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Synthesis and evaluation of original bioisosteres of bacterial type IIA topoisomerases inhibitors

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keywords: bacterial type IIA topoisomerases; bioisosteres; 3-pyrazole carboxylic acid

Abstract

A recently discovered series of inhibitors of the ATPase function of bacterial type IIA topoisomerases featuring a carboxypyrrole component led us to attempt to replace this group with a potentially bioisosteric carboxypyrazole. Accordingly, synthetic pathways to 2-(4-(1*H*-pyrazole-5-carboxamido)piperidin-1-yl)thiazole-5-carboxylic acids or 2-(4-(*N*-methyl-1*H*-pyrazole-5-carboxamido)piperidin-1-yl)thiazole-5-carboxylic acids featuring an array of substituents on the pyrazole ring were explored. Unfortunately, none of the analogues made were effective on the ATPase function of *Mycobacterium tuberculosis* gyrase, as well on the DNA supercoiling activity of the whole gyrase of *M. tuberculosis* and *Escherichia coli*. However, this work is still providing original insights in chemistry as well as in the structure-activity relationships of this series of inhibitors.

Résumé

Une série récente d'inhibiteurs de la fonction ATP-asique des topoisomerases bactériennes de type IIA comportant un noyau carboxypyrrole nous a conduits à tenter de remplacer celui-ci par un noyau carboxypyrazole bioisostère. Ainsi, des accès aux dérivés d'acide 2-(4-(1*H*-pyrazole-5-carboxamido)piperidin-1-yl)thiazole-5-carboxylique, comportant une gamme de substituants, ont été explorés. Malheureusement, aucun des analogues préparés n'a eu d'effet, que ce soit sur la fonction ATP-asique de la gyrase de *Mycobacterium tuberculosis* ou sur l'activité de surenroulement des gyrases de *M. tuberculosis* ou d'*Escherichia coli*. Toutefois, ce travail représente une contribution originale en ce qui concerne la chimie ou les relations structure-activité de cette série d'inhibiteurs.

Keywords: ATPase, topoisomerases; antibiotic; bioisosteres; pyrazole

Introduction

In the recent past, the strategies used to find new antibiotics effective on bacterial strains such as multiple resistant *Mycobacterium tuberculosis*, the Gram-positive methicillin-resistant strains of *Staphylococcus aureus* (MRSA), and the vancomycin-resistant *Enterococcus spp* as well as the Gram-negative *Actinobacter* or *Pseudomonas spp*. currently plaguing hospitals[1] have undergone many changes. A remarkable report from a major actor in the domain, reviewing the results of a full genomic approach, led amongst other conclusions to the following statement: "The only way to overcome the challenges of multifactorial antibacterial lead optimization is to expand the number of chemical derivatives. We now employ roughly two chemists for each biologist in the antibacterial therapeutic area, a fourfold turn-around from the days when genomics dominated our activities".[2] The consequence of this has indeed led to extensive medicinal chemistry in the recent past, which notably led to the discovery of many original series of antibiotics inhibiting bacterial type IIA topoisomerases.[3] Amongst these, series of pyrrole-containing inhibitors of the ATPase function of bacterial type IIA topoisomerases such as the compounds **1a-c** depicted in figure 1 were reported.[4,5] Extensive structure-activity studies led to many analogues including the pyrrolo[2,3-c]pyridine derivative **2**[6] or the more elaborated triazole-bearing analogue **3**.[7] The latter two were actually studied for their antimycobacterial effect on a mice model of *M. tuberculosis* infection.[8] Replacing the carboxypyrrole by a carboxyimidazole group turned out to be possible and imidazole derivatives such as **4** were also claimed for their inhibition of DNA gyrase and topoisomerase IV as well as their antibacterial effect.[9,10] Aside from extensive rescaffolding of the central 4-aminopiperidine with other cyclic amines,[11-16] the 2-(piperidin-1-yl)thiazole component could be replaced, with relative success, by a carboxamide[17] or other heterocycles[18] such as the randomly chosen 4-phenyltriazole **5**[19] and more recently, antibacterials such as the tetrahydrobenzo[1,2-*d*]thiazole **6** were reported.[20] Extensive work

on the carboxylic acid-bearing component of these series led to compound **7** which featured an improved antibacterial activity as well as a lower in vivo clearances and was thus selected for clinical trials.[21] In view of this, along with our simple method for the preparation of carboxypyrazoles,[22] we set to prepare the pyrazole-bearing series of compounds **8** and **9** in order to determine in this case whether a pyrazole nucleus could also be a bioisostere of the carboxy-pyrrole component.

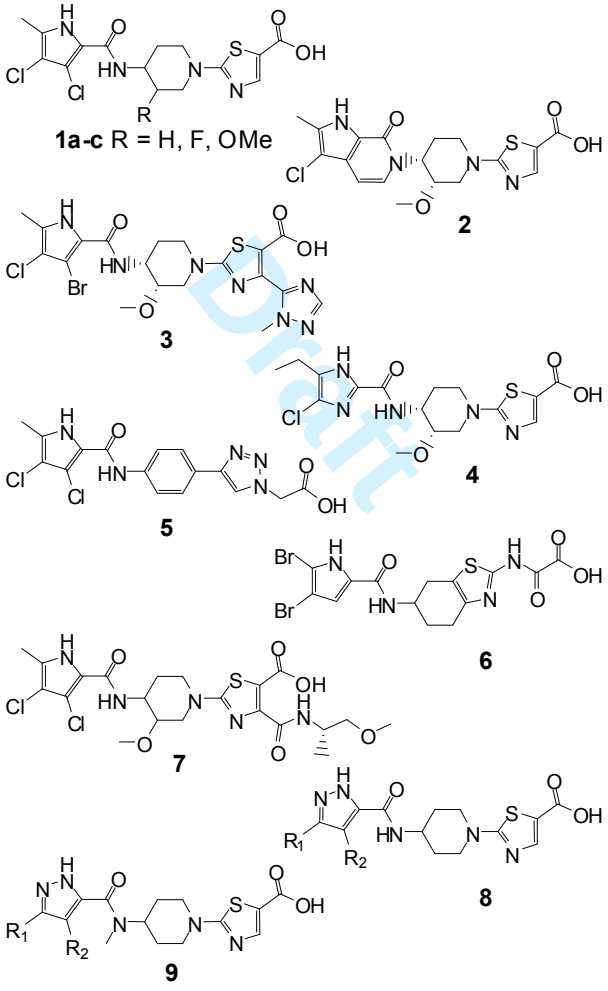
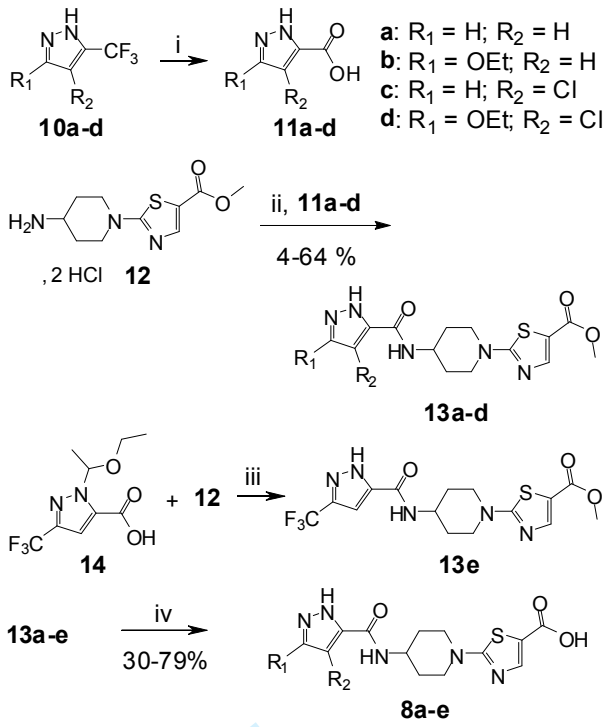


Figure 1. Structures of compounds **1-9**.

Results and discussion

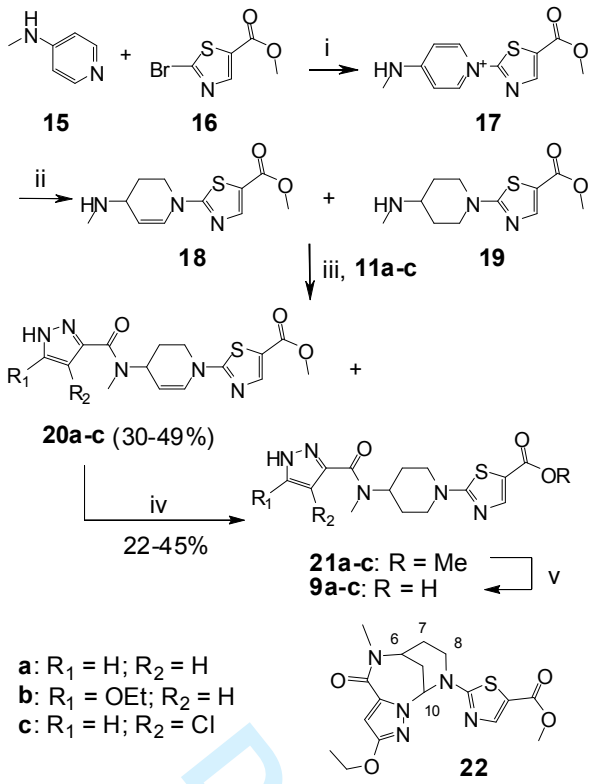
As depicted in scheme 1, the pyrazole acids **11a-d** were obtained by hydrolysis of the corresponding trifluoromethyl pyrazoles **10a-d**, as previously reported[22] or described in the experimental part. Initially, their coupling with the dihydrochloride salt of amine **12**, previously described,[23] to give amides **13a-d** was achieved using a mixture of phosphorus oxychloride and pyridine. This unusual method has been successfully applied in the past for difficult cases[24] including one involving a pyrazole carboxylic acid.[25] However, if the modest yield obtained could be raised with an excess of the acid and phosphorus oxychloride in some cases we realized later on in the course of the preparation of the N-methyl derivatives **9** that *O*-(benzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium tetrafluoroborate (TBTU) was a far better coupling agent. To prepare the trifluoromethyl bearing amide **13e**, we used the *N*-protected acid **14** (preparation described in the experimental part). Coupling of this acid with amine **12** using phosphorus oxychloride and pyridine followed by the cleavage of the ethoxyethyl protecting group gave compound **13e** in an 18 % overall yield. Hydrolysis of esters **13a-e** to give the target acids **8a-e** was achieved using lithium hydroxide as described for the pyrrole series of inhibitors.[23] A relatively large scale (200 mg) and a minimal amount of water were found necessary to isolate, by precipitation, substantial amount of the rather water-soluble acids **8a** and **8b**. Unfortunately, a complete lack of inhibition effect was observed for the analogues **8a-e** on disk-based assays for bacterial strains such as *E. coli* (data not shown). Moreover, to remove the possibility of a lack of membrane solubility which would have explained these results, compounds **8a-e** were also assessed on various bacterial gyrases assays. No effect was seen on the DNA supercoiling activity of the whole DNA gyrases of *M. tuberculosis* and *E. coli*, even at the highest concentration tested (2.5 mM; data not shown).



