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Benzamidobenzoic acids as potent PqsD inhibitors for the treatment of Pseudomonas aeruginosa infections

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Abstract

Targeting PqsD is a promising novel approach to disrupt bacterial cell-to-cell-communication in *Pseudomonas aeruginosa*. In search of selective PqsD inhibitors, two series of benzamidobenzoic acids – one published as RNAP inhibitors and the other as PqsD inhibitors – were investigated for inhibitory activity toward the respective other enzyme. Additionally, novel derivatives were synthesized and biologically evaluated. By this means, the structural features needed for benzamidobenzoic acids to be potent and, most notably, selective PqsD inhibitors were identified. The most interesting compound of this study was the 3-Cl substituted compound 5 which strongly inhibits PqsD ($IC_{50}$ 6.2 µM) while exhibiting no inhibition of RNAP.

Keywords

RNA polymerase inhibitor, PqsD inhibitor, benzamidobenzoic acid, PqsD selectivity, *Pseudomonas aeruginosa*
1. Introduction

Increasing resistance of bacteria against clinically used antibiotics provoke an urgent need for new antibacterial drugs and innovative therapeutic options [1, 2]. An established method is the induction of bacterial growth retardation or cell death by inhibiting pivotal enzymes [3]. A disadvantage of this therapy is selective pressure causing rapid emergence of resistance [3, 4]. This should be different in a more recent approach to combat bacterial infections [5]. By intervening with the bacterial cell-to-cell-communication system the production of virulence factors and the biofilm formation can be reduced [5]. A newly described strategy to interfere with the *Pseudomonas* Quinolone Signal (PQS) communication system in *Pseudomonas aeruginosa*, is the inhibition of PqsD, which is responsible for the biosynthesis of the important signal molecule 2-heptyl-4-quinolone (HHQ) [6].

Recently, we described a series of potent novel RNA polymerase (RNAP) inhibitors [7] derived from a known inhibitor of PqsD [6]. These compounds containing a benzamidobenzoic acid core were shown to inhibit RNAP *in vitro* in the low micromolar range (IC$_{50}$ around 10 µM) [7]. Due to their origin and their structural similarity to the PqsD inhibitors recently published by Weidel et al. [8], we expected this new class of RNAP inhibitors also to inhibit PqsD and tested the compounds in our PqsD *in vitro* assay for inhibitory activity. Indeed, we found most of them to be very potent.

As bacterial cell death caused by potent RNAP inhibition provoking selective pressure is not intended for an anti-virulence concept [3], it is of special interest to look for structural modifications that lead to an increased selectivity of PqsD inhibitors.

In this work we want to elucidate which structural features in the former class of RNAP inhibitors are beneficial for potent PqsD inhibition and which are needed to afford selectivity over RNAP. To make the SAR complete the compounds published by Weidel et al. are tested for RNAP inhibition. For getting an extended insight into this important class of benzamidobenzoic acids and for extension of their structure-activity and structure-selectivity
profile regarding both enzymes several new benzamidobenzoic acid analogues were synthesized and biologically evaluated.

2. Chemistry

Compounds 1–34 and 51–86 were prepared as described earlier [7, 8].

As outlined in Scheme 1 the synthesis of the 6-OH substituted anthranilic acid derivative 35 started from 2-amino-6-methoxybenzoic acid 35c, which was converted into the corresponding hydroxy substituted benzoic acid 35b by reaction with boron tribromide. Subsequently 35b was coupled with [1,1'-biphenyl]-4-carbonyl chloride refluxing in toluene to obtain 35a, which was hydrolyzed with a mixture of 5N LiOH (aq.) and THF, yielding the target compound 35.

Reaction of bromo substituted methyl anthranilate 36b with ammonia in MeOH at room temperature afforded the benzamide 36a. The latter and 37a were coupled with [1,1'-biphenyl]-4-carbonyl chloride in pyridine and catalytic amounts of DMAP at room temperature to obtain products 36 and 37 (Scheme 2).

Compounds 38–50 were obtained as depicted in Scheme 3. For the synthesis of derivatives containing a carboxylic acid group in meta or para position to the amino group (39–42), the methyl esters (39b–42b) were gained by reaction of the starting compounds (39c–42c) with thionyl chloride and MeOH under reflux conditions. The other methyl or ethyl esters used were commercially available or were synthesized as described earlier [9]. A coupling reaction of 38b–49b with [1,1'-biphenyl]-4-carbonyl chloride and 50b with [1,1'-biphenyl]-4-sulfonyl chloride was performed in pyridine with catalytic amounts of DMAP at room temperature, in CH₂Cl₂ with triethylamine at room temperature or in toluene under reflux conditions. The resulting intermediate esters 39a–50a were cleaved with a mixture of MeOH/THF and 1N NaOH (aq.) or 1N KOH (aq.) or 1N LiOH (aq.) to get the free carboxylic acids 39–50.
3. Biological results

The inhibitory activities of compounds 1–50 against RNAP and PqsD are displayed in Table 1 and 2.

The unsubstituted anthranilic acid 1 has already been described as a potent inhibitor of PqsD [6] and RNAP [7]. Removal of the 3′-OPh or 4′-Ph part of the compound led to 2 and 3, which only moderately inhibited the two enzymes. Removal of both moieties resulted in the completely inactive compound 4. In the following, different substituents were introduced into the free positions of the anthranilic acid substructure of 2 or 3. A chloro substituent in 3-position (5) caused a nearly tenfold improvement of PqsD inhibitory activity and a total loss of RNAP inhibition. Lipophilic electron withdrawing substituents in 4-position (6–14) led to an increased inhibition of both enzymes. Compound 15 bearing a phenyl ring in 4-position is slightly more potent against RNAP, but not against PqsD. A 4-methoxy substituent (16) slightly increased activity for both enzymes compared to the unsubstituted compound 2, whereas a 4-,5-di methoxy substitution (17, 18) even decreased PqsD inhibition. A shift of the lipophilic electron withdrawing substituents from 4- to 5-position (19–26) resulted in no substantial changes. The same effect was observed for the phenyl substituent (27). The 5-methyl and the 5-cyano compounds 28 and 29 showed a slightly increased inhibition of RNAP. With respect to PqsD inhibition, this is only true for compound 29. A methoxy substituent in 5-position (30) did not relevantly change the activity compared to the unsubstituted compound 2 for both enzymes. Interestingly, 31, which contains a hydrophilic electron donating hydroxy group in 5-position, that is unfavorable for RNAP inhibition, is a very potent PqsD inhibitor. The introduction of electron withdrawing lipophilic substituents to the 6-position of the anthranilic acid core (32, 33) and also the introduction of a methoxy group in this position (34) led to a drop or even a complete loss of inhibitory activity for both enzymes. By contrast, 6-hydroxy substitution (35) appreciably improved RNAP inhibition and simultaneously led to the most potent PqsD inhibitor of this series (IC\textsubscript{50} 1.3 µM).
Motivated by these initial interesting results several new analogues with further modifications were synthesized and evaluated. In a first step the carboxylic acid group of compound 22, which is a very potent inhibitor of both enzymes, was exchanged by CONH$_2$ (36), CN (37) or COOMe (38). As these modifications led to severe solubility problems, no further COOH-replacements were performed. Shifting the carboxylic acid group of 2 to meta position (39) resulted in a slight increase of activity for RNAP inhibition. However, introduction of a bromo substituent in 4- or 5-position of 39 leading to compounds 40 and 41 reduced inhibitory activity for both enzymes compared to the brominated compounds with the carboxylic acid group in ortho position (11, 12 and 22–24). A shift of the carboxylic acid group of 2 to the para position (42) resulted in a hardly soluble compound with a weak RNAP inhibition.

