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# Synthesis and Structure–Activity Relationship Studies of Pyrido [1,2-*E*]Purine-2,4(1*H*,3*H*)-Dione Derivatives Targeting Flavin-Dependent Thymidylate Synthase in *Mycobacterium Tuberculosis*

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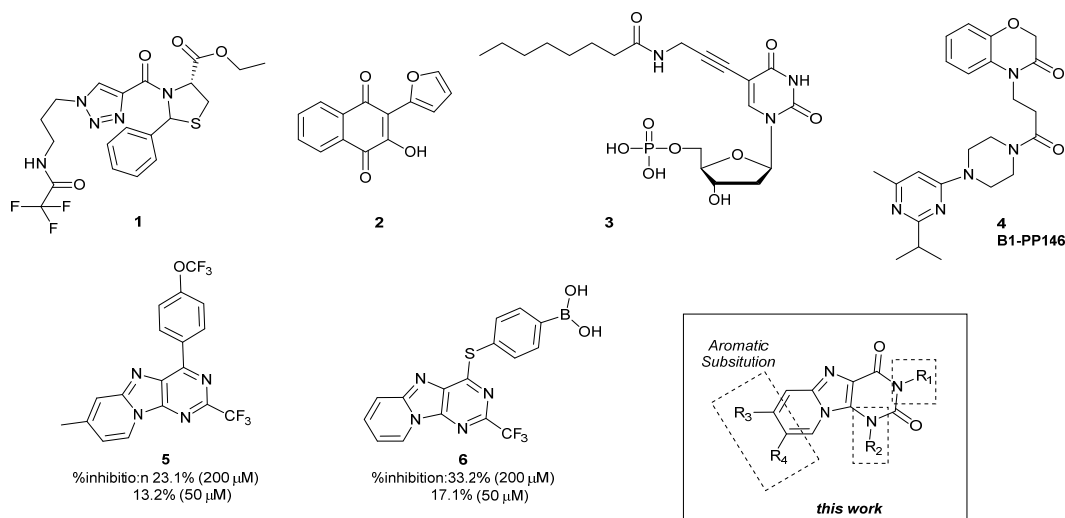
**Abstract:** In 2002, a new class of thymidylate synthase (TS) involved in the de novo synthesis of dTMP named *Flavin-Dependent Thymidylate Synthase (FDTS)* encoded by the *thyX* gene was discovered; *FDTS* is present only in 30% of prokaryote pathogens and not in human pathogens, which makes it an attractive target for the development of new antibacterial agents, especially against multi-resistant pathogens. We report herein the synthesis and structure-activity relationship of a novel series of hitherto unknown *pyrido[1,2-*E*]purine-2,4(1*H*,3*H*)-dione* analogues. Several synthetic efforts were done to optimize regioselective *N*<sup>1</sup>-alkylation through organopalladium cross-coupling. Modelling of potential hits were performed to generate a model of interaction into the active pocket of *FDTS* to understand and guide further synthetic modification. All those compounds were evaluated on an in-house in vitro *NADPH oxidase* assays screening as well as against *Mycobacterium tuberculosis ThyX*. The highest inhibition was obtained for compound **23a** with 84.3% at 200  $\mu$ M without significant cytotoxicity ( $CC_{50} > 100 \mu$ M) on PBM cells.

**Keywords:** flavin-dependent thymidylate synthase; *pyrido[1,2-*e*]purine-2,4(1*H*,3*H*)-dione* analogues; structure-activity relationship; sonogashira and Suzuki–Miyaura cross-coupling

## 1. Introduction

The excessive use of antibiotics in humans and animals has led to the appearance of multi-resistant bacteria (BMRs) and as a consequence to an increased mortality [1,2]. In 2014, the World Health Organization warned of a risk of antibiotic shortages by 2050 if nothing was done. Today, *E. coli*, *K. pneumoniae* and *S. aureus* are resistant to more than 50% of the main antibacterial drugs. From a simple natural genetic evolution, the situation has become a global public health problem, promoting the discovery of new therapeutic targets in order to develop new antibacterial substances. In most eubacteria, plants and eukaryotic cells, *thymidylate synthases* (TS or ThyA) [3] provide the only de novo source of 2'-deoxythymidine-5'-monophosphate (dTMP) required for DNA synthesis. The activity of these enzymes is pivotal for bacterial DNA replication and repair. Reductive methylation of 2'-deoxyuridine-5'-monophosphate (dUMP) to 2'-deoxythymidine-5'-monophosphate (dTMP) was catalyzed by three coupling enzymes of folate metabolism [4]. TS catalyzes methylation by using (6*R*)-*N*<sup>5</sup>,*N*<sup>10</sup>-methylene-5,6,7,8-tetrahydrofolate (CH<sub>2</sub>THF) as a methylene and hydride donor, which results in the formation of dTMP and 7,8-dihydrofolate (DHF). *Dihydrofolate reductase* (DHFR) catalyzes reduction of DHF to THF and

the *serinehydroxymethyl transferase* (SHMT) catalyzes the serine-glycine conversion which is concomitant to the conversion of THF to CH<sub>2</sub>THF as cofactors. TS inhibitors were used as cytotoxic agents, but the lack of selective TS bacterial inhibitors over human has hampered their application. More recently, a new class of TS was discovered [5]. This enzyme is encoded by the *ThyX* gene (formerly *Thy1*) and is absent in the vast majority of eukaryotic cells and only present in approximately 30% of gram-positive or negative pathogenic prokaryotes [6]. It is a *flavin-dependent thymidylate synthase* (*FDTS*), which has a unique mechanism, structure and gene sequence, making it an attractive new therapeutic target for the development of new selective bioactive compounds. *FDTS* uses a FAD as methylene carrier intermediate but also as hydride donor through the reduced form FADH<sub>2</sub> obtained from *NADPH oxidase* activity. Unlike human TS, *FDTS* produces tetrahydrofolate (H<sub>4</sub>folate) indeed of dihydrofolate (H<sub>2</sub>folate) [7]. Thus, *FDTS* can catalyze multiple biotransformation reactions in comparison to the classical TS. So far, few *ThyX* inhibitors have been reported like the 5-fluoro-2'-deoxyuridine-5'-monophosphate (5-FdUMP) and Raltitrexed®, but their poor selectivity between *ThyA* and *ThyX* has hampered their development as new drugs [8]. The thiazolidine analog **1** (Figure 1), reported by *Mylykallio* et al. [9], exhibited an IC<sub>50</sub> of 0.057 μM against *ThyX*. The same group reported a second series of *FDTS* inhibitors discovered from high-throughput screening (HTS) of natural and synthetic compounds [10]. After synthetic modifications and biological evaluation, the benzoquinone analogue **2** was reported to have an inhibitory K<sub>i</sub> value of 28 nM against *ThyX*. Those molecules did not show any mitochondrial toxicity. However, the major matter of discussion is linked to the quinone properties in medicinal chemistry that allow strong redox stress and Michael acceptor which could conduct to cellular damage and cell protein alkylation, [11]. C<sup>5</sup>-modified dUMP analogs were described by *Herdewijn* et al. [12]; among them, compound **3** exhibited an IC<sub>50</sub> value of 0.91 μM. More recently, new series of inhibitors bearing benzo[*b*][1,4]oxazin-3(*4H*)-one scaffold was reported by high-throughput screening of commercially available compounds. Structure activity was performed and led to compound B1-PP146 (**4**) with an IC<sub>50</sub> value of 0.69 μM against *ThyX* [13].



**Figure 1.** Recent inhibitors of *ThyX* and targeted pyrido [1,2-3]purine-2,4(1*H*,3*H*)-dione compounds.

*FDTS* is thus an attractive antibacterial target to the development of new and specific drugs to overcome bacterial resistance. By screening of our in house-synthesized compound libraries, two new pyridopurines analogs **5** and **6** were found to exhibit 23.1% and 33.2% *ThyX* inhibitory effect at 200 μM, respectively. Based on those results, we decided

to use pyrido [1,2-*e*]purine-2,4(1*H*,3*H*)-dione as scaffold to new and more active compounds through diversity-oriented synthesis. The current study aimed at identifying substituents at *N*<sup>1</sup> and/or *N*<sup>3</sup> positions, which could increase *ThyX* inhibitory activity. In this manuscript, the *FDTs* enzyme from *M. tuberculosis* (*MtbThyX*) was chosen for biological and docking assays due the emergence of *multidrug-resistant* strains of *Mycobacterium tuberculosis* (*MDR-TB*) [14].

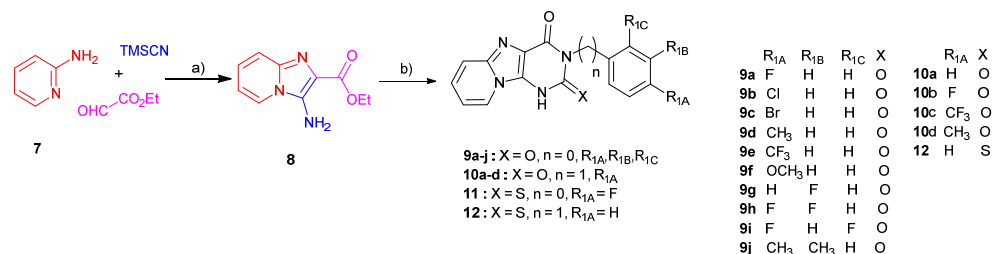
## 2. Results and discussions

### 2.1. In Vitro Mycobacterial *ThyX* Inhibition Assay for Structure–Activity Relationship Studies

To investigate Structure-Activity Relationship (SAR) in a systematic way, we used a *NADPH oxidase* spectrophotometric assay, adapted from Basta et al. [15], to test the in vitro inhibitory activities of synthesized compounds on *Mtb ThyX*, and discuss the influence of substituents. The most active of our compounds was used as reference scaffold for future optimization. Assay reactions, with a final volume of 100  $\mu$ l, consisted of 750  $\mu$ M NADPH, 100  $\mu$ M dUMP, 2 mM MgCl<sub>2</sub>, 1% glycerol, 50  $\mu$ M FAD and 10  $\mu$ M of *ThyX*. The reactions were initiated by injection of NADPH (15  $\mu$ L of a 5 mM solution) to each well of the microtiter 96-well clear flat-bottom plate, followed by rapid shaking of the microplate. *ThyX* activity was determined by following the decrease of absorbance at  $\lambda_{340}$  (due to oxidation of NADPH). All assays used a kinetic mode of a multilabel microplate reader with an injector. The primary screen was performed with molecules dissolved in DMSO, including DMSO alone as low-activity control. B1-PP146 (**4**), with a 1,4-benzoxazine moiety and described as tight-binding inhibitors, was used as reference compound [16]. All screening reactions were performed in duplicates at 200  $\mu$ M concentration.

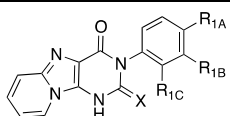
### 2.2. Modification at the *N*<sup>3</sup> Position of Purine Scaffold and SAR Studies

Firstly, *N*<sup>3</sup>-position of pyrido [1,2-*e*]purine-2,4(1*H*,3*H*)-dione scaffold were modified by various *N*-substitutions (arylations, benzylation, etc.). Three different methods were reported to synthesize pyrido [1,2-*e*]purine-2,4(1*H*,3*H*)-dione libraries. Through C–H annulation from anilopyrimidine [17], by a Ugi–Strecker and isocyanate cyclisation [18,19] or by copper catalyzed cyclisation [20]. In order to access to substituted tricyclic structure with *N*<sup>3</sup>-aryl or benzyl diversities, we choose the successive double cyclisation starting from aminopyridine derivatives (Scheme 1). Commercially available 2-aminopyridine **7** and ethyl glyoxylate afforded in moderate yield, the intermediate **8** through Ugi–Strecker reaction in presence of trimethylsilyl cyanide and DABCO under microwave irradiation (MW) at 120 °C during 15 min. Treatment of **8** in presence of EtONa and isocyanates, such as phenyliso- benzyliso- or thioisocyanate derivatives under microwave irradiation afforded the corresponding tricyclic scaffolds (**9a–j**), (**10a–d**), **11** and **12**.



**Scheme 1.** Reagents and conditions: a) DABCO, THF, MW 120 °C, 15 min. b) Phenyl, Benzyl or thioisocyanates, NaOEt, EtOH, MW 120 °C, 20 min.

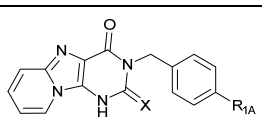
The synthesized compounds, pyrido [1,2-*e*]purine-2,4(1*H*,3*H*)-dione **9a–j** and thio derivative **11** substituted at *N*<sup>3</sup>-position by phenyl, were evaluated for their abilities to inhibit the *ThyX* enzyme (Table 1)

**Table 1.** SAR at the N<sup>3</sup>-position by phenyl derivatives **9a–j** and **11** and the Mtb ThyX inhibition at 200  $\mu$ M by the NADPH oxidase assay.


Compound	R <sub>1A</sub>	R <sub>1B</sub>	R <sub>1C</sub>	X	Inhibition %
<b>9a</b>	F	H	H	O	59.4
<b>9b</b>	Cl	H	H	O	49.8
<b>9c</b>	Br	H	H	O	52.2
<b>9d</b>	CH <sub>3</sub>	H	H	O	55.4
<b>9e</b>	CF <sub>3</sub>	H	H	O	31.7
<b>9f</b>	OCH <sub>3</sub>	H	H	O	8.1
<b>9g</b>	H	F	H	O	12.8
<b>9h</b>	F	F	H	O	22.7
<b>9i</b>	F	H	F	O	6.6
<b>9j</b>	CH <sub>3</sub>	CH <sub>3</sub>	H	O	19.9
<b>11</b>	F	H	H	S	19.8
<b>B1-PP146</b> <sup>a</sup>					95.1 <sup>b</sup>

<sup>a</sup> B1-PP146, a compound with a 1,4 benzoxazine ring, used as positive control; <sup>b</sup> value at 50  $\mu$ M. The standard deviations from two independent experiments is between 3 and 9, as a function of the molecules.

To probe into the optimal scaffold of central heterocycle, the phenyl *para* substitution was studied. With small electron-withdrawing groups, such as fluorine (**9a**), chlorine (**9b**) and bromine (**9c**), a 49–59% inhibition was obtained with the best value for the fluorine derivative. By increasing hydrophobic and electron-withdrawing effects (**9e**), less inhibitory activity was observed (31.7%). Substitution at *para* position with electro-donating group (–OCH<sub>3</sub>) **9f** decreases the activity to 8.1% whereas the methyl group (**9d**) led to 55% inhibition. On the other hand, fluorine analogs of **9a**, by substitution at *meta* position (**9g**) or a second fluorine substitution at *ortho/para* (**9h**) or *meta/para* (**9i**) positions, results in loss of *ThyX* inhibition. Polysubstitution with electron-donating group such as methyl groups (**9j**) also display low enzyme inhibition. We investigate the substitution of oxygen by sulfur atom at 2 position, and we observe that compound **11** decreases drastically the inhibitory effect from 59.4% to 19.8% to compare with **9a**. We also looked at the influence of flexibility induced by benzylic substitution (Table 2). Both benzyl analogs **10a–d** and **12** showed a significant loss of activity (< 25%) in comparison with the aryl derivatives **9**.

**Table 2.** Mtb ThyX inhibition at 200  $\mu$ M by the NADPH oxidase assay of the benzyl derivatives **10** and **12**.


Compound	R <sub>1A</sub>	X	Inhibition %
<b>10a</b>	H	O	22.3
<b>10b</b>	F	O	21.2
<b>10c</b>	CF <sub>3</sub>	O	20.7
<b>10d</b>	CH <sub>3</sub>	O	20.2
<b>12</b>	H	S	0.0
<b>B1-PP146</b> <sup>a</sup>			95.1 <sup>b</sup>

<sup>a</sup> B1-PP146, a compound with a 1,4 benzoxazine ring, used as positive control; <sup>b</sup> value at 50  $\mu$ M. The standard deviations from two independent experiments is between 3 and 9, as a function of the molecules.

Overall, this SAR study reveals that flexibility, sulfur substitution, polysubstitution or large groups decrease inhibitory activities. On phenylic substitution, small electron-withdrawing or electron-donating have shown reasonable inhibition (> 50%). To determine central scaffold hit, other assays were performed by decreasing [FAD] inhibitors concentration, through tritium release assay (Table 3). Without FAD adding, only 1.1–1.5 of 4 active sites were occupied by this natural cofactor. Without excess of FAD, we can detect a competition effect between FAD and potential inhibitors. By decreasing inhibitors concentration to 50  $\mu$ M, we can study the true potential of our molecules. The tritium release assay permitted us to study inhibition on the second mechanism part (methylene transfer).

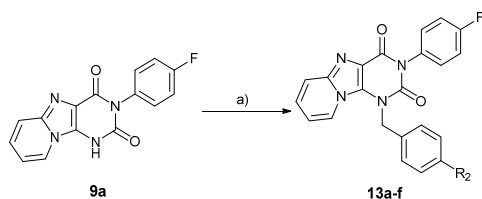
**Table 3.** *ThyX* inhibition assays of **9a,b** and **d** in various conditions.

	Without FAD addition	At 50 $\mu$ m of inhibitors	Tritium release assay
<b>9a</b>	76.1%	18.4%	59.5%
<b>9b</b>	12.6%	2.2%	69.2%
<b>9d</b>	20.4%	2.6%	77.1%

Compound **9a** showed better activity, by increasing inhibition to 76.1% at 200  $\mu$ M in absence of FAD, which was not the case for compounds **9b** and **9d**, for which the inhibition effect had decreased. With a 50  $\mu$ M inhibitor concentration, only compound **9a** provided an equal inhibitory activity to the reference compounds **5** and **6**, and no inhibition was observed for **9b** and **9d**. Through a tritium release assay, compounds **9b** and **9d** exhibited increased inhibitory activities on *ThyX* with 77.1% and 69.2% at 200  $\mu$ M, respectively, which suggested that these molecules would be more active in the methylene transfer mechanism. On the other hand, compound **9a** showed similar inhibition on *Mtb ThyX* (59.5% at 200  $\mu$ M, Table 3) to the NADPH oxidase assay (59.5% at 200  $\mu$ M, Table 1). Because most of the active compounds on the first part of the mechanism were also active on the second part (tritium release assay), NADPH oxidase assay was taken as a reference test to determine inhibitory activities and compound **9a** as the scaffold to perform other modifications in search of better activity.

### 2.3. Modification at the *N*<sup>1</sup> position of compound 9 taken as scaffold and SAR studies

Starting from **9a** as scaffold, a library of molecules with structural modifications through *N*<sup>1</sup>-alkylation with various benzyl groups was obtained (**13a–f**) (Scheme 2). In the first attempt, in the presence of K<sub>2</sub>CO<sub>3</sub>, in DMF at room temperature during 12 h a competition between *N*<sup>1</sup>- and *O*<sup>2</sup>-alkylation was observed (by HMBC-NMR), which decreased the yield and deteriorated the purification. In this case, we investigated the solvent effects (DMF, THF), base influence (K<sub>2</sub>CO<sub>3</sub>, Cs<sub>2</sub>CO<sub>3</sub>, LiH, NaH, etc.,) and activation mode (microwave, sonication) to enhance the *N*<sup>1</sup>-regioisomers. We observed that only the *N*<sup>1</sup>-alkylated regioisomer was obtained under microwave activation at 120 °C during 20 min with potassium carbonate and molecular sieves in presence of various bromobenzyl analogs (Scheme 2).



**Scheme 2.** Reagents and conditions: a)  $K_2CO_3$ , benzyl bromide derivatives, 4 Å MS, DMF, MW 120 °C, 20 min.

The obtained benzylic derivatives **13a–f** were then evaluated against *FDTS* (Table 4).

**Table 4.** Mtb ThyX inhibition at 200  $\mu$ M by the NADPH oxidase assay of benzyl analogues **13a–f**.

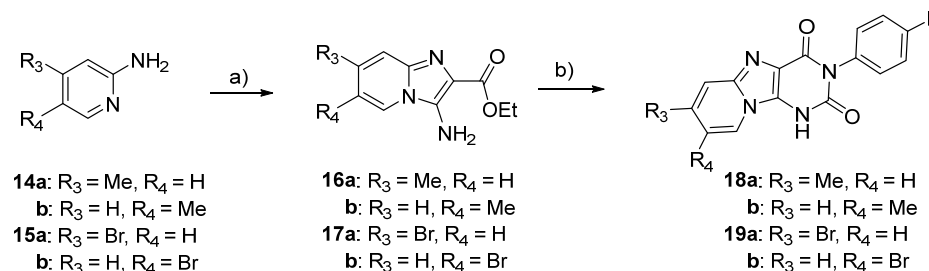
Compound	R <sub>2</sub>	Inhibition %
<b>13a</b>	H	23.2
<b>13b</b>	Me	10.9
<b>13c</b>	CN	26.5
<b>13d</b>	NO <sub>2</sub>	0
<b>13e</b>	COOMe	44.8
<b>13f</b>	PO(OMe) <sub>2</sub>	5.6
<b>B1-PP146</b> <sup>a</sup>		95.1 <sup>b</sup>

<sup>a</sup> B1-PP146, a compound with a 1,4 benzoxazine ring, used as positive control; <sup>b</sup> value at 50  $\mu$ M. The standard deviations from two independent experiments are between 3 and 9, as a function of the molecules.