Scheme 1: i: NaOH, EtOH/H_2O, mw 150 °C. ii: $POCl_3$, pyridine. iii: a) $POCl_3$, pyridine, b) $H_3O^+Cl^-$, EtOH. iv: LiOH, THF/H_2O.

For the synthesis of the N-methylated analogues **9a-j**, a different synthetic approach to the key intermediate **19** was initially explored. By heating equimolar amount of the *N*-methylpyridin-4-amine (**15**) and the methyl 2-bromothiazole-5-carboxylate (**16**) in a microwave oven at 100 °C for 15 minutes, a close to quantitative yield of the pyridinium salt **17** was obtained. Treatment of this salt with sodium borohydride in methanol led to a mixture of the partially hydrogenated dihydropyridine **18** and a smaller proportion of the target piperidine **19**, which could not be separated at this stage. Reduction trials with various borohydrides did not improve this ratio nor atmospheric palladium-catalyzed hydrogenation of the mixture of **18** and **19**. In any case, the coupling of these crude amines with pyrazole carboxylic acids **11a-c**, using the much more efficient TBTU, gave a mixture of the amides **20a-c** and **21a-c** from which the pure 3,4-dihydropyridines **20a-c** could be isolated in 30-49

% yield. The LC/MS monitoring of this reaction actually pointed out the occurrence of various pairs of TBTU adducts (i.e.: *m/z* = 446 and 448 or *m/z* = 419 and 421, when coupling the mixture of **18** and **19** with acid **11a**). This led us to add ammonia in the reaction mixture to decompose them before the reaction work up. The reduction of the dihydropyridine ring of amides **20a-c** turned out to be challenging and the only reagents which provided modest amount of the reduced compounds **21a-c** (22-45 % yield) was the combination of triethylsilane and trifluoromethane sulfonic acid. Extensive investigation of the various products arising from this ionic-based reduction was made and in the case of compound **20b**, we could isolated the tricyclic derivative **22**. As described below, the bridged structure of this compound was fully established by an X-ray crystallography study. The mechanism for the occurrence of this compound is quite straightforward, upon protonation of the 3,4-dihydropyridine double bond of compound **20b**, the resulting cation can either be quenched by the triethylsilane, to give the reduced amide **21b**, or react internally with the nucleophilic nitrogen of the pyrazole ring to give **22**. Hydrolysis of the methyl ester of compounds **21a-c**, to give the target analogues **9a-c**, was then achieved as above using lithium hydroxide. Concerning the ^{1}H NMR characterization of all these *N*-methylamides, their ^{1}H NMR spectra at room temperature pointed out the occurrence of equilibrium between at least two conformations. In every cases, as described in the experimental part, when raising the temperature to 90 °C, all the ^{1}H NMR signals resolved into a single conformation much easier to describe. For the same reason, the ^{13}C NMR spectra obtained at room temperature were intractable and no attempts were made to obtain them at 90 °C.



Scheme 2: i: MeCN, 100 °C, 15 mn. ii: $NaBH_4$, MeOH. iii: a)TBTU, iPr_2EtN, THF, 20 °C, b) NH_4OH. iv: Et_3SiH, CF_3SO_3H, CH_2Cl_2, 20°C, 12h. v: LiOH, MeOH, H_2O, 20 °C, 12h.

The Ortep-3 diagram[26] of compound **22** is shown in Figure 2. The crystallographic data collection and refinement parameters are in table 1 in the experimental part. Compound **22** crystallizes in the triclinic space group P -1, with one molecule in the asymmetric unit. A geometric analysis of all bond distances and angles performed by carrying out a Mogul[27] geometry check implemented in the program Mercury[28] showed that most bond distances, bond angles and torsion angles have typical geometry. Only a couple of valence angles (C2_C1_N1 and O4-C2-C1) concerning the pyrazolo-diazepanone bicycle have an absolute value of z-score slightly above 4. Two planes may be defined through the heteroatom skeleton of the molecule. Plane 1 is formed by the pyrazole ring fused with diazepane ring, which adopts a sofa conformation since the C4 is the most distant atom from the mean plane of the ring (0.775(2)Å). Plane 2 is formed by the piperidine ring to which is attached, equatorially at

the N3 atom, the thiazole ring. Cremer and Pople ring puckering parameters[29] (Q = 0.517(2) Å, θ = 176.9(2)°, φ = 120(5)°) suggest that the piperidine ring is in a chair conformation. The dihedral angle between the two planes is 75.7°.

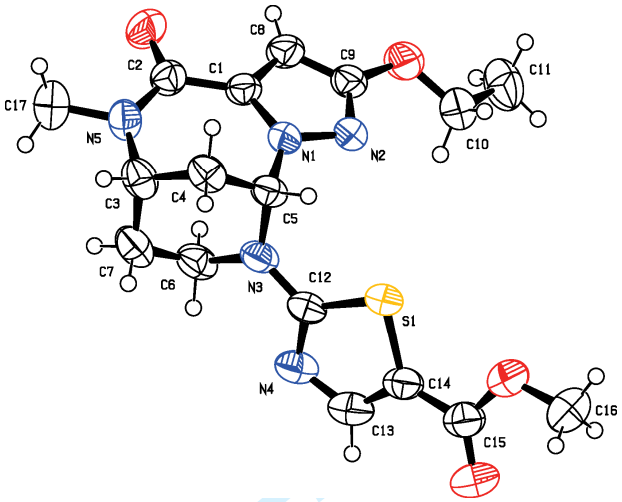
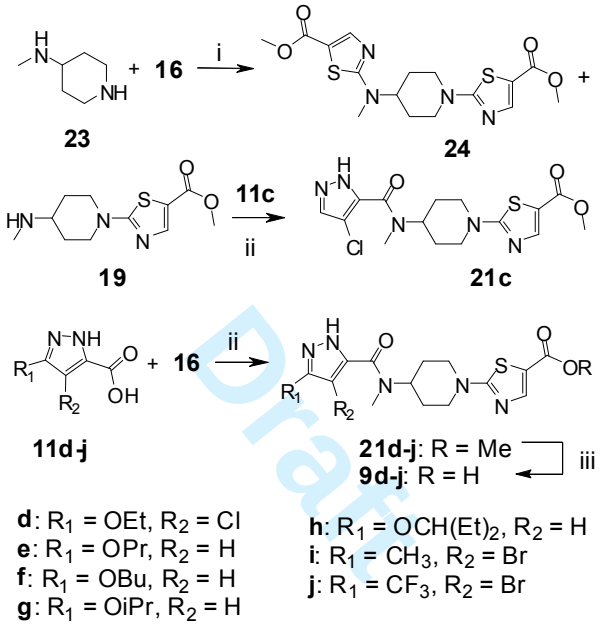


Figure 2. X-ray based structure of compound **22**.

To avoid the difficulties encountered in the reduction of compounds **20a-c**, the alternative pathway, depicted in scheme 3, was investigated for the preparation of analogues **9d-j**. As observed by LC/MS, the condensation of *N*-methylpiperidin-4-amine (**20**) with the 2-bromothiazole **13** led to a mixture of the double and mono-substituted products **21** and **16**. To confirm the reported[30] regioselectivity of such *N*-arylation step in our case, the coupling of the crude mixture of **21** and **16** was first undertaken with the 4-chloropyrazole-5-carboxylic acid (**8c**). In this control experiment, amide **18c** was thus obtained in a 9 % overall yield from the piperidine **20**. Despite this rather low yield, because of the simplicity of this pathway, the amides **18d-j** were then prepared from the corresponding carboxypyrazoles **8d-j** (preparations described in the experimental part). From these esters, their hydrolysis, using lithium hydroxide, gave the target acids **9d-j** in 46-75 % yield. Two types of biochemical assays were

performed: DNA supercoiling and ATPase activity inhibitions. A very weak inhibition was observed using the DNA supercoiling assays (*M. tuberculosis* as well as *E. coli*) for compound **9b** (IC_{50} = 2 mM) in which moxifloxacin displayed an IC_{50} of 2.5 µM but no effect was observed on the ATPase activity. Unfortunately none of the analogues subsequently made had any effect on these two types of biochemical assays.



Scheme 3: i: iPr_2EtN, MeCN, 100 °C, 2h, MW. ii: a) TBTU, iPr_2EtN, THF, 20 °C, b) NH_4OH. iii: LiOH, MeOH, H_2O, 20 °C, 12h.

Conclusion

This work was an attempt to determine whether a carboxypyrazole could, in the present case, be a bioisosteric replacement of a carboxypyrrole. On the chemistry point of view, by using the previously disclosed synthesis of compound **12**,[23] we could couple it with various pyrazole carboxylic acids and this led to the analogues **8a-e**. To synthesize the *N*-methylated derivatives **9a-j**, two synthetic pathways were investigated. The first one used an original access to compound **18** and allowed the preparation of analogues **9a-c**. However, this path

features the drawback of a side reaction leading to bridged products such as the fully identified compound **22**. To avoid this, a second path was sought and we first established that equimolar reaction between the *N*-methylated piperidine **23** and the 2-bromothiazole **16** does provide an access to the key *N*-methyl intermediate **19** along with the bis-substituted compound **24**. This path was used to prepare analogues **9d-j** which, in view of the lipophilic group of most active pyrrole-bearing series depicted in figure 1, were dressed with various combination of lipophilic groups on their pyrazole ring. However, the effect of compounds **9a-j** on the whole DNA gyrases assays of *M. tuberculosis* and *E. coli* turned out to be disappointing. In this regard, the replacement of the lipophilic methyl group of the pyrrole-bearing antibacterials depicted in figure 1 by a polar nitrogen atom is indeed not favorable. Despite this, we hope that this report, which provides some insights in the chemistry of these series as well as in their structure-activity relationships, will still be useful to other investigators.

Experimental part

Disk-based assays. The strain used in this study was *E. coli* CIP 76.24 (susceptible to ofloxacin). For routine use, this strain was grown at 37 °C on trypticase soy agar plates (BioMérieux, La Balme-Les-Grottes, France). The antimicrobial susceptibility test was performed on Mueller-Hinton agar (BioMérieux, La Balme-Les-Grottes, France) by an agar diffusion method, according to the guidelines of the Antibiogram Committee of the French Society for Microbiology.[31] Each compound was solubilized in DMSO to obtain a solution of 50 mg/ml. The *in vitro* susceptibility to 5 compounds was tested by the agar dilution method. These solubilized compounds were used immediately after dilution. The serial dilutions were performed in physiological serum and 10 µL of the dilution were deposited on a blank disk (Biorad). A disk without any compound, and another with 10 µl DMSO were added on the

plates, as negative controls. All plates were incubated at 37°C for 24h. The strain was fully impervious to the compounds **5a-e** tested.

