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A new class of small molecule RNA polymerase inhibitors with activity against Rifampicin-resistant *Staphylococcus aureus*[1]

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Abstract—The RNA polymerase holoenzyme is a proven target for antibacterial agents. A high-throughput screening program based on this enzyme from *Staphylococcus aureus* had previously identified a 2-ureidothiophene-3-carboxylate as a low micromolar inhibitor. An investigation of the relationships between the structures of this class of compounds and their inhibitory- and antibacterial activities is described here, leading to a set of potent RNA polymerase inhibitors with antibacterial activity. Characterization of this bioactivity, including studies of the mechanism of action, is provided, highlighting the power of the reverse chemical genetics approach in providing tools to inhibit the bacterial RNA polymerase.

1. Introduction

Transcription is essential for growth and survival and is catalyzed by the DNA-dependent RNA polymerase (RNAP) core enzyme.[2] In bacteria, this protein complex associates with one of a variety of sigma factors to form the RNA polymerase holoenzyme with specificity for a given set of promoters. The essential role of the RNAP sigma factor has been established through the intracellular production, under regulated expression of bacteriophage inhibitory peptides targeting the sigma factor of *Staphylococcus aureus*,[3] an important pathogen involved in a number of bacteremias.[4] In addition, the RNAP has been validated as a drug target through its inhibition by the Rifamycin class of antibacterial agents, some of which are used clinically.[5] There have been few reports of other classes of RNA polymerase inhibitors as antibacterial agents, but in nearly all cases, these were complex natural products.[6] Small molecule RNAP inhibitors have been recently reported for *Escherichia coli*, but their inability to eradicate the host bacterium was also noted.[7a] Lately a set of inhibitors of the core-sigma factor interaction displaying antibacterial activity have been disclosed.[7b] Herein is described the development of a new class of compounds, with antibacterial activity, acting via the *S. aureus* RNAP holoenzyme.

The screening of a library of 250,000 commercially available compounds against the *S. aureus* RNAP holoenzyme was performed with a functional assay measuring the incorporation of α-[^{32}P]-UTP in acid-precipitable counts. This has allowed the identification of the substituted 2-ureidothiophene-3-carboxylate **1** as a low micromolar inhibitor (Fig. 1).[3] It displayed good antibacterial

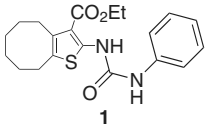

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Figure 1.

Keywords: 2-Ureidothiophene-3-carboxylates; RNA polymerase; Antibacterial agents; Reverse chemical genetics.
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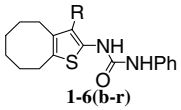
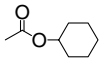
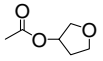
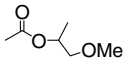
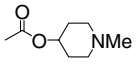
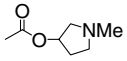
Table 1. Inhibitory activity against the *Staphylococcus aureus* RNAP enzyme in vitro and antibacterial activities of compounds **1** and **6b–r**



1-6(b-r)

Compound	Aminothiophene precursor	Substituent R	IC_{50} (μM), *S. aureus* RNAP	MIC (μg/mL), *S. aureus* ATCC 13709	MIC (μg/mL), *S. aureus* RN4220
1	**5a**	$-CO_2Et$	1	1	>128
6b	**5b**	$-CO_2Me$	2	>16	>16
6c	**5c**	$-CO_2{}^{i}Pr$	0.04	0.5	1
6d	**5d**	$-CO_2{}^{t}Bu$	0.17	1	>128
6e	**5e**	$-CO_2{}^{i}Bu$	0.14	>32	>32
6f	**5f**	$-CO_2Bn$	8	>16	>16
6g	**5g**	$-CO_2All$	0.20	>128	>128
6h	**5h**		0.36	>16	>16
6i	**5i**		0.14	0.5	>16
6j	**5j**		0.21	0.5	1
6k	**5k**		1.25	>8	>8
6l	**5l**		1.6	>128	>128
6m	**5m**		1.24	32	32
6n	**5n**	$-CO{}^{i}Pr$	5	>8	>8
6o	**5o**	$-CO{}^{i}Bu$	1.5	>16	>16
6p	**5p**	–CN	>100	>128	>128
6q	**5q**	$-CONH{}^{i}Pr$	>100	>16	>16
6r		$-CO_2H$	31	64	128
	Rifampicin	—	nd	0.008	0.008
	Vancomycin	—	nd	1	1
	Linezolid	—	nd	2	1

nd, not determined.

activity against *S. aureus* ATCC 13709 and *S. epidermidis* (MICs 1 and 0.25 μg/mL, respectively, Table 1) but it lacked activity against four laboratory strains of *S. aureus* (MIC > 16 μg/mL). Compound **1** lacked Gram-negative activity and the presence of 50% human or mouse serum or 4% human serum albumin abolished all activity. It did not display cytotoxicity in vitro against the HeLa cell line in an MTS assay[8] or against primary human hepatocytes in an ATP assay.[9]

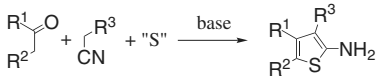
These results made **1** an interesting starting point for the development of antistaphylococcal agents.

2. Chemistry

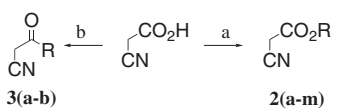
A number of analogs of **1** can be readily prepared via the Gewald reaction,[10] a three-component condensation route to thiophenes, involving a carbonyl compound, an activated nitrile, and elemental sulfur (Scheme 1).

Several such activated nitriles have been prepared for use in this reaction. The cyanoacetic acid esters **2** were prepared from cyanoacetic acid either through an acid catalyzed (Fischer) esterification or via a DCC coupling (Scheme 2).[11] α-Cyanoketones **3** were produced through the condensation of the dilithium salt of cyanoacetic acid with an acid chloride, followed by decarboxylation (Scheme 2).[12]

The cyanoacetamide **4** can be prepared from ethyl cyanoacetate by simple treatment with isopropylamine (Scheme 3).[13]

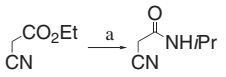


Scheme 1. The Gewald reaction.



3(a-b) **2(a-m)**

Scheme 2. Reagents and conditions: (a) PhH, H_2SO_4, Δ (60%—quant.), or DCC, MeCN (52–79%); (b) 4 equiv ^{n}BuLi, −78 °C, then RCOCl (72%).



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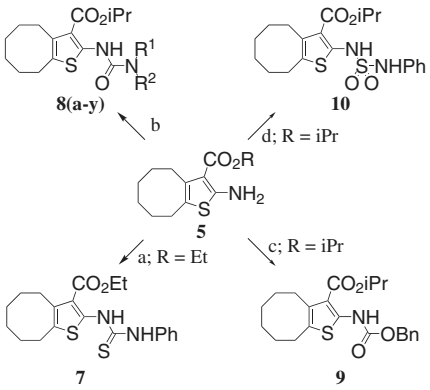
Scheme 3. Reagent: (a) $^{i}PrNH_2$, neat (quant.).

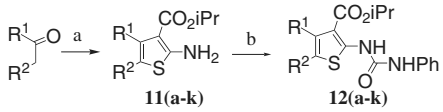
The treatment of cyclooctanone with these easily accessible activated nitriles, as well as with commercially available ones, provides 2-aminothiophenes **5** (Scheme 4). Subsequent reaction of these compounds with phenylisocyanate provides their parent ureas **6**. The condensation to furnish compound **5q** required two steps rather than the single-step Gewald reaction. Ureidothiophene **6m** was prepared from aminothiophene **5m** by treatment with phenylisocyanate, followed by Boc deprotection and reductive methylation. Acid **6r** was obtained by the hydrolysis of the *tert*-butyl ester **6d**.

The aminothiophene **5a** with an ethyl ester substitution at position 3 was treated with phenylthioisocyanate to provide the thiourea **7** (Scheme 5). The aminothiophenes **5a**, **5c**, and **5j** with varying ester substituents at position 3 were also reacted with a number of isocyanates or with triphosgene in the presence of triethylamine,[14] followed by an amine to afford ureas **8** (Scheme 5). Treatment of **5c** with benzyl chloroformate and triethylamine provided the carbamate **9**, whereas reaction with phenylsulfamidyl chloride[15] in the presence of base resulted in the sulfuric diamide **10**.

The Gewald reaction was also used with isopropyl cyanoacetate and a variety of ketones to provide aminothiophenes **11** and eventually ureidothiophenes **12** (Scheme 6).



Scheme 5. Reagents and conditions: (a) phenyl thioisocyanate, pyridine, 50 °C (76%); (b) isocyanate, pyridine, 50 °C (15–77%); or triphosgene, Et_3N, CH_2Cl_2 then amine (17–44%); (c) benzyl chloroformate, Et_3N, CH_2Cl_2 (47%); (d) phenyl sulfamyl chloride, Et_3N, CH_2Cl_2 (36%).



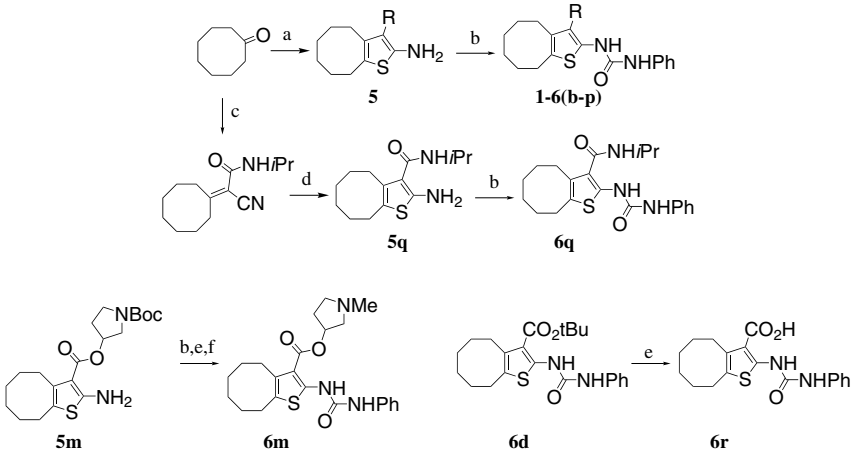
Scheme 6. Reagents and conditions: (a) isopropyl cyanoacetate, S_8, amine base (17–89%); (b) phenylisocyanate, pyridine, 50 °C (15–77%).

3. In vitro evaluation of inhibitor structure–activity relationships

To assay the inhibitory activity of the compounds against the RNAP, the synthesis of RNA by the *S. aureus* RNAP holoenzyme was measured in the presence of the compounds by scintillation counting of TCA-precipitable $\alpha[^{32}P]$-UTP using the same assay format that was used to screen the diversity library.[3,16]

Given the strain dependence of the antibacterial activity of **1**, analogs were screened against both the ATCC 13709 and the RN4220 strains of *S. aureus*.

The activity of the analogs of **1** differentiated by their substituents at position 3 (compounds **1** and **6b–r**) is presented in Table 1. For comparison, the antibacterial activities on these strains for Rifampicin, Vancomycin, and Linezolid are also indicated in this table.



Scheme 4. Reagents and conditions: (a) activated nitrile, S_8, amine base (23–88%); (b) phenylisocyanate, pyridine, 50 °C (25–89%); (c) **4**, NH_4OAc, AcOH (16%); (d) S_8, morpholine, EtOH, 70 °C (91%); (e) TFA/CH_2Cl_2 (quant.); (f) HCO_2H, CH_2O, Δ (10%).

It is clear from this study that bulky esters at position 3 greatly favor the inhibitory activity of these compounds. An optimum is however obtained with the isopropyl ester **6c**. Ketones **6n–o**, the nitrile **6p**, the amide **6q**, and the carboxylate **6r** are inadequate replacements. The antibacterial activity of this class of compounds closely matches the inhibitory activities with all of the submicromolar compounds displaying at least activity against *S. aureus* ATCC 13709. Two compounds, **6c**,**j**, stand out as being active against both strains. Although this does not come as a surprise for **6c**, the most potent inhibitor, the activity of **6j** is slightly more difficult to explain. Thus, the closely related **6i**, with its improved potency against RNAP, fails to display activity against the RN4220 strain. A key difference between these two compounds is their polarity with c$\log D$ values[17] calculated at pH 7.4 to be 5.84 and 5.00 for **6i** and **6j**, respectively.

It is noteworthy that the antibacterial activity does not respond incrementally to the structural modifications. Compounds either display antimicrobial activities of 0.5–1 μg/mL or are without activity.

Derivatives with different substituents at position 2 were evaluated in the same manner (Table 2). Replacement of the urea functionality in **1** by a thiourea (compound **7**) is clearly detrimental, as is its removal (compound **5c**). Differently substituted 2-(phenylureido)thiophenes (**8a–g**) display fairly similar inhibitory activities toward RNAP, but the introduction of substituents abolishes the antibacterial activity. The exceptions are *ortho* substituents, such as **8f** and **8g** which are clearly negatively impacting the inhibitory activity of the compounds. Replacement of the phenyl group in **6c** with a benzyl group (compound **8j**) or by similar aliphatic groups (compounds **8h**,**i**) is also unfavorable.

The trend observed with the ester substituents, whereby higher polarity resulted in improved antibacterial activity, is noticeable in this case as well. Thus, whereas compounds **8a** (c$\log D$ 6.27) and **8c** (c$\log D$ 5.56) display similar inhibitory activities, **8a** has no antibacterial activity, while **8c** is effective against the ATCC13709 strain. Compounds **6c** (c$\log D$ 5.72) and **8d** (c$\log P$ 6.50) display the same tendency.

This trend has directed our attention to heterocyclic substituents as replacements for the phenyl group of **6c**. A 4-pyridyl group (compound **8k**) results in a slight decrease in inhibitory activity, but, interestingly, the 2-pyridyl or 2-pirazinyl substituents (compounds **8l**,**m**) completely abolish all inhibition. This effect may be explained by the ability of these two substituents to form an intramolecular hydrogen bond (Fig. 2) resulting in a presumably unfavorable conformation. The 4-pyridyl substituent, unable to form such a hydrogen bond, is more flexible and can therefore adopt the conformation required by the binding site. This clearly relates to the earlier observation that *ortho*-substituted phenyl groups are also inactive.

The replacement of the phenyl substituent on the urea by the bioisosteric thiophenes[18] (compounds **8n**,**o**) resulted in good inhibitors with antibacterial activities in both strains, as expected. Surprisingly, the use of a thiazolyl substituent led to a compound that, while maintaining the potent RNAP-inhibitory activity associated with the thiophenes, completely lacked antibacterial activity.

The substituted thiophenyl ureas behaved much like the parent aryls, in the sense that 1,4-substitution (compound **8q**) maintained the inhibitory potency but abolished the antibacterial activity, while 1,2-substitution ('*ortho*') (compounds **8r**,**s**) resulted in complete loss of inhibitory activities. The same outcome is expectedly observed with the isoxazole substituent in **8t**.

N,*N*′,*N*′-Trisubstituted ureas such as **8u** and **8v** display a complete lack of inhibition of RNAP, and so does the carbamate **9**, which once more draws attention to the role of the urea in interacting with the binding site. From that perspective, compound **8w** is a little puzzling, as it does display some activity, perhaps pointing to the possibility of an alternative binding mode. The crucial impact of the urea functionality is further demonstrated with the sulfuric diamide **10** whose complete lack of activity clearly stems from either its weaker H-bonding ability or the differences in geometry between the two functionalities.

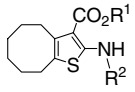
Given the improved antibacterial activities associated with the more polar substituents at positions 2 and 3 of the thiophenes, it was reasoned that the combination of a thiophenyl urea as in **8n** and **8o** and a tetrahydrofuranyl ester as in **6j** would result in active compounds. Indeed, compounds **8x**,**y** do display both good inhibitory and antibacterial activities, although the weaker of the two (**8y**) eradicates only *S. aureus* ATCC 13709.

To further examine the role of the functional groups in relation to the inhibitory and antibacterial activities of this class of compounds, analogs **12a–k**, with replacements for the 8-membered carbocyclic moiety, were also assessed (Table 3).

This table shows that the inhibitory activity of this class of compounds relates to the ring size. The activity is optimal with compounds **6c** (8-membered ring) and **12b** (9-membered ring), and decreases with compounds **12a** (7-membered ring) and **12c** (10-membered ring). The antibacterial activity follows suit with **6c** and **12b**, but not **12a** and **12c**, displaying an ability to eradicate the two strains. The pattern observed previously, with either good (1 μg/mL) antibacterial activity or none at all, is particularly obvious in this case.

