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Synthesis and biological evaluation of benzo[*b*]thiophene acylhydrazones as antimicrobial agents against multidrug-resistant *Staphylococcus aureus*

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Abstract – The benzo[*b*]thiophene nucleus and the acylhydrazone functional group were combined to prepare 3 new series of compounds for screening against *Staphylococcus aureus*. The reaction of substituted benzo[*b*]thiophene-2-carboxylic hydrazide and various aromatic or heteroaromatic aldehydes led to a collection of 26 final products with extensive structural diversification on the aromatic ring and on the position 6 of the benzo[*b*]thiophene. The screening lead to the identification of 8 hits including (*E*)-6-chloro-*N'*-(pyridin-2-ylmethylene)benzo[*b*]thiophene-2-carbohydrazide (**II.b**), a non-cytotoxic derivative showing a minimal inhibitory concentration of 4 µg/mL on three *S. aureus* strains, among which a reference classical strain and two clinically isolated strains resistant to methicillin and daptomycin, respectively.

Key words: Benzothiophene, acylhydrazone, *Staphylococcus aureus*, Antibacterial, MRSA

Introduction

Antimicrobial resistance (AMR) is a public health issue that will continue to worsen in the years to come. At least 700,000 deaths are attributed each year to drug-resistant pathogens, but this could rise to 10,000,000 deaths per year by 2050 [1-3]. Among those pathogens, some are widely spread, such as vancomycin-resistant *Enterococcus faecium* or methicillin-resistant *Staphylococcus aureus* (MRSA) [4]. The development of new antibiotics targeting antibioresistant bacteria is therefore critical. Substituted benzo[*b*]thiophenes are interesting compounds in medicinal chemistry [5] by displaying a broad range of activity including antimicrobial [6, 7], anticancer [8], anti-diabetic [9], anti-depressant [10], anti-inflammatory and analgesic agents [11, 12]. As a part of an ongoing research program aiming at the discovery of new potential antibiotics targeting multidrug-resistant *Staphylococcus aureus* strains, we combined the benzo[*b*]thiophene nucleus with the acylhydrazone functional group which is also relevant in bioactive molecules design [13]. We focused our efforts on the synthesis and biological evaluation of a

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41 collection of acylhydrazones built from various aromatic or heteroaromatic aldehydes and
42 benzo[*b*]thiophene-2-carboxylic hydrazide, readily accessible from benzo[*b*]thiophene-2-carboxylic acid.
43 This sequence allowed an easy structural diversification on both the heteroaromatic benzo[*b*]thiophene
44 nucleus and the aromatic system. The purpose was then to screen the collection of
45 benzo[*b*]thiophene-2-acylhydrazones for identifying new hits against drug-resistant *S. aureus* strains.
46

47 Results and discussion

49 Synthesis and biological evaluation of series I – benzo[*b*]thiophene-2-acylhydrazones

51 Benzo[*b*]thiophene-2-acylhydrazones **I** derived from various aldehydes were first investigated
52 (Figure 1).
53

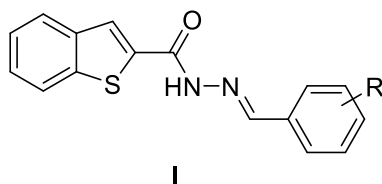
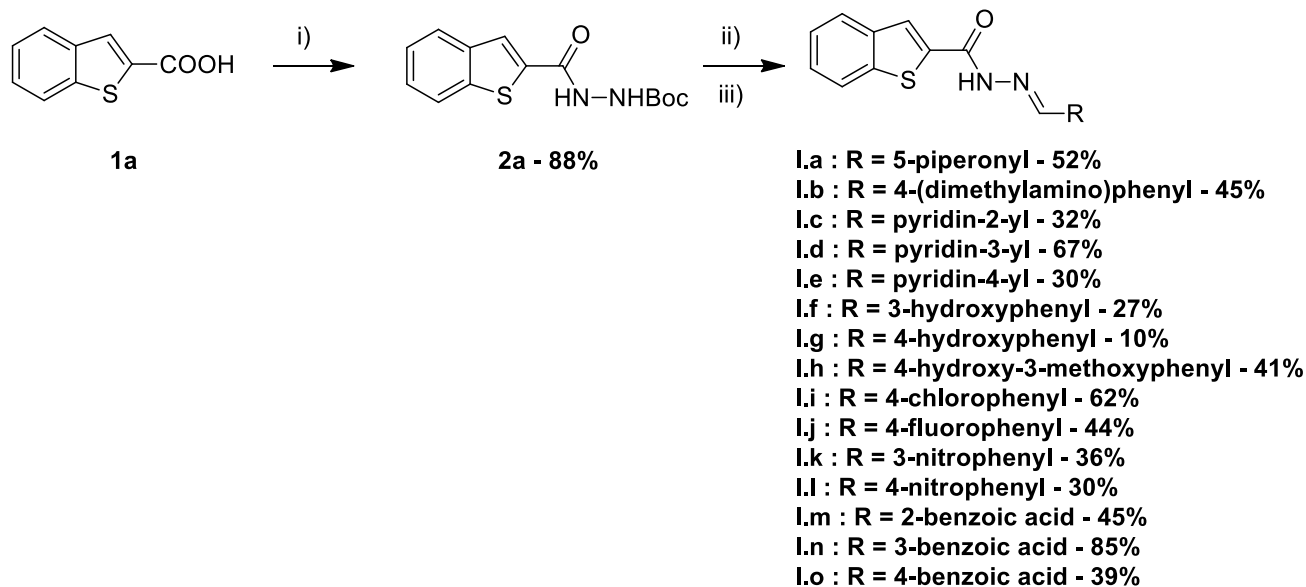


Figure 1 : General structure of set **I** derivatives

56 The synthetic route to these compounds was achieved by conversion of benzothiophene-2-carboxylic
57 acid **1a** into *tert*-butyl 2-(benzothiophene-2-carbonyl)hydrazinecarboxylate **2a** in 88% yield using DCC as
58 coupling reagent. This method was preferred from the substitution of methyl benzothiophene-2-carboxylate
59 with hydrazine leading to a mixture of products difficult to purify [14]. Then, the *tert*-butyl carboxylate
60 group was removed under acidic conditions using TFA to give a crude material which was then engaged
61 without purification in the presence of diversely substituted aromatic or heteroaromatic aldehydes under
62 acidic and refluxing conditions to form the imine bond of the acylhydrazone (

Scheme 1).

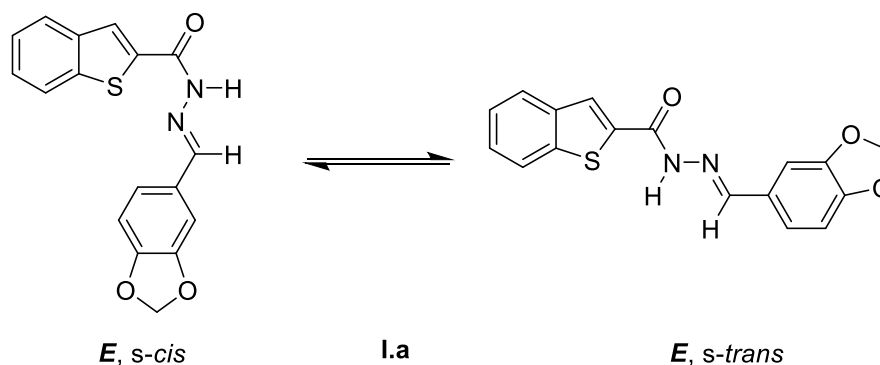


i) Boc-hydrazide, DCC, DMAP, DCM (anh.), r.t., 24 h; ii) TFA, DCM, r.t., 18 h;
 iii) Aldehyde, MeOH (anh.), 65 °C, 2 h;

Scheme 1 : Synthesis of set I derivatives

All the synthesized derivatives **I** were conveniently obtained in satisfactory yields by spontaneous precipitation in pure form in the reaction medium after two hours of reflux. Compounds like the 2-pyridinyl derivative **I.c** required careful removal of all traces of acid after TFA deprotection, by a basic aqueous work-up and extraction with ethyl acetate before reaction with 2-pyridinecarboxaldehyde.

Structural analysis showed the presence of mixtures of geometric isomers for each final compound, as ^1H and ^{13}C NMR signals were split in two. Theoretically, each N-acylhydrazone derivative could present four different isomers due to (*E*)/(*Z*) and *s-cis*/*s-trans* isomerism. Previous studies have shown that only the more stable (*E*)-isomer is generated due to the steric hindrance and under such thermodynamic conditions, and that *s-cis*- and *s-trans*-isomers (rotamers) were identified by ^1H NMR spectroscopy [13, 15, 16]. To demonstrate the presence of rotamers in our case for the piperonyl derivative **I.a**, the temperature of ^1H NMR experiments was gradually increased, and the expected coalescence phenomenon was observed at 330 K and at higher temperature (Figure 2).