DNA supercoiling assay. The *M. tuberculosis* DNA gyrase was purified as described previously.[32] The reaction mixture (total volume, 30 µl) contained DNA gyrase assay buffer (40 mM Tris-HCl [pH 7.5], 25 mM KCl, 6 mM magnesium acetate, 2 mM spermidine, 4 mM dithiothreitol, bovine serum albumin [0.36 µg/mL], 10 mM potassium glutamate, 1 mM ATP [pH 8.0]) and relaxed pBR322 DNA (0.4 µg) as the substrate. Gyrase proteins (180 ng of GyrA and 126 ng of GyrB) were mixed in the presence of increasing concentrations of quinolones for 1 h at 37°C. Reactions were terminated by the addition of 50 % glycerol containing 0.25 % bromophenol blue, and the total reaction mixture was subjected to electrophoresis in a 1 % agarose gel in 0.5X TBE (Tris-borate- EDTA, pH 8.3) buffer. After electrophoresis for 5.5 h at 50 V, the gel was stained with ethidium bromide (0.7 µg/mL). The inhibitory effect of quinolones on DNA gyrase was assessed by determining the concentration of drug required to inhibit the supercoiling activity of the enzyme by 50 % (IC50). Supercoiling activity was assessed by tracing the brightness of the bands corresponding to the supercoiled pBR322 DNA with Molecular Analyst software (Bio-Rad). For the *E. coli* DNA gyrase assay, a similar protocol was used with a commercial available (John Innes Enterprises Ltd) recombinant enzyme.

DNA gyrase ATPase assay. The *M. tuberculosis* DNA gyrase ATPase domain was purified as described previously.[33] The ADP-Glo^{TM} kinase assay (Promega, Madison, WI) was used to test the ATPase inhibition of the compounds having IC_{50} values for supercoiling inhibition below or equal to 2 mM. The ATPase assay was carried out in a 384-well plate with a 5 µL volume containing 0.7 µl of 40 g/l *M. tuberculosis* ATPase, 1 µL Reaction Buffer (Tris 40 mM (pH = 7.5), $MgCl_2$ 20 mM, KCl 50 mM, BSA 0.1 mg/mL) and 0.5 µl ultra pure ATP 1 mM. Reactions in each well were started by adding the ATP solution and kept going for an

hour at 20°C. Then 5 µl of ADP-Glo reagent was added into each well to stop the reaction and consume the remaining ADP within 40 minutes. At the end, 10 µL of the kinase detection reagent was added into the well and incubated for 1 hour and then the luminescence was monitored. Note: the novobiocin used as a reference in this assay had an IC_{50} of 1 µM.

X-ray analysis. X-ray data were collected at 293 K using CuKα (1.54187 Å) on a Rigaku mm007 HF rotating anode diffractometer equipped with Osmic CMF mirror and a curved imaging-plate RapidII detector. The Rigaku CrystalClear-SM Expert 2.0 r15 software package[34] was used for data collection and data reduction. The data were corrected semi-empirically for absorption using multi-scan approach through the Fs Process scaling algorithm. The structure was solved by direct methods using SHELXS-97[35] and refined by full-matrix least squares on F^2 using SHELXL-2014/7.[36] All non-hydrogen atoms were successfully refined using anisotropic displacement parameters. Hydrogen atoms were found in the Fourier difference synthesis and fixed. Crystallographic data for the structure of compound **22** were deposited in the Cambridge Crystallographic Data Centre, with number CCDC 1049028.

Table 1. Crystal data and structure refinement for compound 22

Empirical formula	$C_{17}H_{21}N_5O_4S$
Formula weight	391.45
Temperature	293(2) K
Wavelength	1.54187 Å
Crystal system, Space group	Triclinic, P-1
Unit cell dimensions	a = 8.8695(2) Å a= 113.309(8)° b = 10.7315(3) Å b= 98.373(7)° c = 10.8733(7) Å g = 96.812(7)°
Volume	922.50(9) $Å^3$
Z, Calculated Density	2, 1.409 mg/m^3
Absorption coefficient	1.863 mm^{-1}
F(000)	412
Crystal size	0.380 x 0.340 x 0.270 mm^3
Theta range for data collection	4.530 to 68.248°.

Index ranges	$-10 \leq h \leq 10$, $-12 \leq k \leq 12$ $-13 \leq l \leq 13$
Reflections collected	12730
Independent reflections	3283 [R(int) = 0.0301]
Completeness to theta = 67.687°	96.7 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.61 and 0.47
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	3262 / 0 / 247
Goodness-of-fit on F^2	1.075
Final R indices [$I>2\sigma(I)$]	R1 = 0.0416, wR2 = 0.1101
R indices (all data)	R1 = 0.0527, wR2 = 0.1199
Largest diff. peak and hole	0.217 and -0.174 e.Å^{-3}

Chemistry. A Biotage initiator 2 microwave oven was used for reactions mentioning such heating method. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance 400 spectrometer at 400 MHz and 100 MHz, respectively. Shifts (δ) are given in ppm with respect to the TMS signal and cross-coupling constants (J) are given in hertz. Column chromatography were performed either on Merck silica gel 60 (0.035 - 0.070 mm) or neutral alumina using a solvent pump and an automated collecting system driven by a UV detector set to 254 nm unless required otherwise. Sample deposition was carried out by adsorption of the mixture to be purified on a small amount of the solid phase followed by its deposition on the top of the column. The low resolution mass spectra were obtained on an Agilent 1100 series LC/MSD system using an atmospheric electrospray ionization system and the high resolution mass spectra (HRMS) were obtained using a Waters Micromass Q-Tof with an electrospray ion source. Unless stated otherwise, a purity of at least 95 % was obtained for all the compounds by means of chromatography, recrystallization or distillation and this level of purity was established by TLC, LC/MS and NMR spectroscopy. Moxifloxacin, used as active compound for the supercoiling assays, was provided by Bayer Pharma, Puteaux, France.

General procedure for the hydrolysis of trifluoromethylpyrazole into pyrazole-5-carboxylic acid

As previously described for other cases,[22] in a Biotage tube, the relevant trifluoromethylpyrazole (1 mmol) and sodium hydroxide (0.2 g, 5 mmol) were stirred in ethanol/water 1:3 (1.2 mL). The tube was sealed and heated at 150 °C for 1 h in a microwave oven. The resulting suspension was dissolved in water; the aqueous phase was washed with dichloromethane twice and made acidic with 2N hydrochloric acid. This was extracted with ethyl acetate twice; the organic layer were combined and washed with brine, dried over magnesium sulfate, and concentrated to dryness to yield the corresponding acid as described below. **CAUTION**: the reaction leads to the release of fluorine ions which attack the glass tubes. Never recycle the reaction tubes as their resistance toward pressure and temperature may have been weakened in the process.

4-Chloro-3-ethoxy-1*H*-pyrazole-5-carboxylic acid (**11d**): Step 1, preparation of 4-chloro-3-ethoxy-5-(trifluoromethyl)-1*H*-pyrazole (**10d**): In ethanol (50 mL), 3-ethoxy-5-(trifluoromethyl)-1*H*-pyrazole (1.42 g, 7.89 mmol)[37] and *N*-chlorosuccinimide (1.26 g, 9.46 mmol) were stirred overnight at room temperature and then heated to reflux for 90 minutes. This was concentrated to dryness, the residue was dispersed in cyclohexane and filtrated. The filtrate was washed with water, dried over magnesium sulfate and concentrated to dryness to yield the 4-chloropyrazole which NMR spectra feature two detectable tautomers. ^{1}H NMR ($CDCl_3$): major tautomer, 1.35 (t, 3H, J = 7.0 Hz); 4.35 (q, 2H, J = 7.0 Hz); 10.26 (s(br), 1H); minor tautomer, 1.45 (t, 3H, J = 7.0 Hz); 4.56 (q, 2H, J = 7.0 Hz); 10.12 (s(br), 1H). ^{13}C NMR ($CDCl_3$, D1 set at 5 s): major tautomer, 14.6; 69.9; 97.7; 119.0 (J = 270 Hz); 132.3 (J = 39 Hz); 157.2; minor tautomer, 18.0; 58.5; 66.0; 117.9 (J = 274 Hz); 145.3 (J = 39 Hz); 166.8. HRMS: calcd for $C_6H_7ClF_3N_2O$ + H: 215.0199; found: 215.0135. Step 2, the hydrolysis procedure described above was applied to this compound and led to the acid **11d**

as a beige power (0.66 g, 67 %). ^{1}H NMR (DMSO-*d6*): 1.31 (t, 3H, *J* = 7.0 Hz); 4.23 (q, 2H, *J* = 7.0 Hz); 13.2 (s(br), 2H). ^{13}C NMR (DMSO-*d6*, D1 set at 5 s): 13.0; 65.3; 97.0; 130.9; 158.7; 159.6. HRMS: calcd for $C_6H_7ClN_2O_3$ - H: 189.0067; found: 189.0066.

3-propoxy-1H-pyrazole-5-carboxylic acid (**11e**): Step 1, preparation of 3-propoxy-5-(trifluoromethyl)-1H-pyrazole: In dimethylformamide (100 mL, dried over 4A molecular sieve), 5-(trifluoromethyl)-1H-pyrazol-3-ol[38] (4.04 g, 0.026 mol), cesium carbonate (9.52 g, 0.029 mol) and propyliodide (4.51 g, 0.026 mol) were heated at 80 °C under a moisture-protected atmosphere for 14 hours. This was concentrated to dryness, the residue was dispersed in water and ethyl acetate, the organic layer was washed with water, brine, dried over magnesium sulfate and concentrated. The residue was purified by a chromatography over silica gel (cyclohexane – dichloromethane from 1/2 to 0/1) to yield the 3-propoxy-5-(trifluoromethyl)-1H-pyrazole as a volatile oil (2.72 g, 52 %). ^{1}H NMR ($CDCl_3$): 1.04 (t, 3H, *J* = 7.5 Hz); 1.83 (m, 2H); 4.07 (t, 2H, *J* = 6.5 Hz); 5.87 (s, 1H); 9.69 (s(br), 1H). ^{13}C NMR ($CDCl_3$): 10.1; 22.3; 73.2; 85.1; 120.5 (*J* = 270 Hz); 140.7 (*J* = 39 Hz); 158.4. HRMS: calcd for $C_7H_9F_3N_2O$ + H: 195.0745; found: 195.0715. Step 2: the hydrolysis procedure described above was applied to this compound and led to the acid **11e** (0.64 g, 85 %). ^{1}H NMR (DMSO-*d6*): 0.94 (t, 3H, *J* = 7.3 Hz); 1.69 (m, 2H); 4.01 (t, 2H, *J* = 6.7 Hz); 6.14 (s, 1H); 12.96 (s(br), 2H). ^{13}C NMR (DMSO-*d6*, D1 set at 5 s): 10.2; 21.9; 70.4; 91.9; 135.3; 160.5; 162.4. HRMS: calcd for $C_7H_{10}N_2O_3$ + H: 171.0957; found: 171.0770.

