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Imidazopyrazinones (IPYs): non-quinolone bacterial topoisomerase inhibitors showing partial cross-resistance with quinolones

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ABSTRACT

In our quest for new antibiotics able to address the growing threat of multi-drug resistant infections caused by Gram-negative bacteria, we have investigated an unprecedented series of non-quinolone bacterial topoisomerase inhibitors from the Sanofi patrimony, named IPYs for ImidazoPYrazinones, as part of the Innovative Medicines Initiative (IMI) European Gram Negative Antibacterial Engine (ENABLE) organization. Hybridization of these historical compounds with the quinazolinediones, a known series of topoisomerase inhibitors, led us to a novel series of tricyclic IPYs that demonstrated potential for broad spectrum activity, *in vivo* efficacy and a good developability profile, although later profiling revealed a genotoxicity risk. Resistance studies revealed partial cross-resistance with fluoroquinolones (FQs) suggesting that IPYs bind to the same region of bacterial topoisomerases as FQs and interact with at least some of the keys residues involved in FQ binding.

INTRODUCTION

Bacterial topoisomerases (BTs) are critical enzymes for the control of DNA topological state within bacteria and are essential for replication and transcription[1]. Exploitation of the two related type II BTs, DNA gyrase and topoisomerase IV, as antibacterial targets, has led to different classes of antibiotics[1] such as the highly popular fluoroquinolones (FQs)[2] and the less successful coumarins[3] (Figure 1). Considering the paucity of new validated antibacterial targets, the difficulties to discover new scaffolds active against Gram-negative bacteria, and the increasing levels of resistance to FQs[4], BTs remain attractive targets in the fight against resistant bacteria, provided cross-resistance to FQs can be avoided. In line with this analysis, recent years have witnessed a continuous flow of publications of novel BTs inhibitors (BTIs),

structurally related or not[5] to quinolones (Figure 1), binding to the different sub-units of the two BTs and acting as either cleavage-complex stabilizing agents (like FQs) or catalytic inhibitors (like coumarins). The most advanced compounds[8-10] that have emerged from these endeavors have progressed to clinical trials but none has reached market to date.

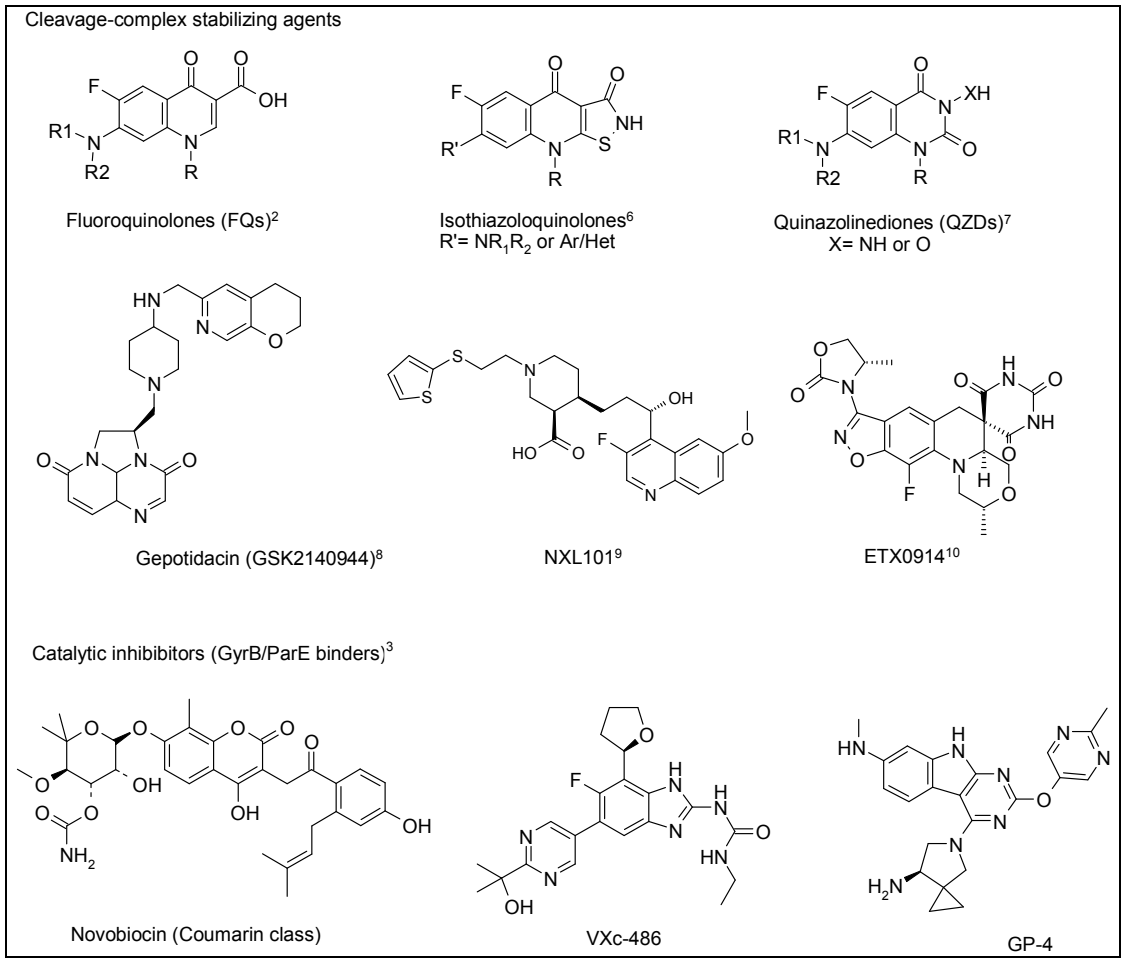


Figure 1. Structures of various Bacterial Topoisomerase Inhibitors (BTIs)

As part of our efforts to identify new antibacterials active on Gram-negative bacteria, we have given a high priority to the discovery of a next-generation of non-quinolone bacterial topoisomerase inhibitors. With this objective in mind, we decided to revisit a proprietary series of tetracyclic imidazopyrazinones (**1**), nicknamed IPYs (Figure 2). This series, structurally unrelated to quinolones, had been identified, in the mid 1990's, by Rhône-Poulenc

Rorer, using the SOS Chromotest[11] (a cell-based SOS induction assay in *E. coli*), an assay that has also delivered other DNA replication inhibitors[12] including the Rhône-Poulenc Rorer hit that was to be ultimately optimized into NXL101[9]. At that time, IPYs had been demonstrated to be topoisomerase inhibitors, stabilizing the gyrase-DNA cleavage complex with some analogs displaying potent antibacterial activities, albeit only against Gram-positive bacteria. The optimization program that was conducted on the initial hit failed to deliver a clinical candidate due to modest *in vivo* efficacy and poor exposures in mice for the most potent analogs. Furthermore, a brief program exploring a simplified bicyclic IPY sub-series (Figure 2) did not yield compounds with reasonable *in vitro* antibacterial activities. As a consequence of these different failures, the project was finally discontinued and the IPY series was never patented nor published.

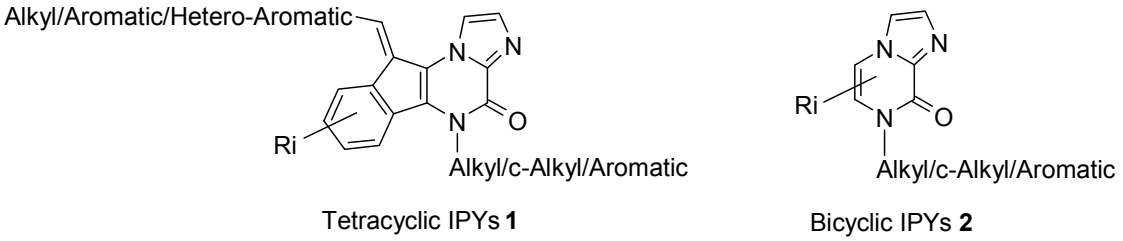


Figure 2. Structures of the different historical IPY sub-series

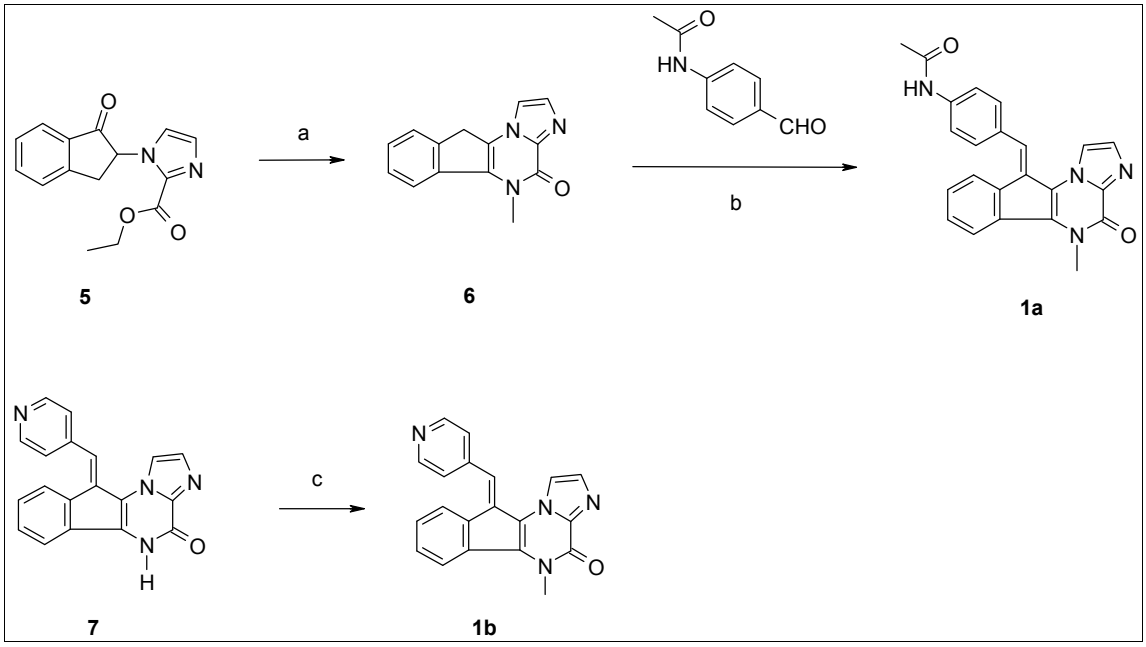
In this paper, we report our recent investigations of the IPY series aimed at identifying novel BTIs active on Gram-negative bacteria. We describe the steps that led us from the initial tetracyclic parent IPYs to the design of a new, promising tricyclic IPY hit that displayed activities against efflux-competent Gram-negative bacteria. We present the exploration program developed around the initial hit, the antibacterial and ADMET profiles of the best analogs as well as the *in vivo* properties (tolerability, efficacy, and PK) of selected analogs and the results of the resistance studies that unfortunately unveiled the partial cross-resistance of the series with FQs. This work was mainly conducted as part of a collaboration between

GSK and Sanofi, within the European IMI New Drugs for Bad Bugs (ND4BB) consortium ENABLE[13].

RESULTS AND DISCUSSION

Chemistry. Compounds **1a** and **1b** were prepared as shown in Scheme 1. Reaction of indanone **5**[14] with methylamine led to the tetracyclic IPY **6** that was directly converted into **1a** by a Knoevenagel-like reaction with 4-acetamido benzaldehyde. Compound **1b** was obtained in one step by N-methylation of the known tetracyclic IPY **7**[15].

Scheme 1. Synthesis of the historical tetracyclic IPYs 1a and 1b[a]

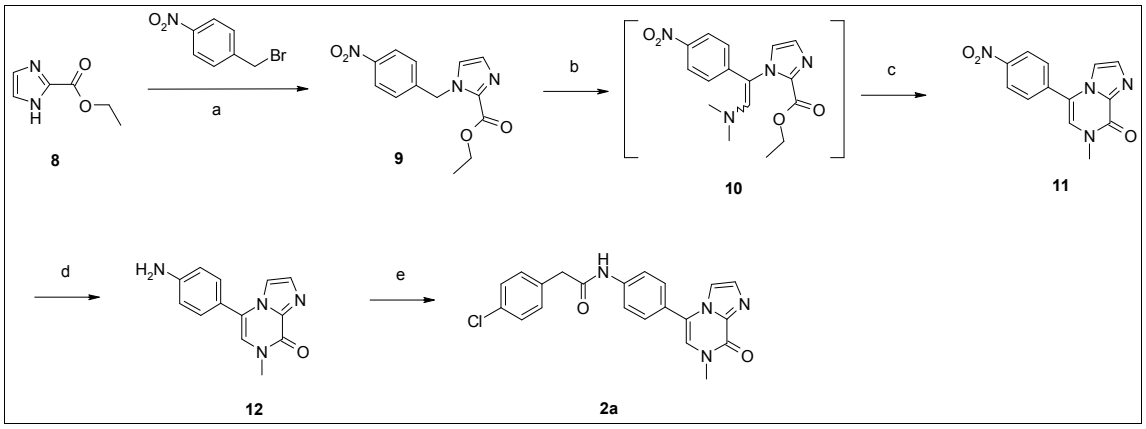


[a]Reagents and conditions: (a) $MeNH_2$, EtOH, AcOH, reflux; (b) Piperidine, MeOH, reflux; (c) NaH, MeI, DMF, 70 °C.

Compound **2a** was synthesized according to Scheme 2. Imidazole carboxylate **8** was N-alkylated to afford compound **9.** Reaction with Bredereck's reagent led to intermediate **10** that

was reacted with methylamine to afford the bicyclic IPY **11**. Palladium-catalyzed transfer hydrogenation followed by acylation successively delivered compounds **12** and **2a**.

Scheme 2. Synthesis of the historical bicyclic IPY 2a[a]

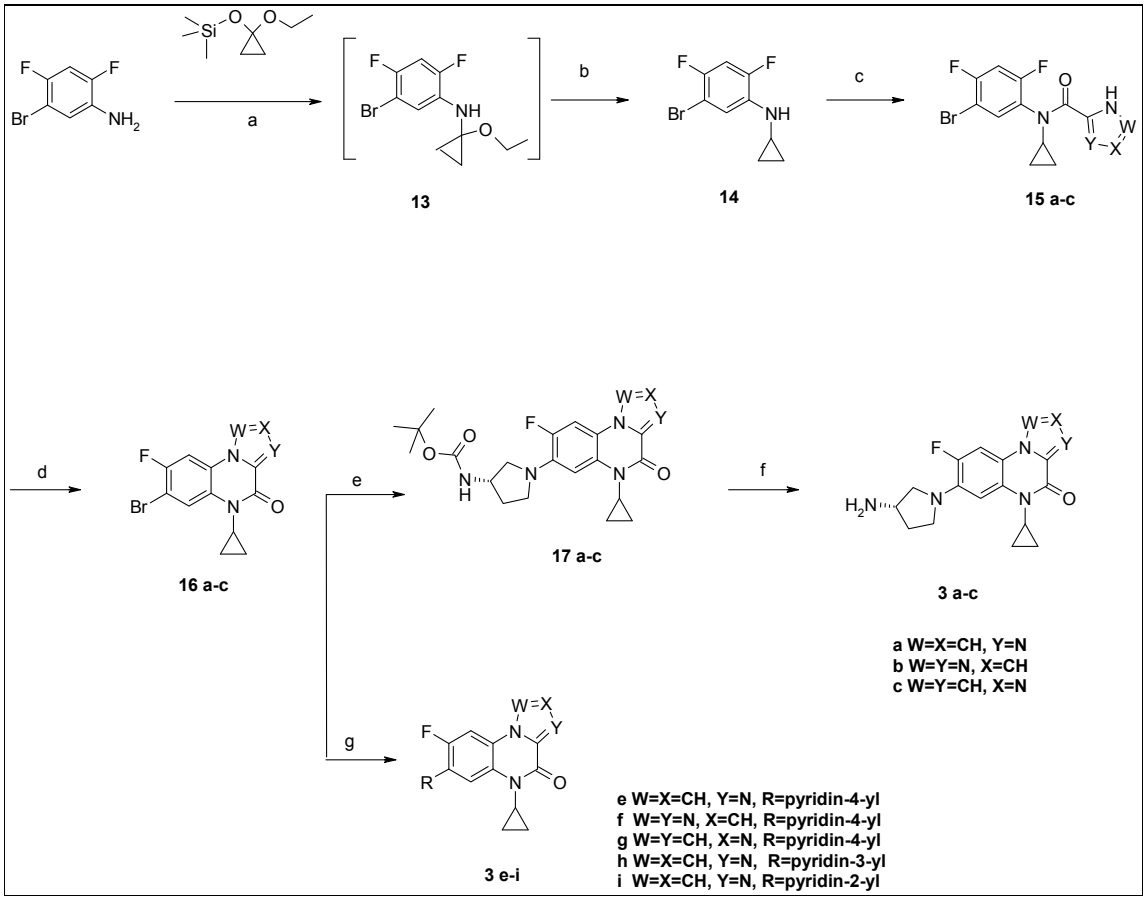


[a]Reagents and conditions: (a) Acetonitrile (MeCN), K_2CO_3, RT; (b) $HC(NMe_2)_2O$*t*Bu (Bredereck's reagent), toluene, 110°C, 20 h; (c) $MeNH_2$, MeOH, AcOH, 85°C, 30 min; (d) Pd/C (cat.), MeOH, HCO_2H, EtOH, 65°C, 4 h; then concentrated HCl; (e) 2-(4-ClPh)CH_2COCl, DCM, pyridine, RT.

The synthetic routes to compounds **3a-c** and **3e-i** are shown in Scheme 3. 5-bromo-2,4-difluoroaniline and (1-ethoxycyclopropoxy)trimethylsilane were first reacted to afford hemi-aminal **13**. The crude reaction mixture was then reacted with sodium borohydride, in the presence of trifluoroboron diethyl etherate, to afford aniline **14** that was then acylated by various alkyl imidazo or triazolo carboxylates, in the presence of trimethylaluminium, to afford amides **15a-c**. Cyclisation of these intermediates into compounds **16a-c** was triggered by reaction with 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU), in DMF. Starting from **16a-c**, we prepared the targeted compounds relying either on Suzuki coupling reactions (compounds **3e-i**) or Buchwald–Hartwig aminations (compounds **17a-c**). In the later cases, compounds **3a-c** were obtained by removing the *t*-butyloxy carbonyl protecting group under acidic conditions. In the cases of triazolo compounds **3b** and **3f**, the ambiguity on the identity of the generated isomers was cleared by an X-Ray structure (see Figure S1 in Supporting

Information) of **3f** that demonstrated that cyclisation into **16b** had occurred through the nitrogen at position 1 of the 1,2,4-triazolo ring.

