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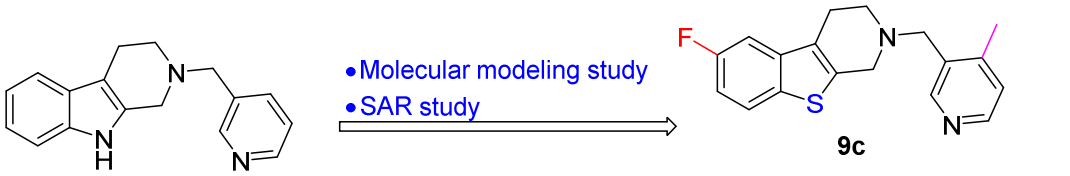
Graphical abstract



hit compound **7**
Rat CYP17 IC_{50} = 228.5 nM

9c
Rat CYP17 IC_{50} = 15.8 nM
Human CYP17 IC_{50} = 20.1 nM
CYP3A4 IC_{50} = 8.5 μM

Discovery of novel 1,2,3,4-tetrahydrobenzo[4, 5]thieno[2, 3-*c*]pyridine derivatives as potent and selective CYP17 inhibitors

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Abstract

The inhibition of CYP17 to block androgen biosynthesis is a well validated strategy for the treatment of prostate cancer. Herein we reported the design, synthesis and structure–activity relationship (SAR) study for a series of novel 1,2,3,4-tetrahydrobenzo[4,5]thieno[2,3-*c*]pyridine derivatives. Some analogs demonstrated a potent inhibition to both rat and human CYP17 protein and reduced testosterone production in human H295R cell line. Some analogs also showed high selectivity against other CYP enzymes such as 3A4, 1A2, 2C9, 2C19 and 2D6, which may limit side effects due to drug-drug interactions. Among these analogs, the most potent compound **9c** showed 1.5 fold more potent against rat and human CYP17 protein than that of abiraterone (IC_{50} = 16 nM and 20 nM vs. 25 nM and 36 nM respectively). In NCI-H295R cells, the inhibitory effect of compound **9c** on testosterone production (52 ± 2%) was also more potent than that of abiraterone (74 ± 15%) at the concentration of 1 μM. Further, it was shown that **9c** reduced plasma testosterone level in a dose-dependent manner in Sprague-Dawley rats. Thus, analog **9c** maybe a potential agent used for the treatment of prostate cancer.

Keywords: Prostate cancer, androgens, CYP17, abiraterone, CYP3A4

1. Introduction

Prostate cancer is the most common malignancy in men and continues to be a major cause of cancer deaths [1-3]. Prostate cancer patients in the early stages can be cured with localized therapies, such as prostatectomy, radiation and cryotherapy [4]. However, men with recurrent or advanced-stage disease, particularly in metastatic state, when treated with hormone therapy, drug resistance occurs rapidly. This leads to a disease state known as castration-resistant prostate cancer (CRPC) [4-6]. Further, traditional androgen deprivation therapies, such as using orchiectomy and GnRH analogues, only eliminate androgen production in testes. The biosynthesis of androgen inside adrenals and tumor cells remains to be not affected [7]. It is well known that androgens produced by the tumor and/or the adrenal gland drive disease progression in CRPC [8]. Thus, reduction or suppression of hormone levels inside adrenals and cancer cells remains a key point in advanced stages of the disease. Therefore, total blockage of the androgen biosynthesis by inhibiting 17α-hydroxylase-17,20-lyse (CYP17) appears to be a superior alternative approach [9-12].

CYP17, expressed in the testes, adrenal glands and prostate cancer cells, is a monooxygenase with a critical role in the synthesis of steroid hormones. This enzyme catalyzes the conversion of 17α-hydroxypregnenolone and 17α-hydroxyprogesterone into the weak androgens, dehydroepiandrosterone and androstenedione, respectively. These weak androgens are subsequently converted into more potent androgens such as testosterone and dihydrotestosterone in prostate cancer cells [9, 13]. The inhibition of CYP17 could completely attenuate the production of androgens in testes, adrenals as well as prostate cancer cells. Therefore, inhibitors of CYP17 are supposed to be more effective for treating androgen-dependent prostate cancer, regardless of androgen receptor mutations.

Several well-characterized CYP17 inhibitors were discovered for the treatment of advanced prostate cancer (Fig.1). Among them, **1** (Ketoconazole), an antifungal agent that inhibits CYP17 activity, has been used clinically in high dose for the treatment of advanced prostate cancer [14]. However, Ketoconazole has been discontinued due to its poor selectivity and severe hepatic toxicity [15]. Compound **2** (Abiraterone

acetate), another approved drug targeting CYP17, exhibits significant beneficial effects in CRPC patients [16]. However, its steroidal scaffold renders abiraterone a promiscuous profile with affinities toward to other steroid receptors, raising the cautions of its side effects. For example, it has been noted that abitaterone acts as a moderate-to-potent inhibitor of several hepatic CYP enzymes including CYP1A2, CYP2D6, CYP2C8, CYP2C9 and CYP3A4 [17]. Recently, steroidal CYP17 inhibitor **3** (TOK-001) [18], also acting as an androgen receptor antagonist, was terminated in its phase III clinical trial due to the lack of clinical efficacy [19]. Thus, these potential adverse effects of steroidal drugs triggered the efforts to develop nonsteroidal CYP17 inhibitors. These efforts have pushed several drug candidates into clinical study, such as **4** (TAK-700) [20, 21], **5** (VT-464) [22, 23], **6 (**YM-116) [24] and CFG920 [25] (Fig. 1). Meanwhile, several other groups also have identified a few CYP17 inhibitors as potential agents for the treatment of prostate carcinoma [26 -31].

Figure 1

Our group has previously synthesized series of metalloenzyme inhibitors [32, 33]. CYP17 enzyme, as one of metalloenzyme family members, has aroused our research interest, especially when the X-ray structure of CYP17 was disclosed by DeVore and Scott in 2012 [34]. Structural analysis of the reported CYP17 inhibitors reveals that most of the compounds consist of two chemical components. One is the metal-binding group that binds to the heme iron, and the second is the scaffold that binds to the substrate pocket of CYP17. Based on this observation, we conducted a screen of compounds from our in-house metalloenzyme inhibitor library and identified compound **7** that could inhibit rat CYP17 lyase activity with IC_{50} of 228 nM (IC_{50} >10000 nM for CYP3A4). Our preliminary modeling study indicated that compound **7** could fit nicely into the CYP17 binding pocket and maintain the key interactions with the residues of CYP17. As shown in Fig. 2, the nitrogen of the pyridine and the tetrahydro-β-carboline core formed a coordination bond and hydrophobic interactions with heme group (Fe atom) and hydrophobic pocket (A113,

F114, S106, A105, V236, L209, I206 and V482 residue), respectively. Since there is unfilled space on the pyridine part in the active site cavity adjacent to V366, A367, I371 and V483 residue, introducing substituent onto the pyridine ring may well fill this space and enhance the potency (Fig. 2). These efforts led to the design and synthesis compounds **8a-i** and **9b-j** and evaluated their CYP17 activity. Preliminary selectivity for hepatic CYP enzymes including 3A4, 1A2, 2C9, 2C19 and 2D6 was also tested for some selected compounds (see supporting document). Cellular activity to inhibit testosterone secretion by CYP17 inhibitors was evaluated in the human adrenal corticocarcinoma H295R cell line. Among these compounds, **9c** showed the best potency against both rat and human CYP17. Compound **9c** was further examined for its potency of reducing plasma testosterone concentration in rats. Molecular modeling study by superposition of **9c** into TOK-001 crystal structure confirmed that the sulfur atom is more likely to bind to hydrophobic pocket than NH, and F and OH group at the scaffold part, which could form hydrogen bond with N202 in the F helix. Keeping these two features, compounds **9a** and **9k-n** were designed, synthesized and evaluated their inhibitory activity against CYP17. This further modification resulted **9m** with a potent CYP17 inhibitory activity, though similar to that of **9c**.

Figure 2

Figure 3

2. Results and discussion

2.1. Chemistry

Compounds **8a-e** were prepared via two different synthetic routes illustrated in Scheme 1. For the synthesis of **8a**, the commercially available material 2-(benzo[b]thiophen-3-yl)acetonitrile (**10**) was used as the starting material. Compound **10** was converted to Boc-protected amine **11** by using $NaBH_4/NiCl_2 \cdot 6H_2O$ in the presence of $(Boc)_2O$ in 48% yield. Intermediate **11** reacted with paraformaldehyde catalyzed by TsOH to afford **12** via Pictet-Spengler reaction in 68% yield. Removal of Boc group in **12** with TFA gave **20a** which reacted with 3-pyridinecarboxaldehyde to afford **8a** via reductive amination in 62% yield (2 steps). For the synthesis of **8b-e**, commercially available 4-substituted benzenethiols **13b-e**

were used as the starting materials. Benzenethiols **13b-e** reacted with ethyl 4-chloroacetoacetate to afford **14b-e** in 86-93% yields, followed by cyclization catalyzed by polyphosphoric acid to give 3-substituted benzo[*b*]thiophenes **15b-e** in 47-62% yields. Treatment of **15b-e** with ammonia in methanol at room temperature afforded the corresponding amides **16b-e** in 72-88% yields. Then intermediates **16b-e** were reduced with borane in tetrahydrofuran to the corresponding amines **17b-e**, followed by reacting with ethyl chloroformate to obtain **18b-e** in 83-91% yields. Similar method to prepare **12** was employed here to convert **18b-e** to **19b-e** via Pictet-Spengler reaction. After deprotection and reductive amination, analogs **8b-e** were prepared from **19b-e** in 2 steps in 48-58% yields.

Scheme 1.

The syntheses of analogs **8f-l** were accomplished by using **8d** as the starting material (Scheme 2). Analog **8f** was obtained via Suzuki coupling of **8d** with methylboronic acid. Through Miyaura boration reaction, **8g** was synthesized from **8d** in 64% yield, which was converted to **8h** by using hydrogen peroxide under basic condition in 54% yield**.** Compound **8d** was transformed to **8i** via palladium-catalyzed coupling reaction in 72% yield. Hydrolysis cyano group in **8i** afforded the corresponding carboxylic acid **8j-1,** which reacted with methylamine to give amide **8j** in 37% yield (2 steps). Compound **8k** was synthesized from **8d** with t-butyl carbamate via Buchwald−Hartwig coupling reaction. The obtained **8k** was further converted to **8l** by deprotection and acylation (69% yield).

Scheme 2.

Analogs **9a-n** were synthesized by using compound **20b** as the common starting material shown in Scheme 3. Analog **9a** was simply prepared by amide bond coupling of **20b** with nicotinic acid under HATU and DIPEA condition in 57% yield. For analogs **9b** and **9h-n**, they were prepared by reductive amination of **20b** with corresponding aldehydes in 44-72% yields. Analogs **9c-f** were obtained via Suzuki coupling of **9b** with the corresponding boronic acids in 54-64% yields. Finally, hydrogenation of **9f** with Pd/C in MeOH afforded **9g** in 69% yield.

Scheme 3.

2.2. Biological results

2.2.1. CYP17 Inhibitory Activity

Inhibition of human or rat CYP17 was determined by performing CYP17 biochemical assay following the procedure as previously described [35, 36]. Human CYP17 gene was cloned and expressed in Adenoviral expression system in A549 cell lines [37]. The purified cell membrane preparations were used as the source for human CYP17 enzyme. For the rat CYP17 assay, we used rat testes microsomes as the enzyme source [38]. The assay was run with 17α-hydroxyprogesterone as substrate at concentration of 10 μM and NADPH as cofactor, separation of substrate and product was accomplished by LCMS using UV detection.

Table 1

Table 2

Initially, we maintained the pyridin-3-ylmethyl group as a metal-binding part and simply replaced with sulfur on **7** instead of nitrogen, the resulting compound **8a** was 4-fold more potent (IC_{50} = 54.9 nM) than its corresponding N analog **7** (IC_{50} = 228.5 nM) for rat CYP17 (Table 1). Further modification on phenyl ring by incorporating a fluoro at R_1 position, compound **8b** enhanced two fold potency (IC_{50} = 28.1 nM) compared with **8a**, which was almost as potent as ABT (IC_{50} = 24.7 nM). When fluoro was replaced with chloro (**8c**) or bromo (**8d**) to increase the substituent volumn, the potency for rat CYP17 was tremendously decreased (16 to 18 fold). Introducing electro-donating groups at this position, such as methoxy (**8e**) or methyl (**8f**) group, did not improve the potency, especially **8e** totally abolished the potency. Other modifications also did not show any benefit, either only having moderate activity (**8g** and **8i**) or totally losing potency (**8j-l**). Interestingly, the hydroxyl substituted analog **8h** was more potent than ABT with IC_{50} value of 20.9 nM. This consisted with our molecular modeling study that F and OH group at the scaffold part may be able to form hydrogen bond with N202 in the F helix. Although compound **8h** showed slightly improved activity, the hydroxyl phenyl group made this compound not very stable, especially in solution. Therefore, we selected compound **8b** as a lead for

further optimizing the heteroaryl substituent on the metal-binding moiety.

The results for SAR study of metal-binding region on nitrogen were shown on Table 2. Acylated compound **9a** lost potency for CYP17, indicating that the conformation between the metal-binding region and metal binding moiety was favored to be linked with a methylene bridge. Thus, methylene linker was kept for further SAR study. Compound **9b** with an ortho substituted bromo kept the potency, though 3-fold decreased compared with **8b**. When replaced bromo with methyl group, compound **9c** showed the most potent inhibitory activity for rat CYP17. Compund **9e** with a cyclopropyl substituent remained a good activity; however, compounds **9f**, **9g** and **9d** decreased the potency or even lost potency when futher increased the substituent volumn. When shifted the substituent from ortho- to meta-position, compounds **9h-j** still kept good to moderate activity, especially **9i** only lost 2-fold potency compared with **9c**. Replacement of 3-pyridine with imidazole ring, analog **9m** also showed very good potency (IC_{50} = 29.8 nM), though both pyrimidine and isoquinoline replacement did not show better activity. Interestly, compound **9l**, which was a 4-pyridine instead of 3-pyridine, totally lost its potency.

Selected compounds (**8b, 8h, 9c, 9h, 9i** and **9m**) along with ABT were evaluated for inhibitory activity for human recombinant $C_{17,20}$-lyase. Similar trend to rat CYP17, these compounds showed potent inhibitory activity for human CYP17 with IC_{50} values of 20–45 nM. Especially, **9c** exhibited 1.5 fold more potent than reference compound ABT (IC_{50} = 35.8 nM) in this assay. Further, all these selected compounds demonstrated great enzyme selectivity against human CYP3A4 (Table 3).

2.2.2. Inhibition of human CYP3A4 and other hepatic CYP enzymes

According to the activity data of rat CYP17 in Table 1 and 2, the most promising compounds (**8b**, **8h**, **9c**, **9h**, **9i** and **9m**) were tested for inhibition of human CYP17, CYP3A4 and other hepatic CYP enzymes. Overall, these compounds exhibited similar activity trend toward to both rat and human CYP17. These compounds showed very weak inhibition of CYP3A4 (IC_{50} > 5000 nM) except imidazol substituted compound **9m** which showed moderate inhibitory activity (IC_{50} = 680 nM). The most potent CYP17 inhibitor **9c** exhibited IC_{50} value of 8.5 μM against CYP3A4,

showing a better selectivity profile than ABT (IC_{50} = 2.7 μM). Compound **9c** also showed no or very weak inhibition against CYP1A2 (16.3 % at 1 μM), CYP2C9 (0 % at 1 μM), CYP2C19 (11.7 % at 1 μM) and CYP2D6 (12.0 % at 1 μM) (see supplementary material).

2.2.3. Inhibitory effects of selected compounds in testosterone production in NCI-H295R cells.

Table 4

Compounds (**8b**, **8h**, **9c**, **9h**, **9i** and **9m**) were also tested their inhibition for testosterone production in NCI-H295R cells that were derived from an adrenocortical carcinoma and commonly studied for autonomous aldosterone secretion. At the concentration of 1 μM, ABT and compound **9c** inhibited testosterone production to 74 ± 15% and 52 ± 2% respectively ($P < 0.05$ comparing with control), suggesting that the inhibitory effect of compound **9c** on testosterone production was more potent than that of ABT (Table 4).

2.2.4. Compound **9c** and ABT were evaluated for their inhibitory effects on steroid biosynthesis in male rats.

Table 5

Following a single oral dose, compound **9c** significantly decreased serum testosterone concentration in a dose-dependent manner. At dose 10 mg/kg, serum testosterone concentration was low to the detectable quantitative limit after 2 h of the post-dose (Table 5).

2.3 Molecular modeling studies.

To better understand the interactions between the newly synthesized compounds and CYP17, the potential binding mode of potent compound **9c** was investigated using the CDOCKER program in Discovery Studio 3.0 software. The published crystal structure of ABT and TOK-001 bound within the active site cavity of CYP17 (PDB ID: 3RUK) served as a useful template for generating proposed binding modes. The docking pose of compound **9c** overlaps well with TOK-001. Compound **9c** forms a coordinate (dative) bond with the heme iron through the nitrogen of the 4-methylpyridine, positioning the tricyclic core above the heme plane at an angle of

~60 degree. The piperidinethiophene ring lies flat against the I helix and forms several hydrophobic and Van de Walls interactions with G301 and A302. As a result, the fluorobenzyl ring is oriented toward to F and G helices. Unlike TOK-001, there is no polar interaction, i.e. hydrogen-bond interactions observed in the docking model. However, substitutions at the ortho-position of the fluorobenzyl moiety could provide hydrogen-bond interactions with the side-chain of N202 and potentially improve the potency.

Figure 4A.

Figure 4B.