We observed that the presence of benzyl group (**13a**), and substituted derivatives at the *para* position by methyl (**13b**) or electron-withdrawing group (**13c–f**) result in lack of inhibition activity compared to the reference **9a** (59.4%). From this study, further modifications at C7 and C8 moiety of compound **9a** with free N<sup>1</sup>-position were thus performed.

#### 2.4. Modification at the C<sup>7</sup> and C<sup>8</sup> position of compound **9a** and SAR studies

After modulation on the pyrimidine-2,4-dione ring, we investigated the influence of various substituents on the aromatic ring at C<sup>7</sup> and C<sup>8</sup> positions. We first synthesized under microwave activation two series of substituted molecules by methyl group (**18a,b**) or bromine (**19a,b**) (Scheme 3). Halogen derivatives **19a,b** were used as starting material to develop a library of 24 molecules through palladium cross-coupling reactions (Scheme 4).



**Scheme 3.** Reagents and conditions: a) DABCO, TMSCN, ethyl glyoxylate, THF, MW 120 °C, 15 min. b) Phenylisocyanate, NaOEt, EtOH, MW 120 °C, 20 min.





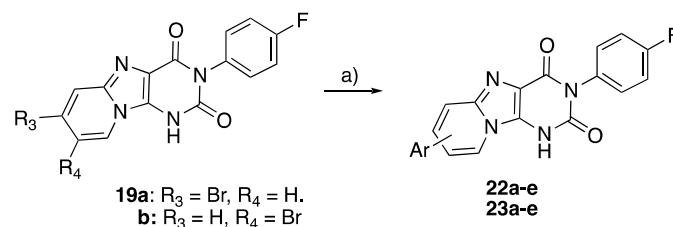
<b>21c</b>	H		0
<b>21d</b>	H		32.5
<b>21e</b>	H		31.7
<b>21f</b>	H		16.9
<b>21g</b>	H		28.2
<b>B1-PP146</b> <sup>a</sup>			95.1 <sup>b</sup>

<sup>a</sup> B1-PP146, a compound with a 1,4 benzoxazine ring, used as positive control; <sup>b</sup> value at 50  $\mu$ M. The standard deviations from two independent experiments are between 3 and 9, as a function of the molecules.

The different substituents on C<sup>7</sup> and C<sup>8</sup> position were chosen for their abilities to generate flexibility, to create a hydrogen bond, hydrophobic interactions and  $\pi$ -stacking interactions with the active site of the enzyme. Modifications at the C<sup>7</sup>-position led to derivatives with aromatic alkynes, which induce flexibility, the presence of aliphatic chain at *para* position (compounds **20a** and **20b** respectively) or aliphatic chain with 5,6 and 7 (compounds **20c**, **20d** and **20e** respectively) which have shown no to low inhibition still below that of the **9a** activity. Compounds **20f** and **20g**, substituted by side-chain presenting hydrogen-bound site with amide and urea moiety (respectively) and hydrophobic chain, did not show more efficiency against ThyX. The same substituents were introduced at C<sup>8</sup>-position of **9a**. Even if most of the molecules do not present an inhibitory activity higher than 33%, this position is however more favorable than that in C<sup>7</sup>. In fact, we observe for the aromatic group derivatives **21a** and **21b**, higher inhibitions than the position C<sup>7</sup>, and especially for **21b** inhibitory activity was higher (69.1% at 200  $\mu$ M) than **9a** (59.4%). Overall, this SAR study reveals that the aromatic ring at C<sup>8</sup>-position increases inhibitory activities as well as maybe some flexibility.  $\pi$ -stacking interactions seem to be predominant in order to increase the activity, which is why we have synthesized a new series of molecules bearing aromatic groups by the Suzuki–Miyaura cross-coupling reaction. Expected molecules were planar and could be intercalated between the different co-factors into the enzyme pocket.

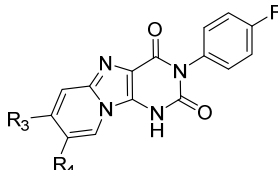
#### 2.4.2. Modifications by the Suzuki–Miyaura cross-coupling reaction



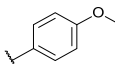
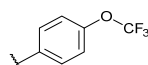



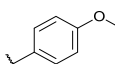
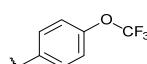

Modifications under the Suzuki–Miyaura cross-coupling conditions [25] were also performed under microwave irradiations [26,27] in presence of Cs<sub>2</sub>CO<sub>3</sub>, Pd(PPh<sub>3</sub>)<sub>4</sub>, and various boronic acid derivatives in DMF at 120 °C for 40 min. A library of **9a** analogs substituted by aromatic derivatives was isolated (**22,23a–e**) in 11 to 50% yield (Scheme 5).



**Scheme 5.** Cs<sub>2</sub>CO<sub>3</sub>, Pd(PPh<sub>3</sub>)<sub>4</sub>, R-B(OH)<sub>2</sub>, a): DMF, MW 120 °C, 40 min.

All those compounds were then evaluated (Table 6).

**Table 6.** SAR of the C<sup>7</sup> and C<sup>8</sup> moiety by the Suzuki–Miyaura cross-coupling and Mtb ThyX inhibition at 200 μM by the NADPH oxidase assay.


Compound	R <sub>3</sub>	R <sub>4</sub>	Inhibition %
22a		H	20.7
22b		H	45.7
22c		H	24.4
22d		H	27.8
22e		H	14.2
23a	H		84.3
23b	H		44.4
23c	H		35.4
23d	H		29.4
23e	H		43.1
<b>B1-PP146</b> <sup>a</sup>			95.1 <sup>b</sup>

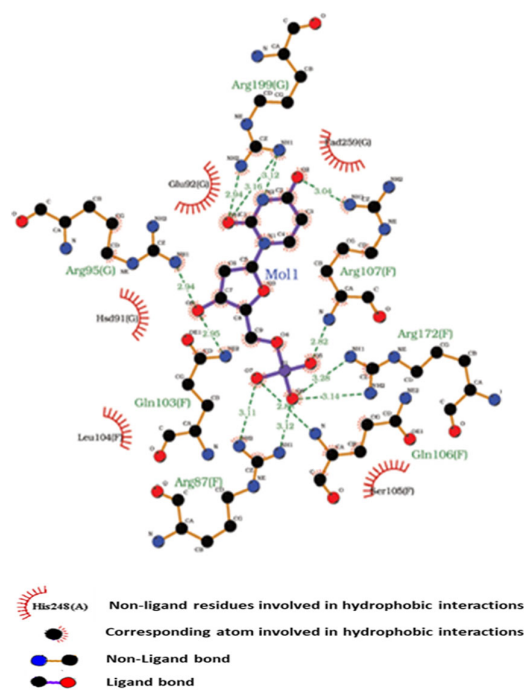
<sup>a</sup> B1-PP146, a compound with a 1,4 benzoxazine ring, used as positive control; <sup>b</sup> value at 50 μM. The standard deviations from two independent experiments is between 3 and 9, as a function of the molecules.

The aromatic substitution at C<sup>7</sup>-position-bearing with hydrogen or methyl (compound **22a** and **22b** respectively) showed low (**22a**: 20.7%) to moderate (**22b**: 45.7%) inhibition. With electron-withdrawing phenylic substitution such as methoxy, trifluoromethyl ether or fluorine (**22c**, **22d** and **22e** respectively) inhibitory activities do not exceed 28%. At C<sup>8</sup> position, the same substitutions were realized with electron-donating substituents at phenylic *para* position (compounds **23a** and **23b**). With **23b** equal inhibition was observed as the C<sup>7</sup> position. For **23a** a promising inhibition was observed with 84.3% at 200 μM. For electron-withdrawing substituents at phenylic *para* position, higher inhibitory activities were shown with methoxy (35.4%) and fluorine (43.1%) group (compound **23c** and **23e** respectively). In the case of trifluoromethyl ether **23d**, equal inhibition was observed. Overall, this SAR study revealed that small electron-withdrawing and donating groups showed only less to reasonable inhibitions. One of those compounds with phenyl

substitution on the C<sup>8</sup> showed the best inhibition of this study at 200  $\mu$ M. Planar structure and  $\pi$ -stacking interactions looked to be the best characteristic to develop potential *Mycobacterium tuberculosis* ThyX inhibitors.

### 2.5. Mycobacterial ThyX docking studies

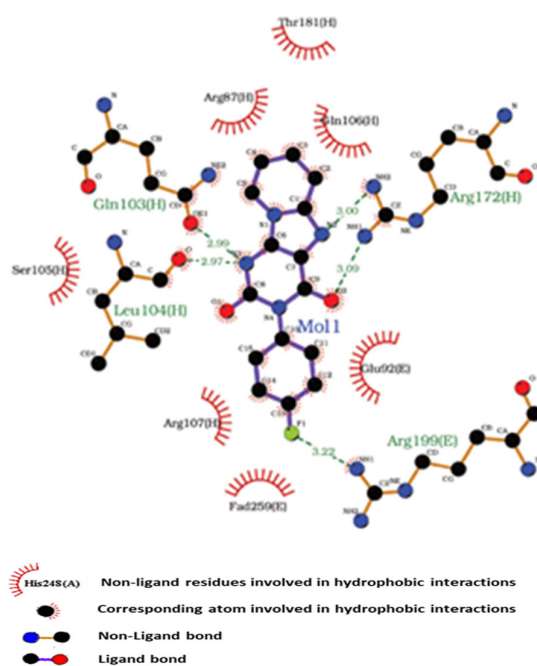
dUMP was docked into the binding site of ThyX from the *Mycobacterium tuberculosis* complex (Figure 2) with FAD, dUMP (PDB: 3GWC) as the starting structure to perform an initial molecular study. dUMP binds in the FDTs pocket by  $\pi$ -stacking with FAD, but also by several hydrogen bonds [28]. On the pyrimidine-2,4-dione ring with four hydrogen bonds: two between C<sup>2</sup>=O and Arg199; one between N<sup>3</sup> and Arg199; and finally one between C<sup>4</sup>=O and Arg107. On the 2'-deoxyribose part, only two hydrogen bonds were created between 3'-OH, Arg95 and Gln103. The last hydrogen bonds were localized on the phosphate moiety of dUMP. This part of the natural substrate acted like an anchor into active site; six hydrogen bonds were reported between oxygens of phosphonic group and Arg87 (2 bonds), Gln106 (1 bond), Arg172 (2 bonds) and Arg107 (1 bond).



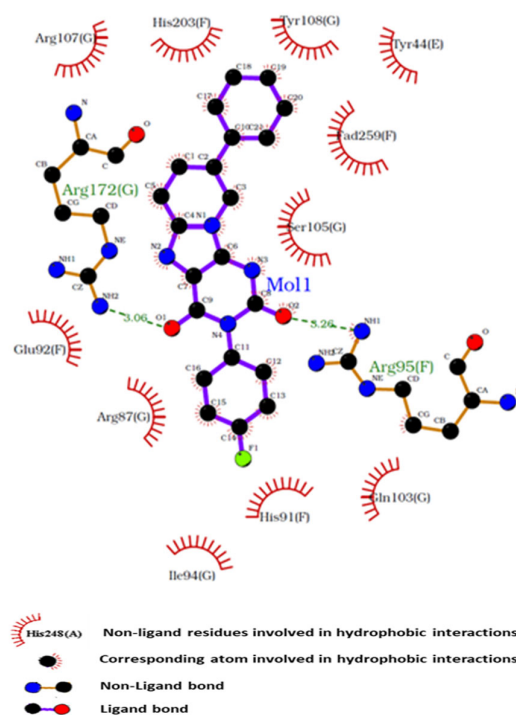
**Figure 2.** Hydrogen bond and hydrophobic interactions between the binding site of ThyX from *Mycobacterium tuberculosis* complex and natural substrate dUMP. The green dotted lines depict the hydrogen bonds whereas the other residues represent the hydrophobic interactions with the respective compounds.

Following this molecular modeling, compounds **9a** (Figure 3) and **23a** (Figure 4) with the highest inhibitions and results at NADPH oxidase assay and tritium release assay, were docked into the FAD and dUMP pocket with 13 amino acid residues. Compound **9a** (Figure 3) was the first docked into *Mycobacterium tuberculosis* pocket. It showed reasonable inhibition at 200  $\mu$ M (59.4%), a reduced activity at 50  $\mu$ M (18.4%). By diminution of [FAD] it showed higher inhibition (76.1%) that suggested possible competition with the natural co-factor. Compound **9a** took place closure than the FAD, its fluorophenyl group near the central ring of FAD, which let assumed  $\pi$ -stacking interactions. On the other hand, 2D representation into the pocket showed several hydrogen bond interactions with amino acid residues. Compound **9a** created five interactions with amino acid responsible of hydrogen bond formation with dUMP. The pyrimidine ring created three interactions with

Leu104 and Gln103 on its  $N^1$  position. One bond was created with the  $C^4=O$  and Arg172. The last two hydrogen bonds were placed on the  $N^5$  with Arg172 and on F with Arg199. The loss of inhibition could be explained by the large size of the S atom of compound 11, which then changes the positioning of the substrate in the active site of the protein. Delocalization **9g** and polysubstitution **9h**, **9i** and **9j** changed the capacity to create the hydrogen bond, but also the ring aromaticity. When fluorine was substituted by trifluoromethyl ether **9e**, the capacity to create bond hydrogen was replaced by a hydrophobic interaction, leading to a loss of inhibitory activity. With a smaller group like methyl **9d**, inhibition stays equal to compound **9a**. Hydrophobic interaction with a small group was equal to hydrogen bond interaction in this case. With methoxy substitution **9f**, the loss of inhibition could result from the change of bond angle. Insertion of carbon between the pyrimidinedione ring and phenyl **10a-d**, **12** increase flexibility of the structure and broke the planar scaffold. The loss of inhibition observed on  $N1$ -alkylated compound **13a-h** could be explained by the loss of the two hydrogen interactions from this position. Compound **23a** (Figure 4) was docked into *Mycobacterium tuberculosis* ThyX pocket. This compound presented the highest inhibition at 200  $\mu$ M.



**Figure 3.** Hydrogen bond and hydrophobic interactions between the binding site of ThyX from *Mycobacterium tuberculosis* complex and **9a**. The green dotted lines depict the hydrogen bonds whereas the other residues represent the hydrophobic interactions with the respective compounds.



**Figure 4.** Hydrogen bond and hydrophobic interactions between the binding site of ThyX from *Mycobacterium tuberculosis* complex with FAD and **23a**. The green dotted lines depict the hydrogen bonds whereas the other residues represent the hydrophobic interactions with the respective compounds.

Compared to **9a**, compound **23a** looked to be less stabilized into the pocket because of three hydrogen bonds interactions' loss. It still conserved two major hydrogen bond interactions with Arg95 (hydrogen bond interaction with 3'OH of dUMP) with C=O and Arg172 (hydrogen bond with phosphonic acid part of dUMP) with C=O. By looking at 3D modeling, we observed three new interactions which could explain biological results. Two  $\pi$ -stacking interactions from the phenyl with Tyr108 and His203. Another  $\pi$ -stacking could be observed on the *p*-fluorophenyl part with His91. The loss of inhibition by decreasing FAD concentration could be explained by the possible  $\pi$ -stacking interaction between isoalloxazine and central ring of pyrido [1,2-*e*]purine-2,4(1*H*,3*H*)-dione. However, one of the biggest difficulties with molecular docking of weak inhibition was the position into the pocket. Some of them were reproducible but a small modification could change everything; the large flexible pocket of *FDTS* was responsible for this issue.

### 3. Materials and methods

#### 3.1. Chemistry General section

Commercially available chemicals were provided as reagent grade and used as received. Some reactions requiring anhydrous conditions were carried out using oven-dried glassware and under an atmosphere of dry Argon. All anhydrous solvents were provided from commercial sources as very dry reagents. The reactions were monitored by thin layer chromatography (TLC) analysis using silica gel precoated plates (Kieselgel 60F254, E. Merck). Compounds were visualized by UV irradiation and/or spraying with sulfuric acid (H<sub>2</sub>SO<sub>4</sub> 5% in ethanol) stain followed by charring at average 150 °C. Flash column chromatography was performed on Silica Gel 60 M (0.040-0.063 mm, E. Merck). The infrared spectra were measured with Perkin-Elmer Spectrometer. The <sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded on BrukerAvance DPX 250 or BrukerAvance 400 Spectrometers. Chemical shifts are given in ppm and are referenced to the deuterated solvent signal or to TMS as

internal standard and multiplicities are reported as s (singlet), d (doublet), t (triplet), q (quartet) and m (multiplet). Carbon multiplicities were assigned by distortion less enhancement by polarization transfer (DEPT) experiments.  $^1\text{H}$  and  $^{13}\text{C}$  signals were attributed on the basis of H–H and H–C correlations. High Resolution Mass spectra were performed on a Bruker Q-TOF MaXis spectrometer by the “Fédération de Recherche” ICOA/CBM (FR2708) platform. LC-MS data were acquired on a Thermo-Fisher UHPLC-MSQ system equipped with an electron spray ionization source (ESI). The temperature of the source was maintained at 350 °C. Initially, the cone voltage was set at 35 V and after 5 min was increased to 75V. In full scan mode, data were acquired between 100 and 1000 m/z in the positive mode with a 1.00 S scan time. In addition, a UV detection was performed with a Diode array detector at three wavelengths 273, 254 and 290 nm, respectively. A water/methanol (70%/30%) solution mixture with 0.1% formic acid was used as mobile phase. The composition of the mobile phase was increased to 100% methanol with 0.1% formic acid with a 7% ramp. The flow rate was set at 0.300 mL.min<sup>-1</sup>. Samples diluted in the mobile phase were injected (3 µL) on a C18 column (X-terra, Waters), 2.1 mm internal diameter, and 100 mm length placed into an oven at 40 °C. Electronic extraction of ions was performed and the subsequent areas under the corresponding chromatographic peaks determined.

### 3.2. General synthetic procedure 1 for Strecker-Ugi cyclization

A mixture of amino pyridine derivatives (200 mg) and ethyl glyoxalate (50% solution in toluene) (1 eq.) was stirred at 25 °C for 2 min. THF (4.5 mL) and 1,4-diazabicyclo [2.2.2] octane (1 eq.) were subsequently added. The reaction mixture was cooled to 0–5 °C and cyanotrimethylsilane (1 eq.) was added. The mixture was heated under microwave irradiation at 120 °C for 15 min. The solvent was evaporated under reduced pressure. The crude product was dissolved with EtOAc, washed with K<sub>2</sub>CO<sub>3</sub>, dried over MgSO<sub>4</sub>, filtered and concentrated under reduced pressure.

Ethyl 3-aminoimidazo [1,2-*a*]pyridine-2-carboxylate (**8**). The title compound was prepared from commercially available **7** to afford after purification the desired product as a yellow solid (50%). CAS # 1487454-00-1.  $^1\text{H}$  NMR (250 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  8.17 (dt, 1H, *J* = 7.1, 1.1 Hz, H<sub>4</sub>), 7.31 (dt, 1H, *J* = 9.3, 1.1 Hz, H<sub>7</sub>), 7.05 (ddd, 1H, *J* = 9.3, 6.5, 1.1 Hz, H<sub>6</sub>), 6.79 (ddd, 1H, *J* = 7.1, 6.5, 1.1 Hz, H<sub>5</sub>), 6.35 (bs, 2H, NH<sub>2</sub>), 4.27 (q, 2H, *J* = 7.1 Hz, O–CH<sub>2</sub>), 1.31 (t, 3H, *J* = 7.1 Hz CH<sub>2</sub>–CH<sub>3</sub>) ppm.

Ethyl 3-amino-7-methylimidazo [1,2-*a*]pyridine-2-carboxylate (**16a**). The title compound was prepared from commercially available **14a** to afford after purification the desired product as an orange solid (45%). CAS # 1216262-11-1.  $^1\text{H}$  NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  7.98 (s, 1H, H<sub>4</sub>), 7.24 (d, 1H, *J* = 9.4 Hz, H<sub>7</sub>), 6.93 (d, 1H, *J* = 9.4 Hz, H<sub>6</sub>), 6.35 (bs, 2H, NH<sub>2</sub>), 4.26 (q, 2H, *J* = 7.0, Hz, O–CH<sub>2</sub>), 2.22 (s, 3H, C–CH<sub>3</sub>), 1.30 (t, 3H, *J* = 7.0, Hz CH<sub>2</sub>–CH<sub>3</sub>) ppm.

Ethyl 3-amino-6-methylimidazo [1,2-*a*]pyridine-2-carboxylate (**16b**). The title compound was prepared from commercially available **14b** to afford after purification the desired product as an orange solid (34%). CAS # 1498691-03-4.  $^1\text{H}$  NMR (400 MHz, Acetone-*d*<sub>6</sub>)  $\delta$  7.62 (d, 1H, *J* = 7.1 Hz, H<sub>4</sub>), 6.62 (s, 1H, H<sub>7</sub>), 6.19 (d, 1H, *J* = 7.1 Hz, H<sub>5</sub>), 5.82 (bs, 2H, NH<sub>2</sub>), 3.80 (q, 2H, *J* = 7.0, Hz, O–CH<sub>2</sub>), 1.82 (s, 3H, C–CH<sub>3</sub>), 0.85 (t, 3H, *J* = 7.0, Hz CH<sub>2</sub>–CH<sub>3</sub>) ppm.