In the next step a bioisosteric replacement of the anthranilic acid phenyl ring by differently substituted thiophenes was performed. The unsubstituted thiophene compounds 43–45 showed a considerably improved PqsD inhibitory potency, especially 43, whereas only for 45 a slightly improved RNAP inhibition was found. Interestingly, adding substituted and unsubstituted phenyl rings to the free positions of the thiophene (46–49) strongly enhanced RNAP inhibition while PqsD inhibitory activity was concurrently diminished.

Finally, we replaced the phenyl rings connecting amide function of highly potent 22 by a sulfonamide linker. The resulting compound 50 exhibited a reasonable increase of PqsD inhibition while the RNAP inhibitory potency was retained.

To make the SAR complete, the potent PqsD inhibitors published by Weidel et al. [8] (51–86, SI) were tested for RNAP inhibition. Interestingly, most of the compounds were found not to inhibit RNAP at a concentration of 200 µM or at their highest soluble concentration (SI). Only some compounds containing a 5-NO$_2$ substituent (64), a substituted phenyl ring in 5-position (74–77), a 3’-SO$_2$NEtBenzyl (86) or a 4’-SO$_2$NEt$_2$ moiety (65) show a weak to moderate inhibition of RNAP (SI).
4. Discussion

In this paper we could demonstrate that it is possible to convert the unsubstituted benzamidobenzoic acid 4 into potent PqsD inhibitors by structural modifications. As already shown for RNAP [7] substituents in 3’- or 4’-position are also a minimum requirement for a moderate inhibition of PqsD (2, 3). The combination of substituents in both positions, however, results in very potent PqsD inhibitor 1 [6]. Another very effective way to increase potency of 2 and 3 is the introduction of lipophilic electron withdrawing substituents or phenyl rings in 4- or 5-position of the anthranilic acid core (6–15, 19–27). The same phenomenon has already been described for the sulfamoylbenzamidobenzoic acids [8].

However, the disadvantage all described modifications have in common is that they do not selectively enhance the inhibitory potency for PqsD, but also for RNAP. This is an unwanted effect of the inhibitors, as antibacterial activity caused by potent RNAP inhibition would provoke selective pressure [3], which is in contradiction with the anti-virulence concept by PqsD inhibition. Therefore it is of particular interest that the presented series of compounds also contain structural modifications which selectively enhance PqsD inhibitory potency and do not affect or even decrease RNAP inhibition.

For example the introduction of a 3-Cl substituent (5), which strongly increases PqsD inhibition, leads to a total loss of activity for RNAP. A similar observation is made for the introduction of a 5-OH group (31), which also results in a very potent and highly PqsD selective compound. The introduction of a OH group in 6-position (35) causes a slight increase in RNAP inhibition, however, improvement of PqsD inhibitory activity is much stronger leading to a compound which is about 50 times more potent on PqsD than on RNAP. Apart from introduction of substituents into the anthranilic acid core, selectivity can be gained by bioisosteric replacement. Via exchange of the anthranilic acid phenyl ring by a thiophene, potent and selective PqsD inhibitors can be obtained without affecting the moderate RNAP inhibitory activity (esp. 43). However, the introduction of a phenyl substituent into 43 should
be avoided, as this modification results in a loss or even an inversion of selectivity. A further option to gain selectivity is the replacement of the amide function by a sulfonamide. This slightly increases PqsD inhibition without affecting RNAP activity.

Regarding the recently published PqsD inhibitors [8] (51–86, SI) it is striking that most of these compounds do not contain any of the structural features that we identified in this study to be causing PqsD selectivity. Instead, many of them contain substituents that are also beneficial for RNAP inhibition in the class of benzamidobenzoic acids, as described earlier [7] (e.g. Br, Cl, CF₃). This raised the question whether these sulfamoylbenzamidobenzoic acids are, beside their strong inhibition of PqsD, also selective. Interestingly, only few of them slightly or moderately inhibit RNAP. This reveals that in the class of benzamidobenzoic acids selectivity between PqsD and RNAP can also be gained by variation of R². Apart from a few exceptions, all aliphatically substituted sulfonamide substituents (e.g. SO₂NEt₂, SO₂N(n-Pr)₂) in 3’ position are suitable to obtain selectivity over RNAP.

As the compounds containing a further substituted 5-Ph or a 5-NO₂ substituent moderately inhibit RNAP, despite of the aliphatically substituted sulfonamide substituent in 3’-position, these RNAP inhibition favoring moieties should be avoided. Compound 86, containing a 3’-SO₂NEtBenzyl rest, inhibits RNAP to a similar extent as 2 and 3, indicating that in general aromatics in this part of the compound are crucial for RNAP inhibition and therefore should not be used as R². Another PqsD inhibitor of the sulfamoylbenzamidobenzoic acid series which shows an RNAP inhibitory activity is compound 65 bearing the SO₂NEt₂ substituent not in 3’- but in 4’-position. Actually, this was not an unexpected observation as earlier we have been able to demonstrate that a very similar inhibitor of transcription/translation (TT) carrying a 4’-SO₂N(n-Pr)₂ substituent is a quite potent RNAP inhibitor [7]. This results in the conclusion that a 4’-sulfonamide substituent in not suitable for selective PqsD inhibition.
5. Conclusion

As emergence of bacterial resistance against clinically used antibiotics is becoming a major public health problem [1], the novel anti-virulence concept by PqsD inhibition is of special interest [10]. For following this strategy and avoiding selective pressure it is important that PqsD inhibitors do not cause bacterial cell death by inhibition of pivotal enzymes [3] like RNAP. In this work we presented a series of new potent PqsD inhibitors of the benzamidobenzoic acid class and identified the structural modifications that are necessary to convert potent RNAP inhibitors into selective PqsD inhibitors. It is striking that already simple bioisosteric replacements led to a notable gain of selectivity. For further optimization of potency and selectivity it is conceivable to combine some of these modifications into one structure. As the in vitro PqsD inhibitory activities of our compounds are promising, they will be further evaluated in vivo.

6. Experimental Section

6.1 General Directions

Chemical names follow IUPAC nomenclature. Starting materials were purchased from Sigma-Aldrich, Acros, Maybridge, Combi Blocks, Fluka, ABCR, Alfa Aesar, Apollo and were used without purification.

Column chromatography (CC) was performed on silica gel (70—200 μm), preparative thin layer chromatography (TLC) on 1 mm SIL G-100 UV254 glass plates (Macherey-Nagel), and reaction progress was monitored by TLC on Alugram SIL G UV254 (Macherey-Nagel).

$^1$H NMR and $^{13}$C NMR spectra were recorded on a Bruker AM500 spectrometer (500 MHz and 125 MHz) at 300 K in CDCl$_3$ or CD$_3$SOCD$_3$. Chemicals shifts are reported in δ values (ppm), the hydrogenated residues of deuterated solvent were used as internal standard
(CDCl₃: δ = 7.27 ppm in ¹H NMR and δ = 77.0 ppm in ¹³C NMR, CD₂SOCD₂: δ = 2.50 ppm in ¹H NMR and δ = 39.5 ppm in ¹³C NMR). Signals are described as s, d, t, dd, ddd, dt and m for singlet, doublet, triplet, doublet of doublet, doublet of doublet of doublet, doublet of triplet and multiplet, respectively. Coupling constants (J) are given in Hertz (Hz).

