopropyl groups was 28° – which is in keeping with previously reported torsion angle data for the aforementioned 2-pyridone, EV-035 (ref. 32 and 42) (Fig. 2D). The implication of this torsion angle suggest that the C-Me may have a steric interaction with the ortho-F of the difluoroaniline from REDX04139. However, there is no observed impact of this potential interaction in our SAR as outlined in this manuscript.

2.2 SAR of aromatic substitution at the R₄ position

We then explored the SAR of the aromatic R₄ substituent (Table 2). Using *S. aureus* as a model (PDB 5CDQ), we predicted that the original incorporation of the difluoroaniline group provided two points of interaction at the active site of *S. aureus* DNA gyrase/topoisomerase IV, via H-bonding of the aniline nitrogen with an adjacent Glu477 residue and a cation- π interaction between a highly conserved Arg458 residue and the R₄-aryl group. Incorporation of electron modulating nitrogen and fluorine substituents can attenuate the electron density on the aryl

ring, which we hypothesised could strengthen the cation- π interaction (Fig. 6). In keeping with this premise, mono fluoro derivatives, (REDX05866 and REDX05412, *ortho* and *meta*, respectively (Table 2)), both had reduced activities compared to REDX04139, while the unsubstituted aniline (REDX05510) showed a further reduction in activity. Introduction of a cyano group (REDX07910) sustained activity, whilst reducing plasma protein binding (PPB) (Table 2).

Nitrogen-containing six-membered heterocycles at R₄ were less active than the parent molecule REDX04139. 2-Amino pyrimidine (REDX05774) displayed MICs against methicillin-sensitive *S. aureus* (MSSA) ATCC 29213 and *Streptococcus pneumoniae* (*S. pneumoniae*) ATCC 49619 of 1 and 4 $\mu\text{g mL}^{-1}$, respectively. The pyrazine (REDX06615) and pyridazine (REDX06062) analogues however, were much less potent, with both displaying activities no better than 64 $\mu\text{g mL}^{-1}$. It should be noted that these derivatives were also much less stable to chemical manipulation, with observations of hydrolytic cleavage of the oxazole moiety.

To further our understanding, we also quantified the inhibition of DNA supercoiling and DNA decatenation, mediated by DNA gyrase and topoisomerase IV, respectively. Previously-reported compounds (REDX04139 and REDX05931) are highly-active against both enzyme targets indicating a dual targeting mechanism of action.³⁴

Next, we evaluated the role of the aniline. Removal of the nitrogen maintained antibacterial potency (REDX05510 vs. REDX05867). Conversion to the difluoro benzoic acid derivative (REDX06189) demonstrated a considerable loss of activity, which could be due to the inherent incompatibility of the acidic environment within the active site, specifically Glu477, but could equally be due to loss in permeability and/or increased efflux. Analogues that retained a H-bond donor functionality, but located further from the core, such as benzylic alcohols and amines (REDX06627 and REDX05853) had MICs of $\leq 0.5 \mu\text{g mL}^{-1}$ against *S. aureus* ATCC 29213 (Table 2). Despite the metabolic risks associated with these unsubstituted benzylic analogues, they provided a good indication that extending this portion of the molecule might be well-tolerated. Fused heterocycles designed to explore this hypothesis, such as the isoindoline analogue (REDX06305) offered an excellent antibacterial activity profile against MSSA, but unfortunately also proved to have a high metabolic turnover (data not shown). Other fully aromatic systems, including benzoxadiazole (REDX06110), benzimidazole (REDX06530), and indazole (REDX05504) had good microbiological profiles and appear to offer a favourable π -stacking interaction with the conserved amino acid Arg458,⁴³ and with DNA bases. However, these compounds all had thermodynamic solubilities of $< 9 \text{ mg L}^{-1}$, $\log D_{7.4}$ values of > 3 and were $> 95\%$ bound in human plasma which precluded them from further analysis. Introduction of an amine in the 2-position of the

Fig. 5 A) 2-D diagram of crystallised structure B) orthogonal view, illustrating torsion angle between methyl/cyclopropyl.