3. Conclusion

In summary, we have designed and synthesized a series of 1,2,3,4-tetrahydrobenzo [4,5]thieno[2,3-*c*]pyridine derivatives. Among them, analogs **8b**, **8h**, **9c**, **9h**, **9i** and **9m**, showed excellent CYP17 activities with IC_{50} values equal or better than marked drug abiraterone both in rat and human enzyme assay. In addition, the most promising compound **9c** showed no or very weak inhibition on other human hepatic CYP enzymes. In addition, compound **9c** significantly reduced testosterone level both in NCI-H295R cells and serum levels in male rats. Further, molecular docking simulation of **9c** into the CYP17 active site was performed. The result demonstrated that **9c** occupies the CYP17 active site, forming interactions with the main amino acid residues. Thus, this type of compounds has the potential to be leads for developing novel CYP17 inhibitors. Further structural optimization and antitumor mechanism study of these derivatives are in progress.

4. Experimental

4.1 Chemistry

4.1.1. General considerations

All starting materials and reagents were either obtained from commercial suppliers or prepared according to literature reported procedures. All purchased chemicals and solvents were used without further purification unless otherwise noted. Flash

chromatography was performed using silica gel (300−400 mesh). All reactions were monitored by thin-layer chromatography (TLC), using silica gel plates with fluorescence F_{254} and visualized under UV light. Melting points (mp) were determined using an X-4 microscope melting point apparatus and were uncorrected. NMR spectra were obtained on a Bruker AV II-400 MHz spectrometer (^{1}H NMR at 400 MHz, ^{13}C NMR at 100 MHz, and ^{19}F NMR at 375 MHz). Chemicals shifts (δ) were reported in parts per million, coupling constants (*J*) values were in hertz, and the splitting patterns were abbreviated as follows: s for singlet; d for doublet; t for triplet; q for quartet; and m for multiplet. Low resolution electrospray ionization (ESI) mass spectra were recorded on an Agilent 1200 HPLC-MSD mass spectrometer and high resolution ESI-MS on a Thermo Fisher Scientific LTQ FT Ultra. EI-MS were performed on an Agilent 5973N instrument for and a Waters Micromass GCT Premier instrument for High-resolution mass. All tested compounds were purified to ≥ 95% purity as determined by high performance liquid chromatography (HPLC).

4.1.2.1. General method A for reductive amination

To a solution of amine (1.0 equiv) in 1,2-dichloroethane (0.1 M) were added aldehyde (1.2 equiv) and acetic acid (0.5 mL), the reaction mixture stirred at rt for 10 mins, followed the addition of sodium triacetoxyborohydride (2.0 equiv). The reaction was stirred at rt under nitrogen for 18 h and concentrated. Sat. $NaHCO_3$ (30 mL) was added and extracted with dichloromethane (20 mL x 3) and the combined organic layers were dried over anhydrous Na_2SO_4, filtered and evaporated under reduced pressure. The crude residue was purified by flash chromatography on silica gel eluting with 0-10% methanol/dichloromethane to give the desired product.

4.1.2.2. General method B for Suzuki coupling

To a solution of the corresponding bromo-derivative (1.0 equiv) in 1,4-Dioxane (10 mL/mmol), an aqueous Na_2CO_3 solution (3.0 equiv) and the corresponding boronic acid (1.5 equiv) were added. The mixture was deoxygenated under reduced pressure and flushed with nitrogen. After having repeated this cycle three times, $Pd(dppf)Cl_2 \cdot CH_2Cl_2$ (0.05 equiv) was added. The resulting suspension was heated

under 100 °C for 8h. After cooling down, ethyl acetate (30 mL) and water (20 mL) were added. The organic layer was separated and the water phase was extracted with ethyl acetate (20 mL x 2). The combined organic layers were dried over Na_2SO_4, concentrated in vacuo, and purified by column chromatography eluting with 0-10% methanol/dichloromethane to give the desired product.

*4.1.3.1. 2-(Pyridin-3-ylmethyl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole (**7**)*

Following the general experimental procedure A, compound **7** was obtained as an off-white solid (280 mg, 71%) from 2,3,4,9-tetrahydro-1H-pyrido[3,4-*b*]indole (258 mg, 1.5 mmol) and 3-pyridinecarboxaldehyde (193 mg, 169 µL, 1.8 mmol). Mp: 171.2 – 173.0 ^{o}C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.85 (s, 1H), 8.57 (s, 1H), 8.50 (d, *J* = 4.8 Hz, 1H), 7.79 (d, *J* = 7.6 Hz, 1H), 7.40 – 7.34 (m, 2H), 7.26 (d, *J* = 8.0 Hz, 1H), 7.01 (t, *J* = 7.4 Hz, 1H), 6.93 (t, *J* = 7.4 Hz, 1H), 3.76 (s, 2H), 3.58 (s, 2H), 2.80 (t, *J* = 5.6 Hz, 2H), 2.69 (t, *J* = 5.0 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 150.0, 148.4, 136.5, 134.1, 132.6, 126.7, 123.5, 120.3, 118.3, 117.4, 110.9, 106.3, 58.4, 50.5, 49.8, 21.1; IR (KBr, ν/cm^{-1}): 3062, 2854, 1577, 1369, 1155, 1029, 843, 769; ESI-MS m/z: 264.1 $[M+H]^+$; HRMS (ESI-TOF) calcd for $C_{17}H_{18}N_3$ $[M+H]^+$: 264.1495, found: 264.1495.

*4.1.3.2. 2-(Pyridin-3-ylmethyl)-1,2,3,4-tetrahydrobenzo[4,5]thieno[2,3-c]pyridine (**8a**)*

Following the general experimental procedure A, compound **8a** was obtained as an off-white solid (204 mg, 73%) from compound **20a** (190 mg, 1.0 mmol) and 3-pyridinecarboxaldehyde (129 mg, 1.2 mmol). Mp: 61.2 – 62.9 ^{o}C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.83 (s, 1H), 8.76 (d, *J* = 4.4 Hz, 1H), 8.15 (d, *J* = 7.6 Hz, 1H), 7.99 (d, *J* = 7.6 Hz, 1H), 7.76 (d, *J* = 7.6 Hz, 1H),7.68 – 7.65 (m, 1H), 7.47 – 7.39 (m, 2H), 4.62 (s, 2H), 4.56 (s, 2H), 3.68 (s, 2H), 3.15 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 158.6, 158.2, 150.6, 149.3, 140.7, 138.3, 137.3, 127.1, 125.1, 124.7, 124.5, 122.8, 121.4, 55.6, 49.6, 48.6, 20.9; IR (KBr, ν/cm^{-1}): 3073, 2531, 1676, 1478, 1193, 1128, 799, 722; ESI-MS m/z: 281.0 $[M+H]^+$; HRMS (ESI-TOF) calcd for $C_{17}H_{17}N_2S$ $[M+H]^+$: 281.1107, found: 281.1106.

*4.1.3.3. 6-Fluoro-2-(pyridin-3-ylmethyl)-1,2,3,4-tetrahydrobenzo[4,5]thieno[2,3-c]pyridine (**8b**)*

Following the general experimental procedure A, compound **8c** was obtained as an off-white solid (110 mg, 73.4%) from compound **20b** (104 mg, 0.50 mmol) and 3-pyridinecarboxaldehyde (64 mg, 0.60 mmol). Mp: 57.3 – 59.1 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.61 (d, *J* = 1.6 Hz, 1H), 8.59 – 8.57 (m, 2H), 7.89 (d, *J* = 8.0 Hz, 1H), 7.70 – 7.67 (m, 1H), 7.35 – 7.32 (m, 1H), 7.26 – 7.23 (m, 1H), 7.08 – 7.03 (m, 1H), 3.87 (s, 2H), 3.85 (s, 2H), 3.03 (s, 2H), 2.88 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.1 (d, *J* = 240.3 Hz), 150.3, 149.2, 140.1 (d, *J* = 9.0 Hz), 137.4, 135.7, 134.0, 132.8, 127.9 (d, *J* = 4.2 Hz), 123.9, 123.7 (d, *J* = 9.3 Hz), 111.8 (d, *J* = 24.9 Hz), 106.7 (d, *J* = 22.9 Hz), 58.6, 52.0, 49.6, 23.4; ^{19}F NMR (375 MHz, $CDCl_3$) δ −118.46 to −118.52 (m, 1F); IR (KBr, ν/cm^{-1}): 2909, 2816, 1654, 1599, 1429, 1177, 1122, 843, 768; ESI-MS m/z: 299.0 $[M+H]^+$; HRMS (ESI-TOF) calcd for $C_{17}H_{16}FN_2S$ $[M+H]^+$: 299.1013, found: 299.1014.

*4.1.3.4. 6-Chloro-2-(pyridin-3-ylmethyl)-1,2,3,4-tetrahydrobenzo[4,5]thieno[2,3-c]pyridine (**8c**)*

Following the general experimental procedure A, compound **8c** was obtained as an off-white solid (63 mg, 66.8%) from compound **20c** (67 mg, 0.30 mmol) and 3-pyridinecarboxaldehyde (39 mg, 0.36 mmol). Mp: 230.2 – 231.9 ^{o}C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.18 (s, 1H), 8.96 (d, *J* = 5.2 Hz, 1H), 8.81 (d, *J* = 8.0 Hz, 1H), 8.05 – 8.02 (m, 2H), 7.87 (d, *J* = 7.6 Hz, 1H), 7.44 – 7.42 (m, 1H), 4.76 (s, 2H), 4.55 (s, 2H), 3.70 (s, 2H), 3.21 (s, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 146.4, 146.3, 144.5, 138.8, 136.8, 130.2, 129.9, 128.7, 126.8, 126.3, 125.0, 124.5, 121.1, 54.4, 48.8, 48.4, 20.6; IR (KBr, ν/cm^{-1}): 3413, 3035, 2553, 2109, 1637, 1429, 1385, 1067, 848, 673; ESI-MS m/z: 315.0 $[M+H]^+$; HRMS (ESI-TOF) calcd for $C_{17}H_{16}ClN_2S$ $[M+H]^+$: 315.0717, found: 315.0719.

*4.1.3.5. 6-Bromo-2-(pyridin-3-ylmethyl)-1,2,3,4-tetrahydrobenzo[4,5]thieno[2,3-c]pyridine (**8d**)*

Following the general experimental procedure A, compound **8d** was obtained as an

off-white solid (524 mg, 72.9%) from compound **20d** (536 mg, 2.0 mmol) and 3-pyridinecarboxaldehyde (257 mg, 2.4 mmol). Mp: 113.2 – 114.9 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.61 (s, 1H), 8.57 – 8.56 (m, 1H), 7.83 (d, *J* = 8.0 Hz, 1H), 7.71 (d, *J* = 1.6 Hz, 1H), 7.62 (d, *J* = 8.0 Hz, 1H), 7.40 – 7.30 (m, 2H), 3.83 (s, 2H), 3.81 (s, 2H), 2.98 (t, *J* = 5.8 Hz, 2H), 2.86 (t, *J* = 5.6 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 150.3, 149.1, 140.7, 137.4, 137.3, 135.5, 133.1, 127.6, 127.2, 123.9, 123.8, 123.7, 118.4, 58.8, 52.0, 49.6, 23.5; IR (KBr, ν/cm^{-1}): 2903, 2821, 1577, 1424, 1330, 1133, 1062, 843, 799; ESI-MS m/z: 358.9/ 360.9 $[M+H]^+$; HRMS (ESI-TOF) calcd for $C_{17}H_{16}BrN_2S$ $[M+H]^+$: 359.0212, found: 359.0211.

4.1.3.6. 6-Methoxy-2-(pyridin-3-ylmethyl)-1,2,3,4-tetrahydrobenzo[4,5]thieno[2,3-c] pyridine ***(8e)***

Following the general experimental procedure A, compound **8e** was obtained as white-off solid (64.5 mg, 69.3%) from compound **6a** (65.8 mg, 0.30 mmol) and 3-pyridinecarboxaldehyde (38.5 mg, 0.36 mmol). Mp: 211.7 – 212.3 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.17 (s, 1H), 8.94 (d, *J* = 5.2 Hz, 1H), 8.77 (d, *J* = 7.6 Hz, 1H), 8.02 – 7.99 (m, 1H), 7.85 (d, *J* = 8.8 Hz, 1H), 7.26 (d, *J* = 2.0 Hz, 1H), 7.04 – 7.01 (m, 1H), 4.75 (s, 2H), 4.51 (s, 2H), 3.84 (s, 3 H), 3.53 (s, 2H), 3.18 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.5, 146.6, 146.0, 144.9, 138.6, 130.2, 128.8, 128.5, 126.9, 126.2, 123.5, 114.6, 104.2, 55.5, 54.5, 48.9, 48.6, 20.8; IR (KBr, ν/cm^{-1}): 3024, 2509, 2317, 2087, 1988, 1610, 1429, 1216, 1150, 1013, 826, 788; ESI-MS m/z: 311.0 $[M+H]^+$; HRMS (ESI-TOF) calcd for $C_{18}H_{19}N_2OS$ $[M+H]^+$: 311.1213, found: 311.1212.

4.1.3.7. 6-Methyl-2-(pyridin-3-ylmethyl)-1,2,3,4-tetrahydrobenzo[4,5]thieno[2,3-c] pyridine ***(8f)***

Following the general experimental procedure B, compound **8f** was obtained as a light yellow oil (50 mg, 56.8%) from **8d** (108 mg, 0.30 mmol) and methylboronic acid (27 mg, 0.45 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.61 (d, *J* = 1.2 Hz, 1H), 8.56 – 8.54 (m, 1H), 8.61 (d, *J* = 1.2 Hz, 1H), 7.77 (d, *J* = 7.6 Hz, 1H), 7.65 (d, *J* = 8.0 Hz, 1H), 7.37 – 7.28 (m, 2H), 7.12 (d, *J* = 8.0 Hz, 1H), 3.78 (s, 2H), 3.77 (s, 2H), 2.93 (t,

J = 5.6 Hz, 2 H), 2.84 (t, *J* = 5.6 Hz, 2 H), 2.46 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 150.4, 148.9, 139.4, 137.0, 135.8, 134.2, 134.0, 133.9, 127.9, 125.7, 123.7, 122.2, 120.9, 59.0, 52.4, 49.8, 23.9, 21.6; IR (KBr, ν/cm^{-1}): 2923, 2456, 1578, 1423, 1302, 1132, 1071, 847, 786; ESI-MS m/z: 295.0 $[M+H]^+$; HRMS (ESI-TOF) calcd for $C_{18}H_{19}N_2S$ $[M+H]^+$: 295.1263, found: 295.1264.

4.1.3.8. 2-(Pyridin-3-ylmethyl)-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1,2,3, 4-tetrahydrobenzo[4,5]thieno[2,3-c]pyridine ***(8g)***

A suspension of **8d** (359 mg, 1.0 mmol), bis(pinacolato)diboron (381 mg, 1.5 mmol) and KOAc (294 mg, 3.0 mmol) in DMF (4.0 mL) was added $Pd(dppf)_2Cl_2$ (37 mg, 0.05 mmol). The resulting reaction mixture was stirred at 100 °C for 3 h. After cooling to rt, the mixture was diluted ethyl acetate (50 mL) and water (20 mL). The organic layer was separated and the aqueous layer was extracted three times with ethyl acetate. The combined organic layers were dried over Na_2SO_4, filtered, and concentrated under a reduced pressure. The residue was then purified by silica gel column chromatography eluting with 0-5% MeOH/DCM to give **8g** (258 mg, 63.5%) as AN OFF-white solid. Mp: 128.7 – 130.2 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.60 (d, *J* = 1.6 Hz, 1H), 8.55 – 8.54 (m, 1H), 8.04 (s, 1H), 7.77 – 7.70 (m, 3H), 7.31 – 7.28 (m, 1H), 3.78 (s, 2H), 3.77 (s, 2H), 2.94 – 2.87 (m, 4H), 1.37 (s, 12H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 150.4, 148.9, 142.0, 138.6, 137.0, 133.8, 133.7, 129.9, 128.6, 127.6, 123.7, 122.0, 84.0, 75.2, 59.1, 52.3, 49.8, 25.0, 24.0; IR (KBr, ν/cm^{-1}): 2969, 2909, 1599, 1451, 1341, 1145, 1089, 821; ESI-MS m/z: 407.1 $[M+H]^+$; HRMS (ESI-TOF) calcd for $C_{23}H_{28}N_2O_2S$ $[M+H]^+$: 407.1959, found: 407.1957.

4.1.3.9. 2-(Pyridin-3-ylmethyl)-1,2,3,4-tetrahydrobenzo[4,5]thieno[2,3-c]pyridin-6-o l ***(8h)***

To a solution of **8g** (204 mg, 0.50 mmol) and NaOH (40 mg, 1.0 mmol) in THF/MeOH (1/1, 18 mL) was added 30% hydrogen peroxide (0.2 mL) at 0 °C. The reaction mixture was allowed to warm to rt and stirred for 2 h. After quenched with water (20 mL), the resulting mixture was extracted with ethyl acetate (20 mL x 2). The organic layer was washed with brine (20 mL), dried over anhydrous Na_2SO_4, and

then concentrated under a reduced pressure. The residue was then purified by silica gel column chromatography eluting with 0-5% MeOH/DCM to give **8h** (80 mg, 53.6%) as an off-white solid. Mp: 207.5 – 208.3 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.55 (d, *J* = 1.2 Hz, 1H), 8.49 – 8.48 (m, 1H), 7.78 (d, *J* = 8.0 Hz, 1H), 7.63 (d, *J* = 8.4 Hz, 1H), 7.38 (t, *J* = 5.6 Hz, 1H), 6.94 (d, *J* = 2.0 Hz, 1H), 6.81 – 6.79 (m, 1H), 3.75 (s, 2H), 3.66 (s, 2H), 2.80 (t, *J* = 5.6 Hz, 2H), 2.69 (t, *J* = 5.2 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.1, 150.1, 148.5, 140.1, 136.7, 134.9, 133.9, 128.2, 127.6, 123.7, 123.2, 114.1, 106.0, 57.9, 51.7, 49.0, 23.4; IR (KBr, ν/cm^{-1}): 3456, 2974, 2834, 1599, 1456, 1226, 1121, 1016, 887; ESI-MS m/z: 297.0 $[M+H]^+$; HRMS (ESI-TOF) calcd for $C_{17}H_{17}N_2OS$ $[M+H]^+$: 297.1056, found: 297.1055.