Scheme 3. Synthesis of the novel tricyclic IPYs 3a, 3b, 3c, 3e, 3f, 3g and 3i[a]

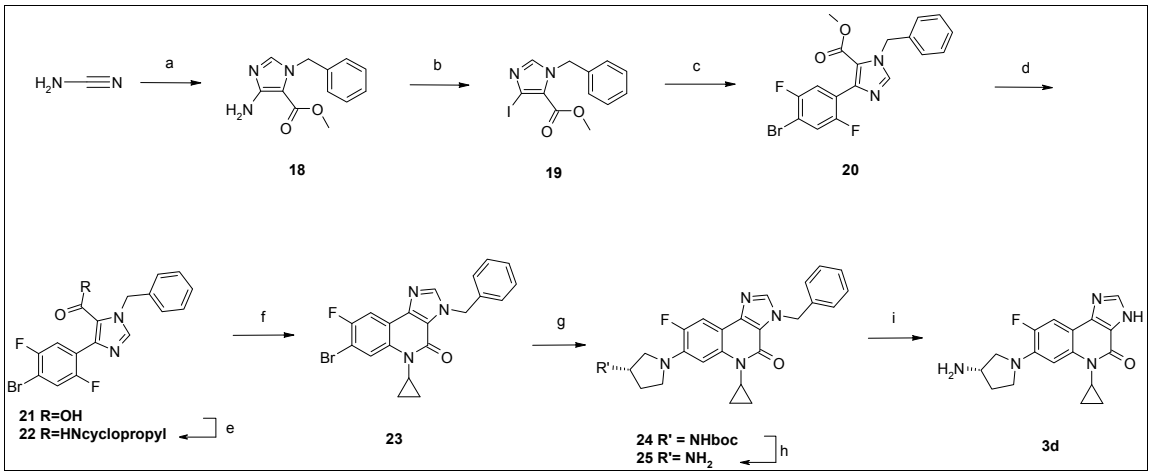


[a]Reagents and conditions: (a) acetic acid, MeOH, reflux 5 h; (b) BF_3-$(C_2H_5)_2O$, $NaBH_4$, THF 5°C to reflux, 4 h; (c) ethyl or methyl imidazole or triazole carboxylate, $AlMe_3$ (2M in toluene), Et_3N, toluene, reflux 2 h; (d) DBU, DMF, 120°C, 3 h; (e) (S)-*tert*-butyl pyrrolidin-3-ylcarbamate, 2,2'-bis(diphenylphosphino)-1,1'-binaphtyle (BINAP), $Pd(OAc)_2$, Cs_2CO_3, toluene, microwaves, 120°C, 20 h; (f) TFA, DCM, RT, 12 h; (g) pyridinyl-4-boronic acid, $Pd(PPh_3)_4$, K_2CO_3, MeOH/Toluene (1:2), microwaves, 120°C, 1 h or pyridin-3-yl boronic acid, $Pd(PPh_3)_4$, Cs_2CO_3, water/1,4-dioxane (1:2), 100°C, 4 h or 2-(tributylstannyl)pyridine, $Pd(PPh_3)_4$, water/1,4-dioxane (1:2), microwaves, 150°C, 2 h.

The synthesis of compound **3d** is shown in Scheme 4. Compound **18** was obtained by reaction of cyanamide, triethyl orthoformate and ethyl 2-(benzylamino)acetate in the presence of

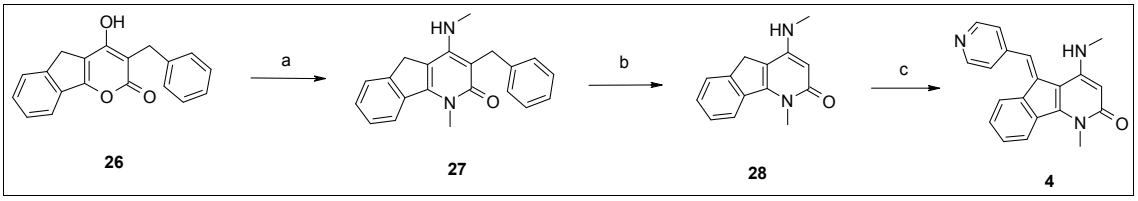
sodium methoxide, followed by *in situ* trans-esterification of the expected ethyl ether into the methyl ester. Nitrosation of the primary amine of **18** afforded the corresponding diazonium salt that was then converted, in the presence of potassium iodide, into the iodo-imidazole **19**. Suzuki coupling then led to ester **20** that was next hydrolyzed into acid **21** which, in turn, was transformed into amide **22** by coupling with cylopropylamine. Cyclisation of the later intermediate was carried out in the presence of DBU to yield compound **23**. Subsequent Buchwald–Hartwig amination and deprotection of the *t*-butyloxy carbonyl protecting group successively provided compounds **24** and **25**. **3d** was finally obtained following hydrogenolysis of the benzylic protecting group still present in compound **25**.

Scheme 4. **Synthesis of the novel tricyclic IPYs 3d**[a]



[a]Reagents and conditions: (a) $HC(OEt)_3$, reflux, 2 h and then ethyl 2-(benzylamino)acetate, Et_2O, NaOMe, RT, 1 h; (b) $NaNO_2$, HCl, KI, water, 0°C to reflux, 24 h; (c) (4-bromo-2,5-difluorophenyl)boronic acid, $Pd(PPh_3)_4$, K_2CO_3, MeOH/PhMe (1:2), microwaves, 1 h, 120°C; (d) NaOH 6M, THF/water (1:1), RT, 1 h; (e) cyclopropylamine, O-(7-azabenzotriazol-1-yl)-N,N,N′,N′-tetramethyluronium hexafluoro phosphate (HATU), DIEA, THF, RT, 12 h; (f) DBU, DMF 120°C, 24 h; (g) (S)-*tert*-butyl pyrrolidin-3-ylcarbamate, BINAP, $Pd(OAc)_2$, Cs_2CO_3, toluene, microwaves, 150°C, 5 h; (h) HCl 2M in Et_2O, RT, 12 h; (i) $H_2CO_2NH_3$, Pd/C, MeOH, reflux, 6 h.

Scheme 5. Synthesis of compound 4[a]



[a]Reagents and conditions: (a) $MeNH_2$, AcONa, reflux, 20 h; (b) $AlCl_3$, xylene, 100°C, 10 min; (c) 4-pyridyl carboxaldehyde, piperidine, DMSO, 100°C, 16 h.

Compound **4** has been prepared as shown in Scheme 5. Compound **26**[16] was reacted with methylamine to afford the tricyclic 2-pyridone **27**. Cleavage of the benzylic side chain by reaction with aluminum trichloride led to **28** that was next converted, upon Knoevenagel-like condensation with 4-pyridyl carboxaldehyde, into compound **4**.

Evaluation of historical IPYs (compounds 1 and 2). We started our investigations with profiling a selection of historical tetracyclic IPYs. Across a primary panel of representative Gram-positive and Gram-negative strains, we confirmed that compounds such as **1a** and **1b** displayed good activities (Table 1) on Gram-positive bacteria such as *Staphylococcus aureus* (see in Table 1, the MIC of **1a** on the Methicillin-resistant *Staphylococcus aureus* (MRSA) strain at 0. 25 μg/ml) but no activities on the efflux-competent *Escherichia coli (E. coli*) or *Pseudomonas aeruginosa (P. aeruginosa*) strains. For the *E. coli* strains, this lack of activity was accounted for by a strong efflux rather than by a lack of affinity for the *E. coli* topoisomerases or by poor influx. This conclusion was suggested, as illustrated for **1a**, by the very low MIC on the efflux-deficient *E. coli* MG1655 Δ*tolC* and by the lack of MIC shift on the hyper-permeable (UDP-3-O-acyl-N-acetylglucosamine deacetylase (*lpxC)* mutant) D22[17a] *E. coli* strain. More generally, all along our work, we used MICs on the pump-deleted *E. coli* strains as surrogates to estimate target affinity, assuming that only efflux limited net permeability of the IPYs in wild-type (wt) *E. coli*. In the case of *P. aeruginosa*, the reason for

the lack of activity against the wt and the partially efflux-deficient PAO750[17b] strains could not be easily ascertained. It could be due to efflux by other pumps (not deleted in PAO750), poor permeability or even poor target binding.

For **1a**, at 8-fold its MIC, the frequency of resistance (FoR) of the MRSA strain ATCC33592 was poor (> 10^{-7}). Cytotoxicity values on the HepG2 cell line, a human hepatocellular carcinoma cell line, were variable across the series but often quite significant (e.g. TC_{50} = 8.4 μM for **1a**). Regarding the ADME and physico-chemical profile (data not shown), the tetracyclic IPYs such as **1a** generally displayed poor solubility in water across a variety of pHs, poor permeability in Caco-2 cells, metabolic instability in the presence of murine or human microsomes and high chemical instability in a DMSO solution in the presence of thiols such as mercapto-ethanol or glutathione (more than 50% degradation within one hour), whereas it was stable in a pH 7.4 buffer solution. These different issues were consistent with our historical failure to optimize the series and reach good *in vivo* efficacy. They were thought to stem from a combination of high hydrophobicity (clogD at pH 7.4 = ~4 for **1a** and **1b**), high planarity and the presence of the reactive exo-methylene moiety prone to act as a Michael acceptor. In addition, compounds such as **1a** displayed significant hERG inhibition (typically >50% inhibition at 10 μM). Overall, there was little hope to optimize the tetracyclic IPY subseries away from the high hydrophobicity that is generally detrimental to activities on Gram-negative bacteria[18].

We next turned our attention to the bicyclic IPY subseries (compounds **2**) that appeared more drug-like than the tetracyclic IPYs **1**. Historically, the few compounds prepared in this sub-series had shown poor activities on Gram-positive bacteria, with MICs for *Staphylococci* strains generally above 32 μg/ml (as for **2a** in Table 1), despite c-logP/c-logD at pH 7.4 values compatible with activities against Gram-positive (clogP = 3 in the case of **2a**). As previously, we first profiled a small selection of historical bicyclic IPYs and confirmed that

these compounds were inactive against our panel of Gram-positive and efflux-competent Gram-negative strains despite significant inhibition of wt *E. coli* gyrase in the DNA supercoiling assay (e.g. IC_{50} = 6.35 μM for **2a**, compared to ciprofloxacin (Cip) IC_{50} = 0.31 μM). Interestingly, compound **2a** displayed poor MIC against the permeable D22 strain but reasonable activities on the pump-deleted *E. coli* strain, suggesting significant target inhibition and, efflux- rather than influx-limited penetration, hence potential to obtain activities on wt *E. coli*, provided efflux could be overcome. In addition, the *E. coli* Δ*tolC* strain showed a satisfactory frequency of resistance (< $5.7\ 10^{-9}$), when challenged by **2a** at 4xMIC (4 μg/ml). Based on this attractive preliminary data and with the objective of enlarging the spectrum toward Gram-negative bacteria, we produced several libraries of bicyclic IPYs displaying an amide, a urea, a carbamate or a sulfonamide instead of the phenylacetamido moiety of **2a**. Unfortunately, the only analogs that displayed activity were carboxamides very similar to **2a** and these were still only active on the efflux pump-deleted strains (data and structures not shown), which led us to discontinue efforts on this series.

Design and antibacterial properties of a new series of tricyclic IPYs. Noting that there was potential structural similarity between the IPYs and the Pfizer quinazolinedione topoisomerase inhibitors[7] (QZD; Figure 1), we theorized that design elements from the QZDs could be beneficial to the IPYs. Even if the hydrogen bond donors OH or NH_2 of the QZDs were exchanged for a hydrogen bond acceptor in our IPYs, the rest of the pyrazinone moiety, and in particular the N1-alkyl group and the C2-carbonyl, superimposed nicely with those of the QZD scaffold. To get a perfect fit with the QZDs, the indene part of the tetracyclic IPYs had to be obviously exchanged for a fused benzo moiety bearing the fluoro and the amino substituents of the QZDs. This reasoning led us to hybridize the tetracyclic IPYs and the QZDs, as shown in Figure 3, to obtain a new series of tricyclic IPYs.

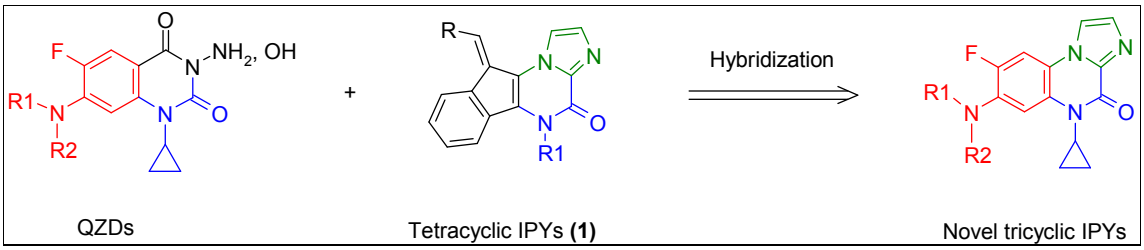
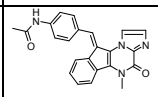
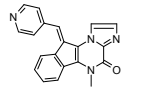
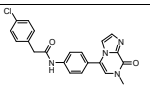
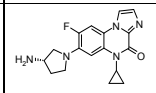
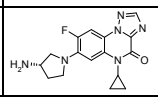
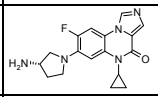
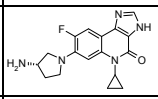
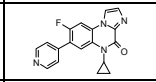
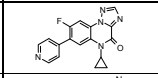
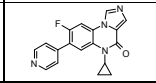
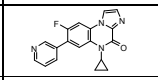
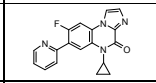
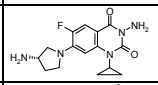
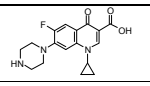


Figure 3. Hybridization of the tetracyclic IPYs with Quinazolinediones[7] (QZDs)

We first synthesized compound **3a** fixing the amino group to (S)-3-amino pyrrolidino (one of the best substituents from the QZD series[7]) to maximize our chance to generate an active compound. Lending credit to our design hypothesis, compound **3a** was found active, albeit less so than QZD1, showing modest MICs on the efflux-competent Gram-negative and the MRSA strain (Table1). Comparing MICs between efflux-competent, efflux-defective, and hyper-permeable strains indicated strong efflux and no significant problem of influx, at least in *E. coli*. In addition, the very low MICs on the two pump-deleted *E. coli* and *P. aeruginosa* strains suggested good inhibition of the corresponding topoisomerases, assuming topoisomerase inhibition-mediated activities. In line with the latter hypothesis, in a wild type *E. coli* gyrase DNA supercoiling assay, an IC_{50} was measured for **3a** at 1.19 µM (0.42 µM for Cip). Moreover, a clear and selective dose-response effect on DNA replication was confirmed for this compound in an *E. coli* macromolecular synthesis assay (Scheme S1).

We immediately prepared a series of analogs of **3a** with modified imidazo moieties (compounds **3a** to **3d**) to probe the influence of this ring (Table 1). As for **3a** (clogD pH 7.4 = 0.3), all these compounds displayed clogD at pH7.4 compatible with activities against Gram-negative[15] (-0.3 to -1.1; Table1). Among this set of "imidazo-like" analogs, the triazolo homolog **3b** had similar activities compared to **3a** on *E. coli* and MRSA and showed higher MICs on the *P. aeruginosa* strains.

Table 1. MICs of representative IPYs on a panel of Gram-positive and Gram-negative strains

Cpd	Structure	logD pH 7.4[a]	MIC (μg/ml)						
			Ec[b]	*Ec*[c]	*Ec*[d]	*Ec*[e]	*Pa*[f]	*Pa*[g]	*Sa*[h]
1a		4.1	>64	>64	<0.125	>64	>64	>64	0.25
1b		3.8	>64	>64	<0.125	>64	>64	>64	>64[i]
2a		3	>32	>32	1	32	>32	>32	>32
3a		0.3	4	8	<0.125	2	32	<0.125	8
3b		-0.3	2	4	<0.125	1	>64	1	4
3c		-1.1	16	32	1	16	>64	1	16
3d		-0.3	16	64	0.5	16	64	0.5	16
3e		3.3	16	32	<0.125	8	>64	8	4
3f		2.6	32	>64	<0.125	32	>64	32	16
3g		1.9	64	>64	0.25	64	>64	32	64
3h		3.4	16	32	<0.125	8	>64	4	64
3i		3.3	>64	>64	4	>64	>64	>64	>64
QZD1		-1.8	1	1	<0.125	0.5	32	<0.125	2
Cip		-0.7	<0.125	<0.125	<0.125	<0.125	0.25	<0.125	0.125

[a]LogD at pH 7.4 as calculated by ACD9; [b]*E. coli* ATCC35218 (ESBL); [c]*E. coli* MG1655 (K12); [d]*E.coli* MG1655 Δ*tolC* (pump-deleted); [e]*E. coli* D22[17a] (hyperpermeable LpxC mutant); [f]*P. aeruginosa* PAO1; [g]*P. aeruginosa* PAO750[17b] (PAO1 with ΔmexAB-oprM ΔmexCD-oprJ Δ mexEF-oprN ΔmexXY ΔopmH ΔmexJK ΔpscC); [h]*S. aureus* ATCC33592 (MRSA); [i]Unreliable result due to poor solubility; Cip = Ciprofloxacin.

Compounds **3c** and **3d** had modest MICs on the MRSA strain similar to that of **3a** whereas they were both less active on the Gram-negative strains than **3a**, may be as a result of decreased permeability. Of note, the lower activities of **3d**, a compound designed to be the closest bio-isostere of the quinolones and quinazolinediones, suggested that the analogy with quinazolinediones was less straightforward than initially envisioned.

