European Journal of Medicinal Chemistry 144 (2018) 116–127

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Research paper

2-Substituted 7-trifluoromethyl-thiadiazolopyrimidones as alkaline phosphatase inhibitors. Synthesis, structure activity relationship and molecular docking study

Behzad Jafari [a], Meirambek Ospanov [a, b], Syeda Abida Ejaz [c], Nazym Yelibayeva [a, b], Shafi Ullah Khan [c], Sayyeda Tayyeba Amjad [c], Sayfidin Safarov [d], Zharylkasyn A. Abilov [b], Mirgul Zh. Turmukhanova [b], Sergey N. Kalugin [b], Peter Ehlers [a, e], Joanna Lecka [f, g], Jean Sévigny [f, g], Jamshed Iqbal [c], Peter Langer [a, e, *]

[a] Institut für Chemie, Universität Rostock, Albert-Einstein-Str. 3a, 18059 Rostock, Germany
[b] Al-Farabi Kazakh National University, Al-Farabi Ave. 71, 050040 Almaty, Kazakhstan
[c] Centre for Advanced Drug Research, COMSATS Institute of Information Technology, Abbottabad, Pakistan
[d] Institute of Chemistry, Tajikistan Academy of Sciences, Ul. Aini 299, Dushanbe, 734063 Tadjikistan
[e] Leibniz Institut für Katalyse an der Universität Rostock e.V. (LIKAT), Albert-Einstein-Str. 29a, 18059 Rostock, Germany
[f] Département de microbiologie-infectiologie et d'immunologie, Faculté de Médecine, Université Laval, Québec, QC, G1V 0A6, Canada
[g] Centre de Recherche du CHU de Québec – Université Laval, Québec, QC, G1V 4G2, Canada

ARTICLE INFO

Article history:
Received 18 October 2017
Received in revised form
23 November 2017
Accepted 24 November 2017
Available online 6 December 2017

Dedicated to Professor Dr. Muhamadsho A. Kukaniev in memory

Keywords:
Suzuki-Miyaura reaction
Thiadiazolopyrimidones
Cross coupling reactions
Ectonucleotidases
Alkaline Phosphatase (AP)

ABSTRACT

Alkaline Phosphatases (APs) play a key role in maintaining a ratio of phosphate to inorganic pyrophosphate (P_i/PP_i) and thus regulate extracellular matrix calcification during bone formation and growth. Among different isozymes of AP, aberrant increase in the level of tissue non-specific alkaline phosphatase (TNAP) is strongly associated with vascular calcification and end-stage renal diseases. In this context, we synthesized a novel series of fluorinated pyrimidone derivatives, *i.e.*, 2-bromo-7-trifluoromethyl-5-oxo-5*H*-1,3,4-thiadiazolepyrimidones. The bromine functionality was further used for derivatisation by nucleophilic aromatic substitution using amines as nucleophiles as well as by Palladium catalysed Suzuki-Miyaura reactions. The synthesized derivatives were found potent but non-selective inhibitors of both isozymes of AP. Arylated thiadiazolopyrimidones exhibited stronger inhibitory activities than 2-amino-thiadiazolopyrimidones. The binding modes and possible interactions of the most active inhibitor within the active site of the enzyme were observed by molecular docking studies.

1. Introduction

Alkaline phosphatases (APs), members of ecto-nucleotidases, are present extensively in various species including bacteria or mammals where they govern the phosphorylation and dephosphorylation of broad variety of substrates [1]. In human, the alkaline phosphatases are encoded by four different genes encoding for various isozymes that may be tissue specific or non-specific alkaline phosphatases [2,3]. Tissue specific isozymes are found in placenta (PLAP), intestine (IAP) and germ cells (GCAP), whereas TNAP are abundantly found in bones, kidney and central nervous system [4]. All three tissue specific isozymes are 90% homologous to each other, while TNAP isozyme share only 50% homology with tissue specific isozymes [5]. TNAP are mainly taking part in the hydrolysis of inorganic pyrophosphate (PPi), into inorganic phosphate (Pi), thus maintaining the level of inorganic phosphate and regulating the deposition of minerals in teeth and bone [6]. TNAP have an important role in the hydroxyapatite crystallization. A mutagenic study of TNAP reveal the accumulation of PPi on bones that leads to hypophosphatasia in adults and rickets in children [7]. However, increased expression of TNAP within the body leads to more calcification in vascular smooth muscle cells [8]. Intestinal

* Corresponding author. Institut für Chemie, Universität Rostock, Albert-Einstein-Str. 3a, 18059 Rostock, Germany.
E-mail address: peter.langer@uni-rostock.de (P. Langer).

https://doi.org/10.1016/j.ejmech.2017.11.068
0223-5234/

alkaline phosphatases (IAP) are present on the brush border of intestine as surfactant like particles. They are responsible for the regulation of lipid absorption, bicarbonate secretion, maintenance of duodenal surface pH and controls the inflammation induced by bacterial endotoxins [9]. Thus, they are involved in the detoxification of intestinal lipopolysaccharide and intestinal homeostasis [10]. As TNAP and IAP share 50% structural similarity very few selective inhibitors are known [11]. Furthermore, the inhibition constants (K_i) of known inhibitors are quite high and lie in the millimolar range (Fig. 1) [12].