The contrast with the positive results, in terms of antibacterial activity, obtained by the introduction of increased polarity in positions 2 and 3, is fairly marked in this case. Smaller rings with polar groups (compounds **12d**,**e**), a heteroatom bridge (**12f**), non-fused rings (compounds **12g**,**h**) or acyclic groups (compounds **12i–k**), all gave a similar decrease in the inhibitory activity back to the micromolar range. Of particular interest is the small difference between **12j** and **12k** in which the removal of

Table 2. Inhibitory activities against the RNAP enzyme and antibacterial activities of compounds **7**, **5c**, **8(a–y), 9,** and **10b**



7, 5c, 8(a-y), 9, 10

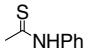
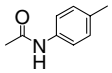
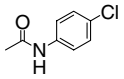
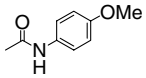
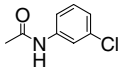
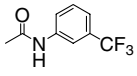
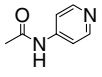
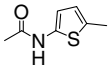
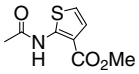
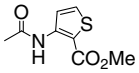
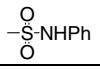
Compound	R^1	R^2	IC_{50} (μM), *S. aureus* RNAP	MIC (μg/mL), *S. aureus* ATCC 13709	MIC (μg/mL), *S. aureus* RN4220
7	Et		32	>32	>32
5c	^{i}Pr	H	>100	>128	32
8a	^{i}Pr		0.12	>128	>128
8b	^{i}Pr		0.11	>128	>128
8c	^{i}Pr		0.12	1	>128
8d	^{i}Pr		0.06	>16	>16
8e	^{i}Pr		0.57	>16	>16
8f	^{i}Pr		3	>8	>8
8g	^{i}Pr		>100	>32	>32
8h	^{i}Pr		0.29	>16	>16
8i	^{i}Pr		0.25	>8	>8
8j	^{i}Pr		1.6	>16	>16
8k	^{i}Pr		0.30	>128	>128
8l	^{i}Pr		>100	>16	>16
8m	^{i}Pr		>100	>16	32
8n	^{i}Pr		0.06	1	2
8o	^{i}Pr		0.20	0.5	1
8p	^{i}Pr		0.14	>16	>16

Table 2 (*continued*)

Compound	R^1	R^2	IC_{50} (μM), *S. aureus* RNAP	MIC (μg/mL), *S. aureus* ATCC 13709	MIC (μg/mL), *S. aureus* RN4220
8q	^{i}Pr		0.49	1	>128
8r	^{i}Pr		>100	>8	>8
8s	^{i}Pr		>100	>4	>4
8t	^{i}Pr		5	>64	>64
8u	^{i}Pr		>100	>8	>8
8v	^{i}Pr		>100	>8	>8
8w	^{i}Pr		6	>16	>16
8x			0.16	1	4
8y			0.25	2	>128
9	^{i}Pr		>100	>32	>32
10	^{i}Pr		>100	>128	>128

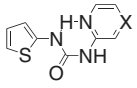


Figure 2.

one lipophilic arm did not impact the inhibitory activity at all. This tends to indicate that the analogs as made are unable to fill the space occupied by the 8-membered ring in **6c** in the binding site, benefiting only from the interactions made by the ester and the urea functionalities. This binding site obviously has a fairly hydrophobic surface associated with it.

4. In vitro characterization of activity

Compound **6c** was selected for further characterization in light of its antibacterial activity. This later was determined against a panel of microbes.

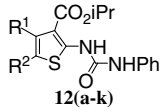
Staphylococcus spp. including *S. epidermidis* and *S. hyicus* were eradicated with MIC values of 0.25–1 μg/mL. The spectrum of activity against *S. aureus* is summarized in Table 4.

The activity of **6c** against the Rifampicin-resistant strains would be indicative of a mechanism of action or a binding site which is different from Rifampicin.

There was no activity of **6c** outside the *Staphylococcus* genus among the Gram-positives in the panel (*S. pyogenes*, *S. pneumoniae*, *E. faecalis*, and *Bacillus subtilis*, not shown). Likewise, **6c** lacked activity against the Gram-negative bacteria (*E. coli*, *P. aeruginosa*, *Salmonella typhimurium*, *Haemophilus influenzae*) and the yeast (*Candida albicans*, and *Candida parapsilosis*) in the panel (not shown).

The impact of serum on the antibacterial activity was established in the ATCC 13709 strain of *S. aureus*. The MIC values of **6c** shift from 0.5 to 128 μg/mL when 50% mouse or human serum is used in the growth medium. If 4% human serum albumin is used the MIC shifts to 16 μg/mL. This detrimental effect of serum and serum albumin is also observed with **6j** (128 μg/mL with 50% serum, 32 μg/mL with 4% albumin), **8n** (>128 μg/mL with 50% serum, 32 μg/mL with 4% albumin), **8x** (128 μg/mL with 50% serum, 32 μg/mL with 4% albumin), and **12b** (>128 μg/mL with 50% serum, 16 μg/mL with 4% albumin).

Table 3. Inhibitory activities against the RNAP enzyme and antibacterial activities of compounds **12a–k**



12(a-k)

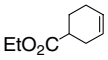
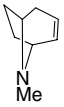
Compound	R^1 / R^2	IC_{50} (μM)	MIC (μg/mL) ATCC 13709	MIC (μg/mL) RN4220
12a		0.12	>64	>64
12b		0.05	0.5	0.5
12c		0.14	>8	>8
12d		17	>16	>16
12e		1.6	>32	>32
12f		2.5	128	128
12g		8.6	>128	>128
12h		2.5	>128	>128
12i		4	>16	>16
12j		5	>16	>16
12k		9	>128	>128

Table 4. In vitro susceptibility of drug- or multidrug-resistant or efflux pump mutants of *Staphylococcus aureus* to compound **6c**

Resistant category	*n*	MIC (μg/mL)		
		MIC or MIC range	MIC_{50}	MIC_{90}
		0.5 (11 strains)		
Mupirocin-resistant	12	>128 (1 strain)	0.5	0.5
Rifampicin-resistant	9	<0.125–1	0.25	0.5
MRSA	14	0.25–2	0.5	1
VISA ATCC 700699	1	0.25	na	na
K2068-MDR	1	0.25	na	na
1199B-NorA	1	1	na	na

The mechanism of antibacterial activity was also confirmed through the impact of **6c** on the synthesis of macromolecules at the cellular level in the RN4220 strain of *S. aureus*.[18] Figure 3 shows that **6c** inhibits the synthesis of both RNA and protein, but not of DNA. This is reminiscent of the mechanism of action of Rifampicin, and likely reflects the immediate cessation of protein synthesis upon inhibition of transcription due to the tight coupling of transcription and translation in bacteria.

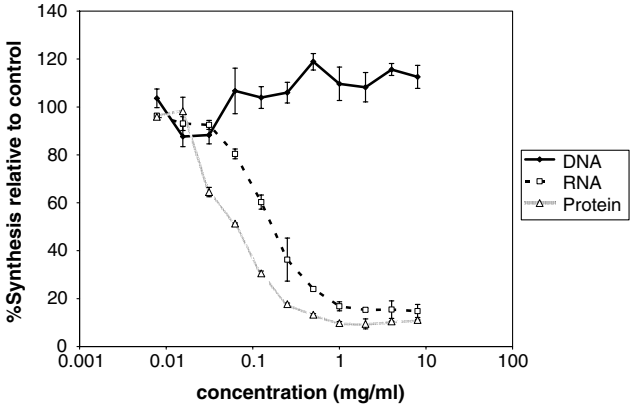


Figure 3. Effect of **6c** on macromolecular biosynthesis.

The time-kill curves (Fig. 4) of **6c** provide another interesting insight into the activity of this compound: an initial dip in the number of viable bacteria is followed by normal growth in the RN4220 strain of *S. aureus*. In the ATCC 13709 strain, the same effect is observed, except at high multiples of the MIC, where a bacteriostatic effect is seen. This provides a basis for the fact that the compounds in this class are either clearly antibacterial or without effect. Indeed, if the bacteria recover from the initial dip, they will be able to grow normally and reach saturation at the endpoint of the MIC assay (18–24 h). Only the most potent compounds, for which the initial killing is more profound and sustained, will result in a measurable MIC.

This phenomenon is in fact reflected in the in vitro frequencies of resistance, measured at 4× MIC, which are 3.2×10^{-5} against RN4220 and 6.8×10^{-8} against ATCC 13709. These are indeed high for RN4220 (the measured frequency of resistance of RN4220 to Rifamycins is $\sim 10^{-7}$), and again display the same strain-dependence.

5. In vivo evaluation

Compound **6c** does not display any cytotoxicity against the HeLa cell line (MTS assay) or human primary hepatocytes (ATP assay), nor is it mutagenic in the Ames test (not shown). It is therefore not surprising that it is well tolerated in vivo, with 100% survival of mice that received up to 2× 100 mg/kg of body weight po in 10%DMSO:90% peanut oil, or 2× 100 mg/kg ip or 50 mg/kg iv both in 15:15:70 solutol:PEG400:PBS.

Not unexpectedly given its large serum shift, **6c** failed to protect mice from lethal *S. aureus* ATCC 13709 infection at 72 h postinfection in the mouse peritonitis

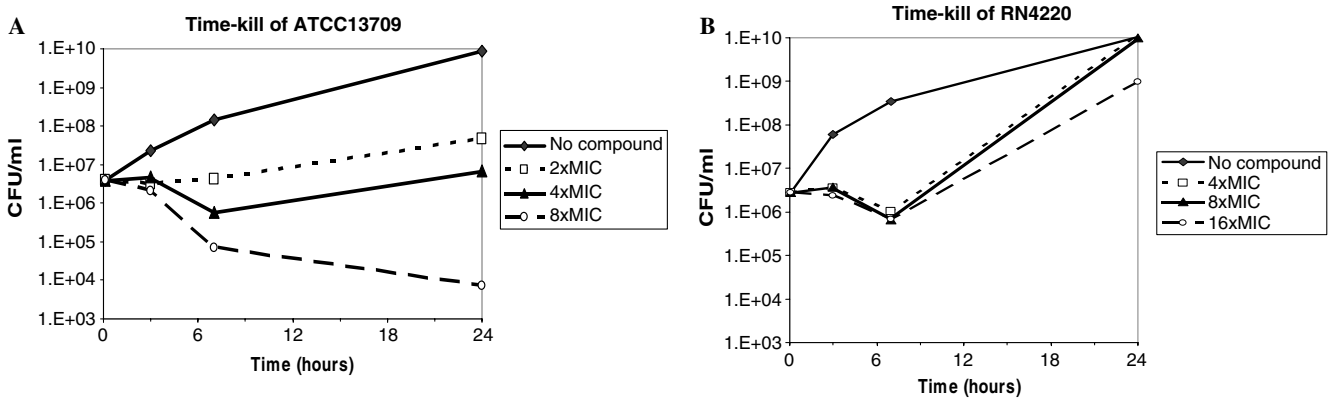


Figure 4. Time-kill curves of 6c against the ATCC 13709 (A) and the RN4220 (B) strains of *Staphylococcus aureus*.

Table 5. Pharmacokinetic parameters for **6c**

Dose (mg/kg)	25	50
Route	iv	po
t_{max} (h)	—	0.50
C_{max} (μg/mL)	—	2.09
AUC (h mg/mL)	36.6	6.18
$t_{1/2}$ (h)	0.52	4.46
V_{ss} (L/kg)	0.31	—
Cl (mL/min/kg)	11.3	—
Bioavailability (%)	—	8.4

model when used iv at up to 50 mg/kg. Statistically significant efficacy (60% survival) was seen only when compound **6c** was administered ip at 2× 100 mg/kg. Pharmacokinetic parameters for plasma concentrations were measured with a 15:15:70 solutol:PEG400:PBS formulation (Table 5).

6. Conclusion

The modifications of the substituents of compound **1** have led to derivatives with potent inhibitory activity toward the *S. aureus* RNAP holoenzyme and antibacterial activity against Staphylococcus spp. More extensive studies on the activity of one of these congeners, compound **6c**, provided a more complete picture in terms of mechanism of action. This inhibitor clearly inhibits transcription in *S. aureus* as judged by its rapid and profound impact on both RNA and protein synthesis as is seen with the Rifamycins. However, the compound maintains its excellent antibacterial activity against Rifampicin-resistant strains of *S. aureus*, suggesting an alternate mechanism of action or binding site on the RNAP holoenzyme compared to the Rifamycins' binding site on the β subunit of the RNAP core.

The transient activity of **6c** as judged by time-kill experiments with *S. aureus* RN4220 and the elevated frequency of resistance of this strain to **6c**, however, remain significant hurdles to further development of these compounds as antibacterial agents.

The very stringent structural requirements needed to confer antibacterial activity do not bode well for tackling the issues associated with serum binding and resistance. Additional studies focusing on the 8-membered macrocyclic portion of the structure may open up a larger chemical space to ultimately circumvent these liabilities.

This study has outlined a strategy to identify useful chemical tools with which to probe the bacterial RNA polymerase. The reverse chemical genetics approach described here started with the desired protein target (the RNAP holoenzyme of *S. aureus*), screened for small molecules that affect its activity, then asked whether the small molecule causes a phenotypic change in *S. aureus*. This approach is analogous to reverse genetics, in which a gene is deliberately mutated or knocked out in order to study the resulting phenotype. Current small molecule- or natural product inhibitors of bacterial RNAP are restricted to the Rifamycins, which act on the β (polymerase) subunit, and recently described synthetic compounds which apparently inhibit the interaction between the core and the sigma (promoter specificity) subunit.[7b] Additional compounds are required to better understand the enzymology of transcription. The well-defined mechanism of action of compound **6c** in inhibiting transcription in growing *S. aureus* cells establishes a chemical platform with which to further probe the workings of a multicomponent enzyme, the bacterial RNAP.

7. Experimental

7.1. General

^{1}H NMR spectra were recorded on a Varian Mercury™ 400 spectrometer. The reported chemical shifts (in parts per million) are referenced using the signals assigned to the residual non-deuterated solvents. Mass spectral analyses were performed on an Agilent mass spectrometer under electron spray ionization (ESI). Reactions were monitored by TLC on Silica gel Gel 60 F254 (0.25 mm, Merck). Column chromatography was performed on Silica gel Gel 60 (70–230 mesh). All reactions were carried out under an argon atmosphere with anhydrous solvents, unless otherwise noted. All chemicals, unless otherwise stated, were obtained from commercial sources.

7.2. Chemistry

7.2.1. Cyanoacetate esters (2a–m). Ethyl 2-cyanoacetate (**2a**), methyl 2-cyanoacetate (**2b**), isopropyl 2-cyanoacetate (**2c**), *tert*-butyl 2-cyanoacetate (**2d**), isobutyl 2-cyanoacetate (**2e**), and allyl 2-cyanoacetate (**2g**) were commercially available. Others were prepared as follows:

Cyanoacetates **2f**,**h**–**k** were prepared via the Fischer esterification procedure as follows: to a solution of cyanoacetic acid (2.0 g, 23.5 mmol) and alcohol (35.3 mmol) in benzene (50 mL) were added a few drops of concd H_2SO_4. The mixture was stirred at reflux for 2.5 h, using a Dean–Stark condenser to remove water. The reaction mixture was concentrated to half its initial volume under reduced pressure, diluted with EtOAc, washed with saturated $NaHCO_3$ solution, water, and brine. The organic layer was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was purified by flash chromatography on silica gel.

7.2.1.1. Benzyl 2-cyanoacetate (2f). Chromatographic eluent: 50% CH_2Cl_2 in hexanes. Yield: 93% (colorless oil). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.49 (s, 2H), 5.24 (s, 2H), 7.36–7.40 (m, 5H).

7.2.1.2. Cyclohexyl 2-cyanoacetate (2h). Chromatographic eluent: 10% EtOAc in hexanes Yield: quantitative (colorless oil). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.26–1.57 (m, 6H), 1.71–1.79 (m, 2H), 1.84–1.90 (m, 2H), 3.43 (s, 2H), 4.83–4.90 (m, 1H).

7.2.1.3. Cyclopentyl 2-cyanoacetate (73). Chromatographic eluent: 20% EtOAc in hexanes. Yield: quantitative (colorless oil). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.59–1.65 (m, 2H), 1.71–1.79 (m, 4H), 1.86–1.93 (m, 2H), 3.41 (s, 2H), 5.24–5.28 (m, 1H).

7.2.1.4. (±)-Tetrahydrofuran-3-yl 2-cyanoacetate (2j). Chromatographic eluent: 50% EtOAc in hexanes. Yield: 60% (colorless oil). ^{1}H NMR (400 MHz, $CDCl_3$) δ 2.03–2.10 (m, 1H), 2.18–2.28 (m, 1H), 3.47 (s, 2H), 5.84–5.97 (m, 4H), 5.37–5.41 (m, 1H).

