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Optimization of physicochemical properties and safety profile of novel bacterial topoisomerase type II inhibitors (NBTIs) with activity against *Pseudomonas aeruginosa*

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ABSTRACT

Type II bacterial topoisomerases are well validated targets for antimicrobial chemotherapy. Novel bacterial type II topoisomerase inhibitors (NBTIs) of these targets are of interest for the development of new antibacterial agents that are not impacted by target-mediated cross-resistance with fluoroquinolones. We now disclose the optimization of a class of NBTIs towards Gram-negative pathogens, especially against drug-resistant *Pseudomonas aeruginosa*. Physicochemical properties (pK_a and log *D*) were optimized for activity against *P. aeruginosa* and for reduced inhibition of the hERG channel. The optimized analogs **9g** and **9i** displayed potent antibacterial activity against *P. aeruginosa*, and a significantly improved hERG profile over previously reported analogs. Compound **9g** showed an improved QT profile in in vivo models and lower clearance in rat over earlier compounds. The compounds show promise for the development of new antimicrobial agents against drug-resistant *Pseudomonas aeruginosa*.
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1. Introduction

The emergence of multi-drug-resistant Gram-negative pathogens is an area of increasing concern in the medical community[1] and provides the impetus to discover new classes of antibacterial agents that are not cross resistant with existing drugs. Efforts aimed at addressing resistance have focused on both novel targets, as well as novel molecules that inhibit established targets through a different mode of action.

We were interested in bacterial type II topoisomerases (DNA gyrase and topoisomerase IV), which are clinically proven antibacterial targets for the fluoroquinolone class of drugs that target the GyrA and ParC subunits of DNA gyrase and topoisomerase IV, respectively. The clinical utility of fluoroquinolones is severely limited by mutations in the quinolone-resistance determining region (QRDR) of the targets.[2,3] The new class of NBTIs (novel bacterial type II topoisomerase inhibitors) engage with the GyrA/ParC subunits of bacterial type II topoisomerases through a different mode of inhibition that is not impacted by QRDR mutations.[4–18] Other classes of inhibitors of the GyrA/ParC subunits of bacterial type II topoisomerases that do not show cross resistance with fluoroquinolones have been described.[19–23]

The pharmacophore of the NBTIs suffers from a hERG liability and the mitigation of this risk has been the focus in the lead optimization phase of our program.[8,10] We recently reported on efforts to optimize physicochemical properties of NBTIs for selective Gram-positive activity, during which the pK_a of the compounds was

Abbreviations: NBTI, novel bacterial type II topoisomerase inhibitor; QRDR, quinolone-resistance determining region; hERG, the potassium ion channel encoded by the 'human Ether-a-go-go-Related Gene'; LHS, bicyclic aromatic left-hand side; RHS, aromatic right-hand side; MIC, minimal inhibitory concentration; MIC_{90}, minimum inhibitory concentration required to inhibit the growth of 90% of organisms; CFU, colony forming units; ND, not determined; fCmax, protein binding adjusted (free) peak plasma concentration.

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reduced and compounds displayed an improved cardiac safety profile.[10] The physicochemical property space for drugs targeting Gram-negative bacteria differs for compounds targeting Gram-positive bacteria, which provides guidelines for different strategies of optimization.[24] In particular, optimization of molecules targeting intracellular targets in Gram-negative pathogens requires consideration of drug permeability through porins of the outer membrane as well as permeability by passive diffusion through the inner membrane. In addition, many Gram-negative pathogens possess sophisticated efflux pump systems.[25] We report herein the optimization of NBTIs against serious Gram-negative pathogens, with focus on the problematic hospital pathogen *Pseudomonas aeruginosa*. In depth biochemical and microbiological characterization of an advanced lead from his series has been described elsewhere.[18]

2. Chemistry

Most final compounds were assembled by reductive aminations of chiral amines **5** with aldehydes **6**[26] or **7**[27] (Scheme 1).[28] The nosyl groups were removed using thiophenol, in most cases in good yields. With fluoro-substituted heterocyclic analogs, especially with naphthyridone **8j**, care had to be taken to minimize substitution of the fluoro group by thiophenol.

The amine building blocks **5a–g** were obtained by alkylation of heterocycles **2a–g**[8,28–34] with the aziridine **3**[28,35] (either racemic or as the *R*-enantiomer), followed by deprotection of the Boc group (Scheme 2). Quinoxalinones **2d** and **2e** were prepared from nitro anilines as outlined in Schemes 3 and 4. The aziridine **3** was obtained by aziridination of olefin **10** with *N*-(*o*-nitrophenylsulfonyl)imino]phenyliodinane and $Cu(OTf)_2$,[36] followed by chiral separation of the enantiomers (Scheme 5). A chiral route to the *R*-enantiomer of **3** was developed as well and has been reported elsewhere.[35]

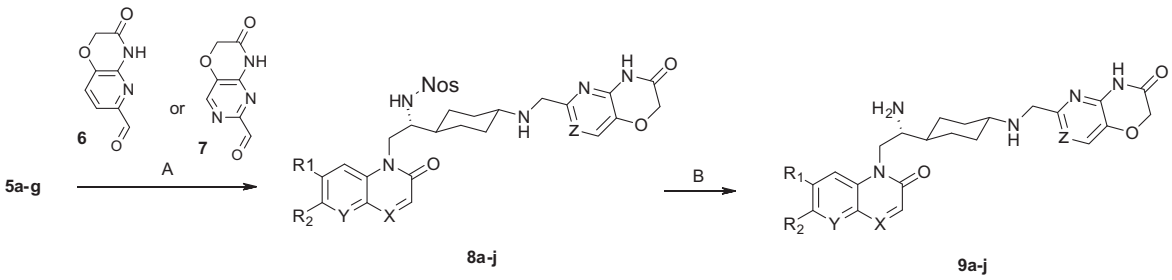
For the N-methylated analog **31**, methylation was performed on nosylated **8h**, via the protocol by Mitsunobu (Scheme 6).

Compound **23**, containing a primary amine on the cyclohexyl bridgehead, was prepared as outlined in Scheme 7. After reduction of the commercially available acid **11** to the alcohol **12**, the amino group was converted to the azide **13** using triflic azide in toluene.[37] The alcohol was protected to **14** and cleavage of the ketal produced the ketone **15** in good yield. Reductive amination with ammonium acetate gave the *cis* isomer as the major product. This is in agreement with the literature, which suggests that small hydride reagents prefer to attack from the axial position.[38] The *cis* and *trans* isomers could be separated chromatographically after Boc-protection of the amino group and removal of the silyl protection of the alcohol. Mesylation of the *cis* isomer **18** gave **19**. Alkylation of **2c** with **19** gave predominantly the O-alkylated product, together with the desired N-alkylated product **20** in low yield (14%). After deprotection of the Boc-group, reductive amination was performed with aldehyde **6** to give **22**. Staudinger reduction of the hindered azide **22** produced the target compound **23** in 12% yield. Under the conditions of the Staudinger reaction, elimination to the cyclohexene derivative was observed as a side reaction.

For the fluorocyclohexyl derivative **45** (Scheme 8), ketone **36** was reacted with trimethylsulfoxonium iodide to give the epoxide **37**, which was opened to the alcohol with HF/pyridine. The *cis*-configured alcohol **38** was obtained in 51% yield, together with the *trans*-configured analog as a minor product. Oxidation of **38** to the aldehyde **39**, followed by Wittig olefination provided the olefin **40**, which was converted to the final racemic compound **45** using the same sequence of conversions as described above.

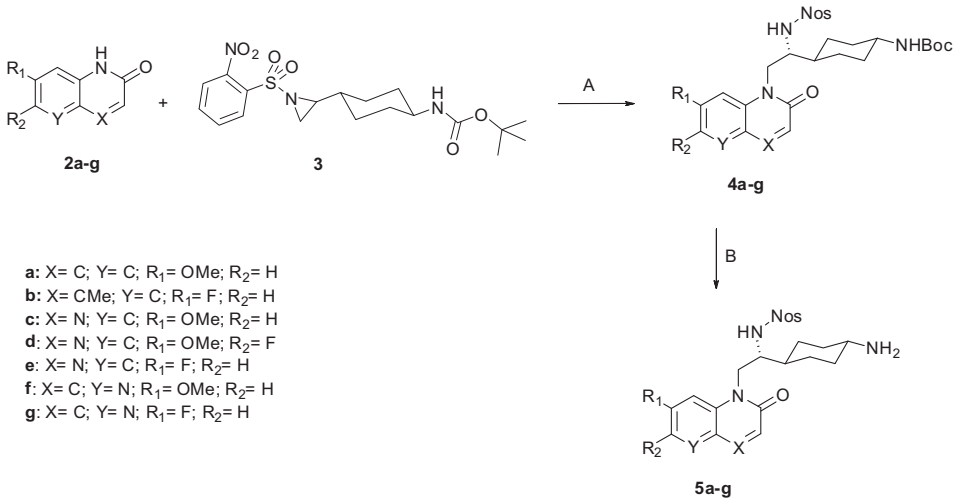
3. Results and discussion

Our lead for this effort was compound **1** (Fig. 1), which displays potent broad spectrum antibacterial activity.[10] However, **1** is inhibiting the hERG cardiac channel (IC_{50} = 35 μM, Table 1), and caused prolongation of the corrected QT (QT_c) interval in a dog toxicology study (Fig. 4). We aimed to reduce the changes in QT_c by reducing hERG inhibition, while retaining Gram-negative activity, especially against multi-drug resistant *P. aeruginosa*. We have previously reported that addressing the hERG issue through reduction of pK_a in analogs of **1** with substitution in the piperidine moiety led to reduced Gram-negative activity.[10] This effect may be due to reduced permeability for less basic compounds through porins in the outer membrane of Gram-negative bacteria, which favor positively charged molecules.[25,39,40] Agents active against Gram-negative organisms generally fall within a lower log*D* range than for drugs against Gram-positive organisms,[24] and this, in our experience, is especially true for *P. aeruginosa*. We chose therefore to explore further reduction in log*D* in our NBTI compounds, specifically through dibasic compounds that may transit better through the porins of Gram-negative bacteria.

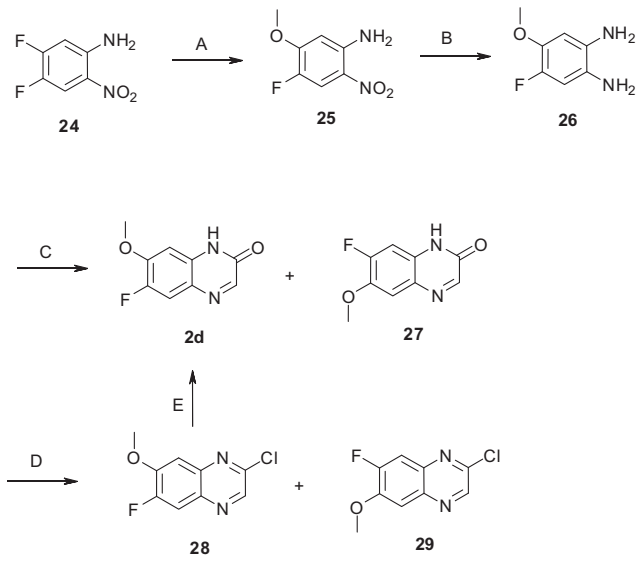


a: X= C; Y= C; Z= N; R_1= OMe; R_2= H
b: X= CMe; Y= C; Z= N; R_1= F; R_2= H
c: X= CMe; Y= C; Z= C; R_1= F; R_2= H
d: X= N; Y= C; Z= N; R_1= OMe; R_2= H
e: X= N; Y= C; Z= C; R_1= OMe; R_2= H
f: X= N; Y= C; Z= C; R_1= F; R_2= H
g: X= N; Y= C; Z= N; R_1= F; R_2= H
h: X= N; Y= C; Z= N; R_1= OMe; R_2= F
i: X= C; Y= N; Z= C; R_1= OMe; R_2= H
j: X= C; Y= N; Z= C; R_1= F; R_2= H

Scheme 1. Reagents and conditions: (A) Molecular sieves 3 Å, $CHCl_3$/MeOH, 70 °C, then $NaBH(OAc)_3$, 0 °C to room temperature; (B) K_2CO_3, thiophenol, DMF, room temperature.



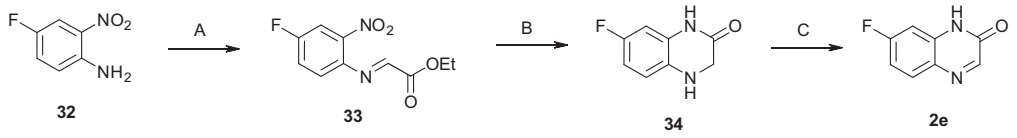
Scheme 2. Reagents and conditions: (A) NaH, DMF, room temperature; (B) TFA/DCM, room temperature.



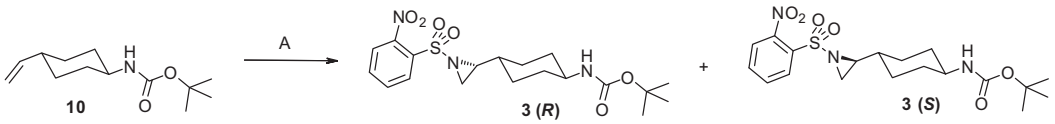
Scheme 3. Reagents and conditions: (A) KOH, MeOH, room temperature, quant; (B) Pd/C, H_2, EtOH/HOAc, room temperature; (C) 2-oxoacetate, toluene, room temperature; (D) $POCl_3$, 100 °C; (E) 5 M HCl, 125 °C, 64%.

In this context, we were interested in the trans amino ethyl cyclohexyl linker in combination with the N-linked left-hand-side (LHS) NBTI scaffold,[9,10,28,41] because this linker allows for introduction of a second basic group in the 2-position of the ethyl bridge such as in compound **9e** (Table 1),[28,41] which also leads to a reduction in log*D* (from 0.6 for **1** to −0.36 for **9e**). The pK_a of the primary amine in compounds **9e** was ~7.5, indicating partial protonation at physiological pH (Table 1). The change from a piperidine to a cyclohexyl linker increased the pK_a of the secondary amine, from 8.42 in **1** to 9.09 in **9e** (the third pK_a value for **9e** at 10.66 is associated with deprotonation of the NH functionality of the oxazinone RHS, denoting a weak acid). The pK_a of the secondary amine in **9e** could be lowered by ~0.8 log units to match the pK_a of the lead **1** by incorporation of pyrimidine right-hand-side (RHS) heterocycle, without large changes in log*D* (compare analogs **9b** and **9d** to **9c** and **9e**). Fine tuning of the pK_a of the more basic secondary amine was aimed at avoiding strongly basic groups that could engender a safety risk[42] and to retain good permeability through the inner membrane of Gram-negative organisms. Introduction of a fluorine in the bridgehead of the cyclohexyl moiety (compound **45**) led to a decrease in pK_a for both amine moieties of **45** relative to the des-fluoro analog **9e** (pK_as 8.57 and 5.87, vs 9.09 and 7.55, respectively).

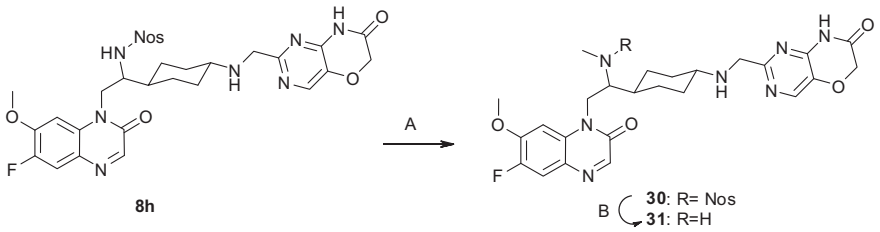
Inhibition of hERG was measured by the IonWorks assay[43] and was found to be significantly reduced with all dibasic amino-cyclohexyl analogs relative to **1**, tracking roughly with the reduced log*D* (Table 1). Compounds were difficult to rank-order by inhibition of hERG, due to solubility limitations in the IonWorks assay at concentrations above 333 μM. However, the most polar analogs such as **9i** and **9j** displayed no detectable inhibition of hERG at the top concentration tested, reinforcing the link between polarity and hERG activity.