Ethyl 3-amino-7-bromoimidazo [1,2-*a*]pyridine-2-carboxylate (**17a**). The title compound was prepared from commercially available **15a** to afford after purification the desired product as a yellow solid (47%). CAS # 1536009-01-4.  $^1\text{H}$  NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  8.16 (d, 1H, *J* = 7.4 Hz, H<sub>4</sub>), 7.86–7.71 (m, 2H, H<sub>7</sub>), 6.96 (dd, 1H, *J* = 7.4, 2.1 Hz, H<sub>6</sub>), 6.47 (bs, 2H, NH<sub>2</sub>), 4.27 (q, 2H, *J* = 7.1 Hz, O–CH<sub>2</sub>), 1.30 (t, 3H, *J* = 7.1 Hz, CH<sub>2</sub>–CH<sub>3</sub>) ppm.

Ethyl 3-amino-6-bromoimidazo [1,2-*a*]pyridine-2-carboxylate (**17b**). The title compound was prepared from commercially available **15b** to afford after purification the desired product as a yellow solid (44%). CAS # 82193-31-5.  $^1\text{H}$  NMR (250 MHz, DMSO-*d*<sub>6</sub>)  $\delta$

8.55 (s, 1H,  $J = 1.8, 0.9$  Hz, H<sub>7</sub>), 7.32 (dd, 1H,  $J = 9.7, 0.9$  Hz, H<sub>4</sub>), 7.13 (dd, 1H,  $J = 9.7, 1.8$  Hz, H<sub>5</sub>), 6.47 (bs, 2H, NH<sub>2</sub>), 4.27 (q, 2H,  $J = 7.1$  Hz, O-CH<sub>2</sub>), 1.30 (t, 3H,  $J = 7.1$  Hz, CH<sub>2</sub>-CH<sub>3</sub>) ppm.

### 3.3. General synthetic procedure 2 for isocyanate cyclisation.

To a solution of amino ester compound (350 mg) in anhydrous ethanol (4.5 mL), were added subsequently isocyanate (2 eq.) and sodium ethoxide (2 eq.) under inert atmosphere. The reaction mixture was heated at 120 °C under microwave irradiation for 20 min. The solvent was evaporated under vacuum. Pure compounds were obtained after purification by flash column chromatography with DCM/MeOH (92:8) as eluent.

3-(4-Fluorophenyl)pyrido [1,2-*e*]purine-2,4(1*H*,3*H*)-dione (**9a**). The title compound was prepared from commercially available **8** to afford after purification the desired product as a light yellow solid (75%). CAS # 1842362-44-0. <sup>1</sup>H NMR (400 MHz, Methanol-*d*<sub>4</sub>) δ 8.36 (d, 1H,  $J = 7.0$  Hz, H<sub>6</sub>), 7.55 (d, 1H,  $J = 9.4$  Hz, H<sub>9</sub>), 7.41–7.37 (m, 1H, H<sub>8</sub>), 7.35–7.28 (m, 2H, 2 × H<sub>arom</sub>), 7.26–7.18 (m, 2H, 2 × H<sub>arom</sub>), 7.00 (t, 1H,  $J = 7.0$ , Hz, H<sub>7</sub>) ppm (see Supplementary Materials).

3-(4-Chlorophenyl)pyrido [1,2-*e*]purine-2,4(1*H*,3*H*)-dione (**9b**). The title compound was prepared from commercially available **8** to afford after purification the desired product as a light-yellow solid (39%). CAS # 1842362-41-7. <sup>1</sup>H NMR (250 MHz, DMSO-*d*<sub>6</sub>) δ 8.47 (d, 1H,  $J = 6.9$  Hz, H<sub>6</sub>), 7.56–7.48 (m, 3H, H<sub>9</sub>, 2 × H<sub>arom</sub>), 7.34–7.23 (m, 3H, H<sub>8</sub>, 2 × H<sub>arom</sub>), 7.00 (t, 1H,  $J = 6.9$  Hz, H<sub>7</sub>) ppm.

3-(4-Bromophenyl)pyrido [1,2-*e*]purine-2,4(1*H*,3*H*)-dione (**9c**). The title compound was prepared from commercially available **8** to afford after purification the desired product as a white/grey solid (23%). <sup>1</sup>H NMR (250 MHz, DMSO-*d*<sub>6</sub>) δ 8.54 (d, 1H,  $J = 7.0$  Hz, H<sub>6</sub>), 7.70–7.61 (m, 2H, 2 × H<sub>arom</sub>), 7.52 (d, 1H,  $J = 9.4$  Hz, H<sub>9</sub>), 7.25 (td, 3H,  $J = 6.2, 2.4$  Hz, H<sub>8</sub>, 2 × H<sub>arom</sub>), 6.96 (t, 1H,  $J = 7.0$  Hz, H<sub>7</sub>) ppm. <sup>13</sup>C NMR (63 MHz, DMSO-*d*<sub>6</sub>) δ 158.9 (C=O), 151.7 (C=O), 141.5 (C<sub>quat</sub>), 136.5 (C<sub>quat</sub>), 135.7 (C<sub>quat</sub>), 131.8 (4 × C<sub>arom</sub>), 126.1 (C<sub>arom</sub>), 124.2 (C<sub>arom</sub>), 120.4 (C<sub>quat</sub>), 118.1 (C<sub>arom</sub>), 117.6 (C<sub>quat</sub>), 112.3 (C<sub>arom</sub>) ppm. HRMS-ESI (*m/z*) [M+H]<sup>+</sup> calcd. for C<sub>15</sub>H<sub>9</sub>BrN<sub>4</sub>O<sub>2</sub>: 356.9981 found: 356.9980. λ<sub>abs</sub>: 263 nm λ<sub>em</sub>: 527nm λ<sub>exc</sub>: 286 nm. R<sub>f</sub> 0.43 (DCM/MeOH, 9:1).

3-(*p*-Tolyl)pyrido [1,2-*e*]purine-2,4(1*H*,3*H*)-dione (**9d**). The title compound was prepared from commercially available **8** to afford after purification the desired product as a light yellow solid (24%). CAS # 1842362-40-6. <sup>1</sup>H NMR (250 MHz, DMSO-*d*<sub>6</sub>) δ 8.52 (d, 1H,  $J = 7.0$  Hz, H<sub>6</sub>), 7.55 (d, 1H,  $J = 9.4$  Hz, H<sub>9</sub>), 7.33–7.22 (m, 3H, H<sub>8</sub>, 2 × H<sub>arom</sub>), 7.14 (d, 2H,  $J = 8.2$  Hz, 2 × H<sub>arom</sub>), 6.99 (t, 1H,  $J = 7.0$  Hz, H<sub>7</sub>), 2.36 (s, 3H, C-CH<sub>3</sub>) ppm.

3-(4-(Trifluoromethyl)phenyl)pyrido [1,2-*e*]purine-2,4(1*H*,3*H*)-dione (**9e**). The title compound was prepared from commercially available **8** to afford after purification the desired product as a yellow solid (58%). <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 8.38 (d, 1H,  $J = 6.9$  Hz, H<sub>6</sub>), 7.80 (d, 2H,  $J = 8.2$  Hz, 2 × H<sub>arom</sub>), 7.49–7.39 (m, 3H, H<sub>9</sub>, 2 × H<sub>arom</sub>), 7.23–7.14 (m, 1H, H<sub>8</sub>), 6.85 (t, 1H,  $J = 6.9$  Hz, H<sub>7</sub>) ppm. <sup>13</sup>C NMR (101 MHz, DMSO-*d*<sub>6</sub>) δ 160.1 (C=O), 154.5 (C=O), 143.0 (C<sub>quat</sub>), 141.7 (C<sub>quat</sub>), 131.0 (C<sub>arom</sub>), 127.9 (d,  $J_{C-F} = 31.6$  Hz, C<sub>quat</sub>), 126.2 (d,  $J_{C-F} = 22.7$  Hz, C<sub>arom</sub>), 126.1 (C<sub>quat</sub>), 125.9 (d,  $J_{C-F} = 3.7$  Hz, 2 × C<sub>arom</sub>), 124.5 (C<sub>arom</sub>), 123.4 (C<sub>quat</sub>), 118.6 (2 × C<sub>arom</sub>), 117.6 (C<sub>quat</sub>), 111.8 (C<sub>arom</sub>) ppm. <sup>19</sup>F NMR (376 MHz, DMSO-*d*<sub>6</sub>) δ –60.86 ppm. HRMS-ESI (*m/z*) [M+H]<sup>+</sup> calcd. for C<sub>16</sub>H<sub>9</sub>F<sub>3</sub>N<sub>4</sub>O<sub>2</sub>: 347.0750 found: 347.0751. λ<sub>abs</sub>: 264 nm λ<sub>em</sub>: 486 nm λ<sub>exc</sub>: 266 nm. R<sub>f</sub> 0.49 (DCM/MeOH, 9:1).

3-(*p*-Methoxyphenyl)pyrido [1,2-*e*]purine-2,4(1*H*,3*H*)-dione (**9f**). The title compound was prepared from commercially available **8** to afford after purification the desired product as a beige solid (34%). CAS # 1842362-42-8. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 8.53 (d, 1H,  $J = 6.8$  Hz, H<sub>6</sub>), 7.55 (d, 1H,  $J = 9.3$  Hz, H<sub>9</sub>), 7.33–7.25 (t, 1H,  $J = 9.3$  Hz, H<sub>8</sub>), 7.17 (d, 2H,  $J = 8.7$  Hz, 2 × H<sub>arom</sub>), 7.06–6.96 (m, 3H, H<sub>7</sub>, 2 × H<sub>arom</sub>), 3.81 (s, 3H, O-CH<sub>3</sub>) ppm.

3-(3-Fluorophenyl)pyrido [1,2-*e*]purine-2,4(1*H*,3*H*)-dione (**9g**). The title compound was prepared from commercially available **8** to afford after purification the desired product as a yellow solid (19%). <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 8.50 (d, 1H,  $J = 6.2$  Hz, H<sub>6</sub>),

7.55–7.49 (m, 2H, H<sub>9</sub>, H<sub>arom</sub>), 7.33–7.24 (m, 3H, H<sub>8</sub>, 2 × H<sub>arom</sub>), 7.20 (d, 1H, *J* = 9.7 Hz, H<sub>arom</sub>), 7.14 (d, 1H, *J* = 7.3 Hz, H<sub>arom</sub>), 6.97 (t, 1H, *J* = 6.2 Hz, H<sub>7</sub>) ppm. <sup>13</sup>C NMR (101 MHz, DMSO-*d*<sub>6</sub>) δ 163.3 (C=O), 160.8 (C<sub>quat</sub>), 158.9 (d, *J* = 11.1 Hz, C<sub>quat</sub>), 151.7 (C=O), 141.6 (C<sub>quat</sub>), 130.0 (C<sub>quat</sub>), 129.9 (d, *J*<sub>C-F</sub> = 9.3 Hz, C<sub>arom</sub>), 126.2 (C<sub>arom</sub>), 125.7 (d, *J*<sub>C-F</sub> = 3.03 Hz, C<sub>arom</sub>), 124.1 (C<sub>arom</sub>), 118.3 (C<sub>arom</sub>), 117.6 (C<sub>quat</sub>), 116.7 (d, *J*<sub>C-F</sub> = 22.7 Hz, C<sub>arom</sub>), 114.4 (d, *J*<sub>C-F</sub> = 20.2 Hz, C<sub>arom</sub>), 112.4 (C<sub>arom</sub>) ppm. <sup>19</sup>F NMR (376 MHz, DMSO-*d*<sub>6</sub>) δ −113.60 ppm. HRMS-ESI (*m/z*) [M+H]<sup>+</sup> calcd. for C<sub>15</sub>H<sub>9</sub>FN<sub>4</sub>O<sub>2</sub>: 297.0782, found: 297.079. λ<sub>abs</sub>: 263 nm λ<sub>em</sub>: 498 nm λ<sub>exc</sub>: 266 nm. R<sub>f</sub> 0.29 (DCM/MeOH 9:1).

3-(3,4-Difluorophenyl)pyrido [1,2-*e*]purine-2,4(1*H*,3*H*)-dione (**9h**). The title compound was prepared from commercially available **8** to afford after purification the desired product as a white/yellow solid (38%). <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 8.44 (d, 1H, *J* = 6.2 Hz, H<sub>6</sub>), 7.55–7.47 (m, 2H, 2 × H<sub>arom</sub>), 7.44 (dd, 1H, *J* = 7.4, 2.3 Hz, H<sub>arom</sub>), 7.25 (dd, 1H *J* = 9.4, 6.6 Hz, H<sub>8</sub>), 7.17–7.11 (m, 1H, H<sub>arom</sub>), 6.93 (t, 1H, *J* = 6.2 Hz, H<sub>7</sub>) ppm. <sup>13</sup>C NMR (101 MHz, DMSO-*d*<sub>6</sub>) δ 159.2 (C=O), 152.5 (C=O), 150.1 (dd, *J* = 38.2, 12.9 Hz, C<sub>quat</sub>), 147.7 (dd, *J*<sub>C-F</sub> = 38.4, 12.8 Hz, C<sub>quat</sub>), 141.5 (C<sub>quat</sub>), 137.1 (C<sub>quat</sub>), 134.3 (dd, *J*<sub>C-F</sub> = 8.6, 3.5 Hz, C<sub>quat</sub>), 126.6 (dd, *J*<sub>C-F</sub> = 6.6, 3.4 Hz, C<sub>arom</sub>), 126.0 (C<sub>arom</sub>), 124.0 (C<sub>arom</sub>), 118.9 (d, *J*<sub>C-F</sub> = 18.2 Hz, C<sub>arom</sub>), 118.2 (C<sub>arom</sub>), 117.5 (C<sub>quat</sub>), 117.0 (d, *J*<sub>C-F</sub> = 17.9 Hz, C<sub>arom</sub>), 112.1 (C<sub>arom</sub>) ppm. <sup>19</sup>F NMR (376 MHz, DMSO-*d*<sub>6</sub>) δ −140.24, −138.54 ppm. HRMS-ESI (*m/z*) [M+H]<sup>+</sup> calcd. for C<sub>15</sub>H<sub>9</sub>F<sub>2</sub>N<sub>4</sub>O<sub>2</sub>: 315.0688, found: 315.0687. λ<sub>abs</sub>: 261 nm λ<sub>em</sub>: 49 nm λ<sub>exc</sub>: 266 nm. R<sub>f</sub> 0.27 (DCM/MeOH 9:1).

3-(2,4-Difluorophenyl)pyrido [1,2-*e*]purine-2,4(1*H*,3*H*)-dione (**9i**). The title compound was prepared from commercially available **8** to afford after purification the desired product as a yellow solid (64%). <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 8.45 (d, 1H, *J* = 6.9 Hz, H<sub>6</sub>), 7.58–7.36 (m, 3H, H<sub>9</sub>, 2 × H<sub>arom</sub>), 7.28 (t, 1H, *J* = 6.8 Hz, H<sub>8</sub>), 7.20 (t, 1H, *J* = 8.4 Hz, H<sub>arom</sub>), 6.95 (t, 1H, *J* = 6.9 Hz, H<sub>7</sub>) ppm. <sup>13</sup>C NMR (101 MHz, DMSO-*d*<sub>6</sub>) δ 162.8 (dd, *J* = 46.5, 11.7 Hz, C=O), 158.5 (C<sub>quat</sub>), 159.6–156.3 (dd, *J* = 251.49, 13.2 Hz, C<sub>quat</sub>), 151.7 (C=O), 141.7 (C<sub>quat</sub>), 136.7 (C<sub>quat</sub>), 132.7 (dd, *J*<sub>C-F</sub> = 10.3, 2.4 Hz, C<sub>arom</sub>), 126.3 (C<sub>arom</sub>), 124.1 (C<sub>arom</sub>), 121.3 (dd, *J*<sub>C-F</sub> = 13.6, 4.0 Hz, C<sub>quat</sub>), 118.2 (C<sub>arom</sub>), 117.2 (C<sub>quat</sub>), 112.3 (C<sub>arom</sub>), 111.6 (dd, *J*<sub>C-F</sub> = 22.4, 3.6 Hz, C<sub>arom</sub>), 104.4 (dd, *J*<sub>C-F</sub> = 26.9, 24.6 Hz, C<sub>arom</sub>) ppm. <sup>19</sup>F NMR (376 MHz, DMSO-*d*<sub>6</sub>) δ −110.21, −117.12 ppm. HRMS-ESI (*m/z*) [M+H]<sup>+</sup> calcd. for C<sub>15</sub>H<sub>9</sub>F<sub>2</sub>N<sub>4</sub>O<sub>2</sub>: 315.0687, found: 315.0688. λ<sub>abs</sub>: 264 nm λ<sub>em</sub>: 490 nm λ<sub>exc</sub>: 266 nm. R<sub>f</sub> 0.32 (DCM/MeOH 9:1).

3-(3,4-Dimethylphenyl)pyrido [1,2-*e*]purine-2,4(1*H*,3*H*)-dione (**9j**). The title compound was prepared from commercially available **8** to afford after purification the desired product as a light-yellow solid (56%). <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 8.46 (d, 1H, *J* = 6.9 Hz, H<sub>6</sub>), 7.54 (d, 1H, *J* = 9.3 Hz, H<sub>9</sub>), 7.28 (t, 1H, *J* = 8.0 Hz, H<sub>arom</sub>), 7.21 (d, 1H, *J* = 9.3 Hz, H<sub>8</sub>), 7.00–6.92 (m, 3H, H<sub>7</sub>, 2 × H<sub>arom</sub>), 2.27 (s, 3H, C-CH<sub>3</sub>), 2.24 (s, 3H, C-CH<sub>3</sub>) ppm. <sup>13</sup>C NMR (101 MHz, DMSO-*d*<sub>6</sub>) δ 159.0 (C=O), 151.7 (C=O), 141.5 (C<sub>quat</sub>), 136.5 (C<sub>quat</sub>), 135.6 (C<sub>quat</sub>), 134.4 (C<sub>quat</sub>), 129.9 (C<sub>arom</sub>), 129.6 (C<sub>arom</sub>), 129.3 (C<sub>arom</sub>), 126.1 (C<sub>arom</sub>), 124.0 (C<sub>arom</sub>), 118.3 (C<sub>arom</sub>), 117.8 (C<sub>quat</sub>), 112.5 (C<sub>arom</sub>), 19.2 (C-CH<sub>3</sub>), 19.0 (C-CH<sub>3</sub>) ppm. HRMS-ESI (*m/z*) [M+H]<sup>+</sup> calcd. for C<sub>17</sub>H<sub>15</sub>N<sub>4</sub>O<sub>2</sub>: 307.1189, found: 307.1190. λ<sub>abs</sub>: 263 nm λ<sub>em</sub>: 496 nm λ<sub>exc</sub>: 266 nm. R<sub>f</sub> 0.68 (DCM/MeOH, 9:1).

3-(4-Fluorophenyl)-2-thioxo-2,3-dihydropyrido [1,2-*e*]purin-4(1*H*)-one (**11**). The title compound was prepared from commercially available **8** to afford after purification the desired product as a light-yellow solid (74%). <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 8.43 (d, 1H, *J* = 6.8 Hz, H<sub>6</sub>), 7.47 (d, 1H, *J* = 9.2 Hz, H<sub>9</sub>), 7.30–7.23 (m, 1H, H<sub>8</sub>), 7.20 (t, 2H, *J* = 8.5 Hz, 2 × H<sub>arom</sub>), 7.07–7.06 (m, 2H, 2 × H<sub>arom</sub>), 6.88 (t, 1H, *J* = 6.8 Hz, H<sub>7</sub>) ppm. <sup>13</sup>C NMR (101 MHz, DMSO-*d*<sub>6</sub>) δ 175.6 (C=S), 161.9 (C=O), 159.5 (d, *J* = 10.9 Hz, C<sub>quat</sub>), 142.3 (C<sub>quat</sub>), 142.1 (C<sub>quat</sub>), 139.0 (C<sub>quat</sub>), 131.4 (d, *J*<sub>C-F</sub> = 8.6 Hz, 2 × C<sub>arom</sub>), 126.8 (C<sub>arom</sub>), 124.2 (C<sub>arom</sub>), 121.0 (C<sub>quat</sub>), 118.0 (C<sub>arom</sub>), 115.1 (d, *J*<sub>C-F</sub> = 22.5 Hz, 2 × C<sub>arom</sub>), 111.4 (C<sub>arom</sub>) ppm. <sup>19</sup>F NMR (376 MHz, DMSO-*d*<sub>6</sub>) δ −116.93 ppm. HRMS-ESI (*m/z*) [M+H]<sup>+</sup> calcd. for C<sub>15</sub>H<sub>10</sub>FN<sub>4</sub>OS: 313.0553, found: 313.0553. λ<sub>abs</sub>: 340 nm λ<sub>em</sub>: 492 nm λ<sub>exc</sub>: 341 nm. R<sub>f</sub> 0.11 (DCM/MeOH 9:1).

3-(Benzyl)pyrido [1,2-*e*]purine-2,4(1*H*,3*H*)-dione (**10a**). The title compound was prepared from commercially available **8** to afford after purification the desired product as a light yellow solid (71%). CAS # 1842362-38-2. <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 8.29 (d, 1H, *J* = 7.0 Hz, H<sub>6</sub>), 7.52 (dd, 1H, *J* = 8.3, 5.5 Hz, H<sub>arom</sub>), 7.41 (d, 1H, *J* = 9.3 Hz, H<sub>9</sub>), 7.35 (dd, 2H,



$J = 8.3, 5.5 \text{ Hz}, 2 \times \text{H}_{\text{arom}}$ ), 7.24 (t, 1H,  $J = 8.3 \text{ Hz}, \text{H}_{\text{arom}}$ ), 7.16 (dd, 1H,  $J = 9.3, 6.75 \text{ Hz}, \text{H}_8$ ), 7.08 (t, 2H,  $J = 8.8 \text{ Hz}, 2 \times \text{H}_{\text{arom}}$ ), 6.81 (t, 1H,  $J = 6.7 \text{ Hz}, \text{H}_7$ ), 5.09 (s, 2H, N-CH<sub>2</sub>) ppm.