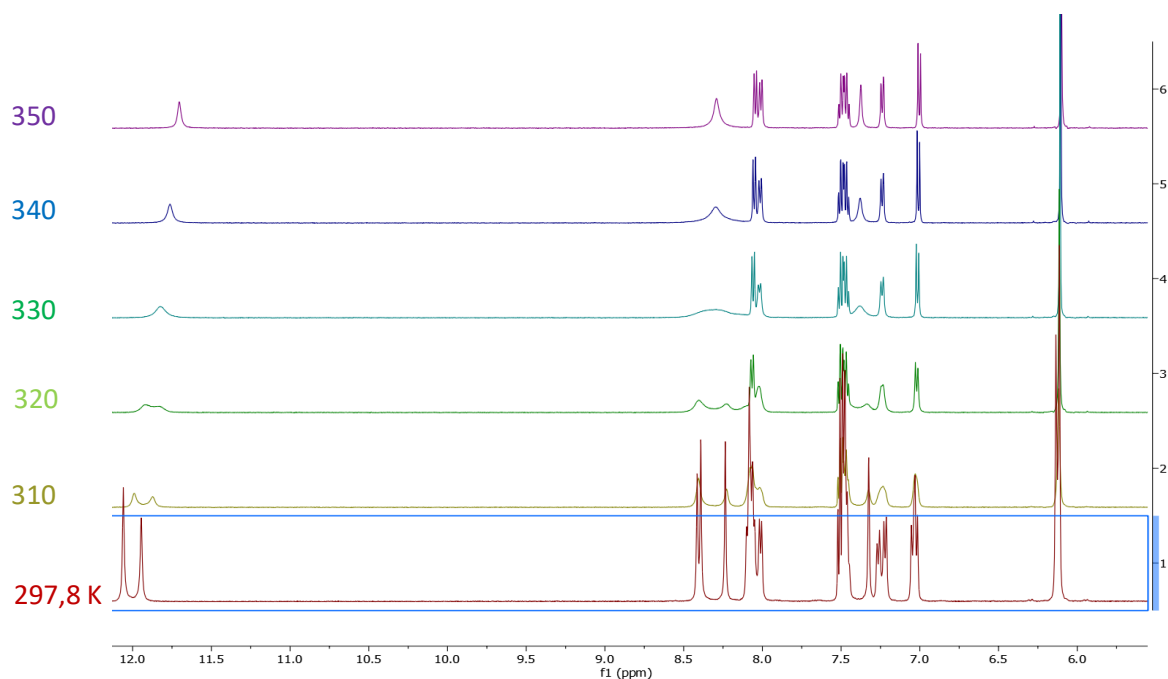
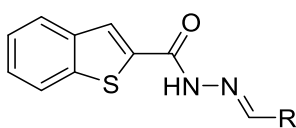




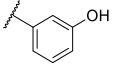
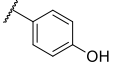
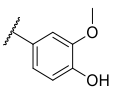


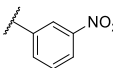
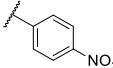

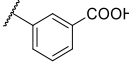
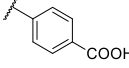

Figure 2 : ^1H NMR spectra of **I.a** at different temperatures

This first set of compounds was then tested on three strains of *Staphylococcus aureus*, including a reference (ATCC29213) and two clinically isolated strains respectively resistant to methicillin (SF8300) and daptomycin (ST20171643). The purpose of this experiment was to establish the minimum inhibitory concentration (MIC), which is the minimum concentration of compound needed to prevent the growth of a standardized bacterial inoculum, for the benzo[*b*]thiophene derived acylhydrazones. The results are presented in Table 1.

Table 1 : Minimum inhibitory concentrations of set **I** molecules against *S. aureus* strains



Name	R	LogP	MIC ($\mu\text{g/mL}$)		
			ATCC 29213 ^a	SF8300 ^b	ST20171643 ^c
I.a		4.20	32	64	16
I.b		4.41	> 256	> 256	> 256
I.c		3.13	16	16	16

I.d		3.07	> 256	> 256	> 256
I.e		3.02	> 256	> 256	> 256
I.f		3.80	> 256	> 256	ND
I.g		3.83	> 256	> 256	> 256
I.h		3.64	> 256	> 256	> 256
I.i		4.98	128	128	128
I.j		4.47	> 256	> 256	128
I.k		4.24	> 256	> 256	ND
I.l		4.26	64	64	32
I.m		3.83	256	> 256	> 256
I.n		4.19	> 256	> 256	256
I.o		4.22	> 256	> 256	256
I.p		2.33	64	64	64

97 ^a : Methicillin-sensitive *S. aureus* ; ^b : Methicillin-resistant *S. aureus* ; ^c : Daptomycin-resistant *S.*
98 *aureus*

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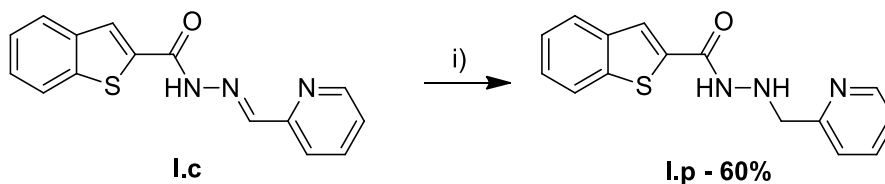
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112

In this series, the two most active compounds are the piperonyl derivative **I.a** which shows a MIC of 32 $\mu\text{g/mL}$ for ATCC29213 and a range from 16 to 64 $\mu\text{g/mL}$ for the two strains of antibiotic-resistant *S. aureus*, and the pyridinyl compound **I.c**, which shows a MIC of 16 $\mu\text{g/mL}$ for every strain tested, making it the most potent molecule in this series. Interestingly, the position of the nitrogen atom in position 2 of the pyridine moiety in **I.c** was found to be important, as the other pyridinyl compounds **I.d** and **I.e**, in which the nitrogen is respectively in position 3 and 4, were found to be inactive. In this set, another position of interest in the phenyl ring is position 4 (**I.i**, **I.l** and, to a lesser extent **I.a**), with interesting MICs for the products, while the other ones had no biological activity.

To examine the effect of the reduction of the imine bond and its consequences on free rotation and conjugation, the pyridinyl compound **I.c** was reduced using palladium over carbon under H_2 atmosphere with a 60% yield (Scheme 2). Reduced compound **I.p** exhibited a MIC four times higher than the one of **I.c** (Table 1). It is therefore suggested that preventing the free rotation of the pyridine ring and allowing

113 conjugation due to the tautomeric form of the amide bond is important for the activity of **I.c**. An effect of
114 hydrophobicity is also observe, decreasing the LogP from 3.13 for **I.c** to 2.33 for **I.p**.

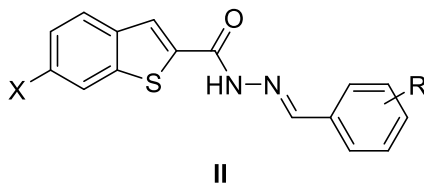


116 i) H₂, Pd/C, MeOH, EtOAc, r.t., 7 h

117 **Scheme 2** : Synthesis of compound **I.p**

118 **Synthesis and biological evaluation of series II – 6-halogenobenzo[*b*]thiophene-2-acylhydrazones**

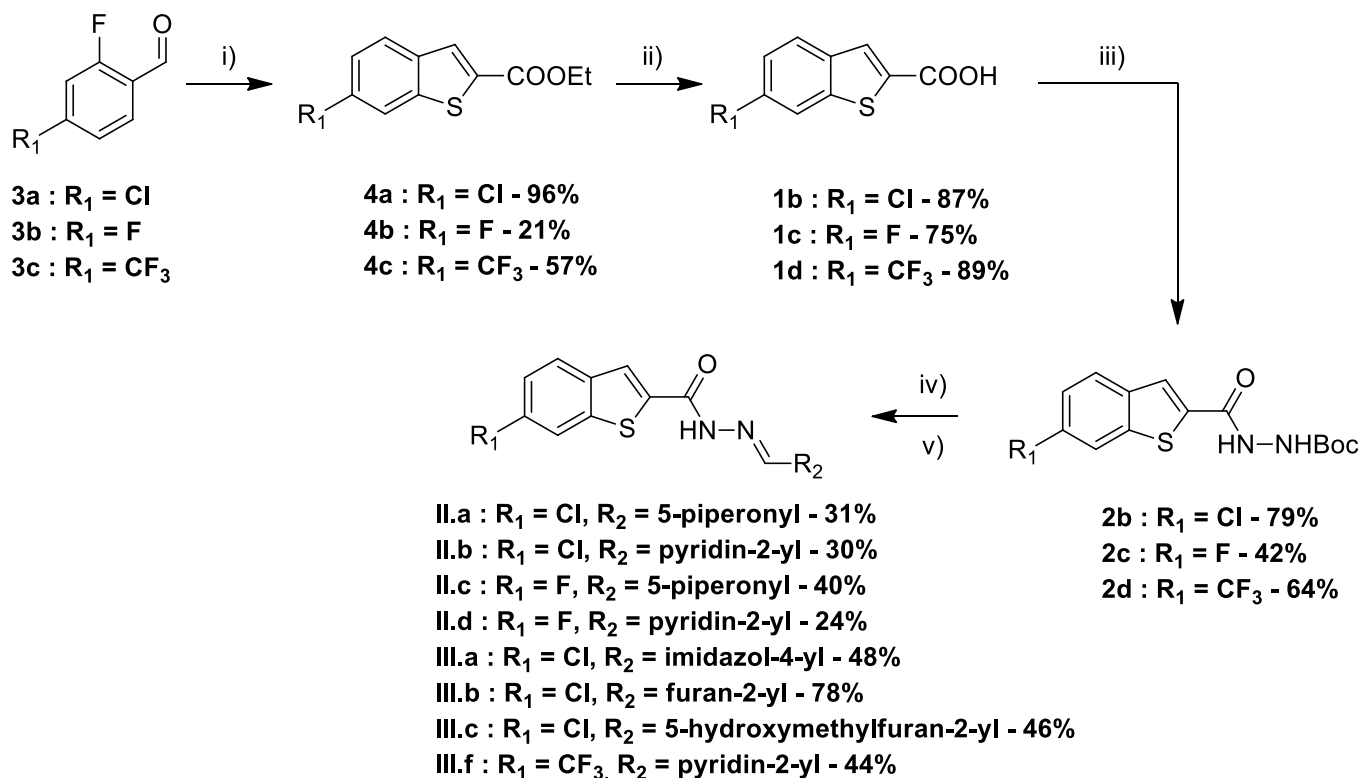
119
120
121 Based on this first set of results, we selected the two compounds **I.a** and **I.c** (respectively bearing a
122 5-piperonyl and a 2-pyridinyl moiety) as hits to improve their activity. 6-halogenobenzo[*b*]thiophene rings
123 being easily accessible using 2-fluoro-4-halogenobenzaldehyde as precursor, we prepared a series of four
124 compounds, namely two piperonyl or pyridinyl compounds bearing a fluorine or chlorine atom respectively.



126 **Figure 3** : General structure of set **II** derivatives

127
128 In order to synthesize the acylhydrazones, 6-chloro- and 6-fluorobenzo[*b*]thiophene-2-carboxylic
129 acids **1b** and **1c** were thus prepared. First, 2-fluoro-4-halogenobenzaldehyde reacted with ethyl thioglycolate
130 by nucleophilic substitution followed by an intramolecular cyclization to obtain compounds **4a** and **4b**, as
131 described in the literature [17]. These esters were then saponified with sodium hydroxide to give the desired
132 carboxylic acids **1b** and **1c**. The general procedure used for the first set was applied to the latter to obtain
133 compounds **II.a-d** with similar yields, as shown in Scheme 3.