4.1.3.10. 2-(Pyridin-3-ylmethyl)-1,2,3,4-tetrahydrobenzo[4,5]thieno[2,3-c]pyridine-6-carbonitrile ***(8i)***

A mixture of compound **8d** (450 mg, 1.25 mmol), zinc cyanide (176 mg, 1.50 mmol), and tetrakis(triphenylphosphine)palladium (0) (145 mg, 0.125 mmol) in DMF (10 mL) was stirred at 100 °C for 6 h under a nitrogen atmosphere. After cooling down, it was poured onto aq. $NaHCO_3$ solution (30 mL). After extracted with ethyl acetate (20 mL x 2), the combined organic layers were washed with brine, dried over Na_2SO_4 and evaporated under vacuum. The residue was then purified by silica gel column chromatography eluting with 0-5% MeOH/DCM to give **8i** (274 mg, 71.8%) as an off-white solid. Mp: 131.7 – 132.9 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.61(s, 1H), 8.56 (d, *J* = 3.6 Hz, 1H), 7.88 – 7.78 (m, 3H), 7.51 (dd, *J* = 8.4 Hz, *J* = 1.6 Hz, 1H), 7.33 – 7.30 (m, 1H), 3.82 (s, 2H), 3.80 (s, 2H), 2.98 (t, *J* = 5.6 Hz, 2H), 2.90 (t, *J* = 5.2 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 154.9, 152.3, 150.4, 149.1, 143.1, 139.0, 137.0, 133.4, 128.3, 126.3, 125.1, 123.8, 123.5, 119.7, 107.9, 59.0, 52.1, 49.5, 23.7; IR (KBr, ν/cm^{-1}): 2956, 2225, 1694, 1570, 1411, 1126, 1012, 812, 779; ESI-MS m/z: 306.0$[M+H]^+$; HRMS (ESI-TOF) calcd for $C_{18}H_{16}N_3S$ $[M+H]^+$: 306.1059, found: 306.1059.

4.1.3.11. N-Methyl-2-(pyridin-3-ylmethyl)-1,2,3,4-tetrahydrobenzo[4,5]thieno[2,3-c]pyridine-6-carboxamide ***(8j)***

To a solution of **8i** (210 mg, 0.68 mmol) in ethanol (5 mL) and water (5 mL) was added sodium hydroxide potassium (220 mg, 5.51 mmol). The resulting mixture was stirred under nitrogen at reflux for 12 hours and then evaporated in vacuo. The residue was dissolved in ethyl acetate (30 mL) and washed with 2M aqueous hydrogen chloride solution (10 mL). The organic extracts were dried over anhydrous Na_2SO_4 and evaporated in vacuo to afford crude **8j-1** which was used directly for the next step. Compound **8j-1** was dissolved in DMF (6 mL), then methylamine hydrochloride (210 mg, 1.36 mmol), HATU (775.6 mg, 2.04 mmol) and DIPEA (0.71 mL, 4.08 mmol) were added at 0 °C. The reaction mixture was allowed to warm to rt and stirred for 12 h. After quenched with water (20 mL), the resulting mixture was extracted with ethyl acetate (15 mL x 2). The organic layer was washed with brine, dried over anhydrous Na_2SO_4, and concentrated under a reduced pressure. The residue was then purified by silica gel column chromatography eluting with 0-10% MeOH/DCM to give **8j** (84 mg, 36.5%, two steps) as an off-white solid. Mp: 175.7 – 177.2 ^oC; ^1H NMR (400 MHz, DMSO-d_6) δ 8.58 (s, 1H), 8.52 (d, *J* = 3.6 Hz, 1H), 8.02 (s, 1H), 7.75 – 7.61 (m, 3H), 7.29 – 7.26 (m, 1H), 6.65 (d, *J* = 3.6 Hz, 1H), 3.75 (s, 2H), 3.73 (s, 2H), 3.01 (d, *J* = 4.8 Hz, 3H), 2.88 (t, *J* = 5.6 Hz, 2H), 2.81 (t, *J* = 5.2 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 168.7, 150.3, 149.0, 141.6, 139.0, 136.9, 135.6, 133.7, 130.9, 128.7, 123.7, 122.5, 122.1, 119.8, 59.1, 52.2, 49.7, 27.0, 23.8; IR (KBr, ν/cm^{-1}): 3325, 2909, 2805, 1649, 1539, 1440, 1308, 1134, 1117, 1023, 812; ESI-MS m/z: 338.0 $[M+H]^+$; HRMS (ESI-TOF) calcd for $C_{19}H_{20}N_3OS$ $[M+H]^+$: 338.1322, found: 338.1323.

*4.1.3.12. Tert-butyl(2-(pyridin-3-ylmethyl)-1,2,3,4-tetrahydrobenzo[4,5]thieno[2,3-c] pyridin-6-yl)carbamate (**8k**)*

To a round bottom flask under dry nitrogen were added $Pd_2(dba)_3$ (91.5 mg, 0.1 mmol), Xantphos (174 mg, 0.30 mmol), **8d** (359 mg, 1.0 mmol), t-butyl carbamate (176 mg, 1.5 mmol), cesium carbonate (652 mg, 2.0 mmol) and 1,4-dioxane (15 mL). After degass three times, the mixture was stirred at 100 °C under dry nitrogen for 6 h. The reaction mixture was cooled to rt, diluted with water (20 mL) and extracted with ethyl acetate (15 mL x 3). The combined extracts were washed with brine, dried over

sodium sulfate, filtered and concentrated under reduced pressure. The crude mixture was purified by silica gel chromatography eluting with 0-10% MeOH/DCM to afford **8k** (247 mg, 62.5%) as an off-white solid. Mp: 181.7 – 182.5 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.61 (s, 1H), 8.55 (d, *J* = 4.4 Hz, 1H), 7.80 – 7.58 (m, 2H), 7.32 – 7.28 (m, 1H), 6.60 (s, 1H), 3.79 (s, 2H), 3.77 (s, 2H), 2.95 (t, *J* = 5.6 Hz, 2H), 2.87 (t, *J* = 5.2 Hz, 2H), 1.53 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 153.1, 150.5, 149.1, 139.8, 137.0, 135.5, 135.0, 133.5, 133.3, 128.2, 123.7, 122.8, 116.3, 110.4, 80.7, 58.9, 52.3, 49.8, 28.5, 23.8; IR (KBr, ν/cm^{-1}): 3227, 3080, 2821, 1719, 1572, 1456, 1358, 1248, 1156, 1029, 792; ESI-MS m/z: 396.1 $[M+H]^+$; HRMS (ESI-TOF) calcd for $C_{22}H_{26}N_3O_2S$ $[M+H]^+$: 396.1740, found: 396.1740.

*4.1.3.13. N-(2-(Pyridin-3-ylmethyl)-1,2,3,4-tetrahydrobenzo[4,5]thieno[2,3-c]pyridin -6-yl)acetamide **(8l)***

To the solution of **8k** (138.5 mg, 0.35 mmol) in DCM (5 mL) was added trifluoracetic acid (1 mL) at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred for 2 h. After the solvent was removed under reduced pressure, the residue was dissolved in DCM (5 mL). To this solution, DIPEA (0.24 mL, 1.38 mmol) and acetic anhydride (0.13 mL, 1.38 mmol) were added at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred for 12 h, then sat. $NaHCO_3$ was added (20 mL). After extracted with CH_2Cl_2 (15 mL x 3), the combined organics were dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. The residue was purified by silica gel column chromatography eluting with 5% MeOH/DCM to give **8l** as an off-white solid (81 mg, 68.7%). Mp: 168.6 – 169.9 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.61 (s, 1H), 8.55 (d, *J* = 3.6 Hz, 1H), 7.90 (s, 1H), 7.76 (d, *J* = 7.6 Hz, 1H), 7.66 (d, *J* = 8.4 Hz, 1H), 7.48 (s, 1H), 7.31 – 7.28 (m, 2H), 3.77 (s, 2H), 3.75 (s, 2H), 2.91 (t, *J* = 5.6 Hz, 2H), 2.82 (t, *J* = 5.2 Hz, 2H), 2.20 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.5, 150.5, 149.0, 139.7, 136.9, 135.4, 134.9, 134.5, 133.8, 128.3, 123.7, 122.8, 117.2, 112.2, 59.1, 52.4, 49.9, 24.8, 23.9; IR (KBr, ν/cm^{-1}): 2953, 2761, 1676, 1533, 1456, 1330, 1145, 1034, 812; ESI-MS m/z: 338.0 $[M+H]^+$; HRMS (ESI-TOF) calcd for $C_{19}H_{20}N_3OS$ $[M+H]^+$: 338.1322, found:

338.1323.

*4.1.3.14. (6-Fluoro-3,4-dihydrobenzo[4,5]thieno[2,3-c]pyridin-2(1H)-yl)(pyridin-3-yl)methanone (**9a**)*

To the solution of **20b** (62 mg, 0.30 mmol) and nicotinic acid (45 mg, 0.36 mmol) in DMF (5 mL) were added HATU (229 mg, 0.60 mmol) and DIPEA (0.16 mL, 0.90 mmol) at 0 °C. The reaction mixture was allowed to warm to rt and stirred for 12 h. After quenched with water (20 mL) and extracted with ethyl acetate (15 mL x 3), the organic layers were washed with brine (15 mL), dried over anhydrous Na_2SO_4, and concentrated under a reduced pressure. The residue was then purified by silica gel column chromatography eluting with 0-10% MeOH/DCM to give **9a** (53 mg, 56.7%) as an off-white solid. Mp: 159.7 – 161.1 ^oC; ^1H NMR (400 MHz, $CDCl_3$) δ 8.76 – 8.72 (m, 2H), 7.83 (d, *J* = 6.8 Hz, 1H), 7.72 (s, 1H), 7.46 – 7.20 (m, 2H), 7.11 – 7.07 (m, 1H), 5.04 – 3.80 (m, 4H), 2.95 – 2.83 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.4, 161.2 (d, *J* = 241.0 Hz), 151.4, 147.9, 139.7, 138.4, 135.1, 134.0, 131.7, 123.9, 123.8, 113.2 (d, *J* = 23.4 Hz), 106.8 (d, *J* = 23.7 Hz), 45.0, 42.8, 24.9; ^{19}F NMR (375 MHz, $CDCl_3$) δ −117.83 to −118.06 (m, 1F); IR (KBr, ν/cm^{-1}): 2936, 1637, 1572, 1429, 1256, 1117, 835; ESI-MS m/z: 313.0 $[M+H]^+$; HRMS (ESI-TOF) calcd for $C_{17}H_{14}FN_2OS$ $[M+H]^+$: 313.0805, found: 313.0805.

*4.1.3.15. 2-[(4-Bromopyridin-3-yl)methyl]-6-fluoro-1,2,3,4-tetrahydrobenzo[4,5]thieno[2,3-c]-pyridine (**9b**)*

Following the general experimental procedure A, compound **9b** was obtained as an off-white solid (517 mg, 68.5%) from **20b** (415 mg, 2.0 mmol) and 4-bromonicotinaldehyde (447 mg, 2.4 mmol). Mp: 121.8 – 123.1 ^oC; ^1H NMR (400 MHz, $CDCl_3$) δ 8.66 (s, 1H), 8.33 (d, *J* = 5.2 Hz, 1H), 7.70 – 7.67 (m, 1H), 7.53 (d, *J* = 5.2 Hz, 1H), 7.24 (d, *J* = 7.6 Hz, 1H), 7.07 – 7.02 (m, 1H), 3.88 (s, 2H), 3.86 (s, 2H), 3.00(t, *J* = 5.8 Hz, 2H), 2.84 (t, *J* = 5.8 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.9 (d, *J* = 240.1 Hz) , 151.7, 149.3, 140.3 (d, *J* = 9.0 Hz), 136.8, 135.4, 133.9, 133.7, 128.1 (d, *J* = 3.2 Hz), 124.7, 123.6 (d, *J* = 9.3 Hz), 112.6 (d, *J* = 24.9 Hz), 106.7 (d, *J* = 22.9 Hz), 58.3, 52.2, 49.8, 23.8; IR (KBr, ν/cm^{-1}): 2887, 2756, 1556, 1440, 1407,

1183, 1117, 1067, 1045, 859, 772; ESI-MS m/z: 376.9/378.9 $[M+H]^+$; HRMS (ESI-TOF) calcd for $C_{17}H_{15}BrFN_2S$ $[M+H]^+$: 377.0118, found: 377.0118.

*4.1.3.16. 6-Fluoro-2-((4-methylpyridin-3-yl)methyl)-1,2,3,4-tetrahydrobenzo[4,5]thieno[2,3-c]pyridine (**9c**)*

Following the general experimental procedure B, compound **9c** was obtained as a white solid (41 mg, 43.5%) from **9b** (113 mg, 0.30 mmol) and methylboronic acid (27 mg, 0.45 mmol). Mp: 113.6 – 115.1 ^oC; ^1H NMR (400 MHz, $CDCl_3$) δ 8.67 – 8.34 (m, 2H), 7.68 (dd, *J* = 8.8 Hz, *J* = 4.8 Hz, 1H), 7.25 – 7.22 (m, 2H), 7.07 – 7.02 (m, 1H), 3.80 (s, 2H), 3.78 (s, 2H), 2.97 (t, *J* = 5.6 Hz, 2H), 2.82 (t, *J* = 5.6 Hz, 2H), 2.48 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.1 (d, *J* = 240.1 Hz), 150.4, 148.1, 146.6, 140.2, 140.1, 136.4, 133.9, 128.1 (d, *J* = 4.1 Hz), 123.7, 123.6, 112.7 (d, *J* = 24.9 Hz), 106.7 (d, *J* = 22.8 Hz), 56.8, 52.2, 49.8, 23.7, 19.3; ^{19}F NMR (375 MHz, $CDCl_3$) δ −118.61 to −118.67 (m, 1F); IR (KBr, ν/cm^{-1}): 2909, 2827, 1604, 1451, 1199, 1117, 881, 854; ESI-MS m/z: 313.0 $[M+H]^+$; HRMS (ESI-TOF) calcd for $C_{18}H_{18}FN_2S$ $[M+H]^+$: 313.1169, found: 313.1169.

*4.1.3.17.6-Fluoro-2-((4-phenylpyridin-3-yl)methyl)-1,2,3,4-tetrahydrobenzo[4,5]thieno[2,3-c]pyridine (**9d**)*

Following the general experimental procedure B, compound **9d** was obtained as a white solid (63 mg, 55.8%) from **9b** (113.2 mg, 0.30 mmol) and phenylboronic acid (54.8 mg, 0.45 mmol). Mp: 141.3 – 142.8 ^oC; ^1H NMR (400 MHz, $CDCl_3$) δ 8.85 (s, 1H), 8.61 (d, *J* = 4.4 Hz, 1H), 7.67 (dd, *J* = 8.8 Hz, *J* = 4.8 Hz, 1H), 7.46 – 7.40 (m, 5H), 7.30 (d, *J* = 5.2 Hz, 1H), 7.21 (dd, *J* = 9.2 Hz, *J* = 2.4 Hz, 1H), 7.06 – 7.01 (m, 1H), 3.76 (s, 2H), 3.74 (s, 2H), 2.88 (t, *J* = 5.6 Hz, 2H), 2.77 (t, *J* = 5.4 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.9 (d, *J* = 240.2 Hz), 151.6, 150.8, 147.8, 140.2, 140.1, 138.2, 136.3, 133.9, 129.1, 128.7, 128.6, 128.1, 128.0, 125.1, 123.7, 123.6, 112.7 (d, *J* = 24.9 Hz), 106.7 (d, *J* = 22.8 Hz), 56.2, 51.8, 49.4, 23.8; ^{19}F NMR (375 MHz, $CDCl_3$) δ −118.67 to −118.73 (m, 1F); IR (KBr, ν/cm^{-1}): 3062, 2914, 2810, 2767, 1599, 1445, 1117, 892, 887, 832; ESI-MS m/z: 375.0 $[M+H]^+$; HRMS (ESI-TOF) calcd for $C_{23}H_{20}FN_2S$ $[M+H]^+$: 375.1326, found: 375.1327.

*4.1.3.18. 2-((4-cyclopropylpyridin-3-yl)methyl)-6-fluoro-1,2,3,4-tetrahydrobenzo[4,5] thieno[2,3-c]pyridine (**9e**)*

Following the general experimental procedure B, compound **9e** was obtained as a white solid (65 mg, 63.5%) from **9b** (113 mg, 0.30 mmol) and cyclopropylboronic acid (39 mg, 0.45 mmol). Mp: 138.1 – 140.3 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.60 – 8.34 (m, 2H), 7.68 (dd, *J* = 8.8 Hz, *J* = 4.8 Hz, 1H), 7.24 (dd, *J* = 9.2 Hz, *J* = 2.4 Hz, 1H), 7.07 – 7.02 (m, 1H), 6.90 – 6.86 (m, 1H), 3.95 (s, 2H), 3.83 (s, 2H), 2.99 (t, *J* = 5.6 Hz, 2H), 2.83 (t, *J* = 5.6 Hz, 2H), 2.39 – 2.32 (m, 1H), 1.19 – 1.14 (m, 1H), 0.86 – 0.82 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.1 (d, *J* = 240.1 Hz) , 156.8, 147.3, 146.4, 140.2 (d, *J* = 8.9 Hz), 136.4, 133.9, 133.5, 128.1 (d, *J* = 4.2 Hz), 123.7 (d, *J* = 9.3 Hz), 119.4, 112.7 (d, *J* = 24.9 Hz), 106.7 (d, *J* = 22.9 Hz), 56.5, 52.3, 49.7, 23.7, 12.2, 10.5; ^{19}F NMR (375 MHz, $CDCl_3$) δ −118.63 to −118.69 (m, 1F); IR (KBr, ν/cm^{-1}): 2925, 2843, 2777, 1599, 1435, 1363, 1117, 793; ESI-MS m/z: 339.1 $[M+H]^+$; HRMS (ESI-TOF) calcd for $C_{20}H_{20}FN_2S$ $[M+H]^+$: 339.1326, found: 339.1327.