We report here the synthesis of novel 2-substituted 7-trifluoromethyl-thiadiazolopyrimidones as a new class of alkaline phosphatases *i.e.,* TNAP and IAP inhibitors. Thiadiazolopyrimidones are of high value for pharmaceutical research, due their diverse biological effects. For instance, they are known as inhibitors of platelet deposition for the treatment or prophylaxes of thrombotic disorders. Moreover, they are reported as anticancer, antiviral, antihypertensive, analgesic as well as anti-inflammatory agents, to name just a few applications [13–16]. Recently, we have reported 2-arylated thiadiazolopyrimidones as novel nucleotide pyrophosphatase (NPPs) inhibitors [13a].

Fluorinated compounds are the least abundant naturally occurring organohalides [17]. Until the middle of the previous century, there were practically no drugs containing fluorine in their structure. In recent years, more than a hundred fluorinated drugs have reached market and nowadays almost twenty percent of all pharmaceuticals include fluorine, even up to thirty percent for agrochemicals, due to the effect of fluorine on pharmacokinetics, metabolic stability and activity [18–21]. For example, fluoxetine, an antidepressant, or the cholesterol-lowering drug atorvastatin are among the most prescribed in the world [22,23]. Despite the importance of the CF_3-group in pharmaceutical research, CF_3-substituted thiadiazolopyrimidones have only scarcely been reported [24]. Herein, we report the synthesis of 2-substituted 7-trifluoromethyl-5*H*-1,3,4-thiadiazolo[3,2-*a*]pyrimidin-5-ones. Our strategy is based on the synthesis of 2-bromo-7-trifluoromethyl-5*H*-1,3,4-thiadiazolo[3,2-*a*]pyrimidin-5-one and subsequent functionalization by reaction with amines and by palladium catalysed Suzuki reactions. Most of the products were found to be inhibitors of TNAP at very low concentrations and, thus, may be used as lead molecules for specific targets.

2. Results and discussion

Our study started with the synthesis of 2-bromo-7-trifluoromethyl-5*H*-1,3,4-thiadiazolo[3,2-*a*]pyrimidin-5-one (**3**) from commercially available 5-bromo-2-amino-1,3,5-thiadiazole (**1**) and trifluoromethyl acetoacetate (**2**) (Scheme 1). The synthesis was carried out according to known procedures for the synthesis of 1,3,4-thiadiazolo[3,2-*a*]pyrimidin-5-one [25–30].

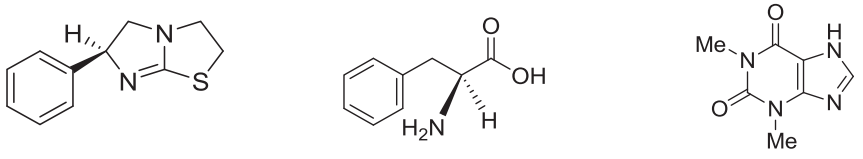
The bromine atom located at position 2 gives access to a wide range of possible functionalization reactions. Thus, we studied aromatic nucleophilic substitution with various amines as well as Palladium catalysed Suzuki-Miyaura reactions. Compound **3** readily reacts with anilines or alkylamines without the need of an additional base. It is important to note, that the products could be easily isolated and purified by simple filtration from the reaction mixture (Table 1).

Generally, anilines gave the corresponding products in very good yields, while the use of alkylamines, methylhydrazine or *N*-substituted anilines led to lower yields. Increasing the number of carbons within the aliphatic chain of amines drastically improved the yields (cf. **4h**-**4j**).

As a next step, we engaged compound **3** in Suzuki-Miyaura reactions using $Pd(OAc)_2$ in the presence of the bidentate ligand Xantphos. Various arylboronic acids were reacted giving moderate to excellent yields of the coupling products (Table 2). Generally, the reactivity is affected by sterical constraints resulting in reduced yields for *ortho*-substituted arylboronic acids. Moreover, electron-rich arylboronic acids led to higher yields, due to their higher nucleophilicity in cross-coupling reactions.

