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Potent and selective inhibitors of *Helicobacter pylori* glutamate racemase (MurI): Pyridodiazepine amines

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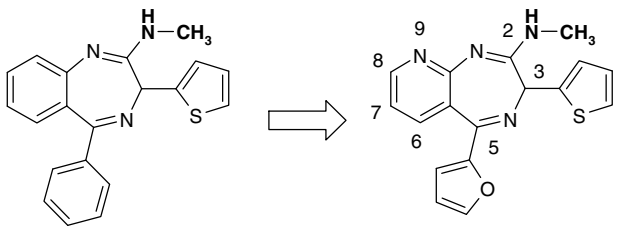
ABSTRACT

An SAR study of an HTS screening hit generated a series of pyridodiazepine amines as potent inhibitors of *Helicobacter pylori* glutamate racemase (MurI) showing highly selective anti-*H. pylori* activity, marked improved solubility, and reduced plasma protein binding. X-ray co-crystal E–I structures were obtained. These uncompetitive inhibitors bind at the MurI dimer interface.

It is well documented that *Helicobacter pylori* infection can cause chronic peptic ulceration and can increase the risk of gastric adenocarcinoma.[1] Novel anti-*H. pylori* agents with a new mode-of-action (MOA) would be highly beneficial for the patient community, as current therapies combining two antibiotics with a proton pump inhibitor (Triple Therapy) have numerous drawbacks, most notably broad-spectrum antibacterial activity leading to the disruption of commensal bacteria in the gastrointestinal (GI) tract and poor patient compliance.[2] Additionally, ever emerging resistant *H. pylori* strains have diminished the effectiveness of the combination therapies. Toward meeting this medical need, we chose a novel enzyme target for the design of an anti-*H. pylori* drug, namely that of glutamate racemase (MurI).[3] MurI is a bacterial cytoplasmic enzyme that catalyzes the conversion of L-glutamate to D-glutamate, one of the essential amino acids in peptidoglycan synthesis.[4] The disruption of peptidoglycan biosynthesis is lethal to bacteria and therefore inhibitors of glutamate racemase should be useful as antibacterials. The *murI* gene is conserved in all bacterial species[5,6] that synthesize peptidoglycan and its essentiality has been well-demonstrated in a number of bacteria.[7] The unique biophysical and biochemical properties of *H. pylori* MurI relative to the MurI of other bacteria could allow for the discovery and development of specific MurI inhibitors.[7] Such specificity would reduce the chances of disturbing off target commensal GI flora and diminish resistance development in other bacterial species as has been seen with the current therapies.

Previously, we reported potent and selective pyrazolopyrimidinedione *H. pylori* MurI inhibitors that were originally identified via high-throughput screening (HTS) of the former Astra compound collection.[3,7] The inhibitors additionally prevented *H. pylori* growth, but were not suitable for progression into animal efficacy experiments, as high plasma protein binding (PPB) compromised their capability to control disease in vivo.[3a]

Herein we report a second scaffold represented by the novel benzodiazepine amine **2** (Fig. 1) discovered by an HTS campaign



2 Hit **15** Lead

Figure 1. HTS hit **2** and lead **15**.