We then tested **3a** and **3b** against a small secondary panel of Gram-negative) strains (Table S2) to provide a more complete picture of the breadth of activities of these compounds against Gram-negative bacteria. Similar or slightly better activities were confirmed on the *E. coli* and *P. aeruginosa* strains while the efflux-competent *Acinetobacter baumannii* (*A. baumannii*) and *Klebsiella pneumoniae* (*K. pneumoniae*) strains were less sensitive (MICs = 32 or > 64 μg/ml). MICs on the corresponding pump-defective strains suggested potential on *K. pneumoniae* strains (MICs = 0.5 μg/ml), provided efflux could be overcome, but less so on *A. baumannii* strains (MICs = 4-8 μg/ml). This difference may reflect different affinity of the test compounds for the BTs of these two species or differences in penetration across species.

We also exchanged the pyrrolidino substituent for a pyridine ring, as reported in the isothiazoloquinolone BTI series[6]. **3e**, the direct analog of **3a** (Table 1), showed a similar MIC on the MRSA strain. **3e** also displayed activity on the *E. coli* Δ*tolC* strain, as good as **3a** suggesting similar affinity for the topoisomerases upon substitution of the amino pyrrolidino substituent present in **3a** for the 4-pyridyl ring of **3e**. This deduction was confirmed by the result of the DNA wild type *E. coli* gyrase supercoiling assay: **3e** showed an IC_{50} of 2.5 μM, very close to that of **3a** (IC_{50} = 1.19 μM) but significantly less potent than that of Cip (IC_{50} = 0.42 μM). Unfortunately, good target affinity failed to translate into good MICs on the efflux-competent *E. coli* strains, possibly due to increased efflux or decreased permeability. Compared to **3e**, compounds **3f** to **3i** were much less active on the MRSA strain. Moreover, **3f**

and **3g** only showed, at best, modest MICs on the efflux-competent *E. coli* strains probably due to either increased efflux (for **3f**) or reduced affinity for the target (for **3g**). Interestingly, compound **3h** and **3i** showed contrasting properties: while **3h** retained similar antibacterial activities on the efflux-competent and the *ΔtolC E. coli* strains compared to **3e**, **3i** was totally inactive (MIC > 64 μg/ml) on the efflux-competent *E. coli* strains and displayed modest activity on the *ΔtolC E. coli* strain. These differences suggested efficient interactions of the pyridyl nitrogens of **3e** and **3h** with the *E. coli* topoisomerases that are lost when the nitrogen atom is shifted to the 2-position of the pyridyl moiety. Moreover, in comparison to **3a**, compounds **3e** to **3i** all showed a lack of activity on the efflux-competent *P. aeruginosa* PAO1 and much higher MICs on the pump-deleted *P. aeruginosa* PAO750, suggesting in all cases a large drop of affinity for the targets with respect to **3a** and stressing the importance of the amino-pyrrolidine moiety of **3a** for good affinity for the *P. aeruginosa* topoisomerases.

Finally, we looked at the serum effect across the new tricyclic IPY series. In the presence of either 50% calf fetal serum or 50% mouse serum (Table S3), there were no significant MIC shifts for **3a-e**, whereas **1a** displayed a strong serum effect (MIC shift >32 fold). These results, consistent with the hydrophobicity of these different compounds, boded well for *in vivo* efficacy (see below) of the most potent tricyclic IPYs.

Developability profiling of the new tricyclic IPYs. At this stage, we profiled our best tricyclic IPYs in various ADMET and physico-chemical assays in order to determine the developability potential of the series (Table 2). Overall, the profile of the 8-amino derivatives **3a** and **3b** was good. In addition to their small molecular weights (~330), these compounds displayed good aqueous solubility even at pH 7.4, low metabolism in microsomes across species, stability in rodent plasma, minimal CYP3A4 inhibition and even potential for oral absorption (see **3b**). In terms of early toxicity signals, **3a** and **3b** were devoid of cytotoxicity

in HepG2 cells and only weakly inhibited the hERG channel (IC_{50}s > 30 μM). In addition, an *in vitro* pharmacology assessment of **3b** showed minimal issues with only two targets (M1 and M3) out of a panel of 33 enzymes, receptors and transporters inhibited at 10 μM, in the 50-60% range. The only serious alert that was detected in this profiling was genotoxicity in a micronucleus test (MNT) assay, with or without S9 metabolic activation. This result may be linked to the mechanism of action of the IPYs (topoisomerase inhibition) and is a usual concern in topoisomerase inhibitor programs[19].

Table 2. Physico-chemistry and ADMET profile of representative IPYs

Compound	3a	3b	3e
Aqueous solubility @ pH 7.4[a]	1132 μg/ml	926 μg/ml	10 μg/ml
Caco-2 Papp ($nm.s^{-1}$)[b]	4	32	216
Microsome lability h/m/r (%)[c]	6/8/11	0/8/8	13/81/68
CYP3A4 IC_{50} M/T[d]	>30/>30 μM	>30/>30 μM	15/13 μM
hERG IC_{50}[e]	>30 μM	>30 μM	>30 μM
Plasma stability (m, r)[f]	Stable at 4 h	Stable at 4 h	Stable at 4 h
HepG2 (TC_{50})[g]	>30 μM	>30 μM	>30 μM
MNT[h] w/wo metabolic activation	Positive w/wo	Positive w/wo	Positive w/wo

[a]Thermodynamic solubility. [b]Apparent permeability; results above 20 $nm.s^{-1}$ suggest good oral absorption in humans. [c]Percentage metabolized by human/mouse/rat liver microsomes following 20 min of incubation at 5 μM in the presence of 1mM NADPH. [d]Inhibition in human liver microsomes of the CYP3A4-mediated metabolism of ether midazolam (M) or testosterone (T). [e]Inhibition of the hERG channel transfected in CHO cells in an automatic patch clamp format, at 20°C. [f]Measured in mouse and rat plasma after 4 h of incubation of 1 μM of the test compounds at 37 °C; the test compounds were considered stable at 4 h if the percentage of stability was within the 80 - 120% range. [g]Cytotoxicity in the HepG2 cell line after 40 h of incubation. [h]L5178Y mouse lymphoma cells incubated with increasing concentrations of test articles for 23 h (without S9 fraction) or 3 h (in the presence of S9).

The profile of **3e** was less satisfactory than that of **3a** and **3b** but still acceptable at this early stage. Probably due to its higher hydrophobicity (clogD pH 7.4 = 3.3), Caco-2 permeability was excellent and aqueous solubility was much lower while metabolic stability in the presence of rodent microsomes was poorer and CYP3A4 inhibition was stronger. As for **3a** and **3b**, **3e** was found to be positive in the MNT assay, with or without S9 metabolic

activation. This general genotoxicity alert was perceived as a setback in the otherwise promising developability profile of the IPYs.

***In vivo* profiling of the new tricyclic IPYs.** Considering the good *in vitro* ADME properties of the best tricyclic IPYs, we decided to progress compounds **3b** to *in vivo* mice studies in order to get an assessment of the tolerability and efficacy of the series, two key hurdles for any new scaffold active on Gram-negative bacteria. Compound **3b** was well tolerated at 30 mg/kg following iv bolus administration. In a septicemia model using *E. coli* ATCC35218, **3b** (MIC = 4 μg/ml) showed no significant effect on survival and on bacterial burden, following iv administration of 2 x15 mg/kg (Table 3). At 2 x30 mg/kg, compound **3b** reduced bacterial burden in the blood (Table 3) but with a limited impact on survival at 96 h. Higher doses could not be tested because of solubility limitations in the simple aqueous formulation used.

Table 3. *In vivo* efficacy of compound 3b[a] in an *E. coli* ATCC35218 mouse septicemia model[b]

	Survival @ 96 h (n = 10 mice/group)	Log_{10} CFU/ml of blood (n = 5 mice/group)	Δ logCFU
Vehicle	0%	7.6	-
Tienam® - 15 mg/kg/d	90%	1.9	5.8[c]
3b – 2 x15 mg/kg	0%	6.1	1.6[d]
3b – 2 x30 mg/kg	30%	4.5	3.2[e]

[a]Formulated in sterile water; [b]Infection ip at T_0, iv administration of **3b** at T_0+1 h and T_0 + 3 h, blood sampling for CFU count at T_0 + 4 h and follow up of mice up to T_0 + 96 h. [c]p = 0.0002 compared with vehicle group; [d]p = 0.0432 compared with vehicle group; [e]p = 0.0233 compared with vehicle group.

To shed light on these results, we first measured mouse protein binding by rapid equilibrium dialysis and found it to be moderate at 62%. We then compared this to a single

dose PK data in mice (iv; 30 mg/kg of **3b** formulated in sterile water; see Table S6). Exposure in plasma ($AUC_{0\text{-last}}$ = 7200 ng.h/ml) was reasonable but half-life was short (1 h) and the time during which the free concentration of **3b** remained above or close to MIC (4 µg/ml) was no more than a few minutes (unbound concentration in plasma at 5 min = 2.5 µg/ml; mean value for 3 animals), which may explain the poor efficacy of **3b** in the septicemia model, at tested doses. Of note, tissue concentrations in lung, liver and kidney were much higher than those recorded in plasma. For instance, in all sampled tissues, assuming similar protein binding as in plasma, unbound concentrations remained above MIC for ~2 h, suggesting that efficacy in tissue infection models might be better, at comparable doses, than that seen in the septicemia model. However, this study was never conducted due to the resistance issue associated to the series (see below).

Resistance studies. In parallel with the *in vitro* and *in vivo* profiling discussed above, we moved to resistance and cross-resistance studies. In order to confirm the good frequency of resistance of the *E. coli* Δ*tolC* with **2a**, we first measured frequencies of resistance for various wild-type efflux-competent Gram-negative strains challenged by **3a** and **3e** (see Table 4). After 16 h and 48 h of culture in the presence of either test compounds, *E. coli, K. pneumoniae* and *A. baumannii* wild-type strains gave modest to good FoR rates, ranging from ~10^{-7} (*A. baumanii* 48 h) to ~ 10^{-9} (*E. coli*). Results were similar between **3a** and **3e** for the *E. coli* strain (≤ 3.6 10^{-9}) while there was a one-log difference in favor of **3a** for the *K. pneumoniae* strain (≤ 2.8 10^{-9} compared to 3.9 10^{-8}). On the other hand, for both compounds, the pump-deleted *(ΔtolC*) and permeable (*lpxC*-mutant) *E. coli* strains unexpectedly displayed, for unclear reasons, significantly higher FoR than the more relevant, wt *E. coli* strains (~10^{-7} compared to ~10^{-9}). This difference of behavior couldn't be confirmed for *P. aeruginosa* since MIC levels allowed us to test only the pump-deleted strain. However, for the latter, FoR were also in the 10^{-7} to 10^{-8} range for both compounds.

Table 4. Frequencies of resistance of various Gram-negative bacteria at concentrations (Conc) of 4xMICs of 3a or 3e

Cpd	Species	Genotype	Conc. (μg/ml)	16 h frequency	48 h frequency
3a	*E. coli*	ATCC 25922 WT parent	32	$\leq 3.6\ 10^{-9}$	$3.6\ 10^{-9}$
	E. coli	ATCC 25922 Δ*tolC*	1	$5.3\ 10^{-7}$	-
	E. coli	CGSC 5163, D22 *lpxC* mutant	8	$2.6\ 10^{-7}$	$6.7\ 10^{-7}$
	P. aeruginosa	PAO750 Efflux-defective	4	$1.6\ 10^{-8}$	$3.4\ 10^{-7}$
	K. pneumoniae	ATCC 13883 WT	64	$\leq 2.8\ 10^{-9}$	$\leq 2.8\ 10^{-9}$
3e	*E. coli*	ATCC 25922 WT parent	32	$\leq 3.6\ 10^{-9}$	$\leq 3.6\ 10^{-9}$
	E. coli	ATCC 25922 Δ*tolC*	0.5	$5.3\ 10^{-7}$	-
	E. coli	CGSC 5163, D22 *lpxC* mutant	2	$2.6\ 10^{-7}$	$4.5\ 10^{-7}$
	P. aeruginosa	PAO750 Efflux-defective	8	$3.7\ 10^{-8}$	$8.2\ 10^{-8}$
	K. pneumoniae	ATCC 13883 WT	64	$3.1\ 10^{-8}$	$3.9\ 10^{-8}$
	A. baumannii	ATCC 19606 WT	64	$4.5\ 10^{-9}$	$4.5\ 10^{-7}$

We further characterized some of the mutant strains selected in these resistance experiments (see details in Table S4). We sequenced the whole genomes of five resistant strains selected from *E. coli* ATCC25922 (one mutant selected by **3a**) and the Δ*tolC E. coli* strain (2 mutants each for **3a** and **3e**) and looked at the corresponding MIC-fold increases. Mutations could be identified only in two out of the three mutant strains selected by **3a** and were found to have no connection with topoisomerases. In one case (Δ*tolC E. coli*), resistance was linked to efflux regulation and resulted in a 32-fold MIC shift. In the other case (*E. coli* wild-type ATCC25922), a point mutation was identified in histidine tRNA synthetase *hisS* (V334E) and was not associated with any MIC shift. For the mutant where no mutation could be identified (selected in Δ*tolC E. coli*), the MIC shift was 8-fold.

We next turned our attention to cross-resistance of the IPYs to FQs and to non-quinolone bacterial topoisomerase inhibitors related to NXL101[8]. When tested against a panel of isogenic *S. aureus* strains engineered to be resistant or hyper-susceptible to such compounds[20], the MICs of **3a, 3b, 3c** but also those of **1a** showed no or much smaller changes

with respect to the parent strain compared to NXL101[8] (Table S5), suggesting that IPYs interact with gyrase and topo IV at a different site in comparison to compounds such as NXL101. We then looked at the activity of **3a** on a panel of *S. aureus*, *K. pneumoniae* and *E. coli* strains engineered to be resistant to FQs[21] (Table 5). To our surprise, **3a** showed cross-resistance with FQs. However, the levels of cross-resistance remained low to moderate (fold shifts ranging from 2 to 64) even for those strains that were resistant to highly resistant to Cip (fold shifts from >16 to 2000). Contrary to our initial hypothesis, **3a** behaved more like FQs than QZDs since these later compounds have been reported to display no cross-resistance with FQs[7]. Results in Table 5 also suggested that **3a** targets both gyrase and topo IV. Furthermore, similar to FQs, topo IV seems to be the primary target in *S. aureus* whereas gyrase appears as the primary target in *K. pneumoniae*.

Table 5. MICs of 3a and of Cip on a panel of efflux-competent *S. aureus, K. pneumoniae* and *E. coli* strains engineered to be resistant to FQs

Strain	MIC (μg/ml)		Fold shift vs wt strain	
	3a	Cip	**3a**	Cip
S. aureus				
RN4220 wt parent	4	0.25	-	-
RN4220 GyrA D83N	4	0.25	1	1
RN4220 GyrA D83N ParC D79N	16	1	4	4
RN4220 GyrB D437N	4	0.25	1	1
RN4220 ParC S80F	8	1-2	2	4-8
RN4220 ParC S80F GyrA S84L	16	64	4	256
K. pneumoniae				
1161486 wt parent	16	<0.063	-	-
1161486 GyrA S83I	64	1	4	>16
1161486 GyrA S83I ParC S80I	128	8	8	>128
1161486 GyrA S83F D87V ParC S80I	64	8	4	>128
E. coli				
MG1655 wt parent	8	0.016	-	-
MG1655 GyrA S83L D87N ParC S80I	512	32	64	2000

Table 6. MICs of selected IPYs and Cip on one FQ-sensitive and one highly FQ-resistant *E. coli* Δ*tolC* clinical isolates

Compound	MIC (μg/ml)		Fold shift
	7623 Δ*tolC* FQ^S (gyrase wt)	W4753 Δ*tolC* FQ^R (GyrA S83L D87N ParC S80I ParE S458A)	
Cip	0.002	10[a]	5000
1a	0.063	2	32
1b	0.016	0.25	16
2a	0.125	4	32
3a	0.125	2	16
3e	0.063	0.5	8
4	1	2	2

[a]Average of two experiments.

To confirm these results, we screened a series of representative IPYs on two *E. coli* clinical isolates, engineered to harbor a *tolC* deletion that make them efflux-deficient (Table 6). The first strain is FQ-sensitive (FQ^S) while the second one is highly resistant to FQs (FQ^R), due to an accumulation of mutations in GyrA, ParC and ParE. The ratio (or fold shift) between the MICs of these two strains was used as a surrogate to estimate the level of cross-resistance with FQs. Under these conditions, Cip demonstrated an impressive 5000-fold shift. On the other hand, compound **3a** displayed a more modest 16-fold shift, confirming partial cross-resistance with FQs. Similar fold shifts were seen for **3e** as well as for **1a**, **1b** and **2a**.

The observed cross-resistance suggested that IPYs, occupy the same binding pockets in topoisomerases as quinolones. Unfortunately, contrary to quinazolinediones that also occupy the FQ-binding pocket, the molecular interactions of IPYs with their targets apparently involve residues that have a key role in the binding of quinolones, thereby conferring cross-resistance to FQs if mutated. Interestingly, upon screening historical compounds related to the parent IPYs on the same two above strains, we discovered that some "non-imidazo" analogs showed much reduced fold shifts, as low as 1-2 fold, although generally at the expense of the activities on the pump-deleted *E. coli* strain, suggesting lower affinity for the topoisomerase

targets. Compound **4** (Scheme 5) was a typical such example with a 2-fold shift, a modest MIC (1 μg/ml) on the wt gyrase 7623 Δ*tolC* Q^S strain and a lack of activity on the MG1655 efflux-competent strain (Table 6). The comparison of the fold shifts and the MICs on the wt gyrase-expressing strain for **4** and **1b**, two compounds that differ only by the imidazo moiety, suggests that an important part of the affinity for the targets as well as the cross-resistance of the bicyclic, tricyclic and tetracyclic IPYs with FQs could be due to this heterocyclic moiety. As a consequence of this hypothesis, we speculate that cross-resistance of IPYs might be abolished by substituting the imidazo moiety for simpler substituents (as in compound **4**). This latter conclusion echoes a recent patent that claims 2-quinolones with simple 3 and 4 substituents related to those seen in compound **4**[22]. These compounds display antibacterial activities on FQ-sensitive and resistant strains[22].