134

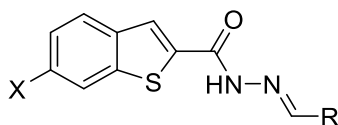


i) Ethyl thioglycolate, Et₃N, DMSO (anh.), 80 °C, 2 h then r.t., 18 h; ii) NaOH, H₂O, EtOH, r.t., 18 h; iii) Boc-hydrazide, DCC, DMAP, DCM (anh.), r.t., 24 h; iv) TFA, DCM (anh.), r.t., 18 h; v) Aldehyde, MeOH (anh.), 65 °C, 2 h;

Scheme 3 : Synthesis of set II and III derivatives

These compounds were also tested to establish their MIC against *S. aureus* with the same method used for set I, and the results are presented in Table 2.

Table 2: Minimum inhibitory concentrations of set II molecules against *S. aureus* strains



Name	X	R	LogP	MIC (µg/mL)		
				ATCC 29213 ^a	SF8300 ^b	ST20171643 ^c
II.a	Cl		4.85	> 256	> 256	> 256
II.b	Cl		3.79	4	4	4
II.c	F		4.33	> 256	> 256	> 256
II.d	F		3.27	32	16	16

^a : Methicillin-sensitive *S. aureus* ; ^b : Methicillin-resistant *S. aureus* ; ^c : Daptomycin-resistant *S.*

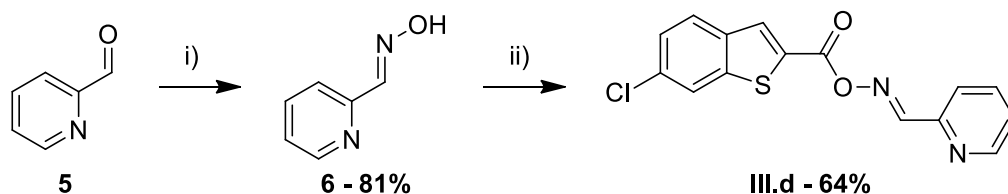
aureus

This short series allowed to identify the chloro pyridinyl compound **II.b** as significantly more active compared to its non-halogenated counterpart **I.c**, reaching 4 µg/mL for the three strains. The presence of a fluorine atom in position 6 of the benzothiophene for the pyridinyl compound did not change its activity. Both halogenated compounds derived from the piperonyl compound **I.a** lost their biological activity (**II.a** and **II.c**).

Synthesis and biological evaluation of series III – structural modifications of II.b

Attempts for improving the activity of the chloro pyridinyl compound **II.b** was then undertaken by replacing the pyridine ring by three five-bond rings, namely imidazole, furan and 5-hydroxymethylfuran. The procedure used was the same as the one used for chlorinated derivatives **II.a** and **II.b** (Scheme 3). As shown in Table 3, the activity remains better for compound **II.b**, confirming the importance of the 2-pyridinyl group.

Then the acylhydrazone was replaced by an acyloxime moiety, leading to a more flexible molecule and losing the *s-cis/s-trans* isomerism. Compound **III.d** was obtained through the conversion of pyridine-2-carboxaldehyde into the corresponding oxime **6**, followed by the acylation from 6-chlorobenzo[*b*]thiophene-2-carboxylic acid **1b** (Erreur ! Source du renvoi introuvable.).

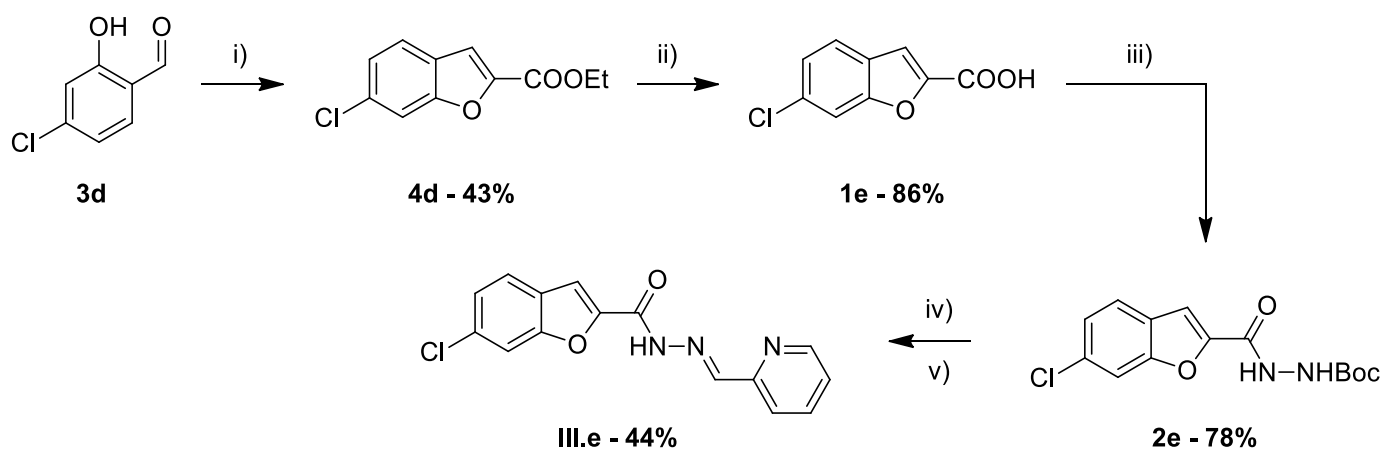


i) HO-NH₂.HCl, NaHCO₃, H₂O, 65°C, 3 h; ii) **1b**, DCC, DMAP, DCM (anh.), 0°C then r.t., 24 h

Scheme 4 : Synthesis of **III.d**

Compound **III.d** showed no anti-staphylococcal activity at concentration lower or equal to 256 µg/mL (Table 3). Therefore, the rigidity imposed by the acylhydrazone moiety is critical to the biological activity.

Then, the effect of the hydrophobicity of the compounds on the antibacterial activity was investigated using the benzo[*b*]furan counterpart of **II.b** (Scheme 5). First, 4-chloro-2-hydroxybenzaldehyde **3d** was converted into the ester **4d** using diethyl 2-bromomalonate as described [18]. Then, the synthetic route was the same as described before for benzo[*b*]thiophene acylhydrazone derivatives.



i) Diethyl 2-bromomalonate, K_2CO_3 , butan-2-one, 90 °C, 7 h then r.t., 18 h; ii) NaOH, H_2O , EtOH, r.t., 2 h; iii) Boc-hydrazide, DCC, DMAP, DCM (anh.), r.t., 24 h; iv) TFA, DCM, r.t., 18 h; v) Pyridine-2-carboxaldehyde, MeOH (anh.), 65 °C, 2 h;

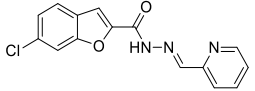
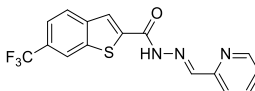
Scheme 5 : Synthesis of III.e

As shown in Table 3, compound III.e showed a weak activity against *S. aureus* with MIC values \geq 128 $\mu\text{g/mL}$. Therefore, the hydrophobicity of the benzo[*b*]thiophene ring is critical for the biological activity. As expected, the replacement of the sulfur by an oxygen atom decreases the overall LogP of the compound (respectively 3.79 for II.b and 3.15 for III.e).

Finally, the chlorine atom was replaced by a bioisostere, a trifluoromethyl group, using the same synthetic strategy (Scheme 3), leading to compound III.f. Even if the MICs are interesting (Table 3), the chlorinated derivative II.b remains the most active compound.

Table 3 : Minimum inhibitory concentrations of set III molecules against *S. aureus* strains

Name	Structure	LogP	MIC ($\mu\text{g/mL}$)		
			ATCC 29213 ^a	SF8300 ^b	ST20171643 ^c
III.a		3.19	256	64	64
III.b		4.22	> 256	256	256
III.c		3.79	> 256	256	256
III.d		3.89	> 256	> 256	> 256

III.e		3.15	> 256	256	128
III.f		4.01	32	8	16

^a : Methicillin-sensitive *S. aureus* ; ^b : Methicillin-resistant *S. aureus* ; ^c : Daptomycin-resistant *S.*

aureus

Cytotoxicity assay of II.b

Due to its interesting antistaphylococcal activity, the potential mammalian cytotoxicity of the chloro pyridinyl derivative **II.b** was examined. Therefore, a 7 h propidium iodide (PI)-based assay was used on adenocarcinomic human alveolar basal epithelial cells (A549), with recombinant *S. aureus* α -hemolysin as positive control. As depicted in Figure 4, no cytotoxicity was found for **II.b** after 7 h at a concentration of 128 $\mu\text{g/mL}$ (32 times the MIC).