*4.1.3.19. 6-Fluoro-2-((4-(prop-1-en-2-yl)pyridin-3-yl)methyl)-1,2,3,4-tetrahydrobenz o[4,5]thieno[2,3-c]pyridine (**9f**)*

Following the general experimental procedure B, compound **9f** was obtained as a pale yellow oil (91 mg, 53.5%) from **9b** (189 mg, 0.50 mmol) and isopropenylboronic acid (65 mg, 0.75 mmol). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.67 (s, 1H), 8.46 (d, *J* = 5.2 Hz, 1H), 7.67 (dd, *J* = 8.4 Hz, *J* = 4.8 Hz, 1H), 7.22 (dd, *J* = 8.8 Hz, *J* = 2.4 Hz, 1H), 7.07 (d, *J* = 5.2 Hz, 1H), 7.05 – 7.00 (m, 1H), 5.25 (t, *J* = 1.6 Hz, 1H), 4.98 (d, *J* = 0.4 Hz, 1H), 3.77 (s, 2H), 3.74 (s, 2H), 2.89 (t, *J* = 5.8 Hz, 2H), 2.77 (t, *J* = 5.6 Hz, 2H), 2.05 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.1 (d, *J* = 239.9 Hz), 152.4, 151.3, 148.6, 142.9, 140.3 (d, *J* = 8.8 Hz), 137.0, 133.9, 130.4, 128.2 (d, *J* = 4.1 Hz), 123.6 (d, *J* = 9.3 Hz), 122.9, 116.3, 112.5 (d, *J* = 24.9 Hz), 106.6 (d, *J* = 22.8 Hz), 56.6, 52.3, 49.6, 24.4, 24.0; ^{19}F NMR (375 MHz, $CDCl_3$) δ −118.84 to −118.90 (m, 1F); IR (KBr, ν/cm^{-1}): 2913, 2763, 1576, 1421, 1263, 1121, 992, 915; 805; ESI-MS m/z: 339.1 $[M+H]^+$; HRMS (ESI-TOF) calcd for $C_{20}H_{20}FN_2S$ $[M+H]^+$: 339.1326, found: 339.1325.

*4.1.3.20. 6-Fluoro-2-((4-isopropylpyridin-3-yl)methyl)-1,2,3,4-tetrahydrobenzo[4,5]thieno[2,3-c]pyridine (**9g**)*

A mixture of **9f** (60 mg, 0.18 mmol) and 20% $Pd(OH)_2$ (30 mg) in MeOH (3 mL) and EtOAc (3 mL) was stirred under H_2 balloon at r.t. After 6 h, the mixture was filtered through Celite. The filtrate was concentrated in vacuo. The residue was then purified by silica gel column chromatography eluting with 0-10% MeOH/DCM to give **9g** (42 mg, 68.5%) as a pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.55 – 8.45 (m, 2H), 7.68 (dd, *J* = 8.8 Hz, *J* = 4.8 Hz, 1H), 7.27 (d, *J* = 5.3 Hz, 1H), 7.22 (dd, *J* = 9.6 Hz, *J* = 2.4 Hz, 1H), 7.05 – 7.00 (m, 1H), 3.78 (s, 2H), 3.75 (s, 2H), 3.44 – 3.37 (m, 1H), 2.90 (t, *J* = 5.6 Hz, 2H), 2.78 (t, *J* = 5.6 Hz, 2H), 1.22 (d, *J* = 6.8 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.1 (d, *J* = 240.0 Hz), 159.0 (d, *J* = 6.2 Hz), 150.2 (d, *J* = 5.5 Hz), 148.6 (d, *J* = 5.4 Hz), 140.3 (d, *J* = 8.9 Hz), 136.8, 133.9 (d, *J* = 1.5 Hz), 131.0, 128.2 (d, *J* = 4.2 Hz), 123.6 (d, *J* = 9.3 Hz), 121.2, 112.5 (d, *J* = 24.9 Hz), 106.6 (d, *J* = 22.9 Hz), 56.9, 52.4, 49.5, 28.7, 23.9, 23.4; ^{19}F NMR (375 MHz, $CDCl_3$) δ −118.75 to −118.81 (m, 1F); IR (KBr, ν/cm^{-1}): 2925, 2802, 1573, 1435, 1213, 1117, 1035, 805; ESI-MS m/z: 341.1 $[M+H]^+$; HRMS (ESI-TOF) calcd for $C_{20}H_{22}FN_2S$ $[M+H]^+$: 341.1482, found: 341.1481.

*4.1.3.21. 6-Fluoro-2-((5-fluoropyridin-3-yl)methyl)-1,2,3,4-tetrahydrobenzo[4,5]thieno[2,3-c]pyridine (**9h**)*

Following the general experimental procedure A, compound **9h** was obtained as a white solid (56 mg, 58.5%) from **20b** (63 mg, 0.30 mmol) and 5-fluoronicotinaldehyde (46 mg, 0.36 mmol). Mp: 60.8 – 62.6 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.42 – 8.41 (m, 2H), 7.68 (dd, *J* = 8.8 Hz, *J* = 4.8 Hz, 1H), 7.54 – 7.51 (m, 1H), 7.24 (dd, *J* = 9.2 Hz, *J* = 2.4 Hz, 1H), 7.07 – 7.02 (m, 1H), 3.80 (s, 2H), 3.78 (s, 2H), 2.94 (t, *J* = 5.8 Hz, 2H), 2.83 (t, *J* = 5.6 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.1 (d, *J* = 240.1 Hz), 159.9 (d, *J* = 255.4 Hz), 146.0 (d, *J* = 3.7 Hz), 140.2 (d, *J* = 8.9 Hz), 137.5 (d, *J* = 23.2 Hz), 136.6, 136.0 (d, *J* = 3.2 Hz), 133.9 (d, *J* = 1.5 Hz), 128.1 (d, *J* = 4.2 Hz), 123.2 (d, *J* = 17.9 Hz), 112.7 (d, *J* = 24.9 Hz), 106.7 (d, *J* = 22.9 Hz), 58.2, 52.4, 49.8, 23.8; ^{19}F NMR (375 MHz, $CDCl_3$) δ −118.65 to −118.71 (m,

1F), −127.00, - 127.02 (d, 1F); IR (KBr, ν/cm^{-1}): 2914, 2816, 2767, 1600, 1430, 1121, 1023, 887, 793; ESI-MS m/z: 317.0 $[M+H]^+$; HRMS (ESI-TOF) calcd for $C_{17}H_{15}F_2N_2S$ $[M+H]^+$: 317.0919, found: 317.0917.

*4.1.3.22. 6-Fluoro-2-[(5-methoxypyridin-3-yl)methyl]-1,2,3,4-tetrahydrobenzo[4,5]thieno[2,3-c]-pyridine (**9i**)*

Following the general experimental procedure A, compound **9i** was obtained as a white solid (67 mg, 68.3%) from **20b** (63 mg, 0.30 mmol) and 5-methoxynicotinaldehyde (50 mg, 0.36 mmol). Mp: 81.3 – 83.1 ^oC; ^1H NMR (400 MHz, $CDCl_3$) δ 8.25 (d, *J* = 2.8 Hz, 1H), 8.20 (d, *J* = 1.2 Hz, 1H), 7.67 (dd, *J* = 8.8 Hz, *J* = 4.8 Hz, 1H), 7.30 (t, *J* = 2.4 Hz, 1H), 7.24 (dd, *J* = 9.6 Hz, *J* = 2.4 Hz, 1H), 7.06 – 7.01 (m, 1H), 3.86 (s, 3H), 3.77 (s, 2H), 3.76 (s, 2H), 2.93 (t, *J* = 5.6 Hz, 2H), 2.82 (t, *J* = 5.6 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.1 (d, *J* = 240.0 Hz), 156.0, 142.6, 140.3 (d, *J* = 8.9 Hz), 137.2, 136.9, 134.5, 133.9, 128.1 (d, *J* = 4.2 Hz), 123.6 (d, *J* = 9.3 Hz), 120.8, 112.6 (d, *J* = 24.9 Hz), 106.6 (d, *J* = 22.8 Hz), 55.8, 55.7, 52.4, 49.7, 23.9; ^{19}F NMR (375 MHz, $CDCl_3$) δ −118.75 to −118.81 (m, 1F); IR (KBr, ν/cm^{-1}): 1914, 2832, 1593, 1588, 1445, 1287, 1171, 1111, 1051, 836, 795; ESI-MS m/z: 329.0 $[M+H]^+$; HRMS (ESI-TOF) calcd for $C_{18}H_{18}FN_2OS$ $[M+H]^+$: 329.1118, found: 329.1118.

*4.1.3.23. 6-Fluoro-2-[(5-methylpyridin-3-yl)methyl]-1,2,3,4-tetrahydrobenzo[4,5]thieno[2,3-c]pyridine (**9j**)*

Following the general experimental procedure A, compound **9j** was obtained as a white solid (64 mg, 67.8%) from **20b** (63 mg, 0.30 mmol) and 5-methylnicotinaldehyde (44 mg, 0.36 mmol). Mp: 120.8 – 122.3 ^oC; ^1H NMR (400 MHz, $CDCl_3$) δ 8.40 – 8.38 (m, 2H), 7.66 (dd, *J* = 8.4 Hz, *J* = 4.8 Hz, 1H), 7.60 (s, 1H), 7.22 (dd, *J* = 9.6 Hz, *J* = 2.4 Hz, 1H), 7.05 – 7.00 (m, 1H), 3.76 (s, 2H), 3.75 (s, 2H), 2.94 (t, *J* = 5.8 Hz, 2H), 2.82 (t, *J* = 5.6 Hz, 2H); 2.34 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.0 (d, *J* = 240.0 Hz), 149.6, 147.5, 140.2 (d, *J* = 8.9 Hz), 137.6, 136.6, 133.9 (d, *J* = 1.5 Hz), 133.3, 132.9, 128.0 (d, *J* = 4.2 Hz), 123.6 (d, *J* = 9.3 Hz), 112.6 (d, *J* = 24.8 Hz), 106.6 (d, *J* = 22.8 Hz), 58.9, 52.3, 49.7, 23.7, 18.5; ^{19}F NMR

(375 MHz, $CDCl_3$) δ −118.68 to −118.74 (m, 1F); IR (KBr, ν/cm^{-1}): 3073, 2799, 1604, 1456, 1352, 1133, 892, 804; ESI-MS m/z: 313.0 $[M+H]^+$; HRMS (ESI-TOF) calcd for $C_{18}H_{18}FN_2S$ $[M+H]^+$: 313.1169, found: 313.1168.

*4.1.3.24. 6-Fluoro-2-(pyrimidin-5-ylmethyl)-1,2,3,4-tetrahydrobenzo[4,5]thieno[2,3-c]pyridine (**9k**)*

Following the general experimental procedure A, compound **9k** was obtained as a white solid (64 mg, 71.5%) from **20b** (63 mg, 0.30 mmol) and pyrimidine-5-carbaldehyde (39 mg, 0.36 mmol). Mp: 109.8 – 111.2 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 9.17 (s, 1H), 8.78 (s, 1H), 7.69 (dd, *J* = 8.8 Hz, *J* = 4.8 Hz, 1H), 7.24 (dd, *J* = 9.2 Hz, *J* = 2.4 Hz, 1H), 7.07 – 7.02 (m, 1H), 3.80 (s, 2H), 3.79 (s, 2H), 2.95 (t, *J* = 5.8 Hz, 2H), 2.83 (t, *J* = 5.6 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.1 (d, *J* = 240.3 Hz), 158.3, 157.5, 140.2 (d, *J* = 9.0 Hz), 136.3, 133.9, 131.7, 128.0 (d, *J* = 4.2 Hz), 123.7 (d, *J* = 9.3 Hz), 112.7 (d, *J* = 24.9 Hz), 106.7 (d, *J* = 22.9 Hz), 56.5, 52.4, 49.0, 23.8; ^{19}F NMR (375 MHz, $CDCl_3$) δ −118.58 to −118.64 (m, 1F); IR (KBr, ν/cm^{-1}): 3013, 2821, 1566, 1435, 1402, 1177, 1112, 867, 799; ESI-MS m/z: 300.0$[M+H]^+$; HRMS (ESI-TOF) calcd for $C_{16}H_{15}FN_3S$ $[M+H]^+$: 300.0965, found: 300.0965.

*4.1.3.25. 6-Fluoro-2-(pyridin-4-ylmethyl)-1,2,3,4-tetrahydrobenzo[4,5]thieno[2,3-c] pyridine (**9l**)*

Following the general experimental procedure A, compound **9l** was obtained as a pale yellow oil (65 mg, 71.8%) from **20b** (63 mg, 0.30 mmol) and isonicotinaldehyde (39 mg, 0.36 mmol). ^1H NMR (400 MHz, $CDCl_3$) δ 8.60 (d, *J* = 5.6 Hz, 2H), 7.71 (dd, *J* = 8.4 Hz, *J* = 4.8 Hz, 1H), 7.37 (d, *J* = 6.0 Hz, 2H), 7.27 (dd, *J* = 9.6 Hz, *J* = 2.4 Hz, 1H), 7.08 – 7.03 (m, 1H), 3.79 (s, 4H), 2.95 (t, *J* = 5.8 Hz, 2H), 2.85 (t, *J* = 5.6 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.1 (d, *J* = 240.1 Hz), 150.0, 147.7, 140.3 (d, *J* = 8.9 Hz), 136.7, 133.9 (d, *J* = 1.5 Hz), 128.1 (d, *J* = 4.2 Hz), 123.9, 123.6 (d, *J* = 9.2 Hz), 112.6 (d, *J* = 24.9 Hz), 106.6 (d, *J* = 22.7 Hz), 60.6, 52.5, 49.9, 23.8; ^{19}F NMR (375 MHz, $CDCl_3$) δ −118.66 to −118.73 (m, 1F); IR (KBr, ν/cm^{-1}): 2923, 2782, 1568, 1421, 1215, 1034, 893, 785; ESI-MS m/z: 299.0 $[M+H]^+$; HRMS (ESI-TOF) calcd for

$C_{17}H_{16}FN_2S$ $[M+H]^+$: 299.1013, found: 299.1012.

*4.1.3.26. 2-[(1H-Imidazol-4-yl)methyl]-6-fluoro-1,2,3,4-tetrahydrobenzo[4,5]thieno [2,3-c]-pyridine (**9m**)*

Following the general experimental procedure A, compound **9m** was obtained as a white solid (38 mg, 43.8%) from **20b** (63 mg, 0.30 mmol) and 1H-imidazole-4-carbaldehyde (35 mg, 0.36 mmol). Mp: 173.6 – 175.7 ^oC; ^1H NMR (400 MHz, $CDCl_3$) δ 7.66 (dd, *J* = 8.8 Hz, *J* = 4.8 Hz, 1H), 7.62 (s, 1H), 7.22 (dd, *J* = 9.6 Hz, *J* = 2.4 Hz, 1H), 7.05 – 7.00 (m, 2H), 3.80 (s, 2H), 3.79 (s, 2H), 2.96 (t, *J* = 5.6 Hz, 2H), 2.81 (t, *J* = 5.6 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.1 (d, *J* = 240.0 Hz), 140.3, 140.2, 136.9, 135.3, 133.9, 128.1 (d, *J* = 4.1 Hz), 123.7, 123.6, 112.6 (d, *J* = 24.8 Hz), 106.6 (d, *J* = 22.7 Hz), 53.2, 52.1, 49.7, 23.9; ^{19}F NMR (375 MHz, $CDCl_3$) δ −118.77 to −118.83 (m, 1F); IR (KBr, ν/cm^{-1}): 2805, 2755, 1610, 1577, 1445, 1182, 980, 886, 753; ESI-MS m/z: 288.0 $[M+H]^+$; HRMS (ESI-TOF) calcd for $C_{15}H_{15}FN_3S$ $[M+H]^+$: 288.0965, found: 288.0967.

*4.1.3.27. 6-Fluoro-2-(isoquinolin-4-ylmethyl)-1,2,3,4-tetrahydrobenzo[4,5]thieno[2,3 -c]-pyridine (**9n**)*

Following the general experimental procedure A, compound **27** was obtained as a white solid (67 mg, 63.6%) from **20b** (63 mg, 0.30 mmol) and isoquinoline-4-carbaldehyde (57 mg, 0.36 mmol). Mp: 160.8 – 162.1 ^oC; ^1H NMR (400 MHz, $CDCl_3$) δ 9.25 (s, 1H), 8.51 (s, 1H), 8.35 (d, *J* = 8.4 Hz, 1H), 8.02 (d, *J* = 8.0 Hz, 1H), 7.77 – 7.62 (m, 1H), 7.22 (dd, *J* = 9.2 Hz, *J* = 2.4 Hz, 1H), 7.05 – 7.00 (m, 1H), 4.16 (s, 2H), 3.84 (s, 2H), 3.03 (t, *J* = 5.8 Hz, 2H), 2.82 (t, *J* = 5.8 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 160.1 (d, *J* = 240.1 Hz), 152.8, 143.1, 140.3 (d, *J* = 8.5 Hz), 136.7, 135.6, 133.9, 131.0, 128.2, 128.1, 127.6, 124.2, 123.7, 123.6, 112.6 (d, *J* = 24.9 Hz), 106.6 (d, *J* = 22.8 Hz), 57.2, 52.4, 49.8, 23.8; ^{19}F NMR (375 MHz, $CDCl_3$) δ −118.71 to −118.77 (m, 1F); IR (KBr, ν/cm^{-1}): 2909, 2859, 1583, 1435, 1358, 1091, 854, 788; ESI-MS m/z: 349.0 $[M+H]^+$; HRMS (ESI-TOF) calcd for $C_{21}H_{18}FN_2S$ $[M+H]^+$: 349.1169, found: 349.1169.