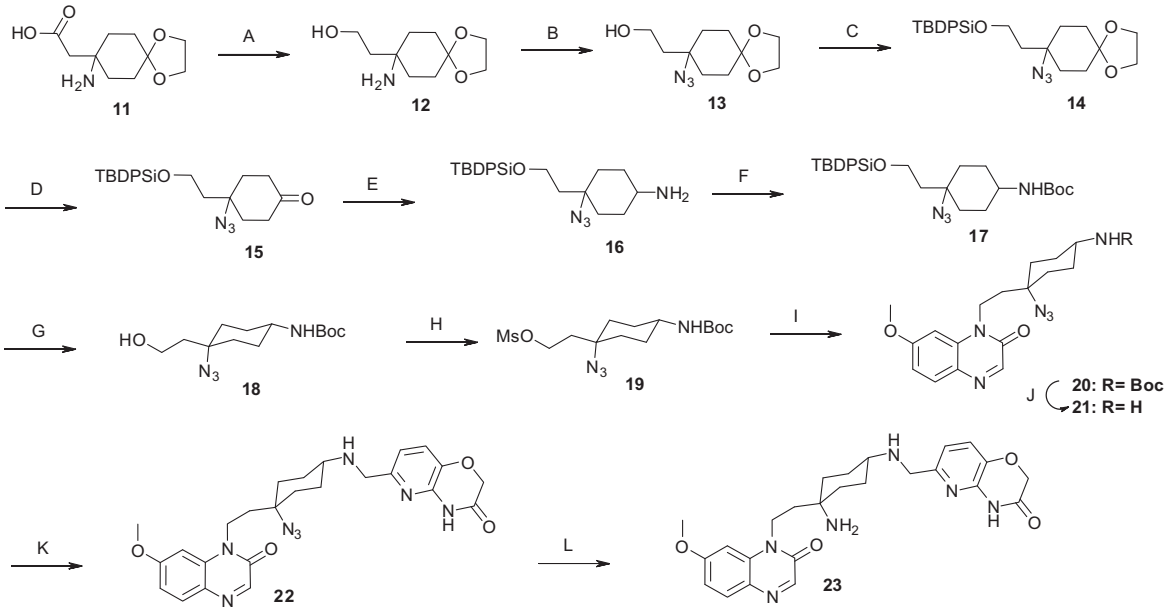
Scheme 4. Reagents and conditions: (A) Ethylglyoxylate, toluene, reflux; (B) Pd/C, H_2, EtOH; (C) NaOH, H_2O_2, 80 °C.



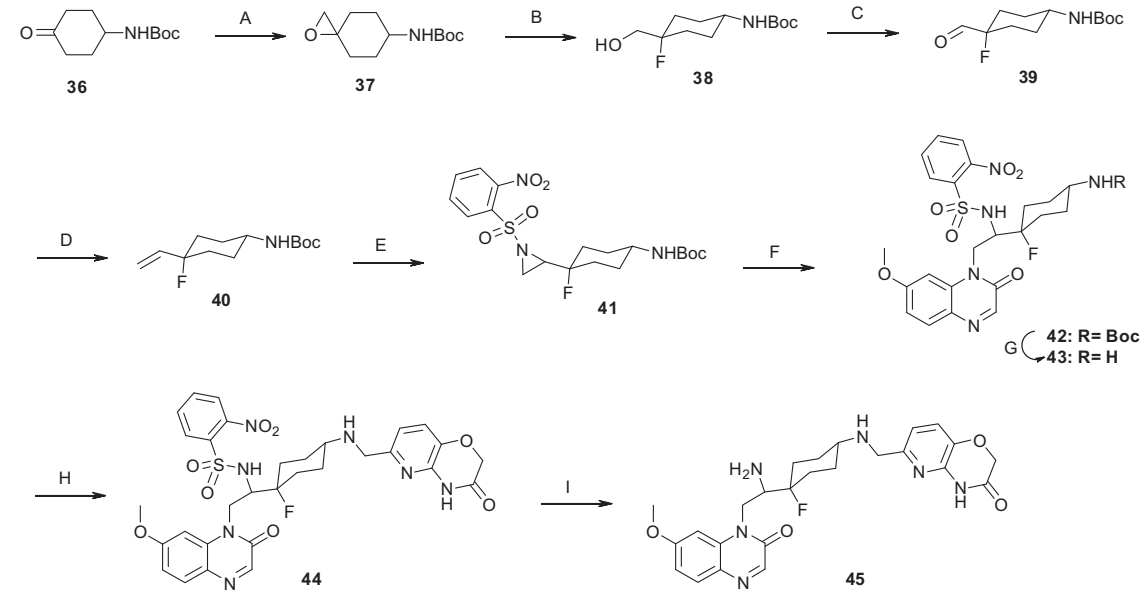
Scheme 5. Reagents and conditions: (A) [*N*-(*o*-nitrophenylsulfonyl)imino]phenyliodinane,[36] Cu(OTf)$_2$, CH_3CN, room temperature.



Scheme 6. Reagents and conditions: (A) $P(Ph)_3$, DIAD, THF/MeOH, room temperature; (B) K_2CO_3, thiophenol, DMF, room temperature.



Scheme 7. Reagents and conditions: (A) LAH, THF, reflux, 91%; (B) $TfIN_3$, $NaHCO_3$, $CuSO_4$, toluene/DMF/water, room temperature, 79%; (C) TBDPSCl, imidazole, DMF, room temperature, quant; (D) *p*-TsOH, acetone, 80 °C, 75%; (E) NH_4OAc, $NaBH_3CN$, MeOH, room temperature, quant; (F) $NaHCO_3$, Boc_2O, EtOAc/water, room temperature, 78%; (G) TBAF, THF, room temperature, 52%; (H) MsCl, NEt_3, CH_2Cl_2, 0 °C; (I) **2c**,[8,31,32] NaH, DMF, room temperature −40 °C, 14%; (J) TFA, CH_2Cl_2, 0 °C, 98%; (K) **6**,[26] molecular sieves 3 Å, DMF, 60 °C, then $NaBH(OAc)_3$, 0 °C to room temperature, 69%; (L) $P(Ph)_3$, CH_3CN/water, room temperature, 12%.



Scheme 8. Reagents and conditions: (A) ^tBuOK, $(CH_3)_3SOI$, THF, 98%; (B) 70% HF/pyridine, DCM, −78 °C, 51%; (C) Dess–Martin, DCM, 0 °C to rt, 56%; (D) $Ph_3P(CH_3)Br$, $K((CH_3)_3Si)_2N$, 0 °C, 57%; (E) *o*-NO_2 $PhSO_2$ NIPh, $Cu(OTf)_2$, molecular sieves, CH_3CN, 15%; (F) **2c**, NaH, DMF, 54%; (G) TFA, DCM, quant; (H) **6**, $NaBH(OAc)_3$, DIEA, DMF, 80%; (I) PhSH, K_2CO_3, DMF, 29%.

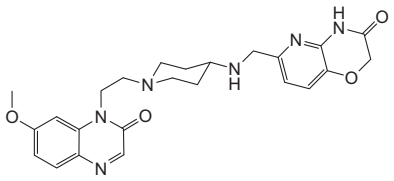


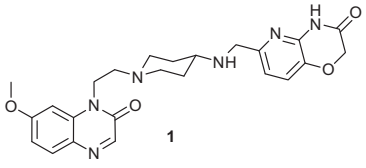
Figure 1. N-linked aminopiperidine lead **1**.

We determined inhibition of the topoisomerase IV target in *Escherichia coli*. Most of the *S*-amino ethyl cyclohexyl analogs showed IC_{50}s in the low nanomolar range, similar to the amino piperidine lead **1** (Table 1). The corresponding *R*-isomers were less active (**35** vs **9i**), as were compounds with substitutions on the primary amine (compare **31** and **9h**). Docking of *N*-methyl analog **31** into the published crystal structure of the *Staphylococcus aureus* gyrase/DNA complex[4] indicated a steric clash of methyl group with the DNA (Fig. 2).

Analogs with either a fluoro- or an amino-substituent on the bridgehead of the cyclohexyl moiety (**45** and **23**) showed good potency against topoisomerase IV.

The antibacterial activity of the compounds was determined against the Gram-negative pathogens *P. aeruginosa* and *E. coli* and the Gram-positive organism *S. aureus* (Table 1). In general, the amino cyclohexyl analogs showed potent activity against the Gram-negative organisms. Activity against *P. aeruginosa* was similar or better compared to the lead **1** for the best analogs (**9c**, **9d**, **9e**, **9f**, **9g**, **9i**). Plasma protein binding was generally lower than for **1**, in the range of 60–70% free fraction for most compounds (Table 1). Activity against *S. aureus* was generally weaker for this set than for lead **1**, and tracked with lower log*D*. Compound **45**, which is less basic and has a relatively high log*D*, was more potent against *S. aureus* than against Gram-negatives. Analog **23**, with an amino group on the bridgehead of the cyclohexyl moiety showed relatively weak antibacterial activity against both Gram-negative organisms and *S. aureus*, despite similar potency against topoisomerase IV. We were unable to measure pK_a and log*D* for **23**, but would expect **23** to be strongly dibasic. This may impair its ability to permeate through the inner membrane of Gram-negative organisms and the lipid bilayer of *S. aureus*. It is interesting to note in this respect that a small molecule X-ray structure of **9g** indicated an intramolecular H-bond between the amino group and the carbonyl

Table 1
SAR of amino ethyl cyclohexyl NBTIs versus amino piperidine **1**



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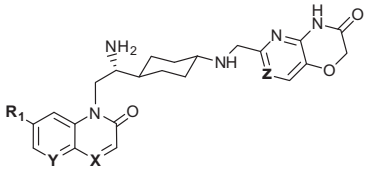
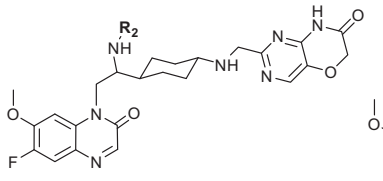
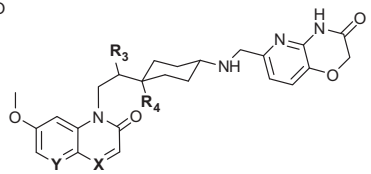


9a-g, i, j
R_1, X, Y, Z see table



9h: (*R*), R_2= H
31: racemic, R= Me



35: R_3= (*S*) NH_2; R_4= H; X= C; Y= N
45: R_3= NH_2; R_4= F; X= N; Y= C
23: R_3= H; R_4= NH_2; X= N; Y= C

Compd	R1	X	Y	Z	*P. aeruginosa*[a] MIC	*E. coli*[b] MIC	*S. aureus*[c] MIC	Fu[d] (%)	*E. coli* TopoIV[e] IC_{50} (nM)	log*D*[f]	pK_a	hERG[g] IC_{50} (μM)
1					1	0.13	0.03	26	2.2	0.60	8.42/5.56/10.46	35
9a	OMe	C	C	N	2	0.5	8	71	5	−0.54	ND	>100
9b	F	CMe	C	N	2	0.5	4	66	2	0.06	8.42/7.59/9.39	>333
9c	F	CMe	C	C	0.5	0.5	1	64	3	−0.05	9.21/7.81/10.58	>333
9d	OMe	N	C	N	0.5	0.2	1	60	2	−0.48	8.33/7.45/9.34	>333
9e	OMe	N	C	C	0.5	0.5	1	70	1	−0.36	9.09/7.55/10.66	>333
9f	F	N	C	C	0.25	<0.06	1	70	28	−0.41	ND	174
9g	F	N	C	N	0.5	<0.06	1	73	3	−0.32	8.25/7.28/9.28	>333
9h					2	2	4	56	4	−0.42	ND	>333
9i	OMe	C	N	C	1	0.5	8	78	2	−0.86	9.09/7.66/10.66	>333
9j	F	C	N	C	2	1	16	ND	34	−1.02	ND	>100
31					>64	16	64	ND	39	ND	ND	ND
35	OMe	C	N	C	16	8	128	>70	33	−0.84	ND	>100
45					8	2	0.5	68	5	0.25	8.57/5.87/10.51	>33
23					8	1	2	ND	2	ND	ND	ND

Minimum inhibitory concentration (MIC, μg/mL): lowest drug concentration that reduced growth by 90% or more.
ND: not determined. Aqueous solubility was >333 μM for all compounds (results not shown).

[a] *Pseudomonas aeruginosa* strain PAO1.
[b] *Escherichia coli* strain W3110.
[c] Methicillin-susceptible *Staphylococcus aureus*.
[d] Fraction unbound, human,% free.
[e] *Escherichia coli* topoisomerase IV IC_{50}.
[f] Partition coefficient at pH 7.4.
[g] hERG IonWorks IC_{50}.[43]

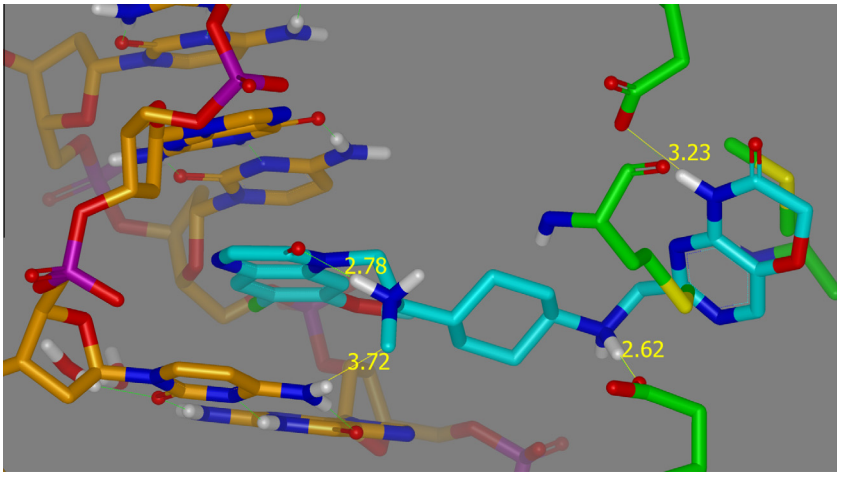


Figure 2. Docking of the *R*-enantiomer of compound **31** into the crystal structure of *S. aureus* gyrase. Intermolecular and intramolecular hydrogen-bonds distances are shown in yellow. The *N*-methyl group in compound **31** has an unfavorable polar-nonpolar interaction with the C11 DNA base. The docking model was generated using Schrodinger's GLIDE software.

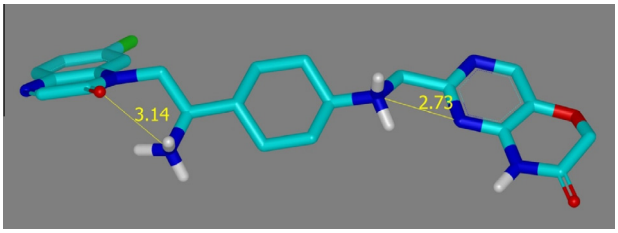


Figure 3. Small molecule X-ray structure of compound **9g**. Intramolecular H-bonds indicated between the carbonyl of the LHS and the primary amine, and between the secondary amine and the pyrimidine nitrogen (indicated by yellow lines) may have relevance for the conformation in solution and may be important for permeability through the inner membrane of Gram-negative bacteria.

Table 2
MIC_{90}[a] (μg/mL) values versus key Gram-negative pathogens

Organism (number of strains)	**1**	**9g**	**9i**	Levofloxacin
Pseudomonas aeruginosa (20)	4	4	4	16
Escherichia coli (20)	0.25	1	0.5	16
Klebsiella pneumoniae (20)	4	16	8	64

[a] MIC_{90} values of clinical isolates of the indicated organisms. MIC_{90} is the concentration of compound that will inhibit at least 90% of the organisms.

functionality (Fig. 3), which would not be possible with **23**. Intramolecular H-bonds can reduce hydration and are known to increase permeability through lipid bilayers.[44,45]

Compounds **9g** and **9i** were selected for further profiling based on an improved hERG IC_{50} and potency against Gram-negative organisms. Both compounds showed excellent activity against a population of *P. aeruginosa* and *E. coli* strains, including fluoroquinolone-resistant isolates (Table 2), but were less active versus *K. pneumoniae*. In our experience, optimal cellular potency against *K. pneumoniae* for this chemical series requires higher log*D* values relative to *P. aeruginosa*.