Table 2 Selected microbiological and *in vitro* DMPK data of R₄ SAR. MICs given in $\mu\text{g mL}^{-1}$ for *S. aureus* ATCC 29213 (MSSA) and *S. pneumoniae* ATCC 49619 – data are based on at least two experimental replicates, shown as modal averages. Mean values are provided for PPB, $\log D_{7.4}$ and thermodynamic solubility (TD Sol)

Compound	R ₄	MIC <i>S. aureus</i>	MIC <i>S. pneumoniae</i>	Cytotoxicity (HepG2, CC ₅₀ $\mu\text{g mL}^{-1}$)	PPB (human)% free	Measured $\log D_{7.4}$	TD Sol (mg L^{-1})
REDX04139		0.03	1	>128	1.2	2.96	<2.5
REDX05412		0.12	2	>128	n.d.	n.d.	n.d.
REDX05504		0.004	0.5	>16	2.4	3.29	1.1
REDX05510		0.5	4	n.d.	3.8	2.59	n.d.
REDX05774		1	4	>32	n.d.	1.32	15
REDX05853		0.06	0.25	>32	2.6	3.14	1.9
REDX05866		0.12	1	16	3.0	3.05	1.1
REDX05867		1	4	n.d.	n.d.	n.d.	n.d.
REDX06062		64	>128	n.d.	>30	1.46	6.8
REDX06110		0.25	4	>32	3	3.03	<9
REDX06189		64	>128	n.d.	n.d.	n.d.	n.d.
REDX06305		0.12	0.5	>64	23.5	0.75	208.6
REDX06530		0.25	0.25	>16	4.7	3.03	<9
REDX06615		>64	>64	n.d.	n.d.	n.d.	n.d.

Table 2 (continued)

Compound	R ₄	MIC <i>S. aureus</i>	MIC <i>S. pneumoniae</i>	Cytotoxicity (HepG2, CC ₅₀ µg mL ⁻¹)	PPB (human)% free	Measured log D _{7.4}	TD Sol (mg L ⁻¹)
REDX06627		0.5	2	>32	26	n.d.	n.d.
REDX07125		0.25	0.25	>32	5.2	2.52	16
REDX07910		0.06	0.25	>4	4.2	2.94	n.d.

5,6 bicyclic systems such as REDX07125 maintained potency but delivered a high frequency of resistance (FoR) of 1.11×10^{-7} (at $4 \times \text{MIC}$) against *S. aureus* ATCC 29213. Comparatively, the FoR of REDX04139 against the same strain was $<3.8 \times 10^{-10}$ (at $4 \times \text{MIC}$). Compounds in this series generally displayed low cytotoxic against the HepG2 cell line, with CC₅₀ values $>16 \mu\text{g mL}^{-1}$. REDX07910 was found to be the only outlier with an CC₅₀ of $>4 \mu\text{g mL}^{-1}$. However, our preliminary kinetic solubility assessment of this compound showed a solubility of $6 \mu\text{M}$ which may account for this result.

2.3 Modifications at the R₄ position

Having shown scope to improve both potency and physicochemical properties through substitutions at the R₄ position, we next explored the 2-position of the oxazole (R₃, Fig. 4). Introduction of a methylene amine (REDX05931) in this position maintained the same

potency against *S. aureus* ATCC 29213 as REDX04139 (Table 3). We predicted the methylene amine could offer a potential interaction with the neighbouring serine residue *via* the NH₂ group, whilst potentially addressing the physicochemical issues associated with REDX04139. REDX05931 displayed low MICs *vs.* Gram-positive and fastidious Gram-negative bacteria, including MDR strains of *N. gonorrhoeae*.³⁴ This compound was then progressed to a murine model of *N. gonorrhoeae* infection with encouraging results.³⁵ However, due to inhibition of several CYP enzymes (Table 3) and a potential interaction with the hERG ion channel, REDX05931 was not developed further. A range of other methylene amine derivatives were prepared. Two are exemplified in Table 3. Replacement of the difluoroaniline with an indazole (REDX07517) maintained potency but did not improve the CYP inhibition or hERG profiles. The aza-indazole (REDX07473), however, showed higher IC₅₀ values for both CYP and hERG inhibition. However, the potency of this compound against FQ-resistant strains was deemed insufficient to warrant further studies.

3. Conclusion

We have described the rational design and synthesis of a range of novel bacterial topoisomerase inhibitors with excellent *in vitro* activity against wild-type and FQR Gram-positive strains. This work contests the well reported SAR that β -keto acids are essential for inhibiting DNA gyrase/topoisomerase IV and offers a subtle strategy to improve the resistance profiles of bacterial topoisomerase targeting therapies. It is interesting to note that in spite of the hypothesised interaction of the methylene amino group of compounds REDX05931, REDX07473 and REDX7517 with Ser84, the

Fig. 6 Proposed C-7 aryl substituent interactions of REDX04139 in *S. aureus* DNA gyrase, modelled using Forge™ from Cresset and PDB 5CDQ.