*4.1.4.1. Tert-butyl [2-(benzo[b]thiophen-3-yl)ethyl]carbamate (**11**)*

To a stirred solution of 2-(benzo[b]thiophen-3-yl)acetonitrile (1.50 g, 8.66 mmol), Nickel (II) chloride hexahydrate (2.06 g, 8.66 mmol), and di-tertbutyl dicarbonate (3.78 g, 17.32 mmol) in methanol (80 mL) under nitrogen was added sodium borohydride (1.63 g , 43.3 mmol) in portions over a period of 10 min. The reaction temperature was kept below 45 °C. The reaction mixture was stirred for 16 h and diluted with ethyl acetate (200 mL), and then water (300 mL) was carefully added. The mixture was filtered through kieselghur and ethyl acetate was added (200 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated in vacuo to afford the crude product, which was purified by flash chromatography eluting with 0-25% ethyl acetate/hexanes to give **11** (1.15 g, 47.9%) as a white solid. M.p.: 71 - 72 ^oC; ^1H NMR (400 MHz, DMSO-d_6) δ 7.96 (d, *J* = 8.8 Hz, 1H), 7.84 (d, *J* = 7.6 Hz, 1H), 7.44 – 7.35 (m, 3H), 6.99 (t, *J* = 5.6 Hz, 1H), 3.26 (q, *J* = 6.8 Hz, 2H), 2.94 (t, *J* = 7.2 Hz, 1H), 1.38 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 155.6, 139.6, 138.8, 133.8, 124.2, 124.0, 122.9, 122.6, 121.7, 77.6, 28.5, 28.3; ESI-MS m/z: 278.1 $[M+H]^+$.

*4.1.4.2. Tert-butyl 3,4-dihydrobenzo[4,5]thieno[2,3-c]pyridine-2(1H)-carboxylate (**12**)*

A solution of compound **11** (1.08 g, 3.89 mmol), powdered paraformaldehyde (234 mg, 7.78 mmol) and *p*-toluenesulfonic acid monohydrate (37.1 mg, 0.20 mmol) in toluene (80 mL) was refluxed under dehydrating conditions for 2 hours. After the reaction mixture was cooled down to rt, the solvent was evaporated under reduced pressure. The residue was diluted with water (60 mL) and then extracted with ethyl acetate (80 mL × 2). The combined organic layers were washed with aqueous $NaHCO_3$ (50 mL) and brine (50 mL). The organic layer was dried over anhydrous Na_2SO_4, and filtrated, and concentrated. The residue was purified by silica gel column chromatography eluting with 0-30% ethyl acetate/petroleum (45-60 ^oC) to give **12** (0.76 g, 67.5%) as a white solid. Mp: 88-89 °C; ^1H NMR (400 MHz, DMSO-d_6) δ 7.93 (d, *J* = 7.6 Hz, 1H), 7.67 (d, *J* = 8.0 Hz, 1H), 7.41 – 7.32 (m, 2H), 4.66 (s, 2H), 3.71 (t, *J* = 5.6 Hz, 2H), 2.80 (t, *J* = 5.6 Hz, 2H), 1.44 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 153.9, 138.4, 137.8, 133.1, 128.4, 124.3, 124.1, 122.6, 120.9, 79.4, 42.9,

41.1, 28.0, 23.4; ESI-MS m/z: 290.1 $[M+H]^+$.

*4.1.4.3. Ethyl 4-[(4-fluorophenyl)thio]-3-oxobutanoate (**14b**)*

To a solution of 4-fluorobenzenethiol (12.8 g, 100.0 mmol) and ethyl 4-chloroacetoacetate (13.5 mL, 100.0 mmol) in CH_2Cl_2 (300 mL) was dropwisely added triethylamine (15.2 mL, 150.0 mmol) at 0 °C. The resulting suspension was stirred at 0 °C for another 2 h, the poured into water (500 mL). After extracted with DCM (200 mL × 2), the combined organic layers were combined and washed consequently with saturated $NaHCO_3$ (100 mL), 0.25 N HCl (80 mL) and brine (100 mL). The separated organic layer was dried (Na_2SO_4), concentrated in vacuo, and purified by flash column chromatography eluting with 0-10% ethyl acetate/ether petroleum (45-60 ^oC) to afford **14b** (24 g, 92.9%) as a pale yellow oil which was used directly for the next step. ^1H NMR (400 MHz, $CDCl_3$) δ 7.39 – 7.34 (m, 2H), 7.03 – 6.97 (m, *2*H), 4.17 (q, *J* = 7.2 Hz, 2H), 3.74 (s, 2H), 3.62 (s, 2H), 1.26 (t, *J* = 7.2 Hz, 3H); EI-MS m/z: 256 $[M]^+$.

*4.1.4.4. Ethyl 4-[(4-chlorophenyl)thio]-3-oxobutanoate (**14c**)*

Compound **14c** was obtained as a pale yellow solid (4.69 g, 86%) from 4-chlorobenzenethiol (2.89 g, 20.0 mmol) in a manner similar to that as described for the preparation of **14b**. The compound was used directly for the next step without further purification. ^1H NMR (400 MHz, $CDCl_3$) δ 7.34 – 7.25 (m, 4H), 4.18 (q, *J* = 7.2 Hz, 2H), 3.79 (s, 2H), 3.62 (s, 2H), 1.26 (t, *J* = 7.2 Hz, 3H); EI-MS m/z: 272 $[M]^+$.

*4.1.4.5. Ethyl 4-[(4-bromophenyl)thio]-3-oxobutanoate (**14d**)*

Compound **14d** was obtained as a pale yellow solid (29.3 g, 92.4%) from 4-chlorobenzenethiol (18.9 g, 100.0 mmol) in a manner similar to that as described for the preparation of **14b**. The compound was used directly for the next step without further purification. ^1H NMR (400 MHz, $CDCl_3$) δ 7.42 – 7.39 (m, 2H), 7.24 – 7.19 (m, *2*H), 4.17 (q, *J* = 7.2 Hz, 2H), 3.80 (s, 2H), 3.62 (s, 2H), 1.26 (t, *J* = 7.2 Hz, 3H); EI-MS m/z: 316 $[M]^+$.

*4.1.4.6. Ethyl 4-[(4-methoxyphenyl)thio]-3-oxobutanoate (**14e**)*

Compound **14e** was obtained as a pale yellow oil (4.6 g, 85.8%) from 4-methoxybenzenethiol (2.8 g, 20.0 mmol) in a manner similar to that as described for the preparation of **14b**. The compound was used directly for the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 – 7.33 (m, 2H), 6.86 – 6.82 (m, *2*H), 4.18 (q, *J* = 7.2 Hz, 2H), 3.79 (s, 3H), 3.67 (s, 2H), 3.63 (s, 2H), 1.26 (t, *J* = 7.2 Hz, 3H); EI-MS m/z: 268 $[M]^+$.

*4.1.4.7. Ethyl 2-(5-fluorobenzo[b]thiophen-3-yl) acetate (**15b**)*

To a solution of PPA (100 g) in toluene (150 mL) at 100 ^{o}C (keep stirring) was added **14b** (23.6 g, 92.1 mmol) in toluene (20 mL). The reaction solution was stirred at 100 ^{o}C overnight under N_2. The reaction mixture was cooled down to rt and then quenched with ice, basified with potassium carbonate (pH~8) and extracted with ethyl acetate (200 mL × 3). The organic layers were combined, washed with brine, dried over anhydrous Na_2SO_4, filtrated and concentrated. The residue was purified by silica gel column chromatography eluting with 0-10% ethyl acetate/petroleum (45-60 ^{o}C) to give **15b** (12.2 g, 55.6%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (dd, *J* = 8.8 Hz, *J* = 4.8 Hz, 1H), 7.46 – 7.43 (m, 2H), 7.14 – 7.09 (m, 1H), 4.19 (q, *J* = 6.8 Hz, 2H), 3.80 (s, 2H), 1.27 (t, *J* = 6.8 Hz, 3H); ^{19}F-NMR (375 MHz, $CDCl_3$) δ -118.42 to -118.49; ^{13}C-NMR (100 MHz, $CDCl_3$) δ 170.5, 161.0 (d, *J* = 240.3 Hz), 139.9 (d, *J* = 9.1 Hz), 135.6 (d, *J* = 1.4 Hz), 128.3 (d, *J* = 4.4 Hz), 127.1, 124.0 (d, *J* = 9.3 Hz), 113.4 (d, *J* = 25.2 Hz), 107.7 (d, *J* = 23.3 Hz), 61.3, 34.6, 14.3; EI-MS m/z: 238 $[M]^+$.

*4.1.4.8. Ethyl 2-(5-chlorobenzo[b]thiophen-3-yl) acetate (**15c**)*

Compound **15c** was obtained as a white solid (2.02 g, 47%) from **14c** (4.60 g, 16.9 mmol) in a manner similar to that as described for the preparation of **15b**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, *J* = 8.0 Hz, 2H), 7.42 (s, 1H), 7.32 (dd, *J* = 8.4 Hz, *J* = 2.0 Hz, 1H), 4.19 (q, *J* = 7.2 Hz, 2H), 3.82 (s, 2H), 1.27 (t, *J* = 7.2 Hz, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 170.3, 139.9, 138.3, 130.7, 127.9, 126.5, 124.9, 123.8, 121.6, 61.2, 34.4, 14.2; EI-MS m/z: 238 $[M]^+$.

*4.1.4.9. Ethyl 2-(5-bromobenzo[b]thiophen-3-yl) acetate (**15d**)*

Compound **15d** was obtained as a white solid (7.7 g, 61.8%) from **14d** (13.2 g, 41.6

mmol) in a manner similar to that as described for the preparation of **15b**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 (d, *J* = 2.0 Hz, 1H), 7.71 (d, *J* = 8.4 Hz, 1H), 7.45 (dd, *J* = 8.4 Hz, *J* = 2.0 Hz, 1H), 7.40 (s, 1H), 4.19 (q, *J* = 7.2 Hz, 2H), 3.81 (s, 2H), 1.28 (t, *J* = 7.2 Hz, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 170.4, 140.4, 139.0, 128.0, 127.6, 126.5, 124.8, 124.3, 118.5, 61.4, 34.5, 14.3; EI-MS m/z: 298 $[M]^+$.

*4.1.4.10. Ethyl 2-(5-methoxybenzo[b]thiophen-3-yl) acetate (**15e**)*

Compound **15e** was obtained as light yellow solid (2.14 g, 53%) from **14e** (4.32 g, 16.1 mmol) in a manner similar to that as described for the preparation of **15b**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 (d, *J* = 8.8 Hz, 1H), 7.37 (s, 1H), 7.21 (d, *J* = 2.4 Hz, 1H), 7.01 (dd, *J* = 8.8 Hz, *J* = 2.4 Hz, 1H), 4.18 (q, *J* = 7.2 Hz, 2H), 3.89 (s, 3H), 3.82 (s, 2H), 1.26 (t, *J* = 7.2 Hz, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 170.8, 157.7, 139.8, 132.7, 128.1, 125.9, 123.6, 114.7, 104.3, 61.2, 55.7, 34.9, 14.4; EI-MS m/z: 250 $[M]^+$.

*4.1.4.11. 2-(5-Fluorobenzo[b]thiophen-3-yl)acetamide (**16b**)*

Compound **15b** (10.2 g, 42.8 mmol) was dissolved in ammonia in methanol (7 M, 183 mL, 1284 mmol) and the reaction mixture was stirred at room temperature for 3 days at 25 ^{o}C. The white solid was precipitated. After removed the solvent and washed with cold water, the solid was sonicated with ether petroleum (45-60 ^{o}C)/ethyl acetate (10 : 1) to afford **16b** (7.8 g, 87.6%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (dd, *J* = 8.8 Hz, *J* = 4.8 Hz, 1H), 7.66 – 7.59 (m, 3H), 7.28 – 7.23 (m, 1H), 7.01 (s, 1H), 3.63 (s, 2H); ^{19}F-NMR: (375 MHz, $CDCl_3$) δ -118.97 to -119.04; ^{13}C-NMR (100 MHz, $CDCl_3$) δ 171.1, 160.1 (d, *J* = 237.3 Hz), 140.2 (d, *J* = 9.2 Hz), 135.2, 130.7 (d, *J* = 4.3 Hz), 127.2, 124.4 (d, *J* = 9.4 Hz), 112.7 (d, *J* = 24.9 Hz), 107.7 (d, *J* = 23.1 Hz), 35.2; ESI-MS m/z: 210.0 $[M+H]^+$.

*4.1.4.12. 2-(5-Chlorobenzo[b]thiophen-3-yl)acetamide (**16c**)*

Compound **16c** was obtained as a white solid (1.32 g, 76.2%) from **15c** (1.95 g, 7.65 mmol) in a manner similar to that as described for the preparation of **15b**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.01 (d, *J* = 8.4 Hz, 1H), 7.90 (d, *J* = 1.6 Hz, 1H), 7.62 (brs, 2H), 7.39 (dd, *J* = 8.4 Hz, *J* = 1.6 Hz, 1H), 7.02 (s, 1H), 3.66 (s, 2H); ^{13}C-NMR (100 MHz, DMSO-d_6) δ 171.1, 140.3, 138.0, 130.4, 129.3, 126.9, 124.5, 124.2, 121.7,

35.1; ESI-MS m/z: 226.0 $[M+H]^+$.

*4.1.4.13. 2-(5-Bromobenzo[b]thiophen-3-yl)acetamide (**16d**)*

Compound **16d** was obtained as a white solid (5.58 g, 82.3%) from **15d** (7.52 g, 25.13 mmol) in a manner similar to that as described for the preparation of **16b**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.88 (d, *J* = 2.0 Hz, 1H), 7.70 (d, *J* = 8.8 Hz, 1H), 7.43 (dd, *J* = 8.4 Hz, *J* = 5.6 Hz, 1H), 7.19 (s, 1H), 3.06 (t, *J* = 7.2 Hz, 2H), 2.95 (t, *J* = 7.2 Hz, 2H), 1.50 (brs, 2H); ^{13}C-NMR (400 MHz, $CDCl_3$) δ 171.1, 140.7, 138.5, 130.3, 126.8, 126.7, 124.8, 124.7, 117.5, 35.1; ESI-MS m/z: 270.0 $[M+H]^+$.

*4.1.4.14. 2-(5-Methoxybenzo[b]thiophen-3-yl)acetamide (**16e**)*

Compound 1**6e** was obtained as a white solid (1.25 g, 72.1%) from **15e** (1.96 g, 7.83 mmol) in a manner similar to that as described for the preparation of **16b**. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.83 (d, *J* = 8.8 Hz, 1H), 7.59 (s, 1H), 7.49 (s, 1H), 7.01 (dd, *J* = 8.8 Hz, *J* = 2.4 Hz, 2H), 3.82 (s, 3H), 3.62 (s, 2H); ^{13}C-NMR (100 MHz, DMSO-d_6) δ 171.1, 156.5, 141.2, 132.3, 129.2, 126.1, 124.1, 115.2, 106.3, 55.6, 35.2; ESI-MS m/z: 222.1 $[M+H]^+$.

*4.1.4.15. Ethyl (2-(5-fluorobenzo[b]thiophen-3-yl)ethyl)carbamate (**18b**)*

Compound **16b** (7.2 g, 34.5 mmol) was dissolved in THF (150 mL) and heated to 80 °C under N_2 protection. After 1M borane in THF (86.1 mL, 86.1mmol) was added dropwise, the reaction mixture was stirred overnight under reflux, then cool down and quenched by dropwised addition of 6N HCl (60 mL). The reaction was continuously stirred for 3 hours at rt. The THF was evaporated from the mixture in vacuum, and then basified with 2N NaOH and extracted with ether (300 mL × 3). The combined ethereal layers were washed with water (50 mL x 2), dried over anhydrous sodium sulfate and concentrated under vacuum to give product **17b** (5.8 g, 86.7%) which was used directly for the next step without further purification. To a solution of **17b** (6.7 g, 29.8 mmoles) and Et_3N (12.5 mL, 89.58 mmole) in DCM (200 mL) was added ethyl carbonochloridate (4.26 mL, 44.79 mmole) dropwise at 0 ^oC, and then the reaction was stirred at room temperature for 2h. After quenched with cold water, washed with NH_4Cl solution, brine, dried(Na_2SO_4), the organic solvent was removed under

vacuum and the residue was purified by column chromatography eluting with 0-10% of ethyl acetate/hexanes to give ethyl (2-(5-fluorobenzo[b]thiophen-3-yl)ethyl)carbamate **18b** (7.1 g, 88.9%) as a pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (dd, *J* = 8.8 Hz, *J* = 4.8 Hz, 1H), 7.42 (dd, *J* = 9.6 Hz, *J* = 2.0 Hz, 1H), 7.24 (s, 1H), 7.13 – 7.08 (m, 1H), 4.84 (s, 1H), 4.15 – 4.09 (m, 2H), 3.52 (q, *J* = 6.4 Hz, 2H), 3.01 (t, *J* = 6.8 Hz, 2H), 1.23 (t, *J* = 6.8 Hz, 3H); ^{19}F-NMR (375 MHz, $CDCl_3$) δ -118.63 to -118.82; ^{13}C-NMR (100 MHz, $CDCl_3$) δ 161.0 (d, *J* = 240.3 Hz), 156.7, 140.1 (d, *J* = 8.8 Hz), 135.9, 133.4, 125.0, 124.1 (d, *J* = 9.3 Hz), 113.3 (d, *J* = 25.1 Hz), 107.4 (d, *J* = 22.8 Hz), 61.0, 40.4, 29.2, 14.7; ESI-MS m/z: 268.1 $[M+H]^+$.