CONCLUSIONS

Starting from a proprietary series of bacterial topoisomerase inhibitors, we have designed by hybridization with the known quinazolinedione series a series of tricyclic imidazopyrazinones that inhibited bacterial topoisomerases and displayed promising preliminary activities against Gram-negative bacteria, acceptable frequencies of resistance and *in vivo* efficacy with concomitant tolerability. During our profiling, we discovered that this new series, as well as the historical parent compounds, displayed partial cross-resistance with fluoroquinolones and that the cross-resistance could be abolished by removing the imidazo moiety, although this led to much weaker inhibition. Considering the cross-resistance issue, our inability to generate improved 8-amino analogues (results not shown) and the MNT alerts across the IPY series, we halted our program at this stage due to the low likelihood of successfully delivering a development candidate, illustrating some of the challenges faced by antibacterial discovery. In a companion paper[23], we report the results of our studies regarding the mechanism of action of the IPYs and X-ray crystallography data confirming that IPYs

bind to the same binding pocket as FQs. Finally, our results suggest that the two core rings of the quinolones could be either rescaffolded to resemble the tricyclic core of the tetracyclic parent IPY series or extended toward the direction occupied by the exo-arylidene or the N-phenylacetamido moieties present respectively in the tetracyclic IPYs **1** and in the bicyclic IPYs such as **2a**.

EXPERIMENTAL SECTION

Chemistry. The nomenclature of the compounds is based on ACDLABS software, version 11.01. All solvents and reagents obtained from commercial sources were used without further purification. The microwave oven was a Biotage Initiator^{TM} 2.0 (400 W, 2450 MHz). Reactions were monitored by thin layer chromatography (TLC) or by LC-MS using an Agilent 1100 or 1260 Infinity (UV detection at 254 nm or 220 nm) relying on a low resonance electrospray mode (ESI). Thin layer chromatography was carried out on Merck silica gel 60 F_{254} pre-coated glass backed plates and the visualization was realized under UV light (254 nm) and/or by staining with aqueous solution of $H_3Mo_{12}O_{40}P$ x H_2O and $Ce(SO_4)_2$ followed by gentle heating with a heat gun. Purifications were performed using pre-packed Redisep silica gel cartridges (20−40 μm) or by flash column chromatography using silica gel 60. HPLC purifications were performed with a Waters AutoPurification HPLC-MS system or a Gilson PLC2020 using RP-18 columns with water/TFA (at respectively 0.1% or 0.05%) as the mobile phase A and MeCN (plus TFA 0.035% in the case of the Gilson HPLC) as the mobile phase B. The product containing fractions were collected and freeze-dried to yield the final products. The ^{1}H and ^{13}C NMR spectra were performed, in DMSO-d_6 or methanol-d_4, at 303K, either on a Bruker Avance I operating at a proton frequency of 600 MHz and a ^{13}C-carbon frequency of 151 MHz or on a Bruker Avance III spectrometers operating at a proton frequency of 500 MHz and a ^{13}C-carbon frequency of 126 MHz. Chemical shifts (δ, expressed

in ppm) were referenced to the solvent signals (2.50 ppm and 39.52 ppm). Coupling constants (J) are given in hertz. The mass spectra were recorded on a Waters UPLC-SQD instrument (mode electrospray ES+ and ES−) using a RP-18 column. Purities of the final compounds are ≥95.0% and were measured by liquid chromatography coupled to high resolution mass spectrometry, using UV detection at 220 nm. High resolution mass spectrometry was performed on an Agilent 6230 TOF LC-MS in combination with an Agilent 1290 Infinity II HPLC or on an LCT Premier XE Waters TOF spectrometer in combination with a Waters Acquity UPLC for the LC part. Following the purification step, solid compounds (intermediate and targeted compounds) were generally not crystallized and used/characterized as such.

N-{4-[(E)-(5-methyl-4-oxo-4,5-dihydro-10H-imidazo[1,2-a]indeno[1,2-e]pyrazin-10-ylidene)methyl]phenyl}acetamide (1a). To a solution of **6** (1.74 g, 7.3 mmol) and 4-acetamido benzaldehyde (1.63 g, 10 mmol) in methanol (50 ml) under nitrogen bubbling, was added piperidine (1 ml, 10 mmol). The reaction mixture was refluxed for 1.5 h. The resulting orange precipitate was then filtered and washed with methanol. The crude solid was crystallized from DMF (50 ml) to afford the expected compound **1a** as an orange solid (2 g, 71%); mp 200 °C. ^{1}H NMR (600 MHz) δ 10.16 (s, 1H), 8.66 (d, *J* = 1 Hz, 1H), 8.06 (s, 1H), 7.95 (d, *J* = 7.8 Hz, 1H), 7.73 (d, *J* = 8.5 Hz, 2H), 7.66 (d, *J* = 1 Hz, 1H), 7.54 (d, *J* = 8.5 Hz, 2H), 7.46 (d, *J* = 7.8 Hz, 1H), 7.31-7.35 (m, 1H), 7.11-7.16 (m, 1H), 3.97 (s, 3H), 2.89 (s, 1H), 2.10 (s, 3H). ^{13}C NMR (151 MHz) δ 169.1, 153.4, 140.4, 137.2, 134.2, 133.8, 133.3, 131.9, 131.1, 130.8, 130.7, 128.9, 128.7, 127.2, 123.8, 122.1, 119.2, 116.6, 116.1, 31.6, 24.6. HRMS (ESI-TOF) m/z: calcd for $C_{23}H_{18}N_4O_2$ $[M+H]^+$, 383.1508; found, 383.1496.

(10E)-5-methyl-10-[(pyridin-4-yl)methylidene]-5,10-dihydro-4H-imidazo[1,2-a]indeno[1,2-e]pyrazin-4-one (1b). To a suspension of (10E)-10-[(pyridin-4-

yl)methylidene]-5,10-dihydro-4H-imidazo[1,2-a]indeno[1,2-e]pyrazin-4-one **7**[15] (1 g, 3.2 mmol) in DMF (30 ml) was added sodium hydride (50% dispersion) (0.154 g, 3.2 mmol). The reaction mixture was first heated at 100°C until a clear solution was obtained and then allowed to cool down to room temperature. Methyl iodide (0.2 ml, 3.2 mmol) in DMF (1 ml) was then added. The resulting suspension was heated at 70°C for 1.5 h and then poured on a water ice mixture. The precipitate was washed successively with water and hot ethanol. The crude solid was then crystallized twice from DMF to afford the title compound **1b** as a solid (0.28 g, 28%); mp 302°C. ^{1}H NMR (600 MHz) δ 8.70-8.76 (m, 2H), 8.66 (d, *J* = 1.2 Hz, 1H), 8.02 (s, 1H), 7.96 (d, *J* = 7.9 Hz, 1H), 7.68 (d, *J* = 1.2 Hz, 1H), 7.53-7.59 (m, 2H), 7.35 (dt, *J* = 1.3, 7.6 Hz, 1H), 7.15-7.19 (m, 1H), 7.10-7.15 (m, 1H), 3.96 (s, 3H). ^{13}C NMR (151 MHz) δ 153.4, 150.5, 144.6, 137.1, 134.3, 134.2, 133.0, 132.4, 130.8, 129.6, 128.1, 127.6, 124.2, 124.2, 122.5, 116.6, 115.5, 31.6. HRMS (ESI-TOF) m/z: calcd for $C_{20}H_{14}N_4O$ $[M+H]^+$, 327.1246; found, 327.1248.

2-(4-chlorophenyl)-N-[4-(7-methyl-8-oxo-imidazo[1,2-a]pyrazin-5-yl)phenyl] acetamide (2a). To a solution of 2-(4-chlorophenyl)acetic acid (70 mg, 0.41 mmol, 1.2 equiv.) in DCM (20 mL) was added oxalyl chloride (0.11 mL, 1.23 mmol, 3.00 equiv.) and a drop of DMF. After stirring overnight at room temperature, the reaction mixture was concentrated *in vacuo* and the resulting acid chloride was used without further purification. To a solution of the hydrochloride salt of **12** (94 mg, 0.34 mmol, 1 equiv.) in DCM (10 mL) and pyridine (0.3 mL) was added the freshly prepared 2-(4-chlorophenyl)acetic acid chloride dissolved in DCM (5 mL) and the reaction mixture was then stirred overnight. The reaction mixture was concentrated *in vacuo*. The residue was dissolved in MeOH (3 mL), filtered and purified by preparative HPLC chromatography (RP-18 column, 15 minutes gradient 10-85% acetonitrile, retention time 7.3 min) to yield **2a** (88 mg, 0.22 mmol; 66% yield) as a colorless solid after freeze drying. ^{1}H NMR (500 MHz): δ 10.45 (s, 1 H), 7.78 (d, ^{3}J = 8.7 Hz, 2 H),

7.72 (s, 1 H), 7.66 (s, 1 H), 7.55 (d, ^{3}J = 8.7 Hz, 2 H), 7.41-7.36 (m, 4 H), 7.33 (s, 1 H), 3.70 (s, 2 H), 3.51 (s, 3 H). ^{13}C NMR (126 MHz): δ 169.08, 151.86, 140.13, 136.09, 134.77, 131.33, 131.04, 130.53, 129.18, 128.24, 124.70, 121.02, 119.36, 118.46, 115.93, 42.45, 35.15. HRMS (ESI-TOF) m/z: calcd for $C_{21}H_{18}ClN_4O_2$ $[M+H]^+$, 393.1113; found, 393.1116.

(S)-7-(3-aminopyrrolidin-1-yl)-5-cyclopropyl-8-fluoroimidazo[1,2-a]quinoxalin-4(5H)-one (3a). Trifluoroacetic acid (0.5 mL) was added to a solution of **17a** (110 mg, 0.257 mmol), in DCM (2 mL). The mixture was stirred for 12 h at room temperature and then concentrated. The residue was washed with a mixture of EtOH and di-isopropyl ether (9/1). The ensuing precipitate was filtered, washed twice with di-isopropyl ether and then dried under reduce pressure to afford the trifluoroacetate salt of **3a** as a white powder (74 mg, 0.168 mmol; 65% yield). ^{1}H NMR (600 MHz) δ 8.36 (d, *J* = 1.1 Hz, 1H), 8.14 (br s, 3H), 8.05 (d, *J* = 13.8 Hz, 1H), 7.52 (d, *J* = 1.1 Hz, 1H), 7.04 (d, *J* = 8.4 Hz, 1H), 3.97 (br s, 1H), 3.73 (ddd, *J* = 2.4, 6.4, 10.6 Hz, 1H), 3.61-3.69 (m, 1H), 3.56 (td, *J* = 2.9, 10.6 Hz, 1H), 3.40-3.48 (m, 1H), 2.91-3.02 (m, 1H), 2.28-2.38 (m, 1H), 2.07 (tdd, *J* = 5.0, 7.8, 12.8 Hz, 1H), 1.20-1.38 (m, 2H), 0.72-0.91 (m, 2H). ^{13}C NMR (151 MHz)+TFA δ ppm 158.33 (q, *J* = 30.9 Hz, 1 C), 153.85 (s, 1 C), 147.50 (d, *J* = 238.7 Hz, 1 C), 136.11 (s, 1 C), 134.92 (d, *J* = 10.6 Hz, 1 C), 133.06 (s, 1 C), 128.38 (s, 1 C), 115.84 (s, 1 C), 113.54 (d, *J* = 10.3 Hz, 1 C), 105.11 (d, *J* = 27.6 Hz, 1 C), 103.76 (d, *J* = 5.0 Hz, 1 C), 53.77 (d, *J* = 6.6 Hz, 1 C), 49.97 (s, 1 C), 47.86 (d, *J* = 4.0 Hz, 1 C), 29.30 (s, 1 C), 26.16 (s, 1 C), 10.50 (d, *J* = 9.0 Hz, 2 C). HRMS (ESI-TOF) m/z: calcd for $C_{17}H_{18}FN_5O$ $[M+H]^+$, 328.1574; found, 328.1578.

(S)-7-(3-aminopyrrolidin-1-yl)-5-cyclopropyl-8-fluoro-[1,2,4]triazolo[1,5-a]quinoxalin-4(5H)-one (3b). The trifluoroacetate salt of **3b** was prepared as described for **3a**, starting from compound **17b** (100 mg, 0.233 mmol), to afford a white powder (69 mg, 0.156 mmol; 67% yield). ^{1}H NMR (600 MHz) δ 8.52 (s, 1H), 8.15 (br s, 3H), 7.84 (d, *J* = 13.1 Hz, 1H), 7.10 (d, *J* = 8.1 Hz, 1H), 3.98 (br s, 1H), 3.78 (ddd, *J* = 2.5, 6.4, 10.9 Hz, 1H), 3.67-3.73 (m, 1H), 3.58

(td, *J* = 3.1, 10.9 Hz, 1H), 3.47-3.53 (m, 1H), 3.01-3.07 (m, 1H), 2.28-2.42 (m, 1H), 2.00-2.18 (m, 1H), 1.28-1.38 (m, 2H), 0.79-0.98 (m, 2H). ^{13}C NMR (151 MHz)+TFA δ ppm 158.41 (q, *J* = 30.8 Hz, 1 C), 153.42 (br s, 1 C), 153.38 (s, 1 C), 147.79 (d, *J* = 240.8 Hz, 1 C), 143.01 (s, 1 C), 136.17 (d, *J* = 10.6 Hz, 1 C), 128.80 (s, 1 C), 115.72 - 119.43 (m, 1 C), 114.10 (d, *J* = 10.6 Hz, 1 C), 104.10 (d, *J* = 27.8 Hz, 1 C), 103.60 (br d, *J* = 5.0 Hz, 1 C), 53.78 (br d, *J* = 6.6 Hz, 1 C), 49.96 (s, 1 C), 47.92 (br d, *J* = 3.7 Hz, 1 C), 29.30 (s, 1 C), 26.60 (s, 1 C), 10.38 (br d, *J* = 5.8 Hz, 2 C). HRMS (ESI-TOF) m/z: calcd for $C_{16}H_{17}FN_6O$ $[M+H]^+$, 329.1526; found, 329.1524.

(S)-7-(3-aminopyrrolidin-1-yl)-5-cyclopropyl-8-fluoroimidazo[1,5-a]quinoxalin-4(5H)-one 2,2,2-trifluoroacetate (3c). The trifluoroacetate salt of **3c** was prepared as described for **3a**, starting from compound **17c** (50 mg, 0.117 mmol), to afford a white powder (40 mg, 0.091 mmol, 78%). ^{1}H NMR (600 MHz) δ 8.86 (s, 1H), 8.03-8.29 (m, 4H), 7.79 (s, 1H), 7.04 (d, *J* = 8.5 Hz, 1H), 3.90-4.04 (m, 1H), 3.38-3.81 (m, 4H), 2.82-3.00 (m, 1H), 2.27-2.41 (m, 1H), 2.00-2.14 (m, 1H), 1.12-1.44 (m, 2H), 0.67-0.97 (m, 2H). ^{13}C NMR (151 MHz)+TFA δ ppm 158.54 (q, *J* = 32.6 Hz, 1 C), 155.56 (s, 1 C), 147.31 (d, *J* = 238.4 Hz, 1 C), 135.13 (d, *J* = 10.3 Hz, 1 C), 132.70 (s, 2 C), 131.12 (s, 1 C), 128.58 (s, 1 C), 122.28 (s, 1 C), 113.46 - 120.93 (m, 1 C), 112.31 (d, *J* = 10.3 Hz, 1 C), 105.00 (d, *J* = 27.8 Hz, 1 C), 104.12 (d, *J* = 5.0 Hz, 1 C), 53.76 (d, *J* = 6.4 Hz, 1 C), 49.91 (d, *J* = 1.3 Hz, 1 C), 47.88 (d, *J* = 4.2 Hz, 1 C), 29.31 (s, 1 C), 25.55 (s, 1 C), 10.37 (d, *J* = 7.4 Hz, 1 C). HRMS (ESI-TOF) m/z: calcd for $C_{17}H_{18}FN_5O$ $[M+H]^+$, 328.1574; found, 328.1563.

(S)-7-(3-aminopyrrolidin-1-yl)-5-cyclopropyl-8-fluoro-3H-imidazo[4,5-c]quinolin-4(5H)-one dihydrochloride (3d). Ammonium formate (42.96 mg, 0.661 mmol) and palladium on carbon (45 mg, 0.042 mmol) were added to a solution of **17** (30 mg, 0.066 mmol) in methanol (3 mL). The resulting mixture was refluxed for 6 h and then concentrated. The residue was suspended in methanol and then filtered through a 0.45 μm RC membrane.

The filtrate was purified via HPLC to afford the trifluoroacetate salt of **3d** as a white powder (2 mg, 0.006 mmol; 1% yield). ^{1}H NMR (600 MHz) δ 8.11 (s, 1H), 7.68 (d, 1H), 7.20 (d, 1H), 3.76-3.81 (m, 1H), 3.71-3.76 (m, 2H), 3.54-3.63 (m, 1H), 3.38-3.45 (m, 1H), 3.02 (tt, 1H), 2.27-2.37 (m, 1H), 1.88-1.99 (m, 1H), 1.41-1.47 (m, 2H), 0.89-0.98 (m, 2H). ^{13}C NMR (151 MHz) δ ppm 158.06 (br s, 1 C), 148.72 (d, *J* = 239.7 Hz, 1 C), 140.72 (br s, 1 C), 137.37 (s, 1 C), 137.30 (s, 1 C), 136.25 (s, 1 C), 110.00 (s, 1 C), 108.08 (d, *J* = 24.4 Hz, 1 C), 105.77 (br d, *J* = 6.4 Hz, 1 C), 102.71 (d, *J* = 5.0 Hz, 1 C), 56.96 (br d, *J* = 5.8 Hz, 1 C), 50.59 (d, *J* = 1.6 Hz, 1 C), 47.60 (s, 1 C), 32.75 (s, 1 C), 26.07 (s, 1 C), 10.17 (d, *J* = 1.9 Hz, 2 C). HRMS (ESI-TOF) m/z: calcd for $C_{17}H_{18}N_5OF$ $[2M+H]^+$, 327.1574; found, 328.1581.