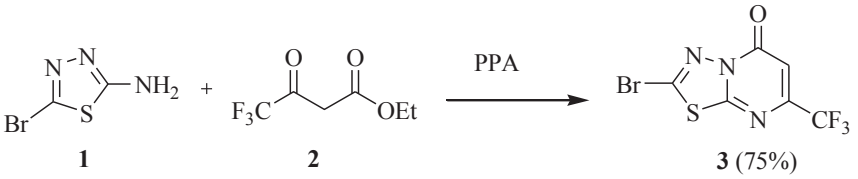
3. Structure-activity relationship

The enzyme inhibitory activity of both series of 7-(trifluoromethyl)-5*H*-[1,3,4]thiadiazolo[3,2-*a*]pyrimidin-5-ones was studied (Table 3). Compounds **4a-4n** contain an amino group at position 2, while derivatives **5a**–**t** have an aryl substituent. In case of compound **4g**, containing an allyl group stronger inhibition of *h*-TNAP was observed compared to *h*-IAP. The selectivity of this compound was found only ≈3 fold. The presence of substituents at the phenyl ring did not improve the inhibitory activity as compared to unsubstituted **4a**. When we compared the activity of **4c**, **4d** and **4f** towards *h*-TNAP, promising increase in the inhibitory effect was noticed with phenyl ring substituted by an amino group (**4f**) as compared to methoxy and ethoxy (**4c, 4d**). The latter compounds exhibited almost equipotent response towards both isozymes and in this case the OCH_3 substituted derivative (**4c**) showed better inhibitory response as compared to OCH_2CH_3 substituted derivative (**4d**). Another effect of the substituent was noticed with the phenyl ring having alkyl substitution, *i.e.,* propyl at the *para* position (**4e**). The introduction of alkyl substituents at other positions was also studied (**4h, 4i, 4j**), but did not result in higher potency. Among these alkyl substituted derivatives, less sterically hindered alkyl substituents did not improve the potency (**4h**), while large alkyl substituents, i.e. **4i** and **4j,** exhibited improved response. An inverse behavior of these derivatives was observed towards *h*-IAP. Small alkyl substituents showed improved response towards *h*-IAP and in this case compound **4h** was identified as the most potent inhibitor, while compound **4j** exhibited less inhibitory response. Similarly, the insertion of a *n*butyl group (**4m**) resulted in strong reduction of the inhibitory potency towards both isozymes. Compound **4l** resulted in equipotent inhibition of both isozymes, but when the structure of **4l** was compared with **4f**, it was observed that the



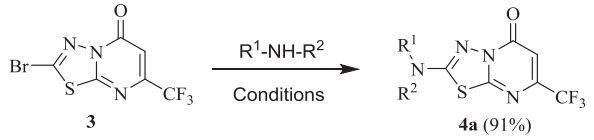
I II III

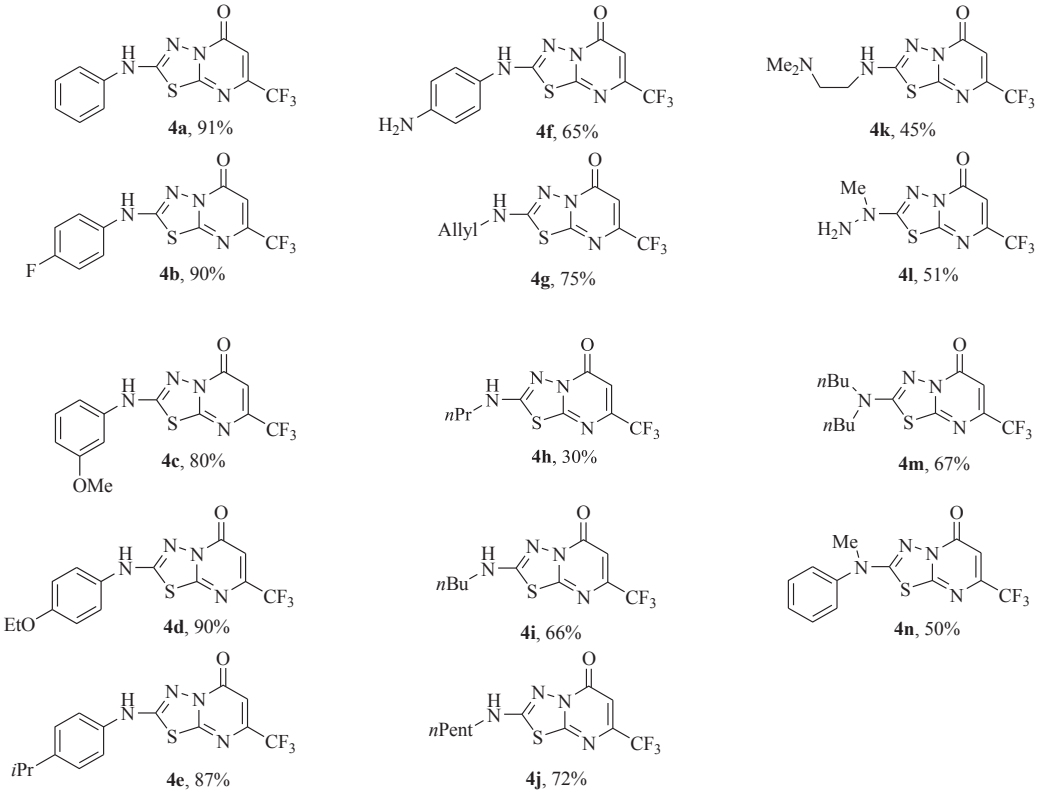
Fig. 1. Few examples of selective Inhibitors of TNAP and IAP: (**I**) Levamisole, an inhibitor of TNAP (IC_{50}±SEM; 19.2 ± 0.1 μM) (**II**) L–phenylalanine, an inhibitor of IAP (IC_{50}±SEM; 80.2 ± 1.1 μM) (**III**) Theophylline, a TNAP inhibitor IC_{50}±SEM; >100 μM).