3-(*p*-Fluorobenzyl)pyrido [1,2-*e*]purine-2,4(1*H*,3*H*)-dione (**10b**). The title compound was prepared from commercially available 8 to afford after purification the desired product as a light-yellow solid (52%). <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 8.29 (d, 1H,  $J = 7.0 \text{ Hz}, \text{H}_6$ ), 7.52 (dd, 1H,  $J = 8.3, 7.5 \text{ Hz}, \text{H}_{\text{arom}}$ ), 7.41 (d, 1H,  $J = 9.3 \text{ Hz}, \text{H}_9$ ), 7.35 (dd, 1H,  $J = 8.3, 5.7 \text{ Hz}, \text{H}_{\text{arom}}$ ), 7.24 (t, 1H,  $J = 7.1 \text{ Hz}, \text{H}_{\text{arom}}$ ), 7.21–7.12 (m, 1H, H<sub>8</sub>), 7.08 (t, 1H,  $J = 8.8 \text{ Hz}, \text{H}_{\text{arom}}$ ), 6.81 (t, 1H,  $J = 7.0 \text{ Hz}, \text{H}_7$ ), 5.09 (s, 2H, N-CH<sub>2</sub>) ppm. <sup>13</sup>C NMR (101 MHz, DMSO-*d*<sub>6</sub>) δ 160.1 (C=O), 154.9 (C=O), 141.6 (C<sub>quat</sub>), 135.8 (C<sub>quat</sub>), 131.6 (d,  $J_{\text{C-F}} = 8.4 \text{ Hz}, \text{C}_{\text{arom}}$ ), 131.1 (C<sub>quat</sub>), 130.0 (d,  $J_{\text{C-F}} = 8.1 \text{ Hz}, 2 \times \text{C}_{\text{arom}}$ ), 129.0 (C<sub>quat</sub>), 126.0 (C<sub>arom</sub>), 124.4 (C<sub>arom</sub>), 118.6 (C<sub>arom</sub>), 117.4 (C<sub>quat</sub>), 115.8 (d,  $J_{\text{C-F}} = 21.1 \text{ Hz}, \text{C}_{\text{arom}}$ ), 115.1 (d,  $J_{\text{C-F}} = 21.5 \text{ Hz}, \text{C}_{\text{arom}}$ ), 111.7 (C<sub>arom</sub>), 42.7 (d,  $J_{\text{C-F}} = 91.0 \text{ Hz}, \text{N-CH}_2$ ) ppm. <sup>19</sup>F NMR (376 MHz, DMSO-*d*<sub>6</sub>) δ -116.00 ppm. HRMS-ESI (*m/z*) [M+H]<sup>+</sup> calcd. for C<sub>16</sub>H<sub>12</sub>FN<sub>4</sub>O<sub>2</sub>: 311.0938, found: 311.0939. λ<sub>abs</sub>: 259 nm λ<sub>em</sub>: 501 nm λ<sub>exc</sub>: 268 nm.  $R_f$  0.37 (DCM/MeOH, 9:1).

3-(*p*-(Trifluoromethyl)benzyl)pyrido [1,2-*e*]purine-2,4(1*H*,3*H*)-dione (**10c**). The title compound was prepared from commercially available 8 to afford after purification the desired product as a white/yellow solid (46%). <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 8.54 (d, 1H,  $J = 6.9 \text{ Hz}, \text{H}_6$ ), 7.66 (d, 2H,  $J = 8.1 \text{ Hz}, 2 \times \text{H}_{\text{arom}}$ ), 7.53 (dd, 3H,  $J = 14.5, 8.8 \text{ Hz}, \text{H}_9, 2 \times \text{H}_{\text{arom}}$ ), 7.31–7.26 (m, 1H, H<sub>8</sub>), 6.98 (t, 1H,  $J = 6.9 \text{ Hz}, \text{H}_7$ ), 5.20 (s, 2H, N-CH<sub>2</sub>) ppm. <sup>13</sup>C NMR (101 MHz, DMSO-*d*<sub>6</sub>) δ 158.6 (C=O), 150.9 (C=O), 142.7 (C<sub>quat</sub>), 141.7 (C<sub>quat</sub>), 127.9 (2 × C<sub>arom</sub>), 127.6 (C<sub>quat</sub>), 126.4 (C<sub>quat</sub>), 126.4 (C<sub>arom</sub>), 125.1 (t,  $J_{\text{C-F}} = 3.7 \text{ Hz}, 2 \times \text{C}_{\text{arom}}$ ), 124.2 (C<sub>arom</sub>), 118.3 (C<sub>arom</sub>), 117.4 (m, C<sub>quat</sub>), 113.5 (C<sub>quat</sub>), 112.6 (C<sub>arom</sub>), 43.1 (N-CH<sub>2</sub>) ppm. <sup>19</sup>F NMR (376 MHz, DMSO-*d*<sub>6</sub>) δ -60.72. HRMS-ESI (*m/z*) [M+H]<sup>+</sup> calcd. for C<sub>17</sub>H<sub>12</sub>F<sub>3</sub>N<sub>4</sub>O<sub>2</sub>: 361.0906, found: 361.0906. λ<sub>abs</sub>: 260 nm λ<sub>em</sub>: 495 nm λ<sub>exc</sub>: 265 nm.  $R_f$  0.35 (DCM/MeOH, 9:1).

3-(*p*-Methylbenzyl)pyrido [1,2-*e*]purine-2,4(1*H*,3*H*)-dione (**10d**). The title compound was prepared from commercially available 8 to afford after purification the desired product as a light yellow solid (44%). <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 8.49 (d, 1H,  $J = 7.0 \text{ Hz}, \text{H}_6$ ), 7.54 (d, 1H,  $J = 9.5 \text{ Hz}, \text{H}_9$ ), 7.28 (dd, 1H,  $J = 9.5, 6.5 \text{ Hz}, \text{H}_8$ ), 7.21 (d, 2H,  $J = 7.9 \text{ Hz}, 2 \times \text{H}_{\text{arom}}$ ), 7.15–7.04 (m, 2H, 2 × H<sub>arom</sub>), 6.97 (dd, 1H,  $J = 7.0, 6.5 \text{ Hz}, \text{H}_7$ ), 5.07 (s, 2H, N-CH<sub>2</sub>), 2.25 (s, 3H, C-CH<sub>3</sub>) ppm. <sup>13</sup>C NMR (101 MHz, DMSO-*d*<sub>6</sub>) δ 161.7 (C=O), 158.7 (C<sub>quat</sub>), 154.5 (C=O), 141.6 (C<sub>quat</sub>), 135.9 (C<sub>quat</sub>), 135.0 (C<sub>quat</sub>), 128.6 (2 × C<sub>arom</sub>), 127.4 (2 × C<sub>arom</sub>), 126.2 (C<sub>arom</sub>), 124.2 (C<sub>arom</sub>), 118.2 (C<sub>arom</sub>), 117.6 (C<sub>quat</sub>), 112.5 (C<sub>arom</sub>), 43.0 (N-CH<sub>2</sub>), 20.6 (C-CH<sub>3</sub>) ppm. HRMS-ESI (*m/z*) [M+H]<sup>+</sup> calcd. for C<sub>17</sub>H<sub>15</sub>N<sub>4</sub>O<sub>2</sub>: 307.1189, found: 307.1191. λ<sub>abs</sub>: 260 nm λ<sub>em</sub>: 496 nm λ<sub>exc</sub>: 265 nm.  $R_f$  0.46 (DCM/MeOH, 9:1).

3-Benzyl-2-thioxo-2,3-dihydropyrido [1,2-*e*]purin-4(1*H*)-one (**12**). The title compound was prepared from commercially available 8 to afford after purification the desired product as a light yellow solid (71%). CAS # 1842362-47-3. <sup>1</sup>H NMR (250 MHz, DMSO-*d*<sub>6</sub>) δ 8.69 (d, 1H,  $J = 6.8 \text{ Hz}, \text{H}_6$ ), 7.58 (d, 1H,  $J = 9.3 \text{ Hz}, \text{H}_9$ ), 7.41–7.15 (m, 6H, H<sub>8</sub>, 5 × H<sub>arom</sub>), 7.01 (t, 1H,  $J = 6.8 \text{ Hz}, \text{H}_7$ ), 5.79 (s, 2H, N-CH<sub>2</sub>) ppm.

3-(*p*-Fluorophenyl)-7-methylpyrido [1,2-*e*]purine-2,4(1*H*,3*H*)-dione (**18a**). The title compound was prepared from commercially available 16a to afford after purification the desired product as a yellow solid (66%). <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 8.17 (s, 1H, H<sub>6</sub>), 7.41 (d, 1H,  $J = 9.4 \text{ Hz}, \text{H}_9$ ), 7.26 (d, 4H,  $J = 6.5 \text{ Hz}, 4 \times \text{H}_{\text{arom}}$ ), 7.11 (d, 1H,  $J = 9.4 \text{ Hz}, \text{H}_8$ ), 2.28 (s, 3H, C-CH<sub>3</sub>) ppm. <sup>13</sup>C NMR (101 MHz, DMSO-*d*<sub>6</sub>) δ 162.8 (C=O), 159.2 (C<sub>quat</sub>), 152.8 (C<sub>quat</sub>), 152.9 (C=O), 141.8 (C<sub>quat</sub>), 136.5 (C<sub>quat</sub>), 133.7 (C<sub>quat</sub>), 131.2 (d,  $J_{\text{C-F}} = 18.6 \text{ Hz}, 2 \times \text{C}_{\text{arom}}$ ), 123.2 (C<sub>arom</sub>), 117.3 (C<sub>quat</sub>), 115.8 (C<sub>arom</sub>), 115.6 (C<sub>arom</sub>), 114.7 (d,  $J_{\text{C-F}} = 29.4 \text{ Hz}, 2 \times \text{C}_{\text{arom}}$ ), 21.0 (C-CH<sub>3</sub>) ppm. <sup>19</sup>F NMR (376 MHz, DMSO-*d*<sub>6</sub>) δ -115.61 ppm. HRMS-ESI (*m/z*) [M+H]<sup>+</sup> calcd for C<sub>16</sub>H<sub>12</sub>FN<sub>4</sub>O<sub>2</sub>: 311.0937, found: 311.0938. λ<sub>abs</sub>: 260 nm λ<sub>em</sub>: 497 nm λ<sub>exc</sub>: 265 nm.  $R_f$  0.28 (DCM/MeOH 9:1).

3-(*p*-Fluorophenyl)-8-methylpyrido [1,2-*e*]purine-2,4(1*H*,3*H*)-dione (**18b**). The title compound was prepared from commercially available 16b to afford after purification the desired product as a yellow solid (69%). <sup>1</sup>H NMR (250 MHz, DMSO-*d*<sub>6</sub>) δ 8.31 (d, 1H,  $J = 7.0 \text{ Hz}, \text{H}_6$ ), 7.31–7.24 (m, 5H, H<sub>9</sub>, 4 × H<sub>arom</sub>), 6.81 (d, 1H,  $J = 7.0 \text{ Hz}, \text{H}_7$ ), 2.35 (s, 3H, C-CH<sub>3</sub>)

ppm.  $^{13}\text{C}$  NMR (63 MHz, DMSO- $d_6$ )  $\delta$  162.4 (C=O), 159.3 (d,  $J = 121.2$  Hz, C<sub>quat</sub>), 151.4 (C=O), 140.9 (C<sub>quat</sub>), 133.7 (C<sub>quat</sub>), 133.0 (d,  $J = 3.0$  Hz, C<sub>quat</sub>), 131.3 (d,  $J_{C-F} = 8.7$  Hz,  $2 \times$  C<sub>arom</sub>), 129.5 (C<sub>arom</sub>), 121.8 (C<sub>quat</sub>), 120.8 (C<sub>arom</sub>), 117.8 (C<sub>quat</sub>), 117.6 (C<sub>arom</sub>), 115.5 (d,  $J_{C-F} = 22.6$  Hz,  $2 \times$  C<sub>arom</sub>), 17.8 (C-CH<sub>3</sub>) ppm.  $^{19}\text{F}$  NMR (376 MHz, DMSO- $d_6$ )  $\delta$  -115.29 ppm. HRMS-ESI ( $m/z$ ) [M+H]<sup>+</sup> calcd for C<sub>16</sub>H<sub>12</sub>FN<sub>4</sub>O<sub>2</sub>: 311.0938, found: 311.0938.  $\lambda_{\text{abs}}$ : 263 nm  $\lambda_{\text{em}}$ : 465 nm  $\lambda_{\text{exc}}$ : 269 nm. R<sub>f</sub> 0.28 (DCM/MeOH 9:1).

7-Bromo-3-(*p*-fluorophenyl)pyrido [1,2-*e*]purine-2,4(1*H*,3*H*)-dione (**19a**). The title compound was prepared from commercially available 17a to afford after purification the desired product as a yellow/orange solid (46%).  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  8.36 (d, 1H,  $J = 7.4$  Hz, H<sub>9</sub>), 7.85–7.77 (m, 1H, H<sub>6</sub>), 7.34–7.20 (m, 4H,  $4 \times$  H<sub>arom</sub>), 7.05 (dd, 1H,  $J = 7.4$ , 1.9 Hz, H<sub>8</sub>) ppm.  $^{13}\text{C}$  NMR (101 MHz, DMSO- $d_6$ )  $\delta$  163.0 (C=O), 159.4 (d,  $J = 17.2$  Hz, C<sub>quat</sub>), 153.7 (C=O), 141.0 (C<sub>quat</sub>), 139.1 (C<sub>quat</sub>), 134.0 (d,  $J_{C-F} = 3.1$  Hz, C<sub>quat</sub>), 131.2 (d,  $J_{C-F} = 8.7$  Hz,  $2 \times$  C<sub>arom</sub>), 125.0 (C<sub>arom</sub>), 120.0 (C<sub>arom</sub>), 118.8 (C<sub>quat</sub>), 117.8 (C<sub>quat</sub>), 115.3 (d,  $J_{C-F} = 22.6$  Hz,  $2 \times$  C<sub>arom</sub>), 115.2 (C<sub>arom</sub>) ppm.  $^{19}\text{F}$  NMR (376 MHz, DMSO- $d_6$ )  $\delta$  -115.56 ppm. HRMS-ESI ( $m/z$ ) [M+H]<sup>+</sup> calcd for C<sub>15</sub>H<sub>9</sub>BrFN<sub>4</sub>O<sub>2</sub>: 374.9887, found: 374.9887.  $\lambda_{\text{abs}}$ : 267 nm  $\lambda_{\text{em}}$ : 492 nm  $\lambda_{\text{exc}}$ : 269 nm. R<sub>f</sub> 0.32 (DCM/MeOH 9:1).

8-Bromo-3-(*p*-fluorophenyl)pyrido [1,2-*e*]purine-2,4(1*H*,3*H*)-dione (**19b**). The title compound was prepared from commercially available 17b to afford after purification the desired product as a yellow/green light solid (51%).  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  8.74 (s, 1H, H<sub>9</sub>), 7.49 (d, 1H,  $J = 9.7$  Hz, H<sub>6</sub>), 7.32 (d, 1H,  $J = 9.7$  Hz, H<sub>7</sub>), 7.30–7.21 (m, 4H,  $4 \times$  H<sub>arom</sub>) ppm.  $^{13}\text{C}$  NMR (101 MHz, DMSO- $d_6$ )  $\delta$  162.8 (C=O), 159.7 (d,  $J = 70.7$  Hz, C<sub>quat</sub>), 153.5 (C=O), 140.1 (C<sub>quat</sub>), 138.0 (C<sub>quat</sub>), 134.1 (d,  $J_{C-F} = 2.0$  Hz, C<sub>quat</sub>), 131.7 (d,  $J_{C-F} = 8.8$  Hz,  $2 \times$  C<sub>arom</sub>), 129.1 (C<sub>arom</sub>), 124.6 (C<sub>arom</sub>), 119.9 (C<sub>arom</sub>), 118.6 (C<sub>quat</sub>), 115.9 (d,  $J_{C-F} = 22.6$  Hz,  $2 \times$  C<sub>arom</sub>), 106.3 (C<sub>quat</sub>) ppm.  $^{19}\text{F}$  NMR (376 MHz, DMSO- $d_6$ )  $\delta$  -115.29 ppm. HRMS-ESI ( $m/z$ ) [M+Na]<sup>+</sup> calcd for C<sub>15</sub>H<sub>9</sub>BrFN<sub>4</sub>NaO<sub>2</sub>: 396.9706, found: 396.9693.  $\lambda_{\text{abs}}$ : 262 nm  $\lambda_{\text{em}}$ : 496 nm  $\lambda_{\text{exc}}$ : 267 nm. R<sub>f</sub> 0.30 (DCM/MeOH 9:1).

### 3.4. General synthetic procedure 3 for the N<sup>1</sup>-alkylation.

To a solution of fluorobenzyl compound (150 mg) in anhydrous DMF (4 mL), were added subsequently potassium carbonate (1.5 eq.) and bromide derivative (1.5 eq.) under inert atmosphere. The reaction mixture was heated at 120 °C under microwave irradiation for 20 min. The mixture was dissolved with EtOAc, washed twice with saturated NH<sub>4</sub>Cl, dried over MgSO<sub>4</sub>, filtrated and concentrated under vacuum. Pure compound was obtained after purification by flash column chromatography using an elution gradient of DCM/MeOH (from 98:2 to 95:5).

1-Benzyl-3-(4-fluorophenyl)purino [9,8-*a*]pyridine-2,4-dione (**13a**). The title compound was prepared from commercially available 9a to afford after purification the desired product as a light orange solid (36%).  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  8.23 (d, 1H,  $J = 7.0$  Hz, H<sub>6</sub>), 7.60 (d, 1H,  $J = 9.3$  Hz, H<sub>9</sub>), 7.50–7.27 (m, 9H,  $5 \times$  H<sub>arom-N1</sub>,  $4 \times$  H<sub>arom-N3</sub>), 7.26–7.20 (m, 1H, H<sub>8</sub>), 6.82 (t, 1H,  $J = 7.0$  Hz, H<sub>7</sub>), 5.73 (s, 2H, N-CH<sub>2</sub>) ppm.  $^{13}\text{C}$  NMR (101 MHz, DMSO- $d_6$ )  $\delta$  160.3 (C=O), 157.9 (C<sub>quat</sub>), 151.1 (C=O), 142.5 (C<sub>quat</sub>), 136.1 (C<sub>quat</sub>), 132.6 (d,  $J_{C-F} = 3.03$  Hz, C<sub>quat</sub>), 132.08 (C<sub>quat</sub>), 131.1 (d,  $J_{C-F} = 8.8$  Hz,  $2 \times$  C<sub>arom-N3</sub>), 129.0 ( $2 \times$  C<sub>arom-N1</sub>), 127.6 (C<sub>arom</sub>), 126.0 (C<sub>arom</sub>), 125.7 ( $2 \times$  C<sub>arom-N1</sub>), 125.0 (C<sub>arom</sub>), 120.1 (C<sub>quat</sub>), 118.9 (C<sub>arom</sub>), 115.7 (d,  $J_{C-F} = 22.8$  Hz,  $2 \times$  C<sub>arom-N3</sub>), 113.4 (C<sub>arom</sub>), 46.9 (N-CH<sub>2</sub>) ppm.  $^{19}\text{F}$  NMR (376 MHz, DMSO- $d_6$ )  $\delta$  -114.07 ppm. HRMS-ESI ( $m/z$ ) [M+H]<sup>+</sup> calcd. for C<sub>22</sub>H<sub>16</sub>FN<sub>4</sub>O<sub>4</sub>: 387.1251, found: 387.1249.  $\lambda_{\text{abs}}$ : 261 nm  $\lambda_{\text{em}}$ : 418 nm  $\lambda_{\text{exc}}$ : 267 nm. R<sub>f</sub> 0.65 (DCM/MeOH 95:5).

3-(*p*-Fluorophenyl)-1-[(*p*-methylphenyl)methyl]purino[9,8-*a*]pyridine-2,4-dione (**13b**). The title compound was prepared from commercially available 9a to afford after purification the desired product as a light yellow solid (20%).  $^1\text{H}$  NMR (250 MHz, DMSO- $d_6$ )  $\delta$  8.20 (d, 1H,  $J = 7.3$  Hz, H<sub>6</sub>), 7.56 (d, 1H,  $J = 9.3$  Hz, H<sub>9</sub>), 7.41 (dd, 2H,  $J = 9.0$ , 5.2 Hz,  $2 \times$  H<sub>arom-N3</sub>), 7.38–7.25 (m, 4H,  $2 \times$  H<sub>arom-N1</sub>,  $2 \times$  H<sub>arom-N3</sub>), 7.24–7.09 (m, 3H, H<sub>8</sub>,  $2 \times$  H<sub>arom-N1</sub>), 6.79 (t, 1H,  $J = 7.3$  Hz, H<sub>7</sub>), 5.64 (s, 2H, N-CH<sub>2</sub>), 2.23 (s, 3H, C-CH<sub>3</sub>) ppm.  $^{13}\text{C}$  NMR (63 MHz, DMSO- $d_6$ )  $\delta$  159.5 (C=O), 157.9 (C<sub>quat</sub>), 151.1 (C=O), 142.5 (C<sub>quat</sub>), 136.7 (C<sub>quat</sub>), 133.0 (C<sub>quat</sub>), 132.7 (d,  $J_{C-F} = 3.1$  Hz, C<sub>quat</sub>), 132.0 (C<sub>quat</sub>), 131.1 (d,  $J_{C-F} = 8.8$  Hz,  $2 \times$  C<sub>arom-N3</sub>), 129.5 ( $2 \times$  C<sub>arom</sub>).