5-cyclopropyl-8-fluoro-7-(pyridin-4-yl)imidazo[1,2-a]quinoxalin-4(5H)-one (3e). Pyridin-4-ylboronic acid (169.59 mg, 1,24 mmol), potassium carbonate (343.23 mg, 2.48 mmol) and tetrakis(triphenylphosphine)palladium(0) (43.05 mg, 0.037 mmol) were added, under an argon atmosphere, to a solution of **16a** (400 mg, 1.24 mmol) in a mixture of methanol (4 ml) and toluene (8 ml). The resulting mixture was heated under micro-wave irradiation for 1 h at 120°C and was then concentrated. The residue was suspended in methanol and then filtered through a 0.45 µm RC membrane. The filtrate was purified by HPLC to afford the trifluoroacetate salt of **3e** as a white powder (65 mg, 0.142 mmol; 12% yield). ^{1}H NMR (500 MHz) δ 8.71-8.77 (m, 2H), 8.54 (d, *J* = 1. 2 Hz, 1H), 8.34 (d, *J* = 11.0 Hz, 1H), 8.04 (d, *J* = 7.1 Hz, 1H), 7.70 (d, *J* = 4.7 Hz, 2H), 7.61 (d, *J* = 1.2 Hz, 1H), 3.04-3.14 (m, 1H), 1.24-1.42 (m, 2H), 0.76-0.96 (m, 2H); ^{13}C NMR (126 MHz) δ ppm 154.74 (d, *J* = 244.7 Hz, 1 C), 152.99 (s, 1 C), 150.15 (s, 2 C), 141.74 (s, 1 C), 136.53 (s, 1 C), 133.09 (s, 1 C), 128.12 (s, 1 C), 123.47 (d, *J* = 3.7 Hz, 2 C), 123.31 (d, *J* = 2.7 Hz, 1 C), 123.21 (s, 1 C), 118.56 (s, 1 C), 116.20 (s, 1 C), 104.68 (br d, *J* = 28.4 Hz, 1 C), 25.96 (s, 1 C), 9.99 (s, 2 C). HRMS (ESI-TOF) m/z: calcd for $C_{18}H_{13}FN_4O$ $[M+H]^+$, 321.1152; found, 321.1155.

5-cyclopropyl-8-fluoro-7-(pyridin-4-yl)-[1,2,4]triazolo[1,5-a]quinoxalin-4(5H)-one (3f). The trifluoroacetate salt of **3f** was prepared as described for **3e**, starting from compound **16b** (200 mg, 0.619 mmol). Purification under conditions similar to **3e** afforded a white powder (109 mg, 0.250 mmol; 41% yield). ^{1}H NMR (600 MHz) δ 8.87 (d, *J* = 6.5 Hz, 2H), 8.67 (s, 1H), 8.18 (d, *J* = 6.8 Hz, 1H), 8.14 (d, *J* = 10.1 Hz, 1H), 7.95 (d, *J* = 5.1 Hz, 2H), 3.07-3.26 (m, 1H), 1.22-1.42 (m, 2H), 0.83-1.06 (m, 2H). ^{13}C NMR (151 MHz) +TFA δ ppm 158.69 (q, *J* = 36.7 Hz, 1 C), 155.46 (d, *J* = 247.4 Hz, 1 C), 154.30 (s, 1 C), 153.11 (s, 1 C), 148.06 (br s, 2 C), 145.13 (br s, 1 C), 144.90 (s, 1 C), 128.88 (d, *J* = 2.1 Hz, 1 C), 125.16 (d, *J* = 3.2 Hz, 2 C), 124.73 (d, *J* = 11.1 Hz, 1 C), 124.48 (d, *J* = 14.0 Hz, 1 C), 119.65 (d, *J* = 2.6 Hz, 1 C), 116.05 (q, *J* = 291.4 Hz, 1 C), 104.34 (d, *J* = 29.1 Hz, 1 C), 26.93 (s, 1 C), 10.40 (s, 2 C). HRMS (ESI-TOF) m/z: calcd for $C_{17}H_{12}FN_5O$ $[M+H]^+$, 322.1104; found, 322.1111.

5-cyclopropyl-8-fluoro-7-(pyridin-4-yl)imidazo[1,5-a]quinoxalin-4(5H)-one (3g). The trifluoroacetate salt of **3g** was prepared as described for **3e**, starting from compound **16c** (200 mg, 0.619 mmol). Purification under conditions similar to **3e** afforded a white powder (110 mg, 0.270 mmol, 41%). ^{1}H NMR (600 MHz) δ 9.10 (d, *J* = 0.7 Hz, 1H), 8.84-8.97 (m, 2H), 8.46 (d, *J* = 11.2 Hz, 1H), 8.01-8.11 (m, 3H), 7.90 (d, *J* = 0.7 Hz, 1H), 2.84-3.24 (m, 1H), 1.07-1.41 (m, 2H), 0.58-1.01 (m, 2H). ^{13}C NMR (151 MHz) +TFA δ ppm 158.75 (q, *J* = 37.1 Hz, 1 C), 155.17 (s, 1 C), 155.36 (d, *J* = 246.4 Hz, 1 C), 146.64 (br s, 2 C), 134.27 (s, 1 C), 131.80 (s, 1 C), 128.99 (d, *J* = 2.4 Hz, 1 C), 125.53 (d, *J* = 3.7 Hz, 2 C), 123.62 (d, *J* = 11.4 Hz, 1 C), 123.21 (s, 1 C), 122.75 - 122.88 (m, 1 C), 122.67 - 122.97 (m, 1 C), 119.48 (d, *J* = 2.7 Hz, 1 C), 115.89 (q, *J* = 290.4 Hz, 1 C), 105.27 (d, *J* = 29.4 Hz, 1 C), 25.90 (s, 1 C), 10.40 (s, 2 C). HRMS (ESI-TOF) m/z: calcd for $C_{18}H_{13}FN_4O$ $[M+H]^+$, 321.1152; found, 321.1163.

5-cyclopropyl-8-fluoro-7-(pyridin-3-yl)imidazo[1,2-a]quinoxalin-4(5H)-one (3h). Pyridin-3-ylboronic acid (125.92 mg, 1.02 mmol), cesium carbonate (546.18 mg, 1.68 mmol) and tetrakis(triphenylphosphine)palladium(0) (53.81 mg, 0.047 mmol) were added, under an

argon atmosphere, to a solution of **16a** (300 mg, 0.931 mmol) in a mixture of 1,4-dioxane (7.5 mL) and water (3 mL). The resulting mixture was stirred for 4 h at 100°C and then concentrated. The residue was dissolved in a mixture of water/DCM. The organic layer was separated, dried over $MgSO_4$ and then concentrated under vacuum. The residue was purified by silica gel flash column chromatography using a gradient DCM to DCM 95/5 MeOH to afford **3h** as a white powder (112 mg, 349.88 µmol; 38% yield). ^{1}H NMR (500 MHz) δ 8.87 (s, 1 H), 8.68 (dd, *J* = 4.9, 1.50 Hz, 1 H), 8.54 (s, 1H), 8.33 (d, *J* = 11.0 Hz, 1 H), 8.09 (dd, *J* = 8.0, 1.50 Hz, 1 H), 8.02 (d, *J* = 7.1 Hz, 1 H), 7.61 (s, 1 H), 7.59 (dd, *J* = 8.0, 4.9 Hz, 1 H), 3.06 - 3.12 (m, 1 H), 1.27 - 1.37 (m, 2 H), 0.83 - 0.91 (m, 2 H). ^{13}C NMR (126 MHz) δ ppm 154.71 (d, *J* = 242.9 Hz, 1 C), 153.00 (s, 1 C), 149.23 (s, 1 C), 149.20 (br s, 1 C), 136.46 (d, *J* = 6.4 Hz, 1 C), 136.45 (s, 1 C), 133.04 (s, 1 C), 130.23 (s, 1 C), 128.06 (d, *J* = 2.7 Hz, 1 C), 123.80 (s, 1 C), 123.01 (d, *J* = 14.7 Hz, 1 C), 122.72 (d, *J* = 11.0 Hz, 1 C), 118.76 (d, *J* = 3.7 Hz, 1 C), 116.15 (s, 1 C), 104.52 (d, *J* = 29.3 Hz, 1 C), 25.98 (s, 1 C), 10.02 (s, 2 C). HRMS (ESI-TOF) m/z: calcd for $C_{18}H_{13}FN_4O$ $[M+H]^+$, 321.1152; found, 321.1141.

5-cyclopropyl-8-fluoro-7-(pyridin-2-yl)imidazo[1,2-a]quinoxalin-4(5H)-one (3i). 2-(tributylstannyl)pyridine (0.429 ml, 1.07 mmol) and tetrakis(triphenylphosphine) palladium(0) (53.81 mg, 0.047 mmol) were added, under argon, to **16a** (300 mg, 0.931 mmol) in 1,4-dioxane (1 ml). The resulting mixture was heated under micro-wave irradiation for 2 h, at 150°C and then the reaction mixture was partitioned between DCM and a 5% KF aqueous solution. The organic layer was successively separated, dried over $MgSO_4$ and concentrated. The residue was purified by silica gel flash column chromatography using a gradient DCM to DCM/MeOH 9/1 to afford **3i** as a white powder (210 mg, 655.59 µmol; 70% yield). ^{1}H NMR (500 MHz) δ 8.76 - 8.86 (m, 1 H), 8.54 (d, *J* = 1.2 Hz, 1 H), 8.48 (d, *J* = 7.1 Hz, 1 H), 8.30 (d, *J* = 11.5 Hz, 1 H), 7.93 - 8.02 (m, 1 H), 7.87 - 7.93 (m, 1 H), 7.61 (d, *J* = 1.2 Hz, 1 H), 7.43 - 7.51 (m, 1 H), 3.03 - 3.17 (m, 1 H), 1.19 - 1.41 (m, 2 H), 0.77 - 0.98 (m, 2 H). ^{13}C NMR (126

MHz) δ ppm 155.45 (d, *J* = 245.6 Hz, 1 C), 153.09 (s, 1 C), 151.42 (d, *J* = 2.7 Hz, 1 C), 150.02 (s, 1 C), 137.17 (s, 1 C), 136.55 (s, 1 C), 133.05 (s, 1 C), 127.83 (s, 1 C), 124.67 (d, *J* = 12.8 Hz, 1 C), 124.30 (d, *J* = 10.1 Hz, 1 C), 123.30 (s, 1 C), 123.13 (d, *J* = 11.0 Hz, 1 C), 118.77 (d, *J* = 3.7 Hz, 1 C), 116.17 (s, 1 C), 104.63 (d, *J* = 30.2 Hz, 1 C), 25.80 (s, 1 C), 9.94 (s, 2 C). HRMS (ESI-TOF) m/z: calcd for $C_{18}H_{13}FN_4O$ $[M+H]^+$, 321.1152; found, 321.1160.

(5E)-1-methyl-4-(methylamino)-5-[(pyridin-4-yl)methylidene]-1,5-dihydro-2H-indeno [1,2-b]pyridin-2-one (4). To a solution of **28** (1.13 g, 5 mmol) in DMSO (20 ml) were added piperidine (1 ml, 10 mmol) and 4-pyridinecarboxaldehyde (0.95 ml, 10 mmol). The reaction mixture was heated at 90°C for 2 h and then at 100°C for 16 h. The resulting mixture was poured into water (200 ml). The precipitate was filtered and washed successively with water (2x20 ml) and ethanol (2x5 ml). Silica gel column chromatography of the residue using a stepwise gradient of methanol (0 to 5%) in dichloromethane followed by trituration in diisopropyl ether afforded the title compound **(4)** as a yellow solid (0.95 g, 60%). ^{1}H NMR (600 MHz) δ 8.66-9.39 (m, 2H), 8.03 (d, *J* = 8.1 Hz, 1H), 7.70 (s, 1H), 7.47 (m, 2H), 7.33-7.39 (m, 1H), 7.15 (d, *J* = 4.0 Hz, 2H), 6.38 (q, *J* = 5.3 Hz, 1H), 5.41 (s, 1H), 3.86 (s, 3H), 2.80 (d, *J* = 5.3 Hz, 3H). ^{13}C NMR (151 MHz) δ 162.9, 153.1, 150.5, 147.5, 145.8, 137.1, 135.3, 135.1, 129.1, 128.3, 126.4, 124.3, 124.2, 123.7, 107.4, 90.6, 31.2, 30.3. HRMS (ESI-TOF) m/z: calcd for $C_{20}H_{17}N_3O$ [M+H]+, 316.1450; found, 316.1462.

5-Methyl-5,10-dihydro-4H-imidazo[1,2-a]indeno[1,2-e]pyrazin-4-one (6). To a solution of ethyl 1-(1-oxo-2,3-dihydro-1H-inden-2-yl)-1H-imidazole-2-carboxylate **5**[14] (100 g, 370 mmol) in acetic acid (300 ml) and absolute ethanol (260 ml) was added dropwise methyl amine (33 wt. % in absolute ethanol) (344 ml, 3700 mmol) over 10 min. The resulting mixture was refluxed for 5 h and then cooled using an ice bath. The insoluble material was filtered, washed with water (3x200 ml) and finally dried at 50°C under vacuum to afford the title compound **6** as a grey solid (77.9 g, 89%). ^{1}H NMR (600 MHz) δ 8.10 (d, *J* = 1.1 Hz, 1H),

8.01 (d, *J* = 7.9 Hz, 1H), 7.78 (d, *J* = 1.1 Hz, 1H), 7.65 (d, *J* = 7.3 Hz, 1H), 7.45 (dt, *J* = 1.3, 7.6 Hz, 1H), 7.35-7.42 (m, 1H), 4.08 (s, 2H), 3.97 (s, 3H). ^{13}C NMR (151 MHz) δ 152.8, 139.6, 136.2, 135.2, 131.2, 130.1, 127.8, 127.0, 125.8, 121.9, 119.7, 116.4, 32.6, 31.4. HRMS (ESI-TOF) m/z: calcd for $C_{14}H_{11}N_3O$ $[M+H]^+$, 238.0980; found, 238.0972.

Ethyl 1-[(4-nitrophenyl)methyl]imidazole-2-carboxylate (9). To a solution of 4-nitrobenzyl bromide (6.63 g, 30.7 mmol, 1 equiv.) and ethyl imidazole-2-carboxylate **8** (4.30 g, 30.7 mmol, 1 equiv.) in MeCN (100 mL) was added K_2CO_3 (4.24 g, 30.7 mmol, 1.00 equiv.). The reaction mixture was stirred overnight at RT. After 16 h, LC-MS analysis indicated complete conversion. EtOAc (500 mL), water (50 ml) and a saturated aqueous. $NaHCO_3$-solution (50 mL) were added. The layers were separated and the organic layer was successively washed with brine (50 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo* to yield **9** (8.46 g, 30.7 mmol, 100%) as a yellow solid which was pure enough to be used for the next reaction. To obtain complete analytical data, a small sample was purified via HPLC (RP-18 column, 15 minutes gradient 15-65% acetonitrile, retention time 7.0 minutes). ^{1}H NMR (500 MHz): δ 8.22-8.20 (m, 2 H), 7.74 (s, 1 H), 7.39-7.35 (m, 2 H), 7.30 (s, 1 H), 5.77 (s, 2 H), 4.24 (q, ^3J = 7.1 Hz, 2 H), 1.22 (t, ^{3} *J* = 7.1 Hz, 3 H). ^{13}C NMR (126 MHz): δ 157.85, 146.86, 144.91, 135.37, 128.55, 127.85, 126.96, 123.79, 61.07, 50.42, 13.93. HRMS (ESI-TOF) m/z: calcd for $C_{13}H_{14}N_3O_4$ $[M+H]^+$, 276.0979; found, 276.0977.

7-methyl-5-(4-nitrophenyl)imidazo[1,2-a]pyrazin-8-one (11). To a solution, heated to 110°C, of crude compound **9** (8.46 g, 30.7 mmol, 1 equiv.) in dry toluene (150 mL) was added in 1 mL portions, over a period of 5 h, the Bredereck's reagent ($HC(NMe_2)_2O$*t*Bu; in total 30 mL). After addition, the reaction mixture was refluxed overnight. To complete the reaction, additional Bredereck's reagent (10 mL in 1 mL portions) was then added over a period of 1 h and the reaction was refluxed for 5 more hours until complete conversion was confirmed via LC-MS. All volatiles were removed *in vacuo* and the crude residue was

dissolved in EtOAc (500 mL). The resulting solution was washed with a saturated aqueous $NaHCO_3$-solution (50 mL) and then brine (50 mL). It was then dried over $MgSO_4$, filtered and concentrated *in vacuo* to afford crude compound **10**. To this material dissolved in EtOH (100 mL) were added methylamine (20 mL, 40% solution in EtOH) and glacial acetic acid (20 mL). The resulting mixture was heated to 70°C and stirred overnight. The reaction mixture was then poured into a mixture of EtOAc (600 mL) and water (100 mL). The resulting precipitate was collected via filtration and washed with water (30 mL). The layers of the filtrate were separated and the organic layer was successively washed with a saturated aqueous. $NaHCO_3$-solution (50 mL), with brine (50 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was combined with the collected precipitate. EtOH (100 mL) was added and the suspension was refluxed for 30 min. After cooling to 0°C and standing overnight, the resulting precipitate was filtered, washed with EtOH (30 mL) and dried *in vacuo* to yield **11** (5.89 g, 21.8 mmol, 71%) as a pale orange solid. ^{1}H NMR (500 MHz): δ 8.40-8.37 (m, 2 H), 7.94-7.91 (m, 2 H), 7.79 (d, ^3J = 1.0 Hz, 1 H), 7.55 (d, ^3J = 1.0 Hz, 1 H), 7.50 (s, 1 H), 3.52 (s, 3 H). ^{13}C NMR (126 MHz): δ 152.67, 147.36, 137.23, 136.96, 132.70, 129.37, 124.27, 122.42, 116.90, 115.73, 35.19. HRMS (ESI-TOF) m/z: calcd for $C_{13}H_{11}N_4O_3$ $[M+H]^+$, 271.0826; found, 271.0823.