3-butoxy-1H-pyrazole-5-carboxylic acid (**11f**): Step 1, preparation of 3-butoxy-5-(trifluoromethyl)-1H-pyrazole: In dimethylformamide (100 mL, dried over 4A molecular sieve), 5-(trifluoromethyl)-1H-pyrazol-3-ol[38] (4.06 g, 0.026 mol), cesium carbonate (9.56 g, 0.029 mol) and butylbromide (3.65 g, 0.026 mol) were heated at 80 °C under a moisture-protected atmosphere for 14 hours. This was concentrated to dryness, the residue was dispersed in water and ethyl acetate, the organic layer was washed with water, brine, dried

over magnesium sulfate and concentrated. The residue was purified by a chromatography over silica gel (cyclohexane – dichloromethane from 4/1 to 0/1) to yield the 3- butoxy-5-(trifluoromethyl)-1H-pyrazole as a volatile oil (2.19 g, 39 %). ^{1}H NMR ($CDCl_3$): 0.98 (t, 3H, *J* = 7.4 Hz); 1.48 (m, 2H); 1.78 (m, 2H); 4.11 (t, 2H, *J* = 6.5 Hz); 5.86 (s, 1H); 10.32 (s(br), 1H). ^{13}C NMR ($CDCl_3$): 13.6; 18.9; 30.9; 71.5; 85.0; 120.7 (*J* = 269 Hz); 140.7 (*J* = 39 Hz); 158.4. HRMS: calcd for $C_8H_{11}F_3N_2O$ + H: 209.0902; found: 209.0817. Step 2: the hydrolysis procedure described above was applied to this compound and led to the acid **11f** (0.86 g, 90 %). ^{1}H NMR (DMSO-*d6*): 0.91 (t, 3H, *J* = 7.3 Hz); 1.40 (m, 2H); 1.66 (m, 2H); 4.05 (t, 2H, *J* = 6.5 Hz); 6.13 (s, 1H); 13.01 (s(br), 2H). ^{13}C NMR (DMSO-*d6*, D1 set at 5 s): 13.6; 18.6; 30.7; 68.6; 91.9; 135.2; 160.5; 162.4. HRMS: calcd for $C_8H_{12}N_2O_3$ + H: 185.0926; found: 185.0884.

3-isopropoxy-1H-pyrazole-5-carboxylic acid (**11g**): Step 1, preparation of 3-isopropoxy-5-(trifluoromethyl)-1H-pyrazole: In dimethylformamide (100 mL, dried over 4A molecular sieve), 5-(trifluoromethyl)-1H-pyrazol-3-ol[38] (5.28 g, 0.034 mol), cesium carbonate (12.4 g, 0.038 mol) and isopropylbromide (4.27 g, 0.034 mol) were heated at 80 °C under a moisture-protected atmosphere for 14 hours. This was concentrated to dryness, the residue was dispersed in water and ethyl acetate, the organic layer was washed with water, brine, dried over magnesium sulfate and concentrated. The residue was purified by a chromatography over silica gel (dichloromethane) to yield the 3-isopropoxy-5-(trifluoromethyl)-1H-pyrazole as a volatile oil (3.59 g, 53 %). ^{1}H NMR ($CDCl_3$): 1.38 (d, 6H, *J* = 6.1 Hz); 4.50 (sept, 1H, *J* = 6.1 Hz); 5.54 (s, 1H); 11.13 (s(br), 1H). ^{13}C NMR ($CDCl_3$): 21.7; 75.2; 85.3; 120.7 (*J* = 267 Hz); 141.0 (*J* = 39 Hz); 157.0. HRMS: calcd for $C_7H_9F_3N_2O$ + H: 195.0745; found: 195.0665. Step 2: the hydrolysis procedure described above was applied to this compound and led to the acid **11g** (0.75 g, 87 %). ^{1}H NMR (DMSO-*d6*): 1.25 (d, 6H, *J* = 6.1 Hz); 4.64 (sept, 1H, *J* = 6.1

Hz); 6.11 (s, 1H); 12.98 (s(br), 2H). ^{13}C NMR (DMSO-*d6*, D1 set at 5 s): 22.3; 72.0; 93.1; 135.8; 161.0; 161.9. HRMS: calcd for $C_7H_{10}N_2O_3$ + H: 171.0770; found: 171.0740.

3-(pentan-3-yloxy)-1H-pyrazole-5-carboxylic acid (**11h**): Step 1, preparation of 3-(pentan-3-yloxy)-5-(trifluoromethyl)-1H-pyrazole: In dimethylformamide (100 mL, dried over 4A molecular sieve), 5-(trifluoromethyl)-1H-pyrazol-3-ol[38] (4.11 g, 0.027 mol), cesium carbonate (9.6 g, 0.029 mol) and 3-bromopentane (4.08 g, 0.027 mol) were heated at 80 °C under a moisture-protected atmosphere for 14 hours. This was concentrated to dryness, the residue was dispersed in water and ethyl acetate, the organic layer was washed with water, brine, dried over magnesium sulfate and concentrated. The residue was purified by a chromatography over silica gel (cyclohexane – dichloromethane 1/2 to 0/1) to yield the 3-(pentan-3-yloxy)-5-(trifluoromethyl)-1H-pyrazole as a volatile oil (2.54 g, 42 %). ^{1}H NMR ($CDCl_3$): 0.98 (t, 6H, J = 7.4 Hz); 1.73 (m, 4H); 4.11 (pent, 1H, J = 5.8 Hz); 5.82 (s, 1H); 8.63 (s(br), 1H). ^{13}C NMR ($CDCl_3$): 9.3; 26.0; 84.9; 85.7; 120.8 (J = 268 Hz); 141.3 (J = 38 Hz); 157.7. HRMS: calcd for $C_9H_{13}F_3N_2O$ + H: 223.1058; found: 223.0955. Step 2: the hydrolysis procedure described above was applied to this compound and led to the acid **11h** (0.82 g, 96 %). ^{1}H NMR (DMSO-*d6*): 1.00 (t, 6H, J = 7.5 Hz); 1.74 (m, 4H); 4.31 (pent, 1H, J = 5.8 Hz); 6.30 (s, 1H); 11.90 (s(br), 2H). ^{13}C NMR (DMSO-*d6*, D1 set at 5 s): 9.4; 21.1; 83.7; 93.9; 135.3; 161.8; 162.7. HRMS: calcd for $C_9H_{14}N_2O_3$ + H: 199.1083; found: 199.1042.

4-Bromo-3-methyl-1*H*-pyrazole-5-carboxylic acid (**11i**): This compound was obtained from 4-bromo-3-methyl-5-(trifluoromethyl)-1*H*-pyrazole[39] using the procedure described above as a white power (0.82 g, 88 %). ^{1}H NMR (DMSO-*d6*): 2.20 (s, 3H); 13.34 (s(br), 2H). ^{13}C NMR (DMSO-*d6*, D1 set at 5s): 11.0; 95.8; 137.5 (br); 143.6 (br); 161.7. HRMS: calcd for $C_5H_5BrN_2O_2$ - H: 202.9456; found: 202.9473.

Synthesis of 4-bromo-3-(trifluoromethyl)-1H-pyrazole-5-carboxylic acid (**11j**). This compound was obtained using a previously described procedure.[40] A solution of 4-bromo-3-

methyl-5-(trifluoromethyl)-1*H*-pyrazole[39] (0.98 g, 4.27 mmol), potassium permanganate (1.7 g, 10.69 mmol) in water/tertbutanol 5-1 (60 mL) was stirred at 75 °C for three days. The suspension was filtered, the filtrate made acid using 37 % hydrochloric acid, the resulting precipitate was filtered and dissolved in a 1N sodium hydroxide solution. This aqueous layer was washed with dichloromethane twice, and made acid again using 37 % hydrochloric acid. This was extracted with ethyl acetate, the organic layer was washed with brine, dried over magnesium sulfate and concentrated to dryness to yield compound **11j** as a white powder (0.64 g, 57 %). ^{1}H NMR (DMSO-*d6*): 14.18 (s(br), H); 14.99 (s(br), H). ^{13}C NMR (DMSO-*d6*, D1 set at 5s): 95.2; 121.0 (270 Hz); 135.0; 140.7 (37 Hz); 159.1. HRMS: calcd for $C_5H_2F_3BrN_2O_2$ - H: 258.9153; found: 258.9208.

General preparation of amides 13a-d, representative synthesis of methyl 2-(4-(1*H*-pyrazole-5-carboxamido)piperidin-1-yl)thiazole-5-carboxylate (**13a**). A calcium chloride-protected solution of methyl 2-(4-aminopiperidin-1-yl)thiazole-5-carboxylate dihydrochloride dihydrate (**12**)[23] (400 mg, 1.14 mmol) and 1*H*-pyrazole-4-carboxylic acid (**11a**) (380 mg, 3.38 mmol) in pyridine (9 mL) was cooled to -15 °C. Phosphorus oxychloride (537 mg, 3.50 mmol) was then slowly added and the solution stirred at -15 °C for 15 min. This was warmed to 50 °C few seconds then cooled back to 0 °C before adding an excess of a saturated solution of sodium hydrogen carbonate. The mixture was extracted four times with ethyl acetate, the organic layers were combined and washed once with brine, dried over magnesium sulfate and concentrated to dryness. The crude residue was purified by chromatography over silica gel (dichloromethane - ethanol, from 97/3 to 95/5) to yield product **13a** as a white solid (240 mg, 63 %). ^{1}H NMR (DMSO-*d6*): note: at high concentration, a minor conformer can also be observed, 1.67 (m, 2H); 1.87 (m, 2H); 3.25 (m, 2H); 3.76 (s, 3H); 3.99 (m, 2H); 4.01 (m, 1H); 6.64 (m, 1H); 7.80 (m, 1H); 7.86 (s, 1H); 8.02 (d, 1H, J = 8.2 Hz); 13.20 (s, 1H). ^{13}C NMR

(DMSO-*d6*): 30.4; 45.2; 47.4; 51.6; 105.0; 114.9; 129.8; 146.6; 148.4; 161.1; 161.6; 173.7. HRMS: calcd for $C_{14}H_{17}N_5O_3S$ + H: 336.1130; found: 336.1112.

Methyl 2-(4-(3-ethoxy-1*H*-pyrazole-5-carboxamido)piperidin-1-yl)thiazole-5-carboxylate (**13b**): By using the procedure described above, this compound was obtained as a white solid (70 mg, 32 %). ^{1}H NMR ($CDCl_3$): 1.41 (t, 3H, *J* = 7.0 Hz); 1.66 (m, 2H); 2.15 (m, 2H); 3.29 (m, 2H); 3.84 (s, 3H); 4.10 (m, 2H); 4.20 (q, 2H, *J* = 7.0 Hz); 4.23 (m, 1H); 6.03 (s, 1H); 6.50 (s(br), 1H); 7.88 (s, 1H). ^{13}C NMR ($CDCl_3$): 15.2; 30.8; 45.9; 47.7; 52.2; 64.7; 89.8; 115.6; 137.7; 148.8; 158.4; 162.1; 163.0; 174.2. LC/MS, m/z = 380 (M+H).

Methyl 2-(4-(4-chloro-1*H*-pyrazole-5-carboxamido)piperidin-1-yl)thiazole-5-carboxylate (**13c**): By using the procedure described above, this compound was obtained as a white solid (150 mg, 49 %). ^{1}H NMR (DMSO-*d6*): 1.67 (m, 2H); 1.88 (m, 2H); 3.28 (m, 2H); 3.75 (s, 3H); 3.99 (m, 2H); 4.07 (m, 1H); 7.86 (s, 1H); 8.04 (m, 2H); 13.51 (s, 1H). ^{13}C NMR (DMSO-*d6*): 30.8; 45.7; 47.8; 52.2; 109.3; 115.5; 129.8; 141.6; 148.9; 160.5; 162.1; 174.2. HRMS: calcd for $C_{14}H_{16}ClN_5O_3S$ + H: 370.0741; found: 370.0757.