*4.1.4.16. Ethyl (2-(5-chlorobenzo[b]thiophen-3-yl)ethyl)carbamate (**18c**)*

Compound **18c** was obtained as a pale yellow solid (1.38 g, 86.7%) from **16c** (1.30 g, 6.14 mmol) in a manner similar to that as described for the preparation of **18b**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, *J* = 8.8 Hz, 1H), 7.73 (d, *J* = 2.0 Hz, 1H), 7.31 (dd, *J* = 8.4 Hz, *J* = 2.0 Hz, 1H), 7.22 (s, 1H), 4.78 (s, 1H), 4.13 (q, *J* = 7.2 Hz, 2H), 3.53 (q, *J* = 6.0 Hz, 2H), 3.03 (t, *J* = 6.8 Hz, 2H), 1.24 (t, *J* = 6.8 Hz, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 156.7, 140.2, 138.7, 133.1, 130.7, 125.0, 124.6, 124.0, 121.5, 61.0, 40.5, 29.1, 14.8; ESI-MS m/z: 284.0 $[M+H]^+$.

*4.1.4.17. Ethyl (2-(5-bromobenzo[b]thiophen-3-yl) ethyl)carbamate (**18d**)*

Compound **18d** was obtained as a pale yellow solid (4.88 g, 90.8%) from **16d** (5.20 g, 19.25 mmol) in a manner similar to that as described for the preparation of **18b**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.89 (d, *J* = 2.0 Hz, 1H), 7.71 (d, *J* = 8.8 Hz, 1H), 7.44 (dd, *J* = 8.4 Hz, *J* = 5.6 Hz, 1H), 7.20 (s, 1H), 4.76 (s, 1H), 4.13 (q, *J* = 7.2 Hz, 2H), 3.53 (q, *J* = 6.4 Hz, 2H), 3.03 (t, *J* = 7.2 Hz, 2H), 1.24 (t, *J* = 7.2 Hz, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 156.7, 140.7, 139.2, 133.0, 127.6, 124.6, 124.4, 124.4, 118.5, 61.0, 40.5, 29.1, 14.8; ESI-MS m/z: 328.0 $[M+H]^+$.

*4.1.4.18. Ethyl (2-(5-methoxybenzo[b]thiophen-3-yl) ethyl) carbamate (**18e**)*

Compound **18e** was obtained as a pale yellow oil (1.04 g, 82.7%) from **16e** (1.20 g, 5.42 mmol) in a manner similar to that as described for the preparation of **18b**. ^{1}H

NMR (400 MHz, $CDCl_3$) δ 7.72 (d, *J* = 8.8 Hz, 1H), 7.22 (s, 1H), 7.17 (s, 1H), 7.01 (dd, *J* = 8.8 Hz, *J* = 2.4 Hz, 1H), 4.78 (brs, 1H), 4.12 (q, *J* = 6.8 Hz, 2H), 3.89 (s, 3H), 3.53 (q, *J* = 6.8 Hz, 2H), 3.03 (t, *J* = 6.8 Hz, 2H), 1.23 (t, *J* = 6.8 Hz, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 157.7, 156.7, 140.0, 133.2, 133.0, 123.8, 123.7, 114.6, 104.2, 60.9, 55.8, 40.4, 29.3, 14.8; ESI-MS m/z: 280.1 $[M+H]^+$.

*4.1.4.19. Ethyl 6-fluoro-3,4-dihydrobenzo[4,5]thieno[2,3-c] pyridine-2(1H)-carbox ylate (**19b**)*

A solution of compound **18b** (7.1 g, 26.56 mmole), powdered paraformaldehyde (1.19 g, 39.84 mmole) and *p*-toluenesulfonic acid monohydrate (252 mg, 1.33 mmole) in toluene (200 mL) was refluxed at 115 °C for 2 h. The reaction mixture was cooled down to rt and the solvent was evaporated under reduced pressure. The residue was diluted with ethyl acetate (400 mL) and water (100 mL) and the organic layer was separated, washed with aqueous $NaHCO_3$ (100 mL), brine (100 mL) and dried over anhydrous Na_2SO_4. After filtered and evaporated, the resulting residue was purified by silica gel column chromatography eluting with 0-10% ethyl acetate/ether petroleum(45-60 ^oC) to give **19b** (6.5 g, 87.8%) as a pale yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 (dd, *J* = 8.4 Hz, *J* = 4.8 Hz, 1H), 7.26 – 7.23 (m, 1H), 7.08 – 7.03 (m, 1H), 4.76 (s, 2H), 4.21 (q, *J* = 6.8 Hz, 2H), 3.85 (t, *J* = 5.6 Hz, 2H), 2.81 (t, *J* = 5.6 Hz, 2H),1.23 (t, *J* = 6.8 Hz, 3H); ^{19}F-NMR (375 MHz, $CDCl_3$) δ -118.37 to -118.57; ^{13}C-NMR (100 MHz, $CDCl_3$) δ 161.1 (d, *J* = 240.4 Hz), 155.7, 140.1 (d, *J* = 8.9 Hz), 135.6, 133.9, 128.7, 123.7 (d, *J* = 9.2 Hz), 112.9 (d, *J* = 24.8 Hz), 106.7 (d, *J* = 23.4 Hz), 61.9, 43.9, 40.9, 23.9, 14.8; ESI-MS m/z: 280.1 $[M+H]^+$.

*4.1.4.20. Ethyl 6-chloro-3,4-dihydrobenzo[4,5]thieno[2,3-c]pyridine-2(1H)-carboxyl ate (**19c**)*

Compound **19c** was obtained as a pale yellow solid (1.04 g, 76.5%) from **18c** (1.30 g, 4.58 mmol) in a manner similar to that as described for the preparation of **19b**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68 (d, *J* = 8.4 Hz, 1H), 7.55 (s, 1H), 7.26 (dd, *J* = 8.4 Hz, *J* = 2.0 Hz, 1H), 4.75 (s, 2H), 4.21 (q, *J* = 7.2 Hz, 2H), 3.84 (s, 2H), 2.81 (s, 2H), 1.30 (t, *J* = 7.2 Hz, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 155.5, 140.0, 136.7, 130.6, 124.6,

123.4, 120.45, 61.8, 43.7, 40.7, 23.7, 14.7; ESI-MS m/z: 296.0 $[M+H]^+$.

*4.1.4.21. Ethyl 6-bromo-3,4-dihydrobenzo[4,5]thieno[2,3-c]pyridine-2(1H)-carboxyl ate (**19d**)*

Compound **19d** was obtained as a pale yellow solid (3.34 g, 79.6%) from **18d** (4.05 g, 12.34 mmol) in a manner similar to that as described for the preparation of **19b**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 (s, 1H), 7.63 (d, *J* = 8.4 Hz, 1H), 7.39 (dd, *J* = 8.4 Hz, *J* = 2.0 Hz, 1H), 4.75 (s, 2H), 4.21 (q, *J* = 7.2 Hz, 2H), 3.84 (s, 2H), 2.81 (s, 2H), 1.30 (t, *J* = 7.2 Hz, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 155.6, 140.6, 137.4, 135.4, 128.2, 127.3, 123.9, 123.7, 118.5, 61.9, 43.8, 40.8, 23.8, 14.8; ESI-MS m/z: 340.0 $[M+H]^+$.

*4.1.4.22. Ethyl 6-methoxy-3,4-dihydrobenzo[4,5]thieno[2,3-c]pyridine-2(1H)-carbox ylate (**19e**)*

Compound **19e** was obtained as a pale yellow solid (0.81 g, 81.6%) from **18e** (0.95 g, 3.40 mmol) in a manner similar to that as described for the preparation of **19b**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.65 (d, *J* = 8.8 Hz, 1H), 7.03 (d, *J* = 2.4 Hz, 1H), 6.95 (dd, *J* = 8.8 Hz, *J* = 2.4 Hz, 1H), 4.75 (s, 2H), 4.20 (q, *J* = 7.2 Hz, 2H), 3.87 (s, 2H), 3.85 (s, 2H), 2.82 (s, 2H), 1.30 (t, *J* = 7.2 Hz, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 157.8, 155.7, 140.0, 130.9, 128.6, 123.7, 123.3, 114.0, 103.8, 61.8, 55.8, 43.9, 40.9, 24.0, 14.9; ESI-MS m/z: 292.1 $[M+H]^+$.

*4.1.4.23. 1,2,3,4-tetrahydrobenzo[4,5]thieno[2,3- c]pyridine (**20a**)*

To a solution of compound **12** (700 mg, 2.42 mmol) in DCM (10 mL) was added TFA (2 mL). The resulting mixture was stirred at rt for 2 h. After the solvent was evaporated under reduced pressure, the residue was diluted with water (30 mL), basified with Na_2CO_3, and extracted with CH_2Cl_2 (50 mL × 2). The combined organic layers were washed with brine (50 mL × 2), dried over anhydrous Na_2SO_4, filtrated, and evaporated under reduced pressure to give compound **20a** as a white solid (390 mg, 85.2%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.89 (d, *J* = 8.0 Hz, 1H), 7.63 (d, *J* = 8.0 Hz, 1H), 7.37 – 7.28 (m, 2H), 3.94 (s, 2H), 3.03 (t, *J* = 5.6 Hz, 2H), 2.68 (t, *J* = 5.6 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 139.3, 137.4, 136.5, 128.5, 124.1, 123.9, 122.5, 120.4, 44.6, 42.2, 25.0.

4.1.4.24. 6-Fluoro-1,2,3,4-tetrahydrobenzo[4,5]thieno [2,3-c]pyridine (**20b**)

Compound **19b** (6.50 g, 23.27 mmole) and NaOH (4.65 g, 116.35 mmole) were suspended in a mixture of EtOH (100 mL) and water (30 mL) at 0 ^{o}C. The resulting reaction mixture was stirred overnight under reflux condition. After cooled down to rt, majority EtOH solvent was removed under reduce vacuum and then diluted with water (100 mL). The mixture was extracted with ethyl acetate (100 mL × 3) and the combined organic layers were dried over Na_2SO_4, filtered and evaporated. The residue was recrystallized with ether petroleum (45-60 ^{o}C)/ethyl acetate (6 : 1) to afford compound **20b** (3.8 g, 79.2%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69 (dd, *J* = 8.4 Hz, *J* = 4.8 Hz, 1H), 7.24 (dd, *J* = 8.8 Hz, *J* = 2.4 Hz, 1H), 7.06 – 7.01 (m, 1H), 4.11 (t, *J* = 1.6 Hz, 2H), 3.23 (t, *J* = 5.8 Hz, 2H), 2.75 – 2.71 (m, 2H), 1.80 (s, 1H); ^{19}F-NMR (375 MHz, $CDCl_3$) δ -118.88 to -118.94; ^{13}C-NMR (100 MHz, $CDCl_3$) δ 161.1 (d, *J* = 239.9 Hz), 140.9 (d, *J* = 8.8 Hz), 138.9, 133.6, 128.3 (d, *J* = 4.2 Hz), 123.6 (d, *J* = 9.3 Hz), 112.6 (d, *J* = 24.9 Hz), 106.4 (d, *J* = 22.8 Hz), 45.5, 43.0, 25.1; ESI-MS m/z: 208.1 $[M+H]^+$.

*4.1.4.25. 6-Chloro-1,2,3,4-tetrahydrobenzo[4,5]thieno[2,3-c]pyridine (**20c**)*

Compound **20c** was obtained as a pale yellow solid (570 mg, 72.5%) from **19c** (1.04 g, 3.52 mmol) in a manner similar to that as described for the preparation of **20b**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.72 (d, *J* = 8.4 Hz, 1H), 7.61 (s, 1H), 7.32 (dd, *J* = 8.4 Hz, *J* = 2.0 Hz, 1H), 4.05 (t, *J* = 2.0 Hz, 2H), 3.16 (t, *J* = 5.8 Hz, 2H), 2.79 – 2.76 (m, 2H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 141.3, 139.8, 130.2, 129.2, 128.3, 126.2, 123.6, 121.4, 45.7, 43.2, 25.0; ESI-MS m/z: 224.0 $[M+H]^+$.

*4.1.4.26. 6-Bromo-1,2,3,4-tetrahydrobenzo[4,5]thieno[2,3-c]pyridine (**20d**)*

Compound **20d** was obtained as a white solid (1.86 g, 71.5%) from **19d** (3.30 g, 9.70 mmol) in a manner similar to that as described for the preparation of **20b**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.75 (d, *J* = 2.0 Hz, 1H), 7.69 (d, *J* = 8.4 Hz, 1H), 7.37(dd, *J* = 8.4 Hz, *J* = 2.0 Hz, 1H), 4.02 (t, *J* = 2.0 Hz, 2H), 3.14 (t, *J* = 5.8 Hz, 2H), 2.77 – 2.73 (m, 2H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 142.5, 138.6, 138.5, 129.0, 128.0, 124.9, 124.3, 119.2, 45.4, 43.3, 25.0; ESI-MS m/z: 268.0 $[M+H]^+$.

*4.1.4.27. 6-Methoxy-1,2,3,4-tetrahydrobenzo[4,5]thieno[2,3-c]pyridine (**20e**)*

Compound **20e** was obtained as a pale yellow solid (417 mg, 73.8%) from **20a** (750 mg, 2.58 mmol) in a manner similar to that as described for the preparation of **20b**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.71 (d, *J* = 8.8 Hz, 1H), 7.08 (d, *J* = 2.0 Hz, 1H), 7.01(dd, *J* = 8.8 Hz, *J* = 2.0 Hz, 1H), 4.01 (t, *J* = 2.0 Hz, 2H), 3.87 (s, 3H), 3.14 (t, *J* = 5.8 Hz, 2H), 2.74 – 2.69 (m, 2H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 156.9, 140.2, 131.3, 129.3, 124.5, 124.8, 115.32, 105.2, 56.1, 45.2, 42.9, 24.9; ESI-MS m/z: 222.1 $[M+H]^+$.

4.2 Biological activity

4.2.1 Animals and materials

Male Sprague-Dawley rats (250–300 g, 60-day-old) were purchased from Shanghai Laboratory Animal Co. Ltd. (Shanghai, China). All animal studies were approved by the Institutional Animal Care and Use Committee of Interdisciplinary Research Center on Biology and Chemistry, Chinese Academy of Sciences (NO. 2016-03-ZJD-01) and were performed in accordance with the Guide for the Care and Use of Laboratory Animals.

4.2.2 CYP17 Preparation and Assay

Microsomal fractions of rat testes were prepared as previously reported [35, 36]. Testes from Sprague Dawley male rats weighing 220–250 g (age of 60-day-old) were minced and homogenized in STKM buffer（0.25 M sucrose, 50 mM Tris-HCl, 2.5 mM KCl, 50 mM MgCl2 (1:4, w/v) then centrifuged at 9000 g for 30 min and the resulting supernatants were further centrifuged at 105,000 g for 1 h, and the pellets were re-suspended in STKM buffer. All the above procedures were performed at 4 °C. The microsomal fraction was stored at -80 °C. The microsomal protein concentrations were determined using the Enhanced BCA Protein Assay Kit (P0010; Beyotime Biotechnology, Shanghai, China) according to manufacturer’s protocol.

Human CYP 17 gene was cloned and expressed in Adenoviral expression system in A549 cell lines. The purified cell membrane preparations were used as the source for

Human CYP17 enzyme. The membrane protein concentrations were determined using the Enhanced BCA Protein Assay Kit (P0010; Beyotime Biotechnology, Shanghai, China) according to manufacturer's protocol.

The lase activity of CYP 17 was determined by measuring the conversion of 17α-hydroxyprogesterone into androstenedione. The assay was performed as follows: A solution of 2 μg microsome/membrane protein in 25 μL phosphate buffer (0.05 M, pH 7.5), and inhibitor was pre-incubated at 25 °C for 5 min. The reaction was started by adding 25 μL of substrate mix in phosphate buffer (10 μM 17α-hydroxyprogesterone, 4.2 mM NADPH). The mixture was incubated for 60 min at 25℃. Subsequently the reaction was stopped with addition of methanol (250 μL). The concentration of reaction product androstenedione was quantified by LC/MS.

4.2.3. Cell-based functional assay for testosterone production

NCI-H295R cells (ATCC No. CRL-2128) were cultured in DMEM medium supplemented with 10% FBS and 1% P/S. Cells were seeded at a density of 1×10^4/100 μL per well in a 96-well plate and left overnight at 37 °C. On the next day, 1 μL solution of compounds in serial dilution was added into the wells and DMSO (1%) was used as the negative control. The plate was then incubated at 37 °C for 72 h. After incubation, the media was collected and the level of testosterone was measured according to the manufacturer's protocol. The experiment was run in triplicate (Testosterone, 64TESPEB, Cisbio).

*4.2.4. Suppression of serum testosterone levels by Compound **9c** in male rats*

Sixty day old adult male Sprague-Dawley rats were randomly assigned into five groups. The reference group was treated with 10 mg/kg of ABT, and the other four groups were treated with **9c** at 1.0, 3.0, 10.0 and 30.0 mg/kg, respectively. The serum level of testosterone was measured 0 h, 2 h, 4 h, 8 h, and 24 h post treatment by using Abcam Elisa kits (cat 108666) according to manufacturer's instructions.

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References

[1]. J. Ferlay, I. Soerjomataram, R. Dikshit, S. Eser, C. Mathers, M. Rebelo, D. M. Parkin, D. Forman, F. B. Ferlay, Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012, Int. J. Cancer. 136 (2015) E359–E386.