The pharmacokinetic properties of **9g** and **9i** following iv dosing were determined in rat and dog (Table 3). Both compounds showed moderate in vivo clearance and high volumes of distribution. Clearance in rat was significantly reduced for **9g** and **9i** relative to **1**. Bioavailability in rat was lower for **9g** than for **1** (2 vs 18% respectively), despite the higher clearance of **1**, likely due to lower permeability through lipid bilayers with **9g** (another indication of this effect is the reduced Gram-positive activity of **9g** relative to **1**).

Compound **9g** was selected for efficacy studies in an immunocompromised mouse thigh model of *P. aeruginosa* infection (Table 4). Dosing with **9g** at 200 mg/kg/day resulted in a 2 $\log_{10}$ reduction in bacterial tissue burden. From a pharmacokinetic–pharmacodynamic (PK–PD) analysis,[46] the AUC/MIC ratio associated with this dose was 21.

Secondary pharmacology against a range of cardiac channels and selected targets from the AstraZeneca secondary pharmacology screen that were hit by **1** is shown in Table 5. Whereas **1** exhibited measurable inhibition of both the hERG and sodium ion-gated channels, as well as against NET, 5-HT4 and SERT, both **9g** and **9i** did not exhibit detectable inhibition of any channel in this panel at the top concentrations tested and showed a cleaner profile against non-ion channel hits. Interestingly, fluorinated analogs such as **9g** displayed consistently a cleaner non-ion channel secondary pharmacology profile than the corresponding methoxy analogs such as **9i**, despite the higher log*D*. Compound **9g** was also better tolerated in rat than **1** or **9i** (results not shown).

Table 3
Pharmacokinetics in rat and dog[a]

	Compound	Clearance (mL/min/kg)	Volume (L/kg)	Half-life (h)	AUC (iv) μg h/mL	Bioavailability (%)
Rat						
	1	252	23	1.9	0.19	18
	9g	46	9	10	3.3	2
	9i	47	13	9	3.9	ND
Dog						
	1	8.5	2	13	6.8	ND
	9g	14	4	9	12	ND
	9i	14	2.7	8	3.8	ND

[a] Pharmacokinetic parameters following administration of 10 mg/kg iv infusion.

Table 4
Exposure and efficacy of compound **9g** in an immunocompromised mouse thigh model[a]

	Compound **9g**		
	Log reduction in CFU/g thigh versus start of treatment[b]	AUC∞ (μg/h/mL)	Free AUC/MIC[c]
Levofloxacin 160 mg/kg/day	3.5	130	65
100 mg/kg/day	0.2	14.8	12
150 mg/kg/day	1.3	20.1	16
200 mg/kg/day	2.5	29.9	23

[a] In vivo activity against *P. aeruginosa* PAO1 (ARC545). Vehicle control grew 1.5 log in the first 10 h post-infection and the mice were sacrificed for humane reasons.
[b] Relative to starting inoculum.
[c] Free AUC/MIC ratios based upon an MIC = 1 μg/mL and a mouse protein binding value of 21.5%.

Table 5
Secondary pharmacology panel results for compound **9g** and **9i** compared to **1**

Secondary pharmacology target	**1** IC_{50} (μM)	**9g** IC_{50} (μM)	**9i** IC_{50} (μM)
Norepinephrine transporter (NET)	61[a]	9[a]	52[a]
Serotonin 5HT 4 receptor (5-HT4)	99[a]	7[a]	32[a]
Serotonin transporter (SERT)	77[a]	32[a]	32[a]
Potassium channel, I_{Kr} (hERG)	35	>333	>333
Potassium channel, I_{Ks} (KvLQTS)	>100	>100	>333
Potassium channel, I_{Kur} (Kv1.5)	>100	>100	ND
Potassium channel, I_{to} (Kv4.3)	>100	>333	>333
Sodium channel (Nav1.5)	92	>333	>333
Calcium channel, (Cav1.2)	>333	>100	>333
Calcium channel, (Cav3.2)	>100	>100	ND
Cyclic nucleotide-gated 4 (HCN4)	>100	>100	ND

[a] % Inhibition of receptor binding at 30 μM test compound; aqueous solubility was >333 μM for all compounds (results not shown).

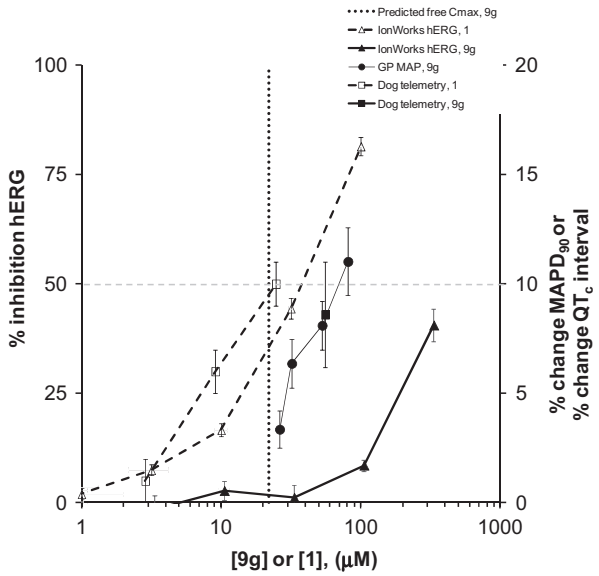


Figure 4. Effects on cardiac repolarization potential ($MAPD_{90}$) of the guinea pig and QT prolongation of the dog for compounds **9g** and **1**: In the guinea pig model, **9g** and vehicle control were infused intravenously over two 17-min periods and MAP duration at 90% repolarization ($MAPD_{90}$) was determined. Increases in action potential duration were observed for **9g** (closed circles), with an EC_{10} of 71 μM free (for in vivo studies *x*-axis concentrations are corrected to account for protein binding). In the dog studies **1** and **9g** were dosed intravenously and data are presented as a percent change from control recordings. Details on the calculations of cardiac parameters, including vehicle control data, can be found in the Section 5. Ion Works hERG dose response data for **1** and **9g** are plotted (triangles) for comparison. The predicted free C_{max} value for the human therapeutic dose of **9g**, 22 μM, is indicated by the vertical dashed black line.

Cardiovascular effects were of particular concern, as they were observed for **1** and related compounds of this class.[10] Therefore, effects of compound **9g** on the cardiac repolarization potential were studied in the anesthetized guinea pig and in dog (Fig. 4). In the guinea pig, **9g** caused a statistically significant change in monophasic action potential duration at 90% repolarization ($MAPD_{90}$) at a free drug concentration of 86 μM (EC_{10}). This effect represents a leftward-shift relative to the hERG IC_{50} value of >333 μM. However, inspection of the dose–response curve for **9g** vs the hERG channel revealed ~40% inhibition at 333 μM. With this in mind, the effect seen in the guinea pig assay is consistent with the hypothesis that 10–20% inhibition of hERG may be sufficient to cause a detectable signal for QT prolongation in vivo.[47] Prolongation of the QT_c interval was next investigated in a telemetered dog study. A statistically significant 9% prolongation effect was seen in the dog with compound **9g** at 56 μM, which is similar to the exposure that produced an effect in the guinea pig model. In the dog model, the concentration where **9g** gave a QT_c signal represents roughly a 3-fold improvement over **1**. For **9g**, the resulting margin from $MAPD_{90}$ EC_{10} and QT_c changes to predicted human fC_{max} was estimated to be in the range of ~2–4, depending on the efficacy endpoint (one to two log reduction of bacterial load in the *P. aeruginosa* thigh model).

4. Conclusions

The NBTI lead **1** was optimized for reduced hERG channel activity and for activity against the Gram-negative organism *P. aeruginosa* by introduction of a second basic group in the linker that both reduces the log*D* of the compounds and allows formation of an intramolecular H-bond. The resulting optimized amino ethyl cyclohexyl NBTI **9g** shows a significantly reduced inhibition of hERG relative to **1**. The effects of compound **9g** on the monophasic action potential (MAP) were studied in the guinea pig model and QT prolongation was studied in dog and the results indicated reduced potential for QT prolongation for **9g** relative to earlier analogs such as **1**.[10]

Compounds **9g** and **9i** have excellent potency against *P. aeruginosa*, including fluoroquinolone-resistant isolates, with MIC_{90}s of 4 μg/mL. Compound **9g** was evaluated in a neutropenic mouse thigh infection model against *P. aeruginosa* and was found to be efficacious with a 2.5 log reduction in CFU associated with a free plasma AUC/MIC ratio of 21, a ratio similar to that seen for fluoroquinolones.[48] The pharmacokinetic properties of **9g** and **9i** were studied in rat and dog and showed moderate clearance and high volumes of distribution. Clearance in rat was significantly reduced with **9g** and **9i** relative to **1**. This compound class thus shows promise for the development of novel antimicrobial agents against drug-resistant *P. aeruginosa*.

5. Experimental section

5.1. Minimum inhibitory concentration testing

Minimum inhibitory concentrations (MICs) were determined by broth microdilution according to the Clinical and Laboratory

Standards Institute guidelines.[49] Bacterial cultures were incubated at 35 °C on blood agar plates (Remel #01202) for 18–20 h prior to MIC determinations. Culture media for bacterial MIC determinations was Mueller Hinton Broth, Difco #275730 in 96 well microtiter plates. Compounds were dissolved in 100% DMSO and diluted to 2% DMSO (v/v) in culture medium in doubling dilutions from 64 to 0.06 μg/mL. After inoculation, plates were incubated for 18–20 h, and optical density of the wells was read by spectrophotometry at 620 nm. The MIC is the concentration at which >90% inhibition of growth (OD) has occurred.

5.2. Topoisomerase IV assay

IC_{50} assays measuring the ATP-ase activity of *E. coli* TopoIV were performed as described previously.[8]

5.3. Plasma protein binding

Plasma protein binding was determined using the Dianorm equilibrium dialysis chamber. Compound (10 μM concentration) was spiked in the plasma chamber (donor side), phosphate buffer was placed in the receiver side. The unit was rotated at 37 °C for 16 h. Drug concentration was determined for the plasma sample that represents the bound fraction, and the buffer sample that represents the free fraction. LC/MS–MS quantitative sample analysis was achieved using an Ace C18 50 × 4.6 mm column (MacMod, PA) and electrospray ionization MRM detection (PE Sciex API 4000 mass spectrometer, Applied Biosystems CA). Plasma samples (50 μL) were treated with methanol (150 μL) containing an internal standard to precipitate the protein. Concentration determination was based on a standard curve (10 nM to 10 μM), data processed by the Analyst Version 1.4.1. software.

5.4. Log*D* determination

The partition coefficient (log*D*) was measured by shake flask method, using 10 mM phosphate buffer at pH 7.4 and *n*-octanol. The samples were allowed to reach equilibrium by shaking for 1 h at 1200 rpm, and sample analysis was done by LC/UV, with MS for mass confirmation.

5.5. pK_a determination

pK_as were determined at Sirius Analytical Instruments Ltd (Forest Row Business Park, Station Road, Forest Row, East Sussex, TH18 5DW) by a Gold Standard pH metric assay on a Sirius T3 automated system in triplicate. The accuracy of the measurement was approximately 0.02 log units.

5.6. Animals

Wistar Han rats for pharmacokinetic studies were obtained from Charles River Laboratories (Raleigh, NC). CD-1 mice were obtained from Charles River Laboratories (Kingston, NY). All animals were housed and acclimated in the animal facility on site before each study. All experimental procedures were conducted in accordance with protocols approved by the Institutional Animal Care and Use Committee.

5.7. Pharmacokinetic studies

Pharmacokinetic properties of selected compounds were studied in male rat and Beagle dog. Plasma pharmacokinetics were determined from 0 to 24 h following 15 min iv infusions at 10 mg/kg. Serial 200 μL samples of whole blood were taken at time intervals. Concentration of compound in plasma was determined by LC–MS/MS and pharmacokinetic parameters were estimated using a non-compartmental model in WinNonLin (Pharsight). Exposure in CD-1 mice was determined for analysis of the efficacy studies. At timed intervals, groups of three mice were sacrificed and whole blood samples collected by cardiac puncture. Plasma samples were prepared and analyzed as described above.

5.8. *Pseudomonas aeruginosa* neutropenic thigh infection model

Compound **9g** was studied in a neutropenic mouse thigh infection model as described in Mills et al.[50] Briefly, mice were rendered neutropenic by injecting cyclophosphamide (Sigma–Aldrich, St. Louis MO) intraperitoneally 4 days (150 mg/kg of body weight) and 1 day (100 mg/kg) before experimental infection. Mice were infected with *P. aeruginosa* PAO1 to achieve a target inoculum of 5×10^5 CFU. Groups of five animals each received an intraperitoneal injection of **9g** at the doses specified in Table 4, prepared in 5% dextrose with lactic acid pH 5.0, on a bid, q12 regime starting 2 h after infection. An additional group of five mice received vehicle alone. Efficacy was determined 24 h after the start of treatment. Thighs were removed, weighed, homogenized and aliquots plated onto tryptic soy agar plates and incubated at 37 °C overnight for CFU determination.

5.9. IonWorks

Electrophysiologic measurements were conducted in ion channel expressing CHO or HEK cells using an automated, 384-well PatchPlate™ device (IonWorks™ (Schroeder et al., 2003)) and based around the method described by Bridgland-Taylor et al., 2006. Initially, a pre-compound current was evoked in each cell by a voltage pulse specific to each channel type. Test compounds, vehicle or positive control were then added to each well and after ~3 min the voltage pulse was re-applied to generate a post-compound current. For each IonWorks™ run, the current amplitude in each cell was compared in the presence of test compound for the same cell. All data were then scaled by defining the effect of 0.33% DMSO as 0% inhibition and the effect of the supra-maximal blocking concentration of the positive control as 100% inhibition. Percentage inhibition data from up to 4 cells for each test concentration was determined from at least two runs.

5.10. Guniea pig

Cardiac electrophysiological characterization of compounds was performed by the method described by.[51] Male guinea pigs were anaesthetized with Nembutal® and tracheotomized for mechanical ventilation. The carotid artery and jugular vein were cannulated, and to eliminate autonomic influence on the heart a bilateral vagotomy was performed and propranolol (0.5 mg/kg) given intravenously. Needle electrodes were positioned for recording of lead II electrocardiogram. The chest was opened and a bipolar electrode was clipped to the left atrial appendage for cardiac pacing. A suction electrode for recording of the monophasic action potential (MAP) was positioned on the left ventricular epicardial wall. For the cardiac electrophysiological characterization of the drug, the MAP duration at 90% repolarization (MAPD90) were recorded during cardiac pacing. The compound was infused intravenously with two 17-min infusion periods and cardiovascular signals were continuously recorded. The first infusion period was administered at 17 mg/kg at a rate of 0.1 mL/kg min; the second infusion period was administered at 51 mg/kg, at a rate of 0.2 mL/kg min. Blood samples were collected at each dose level for determination of plasma concentration. Calculation of% change in MAPD90 was relative to the time zero baseline for each animal.

The vehicle employed was 5% dextrose in water and the change in MAPD90 was less than 2% during vehicle alone infusion.

5.11. Dog telemetry

For compound **1**, non-invasive ECG telemetry data was recorded continuously from a small, lightweight transmitter that was housed in a standard animal jacket. The transmitter was connected to four adhesive skin electrodes producing an approximate Lead II configuration. Each dog was single housed in pens during recordings fitted with appropriate radio antennae to minimize disturbances. For compound **9g**, dogs were surgically implanted with telemetric transmitter in the peritoneal cavity. The electrodes of transmitters were placed in Lead II configuration and a sensor catheter was introduced into the femoral artery to measure blood pressure. For both compounds, recordings were made once during the pre-study period, on Day 1 and at end of the repeat dose period. Heart rate (RR interval) and ECG intervals (PR, QRS, QT, QT_cV and QT_cR) were measured or calculated (QT_cV is QT adjusted for heart rate using Van de Water's correction, QT_cR is QT adjusted for heart rate using an individual animals correction. The% change in QT_cR was calculated relative to the matched time point from the same animal dosed with the vehicle control. Vehicle for compound **1** was 5% w/v dextrose in water, adjusted to pH 4 and route of administration was by intravenous infusion. Dosing duration was 1 h at a rate of 5 mL/kg/h. Vehicle for compound **9g** was 5% mannitol/0.1 M tartaric acid in water, adjusted to pH 4 and route of administration was by intravenous infusion. Dosing duration was 4 h at a rate of 2 mL/kg/h. Data plotted on Figure 4 are from measurements made at completion of infusion, 1 h for compound **1** and 4 h for compound **9g**, when plasma concentrations were at C_{max}.