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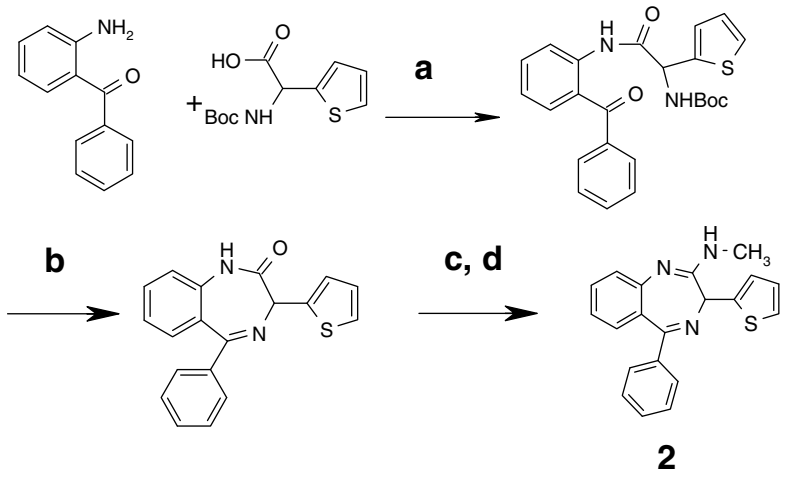
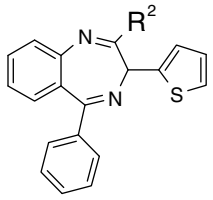
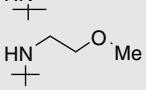
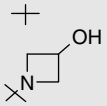


Scheme 1. Reagents and conditions: (a) DCC, CH_2Cl_2; (b) HBr, CH_3CO_2H and NH_4OAc; (c) Lawesson's reagent, dioxane; (d) $MeNH_2$, $HgCl_2$, THF.

of the former Zeneca compound collection. Compound **2** is highly selective against MurI of *H. pylori,* with an IC_{50} of 1.7 μM, versus isozymes from three other bacteria species: *Enterococcus faecalis, Escherichia coli and Staphylococcus aureus*, with IC_{50}s > 400 μM. This selectivity was maintained throughout the analog series described in this letter. Furthermore, we also observed corresponding microbiological selectivity. Compound **2** showed an MIC of 0.5 μg/mL against *H. pylori*, but demonstrated MICs > 64 μg/mL against a broad panel of bacteria that included the three bacteria species above from which the MurI isozymes had been isolated.[7]

Table 1
Effect of R^2 variations (Core = benzene, R^3 = 2′-thienyl, R^5 = phenyl)



Compound	R^2	IC_{50}[a] (μM)	MIC[b] (μg/mL)	Solu[c] (μM)	PPB[d]	Cl int[e] (μL/mL/kg)	
						Human	Mouse
1		400	32	0.6	98.0	>100	>100
2		1.7	0.5	0.5	99.7	43	97
3		1.1	2	4.0	99.7	46	>100
4		4.0	16	NT	NT	30	>100
5		1.1	8	NT	97.0	50	74
6		18.9	32	NT	NT	80	>100
7		0.5	0.25	0.3	99.0	49	>100
8		4.0	8	NT	NT	43	87

[a] MurI protein was expressed from *mur*I gene of chromosomal DNA of wild-type *Helicobacter pylori* strain J 99 (human isolate, Tenn. Genome Therapeutic Corporation, now Oscient Pharmaceuticals, Inc.) IC_{50} is the mean of duplicate determinations.
[b] *Helicobacter pylori* strain SS1, original Sydney strain from U.S.W. Australia; MICs were measured according to NCCLS guidelines and repeat MICs were generally within a twofold dilution range.
[c] Equilibrium solubility; NT, not tested.
[d] Experimental PPB value; NT, not tested.
[e] Liver microsomal clearance.

Substitution of the aromatic groups at C-3 of the benzodiazepine scaffold represents a novel chemotype relative to the plethora of benzodiazepines emanating substantially from CNS research.[15] Another novel feature is the embedded amidine functionality. In an effort to optimize this hit to a lead, we set out to improve solubility (<0.6 μM for **2**) and reduce plasma protein binding (PPB > 99% for **2**) deemed necessary for developing a viable drug for oral administration. Within this context we would need to retain the good enzyme inhibitory potency and antibacterial activity of **2**.

Here, we describe the structure–activity relationships (SAR) that led to the discovery and elaboration of a novel 9-aza lead series of potent and selective anti-*H. pylori* compounds as exemplified by Compound **15** (Fig. 1). The incorporation of a nitrogen atom into the benzodiazepine scaffold, forming a pyridodiazepine, adds another distinguishing feature relative to the vast knowledge base in the literature for this scaffold.