The reported yields are the isolated yields of purified material and are not optimized.

Purity of compounds 35 to 50 was determined using LC/MS as follows:

The SpectraSystems®-LC-system consisted of a pump, an autosampler, and a UV detector. Mass spectrometry was performed on a MSQ® electro spray mass spectrometer (Thermo Fisher, Dreieich, Germany). The system was operated by the standard software Xcalibur®. A RP C18 NUCLEODUR® 100-5 (125 x 3 mm) column (Macherey-Nagel GmbH, Duehren, Germany) was used as stationary phase. All solvents were HPLC grade.

Solvent system: In a gradient run the percentage of acetonitrile (containing 0.1% trifluoroacetic acid) in 0.1% trifluoroacetic acid was increased from an initial concentration of 0% at 0 min to 100% at 15 min and kept at 100% for 5 min.

The injection volume was 10 µL and flow rate was set to 800 µL/min. MS analysis was carried out at a spray voltage of 3800 V, a capillary temperature of 350 °C and a source CID of 10 V. Spectra were acquired in positive mode from 100 to 1000 m/z and at 254 nm for the UV trace.

All tested compounds have >95% chemical purity as measured by HPLC.

Melting points were determined on a Stuart Scientific melting point apparatus SMP3 and are uncorrected.

6.2 Chemistry
6.2.1 General Procedure for the synthesis of methyl aminobenzoates 39b–42b. A solution of the appropriate 2-aminobenzoic acid (1 equiv) in MeOH was cooled to 0 °C followed by a dropwise addition of thionyl chloride (2.5 equiv). The mixture was refluxed for 24 h. After evaporation of the solvent and neutralization by addition of a saturated aqueous NaHCO₃ solution, the mixture was extracted with EtOAc and the combined organic layers were dried over MgSO₄. Purification by CC (n-hexane/EtOAc) provided the title compounds.

**Methyl 3-aminobenzoate** (39b)

yellow oil, yield: 100%. ¹H NMR (500 MHz, CDCl₃) δ = 7.45–7.41 (m, 1 H) 7.38–7.33 (m, 1 H), 7.24–7.19 (m, 1 H), 6.86 (ddd, J = 8.0, 2.4, 0.9 Hz, 1 H), 3.89 (s, 3 H, OCH₃), 3.78 (br. s., 2 H, NH₂) ppm. ¹³C NMR (125 MHz, CDCl₃) δ = 167.3, 146.4, 131.1, 129.2, 119.7, 119.4, 115.7, 52.0 (OCH₃) ppm. LC/MS: m/z = 193 [M + H⁺ + CH₃CN]; tᵣ = 3.44 min; > 99.9% pure (UV).

**Methyl 3-amino-4-bromobenzoate** (40b)

brown solid, yield: 83%. ¹H NMR (500 MHz, CDCl₃) δ = 7.47 (d, J = 8.3 Hz, 1 H), 7.43 (d, J = 2.0 Hz, 1 H), 7.26 (dd, J = 8.3, 2.0 Hz, 1 H), 4.23 (br. s., 2 H, NH₂), 3.89 (s, 3 H, OCH₃) ppm. ¹³C NMR (125 MHz, CDCl₃) δ = 166.7, 144.1, 132.6, 130.3, 120.0, 116.3, 114.2, 52.2 (OCH₃) ppm. LC/MS: m/z = 230 and 232 [M + H⁺], 271 and 273 [M + H⁺ + CH₃CN]; tᵣ = 10.13 min; 98.8% pure (UV).

**Methyl 3-amino-5-bromobenzoate** (41b)

yellow solid, yield: 91%. ¹H NMR (500 MHz, CDCl₃) δ = 7.54–7.52 (m, 1 H), 7.26 (dd, J = 2.2, 1.3 Hz, 1 H), 6.99 (dd, J = 2.2, 1.6 Hz, 1 H), 3.89 (s, 3 H, OCH₃), 3.86 (br. s., 2 H, NH₂) ppm. ¹³C NMR (125 MHz, CDCl₃) δ = 166.0, 147.7, 132.6, 122.9, 122.2, 121.6, 114.6, 52.3 ppm.
Methyl 4-aminobenzoate (42b)
yellow solid, yield: 99%. $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ = 7.89–7.81 (m, 2 H), 6.66–6.60 (m, 2 H), 4.10 (br. s., 2 H, NH$_2$), 3.85 (s, 3 H, OCH$_3$) ppm. $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ = 167.1, 150.8, 131.5, 119.6, 113.7, 51.6 (OCH$_3$) ppm. LC/MS: m/z = 193 [M + H$^+$ + CH$_3$CN]; $t_R$ = 5.21 min; > 99.9% pure (UV).

6.2.2 General Procedures for the synthesis of methyl benzamidobenzoate or methyl benzamidothiophenecarboxylate derivatives 36–38, 39a–49a (amide coupling reactions).

Three different amide coupling procedures were used to obtain the title compounds:

6.2.2.1 General Procedure for the synthesis of 38, 39a, 41a–43a. The appropriate alkyl aminobenzoate or alkyl aminothiophenecarboxylate (1 equiv) was added to a solution of the acyl chloride (1.2 equiv) in CH$_2$Cl$_2$ under a N$_2$ atmosphere. After the addition of TEA (2 equiv) the reaction mixture was stirred for 18 h at room temperature. For purification the solvent was evaporated and the remaining solid was suspended in MeOH. After filtration the precipitate was washed with MeOH to provide the pure compound.

Methyl 2-([1,1'-biphenyl]-4-y lacboxamido)-5-bromobenzoate (38)
slightly yellow solid, yield: 26%. $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ = 12.01 (br. s, 1 H, NH), 8.91 (d, $J$ = 9.1 Hz, 1 H), 8.22 (d, $J$ = 2.5 Hz, 1 H), 8.15–8.08 (m, 2 H), 7.79–7.74 (m, 2 H), 7.71 (dd, $J$ = 9.1, 2.5 Hz, 1 H), 7.68–7.62 (m, 2 H), 7.54–7.46 (m, 2 H), 7.45–7.38 (m, 1 H), 4.00 (OCH$_3$) ppm. LC/MS: m/z = 230 and 232 [M + H$^+$], 271 and 273 [M + H$^+$ + CH$_3$CN]; $t_R$ = 9.22 min; 97.0% pure (UV).
(s, 3 H, OCH₃) ppm. ¹³C NMR (125 MHz, CDCl₃) δ = 168.0, 165.4, 144.9, 141.0, 139.9, 137.5, 133.5, 133.1, 128.9, 128.1, 127.9, 127.5, 127.2, 122.1, 116.6, 115.0, 52.8 (OCH₃) ppm.

LC/MS: m/z = 410 and 412 [M + H⁺], 821 and 823 [2M + H⁺]; tᵣ = 16.41 min; 98.4% pure (UV).