6. General chemical methods

All commercially available solvents and reagents were used without further purification. All moisture-sensitive reactions were carried out under a nitrogen atmosphere in commercially available anhydrous solvents. Column chromatography was performed on 230–400 mesh silica gel 60. Aluminium-backed sheets of silica gel 60 F254 (EM Science) were used for TLC. Melting points were obtained with a Mel-TempII melting point apparatus from Laboratory Devices, Inc and are uncorrected. ^{1}H NMR spectra were recorded at 300 MHz or 400 MHz. Chemical shifts are reported in ppm (δ) relative to solvent. The purity of tested compounds was assessed by LC-MS. Reverse Phase HPLC was carried out using YMC Pack ODS-AQ (100 × 20 mm ID, S-5μ particle size, 12 nm pore size) on Agilent instruments. Mass spectroscopy was performed using a Micromass Quattro Micro mass spectrometer (for ESP) and an Agilent 1100 MSD instrument (for APCI). All compounds tested possessed a purity of at least 95%.

6.1. 6-Fluoro-7-methoxyquinoxalin-2(1*H*)-one (2d)

The reaction mixture of **26** was filtered through a 0.45 μm membrane into a flask containing ethyl 2-oxoacetate, 50% in toluene (31.9 mL, 161.16 mmol). The filter cake was into the reaction mixture with ethanol (30 mL) and the mixture was stirred for 3 days at room temperature. The mixture was filtered through a 0.45 μm membrane and the solid was washed with ethanol (2 × 50 mL) and hexanes (50 mL) to give the product together with the regioisomer **27** as a yellow solid, 11.80 g, ~1:1 mixture. This mixture was converted to the chlorides **28** and **29** as described under the preparation for **28**, at which stage the regioisomers could be separated by chromatography on silica gel. A suspension of **28** (3.37 g, 15.85 mmol) in HCl (5 M, 100 mL) was heated to 125 °C bath temperature for 2 h. The mixture was cooled to room temperature and diluted with water (50 mL). The brown precipitate was collected by filtration and washed with water to give the desired product as a light brown solid, 1.98 g (64%). MS (ESP) m/z 195 (MH^+); ^{1}H NMR (DMSO-d_6) δ: 12.38 (br s, 1H); 8.05 (s, 1H); 7.65 (d, 1H); 6.92 (m, 1H); 3.92 (s, 3H).

6.2. 7-Fluoro-1*H*-quinoxalin-2-one (2e)

To a solution of sodium hydroxide (597.1 g) in water (6.7 L) was added **34** followed by a solution of 3 wt % hydrogen peroxide in water (6.7 L). The reaction mixture was slowly heated to 80 °C and maintained at this temperature for 4 h. Then the heating source was removed and acetic acid (860 mL) was added dropwise. The suspension was stirred overnight at room temperature and the precipitate was collected by suction filtration and dried under vacuum at 60 °C to afford the product as an off-white solid (522 g). MS (ESP) m/z 165 (MH^+); ^{1}H NMR (DMSO-d_6) δ: 12.44 (br s, 1H); 8.13 (s, 1H); 7.83 (m, 1H); 7.79 (m, 1H); 7.16 (m, 1H); 7.04 (m, 1H).

6.3. *trans-tert*-Butyl 4-(1-(2-nitrophenylsulfonyl)aziridin-2-yl)-cyclohexylcarbamate (3)

To a solution of copper(II) triflate (643, 1.78 mmol) in dry acetonitrile (2 mL) was added a solution of commercially available *tert*-butyl 4-vinylcyclohexylcarbamate **10** (4.0 g, 17.75 mmol) in dry acetonitrile (4 mL, heated till dissolved). The mixture was warmed gently to enhance solubility, then cooled to room temperature and [*N*-(*o*-nitrophenylsulfonyl)imino]phenyliodinane[36] (7.2 g, 17.75 mmol) was added. The reaction mixture became exothermic after several minutes. The mixture was stirred at room temperature under nitrogen for 3 h, then concentrated under reduced pressure. The crude product was taken up in dichloromethane and the solids were removed by filtration. The filtrate was chromatographed on silica gel eluting with 10–50% acetone in hexanes to give 2.0 g (27%) of the racemic product as an off-white solid. MS (ESP) m/z 426 (MH^+); ^{1}H NMR (DMSO-d_6) δ: 0.98–1.12 (m, 5H); 1.36 (m, 10H); 1.45–1.53 (m, 1H); 1.60–1.65 (m, 1H); 1.66–1.79 (m, 2H); 2.63–2.69 (m, 1H); 2.68–2.75 (m, 1H); 3.09 (br s, 1H); 6.64 (d, 1H); 7.88–7.95 (m, 1H); 7.96–8.02 (m, 1H); 8.03–8.07 (m, 1H); 8.15 (dd, 1H).

The *R* and *S* enantiomers of **3** were separated by chiral HPLC (Chiralcel OJ column, 20 × 250 mm, 10 μ, 1:1 isopropanol: hexanes, 10 mL/min) to give the (*S*) enantiomer of **3** as first eluting enantiomer and the (*R*) enantiomer of **3** as second eluting enantiomer.

6.4. (*S*)-*trans-tert*-Butyl 4-(1-(2-nitrophenylsulfonyl)aziridin-2-yl)-cyclohexylcarbamate (3,(*S*))

$[\alpha]_{D,S}$ +38 (*c* 0.1, in methanol).

6.5. (*R*)-*trans-tert*-Butyl 4-(1-(2-nitrophenylsulfonyl)aziridin-2-yl)-cyclohexylcarbamate (3,(*R*))

$[\alpha]_{D,R}$ −38 (*c* 0.1, in methanol).

7. General procedure for 4, by alkylation of 2

To a solution of **2** (2 mmol) in DMF (10 mL) was added sodium hydride (120 mg, 3.0 mmol), and the mixture was stirred at room temperature for 15 min under nitrogen. A solution of **3** (850 mg, 2.00 mmol) in DMF (6 mL) was added and the mixture was stirred at room temperature over night. The mixture was quenched with ice water and extracted with ethyl acetate (100 mL). The organic phase was washed with brine twice (100 mL) and dried over magnesium sulfate, then concentrated under reduced pressure.

7.1. (*R*)-*trans*-*tert*-Butyl 4-(2-(7-methoxy-2-oxoquinolin-1(2*H*)-yl)-1-(2-nitrophenylsulfonamido)ethyl)cyclohexylcarbamate (4a)

Compound **2a**[29] was reacted with sodium hydride and **3**, (*R*)-enantiomer, following the general procedure for **4**. Chromatography was done on silica gel with 10–80% ethyl acetate in hexanes to give the product in 83% yield as a pale yellow solid. MS (ESP) *m*/*z* 601 (MH^+); ^{1}H NMR ($CDCl_3$) δ: 1.04–1.22 (m, 2H); 1.22–1.42 (m, 4H); 1.46 (s, 9H); 1.64–1.78 (m, 1H); 1.82–1.95 (m, 1H); 2.06–2.22 (m, 3H); 3.41 (br s, 1H); 3.95 (s, 3H); 4.05 (d, *J* = 14.32 Hz, 1H); 4.38 (br s, 1H); 4.76 (t, *J* = 12.15 Hz, 1H); 6.21 (d, *J* = 7.16 Hz, 1H); 6.26 (d, *J* = 9.42 Hz, 1H); 6.71 (s, 1H); 6.79 (dd, *J* = 8.67, 2.26 Hz, 1H); 7.19–7.26 (m, 1H); 7.28–7.32 (m, 1H); 7.41 (td, *J* = 7.72, 1.32 Hz, 1H); 7.50–7.61 (m, 2H).

7.2. (*R*)-*trans*-*tert*-Butyl 4-(2-(7-fluoro-4-methyl-2-oxoquinolin-1(2*H*)-yl)-1-(2-nitrophenylsulfonamido)ethyl)cyclohexylcarbamate (4b)

7-Fluoro-4-methylquinolin-2(1*H*)-one **2b**[30] (354 mg, 2.00 mmol) was reacted with sodium hydride (120 mg, 3.0 mmol) and **3**, (*R*) isomer, following the general procedure for **4**. Chromatography was done on silica gel with hexanes/acetone 2:1 to give 640 mg (53%) of the product as a colorless solid. MS (ESP) *m*/*z* 601 (MH^+); ^{1}H NMR (DMSO-d_6) δ: 7.40–7.75 (m, 7H); 6.97 (t, 1H); 6.68 (d, 1H); 6.29 (s, 1H); 4.00–4.30 (m, 2H); 3.75 (s, br s, 1H); 3.16 (m, 1H); 2.27 (s, 3H); 1.82 (m, 4H); 1.50 (m, 1H); 1.38 (s, 9H); 1.00–1.25 (m, 4H).

7.3. (*R*)-*trans*-*tert*-Butyl 4-(2-(7-methoxy-2-oxoquinoxalin-1(2*H*)-yl)-1-(2-nitrophenylsulfonamido)ethyl)-cyclohexylcarbamate (4c)

Compound **2c**[8,31,32] was reacted with sodium hydride and **3**, (*R*)-enantiomer, following the general procedure for **4**. Chromatography was done on silica gel with 10–50% acetone in hexanes to give the product in 48% yield as an off-white hard foam. $[\alpha]_D$ +6.0 (*c* 0.1, in methanol). MS (ESP) *m*/*z* 602 (MH^+); ^{1}H NMR (DMSO-d_6) δ: 0.98–1.18 (m, 4H); 1.37 (s, 9H); 1.45–1.58 (m, 1H); 1.72–1.92 (m, 4H); 3.10 (br s, 1H); 3.73 (br s, 1H); 3.92 (s, 3H); 4.13–4.31 (m, 2H); 6.61–6.71 (m, 1H); 6.86 (dd, 1H); 7.04 (s, 1H); 7.46–7.68 (m, 5H); 7.86–7.97 (m, 2H).

7.4. *trans*-*tert*-Butyl-4-(2-(6-fluoro-7-methoxy-2-oxoquinoxalin-1(2*H*)-yl)-1-(2-nitrophenylsulfonamido)ethyl)-cyclohexylcarbamate (4d)

Compound **2d** (0.412 g, 2.12 mmol) was reacted with sodium hydride (84 mg, 2.1 mmol) and **3**, racemic, following the general procedure for **4**. Chromatography was done on silica gel with 20–100% ethyl acetate in hexanes to give 520 mg (48%) of the product as a yellow foam. MS (ESP) *m*/*z* 620 (MH^+); ^{1}H NMR (DMSO-d_6) δ: 7.99 (s, 1H); 7.96 (s, 1H); 7.66 (m, 2H); 7.56 (m, 2H); 7.43 (d, 1H); 7.14 (d, 1H); 6.69 (d, 1H); 4.30 (d, 1H); 4.11 (t, 1H); 4.04 (s, 3H); 3.73 (m, 1H); 1.84 (m, 4H); 1.50 (m, 1H); 1.37 (s, 9H); 1.15 (m, 4H).

7.5. (*R*)-*trans*-*tert*-Butyl 4-(2-(7-fluoro-2-oxoquinoxalin-1(2*H*)-yl)-1-(2-nitrophenylsulfonamido)ethyl)cyclohexylcarbamate (4e)

7-Fluoro-4-methylquinolin-2(1H)-one **2e** (171 mg, 1.04 mmol) was reacted with sodium hydride (50 mg, 1.25 mmol) and **3**, (*R*) isomer, following the general procedure for **4**. Chromatography was done on silica gel with hexanes/acetone 2:1 to give 300 mg (49%) of the product as a colorless solid. MS (ESP) *m*/*z* 612 (MNa^+); ^{1}H NMR (DMSO-d_6) δ: 8.05 (s, 1H); 7.92 (d, 1H); 7.68–7.52 (m, 6H); 7.11 (ddd, 1H); 6.66 (d, 1H); 4.23 (dd, 1H); 4.09 (dd, 1H); 3.69 (m, 1H); 3.12 (m, 1H); 1.86–1.71 (m, 4H); 1.51 (m, 1H); 1.38 (s, 9H); 1.22–0.94 (m, 4H).

7.6. *trans*-*tert*-Butyl 4-(2-(7-methoxy-2-oxo-1,5-naphthyridin-1(2*H*)-yl)-1-(2-nitrophenylsulfonamido)ethyl)-cyclohexylcarbamate (4f)

7-Methoxy-1,5-naphthyridin-2(1*H*)-one **2f**[52] (0.444 g, 2.52 mmol) was reacted with sodium hydride (101 mg, 2.52 mmol) and **3**, racemic, (715 mg, 1.68 mmol) following the general procedure for **4**. Chromatography was done on silica gel with 10–50% acetone in hexanes to give 740 mg (73%) of the product as an off-white solid. MS (ESP) *m*/*z* 602 (MH^+); ^{1}H NMR (DMSO-d_6) δ: 1.01–1.20 (m, 4H); 1.38 (s, 9H); 1.47–1.59 (m, 1H); 1.73–1.94 (m, 4H); 3.14 (br s, 1H); 3.66–3.78 (m, 1H); 3.98 (s, 3H); 4.12 (br s, 1H); 4.27–4.39 (m, 1H); 6.48–6.58 (m, 1H); 6.65–6.75 (m, 1H); 7.39–7.45 (m, 1H); 7.47–7.69 (m, 5H); 7.76–7.89 (m, 1H); 8.11 (s, 1H).

7.7. (*R*)-*trans*-*tert*-Butyl 4-(2-(7-fluoro-2-oxo-1,5-naphthyridin-1(2*H*)-yl)-1-(2-nitrophenylsulfonamido)ethyl)cyclo-hexylcarbamate (4g)

7-Fluoro-1,5-naphthyridin-2(1*H*)-one 2 g[52] (680 mg, 4.14 mmol) was reacted with sodium hydride (199 mg, 4.97 mmol) and **3**, (*R*) isomer, (1.76 g, 4.14 mmol) following the general procedure for **4**. Chromatography was done on silica gel with 20–100% ethyl acetate in hexanes to give 720 mg of the product as a yellow foam. MS (ESP) *m*/*z* 612 (MNa^+); ^{1}H NMR ($CDCl_3$) δ: 8.25 (m, 1H); 7.96 (m, 1H); 7.56–7.40 (m, 5H); 7.33 (m, 1H); 6.59 (d, 1H); 4.56 (m, 1H); 4.39 (m, 1H); 4.14 (m, 1H); 3.88 (m, 1H); 1.96 (m, 2H); 1.75 (m, 2H); 1.58 (m, 2H); 1.36 (s, 9H); 1.20–1.00 (m, 3H).

8. General procedure for 5, by deprotection of 4

A solution of **4** (1 mmol) in dichloromethane (3 mL) was treated with trifluoroacetic acid (1 mL) at room temperature for 2 h. The solvent was removed under reduced pressure and the residue codistilled with methanol twice, to give the products as bis-trifluoroacetate salts.

8.1. (*R*)-*N*-(1-(4-Aminocyclohexyl)-2-(7-methoxy-2-oxoquinolin-1(2*H*)-yl)ethyl)-2-nitrobenzenesulfonamide trifluoroacetate salt (5a)

Compound **4a** (1.14 g, 1.9 mmol) was reacted with trifluoroacetic acid according to the general procedure for **5** to give the product as a pale yellow solid 1.05 g (90%). MS (ESP) *m*/*z* 501 (MH^+); ^{1}H NMR (DMSO-d_6) δ: 1.10–1.36 (m, 4H); 1.49–1.63 (m, 1H); 1.87–2.05 (m, 4H); 2.96 (br s, 1H); 3.76–3.86 (m, 1H); 3.89 (s, 3H); 4.10–4.37 (m, 2H); 6.25 (d, *J* = 9.42 Hz, 1H); 6.74 (dd, *J* = 8.57, 1.98 Hz, 1H); 6.97 (s, 1H); 7.35 (d, *J* = 8.67 Hz, 1H); 7.45 (d, *J* = 3.96 Hz, 2H); 7.50–7.58 (m, 2H); 7.59–7.64 (m, 1H); 7.65–7.87 (m, 4H).