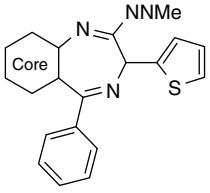
The synthesis of HTS hit **2** is shown in Scheme 1. The DCC amide coupling product of amino benzophenone with the Boc-protected thiophene amino acid was deprotected and cyclized to yield a benzodiazepinone intermediate that was converted to corresponding thioamide using Lawesson's reagent. Conversion to the amidine was achieved with mercury chloride and methylamine in THF. This sequence was applied analogously for the synthesis of the other benzodiazepine amines **1** and **3–9** (Tables 1 and 2).

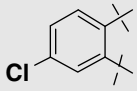
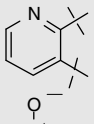
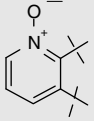
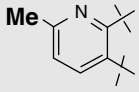
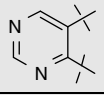
The synthesis of pyridodiazepine analogs is exemplified by that of **14** in Scheme 2. Lithiation of 2-fluoropyridine at the 3-position with LDA was followed by reaction with 2-thiophene carboxaldehyde. The resulting alcohol was oxidized with manganese dioxide[8] to yield the corresponding ketone. Subsequent amination, amide coupling, Boc-deprotection, and cyclization were followed by conversion to the amidine. The conversion to the amidine from pyridodiazepinone intermediate can also be performed in high yield with $TiCl_4$ and methylamine in a microwave reactor.[9,10] The Boc-protected amino acids used in this program are generally known in the literature and are easily prepared according to literature procedures.

Structures of all final products were established by both NMR and mass spectroscopy techniques. The structures of a number of compounds were also confirmed by small molecule X-ray crystallography (data not shown).

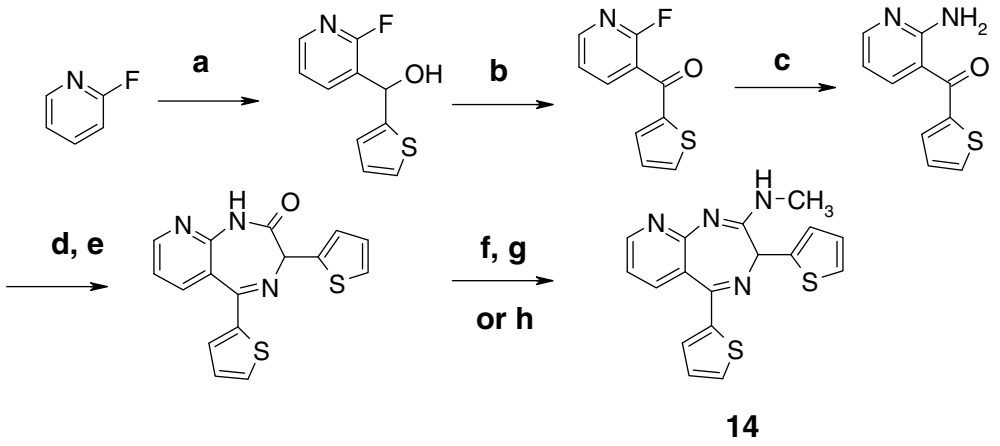
An X-ray structure of *H. pylori* MurI in a ternary complex with **2** and D-glutamate was obtained[16] demonstrating a distinctive non-competitive binding interaction at the enzyme dimer interface (Picture 1 left). Previously, the X-ray structure of a ternary MurI, D-glutamate, pyrazolopyrimidinedione inhibitor complex was described.[7] However, though MurI requires glutamate for binding both the benzodiazepine and pyrazolopyrimidinedione classes of inhibitors, the binding sites for the two are distinct from one another. Unlike other Gram-negative MurI, *H. pylori* MurI exists in a homodimer, more similar to Gram-positive MurI isozymes. However, the dimer interface in the Gram-positive isozymes is situated backside to the glutamate binding site while for *H. pylori* MurI it abuts the substrate glutamate.[7] The rather unusual inhibitor-binding site might not have been recognized *a priori,* but rather required the identification of an inhibitor through HTS. To our knowledge, these are the first of such unique binding interactions of MurI/inhibitor. This result once again demonstrated the power