5-(4-aminophenyl)-7-methyl-imidazo[1,2-a]pyrazin-8-one (12). To a suspension of **11** (5.89 g, 21.8 mmol, 1 equiv.) in MeOH was added Pd/C (10% palladium; 400 mg, 0.38 mmol, 0.02 mol%) and formic acid under an argon atmosphere. The reaction mixture was stirred at 65°C under reflux for 4 h until LC-MS analysis indicated complete conversion. The reaction mixture was cooled to room temperature, filtered through a pad of celite® and concentrated. HCl (5 mL) was added (to avoid quantitative formation of the corresponding formamide upon concentration). The reaction mixture was concentrated *in vacuo* and the remaining brownish solid was suspended in EtOH (20 mL), heated to 60°C for 10 minutes and cooled to 0°C. The

precipitate was filtered to yield the hydrochloride salt of **12** (6.42 g, 20.5 mmol; 94% yield) as a pale yellow solid. ^{1}H NMR (500 MHz): δ 7.99 (d, ^{3}J = 1.4 Hz, 1 H), 7.94 (d, ^{3}J = 1.4 Hz, 1 H), 7.64-7.60 (m, 3 H), 7.42-7.40 (m, 2 H), 3.57 (s, 1 H);. ^{13}C NMR (126 MHz): δ 150.51, 137.53, 134.49, 129.98, 126.96, 125.57, 123.14, 121.80, 118.15, 116.69, 35.43. HRMS (ESI-TOF) m/z: calcd for $C_{13}H_{13}N_4O$ $[M+H]^+$, 241.1084; found, 241.1083.

5-bromo-N-cyclopropyl-2,4-difluoroaniline (13). To a solution of 5-bromo-2,4-difluoroaniline (10 g, 48.08 mmol) in MeOH (44 ml), were added at room temperature acetic acid (8.26 ml, 144.23 mmol) and (1-ethoxycyclopropoxy)trimethylsilane (10.89 g, 62.50 mmol). The mixture was refluxed for 5 h and then concentrated in vacuo. The crude residue was used as such without further purification in the next step.

5-bromo-2,4-difluoro-N-(1-methoxycyclopropyl)aniline (14). A solution of sodium borohydride (2.76 g, 72.12 mmol) and boron trifluoride ethyl etherate (10.24 g, 72.12 mmol), in dry THF (90 ml) was stirred 1 h, at 5 °C, under nitrogen. Crude compound 13, in THF (90mL), was added at 5 °C. The resulting mixture was then stirred for 2 h at room temperature and for 1 h under reflux. The reaction was quenched by addition of water and extracted with ethyl acetate. The organic layer was successively washed with an aqueous solution of $NaHCO_3$, water and brine, dried with $MgSO_4$ and concentrated. The residue was purified by silica gel flash column chromatography, using a gradient heptane to ethyl acetate, to afford **14** as a colorless oil (7.6 g, 30.64 mmol; 64% yield). ^{1}H NMR (600 MHz) δ 7.28 (dd, J = 11.6, 8.7 Hz, 1 H), 7.11 (dd, J = 9, 7.1 Hz, 1 H), 6.14 (s, 1 H), 2.31 - 2.37 (m, 1 H), 0.64 - 0.78 (m, 2 H), 0.36 - 0.47 (m, 2H). ^{13}C NMR (151 MHz) δ ppm 150.15 (dd, J = 26.0, 10.9 Hz, 1 C), 148.57 (dd, J = 17.5, 10.9 Hz, 1 C), 136.01 (dd, J = 12.8, 2.5 Hz, 1 C), 115.01 (d, J = 5.3 Hz, 1 C), 105.03 (dd, J = 27.3, 23.8 Hz, 1 C), 102.91 (dd, J = 21.2, 4.0 Hz, 1 C), 24.82 (s, 1 C), 7.12 (s, 2 C). HRMS (ESI-TOF) m/z: calcd for $C_9H_8BrF_2N$ $[M+H]^+$, 247.9886; found, 247.9881.

N-(5-bromo-2,4-difluorophenyl)-N-cyclopropyl-1H-imidazole-2-carboxamide (15a). To a suspension of compound **14** (3.6 g, 14.51 mmol) in toluene (75ml), were added, at room temperature and under a nitrogen atmosphere, triethylamine (2.03 mL, 14.51 mmol), ethyl 1H-imidazole-2-carboxylate (2.03 g, 14.51 mmol) and trimethylaluminum (2M in toluene; 7.26 ml, 14.51 mmol). The mixture was stirred under reflux for 2 h and then concentrated. The residue was suspended in a solution of water/ethyl acetate and stirred for 30 minutes at room temperature before filtration and washing of the insoluble material with ethyl acetate. The combined filtrates were successively washed three times with an aqueous solution of $NaHCO_3$, water and brine, dried on $MgSO_4$ and concentrated. The residue was purified by silica gel flash column chromatography using a gradient of heptane to ethyl acetate to afford **15a** as a white powder (4.97 g, 3.54 mmol, 24% yield). ^{1}H NMR (600 MHz) δ 12.99 (br s, 1 H), 7.84 (t, *J* = 7.8 Hz, 1 H), 7.56 (t, *J* = 9.4 Hz, 1 H), 7.20 (br s, 1 H), 6.88 (br s, 1 H), 3.31 - 3.41 (m, 1 H), 0.72 - 0.84 (m, 2 H), 0.43 - 0.58 (m, 2 H). ^{13}C NMR (151 MHz) δ ppm 160.67 (s, 1 C), 158.32 - 159.60 (m, 1 C), 156.51 - 157.87 (m, 1 C), 140.55 (s, 1 C), 134.74 (s, 1 C), 129.83 (br s, 1 C), 128.41 (br d, *J* = 11.9 Hz, 1 C), 119.95 (br s, 1 C), 105.93 (t, *J* = 27.0 Hz, 1 C), 102.83 (dd, *J* = 22.0, 4.0 Hz, 1 C), 32.73 (br s, 1 C), 7.88 (br s, 2 C). HRMS (ESI-TOF) m/z: calcd for $C_{13}H_{10}BrF_2N_3O$ $[M+H]^+$, 342.0054; found, 342.0045.

N-(5-bromo-2,4-difluorophenyl)-N-cyclopropyl-1H-1,2,4-triazole-5-carboxamide (15b). **15b** was prepared as **15a**, starting from compound **14** (1.00 g, 4.03 mmol). Purification under conditions similar to **15a** afforded a white powder (200 mg, 0.583 mmol, 15% yield). ^{1}H NMR (600 MHz) δ 14.45 (br s, 1H), 8.42 (br s, 1H), 7.87 (br s, 1H), 7.59 (br t, *J* = 9.0 Hz, 1H), 3.30 (br s, 1H), 0.75 (br s, 2H), 0.50 (br s, 2H). ^{1}H NMR (600 MHz) +TFA δ 8.43 (br s, 1H), 7.86 (br s, 1H), 7.57 (br t, *J* = 9.0 Hz, 1H), 3.29 (br s, 1H), 0.74 (br s, 2H), 0.50 (br s, 2H). ^{13}C NMR (151 MHz) δ ppm 157.16 (br s, 1 C), 157.08 (br s, 1 C), 154.92 (br s, 1 C), 148.85 (br s, 1 C), 145.70 (br s, 1 C), 134.67 (br s, 1 C), 127.56 (br s, 1 C), 106.08 (br t, *J* =

27.0 Hz, 1 C), 103.04 (br d, *J* = 20.9 Hz, 1 C), 32.40 (br s, 1 C), 7.73 (br s, 2 C). HRMS (ESI-TOF) m/z: calcd for $C_{12}H_9BrF_2N_4O$ $[M+H]^+$, 343.0006; found, 342.9999.

N-(5-bromo-2,4-difluorophenyl)-N-cyclopropyl-1H-imidazole-2-carboxamide (15c). 15c was prepared as **15a**, starting from compound **14** (2 g, 8.06 mmol). Purification under conditions similar to **15a** afforded a white powder (718 mg, 2.1 mmol; 26% yield). ^{1}H NMR (500 MHz)TFAδ 9.06 (d, *J* = 0.8 Hz, 1H), 7.91 (t, *J* = 7.7 Hz, 1H), 7.68 (br s, 1H), 7.56 (dd, *J* = 8.8, 9.9 Hz, 1H), 3.27-3.59 (m, 1H), 0.85-1.04 (m, 2H), 0.51-0.76 (m, 2H). ^{13}C NMR (126 MHz): no clear signals likely due to the presence of several tautomers and/or conformations. HRMS (ESI-TOF) m/z: calcd for $C_{13}H_{10}BrF_2N_3O$ $[M+H]^+$, 342.0054; found, 342.0049.

7-bromo-5-cyclopropyl-8-fluoroimidazo[1,2-a]quinoxalin-4(5H)-on (16a). To a solution of **15a** (30 g, 87.68 mmol) in DMF (200 mL), was added, at room temperature, 2,3,4,6,7,8,9,10-octahydropyrimido[1,2-a]azepine (40.05 g, 263.05 mmol). The resulting solution was stirred 3 h at 120°C and then concentrated under reduced pressure. The residue was suspended in a solution of water/ethyl acetate and the mixture was then stirred for 30 min at room temperature before filtration and washing of the insoluble material with ethyl acetate. This afforded **16a** as a white powder (22 g, 68.29 mmol, 78% yield). ^{1}H NMR (600 MHz) δ 8.47 (s, 1 H), 8.34 (d, *J* = 9.2 Hz, 1 H), 8.10 (d, *J* = 6.5 Hz, 1 H), 7.58 (s, 1 H), 2.93 - 3.10 (m, 1 H), 1.19 - 1.44 (m, 2 H), 0.70 - 0.99 (m, 2 H). ^{13}C NMR (151 MHz) δ ppm 154.28 (d, *J* = 240.8 Hz, 1 C), 153.34 (s, 1 C), 136.83 (s, 1 C), 133.52 (s, 1 C), 129.29 (d, *J* = 2.4 Hz, 1 C), 122.93 (d, *J* = 9.8 Hz, 1 C), 121.60 (s, 1 C), 116.67 (s, 1 C), 105.78 (d, *J* = 22.3 Hz, 1 C), 105.29 (d, *J* = 28.6 Hz, 1 C), 26.46 (s, 1 C), 10.40 (s, 2 C). HRMS (ESI-TOF) m/z: calcd for $C_{13}H_9BrFN_3O$ $[M+H]^+$, 321.9991; found, 321.9989.

7-bromo-5-cyclopropyl-8-fluoro-[1,2,4]triazolo[1,5-a]quinoxalin-4(5H)-one (16b). 16b was prepared as **16a**, starting from compound **15b** (750 mg, 2.19 mmol), to afford a white

powder (420 mg, 1.3 mmol, 60% yield). ^{1}H NMR (600 MHz) δ 8.63 (s, 1H), 8.22 (d, *J* = 6.2 Hz, 1H), 8.11 (d, *J* = 8.2 Hz, 1H), 3.04-3.13 (m, 1H), 1.26-1.39 (m, 2H), 0.79-0.97 (m, 2H). ^{13}C NMR (151 MHz) δ ppm 153.61 (s, 1 C), 154.04 (d, *J* = 242.7 Hz, 1 C), 152.50 (s, 1 C), 144.09 (s, 1 C), 128.87 (d, *J* = 2.4 Hz, 1 C), 122.96 (d, *J* = 10.1 Hz, 1 C), 121.53 (s, 1 C), 107.08 (d, *J* = 22.3 Hz, 1 C), 103.79 (d, *J* = 28.9 Hz, 1 C), 26.39 (s, 1 C), 9.80 (s, 2 C). HRMS (ESI-TOF) m/z: calcd for $C_{12}H_8BrFN_4O$ $[M+H]^+$, 322.9944; found, 322.9945.

7-bromo-5-cyclopropyl-8-fluoroimidazo[1,5-a]quinoxalin-4(5H)-one (16c). 16c was prepared as **16a**, starting from compound **15c** (718 mg, 2.10 mmol), to afford a white powder (495 mg, 1.54 mmol, 73% yield). ^{1}H NMR (600 MHz) δ 8.93-9.04 (m, 1H), 8.41 (dd, *J* = 1.0, 9.24 Hz, 1H), 8.04 (d, *J* = 6.5 Hz, 1H), 7.84 (s, 1H), 2.88-2.99 (m, 1H), 1.18-1.32 (m, 2H), 0.66-0.85 (m, 2H). ^{13}C NMR (151 MHz) δ ppm 155.12 (s, 1 C), 154.25 (d, *J* = 240.3 Hz, 1 C), 134.07 (s, 1 C), 131.92 (s, 1 C), 129.45 (d, *J* = 2.6 Hz, 1 C), 122.59 (s, 1 C), 121.91 (d, *J* = 10.1 Hz, 1 C), 121.76 (s, 1 C), 105.93 (d, *J* = 22.3 Hz, 1 C), 105.16 (d, *J* = 29.1 Hz, 1 C), 25.82 (s, 1 C), 10.29 (s, 2 C). HRMS (ESI-TOF) m/z: calcd for $C_{13}H_9BrFN_3O$ $[M+H]^+$, 321.9991; found, 321.9987.

tert-butyl(S)-(1-(5-cyclopropyl-8-fluoro-4-oxo-4,5-dihydroimidazo[1,2-a]quinoxalin-7-yl) pyrrolidin-3-yl) carbamate (17a). To a suspension of **16a** in toluene (12 mL). were added, under argon, *tert*-butyl (S)-pyrrolidin-3-ylcarbamate (450.98 mg, 2.42 mmol), cesium carbonate (1.2 g, 3.63 mmol), (R)-(+)-2,2'-bis(diphenylphosphino)-1,1'-binaphthyl (155.44 mg, 0.242 mmol) and palladium(II) acetate (55.47 mg, 0.242 mmol). The resulting mixture was heated at 150°C, for 5 h, under microwave irradiation and then concentrated. The residue was purified by silica gel flash column chromatography using a gradient dichloromethane to dichloromethane 95/5 methanol to afford **17a** as a white powder (147 mg, 0.344 mmol, 28%). ^{1}H NMR (500 MHz): 8.34 (s, 1H), 8.02 (d, *J* = 13.8 Hz, 1H), 7.50 (s, 1H), 7.20 (br s, 1H), 7.03 (d, *J* = 8.4 Hz, 1H), 4.15 (br s, 1H), 3.66-3.68 (m, 1H), 3.57 (dt, *J* = 13.9, 7.2 Hz, 1H),

3.47(dt, *J* = 13.9, 7.3 Hz, 1H), 3.30 (br s, 1H), 2.96-3.00 (m, 1H), 2.12-2.19(m, 1H), 1.87-1.94 (m, 1H), 1.41 (s, 9H), 1.30 (br s, 2H), 0.83 (br s, 2H). ^{13}C NMR (126 MHz) 154.9 (s, 1 C), 153.0 (s, 1 C), 146.5 (d, *J* = 239 Hz, 1C), 135.2 (s, 1 C), 134.8 (d, *J* = 10 Hz, 1C), 132.1 (s, 1 C), 127.6 (s, 1 C), 114.8 (s, 1 C), 111.9 (d, *J* = 10 Hz, 1C), 104.1 (d, *J* = 28Hz, 1C),102.3 (d, *J* = 5 Hz, 1C), 77.5 (s, 1 C), 54.8 (s, 1 C), 49.4 (s, 1 C), 47.5 (s, 1 C), 30.1 (s, 1 C), 27.9 (s, 3 C), 25.3 (s, 1 C), 9.6 (s, 2 C). HRMS (ESI-TOF) m/z: calcd for $C_{22}H_{26}FN_5O_3$ $[M+H]^+$, 428.2098; found, 428.2088.

tert-butyl(S)-(1-(5-cyclopropyl-8-fluoro-4-oxo-4,5-dihydro-[1,2,4]triazolo[1,5-a] quinoxalin-7-yl)pyrrolidin-3-yl)carbamate (17b). 17b was prepared as **17a**, starting from compound **16b** (200 mg, 0.619 mmol). Purification under conditions similar to **17a** afforded a white powder (109 mg, 0.254 mmol, 41% yield). ^{1}H NMR (600 MHz) δ 8.49 (s, 1H), 7.77 (d, *J* = 13.1 Hz, 1H), 7.22 (br d, *J* = 6.0 Hz, 1H), 7.04 (d, *J* = 8.1 Hz, 1H), 4.04-4.26 (m, 1H), 3.31-3.77 (m, 4H), 2.93-3.10 (m, 1H), 1.80-2.22 (m, 2H), 1.40 (s, 9H), 1.24-1.34 (m, 2H), 0.80-0.93 (m, 2H). ^{13}C NMR (151 MHz) δ ppm 155.73 (br s, 1 C), 153.41 (s, 1 C), 153.27 (s, 1 C), 147.64 (d, *J* = 240.5 Hz, 1 C), 142.81 (s, 1 C), 136.82 (d, *J* = 10.3 Hz, 1 C), 128.81 (s, 1 C), 113.33 (d, *J* = 10.6 Hz, 1 C), 103.94 (d, *J* = 27.6 Hz, 1 C), 102.83 (d, *J* = 5.6 Hz, 1 C), 78.37 (br s, 1 C), 55.62 (br s, 1 C), 50.29 (br s, 1 C), 48.42 (br d, *J* = 4.5 Hz, 1 C), 30.96 (br s, 1 C), 28.71 (s, 3 C), 26.57 (s, 1 C), 10.38 (s, 2 C). HRMS (ESI-TOF) m/z: calcd for $C_{21}H_{25}FN_6O_3$ $[M+H]^+$, 429.2050; found, 429.2057.

tert-butyl(S)-(1-(5-cyclopropyl-8-fluoro-4-oxo-4,5-dihydroimidazo[1,5-a]quinoxalin-7-yl) pyrrolidin-3-yl) carbamate (17c). 17c was prepared as **17a**, starting from compound **16c** (200 mg, 0.621 mmol). Purification under conditions similar to **17a** afforded a white powder (59 mg, 0.138 mmol, 22% yield). ^{1}H NMR (600 MHz) δ 8.81 (d, *J* = 0.8 Hz, 1H), 8.07 (d, *J* = 14.0 Hz, 1H), 7.75 (d, *J* = 0.8 Hz, 1H), 7.20 (br d, *J* = 6.0 Hz, 1H), 6.98 (d, *J* = 8.5 Hz, 1H), 3.94-4.37 (m, 1H), 3.65 (ddd, *J* = 2.6, 6.6, 9.5 Hz, 1H), 3.52-3.58 (m, 1H), 3.41-3.47 (m, 1H),

3.25-3.30 (m, 1H), 2.86-2.92 (m, 1H), 2.06-2.20 (m, 1H), 1.81-1.96 (m, 1H), 1.32-1.47 (m, 9H), 1.18-1.30 (m, 2H), 0.70-0.89 (m, 2H). ^{13}C NMR (151 MHz) δ ppm 155.74 (br s, 1 C), 155.62 (s, 1 C), 147.12 (d, *J* = 238.2 Hz, 1 C), 135.76 (br d, *J* = 10.3 Hz, 1 C), 132.60 (s, 1 C), 131.20 (s, 1 C), 128.52 (s, 1 C), 122.22 (s, 1 C), 111.53 (d, *J* = 10.1 Hz, 1 C), 104.84 (br d, *J* = 27.6 Hz, 1 C), 103.38 (br d, *J* = 5.3 Hz, 1 C), 78.33 (br s, 1 C), 55.62 (br s, 1 C), 50.26 (br s, 1 C), 48.33 (br s, 1 C), 30.94 (br s, 1 C), 28.71 (s, 3 C), 25.52 (s, 1 C), 10.37 (s, 2 C). HRMS (ESI-TOF) m/z: calcd for $C_{22}H_{26}FN_5O_3$ $[M+H]^+$, 428.2098; found, 428.2094.