7.2.1.5. (±)-1-Methoxypropan-2-yl 2-cyanoacetate (2k). Chromatographic eluent: 40% EtOAc in hexanes. Yield: 85% (colorless oil). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.28 (d, J = 6.5 Hz, 3H), 3.37 (s, 3H), 3.43–3.49 (m, 2H), 3.47 (s, 2H), 5.15–5.21 (m, 1H).

Cyanoacetates **2l**,**m** were prepared via DCC coupling as follows: to a solution of DCC (1.29 g, 6.25 mmol) in MeCN (6 mL) was added dropwise a solution of cyanoacetic acid (483 mg, 5.68 mmol) and alcohol (7.10 mmol) in MeCN (6 mL). After stirring for 3.5 h, the suspension was filtered through Celite. The filtrate was concentrated and the crude product was purified by flash chromatography on silica gel.

7.2.1.6. 1-Methylpiperidin-4-yl 2-cyanoacetate (2l). Chromatographic eluent: gradient of 30–40% EtOAc in hexanes. Yield: 52% (yellow oil which slowly solidified). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.73–1.82 (m, 2H), 1.90–1.98 (m, 2H), 2.22–2.32 (m, 5H), 2.59–2.66 (m, 2H), 3.45 (s, 2H), 4.86–4.90 (m, 1H).

7.2.1.7. (*R*)-*tert*-Butyl 3-(2-cyanoacetoyloxy)pyrrolidine-1-carboxylate (2m). To a solution of (*R*)-pyrrolidin-3-ol (0.78 g, 8.9 mmol) in THF (18 mL) at 0 °C was added Boc anhydride (1.94 g, 8.9 mmol). The solution was stirred for 48 h at room temperature, then concentrated to dryness. Purification by flash chromatography on silica gel, using 50% EtOAc/hexanes as eluent, provided (*R*)-*tert*-butyl 3-hydroxypyrrolidine-1-carboxylate as a colorless oil (1.66 g, 99% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.46 (s, 9H), 1.92 (br s, 1H), 1.94–2.04 (m, 2H), 3.30–3.52 (m, 4H), 4.44–4.47 (m, 1H). (*R*)-*tert*-Butyl 3-hydroxypyrrolidine-1-carboxylate (1.0 g, 5.34 mmol) was coupled with cyanoacetic acid using the general procedure above. Cyanoacetate **2m** was obtained as a white solid (858 mg, 79% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.46 (s, 9H), 2.10 (br s, 2H), 3.39–3.61 (m, 6H), 5.37 (br s, 1H) broad signals due to carbamate isomerism.

7.2.2. α-Cyanoketones (3a,b). To a solution of cyanoacetic acid (1 g, 11.8 mmol) in THF (100 mL), cooled in a dry ice/acetone bath, was added a 1.6 M solution of *n*-BuLi in THF (29 mL, 47.0 mmol). The resulting solution was warmed to room temperature over 1 h, stirred for 30 min at this temperature, and cooled back in a dry ice/acetone bath. The acid chloride (11.8 mmol) was added dropwise and the resulting mixture was stirred for 30 min at the same temperature, warmed to room temperature, and stirred for 1 h at this temperature. A 20% aqueous solution of hydrochloric acid (50 mL) was then added and the resulting mixture was vigorously stirred for 2h. Et_2O was added to the mixture, and the organics were collected. The aqueous layer was extracted twice more with Et_2O, the combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure.

7.2.2.1. 4-Methyl-3-oxopentanenitrile (3a). The crude product was purified by flash chromatography on silica gel using 5–20% EtOAc in hexanes (linear gradient) as the eluent. Yield: 72% (pale yellow oil). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.19 (d, J = 6.9 Hz, 6H), 2.83 (m, 1H), 3.53 (s, 2H).

7.2.2.2. 5-Methyl-3-oxohexanenitrile (3b). The product was purified by distillation under reduced pressure (bp = 150 °C/20 mmHg). Yield: 72% (colorless liquid). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.96 (d, J = 6.9 Hz, 6H), 2.83 (m, 1H), 2.50 (d, J = 6.9 Hz, 2H), 3.44 (s, 2H).

7.2.3. 2-Cyano-*N*-isopropylacetamide (4). To isopropylamine (1.85 g, 31.2 mmol) was added slowly ethyl cyanoacetate (1.5 mL, 12.5 mmol). The mixture was stirred for 18 h and concentrated to dryness, yielding **4** as a yellow solid (1.97 g, quantitative). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.20 (d, J = 6.6 Hz, 6H), 3.34 (s, 2H), 4.08 (sept, J = 6.8 Hz, 1H), 5.92 (br s, 1H).

7.2.4. 4,5,6,7,8,9-Hexahydrocycloocta[*b*]thiophen-2-amines (5). To a solution of cyclooctanone (500 mg, 3.96 mmol) in solvent (4 mL) in a pressure tube were

added sulfur (127 mg, 3.96 mmol), 2-cyanoacetate ester (3.96 mmol), and amine base (5.54 mmol). The tube was capped and the mixture was stirred at 70 °C for 18 h, after which the reaction mixture was brought to room temperature. The resulting solution was diluted with EtOAc and washed with H_2O twice and with brine twice. The organic layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by flash chromatography on silica gel.

7.2.4.1. Ethyl 2-amino-4,5,6,7,8,9-hexahydrocycloocta[*b*]thiophene-3-carboxylate (5a). Using ethanol as the solvent, morpholine as the amine base, and cyanoacetate **2a**. Chromatographic eluent: 50% CH_2Cl_2 in hexanes. Yield: 68% (yellow oil). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.28–1.34 (m, 2H), 1.35 (t, J = 7.1 Hz, 3H), 1.43–1.49 (m, 2H), 1.54–1.66 (m, 4H), 2.59–2.62 (m, 2H), 2.81–2.84 (m, 2H), 4.28 (q, J = 7.1 Hz, 2H), 5.91 (br s, 2H).

7.2.4.2. Methyl 2-amino-4,5,6,7,8,9-hexahydrocycloocta[*b*]thiophene-3-carboxylate (5b). Using methanol as the solvent, morpholine as the amine base, and cyanoacetate **2b**. Chromatographic eluent: 50% CH_2Cl_2 in hexanes. Yield: 38% (yellow solid). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.27–1.33 (m, 2H), 1.43–1.48 (m, 2H), 1.54–1.64 (m, 4H), 2.59–2.62 (m, 2H), 2.79–2.82 (m, 2H), 3.80 (s, 3H), 5.90 (br s, 2H).

7.2.4.3. Isopropyl 2-amino-4,5,6,7,8,9-hexahydrocycloocta[*b*]thiophene-3-carboxylate (5c). Using *i*-PrOH as the solvent, morpholine as the amine base, and cyanoacetate **2c**. Chromatographic eluent: 50% CH_2Cl_2 in hexanes. Yield: 63% (pale yellow oil, which solidified over time). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.32 (d, J = 6.2 Hz, 6H), 1.43–1.49 (m, 6H), 1.53–1.66 (m, 2H), 2.59–2.62 (m, 2H), 2.81–2.84 (m, 2H), 5.18 (sept, J = 6.2 Hz, 1H), 5.92 (br s, 2H). Mass calculated for $C_{14}H_{21}NO_2S$: 267, found: 268.0 $(M+H)^+$.

7.2.4.4. *tert*-Butyl 2-amino-4,5,6,7,8,9-hexahydrocycloocta[*b*]thiophene-3-carboxylate (5d). Using EtOH as the solvent, morpholine as the amine base, and cyanoacetate **2d**. Chromatographic eluent: 50% CH_2Cl_2 in hexanes. Yield: 44% (yellow oil). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.28–1.34 (m, 2H), 1.43–1.48 (m, 2H), 1.55 (s, 9H), 1.52–1.64 (m, 4H), 2.59–2.62 (m, 2H), 2.79–2.82 (m, 2H), 5.88 (br s, 2H).

7.2.4.5. Isobutyl 2-amino-4,5,6,7,8,9-hexahydrocycloocta[*b*]thiophene-3-carboxylate (5e). Using DMF as solvent, morpholine as the amine base, and cyanoacetate **2e**. Chromatographic eluent: 50% CH_2Cl_2 in hexanes. Yield: 41% (yellow oil). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.00 (d, J = 6.7 Hz, 6H), 1.31–1.35 (m, 2H), 1.43–1.48 (m, 2H), 1.55–1.66 (m, 4H), 2.03 (sept, J = 6.7 Hz, 1H), 2.60–2.62 (m, 2H), 2.83–2.86 (m, 2H), 4.02 (d, J = 6.4 Hz, 2H), 5.96 (br s, 2H).

7.2.4.6. Benzyl 2-amino-4,5,6,7,8,9-hexahydrocycloocta[*b*]thiophene-3-carboxylate (5f). Using DMF as solvent, morpholine as the amine base, and cyanoacetate **2f**. Chromatographic eluent: 50% CH_2Cl_2 in hexanes. Yield: 37% (yellow oil). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.27–1.32 (m, 2H), 1.41–1.47 (m, 2H), 1.53–1.59 (m, 4H), 2.58–2.61 (m, 2H), 2.79–2.83 (m, 2H), 5.27 (s, 2H), 5.93 (br s, 2H), 7.30–7.42 (m, 5H).

7.2.4.7. Allyl 2-amino-4,5,6,7,8,9-hexahydrocycloocta[*b*]thiophene-3-carboxylate (5g). Using DMF as solvent, morpholine as the amine base, and cyanoacetate **2g**. Chromatographic eluent: 50% CH_2Cl_2 in hexanes. Yield: 42% (yellow oil). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.28–1.34 (m, 2H), 1.43–1.49 (m, 2H), 1.56–1.65 (m, 4H), 2.59–2.62 (m, 2H), 2.82–2.85 (m, 2H), 4.74 (dt, J = 5.7 Hz, 1.3, 2H), 5.25 (dq, J = 10.5, 1.3 Hz, 1H), 5.36 (dq, J = 17.2, 1.5 Hz, 1H), 5.93 (br s, 2H), 5.96–6.05 (m, 1H).

7.2.4.8. Cyclohexyl 2-amino-4,5,6,7,8,9-hexahydrocycloocta[*b*]thiophene-3-carboxylate (5h). Using DMF as solvent, morpholine as the amine base, and cyanoacetate **2h**. Chromatographic eluent: 50% CH_2Cl_2 in hexanes. Yield: 33% (yellow oil). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.29–1.34 (m, 3H), 1.41–1.48 (m, 4H), 1.52–1.59 (m, 5H), 1.62–1.66 (m, 2H), 1.71–1.77 (m, 2H), 1.89–1.95 (m, 2H), 2.59–2.62 (m, 2H), 2.83–2.86 (m, 2H), 4.97 (quint, J = 3.9 Hz, 1H), 5.93 (br s, 2H).

7.2.4.9. Cyclopentyl 2-amino-4,5,6,7,8,9-hexahydrocycloocta[*b*]thiophene-3-carboxylate (5i). Using DMF as solvent, morpholine as the amine base, and cyanoacetate **2i**. Chromatographic eluent: 50% CH_2Cl_2 in hexanes. Yield: ca. 44% (yellow oil). The compound was inseparable from cyclooctanone and was used as such in the next stage. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.28–1.34 (m, 2H), 1.42–1.48 (m, 2H), 1.52–1.67 (m, 6H), 1.74–1.91 (m, 6H), 2.59–2.62 (m, 2H), 2.78–2.81 (m, 2H), 5.34–5.38 (m, 1H), 5.95 (br s, 2H).

7.2.4.10. (±)-Tetrahydrofuran-3-yl 2-amino-4,5,6,7,8,9-hexahydrocycloocta[*b*]thiophene-3-carboxylate (5j). Using DMF as solvent, morpholine as the amine base, and cyanoacetate **2j**. Chromatographic eluent: 80–100% CH_2Cl_2 in hexanes then 10% EtOAc in CH_2Cl_2. Yield: 40% (dark yellow oil). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.28–1.34 (m, 2H), 1.43–1.48 (m, 2H), 1.54–1.65 (m, 4H), 2.09–2.26 (m, 2H), 2.59–2.62 (m, 2H), 2.79–2.82 (m, 2H), 3.90–3.95 (m, 3H), 4.00 (dd, J = 10.4, 4.6 Hz, 1H), 5.47–5.51 (m, 1H), 5.97 (br s, 2H).

7.2.4.11. (±)-1-Methoxypropan-2-yl 2-amino-4,5,6,7,8,9-hexahydrocycloocta[*b*]thiophene-3-carboxylate (5k). Using DMF as solvent, morpholine as the amine base, and cyanoacetate **2k**. Chromatographic eluent: CH_2Cl_2. Yield: 43% (dark orange oil). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.27–1.34 (m, 2H), 1.32 (d, J = 6.4 Hz, 3H), 1.43–1.48 (m, 2H), 1.53–1.66 (m, 4H), 2.59–2.62 (m, 2H), 2.81–2.84 (m, 2H), 3.37 (s, 3H), 3.45 (dd, J = 10.2 Hz, 4.3, 1H), 3.53 (dd, J = 10.2 Hz, 6.0, 1H), 5.20–5.26 (m, 1H), 5.89 (br s, 2H).

7.2.4.12. 1-Methylpiperidin-4-yl 2-amino-4,5,6,7,8,9-hexahydrocycloocta[*b*]thiophene-3-carboxylate (5l). Using DMF as solvent, morpholine as the amine base, and cyanoacetate **2l**. Chromatographic eluent: 0–5% MeOH in

CH_2Cl_2. Yield: 23% (orange gum). ^1H NMR (400 MHz, $CDCl_3$) δ 1.28–1.35 (m, 2H), 1.42–1.47 (m, 2H), 1.54–1.64 (m, 4H), 1.78–1.88 (m, 2H), 1.98–2.06 (m, 2H), 2.31 (s, 3H), 2.28–2.38 (m, 2H), 2.59–2.70 (m, 4H), 2.82–2.85 (m, 2H), 4.99–5.03 (m, 1H), 6.00 (br s, 2H).

7.2.4.13. (*R*)-*N*-Boc-pyrrolidin-3-yl 2-amino-4,5,6,7,8,9-hexahydrocycloocta[*b*]thiophene-3-carboxylate (5m). Using DMF as solvent, morpholine as the amine base, and cyanoacetate **2m**. Chromatographic eluent: 0–5% EtOAc in CH_2Cl_2. Yield: 47% (orange foam). ^1H NMR (400 MHz, $CDCl_3$) δ 1.27–1.33 (m, 2H), 1.40–1.48 (m, 2H), 1.45 (s, 9H), 2.09–2.15 (m, 2H), 2.58–2.61 (m, 2H), 2.73–2.78 (m, 2H), 3.42–3.67 (m, 4H), 5.47 (br s, 1H), 5.99 (br s, 2H).

7.2.4.14. 1-(2-Amino-4,5,6,7,8,9-hexahydrocycloocta-[*b*]thiophen-3-yl)-2-methylpropan-1-one (5n). Using MeOH as solvent, triethylamine as the amine base, and α-cyanoketone **3a**. Chromatographic eluent: 40–70% CH_2Cl_2 in hexanes. The fractions containing the product were used in the next step as such.

7.2.4.15. 1-(2-Amino-4,5,6,7,8,9-hexahydrocycloocta-[*b*]thiophen-3-yl)-3-methylbutan-1-one (5o). Using MeOH as solvent, triethylamine as the amine base, and α-cyanoketone **3b**. Chromatographic eluent: 40–70% CH_2Cl_2 in hexanes. The fractions containing the product were used in the next step as such.

7.2.4.16. 2-Amino-4,5,6,7,8,9-hexahydrocycloocta-[*b*]thiophene-3-carbonitrile (5p). Using EtOH as solvent, morpholine as the amine base, and malononitrile. Chromatographic eluent: 0–40% EtOAc in hexanes. Yield: 88% (yellow solid). ^1H NMR (400 MHz, $CDCl_3$) δ 1.29–1.38 (m, 4H), 1.46–1.57 (m, 4H), 2.48 (t, J = 6.1 Hz, 2H), 2.56 (t. J = 6.3 Hz, 2H) 6.88 (s, 2H).