Table 3 Microbiology and toxicity profiles of REDX05931 derivatives. MICs given in $\mu\text{g mL}^{-1}$; data are based on at least two experimental replicates, shown as model averages

	REDX05931	REDX07473	REDX07517

Comparative data	REDX04139				Ciprofloxacin
<i>S. aureus</i> ATCC 29213 (MSSA)	0.03	0.06	0.25	0.06	0.25
<i>S. aureus</i> NRS74 (MRSA)	0.06	0.06	2	0.5	128
<i>S. aureus</i> VRS8 (MRSA)	0.5	4	2	2	32
CYP IC ₅₀ (μM) ^a	>30, >30, >30, >30,	7.5, 0.3, 0.5, 12.9, 11.3	30.0, 0.8, 2.9, 22.6, 22.8	12.3, 0.2, 0.8, 1.3, 12.7	—
hERG IC ₅₀ (μM)	3% inhibition at 100 μM	7.9	45.08	6.2	—
Cytotoxicity HepG2 CC ₅₀ ($\mu\text{g mL}^{-1}$)	>128	>64	>128	~32	—
TD Sol (mg L^{-1})	<2.5	36	618	10	—

^a CYP inhibition values in order as follows: 1A2, 2C9, 2C19, 3A4, 2D6.

activity of this series of compounds is only diminished seven fold *vs.* MRSA NRS74, with target relevant mutations of GyrA (S84L, E409L) and GrlA (S80F). In contrast, ciprofloxacin has a 500-fold drop-off in activity. The implication being that a) the proposed interaction is not essential to activity, b) the loss of activity may be due to an alternative mechanism(s), or c) the proposed MoA is incorrect. To improve the drug-like properties of this series, the solubility, CYP inhibition and hERG inhibition profiles need to be addressed. In part 2, we discuss the merits of saturated heterocycloalkyls at the R₄ position (Fig. 4). Optimisation of the saturated amine heterocycle at this position, has in the past led to improvements to the antibacterial spectrum of activity, covering Gram-positive, Gram-negative and anaerobic bacteria, whilst also improving *in vitro* pharmacokinetic and physicochemical properties including half-life and solubility.⁴⁴

4. Chemistry

4.1 Derivatisation of core

Our synthetic route began with 2-bromo-3-methylaniline and introduction of cyclopropane moiety as an initial step using (1-ethoxycyclopropoxy)trimethylsilane⁴⁵ followed by borane reduction to give (3). Acylation with oxalyl chloride followed by intra-molecular Friedel–Crafts acylation,⁴⁶ using aluminium trichloride, gave isatin (4). This was then reacted with hydrogen peroxide to provide the anthranilic acid (5).⁴⁷ Treatment of (5) with triphosgene or phosgene

gave the key anhydride (6). At this point in the synthesis, we either carried out a condensation with ethyl nitroacetate⁴⁸ (route A, Scheme 1a, in THF at 60 °C) to give a hydroxy nitro intermediate (7), which, after reduction of the nitro-group and cyclisation using an ortho-formate,⁴⁹ generated the tricyclic core, or in a more direct fashion (route B, Scheme 1a) reacted anhydride (6) with ethyl isocynoacetate to form the tricycle.⁵⁰ In both cases the desired aryl group was added in a Suzuki coupling. Substitution of the oxazole 2-position to give 2-methyl derivatives was achieved *via* cyclisation of amino hydroxyl intermediate (8) with trimethyl orthoacetate. The synthesis was then completed as above using a Suzuki arylation with appropriate aryl boronic acid or pinacol boronic esters. Alternatively, the tricyclic core (9a/b) can undergo Miyura borylation, followed by reaction with the desired aryl halide (REDX06062).

The R₁ = ethyl (Fig. 4) derivative was successfully synthesised using the procedure detailed in Scheme 1b *via* alkylation of the commercially available isatin (12) with ethyl iodide. This was then followed by the procedure detailed in Scheme 1a to give the desired product.