Table 2
Effect of core variations (R^2 = methylamino, R^3 = 2′-thienyl, R^5 = phenyl)



Compound	Core	IC_{50}[a] (μM)	MIC[b] (μg/mL)	Solu[c] (μM)	PPB[d]	Cl int[e] (μL/mL/kg)	
						Human	Mouse
9		13.0	8	NT	NT	52	>100
10		2.2	0.17	98	95.9	40	82
11		67.0	16	NT	64.7	<14	41
12		1.7	0.25	70	96.1	73	>100
13		50	64	NT	84.2	36	26

[a] MurI protein was expressed from *mur*I gene of chromosomal DNA of wild-type *Helicobacter pylori* strain J 99 (human isolate, Tenn. Genome Therapeutic Corporation, now Oscient Pharmaceuticals, Inc.) IC_{50} is the mean of duplicate determinations.
[b] *Helicobacter pylori* strain SS1, original Sydney strain from U.S.W. Australia; MICs were measured according to NCCLS guidelines and repeat MICs were generally within a twofold dilution range.
[c] Equilibrium solubility; NT, not tested.
[d] Experimental PPB value; NT, not tested.
[e] Liver microsomal clearance.



Scheme 2. Reagents and conditions: (a) LDA, THF, −78 °C, 2-thiophene carboxaldehyde; (b) MnO_2, THF reflux; (c) 7 M NH_3, methanol, in sealed tube; d, e, f and g are same as a, b, c and d in Scheme 1; (h) $TiCl_4/MeNH_2$ in CH_2Cl_2.

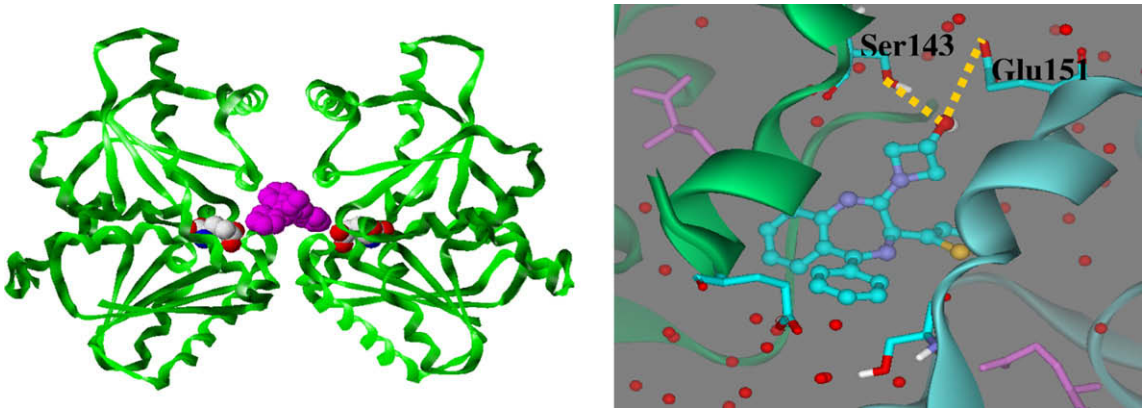
of HTS in discovering inhibitors that otherwise would be difficult to design.