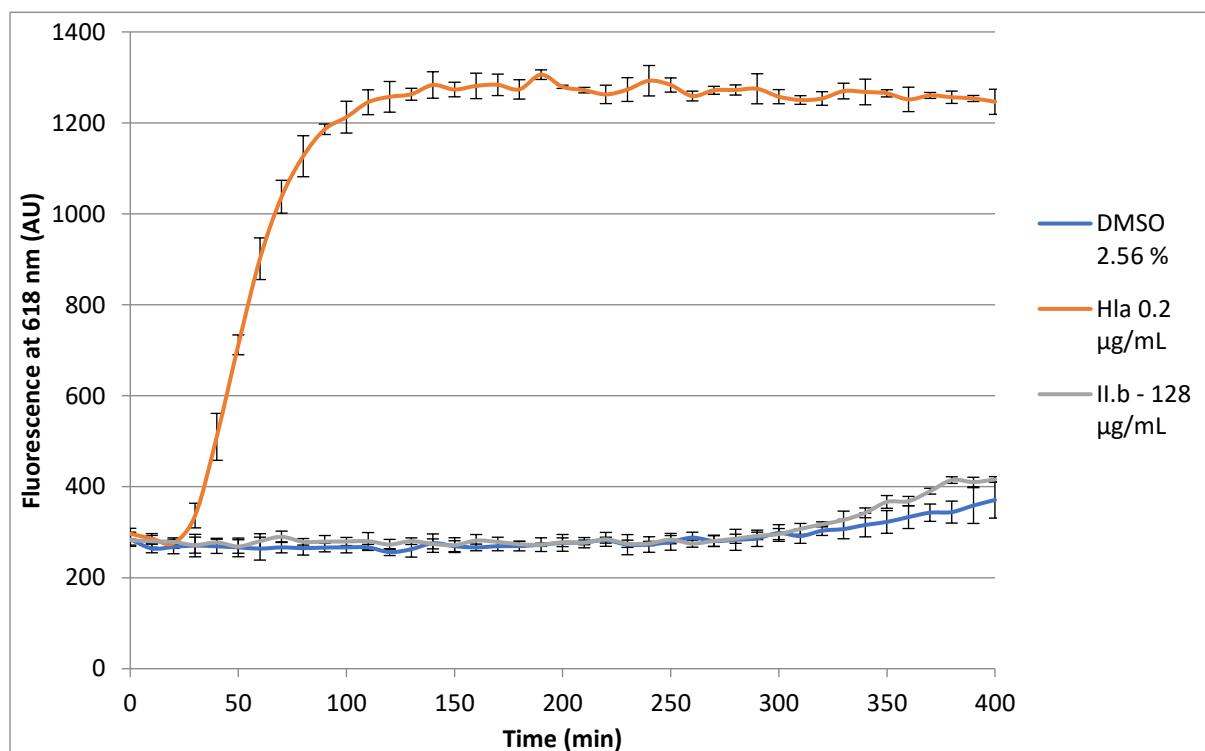


Figure 4 : Effect of **II.b** on the growth of A549 cells

204 **Conclusion**

205 In this study, benzo[*b*]thiophene-based acylhydrazones are shown to be interesting compounds acting
206 as anti-staphylococcal agents. More precisely,
207 (*E*)-6-chloro-*N'*-(pyridin-2-ylmethylene)benzo[*b*]thiophene-2-carbohydrazide **II.b** showed an equal
208 minimum inhibitory concentration of 4 µg/mL for the three strains of *S. aureus*, including two clinical
209 isolated strains of drug resistant *S. aureus*. Moreover, **II.b** showing no cytotoxicity on A549 cells, this
210 suggests that the chloro-pyridinyl benzothiophene acylhydrazone structure is pertinent for future work on
211 chemical diversification and the understanding of the mode of action.

212

213

214 **Experimental**

215 All commercial materials were used as received without further purification. Flash chromatography was
216 carried out using Macherey-Nagel Kieselgel 60 M silica. Analytical thin layer chromatography was realized
217 using aluminum-backed plates coated with Macherey-Nagel Kieselgel 60 XtraSIL G/UV254 and were
218 visualized under UV light (at 254 nm or 365 nm) or stained using ninhydrin. Nuclear magnetic resonance
219 (NMR) spectra were recorded on a Bruker AVL300 or a Bruker AV400 or a Bruker AV500 spectrometer,
220 operating respectively at 300 MHz, 400 MHz and 500 MHz for the proton (¹H) NMR. Carbon (¹³C) NMR
221 spectra were recorded on a Bruker AVL300 or a Bruker AV400 or a Bruker AV500 spectrophotometer,
222 operating respectively at 75 MHz, 100 MHz and 101 MHz. Chemical shifts were reported in parts per million
223 (ppm) in the scale relative to residual solvent signals. Multiplicities are abbreviated as: s, singlet; d, doublet; t,
224 triplet; q, quadruplets; dd, doublet of doublets; dt, doublet of triplets; td, triplet of doublets; ddd, doublet of
225 doublet of doublets; m, multiplet. Coupling constants are measured in Hertz (Hz). High-resolution mass
226 spectra (HRMS) and low-resolution mass spectra were performed by the Centre Commun de Spectrométrie de
227 Masse (CCSM), University of Lyon 1, Lyon, France. LogP calculations were performed using Molinspiration
228 Cheminformatics free web services, <https://www.molinspiration.com>, Slovensky Grob, Slovakia.

229

230 **General procedure for ethyl 6-halogenobenzo[*b*]thiophene-2-carboxylate (4) (adapted from Fedi *et al.***

231 **2007 [17])**

232

233 Under a dried and inert atmosphere (N₂), a solution of 4-chloro-2-fluorobenzaldehyde (14.9 mmol, 1 eq.),
234 ethyl thioglycolate (16.5 mmol, 1.1 eq.) and triethylamine (45 mmol, 3 eq.) in anhydrous DMSO (20 mL) is
235 stirred at 80 °C for 2 h and at room temperature overnight. The mix is then poured into 800 mL of ice/water
236 and stirred vigorously. After 1 h of digestion the formed solid was filtered, washed with water, and dried by
237 suction.

238

239 **Ethyl 6-chlorobenzo[*b*]thiophene-2-carboxylate (4a)**

240 Yellow crystals (3.446 g, 96% yield). ¹H NMR (300 MHz, DMSO-*d*₆) δ 8.25 (d, *J* = 2.0 Hz, 1H), 8.21 (s, 1H),
241 8.04 (d, *J* = 8.6 Hz, 1H), 7.52 (dd, *J* = 8.6, 2.0 Hz, 1H), 4.35 (q, *J* = 7.1 Hz, 2H), 1.33 (t, *J* = 7.1 Hz, 3H).
242 (Spectrum in accordance with patent WO2018/122232 [19])

243

244 **Ethyl 6-fluorobenzo[*b*]thiophene-2-carboxylate (4b)**

245 No crystallisation occurred in water, and the residue was purified by chromatography (eluent: pentane / Et₂O
246 10:1). White powder (710 mg, 21% yield). ¹H NMR (300 MHz, DMSO-*d*₆) δ 8.20 (s, 1H), 8.07 (dd, *J* = 9.1,
247 5.4 Hz, 1H), 7.99 (dd, *J* = 9.1, 2.4 Hz, 1H), 7.37 (td, *J* = 9.1, 2.4 Hz, 1H), 4.35 (q, *J* = 7.1 Hz, 2H), 1.33 (t, *J* =
248 7.1 Hz, 3H).

249 (Spectrum in accordance with Cheng *et al.* [20])

250

251 **Procedure for ethyl 6-(trifluoromethyl)benzo[*b*]thiophene-2-carboxylate (4c) (adapted from patent**
252 **WO2018/122232 [19])**

253

254 Under a dried and inert atmosphere (N₂), a solution of 2-fluoro-4-(trifluoromethyl)benzaldehyde (5.20 mmol,
255 1 eq.), ethyl thioglycolate (6.20 mmol, 1.2 eq.) and K₂CO₃ (5.70 mmol, 1.1 eq.) in anhydrous DMF (10 mL)
256 was stirred at 60 °C for 2 h. The mix was then diluted with water (20 mL), extracted with Et₂O, dried over
257 Na₂SO₄ and concentrated under vacuum. The residue was then recrystallized in MeOH and filtered.

258

259 **Ethyl 6-(trifluoromethyl)benzo[*b*]thiophene-2-carboxylate (4c)**

260 White powder (814 mg, 57% yield). ¹H NMR (300 MHz, Chloroform-*d*) δ 8.16 (q, *J* = 0.8 Hz, 1H), 8.10 (d,
261 *J* = 0.8 Hz, 1H), 7.98 (dt, *J* = 8.4, 0.8 Hz, 1H), 7.63 (dd, *J* = 8.4, 1.6 Hz, 1H), 4.43 (q, *J* = 7.1 Hz, 2H), 1.43
262 (t, *J* = 7.1 Hz, 3H).

263 (Spectrum in accordance with Tan *et al.* [21])

264

265 **Procedure for ethyl 6-chlorobenzofuran-2-carboxylate (4d) [adapted from patent US2015315198 [18]]**

266

267 A solution of 4-chlorosalicylaldehyde (6.39 mmol, 1 eq.), ethyl bromomalonate (13.03 mmol, 2.04 eq.) and
268 K₂CO₃ (19.74 mmol, 3.09 eq.) in 2-butanone (6 mL) was stirred at 90 °C for 7 h then at room temperature
269 for 18 h. The mixture was then diluted with EtOAc, neutralized with HCl 1 N and extracted. The organic
270 phase was washed with saturated NaHCO₃ and brine, dried over Na₂SO₄ and concentrated under vacuum.
271 The residue was then purified by chromatography (eluent: pentane / Et₂O 9:1).

272

273 **Ethyl 6-chlorobenzofuran-2-carboxylate (4d)**

274 White powder (611 mg, 43% yield). ¹H NMR (300 MHz, Chloroform-*d*) δ 7.62 – 7.57 (m, 2H), 7.49 (d, *J*
275 = 1.3 Hz, 1H), 7.29 (dd, *J* = 8.5, 1.3 Hz, 1H), 4.44 (q, *J* = 7.1 Hz, 2H), 1.43 (t, *J* = 7.1 Hz, 3H).

276 (Spectrum in accordance with Chen *et al.* 2017 [22])

277

278 **General procedure for benzo[*b*]thiophene-2-carboxylic acids (1) (adapted from patent WO2018/122232**
279 **[19])**

280

281 To a solution of ethyl 6-chlorobenzo[*b*]thiophene-2-carboxylate (14.1 mmol, 1 eq.) in EtOH (15 mL) was
282 added a solution of NaOH 3N (28.2 mmol, 2 eq.). The solution was stirred at room temperature overnight,
283 concentrated under vacuum, diluted with H₂O (75 mL), acidified with HCl 1 N, extracted with EtOAc, dried
284 over Na₂SO₄ and concentrated under vacuum.

285

286 **6-chlorobenzo[*b*]thiophene-2-carboxylic acid (1b)**

287 White powder (2.616 g, 87% yield). ¹H NMR (300 MHz, DMSO-*d*₆) δ 13.57 (bs, 1H), 8.23 (d, *J* = 2.0 Hz, 1H),
288 8.12 (s, 1H), 8.02 (d, *J* = 8.6 Hz, 1H), 7.50 (dd, *J* = 8.6, 2.0 Hz, 1H); ¹³C NMR (101 MHz, DMSO-*d*₆) δ 163.48,
289 142.6, 137.59, 135.82, 132.07, 129.89, 127.20, 125.84, 122.60.