4.2 Chemistry of X substitution

To determine the contribution of the X= methyl substituent (Fig. 4) to the observed anti-bacterial effect, we initially synthesised X = C–H with R₂ = fluorine derivatives, as depicted in Scheme 2. 1,4-diBromo,2,5-difluorobenzene (14) was treated with butyl lithium at

Scheme 1 1a Reagents and conditions: (a) (1-ethoxycyclopropoxy)-trimethyl-silane, AcOH, MeOH, rt–70 °C, 16 h, quant. (b) BH₃. THF, THF, 0–70 °C, 18 h. (c) (1) Oxalyl chloride, Et₂O, 40 °C; (2) AlCl₃, DCM, 0 °C – rt, 21% (d) H₂O₂, NaOH 0 °C – rt, 2 h, 99% (e) triphosgene, rt, 3 h. (f) Ethyl nitroacetate, THF, 60 °C, 16 h, 27% (g) sodium hydrosulfite, EtOH, H₂O, 77 °C, 1 h. (h) Triethyl orthoformate, reflux, 2 h or trimethyl orthoacetate, cat HCl, reflux, 4 h, 40% (i) aryl pinacol ester, Pd(dppf)Cl₂, Cs₂CO₃, DME, H₂O, MW, 120 °C, 20 min. (j) (1) ethyl isocyanoacetate, THF, 60 °C, 16 h; (2) NaH, DMF, 100 °C, 1 h, 35% (k) bis(pinacolato)diboron, Pd(dppf)Cl₂, K₂CO₃, DME, 75–90 °C, 16–72 h, 85% (l) aryl halide, Pd(dppf)Cl₂, Cs₂CO₃, DME, H₂O, MW, 120 °C, 20 min. 1b Reagents and conditions: (a) Et-I, K₂CO₃, DMF, 100 °C, 1 h, 88%.

–78 °C and carboxylated with carbon dioxide.⁵¹ The resulting acid (15) was converted to acid chloride and then to the oxazole carboxylic acid ethyl ester (16) *via* treatment with ethyl isocyanoacetate and triethylamine.⁵² Saponification with lithium hydroxide afforded (17) which was followed by acid chloride formation (oxalyl chloride, catalytic DMF) and addition of cyclopropylamine to give amide (18).

S_NAr cyclisation by displacement of fluorine using potassium carbonate as base and 18-crown-6 at 140 °C gave the desired fluoro oxazole (19), which was then derivatised *via* Suzuki cross coupling (Scheme 2).

X = C-chloro core was synthesised in an analogous fashion to Scheme 2, using a commercially available carboxylic acid (20, Scheme 3). 8-Aza-tricyclic derivatives were synthesised in a similar manner using 2,6-dichloro-5-fluoronicotinic acid (22) and methyl isonitrile carboxylate, to give the oxazole methyl ester derivative (23).

Saponification, amide coupling and cyclisation gave intermediate (24), which was arylated *via* Suzuki cross coupling, as described previously. The (*S*)-morpholino fused core was synthesised in a similar fashion from 2,3-difluoro-4-bromo benzoic acid (25), using 2-(*S*)-aminopropan-3-ol as the amine coupling partner to give compound (26). This was followed by a double ring closure (firstly, the quinolone ring forms then morpholine ring *via* sequential S_NAr fluorine displacement⁵³). The resulting tetracyclic core (27) was then derivatised using standard Suzuki cross coupling conditions.

R₂ and X unsubstituted derivatives were synthesised using an alternative route, starting with Chan–Lam alkylation⁵⁴ of 6-bromo indole (28) using cyclopropane boronic acid. NBS–DMSO oxidation⁵⁵ produced the isatin (30) and after treatment of (30) with hydrogen peroxide and sodium hydroxide, the anthranilic acid (31) was formed. A one-pot procedure using triphosgene followed

Scheme 2 Reagents and conditions: (a) *n*-BuLi, CO₂, THF, −78 °C, 97% (b) (1) oxalyl chloride, DMF, 0 °C – rt, 2–16 h; (2) ethyl isocyanoacetate, THF, NEt₃, 0 °C – rt 16 h, 89% (c) (1) LiOH, 1,4-dioxane, rt, 16 h, 85% (d) (1) oxalyl chloride, DMF, DCM, 0 °C – rt, 2 h; (2) cyclopropylamine, DCM, rt, 83% (e) K₂CO₃, 18-crown-6, DMSO, MW, 140 °C, 90 min, 26% (f) aryl pinacol ester, Pd(dppf)Cl₂, Cs₂CO₃, DME, H₂O, MW, 120 °C, 20 min.

Scheme 3 Reagents and conditions: (a) (1) LiOH, 1,4-dioxane, 16 h, 94%; (2) cyclopropylamine, DIPEA, HATU, DCM, rt, 16 h, 74% (3) NaH, DMF, 0 °C – rt, 16 h, 90% (b) K₂CO₃, 18-crown-6, DMSO, 140 °C, 40 min, 45%.

by treatment with ethyl isocyanoacetate and sodium hydride gave the tricyclic intermediate (32); which was then arylated under Suzuki cross coupling conditions (Scheme 4).