Scheme 1. Synthesis of 2-bromo-7-trifluoromethyl-5*H*-1,3,4-thiadiazolo[3,2-*a*]pyrimidin-5-one.

Table 1
Synthesis of compounds **4a-n**.



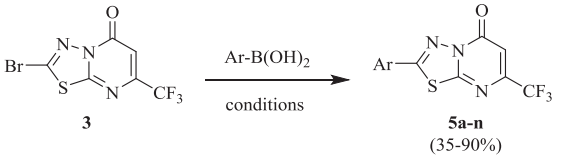


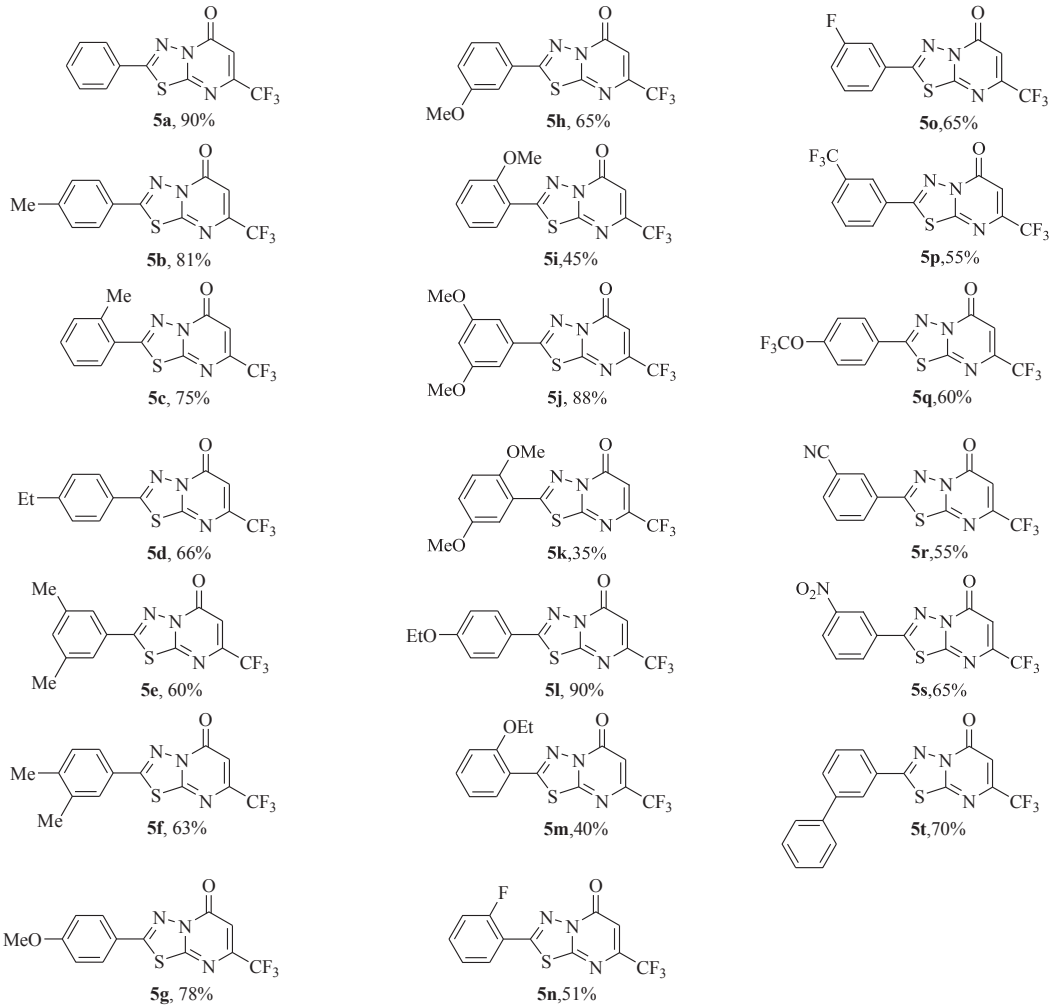
Conditions: Amine (2.0 equiv.), MeOH, 5 h, 20 °C. Yields refer to yields of isolated products.

attachment of a methyl group and insertion of an amino group are not beneficial towards both isozymes.