291 **6-fluorobenzo[*b*]thiophene-2-carboxylic acid (1c)**

292 Yellowish powder (295 mg, 75% yield). ¹H NMR (300 MHz, DMSO-*d*₆) δ 13.46 (s, 1H), 8.12 (d, *J* = 0.8 Hz,
293 1H), 8.05 (dd, *J* = 9.0, 5.4 Hz, 1H), 7.98 (dd, *J* = 9.4, 2.6 Hz, 1H), 7.35 (td, *J* = 9.0, 2.6 Hz, 1H); ¹³C NMR
294 (101 MHz, DMSO-*d*₆) δ 163.48 161.43 (d, *J* = 245.2 Hz), 142.76 (d, *J* = 11.3 Hz), 135.75, 134.96 (d, *J* = 3.9
295 Hz), 129.99, 127.70 (d, *J* = 9.6 Hz), 114.48 (d, *J* = 24.8 Hz), 109.13 (d, *J* = 26.2 Hz).
296 (Spectrum in accordance with Cai *et al.* [23])

298 **6-(trifluoromethyl)benzo[*b*]thiophene-2-carboxylic acid (1d)**

299 White powder (400 mg, 89% yield). ¹H NMR (300 MHz, DMSO-*d*₆) δ 8.59 (q, *J* = 0.8 Hz, 1H), 8.10 (d, *J* =
300 0.8 Hz, 1H), 7.98 (dt, *J* = 8.5, 0.8 Hz, 1H), 7.75 (dd, *J* = 8.5, 1.3 Hz, 1H).
301 (Spectrum in accordance with Tan *et al.* [21])

303 **6-chlorobenzofuran-2-carboxylic acid (1e)**

304 White powder (378 mg, 86% yield). ¹H NMR (300 MHz, DMSO-*d*₆) δ 7.90 (d, *J* = 1.9 Hz, 1H), 7.80 (d, *J* =
305 8.4 Hz, 1H), 7.68 (d, *J* = 1.0 Hz, 1H), 7.40 (dd, *J* = 8.4, 1.9 Hz, 1H).
306 (Spectrum in accordance with Gensini *et al.* [24])

308 **General procedure for *tert*-butyl 2-(benzo[*b*]thiophene-2-carbonyl)hydrazine-1-carboxylates (2)**

309
310 Under a dried and inert atmosphere (N₂), a solution of benzothiophene-2-carboxylic acid (11 mmol, 1.1 eq.)
311 and *tert*-butyl carbazate (10 mmol, 1.0 eq.) in anhydrous DCM (40 mL) was cooled to 0 °C. Then, DMAP (1.3
312 mmol, 0.13 eq.) was added to the mixture. DCC (12 mmol, 1.2 eq.) in anhydrous DCM (10 mL) was added
313 dropwise. The mixture was then stirred at room temperature for 24 h and then filtered on Celite® and washed
314 with DCM. The filtrate was then concentrated, and the viscous yellow oil was purified by chromatography
315 (eluent: pentane / Et₂O, 1:1).

317 ***tert*-butyl 2-(benzo[*b*]thiophene-2-carbonyl)hydrazine-1-carboxylate (2a)**

318 White powder (2.630 g, 88% yield). ¹H NMR (300 MHz, Chloroform-*d*) δ 8.77 (sz, 1H), 7.83 (s, 1H), 7.75 (t,
319 *J* = 6.9 Hz, 2H), 7.47 – 7.31 (m, 2H), 6.82 (s, 1H), 1.50 (s, 9H); ¹³C NMR (75 MHz, Chloroform-*d*) δ 162.4,
320 156.2, 141.1, 138.9, 135.3, 126.6, 126.5, 125.4, 124.8, 122.4, 82.3, 28.1.

322 ***tert*-butyl 2-(6-chlorobenzo[*b*]thiophene-2-carbonyl)hydrazine-1-carboxylate (2b)**

323 Acid **1b** was used. White powder (1.435 g, 79% yield). ¹H NMR (300 MHz, Chloroform-*d*) δ 8.73 (s, 1H),
324 7.75 (s, 1H), 7.71 (s, 1H), 7.67 (d, *J* = 8.6 Hz, 1H), 7.33 (dd, *J* = 8.6, 1.9 Hz, 1H), 6.72 (s, 1H), 1.51 (s, 9H); ¹³C
325 NMR (101 MHz, Acetone-*d*₆) δ 162.42, 156.46, 142.96, 139.20, 138.93, 132.97, 127.41, 126.55, 125.83,
326 123.03, 80.78, 28.39.

328 ***tert*-butyl 2-(6-fluorobenzo[*b*]thiophene-2-carbonyl)hydrazine-1-carboxylate (2c)**

329 Acid **1c** was used. White powder (305 mg, 42% yield). ¹H NMR (300 MHz, Chloroform-*d*) δ 8.41 (s, 1H),
330 7.79 (s, 1H), x7.75 (dd, *J* = 8.9, 5.2 Hz, 1H), 7.45 (d, *J* = 8.9 Hz, 1H), 7.14 (td, *J* = 8.9, 2.4 Hz, 1H), 6.67 (s,
331 1H), 1.51 (s, 9H); ¹³C NMR (101 MHz, DMSO-*d*₆) δ 162.19, 160.52 (d, *J* = 153.3 Hz), 155.39, 141.54 (d, *J* =
332 10.8 Hz), 137.52 (d, *J* = 3.0 Hz), 135.91, 127.24 (d, *J* = 9.4 Hz), 125.14, 114.27 (d, *J* = 24.4 Hz), 108.98 (d, *J*
333 = 26.2 Hz), 79.49, 28.08.

334

335 ***tert*-butyl 2-(6-(trifluoromethyl)benzo[*b*]thiophene-2-carbonyl)hydrazine-1-carboxylate (2d)**

336 Acid **1d** was used. White powder (213 mg, 64% yield). ¹H NMR (300 MHz, Chloroform-*d*) δ 9.11 (s, 1H),
337 7.94 (s, 1H), 7.82 (d, *J* = 8.7 Hz, 1H), 7.80 (s, 1H), 7.56 (dd, *J* = 8.7, 1.7 Hz, 1H), 6.76 (s, 1H), 1.53 (s, 9H);
338 ¹³C NMR (75 MHz, Acetone-*d*₆) δ 162.23, 156.45, 142.86, 142.07, 141.57, 128.48 (q, *J* = 32.3 Hz), 127.07,
339 125.77, 125.4 (q, *J* = 271.5 Hz), 122.16 (q, *J* = 3.5 Hz), 121.22 (q, *J* = 4.5 Hz), 80.88, 28.40.

340

341 ***tert*-butyl 2-(6-chlorobenzofuran-2-carbonyl)hydrazine-1-carboxylate (2e)**

342 Acid **1e** was used. White powder (281 mg, 78% yield). ¹H NMR (300 MHz, Acetone-*d*₆) δ 9.74 (s, 1H), 8.17
343 (s, 1H), 7.77 (d, *J* = 8.4 Hz, 1H), 7.65 – 7.53 (m, 2H), 7.35 (dd, *J* = 8.4, 1.8 Hz, 1H), 1.45 (s, 9H); ¹³C NMR
344 (75 MHz, Acetone-*d*₆) δ 158.77, 156.30, 155.75, 149.81, 133.17, 127.08, 125.38, 124.68, 112.96, 111.27,
345 80.77, 28.39.

346

347 **General procedure for N-acylhydrazones derivatives (I, II and III)**

348

349 **Step 1:** A solution of *tert*-butyl 2-(benzo[*b*]thiophene-2-carbonyl)hydrazine-1-carboxylate (1 mmol, 1 eq.)
350 and TFA (20 mmol, 20 eq.) in anhydrous DCM (3 mL) is stirred at room temperature for 18 h. The mixture
351 was co-evaporated with toluene to afford a white solid which was engaged as crude in the next step.

352 **Step 2:** The crude material was diluted in MeOH (10 mL) before the addition of the corresponding substituted
353 benzaldehyde (2 mmol, 2 eq.) at room temperature. Reflux for 2 h was done, and the final compound
354 crystallized from the reaction mixture. Then, the reaction mixture was cooled to 0 °C, the solid was filtered off
355 and washed with cold MeOH.

356

357 **(*E*)-N'-(benzo[*d*][1,3]dioxol-5-ylmethylene)benzo[*b*]thiophene-2-carbohydrazide (I.a)**

358 Light-yellow solid (127 mg, 52% yield). ¹H NMR (300 MHz, DMSO-*d*₆) δ 12.05 (s, 0.5H), 11.94 (s, 0.5H),
359 8.40 (d, *J* = 7.6 Hz, 1H), 8.23 (s, 0.5H), 8.16 – 7.97 (m, 2.5H), 7.69 – 7.37 (m, 2.5H), 7.32 (s, 0.5H), 7.28 –
360 7.18 (m, 1H), 7.03 (t, *J* = 7.1 Hz, 1H), 6.13 (s, 1H), 6.11 (s, 1H); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 161.9,
361 158.5, 149.7, 149.6, 148.5, 148.4, 145.0, 143.5, 140.8, 139.5, 138.9, 137.7, 133.7, 132.3, 129.0, 128.8, 127.2,
362 127.0, 126.0, 125.8, 125.5, 125.2, 124.2, 124.0, 123.3, 123.1, 109.1, 109.0, 106.0, 105.7, 102.1; HRMS (ESI)
363 *m/z*: calcd. for C₁₇H₁₃N₂O₃S [M+H]⁺ 325.0641, found 325.0641.

364

365 **(*E*)-N'-(4-(dimethylamino)benzylidene)benzo[*b*]thiophene-2-carbohydrazide (I.b)**

366 Yellow solid (99 mg, 45% yield). ¹H NMR (300 MHz, DMSO-*d*₆) δ 11.85 (s, 0.5H), 11.78 (s, 0.5H), 8.40 (s,
367 0.5H), 8.32 (s, 0.5H), 8.20 (s, 0.5H), 8.13 – 7.94 (m, 2.5H), 7.68 (d, *J* = 8.7 Hz, 1H), 7.57 (d, *J* = 8.7 Hz, 1H),
368 7.52 – 7.41 (m, 2H), 6.82 (d, *J* = 8.7 Hz, 1H), 6.77 (d, *J* = 8.7 Hz, 1H), 3.00 (s, 3H), 2.99 (s, 3H); MS (ESI) *m/z*
369 = 324.1 [M+H]⁺, 346.1 [M+Na]⁺.