Methyl 2-(4-(4-chloro-3-ethoxy-1*H*-pyrazole-5-carboxamido)piperidin-1-yl)thiazole-5-carboxylate (**13d**): By using the procedure described above, this compound was obtained as a white solid (60 mg, 4 %). ^{1}H NMR (DMSO-*d6*): 1.32 (t, 2H, *J* = 7.0 Hz); 1.65 (m, 2H); 1.93 (m, 2H); 3.33 (m, 3H); 3.75 (s, 3H); 3.96 (m, 2H); 4.08 (m, 1H); 4.23 (q, 2H, *J* = 7.0 Hz); 7.87 (m, 2H); 12.91 (s, 1H). ^{13}C NMR (DMSO-*d6*): 15.1; 30.5; 46.1; 47.4; 52.2; 65.3; 93.7; 115.6; 134.4; 148.8; 157.5; 158.2; 162.1; 174.2. HRMS: calcd for $C_{16}H_{20}ClN_5O_4S$ + H: 414.1003; found: 414.0985.

1-(1-Ethoxyethyl)-3-(trifluoromethyl)-1*H*-pyrazole-5-carboxylic acid (**14**): Step 1, preparation of 3-(trifluoromethyl)-1*H*-pyrazole. Ethoxyethene (19.3 g, 0.267 mol) was dissolved in pentane (120 mL). This was cooled to 0 °C with an ice bath and trifluoroacetic anhydride (37.6 mL, 0.270 mol) was added slowly. This was stirred overnight at 0 °C and

then stirred 3 more hours at room temperature to yield a solution of 4-ethoxy-1,1,1-trifluorobut-3-en-2-one which was directly used in the next step. Nota as previously described,[41] the use of pentane was really optimal for this step. To this pentane solution was added ethanol (100 mL) and this was cooled to 0°C with a large ice bath. Hydrazine hydrate (15.6 mL, 0.321 mol) was slowly added and after an additional 30 minutes of stirring at room temperature, the solution was concentrated to dryness. The residue was dissolved in ethyl acetate and a saturated solution of sodium hydrogen carbonate. The organic layer was washed with a saturated solution of sodium hydrogen carbonate, brine, dried over magnesium sulfate and concentrated to dryness. A distillation at 30 mbar in the rotary evaporator heating with a heat gun and trapping the distillate in a "no return" bulb gave 3-(trifluoromethyl)-1*H*-pyrazole as a white solid (27.63 g, 75 %). ^{1}H NMR ($CDCl_3$): 6.68 (d, 1H, J = 2.2 Hz); 7.73 (m, 1H); 12.85 (s(br), 1H). Step 2, preparation of 1-(1-ethoxyethyl)-3-(trifluoromethyl)-1*H*-pyrazole: 3-(trifluoromethyl)-1*H*-pyrazole (1.02 g, 7.50 mmol), ethoxyethene (1.58 g, 21.91 mmol), and pyridinium paratoluenesulfonic acid salt (36 mg, 0.14 mmol) were stirred at room temperature in dichloromethane (30 mL) for 30 min. The mixture was extracted with dichloromethane, the organic layers were combined and washed with water, brine, and then dried over magnesium sulfate and concentrated to dryness to yield the *N*-protected pyrazole as a yellow liquid (770 mg, 49 %) which was used without further purification. ^{1}H NMR ($CDCl_3$): 1.18 (t, 3H, J = 7.0 Hz); 1.68 (d, 3H; J = 6.0 Hz); 3.37 (m, 1H); 3.51 (m, 1H); 5.59 (q, 1H, J = 6.0 Hz); 6.61 (d, 1H, J = 2.4 Hz); 7.67 (m, 1H). Step 3, preparation of 1-(1-ethoxyethyl)-3-(trifluoromethyl)-1*H*-pyrazole-5-carboxylic acid (**14**). To a solution of 1-(1-ethoxyethyl)-3-(trifluoromethyl)-1*H*-pyrazole (770 mg, 3.70 mmol) in dry tetrahydrofuran (50 mL) at -78 °C under an atmosphere of argon was slowly added *n*-butyl lithium 2M (2.3 mL, 4.60 mmol). This was stirred for 10 minutes and the orange mixture was poured on dry ice dispersed in ether. This was allowed to warm to room temperature and the organic layer was extracted

with water. The aqueous layer was made acidic with 2N hydrochloric acid and extracted with ethyl acetate. The resulting organic layer was washed with water, dried over magnesium sulfate and concentrated to dryness to yield compound **14** as a brown oil (540 mg, 58 %) which was used without further purification.

Methyl 2-(4-(3-(trifluoromethyl)-1*H*-pyrazole-5-carboxamido)piperidin-1-yl)thiazole-5-carboxylate (**13e**): A calcium chloride protected solution of methyl 2-(4-aminopiperidin-1-yl)thiazole-5-carboxylate dihydrochloride dihydrate (**12**)[23] (450 mg, 1.30 mmol) and 1-(1-ethoxyethyl)-3-(trifluoromethyl)-1*H*-pyrazole-5-carboxylic acid (**14**) (420 mg, 1.65 mmol) in pyridine (14 mL) was cooled to -15 °C. Phosphorus oxychloride (0.51 mL, 5.56 mmol) was then slowly added and the solution stirred at -15 °C for 15 min. This was warmed to 50 °C few seconds then cooled back to 0 °C before adding an excess of a solution of saturated sodium hydrogenocarbonate. The mixture was extracted four times with ethyl acetate, the organic layers were combined and washed once with brine, dried over magnesium sulfate and concentrated to dryness. The resulting crude residue was treated with hydrochloric acid 2M (5 mL) in ethanol (4 mL) at room temperature overnight. The yellow solution was extracted with ethyl acetate, the organic layer washed with water, brine, dried over magnesium sulfate and concentrated to dryness. The residue was purified by chromatography over silica gel (dichloromethane - ethanol, from 99/1 to 97/3) to yield compound **13e** as a pale yellow solid (94 mg, 18 %). ^{1}H NMR (DMSO-*d6*): 1.60 (m, 2H); 1.95 (m, 2H); 3.33 (m, 2H); 3.75 (s, 3H); 3.99 (d, 2H); 4.12 (m, 1H); 7.31 (s, 1H); 7.87 (s, 1H); 8.49 (m, 1H); 14.43 (s(br), 1H). ^{13}C NMR (DMSO-*d6*): 30.1; 45.6; 47.1; 54.8; 103.5; 115.1; 121.3 (q, J = 267 Hz); 138.8; 141.1 (q, J = 39 Hz); 148.3; 157.0; 161.6; 173.7. HRMS: calcd for $C_{15}H_{16}F_3N_5O_3S$ + H: 404.1004; found: 404.0940.

Preparation of acids **8a**-**e**, representative synthesis of 2-(4-(1*H*-pyrazole-5-carboxamido)piperidin-1-yl)thiazole-5-carboxylic acid (**8a**): To a solution of methyl 2-(4-

(1*H*-pyrazole-3-carboxamido)piperidin-1-yl)thiazole-5-carboxylate (**13a**) (187 mg, 0.56 mmol) in tetrahydrofuran (20 mL) was added a solution of lithium hydroxide (59 mg, 2.46 mmol) in water (6 mL). The mixture was stirred at room temperature overnight. The solvent was removed under vacuum. The mixture was then diluted in water and the aqueous layer washed with ether. Hydrochloric acid 2N was added to the aqueous layer added until pH 3, which was then concentrated under vacuum (water pump) at room temperature. The residue was taken up in water and concentrated under vacuum again. This step was repeated three times. The residue was then suspended in a small amount of water at 4 °C and this was filtered, washed with cold water (4 °C) and dried under vacuum to yield the desired product (**8a**) as a white powder (110 mg, 61 %). ^{1}H NMR (DMSO-*d6*): 1.67 (m, 2H); 1.88 (m, 2H); 3.25 (m, 2H); 3.98 (d, 2H); 4.08 (m, 1H); 6.68 (d, 1H, *J* = 2.2 Hz); 7.74 (d, 1H, *J* = 2.2 Hz); 7.77 (s, 1H); 8.06 (d, 1H, *J* = 8.1 Hz); 12.94 (s, 2H). ^{13}C NMR (DMSO-*d6*): 30.9; 45.8; 47.8; 105.5; 117.3; 132.2; 145.3; 148.2; 161.0; 163.1; 174.0. HRMS: calcd for $C_{13}H_{15}N_5O_3S$ - H: 320.0817; found: 320.0852.

2-(4-(3-Ethoxy-1*H*-pyrazole-5-carboxamido)piperidin-1-yl)thiazole-5-carboxylic acid (**8b**): By using the procedure described above, this compound was obtained as a solid (21 mg, 32 %). ^{1}H NMR (DMSO-*d6*): 1.29 (t, 3H, *J* = 7.0 Hz); 1.58 (m, 2H); 1.90 (m, 2H); 3.27 (m, 2H); 3.98 (m, 2H); 4.07 (m, 1H); 4.10 (q, 2H, *J* = 7.0 Hz); 6.21 (s, 1H); 7.78 (s, 1H); 8.15 (d, 1H, *J* = 8.1 Hz); 12.62 (s, 1H). ^{13}C NMR (DMSO-*d6*): (one signal missing); 15.1; 30.8; 45.9; 47.7; 65.1; 89.15; 117.5; 148.1; 158.9; 162.2; 163.1; 174.0. HRMS: calcd for $C_{15}H_{19}N_5O_4S$ - H: 364.1096; found: 364.1080.

2-(4-(4-Chloro-1*H*-pyrazole-5-carboxamido)piperidin-1-yl)thiazole-5-carboxylic acid (**8c**): By using the procedure described above, this compound was obtained as a solid (107 mg, 14 %). ^{1}H NMR (DMSO-*d6*): 1.67 (m, 2H); 1.88 (m, 2H); 3.25 (m, 2H); 3.97 (d, 2H); 4.06 (m, 1H); 7.77 (s, 1H); 8.02 (s, 1H); 8.09 (d, 1H, *J* = 8.1 Hz); 12.67 (s, 1H); 13.50 (s, 1H). ^{13}C

NMR (DMSO-*d6*): 30.2; 45.3; 47.2; 108.8; 116.8; 130.0; 141.1; 147.7; 159.7; 162.6; 173.5. HRMS: calcd for $C_{13}H_{14}ClN_5O_3S$ - H: 354.0461; found: 354.0428.

2-(4-(4-Chloro-3-ethoxy-1*H*-pyrazole-5-carboxamido)piperidin-1-yl)thiazole-5-carboxylic acid (**8d**): By using the procedure described above, this compound was obtained as a solid (48 mg, 71 %). ^{1}H NMR (DMSO-*d6*): 1.32 (t, 3H, *J* = 7.0 Hz); 1.65 (m, 2H); 1.93 (m, 2H); 3.31 (m, 2H); 3.95 (m, 2H); 4.08 (m, 1H); 4.23 (q, 2H, *J* = 7.0 Hz); 7.77 (s, 1H); 7.89 (d, 1H, *J* = 8.1 Hz); 12.90 (s, 1H). ^{13}C NMR (DMSO-*d6*): 14.6; 30.0; 45.7; 46.6; 64.9; 93.1; 116.9; 134.1; 147.6; 157.1; 157.8; 162.6; 173.5. HRMS: calcd for $C_{15}H_{18}ClN_5O_4S$ - H: 398.0707; found: 398.0690.