7.2.4.17. *N*-Isopropyl 2-amino-4,5,6,7,8,9-hexahydrocycloocta[*b*]thiophene-3-carboxamide (5q). To a solution of cyclooctanone (300 mg, 2.38 mmol) and cyanoacetamide **4** (300 mg, 2.38 mmol) in benzene (10 mL) were added ammonium acetate (37 mg, 0.48 mmol) and acetic acid (109 μL, 1.90 mmol). The mixture was stirred under reflux, with 4 Å molecular sieves and a Dean–Stark condenser, for 48 h, after which it was diluted with EtOAc, washed with H_2O twice and with brine, dried ($MgSO_4$), filtered, and concentrated. The crude product was purified by column chromatography on silica gel using 10% EtOAc/hexanes as eluent. 2-Cyano-2-cyclooctylidene-*N*-isopropylacetamide was obtained as an off-white solid (90 mg, 16% yield). ^1H NMR (400 MHz, $CDCl_3$) δ 1.20 (d, J = 6.5 Hz, 6H), 1.34–1.40 (m, 2H), 1.47–1.56 (m, 4H), 1.82–1.92 (m, 4H), 2.65–2.68 (m, 2H), 2.94–2.97 (m, 2H), 4.09 (sept, J = 6.5 Hz, 1H), 5.92 (br s, 1H). To a solution of 2-cyano-2-cyclooctylidene-*N*-isopropylacetamide (88 mg, 0.38 mmol) in EtOH (1 mL) were added sulfur powder (14 mg, 0.45 mmol) and morpholine (26 μL, 0.30 mmol). The mixture was stirred at 70 °C for 18 h in a pressure tube. After concentration to dryness, the crude product was purified by column chromatography on silica gel using 20% EtOAc/CH_2Cl_2 as eluent, providing compound **5q** as a beige solid (92 mg, 91% yield). ^1H NMR (400 MHz, $CDCl_3$) δ 1.22 (dd, J = 6.5, 1.0 Hz, 6H), 1.36–1.42 (m, 2H), 1.44–1.50 (m, 2H), 1.56–1.68 (m, 4H), 2.61–2.64 (m, 2H), 2.70–2.74 (m, 2H), 4.20 (sept, J = 6.4 Hz, 1H), 5.68 (br s, 3H).

7.2.5. 3-Substituted 4,5,6,7,8,9-hexahydro-2-(3-phenylureido)cycloocta[*b*]thiophenes. Three procedures were used:

Procedure A. To a solution of 3-substituted 2-amino-4,5,6,7,8,9-hexahydrocycloocta[*b*]thiophene (2.98 mmol) in pyridine (6 mL) was added phenylisocyanate (747 μL, 6.85 mmol). The mixture was heated in a pressure tube at 65 °C for 18 h, after which the reaction mixture was brought to room temperature and concentrated under reduced pressure. The crude material was stirred in EtOAc, undissolved diphenyl urea was removed by filtration, and the filtrate was concentrated and purified by SiO_2 chromatography.

Procedure B. To a solution of 3-substituted 2-amino-4,5,6,7,8,9-hexahydrocycloocta[*b*]thiophene (100 mg, 0.29 mmol) in $CHCl_3$ (1.5 mL) were added phenylisocyanate (63 μL, 0.58 mmol) and a catalytic amount of DMAP. The mixture was heated in a pressure tube at 70 °C for 18 h, after which the reaction mixture was brought to room temperature and concentrated under reduced pressure. The crude material was purified by flash chromatography on silica gel.

Procedure C. To a stirred solution of 3-substituted 2-amino-4,5,6,7,8,9-hexahydrocycloocta[*b*]thiophene (0.757 mmol) in $CHCl_3$ were added phenylisocyanate (82 μL, 0.757 mmol) and DMAP (~10 mg). The resulting solution was stirred at reflux for 12 h before another portion of phenylisocyanate (82 μL, 0.757 mmol) was added. After another 12 h at reflux, the solution was cooled to room temperature, diluted with CH_2Cl_2 and H_2O. The organics were collected and the aqueous layer was extracted twice more with CH_2Cl_2. The combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated under reduced pressure. The crude product was purified by two consecutive column chromatographies on silica gel.

7.2.5.1. Ethyl 4,5,6,7,8,9-hexahydro-2-(3-phenylureido)cycloocta[*b*]thiophene-3-carboxylate (1). Procedure A used. Chromatographic eluent: 50–100% CH_2Cl_2 in hexanes. Yield: 85% (white foam). ^1H NMR (400 MHz, $CDCl_3$) δ 1.25–1.30 (m, 2H), 1.35 (t, J = 7.1 Hz, 3H), 1.44–1.49 (m, 2H), 1.59–1.65 (m, 4H), 2.70–2.73 (m, 2H), 2.86–2.89 (m, 2H), 4.29 (q, J = 7.1 Hz, 2H), 6.76 (s, 1H), 7.14 (t, J = 7.4 Hz, 1H), 7.35 (t, J = 8.3 Hz, 2H), 7.44 (d, J = 8.4 Hz, 2H), 10.93 (s, 1H). Mass calculated for $C_{20}H_{24}N_2O_3S$: 372, found: 373.1 $(M+H)^+$.

7.2.5.2. Methyl 4,5,6,7,8,9-hexahydro-2-(3-phenylureido)cycloocta[*b*]thiophene-3-carboxylate (6b). Procedure A used. Chromatographic eluent: 50% CH_2Cl_2 in hexanes. Yield: 40% (white foam). ^1H NMR (400 MHz, $CDCl_3$) δ 1.25–1.30 (m, 2H), 1.44–1.49 (m, 2H), 1.59–1.65 (m, 4H), 2.69–2.72 (m, 2H), 2.85–2.88 (m, 2H),

3.81 (s, 3H), 6.83 (s, 1H), 7.14–7.17 (m, 1H), 7.34–7.38 (m, 2H), 7.42–7.45 (m, 2H), 10.84 (s, 1H). Mass calculated for $C_{19}H_{22}N_2O_3S$: 358, found: 359.1 $(M+H)^+$.

7.2.5.3. Isopropyl 4,5,6,7,8,9-hexahydro-2-(3-phenylureido)cycloocta[*b*]thiophene-3-carboxylate (6c). Procedure A used. Chromatographic eluent: 50–60% CH_2Cl_2 in hexanes. Yield: 89% (white foam). ^1H NMR (400 MHz, $CDCl_3$) δ 1.24–1.30 (m, 2H), 1.32 (d, J = 6.2 Hz, 6H), 1.43–1.49 (m, 2H), 1.58–1.66 (m, 4H), 2.69–2.72 (m, 2H), 2.86–2.89 (m, 2H), 5.16 (sept, J = 6.2 Hz, 1H), 7.03 (s, 1H), 7.12 (t, J = 7.4 Hz, 1H), 7.34 (t, J = 7.5 Hz, 2H), 7.45 (d, J = 7.5 Hz, 2H), 10.99 (s, 1H). Mass calculated for $C_{21}H_{26}N_2O_3S$: 386, found: 387.1 $(M+H)^+$.

7.2.5.4. *tert*-Butyl 4,5,6,7,8,9-hexahydro-2-(3-phenylureido)cycloocta[*b*]thiophene-3-carboxylate (6d). Procedure A used. Chromatographic eluent: 50% CH_2Cl_2 in hexanes. Yield: 68% (white foam). ^1H NMR (400 MHz, $CDCl_3$) δ 1.26–1.31 (m, 2 H), 1.43–1.49 (m, 2H), 1.56 (s, 9H), 1.59–1.65 (m, 4H), 2.69–2.72 (m, 2H), 2.85–2.88 (m, 2H), 6.81 (s, 1H), 7.09–7.13 (m, 1H), 7.31–7.35 (m, 2H), 7.44–7.46 (m, 2H), 11.06 (s, 1H). Mass calculated for $C_{22}H_{28}N_2O_3S$: 400, found: 401.1 $(M+H)^+$.

7.2.5.5. Isobutyl 4,5,6,7,8,9-hexahydro-2-(3-phenylureido)cycloocta[*b*]thiophene-3-carboxylate (6e). Procedure A used. Chromatographic eluent: 50–60% CH_2Cl_2 in hexanes. Yield: 78% (white foam). ^1H NMR (400 MHz, $CDCl_3$) δ (d, J = 6.7 Hz, 6H), 1.26–1.32 (m, 2H), 1.43–1.48 (m, 2H), 1.59–1.67 (m, 4H), 2.03 (sept, J = 6.7 Hz, 1H), 2.70–2.73 (m, 2H), 2.89–2.92 (m, 2H), 4.02 (d, J = 6.5 Hz, 2H), 6.91 (s, 1H), 7.11–7.15 (m, 1H), 7.35 (t, J = 7.5 Hz, 2H), 7.44 (d, J = 7.7 Hz, 2H), 11.00 (s, 1H). Mass calculated for $C_{22}H_{28}N_2O_3S$: 400, found: 401.1 $(M+H)^+$.

7.2.5.6. Benzyl 4,5,6,7,8,9-hexahydro-2-(3-phenylureido)cycloocta[*b*]thiophene-3-carboxylate (6f). Procedure A used. Chromatographic eluent: 70% CH_2Cl_2 in hexanes. Yield: 46% (white foam). ^1H NMR (400 MHz, $CDCl_3$) δ 1.21–1.28 (m, 2H), 1.40–1.46 (m, 2H), 1.51–1.63 (m, 4H), 2.69–2.72 (m, 2H), 2.84–2.87 (m, 2H), 5.28 (s, 2H), 6.66 (s, 1H), 7.11–7.15 (m, 1H), 7.30–7.43 (m, 9H), 10.86 (s, 1H). Mass calculated for $C_{25}H_{26}N_2O_3S$: 434, found: 435.1 $(M+H)^+$.

7.2.5.7. Allyl 4,5,6,7,8,9-hexahydro-2-(3-phenylureido)cycloocta[*b*]thiophene-3-carboxylate (6g). Procedure A used. Chromatographic eluent: 50–60% CH_2Cl_2 in hexanes. Yield: 60% (white foam). ^1H NMR (400 MHz, $CDCl_3$) δ1.25–1.30 (m, 2H), 1.44–1.49 (m, 2H), 1.59–1.66 (m, 4H), 2.70–2.73 (m, 2H), 2.87–2.90 (m, 2H), 4.73 (dt, J = 5.7 Hz, 1.3, 2H), 5.26–5.29 (m, 1H), 5.33–5.39 (m, 1H), 5.93–6.03 (m, 1H), 6.80 (s, 1H), 7.12–7.16 (m, 1H), 7.33–7.38 (m, 2H), 7.42–7.45 (m, 2H), 10.89 (s, 1H). Mass calculated for $C_{21}H_{24}N_2O_3S$: 384, found: 385.0 $(M+H)^+$.

7.2.5.8. Cyclohexyl 4,5,6,7,8,9-hexahydro-2-(3-phenylureido)cycloocta[*b*]thiophene-3-carboxylate (6h). Procedure A. Chromatographic eluent: 50% CH_2Cl_2 in hexanes. Yield: 78% (white foam). ^1H NMR (400 MHz, $CDCl_3$) δ 1.27–1.31 (m, 2H), 1.35–1.49 (m, 5H), 1.52–1.68 (m, 7H), 1.72–1.80 (m, 2H), 1.89–1.95 (m, 2H), 2.70–2.73 (m, 2H), 2.89–2.92 (m, 2H), 4.94–4.99 (m, 1H), 6.78 (s, 1H), 7.10–7.14 (m, 1H), 7.31–7.37 (m, 2H), 7.42–7.45 (m, 2H), 11.05 (s, 1H). Mass calculated for $C_{24}H_{30}N_2O_3S$: 426, found: 427.2 $(M+H)^+$.

7.2.5.9. Cyclopentyl 4,5,6,7,8,9-hexahydro-2-(3-phenylureido)cycloocta[*b*]thiophene-3-carboxylate (6i). Procedure B used. Chromatographic eluent: 50–60% CH_2Cl_2 in hexanes. Yield: 79% (white foam). ^1H NMR (400 MHz, $CDCl_3$) δ 1.25–1.31 (m, 2H), 1.43–1.48 (m, 2H), 1.59–1.70 (m, 6H), 1.75–1.92 (m, 6H), 2.69–2.72 (m, 2H), 2.84–2.87 (m, 2H), 5.35–5.38 (m, 1H), 6.78 (s, 1H), 7.10–7.14 (m, 1H), 7.32–7.36 (m, 2H), 7.43–7.46 (m, 2H), 11.05 (s, 1H). Mass calculated for $C_{23}H_{28}N_2O_3S$: 412, found: 413.1 $(M+H)^+$.

7.2.5.10. (±)-Tetrahydrofuran-3-yl 4,5,6,7,8,9-hexahydro-2-(3-phenylureido)cycloocta[*b*]thiophene-3-carboxylate (6j). Procedure A used. Chromatographic eluent: 5% EtOAc in CH_2Cl_2/hexanes (1:1). Yield: 37% (white foam). ^1H NMR (400 MHz, $CDCl_3$) δ 1.25–1.31 (m, 2H), 1.43–1.48 (m, 2H), 1.59–1.66 (m, 4H), 2.11–2.27 (m, 2H), 2.69–2.72 (m, 2H), 2.85–2.88 (m, 2H), 3.92–4.00 (m, 4H), 5.48–5.51 (m, 1H), 6.94 (s, 1H), 7.11–7.15 (m, 1H), 7.32–7.36 (m, 2H), 7.43–7.46 (m, 2H), 10.82 (s, 1H). Mass calculated for $C_{22}H_{26}N_2O_4S$: 414, found: 415.2 $(M+H)^+$.

7.2.5.11. (±)-1-Methoxypropan-2-yl 4,5,6,7,8,9-hexahydro-2-(3-phenylureido)cycloocta[*b*]thiophene-3-carboxylate (6k). Procedure B used. Chromatographic eluent (two columns used): CH_2Cl_2 (first column) then 25% Et_2O in hexanes (second column). Yield: 25% (white foam). ^1H NMR (400 MHz, $CDCl_3$) δ 1.27–1.33 (m, 2H), 1.37 (d, J = 6.4 Hz, 3H), 1.43–1.49 (m, 2H), 1.59–1.69 (m, 4H), 2.71–2.74 (m, 2H), 2.87–2.90 (m, 2H), 3.45 (s, 3H), 3.55 (dd, J = 10.9, 3.7 Hz, 1H), 3.65 (dd, J = 10.9 Hz, 7.0, 1H), 5.23–5.27 (m, 1H), 6.89 (s, 1H), 7.07–7.12 (m, 1H), 7.30–7.34 (m, 2H), 7.43–7.46 (m, 2H), 10.61 (s, 1H). Mass calculated for $C_{22}H_{28}N_2O_4S$: 416, found: 417.1 $(M+H)^+$.

7.2.5.12. 1-Methylpiperidin-4-yl 4,5,6,7,8,9-hexahydro-2-(3-phenylureido)cycloocta[*b*]thiophene-3-carboxylate (6l). Procedure B used. Chromatographic eluent: 0–5% MeOH in CH_2Cl_2. Yield: 62% (beige foam). ^1H NMR (400 MHz, $CDCl_3$) δ 1.25–1.32 (m, 2H), 1.43–1.49 (m, 2H), 1.59–1.66 (m, 4H), 1.80–1.89 (m, 2H), 1.97–2.05 (m, 2H), 2.31 (s, 3H), 2.33–2.38 (m, 2H), 2.62–2.69 (m, 2H), 2.70–2.73 (m, 2H), 2.89–2.92 (m, 2H), 4.98–5.02 (m, 1H), 6.91 (s, 1H), 7.11–7.15 (m, 1H), 7.32–7.37 (m, 2H), 7.43–7.46 (m, 2H), 11.00 (s, 1H). Mass calculated for $C_{24}H_{31}N_3O_3S$: 441, found: 442.2 $(M+H)^+$.