8.2. (*R*)-*trans*-*N*-(1-(4-Aminocyclohexyl)-2-(7-fluoro-4-methyl-2-oxoquinolin-1(2*H*)-yl)ethyl)-2-nitrobenzenesulfonamide, trifluoroacetic acid salt (5b)

Compound **4b** (640 mg, 1.06 mmol) was reacted with trifluoroacetic acid according to the general procedure for **5** to give the product as a colorless solid (740 mg). MS (ESP) *m*/*z* 503 (MH^+); ^{1}H NMR (DMSO-d_6) δ: 1.26 (m, 4H); 1.50–1.66 (m, 3H); 1.79–2.04 (m, 4H); 2.26 (s, 3H); 3.00 (m 1H); 3.79 (m, 1H); 4.10 (m, 2H); 6.28 (s, 1H); 6.80–7.04 (m, 1H); 7.30–7.76 (m, 10H).

8.3. (*R*)-*trans*-*N*-(1-(4-Aminocyclohexyl)-2-(7-methoxy-2-oxoquinoxalin-1(2*H*)-yl)ethyl)-2-nitrobenzenesulfonamide, trifluoroacetic acid salt (5c)

Compound **4c** (570 mg, 0.95 mmol) was reacted with trifluoroacetic acid according to the general procedure for **5** to give the product as colorless hard foam, 580 mg (quant). MS (ESP) *m*/*z* 502 (MH^+).

8.4. *N*-*trans*-(1-(4-Aminocyclohexyl)-2-(6-fluoro-7-methoxy-2-oxoquinoxalin-1(2*H*)-yl)ethyl)-2-nitrobenzenesulfonamide (5d)

Compound **4d** (520 mg, 0.84 mmol) was reacted with trifluoroacetic acid according to the general procedure for **5** to give the product as an orange foam (quant). MS (ESP) *m*/*z* 520 (MH^+).

8.5. (*R*)-*trans*-*N*-(1-(4-Aminocyclohexyl)-2-(7-fluoro-2-oxoquinoxalin-1(2*H*)-yl)ethyl)-2-nitrobenzenesulfonamide, trifluoroacetic acid salt (5e)

Compound **4e** (295 mg, 0.50 mmol) was reacted with trifluoroacetic acid according to the general procedure for **5** to give the product as a colorless oil, 233 mg (quant). MS (ESP) *m*/*z* 490 (MH^+).

8.6. *trans*-*N*-(1-(4-Aminocyclohexyl)-2-(7-methoxy-2-oxo-1,5-naphthyridin-1(2*H*)-yl)ethyl)-2-nitrobenzenesulfonamide, trifluoroacetic acid salt (5f)

Compound **4f** (655 mg, 1.09 mmol) was reacted with trifluoroacetic acid according to the general procedure for **5** to give the product as a colorless oil, 670 mg (quant). MS (ESP) *m*/*z* 502 (MH^+).

8.7. (*R*)-*trans*-*N*-(1-(4-Aminocyclohexyl)-2-(7-fluoro-2-oxo-1,5-naphthyridin-1(2*H*)-yl)ethyl)-2-nitrobenzenesulfonamide (5g)

Compound **4g** (582 mg, 0.99 mmol) was reacted with trifluoroacetic acid according to the general procedure for **5** to give the product as a colorless oil, 483 mg (quant). MS (ESP) *m*/*z* 490 (MH^+).

9. General procedure for 8, by reductive amination of 5

A solution of **5** (0.20 mmol) and 1 equiv aldehyde, either 3-oxo-3,4-dihydro-2*H*-pyrido[3,2-*b*][1,4]oxazine-6-carbaldehyde **6**[26] or 7-oxo-7,8-dihydro-6*H*-pyrimido[5,4-*b*][1,4]oxazine-2-carbaldehyde **7**[53] in dry DMF (2 mL) were heated over freshly activated 3 Å molecular sieves (pearled) at 60 °C for 3 h. The reaction mixture was cooled to 0 °C, and sodium triacetoxy borohydride (0.6 mmol) was added. The reaction mixture was stirred at room temperature for 30 min, then filtered. The filtrate was concentrated to dryness under reduced pressure. The residue was taken up in dichloromethane/methanol (9:1, 50 mL) and saturated aqueous sodium hydrogencarbonate solution (5 mL). The aqueous phase was back extracted twice with dichloromethane/methanol (9:1, 2 × 20 mL) and the combined organic phases were dried over sodium sulfate and concentrated under reduced pressure. Chromatography was done on silica gel with a gradient of 0–20% methanol in CH_2Cl_2, containing 0.25% NH_4OH.

9.1. (*R*)-*N*-(2-(7-Methoxy-2-oxoquinolin-1(2*H*)-yl)-1-(4-((7-oxo-7,8-dihydro-6*H*-pyrimido[5,4-*b*][1,4]oxazin-2-yl)methylamino)-cyclohexyl)ethyl)-2-nitrobenzenesulfonamide (8a)

Compound **5a** (500 mg, 0.8 mmol), *N*,*N*-diisopropylethylamine, **7**[53] and sodium triacetoxyborohydride were reacted following the general procedure for **8** to give 0.36 g (67%) of the product as a pale yellow solid. MS (ESP) *m*/*z* 664 (MH^+); ^{1}H NMR (DMSO-d_6) δ: 0.90–1.20 (m, 4H); 1.47–1.62 (m, 1H); 1.79–2.01 (m, 4H); 2.35 (t, *J* = 10.27 Hz, 1H); 3.70–3.84 (m, 3H); 3.89 (s, 3H); 4.24 (br s, 2H); 4.72 (s, 2H); 6.25 (d, *J* = 9.42 Hz, 1H); 6.73 (dd, *J* = 8.67, 1.88 Hz, 1H); 6.95 (br s, 1H); 7.35 (d, *J* = 8.67 Hz, 1H); 7.42–7.49 (m, 2H); 7.49–7.58 (m, 2H); 7.58–7.65 (m, 1H); 8.23 (s, 1H).

9.2. (*R*)-*N*-(2-(7-Fluoro-4-methyl-2-oxoquinolin-1(2*H*)-yl)-1-*trans*-(4-((7-oxo-7,8-dihydro-6*H*-pyrimido[5,4-*b*][1,4]oxazin-2-yl)methylamino)cyclohexyl)ethyl)-2-nitrobenzenesulfonamide (8b)

Compound **5b** (725 mg, 1.44 mmol), *N*,*N*-diisopropylethylamine (0.25 mL, 1.6 mmol), **7**[53] (0.257 g, 1.44 mmol) and sodium triacetoxyborohydride (1.523 g, 7.19 mmol) were reacted following the general procedure for **8** to give 550 mg of the product as a colorless solid (58%). MS (ESP) *m*/*z* 666 (MH^+); ^{1}H NMR (methanol-d_4) δ: 1.16 (m, 5H); 1.58 (m, 1H); 1.79 (m, 1H); 1.99 (m, 3H); 2.17 (s, 3H); 2.44 (m, 1H); 3.78–3.93 (m, 6H); 4.61 (s, 2H); 6.10 (s, 1H); 6.83 (t, *J* = 9.8 Hz,1H); 6.94 (d, *J* = 9.8 Hz, 1H), 7.14 (t, *J* = 7.5 Hz, 1H); 7.30–7.50 (m, 4H); 8.08 (s, 1H).

9.3. (*R*)-*N*-(2-(7-Fluoro-4-methyl-2-oxoquinolin-1(2*H*)-yl)-1-*trans*-(4-((3-oxo-3,4-dihydro-2*H*-pyrido[3,2-*b*][1,4]oxazin-6-yl)methylamino)cyclohexyl)ethyl)-2-nitrobenzenesulfonamide (8c)

Compound **5b** (620 mg, 0.99 mmol), triethylamine (1 mL, 0.71 mmol), **6**[26] and sodium triacetoxyborohydride were reacted following the general procedure for **8** to give 237 mg of the product as a colorless hard foam (29%). MS (ESP) *m*/*z* 665 (MH^+); ^{1}H NMR (DMSO-d_6) δ: 11.15 (br s, 1H); 7.25–7.60 (m, 7H); 6.90–7.05 (m, 2H); 6.28 (s, 1H); 4.61 (s, 2H); 4.24 (m, 1H); 4.07 (m, 1H); 3.75 (m, 3H); 3.17 (d, 1H); 2.33 (m, 1H); 2.26 (m, 3H); 1.93 (m, 4H); 1.51 (m, 1H); 1.05 (m, 4H).

9.4. (*R*)-*trans*-*N*-(2-(7-Methoxy-2-oxoquinoxalin-1(2*H*)-yl)-1-(4-((7-oxo-7,8-dihydro-6*H*-pyrimido[5,4-*b*][1,4]oxazin-2-yl) methylamino)-cyclohexyl)ethyl)-2-nitrobenzenesulfonamide (8d)

Compound **5c** (725 mg, 1.44 mmol), *N*,*N*-diisopropylethylamine, **7**[53] and sodium triacetoxyborohydride were reacted following the procedure for **8b** to give the product in 80% yield as a colorless solid. MS (ESP) *m*/*z* 665 (MH^+); ^{1}H NMR (DMSO-d_6) δ: 0.93–1.18 (m, 4H); 1.55 (br s, 1H); 1.80–1.98 (m, 4H); 2.35 (br s, 1H); 3.69–3.80 (m, 2H); 3.92 (s, 3H); 4.10–4.31 (m, 2H); 4.72 (s, 2H); 6.84 (dd, 1H); 7.04 (s, 1H); 7.43–7.66 (m, 5H); 7.89 (s, 1H); 7.96 (s, 1H); 8.23 (s, 1H).

9.5. (*R*)-*trans*-*N*-(2-(7-Methoxy-2-oxoquinoxalin-1(2*H*)-yl)-1-(4-((3-oxo-3,4-dihydro-2*H*-pyrido[3,2-*b*][1,4]oxazin-6-yl)methyl-amino)-cyclohexyl)ethyl)-2-nitrobenzenesulfonamide (8e)

Compound **5b** ethyldiisopropylamine, **6**[26] and sodium triacetoxyborohydride were reacted following the procedure for **8b** to give the product in 71% yield as a colorless solid. $[\alpha]_D$ +20 (*c* 0.1, in methanol); MS (ESP) *m*/*z* 664 (MH^+); ^{1}H NMR (DMSO-d_6) δ: 0.94–1.12 (m, 4H); 1.50–1.61 (m, 1H); 1.83–1.98 (m, 4H); 2.25–2.37 (m, 1H); 3.67–3.76 (m, 2H); 3.92 (s, 3H); 4.12–4.30 (m, 2H); 4.61 (s, 2H); 6.81–6.88 (m, 1H); 6.98–7.06 (m, 2H); 7.27–7.32 (m, 1H); 7.45–7.64 (m, 6H); 7.89 (s, 1H); 11.19 (br s, 1H).

9.6. (*R*)-*trans*-*N*-(2-(7-Fluoro-2-oxoquinoxalin-1(2*H*)-yl)-1-(4-((3-oxo-3,4-dihydro-2*H*-pyrido[3,2-*b*][1,4]oxazin-6-yl)methylamino)cyclohexyl)ethyl)-2-nitrobenzenesulfonamide (8f)

Compound **5e** ethyldiisopropylamine, **6**[26] and sodium triacetoxyborohydride were reacted following the procedure for **8b** to give the product in 71% yield as a colorless solid. $[\alpha]_D$ +20 (*c* 0.1, in methanol); MS (ESP) *m*/*z* 652 (MH^+); ^1H NMR (DMSO-d_6) δ: 11.15 (br s, 1H); 8.04 (s, 1H); 7.89 (br s, 1H); 7.66–7.49 (m, 6H); 7.30 (d, 1H); 7.09 (m, 1H); 7.02 (d, 1H); 4.61 (s, 2H); 4.24 (m, 1H); 4.08 (m, 1H); 3.71 (m, 3H); 2.31 (m, 1H); 1.99–1.75 (m, 4H); 1.57 (m, 1H); 1.16–0.88 (m, 4H).

9.7. (*R*)-*trans*-*N*-(2-(7-Fluoro-2-oxoquinoxalin-1(2*H*)-yl)-1-(4-((7-oxo-7,8-dihydro-6*H*-pyrimido[5,4-*b*][1,4]oxazin-2-yl)methylamino)cyclohexyl)ethyl)-2-nitrobenzenesulfonamide (8g)

Compound **5e** (143 mg, 0.24 mmol), *N*,*N*-diisopropylethylamine, **7**[53] and sodium triacetoxyborohydride were reacted following the procedure for **8b** to give 106 mg (68%) of the product as a colorless hard foam. MS (ESP) *m*/*z* 653 (MH^+); ^1H NMR (DMSO-d_6) δ: 8.25 (s, 1H); 8.04 (s, 1H); 7.87 (br s, 1H); 7.66–7.49 (m, 6H); 7.08 (ddd, 1H); 4.73 (s, 2H); 4.23 (dd, 1H); 4.08 (m, 1H); 3.76 (s, 2H); 3.69 (m, 1H); 2.35 (m, 1H); 1.99–1.77 (m, 4H); 1.58 (m, 1H); 1.18–0.91 (m, 4H).

9.8. *N*-(2-(6-Fluoro-7-methoxy-2-oxoquinoxalin-1(2*H*)-yl)-1-*trans*-(4-((7-oxo-7,8-dihydro-6*H*-pyrimido[5,4-*b*][1,4]oxazin-2-yl)methylamino)cyclohexyl)ethyl)-2-nitrobenzenesulfonamide (8h)

Compound **5h** (520 mg, 1.00 mmol), **7**[53] (0.179 g, 1.00 mmol) and sodium triacetoxyborohydride (528 mg, 2.5 mmol) were reacted following the general procedure for **8** to give 295 mg of the product as an off white solid (44%). MS (ESP) *m*/*z* 683 (MH^+); ^1H NMR (DMSO-d_6) δ: 0.8–1.2 (m, 4H); 1.52 (m, 1H); 1.90 (m, 4H); 2.36 (m, 1H); 3.17 (s, 1H); 3.71 (m, 1H); 3.76 (s, 2H); 4.04 (d, 2H); 4.05–4.29 (m, 3H); 4.73 (s, 2H); 7.14 (d, 1H); 7.37–7.65 (m, 5H); 7.97 (s, 1H); 8.25 (s, 1H).

9.9. *trans*-*N*-(2-(7-Methoxy-2-oxo-1,5-naphthyridin-1(2*H*)-yl)-1-(4-((3-oxo-3,4-dihydro-2*H*-pyrido[3,2-*b*][1,4]oxazin-6-yl)methylamino)-cyclohexyl)ethyl)-2-nitrobenzenesulfonamide (8i)

Compound **5f** (795 mg, 1.09 mmol), ethyldiisopropylamine (0.758 mL, 4.36 mmol), **6**[26] (194 mg, 1.09 mmol) and sodium triacetoxyborohydride (693 mg, 3.27 mmol) were reacted following the procedure for **8b** to give 555 mg of the product as an off-white solid (77%). MS (ESP) *m*/*z* 664 (MH^+); ^1H NMR (DMSO-d_6) δ: 0.98–1.13 (m, 4H); 1.57 (br s, 1H); 1.86–2.01 (m, 4H); 2.30 (br s, 1H); 3.68–3.77 (m, 2H); 3.99 (s, 3H); 4.12 (br s, 1H); 4.26–4.37 (m, 1H); 4.61 (s, 2H); 6.52 (d, 1H); 7.01 (d, 1H); 7.30 (d, 1H); 7.40–7.64 (m, 6H); 7.77 (br s, 1H); 8.09 (d, 1H); 11.15 (br s, 1H).

9.10. (*R*)-*trans*-*N*-(2-(7-Fluoro-2-oxo-1,5-naphthyridin-1(2*H*)-yl)-1-(4-((3-oxo-3,4-dihydro-2*H*-pyrido[3,2-*b*][1,4]oxazin-6-yl)methylamino)cyclohexyl)ethyl)-2-nitrobenzenesulfonamide (8j)

Compound **5g** (483 mg, 0.99 mmol), ethyldiisopropylamine (0.652 mL, 3.95 mmol), **6**[26] (211 mg, 1.18 mmol) and sodium triacetoxyborohydride (627 mg, 2.96 mmol) were reacted following the procedure for **8b** to give 215 mg of the product as an off-white solid (41%). MS (ESP) *m*/*z* 652 (MH^+); ^1H NMR (MeOD) δ: 8.41 (d, 1H); 7.98 (m, 1H); 7.76 (d, 1H); 7.63 (m, 3H); 7.55 (m, 1H); 7.31 (d, 1H); 7.01 (d, 1H); 6.76 (d, 1H); 4.69 (s, 2H); 4.40 (m, 2H); 3.95 (m, 1H); 3.83 (s, 2H); 2.49 (m, 1H); 2.06 (m, 4H); 1.68 (m, 1H); 1.33–1.12 (m, 4H).