370

371 **(*E*)-N'-(pyridin-2-ylmethylene)benzo[*b*]thiophene-2-carbohydrazide (I.c)**

372 Intermediate **2a** (938 mg) was deprotected under the reported acidic conditions (TFA) to afford, after an
373 aqueous workup with NaHCO₃ and extractions with EtOAc, a white solid (615 mg) which was directly
374 engaged in the presence of 2-pyridinecarboxaldehyde (6.40 mmol, 2.0 eq.) in MeOH (20 mL). After 2 hours
375 reflux, the reaction mixture was concentrated *in vacuo* to give a brown viscous oil which was purified by
376 recrystallization in EtOAc to give product. White powder (292 mg, 32% yield). ¹H NMR (300 MHz,
377 DMSO-*d*₆) δ 12.34 (s, 1H, 0.5H), 12.26 (s, 0.5H), 8.65 (d, *J* = 4.5 Hz, 1H), 8.49 (d, *J* = 10.6 Hz, 1H), 8.29 (s,
378 0.5H), 8.23 (s, 1H), 8.16 – 7.85 (m, 3.5H), 7.61 – 7.37 (m, 3H); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 162.3,
379 158.9, 153.5, 153.4, 150.1, 148.7, 145.6, 143.4, 140.9, 139.5, 138.4, 137.8, 137.6, 137.4, 133.5, 132.6, 127.3,
380 127.2, 126.6, 126.1, 126.0, 125.6, 125.3, 125.0, 123.3, 123.1, 120.8, 120.5; HRMS (ESI) *m/z*: calcd. for
381 C₁₅H₁₂N₃OS [M+H]⁺ 282.0696, found 282.0682.

382

383 **(*E*)-N'-(pyridin-3-ylmethylene)benzo[*b*]thiophene-2-carbohydrazide (I.d)**

384 White powder (129 mg, 67% yield). ¹H NMR (300 MHz, DMSO-*d*₆) δ 12.32 (s, 0.5H), 12.21 (s, 0.5H), 8.99 (s,
385 0.5H), 8.89 (s, 0.5H), 8.74 – 8.60 (m, 1H), 8.52 (s, 0.5H), 8.44 (s, 0.5H), 8.27 (s, 1H), 8.24 – 8.14 (m, 1H), 8.13
386 – 7.98 (m, 2H), 7.59 – 7.39 (m, 3H); ¹³C NMR (126 MHz, DMSO-*d*₆) δ 161.86, 161.44, 158.42, 150.89,
387 150.69, 148.91, 145.43, 143.10, 142.06, 140.49, 140.39, 139.08, 138.09, 137.36, 137.10, 134.08, 133.62,
388 132.15, 130.14, 130.06, 126.87, 126.73, 126.11, 126.03, 125.69, 125.55, 125.20, 124.90, 124.11, 122.92,
389 122.67; HRMS (ESI) *m/z*: calcd. for C₁₅H₁₂N₃OS [M+H]⁺ 282.0696, found 282.0694.

390

391 **(*E*)-N'-(pyridin-4-ylmethylene)benzo[*b*]thiophene-2-carbohydrazide (I.e)**

392 Crystallized very slowly in the reaction mixture after the reflux. The mixture was concentrated and then
393 engaged in recrystallization with MeOH and EtOAc. Light-yellow powder (92 mg, 30% yield). ¹H NMR (300
394 MHz, DMSO-*d*₆) δ 12.41 (s, 0.5H), 12.32 (s, 0.5H), 8.70 (s, 2H), 8.46 (s, 1H), 8.29 (s, 0.5H), 8.20 – 7.98 (m,
395 2.5H), 7.80 (s, 1H), 7.71 (s, 1H), 7.58 – 7.43 (m, 2H); MS (ESI) *m/z* = 282.0 [M+H]⁺, 304.0 [M+Na]⁺.

396

397 **(*E*)-N'-(3-hydroxybenzylidene)benzo[*b*]thiophene-2-carbohydrazide (I.f)**

398 Did not crystallize in the reaction mixture after the reflux. The mixture was concentrated and then engaged in
399 recrystallization with MeOH and EtOAc (8:2). Light-brown powder (47 mg, 27% yield). ¹H NMR (300 MHz,
400 DMSO-*d*₆) δ 12.10 (s, 0.5H), 11.99 (s, 0.5H), 9.75 (s, 0.5H), 9.67 (s, 0.5H), 8.41 (d, *J* = 11.2 Hz, 1H), 8.25 (s,
401 0.5H), 8.14 – 7.95 (m, 2.5H), 7.57 – 7.44 (m, 2H), 7.38 – 7.21 (m, 2.5H), 7.14 (d, *J* = 7.7 Hz, 0.5H), 6.88 (s,
402 1H); MS (ESI) *m/z* = 297.1 [M+H]⁺, 319.0 [M+Na]⁺.

403

404 **(*E*)-N'-(4-hydroxybenzylidene)benzo[*b*]thiophene-2-carbohydrazide (I.g)**

405 White powder (32 mg, 10% yield). ¹H NMR (300 MHz, DMSO-*d*₆) δ 11.95 (s, 0.5H), 11.85 (s, 0.5H), 10.00 (s,
406 0.5H), 9.97 (s, 0.5H), 8.41 (s, 0.5H), 8.36 (s, 0.5H), 8.22 (s, 0.5H), 8.11 – 7.96 (m, 2.5H), 7.69 (d, *J* = 8.3 Hz,
407 1H), 7.59 (d, *J* = 8.3 Hz, 1H), 7.55 – 7.39 (m, 2H), 6.90 (d, *J* = 8.3 Hz, 1H), 6.85 (d, *J* = 8.3 Hz, 1H); MS (ESI)
408 *m/z* = 297.1 [M+H]⁺, 319.0 [M+Na]⁺.

409

410 **(*E*)-N'-(4-hydroxy-3-methoxybenzylidene)benzo[*b*]thiophene-2-carbohydrazide (I.h)**

411 White powder (141 mg, 41% yield). ¹H NMR (300 MHz, DMSO-*d*₆) δ 11.97 (s, 0.5H), 11.93 (s, 0.5H), 9.62 (s,
412 0.5H), 9.60 (s, 0.5H), 8.42 (s, 0.5H), 8.36 (s, 0.5H), 8.22 (s, 0.5H), 8.10 – 7.96 (m, 2.5H), 7.58 – 7.41 (m,
413 2.5H), 7.33 (s, 0.5H), 7.20 (d, *J* = 8.2 Hz, 0.5H), 7.13 (d, *J* = 8.2 Hz, 0.5H), 6.87 (t, *J* = 7.2 Hz, 1H), 3.92 (s,
414 1.5H), 3.84 (s, 1.5H); MS (ESI) *m/z* = 327.1 [M+H]⁺, 349.0 [M+Na]⁺.

415

416 **(E)-N'-(4-chlorobenzylidene)benzo[*b*]thiophene-2-carbohydrazide (I.i)**
417 White solid (216 mg, 62% yield). ¹H NMR (300 MHz, DMSO-*d*₆) δ 12.22 (s, 0.5H), 12.10 (s, 0.5H), 8.44 (d, *J*
418 = 11.4 Hz, 1H), 8.26 (s, 0.5H), 8.16 (s, 0.5H), 8.10 – 7.97 (m, 2H), 7.88 (d, *J* = 8.0 Hz, 1H), 7.79 (d, *J* = 8.0 Hz,
419 1H), 7.63 – 7.39 (m, 4H); MS (ESI) *m/z* = 315.0 [M+H]⁺, 337.0 [M+Na]⁺.

420
421 **(E)-N'-(4-fluorobenzylidene)benzo[*b*]thiophene-2-carbohydrazide (I.j)**
422 White solid (173 mg, 44% yield). ¹H NMR (300 MHz, DMSO-*d*₆) δ 12.17 (s, 0.5H), 12.06 (s, 0.5H), 8.45 (d, *J*
423 = 14.0 Hz, 1H), 8.25 (s, 0.5H), 8.17 (s, 0.5H), 8.13 – 7.99 (m, 2H), 7.92 (t, *J* = 6.8 Hz, 1H), 7.83 (t, *J* = 6.8 Hz,
424 1H), 7.56 – 7.42 (m, 2H), 7.40 – 7.27 (m, 2H); MS (ESI) *m/z* = 299.1 [M+H]⁺, 321.0 [M+Na]⁺.

425
426 **(E)-N'-(3-nitrobenzylidene)benzo[*b*]thiophene-2-carbohydrazide (I.k)**
427 Yellowish solid (111 mg, 36% yield). ¹H NMR (300 MHz, DMSO-*d*₆) δ 12.41 (s, 0.5H), 12.30 (s, 0.5H), 8.68
428 (s, 0.5H), 8.58 (s, 1H), 8.44 (s, 0.5H), 8.36 – 8.16 (m, 3H), 8.05 (s, 2H), 7.87 – 7.68 (m, 1H), 7.58 – 7.41 (m,
429 2H); MS (ESI) *m/z* = 326.1 [M+H]⁺, 348.0 [M+Na]⁺.

430
431 **(E)-N'-(4-nitrobenzylidene)benzo[*b*]thiophene-2-carbohydrazide (I.l)**
432 Yellowish solid (110 mg, 30% yield). ¹H NMR (300 MHz, DMSO-*d*₆) δ 12.43 (s, 0.5H), 12.35 (s, 0.5H), 8.56
433 (s, 0.5H), 8.44 (s, 0.5H), 8.33 (d, *J* = 8.4 Hz, 3H), 8.18 – 7.94 (m, 4H), 7.59 – 7.39 (m, 2H); ¹³C NMR (126
434 MHz, DMSO-*d*₆) δ 161.98, 158.47, 147.90, 145.52, 142.41, 140.44, 138.97, 137.85, 137.34, 132.22, 128.35,
435 128.14, 126.25, 125.58, 125.18, 124.91, 124.12, 122.89, 122.71; HRMS (ESI) *m/z*: calcd. for C₁₆H₁₀N₃O₃S
436 [M-H]⁻ 324.0448, found 324.0449.

437
438 **(E)-2-((2-(benzo[*b*]thiophene-2-carbonyl)hydrazono)methyl)benzoic acid (I.m)**
439 White solid (88 mg, 45% yield). ¹H NMR (300 MHz, DMSO-*d*₆) δ 13.40 (s, 1H), 12.37 (s, 0.5H), 12.18 (s,
440 0.5H), 9.23 (s, 0.5H), 8.94 (s, 0.5H), 8.42 (s, 0.5H), 8.32 (s, 0.5H), 8.28 – 7.97 (m, 3H), 7.93 (dd, *J* = 7.7, 1.4
441 Hz, 1H), 7.81 – 7.61 (m, 1H), 7.61 – 7.42 (m, 3H); MS (ESI) *m/z* = 325.1 [M+H]⁺, 347.0 [M+Na]⁺.