**Methyl 3-([1,1'-biphenyl]-4-ylcarboxamido)benzoate (39a)**

white solid, yield: 62%. ¹H NMR (500 MHz, DMSO-d₆) δ = 10.51 (s, 1 H, NH), 8.50 (s, 1 H), 8.13–8.07 (m, 3 H), 7.85 (d, J = 8.3 Hz, 2 H), 7.77 (d, J = 7.3 Hz, 2 H), 7.74–7.68 (m, 1 H), 7.55–7.48 (m, 3 H), 7.47–7.41 (m, 1 H), 3.88 (s, 3 H, OCH₃) ppm. ¹³C NMR (125 MHz, DMSO-d₆) δ = 166.1, 165.3, 143.3, 139.6, 139.0, 133.3, 130.0, 129.1, 129.1, 128.4, 128.2, 126.9, 126.6, 124.7, 124.2, 120.8, 52.2 (OCH₃) ppm. LC/MS: m/z = 332 [M + H⁺], 373 [M + H⁺ + CH₃CN], 663 [2M + H⁺]; tᵣ = 12.50 min; 97.7% pure (UV).

**Methyl 3-([1,1'-biphenyl]-4-ylcarboxamido)-5-bromobenzoate (41a)**

beige solid, yield: 51%. ¹H NMR (500 MHz, CDCl₃) δ = 8.37–8.33 (m, 1 H), 8.23 (s, 1 H, NH), 8.12–8.07 (m, 1 H), 7.99–7.92 (m, 3 H), 7.72–7.69 (m, 2 H), 7.64–7.61 (m, 2 H), 7.50–7.45 (m, 2 H), 7.45–7.38 (m, 1 H), 3.91 (s, 3 H, OCH₃) ppm. ¹³C NMR (125 MHz, CDCl₃) δ = 165.5, 165.5, 145.1, 139.6, 139.4, 132.6, 132.3, 129.0, 128.3, 128.2, 127.7, 127.5, 127.3, 127.2, 122.9, 119.7, 52.5 (OCH₃) ppm. LC/MS: m/z = 410 and 412 [M + H⁺], 451 and 453 [M + H⁺ + CH₃CN], 821 and 823 [2M + H⁺]; tᵣ = 14.91 min; 97.5% pure (UV).

**Methyl 4-([1,1'-biphenyl]-4-ylcarboxamido)benzoate (42a)**

slightly yellow solid, yield: 43%. ¹H NMR (500 MHz, DMSO-d₆) δ = 10.61 (s, 1 H, NH), 8.08 (d, J = 8.3 Hz, 2 H), 7.98 (s, 4 H), 7.86 (d, J = 8.3 Hz, 2 H), 7.81–7.74 (m, 2 H), 7.55–7.48 (m, 2 H), 7.47–7.40 (m, 1 H), 3.84 (s, 3 H, OCH₃) ppm. ¹³C NMR (125 MHz,
DMSO-$d_6$ $\delta =$ 165.8, 165.6, 143.7, 143.4, 139.0, 133.3, 130.1, 129.1, 128.5, 128.2, 126.9, 126.6, 124.3, 119.6, 51.9 (OCH$_3$) ppm. LC/MS: m/z = 332 [M + H$^+$], 373 [M + H$^+$ + CH$_3$CN], 663 [2M + H$^+$]; $t_R =$ 12.59 min; > 99.9% pure (UV).

*Methyl 3-[(1,1'-biphenyl]-4-ylcarboxamido)thiophene-2-carboxylate (43a)*

beige solid, yield: 19%. $^1$H NMR (500 MHz, CDCl$_3$) $\delta =$ 11.25 (s, 1 H, NH), 8.34 (d, $J =$ 5.5 Hz, 1 H), 8.13–8.08 (m, 2 H), 7.78–7.72 (m, 2 H), 7.67–7.64 (m, 2 H), 7.55 (d, $J =$ 5.5 Hz, 1 H), 7.52–7.46 (m, 2 H), 7.45–7.37 (m, 1 H), 3.95 (s, 3 H, OCH$_3$) ppm. $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta =$ 165.2, 163.9, 145.3, 145.1, 139.9, 132.3, 131.9, 128.9, 128.1, 127.5, 127.2, 122.4, 110.4, 52.1 (OCH$_3$) ppm. LC/MS: m/z = 338 [M + H$^+$], 379 [M + H$^+$ + CH$_3$CN], 675 [2M + H$^+$]; $t_R =$ 14.87 min; 94.9% pure (UV).

6.2.2.2 General Procedure for the synthesis of 37, 40a, 44a–47a, 49a. The appropriate alkyl aminobenzoate or alkyl aminothiophenecarboxylate (1 equiv) and a catalytic amount of DMAP were added to a suspension of the acyl chloride (1.5 equiv) in pyridine under a N$_2$ atmosphere. The reaction mixture was stirred for 18 h at room temperature and 2 M HCl was added. The mixture was extracted with EtOAc, the combined organic layers washed with saturated NaHCO$_3$ and dried over MgSO$_4$. For purification the solvent was evaporated and the remaining solid was suspended in MeOH. After filtration the precipitate was washed with MeOH (and EtOAc in case of 45a) to provide the pure compound

*N-(4-Bromo-2-cyanophenyl)-[1,1'-biphenyl]-4-carboxamide (37)*

white solid, yield: 35%. Mp: 232–233 °C. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta =$ 10.72 (s, 1 H, NH), 8.19 (d, $J = 2.3$ Hz, 1 H), 8.12–8.08 (m, 2 H), 7.95 (dd, $J =$ 8.8, 2.3 Hz, 1 H), 7.91–7.86
(m, 2 H), 7.80–7.75 (m, 2 H), 7.58 (d, J = 8.8 Hz, 1 H), 7.55–7.49 (m, 2 H), 7.47–7.40 (m, 1 H) ppm. $^{13}$C NMR (125 MHz, DMSO-$d_6$) δ = 165.2, 143.8, 139.9, 138.9, 136.8, 135.2, 132.0, 129.1, 128.6, 128.4, 128.3, 127.0, 126.8, 117.9, 115.6, 111.0 ppm. LC/MS: m/z = 377 and 379 [M + H$^+$]; $t_R$ = 13.63 min; 98.0% pure (UV).

**Methyl 3-([1,1'-biphenyl]-4-ylcarboxamido)-4-bromobenzoate (40a)**

white solid, yield: 71%. $^1$H NMR (500 MHz, CDCl$_3$) δ = 9.21 (d, J = 2.2 Hz, 1 H), 8.53 (s, 1 H, NH), 8.05–8.02 (m, 2 H), 7.77–7.64 (m, 6 H), 7.52–7.47 (m, 2 H), 7.45–7.40 (m, 1 H), 3.95 (s, 3 H, OCH$_3$) ppm. $^{13}$C NMR (125 MHz, CDCl$_3$) δ = 166.2, 164.9, 145.3, 139.7, 136.0, 132.8, 132.3, 130.7, 129.0, 128.2, 127.7, 127.6, 127.2, 126.2, 122.5, 118.7, 52.4 (OCH$_3$) ppm. LC/MS: m/z = 410 and 412 [M + H$^+$], 451 and 453 [M + H$^+$ + CH$_3$CN], 821 and 823 [2M + H$^+$]; $t_R$ = 14.62 min; 99.0% pure (UV).