10. General procedure for 9 by deprotection of 8

To a solution of **8** (0.68 mmol) in anhydrous DMF (5 mL) was added anhydrous K_2CO_3 (467 mg, 3.38 mmol) and thiophenol (0.348 mL, 3.38 mmol). The mixture was stirred at room temperature for 2 h under a blanket of nitrogen. The volatile portion of the mixture was removed by rotary evaporation and saturated aqueous $NaHCO_3$ (20 mL) was added to the resulting residue. The residue was extracted with MeOH/CH_2Cl_2 (10%, twice with 100 mL). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure.

10.1. (*R*)-2-(*trans*-(4-(1-Amino-2-(7-methoxy-2-oxoquinolin-1(2*H*)-yl)ethyl)cyclohexylamino)methyl)-6*H*-pyrimido[5,4-*b*][1,4]oxazin-7(8*H*)-one (9a)

Compound **8a** (420 mg, 0.60 mmol) was reacted following the general procedure for **9**. Chromatography was done on silica gel with a gradient of 0–25% MeOH in CH_2Cl_2, containing 0.25% NH_4OH, to give the product as a colorless solid (214 mg, 71%). MS (ESP) *m*/*z* 479 (MH^+); ^1H NMR (DMSO-d_6) δ: 1.29–1.53 (m, 4H); 1.53–1.69 (m, 1H); 1.84 (br s, 1H); 2.07 (d, *J* = 14.51 Hz, 1H); 2.13–2.30 (m, 2H); 3.06 (t, *J* = 11.21 Hz, 1H); 3.48 (dd, *J* = 9.32, 5.56 Hz, 2H); 3.94 (s, 3H); 4.22 (s, 2H); 4.35–4.52 (m, 1H); 4.52–4.68 (m, 1H); 4.79 (s, 2H); 6.46 (d, *J* = 9.42 Hz, 1H); 6.93 (dd, *J* = 8.67, 1.88 Hz, 1H); 7.02 (s, 1H); 7.68 (d, *J* = 8.67 Hz, 1H); 7.87 (d, *J* = 9.61 Hz, 1H); 8.36 (s, 1H).

10.2. (*R*)-2-(*trans*-[4-(1-Amino-2-(7-fluoro-4-methyl-2-oxoquinolin-1(2*H*)-yl)ethyl)cyclohexylamino]-methyl)-6*H*-pyrimido[5,4-*b*][1,4]oxazin-7(8*H*)-one (9b)

Compound **8b** (450 mg, 0.68 mmol) was reacted following the general procedure for **9**. Chromatography was done on silica gel with a gradient of 0–25% MeOH in CH_2Cl_2, containing 0.25% NH_4OH, to give the product as a colorless solid (130 mg, 40.0%). [α] +43 (*c* 0.1, MeOH). MS (ESP) *m*/*z* 481 (MH^+); ^1H NMR (DMSO-d_6) δ: 0.87–1.18 (m, 5H); 1.48 (m, 1H); 1.70–1.98 (m, 4H); 2.18–2.38 (m, 4H); 2.77 (m, 1H); 3.10 (s, 1H); 3.69 (s, 2H); 3.90–4.08 (m, 1H); 4.09–4.23 (m, 1H); 4.65 (s, 2H); 6.40 (s, 1H); 7.05 (m, 1H); 7.37 (dd, 1H); 7.74 (dd, 1H), 8.16 (s, 1H).

10.3. (*R*)-*trans*-6-((4-(1-Amino-2-(7-fluoro-4-methyl-2-oxoquinolin-1(2*H*)-yl)ethyl)cyclohexylamino)methyl)-2*H*-pyrido[3,2-*b*][1,4]oxazin-3(4*H*)-one (9c)

Compound **8c** (235 mg, 0.35 mmol) was reacted following the general procedure for **9**. The crude product was then purified by reverse phase HPLC using water/trifluoroacetic acid (0.1%) with acetonitrile gradient of 5–95%. The trifluoroacetic acid salt of the product was redissolved in dichloromethane (100 mL) and basified by a potassium carbonate solution (20 mL) and extracted with dichloromethane (2 × 100 mL), the organic was dried over magnesium sulfate and concentrated under reduced pressure to give the product as a colorless solid, 42 mg (24.8%). MS (ESP) *m*/*z* 480 (MH^+); ^1H NMR (DMSO-d_6) δ: 0.90–1.42 (m, 6H); 1.55 (m, 1H); 1.92 (m, 3H); 2.30 (t, 1H); 2.42 (s, 3H); 2.84 (s, 1H); 3.69 (s, 2H); 4.00–4.40 (m, 2H); 4.60 (s, 2H); 6.48 (s, 1H); 7.02 (d, 1H); 7.13 (t, 1H); 7.28 (d, 1H); 7.44 (d, 1H); 7.81 (t, 1H).

10.4. (*R*)-*trans*-2-((4-(1-Amino-2-(7-methoxy-2-oxoquinoxalin-1(2*H*)-yl)ethyl)cyclohexylamino)methyl)-6*H*-pyrimido[5,4-*b*][1,4]oxazin-7(8*H*)-one (9d)

Compound **8d** was reacted following the general procedure for **9**. Chromatography was done on silica gel with a gradient of 5–15% methanol in dichloromethane containing 0.25% ammonium hydroxide to give the product as a colorless solid in 45% yield. MS (ESP) *m*/*z* 480 (MH^+); ^{1}H NMR (DMSO-d_6) δ: ^{1}H NMR (DMSO-d_6) δ: ppm 0.95–1.36 (m, 6H); 1.62–1.75 (m, 1H); 1.83–2.03 (m, 4H); 2.29–2.46 (m, 2H); 2.91 (br s, 1H); 3.78 (s, 2H); 3.90 (s, 3H); 4.12–4.23 (m, 2H); 4.73 (s, 2H); 6.93–7.04 (m, 2H); 7.74 (d, 1H); 8.04 (s, 1H); 8.24 (s, 1H).

10.5. (*R*)-*trans*-6-((4-(1-Amino-2-(7-methoxy-2-oxoquinoxalin-1(2*H*)-yl)ethyl)cyclohexylamino)methyl)-2*H*-pyrido[3,2-*b*][1,4]oxazin-3(4*H*)-one (9e)

Compound **8e** was reacted following the general procedure for **9**. Chromatography was done on silica gel with a gradient of 5–15% methanol in dichloromethane containing 0.25% ammonium hydroxide to give the product as a colorless solid in 58% yield. MS (ESP) *m*/*z* 479 (MH^+); ^{1}H NMR (DMSO-d_6) δ: 0.94–1.35 (m, 6H); 1.62–1.74 (m, 1H); 1.84–2.03 (m, 4H); 2.23–2.40 (m, 2H); 2.90 (br s, 1H); 3.71 (s, 2H); 3.90 (s, 3H); 4.17 (d, 2H); 4.61 (s, 2H); 6.95–7.06 (m, 3H); 7.30 (d, 1H); 7.74 (d, 1H); 8.03 (s, 1H); 11.15 (br s, 1H).

10.6. (*R*)-*trans*-6-((4-(1-Amino-2-(7-fluoro-2-oxoquinoxalin-1(2*H*)-yl)ethyl)cyclohexylamino)methyl)-2*H*-pyrido[3,2-*b*][1,4]oxazin-3(4*H*)-one (9f)

Compound **8f** (2.65 g, 4.07 mmol) was reacted following the general procedure for **9**. Chromatography was done on silica gel with a gradient of 5–15% methanol in dichloromethane containing 0.25% ammonium hydroxide. The crude product was triturated in a mixture of EtOH/hexanes overnight, while stirring vigorously. The solid was collected by filtration to give the product as an off-white solid, 958 mg (51%). $[\alpha]_D$ +31.9 (*c* 1, methanol). MS (ESP) *m*/*z* 467 (MH^+); ^{1}H NMR (DMSO-d_6) δ: 11.16 (br s, 1H); 8.18 (s, 1H); 7.86 (dd, 1H); 7.56 (dd, 1H); 7.30 (d, 1H); 7.22 (dd, 1H); 7.03 (d, 1H); 4.61 (s, 2H); 4.21–4.05 (m, 2H); 3.72 (s, 2H); 2.88 (m, 1H); 2.35 (m, 1H); 2.02–1.83 (m, 4H); 1.64 (m, 1H); 1.35–0.93 (m, 4H).

10.7. (*R*)-*trans*-2-((4-(1-Amino-2-(7-fluoro-2-oxoquinoxalin-1(2*H*)-yl)ethyl)cyclohexylamino)methyl)-6*H*-pyrimido[5,4-*b*][1,4]oxazin-7(8*H*)-one (9g)

Compound **8g** (103 mg, 0.16 mmol) was reacted following the general procedure for **9**. Chromatography was done on silica gel with a gradient of 5–15% methanol in dichloromethane containing 0.25% ammonium hydroxide to give 30 mg (40%) of the product as a slightly yellow solid. $[\alpha]_D$ +34.8 (*c* 1, methanol). MS (ESP) *m*/*z* 468 (MH^+); ^{1}H NMR (DMSO-d_6) δ: 8.25 (s, 1H); 8.18 (s, 1H); 7.86 (dd, 1H); 7.56 (m, 1H); 7.23 (ddd, 1H); 4.73 (s, 2H); 4.13 (m, 2H); 3.78 (s, 2H); 2.86 (m, 1H); 2.37 (m, 1H); 2.02–1.82 (m, 4H); 1.64 (m, 1H); 1.38–0.95 (4H).

10.8. (*R*)-2-(*trans*-(4-(1-Amino-2-(6-fluoro-7-methoxy-2-oxoquinoxalin-1(2*H*)-yl)ethyl)cyclohexylamino)methyl)-6*H*-pyrimido[5,4-*b*][1,4]oxazin-7(8*H*)-one (9h)

Compound **8h** (300 mg, 0.44 mmol) was reacted following the general procedure for **9**. Chromatography was done on silica gel with 20% methanol in dichloromethane, containing 1% ammonium hydroxide to give the product as a light yellow solid, 85 mg (39%). The racemic material was separated on a Chiralpak IB column with 50% hexanes in EtOH/MeOH (1:1), containing 0.1% diethylamine. The desired *R*-enantiomer eluted second. MS (ESP) *m*/*z* 498 (MH^+); ^{1}H NMR (DMSO-d_6) δ: 0.8–1.4 (m, 7H); 1.73 (d, 1H); 1.92 (m, 3H); 2.35 (m, 1H); 2.89 (m, 1H); 3.75 (s, 2H); 4.00 (s, 3H); 4.21 (d, 2H); 4.72 (s, 2H); 7.17 (d, 1H); 7.68 (d, 1H); 8.10 (s, 1H); 8.24 (s, 1H).

10.9. (*R*)-*trans*-6-((4-(1-Amino-2-(7-methoxy-2-oxo-1,5-naphthyridin-1(2*H*)-yl)ethyl)cyclohexylamino)methyl)-2*H*-pyrido[3,2-*b*][1,4]oxazin-3(4*H*)-one (9i) and (*S*)-*trans*-6-((4-(1-amino-2-(7-methoxy-2-oxo-1,5-naphthyridin-1(2*H*)-yl)ethyl)cyclohexylamino)methyl)-2*H*-pyrido[3,2-*b*][1,4]oxazin-3(4*H*)-one (35)

Compound **8i** (550 mg, 0.83 mmol) was reacted following the general procedure for **9**. Chromatography was done on silica gel with 20% methanol in dichloromethane, containing 1% ammonium hydroxide to give 255 mg of the racemic product as a light yellow solid, (64%). MS (ESP) *m*/*z* 479 (MH^+); ^{1}H NMR (DMSO-d_6) δ: 0.96–1.32 (m, 6H); 1.61–1.71 (m, 1H); 1.84–2.02 (m, 4H); 2.24–2.40 (m, 2H); 2.79–2.89 (m, 1H); 3.70 (s, 2H); 3.96 (s, 3H); 4.12–4.31 (m, 2H); 4.61 (s, 2H); 6.65 (d, 1H); 7.02 (d, 1H); 7.29 (d, 1H); 7.43 (s, 1H); 7.85 (d, 1H); 8.27 (d, 1H); 11.12 (br s, 1H).

The racemic material was separated on a Chiralcel OJ column, 20 × 250 mm, 10 μ, with 40% 1:1 methanol/ethanol, 60% hexanes, 10 mL/min) to give **9i** as the first eluting enantiomer, followed by **35**.

10.10. (*R*)-*trans*-6-((4-(1-Amino-2-(7-fluoro-2-oxo-1,5-naphthyridin-1(2*H*)-yl)ethyl)cyclohexylamino)methyl)-2*H*-pyrido[3,2-*b*][1,4]oxazin-3(4*H*)-one (9j)

Compound **8j** (72 mg, 0.11 mmol) was reacted following the general procedure for **9**, except the temperature was 0 °C, and the reaction time 3 h. Chromatography was done on silica gel with 0–10% methanol in dichloromethane, containing 0.25% ammonium hydroxide to give 14 mg (27%) of the product as a light yellow foam. MS (ESP) *m*/*z* 467 (MH^+); ^{1}H NMR (MeOD) δ: 8.52 (d, 1H); 7.98 (m, 2H); 7.29 (d, 1H); 6.96 (m, 2H); 4.67 (s, 2H); 4.36 (m, 2H); 3.82 (m, 2H); 3.07 (m, 1H); 2.51 (m, 1H); 2.11 (m, 3H); 1.81 (m, 1H); 1.35 (m, 6H).

10.11. 2-(8-Amino-1,4-dioxaspiro[4.5]decan-8-yl)ethanol (12)

To a mixture of 2-(8-amino-1,4-dioxaspiro[4.5]decan-8-yl)acetic acid (from Discovery Chemscience) (4.2 g, 19.51 mmol) in THF (100 mL) was added LAH (1.851 g, 48.78 mmol). After the exothermic reaction had subsided, the mixture was heated to reflux for 3 h. The mixture was cooled to 0 °C and water (5 mL, in 20 mL THF) was added dropwise under nitrogen. The mixture was stirred for 30 min, then filtered through a 0.45 μm membrane and washed with ethyl acetate (3 × 10 mL). Filtrate and wash were combined and concentrated under reduced pressure. Chromatography was done on silica gel with CH_2Cl_2/MeOH 4:1, containing 1% ammonium hydroxide to give 3.56 g of the product as a colorless solid (91%). MS (ESP) *m*/*z* 202 (MH^+); ^{1}H NMR (DMSO-d_6) δ: 3.83 (s, 4H); 3.56 (m, 2H); 1.72 (m, 2H); 1.50–1.35 (m, 8H). (The OH and NH_2 protons were exchanged).

10.12. 2-(8-Azido-1,4-dioxaspiro[4.5]decan-8-yl)ethanol (13)

A triflic azide stock solution was prepared from sodium azide (4.60 g, 70.75 mmol) and triflic anhydride (5.98 mL, 35.38 mmol) in toluene according to the protocol by Titz et al.[37] (caution! Triflic azide is potentially explosive.) To a mixture of **12** (3.56 g,

17.69 mmol), sodium bicarbonate (5.94 g, 70.75 mmol) and copper(II) sulfate pentahydrate (0.177 g, 0.71 mmol) in water (23 mL) was added the triflic azide solution, followed by addition of methanol (170 mL). The mixture was stirred over night at room temperature, then filtered and washed with methanol (2 × 50 mL). Filtrate and wash were combined and concentrated under reduced pressure to ~30 mL volume. This aqueous mixture was extracted with dichloromethane three times (3 × 70 mL) and the combined organic phases were dried over sodium sulfate and concentrated under reduced pressure, keeping the temperature of the water bath below 25 °C. The residue was chromatographed on silica gel with hexanes/acetone 4:1 to give the product as a colorless oil, 3.16 g (79%). ^{1}H NMR (DMSO-d_6) δ: 4.51 (t, 1H); 3.86 (s, 4H); 3.54 (m, 2H); 1.75 (t, 2H); 1.60–1.50 (m, 8H).