From the set of arylated derivatives of 7-(trifluoromethyl)-5*H*-[1,3,4]thiadiazolo[3,2-*a*]pyrimidin-5-ones (**5a-t;** except **5c, 5h**, **5k),** compounds **5i** and **5m** were identified as the selective inhibitors of *h*-TNAP while compounds **5d** and **5q** exhibited the selective inhibition on *h*-IAP. Compound **5a** (*h*-TNAP; 0.21 ± 0.02 μM, *h*-IAP; 0.43 ± 0.07 μM) and **5g** (*h*-TNAP; 0.28 ± 0.02 μM, *h*-IAP; 0.48 ± 0.02 μM) exhibited almost equipotent inhibitory effects on both APs. The detailed structure activity relationship of these two derivatives suggested that the unsubstituted derivatives (**5a**) exhibited the most promising effects than 4-methoxy derivative (**5g**) and was identified as the most effective inhibitor of *h*-TNAP. The compounds having dimethyl group substitutions (**5e** and **5f**), showed a higher improvement of the effective inhibitory potential as that of mono-substituted methyl derivative **5b** (IC_{50} ± SEM = 1.06 ± 0.05 μM). Remarkably, 3,5-dimethyl substitution revealed a high inhibition profile *i.e.,* IC_{50}±SEM = 0.52 ± 0.08 μM. Moreover, the activity was moderately reduced with the shift of one *meta*-methyl group to the *para*-position of phenyl ring (**5f**), i.e. IC_{50} ± SEM = 0.54 ± 0.09 μM. The attachment of a methyl group at the *meta*-position seems to be

Table 2
Synthesis of compounds (**5a-t**).





Conditions: Ar-B(OH)$_2$ (1.1 equiv.), Pd(OAc)$_2$ (10 mol%), Xantphos (20 mol%), K_2CO_3 (2.0 equiv.), 1,4-dioxane, 100 °C, 16 h. Yields refer to isolated products.

essential for the *h*-TNAP activity. Inverse activities were perceived in case of *h*-IAP: the mono-substituted methyl derivative (**5b**) resulted in an improved inhibitory value towards *h*-IAP. Insertion of a methoxy group resulted in a significant inhibition of both isozymes but less than that of compound **5a.** Introduction of methoxy group located at the *para*-position of the phenyl ring was favorable for the inhibition of both *h*-TNAP and *h*-IAP, but more selective towards *h*-TNAP. The shifting of the methoxy group from the *para* to the *ortho*-position resulted in a lower inhibition of *h*-TNAP and complete loss of inhibition of *h*-IAP. Likewise, for compound **5e**, the presence of methyl groups at the *meta* position is more favorable for inhibition as compared to other positions (except compound **5c**). When the phenyl ring contains a fluorine atom, the position of the substituent plays an important role. In case of compound **5n,** where the fluorine atom is located at the *ortho*-postion, resulted in loss of the activity towards both isozymes. In contrast, compound **5o,** containing a fluorine atom located at the *meta*-position, resulted in significant inhibition of both isozymes. In comparison to compound **5o** and **5p**, an interesting observation was made in the case of a trifluoromethoxy group (F_3CO) located at the *para*-position (**5q**). This compound was found to be the most potent as well as selective inhibitor of *h*-IAP. The presence of F_3CO makes this compound more lipophilic and more selective for *h*-IAP. The compounds **5r**, **5s** and **5t** with NO_2, NC and phenyl substitution,

Table 3
Alkaline Phosphatase (*h*-TNAP & *h*-IAP) inhibition.

Sr. No.	Codes	*h*-TNAP	*h*-IAP
		IC_{50} ±SEM (μM)	
1	**4c**	0.38 ± 0.06	0.44 ± 0.07
2	**4d**	1.58 ± 0.11	2.02 ± 0.16
3	**4e**	1.98 ± 0.12	31.5 ± 3.67
4	**4f**	0.33 ± 0.04	0.71 ± 0.08
5	**4g**	0.29 ± 0.03	0.89 ± 0.07
6	**4h**	1.44 ± 0.14	0.31 ± 0.01
7	**4i**	0.88 ± 0.09	0.71 ± 0.07
8	**4j**	0.61 ± 0.05	0.76 ± 0.06
9	**4k**	0.45 ± 0.04	3.75 ± 1.02
10	**4l**	2.02 ± 0.17	2.22 ± 0.06
11	**4m**	1.53 ± 0.13	4.63 ± 1.16
12	**5a**	0.21 ± 0.02	0.43 ± 0.07
13	**5b**	1.06 ± 0.05	0.36 ± 0.04
14	**5d**	>100	4.55 ± 1.08
15	**5e**	0.52 ± 0.08	0.46 ± 0.05
16	**5f**	0.54 ± 0.11	1.75 ± 0.14
17	**5g**	0.28 ± 0.02	0.48 ± 0.02
18	**5i**	3.48 ± 0.25	>100
19	**5j**	1.15 ± 0.16	0.76 ± 0.12
20	**5l**	0.93 ± 0.12	0.79 ± 0.15
21	**5m**	4.52 ± 0.91	>100
22	**5n**	>100	>100
23	**5o**	1.39 ± 0.1	0.74 ± 0.09
24	**5p**	0.52 ± 0.08	0.68 ± 0.03
25	**5q**	>100	0.24 ± 0.02
26	**5r**	0.56 ± 0.04	1.12 ± 0.26
27	**5s**	0.62 ± 0.02	0.48 ± 0.04
28	**5t**	1.12 ± 0.25	1.67 ± 0.25
Levamisole		19.2 ± 0.1	–
L-Phenyl alanine		–	80.2 ± 1.1

Values are expressed as mean ± SEM of n = 3. The IC_{50} is the concentration at which 50% of the enzyme activity is inhibited.

respectively, also influence the inhibition of *h*-IAP, but the activity was lower in comparison to **5q**.