2-(4-(3-(Trifluoromethyl)-1*H*-pyrazole-5-carboxamido)piperidin-1-yl)thiazole-5-carboxylic acid (**8e**): By using the procedure described above, this compound was obtained as a solid (92 mg, 79 %). ^{1}H NMR (DMSO-*d6*): 1.60 (m, 2H); 1.95 (m, 2H); 3.30 (m, 2H); 3.99 (m, 2H); 4.11 (m, 1H); 7.31 (s, 1H); 7.78 (s, 1H); 8.49 (d, 1H, *J* = 8.1 Hz); 12.64 (s, 1H); 14.44 (s, 1H). ^{13}C NMR (DMSO-*d6*): 30.7; 46.2; 47.5; 104.0; 117.6; 121.3 (q, *J* = 267 Hz); 139.4; 141.6 (q, *J* = 38 Hz); 158.1; 157.5; 163.1; 174.0. HRMS: calcd for $C_{14}H_{14}F_3N_5O_3S$ - H: 388.0728; found: 388.0691.

Preparation of 1-(5-(methoxycarbonyl)thiazol-2-yl)-4-(methylamino)pyridinium bromide (**17**): In a vial adapted for microwave heating, *N*-methylpyridin-4-amine (**15**) (1.04 g, 9.6 mmol) and methyl 2-bromothiazole-5-carboxylate (**16**) (2.14 g, 9.6 mmol) were dissolved in dry acetonitrile (15 mL, dried over 4Å molecular sieve). This was heated in a microwave oven for 15 minutes at 100 °C. The resulting suspension was dissolved in methanol and concentrated to dryness to give a brown powder (3.16 g) still containing solvents which was used without further purification in the next step. ^{1}H NMR (DMSO-*d6*): Major tautomer: 3.06 (s, 3H); 3.91 (s, 3H); 7.1 (m, 1H); 7.20 (m, 1H); 8.40 (s, 1H); 8.76 (m, 1H); 8.89 (m, 1H);

10.02 (s, 1H). ^{13}C NMR (DMSO-*d6*): 30.3; 53.0; 106.45; 111.1; 128.4; 138.2; 140.4; 146.6; 159.4; 160.9; 164.6. HRMS: calcd for $C_{11}H_{12}N_3O_2S^+$: 250.0589; found: 250.0650.

Preparation of the mixture of methyl 2-(4-(methylamino)-3,4-dihydropyridin-1(2*H*)-yl)thiazole-5-carboxylate (**18**) and methyl 2-(4-(methylamino)piperidin-1-yl)thiazole-5-carboxylate (**19**): The crude compound **17** (3.16 g) was dissolved in methanol (100 mL) and sodium borohydride (2.39 g, 63.2 mmol) was added portion-wise. The resulting solution was stirred for 30 minutes at room temperature, concentrated to dryness and the residue dispersed in water. The aqueous layer was saturated with sodium chloride and extracted four times with ethyl acetate. The organic layers were combined and washed with brine, dried over magnesium sulfate and concentrated to dryness to yield an orange glass (1.89 g, 59 % from compound **15**). As seen by ^{1}H NMR and LC/MS, this solid contained a 5/1 ratio of compounds **18** and **19**, and this was used in the next step without further purification. ^{1}H NMR ($CDCl_3$): minor compound **19** 1.50 (m, 2H); 1.91 (m, 2H); 2.04 (m, 2H); 2.49 (s, 3H); 3.21 (m, 2H); 3.84 (s, 3H); 4.03 (m, 1H); 7.87 (s, 1H); major compound **18** 1.92 (m, 1H); 2.07 (m, 1H); 2.51 (s, 3H); 3.29 (m, 1H); 3.78 (m, 2H); 3.86 (s, 3H); 5.17 (m, 1H); 6.90 (m, 1H); 7.93 (s, 1H). LC/MS, main peak at *m/z* = 223 (M - CH_3NH) and secondary peak at *m/z* = 256 (M + H).

Preparation of the dihydropyridine amides 20a-c, representative synthesis of methyl 2-(4-(*N*-methyl-1*H*-pyrazole-3-carboxamido)-3,4-dihydropyridin-1(2*H*)-yl)thiazole-5-carboxylate (**17a**): Under an argon atmosphere, the mixture of compound **18** and **19** (0.57 g, 2.24 mmol), 1*H*-pyrazole-3-carboxylic acid (**11a**) (0.25 g, 2.2 mmol) and *O*-(benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium tetrafluoroborate (TBTU) (1.51 g, 4.7 mmol) were dispersed in tetrahydrofuran (50 mL, dried over 4Å molecular sieves). Diisopropylethylamine (1.60 mL, 9.4 mmol) was added and the suspension was stirred at room temperature for four hours. Concentrated ammonia (10 mL, 22 %) was added and this was stirred at room temperature for

30 minutes before removing the tetrahydrofuran under vacuum. The residue was diluted in ethyl acetate and a saturated solution of sodium hydrogen carbonate. The organic layer was washed with a saturated solution of sodium hydrogen carbonate, brine, dried over magnesium sulfate and concentrated to dryness. The residue was purified by a chromatography over silica gel (dichloromethane – ethanol 95/5) to yield compound **20a** as a hard foam (0.38 g, 49 %), a sample was recrystallized in toluene. ^{1}H NMR (DMSO-*d6* at 90 °C): 2.15 (m, 2H); 3.01 (s, 3H); 3.7 (m, 1H); 3.8 (s, 3H); 3.97 (m, 1H); 5.04 (m, 1H); 5.40 (s(br), 1H); 6.57 (d, 1H, J = 2.2 Hz);7.08 (m, 1H); 7.69 (s(br), 1H); 7.92 (s, 1H); 12.9 (s(br), 1H). HRMS: calcd for $C_{15}H_{17}N_5O_3S$ + H: 348.1130; found: 348.1185.

Methyl 2-(4-(5-ethoxy-*N*-methyl-1*H*-pyrazole-3-carboxamido)-3,4-dihydropyridin-1(2*H*)-yl)thiazole-5-carboxylate (**20b**): By using the procedure described above, this compound was obtained as a pale yellow powder (0.76 g, 30 %). ^{1}H NMR (DMSO-*d6* at 90 °C): 1.33 (t, 3H, J = 7.1 Hz); 2.13 (m, 2H); 2.98 (s, 3H); 3.81 (s, 3H); 3.96 (m, 2H); 4.16 (q, 2H, J = 7.1 Hz); 5.01 (dd, 1H, J = 2.8, 8.3 Hz); 5.15 (s(br), 1H); 5.97 (s, 1H); 7.10 (d, 1H, J = 8.3 Hz); 7.92 (s, 1H); 12.20 (s, 1H). HRMS: calcd for $C_{17}H_{21}N_5O_4S$ + H: 392.1393; found: 392.1490.

Methyl 2-(4-(4-chloro-*N*-methyl-1*H*-pyrazole-3-carboxamido)-3,4-dihydropyridin-1(2*H*)-yl)thiazole-5-carboxylate (**20c**): By using the procedure described above, this compound was obtained, after a recrystallization in toluene, as a pale yellow powder still containing traces of toluene (0.53 g, 43 %). ^{1}H NMR (DMSO-*d6* at 90 °C): 2.15 (m, 2H); 2.90 (s, 3H); 3.69 (m, 1H); 3.80 (s, 3H); 3.96 (m, 1H); 5.00 (m, 2H); 7.09 (m, 1H); 7.87 (s(br), 1H); 7.91 (s, 1H); 13.99 (s, 1H). HRMS: calcd for $C_{17}H_{21}N_5O_4S$ + H: 382.0706; found: 382.0741.

General reduction of compounds **20a**-**c**, representative synthesis of methyl 2-(4-(*N*-methyl-1*H*-pyrazole-5-carboxamido)piperidin-1-yl)thiazole-5-carboxylate (**21a**): Under a calcium chloride-protected atmosphere, to a solution of compound **20a** (0.37 g, 1.06 mmol) in dichloromethane (50 mL) were added trifluoromethane sulfonic acid (0.47 mL, 5.33 mmol)

and triethylsilane (0.68 ml, 8.5 mmol). This was stirred overnight and concentrated to dryness. The residue was dissolved in ethyl acetate, the organic layer was washed with a saturated solution of sodium hydrogen carbonate, brine, dried over magnesium sulfate and concentrated to dryness. A chromatography over silica gel (dichloromethane – ethanol 97/3) gave compound **21a** as a white powder (0.14 g, 38 %). ^{1}H NMR (DMSO-*d6* at 90 °C): 1.82 (m, 2H); 1.91 (m, 2H); 2.98 (s, 3H); 3.19 (m, 2H); 3.77 (s, 3H); 4.10 (m, 2H); 4.62 (m, 1H); 6.54 (d, 1H, *J* = 2.2 Hz); 7.76 (d, 1H, *J* = 2.2 Hz); 7.85 (s, 1H). HRMS: calcd for $C_{15}H_{19}N_5O_3S$ + H: 350.1262; found: 350.1287.

Methyl 2-(4-(3-ethoxy-*N*-methyl-1*H*-pyrazole-5-carboxamido)piperidin-1-yl)thiazole-5-carboxylate (**21b**): By using the procedure described above, compound **21b** was obtained as glass (0.10 g, 22 %). ^{1}H NMR (DMSO-*d6* at 90 °C): 1.30 (t, 3H, *J* = 7.1 Hz); 1.80 (m, 2H); 1.92 (m, 2H); 2.96 (s, 3H); 3.27 (m, 2H); 3.75 (s, 3H); 4.10 (m, 2H); 4.16 (q, 2H, *J* = 7.1 Hz); 4.49 (m, 1H); 6.01 (s, 1H); 7.86 (s, 1H); 12.54 (s, 1H). HRMS: calcd for $C_{17}H_{23}N_5O_4S$ + H: 394.1549; found: 394.1569. Moreover, another chromatographic fraction contained a substance which was recrystallized in a mixture of cyclohexane and toluene to yield methyl 2-(2-ethoxy-5-methyl-4-oxo-5,6,7,8-tetrahydro-4H-6,10-methanopyrazolo[1,5-c][1,3,6]triazonin-9(10H)-yl)thiazole-5-carboxylate (**22**) as white crystals (0.08 g, 16 %). The use of HMQC and HMBC experiment allowed to following signals assignment. ^{1}H NMR (DMSO-*d6*): 1.23 (t, *J* = 7.0 Hz, 3H, $OCH_2\mathit{CH_3}$); 1.95 (m, 2H, CH_2-7); 2.54 (m, 1H, ½ CH-$\mathit{CH_2}$-CH); 2.66 (m, 1H, ½ CH-$\mathit{CH_2}$-CH); 2.85 (m, 1H, ½ CH_2-8); 3.08 (s, 3H, NMe); 3.78 (s, 3H, OMe); 3.94 (m, 2H, CH-6 and ½ CH_2-8); 4.03 (m, 2H, $O\mathit{CH_2}CH_3$); 6.39 (s, 1H,CH-3); 6.49 (m, 1H,CH-10); 7.92 (s, 1H, CH-4 pyrazole). ^{13}C NMR (DMSO-*d6*): 14.4 ($OCH_2\mathit{CH_3}$); 27.0 (CH-$\mathit{CH_2}$-CH); 30.0 (CH-7); 36.6 (N-CH_3); 39.5 (CH-8); 51.6 (CH-6); 51.9 (OCH_3); 64.5 ($O\mathit{CH_2}CH_3$); 69.4 (CH-10); 98.2 (CH-3); 116.6 (C5'); 139.7 (C3a); 147.2 (CH-4'); 156.8

(C-4); 160.9 (C2); 161.5 (C2'); 172.7 (CO_2Me). HRMS: calcd for $C_{17}H_{21}N_5O_4S$ + H: 392.1414; found: 392.1393.