10.13. (2-(8-Azido-1,4-dioxaspiro[4.5]decan-8-yl)ethoxy)(*tert*-butyl)diphenylsilane (14)

To a solution of **13** (250 mg, 1.10 mmol) in DMF (2 mL) was added a solution of imidazole (195 mg, 2.86 mmol) and *tert*-butyldiphenylsilyl chloride (0.367 mL, 1.43 mmol) in DMF (1 mL) at room temperature. After 30 min the reaction mixture was diluted with ethyl acetate (30 mL) and washed with potassium phosphate buffer pH 7 (1 M, 20 mL), then washed with water (30 mL). The organic phase was dried over sodium sulfate and concentrated under reduced pressure to give 610 mg of the product as a colorless oil (quant). The crude product was taken to the next step without further purification. NMR (DMSO-d_6) δ: 7.75–7.45 (m, 10H); 3.92 (s, 4H); 3.82 (t, 2H); 1.94 (t, 2H); 1.75–1.55 (m, 8H); 1.05 (s, 9H).

10.14. 4-Azido-4-(2-(*tert*-butyldiphenylsilyloxy)-ethyl)cyclohexanone (15)

A solution of **14** (6.5 g, 13.96 mmol) and p-TsOH (0.425 g, 2.23 mmol) in acetone (300 mL) was heated to 80 °C bath temperature over night. The mixture was cooled with an ice bath and pyridine (0.271 mL, 3.35 mmol) was added once the temperature reached room temperature. The mixture was concentrated under reduced pressure, the residue taken up in ethyl acetate (200 mL) and washed with phosphate buffer pH 7 (1 M, 50 mL), then with water (50 mL), dried over sodium sulfate and concentrated under reduced pressure. Chromatography was done on silica gel with hexanes/acetone 9:1 to give the product as a colorless oil, 4.41 g (75%). MS (ESP) m/z 422 (MH^+); ^{1}H NMR (DMSO-d_6) δ: 7.64 (m, 4H); 7.46 (m, 6H); 3.82 (t, 2H); 2.41 (m, 2H); 2.18 (m, 2H); 1.99 (m, 6H); 1.00 (s, 9H).

10.15. 4-Azido-4-(2-(*tert*-butyldiphenylsilyloxy)-ethyl)cyclohexanamine (16)

A mixture of **15** (4.41 g, 10.46 mmol) and ammonium acetate (8.06 g, 104.60 mmol) in methanol (100 mL) was treated with sodium cyanoborohydride (0.657 g, 10.46 mmol) and the mixture was stirred at room temperature for 18 h. The mixture was concentrated under reduced pressure, the residue was taken up in dichloromethane (300 mL) and washed with saturated sodium bicarbonate solution (50 mL, pH adjusted to 10 with 15% NaOH). The aqueous phase was back-extracted twice with dichloromethane (2 × 100 mL) and the combined organic phases were dried over sodium sulfate and concentrated under reduced pressure to give the product as a colorless oil, 4.5 g (quant). MS (ESP) m/z 423 (MH^+); ^{1}H NMR (DMSO-d_6) δ: 7.65 (m, 4H); 7.43 (m, 6H); 3.77 (m, 2H); 1.86 (m, 2H); 1.80–1.50 (m, 4H); 1.44 (m, 3H); 1.15 (m, 2H); 1.00 (s, 9H).

10.16. *tert*-Butyl 4-azido-4-(2-(*tert*-butyldiphenylsilyloxy)-ethyl)cyclohexylcarbamate (17)

To a biphasic mixture of **16** (4.42 g, 10.46 mmol) in ethyl acetate (80 mL) and sodium bicarbonate (2.64 g, 31.38 mmol) in water (80 mL) was added BOC_2O (2.91 mL, 12.55 mmol) (in ethyl acetate, 30 mL) and the mixture was rapidly stirred at room temperature for 3 d. The organic phase was separated, the aqueous phase extracted once with ethyl acetate (30 mL) and the combined organic phases were tried over sodium sulfate and concentrated under reduced pressure. Chromatography was done on silica gel with hexanes/ethyl acetate 10:1 to give the product as a colorless oil, 4.28 g (78%). The product was obtained as a mixture of ~1:2 of the *cis*- and *trans*-isomers. MS (ESP) m/z 523 (MH^+); ^{1}H NMR (DMSO-d_6) δ: 7.66 (m, 4H); 7.42 (m, 7H); 3.76 (m, 2H); 1.85 (m, 2H); 1.75–1.50 (m, 4H); 1.50–1.20 (m, 5H); 1.37 (s, 9H); 1.00 (s, 9H).

10.17. *cis-tert*-Butyl 4-azido-4-(2-hydroxyethyl)-cyclohexylcarbamate (18)

To a solution of **17** (4.25 g, 8.13 mmol) in THF (60 mL) was added a solution of tetrabutylammonium fluoride in THF (1 M, 10.57 mL, 10.57 mmol) at room temperature. After 1d, the mixture was concentrated under reduced pressure, the residue was taken up in ethyl acetate (200 mL) and washed with potassium phosphate buffer pH7 (1 M, 100 mL), then dried over sodium sulfate and concentrated under reduced pressure. Chromatographed was done on silica gel with hexanes/acetone 4:1. The first eluting peak was the product with the *trans*-configuration (0.57 g, 25%). The second eluting peak was the desired *cis*-isomer (azido- and –NHBoc groups on the same side of the ring) and was collected to give the product as a colorless oil, 1.212 g (52%). MS (ESP) m/z 523 (MH^+); ^{1}H NMR (DMSO-d_6) δ: 6.75 (d, 1H); 4.50 (t, 1H); 3.53 (m, 2H); 3.20 (m, 1H); 1.74–1.25 (m, 10H); 1.37 (s, 9H). The *cis* configuration for the major product was confirmed by NOE NMR studies.

10.18. *cis*-2-(1-Azido-4-(*tert*-butoxycarbonylamino)cyclohexyl)ethyl methanesulfonate (19)

To a solution of **18** (600 mg, 2.11 mmol) in dichloromethane (10 mL) was added TEA (0.412 mL, 2.95 mmol) and the mixture was cooled to 0 °C. A solution of MsCl (0.197 mL, 2.53 mmol) in dichloromethane (3 mL) was added dropwise. After one h the mixture was quenched with phosphate buffer pH 7 (1 M, ~50 mL) and dichloromethane was removed under reduced pressure. The aqueous mixture was extracted with ethyl acetate (50 mL) and the extract was washed once more with phosphate buffer as above, then dried over sodium sulfate and concentrated under reduced pressure. The colorless oil was taken up in DMF (5 mL) and used without further purification directly in the next step. MS (ESP) m/z 385 (MNa^+).

10.19. *cis-tert*-Butyl 4-azido-4-(2-(7-methoxy-2-oxoquinoxalin-1(2*H*)-yl)ethyl)cyclohexylcarbamate (20)

To a suspension of 7-methoxyquinoxalin-2(1*H*)-one **2c**[8,31,32] (558 mg, 3.17 mmol) in DMF (10 mL) at 0 °C was added NaH, 60% in oil (135 mg, 3.38 mmol) and the mixture was stirred for 30 min. A solution of **18** (765 mg, 2.11 mmol) in DMF (5 mL) was added and the mixture was stirred at room temperature. The mixture was stirred for 2 days at room temperature and then for 5 d at 40 °C. The mixture was cooled to room temperature, diluted with ethyl acetate (100 mL) and quenched with potassium phosphate buffer pH7 (1 M, 50 mL). The organic phase was washed with water (2 × 50 mL), dried over sodium sulfate and concentrated

under reduced pressure. Chromatography was done on silica gel with hexanes/acetone 3:1. The first eluting spot was O-alkylated material which was the major product, the desired N-alkylated product eluted second, 134 mg colorless hard foam (14%). MS (ESP) *m*/*z* 465 (MNa^+); ^{1}H NMR (DMSO-d_6) δ: 8.05 (s, 1H); 7.78 (d, 1H); 7.02 (dd, 1H); 6.92 (m, 1H); 6.80 (d, 1H); 4.31 (m, 2H); 3.94 (s, 3H); 1.95–1.30 (m, 11H); 1.39 (s, 9H).

10.20. *cis*-1-(2-(4-Amino-1-azidocyclohexyl)ethyl)-7-methoxyquinoxalin-2(1*H*)-one (21)

To a solution of **20** (133 mg, 0.30 mmol) in dichloromethane (2 mL) was added trifluoroacetic acid (1.0 mL) at 0 °C. The cooling bath was removed and the mixture was stirred at room temperature for 2 h. The mixture was concentrated under reduced pressure and the residue was codistilled with dichloromethane twice. The residue was taken up in dichloromethane (50 mL) and washed with saturated sodium bicarbonate solution (7 mL, 1 M, pH adjusted to ~10 with 15% NaOH). The aqueous phase was back extracted with dichloromethane twice (2 × 50 mL) and the combined organic phases were dried over sodium sulfate and concentrated under reduced pressure to give the product as a slightly yellow oil, 101 mg (98%). MS (ESP) *m*/*z* 343 (MH^+).

10.21. *cis*-6-((4-Azido-4-(2-(7-methoxy-2-oxoquinoxalin-1(2*H*)-yl)ethyl)cyclohexylamino)methyl)-2*H*-pyrido[3,2-*b*][1,4]oxazin-3(4*H*)-one (22)

Compound **21** (101 mg, 0.29 mmol), 3-oxo-3,4-dihydro-2*H*-pyrido[3,2-*b*][1,4]oxazine-6-carbaldehyde **6**[26] (52.5 mg, 0.29 mmol) and sodium triacetoxyborohydride (156 mg, 0.74 mmol) were reacted following the general procedure for **8**. Chromatography was done on silica gel with dichloromethane/methanol 10:1 to give the product as a colorless hard foam, 130 mg (69%). MS (ESP) *m*/*z* 505 (MH^+); ^{1}H NMR (DMSO-d_6) δ: 11.18 (s, 1H); 8.05 (s, 1H); 7.78 (d, 1H); 7.31 (m, 1H); 7.04 (m, 2H); 6.91 (m, 1H); 4.63 (s, 2H); 4.32 (m, 2H); 3.93 (s, 3H); 3.80 (br s, 2H); 3.30 (m, 2H); 1.90 (m, 5H); 1.56 (m, 2H); 1.35 (m, 2H).

10.22. *cis*-6-((4-Amino-4-(2-(7-methoxy-2-oxoquinoxalin-1(2*H*)-yl)ethyl)cyclohexylamino)methyl)-2*H*-pyrido[3,2-*b*][1,4]oxazin-3(4*H*)-one (23)

A solution of **22** (80 mg, 0.16 mmol) and triphenylphosphine (49.9 mg, 0.19 mmol) in acetonitrile (5 mL) and water (0.55 mL) was stirred at room temperature for 5 days. The temperature was increased to 45 °C and the mixture was stirred for another 3 days. The reaction mixture was cooled to room temperature and concentrated under reduced pressure. The residue was codistilled with methanol twice to remove water. Chromatography was done on silica gel with CH_2Cl_2/MeOH 8:1, containing 1% ammonium hydroxide. This gave 9 mg (12%) of the desired product as a colorless hard film. MS (ESP) *m*/*z* 505 (MH^+); ^{1}H NMR (MeOD) δ: 8.05 (s, 1H); 7.80 (m, 1H); 7.33 (m, 1H); 7.09–7.01 (m, 3H); 4.67 (m, 2H); 4.40 (m, 2H); 4.05 (m, 2H); 3.97 (m, 3H); 2.92 (m, 1H); 2.02 (m, 2H); 1.91 (m, 4H); 1.69 (m, 4H).

10.23. 4-Fluoro-5-methoxy-2-nitroaniline (25)

To a suspension of commercially available 4,5-difluoro-2-nitroaniline **24** (10 g, 57.44 mmol) in methanol (200 mL) was added KOH, 2 M (60 mL, 120 mmol) and the mixture was stirred vigorously at room temperature for 20 h. The mixture was concentrated under reduced pressure, the residue was taken up in dichloromethane (500 mL) and washed with water (100 mL), dried over sodium sulfate and concentrated under reduced pressure to give the product as a yellow solid, 10.77 g (quant). MS (ESP) *m*/*z* 187 (MH^+); ^{1}H NMR (DMSO-d_6) δ: 7.75 (d, 1H); 7.52 (br s, 2H); 6.63 (d, 1H); 3.87 (s, 3H).

10.24. 4-Fluoro-5-methoxybenzene-1,2-diamine (26)

A mixture of **25** (4 g, 21.49 mmol) in ethanol (100 mL) and acetic acid (10.0 mL) was hydrogenated on Pd/C, wet, 10% (1 g, 9.40 mmol) at normal pressure and room temperature for 3 h. The mixture was filtered through a 0.45 μm membrane directly into the reaction mixture for **2d** MS (ESP) *m*/*z* 157 (MH^+).

10.25. 2-Chloro-6-fluoro-7-methoxyquinoxaline (28)

A suspension of a mixture of **2d** (5.9 g, 30.39 mmol) and 7-fluoro-6-methoxyquinoxalin-2(1*H*)-one **27** (5.9 g, 30.39 mmol) in $POCl_3$ (70 mL, 750.98 mmol) was heated at 100 °C bath temperature for 1 h. The mixture was cooled to room temperature and poured onto ice and dichloromethane (~200 mL) was added. The pH of the mixture was brought to ~8 by addition of solid sodium carbonate in portions under stirring, while maintaining the temperature of the mixture between room temperature and 10 °C with ice. The mixture brought to room temperature and was diluted with dichloromethane (400 mL) and with water (400 mL). The organic phase was separated and washed once with saturated aqueous bicarbonate solution (300 mL), then dried over sodium sulfate. Chromatography was done on silica gel with hexanes/ethyl acetate 10:1. The higher migrating product was isolated to give a colorless solid, 3.37 g. MS (ESP) *m*/*z* 213/215 (MH^+); ^{1}H NMR (DMSO-d_6) δ: 8.87 (s, 1H); 7.99 (d, 1H); 7.66 (d, 1H); 4.05 (s, 3H).

10.26. *N*-(*trans*-2-(6-Fluoro-7-methoxy-2-oxoquinoxalin-1(2*H*)-yl)-1-((1s,4*R*)-4-((7-oxo-7,8-dihydro-6*H*-pyrimido[5,4-*b*][1,4]oxazin-2-yl)methylamino)cyclohexyl)ethyl)-*N*-methyl-2-nitrobenzenesulfonamide (30)

To a mixture of **8h** (100 mg, 0.15 mmol) in THF (5 mL) and triphenylphosphine (45 mg, 0.17 mmol) and methanol (18 μL, 0.44 mmol) was added diisopropylazodicarboxylate (28.8 μL, 0.15 mmol) at room temperature. After 2 h, additional 20 μL of diisopropylazodicarboxylate (20 μL) and triphenylphosphine (20 mg) were added. The reaction mixture was concentrated under reduced pressure. The residue was purified by reverse phase HPLC, eluting with methanol/TFA to give the TFA salt of the product as a red foam, 101 mg. MS (ESP) *m*/*z* 697 (MH^+).

10.27. *trans*-2-((4-(2-(6-Fluoro-7-methoxy-2-oxoquinoxalin-1(2*H*)-yl)-1-(methylamino)ethyl)cyclohexylamino)methyl)-6*H*-pyrimido[5,4-*b*][1,4]oxazin-7(8*H*)-one (31)

Compound **30** (100 mg, 0.14 mmol), potassium carbonate (40 mg, 0.29 mmol) and benzenethiol (30 mg, 0.27 mmol) were reacted following the general procedure for **9**. The crude material was purified by reverse phase HPLC with methanol/ammonia to give the product as a yellow solid, 11 mg (15%). MS (ESP) *m*/*z* 512 (MH^+); ^{1}H NMR (DMSO-d_6) δ: 0.9–1.4 (m, 6H); 1.74 (d, 1H); 1.98 (m, 3H); 2.39 (m, 1H); 2.90 (m, 2H); 3.84 (s, 2H); 4.00 (s, 3H); 4.22 (d, 2H); 4.85 (s, 2H); 7.17 (d, 1H); 7.68 (d, 1H); 8.10 (s, 1H); 8.28 (s, 1H).