442
443 **(E)-3-((2-(benzo[*b*]thiophene-2-carbonyl)hydrazineylidene)methyl)benzoic acid (I.n)**
444 White powder (188 mg, 85% yield). ¹H NMR (300 MHz, DMSO-*d*₆) δ 12.29 (s, 0.5H), 12.16 (s, 0.5H), 8.52
445 (s, 0.5H), 8.49 – 8.30 (m, 1.5H), 8.32 – 7.91 (m, 5H), 7.71 – 7.57 (m, 1H), 7.57 – 7.41 (m, 2H); ¹³C NMR (101
446 MHz, DMSO-*d*₆) δ 166.97, 161.82, 161.41, 158.41, 147.15, 143.87, 143.00, 140.46, 139.08, 138.17, 137.40,
447 134.68, 132.01, 131.65, 131.51, 131.11, 130.78, 129.36, 128.54, 127.54, 126.91, 126.72, 125.97, 125.56,
448 125.20, 124.96, 122.93, 122.49; HRMS (ESI) *m/z*: calcd. for C₁₇H₁₃N₂O₃S [M+H]⁺ 325.0641, found
449 325.0626

450
451 **(E)-4-((2-(benzo[*b*]thiophene-2-carbonyl)hydrazono)methyl)benzoic acid (I.o)**
452 White solid (92 mg, 39% yield). ¹H NMR (300 MHz, DMSO-*d*₆) δ 13.14 (s, 1H), 12.30 (s, 0.5H), 12.19 (s,
453 0.5H), 8.53 (s, 0.5H), 8.44 (s, 0.5H), 8.28 (s, 0.5H), 8.23 (s, 0.5H), 8.13 – 7.81 (m, 6H), 7.57 – 7.40 (m, 2H);
454 MS (ESI) *m/z* = 325.1 [M+H]⁺, 347.0 [M+Na]⁺.

455
456 **(E)-N'-(benzo[*d*][1,3]dioxol-5-ylmethylene)-6-chlorobenzo[*b*]thiophene-2-carbohydrazide (II.a)**
457 Intermediate **2b** was deprotected under the reported acidic conditions (TFA) to afford a white solid which was
458 engaged without further purification for the next step. White powder (75 mg, 31% yield). ¹H NMR (300 MHz,
459 DMSO-*d*₆) δ 12.08 (s, 0.5H), 11.97 (s, 0.5H), 8.39 (d, *J* = 8.3 Hz, 1H), 8.33 – 7.97 (m, 3H), 7.48 (d, *J* = 9.3

460 Hz, 1.5H), 7.31 (s, 0.5H), 7.23 (t, $J = 9.9$ Hz, 1H), 7.10 – 6.97 (m, 1H), 6.13 (s, 1H), 6.11 (s, 1H); ^{13}C NMR
461 (101 MHz, DMSO- d_6) δ 161.18, 157.85, 149.26, 148.16, 144.90, 144.40, 141.58, 139.44, 137.86, 136.02,
462 134.19, 131.37, 128.52, 128.28, 127.01, 126.83, 125.77, 125.48, 125.07, 123.96, 123.67, 122.48, 122.24,
463 108.65, 105.64, 105.27, 101.68; HRMS (ESI) m/z : calcd. for $\text{C}_{17}\text{H}_{12}\text{ClN}_2\text{O}_3\text{S}$ $[\text{M}+\text{H}]^+$ 359.0252, found
464 359.0254.

465

466 **(E)-6-chloro-N'-(pyridin-2-ylmethylene)benzo[*b*]thiophene-2-carbohydrazide (II.b)**

467 Intermediate **2b** was deprotected under the reported acidic conditions (TFA) to afford, after an aqueous
468 workup with NaHCO_3 and extractions with EtOAc, a white solid which was engaged without further
469 purification for the next step. White solid (52 mg, 30% yield). ^1H NMR (300 MHz, DMSO- d_6) δ 12.08 (s,
470 0.5H), 11.97 (s, 0.5H), 8.39 (d, $J = 8.3$ Hz, 1H), 8.33 – 7.97 (m, 3H), 7.48 (d, $J = 9.3$ Hz, 1.5H), 7.31 (s, 0.5H),
471 7.23 (t, $J = 9.9$ Hz, 1H), 7.10 – 6.97 (m, 1H), 6.13 (s, 1H), 6.11 (s, 1H); ^{13}C NMR (126 MHz, DMSO- d_6) δ
472 161.52, 158.24, 153.04, 152.82, 149.69, 148.52, 145.41, 144.33, 141.70, 138.90, 137.81, 137.07, 136.13,
473 133.96, 131.69, 127.20, 126.99, 125.88, 125.68, 125.58, 124.63, 122.54, 122.24, 120.51, 120.16; HRMS (ESI)
474 m/z : calcd. for $\text{C}_{15}\text{H}_{11}\text{ClN}_3\text{OS}$ $[\text{M}+\text{H}]^+$ 316.0306, found 316.0315

475

476 **(E)-N'-(benzo[*d*][1,3]dioxol-5-ylmethylene)-6-fluorobenzo[*b*]thiophene-2-carbohydrazide (II.c)**

477 Intermediate **2c** was deprotected under the reported acidic conditions (TFA) to afford a white solid which was
478 engaged without further purification for the next step. White powder (44 mg, 40% yield). ^1H NMR (300
479 MHz, DMSO- d_6) δ 12.05 (s, 0.5H), 11.93 (s, 0.5H), 8.38 (d, $J = 8.7$ Hz, 1H), 8.21 (s, 0.5H), 8.16 – 7.89 (m,
480 2.5H), 7.46 (s, 0.5H), 7.41 – 7.28 (m, 1.5H), 7.23 (t, $J = 8.9$ Hz, 1H), 7.11 – 6.92 (m, 1H), 6.13 (s, 1H), 6.11 (s,
481 1H); ^{13}C NMR (101 MHz, DMSO- d_6) δ 157.89, 149.33, 149.24, 148.14, 148.08, 148.06, 148.01, 144.80,
482 138.58, 138.55, 135.97, 134.17, 133.40, 131.45, 128.56, 128.31, 127.48, 125.09, 123.92, 123.62, 114.20,
483 109.15, 108.73, 108.63, 108.56, 108.49, 105.57, 105.26, 101.67; HRMS (ESI) m/z : calcd. for $\text{C}_{17}\text{H}_{12}\text{FN}_2\text{O}_3\text{S}$
484 $[\text{M}+\text{H}]^+$ 343.0547, found 343.0550

485

486 **(E)-6-fluoro-N'-(pyridin-2-ylmethylene)benzo[*b*]thiophene-2-carbohydrazide (II.d)**

487 Intermediate **2c** was deprotected under the reported acidic conditions (TFA) to afford, after an aqueous
488 workup with NaHCO_3 and extractions with EtOAc, a white solid which was engaged without further
489 purification for the next step. White solid (23 mg, 24% yield). ^1H NMR (300 MHz, DMSO- d_6) δ 12.31 (s, 1H),
490 8.64 (s, 0.5H), 8.54 – 7.71 (m, 6.5H), 7.59 – 7.14 (m, 2H); ^{13}C NMR (126 MHz, DMSO- d_6) δ 162.02, 160.08,
491 158.80, 150.13, 145.75, 144.71, 144.62, 141.43, 141.34, 140.47, 137.50, 137.04, 136.76, 136.33, 135.04,
492 132.22, 129.70, 127.83, 127.28, 127.20, 127.15, 127.07, 126.16, 125.03, 123.37, 120.85, 120.60, 118.72,
493 114.78, 114.43, 114.24, 114.01, 113.82, 109.37, 109.16, 108.79, 108.59; HRMS (ESI) m/z : calcd. for
494 $\text{C}_{15}\text{H}_{11}\text{FN}_3\text{OS}$ $[\text{M}+\text{H}]^+$ 300.0601, found 300.0603.

495

496 **(E)-N'-((1H-imidazol-4-yl)methylene)-6-chlorobenzo[*b*]thiophene-2-carbohydrazide (III.a)**

497 Intermediate **2b** was deprotected under the reported acidic conditions (TFA) to afford, after an aqueous
498 workup with NaHCO_3 and extractions with EtOAc, a white solid which was engaged without further
499 purification for the next step. Beige powder (90 mg, 48% yield). ^1H NMR (300 MHz, DMSO- d_6) δ 12.92 (s,
500 0.5H), 12.52 (s, 0.5H), 11.92 (s, 0.5H), 11.85 (s, 0.5H), 8.53 – 8.35 (m, 1H), 8.31 – 8.13 (m, 1.5H), 8.13 – 7.96
501 (m, 1.5H), 7.90 – 7.67 (m, 1.5H), 7.67 – 7.40 (m, 1.5H); ^{13}C NMR (101 MHz, DMSO- d_6) δ 157.66, 144.16,
502 141.51, 139.58, 137.86, 136.71, 131.38, 130.92, 126.77, 125.75, 125.34, 124.90, 122.48, 122.08; MS (ESI)
503 $m/z = 305.1$ $[\text{M}+\text{H}]^+$, 327.0 $[\text{M}+\text{Na}]^+$.

504

(E)-6-chloro-N'-(furan-2-ylmethylene)benzo[b]thiophene-2-carbohydrazide (III.b)

505 Intermediate **2b** was deprotected under the reported acidic conditions (TFA) to afford a white solid which was
506 engaged without further purification for the next step. Beige powder (145 mg, 78% yield). ¹H NMR (300 MHz,
507 DMSO-*d*₆) δ 12.12 (s, 0.5H), 12.04 (s, 0.5H), 8.43 (s, 0.5H), 8.34 (s, 0.5H), 8.30 – 8.12 (m, 1.5H), 8.12 – 7.96
508 (m, 1.5H), 7.88 (s, 1H), 7.57 – 7.41 (m, 1H), 7.06 (s, 0.5H), 6.99 (s, 0.5H), 6.67 (s, 1H); ¹³C NMR (101 MHz,
509 DMSO-*d*₆) δ 158.34, 149.66, 145.94, 142.04, 139.68, 138.42, 138.25, 135.33, 134.70, 131.97, 131.65, 127.49,
510 127.30, 126.24, 125.90, 125.64, 122.96, 122.56, 114.70, 114.23, 112.79; MS (ESI) *m/z* = 305.0 [M+H]⁺,
511 327.0 [M+Na]⁺.