[2]. G. Attard, C. Parker, R. A. Eeles, F. Schröder, S. A. Tomlins, I. Tannock, C. G. Drake, J. S. de Bono, Prostate cancer, Lancet. 387 (2016) 70–82.

[3]. R. L. Siegel, K. D. Miller, A. Jemal, Cancer Statistics, 2016, CA. Cancer. J Clin. 66 (2016) 7–30.

[4]. L. Yin, Q. Hu, R.W. Hartmann, Recent progress in pharmaceutical therapies for castration-resistant prostate cancer, Int. J. Mol. Sci. 14 (2013) 13958-13978.

[5]. R. Siegel, C. DeSantis, K. Virgo, K. Stein, A. Mariotto, T. Smith, D. Cooper, T. Gansler, C. Lerro, S. Fedewa, Cancer treatment and survivorship statistics, CA. Cancer. J. Clin. 62 (2012) 220–241.

[6]. Y. Zong, A. S. Goldstein, Adaptation or selection-mechanisms of castration-resistant prostate cancer, Nat Rev Urol 10 (2013) 90–98.

[7]. J. Geller, Basis for hormonal management of advanced prostate cancer, Cancer 71 (1993) 1039–1045.

[8]. M.A. Titus, M.J. Schell, F.B. Lih, K.B. Tomer, J.L. Mohler, Testosterone and dihydrotestosterone tissue levels in recurrent prostate cancer, Clin. Cancer Res. 11 (2005) 4653–4657.

[9]. T.A. Yap, C.P. Carden, G. Attard, J.S. de Bono, Targeting CYP17: established and novel approaches in prostate cancer, Curr. Opin. Pharmacol. 8 (2008) 449–457.

[10]. L. Yin, Q. Hu, CYP17 inhibitors abiraterone, C17, 20-lyase inhibitors and multi-targeting agents, Nat. Rev. Urol. 11 (2014) 32–42.

[11]. L. Gomez, J.R. Kovac, D.J. Lamb, CYP17A1 inhibitors in castration-resistant prostate cancer, Steroids. 95 (2015) 80-87.

[12]. A.B. Alex, S.K. Pal, N. Agarwal, CYP17 inhibitors in prostate cancer: latest evidence and clinical potential, Ther. Adv. Med. Oncol. 8(4) (2016) 267-275.

[13]. A.H. Payne, D.B. Hales, Overview of steroidogenic enzymes in the pathway from cholesterol to active steroid hormones, Endocr. Rev. 25 (2004) 947–970.

[14]. R.J. Santen, H. Van den Bossche, J. Symoens, J. Brugmans, R. De Coster, Site of action of low dose ketoconazole on androgen biosynthesis in men, J. Clin. Endocrinol. Metab. 57 (1983) 732–736.

[15]. G. Lake-Bakaar, P. J. Scheuer, S. Sherlock, Hepatic reactions associated with ketoconazole in the United Kingdom, Br. Med. J. 294 (1987) 419–422.

[16]. J.S. de Bono, C.J. Logothetis, A. Molina, K. Fizazi, S. North, L. Chu, K.N. Chi, R.J. Jones, O.B. Goodman Jr., F. Saad, J.N. Staffurth, P. Mainwaring, S. Harland, T.W. Flaig, T.E. Hutson, T. Cheng, H. Patterson, J.D. Hainsworth, C.J. Ryan, C.N. Sternberg, S.L. Ellard, A. Flechon, M. Saleh, M. Scholz, E. Efstathiou, A. Zivi, D.Bianchini, Y. Loriot, N. Chieffo, T. Kheoh, C.M. Haqq, H.I. Scher, C.-A.-Investigators, Abiraterone and increased survival in metastatic prostate cancer, N. Engl. J. Med. 364 (2011) 1995–2005.

[17]. Janseen. Highlights of prescribing information: Zytiga® [online], http://www.zytigahcp.com/pdf/full_prescribing_info.pdf (2013).

[18]. V.C.O. Njar, A.M.H. Brodie, Discovery and development of galeterone (TOK-001 or VN/124-1) for the treatment of all stages of prostate cancer, J. Med.Chem. 58 (2015) 2077-2087.

[19]. Tokai announced the discontinuation of ARMOR3-SV, after a data monitoring committee determined that the trial was unlikely to meet its endpoint. [Press release]; (2016) http://investors.tokaipharmaceuticals.com/phoenix.zhtml?c=252857&p=irol-newsArticle&ID=2188163.

[20]. T. Kaku, T. Hitaka, A. Ojida, N. Matsunaga, M. Adachi, T. Tanaka, T. Hara, M. Yamaoka, M. Kusaka, T. Okuda, S. Asahi, S. Furuya, A. Tasaka, Discovery of orteronel (TAK-700), a naphthylmethylimidazole derivative, as a highly selective 17,20-lyase inhibitor with potential utility in the treatment of prostate cancer, Bioorg. Med. Chem. 19 (2011) 6383-6399.

[21]. M. Yamaoka. et al. Orteronel (TAK-700), a novel non-steroidal 17,20‑lyase inhibitor: effects on steroid synthesis in human and monkey adrenal cells and serum steroid levels in cynomolgus monkeys. J. Steroid Biochem. Mol. Biol. 129 (2012)115–128.

[22]. S. W. Rafferty, J. R. Eisner, W. R. Moore, R. J. Schotzinger, W. J. Hoekstra, Highly-selective 4-(1,2,3-triazole)-based P450c17a 17,20-lyase inhibitors, Bioorg. Med. Chem. Lett. 24 (2014) 2444-2447.

[23]. P. J. Toren, S. Kim, S. Pham, A. Mangalji, H. Adomat, E. S. T. Guns, A. Zoubeidi, W. Moore, M. E. Gleave, Anticancer activity of a novel selective CYP17A1 inhibitor in preclinical models of castrate resistant prostate cancer, Mol. Cancer. Ther. 14(1) (2015) 59-69.

[24]. Y.Ideyama, M. Kudoh, K. Tanimoto, Y. Susaki, T. Nanya, T. Nakahara, H. Ishikawa, T. Yoden, M. Okada, T. Fujikura, H. Akaza, H. Shikama, Novel nonsteroidal inhibitor of cytochrome P45017a(17a-Hydroxylase/C17–20 Lyase),YM116, decreased prostatic weights by reducing serum concentrations of testosterone and adrenal androgens in rats, Prostate. 37 (1998) 10-18.

[25]. G. Christoph, M. Prakash, M. Henrik, P. Mark, G. Bjoern, G. Nelson, H. Wolfgang, W. Peter, B. Estelle, B. Mark G, S. Saumitra, R. Venkateshwar, R. Murali, A. Thomas, N. Kishore, D. Samiulla, B. Aravind, C. Shekar, Discovery of CFG920, a dual CYP17/CYP11B2 inhibitor, for the treatment of castration resistant prostate cancer. Abstract MEDI-341, 250th ACS National Meeting & Exposition, August 16-20, 2015. Boston, MA, USA.

[26]. U. E. Hille, Q. Hu, C. Vock, M. Negri, M. Bartels, U. M. Vieira, T. Lauterbach, R.W. Hartmann, Novel CYP17 inhibitors: Synthesis, biological evaluation, structure–activity relationships and modelling of methoxy- and hydroxy-substituted methyleneimidazolyl biphenyls, Eur. J. Med. Chem. 44 (2009) 2765–2775.

[27]. Q. Hu, C. Jagusch, UE, Hille, J. Haupenthal, R.W. Hartmann, Replacement of imidazolyl by pyridyl in biphenylmethylenes results in selective CYP17 and dual CYP17/CYP11B1 inhibitors for the treatment of prostate cancer, J. Med. Chem. 53 (2010) 5749–5758.

[28]. Q. Hu, L. Yin, C. Jagusch, U.E. Hille, R.W. Hartmann, Isopropylidene substitution increases activity and selectivity of biphenylmethylene 4-pyridine type CYP17 inhibitors, J. Med. Chem. 53 (2010) 5049–5053.

[29]. M. Mendieta, Q. Hu, M. Engel, R.W. Hartmann, Highly potent and selective nonsteroidal dual inhibitors of CYP17/CYP11B2 for the treatment of prostate cancer to reduce risks of cardiovascular diseases, J. Med. Chem. 56 (2013) 6101–6107.

[30]. D. Kovács, J. Wölfling, N. Szabó, I. Kovócs, I. Zupkó, É. Frank, An efficient approach to novel

17-5′-(1′,2′,4′)-oxadiazolyl androstenes via the cyclodehydration of cytotoxic O-steroidacylamidoximes, and an evaluation of their inhibitory action on 17α-hydroxylase/$C_{17,20}$-lyase, Eur. J. Med. Chem. 70 (2013) 649-660.

[31]. D. Kovács, J. Wölfling, N. Szabó, M. Szécsi, Z. Schelz, I. Zupkó, É. Frank, Synthesis of novel 17-(4′-formyl)pyrazolylandrosta-5,16-dienes and their derivatives as potent 17a-hydroxylase/C17,20-lyase inhibitors or antiproliferative agents depending on the substitution pattern of the heteroring, Eur. J. Med. Chem. 120 (2016) 284-295.

[32]. Q. Yang, J. Chang, J. Song, M. Qian, J. Yu, X. Sun, Synthesis and in vitro antitumor activity of novel iridium (III) complexes with enantiopure C2-symmetrical vicinal diamine ligands, Bioorg. Med. Chem. Lett. 16 (2013) 4602-4607.

[33]. J. Song, P. Peng, J. Chang, M. Liu, J. Yu, L. Zhou, X. Sun, Selective non-zinc binding MMP-2 inhibitors: Novel benzamide Ilomastat analogs with anti-tumor metastasis, Bioorg. Med. Chem. Lett. 26 (2016) 2174–2178.

[34]. N.M. DeVore, E.E. Scott, Structures of cytochrome P450 17A1 with prostate cancer drugs abiraterone and TOK-001, Nature 482 (2012) 116-120.

[35]. J. Li, Y. Li, C. Son, P. Banks, A. Brodie, 4-pregnene-3-one-20βcarboxaldehyde: a potent inhibitor of 17α-hydroxylase/$C_{17,20}$-lyase and of 5α-reductase, J. Steroid Biochem. Molec. Biol. 42 (1992) 313-320.

[36]. D.N. Grigoryev, K. Kato, V.C. Njar, B.J. Long, Y.Z. Ling, X. Wang, J. Mohler, A.M. Brodie, Cytochrome P450c17-expressing Escherichia coli as a first-step screening system for 17alphahydroxylase-C17,20-lyase inhibitors, Anal. Biochem. 267 (2) (1999) 319−330.

[37]. M.G. Bock, C. Gaul, G.V. Rao, S. Saumitra, 17α-hydroxylase/$C_{17,20}$-lyase inhibitors, WO Patent WO2012149413A1.

[38]. S.J. Kruga, Q. Hua, R.W. Hartmanna. Hits identified in library screening demonstrate selective CYP17A1 lyase inhibition, J. Steroid. Biochem. Mol. Biol. 134 (2013) 75-79.

Figure captions:

1. **Figure 1**. Examples of chemical structures of CYP17 inhibitors.
2. **Figure 2**.The proposed binding mode of compound **7** in the active site of CYP17.
3. **Figure 3.** Design strategy of potential new CYP17 inhibitors.
4. **Figure 4A.** Superposition of compound **9c** with TOK-001 crystal structure by docking (pdb code 3swz).
5. **Figure 4B**. Compound **9c** binding mode.
6. **Scheme 1.** Synthesis of compounds **8a-e**.
7. **Scheme 2**. Synthesis of compounds **8f-i**.
8. **Scheme 3**. Synthesis of compounds **9a-n**.
9. **Table 1.** IC_{50} values of compounds **7** and **8a-l** toward rat CYP17.
10. **Table 2.** IC_{50} values of compounds **9a-n** toward rat CYP17.
11. **Table 3.** IC_{50} values of selected compounds toward CYP17 and CYP3A4.
12. **Table 4.** Inhibitory activity of selected compounds toward testosterone production in NCI-H295R cells.
13. **Table 5**. Reduction of plasma testosterone levels by compound **9c** and ABT in male rats.

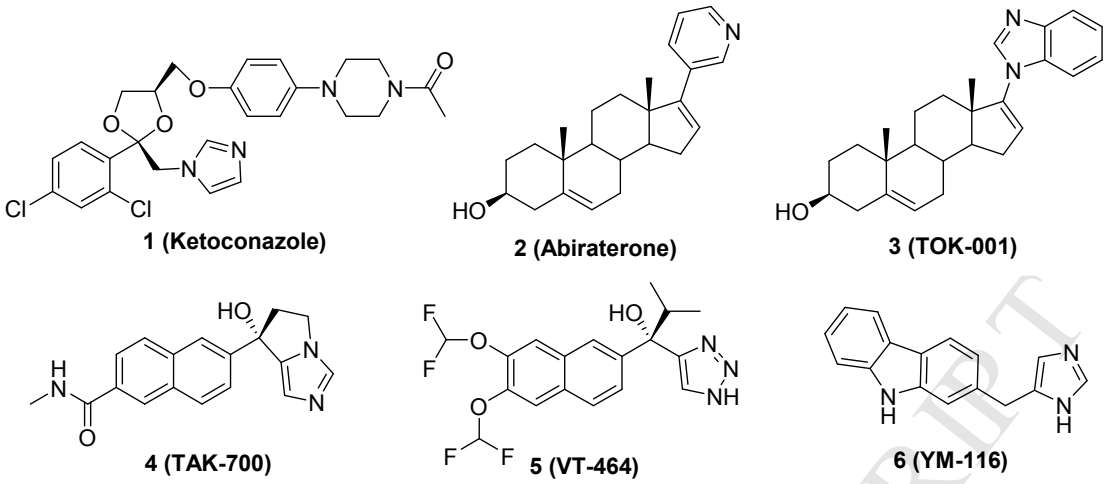


Figure 1. Examples of chemical structures of CYP17 inhibitors.

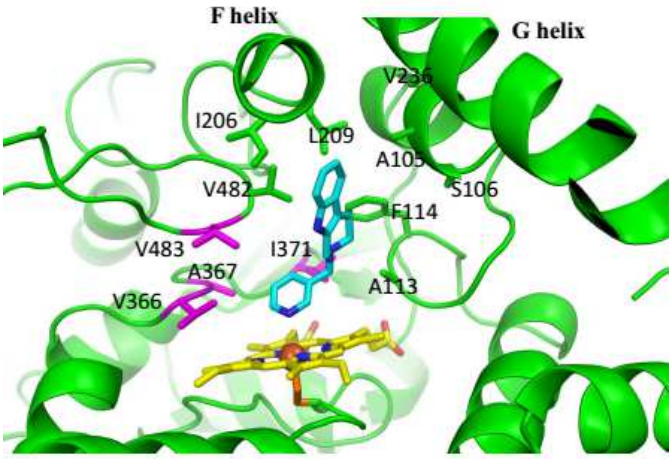


Figure 2. Proposed binding mode of compound **7** in the active site of CYP17.

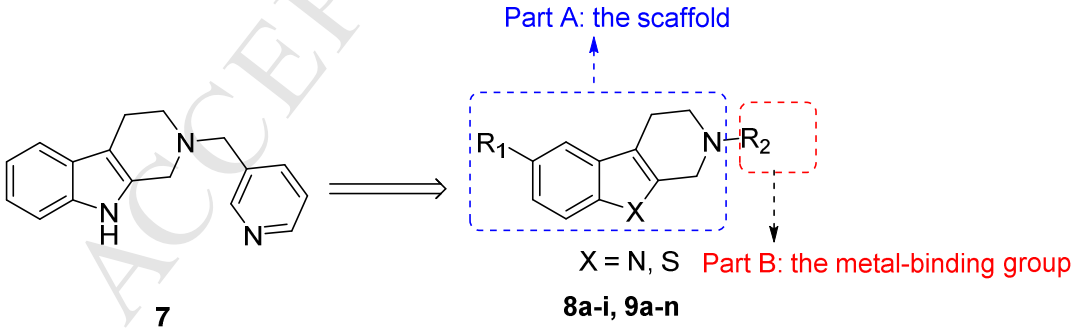


Figure 3. Design strategy of potential new CYP17 inhibitors.

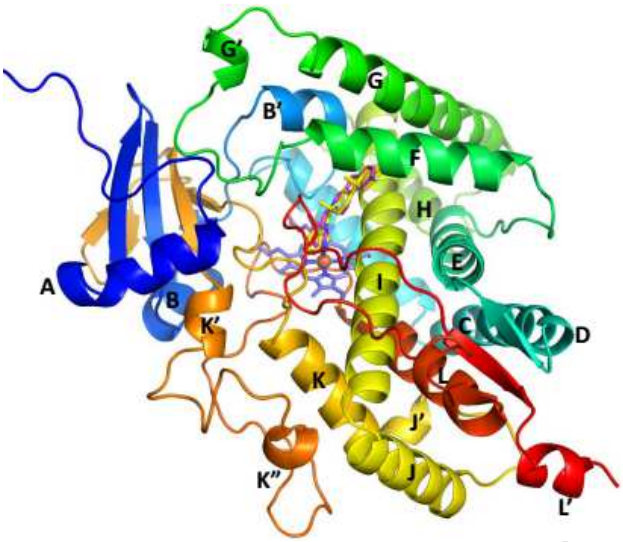


Figure 4A. Superposition of compound **9c** with TOK-001 crystal structure by docking (pdb code 3swz). CYP17A1 is represented by color scheme from blue (N terminus) to red (C terminus). All ligands are represented by stick and sphere model, with Fe in gold sphere, haem in blue sticks, TOK-001 in yellow sticks, and Lig001 in magenta sticks.