7.2.5.13. (*R*)-1-Methylpyrrolidin-3-yl 4,5,6,7,8,9-hexahydro-2-(3-phenylureido)cycloocta[*b*]thiophene-3-carboxylate (6m). Procedure B was used, with **5m** and phenylisocyanate. The crude material was purified by flash chromatography on silica gel using a gradient of

0–5% EtOAc/hexanes. (*R*)-*N*-Boc-pyrrolidin-3-yl 4,5,6,7,8,9-hexahydro-2-(3-phenylureido)cycloocta[*b*]thiophene-3-carboxylate was obtained as a pale yellow foam in 50% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.24–1.29 (m, 2H), 1.46 (s, 11H), 1.57–1.69 (m, 4H), 2.16 (br s, 2H), 2.69–2.71 (m, 2H), 2.80–2.83 (m, 2H), 3.46–3.61, 3.75–3.79 (2m, 4H), 5.41–5.47 (m, 1H), 7.09–7.14 (m, 1H), 7.18 (br s, 1H), 7.32–7.36 (m, 2H), 7.47–7.48 (m, 2H), 10.63, 10.75 (2s, 1H), rotamers due to carbamate isomerism. Mass calculated for $C_{27}H_{35}N_3O_5S$: 513, found: 414.1 $(M-Boc+H)^+$. It was then treated with a solution of TFA/CH_2Cl_2 (40%, v/v) and stirred during 40 minutes and concentrated to dryness. After two co-evaporations with benzene, the TFA salt was obtained as a beige solid in quantitative yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.21–1.29 (m, 2H), 1.40–1.46 (m, 2H), 1.53–1.65 (m, 4H), 2.20–2.27 (m, 2H), 2.69–2.72 (m, 2H), 2.80–2.87 (m, 2H), 3.22–3.28 (m, 1H), 3.39–3.47 (m, 2H), 3.54–3.60 (m, 1H), 5.51–5.55 (m, 1H), 7.00–7.05 (m, 1H), 7.30–7.33 (m, 2H), 7.48–7.50 (m, 2H), 9.04 (br s, 1H), 9.13 (br s, 1H), 10.24 (s, 1H), 10.66 (s, 1H). Mass calculated for $C_{22}H_{27}N_3O_5S$: 413, found: 414.1 $(M+H)^+$. The TFA salt (121 mg, 0.23 mmol) was dissolved in a solution of formic acid (1.0 mL) and formaldehyde (1.0 mL), and stirred under reflux for 30 min, after which it was concentrated to dryness. The residue was poured into sat. $NaHCO_3$ solution and extracted three times with a mixture of $CHCl_3$/*i*-PrOH (4:1), the combined organic layers were dried ($MgSO_4$), filtered, and concentrated. Crude **6m** was purified by flash chromatography, 0–3% MeOH/CH_2Cl_2, then reverse phase on C18, 20–100% MeCN/H_2O, followed by prep. TLC, 10% MeOH/EtOAc. Compound **6m** was obtained as a white foam (10 mg, 10% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.25–1.32 (m, 2H), 1.42–1.48 (m, 2H), 1.58–1.67 (m, 4H), 1.97–2.05 (m, 1H), 2.30–2.39 (m, 1H), 2.46–2.52 (m, 1H), 2.48 (s, 3H), 2.70–2.73 (m, 2H), 2.79–2.95 (m, 5H), 5.40–5.45 (m, 1H), 7.08–7.12 (m, 2H), 7.33 (t, J = 7.5 Hz, 2H), 7.45 (d, J = 7.5 Hz, 2H), 10.62 (s, 1H). Mass calculated for $C_{23}H_{29}N_3O_3S$: 427, found: 428.2 $(M+H)^+$.

7.2.5.14. 1-(4,5,6,7,8,9-Hexahydro-3-(isobutyryl)-cycloocta[*b*]thiophen-2-yl)-3-phenylurea (6n). Procedure C used. Chromatographic eluent (two columns used): 40–70% CH_2Cl_2 in hexanes (first column), 5–20% EtOAc in hexanes (second column). Yield: 82% (white solid). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.20 (d, J = 6.7 Hz, 6H), 1.33 (m, 2H), 1.50 (m, 2H), 1.62–1.72 (m, 4H), 2.75 (br t, J = 5.9 Hz, 2H), 2.88 (br t, J = 8.7 Hz, 2H), 3.36 (m, 1H), 6.76 (br s, 1H), 7.12 (dt, J = 7.4, 0.8 Hz, 1H), 7.35 (m, 2H), 7.46 (m, 2H), 12.42 (br s, 1H). Mass calculated for $C_{11}H_{26}N_2O_2S$: 370, found: 371.5 $(M+H)^+$.

7.2.5.15. 1-(3-(3-Methylbutanoyl)-4,5,6,7,8,9-hexahydrocycloocta[*b*]thiophen-2-yl)-3-phenylurea (6o). Procedure C used. Chromatographic eluent (two columns used): 40–70% CH_2Cl_2 in hexanes (first column), 5–20% EtOAc in hexanes (second column). Yield: 88% (white foam). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.93 (d, J = 6.7 Hz, 6H), 1.32 (m, 2H), 1.48 (m, 2H), 1.59–1.69 (m, 4H), 2.19 (m, 1H), 2.67–2.72 (m, 4H), 2.87 (br t, J = 6.2 Hz, 2H), 7.11 (dt, J = 8.5, 1.5 Hz, 1H), 7.33 (m, 2H), 7.47 (m, 2H), 7.83 (br s, 1H), 12.42 (br s, 1H). Mass calculated for $C_{22}H_{28}N_2O_2S$: 384, found: 385.2 $(M+H)^+$.

7.2.5.16. 1-(3-Cyano-4,5,6,7,8,9-hexahydrocycloocta[*b*]thiophen-2-yl)-3-phenylurea (6p). A solution of **5p** (0.91 g, 4.4 mmol) and phenylisocyanate (0.97 mL, 8.8 mmol) in DCM (10 mL) was stirred at room temperature for 20 hr. The reaction was quenched by the addition of water. After stirring the mixture at room temperature for 1 h, the precipitate was collected by filtration and the crude product was recrystallized from EtOAc/hexanes, yielding 750 mg (52%) of **6p** as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.38–1.46 (m, 4H), 1.62–1.75 (m, 4H), 2.70–2.74 (m, 4H), 7.10–7.14 (m, 1H), 7.35 (dd, J = 8.6, 7.5 Hz, 2H), 7.47 (dd, J = 8.7, 1.1 Hz, 2H), 7.54 (s, 1H), 8.57 (s, 1H). Mass calculated for $C_{18}H_{19}N_3OS$: 325, found: 326.1 $(M+H)^+$.

7.2.5.17. *N*-Isopropyl 4,5,6,7,8,9-hexahydro-2-(3-phenylureido)cycloocta[*b*]thiophene-3-carboxamide (6q). Procedure B was used, with **5q** and phenylisocyanate. The crude material was purified by flash chromatography on silica gel using a gradient of 0–2% EtOAc/CH_2Cl_2, followed by HPLC prep. on XTerra Prep RP18 (10× 300 mm), gradient of MeCN in water (containing 0.05% TFA) 20–75% (20 min) then isocratic 75% (15 min). Compound **6q** was obtained as a white solid (34 mg, 27% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.25 (d, J = 6.5 Hz, 6H), 1.37–1.43 (m, 2H), 1.46–1.52 (m, 2H), 1.62–1.71 (m, 4H), 2.73–2.76 (m, 2H), 2.78–2.81 (m, 2H), 4.21 (sept, J = 6.6 Hz, 1H), 5.91 (d, J = 6.6 Hz, 1H), 6.98 (s, 1H), 7.04–7.094 (m, 1H), 7.27–7.32 (m, 2H), 7.42–7.44 (m, 2H), 11.41 (s, 1H). Mass calculated for $C_{21}H_{27}N_3O_2S$: 385, found: 386.1 $(M+H)^+$.

7.2.6. 4,5,6,7,8,9-Hexahydro-2-(3-phenylureido)cycloocta[*b*]thiophene-3-carboxylic acid (6r). *tert*-Butyl ester **6d** (55 mg, 0.14 mmol) was stirred for 1 h in a solution of TFA/CH_2Cl_2 (40%, v/v, 4 mL), after which the reaction mixture was concentrated under reduced pressure, to afford 35 mg (74%) of **6r** as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.18–1.25 (m, 2H), 1.38–1.45 (m, 2H), 1.51–1.61 (m, 4H), 2.67–2.71 (m, 2H), 2.84–2.89 (m, 2H), 6.99–7.03 (m, 1H), 7.28–7.32 (m, 2H), 7.48–7.50 (m, 2H), 10.15 (s, 1H), 10.72 (s, 1H), 12.81 (br s, 1H). Mass calculated for $C_{18}H_{20}N_2O_3S$: 344, found: 345.1 $(M+H)^+$.

7.2.7. Ethyl 4,5,6,7,8,9-hexahydro-2-(3-phenylthioureido)cycloocta[*b*]thiophene-3-carboxylate (7). Prepared as for **1** but substituting phenyl isothiocyanate for phenylisocyanate. The reaction mixture was diluted in EtOAc, washed with 5% HCl solution twice, saturated $NaHCO_3$ solution, and satd NaCl solution. The organic layer was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was purified by flash chromatography on silica gel, using 50% CH_2Cl_2 in hexanes as eluent. Compound **7** was obtained in 76% yield,

as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.22–1.28 (m, 2H), 1.25 (t, J = 7.1 Hz, 3H), 1.43–1.48 (m, 2H), 1.57–1.67 (m, 4H), 2.72–2.75 (m, 2H), 2.83–2.86 (m, 2H), 7.13 (q, J = 7.1 Hz, 2H), 7.34 (d, J = 7.2 Hz, 2H), 7.39 (d, J = 7.4 Hz, 1H), 7.48 (t, J = 7.9 Hz, 2H), 7.74 (s, 1H), 12.23 (s, 1H). Mass calculated for $C_{20}H_{24}N_2O_2S_2$: 388, found: 389.1 $(M+H)^+$.

7.2.8. Susbstituted 4,5,6,7,8,9-hexahydro-2-ureidocycloocta[*b*]thiophene-3-carboxylates (8a–y). Two procedures were used:

Procedure A. To a solution of **5c/5j** (2.98 mmol) in pyridine (6 mL) was added an isocyanate (6.85 mmol). The mixture was heated in a pressure tube at 65 °C for 18 h, after which the reaction mixture was brought to room temperature and concentrated under reduced pressure. The crude material was purified by SiO_2 chromatography.

Procedure B. To a solution of **5c/5j** (0.37 mmol) in CH_2Cl_2 (7 mL) was added dropwise a solution of triphosgene (110 mg, 0.37) in CH_2Cl_2 (1 mL), followed by dropwise addition of triethylamine (80 μL, 0.59 mmol) at 0 °C. After stirring for 2 h at room temperature, the appropriate amine (3.0 mmol) was added and the reaction mixture was stirred for 1 h after which H_2O was added. The resulting solution was extracted three times with CH_2Cl_2. The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated. The crude product was purified by flash chromatography on silica gel.

7.2.8.1. Isopropyl 4,5,6,7,8,9-hexahydro-2-(3-*p*-tolylureido)cycloocta[*b*]thiophene-3-carboxylate (8a). Procedure A was used with **5c** and *p*-tolylisocyanate. Chromatographic eluent: 50–60% CH_2Cl_2 in hexanes. Yield: 54% (light pink foam). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.25–1.31 (m, 2H), 1.33 (d, J = 6.3 Hz, 6H), 1.44–1.48 (m, 2H), 1.59–1.65 (m, 4H), 2.33 (s, 3H), 2.70–2.73 (m, 2H), 2.86–2.89 (m, 2H), 5.18 (sept, J = 6.2 Hz, 1H), 6.59 (s, 1H), 7.14–7.17 (m, 2H), 7.30–7.33 (m, 2H), 10.96 (s, 1H). Mass calculated for $C_{22}H_{28}N_2O_3S$: 400, found: 401.1 $(M+H)^+$.

7.2.8.2. Isopropyl 2-(3-(4-chlorophenyl)ureido)-4,5,6,7,8,9-hexahydrocycloocta[*b*]thiophene-3-carboxylate (8b). Procedure A was used with **5c** and 4-chlorophenylisocyanate. Chromatographic eluent: 50% CH_2Cl_2 in hexanes. Yield: 57% (light pink foam). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.25–1.30 (m, 2H), 1.35 (d, J = 6.3 Hz, 6H), 1.44–1.48 (m, 2H), 1.60–1.65 (m, 4H), 2.70–2.73 (m, 2H), 2.86–2.89 (m, 2H), 5.19 (sept, J = 6.3 Hz, 1H), 6.77 (s, 1H), 7.28–7.30 (m, 2H), 7.39–7.42 (m, 2H), 11.05 (s, 1H). Mass calculated for $C_{21}H_{25}ClN_2O_3S$: 420, found: 421.1 $(M+H)^+$.

7.2.8.3. Isopropyl 4,5,6,7,8,9-hexahydro-2-(3-(4-methoxyphenyl)ureido)cycloocta[*b*]thiophene-3-carboxylate (8c). Procedure A was used with **5c** and 4-methoxyphenylisocyanate. Chromatographic eluent: 75–80% CH_2Cl_2 in hexanes. Yield: 77% (beige foam). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.25–1.30 (m, 2H), 1.31 (d, J = 6.3 Hz, 6H), 1.44–1.47 (m, 2H), 1.58–1.64 (m, 4H), 2.69–2.72 (m, 2H), 2.85–2.88 (m, 2H), 3.81 (s, 3H), 5.15 (sept, J = 6.2 Hz, 1H), 6.58 (s, 1H), 6.89–6.91 (m, 2H), 7.31–7.34 (m, 2H), 10.89 (s, 1H). Mass calculated for $C_{22}H_{28}N_2O_4S$: 416, found: 417.1 $(M+H)^+$.

7.2.8.4. Isopropyl 2-(3-(3-chlorophenyl)ureido)-4,5,6,7,8,9-hexahydrocycloocta[*b*]thiophene-3-carboxylate (8d). Procedure A was used with **5c** and 3-chlorophenylisocyanate. Chromatographic eluent: 50–60% CH_2Cl_2 in hexanes. This was followed by trituration in hexanes. Yield: 38% (white solid). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.27–1.30 (m, 2H), 1.36 (d, J = 6.3 Hz, 6H), 1.44–1.48 (m, 2H), 1.60–1.65 (m, 4H), 2.71–2.73 (m, 2H), 2.87–2.90 (m, 2H), 5.19 (sept, J = 6.3 Hz, 1H), 6.87 (s, 1H), 7.06–7.09 (m, 1H), 7.24–7.26 (m, 2H), 7.61–7.62 (m, 1H), 11.06 (s, 1H). Mass calculated for $C_{21}H_{25}ClN_2O_3S$: 420, found: 421.0 $(M+H)^+$.

7.2.8.5. Isopropyl 2-(3-(3-(trifluoromethyl)phenyl)ureido)-4,5,6,7,8,9-hexahydrocycloocta[*b*]thiophene-3-carboxylate (8e). Procedure A was used with **5c** and α,α,α-trifluoro-*m*-tolylisocyanate. Chromatographic eluent: 80% CH_2Cl_2 in hexanes. Yield: 36% (white solid). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.25–1.28 (m, 2H), 1.35–1.36 (d, J = 6.3 Hz, 6H), 1.44–1.49 (m, 2H), 1.59–1.67 (m, 4H), 2.71–2.73 (m, 2H), 2.87–2.90 (m, 2H), 5.15–5.22 (m, 1H), 6.96 (s, 1H), 7.34–7.36 (d, J = 7.6 Hz, 1H), 7.42–7.46 (t, J = 8.2 Hz, 1H), 7.59–7.61 (d, J = 8.4 Hz, 1H), 7.82 (s, 1H), 11.12 (s, 1H). Mass calculated for $C_{22}H_{25}F_3N_2O_3S$: 454, found: 455.1 $(M+H)^+$.

7.2.8.6. Isopropyl 2-(3-(2-chlorophenyl)ureido)-4,5,6,7,8,9-hexahydrocycloocta[*b*]thiophene-3-carboxylate (8f). Procedure A was used with **5c** and 2-chlorophenylisocyanate. Chromatographic eluent: 50–60% CH_2Cl_2 in hexanes. Yield: 45% (yellow foam). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.27–1.30 (m, 2H), 1.37–1.38 (d, J = 6.3 Hz, 6H), 1.45–1.49 (m, 2H), 1.60–1.66 (m, 4H), 2.71–2.74 (m, 2H), 2.88–2.89 (m, 2H), 5.22 (m, 1H), 7.00–7.05 (m, 2H), 7.26–7.30 (m, 1H), 7.36–7.38 (d, J = 8.4 Hz, 1H), 8.21–8.23 (d, J = 8.4 Hz, 1H), 11.11 (s, 1H). Mass calculated for $C_{21}H_{25}ClN_2O_3S$: 420, found: 421.0 $(M+H)^+$.

7.2.8.7. Isopropyl 2-(3-(2,6-dichlorophenyl)ureido)-4,5,6,7,8,9-hexahydrocycloocta[*b*]thiophene-3-carboxylate (8g). Procedure A was used with **5c** and 2,6-dichlorophenylisocyanate. Chromatographic eluent: 50–60% CH_2Cl_2 in hexanes. Yield: 31% (white foam). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.27–1.28 (m, 2H), 1.31–1.32 (d, J = 6.3 Hz, 6H), 1.45 (m, 2H), 1.56–1.62 (m, 4H), 2.68–2.70 (m, 2H), 2.85–2.88 (m, 2H), 5.15 (m, 1H), 6.47 (s, 1H), 7.17–7.23 (m, 1H), 7.42 (d, J = 5.6 Hz, 2H), 10.97 (s, 1H). Mass calculated for $C_{21}H_{24}Cl_2N_2O_3S$: 455, found: 456.1 $(M+H)^+$.