N1), 126.0 (Carom), 125.5 (2 × Carom-N1), 125.1 (Carom), 120.1 (Cquat), 118.9 (Carom), 115.7 (d,  $J_{C-F}$  = 23.3 Hz, 2 × Carom-N3), 113.4 (Carom), 46.7 (N-CH<sub>2</sub>), 20.6 (C-CH<sub>3</sub>) ppm. <sup>19</sup>F NMR (376 MHz, DMSO-*d*<sub>6</sub>) δ -114.08 ppm. HRMS-ESI (*m/z*) [M+H]<sup>+</sup> calcd. for C<sub>23</sub>H<sub>18</sub>FN<sub>4</sub>O<sub>2</sub>: 401.1708, found: 401.1405. λ<sub>abs</sub>: 260 nm λ<sub>em</sub>: 418 nm λ<sub>exc</sub>: 261 nm. R<sub>f</sub> 0.5 (DCM/MeOH 95:5).

4-[[3-(*p*-Fluorophenyl)-2,4-dioxo-purino[9,8-*a*]pyridin-1-yl]methyl]benzotrile (13c). The title compound was prepared from commercially available 9a to afford after purification the desired product as a light yellow solid (30%). <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 8.13 (d, 1H,  $J$  = 7.3 Hz, H<sub>6</sub>), 7.86 (d, 2H,  $J$  = 8.1 Hz, 2 × H<sub>arom-N1</sub>), 7.69 (d, 2H,  $J$  = 8.1 Hz, 2 × H<sub>arom-N1</sub>), 7.62 (d, 1H,  $J$  = 9.3 Hz, H<sub>9</sub>), 7.45–7.43 (m, 2H, 2 × H<sub>arom-N3</sub>), 7.34 (t, 2H,  $J$  = 8.7 Hz, 2 × H<sub>arom-N3</sub>), 7.29–7.21 (m, 1H, H<sub>8</sub>), 6.83 (t, 1H,  $J$  = 7.3 Hz, H<sub>7</sub>), 5.80 (s, 2H, N-CH<sub>2</sub>) ppm. <sup>13</sup>C NMR (101 MHz, DMSO-*d*<sub>6</sub>) δ 160.3 (C=O), 157.9 (C<sub>quat</sub>), 151.1 (C=O), 142.5 (C<sub>quat</sub>), 142.0 (C<sub>quat</sub>), 132.8 (2 × Carom-N1), 132.6 (d,  $J_{C-F}$  = 3.0 Hz, C<sub>quat</sub>), 131.8 (C<sub>quat</sub>), 131.1 (d,  $J_{C-F}$  = 8.8 Hz, 2 × Carom-N3), 126.9 (2 × Carom-N1), 126.1 (Carom), 124.7 (Carom), 120.3 (C<sub>quat</sub>), 119.0 (Carom), 118.5 (C<sub>quat</sub>), 115.7 (d,  $J_{C-F}$  = 22.8 Hz, 2 × Carom-N3), 113.7 (Carom), 110.4 (C<sub>quat</sub>), 47.0 (N-CH<sub>2</sub>) ppm. <sup>19</sup>F NMR (376 MHz, DMSO-*d*<sub>6</sub>) δ -114.00 ppm. HRMS-ESI (*m/z*) [M+H]<sup>+</sup> calcd. for C<sub>23</sub>H<sub>15</sub>FN<sub>5</sub>O<sub>2</sub>: 412.1203, found: 412.1204. λ<sub>abs</sub>: 259 nm λ<sub>em</sub>: 418 nm λ<sub>exc</sub>: 259 nm. R<sub>f</sub> 0.5 (DCM/MeOH 95:5).

3-(*p*-Fluorophenyl)-1-[(*p*-nitrophenyl)methyl]purino [9,8-*a*]pyridine-2,4-dione (13d). The title compound was prepared from commercially available 9a to afford after purification the desired product as a yellow solid (28%). <sup>1</sup>H NMR (250 MHz, DMSO-*d*<sub>6</sub>) δ 8.19 (d, 2H,  $J$  = 8.8 Hz, 2 × H<sub>arom-N1</sub>), 7.95 (d, 1H,  $J$  = 7.3 Hz, H<sub>6</sub>), 7.77 (d, 2H,  $J$  = 8.8 Hz, 2 × H<sub>arom-N1</sub>), 7.63 (d, 1H,  $J$  = 9.3 Hz, H<sub>9</sub>), 7.50–7.20 (m, 5H, H<sub>8</sub>, 4 × H<sub>arom-N3</sub>), 6.84 (t, 1H,  $J$  = 7.3 Hz, H<sub>7</sub>), 5.85 (s, 2H, N-CH<sub>2</sub>) ppm. <sup>13</sup>C NMR (63 MHz, DMSO-*d*<sub>6</sub>) δ 159.5 (C=O), 157.9 (C<sub>quat</sub>), 151.1 (C=O), 147.0 (C<sub>quat</sub>), 144.0 (C<sub>quat</sub>), 142.5 (C<sub>quat</sub>), 132.5 (d,  $J_{C-F}$  = 3.1 Hz, C<sub>quat</sub>), 131.8 (C<sub>quat</sub>), 131.1 (d,  $J_{C-F}$  = 8.8 Hz, 2 × Carom-N3), 127.2 (2 × Carom-N1), 126.1 (Carom), 124.7 (Carom), 123.9 (2 × Carom-N1), 120.3 (C<sub>quat</sub>), 119.06 (Carom), 115.7 (d,  $J_{C-F}$  = 23.3 Hz, 2 × Carom-N3), 113.7 (Carom), 46.9 (N-CH<sub>2</sub>) ppm. <sup>19</sup>F NMR (376 MHz, DMSO-*d*<sub>6</sub>) δ -113.96 ppm. HRMS-ESI (*m/z*) [M+H]<sup>+</sup> calcd. for C<sub>22</sub>H<sub>15</sub>FN<sub>5</sub>O<sub>4</sub>: 432.1002, found: 432.1100. λ<sub>abs</sub>: 269.5 nm λ<sub>em</sub>: 551 nm λ<sub>exc</sub>: 275 nm. R<sub>f</sub> 0.5 (DCM/MeOH 95:5).

Methyl 4-[[3-(*p*-fluorophenyl)-2,4-dioxo-purino [9,8-*a*]pyridin-1-yl]methyl]benzoate (13e). The title compound was prepared from commercially available 9a to afford after purification the desired product as a white solid (65%). <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 8.14 (d, 1H,  $J$  = 7.3 Hz, H<sub>6</sub>), 7.95 (d, 2H,  $J$  = 7.9 Hz, 2 × H<sub>arom-N1</sub>), 7.61 (d, 3H,  $J$  = 7.9 Hz, H<sub>9</sub>, 2 × H<sub>arom-N1</sub>), 7.45 (dd, 2H,  $J$  = 8.6, 5.3 Hz, 2 × H<sub>arom-N3</sub>), 7.35 (t, 2H,  $J$  = 8.6 Hz, 2 × H<sub>arom-N3</sub>), 7.28–7.20 (m, 1H, H<sub>8</sub>), 6.82 (t, 1H,  $J$  = 7.3 Hz, H<sub>7</sub>), 5.80 (s, 2H, N-CH<sub>2</sub>), 3.83 (s, 3H, O-CH<sub>3</sub>) ppm. <sup>13</sup>C NMR (101 MHz, DMSO-*d*<sub>6</sub>) δ 165.8 (C=O), 157.9 (C=O), 151.1 (C=O), 142.5 (C<sub>quat</sub>), 141.8 (C<sub>quat</sub>), 132.6 (d,  $J_{C-F}$  = 3.2 Hz, C<sub>quat</sub>), 131.9 (C<sub>quat</sub>), 131.1 (d,  $J_{C-F}$  = 8.9 Hz, 2 × Carom-N3), 129.7 (2 × Carom-N1), 128.9 (C<sub>quat</sub>), 126.2 (2 × Carom-N1), 126.1 (Carom), 125.0 (C<sub>quat</sub>), 124.8 (Carom), 120.2 (C<sub>quat</sub>), 119.0 (Carom), 115.7 (d,  $J_{C-F}$  = 22.7 Hz, 2 × Carom-N3), 113.6 (Carom), 52.1 (O-CH<sub>2</sub>), 47.01 (N-CH<sub>3</sub>) ppm. <sup>19</sup>F NMR (376 MHz, DMSO-*d*<sub>6</sub>) δ -114.03 ppm. HRMS-ESI (*m/z*) [M+H]<sup>+</sup> calcd. for C<sub>24</sub>H<sub>18</sub>FN<sub>4</sub>O<sub>4</sub>: 445.1306 found: 445.1305. λ<sub>abs</sub>: 259 nm λ<sub>em</sub>: 492 nm λ<sub>exc</sub>: 266 nm. R<sub>f</sub> 0.45 (DCM/MeOH 95:5).

1-[[*p*-(Dimethoxyphosphorylmethyl)phenyl]methyl]-3-(4-fluorophenyl)purino [9,8-*a*]pyridine-2,4-dione (13f). The title compound was prepared from commercially available 9a to afford after purification the desired product as a yellow foam (16%). <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 8.22 (d, 1H,  $J$  = 7.3 Hz, H<sub>6</sub>), 7.60 (d, 1H,  $J$  = 9.3 Hz, H<sub>9</sub>), 7.46 (dd, 2H,  $J$  = 7.8, 5.3 Hz, 2 × H<sub>arom-N3</sub>), 7.41–7.31 (m, 4H, 2 × H<sub>arom-N1</sub>, 2 × H<sub>arom-N3</sub>), 7.31–7.20 (m, 3H, H<sub>8</sub>, 2 × H<sub>arom-N1</sub>), 6.81 (t, 1H,  $J$  = 7.3 Hz, H<sub>7</sub>), 5.71 (s, 2H, N-CH<sub>2</sub>), 3.55 (s, 3H, O-CH<sub>3</sub>), 3.53 (s, 3H, O-CH<sub>3</sub>), 3.33 (s, 2H, C-CH<sub>2</sub>) ppm. <sup>13</sup>C NMR (101 MHz, DMSO-*d*<sub>6</sub>) δ 160.8 (C=O), 158.4 (C<sub>quat</sub>), 151.6 (C=O), 143.0 (C<sub>quat</sub>), 134.9 (d,  $J$  = 3.7 Hz, C<sub>quat</sub>), 133.1 (d,  $J_{C-F}$  = 3.0 Hz, C<sub>quat</sub>), 132.5 (C<sub>quat</sub>), 131.9 (d,  $J_{C-F}$  = 9.1 Hz, C<sub>quat</sub>), 131.6 (d,  $J_{C-F}$  = 9.0 Hz, 2 × Carom-N3), 130.8 (d,  $J_{C-P}$  = 6.5 Hz, 2 × Carom-N1), 126.5 (Carom), 126.2 (d,  $J_{C-P}$  = 3.0 Hz, 2 × Carom-N1), 125.5 (Carom), 120.6 (C<sub>quat</sub>), 119.4 (Carom), 116.2 (d,  $J_{C-F}$  = 22.9 Hz, 2 × Carom-N3), 113.9 (Carom), 52.8 (O-CH<sub>3</sub>), 52.7 (O-CH<sub>3</sub>), 47.2 (N-CH<sub>2</sub>), 29.52 (d,  $J_{C-P}$  = 108.5 Hz, P-CH<sub>2</sub>) ppm. <sup>19</sup>F NMR (376 MHz, DMSO-

$d_6$ )  $\delta$  -114.07 ppm.  $^{31}\text{P}$  NMR (162 MHz, DMSO- $d_6$ )  $\delta$  28.89 ppm. HRMS-ESI ( $m/z$ )  $[\text{M}+\text{H}]^+$  calcd. for  $\text{C}_{24}\text{H}_{21}\text{FN}_4\text{O}_5\text{P}$ : 509.1384, found: 509.1380.  $\lambda_{\text{abs}}$ : 264 nm  $\lambda_{\text{em}}$ : 415 nm  $\lambda_{\text{exc}}$ : 265 nm.  $R_f$  0.2 (DCM/MeOH 95:5).

### 3.5. General synthetic procedure 4 for the Sonogashira coupling cross coupling.

Under inert atmosphere, to a solution of deprotected Br-compound (1 eq.) in dry DMF (0.082 M) were successively added copper iodide (0.2 eq.), triethylamine (3 eq.), alkynyl substrate (3 eq.), and  $\text{Pd}(\text{PPh}_3)_4$  (10 mol%). The reaction mixture was heated under microwave irradiation at 110 °C for 15 min. The reaction was quenched with EtOAc and co-evaporated with heptane. Pure compounds were obtained after purification by flash column chromatography with DCM/MeOH as eluent.

3-(*p*-Fluorophenyl)-7-(4-phenylbut-1-ynyl)-1*H*-purino [9,8-*a*]pyridine-2,4-dione (**20a**). The title compound was prepared from commercially available **19a** to afford after purification the desired product as an orange/yellow solid (70%).  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  8.35 (d, 1H,  $J = 7.2$  Hz,  $\text{H}_9$ ), 7.45 (s, 1H,  $\text{H}_6$ ), 7.35–7.30 (d, 4H,  $4 \times \text{H}_{\text{arom}}$ ), 7.29–7.22 (m, 5H,  $4 \times \text{H}_{\text{arom-N3}}$ ,  $\text{H}_{\text{arom}}$ ), 6.77 (dd, 1H,  $J = 7.2, 1.8$  Hz,  $\text{H}_8$ ), 2.88 (t, 2H,  $J = 7.2$  Hz, C- $\text{CH}_2$ ), 2.76 (t, 2H,  $J = 7.2$  Hz,  $\text{CH}_2$ - $\text{CH}_2$ ) ppm.  $^{13}\text{C}$  NMR (101 MHz, DMSO- $d_6$ )  $\delta$  162.3 (C=O), 159.9 ( $\text{C}_{\text{quat}}$ ), 159.2 ( $\text{C}_{\text{quat}}$ ), 153.2 (C=O), 140.7 ( $\text{C}_{\text{quat}}$ ), 140.3 ( $\text{C}_{\text{quat}}$ ), 134.0–133.6 (m,  $\text{C}_{\text{quat}}$ ), 131.2 (d,  $J_{\text{C-F}} = 8.8$  Hz,  $2 \times \text{C}_{\text{arom-N3}}$ ), 128.5 ( $2 \times \text{C}_{\text{arom}}$ ), 128.2 ( $2 \times \text{C}_{\text{arom}}$ ), 126.2 ( $\text{C}_{\text{arom}}$ ), 123.9 ( $\text{C}_{\text{arom}}$ ), 120.4 ( $\text{C}_{\text{quat}}$ ), 119.9 ( $\text{C}_{\text{arom}}$ ), 118.6 ( $\text{C}_{\text{quat}}$ ), 115.3 (d,  $J_{\text{C-F}} = 22.7$  Hz,  $2 \times \text{C}_{\text{arom-N3}}$ ), 114.0 ( $\text{C}_{\text{arom}}$ ), 93.9 (CH $_2$ -C), 80.0 (C=C), 34.0 (CH $_2$ -C), 21.0 (C-CH $_2$ ) ppm.  $^{19}\text{F}$  NMR (376 MHz, DMSO- $d_6$ )  $\delta$  -115.39 ppm. HRMS-ESI ( $m/z$ )  $[\text{M}+\text{H}]^+$  calcd. for  $\text{C}_{25}\text{H}_{18}\text{FN}_4\text{O}_2$ : 425.1408, found: 425.1409.  $\lambda_{\text{abs}}$ : 266 nm  $\lambda_{\text{em}}$ : 513 nm  $\lambda_{\text{exc}}$ : 278 nm.  $R_f$  0.34 (DCM/MeOH 95:5).

3-(*p*-Fluorophenyl)-7-[2-(4-propylphenyl)ethynyl]-1*H*-purino [9,8-*a*]pyridine-2,4-dione (**20b**). The title compound was prepared from commercially available **19a** to afford after purification the desired product as a yellow solid (74%).  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  9.57 (s, 1H,  $\text{H}_1$ ), 8.39 (d, 1H,  $J = 7.3$  Hz,  $\text{H}_9$ ), 7.71 (s, 1H,  $\text{H}_6$ ), 7.52 (d, 2H,  $J = 7.8$  Hz,  $2 \times \text{H}_{\text{arom}}$ ), 7.28 (dd, 6H,  $J = 7.5, 3.5$  Hz,  $4 \times \text{H}_{\text{arom-N3}}$ ,  $2 \times \text{H}_{\text{arom}}$ ), 6.99 (d, 1H,  $J = 7.3$  Hz,  $\text{H}_8$ ), 2.60 (t, 2H,  $J = 7.5$  Hz, C-CH $_2$ ), 1.61 (h, 2H,  $J = 7.5$  Hz,  $\text{CH}_2$ -CH $_2$ ), 0.90 (t, 3H,  $J = 7.5$  Hz, CH $_2$ -CH $_3$ ) ppm.  $^{13}\text{C}$  NMR (101 MHz, DMSO- $d_6$ )  $\delta$  162.3 (C=O), 159.9 ( $\text{C}_{\text{quat}}$ ), 159.2 ( $\text{C}_{\text{quat}}$ ), 154.3 (C=O), 143.6 ( $\text{C}_{\text{quat}}$ ), 140.6 ( $\text{C}_{\text{quat}}$ ), 133.6 ( $\text{C}_{\text{quat}}$ ), 131.4 ( $2 \times \text{C}_{\text{arom}}$ ), 131.2 (d,  $J_{\text{C-F}} = 8.7$  Hz,  $2 \times \text{C}_{\text{arom-N3}}$ ), 128.8 ( $2 \times \text{C}_{\text{arom}}$ ), 123.9 ( $\text{C}_{\text{arom}}$ ), 120.9 ( $\text{C}_{\text{arom}}$ ), 119.0 ( $\text{C}_{\text{quat}}$ ), 118.9 ( $\text{C}_{\text{quat}}$ ), 115.3 (d,  $J_{\text{C-F}} = 22.3$  Hz,  $2 \times \text{C}_{\text{arom-N3}}$ ), 113.8 ( $\text{C}_{\text{arom}}$ ), 92.6 ( $\text{C}_{\text{quat}}$ ), 90.1 (CH $_2$ -C), 87.6 (C=C), 37.0 (C-CH $_2$ ), 23.7 (CH $_2$ -CH $_2$ ), 13.5 (CH $_2$ -CH $_3$ ) ppm.  $^{19}\text{F}$  NMR (376 MHz, DMSO- $d_6$ )  $\delta$  -115.43 ppm. HRMS-ESI ( $m/z$ )  $[\text{M}+\text{H}]^+$  calcd. for  $\text{C}_{26}\text{H}_{20}\text{FN}_4\text{O}_2$ : 439.1564, found: 439.1563.  $\lambda_{\text{abs}}$ : 300 nm  $\lambda_{\text{em}}$ : 528 nm  $\lambda_{\text{exc}}$ : 307 nm.  $R_f$  0.35 (DCM/MeOH 95:5).

3-(*p*-Fluorophenyl)-8-hex-1-ynyl-1*H*-purino [9,8-*a*]pyridine-2,4-dione (**20c**). The title compound was prepared from commercially available **19a** to afford after purification the desired product as a yellow/orange solid (91%).  $^1\text{H}$  NMR (400 MHz, DMSO- $d_6$ )  $\delta$  12.95 (bs, 1H,  $\text{H}_1$ ), 8.40 (d, 1H,  $J = 7.2$  Hz,  $\text{H}_9$ ), 7.61 (s, 1H,  $\text{H}_6$ ), 7.39–7.25 (m, 4H,  $4 \times \text{H}_{\text{arom}}$ ), 6.95 (dd, 1H,  $J = 7.2, 1.6$  Hz,  $\text{H}_8$ ), 2.51–2.47 (m, 2H, CH $_2$ ), 1.59–1.52 (m, 2H, CH $_2$ ), 1.50–1.39 (m, 2H, CH $_2$ ), 0.93 (t, 3H,  $J = 7.3$  Hz, CH $_2$ -CH $_3$ ) ppm.  $^{13}\text{C}$  NMR (101 MHz, DMSO- $d_6$ )  $\delta$  161.6 (C=O), 159.5 ( $\text{C}_{\text{quat}}$ ), 150.0 (C=O), 141.1 ( $\text{C}_{\text{quat}}$ ), 132.4 ( $\text{C}_{\text{quat}}$ ), 131.3 (d,  $J_{\text{C-F}} = 9.0$  Hz,  $2 \times \text{C}_{\text{arom}}$ ), 123.8 ( $\text{C}_{\text{arom}}$ ), 120.7 ( $\text{C}_{\text{quat}}$ ), 120.5 ( $\text{C}_{\text{arom}}$ ), 119.9 ( $\text{C}_{\text{quat}}$ ), 115.6 (d,  $J_{\text{C-F}} = 23.2$  Hz,  $2 \times \text{C}_{\text{arom}}$ ), 115.3 ( $\text{C}_{\text{arom}}$ ), 113.7 ( $\text{C}_{\text{quat}}$ ), 95.0 (CH $_2$ -C), 79.1 (C=C), 30.0 (CH $_2$ -CH $_2$ ), 21.4 (CH $_2$ -CH $_2$ ), 18.4 (CH $_2$ -CH $_2$ ), 13.4 (CH $_2$ -CH $_3$ ) ppm.  $^{19}\text{F}$  NMR (376 MHz, DMSO- $d_6$ )  $\delta$  -114.41 ppm. HRMS-ESI ( $m/z$ )  $[\text{M}+\text{H}]^+$  calcd. for  $\text{C}_{21}\text{H}_{18}\text{FN}_4\text{O}_2$ : 377.1408, found: 377.1406.  $\lambda_{\text{abs}}$ : 263 nm  $\lambda_{\text{em}}$ : 513 nm  $\lambda_{\text{exc}}$ : 278 nm.  $R_f$  0.33 (DCM/MeOH 95:5).