**Methyl 4-([1,1'-biphenyl]-4-ylcarboxamido)thiophene-3-carboxylate (44a)**

beige solid, yield: 58%. $^1$H NMR (500 MHz, CDCl$_3$) δ = 11.06 (s, 1 H, NH), 8.24 (d, J = 3.5 Hz, 1 H), 8.12 (d, J = 3.5 Hz, 1 H), 8.10–8.07 (m, 2 H), 7.76–7.74 (m, 2 H), 7.67–7.64 (m, 2 H), 7.51–7.47 (m, 2 H), 7.44–7.38 (m, 1 H), 3.97 (s, 3 H, OCH$_3$) ppm. $^{13}$C NMR (125 MHz, CDCl$_3$) δ = 164.9, 164.4, 144.7, 140.0, 136.7, 132.6, 128.9, 128.0, 127.7, 127.5, 127.2, 121.6, 110.8, 52.1 (OCH$_3$) ppm. LC/MS: m/z = 338 [M + H$^+$], 379 [M + H$^+$ + CH$_3$CN], 675 [2M + H$^+$]; $t_R$ = 15.41 min; 94.2% pure (UV).

**Ethyl 2-([1,1'-biphenyl]-4-ylcarboxamido)thiophene-3-carboxylate (45a)**

brown solid, yield: 42%. $^1$H NMR (500 MHz, CDCl$_3$) δ = 12.09 (br. s., 1 H, NH), 8.15–8.09 (m, 2 H), 7.79–7.74 (m, 2 H), 7.68–7.63 (m, 2 H), 7.53–7.47 (m, 2 H), 7.45–7.39 (m, 1 H), 7.29 (d, J = 5.7 Hz, 1 H), 6.81 (d, J = 5.7 Hz, 1 H), 4.42 (q, J = 7.2 Hz, 2 H, OCH$_2$), 1.44 (t, J
= 7.2 Hz, 3 H, CH₃) ppm. ¹³C NMR (125 MHz, CDCl₃) δ = 166.1, 163.3, 149.3, 145.4, 139.7, 130.7, 129.0, 128.2, 128.0, 127.6, 127.2, 123.9, 116.2, 113.3, 60.8 (OCH₂), 14.4 (CH₃) ppm. LC/MS: m/z = 352 [M + H⁺], 393 [M + H⁺ + CH₃CN], 703 [2M + H⁺]; tᵢₜ = 16.46 min; 97.2% pure (UV).

*Methyl 3-([1,1′-biphenyl]-4-y1carboxamido)-5-phenylthiophene-2-carboxylate (46a)*
beige solid, yield: 43%. ¹H NMR (500 MHz, CDCl₃) δ = 11.28 (s, 1 H, NH), 8.60 (s, 1 H), 8.15–8.09 (m, 2 H), 7.78–7.73 (m, 4 H), 7.68–7.64 (m, 2 H), 7.52–7.39 (m, 6 H), 3.97 (s, 3 H, OCH₃) ppm. ¹³C NMR (125 MHz, CDCl₃) δ = 165.2, 164.0, 150.2, 145.7, 145.1, 139.9, 133.2, 132.3, 129.3, 129.1, 129.0, 128.2, 128.0, 127.6, 127.2, 126.2, 118.1, 109.1, 52.1 (OCH₃) ppm. LC/MS: m/z = 414 [M + H⁺], 827 [2M + H⁺]; tᵢₜ = 17.93 min; 97.1% pure (UV).

*Methyl 3-([1,1′-biphenyl]-4-y1carboxamido)-5-(4-chlorophenyl)thiophene-2-carboxylate (47a)*
beige solid, yield: 28%. ¹H NMR (500 MHz, CDCl₃) δ = 11.26 (s, 1 H, NH), 8.58 (s, 1 H), 8.13–8.09 (m, J = 8.2 Hz, 2 H), 7.78–7.75 (m, J = 8.5 Hz, 2 H), 7.68–7.62 (m, 4 H), 7.52–7.48 (m, 2 H), 7.46–7.38 (m, 3 H), 3.97 (s, 3 H, OCH₂) ppm. ¹³C NMR (125 MHz, CDCl₃) δ = 165.1, 164.0, 148.6, 145.7, 145.2, 139.8, 135.3, 132.1, 131.7, 129.3, 129.0, 128.2, 128.0, 127.6, 127.4, 127.2, 118.4, 109.3, 52.2 (OCH₃) ppm. LC/MS: m/z = 448 and 450 [M + H⁺], 895 and 899 [2M + H⁺]; tᵢₜ = 18.42 min; 99.1% pure (UV).

*Ethyl 2-([1,1′-biphenyl]-4-y1carboxamido)-5-phenylthiophene-3-carboxylate (49a)*
yellow solid, yield: 40%. ¹H NMR (500 MHz, CDCl₃) δ = 12.10 (s, 1 H, NH), 8.16–8.10 (m, 2 H), 7.80–7.77 (m, 2 H), 7.68–7.62 (m, 4 H), 7.52–7.48 (m, 3 H), 7.46–7.38 (m, 3 H), 7.33–7.28 (m, 1 H), 4.45 (q, J = 7.3 Hz, 2 H, OCH₂), 1.47 (t, J = 7.3 Hz, 3 H, CH₃) ppm. ¹³C
NMR (125 MHz, CDCl$_3$) $\delta = 166.0, 163.3, 148.6, 139.7, 134.0, 133.8, 130.7, 129.0, 129.0, 128.3, 128.1, 127.6, 127.5, 127.3, 125.5, 119.3, 114.1, 61.0$ (OCH$_3$), 14.4 (CH$_3$) ppm.

LC/MS: m/z = 428 [M + H$^+$], 469 [M + H$^+$ + CH$_3$CN], 855 [2M + H$^+$]; $t_R = 18.34$ min; > 99.9% pure (UV).

6.2.2.3 General Procedure for the synthesis of 36 and 48a. The appropriate alkyl aminobenzoate or alkyl aminothiophenecarboxylate (1 equiv) and the acyl chloride (1.2 equiv) were dissolved in toluene and refluxed for 4 h (36) or 18 h (48a). For purification the solvent was removed under reduced pressure and the remaining solid suspended in MeOH. After filtration the precipitate was washed with MeOH to yield the pure compound.

**N-(4-Bromo-2-carbamoylphenyl)-[1,1'-biphenyl]-4-carboxamide (36)**

white solid, yield: 79%. Mp: 254–256 °C. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta = 12.93$ (s, 1 H, NH), 8.68 (d, $J = 9.1$ Hz, 1 H), 8.54 (br. s., 1 H), 8.11 (d, $J = 2.5$ Hz, 1 H), 8.04–7.97 (m, 3 H), 7.90–7.86 (m, 2 H), 7.79–7.74 (m, 3 H), 7.54–7.48 (m, 2 H), 7.46–7.41 (m, 1 H) ppm. $^{13}$C NMR (125 MHz, DMSO-$d_6$) $\delta = 169.8, 164.2, 143.7, 139.4, 138.9, 135.2, 133.1, 131.2, 129.1, 128.3, 127.7, 127.2, 127.0, 122.1, 121.1, 114.4$ ppm. LC/MS: m/z = 395 and 397 [M + H$^+$], 789 and 791 [2M + H$^+$]; $t_R = 14.07$ min; > 99.9% pure (UV).