The SAR around **2** was first explored through selective variation of substituents around the diazepine scaffold and benzene core as allowed by the constraints of the inhibitor-binding pocket delineated in the X-ray structure. As shown in Table 1 (compounds **1–8**), variations of the 2-position amine (R^2) did not produce any compounds that markedly improved solubility or decreased PPB. For inhibitory potency, only small alkylamines were tolerated, with methylamino remaining the best. Inhibitor potency and antibacterial activity were retained with the hydroxy azetidine substituent in compound **7** in an attempt to build an H-bonding interaction. An X-ray co-crystal structure of **7** with MurI revealed H-bonding interactions between the azetine hydroxyl and both Ser143 and Glu151 (Picture 1, right).[16] Initial SAR activity at R^2 aiming to address microsomal stability issues of the series, was unsuccessful (data not shown).

It should be noted that compounds **1–25** are all racemic. The *R*- and *S*-isomers of **2** were separated by chiral chromatography with the greater MurI enzyme inhibitory potency residing in the one enantiomer (sevenfold difference). However, the two optical antipodes showed identical MICs. For compound **10,** the same trend was observed with a 42-fold difference in IC_{50} and only four-fold difference in MIC. We speculated this observation might be the result of racemization and it was shown that racemization did occur slowly (28% racemization over a 3-week period in methanol at room temperature for compound **2** vs 11% for compound **10** over same period), and the racemization may well be faster under physiological conditions. Introduction of a second substitution at C3 was attempted, to block the racemization,[14] without success.

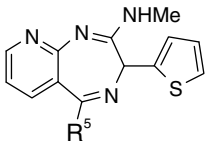
Addition of a single chlorine atom to the 8-position of **2** (compound **9**) (Table 2) is not well tolerated, in contrast to the established SAR[15] trends for CNS-active benzodiazepines where 8-substitution offers improvement. By way of contrast, a single methyl substitution in the analogous 7-position (exemplified in pyridodiazepine **12** relative to **10**) is well tolerated and gives an improvement in MurI activity. Selected benzodiazepines and pyridodiazepines herein did not inhibit GABA receptors.

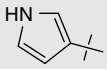
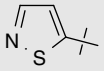
To improve solubility, a nitrogen atom was incorporated into the core scaffold affording a pyridodiazepine amine (compound **10**) with a reduced Log*D* value (2.7 vs 4.1 of compound **2**). MurI inhibitory potency and antibacterial activity were retained. More importantly, the solubility and PPB characteristics are markedly improved (compare **2** with **10**). An X-ray co-crystal structure of **10** with MurI shows a water molecule bridging the pyridine nitrogen to bound D-Glu product in neighboring active site. Hence, the *N*-oxide analog (**11**) was considerably less potent as an inhibitor, disrupting the water-to-glutamate bridge. The 8-Me substituted analog (**12**), in which the methyl may increase the hydrogen bonding capability of 9-aza nitrogen lone pair, was slightly more active compared with **10**. Interestingly, introduction of two nitrogen atoms (**13**), which was expected to in-



Picture 1. (Left) Co-crystal structure shows that compound **2** (purple) binds at MurI enzyme (green) dimer interface. Two D-Glu products are shown at the active sites (Red, white, blue balls). (right): Weak H-bonds (yellow dotted lines) between the hydroxyl group of compound **7** and both Serine-143 and Glutamate-151 of MurI enzyme. The discrete red balls are water molecules.

Table 3
Effect of R^5 variations (Core = pyridine, R^2 = methylamino, R^3 = 2′-thienyl)



Compound	R^5	IC_{50}[a] (μM)	MIC[b] (μg/mL)	Solu[c] (μM)	PPB[d]	Cl int[e] (μL/mL/kg)	
						Human	Mouse
14		0.7	0.25	227	97.0	30	>100
15		2.0	0.19	1365	81.8	13	30
16		1.7	1.0	39	83.0	<14	33
17		7.7	1.0	211	74.3	<5	19
18		0.6	0.40	1730	75.4	19	36
19		4.1	8	16	95.2	59	>100
20		1.1	0.32	176	75.9	18	37

[a] MurI protein was expressed from *mur*I gene of chromosomal DNA of wild-type *Helicobacter pylori* strain J 99 (human isolate, Tenn. Genome Therapeutic Corporation, now Oscient Pharmaceuticals, Inc.) IC_{50} is the mean of duplicate determinations.
[b] *Helicobacter pylori* strain SS1, original Sydney strain from U.S.W. Australia; MICs were measured according to NCCLS guidelines and repeat MICs were generally within a twofold dilution range.
[c] Equilibrium solubility; NT, not tested.
[d] Experimental PPB value; NT, not tested.
[e] Liver microsomal clearance.

crease solubility and reduce PPB, diminished both MurI enzymatic and antibacterial activity.