513

(E)-6-chloro-N'-((5-(hydroxymethyl)furan-2-yl)methylene)benzo[b]thiophene-2-carbohydrazide (III.c)

514 Intermediate **2b** was deprotected under the reported acidic conditions (TFA) to afford a white solid which was
515 engaged without further purification for the next step. Beige powder (94 mg, 46% yield). ¹H NMR (300 MHz,
516 DMSO-*d*₆) δ 12.09 (s, 0.5H), 12.03 (s, 0.5H), 8.44 (s, 0.5H), 8.36 – 8.15 (m, 2H), 8.12 – 7.94 (m, 1.5H), 7.50
517 (d, *J* = 8.4 Hz, 1H), 7.15 – 6.85 (m, 1H), 6.47 (s, 1H), 5.44 (t, *J* = 6.3 Hz, 1H), 4.63 – 4.29 (m, 2H); ¹³C NMR
518 (101 MHz, DMSO-*d*₆) δ 158.22, 157.89, 148.44, 141.61, 139.28, 137.89, 136.46, 135.18, 134.30, 131.54,
519 131.16, 127.07, 126.86, 125.82, 125.45, 125.19, 122.51, 122.11, 115.54, 114.25, 109.38, 55.82; MS (ESI) *m/z*
520 = 335.0 [M+H]⁺, 357.0 [M+Na]⁺.

522

(E)-6-chloro-N'-(pyridin-2-ylmethylene)benzofuran-2-carbohydrazide (III.e)

523 Intermediate **2e** was deprotected under the reported acidic conditions (TFA) to afford, after an aqueous
524 workup with NaHCO₃ and extractions with EtOAc, a white solid which was engaged without further
525 purification for the next step. White powder (63 mg, 44% yield). ¹H NMR (300 MHz, DMSO-*d*₆) δ 12.45 (s,
526 1H), 8.63 (dd, *J* = 4.8, 1.5 Hz, 1H), 8.54 (s, 1H), 8.03 – 7.73 (m, 5H), 7.49 – 7.41 (m, 2H); ¹³C NMR (101
527 MHz, DMSO-*d*₆) δ 154.59, 153.05, 149.11, 148.57, 137.00, 131.93, 126.04, 124.67, 124.27, 120.17, 112.29,
528 111.20; HRMS (ESI) *m/z*: calcd. for C₁₅H₁₁ClN₃O₂ [M+H]⁺ 300.0534, found 300.0533.

530

(E)-N'-(pyridin-2-ylmethylene)-6-(trifluoromethyl)benzo[b]thiophene-2-carbohydrazide (III.f)

531 Intermediate **2d** was deprotected under the reported acidic conditions (TFA) to afford, after an aqueous
532 workup with NaHCO₃ and extractions with EtOAc, a white solid which was engaged without further
533 purification for the next step. White powder (64 mg, 44% yield). ¹H NMR (300 MHz, DMSO-*d*₆) δ 12.42 (s,
534 1H), 8.70 – 8.59 (m, 2H), 8.52 (d, *J* = 13.4 Hz, 1H), 8.39 – 8.18 (m, 3H), 8.07 – 7.85 (m, 2H), 7.77 (d, *J* =
535 7.9 Hz, 1H), 7.46 (d, *J* = 6.5 Hz, 1H). ¹³C NMR (101 MHz, DMSO-*d*₆) δ 161.37, 158.04, 152.99, 152.78,
536 149.68, 148.84, 145.64, 142.91, 141.86, 141.72, 140.29, 139.92, 137.13, 137.02, 136.72, 131.50, 126.66,
537 126.51, 125.55, 124.69, 124.61, 121.45, 121.09, 120.88, 120.83, 120.77, 120.71, 120.68, 120.59, 120.19;
538 HRMS (ESI) *m/z*: calcd. for C₁₆H₁₁F₃N₃OS [M+H]⁺ 350.0569, found 350.0572.

540

541

Procedure for N'-(pyridin-2-ylmethyl)benzo[b]thiophene-2-carbohydrazide (I.p) (adapted from Kim *et***al. [25])**

544

545 Under H₂ atmosphere, a solution of (E)-N'-(pyridin-2-ylmethylene)benzo[b]thiophene-2-carbohydrazide
546 (0.12 mmol, 1 eq.) and palladium on carbon 10% (0.019 mmol, 15 mol%) in 5 mL of EtOAc and 1 mL of

547 MeOH is stirred at room temperature for 7 h. The mixture is filtered on Celite[®], washed with EtOAc and the
548 residue is concentrated under vacuum and purified by chromatography (eluent: EtOAc / MeOH 98:2).

549
550 ***N'*-(pyridin-2-ylmethyl)benzo[*b*]thiophene-2-carbohydrazide (I.p)**

551 White powder (30 mg, 60% yield). ¹H NMR (300 MHz, DMSO-*d*₆) δ 10.34 (d, *J* = 5.1 Hz, 1H), 8.63 – 8.40 (m,
552 1H), 8.04 – 7.98 (m, 2H), 7.95 – 7.88 (m, 1H), 7.78 (td, *J* = 7.7, 1.8 Hz, 1H), 7.54 (d, *J* = 7.7 Hz, 1H), 7.49 –
553 7.38 (m, 2H), 7.32 – 7.21 (m, 1H), 5.76 (q, *J* = 5.1 Hz, 1H), 4.12 (d, *J* = 5.1 Hz, 2H); ¹³C NMR (101 MHz,
554 DMSO-*d*₆) δ 161.02, 158.23, 148.90, 140.04, 139.12, 138.16, 136.59, 126.25, 125.20, 125.00, 124.69, 122.82,
555 122.36, 122.32, 56.21; HRMS (ESI) *m/z*: calcd. for C₁₅H₁₄N₃OS [M+H]⁺ 284.0852, found 284.0858

556
557 **Procedure for pyridine 2-aldoxime (6) (adapted from Younus Wani *et al.* 2011 [26])**

558
559 To a solution of pyridine-2-carboxaldehyde (1.87 mmol, 1 eq.) and hydroxylamine hydrochloride (2.33
560 mmol, 1.25 eq.) in water (5 mL) was added dropwise a solution of sodium bicarbonate (2.33 mmol, 1.25 eq.)
561 in water (10 mL) and the mixture was stirred for 3 h at room temperature. The solution was then extracted
562 with EtOAc and the organic layer dried over Na₂SO₄ and concentrated under vacuum.

563
564 **Pyridine 2-aldoxime (6)**

565 White powder (186 mg, 81%). ¹H NMR (300 MHz, Chloroform-*d*) δ 8.99 (s, 1H), 8.63 (ddd, *J* = 4.9, 1.8,
566 1.0 Hz, 1H), 8.31 (s, 1H), 7.82 (dt, *J* = 8.1, 1.0 Hz, 1H), 7.72 (td, *J* = 8.1, 1.8 Hz, 1H), 7.29 (ddd, *J* = 8.1, 4.9,
567 1.0 Hz, 1H).
568 (Spectrum in accordance to Zhang *et al.* [27]).

569
570 **Procedure for (*E*)-picolinaldehyde O-(6-chlorobenzo[*b*]thiophene-2-carbonyl) oxime (III.d)**

571
572 Under a dried and inert atmosphere (N₂), a solution of 6-chlorobenzothiophene-2-carboxylic acid
573 (1.06 mmol, 1.3 eq.) and pyridine 2-aldoxime (0.82 mmol, 1.0 eq.) in anhydrous DCM (3 mL) was cooled to
574 0 °C. Then, DMAP (0.12 mmol, 0.15 eq.) and DCC (1.06 mmol, 1.3 eq.) were gradually added to the
575 mixture. The mixture was then stirred at room temperature overnight and then filtered on Celite[®] and
576 washed with DCM. The filtrate was then concentrated, and the residue was purified by column
577 chromatography (eluent: pentane / EtOAc 2:1).

578
579 **(*E*)-picolinaldehyde O-(6-chlorobenzo[*b*]thiophene-2-carbonyl) oxime (III.d)**

580 Orange powder (166 mg, 64% yield). ¹H NMR (300 MHz, DMSO-*d*₆) δ 8.85 (s, 1H), 8.77 – 8.73 (m, 1H),
581 8.43 (d, *J* = 0.7 Hz, 1H), 8.33 (dt, *J* = 1.9, 0.7 Hz, 1H), 8.10 (dd, *J* = 8.5, 0.7 Hz, 1H), 8.04 – 7.95 (m, 2H),
582 7.62 – 7.54 (m, 2H); ¹³C NMR (126 MHz, DMSO-*d*₆) δ 159.04, 158.37, 150.12, 148.94, 142.67, 137.31,
583 137.04, 132.61, 131.55, 131.02, 127.37 (2C), 126.05, 122.57, 122.49; HRMS (ESI) *m/z*: calcd. for
584 C₁₅H₁₀ClN₂O₂S [M+H]⁺ 317.0146, found 317.0136.

585
586 **Minimum inhibitory concentration (MIC) evaluation**

588 MICs were evaluated in CaMHB (cation-adjusted Mueller-Hinton broth) by the method of microdilution in
589 liquid medium which follows the CLSI recommendations (Clinical and Laboratory Standards Institute (CLSI),
590 methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard
591 seventh edition. Clinical and Laboratory Standards Institute, Wayne, PA). Evaluated compounds were diluted
592 in DMSO with a concentration of 5 mg/mL and then further diluted in CaMHB. The 0.5 MacFarland bacterial
593 suspensions were made from colonies previously grown on blood agar plate (COS, Biomérieux) in a saline
594 solution (0.45% NaCl), they were diluted in CaMHB (1/100) before addition into 96-well microplates. MICs
595 were done in triplicate and determined after 18 h of incubation at 37 °C. The median values were reported.

596

597 **Cytotoxicity assay**

598

599 Cytotoxicity was evaluated using a propidium iodide assay. Briefly, human lung adenocarcinomic A549
600 cells were plated on black flat 96-well plates (Greiner) at a concentration of 0.5×10^6 cells / mL in DMEM
601 GlutaMAX (Gibco) supplemented with 10% fetal bovine serum for 24 h in a humidified atmosphere at
602 37 °C and 5% CO₂. The medium was then removed and fresh medium with 2.5 µg/mL of propidium iodide
603 and tested compounds (128 µg/mL of II.b and 0.2 µg/mL for recombinant Hla) was added. Absorbance at
604 618 nm was measured every 10 min over 7 h. Each concentration was made as a triplicate and mean values
605 are displayed with standard deviation.

606

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610

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615

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618

619 **Data Availability Statement**

620 Data can be found in the manuscript.

621

622 **Conflicts of Interest**

623 The authors declare no conflict of interest.

624

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