**Methyl 3-([1,1'-biphenyl]-4-ylcarboxamido)-5-(4-methoxyphenyl)thiophene-2-carboxylate (48a)**

yellow solid, yield: 82%. $^1$H NMR (500 MHz, CDCl$_3$) $\delta = 11.29$ (s, 1 H, NH), 8.49 (s, 1 H), 8.15–8.09 (m, 2 H), 7.78–7.74 (m, 2 H), 7.70–7.63 (m, 4 H), 7.54–7.47 (m, 2 H), 7.46–7.39 (m, 1 H), 7.00–6.92 (m, 2 H), 3.96 (s, 3 H, OCH$_3$), 3.87 (s, 3 H, OCH$_3$) ppm. $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta = 166.0, 163.3, 148.6, 139.7, 134.0, 133.8, 130.7, 129.0, 128.3, 128.1, 127.6, 127.5, 127.3, 125.5, 119.3, 114.1, 61.0$ (OCH$_3$), 14.4 (CH$_3$) ppm.
MHz, CDCl$_3$) $\delta = 165.3$, 164.0, 160.6, 150.4, 145.8, 145.1, 139.9, 132.3, 129.0, 128.1, 128.0, 127.6, 127.5, 127.2, 125.9, 117.0, 114.5, 108.0, 55.4 (OCH$_3$), 52.0 (OCH$_3$) ppm. LC/MS: m/z = 444 [M + H$^+$], 485 [M + H$^+$ + CH$_3$CN], 887 [2M + H$^+$]; $t_R = 17.96$ min; 97.3% pure (UV).

6.2.3 General Procedure for the synthesis of benzamidobenzoate or benzamidothiophenecarboxylate derivatives 39−47, 50 (ester cleavage). The methyl or ethyl esters of the title compounds (39a−47a, 50a) were hydrolyzed with 5 M NaOH in THF/MeOH (2:1) at room temperature (18 h). The mixture was acidified by the addition of 1 M HCl, filtered and the precipitate was washed with 1 M HCl to provide the title compounds (39, 50). If the compound was not pure at this stage of procedure it was washed with CH$_2$Cl$_2$ (41−43) or CH$_2$Cl$_2$ and MeOH (40, 44−47).

3-([1,1'-Biphenyl]-4-ylcarboxamido)benzoic acid (39)

white solid, yield: 42%. Mp: 288−289 °C. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta = 10.49$ (s, 1 H, NH), 8.47 (s, 1 H), 8.15−8.05 (m, 3 H), 7.84 (d, $J = 8.3$ Hz, 2 H), 7.76 (d, $J = 7.3$ Hz, 2 H), 7.72−7.65 (m, 1 H), 7.56−7.47 (m, 3 H), 7.47−7.36 (m, 1 H) ppm. $^{13}$C NMR (125 MHz, DMSO-$d_6$) $\delta = 167.2$, 165.3, 143.3, 139.5, 139.1, 133.4, 131.3, 129.1, 128.9, 128.4, 128.2, 126.9, 126.6, 124.5, 121.2, 116.9 ppm. LC/MS: m/z = 318 [M + H$^+$], 359 [M + H$^+$ + CH$_3$CN]; $t_R = 11.17$ min; 99.0% pure (UV).

3-([1,1'-Biphenyl]-4-ylcarboxamido)-4-bromobenzoic acid (40)

white solid, yield: 89%. Mp: 281−282 °C. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta = 10.24$ (s, 1 H, NH), 8.15−8.12 (m, 2 H), 8.12−8.09 (m, 1 H), 7.90−7.85 (m, 3 H), 7.80−7.74 (m, 3 H), 7.55−7.49 (m, 2 H), 7.46−7.40 (m, 1 H) ppm. $^{13}$C NMR (125 MHz, DMSO-$d_6$) $\delta = 166.3$, 165.3, 150.8, 145.1, 139.9, 132.3, 129.0, 128.1, 128.0, 127.6, 127.5, 127.2, 125.9, 117.0, 114.5, 108.0, 55.4 (OCH$_3$), 52.0 (OCH$_3$) ppm. LC/MS: m/z = 444 [M + H$^+$], 485 [M + H$^+$ + CH$_3$CN], 887 [2M + H$^+$]; $t_R = 17.96$ min; 97.3% pure (UV).
165.1, 143.5, 139.1, 136.9, 133.2, 132.5, 130.8, 129.1, 129.0, 128.4, 128.2, 128.2, 127.0, 126.7, 125.5 ppm. LC/MS: m/z = 396 and 398 [M + H\(^+\)], 437 and 439 [M + H\(^+\) + CH\(_3\)CN]; t\(_R\) = 12.60 min; 97.3% pure (UV).

3-([1,1'-Biphenyl]-4-ylcarboxamido)-5-bromobenzoic acid (41)
beige solid, yield: 94%. Mp: 285–286 °C. \(^1\)H NMR (500 MHz, DMSO-\(d_6\)) \(\delta = 10.60\) (s, 1 H, NH), 8.46–8.37 (m, 2 H), 8.12–8.06 (m, 2 H), 7.89–7.84 (m, 2 H), 7.81–7.73 (m, 3 H), 7.54–7.48 (m, 2 H), 7.48–7.39 (m, 1 H) ppm. \(^{13}\)C NMR (125 MHz, DMSO-\(d_6\)) \(\delta = 165.9, 165.5, 143.5, 141.0, 139.0, 133.2, 132.9, 129.1, 128.5, 128.2, 126.9, 126.7, 126.5, 126.2, 121.4, 119.9 ppm. LC/MS: m/z = 396 and 398 [M + H\(^+\)], 437 and 439 [M + H\(^+\) + CH\(_3\)CN]; t\(_R\) = 13.01 min; 99.4% pure (UV).

4-([1,1'-Biphenyl]-4-ylcarboxamido)benzoic acid (42) [12]
beige solid, yield: 92%. Mp: 337 °C (decomposition). \(^1\)H NMR (500 MHz, DMSO-\(d_6\)) \(\delta = 10.58\) (s, 1 H, NH), 8.08 (d, \(J = 8.3\) Hz, 2 H), 7.99–7.91 (m, 4 H), 7.85 (d, \(J = 8.3\) Hz, 2 H), 7.80–7.74 (m, 2 H), 7.54–7.48 (m, 2 H), 7.46–7.40 (m, 1 H) ppm. \(^{13}\)C NMR (125 MHz, DMSO-\(d_6\)) \(\delta = 167.0, 165.6, 143.4, 143.3, 139.1, 133.4, 130.3, 129.1, 128.5, 128.2, 127.0, 126.6, 125.5, 119.5 ppm. LC/MS: m/z = 318 [M + H\(^+\)], 359 [M + H\(^+\) + CH\(_3\)CN]; t\(_R\) = 11.29 min; 99.4% pure (UV).

3-([1,1'-Biphenyl]-4-ylcarboxamido)thiophene-2-carboxylic acid (43) [13]
yellow solid, yield: 87%. Mp: 242–243 °C. \(^1\)H NMR (500 MHz, DMSO-\(d_6\)) \(\delta = 11.27\) (s, 1 H, NH), 8.13 (d, \(J = 5.5\) Hz, 1 H), 8.01 (d, \(J = 8.5\) Hz, 2 H), 7.95–7.87 (m, 3 H), 7.80–7.73 (m, 2 H), 7.54–7.48 (m, 2 H), 7.47–7.39 (m, 1 H) ppm. \(^{13}\)C NMR (125 MHz, DMSO-\(d_6\)) \(\delta = 165.5, 163.0, 144.0, 143.8, 138.8, 132.6, 132.1, 129.1, 128.4, 127.8, 127.3, 127.0, 121.8, 112.0 ppm.
4-(1,1'-Biphenyl)-4-ylcarboxamido)thiophene-3-carboxylic acid (44) [13]  
**Yellow solid, yield: 90%.** Mp: 265–265 °C. $^{1}$H NMR (500 MHz, DMSO-$d_6$) $\delta$ = 11.23 (s, 1 H, NH), 8.39 (d, $J$ = 3.5 Hz, 1 H), 8.10 (d, $J$ = 3.5 Hz, 1 H), 8.01–7.97 (m, 2 H), 7.93–7.87 (m, 2 H), 7.79–7.73 (m, 2 H), 7.55–7.48 (m, 2 H), 7.46–7.41 (m, 1 H) ppm. $^{13}$C NMR (125 MHz, DMSO-$d_6$) $\delta$ = 165.7, 163.2, 143.7, 138.9, 136.0, 133.9, 132.4, 129.1, 128.3, 127.4, 127.3, 126.9, 122.7, 110.6 ppm. LC/MS: $m/z$ = 324 [M + H$^+$], 647 [2M + H$^+$]; $t_R$ = 12.82 min; 99.5% pure (UV).