These results show that most of the derivatives from both series had IC_{50}s towards both APs in the low micromolar range. Moreover the selective inhibitors from arylated derivatives of 7-(trifluoromethyl)-5*H*-[1,3,4]thiadiazolo[3,2-*a*]pyrimidin-5-ones represent an important starting point for the development of some more potent and selective inhibitors of APs.

4. Molecular docking

Molecular docking studies were performed to find a possible binding orientation of the most potent compounds in the homology models of both isozymes. Fig. 2 exhibits the postulated binding interactions of compound **5a** with the amino acid residue of the *h*-TNAP model. The model revealed seven bonding interactions, four being hydrogen bonds, as shown by the green dashed line, while others are π-π stacked as shown by the pink dashed line. The carbonyl group of the pyrimidone forms two hydrogen bonds with residues His154 and Arg167. Two other hydrogen bonds are formed between the nitrogen atoms of the thiadiazole and pyrimidine rings with the residue of Arg151 and His434. These strong binding interactions of different side chains of compound **5a** are presumably the main reason for the strong inhibitory action against *h*-TNAP. The docking studies of compound **5q** against *h*-IAP (Fig. 3) revealed three strong hydrogen bonds and four π-π stacking interactions with various amino acid residue of *h*-IAP. Four π-π stacking interactions were formed between the phenyl, pyrimidine and thiadizole ring and the residue of His320 and His317. The carbonyl group of compound **5q** also forms two hydrogen bonds with the amino acid residue of Arg150 and His153. The oxygen

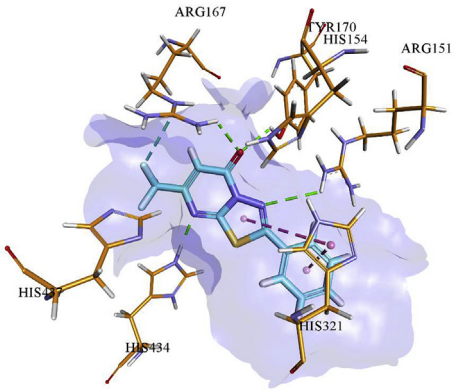


Fig. 2. Putative binding interactions of 5a (cyan) within the active site of *h*-TNAP (golden). Hydrogen bonds and π-π interactions are depicted as green and pink dashed lines, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

atom of the methoxy group is involved in a third hydrogen bond with the amino acid residue of Gln108. The fluorine atom of both compounds **5a** and **5q** has strong binding interactions with their respective enzymes. The fluorine atom of compound **5q** displayed more interactions with *h*-IAP as compared to the binding interaction of compound **5a** with *h*-TNAP as shown in Figs. 2 and 3.

5. Conclusions

In conclusion, 2-bromo-7-trifluoromethyl-thiadiazolopyrimidone has been used as template and engaged in S_NAr-reaction and Suzuki-Miyaura reactions giving corresponding 2-substituted 7-trifluoromethyl-thiadiazolopyrimidones. Almost all derivatives composing the library were potent, but non-selective inhibitors of both isozymes of alkaline phosphatase with IC_{50} values in the micro-molar range. These new AP inhibitors may further be used as lead compounds in medicinal chemistry. Especially compounds **4h** and **5b** show promising activity for further investigations. Docking studies allowed establishing an understanding of a possible binding mode of the compounds in the active site of the enzyme.

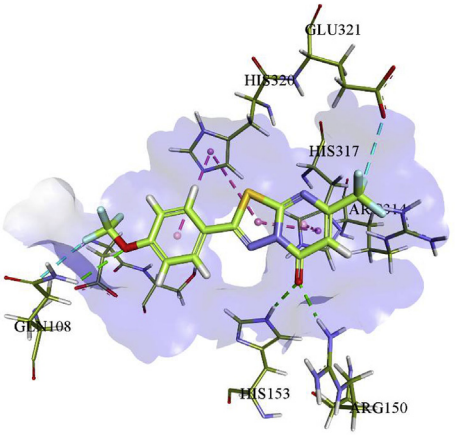


Fig. 3. Putative binding interactions of **5q** (light green) within the active site of *h*-IAP (olive). Hydrogen bonds and π-π interactions are depicted as green and pink dashed lines, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

6. Experimental section

6.1. Synthesis of 2-bromo-7-trifluoromethyl-5H-1,3,4-thiadiazolo [3,2-a]pyrimidin-5-one (**3**)

2-bromo-5-amino-1,3,4-thiadiazole (1.0 equiv., 0.013 mmol) was dissolved in 15 g polyphosphoric acid (PPA) then 2.4 ml of ethyl 4,4,4-trifluoroacetoacetate was added dropwise over 15 min. Afterwards the reaction mixture was refluxed for 8 h. After cooling to room temperature, the reaction mixture washed with ice-water and precipitate formed was filtrated and dried.