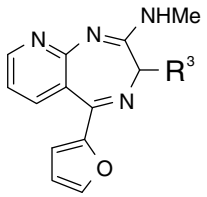
Variation of the substituent in the 5-position (R^5) with 5- and 6-membered aromatic rings generally retains inhibitory potency and antibacterial activity (see Table 3, compounds **14–20**). Both cylic (**19**) and acyclic (data not shown) alkyl substituents at R^5 diminished inhibitory potency considerably. This may suggest that this site favors a flat electron-rich π system.[11,12] Five-membered aromatic heterocycles oftentimes improved solubility and increased the free fraction (compare **10** with **15**).[12] There was also a general improvement in microsomal stability in moving from benzene to heterocyclic R^5 subsituents.

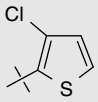
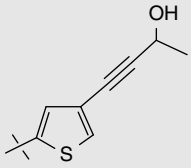
The substitution at 3-position (R^3) (see Table 4, compounds **21–25**) tolerated a variety of 5-membered aromatic rings. Phenyl and pyridyl rings are less active. This region of the R3 binding site is quite constrained and can only tolerate small substituents except where there is a 1,3-relationship to the azepine ring bond connection, such as 4′-position substituted thiophene. The region here leads to a hydrophilic water channel that further leads to solvent. Larger substituents occupying this region (as in **22**) are tolerated but less active. Overall, considering MICs, solubility and PPB, the unsubstituted 2-thiophene group at R^3 was viewed to be preferred. However, thiophenes can often be metabolic and safety liabilities.[13] An extensive effort of searching for an alternative to the direct C3-linked 2′-thiophene group was conducted, unfortunately, in most cases, the modification resulted in both enzymatic and antibacterial potency losses. This was mirrored by the early SAR studies at this position in the original hit series where core = benzene, R^5 = phenyl, R^2 = MeNH, R^3 = alkyls and aromatic substituted alkyls (data not shown).

In general, a good correlation between MurI enzyme inhibition (IC_{50}) and whole-cell growth inhibition (MIC) was observed. In contrast to the previously reported pyrazolopyrimidinedione series,[3] the current series was not affected by efflux pumps (such as *hefB*$^-$), therefore an excellent whole cell activity was observed in spite of moderate MurI enzyme potency. Studies to evaluate emergence of resistance demonstrated the series has a moderate tendency of spontaneous resistance generation (resistance rate $<10^{-7}$). Mode-of-action (MOA) studies using both a MurI-overexpressing strain and strains with point mutations in MurI confirmed the whole cell activity was the result of inhibition of MurI enzyme in the cell. The cidal killing kinetic was comparable to the β-lactam Amoxicillin.[17]

In summary, we discovered a novel series of uncompetitive MurI inhibitors with potent and selective anti-*H. pylori* activities and a unique binding interaction. A privileged pyridodiazepine scaffold provided a drugable lead that could be further developed into a viable mono-therapeutic agent. Combination of the pyridine core with 5-membered aromatic R^5 heterocycles improved physical properties while retaining enzymatic and antibacterial potency and selectivity. A set of compounds with reasonably low protein binding and improved solubility (**15**, **18** and **20**) were identified.