Methyl 4-amino-1-benzyl-1H-imidazole-5-carboxylate (18). A mixture of cyanamide (4 g, 95.15 mmol) and triethoxyethane (30 ml) was refluxed for 2 h and then distilled under reduce pressure to afford a colorless oil that was then dissolved in Et_2O (8 mL). Following addition of ethyl 2-(benzylamino)acetate (15.53 ml, 82.80 mmol), the resulting mixture was stirred for 1 h at room temperature and then concentrated. The residue was dissolved in MeOH (80 mL) and sodium methoxide (16 mL, 86.4 mmol) was added. This mixture was stirred for 1 h at room temperature and then filtered. The insoluble material was washed with cold MeOH to afford **18** as a white powder (12 g, 51.89 mmol; 55% yield). ^{1}H NMR (600 MHz) δ 7.69 (s, 1 H), 7.34 - 7.29 (m, 2 H), 7.27 - 7.22 (m, 1 H), 7.16 - 7.11 (m, 2 H), 5.69 (s, 2 H), 5.30 (s, 2 H), 3.64 (s, 3 H). ^{13}C NMR (151 MHz) δ 161.20, 156.85, 141.37, 138.54, 128.91, 127.82, 127.24, 100.04, 50.68, 49.70. HRMS (ESI-TOF) m/z: calcd for $C_{12}H_{13}N_3O_2$ $[M+H]^+$, 232.1086; found, 232.1078.

Methyl 1-benzyl-4-iodo-1H-imidazole-5-carboxylate (19). To a solution of **18** (5 g, 21.62 mmol) in water (6 mL) were added, at 0°C, hydrogen chloride (6 M in water; 39.64 ml, 237.84 mmol) and sodium nitrite (3.73 g, 54.05 mmol). The resulting mixture was stirred at 0°C for 1 h and then refluxed for 1 h. A solution of potassium iodide (14.36 g, 86.49 mmol) in water (5 mL) was then added at room temperature. The ensuing mixture was refluxed for 1 h and then stirred at room temperature overnight. The insoluble fraction was filtered and

washed with water to afford **19** (3.92 g, 11.45 mmol; 53% yield), as a white powder. ^{1}H NMR (600 MHz) δ 8.14 (s, 1H), 7.30-7.36 (m, 2H), 7.25-7.30 (m, 1H), 7.10-7.16 (m, 2H), 5.52 (s, 2H), 3.72 (s, 3H). ^{13}C NMR (151 MHz) δ 159.7, 145.3, 137.5, 129.1, 128.2, 127.3, 124.1, 95.9, 51.9, 50.8. HRMS (ESI-TOF) m/z: calcd for $C_{12}H_{11}IN_2O_2$ $[M+H]^+$, 342.9943; found, 342.9938.

Methyl 1-benzyl-4-(4-bromo-2,5-difluorophenyl)-1H-imidazole-5-carboxylate (20). Compound **19** (86.69 mg, 0.253 mmol), potassium carbonate (93.38 mg, 0.676 mmol) and tetrakis(triphenylphosphine)palladium(0) (14.64 mg, 0.013 mmol) were added, under argon, to (4-bromo-2,5-difluorophenyl)boronic acid (50 mg, 0.211 mmol) in a mixture of MeOH (0.5 ml) and toluene (1 ml). This mixture was heated for 1 h, at 120°C, under microwave irradiation and then concentrated. The residue was suspended in methanol and then filtered through a 0.45 µm RC membrane. The filtrate was purified by HPLC to afford **20** as a white powder (15 mg, 0.0368 mmol; 17% yield). ^{1}H NMR (600 MHz) δ 8.28 (s, 1H), 7.76 (dd, *J* = 5.8, 9.10 Hz, 1H), 7.54 (dd, *J* = 6.2, 9.0 Hz, 1H), 7.32-7.39 (m, 2H), 7.27-7.31 (m, 1H), 7.18-7.23 (m, 2H), 5.54 (s, 2H), 3.60 (s, 3H). ^{13}C NMR (151 MHz) δ ppm 160.43 (s, 1 C), 155.63 (d, *J* = 247.4 Hz, 1 C), 154.99 (d, *J* = 242.7 Hz, 1 C), 143.02 (s, 1 C), 140.06 (s, 1 C), 137.56 (s, 1 C), 129.15 (s, 2 C), 128.23 (s, 1 C), 127.51 (s, 2 C), 124.34 (br dd, *J* = 17.4, 7.8 Hz, 1 C), 120.69 (br d, *J* = 28.6 Hz, 1 C), 120.49 (s, 1 C), 118.46 (br dd, *J* = 25.4, 2.9 Hz, 1 C), 108.55 (br dd, *J* = 23.6, 10.6 Hz, 1 C), 52.04 (s, 1 C), 50.08 (s, 1 C). HRMS (ESI-TOF) m/z: calcd for $C_{18}H_{13}BrF_2N_2O_2$ $[M+H]^+$, 407.0207; found, 407.0212.

1-benzyl-4-(4-bromo-2,5-difluorophenyl)-1H-imidazole-5-carboxylic acid (21). To a solution of **20** (650 mg, 1.60 mmol) in a mixture of THF (6 ml) and water (6 ml) was added sodium hydroxide 6 M (0.399 mL, 2.39 mmol). This mixture was stirred for 12 h at room temperature and then acidified by addition of HCl 2 M (1.195 mL, 2.39 mmol). The resulting mixture was concentrated to afford **21** as a white powder (447 mg, 1.14 mmol; 71% yield). ^{1}H

NMR (600 MHz) δ 12.58-13.42 (m, 1H), 8.20 (s, 1H), 7.74 (dd, *J* = 5.7, 9.0 Hz, 1H), 7.50 (dd, *J* = 6.2, 9.0 Hz, 1H), 7.32-7.38 (m, 2H), 7.26-7.31 (m, 1H), 7.18-7.22 (m, 2H), 5.57 (s, 2H). ^{13}C NMR (151 MHz) δ ppm 161.42 (s, 1 C), 155.71 (d, *J* = 247.4 Hz, 1 C), 154.84 (d, *J* = 241.1 Hz, 1 C), 142.53 (s, 1 C), 139.73 (s, 1 C), 137.93 (s, 1 C), 129.10 (s, 2 C), 128.12 (s, 1 C), 127.50 (s, 2 C), 124.91 (dd, *J* = 17.5, 7.9 Hz, 1 C), 121.47 (s, 1 C), 120.55 (d, *J* = 28.3 Hz, 1 C), 118.65 (dd, *J* = 25.3, 3.6 Hz, 1 C), 108.25 (dd, *J* = 23.4, 10.5 Hz, 1 C), 49.84 (s, 1 C). HRMS (ESI-TOF) m/z: calcd for $C_{17}H_{11}BrF_2N_2O_2$ $[M+H]^+$, 393.0050; found, 393.0047.

1-benzyl-4-(4-bromo-2,5-difluorophenyl)-N-cyclopropyl-1H-imidazole-5-carboxamide (22). To a solution of **21** (800 mg, 2.03 mmol) in THF (10 ml) were added N,N-diisopropylethylamine (1.06 ml, 6.10 mmol), cyclopropylamine (285.43 µl, 4.07 mmol) and HATU (0.774 g, 2.03 mmol). The resulting mixture was stirred for 12 h at room temperature. The reaction was quenched by addition of aqueous $NaHCO_3$ and the resulting mixture was extracted with ethyl acetate. The organic layer was successively separated, washed with $NaHCO_3$, water and brine, dried with $MgSO_4$ and concentrated. The residue was purified by silica gel flash column chromatography using a gradient heptane to ethyl acetate to afford **22** as a white powder (600 mg, 1.39 mmol; 68% yield). ^{1}H NMR (600 MHz) δ 8.08 - 8.11 (m, 1 H), 8.06 (s, 1 H), 7.70 (dd, *J* = 9.4, 5.9 Hz, 1 H), 7.50 (dd, *J* = 9.2, 6.2 Hz, 1 H), 7.31 - 7.36 (m, 2 H), 7.26 - 7.30 (m, 1 H), 7.21 - 7.25 (m, 2 H), 5.37 (s, 2 H), 2.54 - 2.68 (m, 1 H), 0.44 - 0.63 (m, 2 H). ^{13}C NMR (151 MHz) δ ppm 162.03 (s, 1 C), 155.26 (d, *J* = 240.3 Hz, 1 C), 154.93 (d, *J* = 247.7 Hz, 1 C), 139.86 (s, 1 C), 137.48 (s, 1 C), 133.28 (s, 1 C), 129.06 (s, 2 C), 128.31 (s, 1 C), 128.15 (s, 2 C), 126.23 (s, 1 C), 123.95 (dd, *J* = 16.8, 7.8 Hz, 1 C), 120.76 (d, *J* = 28.1 Hz, 1 C), 117.75 (dd, *J* = 25.7, 4.5 Hz, 1 C), 107.26 (dd, *J* = 23.7, 10.5 Hz, 1 C), 49.07 (s, 1 C), 22.80 (s, 1 C), 5.74 (s, 2 C). HRMS (ESI-TOF) m/z: calcd for $C_{20}H_{16}BrF_2N_3O$ $[M+H]^+$, 432.0523; found, 432.0508.

3-benzyl-7-bromo-5-cyclopropyl-8-fluoro-3H-imidazo[4,5-c]quinolin-4(5H)-one (23). To a solution of **22** (900 mg, 2.08 mmol) in DMF (1.5 mL) was added 2,3,4,6,7,8,9,10-octahydropyrimido[1,2-a]azepine (1.27g, 8.3 mmol). The resulting solution was stirred 24 h at 120°C and then concentrated. The residue was purified by silica gel flash column chromatography using a gradient DCM to DCM/methanol (98/2) to afford **23** as a white powder (671 mg, 1.63 mmol; 78% yield). ^{1}H NMR (600 MHz) δ 8.48 (s, 1 H), 8.17 (d, *J* = 6.0 Hz, 1 H), 7.91 (d, *J* = 8.5 Hz, 1 H), 7.23 - 7.38 (m, 5 H), 5.70 (s, 2 H), 2.97 - 3.08 (m, 1 H), 1.22 - 1.36 (m, 2 H), 0.70 - 0.86 (m, 2 H). ^{13}C NMR (151 MHz) δ ppm 156.05 (s, 1 C), 154.23 (d, *J* = 240.8 Hz, 1 C), 145.59 (s, 1 C), 142.82 (d, *J* = 2.7 Hz, 1 C), 137.97 (s, 1 C), 136.26 (s, 1 C), 129.12 (s, 2 C), 128.26 (s, 1 C), 128.03 (s, 2 C), 121.69 (s, 1 C), 120.81 (s, 1 C), 118.35 (d, *J* = 7.9 Hz, 1 C), 108.54 (d, *J* = 24.4 Hz, 1 C), 108.01 (d, *J* = 22.8 Hz, 1 C), 49.20 (s, 1 C), 26.67 (s, 1 C), 10.84 (s, 2 C). HRMS (ESI-TOF) m/z: calcd for $C_{20}H_{15}BrFN_3O$ $[M+H]^+$, 412.0461; found, 412.0470.

(S)-tert-butyl (1-(3-benzyl-5-cyclopropyl-8-fluoro-4-oxo-4,5-dihydro-3H-imidazo[4,5-c]quinolin-7-yl)pyrrolidin-3-yl)carbamate (24). To a suspension of **23** (175 mg, 0.424 mmol) in toluene (13 ml) were added, under argon, (S)-*tert*-butyl pyrrolidin-3-ylcarbamate (0.165 g, 0.849 mmol), cesium carbonate (0.415 g, 1.27 mmol), 2,2'-bis(diphenylphosphino)-1,1'-binaphthalene (0.055 g, 0.085 mmol) and diacetoxypalladium (0) (0.019 g, 0.085mmol). The resulting solution was heated for 5 h, at 150°C, under micro-wave irradiation and then concentrated. The residue was purified by silica gel flash column chromatography using a gradient heptane to ethyl acetate to afford **24** (142 mg, 0.274 mmol; 65% yield). ^{1}H NMR (600 MHz) δ 8.33 (s, 1H), 7.61 (d, *J* = 13.7 Hz, 1H), 7.22-7.43 (m, 5H), 7.20 (br d, *J* = 5.9 Hz, 1H), 7.05 (d, *J* = 8.1 Hz, 1H), 5.66 (s, 2H), 4.08-4.18 (m, 1H), 3.62-3.75 (m, 1H), 3.53-3.61 (m, 1H), 3.42-3.52 (m, 1H), 3.30-3.35 (m, 1H), 2.91-2.97 (m, 1H), 2.09-2.21 (m, 1H), 1.82-1.95 (m, 1H), 1.39 (s, 9H), 1.25-1.33 (m, 2H), 0.69-0.83 (m, 2H). ^{13}C NMR (151 MHz)

δ ppm 156.63 (s, 1 C), 155.74 (br s, 1 C), 148.25 (d, *J* = 238.2 Hz, 1 C), 144.97 (s, 1 C), 144.27 (d, *J* = 2.4 Hz, 1 C), 138.35 (s, 1 C), 136.61 (s, 1 C), 129.07 (s, 2 C), 128.13 (s, 1 C), 128.02 (s, 2 C), 125.77 (s, 1 C), 118.32 (s, 1 C), 108.36 (d, *J* = 23.3 Hz, 1 C), 107.51 (d, *J* = 8.5 Hz, 1 C), 102.80 (d, *J* = 4.8 Hz, 1 C), 78.31 (br s, 1 C), 55.62 (br s, 1 C), 50.29 (br s, 1 C), 49.00 (s, 1 C), 48.37 (br d, *J* = 4.0 Hz, 1 C), 30.98 (br s, 1 C), 28.71 (s, 3 C), 26.34 (s, 1 C), 10.98 (s, 2 C). HRMS (ESI-TOF) m/z: calcd for $C_{29}H_{32}FN_5O_3$ $[2M+H]^+$, 1035.5057; found, 1035.5089.

(S)-7-(3-aminopyrrolidin-1-yl)-3-benzyl-5-cyclopropyl-8-fluoro-3,5-dihydro-4H-imidazo[4,5-c]quinolin-4-one (25). To a solution of **24** (253 mg, 0.489 mmol) in ethyl acetate (7 mL) was added hydrogen chloride 2 M in Et_2O (1.22 mL, 2.44 mmol). The resulting solution was stirred at room temperature for 18 h and then filtered. The residue was washed with di-isopropyl ether and then dried under vacuum to afford **25** as a white powder (200 mg, 0.441 mmol; 90% yield). ^{1}H NMR (600 MHz) δ 8.84 (br s, 1H), 8.44 (br s, 3H), 7.87 (d, *J* = 13.7 Hz, 1H), 7.38-7.42 (m, 2H), 7.32-7.37 (m, 2H), 7.25-7.31 (m, 1H), 7.10 (d, *J* = 7.9 Hz, 1H), 5.72 (s, 2H), 3.88-3.97 (m, 1H), 3.76-3.83 (m, 1H), 3.72 (q, *J* = 7.7 Hz, 1H), 3.61-3.66 (m, 1H), 3.47- 3.54 (m, 1H), 2.94-3.00 (m, 1H), 2.25-2.37 (m, 1H), 2.08-2.18 (m, 1H), 1.26-1.40 (m, 2H), 0.68-0.84 (m, 2H). ^{13}C NMR (151 MHz) δ ppm 156.06 (s, 1 C), 148.16 (d, *J* = 238.7 Hz, 1 C), 143.59 (br s, 1 C), 137.41 (s, 1 C), 137.14 (s, 1 C), 137.07 (s, 1 C), 137.03 (s, 1 C), 129.14 (s, 2 C), 128.43 (s, 1 C), 128.25 (s, 2 C), 118.02 (s, 1 C), 109.16 (br d, *J* = 24.1 Hz, 1 C), 105.18 - 105.96 (m, 1 C), 103.33 (d, *J* = 4.8 Hz, 1 C), 53.69 (d, *J* = 6.6 Hz, 1 C), 49.84 (d, *J* = 1.9 Hz, 1 C), 49.74 (s, 1 C), 47.87 (d, *J* = 4.2 Hz, 1 C), 29.27 (s, 1 C), 26.55 (s, 1 C), 10.99 (d, *J* = 2.6 Hz, 2 C). HRMS (ESI-TOF) m/z: calcd for $C_{24}H_{24}FN_5O$ $[2M+H]^+$, 835.4008; found, 835.4009.

3-benzyl-1-methyl-4-(methylamino)-1,5-dihydro-2H-indeno[1,2-b]pyridin-2-one (27). To a suspension of 3-benzyl-4-hydroxyindeno[1,2-b]pyran-2(5H)-one (24.7 g, 85 mmol) **26**[16]

in acetic acid (255 ml) was added methylamine (106 ml, 85 mmol) . The resulting mixture was refluxed for 20 hours and filtered. The precipitate was successively washed with ethanol and diisopropyl ether. The filtrate was concentrated under reduced pressure, taken up in water followed by the addition of ethyl acetate and dichloromethane (3/1). A sodium hydroxide solution (30% solution) was then added up to pH 8. The organic phase was washed with brine and then dried over magnesium sulfate, filtered and evaporated to dryness. Silica gel column chromatography of the residue using a stepwise gradient of methanol (0–2%) in dichloromethane followed by trituration using diisopropyl ether gave the expected compound **(27)** (8 g, 30%). ^{1}H NMR (600 MHz) δ 8.01-8.10 (m, 1H), 7.58-7.65 (m, 1H), 7.34-7.48 (m, 2H), 7.22-7.26 (m, 2H), 7.18-7.22 (m, 2H), 7.07-7.12 (m, 1H), 5.62 (q, *J* = 5.2 Hz, 1H), 4.04 (s, 2H), 3.89 (s, 5H), 3.09 (d, *J* = 5.2 Hz, 3H). ^{13}C NMR (151 MHz) δ 162.9, 151.6, 145.2, 144.7, 141.8, 136.8, 128.6, 128.3, 127.5, 127.3, 125.8, 125.3, 123.0, 110.7, 103.3, 36.0, 32.3, 31.9, 30.6. HRMS (ESI-TOF) m/z: calcd for $C_{21}H_{20}N_2O$ $[M+H]^+$, 317.1654; found, 317.1650.