7.2.8.8. Isopropyl 2-(3-cyclohexylureido)-4,5,6,7,8,9-hexahydrocycloocta[*b*]thiophene-3-carboxylate (8h). Procedure A was used with **5c** and cyclohexylisocyanate. Chromatographic eluent: 80% CH_2Cl_2 in hexanes. Yield: 15% (colorless oil). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.12–1.44 (m, 16H), 1.60–1.61 (m, 4H), 1.70–1.73 (m, 2H), 1.97–1.99 (m, 2H), 2.67–2.70 (m, 2H), 2.84–2.87

(m, 2H), 3.64 (m, 1H), 4.70 (d, J = 8.0 Hz, 1H), 5.14–5.21 (m, 1H), 10.67 (s, 1H). Mass calculated for $C_{21}H_{32}N_2O_3S$: 392, found: 393.1 $(M+H)^+$.

7.2.8.9. Isopropyl 2-(3-cyclopentylureido)-4,5,6,7,8,9-hexahydrocycloocta[*b*]thiophene-3-carboxylate (8i). Procedure A was used with **5c** and cyclopentylisocyanate. Chromatographic eluent: 80% CH_2Cl_2 in hexanes. Yield: 17% (colorless oil). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.26–1.46 (m, 12H), 1.60–1.69 (m, 8H), 2.00–2.04 (m, 2H), 2.67–2.70 (m, 2H), 2.84–2.87 (m, 2H), 4.09 (m, 1H), 4.80 (m, 1H), 5.16–5.19 (m, 1H), 10.71 (s, 1H). Mass calculated for $C_{20}H_{30}N_2O_3S$: 378, found: 379.2 $(M+H)^+$.

7.2.8.10. Isopropyl 2-(3-benzylureido)-4,5,6,7,8,9-hexahydrocycloocta[*b*]thiophene-3-carboxylate (8j). Procedure A was used with **5c** and benzylisocyanate. Chromatographic eluent: 80% CH_2Cl_2 in hexanes. Yield: 28% (colorless oil). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.24–1.27 (m, 2H), 1.29–1.31 (d, J = 6.3 Hz, 6H), 1.42–1.47 (m, 2H), 1.56–1.64 (m, 4H), 2.66–2.68 (m, 2H), 2.84–2.87 (m, 2H), 4.47 (d, J = 5.6 Hz, 2H), 5.04–5.11 (m, 1H), 5.58 (s, 1H), 7.24–7.33 (m, 5H), 10.80 (s, 1H). Mass calculated for $C_{22}H_{28}N_2O_3S$: 400, found: 401.2 $(M+H)^+$.

7.2.8.11. Isopropyl 4,5,6,7,8,9-hexahydro-2-(3-(pyridin-4-yl)ureido)cycloocta[*b*]thiophene-3-carboxylate (8k). Procedure B was used with **5c** and 4-aminopyridine. Chromatographic eluent: 10% hexanes in CH_2Cl_2. Yield: 30% (white powder). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.25–1.30 (m, 2H), 1.32–1.34 (d, J = 6.3 Hz, 6H), 1.44–1.47 (m, 2H), 1.62–1.66 (m, 4H), 2.71–2.74 (m, 2H), 2.85–2.89 (m, 2H), 5.11–5.17 (m, 1H), 7.47 (d, J = 6.1 Hz, 2H), 8.34 (s, 1H), 8.47 (s, 2H), 11.16 (s, 1H). Mass calculated for $C_{20}H_{25}N_3O_3S_2$: 387, found: 388.1 $(M+H)^+$.

7.2.8.12. Isopropyl 4,5,6,7,8,9-hexahydro-2-(3-(pyridin-2-yl)ureido)cycloocta[*b*]thiophene-3-carboxylate (8l). Procedure B was used with **5c** and 2-aminopyridine. Chromatographic eluent: 10% hexanes in CH_2Cl_2. Yield: 34% (white powder). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.24–1.31 (m, 2H), 1.32–1.34 (d, J = 6.3 Hz, 6H), 1.43–1.47 (m, 2H), 1.61–1.67 (m, 4H), 2.71–2.74 (m, 2H), 2.85–2.89 (m, 2H), 5.25–5.33 (m, 1H), 7.01–7.10 (m, 2H), 7.67–7.69 (m, 1H), 8.43 (s, 1H), 11.16 (s, 1H). Mass calculated for $C_{20}H_{25}N_3O_3S_2$: 387, found: 388.1 $(M+H)^+$.

7.2.8.13. Isopropyl 4,5,6,7,8,9-hexahydro-2-(3-(pyrazin-2-yl)ureido)cycloocta[*b*]thiophene-3-carboxylate (8m). Procedure B was used with **5c** and 2-aminopyrazine. Chromatographic eluent: 10% hexanes in CH_2Cl_2. Yield: 30% (white powder). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.25–1.31 (m, 2H), 1.37–1.39 (d, J = 6.3 Hz, 6H), 1.45–1.51 (m, 2H), 1.63–1.69 (m, 4H), 2.75–2.79 (m, 2H), 2.90–2.95 (m, 2H), 5.28–5.34 (m, 1H), 8.27 (s, 1H), 8.37 (s, 1H), 8.64 (s, 1H), 9.21 (s, 1H), 13.32 (s, 1H). Mass calculated for $C_{19}H_{24}N_4O_3S$: 388, found: 389.1 $(M+H)^+$.

7.2.8.14. Isopropyl 4,5,6,7,8,9-hexahydro-2-(3-(thiophen-2-yl)ureido)cycloocta[*b*]thiophene-3-carboxylate (8n). Procedure A was used with **5c** and 2-thienylisocyanate. Chromatographic eluent: 5% MeOH in CH_2Cl_2. Yield: 33% (colorless oil). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.25–1.31 (m, 8H), 1.44–1.46 (m, 2H), 1.58–1.63 (m, 4H), 2.68–2.71 (m, 2H), 2.84–2.88 (m, 2H), 5.13–5.30 (m, 1H), 6.80 (s, 1H), 6.90–6.92 (m, 1H), 7.01–7.03 (m, 1H), 7.26 (br s, 1H), 11.02 (s, 1H). Mass calculated for $C_{19}H_{24}N_2O_3S_2$: 392, found: 393.1 $(M+H)^+$.

7.2.8.15. Isopropyl 4,5,6,7,8,9-hexahydro-2-(3-(thiophen-3-yl)ureido)cycloocta[*b*]thiophene-3-carboxylate (8o). Procedure A was used with **5c** and 3-thienylisocyanate. Chromatographic eluent: 5% MeOH in CH_2Cl_2. Yield: 28% (colorless oil). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.23–1.30 (m, 8H), 1.42–1.47 (m, 2H), 1.56–1.63 (m, 4H), 2.66–2.69 (m, 2H), 2.84–2.87 (m, 2H), 5.09–5.14 (m, 1H), 7.02 (d, J = 5.1 Hz, 1H), 7.22–7.26 (m, 1H), 7.35 (s, 1H), 7.93 (s, 1H), 10.94 (s, 1H). Mass calculated for $C_{19}H_{24}N_2O_3S_2$: 392, found: 393.1 $(M+H)^+$.

7.2.8.16. Isopropyl 4,5,6,7,8,9-hexahydro-2-(3-(thiazol-2-yl)ureido)cycloocta[*b*]thiophene-3-carboxylate (8p). Procedure B was used with **5c** and 2-aminothiazole. Chromatographic eluent: 5% MeOH in CH_2Cl_2. Yield: 44% (colorless oil). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.25–1.30 (m, 2H), 1.36–1.38 (d, J = 6.3 Hz, 6H), 1.46–1.48 (m, 2H), 1.62–1.67 (m, 4H), 2.72–2.75 (m, 2H), 2.88–2.92 (m, 2H), 5.21–5.28 (m, 1H), 6.93 (d, J = 3.7 Hz, 1H), 7.78 (d, J = 3.7 Hz, 1H), 11.35 (s, 1H). Mass calculated for $C_{18}H_{23}N_3O_3S_2$: 393, found: 394.1 $(M+H)^+$.

7.2.8.17. Isopropyl 4,5,6,7,8,9-hexahydro-2-(3-(5-methylthiophen-2-yl)ureido)cycloocta[*b*]thiophene-3-carboxylate (8q). To a solution of 5-methylthiophene-2-carboxylic acid (71 mg, 0.50 mmol) in benzene (50 mL) was added dropwise diphenyl azidophosphate (110 μL, 0.50 mmol), followed by the dropwise addition of triethylamine (110 μL, 0.75 mmol) at room temperature. After stirring for 3 h at reflux, the amine **5c** (134 mg, 0.50 mmol) was added and the reaction mixture was stirred and refluxed overnight. The resulting solution was evaporated to dryness and the crude product was purified by flash chromatography on silica gel using 10% hexanes in CH_2Cl_2. Yield 55% (dark powder). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.26–1.29 (m, 8H), 1.42–1.47 (m, 2H), 1.57–1.64 (m, 4H), 2.44 (s, 3H), 2.67–2.70 (m, 2H), 2.84–2.87 (m, 2H), 5.10–5.30 (m, 1H), 6.56 (s, 1H), 6.65 (s, 1H), 7.26 (s, 1H), 10.94 (s, 1H). Mass calculated for $C_{20}H_{26}N_2O_3S_2$: 406, found: 407.1 $(M+H)^+$.

7.2.8.18. Isopropyl 4,5,6,7,8,9-hexahydro-2-(3-(3-(methylcarboxy)thiophen-2-yl)ureido)cycloocta[*b*]thiophene-3-carboxylate (8r). Procedure B was used with **5c** and methyl 5-aminothiophene-2-carboxylate. Chromatographic eluent: 10% hexanes in CH_2Cl_2. Yield: 27% (white powder). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.26–1.31 (m, 4H), 1.37–1.38 (d, J = 6.3 Hz, 6H), 1.43–1.49 (m, 2H), 1.60–1.67 (m, 4H), 2.71–2.74 (m, 2H), 2.88–2.91 (m, 2H), 3.88 (s, 3H), 5.20–5.30 (m, 1H), 6.68–6.69 (d, J = 6.3 Hz, 1H), 7.16–7.17 (d, J = 6.3 Hz, 1H), 10.66 (s, 1H), 11.24 (s, 1H). Mass calculated for $C_{21}H_{26}N_2O_5S_2$: 450, found: 451.1 $(M+H)^+$.

7.2.8.19. Isopropyl 4,5,6,7,8,9-hexahydro-2-(3-(2-(methylcarboxy)thiophen-3-yl)ureido)cycloocta[*b*]thiophene-3-carboxylate (8s). Procedure A was used with **5c** and 3-thienylisocyanate. Chromatographic eluent: 10% hexanes in CH_2Cl_2. Yield: 21% (dark powder). ^1H NMR (400 MHz, $CDCl_3$) δ 1.23–1.27 (m, 8H), 1.44–1.47 (m, 2H), 1.56–1.63 (m, 4H), 2.66–2.69 (m, 2H), 2.84–2.89 (m, 2H), 5.09–5.14 (m, 1H), 7.22–7.24 (d, $J = 6.3$ Hz, 1H), 7.22–7.24 (m, 1H), 7.34–7.36 (d, $J = 6.3$ Hz, 1H), 7.93 (s, 1H), 10.94 (s, 1H). Mass calculated for $C_{19}H_{24}N_2O_3S_2$: 392, found: 393.1 $(M+H)^+$.

7.2.8.20. Isopropyl 4,5,6,7,8,9-hexahydro-2-(3-(3,5-dimethylisoxazol-4-yl)ureido)cycloocta[*b*]thiophene-3-carboxylate (8t). Procedure A was used with **5c** and 3,5-dimethylisoxazol-4-ylisocyanate. Chromatographic eluent: 10% MeOH in CH_2Cl_2. Yield: 15% (colorless oil). ^1H NMR (400 MHz, $CDCl_3$) δ 1.28–1.30 (m, 8H), 1.45 (m, 2H), 1.61 (m, 4H), 2.24 (s, 3H), 2.39 (s, 3H), 2.67–2.70 (m, 2H), 2.84–2.87 (m, 2H), 5.11–5.14 (m, 1H), 6.25 (s, 1H), 10.93 (s, 1H). Mass calculated for $C_{20}H_{27}N_3O_4S$: 405, found: 406.2 $(M+H)^+$.

7.2.8.21. Isopropyl 4,5,6,7,8,9-hexahydro-2-(3-methyl-3-phenylureido)cycloocta[*b*]thiophene-3-carboxylate (8u). Procedure B was used with **5c** and *N*-methylanilinium trifluoroacetate. Chromatographic eluent: 10% hexanes in CH_2Cl_2. Yield: 19% (white powder). ^1H NMR (400 MHz, $CDCl_3$) δ 1.17–1.19 (d, $J = 6.3$ Hz, 6H), 1.21–1.24 (m, 2H), 1.41–1.44 (m, 2H), 1.60–1.67 (m, 4H), 2.66–2.69 (m, 2H), 2.79–2.82 (m, 2H), 3.37 (s, 3H), 4.97–5.00 (m, 1H), 7.31 (d, $J = 6.3$ Hz, 2H), 7.39 (t, $J = 6.3$ Hz, 1H), 7.46–7.50 (t, 2H), 10.64 (s, 1H). Mass calculated for $C_{22}H_{28}N_2O_3S$: 400, found: 401.1 $(M+H)^+$.

7.2.8.22. Isopropyl 4,5,6,7,8,9-hexahydro-2-(piperidine-1-carboxamido)cycloocta[*b*]thiophene-3-carboxylate (8v). Procedure B was used with **5c** and piperidine. Chromatographic eluent: 10% hexanes in CH_2Cl_2. Yield: 35% (white powder). ^1H NMR (400 MHz, $CDCl_3$) δ 1.19–1.25 (m, 2H), 1.38 (d, $J = 6.3$ Hz, 6H), 1.40–1.45 (m, 2H), 1.57–1.68 (m, 10H), 2.65–2.68 (m, 2H), 2.82–2.88 (m, 2H), 3.47 (s, 4H), 5.17–5.23 (m, 1H), 11.18 (s, 1H). Mass calculated for $C_{20}H_{30}N_2O_3S$: 378, found: 379.2 $(M+H)^+$.

7.2.8.23. Isopropyl 4,5,6,7,8,9-hexahydro-2-(morpholine-4-carboxamido)cycloocta[*b*]thiophene-3-carboxylate (8w). Procedure B was used with **5c** and morpholine. Chromatographic eluent: 10% hexanes in CH_2Cl_2. Yield: 32% (white powder). ^1H NMR (400 MHz, $CDCl_3$) δ 1.25–1.27 (m, 2H), 1.35 (d, $J = 6.3$ Hz, 6H), 1.43–1.46 (m, 2H), 1.59–1.64 (m, 4H), 2.69–2.72 (m, 2H), 2.86–2.89 (m, 2H), 3.52–3.55 (m, 4H), 3.73–3.76 (m, 4H), 5.18–5.21 (m, 1H), 11.28 (s, 1H). Mass calculated for $C_{19}H_{28}N_2O_4S$: 380, found: 381.1 $(M+H)^+$.

7.2.8.24. (±)-Tetrahydrofuran-3-yl 4,5,6,7,8,9-hexahydro-2-(3-(thiophen-2-yl)ureido)cycloocta[*b*]thiophene-3-carboxylate (8x). Procedure B was used, but a mixture of desired product and product of the addition of two isocyanates was obtained. Purification by flash chromatography on silica gel using a gradient of 0–5% EtOAc in CH_2Cl_2 as eluent, then reverse phase chromatography on Biotage (C18, 25+M), using a gradient of MeCN in H_2O could not separate the two products. The mixture obtained after those separation attempts was treated with a solution of 5%, v/v Et_3N in MeOH, at 65 °C during 5 h, then concentrated. Flash chromatography on silica gel using a gradient of 0–5% EtOAc in CH_2Cl_2 as eluent, then recrystallization in Et_2O/CH_2Cl_2 finally provided **8x** as a beige solid (29 mg, 16% yield). ^1H NMR (400 MHz, $CDCl_3$) δ 1.25–1.30 (m, 2H), 1.42–1.48 (m, 2H), 1.57–1.63 (m, 4H), 2.08–2.24 (m, 2H), 2.68–2.71 (m, 2H), 2.83–2.86 (m, 2H), 3.87–3.98 (m, 4H), 5.44–5.48 (m, 1H), 6.76–6.78 (m, 1H), 6.88–6.91 (m, 1H), 7.01–7.02 (m, 1H), 7.63 (br s, 1H), 10.86 (s, 1H). Mass calculated for $C_{20}H_{24}N_2O_4S_2$: 420, found: 421.0 $(M+H)^+$.