3-(*p*-Fluorophenyl)-8-hept-1-ynyl-1*H*-purino [9,8-*a*]pyridine-2,4-dione (**20d**). The title compound was prepared from commercially available **19a** to afford after purification the desired product as an orange solid (95%).  $^1\text{H}$  NMR (250 MHz, DMSO- $d_6$ )  $\delta$  12.95 (s, 1H,  $\text{H}_1$ ), 8.43 (d, 1H,  $J = 7.3$  Hz,  $\text{H}_9$ ), 7.62 (s, 1H,  $\text{H}_6$ ), 7.42–7.24 (m, 4H,  $4 \times \text{H}_{\text{arom}}$ ), 6.97 (dd, 1H,  $J = 7.3, 1.6$  Hz,  $\text{H}_8$ ), 2.51–2.47 (m, 2H, CH $_2$ ), 1.57 (q, 2H,  $J = 7.0$  Hz, CH $_2$ -CH $_2$ ), 1.46–1.28 (m, 4H,  $2 \times \text{CH}_2$ ), 0.90 (t, 3H,  $J = 7.0$  Hz, CH $_2$ -CH $_3$ ) ppm.  $^{13}\text{C}$  NMR (63 MHz, DMSO- $d_6$ )  $\delta$

163.4 (C=O), 158.2 (C<sub>quat</sub>), 150.2 (C=O), 141.1 (C<sub>quat</sub>), 132.3 (d,  $J_{C-F}$  = 3.7 Hz, C<sub>quat</sub>), 131.3 (d,  $J_{C-F}$  = 8.8 Hz, 2 × C<sub>arom</sub>), 128.6 (C<sub>quat</sub>), 123.8 (C<sub>arom</sub>), 120.8 (C<sub>arom</sub>), 120.5 (C<sub>quat</sub>), 119.0 (C<sub>quat</sub>), 115.9 (2 × C<sub>arom</sub>), 115.5 (C<sub>arom</sub>), 95.2 (CH<sub>2</sub>-C), 79.0 (C≡C), 30.4 (C-CH<sub>2</sub>), 27.5 (CH<sub>2</sub>-CH<sub>2</sub>), 21.5 (CH<sub>2</sub>-CH<sub>2</sub>), 18.8 (CH<sub>2</sub>-CH<sub>2</sub>), 13.8 (CH<sub>2</sub>-CH<sub>3</sub>) ppm. <sup>19</sup>F NMR (376 MHz, DMSO-*d*<sub>6</sub>) δ -114.36 ppm. HRMS-ESI (*m/z*) [M+H]<sup>+</sup> calcd. for C<sub>22</sub>H<sub>20</sub>FN<sub>4</sub>O<sub>2</sub>: 391.1564, found: 391.1563. λ<sub>abs</sub>: 267 nm λ<sub>em</sub>: 517 nm λ<sub>exc</sub>: 277 nm. R<sub>f</sub> 0.33 (DCM/MeOH 95:5).

3-(*p*-Fluorophenyl)-7-oct-1-ynyl-1*H*-purino [9,8-*a*]pyridine-2,4-dione (**20e**). The title compound was prepared from commercially available 19a to afford after purification the desired product as a yellow solid (92%). <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 8.41 (d, 1H,  $J$  = 7.7 Hz, H<sub>9</sub>), 7.61 (s, 1H, H<sub>6</sub>), 7.39–7.27 (m, 4H, 4 × H<sub>arom</sub>), 6.96 (dd, 1H,  $J$  = 7.7, 1.5 Hz, H<sub>8</sub>), 2.51–2.40 (m, 2H, CH<sub>2</sub>), 1.57 (p, 2H,  $J$  = 7.0 Hz, CH<sub>2</sub>), 1.43 (dt, 2H,  $J$  = 13.3, 7.0 Hz, CH<sub>2</sub>-CH<sub>2</sub>), 1.30 (dt, 4H,  $J$  = 7.0, 3.8 Hz, 2 × CH<sub>2</sub>), 0.89 (t, 3H,  $J$  = 7.0 Hz, CH<sub>2</sub>-CH<sub>3</sub>) ppm. <sup>13</sup>C NMR (101 MHz, DMSO-*d*<sub>6</sub>) δ 162.7 (C=O), 158.4 (C<sub>quat</sub>), 150.3 (C=O), 141.2 (C<sub>quat</sub>), 132.4 (C<sub>quat</sub>), 132.3 (d,  $J_{C-F}$  = 3.1 Hz, C<sub>quat</sub>), 131.2 (d,  $J_{C-F}$  = 8.8 Hz, 2 × C<sub>arom</sub>), 123.8 (C<sub>arom</sub>), 120.8 (C<sub>quat</sub>), 120.5 (C<sub>arom</sub>), 118.9 (C<sub>quat</sub>), 115.6 (d,  $J_{C-F}$  = 22.7 Hz, 2 × C<sub>arom</sub>), 115.4 (C<sub>arom</sub>), 95.1 (CH<sub>2</sub>-C), 79.1 (C≡C), 30.7 (C-CH<sub>2</sub>), 27.9 (CH<sub>2</sub>-CH<sub>2</sub>), 27.8 (CH<sub>2</sub>-CH<sub>2</sub>), 21.9 (CH<sub>2</sub>-CH<sub>2</sub>), 18.7 (CH<sub>2</sub>-CH<sub>2</sub>), 13.9 (CH<sub>2</sub>-CH<sub>3</sub>) ppm. <sup>19</sup>F NMR (376 MHz, DMSO-*d*<sub>6</sub>) δ -114.38 ppm. HRMS-ESI (*m/z*) [M+H]<sup>+</sup> calcd. for C<sub>23</sub>H<sub>22</sub>FN<sub>4</sub>O<sub>2</sub>: 405.1721, found: 405.1718. λ<sub>abs</sub>: 268 nm λ<sub>em</sub>: 512 nm λ<sub>exc</sub>: 276 nm. R<sub>f</sub> 0.33 (DCM/MeOH 95:5).

*N*-[3-[3-(*p*-fluorophenyl)-2,4-dioxo-1*H*-purino [9,8-*a*]pyridin-7-yl]prop-2-ynyl]octanamide (**20f**). The title compound was prepared from commercially available 19a to afford after purification the desired product as an orange solid (95%). <sup>1</sup>H NMR (250 MHz, DMSO-*d*<sub>6</sub>) δ 8.43–8.31 (m, 1H, H<sub>9</sub>), 7.52 (s, 1H, H<sub>6</sub>), 7.26 (t, 4H,  $J$  = 7.1 Hz, 4 × H<sub>arom</sub>), 6.82 (t, 1H,  $J$  = 8.0 Hz, H<sub>8</sub>), 4.23 (d, 2H,  $J$  = 11.3 Hz, C-CH<sub>2</sub>), 2.10 (q, 2H,  $J$  = 12.5, 9.9 Hz, CO-CH<sub>2</sub>), 1.65 (q, 2H,  $J$  = 7.1 Hz, CH<sub>2</sub>-CH<sub>2</sub>), 1.24 (q, 8H,  $J$  = 9.6, 7.1 Hz, 4 × CH<sub>2</sub>), 0.85 (tq, 3H,  $J$  = 11.1, 6.5, 5.6 Hz, CH<sub>2</sub>-CH<sub>3</sub>) ppm. <sup>13</sup>C NMR (63 MHz, DMSO-*d*<sub>6</sub>) δ 167.0 (C=O), 162.7 (C=O), 159.3 (C<sub>quat</sub>), 152.03 (C=O), 136.1 (C<sub>quat</sub>), 134.0 (C<sub>quat</sub>), 133.8 (d,  $J_{C-F}$  = 3.2 Hz, C<sub>quat</sub>), 131.3 (d,  $J_{C-F}$  = 11.6 Hz, 2 × C<sub>arom</sub>), 124.1 (C<sub>arom</sub>), 120.9 (C<sub>arom</sub>), 119.0 (C<sub>quat</sub>), 118.8 (C<sub>quat</sub>), 115.2 (2 × C<sub>arom</sub>), 113.7 (C<sub>arom</sub>), 90.9 (CH<sub>2</sub>-C), 79.6 (C≡C), 45.3 (N-CH<sub>2</sub>), 34.6 (CO-CH<sub>2</sub>), 31.0 (CH<sub>2</sub>-CH<sub>2</sub>), 28.5 (CH<sub>2</sub>-CH<sub>2</sub>), 28.2 (CH<sub>2</sub>-CH<sub>2</sub>), 24.9 (CH<sub>2</sub>-CH<sub>2</sub>), 22.1 (CH<sub>2</sub>-CH<sub>2</sub>), 13.7 (CH<sub>2</sub>-CH<sub>3</sub>) ppm. <sup>19</sup>F NMR (376 MHz, DMSO-*d*<sub>6</sub>) δ -115.58 ppm. HRMS-ESI (*m/z*) [M+H]<sup>+</sup> calcd. for C<sub>26</sub>H<sub>27</sub>FN<sub>5</sub>O<sub>2</sub>: 476.2092, found: 476.2089. λ<sub>abs</sub>: 271 nm λ<sub>em</sub>: 510 nm λ<sub>exc</sub>: 279 nm. R<sub>f</sub> 0.32 (DCM/MeOH 95:5).

1-[3-[3-(*p*-Fluorophenyl)-2,4-dioxo-1*H*-purino [9,8-*a*]pyridin-7-yl]prop-2-ynyl]-3-hexyl-urea (**20g**). The title compound was prepared from commercially available 19a to afford after purification the desired product as an orange solid (92%). <sup>1</sup>H NMR (250 MHz, DMSO-*d*<sub>6</sub>) δ 8.29 (d, 1H,  $J$  = 7.5 Hz, H<sub>9</sub>), 7.48 (s, 1H, H<sub>6</sub>), 7.41–7.11 (m, 4H, 4 × H<sub>arom-N3</sub>), 6.73 (t, 1H,  $J$  = 7.5 Hz, H<sub>8</sub>), 4.09 (d, 1H,  $J$  = 5.7 Hz, NH-CH<sub>2</sub>), 1.52–0.99 (m, 10H, 5 × CH<sub>2</sub>), 0.85 (t, 3H,  $J$  = 6.6 Hz, CH<sub>2</sub>-CH<sub>3</sub>) ppm. <sup>13</sup>C NMR (63 MHz, DMSO-*d*<sub>6</sub>) δ 163.8 (C=O), 158.0 (C=O), 151.4 (C=O), 140.7 (C<sub>quat</sub>), 136.9 (C<sub>quat</sub>), 135.3 (C<sub>quat</sub>), 131.6 (d,  $J_{C-F}$  = 9.9 Hz, 2 × C<sub>arom</sub>), 124.3 (C<sub>arom</sub>), 121.2 (C<sub>arom</sub>), 118.9 (C<sub>quat</sub>), 115.7 (C<sub>quat</sub>), 115.2 (2 × C<sub>arom</sub>), 113.3 (C<sub>arom</sub>), 96.3 (C<sub>quat</sub>), 92.1 (CH<sub>2</sub>-C), 80.6 (C≡C), 39.7 (N-CH<sub>2</sub>), 31.4 (CH<sub>2</sub>-CH<sub>2</sub>), 30.4 (CH<sub>2</sub>-CH<sub>2</sub>), 30.0 (CH<sub>2</sub>-CH<sub>2</sub>), 26.4 (CH<sub>2</sub>-CH<sub>2</sub>), 22.4 (CH<sub>2</sub>-CH<sub>2</sub>), 14.2 (CH<sub>2</sub>-CH<sub>3</sub>) ppm. <sup>19</sup>F NMR (376 MHz, DMSO-*d*<sub>6</sub>) δ -116.27 ppm. HRMS-ESI (*m/z*) [M+H]<sup>+</sup> calcd. for C<sub>25</sub>H<sub>26</sub>FN<sub>6</sub>O<sub>3</sub>: 477.2044, found: 477.2042. λ<sub>abs</sub>: 279 nm λ<sub>em</sub>: 515 nm λ<sub>exc</sub>: 278 nm. R<sub>f</sub> 0.30 (DCM/MeOH 95:5).

3-(*p*-Fluorophenyl)-8-(4-phenylbut-1-ynyl)-1*H*-purino [9,8-*a*]pyridine-2,4-dione (**21a**). The title compound was prepared from commercially available 19b to afford after purification the desired product as an orange solid (80%). <sup>1</sup>H NMR (250 MHz, DMSO-*d*<sub>6</sub>) δ 8.44 (s, 1H, H<sub>9</sub>), 7.45 (d, 1H,  $J$  = 9.6 Hz, H<sub>6</sub>), 7.33 (d, 4H,  $J$  = 4.3 Hz, 4 × H<sub>arom</sub>), 7.25 (h, 5H,  $J$  = 4.0 Hz, 4 × H<sub>arom-N3</sub>, H<sub>arom</sub>), 7.07 (d, 1H,  $J$  = 9.6 Hz, H<sub>7</sub>), 2.89 (t, 2H,  $J$  = 6.9 Hz, C-CH<sub>2</sub>), 2.75 (t, 2H,  $J$  = 6.9 Hz, CH<sub>2</sub>-CH<sub>2</sub>) ppm. <sup>13</sup>C NMR (63 MHz, DMSO-*d*<sub>6</sub>) δ 162.4 (C=O), 159.3 (C<sub>quat</sub>), 153.7 (C=O), 151.2 (C<sub>quat</sub>), 140.3 (C<sub>quat</sub>), 139.8 (C<sub>quat</sub>), 133.9 (C<sub>quat</sub>), 131.2 (2 × C<sub>arom</sub>), 128.5 (d,  $J_{C-F}$  = 8.8 Hz, 2 × C<sub>arom-N3</sub>), 128.2 (2 × C<sub>arom</sub>), 127.9 (C<sub>arom</sub>), 126.3 (C<sub>arom</sub>), 126.2 (C<sub>arom</sub>), 118.3 (C<sub>arom</sub>), 118.0 (C<sub>quat</sub>), 115.3 (d,  $J_{C-F}$  = 22.3 Hz, 2 × C<sub>arom-N3</sub>), 107.8 (C<sub>quat</sub>), 91.7 (CH<sub>2</sub>-C),

77.4 (C≡C), 34.0 (C–CH<sub>2</sub>), 20.8 (CH<sub>2</sub>–C) ppm. <sup>19</sup>F NMR (376 MHz, DMSO-*d*<sub>6</sub>) δ –115.57 ppm. HRMS-ESI (*m/z*) [M+H]<sup>+</sup> calcd. for C<sub>25</sub>H<sub>18</sub>FN<sub>4</sub>O<sub>2</sub>: 425.1408, found: 425.1406. λ<sub>abs</sub>: 272 nm λ<sub>em</sub>: 443 nm λ<sub>exc</sub>: 286 nm. R<sub>f</sub> 0.33 (DCM/MeOH 95:5).

3-(*p*-Fluorophenyl)-8-[2-(4-propylphenyl)ethynyl]-1*H*-purino [9,8-*a*]pyridine-2,4-dione (**21b**). The title compound was prepared from commercially available **19b** to afford after purification the desired product as a beige solid (67%). <sup>1</sup>H NMR (250 MHz, DMSO-*d*<sub>6</sub>) δ 8.81 (s, 1H, H<sub>9</sub>), 7.86–7.72 (m, 1H, H<sub>6</sub>), 7.68–7.61 (m, 3H, H<sub>7</sub>, 2 × H<sub>arom</sub>), 7.55–7.48 (m, 2H, 2 × H<sub>arom</sub>), 7.44–7.23 (m, 4H, 4 × H<sub>arom-N3</sub>), 2.61 (t, 2H, *J* = 7.6 Hz, C–CH<sub>2</sub>), 1.59 (ddt, 2H, *J* = 12.8, 10.0, 6.7 Hz, CH<sub>2</sub>–CH<sub>2</sub>), 0.98–0.80 (m, 3H, CH<sub>2</sub>–CH<sub>3</sub>) ppm. <sup>13</sup>C NMR (63 MHz, DMSO-*d*<sub>6</sub>) δ 163.4 (C=O), 159.6 (C<sub>quat</sub>), 150.0 (C=O), 143.6 (C<sub>quat</sub>), 140.4 (C<sub>quat</sub>), 133.7 (C<sub>quat</sub>), 132.2 (C<sub>quat</sub>), 131.4 (2 × C<sub>arom</sub>), 131.3 (d, *J*<sub>C-F</sub> = 10.3 Hz, 2 × C<sub>arom-N3</sub>), 128.8 (2 × C<sub>arom</sub>), 128.8 (C<sub>arom</sub>), 126.9 (C<sub>arom</sub>), 118.7 (C<sub>arom</sub>), 118.5 (C<sub>quat</sub>), 118.5 (C<sub>quat</sub>), 115.5 (d, *J*<sub>C-F</sub> = 24.9 Hz, 2 × C<sub>arom-N3</sub>), 108.5 (C<sub>quat</sub>), 91.2 (CH<sub>2</sub>–C), 84.7 (C≡C), 37.0 (CH<sub>2</sub>–CH<sub>2</sub>), 23.6 (CH<sub>2</sub>–CH<sub>2</sub>), 13.4 (CH<sub>2</sub>–CH<sub>3</sub>) ppm. <sup>19</sup>F NMR (235 MHz, DMSO-*d*<sub>6</sub>) δ –114.29 ppm. HRMS-ESI (*m/z*) [M+H]<sup>+</sup> calcd. for C<sub>26</sub>H<sub>20</sub>FN<sub>4</sub>O<sub>2</sub>: 439.1564, found: 439.1562. λ<sub>abs</sub>: 302 nm λ<sub>em</sub>: 454 nm λ<sub>exc</sub>: 288 nm. R<sub>f</sub> 0.35 (DCM/MeOH 95:5).

3-(*p*-Fluorophenyl)-8-hex-1-ynyl-1*H*-purino [9,8-*a*]pyridine-2,4-dione (**21c**). The title compound was prepared from commercially available **19b** to afford after purification the desired product as a beige solid (42%). <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 12.80 (s, 1H, H<sub>1</sub>), 8.64 (s, 1H, H<sub>9</sub>), 7.56 (d, 1H, *J* = 9.5 Hz, H<sub>6</sub>), 7.38–7.27 (m, 4H, 4 × H<sub>arom</sub>), 7.23 (dd, 1H, *J* = 9.5, 1.6 Hz, H<sub>8</sub>), 2.48 (d, 2H, *J* = 7.0 Hz, C–CH<sub>2</sub>), 1.61–1.51 (m, 2H, CH<sub>2</sub>–CH<sub>2</sub>), 1.51–1.41 (m, 2H, CH<sub>2</sub>–CH<sub>2</sub>), 0.94 (t, 3H, *J* = 7.0 Hz, CH<sub>2</sub>–CH<sub>3</sub>) ppm. <sup>13</sup>C NMR (101 MHz, DMSO-*d*<sub>6</sub>) δ 160.3 (C=O), 158.4 (C<sub>quat</sub>), 150.2 (C=O), 140.3 (C<sub>quat</sub>), 132.3 (C<sub>quat</sub>), 132.2 (C<sub>quat</sub>), 131.2 (d, *J*<sub>C-F</sub> = 8.8 Hz, 2 × C<sub>arom</sub>), 128.7 (C<sub>arom</sub>), 126.5 (C<sub>arom</sub>), 118.3 (C<sub>quat</sub>), 118.3 (C<sub>arom</sub>), 115.6 (d, *J*<sub>C-F</sub> = 22.8 Hz, 2 × C<sub>arom</sub>), 109.2 (C<sub>quat</sub>), 92.8 (CH<sub>2</sub>–C), 76.4 (C≡C), 30.0 (CH<sub>2</sub>–CH<sub>2</sub>), 21.3 (CH<sub>2</sub>–CH<sub>2</sub>), 18.2 (CH<sub>2</sub>–CH<sub>2</sub>), 13.4 (CH<sub>2</sub>–CH<sub>3</sub>) ppm. <sup>19</sup>F NMR (376 MHz, DMSO-*d*<sub>6</sub>) δ –114.37 ppm. HRMS-ESI (*m/z*) [M+H]<sup>+</sup> calcd. for C<sub>21</sub>H<sub>18</sub>FN<sub>4</sub>O<sub>2</sub>: 377.1408, found: 377.1404. λ<sub>abs</sub>: 273 nm λ<sub>em</sub>: 498 nm λ<sub>exc</sub>: 286 nm. R<sub>f</sub> 0.33 (DCM/MeOH 95:5).