Table 4
Effect of R^3 variations (Core = pyridine, R^2 = methylamino, R^5 = 2′-furyl)



Compound	R^3	IC_{50}[a] (μM)	MIC[b] (μg/mL)	Solu[c] (μM)	PPB[d]	Cl int[e] (μL/mL/kg)	
						Human	Mouse
21		2.8	0.5	191	98.7	74	>150
22		4.9	4.0	201	90.9	7	17
23		8.6	4.0	196	60.9	22	32
24		8.7	2.0	>1000	73.6	26	27
25		18.5	8.0	192	80.7	9	19

[a] MurI protein was expressed from *mur*I gene of chromosomal DNA of wild-type *Helicobacter pylori* strain J 99 (human isolate, Tenn. Genome Therapeutic Corporation, now Oscient Pharmaceuticals, Inc.) IC_{50} is the mean of duplicate determinations.
[b] *Helicobacter pylori* strain SS1, original Sydney strain from U.S.W. Australia; MICs were measured according to NCCLS guidelines and repeat MICs were generally within a twofold dilution range.
[c] Equilibrium solubility; NT, not tested.
[d] Experimental PPB value; NT, not tested.
[e] Liver microsomal clearance.

Improvement in both in vitro and in vivo pharmacokinetic profiles is necessary to optimize the lead toward the design of a viable drug for human utility.[18]

Acknowledgments

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References and notes

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10. Typical synthesis of pyridodiazepine Amine (**14**).*Step a:* (2-Fluoropyridin-3-yl)(2-thienyl)methanol To a solution of 2-fluoropyridine (2.0 g, 20.6 mmol) in anhydrous THF (5 mL) was added a solution of 2 M LDA (heptane/THF, 12 mL, 24 mmol) at −78 °C under nitrogen. An orange suspension was formed. To the above suspension was added a solution of 2-thiophene carboxaldehyde in THF (5 mL). The reaction was stirred at −78 °C for 2 h and was allowed to warm to room temperature overnight. The reaction was worked up as follows: the reaction mixture was poured into an ice water mixture and was extracted with ethyl acetate (3 × 100 mL). The combined organic layers were dried over magnesium sulfate and purified using Flash Master silica-gel chromatography with ethyl acetate/hexane as eluent. The product was dried under reduced pressure to yield a yellow oil (2.6 g, yield, 63%). MS (ES^+) 210 (MH^+) for $C_{10}H_8FN$ OS.*Step b:* (2-Fluoropyridin-3-yl)(2-thienyl)methanone *S*(2-fluoropyridin-3-yl)(2-thienyl)methanol (2.6 g, 12.3 mmol) was refluxed with manganese (IV) oxide (3.0 g, 33.2 mmol) in THF (14 mL) for 48 h. The reaction mixture was filtered and the product was purified by silica-gel chromatography using ethyl acetate/hexane as eluant. The desired fractions were collected, concentrated and dried under reduced pressure to give (2-fluoropyridin-3-yl)(2-thienyl)methanone as a yellow solid (2.25 g, yield,