Methyl 2-(4-(4-chloro-*N*-methyl-1*H*-pyrazole-5-carboxamido)piperidin-1-yl)thiazole-5-carboxylate (**21c**): By using the procedure described above, this compound was obtained as a glass (0.07 g, 46 %). ^{1}H NMR (DMSO-*d6* at 90 °C): 1.86 (m, 4H); 2.87 (s, 3H); 3.18 (m, 2H); 3.77 (s, 3H); 4.09 (m, 2H); 4.29 (s, 1H); 7.82 (s, 1H); 7.87 (s, 1H); 13.16 (s, 1H). HRMS: calcd for $C_{15}H_{18}ClN_5O_3S$ + H: 384.0897; found: 384.0928.

Alternative preparation of methyl 2-(4-(methylamino)piperidin-1-yl)thiazole-5-carboxylate (**19**): In a vial adapted for microwave heating, *N*-methylpiperidin-4-amine bishydrochloride (**23**) (1.0 g, 5.34 mmol), methyl 2-bromothiazole-5-carboxylate (**16**) (1.18 g, 5.34 mmol) and diisopropylethylamine (2.11 g, 16.29 mmol) were dissolved in dry acetonitrile (15 mL, dried over 4Å molecular sieve). This was heated in a microwave oven for 2 hours at 100 °C and the resulting mixture was concentrated to dryness. The residue was dispersed in brine, a saturated solution of sodium hydrogen carbonate and ethyl acetate. The aqueous layer was extracted four times with ethyl acetate, the organic layers were combined and washed once with brine, dried over sodium carbonate and concentrated to dryness to give an oil (0.79 g) which was used without further purification in the next step. An LC/MS analysis pointed out the occurrence of the expected *m/z* = 256 (compound **19**) as well as, to a lesser degree, *m/z* = 397 (compound **24**).

Preparation of compounds 21c-j representative synthesis of methyl 2-(4-(4-chloro-*N*-methyl-1*H*-pyrazole-5-carboxamido)piperidin-1-yl)thiazole-5-carboxylate (**21c**): The crude mixture containing compound **24** and **19** (0.28 g, 1.09 mmol), 4-chloro-1*H*-pyrazole-5-carboxylic acid (**11c**) (0.16 g, 1.09 mmol), and diisopropylethylamine (0.59 g, 4.58mmol) were dissolved in dry tetrahydrofuran (30 mL, dried over 4Å molecular sieves). The *O*-(benzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium tetrafluoroborate (0.74 g, 2.29 mmol) was

added and the suspension was stirred at room temperature under a calcium chloride-protected atmosphere overnight. Concentrated ammonia (10 mL, 22 %) was added and this was stirred for two hours. The resulting suspension was diluted in ethyl acetate and a saturated solution of sodium hydrogen carbonate. The organic layer was washed with water, brine, dried over magnesium sulfate and concentrated to dryness. In this specific case, the resulting residue was purified by two consecutive chromatography, the first one over silica gel (dichloromethane-ethanol 95/5), the second one over alumina containing 1.5 % of water (dichloromethane-ethanol 95/5) to yield compound **21c** as a white foam (0.10 g, 9 % from *N*-methylpiperidin-4-amine dihydrochloride (**23**)). The ^{1}H NMR (DMSO-*d6* at 90 °C) spectra was identical to the one reported above.

Methyl 2-(4-(4-chloro-3-ethoxy-N-methyl-1H-pyrazole-5-carboxamido)piperidin-1-yl)thiazole-5-carboxylate (**21d**): By following the protocol described above, a residue was obtained which was in this case purified by two consecutive chromatography, the first one over silica gel (dichloromethane-ethanol 97/3), the second one over alumina containing 1.5 % of water (dichloromethane-ethanol 96/4) to yield compound **21d** as a glass (0.17 g, 16 % from *N*-methylpiperidin-4-amine dihydrochloride). ^{1}H NMR (DMSO-*d6* at 90 °C): 1.35 (t, 3H, J = 7.2 Hz); 1.80 (m, 2H); 1.95 (m, 2H); 2.87 (s, 3H); 3.21 (m, 2H); 3.77 (s, 3H); 4.10 (m, 2H); 4.23 (s(br), 1H); 4.27 (q, 2H, J = 7.2; Hz); 7.82 (s, 1H); 12.44 (s(br), 1H). HRMS: calcd for $C_{17}H_{22}ClN_5O_4S$ + H: 428.1159; found: 428.1169.

Methyl 2-(4-(N-methyl-3-propoxy-1H-pyrazole-5-carboxamido)piperidin-1-yl)thiazole-5-carboxylate (**21e**): By following the protocol described above, a residue was obtained which was in this case purified by two consecutive chromatography, the first one over silica gel (dichloromethane-ethanol from 97/3), the second one over alumina containing 1.5 % of water (dichloromethane-ethanol 96/4) to yield compound **21e** as a glass (0.14 g, 14 % from *N*-methylpiperidin-4-amine dihydrochloride). ^{1}H NMR (DMSO-*d6* at 90 °C): 0.98 (t, 3H, J =

7.5 Hz); 1.72 (m, 2H); 1.80 (m, 2H); 1.91 (m, 2H); 2.91 (s(br), 1/5 of 3H); 2.96 (s (br), 4/5 of 3H); 3.24 (m, 2H); 3.77 (s, 3H); 4.08 (m, 4H); 4.42 (s(br), 4/5 of 1H); 4.67 (s(br), 1/5 of 1H); 5.96 (s(br), 1H); 7.82 (s, 1H); 12.12 (s(br), 4/5 of 1H); 12.45 (s(br), 1/5 of 1H). HRMS: calcd for $C_{18}H_{25}N_5O_4S$ + H: 408.1706; found: 408.1729.

Methyl 2-(4-(3-butoxy-N-methyl-1H-pyrazole-5-carboxamido)piperidin-1-yl)thiazole-5-carboxylate (**21f**): By following the protocol described above, a residue was obtained which was in this case purified by two consecutive chromatography, the first one over silica gel (dichloromethane-ethanol from 97/3), the second one over alumina containing 1.5 % of water (dichloromethane-ethanol 96/4) to yield compound **21f** as a glass (0.22 g, 18 % from *N*-methylpiperidin-4-amine dihydrochloride). ^{1}H NMR (DMSO-*d6* at 90 °C): 0.95 (t, 3H, *J* = 7.4 Hz); 1.45 (m, 2H); 1.71 (m, 2H); 1.81 (m, 2H); 1.92 (m, 2H); 2.95 (s(br), 3H); 3.24 (m, 2H); 3.77 (s, 3H); 4.11 (m, 4H); 4.42 (s(br), 4/5 of 1H); 4.70 (s(br), 1/5 of 1H); 5.96 (s(br), 1H); 7.82 (s, 1H); 12.13 (s(br), 4/5 of 1H); 12.46 (s(br), 1/5 of 1H). HRMS: calcd for $C_{19}H_{27}N_5O_4S$ + H: 422.1862; found: 422.1879.

Methyl 2-(4-(3-isopropoxy-N-methyl-1H-pyrazole-5-carboxamido)piperidin-1-yl)thiazole-5-carboxylate (**21g**): By following the protocol described above, a residue was obtained which was in this case purified by two consecutive chromatography, the first one over silica gel (dichloromethane-ethanol from 97/3 to 96/4), the second one over alumina containing 1.5 % of water (dichloromethane-ethanol 96/4) to yield compound **21g** as a glass (0.26 g, 21 % from *N*-methylpiperidin-4-amine dihydrochloride). ^{1}H NMR (DMSO-*d6* at 90 °C): 1.30 (d, 6H, *J* = 6.1 Hz); 1.80 (m, 2H); 1.91 (m, 2H); 2.92 (s(br), 1/6 of 3H); 2.96 (s (br), 5/6 of 3H); 3.23 (m, 2H); 3.78 (s, 3H); 4.10 (m, 2H); 4.44 (s(br), 1H); 4.68 (s(br), 1H); 5.93 (s(br), 1H); 7.82 (s, 1H); 12.13 (s(br), 5/6 of 1H); 12.43 (s(br), 1/6 of 1H). HRMS: calcd for $C_{18}H_{25}N_5O_4S$ + H: 408.1706; found: 408.1739.

Methyl 2-(4-(N-methyl-3-(pentan-3-yloxy)-1H-pyrazole-5-carboxamido)piperidin-1-yl)thiazole-5-carboxylate (**21h**): By following the protocol described above, a residue was obtained which was in this case purified by two consecutive chromatography, the first one over silica gel (dichloromethane-ethanol from 97/3), the second one over alumina containing 1.5 % of water (dichloromethane-ethanol 96/4) to yield compound **21h** as a glass (0.30 g, 26 % from *N*-methylpiperidin-4-amine dihydrochloride). ^{1}H NMR (DMSO-*d6* at 90 °C): 0.93 (t, 6H, *J* = 7.6 Hz); 1.66 (m, 4H); 1.80 (m, 2H); 1.91 (m, 2H); 2.92 (s(br), 1/6 of 3H); 2.96 (s (br), 5/6 of 3H); 3.23 (m, 2H); 3.77 (s, 3H); 4.10 (m, 2H); 4.35 (s(br), 1H); 4.43 (s(br), 1H); 5.95 (s(br), 1H); 7.82 (s, 1H); 12.09 (s(br), 5/6 of 1H); 12.42 (s(br), 1/6 of 1H). HRMS: calcd for $C_{20}H_{29}N_5O_4S$ + H: 436.2019; found: 436.2049.

Methyl 2-(4-(4-bromo-*N*,3-dimethyl-1*H*-pyrazole-5-carboxamido)piperidin-1-yl)thiazole-5-carboxylate (**21i**): By following the protocol described above, a residue was obtained which was in this case purified by two consecutive chromatography, the first one over silica gel (dichloromethane-ethanol 95/5), the second one over alumina containing 1.5 % of water (dichloromethane-ethanol 95/5) to yield compound **21i** as a glass (0.18 g, 15 % from *N*-methylpiperidin-4-amine dihydrochloride). ^{1}H NMR (DMSO-*d6* at 90 °C): 1.80 (m, 2H); 1.92 (m, 2H); 2.24 (s, 3H); 2.86 (s, 3H); 3.23 (m, 2H); 3.77 (s, 3H); 4.10 (m, 2H); 4.29 (s(br), 1H); 7.82 (s, 1H); 12.90 (s(br), 1H). HRMS: calcd for $C_{16}H_{20}BrN_5O_3S$ + H: 442.0548; found: 442.0555.

Methyl 2-(4-(4-bromo-*N*-methyl-3-(trifluoromethyl)-1*H*-pyrazole-5-carboxamido)piperidin-1-yl)thiazole-5-carboxylate (**21j**): By following the protocol described above, a residue was obtained which was in this case purified by a chromatography over silica gel (dichloromethane-ethanol 96/4) followed by a dispersion in boiling toluene which led to pure compound **21j** (0.26 g, 19 % from *N*-methylpiperidin-4-amine dihydrochloride) as a white powder. ^{1}H NMR (DMSO-*d6* at 90 °C): 1.81 (m, 2H); 1.97 (m, 2H); 2.87 (s, 3H); 3.23 (m,

2H); 3.77 (s, 3H); 4.10 (m, 2H); 4.23 (s(br), 1H); 7.82 (s, 1H); 14.31 (s, 1H). HRMS: calcd for $C_{16}H_{17}BrF_3N_5O_3S$ + H: 496.0266; found: 496.0331.