2-(1,1'-Biphenyl)-4-ylcarboxamido)thiophene-3-carboxylic acid (45)  
**Brown solid, yield: 92%.** Mp: 242–244 °C. $^{1}$H NMR (500 MHz, DMSO-$d_6$) $\delta$ = 12.12 (s, 1 H, NH), 8.03–8.00 (m, 2 H), 7.95–7.91 (m, 2 H), 7.79–7.75 (m, 2 H), 7.54–7.50 (m, 2 H), 7.48–7.41 (m, 1 H), 7.24 (d, $J$ = 5.7 Hz, 1 H), 7.08 (d, $J$ = 5.7 Hz, 1 H) ppm. $^{13}$C NMR (125 MHz, DMSO-$d_6$) $\delta$ = 166.8, 162.5, 147.9, 144.3, 138.7, 130.6, 129.1, 128.4, 127.8, 127.4, 127.0, 124.3, 116.9, 114.1 ppm. LC/MS: $m/z$ = 324 [M + H$^+$], 365 [M + H$^+$ + CH$_3$CN], 647 [2M + H$^+$]; $t_R$ = 13.53 min; 96.2% pure (UV).

3-(1,1'-Biphenyl)-4-ylcarboxamido)-5-phenylthiophene-2-carboxylic acid (46)  
**Beige solid, yield: 81%.** Mp: 224–225 °C. $^{1}$H NMR (500 MHz, DMSO-$d_6$) $\delta$ = 11.31 (s, 1 H, NH), 8.46 (s, 1 H), 8.05–8.02 (m, 2 H), 7.93–7.90 (m, 2 H), 7.78–7.75 (m, 4 H), 7.53–7.49 (m, 4 H), 7.47–7.42 (m, 2 H) ppm. $^{13}$C NMR (125 MHz, DMSO-$d_6$) $\delta$ = 165.3, 163.0, 148.0, 144.2, 144.0, 138.8, 132.5, 132.0, 129.4, 129.1, 128.4, 127.8, 127.3, 127.0, 125.8, 117.8,
111.1, 92.8 ppm. LC/MS: m/z = 400 [M + H+], 441 [M + H+ + CH3CN], 799 [2M + H+]; tR = 15.73 min; 98.2% pure (UV).

3-([1,1'-Biphenyl]-4-ylcarboxamido)-5-(4-chlorophenyl)thiophene-2-carboxylic acid (47) grey-green solid, yield: 81%. Mp: 225–226 °C. 1H NMR (500 MHz, DMSO-d6) δ = 11.26 (s, 1 H, NH), 8.46 (s, 1 H), 8.03–8.01 (m, 2 H), 7.92–7.89 (m, 2 H), 7.79–7.75 (m, 4 H), 7.55–7.50 (m, 4 H), 7.46–7.41 (m, 1 H) ppm. 13C NMR (125 MHz, DMSO-d6) δ = 165.2, 163.0, 146.5, 144.2, 144.1, 138.8, 134.0, 131.9, 131.3, 129.4, 129.1, 128.4, 127.8, 127.6, 127.3, 127.0, 118.3, 111.4 ppm. LC/MS: m/z = 434 and 436 [M + H+], 867 and 869 [2M + H+]; tR = 16.31 min; > 99.9% pure (UV).

2-([1,1'-Biphenyl]-4-ylsulfonamido)-5-bromobenzoic acid (50) [14] beige solid, yield: 96%. Mp: 218–219 °C. 1H NMR (500 MHz, DMSO-d6) δ = 11.12 (br. s., 1 H, NH), 7.98 (d, J = 2.5 Hz, 1 H), 7.92–7.85 (m, 4 H), 7.75 (dd, J = 8.8, 2.5 Hz, 1 H), 7.72–7.67 (m, 2 H), 7.54–7.47 (m, 3 H), 7.47–7.40 (m, 1 H) ppm. 13C NMR (125 MHz, DMSO-d6) δ = 168.4, 145.0, 138.9, 138.0, 137.2, 137.0, 133.6, 129.1, 128.8, 127.7, 127.6, 127.1, 120.7, 119.1, 115.1 ppm. LC/MS: m/z = 430 and 432 [M + H+] (negative mode); tR = 13.70 min; > 99.9% pure (UV).

6.2.4. Compounds prepared by different procedures (35b, 35a, 35, 36a, 48, 49, 50a).

2-Amino-6-hydroxybenzoic acid (35b). To a solution of 2-amino-6-methoxybenzoic acid (1 equiv) in anhydrous CH2Cl2 at −78 °C (dry ice/acetone bath), boron tribromide (1 M in CH2Cl2, 3 equiv) was added dropwise. The reaction mixture was stirred for 18 h at room
temperature under a nitrogen atmosphere. After addition of EtOH and MeOH the solvents were evaporated to provide a brown solid which was used for the next reaction step without further purification; brown solid, yield: 75%.

2-((1,1’-Biphenyl]-4-yl)-5-hydroxy-4H-benzo[d][1,3]oxazin-4-one (35a) was prepared according to 6.2.2.3. The solvent was evaporated and the remaining solid was suspended in MeOH. After filtration the precipitate was washed with MeOH to provide a mixture of the title compound (80%) and 2-((1,1’-biphenyl]-4-yl)-4-oxo-4H-benzo[d][1,3]oxazin-5-yl [1,1’-biphenyl]-4-carboxylate (20%). This beige solid was used for the next reaction step without further purification; beige solid, yield: 50%.

2-((1,1’-Biphenyl]-4-ylcarboxamido)-6-hydroxybenzoic acid (35). 2-((1,1’-biphenyl]-4-yl)-5-hydroxy-4H-benzo[d][1,3]oxazin-4-one (35a) was dissolved in THF and hydrolyzed by an aqueous solution containing 1 M LiOH at room temperature (18 h). The mixture was acidified by the addition of 1 M HCl, filtered and the precipitate was successively washed with 1 M HCl and MeOH to provide the title compound. Sufficient purity was achieved without further purification; brown solid, yield: 61%. Mp: 209–210 °C. ¹H NMR (500 MHz, DMSO-**d**₆) δ = 11.99 (s, 1 H, NH), 8.04–8.02 (m, 2 H), 7.99 (dd, J = 8.2, 1.1 Hz, 1 H), 7.87–7.85 (m, 2 H), 7.77–7.75 (m, 2 H), 7.52–7.49 (m, 2 H), 7.44–7.39 (m, 2 H), 6.69 (dd, J = 8.2, 1.1 Hz, 1 H) ppm. ¹³C NMR (125 MHz, DMSO-**d**₆) δ = 171.8, 164.4, 161.3, 143.5, 140.6, 139.0, 133.7, 133.7, 129.2, 128.3, 127.9, 127.1, 127.0, 112.3, 111.5, 105.9 ppm. LC/MS: m/z = 334 [M + H⁺], 667 [2M + H⁺]; tᵣ = 11.00 min; 96.2% pure (UV).