87%). MS (ES+) 208 (MH+) for $C_{10}H_6FN$ OS. *Step c:* (2-aminopyridin-3-yl)(2-thienyl)methanone (2-fluoropyridin-3-yl)(2-thienyl)methanone (2.25 g, 10.87 mmol) was treated with ammonia methanol solution (7 N) with moderate heating (or conducted in a bomb reaction at 40 °C). After heating overnight, the reaction mixture was concentrated and purified on Flash Master silica-gel chromatography using ethyl acetate/hexane as eluant. The desired fractions were collected, concentrated and dried under reduced pressure to give (2-aminopyridin-3-yl)(2-thienyl)methanone as a yellow solid (1.73 g, yield, 78%). MS (ES^+) 205 (MH^+) for $C_{10}H_8N_2$ OS. *Step d and e:* 3,5-Di-2-thienyl-1, 3-dihydro-2H-pyrido[2,3-e][1,4]diazepin-2-one to a solution of (2-aminopyridin-3-yl)(2-thienyl)methanone (0.88 g, 4.31 mmol) and N-Boc amino (2-thienyl)acetic acid (2.37 g, 9.22 mmol) in anhydrous dichloromethane (15 mL) was added a solution of DCC (2.97 g, 8 mmol) in anhydrous dichloromethane (10 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 3 h and was allowed to warm to room temperature overnight. The suspension was filtered and the filtrate was concentrated and purified on Flash master silica-gel chromatography using ethyl acetate/hexane as eluant yielding a white solid.The coupling product was then treated with a TFA solution (30 mL, 30%) in dichloromethane at 0 °C for 1 h and continued stirring for 1 h at room temperature. After deprotection was complete (monitored by LC-MS) the reaction mixture was concentrated to remove all solvent and TFA. The oil like deprotected product was then dissolved with 30 mL of acetic acid and mixed with ammonium acetate solid. The suspension was heated to 60 °C for 12 h. After removing the acetic acid, product 3,5-di-2-thienyl-1, 3-dihydro-2H-pyrido[2,3-*e*][1,4]diazepin-2-one was purified on Flash master silica-gel chromatography using ethyl acetate/hexane as eluant and dried under reduced pressure to give a brown solid (0.48 g, yield, 69%) 1H NMR (300 MHz, DMSO-d_6) δ (ppm): 5.55 (d, 1 H); 7.15–7.70 (m, 4H); 7.37 (m, 1H); 7.52 (d, 1H); 7.80 (d, 1H); 8.20 (d, 1H); 8.60 (d, 1H); 11.19 (s, 1H). MS (ES+) 326 (MH+) for $C_{16}H_{11}N_3$ OS_2. *Step f:* 3,5-Di-2-thienyl-1,3-dihydro-2*H*-pyrido[2,3-*e*][1,4] diazepine-2-thione 3,5-di-2-thienyl-1,3-dihydro-2*H*-pyrido[2,3-*e*][1,4]-diazepin-2-one (0.48 g, 1.476 mmol) was refluxed with Lawesson's reagent (1.0 g, 2.47 mmol) in dioxane for 12 h. The reaction mixture was then concentrated and the product was purified on Flash master silica-gel chromatography using ethyl acetate/hexane as eluant. The desired fractions were dried under reduced pressure to give a yellow solid (0.19 g, yield, 38%). MS (ES^+) 342 (MH^+) for $C_{16}H_{11}N_3S_3$. *Step g:* *N*-Methyl-3,5-di-2-thienyl-3*H*-pyrido[2,3-*e*][1,4]diazepin-2-amine (compound **14**) 3,5-di-2-thienyl-1, 3-dihydro-2*H*-pyrido[2,3-*e*][1,4]diazepine-2-thione (0.19 g, 0.557 mmol) was dissolved in a 2 M methylamine solution (10 mL, 20 mmol) in THF. The mixture was heated to 60 °C for 15 min. The reaction mixture was concentrated and purified on Flash Master silica-gel chromatography using ethyl acetate/hexane as eluant and dried under reduced pressure to give a white solid (55 mg, yield, 31%) ^{1}H NMR (300 MHz, CHLOROFORM-d) δ (ppm): 2.94 (d, J = 4.58 Hz, 3H); 4.92(s, 1H); 5.13 (s, br, 1H); 7.0–7.2 (m, 5H); 7.47 (dd, J = 13.6, 4.54 Hz, 2H); 8.05 (d, J = 7.59 Hz, 1H); 8.71 (d, J = 3.70 Hz, 1H). MS (ES^+) 339 (MH^+) for $C_{17}H_{14}N_4S_2$.For more experimental details of other compounds described in this letter, see: Basarab, G.; Eyermann, C.; Geng, B.; Gowravaram, M.; Loch, J.; MacPherson, L.; Morningstar, M.; Mullen, G.; Satz, A.; Kiely, A. PCT Int. Appl. WO200511101, **2005**.

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