7.2.8.25. (±)-Tetrahydrofuran-3-yl 4,5,6,7,8,9-hexahydro-2-(3-(thiophen-3-yl)ureido)cycloocta[*b*]thiophene-3-carboxylate (8y). To a solution of **5j** (133 mg, 0.45 mmol), in EtOAc (2.3 mL), was added 3-thienylisocyanate (113 mg, 0.90 mmol). The mixture was stirred at room temperature during 18 h. After concentrating to dryness, crude **8y** was purified by flash chromatography on silica gel using a gradient of 0–5% EtOAc in CH_2Cl_2 as eluent, followed by triturations: first in EtO_2 (dithienylurea insoluble), then in hexanes (product insoluble), providing **8y** as an off-white solid (113 mg, 70% yield). ^1H NMR (400 MHz, $CDCl_3$) δ 1.26–1.31 (m, 2H), 1.43–1.49 (m, 2H), 1.59–1.66 (m, 4H), 2.11–2.25 (m, 2H), 2.69–2.72 (m, 2H), 2.81–2.87 (m, 2H), 3.91–4.00 (m, 4H), 5.47–5.50 (m, 1H), 7.01 (dd, $J = 5.2$ Hz, 1.4 Hz, 1H), 7.14 (br s, 1H), 7.26–7.28 (m, 1H), 7.36–7.37 (m, 1H), 10.82 (s, 1H). Mass calculated for $C_{20}H_{24}N_2O_4S_2$: 420, found: 421.0 $(M+H)^+$.

7.2.9. Benzyl 3-(isopropoxycarbonyl)-4,5,6,7,8,9-hexahydrocycloocta[*b*]thiophen-2-ylcarbamate (9). To a solution of **5c** (100 mg, 0.375 mmol) in pyridine (0.8 mL) was added benzylchloroformate (120 μL, 0.86 mmol). The mixture was heated in a pressure tube at 65 °C for 18 h, after which the reaction mixture was brought to room temperature and concentrated under reduced pressure. The crude material was purified by SiO_2 chromatography, using 10% hexanes in CH_2Cl_2 as the eluent to furnish 141 mg of product **9** (0.35 mmol, 47% yield) as a colorless oil. ^1H NMR (400 MHz, $CDCl_3$) δ 1.24–1.32 (m, 8H), 1.44 (m, 2H), 1.60 (m, 4H), 2.70 (m, 2H), 2.86 (m, 2H), 5.11–5.22 (m, 3H), 7.21–7.42 (m, 5H), 10.63 (s, 1H). Mass calculated for $C_{22}H_{27}NO_4S$: 401, found: 402.1 $(M+H)^+$.

7.2.10. 1-(3-(isopropoxycarbonyl)-4,5,6,7,8,9-hexahydrocycloocta[*b*]thiophen-2-yl)-3-phenyl sulfuric diamide (10). To a solution of **5c** (257 mg, 0.96 mmol) in CH_2Cl_2 were added phenylsulfamoyl chloride (460 mg, 2.4 mmol) and triethylamine (0.33 mL, 2.4 mmol). The mixture was refluxed for 18 h, after which the reaction mixture was brought to room temperature and concentrated under reduced pressure. The crude material was purified by SiO_2 chromatography, using 50/47/3 CH_2Cl_2–hexanes–methanol as the eluent to furnish 114 mg of product **10** (0.27 mmol, 36% yield) as a colorless oil. ^1H NMR (400 MHz, $CDCl_3$) δ 1.24–1.31 (m, 8H), 1.43–1.48 (m,

2H), 1.55–1.64 (m, 4H), 2.69–2.72 (m, 2H), 2.78–2.81 (m, 2H), 4.99–5.06 (m, 1H), 6.93 (s, 1H), 7.06–7.10 (m, 3H), 7.22–7.26 (m, 2H), 10.49 (s, 1H). Mass calculated for $C_{20}H_{26}N_2O_4S_2$: 422, found: 423.1 $(M+H)^+$.

7.2.11. Substituted isopropyl 2-aminothiophene-3-carboxylates (11). To a solution of ketone (3.96 mmol) in solvent (4 mL) were added sulfur (150 mg, 4.68 mmol of elemental S), isopropyl cyanoacetate (500 μL, 3.96 mmol), and base (5.54 mmol). The mixture was stirred at the indicated temperature for 18 h. The mixture was concentrated under reduced pressure and purified as indicated.

7.2.11.1. Isopropyl 2-amino-5,6,7,8-tetrahydro-4*H*-cyclohepta[*b*]thiophene-3-carboxylate (11a). Prepared from cycloheptanone using morpholine in isopropanol at 70 °C. The product was purified by flash chromatography on silica gel, using 50% CH_2Cl_2 in hexanes as eluent. Yield: 78% (yellow oil). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.33 (d, J = 6.3 Hz, 6H), 1.58–1.67 (m, 4H), 1.78–1.84 (m, 2H), 2.56–2.59 (m, 2H), 2.96–2.98 (m, 2H), 5.18 (sept, J = 6.1 Hz, 1H), 5.74 (br s, 2H).

7.2.11.2. Isopropyl 2-amino-5,6,7,8,9,10-hexahydro-4*H*-cyclonona[*b*]thiophene-3-carboxylate (11b). Prepared from cyclononanone using morpholine in isopropanol at 70 °C. The product was purified by flash chromatography on silica gel, using a gradient of 20–50% CH_2Cl_2 in hexanes as eluent. This was obtained as mixture of product and cyclononanone, 2:1 ratio, which was used directly for the next step. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.26–1.30 (m, 2H), 1.32 (d, J = 6.2, 6H), 1.37–1.41 (m, 2H), 1.44–1.48 (m, 4H), 1.54–1.61 (m, 4H), 2.62–2.65 (m, 2H), 2.79–2.82 (m, 2H), 5.18 (sept, J = 6.3, 1H), 5.96 (br s, 2H).

7.2.11.3. Isopropyl 2-amino-4,5,6,7,8,9,10,11-octahydrocyclodeca[*b*]thiophene-3-carboxylate (11c). Prepared from cyclodecanone using morpholine in isopropanol at 90 °C for four days. The product was purified by flash chromatography on silica gel, using 70% CH_2Cl_2 in hexanes as eluent, followed by another flash chromatography on silica gel, using 50% CH_2Cl_2 in hexanes as eluent, providing a mixture of desired compound and starting cyclodecanone, the latter was removed on Kugelrohr apparatus (80 °C, hi-vacuum), and pure **11c** was obtained as a dark yellow oil (207 mg, 21% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.12–1.17 (m, 2H), 1.32 (d, J = 6.3 Hz, 6H), 1.41–1.44 (m, 4H), 1.57 (br s, 2H), 1.62–1.72 (m, 4H), 2.77–2.84 (m, 4H), 5.18 (sept, J = 6.3 Hz, 1H), 6.00 (br s, 2H).

7.2.11.4. 6-Ethyl 3-isopropyl 2-amino-4,5,6,7-tetrahydrobenzo[*b*]thiophene-3,6-dicarboxylate (11d). Prepared from ethyl 4-oxocyclohexane carboxylate using morpholine in DMF at 70 °C. Crude mixture was diluted with EtOAc, washed with 2× H_2O, 2× brine, dried (Na_2SO_4), filtered, and concentrated. The product was purified by flash chromatography on silica gel using a gradient of 80–100% CH_2Cl_2 in hexanes as eluent. Yield: 89% (white solid). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.27 (dt, J = 7.2, 1.0 Hz, 3H), 1.29–1.32 (m, 6H), 1.74–1.84 (m, 1H), 2.14–2.20 (m, 1H), 2.60–2.79 (m, 4H), 2.90–2.97 (m, 1H), 4.16 (q, J = 7.2 Hz, 2H), 5.15 (d sept, J = 6.3, 1.0 Hz, 1H), 5.95 (br s, 2H).

7.2.11.5. Isopropyl 2-amino-5,7-dihydro-4*H*-thieno[2,3-*c*]pyran-3-carboxylate (11e). Prepared from tetrahydropyran-4-one using morpholine in isopropanol at 70 °C. The product was purified by flash chromatography on silica gel using a gradient of 20–25% EtOAc in hexanes containing 1% Et_3N as eluent. Yield: 82% (white solid). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.31 (d, J = 6.3 Hz, 6H), 2.82 (m, 2H), 3.91 (t, J = 5.7 Hz, 2H), 4.56 (t, J = 2.1 Hz, 2H), 5.16 (sept, J = 6.3 Hz, 1H), 5.98 (br s, 2H).

7.2.11.6. Isopropyl 2-amino-8-methyl-8-aza-thieno[2,3-*c*]bicyclo[3.2.1]octane-3-carboxylate (11f). Prepared from tropinone using morpholine in DMF at room temperature. The residue slowly crystallized, and the resulting crystals were washed with cold CH_2Cl_2. Yield: 17% (light brown solid). ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.25–1.28 (m, 6H), 1.77 (m, 1H), 2.01 (m, 1H), 2.28 (m, 2H), 2.61 (2 s, 3H), 3.06 (m, 1H), 3.78 (m, 1H), 3.92 (br s, 1H), 4.45 (br m, 1H), 5.00 (sept, J = 6.2 Hz, 1H), 7.42 (s, 2H).

7.2.11.7. Isopropyl 2-amino-4-cyclohexylthiophene-3-carboxylate (11g). Prepared from cyclohexyl methyl ketone using diethylamine in pyridine at 80 °C. The product was purified by flash chromatography on silica gel using a gradient of 5% EtOAc in hexanes as eluent. Yield: 24%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.16–1.42 (m, 5H), 1.33 (d, J = 6.3 Hz, 6H), 1.72–1.97 (m, 5H), 3.05 (m, 1H), 5.19 (sept, J = 6.3 Hz, 1H), 5.85 (s, 1H), 6.10 (br s, 2H).

7.2.11.8. Isopropyl 2-amino-4-cyclopentylthiophene-3-carboxylate (11h). Prepared from cyclopentyl methyl ketone using diethylamine in pyridine at 80 °C. The product was purified by flash chromatography on silica gel using 8% EtOAc in hexanes as eluent. Yield: 28%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.33 (d, J = 6.3 Hz, 6H), 1.43–1.53 (m, 2H), 1.58–1.75 (m, 4H), 2.04 (m, 2H), 3.43 (quintet, J = 7.43 1H), 5.20 (sept, J = 6.3 Hz, 1H), 5.89 (s, 1H), 6.05 (br s, 2H).

7.2.11.9. Isopropyl 2-amino-4-butyl-5-propylthiophene-3-carboxylate (11i). Prepared from nonanone using morpholine in isopropanol at 70 °C. The product was purified by flash chromatography on silica gel using a gradient of 20–25% EtOAc in hexanes containing 1% Et_3N as eluent. Yield: 45% (yellow oil). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.92 (t, J = 7.0 Hz, 3H), 0.95 (t, J = 7.4 Hz, 3H), 1.32 (d, J = 6.4 Hz, 6H), 1.32–1.58 (m, 6H), 2.52 (t, J = 7.6 Hz, 2H), 2.63 (t, J = 7.8 Hz, 2H), 5.20 (sept, J = 6.2 Hz, 1H), 5.96 (br s, 2H).

7.2.11.10. 2-Ethyl 4-isopropyl 5-amino-3-propylthiophene-2,4-dicarboxylate (11j). Prepared from ethyl 3-oxohexanoate using morpholine in isopropanol at 70 °C. The product was purified by flash chromatography on silica gel using a gradient of 20–25% EtOAc in hexanes

containing 1% Et_3N as eluent. Yield: 41% (yellow oil). ^1H NMR (400 MHz, $CDCl_3$) δ 0.98 (t, $J = 7.2$ Hz, 3H), 1.31 (t, $J = 7.2$ Hz, 3H), 1.33 (d, $J = 6.4$ Hz, 6H), 1.48–1.57 (m, 2H), 3.19 (m, 2H), 4.24 (q, $J = 7.1$ Hz, 2H), 5.18 (sept, $J = 6.3$ Hz, 1H), 5.96 (br s, 2H).

7.2.11.11. 2-Ethyl 4-isopropyl 5-amino-3-methylthiophene-2,4-dicarboxylate (11k). Prepared from ethyl acetoacetate using morpholine in isopropanol at 70 °C. The product was purified by flash chromatography on silica gel using 20% EtOAc in hexanes containing 1% Et_3N as eluent. Yield: 71% (white solid). ^1H NMR (400 MHz, $CDCl_3$) δ 1.31 (t, $J = 7.2$ Hz, 3H) overlapping with 1.33 (d, $J = 6.0$ Hz, 6H), 2.68 (s, 3H), 4.24 (q, $J = 7.1$ Hz, 2H), 5.18 (sept, $J = 6.3$ Hz, 1H), 6.59 (br s, 2H).

7.2.12. Substituted isopropyl 2-(phenylureido)thiophene-3-carboxylates (12a–k). Three procedures were used:

Procedure A. To a solution of isopropyl 2-aminothiophene-3-carboxylate (2.98 mmol) in pyridine (6 mL) was added phenylisocyanate (747 μL, 6.85 mmol). The mixture was heated in a pressure tube at 65 °C for 18 h, after which the reaction mixture was brought to room temperature and concentrated under reduced pressure.

Procedure B. To a solution of isopropyl 2-aminothiophene-3-carboxylate (100 mg, 0.29 mmol) in $CHCl_3$ (1.5 mL) were added phenylisocyanate (63 μL, 0.58 mmol) and a catalytic amount of DMAP. The mixture was heated in a pressure tube at 70 °C for 18 h, after which the reaction mixture was brought to room temperature and concentrated under reduced pressure. The crude material was purified by flash chromatography on silica gel.

Procedure C. To a solution of isopropyl 2-aminothiophene-3-carboxylate (470 mg, 1.76 mmol) in $CHCl_3$ (5 mL) were added phenylisocyanate (0.38 mL, 3.52 mmol) and triethylamine (0.29 mL, 2.11 mmol), and the mixture was heated at 70 °C for 5 h, after which the reaction mixture was brought to room temperature and concentrated under reduced pressure

7.2.12.1. Isopropyl 5,6,7,8-tetrahydro-2-(3-phenylureido)-4*H*-cyclohepta[*b*]thiophene-3-carboxylate (12a). Procedure A. The product was purified by flash chromatography on silica gel, using a gradient of 50–80% CH_2Cl_2 in hexanes as eluent. Yield: 66% (white foam). ^1H NMR (400 MHz, $CDCl_3$) δ 1.33 (d, $J = 6.3$ Hz, 6H), 1.60–1.69 (m, 4H), 1.81–1.86 (m, 2H), 2.68–2.71 (m, 2H), 2.99–3.02 (m, 2H), 5.16 (sept, $J = 6.2$ Hz, 1H), 6.72 (s, 1H), 7.10–7.14 (m, 1H), 7.32–7.36 (m, 2H), 7.42–7.44 (m, 2H), 10.83 (s, 1H). Mass calculated for $C_{20}H_{24}N_2O_3S$: 372, found: 373.1 $(M+H)^+$.

7.2.12.2. Isopropyl 5,6,7,8,9,10-hexahydro-2-(3-phenylureido)-4*H*-cyclonona[*b*]thiophene-3-carboxylate (12b). Procedure A. The crude product was purified by flash chromatography on silica gel, using a gradient of 50–60% CH_2Cl_2 in hexanes as eluent. Yield: 45% (white foam). ^1H NMR (400 MHz, $CDCl_3$) δ 1.23–1.28 (m, 2H), 1.34 (d, $J = 6.3$ Hz, 6H), 1.44–1.47 (m, 4H), 1.61–1.67 (m, 4H), 2.73–2.76 (m, 2H), 2.85–2.88 (m, 2H), 5.19 (sept, $J = 6.1$ Hz, 1H), 6.67 (s, 1H), 7.10–7.14 (m, 1H), 7.31–7.36 (m, 2H), 7.43–7.45 (m, 2H), 11.08 (s, 1H). Mass calculated for $C_{22}H_{28}N_2O_3S$: 400, found: 401.1 $(M+H)^+$.

7.2.12.3. Isopropyl 4,5,6,7,8,9,10,11-octahydro-2-(3-phenylureido)cyclodeca[*b*]thiophene-3-carboxylate (12c). Procedure C. Heated at 80 °C during 72 h. The crude product was purified by flash chromatography on silica gel using CH_2Cl_2 as eluent, then reverse phase chromatography on Biotage (C18, 12+M), using a gradient of MeCN in H_2O. Yield: 77%. ^1H NMR (400 MHz, $CDCl_3$) δ 1.07–1.13 (m, 2H), 1.30–1.36 (m, 2H), 1.34 (dd, $J = 6.3$ Hz, 0.8 Hz, 6H), 1.43–1.45 (m, 4H), 1.62–1.69 (m, 2H), 1.77–1.84 (m, 2H), 2.87–2.90 (m, 4H), 5.18 (d sept, $J = 6.3$ Hz, 0.7 Hz, 1H), 6.85 (s, 1H), 7.10–7.15 (m, 1H), 7.31–7.36 (m, 2H), 7.44–7.47 (m, 2H), 11.14 (s, 1H). Mass calculated for $C_{23}H_{30}N_2O_3S$: 414, found: 415.2 $(M+H)^+$.