10.28. 4-Fluoro-2-nitro-phenylimino)-acetic acid ethyl ester (33)

A solution of 4-fluoro-2-nitroaniline (1.50 kg, 9.6 mol) and ethyl glyoxylate (1.96 kg, 50 wt % in toluene, 9.6 mol) in toluene (12.6 L) was refluxed in a 22-L flask equipped with a Dean–Stark apparatus for 48 h and evaporated in vacuo to give the product as a dark

brown oil that was used directly for the next step without further purification.

10.29. 7-Fluoro-3,4-dihydro-1*H*-quinoxalin-2-one (34)

In a 18-L Parr apparatus a suspension of **33** generated above in ethanol was hydrogenated at 50 psi in the presence of 20 wt % Pd/C (240 g, containing ca. 50 wt % water) until no hydrogen was consumed. (Note: The reaction was strongly exothermic and the temperature should be controlled at around 60 °C by adjusting the rate of recharging hydrogen and by a cooling system). The reaction mixture was discharged, filtered over a Celite cake, and evaporated in vacuo to give a crude solid that was triturated with MTBE (8 L) to afford the product (600 g) as a tan solid.

10.30. *tert*-Butyl 1-oxaspiro[2.5]octan-6-ylcarbamate (37)[54]

To a suspension of potassium *tert*-butoxide (95%, 2.4 g, 20.4 mmol) in dry THF (70 mL) was added trimethylsulfoxonium iodide (4.75 g, 21 mmol) in one portion and the pale yellow mixture was heated to reflux for 2 h. The mixture was cooled to room temperature and a solution of *tert*-butyl 4-oxocyclohexylcarbamate (3.0 g, 14.1 mmol) in dry THF (20 mL) was added dropwise over 5 min. The resulting pale orange mixture was heated to reflux and stirred for 2 h. The yellow mixture was allowed to cool to room temperature, partitioned between water (100 mL) and ethyl acetate (200 mL) and the phases separated. The aqueous phase was extracted twice with ethyl acetate, and the combined organic phases were washed with brine, then dried over sodium sulfate and concentrated under reduced pressure to give 3.13 g (13.8 mmol, 98%) of the product as a waxy colorless solid. The crude product was taken to the next step without further purification. MS (ESP) *m*/*z* 250 ($M+Na^+$); ^{1}H NMR ($CDCl_3$) δ: 1.29–1.41 (m, 2H); 1.41–1.50 (m, 11H); 1.86–2.07 (m, 4H); 2.67 (s, 2H); 3.58 (d, J = 9.98 Hz, 1H); 4.46 (br s, 1H).

10.31. (*Z*)-*tert*-Butyl-4-fluoro-4-(hydroxymethyl)cyclohexylcarbamate (38)

To a 125 mL polypropylene flask was added a 70% HF solution in pyridine (5 mL) under dry nitrogen with stirring, and the colorless solution cooled to −78 °C. To this was added a solution of **37** (1.0 g, 4.4 mmol) in dichloromethane (5 mL) dropwise over 10 min. The resulting solution was stirred at −78 °C for 5 h, then poured into an ice-cold 2 N ammonium hydroxide solution (50 mL) and the pH of the mixture was made alkaline by addition of conc. ammonium hydroxide. The mixture was extracted three times with dichloromethane, the combined organic phases were washed with brine, dried over sodium sulfate and concentrated under reduced pressure. Chromatography was done on silica gel with a gradient of 10–20% ethyl acetate in hexanes to give 0.55 g of the desired product (2.2 mmol, 51%) as a white solid. The E isomer was also isolated and its configuration was determined by a 2D proton NMR NOE-experiment. (*E*)-*tert*-Butyl-4-fluoro-4-(hydroxymethyl)cyclohexylcarbamate: ^{1}H NMR ($CDCl_3$) δ: 1.37–1.56 (m, 11H); 1.69–1.85 (m, 4H); 1.86–2.01 (m, 2H); 3.60–3.78 (m, 3H); 4.55 (br s, 1H); ^{19}F NMR ($CDCl_3$) δ: −160.68 (s, 1F).

(Z)-tert-Butyl-4-fluoro-4-(hydroxymethyl)cyclohexylcarbamate: ^{1}H NMR ($CDCl_3$) δ: 1.30–1.43 (m, 2H), 1.43–1.49 (m, 11H), 1.85–2.09 (m, 4H), 3.47 (d, J = 7.72 Hz, 1H), 3.57 (d, J = 19.97 Hz, 2H), 4.43 (br s, 1H); ^{19}F NMR ($CDCl_3$) δ: −169.91 (s, 1F).

10.32. (*Z*)-*tert*-Butyl-4-fluoro-4-formylcyclohexylcarbamate (39)

A solution of **38** (0.8 g, 3.2 mmol) in dichloromethane (40 mL) was cooled to 0 °C and Dess–Martin periodinane (2.75 g, 6.5 mmol) was added in portions over 30 min. The mixture was stirred at 0 °C for 90 min, then the cooling bath was removed and the reaction stirred at room temperature for 2 h. The mixture was diluted with dichloromethane, washed once with an aqueous 10% sodium thiosulfate solution, once with a saturated aqueous sodium bicarbonate solution, dried over sodium sulfate and concentrated under reduced pressure. Chromatography was done on silica gel with a gradient of 10–15% ethyl acetate in hexanes to give 0.44 g (1.8 mmol, 56%) of the desired product as a colorless solid. ^{1}H NMR ($CDCl_3$) δ: 1.46 (s, 11H); 1.57–1.70 (m, 1H); 1.77 (td, J = 14.03, 4.52 Hz, 1H); 1.89–2.05 (m, 4H); 3.52 (br s, 1H); 4.46 (br s, 1H); 9.75 (d, J = 5.27 Hz, 1H); ^{19}F NMR ($CDCl_3$) δ: −172.48 (s, 1F).

10.33. (Z)-*tert*-Butyl-4-fluoro-4-vinylcyclohexylcarbamate (40)

A suspension of methyltriphenylphosphonium bromide (0.44 g, 1.2 mmol) in dry THF (5 mL) was cooled to 0 °C and a potassium hexamethyldisilazide solution in THF (1 M, 1.2 mL, 1.2 mmol) added dropwise to give a bright yellow mixture. The mixture was stirred at 0 °C for 60 min, then a solution of (*Z*)-*tert*-butyl (1*S*,4*S*)-4-fluoro-4-formylcyclohexylcarbamate (0.15 g, 0.6 mmol) in dry THF (5 mL) added and the yellow mixture stirred at 0 °C for 60 min. The reaction was quenched with 5 mL of a saturated aqueous ammonium chloride solution and extracted twice with ethyl acetate. The combined organic phases were washed with brine, dried over sodium sulfate and concentrated under reduced pressure. Chromatography was done on silica gel with a gradient of 0–5% EtOAc in hexanes to give 105 mg (0.43 mmol, 71%) of the desired product as a colorless solid. ^{1}H NMR ($CDCl_3$-*d*) δ: 1.44 (s, 9H); 1.46–1.71 (m, 4H); 1.81–1.99 (m, 4H); 3.44 (br s, 1H); 4.46 (br s, 1H); 5.10 (dd, J = 11.11, 0.94 Hz, 1H); 5.27 (dt, J = 17.52, 1.41 Hz, 1H); 5.88 (td, J = 17.05, 10.93 Hz, 1H); ^{19}F NMR (282 MHz, $CDCl_3$-*d*) δ: −159.82 (s, 1F).

10.34. (*Z*)-*tert*-Butyl-4-fluoro-4-(1-(2-nitrophenylsulfonyl)aziridin-2-yl)cyclohexylcarbamate (41)

To a solution of **40** (0.308 g, 1.3 mmol) in dry acetonitrile (10 mL) was added activated molecular sieves, followed by copper (II) triflate (46 mg, 0.13 mmol) to give a green/blue mixture. To this was added (*N*-(2-nitrobenzylsulfonyl)imino)phenyliodinane (0.77 g, 1.9 mmol) and the pale green mixture was flushed with dry nitrogen and stirred at room temperature for 2 h. An additional 46 mg (0.13 mmol) copper (II) triflate was added and the tan mixture stirred overnight. The reaction mixture was filtered through Celite, the filter solids washed with acetonitrile, then with ethyl acetate, the filtrate was concentrated under reduced pressure and the residue dissolved in dichloromethane and filtered through Celite. The filtrate was concentrated under reduced pressure, redissolved in minimal dichloromethane and filtered through a cotton plug. Chromatography of the filtrate was done on silica gel with a gradient of 0–10% acetone in hexanes to give 163 mg (0.67 mmol) recovered olefin and 83 mg (0.19 mmol, 15%) of the desired product as a colorless hard foam. MS (ESP) *m*/*z* 466 (MNa^+); ^{1}H NMR ($CDCl_3$) δ: 1.41–1.52 (m, 12H); 1.60–1.70 (m, 1H); 1.84–1.99 (m, 4H); 2.62 (d, J = 4.71 Hz, 1H); 2.89 (d, J = 7.35 Hz, 1H); 3.08 (ddd, J = 15.12, 7.21, 4.80 Hz, 1H); 3.46 (br s, 1H); 4.40 (br s, 1H); 7.71–7.82 (m, 3H); 8.18–8.26 (m, 1H); ^{19}F NMR ($CDCl_3$) δ: −171.33 (s, 1F).

10.35. (*Z*)-*tert*-Butyl-4-fluoro-4-(2-(7-methoxy-2-oxoquinoxalin-1(2*H*)-yl)-1-(2-nitrophenylsulfonamido)ethyl)-cyclohexylcarbamate (42)

Compound **2c**[8,31,32] (41 mg, 0.23 mmol) was reacted with sodium hydride (11 mg, 0.28 mmol) and **41** (103 mg, 0.23 mmol)

following the general procedure for **4**. Chromatography was done on silica gel with 20–80% ethyl acetate in hexanes to give 78 mg of the desired product as an off-white solid (54%). MS (ESP) *m*/*z* 618 ($M-H^-$); ^1H NMR ($CDCl_3$) δ: 1.46 (s, 9H); 1.51–1.66 (m, 2H); 1.70–1.93 (m, 2H); 1.98–2.08 (m, 2H); 2.09–2.20 (m, 1H); 2.20–2.34 (m, 1H); 3.51 (br s, 1H); 3.92 (s, 3H); 4.20 (d, *J* = 13.56 Hz, 1H); 4.26–4.43 (m, 1H); 4.43–4.56 (m, 1H); 4.56–4.87 (m, 1H); 6.06 (d, *J* = 9.04 Hz, 1H); 6.70 (d, *J* = 2.45 Hz, 1H); 6.86 (dd, *J* = 8.85, 2.26 Hz, 1H); 7.30–7.49 (m, 2H); 7.51–7.60 (m, 2H); 7.60–7.72 (m, 2H); ^{19}F NMR (CD_2Cl_2) δ: −170.89 (s, 1F).

10.36. (*Z*)-*N*-(1-(4-Amino-1-fluorocyclohexyl)-2-(7-methoxy-2-oxoquinoxalin-1(2*H*)-yl)ethyl)-2-nitrobenzenesulfonamide (43)

Compound **42** was reacted with trifluoroacetic acid according to the general procedure for **5** to give the desired product as a pale orange solid in quantitative yield. MS (ESP) *m*/*z* 520 (MH^+). ^1H NMR (acetonitrile-d_3) δ: 1.56 (br s, 1H); 1.61–1.87 (m, 4H); 1.99–2.12 (m, 2H); 2.19–2.37 (m, 2H); 3.30 (br s, 1H); 3.93 (s, 3H); 4.51–4.70 (m, 1H); 6.30 (d, *J* = 9.42 Hz, 1H); 6.57 (br s, 3H); 6.80 (d, *J* = 2.45 Hz, 1H); 6.89 (dd, *J* = 8.95, 2.54 Hz, 1H); 7.29–7.45 (m, 2H); 7.45–7.66 (m, 4H); 7.66–7.77 (m, 1H); ^{19}F NMR (acetonitrile-d_3) δ: −171.16 (s, 1F); −76.73 (s, 3F).

10.37. (*Z*)-*N*-(1-(1-Fluoro-4-((3-oxo-3,4-dihydro-2*H*-pyrido[3,2-*b*][1,4]oxazin-6-yl)methylamino)cyclohexyl)-2-(7-methoxy-2-oxoquinoxalin-1(2*H*)-yl)ethyl)-2-nitrobenzenesulfonamide (44)

Compound **43** (80 mg, 0.13 mmol), ethyldiisopropylamine, **6**[26] (22 mg, 0.13 mmol) and sodium triacetoxyborohydride were reacted following the procedure for **8b** to give 69 mg (80%) of the desired product as a colorless solid. MS (ESP) *m*/*z* 682 (MH^+); ^1H NMR (DMF-d_7) δ: 1.36–1.52 (m, 2H); 1.66–1.80 (m, 1H); 1.84–1.99 (m, 3H); 2.00–2.34 (m, 3H); 2.44–2.62 (m, 1H); 3.80 (s, 2H); 3.99 (s, 3H); 4.23 (ddd, *J* = 17.80, 10.36, 2.17 Hz, 1H); 4.39–4.48 (m, 1H); 4.58 (d, *J* = 12.24 Hz, 1H); 4.70 (s, 2H); 6.92 (dd, *J* = 8.95, 2.35 Hz, 1H); 7.10 (d, *J* = 8.10 Hz, 1H); 7.16–7.22 (m, 1H); 7.33 (d, *J* = 8.10 Hz, 1H); 7.51–7.59 (m, 2H); 7.59–7.64 (m, 1H); 7.66–7.72 (m, 2H); 7.86 (s, 1H), (secondary amine and lactam protons not seen); ^{19}F NMR (282 MHz, DMF-d_7) δ: −167.29 (s, 1F).

10.38. (*Z*)-6-((4-(1-Amino-2-(7-methoxy-2-oxoquinoxalin-1(2*H*)-yl)ethyl)-4-fluorocyclohexylamino)methyl)-2*H*-pyrido[3,2-*b*][1,4]oxazin-3(4*H*)-one (45)

To a suspension of **44** (69 mg, 0.1 mmol) in DMF (2 mL) was added 168 mg (1.2 mmol) powdered potassium carbonate (168 mg, 1.2 mmol), followed by addition of thiophenol (0.126 mL, 1.2 mmol) and the mixture stirred vigorously at room temperature for 12 h. DMF was removed by azeotroping with tetrachloroethylene. The residue was triturated with methanol, filtered through Celite, the filter solids washed with methanol, then with dichloromethane, and the filtrate concentrated under reduced pressure. The residue was suspended in a mixture of brine and a saturated aqueous sodium bicarbonate solution (1:1, 10 mL), the mixture was extracted five times with 10% methanol in DCM, and the combined organic phases were dried over sodium sulfate and concentrated under reduced pressure. Chromatography was done on silica gel with a gradient of 0–4% methanolic 7 N ammonia in dichloromethane to give 30 mg of a red oil. This was dissolved in minimal dichloromethane and diethyl ether added dropwise until a red precipitate formed. This was filtered off and the filtrate diluted with ether to turbidity. The mixture was placed at 0 °C for 30 min, the supernatant pipetted away from a precipitated solid and filtered. The filter solids and flask solids were dissolved in methanol, filtered through a 0.45 μm syringe filter and conc. to give 14.3 mg (0.03 mmol, 29%) of the desired product as a pale pink hard foam. MS (ESP) *m*/*z* 497.1 (MH^+); ^1H NMR ($CDCl_3$-*d*) δ: 1.55–1.69 (m, 2H); 1.76–1.88 (m, 2H); 1.88–2.05 (m, 4H); 2.59 (t, *J* = 10.74 Hz, 1H); 3.20–3.29 (m, 1H); 3.48 (s, 2H); 3.84–3.95 (m, 6H); 4.14 (dd, *J* = 13.94, 1.70 Hz, 1H); 4.64 (s, 2H); 4.70 (dd, *J* = 13.94, 10.36 Hz, 1H); 6.90–6.98 (m, 3H); 7.21 (d, *J* = 7.91 Hz, 1H); 7.79 (d, *J* = 9.42 Hz, 1H); 8.15 (s, 1H) (lactam proton not seen); ^{19}F NMR (282 MHz, $CDCl_3$-*d*) δ: −165.14 (s, 1F).

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