1-methyl-4-(methylamino)-1,5-dihydro-2H-indeno[1,2-b]pyridin-2-one (28). A mixture of **27** (3.16 g, 10 mmol), aluminum chloride (4 g, 30 mmol), and xylene (30 ml) was heated to reflux for 10 min. Ethyl acetate (100 ml) and cold water (50 ml) were then added. The resulting precipitate was filtered, and washed successively with an aqueous sodium hydrogen carbonate saturated solution, water, ethyl acetate and diisopropyl ether. Silica gel column chromatography of the residue using a stepwise gradient of methanol (5 to 10%) in dichloromethane followed by trituration in diisopropyl ether afforded the title compound (**28)** as a white solid (1.91 g, 84%). ^{1}H NMR (600 MHz) δ 7.98-8.05 (m, 1H), 7.60-7.64 (m, 1H), 7.36-7.45 (m, 2H), 6.39 (q, *J* = 4.8 Hz, 1H), 5.26 (s, 1H), 3.80 (s, 3H), 3.48-3.60 (m, 2H), 2.74 (d, *J* = 4.8 Hz, 3H). ^{13}C NMR (151 MHz) δ 163.9, 153.1, 145.2, 144.1, 137.5, 127.5, 127.3, 125.7, 122.9, 111.7, 88.7, 32.8, 30.7, 29.4. HRMS (ESI-TOF) m/z: calcd for $C_{14}H_{14}N_2O$ $[M+H]^+$, 227.1184; found, 227.1188.

DNA gyrase supercoiling inhibition assay. Supercoiling reactions were carried out as described previously[24] with minor adjustments. 500 ng of relaxed pNO1[25] plasmid was used as a substrate for each 30 µl reaction. The amount of gyrase (A_2B_2) to add was assessed by testing various dilution of the stock (A_2B_2 containing 0.5 mg/ml of each subunit) without compound. A limiting amount of enzyme was used when testing compounds (ie an amount sufficient to supercoil only a fraction of the substrate, typically 50%). The individual gyrase subunits were either prepared in the lab as described[24] or purchased from Inspiralis. The IC_{50} (compound concentration giving only 50% of the supercoiled substrate obtained with the uninhibited enzyme) was determined by plotting the quantified (using ImageJ) proportion of supercoiled DNA to the total of the lane against the compound concentration and fitting it to a four-parameter binding curve (y = Min + ((Max - Min)/(1 + (x/IC_{50})^HillSlope)) with Scipy[26]. The measured value was the best fit for the IC_{50} parameter.

Cytotoxicity in HepG2 cells. Cytotoxicity was assessed in 96 well plates using 10^4 HepG2 cells per well in DMEM F-12 medium (Gibco), in the presence of 5% fetal bovin serum, of 0.1 mM non essential amino acids (Gibco), of 1 mM Na-pyruvate (Gibco) and of the compounds at various concentrations in 1% DMSO final. Viability of the cells was measured after 40 h incubation at 37°C, under 5% CO_2, using the Celltiter Glo assay (Promega).

Bacterial strains. The primary screening panel (see Table 1) was composed of the following strains obtained from ATCC or from the *Escherichia coli* Genetic Stock center: *E. coli* ATCC 35218 (NCLSI standard); *E. coli* MG1655 (F- lambda- *ilvG*- *rfb*-50 *rph*-1); isogenic *E. coli* MG1655 (*tolC*::Tn10); *E. coli* D22 (F-, *lpxC*101, *proA*23, *lac*-28, *tsx*-81, trp-30, *his*-51, *tufA*1, *rpsL*173(strR), *ampC*p-1), an *lpxC* mutant derived from the *E. coli* D21

strain[17]; *P. aeruginosa* PAO1 and isogenic *P. aeruginosa* PAO750 (Δ*mexAB-oprM* Δ*mexCD-oprJ* Δ*mexEF-oprN* Δ*mexJK* Δ*mexXY* Δ*opmH* Δ*pscC*); *S. aureus* ATCC 33592 (MRSA).

Additional strains used for FoR experiments (see Table 4): *E. coli* ATCC 25922 (wt) and *K. pneumonia* ATCC 13883 (wt), *A. baumannii* ATCC 19606 (wt) were obtained from ATCC; *E. coli* ATCC 25922 Δ*tolC* (CH3130) was constructed by Lambda-red recombineering; *E. coli* CGSC 5163 (lpxC101), a D22 *lpxC* mutant (proA23 lac-28 tsx-81 trp-30 his-51 tufA1 rpsL173(stR) ampCp-1) obtained from the *E. coli* Genetic Stock Center at Yale University (strain #5163).

Additional strains used in FQ-cross-resistance studies (see Tables 5 and 6): *S. aureus* RN4220 (wt)[27]; *S. aureus* RN4220 isogenic strains were described in references 20a and 28 and/or originated from GSK culture collection; *K. pneumoniae* 1161486 strains (wt and isogenic strains)[21]; *E. coli ΔtolC* strains 7623 and W4753 originated from GSK culture collection; MG1655 *gyrA* S83L D87N, *parC* S80I (LM693) was constructed by Lambda-red recombineering[29].

Additional strains used in cross-resistance studies with non-quinolone bacterial topoisomerase inhibitors (see Table S5) have been described in reference 20 or are from GSK collection.

Additional strains used in the secondary screening panel (see Table S2): *E. coli* ATCC 25922 obtained from ATCC; *E. coli* JC7623[30] (*E. coli* K12 AB1157 recB21 recC22 sbcB15); isogenic *A. baumannii* BM4454[31] and *A. baumannii* BM4652[32] (Δ*adeABC* Δ*adeIJK*) strains; *K. pneumoniae* Δ*tolC* 1161486a[33], isogenic to *K. pneumoniae* 1161486[21]; *K. pneumoniae* NCTC 13443 and *K. pneumoniae* NCTC 13438 originated from the Health Protection Agency [HPA], London, United Kingdom; *P. aeruginosa* 18S/H[34], a strain that constitutively synthesizes Type 1d β-lactamase.

Minimum Inhibitory Concentration Testing. MICs were evaluated following the CLSI and EUCAST guidelines. For the primary and the secondary panels of strains as well as the panel of stains used for the cross-resistance studies, bacterial inoculum were prepared by overnight culture in cation-adjusted Mueller-Hinton II (MHII) medium from colonies isolated from isolated colonies on agar plates, then diluted to evaluate the colony forming units (cfu) using the McFarland turbidity standard. A 96 well plate was inoculated with 100 µl of 5 x 10^5 cfu/ml in MHII, and test compounds prepared by serial dilution at 100X concentration in DMSO. After a 20-22 h incubation, optical density was evaluated on a microplate reader and the MIC endpoint was determined as the lowest concentration of antibiotic at which there is no visible growth in duplicates, meaning that optical density differs no more from negative controls than 10% of the difference in optical density between negative controls (medium, no bacteria) and positive control (bacteria in medium, no antibiotics). Each compound was assayed at least in duplicate. To evaluate the serum effects, test media were prepared by mixing either 50% mouse serum or 50% calf fetal serum with 50% 2-fold concentrated MH2 medium.

For the panel of strains used in the FoR experiments, MICs were assayed in Mueller-Hinton II broth, cation-adjusted (from BD, ref: 212322) (MHII). Compounds were assayed in 2-fold dilution steps from 64 µg/mL. The assay was performed in 96-well (round-bottomed) plates. Assay volume was 100 µl/well, the initial bacterial concentration was ≅ 0.5-1x10^6 CFU/mL, and the incubation time and temperature were 18-20 h at 35°C ± 2°C. MIC was read visually, as detection of complete inhibition of growth by the unaided eye, using medium only as the control. Each compound was assayed in duplicate (Independent plates).

Frequency of resistance. For the determination of the FoR of **1a** and **2a**, MICs of test compounds were first evaluated on solid media by preparing Tryptic Soy Agar (TSA) in 6

well plates with serial dilutions of test compounds. 2 spots of 10^5 CFU and 10^6 CFU were seeded on the agar surface in each well. The MIC endpoint was determined as the lowest concentration of antibiotic at which there was no visible growth from these spots. Then 10 cm plates were prepared with TSA containing 4X or 8X solid MIC concentrations of test compounds and seeded with 100 µl of a bacterial preparation concentrated by centrifugation from overnight cultures and resuspended in TSA corresponding to 10^8 CFU and 10^9 CFU per plate. Since CFU were evaluated using the McFarland turbidity standard, the exact CFU number was evaluated by plating of serial dilutions of inoculum preparation on TSA agar plates. The frequencies of resistance were calculated by counting the number of colonies growing on antibiotic preparations divided by the evaluated number of CFU in the inoculum.

For the determination of the FoR of **3a** and **3e**, bacterial strains for testing were streaked out from frozen stocks onto MH-II plates and grown overnight at 37°C. The following day, single colonies were picked and inoculated into 2 mL MH-II broth and grown overnight at 37°C with vigorous shaking. To measure frequency of resistance, ~2 x 10^8 cells of each strain (100 µL of an overnight culture) were plated onto appropriate agar plates (25 mL MH-II agar plates containing dissolved compound at 4xMIC concentration) using glass bead spreading. Plates were incubated at 37°C and colonies were counted at 16 h and 48 h post-plating. To determine the viable cell density of the original cultures, dilutions were made of these overnight cultures in 0.9% NaCl, plated onto MH-II plates, and incubated overnight at 37°C.

Sequencing of resistant mutants. Individual colonies from the frequency of resistance experiment were picked and directly frozen into 10% glycerol in LB and stocked at -80°C. These frozen cultures were used to directly inoculate liquid MH-II cultures containing the same concentration of compound used in their selection. These liquid cultures were used to prepare genomic DNA for whole genome sequencing, using an Epicentre Masterpure DNA purification kit according to the manufacturer's instructions. DNA was resuspended in EB

buffer (Qiagen) and subsequently diluted in molecular biology grade water to 0.2 μg/μL. Initial DNA concentration was assayed using Nanodrop. For diluted DNA the concentration was assayed using a Qubit device. Genomic DNA libraries were prepared using Illumina Nextera XT kit and Nextera XT index primers. A fraction of each library was assayed for fragment length using Agilent Tapestation. All the assayed samples showed fragment lengths within expected parameters (broad distribution between 400 bp and 1500 bp). The libraries were sequenced on an Illumina Miseq using the Miseq V3 600 cycle reagent kit. Mutations were identified in only 2 of the 5 sequenced genomic samples (Table S4).

Protein binding. Mouse protein binding was measured using the RED (rapid equilibrium dialysis) Device Inserts from ThermoFischer Scientific.

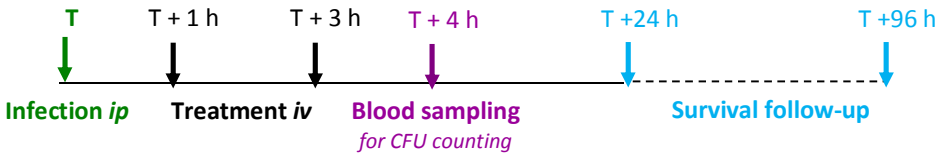
***In vivo* studies in mice (Pharmacokinetics, Tolerability and Efficacy model).** Male Swiss mice (23-35 g; 5 to 6-week old), from Janvier laboratories (Route des chênes secs, 53940 Le Genest Saint Ile, France) were maintained at temperature of 22 ± 2°C, with relative humidity of 40 – 70%, and light cycle of 12/12 hours. The animals were allowed at least 5 days of acclimatization before the start of the experiment. Animals were housed 5 per cage and they were allowed access to water and to standard diet (AO4 C standard diet (SAFE)) ad libitum. All *in vivo* experiments were conducted under protocols approved by the local Sanofi animal welfare Committee "Comité d'Éthique pour la Protection de l'Animal de Laboratoire".

Pharmacokinetics (iv; mice). Pharmacokinetics parameters of compound **3b** were determined after single iv bolus injections of 30 mg/kg. Blood samples were collected at 5 min, as well as 0.5, 1, 2, 4, 6, 8, 12, and 24 h after compound administration. After centrifugation of the heparinized blood samples, the plasma was immediately separated and frozen at 20°C until analysis. Plasma concentrations of compound **3b** were determined by using a liquid chromatography/tandem mass spectrometry (LC/MS-MS) assay. The PK

parameters were calculated using the non-compartmental models Phoenix (WinNonLin version 6.4).

Tolerability. For evaluation of tolerability, mice (3 animals per dose/compound) were treated intravenously with either compound **3a** or compound **3b** (30 mg/kg; twice a day, at 2-hour intervals) and they were then observed daily for 5 days.

In vivo efficacy in an E. coli ATCC35218 septicemia model.



Male Swiss mice were inoculated intraperitoneally with 1.10^6 CFU/mouse of *E. coli* (ATCC 35218 in NaCl 0.9% / 5% hog gastric mucine) and treated intravenously with compounds **3a** or **3b** or Tienam 1 hour and 3 hours post-infection. Four hours post-infection, five animals in each group were euthanized and blood samples collected by retro-orbital puncture for CFU counting. The other mice (10 animals per group) were observed daily up to 96 h post-infection and mortality was noted. Data are expressed as Mean +/- sem and reductions of bacterial counts in target organ were analyzed by a one-way analysis of variance (ANOVA), followed by Dunnett's adjustment for multiplicity, using the SAS v9.2 software.

ASSOCIATED CONTENT

Supporting Information Available

The Supporting Information is available free of charge on the ACS Publications website at http://pubs.acs.org.

Proof of molecular structure for compound **3f** from X-Ray Single Crystal Diffraction data of the TFA salt of compound **3f** in Figure S1; Crystallographic data of the TFA salt of compound **3f** in Table S1; Activities of **1a**, **2a**, **3a** and **3b** on a secondary panel of Gram-negative strains in Table S2; Serum effect on the MICs of representative IPYs for MRSA ATCC33592 in Table S3; Results of the MacroMolecular Synthesis assay (MMS) assay for **3a** using a Δ*tolC E.coli* strain (MIC = 1 µg/ml) in Figure S2; Characterization of selected *E. coli* ATCC25922 and ATCC25922 Δ*tolC* mutant strains resistant to either **3a** or **3e** in Table S4; MICs of selected IPYs on a panel of *S. aureus* strains resistant or hyper-susceptible to non-quinolone bacterial topoisomerase inhibitors related to NXL101[8] in Table S5; Pharmacokinetic parameters of **3b** in Table S6; ^{1}H and ^{13}C NMR spectra of all final and intermediate compounds in Figures S3 to S77; Molecular strings of compounds 1a to 28 without biological data in Table S7; Molecular strings of compounds 1a, 1b, 2a, 3a, 3, 3c, 3d, 3e, 3f, 3g, 3h, 3i and 4 with biological data in Table S8.

CCDC ID Codes

Crystal data for compound **3f** has been deposited in the Cambridge Crystallographic Data Centre with the number CCDC 1526537.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

A. baumannii, Acinetobacter baumannii; BINAP, 2,2'-bis(diphenylphosphino)-1,1'-binaphtyle; BT, Bacterial Topoisomerase; BTI, Bacterial Topoisomerase Inhibitor; CFS, Calf fetal serum; FCCP, carbonyl cyanide-4-(trifluoromethoxy)phenylhydrazone); CFU, Colony Forming Unit; clogD, calculated logD; Cip, Ciprofloxacin; DBU, 1,8-Diazabicyclo[5.4.0]undec-7-ene; *E. coli, Escherichia coli*; ENABLE, European Gram Negative Antibacterial Engine; EtOAc, ethyl acetate; FoR, Frequency of Resistance; FQ, Fluoroquinolone;; FQ^S and FQ^R, Fluoro-quinolone-sensitive or resistant; HATU, O-(7-azabenzotriazol-1-yl)-N,N,N′,N′-tetramethyluronium hexafluoro phosphate; IMI, Innovative Medicines Initiative; IPY, ImidazoPyrazinone; iv, intravenous; *K. pneumoniae, Klebsiella pneumoniae;* LOQ, Limit Of Quantification; LpxC, UDP-3-O-acyl-N-acetylglucosamine deacetylase; MeCN, acetonitrile; MMS, MacroMolecular Synthesis assay; MNT, Micronucleus Test; ND4BB, New Drugs for Bad Bugs; QZD, Quinazolinedione; *P. aeruginosa, Pseudomonas aeruginosa; S. aureus, Staphylococcus aureus; S. pneumoniae, Streptococcus pneumoniae;* TCA, trichloroacetic acid; Vss, Volume of distribution; wt, wild type.

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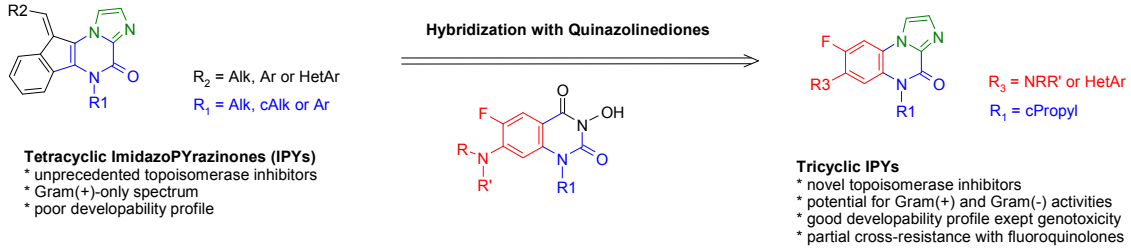
Table of Contents graphic



Tetracyclic ImidazoPYrazinones (IPYs)
* unprecedented topoisomerase inhibitors
* Gram(+)-only spectrum
* poor developability profile

Tricyclic IPYs
* novel topoisomerase inhibitors
* potential for Gram(+) and Gram(-) activities
* good developability profile exept genotoxicity
* partial cross-resistance with fluoroquinolones