2-(4-(*N*-Methyl-1*H*-pyrazole-5-carboxamido)piperidin-1-yl)thiazole-5-carboxylic acid (**9a**): Compound **21a** (0.11 g, 0.32 mmol) and lithium hydroxide (0.13 g, 3.1 mmol) were stirred in water (10 mL) and tetrahydrofuran (30 mL) overnight. This was concentrated to dryness, made acidic with hydrochloric acid and, since there was no precipitate in these cases, the aqueous phase was extracted with ethyl acetate five times. The organic layers were combined and washed with brine once, dried over magnesium sulfate and concentrated to dryness to yield compound **9a** as a white powder (0.01 g, 10 %). ^{1}H NMR (DMSO-*d6* at 90 °C): 1.91 (m, 4H); 2.99 (s, 3H); 3.18 (m, 2H); 4.10 (m, 2H); 4.62 (m, 1H); 6.55 (d, 1H, *J* = 2.2 Hz); 7.67 (d, 1H, *J* = 2.2 Hz); 7.75 (s 1*H*); 12.88 (s, 1H). HRMS: calcd for $C_{14}H_{17}N_5O_3S$ + H: 336.1130; found: 336.1145.

2-(4-(3-Ethoxy-*N*-methyl-1*H*-pyrazole-5-carboxamido)piperidin-1-yl)thiazole-5-carboxylic acid (**9b**): By using the procedure described above, this compound was obtained as a pale yellow powder (0.06 g, 89 %). ^{1}H NMR (DMSO-*d6* at 90 °C): 1.33 (t, 3H, *J* = 7.1 Hz); 1.91 (m, 4H); 2.96 (s, 3H); 3.21 (m, 2H); 4.09 (m, 2H); 4.16 (q, 2H, *J* = 7.1 Hz); 4.48 (m, 1H); 5.94 (s, 1H); 7.76 (s, 1H); 12.58 (s, 1H). HRMS: calcd for $C_{16}H_{21}N_5O_4S$ + H: 380.1393; found: 380.1395.

2-(4-(4-Chloro-*N*-methyl-1*H*-pyrazole-5-carboxamido)piperidin-1-yl)thiazole-5-carboxylic acid (**9c**): By using the procedure described above, this compound was obtained as a white powder (0.04 g, 59 %). ^{1}H NMR (DMSO-*d6* at 90 °C?) 1.81 (m, 2H); 1.90 (m, 2H); 2.86 (s, 3H); 3.15 (m, 2H); 4.08 (m, 2H); 4.27 (s(br), 1H); 7.74 (s, 1H); 7.83 (s, 1H). HRMS: calcd for $C_{14}H_{16}ClN_5O_3S$ + H: 370.0741; found: 370.0741.

2-(4-(4-chloro-3-ethoxy-N-methyl-1H-pyrazole-5-carboxamido)piperidin-1-yl)thiazole-5-carboxylic acid (**9d**): Compound **21d** (0.15 g, 0.35 mmol), lithium hydroxide (0.15 g, 3.5

mmol) were stirred in water (5 mL) and tetrahydrofuran (10 mL) overnight. This was concentrated to dryness. The residue was dissolved in a small amount of water saturated with sodium chloride and made acid with the minimum amount of 1N hydrochloric acid. The resulting suspension was extracted with ethyl acetate, the organic layer was washed with brine, dried over magnesium sulfate and concentrated to dryness. The residue was dispersed in a small amount of water, this was filtered and dried to give compound **9d** as a white powder (0.11 g, 75 %). ^{1}H NMR (DMSO-*d6* at 90 °C): 1.35 (t, 3H, *J* = 7.1 Hz); 1.81 (m, 2H); 1.92 (m, 2H); 2.87 (s, 3H); 3.18 (m, 2H); 4.08 (m, 2H); 4.22 (s(br), 1H); 4.27 (q, 2H, *J* = 7.1; Hz); 7.74 (s, 1H); 12.28 (s(br), 1H). HRMS: calcd for $C_{16}H_{20}ClN_5O_4S$ + H: 414.1003; found: 414.1021.

2-(4-(N-methyl-3-propoxy-1H-pyrazole-5-carboxamido)piperidin-1-yl)thiazole-5-carboxylic acid (**9e**): Compound **21e** (0.12 g, 0.29 mmol), lithium hydroxide (0.12 g, 2.8 mmol) were stirred in water (5 mL) and tetrahydrofuran (10 mL) overnight. This was concentrated to dryness. The residue was dissolved in a small amount of water saturated with sodium chloride and made acid with the minimum amount of 1N hydrochloric acid. The resulting suspension was extracted with ethyl acetate, the organic layer was washed with brine, dried over magnesium sulfate and concentrated to dryness to yield compound **9e** as a white powder (0.07 g, 60 %). ^{1}H NMR (DMSO-*d6* at 90 °C): 0.94 (t, 3H, *J* = 7.4 Hz); 1.73 (m, 2H); 1.80 (m, 2H); 1.91 (m, 2H); 2.96 (s, 3H); 3.20 (m, 2H); 4.06 (t, 2H, *J* = 6.6 Hz); 4.09 (m, 2H); 4.48 (m, 1H); 5.94 (s, 1H); 7.75 (s, 1H); 12.16 (s(br), 1H). HRMS: calcd for $C_{17}H_{23}N_5O_4S$ + H: 394.1549; found: 394.1538.

2-(4-(3-butoxy-N-methyl-1H-pyrazole-5-carboxamido)piperidin-1-yl)thiazole-5-carboxylic acid (**9f**): Compound **21f** (0.2 g, 0.47 mmol), lithium hydroxide (0.2 g, 4.7 mmol) were stirred in water (5 mL) and tetrahydrofuran (10 mL) overnight. This was concentrated to dryness. The residue was dissolved in a small amount of water saturated with sodium chloride and

made acid with the minimum amount of 1N hydrochloric acid. The resulting suspension was extracted with ethyl acetate, the organic layer was washed with brine, dried over magnesium sulfate and concentrated to dryness to yield compound **9f** as a white powder (0.09 g, 46 %). ^{1}H NMR (DMSO-*d6* at 90 °C): 0.94 (t, 3H, *J* = 7.3 Hz); 1.44 (m, 2H); 1.70 (m, 2H); 1.79 (m, 2H); 1.91 (m, 2H); 2.96 (s, 3H); 3.21 (m, 2H); 4.09 (m, 2H); 4.10 (t, 2H, *J* = 6.6 Hz); 4.48 (m, 1H); 5.94 (s, 1H); 7.75 (s, 1H); 11.80 (s(br), 1H). HRMS: calcd for $C_{18}H_{25}N_5O_4S$ + H: 408.1706; found: 408.1734.

2-(4-(3-isopropoxy-N-methyl-1H-pyrazole-5-carboxamido)piperidin-1-yl)thiazole-5-carboxylic acid (**9g**): Compound **21g** (0.24 g, 0.58 mmol), lithium hydroxide (0.24 g, 5.7 mmol) were stirred in water (5 mL) and tetrahydrofuran (10 mL) overnight. This was concentrated to dryness. The residue was dissolved in a small amount of water saturated with sodium chloride and made acid with the minimum amount of 1N hydrochloric acid. The resulting suspension was extracted with ethyl acetate, the organic layer was washed with brine, dried over magnesium sulfate and concentrated to dryness to yield compound **9g** as a white powder (0.13 g, 56 %). ^{1}H NMR (DMSO-*d6* at 90 °C): 1.30 (d, 6H, *J* = 6.3 Hz); 1.80 (m, 2H); 1.91 (m, 2H); 2.96 (s, 3H); 3.20 (m, 2H); 4.08 (m, 2H); 4.48 (m, 1H); 4.63 (sept, 1H, *J* = 6.3 Hz); 5.92 (s, 1H); 7.75 (s, 1H); 12.02 (s(br), 1H). HRMS: calcd for $C_{17}H_{23}N_5O_4S$ + H: 394.1549; found: 394.1562.

2-(4-(N-methyl-3-(pentan-3-yloxy)-1H-pyrazole-5-carboxamido)piperidin-1-yl)thiazole-5-carboxylic acid (**9h**): Compound **21h** (0.28 g, 0.64 mmol), lithium hydroxide (0.28 g, 6.6 mmol) were stirred in water (5 mL) and tetrahydrofuran (10 mL) overnight. This was concentrated to dryness. The residue was dissolved in a small amount of water saturated with sodium chloride and made acid with the minimum amount of 1N hydrochloric acid. The resulting suspension was dispersed in ethyl acetate and this now biphasic suspension was filtered, washed with water and ethyl acetate and dried under vacuum to yield compound **9h**

as a white powder (0.19 g, 70 %). ^{1}H NMR (DMSO-*d6* at 90 °C): 0.92 (t, 6H, *J* = 7.2 Hz); 1.65 (m, 4H); 1.74 (m, 2H); 1.89 (m, 2H); 2.95 (s(br), 1H); 3.0 (m, 2H); 4.01 (m, 2H); 4.27 (pent, 1H, *J* = 5.8 Hz); 4.43 (m, 1H); 5.90 (s, 1H); 7.26 (s, 1H); 12.24 (s(br), 1H). HRMS: calcd for $C_{19}H_{27}N_5O_4S$ + H: 422.1862; found: 422.1862.

2-(4-(4-Bromo-*N*,3-dimethyl-1*H*-pyrazole-5-carboxamido)piperidin-1-yl)thiazole-5-carboxylic acid (**9i**): Compound **21i** (0.13 g, 0.29 mmol), lithium hydroxide (0.12 g, 2.8 mmol) were stirred in water (10 mL) and tetrahydrofuran (30 mL) overnight. This was concentrated to dryness. The residue was dissolved in a small amount of water and made acid with the minimum amount of 1N hydrochloric acid. The resulting precipitate was filtered and dried under vacuum at 50 °C to yield acid **9i** as a white powder (0.06 g, 47 %). ^{1}H NMR (DMSO-*d6* at 90 °C): 1.80 (m, 2H); 1.92 (m, 2H); 2.23 (s, 3H); 2.85 (s, 3H); 3.15 (m, 2H); 4.07 (m, 2H); 4.28 (s(br), 1H); 7.74 (s, 1H). HRMS: calcd for $C_{15}H_{18}BrN_5O_3S$ + H: 428.0392; found: 428.0397.

2-(4-(4-Bromo-*N*-methyl-3-(trifluoromethyl)-1*H*-pyrazole-5-carboxamido)piperidin-1-yl)thiazole-5-carboxylic acid (**9j**): By following the protocol described above, this acid was obtained as a white powder (0.14 g, 72 %). ^{1}H NMR (DMSO-*d6* at 90 °C): 1.80 (m, 2H); 1.95 (m, 2H); 2.87 (s, 3H); 3.20 (m, 2H); 4.10 (m, 2H); 4.22 (s(br), 1H); 7.75 (s, 1H). HRMS: calcd for $C_{15}H_{15}BrF_3N_5O_3S$ + H: 482.0109; found: 482.0146.

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