6.2. General procedure for the synthesis of 2-(substituted)amino-7-trifluoromethyl-5H-1,3,4-thiadiazolo[3,2-a]pyrimidin-5-one (**4a-n**)

2-bromo-7-trifluoromethyl-5*H*-1,3,4-thiadiazolo[3,2-*a*]pyrimidin-5-one (1.0 equiv., 0.334 mmol) was dissolved in methanol then 2.0 equiv. of aniline or amine were added. After 5 h stirring at room temperature, the reaction mixture was refluxed for 20 min. After cooling to room temperature, the solution was treated with ice-water. The precipitate formed was filtrated and dried.

6.3. General procedure for the synthesis of 2-aryl-7-trifluoromethyl-5H-1,3,4-thiadiazolo[3,2-a]pyrimidin-5-one(**5a-t**)

A mixture of 2-bromo-7-trifluoromethyl-5*H*-1,3,4-thiadiazolo [3,2-*a*]pyrimidin-5-one (1.0 equiv., 0.335 mmol), arylboronic acid (1.1 equiv.), Palladium(II)acetate (0.1 equiv.), Xantphos (0.2 equiv.) and Potassium carbonate (2.0 equiv.) was vigorously stirred and heated in dry 1,4-dioxane (2 ml) at 100 °C for 16 h. After cooling to room temperature, the reaction mixture was diluted with water and extracted into ethyl acetate. The organic layer was dried with anhydrous sodium sulfate and the solvent was evaporated. The crude compound was purified by flash column chromatography on silicagel (ethyl acetate:heptane).

6.4. 2-Bromo-7-trifluoromethyl-5H-1,3,4-thiadiazolo[3,2-a] pyrimidin-5-one (**3**)

2.3 g of 2-bromo-5-amino-1,3,4-thiadiazole **3** (75%) was obtained as a yellow solid; mp (133–134 °C); ^{1}HNMR (300 MHz, $CDCl_3$) δ 6.32 (s, 1H, $CH_{Het\text{-}Ar}$); ^{13}C NMR (62 MHz, $CDCl_3$) δ 163.96 (C_{Ar}), 157.78 (C_{Ar}), 158.78 (C_{Ar}),150.99 (q, ^2J = 36.53 Hz, *C*-CF_3), 120.01 (q, ^1J = 275.57 Hz, CF_3), 108.43 (q, ^3J = 3.17 Hz, $CH_{Het\text{-}Ar}$); IR (ATR) υ 3067 (w), 1709 (s), 1650 (w), 1581 (w), 1523 (s), 1488 (s), 1423 (m), 1281 (s), 1207 (w), 1188 (m), 1144 (s), 1056 (s), 964 (w), 887 (m), 854 (m), 792 (w), 701 (m), 681 (m), 562 (w), 532 (m) cm^{-1}; MS *m*/*z* 299 (M^+, 57), 273(23), 271(22), 180(12), 166(100), 162(26), 139(16), 125(24), 123(24), 108(10), 93(47); HRMS calcd for $C_6HON_3BrF_3S$ 298.89703; found 298.89708. Anal. Calcd for $C_6HON_3BrF_3S$: C, 24.02; H, 0.34; N, 14.00; found: C, 24.40; H, 0.24; N, 13.66.

6.5. 2-Phenylamino-7-trifluoromethyl-5H-1,3,4-thiadiazolo[3, 2-a] pyrimidin-5-one (**4a**)

According to the general procedure, aniline afforded 95 mg of product **4a** (91%) as a yellow solid; mp (207–208 °C); ^{1}HNMR (250 MHz, DMSO-d_6) δ 10.85 (s, 1H, NH), 7.62 (dd, ^3J = 8.75 Hz, ^4J = 1.12 Hz, 2H, CH_{Ar}), 7.43 (pt, ^3J = 7.81 Hz, 2H, CH_{Ar}), 7.12 (pt, ^3J = 7.36 Hz, 1H, CH_{Ar}), 6.86 (s, 1H, $CH_{Het\text{-}Ar}$); ^{13}C NMR (62 MHz, DMSO-d_6) δ 161.18 (C_{Ar}), 154.87 (C_{Ar}), 154.18 (C_{Ar}), 148.11 (q, ^2J = 34.78 Hz, *C*-CF_3), 138.97 (C_{Ar}), 129.43 (CH_{Ar}), 123.62 (CH_{Ar}), 120.78 (q, ^1J = 274.68 Hz, CF_3), 118.43 (CH_{Ar}), 107.03 (q, ^3J = 3.37 Hz, $CH_{Het\text{-}Ar}$); IR (ATR) υ 3262 (w), 3206 (w), 3092 (w), 1953 (w), 1795 (w), 1672 (m), 1658 (s), 1614 (m), 1556 (m), 1498 (s), 1419 (m), 1333 (w), 1276 (m), 1268 (m), 1181 (s), 1088 (m), 1009 (m), 915 (m), 844 (s), 752 (s), 690 (m), 657 (m), 584 (m) cm^{-1}; MS *m*/*z* 312 (M^+, 100), 293(5), 243(3) 180(5), 166(12), 136(29), 118(9), 109(12); HRMS calcd for $C_{12}H_7ON_4F_3S$ 312.02872; found 312.02855. Anal. Calcd for $C_{12}H_7ON_4F_3S$: C, 46.16; H, 2.26; N, 17.94; found: C, 45.93; H, 2.03; N, 17.85.