2-Amino-5-bromobenzamide (36a). Methyl 2-amino-5-bromobenzoate (1 equiv) was added to a solution of NH₃ in MeOH (7 M, 10 equiv) under a N₂ atmosphere. The reaction mixture was
stirred for 2 weeks at room temperature. After evaporation of the solvent the remaining solid was purified by CC (n-hexane/EtOAc 1:1) to provide the pure compound; white solid, yield: 11%. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ = 7.84 (br. s., 1 H), 7.70 (d, $J = 2.3$ Hz, 1 H), 7.25 (dd, $J = 8.8$, 2.3 Hz, 1 H), 7.22–7.09 (m, 1 H), 6.70 (s, 2 H, NH$_2$), 6.66 (d, $J = 8.8$ Hz, 1 H) ppm. $^{13}$C NMR (125 MHz, DMSO-$d_6$) $\delta$ = 169.9, 149.4, 134.3, 130.8, 118.5, 115.2, 104.7 ppm. LC/MS: m/z = 215 and 217 [M + H$^+$], 198 and 200 [M$^+$ – NH$_2$]; $t_R$ = 6.65 min; 97.0% pure (UV).

3-[[1,1'-Biphenyl]-4-ylcarboxamido]-5-(4-methoxyphenyl)thiophene-2-carboxylic acid (48).

Methyl 3-[[1,1'-biphenyl]-4-ylcarboxamido]-5-(4-methoxyphenyl)thiophene-2-carboxylate (48a) was dissolved in THF/MeOH (2:1). An aqueous solution containing 1 M NaOH and 5 M LiOH was added and the mixture was stirred at 80 °C for 8 h. After acidification by the addition of 1 M HCl the resulting suspension was filtered and the precipitate was washed with 1 M HCl to provide the title compound. Sufficient purity was achieved without further purification; yellow solid, yield: 37%. Mp: 210–212 °C. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ = 11.29 (s, 1 H, NH), 8.34 (s, 1 H), 8.04–8.01 (m, 2 H), 7.93–7.90 (m, 2 H), 7.78–7.75 (m, 2 H), 7.72–7.67 (m, 2 H), 7.54–7.50 (m, 2 H), 7.46–7.41 (m, 1 H), 7.08–7.02 (m, 2 H), 3.81 (s, 3 H, OCH$_3$) ppm. $^{13}$C NMR (125 MHz, DMSO-$d_6$) $\delta$ = 165.5, 163.1, 160.4, 148.5, 144.5, 144.1, 138.8, 132.1, 129.2, 128.5, 127.9, 127.4, 127.4, 127.0, 125.1, 116.6, 114.9, 109.7, 55.4 (OCH$_3$) ppm. LC/MS: m/z = 430 [M + H$^+$], 859 [2M + H$^+$]; $t_R$ = 15.43 min; 95.4% pure (UV).

2-[[1,1'-Biphenyl]-4-ylcarboxamido]-5-phenylthiophene-3-carboxylic acid (49).

Ethyl 2-[[1,1'-biphenyl]-4-ylcarboxamido]-5-phenylthiophene-3-carboxylate (49a) was dissolved in THF/MeOH (2:1) and hydrolyzed at room temperature with an aqueous solution containing 5 M NaOH and 5 M KOH (18 h). The mixture was acidified by the addition of 1 M HCl,
filtered and the precipitate was washed with 1 M HCl to provide the title compounds.
Sufficient purity was achieved without further purification; yellow solid, yield: 62%. Mp:
249–250 °C. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta = 12.18$ (s, 1 H, NH), 8.05–8.01 (m, 2 H),
7.97–7.91 (m, 2 H), 7.80–7.75 (m, 2 H), 7.71–7.66 (m, 2 H), 7.60 (s, 1 H), 7.55–7.51 (m, 2 H), 7.47–7.41 (m, 3 H), 7.35–7.29 (m, 1 H) ppm. $^{13}$C NMR (125 MHz, DMSO-$d_6$) $\delta = 166.7,
162.5, 147.0, 144.4, 138.7, 133.2, 132.6, 130.4, 129.2, 129.1, 128.5, 127.9, 127.6, 127.4,
127.0, 125.1, 120.2, 115.3 ppm. LC/MS: m/z = 400 [M + H$^+$], 441 [M + H$^+$ + CH$_3$CN], 799
[2M + H$^+$]; $t_R = 14.64$ min; 98.2% pure (UV).

*Methyl 2-([1,1'-biphenyl]-4-ylsulfonamido)-5-bromobenzoate (50a).* Methyl 2-amino-5-
bromobenzoate (1 equiv) and a catalytic amount of DMAP were added to a suspension of
[1,1'-biphenyl]-4-sulfonyl chloride (1.5 equiv) in pyridine under a N$_2$ atmosphere. The
reaction mixture was stirred for 6 days at room temperature and 2 M HCl was added. The
mixture was extracted with EtOAc, the combined organic layers washed with saturated
NaHCO$_3$ and dried over MgSO$_4$. For purification the solvent was evaporated and the
remaining solid was suspended in MeOH. After filtration the precipitate was washed with
MeOH to provide the pure compound; slightly yellow solid, yield: 78%. $^1$H NMR (500 MHz,
CDCl$_3$) $\delta = 10.59$ (s, 1 H, NH), 8.06 (d, $J = 2.5$ Hz, 1 H), 7.92–7.89 (m, 2 H), 7.68–7.65 (m, 3 H),
7.59–7.55 (m, 3 H), 7.49–7.44 (m, 2 H), 7.43–7.38 (m, 1 H), 3.89 (s, 3 H, OCH$_3$) ppm.
$^{13}$C NMR (125 MHz, CDCl$_3$) $\delta = 167.1, 146.1, 139.5, 139.0, 137.6, 137.3, 133.8, 129.0,
128.6, 127.7, 127.2, 120.8, 117.4, 115.7, 52.8 (OCH$_3$) ppm. LC/MS: m/z = 446 and
448 [M + H$^+$], 487 and 489 [M + H$^+$ + CH$_3$CN]; $t_R = 15.61$ min; 95.4% pure (UV).
6.3 Biology

**RNAP transcription inhibition assay.** RNAP transcription inhibition assay was performed as described previously [9, 11].

**PqsD inhibition assay.** PqsD inhibition assay was performed as described previously [8].

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**Conflict of Interest**

The authors have declared no conflict of interest.
References

**Figure Captions**

**Figure 1.** A recently described series of potent novel RNA polymerase (RNAP) inhibitors (II) [7] was derived from a known PqsD inhibitor (I) [6]. The compounds consisting of a benzamidobenzoic acid core are structurally very similar to the class of PqsD inhibitors (III) published by Weidel et al. [8]. This raised the question about selectivity: Do the compounds from class II also inhibit PqsD and are the molecules from class III inhibitors of RNAP?

**Figure 2.** Structural modifications generating selectivity in favor of PqsD inhibition over RNAP inhibition in the class of benzamidobenzoic acids