3-(*p*-Fluorophenyl)-8-hept-1-ynyl-1*H*-purino [9,8-*a*]pyridine-2,4-dione (**21d**). The title compound was prepared from commercially available **19b** to afford after purification the desired product as an orange solid (50%). <sup>1</sup>H NMR (250 MHz, DMSO-*d*<sub>6</sub>) δ 8.56 (s, 1H, H<sub>9</sub>), 7.93–7.79 (m, 1H, H<sub>6</sub>), 7.57–7.41 (m, 1H, H<sub>7</sub>), 7.24 (dd, 4H, *J* = 28.7, 8.3 Hz, 4 × H<sub>arom</sub>), 2.60–2.45 (m, 2H, C–CH<sub>2</sub>), 1.58 (t, 2H, *J* = 7.2 Hz, CH<sub>2</sub>–CH<sub>2</sub>), 1.52–1.30 (m, 4H, 2 × CH<sub>2</sub>), 0.88 (dt, 3H, *J* = 13.5, 6.5 Hz, CH<sub>2</sub>–CH<sub>3</sub>) ppm. <sup>13</sup>C NMR (63 MHz, DMSO-*d*<sub>6</sub>) δ 163.4 (C=O), 158.8 (C<sub>quat</sub>), 151.7 (C=O), 146.8 (C<sub>quat</sub>), 140.0 (C<sub>quat</sub>), 133.1 (d, *J*<sub>C-F</sub> = 3.2 Hz, C<sub>quat</sub>), 131.3 (d, *J*<sub>C-F</sub> = 11.7 Hz, 2 × C<sub>arom</sub>), 128.4 (C<sub>arom</sub>), 126.2 (C<sub>arom</sub>), 118.2 (C<sub>arom</sub>), 118.1 (C<sub>quat</sub>), 115.5 (d, *J*<sub>C-F</sub> = 24.3 Hz, 2 × C<sub>arom</sub>), 108.3 (C<sub>quat</sub>), 92.31 (CH<sub>2</sub>–C), 76.7 (C≡C), 30.4 (CH<sub>2</sub>–CH<sub>2</sub>), 27.6 (CH<sub>2</sub>–CH<sub>2</sub>), 21.5 (CH<sub>2</sub>–CH<sub>2</sub>), 18.6 (CH<sub>2</sub>–CH<sub>2</sub>), 13.8 (CH<sub>2</sub>–CH<sub>3</sub>) ppm. <sup>19</sup>F NMR (376 MHz, DMSO-*d*<sub>6</sub>) δ –114.97 ppm. HRMS-ESI (*m/z*) [M+H]<sup>+</sup> calcd. for C<sub>22</sub>H<sub>20</sub>FN<sub>4</sub>O<sub>2</sub>: 391.1564, found: 391.1563. λ<sub>abs</sub>: 271 nm λ<sub>em</sub>: 515 nm λ<sub>exc</sub>: 284 nm. R<sub>f</sub> 0.33 (DCM/MeOH 95:5).

3-(*p*-Fluorophenyl)-8-oct-1-ynyl-1*H*-purino [9,8-*a*]pyridine-2,4-dione (**21e**). The title compound was prepared from commercially available **19b** to afford after purification the desired product as an orange solid (74%). <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 12.79 (s, 1H, H<sub>1</sub>), 8.63 (s, 1H, H<sub>9</sub>), 7.57 (d, 1H, *J* = 9.6 Hz, H<sub>6</sub>), 7.41–7.28 (m, 4H, 4 × H<sub>arom</sub>), 7.23 (dd, 1H, *J* = 9.6, 1.6 Hz, C–CH<sub>2</sub>), 2.48–2.42 (m, 2H, CH<sub>2</sub>–CH<sub>2</sub>), 1.62–1.52 (m, 2H, CH<sub>2</sub>–CH<sub>2</sub>), 1.48–1.38 (m, 2H, CH<sub>2</sub>–CH<sub>2</sub>), 1.32 (dq, 4H, *J* = 7.1, 3.3 Hz, 2 × CH<sub>2</sub>), 0.95–0.79 (m, 3H, CH<sub>2</sub>–CH<sub>3</sub>) ppm. <sup>13</sup>C NMR (101 MHz, DMSO-*d*<sub>6</sub>) δ 162.7 (C=O), 160.3 (C<sub>quat</sub>), 158.4 (C<sub>quat</sub>), 150.2 (C=O), 140.3 (C<sub>quat</sub>), 132.3 (d, *J* = 4.3 Hz, C<sub>quat</sub>), 131.2 (d, *J*<sub>C-F</sub> = 9.9 Hz, 2 × C<sub>arom</sub>), 128.7 (C<sub>arom</sub>), 126.4 (C<sub>arom</sub>), 118.5 (C<sub>quat</sub>), 118.3 (C<sub>arom</sub>), 115.6 (d, *J*<sub>C-F</sub> = 22.6 Hz, 2 × C<sub>arom</sub>), 109.2 (C<sub>quat</sub>), 92.9 (CH<sub>2</sub>–C), 76.4 (C≡C), 30.7 (CH<sub>2</sub>–CH<sub>2</sub>), 27.9 (CH<sub>2</sub>–CH<sub>2</sub>), 27.9 (CH<sub>2</sub>–CH<sub>2</sub>), 21.9 (CH<sub>2</sub>–CH<sub>2</sub>), 18.5 (CH<sub>2</sub>–CH<sub>2</sub>), 13.9 (CH<sub>2</sub>–CH<sub>3</sub>) ppm. <sup>19</sup>F NMR (376 MHz, DMSO-*d*<sub>6</sub>) δ –114.34 ppm. HRMS-ESI (*m/z*) [M+H]<sup>+</sup> calcd. for C<sub>23</sub>H<sub>22</sub>FN<sub>4</sub>O<sub>2</sub>: 405.1721, found: 405.1720. R<sub>f</sub> 0.33 (DCM/MeOH 95:5). λ<sub>abs</sub>: 275 nm λ<sub>em</sub>: 503 nm λ<sub>exc</sub>: 286 nm.

*N*-[3-[3-(*p*-fluorophenyl)-2,4-dioxo-1*H*-purino [9,8-*a*]pyridin-8-yl]prop-2-ynyl]octanamide (**21f**). The title compound was prepared from commercially available 19b to afford after purification the desired product as an orange solid (40%). <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 8.49 (s, 1H, H<sub>9</sub>), 7.39 (d, 1H, *J* = 9.4 Hz, H<sub>6</sub>), 7.28–7.17 (m, 4H, 4 × H<sub>arom</sub>), 7.06 (d, 1H, *J* = 9.4 Hz, H<sub>7</sub>), 4.07 (s, 1H, NH–CH<sub>2</sub>), 3.11–2.87 (m, 2H, CO–CH<sub>2</sub>), 1.43–1.28 (m, 2H, CH<sub>2</sub>–CH<sub>2</sub>), 1.21 (bs, 8H, 4 × CH<sub>2</sub>), 0.84 (t, 3H, *J* = 6.8 Hz, CH<sub>2</sub>–CH<sub>3</sub>) ppm. <sup>13</sup>C NMR (63 MHz, DMSO-*d*<sub>6</sub>) δ 175.0 (C=O), 162.0 (C=O), 159.5 (C<sub>quat</sub>), 150.4 (C=O), 139.6 (C<sub>quat</sub>), 134.9 (C<sub>quat</sub>), 134.4 (C<sub>quat</sub>), 131.2 (d, *J*<sub>C–F</sub> = 16.8 Hz, 2 × C<sub>arom</sub>), 127.4 (C<sub>arom</sub>), 126.9 (C<sub>arom</sub>), 118.2 (C<sub>arom</sub>), 117.5 (C<sub>quat</sub>), 115.2 (d, *J*<sub>C–F</sub> = 14.4 Hz, 2 × C<sub>arom</sub>), 106.4 (C<sub>quat</sub>), 89.7 (CH<sub>2</sub>–C), 77.8 (C≡C), 45.4 (N–CH<sub>2</sub>), 35.0 (C–CH<sub>2</sub>), 31.0 (CH<sub>2</sub>–CH<sub>2</sub>), 29.9 (CH<sub>2</sub>–CH<sub>2</sub>), 29.5 (CH<sub>2</sub>–CH<sub>2</sub>), 26.0 (CH<sub>2</sub>–CH<sub>2</sub>), 22.0 (CH<sub>2</sub>–CH<sub>2</sub>), 13.9 (CH<sub>2</sub>–CH<sub>3</sub>) ppm. <sup>19</sup>F NMR (376 MHz, DMSO-*d*<sub>6</sub>) δ –115.90 ppm. HRMS-ESI (*m/z*) [M+H]<sup>+</sup> calcd. for C<sub>26</sub>H<sub>27</sub>FN<sub>5</sub>O<sub>2</sub>: 476.2092, found: 476.2091. λ<sub>abs</sub>: 273 nm λ<sub>em</sub>: 515 nm λ<sub>exc</sub>: 284 nm. R<sub>f</sub> 0.32 (DCM/MeOH 95:5).

1-[3-[3-(*p*-Fluorophenyl)-2,4-dioxo-1*H*-purino [9,8-*a*]pyridin-8-yl]prop-2-ynyl]-3-hexyl-urea (**21g**). The title compound was prepared from commercially available 19b to afford after purification the desired product as an orange solid (92%). <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 8.49 (s, 1H, H<sub>9</sub>), 7.39 (d, 1H, *J* = 9.5 Hz, H<sub>6</sub>), 7.31–7.16 (m, 4H, 4 × H<sub>arom</sub>), 7.06 (d, 1H, *J* = 9.5 Hz, H<sub>7</sub>), 4.07 (d, 2H, *J* = 5.7 Hz, CH<sub>2</sub>), 1.45–1.03 (m, 10H, 5 × CH<sub>2</sub>), 0.84 (q, 3H, *J* = 5.7 Hz, CH<sub>3</sub>) ppm. <sup>13</sup>C NMR (101 MHz, DMSO-*d*<sub>6</sub>) δ 162.0 (C=O), 159.9 (C<sub>quat</sub>), 157.58 (C=O), 150.4 (C=O), 139.6 (C<sub>quat</sub>), 134.8 (C<sub>quat</sub>), 131.3 (C<sub>quat</sub>), 131.1 (2 × C<sub>arom</sub>), 127.3 (C<sub>arom</sub>), 126.9 (C<sub>arom</sub>), 118.2 (C<sub>arom</sub>), 117.74 (C<sub>quat</sub>), 115.1 (2 × C<sub>arom</sub>), 106.4 (C<sub>quat</sub>), 89.7 (CH<sub>2</sub>–C), 77.8 (C≡C), 45.4 (N–CH<sub>2</sub>), 31.0 (CH<sub>2</sub>–CH<sub>2</sub>), 29.9 (CH<sub>2</sub>–CH<sub>2</sub>), 28.5 (CH<sub>2</sub>–CH<sub>2</sub>), 26.0 (CH<sub>2</sub>–CH<sub>2</sub>), 22.0 (CH<sub>2</sub>–CH<sub>2</sub>), 14.2 (CH<sub>2</sub>–CH<sub>3</sub>) ppm. <sup>19</sup>F NMR (376 MHz, DMSO-*d*<sub>6</sub>) δ –116.17 ppm. HRMS-ESI (*m/z*) [M+H]<sup>+</sup> calcd. for C<sub>25</sub>H<sub>26</sub>FN<sub>6</sub>O<sub>3</sub>: 477.2045, found: 477.2045. λ<sub>abs</sub>: 285 nm λ<sub>em</sub>: 512 nm λ<sub>exc</sub>: 286 nm. R<sub>f</sub> 0.30 (DCM/MeOH 95:5).

### 3.6. General synthetic procedure 1 for the Suzuki-Miyaura coupling cross coupling.

A mixture of Br-compound (1 eq.), boronic acid (1.5 eq.), cesium carbonate (2 eq.), Pd(PPh<sub>3</sub>)<sub>4</sub> (0.1 eq.) in anhydrous DMF (0.1 M) under inert atmosphere was heated under microwave irradiation at 120 °C for 40 min. Pure compounds were obtained after purification by flash column chromatography with DCM/MeOH as eluent.

3-(*p*-Fluorophenyl)-7-phenyl-1*H*-purino [9,8-*a*]pyridine-2,4-dione (**22a**). The title compound was prepared from commercially available 19a to afford after purification the desired product as a yellow solid (45%). <sup>1</sup>H NMR (250 MHz, DMSO-*d*<sub>6</sub>) δ 8.52 (d, 1H, *J* = 7.3 Hz, H<sub>9</sub>), 7.85 (d, 3H, *J* = 6.9 Hz, H<sub>6</sub>, 2 × H<sub>arom</sub>), 7.60–7.34 (m, 4H, H<sub>8</sub>, 3 × H<sub>arom</sub>), 7.30 (d, 4H, *J* = 7.1 Hz, 4 × H<sub>arom-N3</sub>) ppm. <sup>13</sup>C NMR (63 MHz, DMSO-*d*<sub>6</sub>) δ 163.1 (C=O), 159.2 (C<sub>quat</sub>), 159.0 (C<sub>quat</sub>), 152.1 (C=O), 142.0 (C<sub>quat</sub>), 137.2 (d, *J* = 14.2 Hz, C<sub>quat</sub>), 135.7 (C<sub>quat</sub>), 133.3 (d, *J*<sub>C–F</sub> = 9.3 Hz, C<sub>quat</sub>), 131.2 (d, *J*<sub>C–F</sub> = 9.1 Hz, 2 × C<sub>arom-N3</sub>), 128.9 (2 × C<sub>arom</sub>), 128.4 (C<sub>arom</sub>), 126.3 (2 × C<sub>arom</sub>), 123.9 (C<sub>arom</sub>), 118.4 (C<sub>quat</sub>), 115.4 (d, *J*<sub>C–F</sub> = 24.2 Hz, 2 × C<sub>arom-N3</sub>), 114.1 (C<sub>arom</sub>), 111.7 (C<sub>arom</sub>) ppm. <sup>19</sup>F NMR (376 MHz, DMSO-*d*<sub>6</sub>) δ –115.15 ppm. HRMS-ESI (*m/z*) [M+H]<sup>+</sup> calcd. for C<sub>21</sub>H<sub>14</sub>FN<sub>4</sub>O<sub>2</sub>: 373.1095, found: 373.1099. λ<sub>abs</sub>: 264 nm λ<sub>em</sub>: 517 nm λ<sub>exc</sub>: 285 nm. R<sub>f</sub> 0.31 (DCM/MeOH 95:5).

3-(*p*-Fluorophenyl)-7-(*p*-tolyl)-1*H*-purino [9,8-*a*]pyridine-2,4-dione (**22b**). The title compound was prepared from commercially available 19a to afford after purification the desired product as an orange solid (44%). <sup>1</sup>H NMR (250 MHz, DMSO-*d*<sub>6</sub>) δ 8.33 (d, 1H, *J* = 7.3 Hz, H<sub>9</sub>), 7.72 (d, 2H, *J* = 8.1 Hz, 2 × H<sub>arom</sub>), 7.67 (s, 1H, H<sub>6</sub>), 7.31 (d, 2H, *J* = 8.0 Hz, 2 × H<sub>arom</sub>), 7.26–7.17 (m, 5H, H<sub>8</sub>, 4 × H<sub>arom-N3</sub>), 2.37 (s, 3H, C–CH<sub>3</sub>) ppm. <sup>13</sup>C NMR (63 MHz, DMSO-*d*<sub>6</sub>) δ 159.9 (C=O), 158.7 (C<sub>quat</sub>), 152.3 (C=O), 141.5 (C<sub>quat</sub>), 138.0 (C<sub>quat</sub>), 137.7 (C<sub>quat</sub>), 136.3 (C<sub>quat</sub>), 135.1 (d, *J*<sub>C–F</sub> = 2.5 Hz, C<sub>quat</sub>), 134.9 (C<sub>quat</sub>), 131.2 (d, *J*<sub>C–F</sub> = 8.8 Hz, 2 × C<sub>arom-N3</sub>), 129.6 (2 × C<sub>arom</sub>), 126.4 (C<sub>quat</sub>), 126.1 (2 × C<sub>arom</sub>), 123.8 (C<sub>arom</sub>), 115.0 (d, *J*<sub>C–F</sub> = 22.6 Hz, 2 × C<sub>arom-N3</sub>), 113.5 (C<sub>arom</sub>), 110.3 (C<sub>arom</sub>), 20.7 (C–CH<sub>3</sub>) ppm. <sup>19</sup>F NMR (376 MHz, DMSO-*d*<sub>6</sub>) δ –116.31 ppm. HRMS-ESI (*m/z*) [M+H]<sup>+</sup> calcd. for C<sub>22</sub>H<sub>16</sub>FN<sub>4</sub>O<sub>2</sub>: 387.1251, found: 387.1255. λ<sub>abs</sub>: 276 nm λ<sub>em</sub>: 519 nm λ<sub>exc</sub>: 279 nm. R<sub>f</sub> 0.32 (DCM/MeOH 95:5).

3-(*p*-Fluorophenyl)-7-(*p*-methoxyphenyl)-1*H*-purino [9,8-*a*]pyridine-2,4-dione (**22c**). The title compound was prepared from commercially available **19a** to afford after purification the desired product as a yellow solid (15%). <sup>1</sup>H NMR (250 MHz, DMSO-*d*<sub>6</sub>) δ 8.50 (d, 1H, *J* = 7.2 Hz, H<sub>9</sub>), 7.81 (d, 2H, *J* = 8.4 Hz, 2 × H<sub>arom</sub>), 7.77 (s, 1H, H<sub>6</sub>), 7.40–7.06 (m, 5H, H<sub>s</sub>, 4 × H<sub>arom-N3</sub>), 7.07 (d, 2H, *J* = 8.4 Hz, 2 × H<sub>arom</sub>), 3.82 (s, 3H, O–CH<sub>3</sub>) ppm. <sup>13</sup>C NMR (63 MHz, DMSO-*d*<sub>6</sub>) δ 159.6 (C=O), 159.1 (C<sub>quat</sub>), 152.7 (C=O), 142.4 (C<sub>quat</sub>), 140.1 (C<sub>quat</sub>), 136.0 (C<sub>quat</sub>), 133.9 (C<sub>quat</sub>), 133.6 (d, *J*<sub>C-F</sub> = 4.4 Hz, C<sub>quat</sub>), 131.2 (d, *J*<sub>C-F</sub> = 8.19 Hz, 2 × C<sub>arom-N3</sub>), 129.6 (C<sub>quat</sub>), 127.7 (2 × C<sub>arom</sub>), 123.8 (C<sub>arom</sub>), 118.2 (C<sub>quat</sub>), 115.3 (d, *J*<sub>C-F</sub> = 22.6 Hz, 2 × C<sub>arom-N3</sub>), 114.4 (2 × C<sub>arom</sub>), 112.8 (C<sub>arom</sub>), 111.5 (C<sub>arom</sub>), 55.2 (O–CH<sub>3</sub>) ppm. <sup>19</sup>F NMR (376 MHz, DMSO-*d*<sub>6</sub>) δ –115.28 ppm. HRMS-ESI (*m/z*) [M+H]<sup>+</sup> calcd. for C<sub>22</sub>H<sub>16</sub>FN<sub>4</sub>O<sub>3</sub>: 403.1200, found: 403.1202. λ<sub>abs</sub>: 275 nm λ<sub>em</sub>: 522 nm λ<sub>exc</sub>: 278 nm. R<sub>f</sub> 0.32 (DCM/MeOH 95:5).

3-(*p*-fluorophenyl)-7-[*p*-(trifluoromethoxy)phenyl]-1*H*-purino [9,8-*a*]pyridine-2,4-dione (**22d**). The title compound was prepared from commercially available **19a** to afford after purification the desired product as an orange solid (15%). <sup>1</sup>H NMR (250 MHz, DMSO-*d*<sub>6</sub>) δ 8.53 (d, 1H, *J* = 7.4 Hz, H<sub>9</sub>), 7.98 (d, 2H, *J* = 8.5 Hz, 2 × H<sub>arom</sub>), 7.88 (s, 1H, H<sub>6</sub>), 7.49 (d, 2H, *J* = 8.5 Hz, 2 × H<sub>arom</sub>), 7.39 (d, 1H, *J* = 7.4 Hz, H<sub>s</sub>), 7.30 (d, 4H, *J* = 7.0 Hz, 4 × H<sub>arom-N3</sub>) ppm. <sup>13</sup>C NMR (63 MHz, DMSO-*d*<sub>6</sub>) δ 163.1 (C=O), 159.3 (C<sub>quat</sub>), 159.0 (C<sub>quat</sub>), 152.2 (C=O), 148.4 (C<sub>quat</sub>), 141.7 (C<sub>quat</sub>), 136.7 (C<sub>quat</sub>), 136.1 (C<sub>quat</sub>), 135.6 (C<sub>quat</sub>), 133.4 (C<sub>quat</sub>), 131.2 (d, *J*<sub>C-F</sub> = 8.7 Hz, 2 × C<sub>arom-N3</sub>), 128.5 (2 × C<sub>arom</sub>), 124.1 (C<sub>arom</sub>), 121.4 (2 × C<sub>arom</sub>), 118.6 (C<sub>quat</sub>), 115.3 (d, *J*<sub>C-F</sub> = 22.6 Hz, 2 × C<sub>arom-N3</sub>), 114.8 (C<sub>arom</sub>), 111.6 (C<sub>arom</sub>) ppm. <sup>19</sup>F NMR (376 MHz, DMSO-*d*<sub>6</sub>) δ –56.71 (–OCF<sub>3</sub>), –115.18 ppm. HRMS-ESI (*m/z*) [M+H]<sup>+</sup> calcd. for C<sub>22</sub>H<sub>13</sub>F<sub>4</sub>N<sub>4</sub>O<sub>3</sub>: 457.0918, found: 457.0917. λ<sub>abs</sub>: 274 nm λ<sub>em</sub>: 536 nm λ<sub>exc</sub>: 279 nm. R<sub>f</sub> 0.3 (DCM/MeOH 95:5).