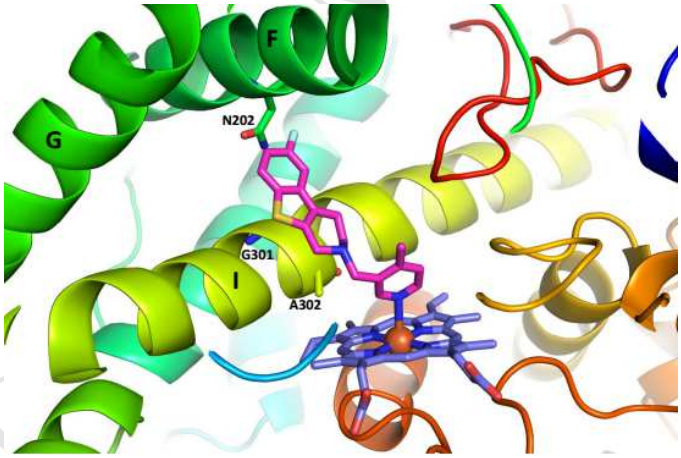
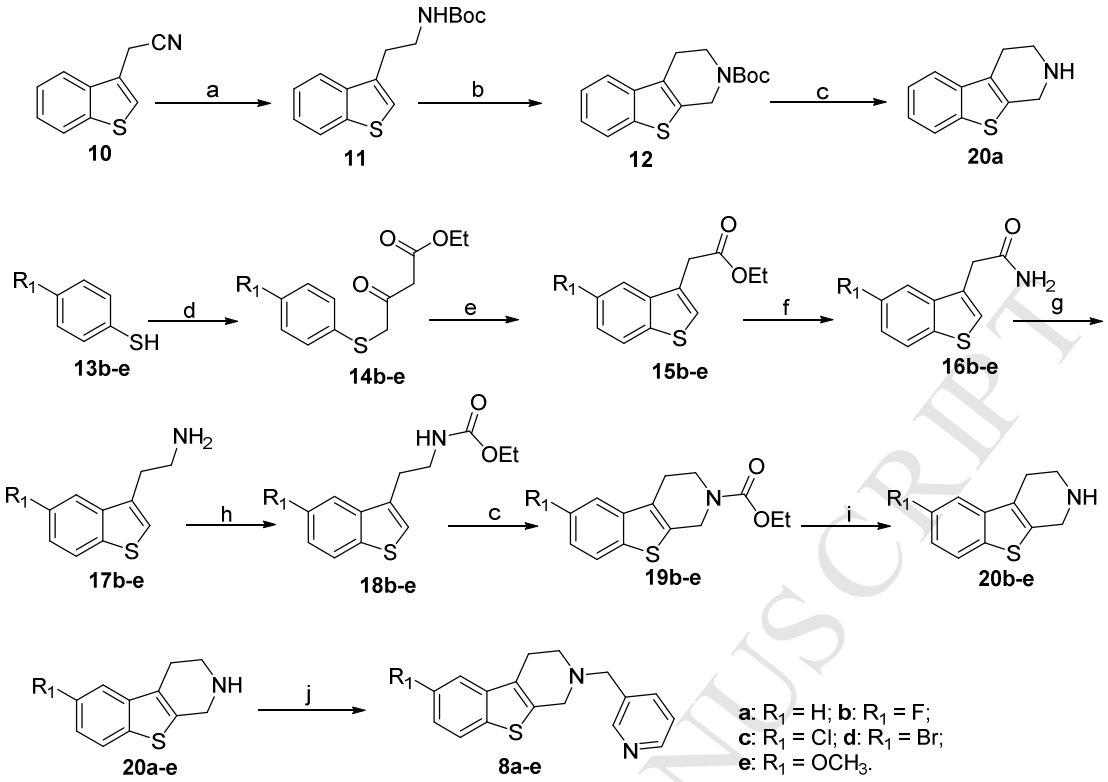
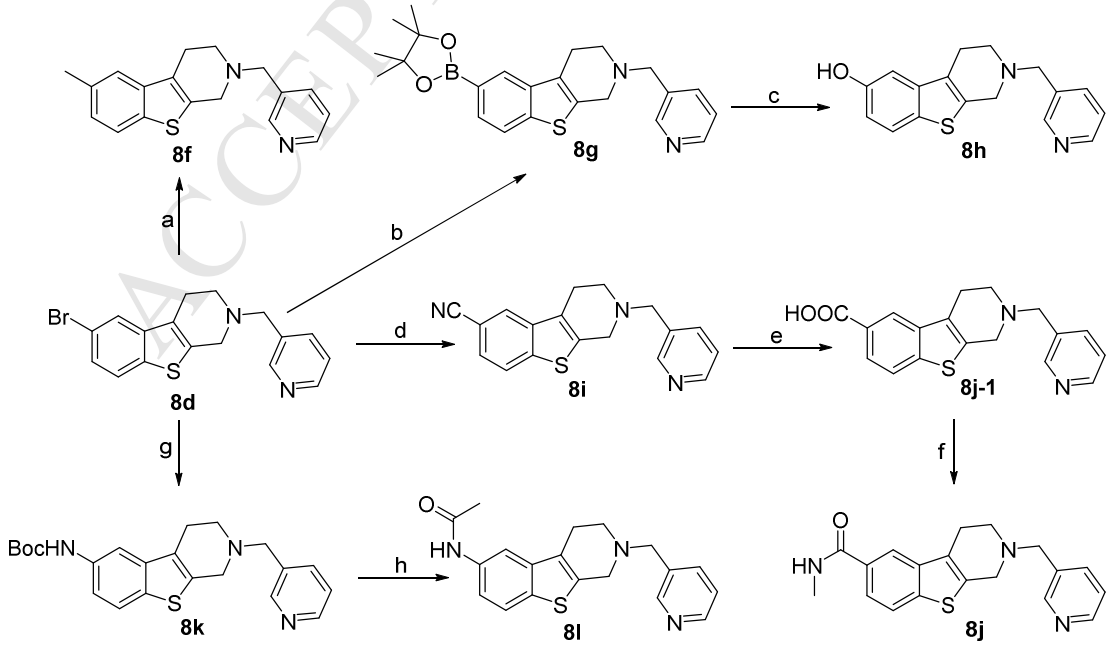


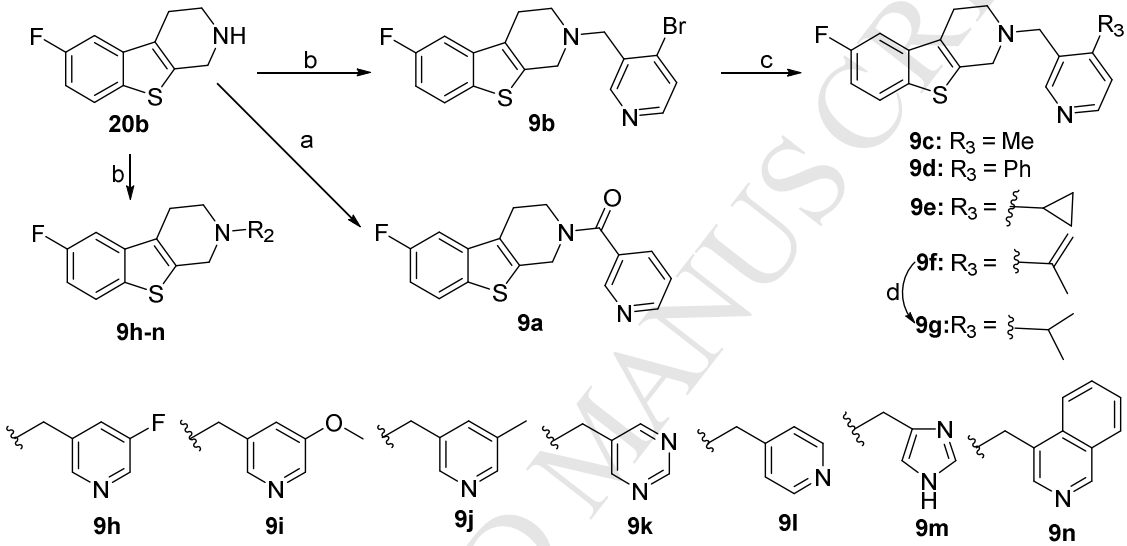
Figure 4B. Compound **9c** binding mode. Compound **9c** is colored in magenta, Haem is colored in blue, and Fe is colored in gold.



Scheme 1. Synthesis of compounds **8a-e**. Reagents and reaction condition: (a) $NaBH_4$, $NiCl_2 \cdot 6H_2O$, MeOH, $(Boc)_2O$, 23 ^oC, 3h; (b) paraformaldehyde, TsOH, toluene, reflux, 2h; (c)TFA/DCM, 0 ^oC, 2h; (d) TEA, DCM, 0 ^oC, 2h; (e) PPA, toluene, 100 ^oC, 16h; (f) 7N NH_3/MeOH, 23 ^oC, 72h; (g) BH_3, THF, reflux, 12h; (h) TEA, DCM, 0 ^oC, 2h; (i) NaOH, ethanol, H_2O, reflux, 12h; (j) 3-pyridinecarboxaldehyde, $NaBH(OAc)_3$, acetic acid, DCE, 23 ^oC, 12h.

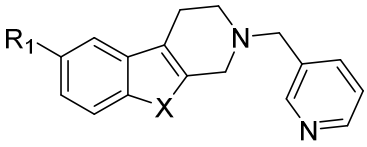


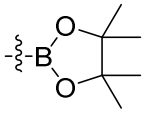
Scheme 2. Synthesis of compounds **8f-i**. Reagents and reaction condition: (a) $PdCl_2(dppf)\cdot CH_2Cl_2$, Na_2CO_3, methylboronic acid, dioxane/H_2O, 100 ^oC, 8h; (b) $PdCl_2(dppf).CH_2Cl_2$, KOAc, bis(pinacolato)diboron, DMF, 100 ^oC, 3h; (c) NaOH, H_2O_2 (30%), THF/MeOH, 0 ^oC to rt, 2h; (d) $Pd(PPh_3)_4$, $Zn(CN)_2$, DMF, 100 ^oC, 6h; (e) NaOH, EtOH/H_2O, reflux, 12h; (f) methylamine hydrochloride, HATU, DIPEA, DMF, rt, 12h; (g) NH_2Boc, $Pd_2(dba)_3$, Xantphos, Cs_2CO_3, dioxane, 100 ^oC, 6h; (h) (i) TFA/DCM, rt, 2h; (ii) $(Ac)_2O$, DIPEA, DCM, rt, 12h.



Scheme 3. Synthesis of compounds **9a-n**. Reagents and reaction condition: (a) nicotinic acid, HATU, DIPEA, DMF, rt, 12h; (b) aldehyde, $NaBH(OAc)_3$, acetic acid, DCE, 23 ^oC, 12h; (c) boronic acid, Na_2CO_3, $PdCl_2(dppf)\cdot CH_2Cl_2$, dioxane/$H_2O$, 100 ^oC, 8h; (d) Pd/C, MeOH, rt, 4h.

Table 1. IC_{50} values of compounds **7** and **8a-l** toward rat CYP17[a,b,c]



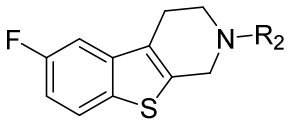
Compd	X	R1	Rat CYP17 IC_{50} (nM)
7	N	H	228.5
8a	S	H	54.9
8b	S	F	28.1
8c	S	Cl	448.6
8d	S	Br	516.9
8e	S	OCH_3	>1000
8f	S	CH_3	257.1
8g	S		115.0
8h	S	OH	20.9
8i	S	CN	257.1
8j	S	CONHMe	>1000
8k	S	NHBoc	>1000
8l	S	$NHCOCH_3$	>1000
ABT			24.7

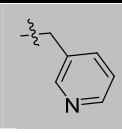
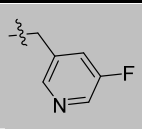
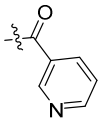
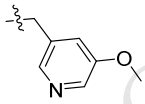
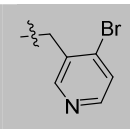
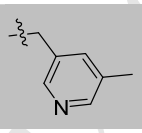
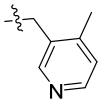
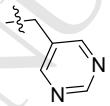
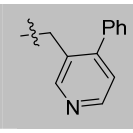
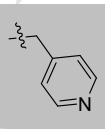
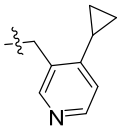
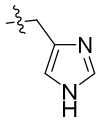
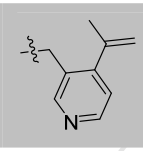
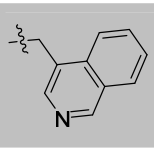
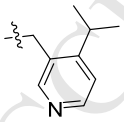
[a] Concentration of 17α-hydroxyprogesterone (substrate): 10 μM; standard deviations were with < ± 5%.

[b] The given values are mean values of at least three experiments. The deviations were with < ± 5%.

[c] ABT: Abiraterone (reference compound).

Table 2. IC_{50} values of compounds **9a-n** toward rat CYP17[a,b,c]



Compd	R_2	Rat CYP17 IC_{50} (nM)	Compd	R_2	Rat CYP17 IC_{50} (nM)
8b		28.1	**9h**		56.5
9a		>1000	**9i**		33.9
9b		75.8	**9j**		120.6
9c		15.8	**9k**		80.59
9d		>1000	**9l**		>1000
9e		61.1	**9m**		29.8
9f		979.8	**9n**		389.8
9g		535.1	**ABT**		24.7

[a] Concentration of 17α-hydroxyprogesterone (substrate): 10 μM; standard deviations were with < ± 5%.

[b] The given values are mean values of at least three experiments. The deviations were with < ± 10%.

[c] ABT: Abiraterone (reference compound).

Table 3. IC_{50} values of selected compounds toward CYP17 and CYP3A4[a,b,c]

Compd	Rat CYP17 IC_{50} (nM)	Human CYP17 IC_{50} (nM)	CYP3A4 IC_{50} (nM)
8b	28.1	29.0	7897
8h	20.9	23.7	5282
9c	15.8	20.1	8561
9h	56.5	45.4	9032
9i	33.9	45.0	6526
9m	29.8	24.3	685
ABT	24.7	35.8	2700

[a] Concentration of 17α-hydroxyprogesterone (substrate): 10 μM; standard deviations were with < ± 5%.
[b] The given values are mean values of at least three experiments. The deviations were with < ± 10%.
[c] ABT: Abiraterone (reference compound).

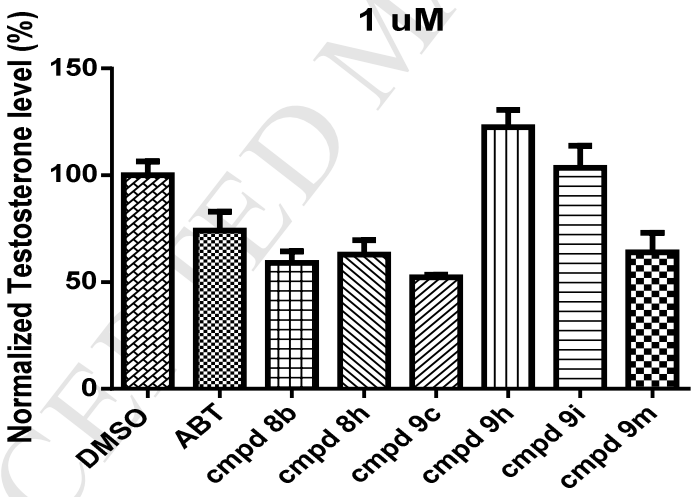


Table 4. Inhibitory activity of selected compounds toward testosterone production in NCI-H295R cells.

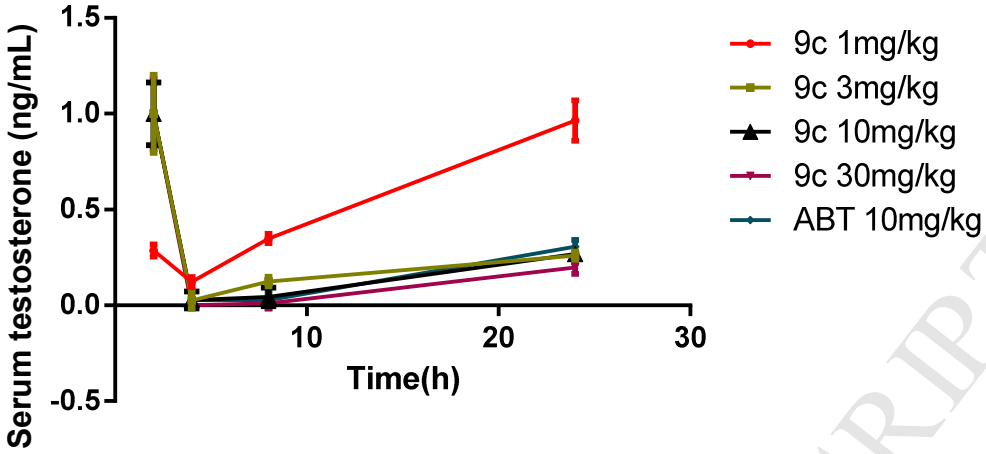


Table 5. Reduction of plasma testosterone levels by compound **9c** and ABT in male rats. Male rats were treated orally with compounds and the blood sample was obtained before and 2, 4, 8 and 24 h after dosing to measure the serum testosterone levels. Compound **9c** was applied at dose at 1 mg/kg, 3 mg/kg, 10 mg/kg and 30 mg/kg, ABT (abirateron) was administrated at dose at 10 mg/kg. Serum testosterone levels were determined by Testosterone ELISA and were expressed as percentages of the mean pretreatment values. Each value represents mean ± standard error of the mean (SEM [n = 6]).

Research highlights

- Novel potent and selective CYP17 inhibitors have been discovered.
- Compound **9c** showed more potent inhibitory activity than abiraterone for rat and human CYP17.
- Compound **9c** reduced testosterone production in both NCI-H295R cells and male rat.