7.2.12.4. 6-Ethyl 3-isopropyl 4,5,6,7-tetrahydro-2-(3-phenylureido)benzo[*b*]thiophene-3,6-dicarboxylate (12d). Procedure B. The crude product was purified by flash chromatography on silica gel using a gradient of 80–100% CH_2Cl_2 in hexanes as eluent, then another flash chromatography on silica gel using a gradient of 0–5% Et_2O in CH_2Cl_2/hexanes (1:1) as eluent. Yield: 34%. ^1H NMR (400 MHz, $CDCl_3$) δ 1.28 (t, $J = 7.2$ Hz, 3H), 1.32 (dd, $J = 6.2$, 4.2 Hz, 6H), 1.80–1.88 (m, 1H), 2.18–2.23 (m, 1H), 2.68–2.75 (m, 2H), 2.86–2.89 (m, 2H), 2.94–3.00 (m, 1H), 4.18 (q, $J = 7.2$ Hz, 2H), 5.15 (sept, $J = 6.2$ Hz, 1H), 6.88 (s, 1H), 7.11–7.15 (m, 1H), 7.32–7.36 (m, 2H), 7.42–7.46 (m, 2H), 10.92 (s, 1H). Mass calculated for $C_{22}H_{26}N_2O_5S$: 430, found: 431.1 $(M+H)^+$.

7.2.12.5. Isopropyl 5,7-dihydro-2-(3-phenylureido)-4*H*-thieno[2,3-*c*]pyran-3-carboxylate (12e). Procedure A. The crude product was purified by flash chromatography on silica gel using a gradient of 50–100% CH_2Cl_2 in hexanes as eluent, followed by prep HPLC on XTerra PrepRP18 column (gradient of 20–90% MeCN in water containing 0.005% TFA, over 25 min). Yield: 15% (white solid). ^1H NMR (400 MHz, $CDCl_3$) δ 1.33 (d, $J = 6.3$, 6H), 2.86 (t, $J = 5.6$ Hz, 2H), 3.94 (t, $J = 5.6$ Hz, 2H), 4.68 (t, $J = 1.8$ Hz, 2H), 5.16 (sept, $J = 6.3$ Hz, 1H), 6.95 (br s, 1H), 7.14 (t, $J = 7.4$ Hz, 1H), 7.35 (t, $J = 8.0$ Hz, 2H), 7.44 (d, $J = 7.5$ Hz, 2H), 10.87 (s, 1H). Mass calculated for $C_{18}H_{20}N_2O_4S$: 360, found: 361.0 $(M+H)^+$.

7.2.12.6. Isopropyl 8-methyl-8-aza-2-(3-phenylureido)thieno[2,3-*c*]bicyclo[3.2.1]octane-3-carboxylate, TFA salt (12f). Procedure A. The crude product was purified by flash chromatography on silica gel using a gradient of 50–100% CH_2Cl_2 in hexanes as eluent, followed by prep HPLC on XTerra PrepRP18 column (gradient of 20–90% MeCN in water containing 0.005% TFA, over 25 min). Yield: 27% (TFA salt, fluffy white sold). ^1H NMR (400 MHz, DMSO-d_6) δ 1.35–1.40 (m, 6H), 1.98–2.03 (m, 1H), 2.31–2.58 (m, 3H), 2.85 and 2.90 (2s, 3H), 3.07 (dd, $J = 7.7$ Hz, 18.0, 1H), 3.47 (dd,

J = 4.9 Hz, 18.0, 1H), 4.16–4.22 (m, 1H), 4.75–4.82 (m, 1H), 5.20–5.26 (m, 1H), 7.06 (t, J = 7.4 Hz, 1H), 7.30 (t, J = 7.2 Hz, 2H), 7.47 (d, J = 8.6 Hz, 2H), 9.92 (s, 1H), 10.83 and 10.87 (2s, 1H). Mass calculated for $C_{21}H_{25}N_3O_3S$: 399, found: 400.2 $(M+H)^+$.

7.2.12.7. Isopropyl 4-cyclohexyl-2-(3-phenylureido)thiophene-3-carboxylate (12g). Procedure C. The crude product was purified by flash chromatography on silica gel using 8% EtOAc in hexanes as eluent. Yield: 75%. ^1H NMR (400 MHz, $CDCl_3$) δ 1.21–1.41 (m, 5H), 1.33 (d, J = 6.3 Hz, 6H), 1.72–1.97 (m, 5H), 3.05 (m, 1H), 5.19 (sept, J = 6.3 Hz, 1H), 6.32 (s, 1H), 7.04 (s, 1H), 7.12 (t, J = 7.23 Hz, 1H), 7.33 (t, J = 8.21 Hz, 2H), 7.45 (d, J = 8.41 Hz, 2H), 11.11 (s, 1H). Mass calculated for $C_{21}H_{26}N_2O_3S$: 386, found: 387.1 $(M+H)^+$.

7.2.12.8. Isopropyl 4-cyclopentyl-2-(3-phenylureido)thiophene-3-carboxylate (12h). Procedure C. The crude product was purified by flash chromatography on silica gel using 5% EtOAc in hexanes as eluent. Yield: 75%. ^1H NMR (400 MHz, $CDCl_3$) δ 1.33 (d, J = 6.3 Hz, 6H), 1.49–1.76 (m, 6H), 2.0 (m, 2H), 3.47 (quint, J = 7.43 1H), 5.17 (sept, J = 6.3 Hz, 1H), 6.37 (s, 1H), 7.04 (br s, 1H), 7.12 (t, J = 7.23 Hz, 1H), 7.33 (t, J = 8.41 Hz, 2H), 7.45 (d, J = 8.41 Hz, 2H), 11.10 (s, 1H). Mass calculated for $C_{20}H_{24}N_2O_3S$: 372, found: 373.1 $(M+H)^+$.

7.2.12.9. Isopropyl 4-butyl-2-(3-phenylureido)-5-propylthiophene-3-carboxylate (12i). Procedure A. The crude product was purified by flash chromatography on silica gel using a gradient of 50–80% CH_2Cl_2 in hexanes as eluent. Yield: 72% (light yellow oil which slowly solidified). ^1H NMR (400 MHz, $CDCl_3$) δ 0.93 (t, J = 7.1 Hz, 3H), 0.96 (t, J = 7.3 Hz, 3H), 1.33 (d, J = 6.3 Hz, 6H), 1.35–1.49 (m, 4H), 1.62 (q, J = 7.5 Hz, 2H), 2.60 (t, J = 7.6 Hz, 2H), 2.68 (dd, J = 6.8 Hz, 8.6, 2H), 5.19 (sept, J = 6.3 Hz, 1H), 7.03 (br s, 1H), 7.11 (t, J = 7.4 Hz, 1H), 7.33 (t, J = 8.0 Hz, 2H), 7.45 (d, J = 7.5 Hz, 2H), 11.06 (s, 1H). Mass calculated for $C_{22}H_{30}N_2O_3$: 402, found: 403.2 $(M+H)^+$.

7.2.12.10. 2-Ethyl 4-isopropyl 5-(3-phenylureido)-3-propylthiophene-2,4-dicarboxylate (12j). Procedure A. The crude product was purified by flash chromatography on silica gel using 15% EtOAc in hexanes as eluent. Yield: 38% (light yellow gum). ^1H NMR (400 MHz, $CDCl_3$) δ 0.98 (t, J = 7.3 Hz, 3H), 1.32 (t, J = 7.1 Hz, 3H), 1.33 (d, J = 6.3 Hz, 6H), 1.50–1.60 (m, 2H), 3.24 (m, 2H), 4.27 (q, J = 7.1 Hz, 2H), 5.17 (sept, J = 6.3 Hz, 1H), 7.14 (t, J = 7.4 Hz, 1H), 7.34 (t, J = 8.0 Hz, 2H), 7.43 (br s, 1H), 7.47 (d, J = 7.6 Hz, 2H), 11.31 (s, 1H). Mass calculated for $C_{21}H_{26}N_2O_5S$: 418, found: 419.1 $(M+H)^+$.

7.2.12.11. 2-Ethyl 4-isopropyl 5-(3-phenylureido)-3-methylthiophene-2,4-dicarboxylate (12k). Procedure A. The crude product was purified by flash chromatography on silica gel using a gradient of 50% CH_2Cl_2 in hexanes to neat CH_2Cl_2 as eluent. Yield: 49% (light yellow gum). ^1H NMR (400 MHz, $CDCl_3$) δ 1.33 (t, J = 7.2 Hz, 3H) overlapping with 1.35 (d, J = 6.0 Hz, 6H), 2.73 (s, 3H), 4.28 (q, J = 7.2 Hz, 2H), 5.17 (sept, J = 6.3 Hz, 1H), 7.15 (t, J = 7.4 Hz, 1H), 7.35 (t, J = 8.0 Hz, 2H), 7.47 (d, J = 8.0 Hz, 2H), 11.25 (s, 1H). Mass calculated for $C_{19}H_{22}N_2O_5S$: 390, found: 391.0 $(M+H)^+$.

7.3. Biology

7.3.1. Determination of inhibitory activity toward the RNA polymerase enzyme. In vitro transcription assays used 12.5 nM of *S. aureus* sigma factor and 12.5 nM of *S. aureus* core RNA polymerase in a total volume of 25 μL containing 40 mM Tris–acetate, pH 7.9, 100 mM NaCl, 5 mM $MgCl_2$, 1 mM DTT, 0.1 mg/mL BSA, 150 μM each of ATP, GTP, CTP, 30 μM UTP, 200,000–300,000 cpm of α-[^{32}P] UTP (3000 Ci/mmol), 1 U RNasin (Promega), and 40 ng of pTMSM template DNA (a vector derived by modification of pTOO21[19]). To measure 50% inhibitory concentrations (IC_{50}s), compounds were prepared in serial dilution typically ranging from 1 nM to 100 μM and added to the reaction mixture. The final concentration of DMSO in the reaction mixture was 2%. Rifampicin was used as a positive control and DMSO alone was used as a negative control. The reaction mixture was incubated for 1 h at 37 °C in a 96-well PCR-plate (BD-Falcon™). Samples were transferred to a 96-well Multiscreen™ GF-B plate (Millipore) and subjected to a 10% TCA precipitation step for 1 h at 4 °C in the presence of 10 μg of single strand DNA as a carrier in a total volume of 180 μL. After filtration, the wells were washed three times (225 μL per wash) with 10% TCA, two times (225 μL per wash) with 95% ethanol. Liquid scintillation cocktail (75 μL) was then added and the plates were counted for 15 s in a Trilux™ Microbeta counter (Perkin-Elmer-Packard).

7.3.2. Determination of cell based activities. The antimicrobial activity of the compounds was tested against *S. aureus* strains RN4220 and ATCC 13709. Minimum inhibitory concentration (MIC) testing was performed by the microdilution method according to the guidelines set by the Clinical and Laboratory Standards Institute (formerly the National Committee for Clinical Laboratory Standards).[20]

The cytotoxicities of compounds **1** and **6c** were determined by the MTS and the ATP assays as reported. The mutagenicity of compound **6c** against *S. typhimurium* was determined as described.[21]

For resistance frequency determination, overnight cultures of *S. aureus* strains RN4220 and ATCC 13709 in TSB were diluted or concentrated appropriately in saline and plated on TSA without any compound or containing compound 6c at two- and fourfold the MIC. Plates were incubated at 37 °C for 48 h. Mutation frequencies were calculated as the ratio of the number of resistant colonies at 48 h to the number of CFU inoculated. To purify, selected colonies were streaked once on TSA plates containing the selected concentration of compound.

Time-kill curves were generated by growing *S. aureus* strains RN4220 and ATCC 13709 to mid-logarithmic phase at 37 °C with vigorous shaking. Compound **6c**

was added at the indicated folds over MIC and the incubation was continued. To assess the number of surviving bacteria, samples were taken immediately prior to drug exposure and at the indicated time points thereafter. Samples were diluted in saline and were plated onto cation-adjusted Mueller–Hinton agar.

7.3.3. Effect of 6c on macromolecular synthesis. The synthesis of DNA, RNA, and proteins was evaluated by the incubation of radiolabeled macromolecule precursors into bacteria followed by trichloroacetic acid (TCA) precipitation of radiolabeled macromolecules. *S. aureus* RN4220 was grown overnight at 37 °C in CAMHB. The overnight culture was diluted 1:100 in fresh CAMHB and grown to $OD_{565} = 0.3$. Broth was removed from the bacteria by centrifugation for 15 min at 3500*g*. The *S. aureus* pellet was washed twice in synthetic medium comprising M63[22] supplemented with 0.5% casamino acids, 3 μg/mL thymine, 6.25 ng/mL biotin, 3.1 μg/mL sodium pantothenate, and 1.55 μg/mL nicotinic acid. The pellet was resuspended in the supplemented synthetic medium and diluted to $OD_{565} = 0.3$. Macromolecular synthesis was measured by pre-incubating cells with different amounts of compound **6c** for 5 min, followed by labeling 100 μL of cells with 10 μL of ^{3}H-thymidine (0.01 mCi/mL), 10 μL of ^{3}H-uridine (0.01 mCi/mL), and 4 μL of ^{35}S-methionine (0.05 mCi/mL) for 10 min at 37 °C. The labeling reactions were stopped by adding 10 μL of 1.23 mM cold label (thymidine, uridine or methionine) containing 0.2% sodium azide. The reaction mixtures were precipitated with 110 μL of cold 10% TCA for 30 min on ice and filtered through a 96-well multiscreen plate (MAFBNOB10, Millipore Corporation, Bedford, MA, USA). The samples were washed sequentially with 200 μL of cold 5% TCA, 1% TCA, and 95% ethanol, and dried. After addition of 100 μL of scintillation fluid, the samples were counted in a liquid scintillation counter (Trilux 1450™ Microbeta counter, Perkin-Elmer Biosciences, Turku, Finland).

7.3.4. In vivo evaluation

7.3.4.1. Acute tolerability in mice. All animal experiments conformed to Institutional Animal Care and Use Guidelines and were designed to minimize the number of animals while maintaining statistical power. Female CD-1 mice (age, 4–6 weeks; $n = 3$/group; Charles River, St-Constant, Canada) received selected compounds and controls iv bolus in the lateral tail vein, intraperitoneally, subcutaneously, or orally by gavage. The compounds were administered twice, at time 0 and 45 min later. The mice were monitored for clinical signs for 72 h and the number of surviving mice was recorded.

7.3.4.2. Mouse sepsis model. Female CD-1 mice (age, 4–6 weeks; $n = 10$/group; Charles River, St-Constant, Canada) were infected with an intraperitoneal dose of 0.2 mL of a bacterial suspension (*S. aureus* ATCC 13709, ca. 4×10^{7} CFU/mouse; 10 times the 100% lethal dose). Compound **6c** was injected as indicated. Moxifloxacin (positive control) was injected once (ip 15 min postinfection). The number of surviving mice was recorded at 72 h postinfection.

7.3.4.3. Determination of 6c in plasma. Female CD-1 mice were administered with **6c** intravenously (a single bolus dose of 25 mg/kg of body weight), or orally (1 dose of 50 mg/kg of body weight). Animals were sacrificed by CO_2 inhalation at specified time points after dosing. Blood samples were collected by cardiac puncture and transferred in BD vacutainer tubes (green cap) for plasma isolation. The plasma components were obtained by centrifugation of these samples and they were stored at −80 °C until analysis. Plasma samples (100 μL) were mixed with 900 μL of acetonitrile. The mixture was vortexed for 30 min and centrifuged for 15 min at 10,000*g*. The supernatant was evaporated to dryness under a nitrogen stream and the dried residue was reconstituted in 200 μL water–acetonitrile 1/4. The amount of compound **6c** was evaluated from a 8-point calibration curve by injecting 20 μL of the reconstituted sample into a LC analyzer (Agilent 1100 LC). The analysis was done on a Zorbax™ SB-C18 column (2.1× 30 mm, 3.5 μL) using ammonium acetate (10 mM, pH 6.8) and acetonitrile as eluents, in isocratic conditions (65% acetonitrile, run time 8 min) at a flow rate of 0.3 mL/min. The diode array detector was set at 254 nm.

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