3,7-Bis(*p*-fluorophenyl)-1*H*-purino [9,8-*a*]pyridine-2,4-dione (**22e**). The title compound was prepared from commercially available **19a** to afford after purification the desired product as a yellow solid (45%). <sup>1</sup>H NMR (250 MHz, DMSO-*d*<sub>6</sub>) δ 8.50 (d, 1H, *J* = 7.4 Hz, H<sub>9</sub>), 7.97–7.86 (m, 2H, 2 × H<sub>arom-N3</sub>), 7.82 (s, 1H, H<sub>6</sub>), 7.44–7.22 (m, 7H, H<sub>s</sub>, 2 × H<sub>arom-N3</sub>, 4 × H<sub>arom-N3</sub>) ppm. <sup>13</sup>C NMR (63 MHz, DMSO-*d*<sub>6</sub>) δ 160.3 (C=O), 159.1 (C<sub>quat</sub>), 159.0 (C<sub>quat</sub>), 152.4–152.1 (m, C=O, C<sub>quat</sub>), 141.8 (C<sub>quat</sub>), 136.0 (C<sub>quat</sub>), 133.8 (d, *J*<sub>C-F</sub> = 3.1 Hz, C<sub>quat</sub>), 133.5 (C<sub>quat</sub>), 131.2 (d, *J*<sub>C-F</sub> = 8.8 Hz, 2 × C<sub>arom-N3</sub>), 128.6 (d, *J*<sub>C-F</sub> = 8.1 Hz, 2 × C<sub>arom</sub>), 124.5 (C<sub>quat</sub>), 124.0 (C<sub>arom</sub>), 118.4 (C<sub>quat</sub>), 115.8 (d, *J*<sub>C-F</sub> = 21.4 Hz, 2 × C<sub>arom-N3</sub>), 115.3 (d, *J*<sub>C-F</sub> = 22.6 Hz, 2 × C<sub>arom</sub>), 114.1 (C<sub>arom</sub>), 111.6 (C<sub>arom</sub>) ppm. <sup>19</sup>F NMR (235 MHz, DMSO-*d*<sub>6</sub>) δ –113.66, –115.26 ppm. HRMS-ESI (*m/z*) [M+H]<sup>+</sup> calcd. for C<sub>21</sub>H<sub>13</sub>F<sub>2</sub>N<sub>4</sub>O<sub>2</sub>: 391.1002, found: 391.1001. λ<sub>abs</sub>: 274 nm λ<sub>em</sub>: 539 nm λ<sub>exc</sub>: 277 nm. R<sub>f</sub> 0.26 (DCM/MeOH 95:5).

3-(*p*-Fluorophenyl)-8-phenyl-1*H*-purino [9,8-*a*]pyridine-2,4-dione (**23a**). The title compound was prepared from commercially available **19b** to afford after purification the desired product as a yellow solid (50%). <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 8.86 (s, 1H, H<sub>9</sub>), 7.75–7.72 (m, 2H, 2 × H<sub>arom</sub>), 7.67 (qd, 2H, *J* = 9.7, 1.4 Hz, H<sub>6</sub>, H<sub>arom</sub>), 7.54 (t, 2H, *J* = 7.7 Hz, 2 × H<sub>arom</sub>), 7.47–7.41 (m, 1H, H<sub>7</sub>), 7.36–7.27 (m, 4H, 4 × H<sub>arom-N3</sub>) ppm. <sup>13</sup>C NMR (101 MHz, DMSO-*d*<sub>6</sub>) δ 162.5 (C=O), 160.1 (C<sub>quat</sub>), 158.9 (C<sub>quat</sub>), 151.5 (C=O), 140.8 (C<sub>quat</sub>), 136.0 (C<sub>quat</sub>), 134.0 (C<sub>quat</sub>), 133.1 (d, *J*<sub>C-F</sub> = 2.8 Hz, C<sub>quat</sub>), 131.3 (d, *J*<sub>C-F</sub> = 9.1 Hz, 2 × C<sub>arom-N3</sub>), 129.2 (2 × C<sub>arom</sub>), 128.1 (C<sub>arom</sub>), 126.2 (2 × C<sub>arom</sub>), 125.2 (C<sub>quat</sub>), 120.9 (C<sub>arom</sub>), 118.3 (C<sub>arom</sub>), 115.4 (d, *J*<sub>C-F</sub> = 22.7 Hz, 2 × C<sub>arom-N3</sub>) ppm. <sup>19</sup>F NMR (376 MHz, DMSO-*d*<sub>6</sub>) δ –114.90 ppm. HRMS-ESI (*m/z*) [M+H]<sup>+</sup> calcd. for C<sub>21</sub>H<sub>14</sub>FN<sub>4</sub>O<sub>2</sub>: 373.1095, found: 373.1094. λ<sub>abs</sub>: 274 nm λ<sub>em</sub>: 441 nm λ<sub>exc</sub>: 288 nm. R<sub>f</sub> 0.31 (DCM/MeOH 95:5).

3-(*p*-Fluorophenyl)-8-(*p*-tolyl)-1*H*-purino [9,8-*a*]pyridine-2,4-dione (**23b**). The title compound was prepared from commercially available **19b** to afford after purification the desired product as a yellow/beige solid (31%). <sup>1</sup>H NMR (250 MHz, DMSO-*d*<sub>6</sub>) δ 8.82 (s, 1H, H<sub>9</sub>), 7.62 (d, 5H, *J* = 7.9 Hz, H<sub>6</sub>, 4 × H<sub>arom</sub>), 7.33 (t, 7H, *J* = 6.7 Hz, H<sub>7</sub>, 2 × H<sub>arom</sub>, 4 × H<sub>arom-N3</sub>), 2.37 (s, 3H, C–CH<sub>3</sub>) ppm. <sup>13</sup>C NMR (63 MHz, DMSO-*d*<sub>6</sub>) δ 158.9 (C=O), 157.0 (C<sub>quat</sub>), 151.6 (C=O), 150.8 (C<sub>quat</sub>), 140.8 (C<sub>quat</sub>), 137.5 (C<sub>quat</sub>), 135.0 (C<sub>quat</sub>), 133.1 (d, *J*<sub>C-F</sub> = 3.7 Hz, C<sub>quat</sub>), 133.0 (C<sub>quat</sub>), 131.3 (d, *J*<sub>C-F</sub> = 8.8 Hz, 2 × C<sub>arom-N3</sub>), 129.7 (2 × C<sub>arom</sub>), 126.2 (C<sub>arom</sub>), 126.0 (2 × C<sub>arom</sub>), 125.0 (C<sub>quat</sub>), 120.4 (C<sub>arom</sub>), 118.2 (C<sub>arom</sub>), 115.4 (d, *J*<sub>C-F</sub> = 22.6 Hz, 2 × C<sub>arom-N3</sub>), 20.6 (C–CH<sub>3</sub>) ppm. <sup>19</sup>F NMR (376 MHz, DMSO-*d*<sub>6</sub>) δ –114.93 ppm. HRMS-ESI (*m/z*) [M+H]<sup>+</sup> calcd. for



C<sub>22</sub>H<sub>16</sub>FN<sub>4</sub>O<sub>2</sub>: 387.1251, found: 387.1253.  $\lambda_{\text{abs}}$ : 244 nm  $\lambda_{\text{em}}$ : 444 nm  $\lambda_{\text{exc}}$ : 274 nm. R<sub>f</sub> 0.32 (DCM/MeOH 95:5).

3-(*p*-Fluorophenyl)-8-(*p*-methoxyphenyl)-1*H*-purino [9,8-*a*]pyridine-2,4-dione (**23c**). The title compound was prepared from commercially available **19b** to afford after purification the desired product as a yellow/beige solid (31%). <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  8.78 (s, 1H, H<sub>9</sub>), 7.70–7.57 (m, 4H, H<sub>6</sub>, H<sub>7</sub>, 2 × H<sub>arom</sub>), 7.33–7.30 (m, 4H, 4 × H<sub>arom-N3</sub>), 7.10 (d, 2H, *J* = 8.7 Hz, 2 × H<sub>arom</sub>), 3.82 (s, 3H, O–CH<sub>3</sub>) ppm. <sup>13</sup>C NMR (101 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  162.5 (C=O), 162.5 (C<sub>quat</sub>), 159.3 (C<sub>quat</sub>), 151.6 (C=O), 140.7 (C<sub>quat</sub>), 140.0 (C<sub>quat</sub>), 133.1 (C<sub>quat</sub>), 131.3 (d, *J*<sub>C-F</sub> = 9.0 Hz, 2 × C<sub>arom-N3</sub>), 128.2 (C<sub>quat</sub>), 127.4 (2 × C<sub>arom</sub>), 126.3 (C<sub>arom</sub>), 124.9 (C<sub>quat</sub>), 119.9 (C<sub>arom</sub>), 118.2 (C<sub>arom</sub>), 115.4 (d, *J*<sub>C-F</sub> = 22.2 Hz, 2 × C<sub>arom-N3</sub>), 114.6 (2 × C<sub>arom</sub>), 99.4 (C<sub>quat</sub>), 55.2 (O–CH<sub>3</sub>) ppm. <sup>19</sup>F NMR (376 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  –114.96 ppm. HRMS-ESI (*m/z*) [M+H]<sup>+</sup> calcd. for C<sub>22</sub>H<sub>16</sub>FN<sub>4</sub>O<sub>3</sub>: 403.1200, found: 403.1201.  $\lambda_{\text{abs}}$ : 274 nm  $\lambda_{\text{em}}$ : 438 nm  $\lambda_{\text{exc}}$ : 286 nm. R<sub>f</sub> 0.32 (DCM/MeOH 95:5).

3-(*p*-Fluorophenyl)-8-[*p*-(trifluoromethoxy)phenyl]-1*H*-purino [9,8-*a*]pyridine-2,4-dione (**23d**). The title compound was prepared from commercially available **19b** to afford after purification the desired product as a yellow/beige solid (43%). <sup>1</sup>H NMR (250 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  8.68 (s, 1H, H<sub>9</sub>), 7.94–7.84 (m, 2H, 2 × H<sub>arom</sub>), 7.48 (d, 1H, *J* = 9.7 Hz, H<sub>6</sub>), 7.39–7.22 (m, 7H, H<sub>7</sub>, 2 × H<sub>arom</sub>, 4 × H<sub>arom-N3</sub>) ppm. <sup>13</sup>C NMR (101 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  164.6 (C=O), 159.3 (C<sub>quat</sub>), 159.0 (C<sub>quat</sub>), 152.5 (C=O), 146.2 (C<sub>quat</sub>), 139.7 (C<sub>quat</sub>), 138.9 (C<sub>quat</sub>), 136.1 (2 × C<sub>arom</sub>), 133.3 (C<sub>quat</sub>), 131.2 (d, *J*<sub>C-F</sub> = 3.1 Hz, C<sub>quat</sub>), 131.9 (C<sub>quat</sub>), 131.2 (d, *J*<sub>C-F</sub> = 8.8 Hz, 2 × C<sub>arom</sub>), 128.7 (C<sub>arom</sub>), 124.0 (C<sub>arom</sub>), 119.5 (d, *J*<sub>C-F</sub> = 5.5 Hz, 2 × C<sub>arom</sub>), 118.3 (C<sub>quat</sub>), 115.3 (d, *J*<sub>C-F</sub> = 22.8 Hz, 2 × C<sub>arom</sub>), 106.0 (C<sub>quat</sub>) ppm. <sup>19</sup>F NMR (376 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  –56.72 (–O–CF<sub>3</sub>), –114.27 ppm. HRMS-ESI (*m/z*) [M+H]<sup>+</sup> calcd. for C<sub>22</sub>H<sub>13</sub>F<sub>4</sub>N<sub>4</sub>O<sub>3</sub>: 457.0919, found: 457.0919.  $\lambda_{\text{abs}}$ : 273 nm  $\lambda_{\text{em}}$ : 510 nm  $\lambda_{\text{exc}}$ : 294 nm. R<sub>f</sub> 0.3 (DCM/MeOH 95:5).

3,8-Bis(*p*-Fluorophenyl)-1*H*-purino [9,8-*a*]pyridine-2,4-dione (**23e**). The title compound was prepared from commercially available **19b** to afford after purification the desired product as a yellow/beige solid (11%). <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  8.90 (s, 1H, H<sub>9</sub>), 7.82–7.73 (m, 2H, 2 × H<sub>arom</sub>), 7.69–7.58 (m, 2H, H<sub>6</sub>, H<sub>7</sub>), 7.38 (t, 2H, *J* = 8.8 Hz, 2 × H<sub>arom</sub>), 7.32–7.22 (m, 4H, 4 × H<sub>arom-N3</sub>) ppm. <sup>13</sup>C NMR (101 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  160.8 (C=O), 160.0 (C<sub>quat</sub>), 159.1 (C<sub>quat</sub>), 151.9 (C=O), 140.7 (C<sub>quat</sub>), 136.4 (C<sub>quat</sub>), 135.9 (C<sub>quat</sub>), 132.5 (d, *J*<sub>C-F</sub> = 3.5 Hz, C<sub>quat</sub>), 131.5–13.4 (m, C<sub>quat</sub>), 131.3 (d, *J*<sub>C-F</sub> = 8.4 Hz, 2 × C<sub>arom</sub>), 128.4 (d, *J*<sub>C-F</sub> = 8.3 Hz, 2 × C<sub>arom-N3</sub>), 126.1 (C<sub>arom</sub>), 124.0 (C<sub>quat</sub>), 121.0 (C<sub>arom</sub>), 118.3 (C<sub>arom</sub>), 116.0 (d, *J*<sub>C-F</sub> = 21.8 Hz, 2 × C<sub>arom</sub>), 115.4 (d, *J*<sub>C-F</sub> = 22.7 Hz, 2 × C<sub>arom-N3</sub>) ppm. <sup>19</sup>F NMR (376 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  –114.36, –115.10 ppm. HRMS-ESI (*m/z*) [M+H]<sup>+</sup> calcd. for C<sub>21</sub>H<sub>13</sub>F<sub>2</sub>N<sub>4</sub>O<sub>2</sub>: 391.1001, found: 391.1006.  $\lambda_{\text{abs}}$ : 274 nm  $\lambda_{\text{em}}$ : 441 nm  $\lambda_{\text{exc}}$ : 273 nm. R<sub>f</sub> 0.26 (DCM/MeOH 95:5).

### 3.7. Biological assays

#### 3.7.1. *Mtb* ThyX protein expression and purification [29].

The *M. tuberculosis* ThyX enzyme was expressed in *E. coli* BL21(DE3)/pLysS strains containing the recombinant pET24d plasmid carrying the *M. tuberculosis* H37Rv thyX gene (Rv2754c) as previously described. Before the purification step, 200  $\mu$ M of flavin-adenine dinucleotide (FAD) cofactor was added to the supernatant after the lysis step to increase the amount of FAD bound to *Mtb* ThyX protein. The solubilized protein extract was loaded on a Hi-Trap Talon 5 mL column (GE Healthcare) previously equilibrated with equilibration buffer containing 30 mM Hepes and 300 mM NaCl at pH 8.0. The His-tagged ThyX protein was eluted with elution buffer (30 mM Hepes pH 8.0, 300 mM NaCl, 500 mM imidazol). The fractions containing *Mtb* ThyX enzyme were pooled, buffer-exchanged on Econo-Pac PD-10 columns (Bio-rad) with the equilibration buffer, concentrated to a final concentration of 480  $\mu$ M and stored at –20 °C for further use. The measured absorbance of FAD bound to *Mtb* ThyX at 450 nm showed a ratio FAD to ThyX of 1 to 3 for the purified *Mtb* ThyX chain.

#### 3.7.2. *M. Tuberculosis* ThyX NADPH oxidase assay

The NADPH oxidation assay for *M. tuberculosis* ThyX activity in 96-well plates was used to screen the synthesized compounds at a final concentration of 200  $\mu\text{M}$ . All molecules were solubilized in dimethylsulfoxide (DMSO) and used at a 1% final concentration of DMSO during the test. One hundred microlitres of standard reaction mixture contained HEPES 50 mM pH 8, NaCl 30 mM, FAD 50  $\mu\text{M}$ ,  $\beta$ -mercaptoethanol 1.43 mM, dUMP 100  $\mu\text{M}$ , NADPH 750  $\mu\text{M}$ , and 10  $\mu\text{M}$  of purified *MtbThyX*. Microtiter plates were prepared and transferred to the microplate reader Chameleon II (Hidex). Molecules at 200  $\mu\text{M}$  were incubated with *MtbThyX* in the standard reaction mixture for 10 min at 25  $^{\circ}\text{C}$  before starting measurements. The reactions were started by automatically injecting NADPH into individual wells and ThyX activity was determined by following a decrease in absorbance at 340 nm for up to 20 min at 25  $^{\circ}\text{C}$ . The experiment was done in duplicates and samples with added DMSO and enzyme-free reactions were used as positive and negative controls, respectively. % of inhibition was calculated using the following equation:  $(1 - V_i/V_o) \times 100$ ;  $V_o$  and  $V_i$  are, respectively, the initial rates of the reaction without or with addition of molecule in the assay.

### 3.7.3. M. Tuberculosis ThyX tritium release assays

*MtbThyX* tritium release assays consist to measure “deprotonation” of [5- $^3\text{H}$ ]-dUMP *in vitro*. Reaction mixture included 10 mM  $\text{MgCl}_2$ , 500  $\mu\text{M}$  FAD, 10% (v/v) glycerol, 2 mM NADPH, 1 mM  $\text{CH}_2\text{H}_4\text{folate}$ , 10 mM  $\beta$ -mercaptoethanol, bovine serum albumin (200  $\mu\text{g}/\text{mL}$ ), 100  $\mu\text{M}$  dUMP, [5- $^3\text{H}$ ]dUMP and 2  $\mu\text{M}$  *MtbThyX* in 50 mM HEPES pH 8. Molecules at 200  $\mu\text{M}$  *in DMSO* were incubated with *MtbThyX* in the standard reaction mixture for 10 min at 25  $^{\circ}\text{C}$  before starting measurements. DMSO concentration was maintained constant at 1%. Reactions were initiated by addition of NADPH (1 mM) at 37  $^{\circ}\text{C}$  and were stopped after 20 min. The specific activity of tritiated [5- $^3\text{H}$ ]dUMP (diammonium salt) stock was 15–30 Ci  $\text{mmol}^{-1}$  (Moravek Biochemicals, CA, USA). 700  $\mu\text{L}$  of activated charcoal (10% (w/v), Norit A in 2% trichloroacetic acid) was added to the reaction mixture to stop the reaction and removal of radioactive nucleotides from the solution. The suspension was centrifuged at 12,000 rpm for 2 min, and 450  $\mu\text{L}$  of the supernatant were collected before addition of 5 mL of scintillation solution. The radioactivity remaining in the supernatant was measured for scintillation counting.

### 3.7.4. Cytotoxicity assays

Assays were performed in human peripheral blood mononuclear (PBM) cells via MTS assay using the CellTiter 96<sup>®</sup> Non-Radioactive Cell Proliferation (Promega) kit. Cytotoxicity was expressed as the concentration of test compounds that inhibited cell growth by 50% ( $\text{CC}_{50}$ ).

### 3.8. Virtual docking

The *MtbThyX* protein structure from PDB code 3GWC26 was used to perform *in silico* molecular docking with the QuickVina 2 software [30]. The A to D chains, water oxygen atoms and UFP cofactor were removed from the structure, keeping only chains F to G and their FAD molecule. Atomic partial charges were assigned and polar hydrogen atoms were added with the Pymol [31] Vina plugin [32]. A cubic search volume of  $35 \times 35 \times 35 \text{ \AA}$  centered on each active site of the four chains was used. The Arg87, Gln103, Ser105, Arg107, Tyr108 and Arg199 amino acids were chosen to be flexible during the docking attempts. Ten docking poses were generated and the pose with the best score was used to further analysis.

## 4. Conclusion

In summary, starting from lead compounds 5 and 6, we have synthesized various hitherto unknown heterocycles with a pyrido [1,2-*e*]purine-2,4(1*H*,3*H*)-dione scaffold. Structure-activity relationship was performed in order to guide the design of new *FDTs*

inhibitors. Several pharmacomodulations through cyclization, and regioselective *N*-alkylation and organopalladium cross-coupling reactions were performed to synthesize *N*<sup>3</sup>, *N*<sup>1</sup>, *C*<sup>7</sup> and *C*<sup>8</sup> alkylated derivatives. Unfortunately no compound showed total inhibition at low concentration; the highest inhibition was obtained for compound 23a with 84.3% at 200 μM. IC<sub>50</sub> was calculated at 95 μM for central fluorine structure 9a. It was also evaluated on viral pathogens and showed some promiscuous activity without toxicity. Docking was performed to understand the main interactions of those compounds in *ThyX*. Altogether, this paves the route to more potent *Flavin-Dependent Thymidylate Synthase* inhibitors.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/molecules27196216/s1>, <sup>1</sup>H-, <sup>13</sup>C- and <sup>1</sup>H-<sup>13</sup>C HMBC NMR spectra of representative compounds from isocyanate cyclisation compounds (**9c**, **9e**, **9g-j**, **10b-d**) and from final molecules which exhibit Mtb Thy X inhibition > 35%

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