4.3 Chemistry of REDX05237

Synthesis of REDX05237 began with the formation of the isatin (34) from 4-fluoro-2-methyl-aniline (33) and chloral hydrate in HCl, followed by the addition of hydroxylamine hydrochloride.⁵⁶ As we were unable to selectively mono-

brominate at the 6 position of the isatin, this was bis-brominated with NBS to give the (35) which was then selectively mono-debrominated with the use of copper and propionic acid.⁵⁷ The regioselectivity of the mono-debrominated product (36) was confirmed by NMR. Compound (36) was alkylated with ethyl iodide in the presence of K₂CO₃ to give isatin (37). This was then reacted with hydrogen peroxide/sodium hydroxide to provide the anthranilic acid (38). Treatment of (38) with triphosgene followed by NEt₃ and ethyl isocyanoacetate formed the oxazole (39). Cyclisation to the tricyclic core (40) was achieved

Scheme 4 Reagents and conditions: (a) cyclopropylboronic acid, Na_2CO_3 , 2,2'-bipyridyl, $\text{Cu}(\text{OAc})_2$, DCE, 70 °C, 5 h, 73% (b) NBS, DMSO, 60 °C, 18 h. (c) NaOH , H_2O_2 , 0 °C – rt, 2 h, 99% (d) (1) triphosgene, THF, rt, 3 h; (2) ethyl isocynoacetate, NaH , DMF, 100 °C, 2 h. (e) Aryl pinacol ester, $\text{Pd}(\text{dppf})\text{Cl}_2$, Cs_2CO_3 , DME, H_2O , MW, 70 °C, 2 h.

with use of NaH in DMF, which was then derivatised *via* Suzuki cross coupling to give REDX05237 (Scheme 5).

4.4 Chemistry of 2'-methylene amine derivatives

Synthesis of the 2'-methylene amine derivatives (REDX05931, REDX07473 and REDX07517) began with the ring closure of the amino hydroxy intermediate (8) with 2-chloro-1,1,1-trimethoxyethane in the presence of boron trifluoride diethyl etherate to form the '2'-methylene chloro' intermediate (41). Sodium azide was then used to displace the chlorine at room temperature to form intermediate (42) which, for the synthesis of REDX05931

underwent Suzuki arylation with 2,5-difluoro-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline to form the azide precursor (43). Intermediate (43) was then subject to standard Staudinger reduction conditions to form REDX05931. To synthesise REDX07473 and REDX07517, intermediate (42) was reduced with PPh_3 (Staudinger) to give the 2'-methylene amine intermediate (44), which was then BOC protected (45) and functionalised with an aryl pinacol ester (46). Finally, a BOC deprotection of intermediate(s) (46) with TFA in DCM furnished the desired products as TFA salts, which were then be neutralised/purified by column chromatography to elicit the final compounds as free bases (Scheme 6).

Scheme 5 Reagents and conditions: (a) (1) chloral hydrate, Na_2SO_4 , HCl followed by hydroxylamine hydrochloride, rt – reflux; (2) H_2SO_4 , 50–80 °C, 65% (b) NBS, H_2SO_4 , 0 °C – rt, 6 h. (c) Cu , propionic acid, 130 °C, 83% (d) ethyl iodide, K_2CO_3 , DMF, 100 °C, 1 h. (e) NaOH , H_2O_2 , rt, 2 h, 87% (f) (1) triphosgene, THF, rt, 3 h; (2) ethyl isocynoacetate, NET_3 , THF, 60 °C, 18 h, 84% (g) NaH , DMF, 100 °C, 1 h, 21% (h) aryl pinacol ester, $\text{Pd}(\text{dppf})\text{Cl}_2$, Cs_2CO_3 , DME, H_2O , MW, 70 °C, 2 h.

Scheme 6 Reagents and conditions: (a) 2-chloro-1,1,1-trimethoxyethane, boron trifluoride diethyl etherate, DCM, 0 °C – rt, 44% (b) NaN₃, DMSO, rt, 16 h, 86% (c) 2,5-difluoro-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline, Pd(dppf)Cl₂, Cs₂CO₃, DME, H₂O, MW, 120 °C, 20 min, 47% (d) PPh₃, THF/H₂O, 75 °C, 2 h, 86% (e) PPh₃, THF/H₂O, 75 °C, 1 h, 87% (f) di-tert-butyl dicarbonate, NEt₃, DCM, rt, 2 h, 74% (g) aryl pinacol ester, Pd(dppf)Cl₂, Cs₂CO₃, DME, H₂O, MW, 70 °C, 2 h (h) trifluoroacetic acid, DCM, 2 h.

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Conflicts of interest

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

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