6.6. 2-(4-Fluorophenyl)amino-7-trifluoromethyl-5H-1,3,4-thiadiazolo[3,2-a]pyrimidin-5-one (**4b**)

According to the general procedure, 4-fluoroaniline afforded 99 mg of product **4b** (90%) as a yellow solid; mp (320–321 °C); ^{1}HNMR (300 MHz, DMSO-d_6) δ 10.87 (s, 1H, NH), 7.60–7.67 (m, 2H, CH_{Ar}), 7.24–7.32 (m, 2H, CH_{Ar}), 6.86 (s, 1H, $CH_{Het\text{-}Ar}$); ^{13}C NMR (62 MHz, DMSO-d_6) δ 160.75 (C_{Ar}), 157.79 (d, ^1J = 240.31 Hz, C-F), 154.42 (C_{Ar}), 153.83 (C_{Ar}), 147.70 (q, ^2J = 35.21 Hz, *C*-CF_3), 135.01 (d, ^4J = 2.75 Hz, C_{Ar}), 120.36 (q, ^1J = 275.71 Hz, CF_3), 119.89 (d, ^3J = 7.96 Hz, CH_{Ar}), 115.67 (d, ^2J = 22.67 Hz, CH_{Ar}), 106.65 (q, ^3J = 3.10 Hz, $CH_{Het\text{-}Ar}$); IR (ATR) υ 3264 (w), 3220 (w), 3080 (m), 3027 (w), 1582 (m), 1557 (m), 1495 (s), 1420 (s), 1320 (w), 1278 (s), 1238 (m), 1196 (m), 1133 (m), 1086 (m), 1010 (m), 918 (m), 836 (s), 774 (m), 685 (m), 539 (w) cm^{-1}; MS *m*/*z* 330 (M^+, 100), 302(2), 261(2), 195(3), 166(17), 154(29), 136(11), 127(11), 109(5); HRMS calcd for $C_{12}H_6ON_4F_3S$ 330.01930; found 330.01929. Anal. Calcd for $C_{12}H_6ON_4F_3S$: C, 43.64; H, 1.83; N, 16.96; found: C, 43.39; H, 1.58; N, 16.72.

6.7. 2-(3-Methoxyphenyl)amino-7-trifluoromethyl-5H-1,3,4-thiadiazolo[3,2-a]pyrimidin-5-one (**4c**)

According to the general procedure, 3-methoxyaniline afforded 92 mg of product **4c** (91%) as a white solid; mp (297–299 °C); ^{1}HNMR (300 MHz, DMSO-d_6) δ 10.85 (s, 1H, NH), 7.28–7.35 (m, 2H, CH_{Ar}), 7.12 (dd, ^3J = 8.07 Hz, ^4J = 1.44 Hz, 1H, CH_{Ar}), 6.86 (s, 1H, $CH_{Het\text{-}Ar}$), 6.72 (dd, ^3J = 8.14 Hz, ^4J = 1.99 Hz, 1H, CH_{Ar}), 3.77 (s, 3H, OCH_3); ^{13}C NMR (75 MHz, DMSO-d_6) δ 161.05 (C_{Ar}), 159.92 (C_{Ar}), 154.75 (C_{Ar}), 154.04 (C_{Ar}), 148.02 (q, ^2J = 34.91 Hz, *C*-CF_3), 139.91 (CH_{Ar}), 130.20 (CH_{Ar}), 120.68 (q, ^1J = 274.64 Hz, CF_3),110.74 (CH_{Ar}), 108.56 (CH_{Ar}), 106.95 (q, ^3J = 3.32 Hz, $CH_{Het\text{-}Ar}$), 104.68 (C_{Ar}), 55.10 (OCH_3); IR (ATR) υ 3275 (w), 3222 (w), 3092 (w), 2954 (w), 1663 (s), 1567 (s), 1497 (s), 1421 (m), 1337 (w), 1298 (w), 1273 (s), 1149 (s), 1089 (w), 1042 (w), 955 (m), 833 (m), 819 (m), 703 (m), 659 (m), 550 (w) cm^{-1}; MS *m*/*z* 342 (M^+, 100), 341(26), 312(5), 180(10), 166(21), 139(7), 107(12); HRMS calcd for $C_{13}H_9O_2N_4F_3S$ 342.03928; found 342.03926. Anal. Calcd for $C_{13}H_9O_2N_4F_3S$: C, 45.62; H, 2.65; N, 16.37; found: C, 45.56; H, 2.39; N, 16.05.

6.8. 2-(4-Ethoxyphenyl)amino-7-trifluoromethyl-5H-1,3,4-thiadiazolo[3,2-a]pyrimidin-5-one (**4d**)

According to the general procedure, 4-ethoxyaniline afforded 106 mg of product **4d** (90%) as a yellow solid; mp (286–287 °C); ^{1}HNMR (300 MHz, DMSO-d_6) δ 10.66 (s, 1H, NH), 7.51 (d, ^3J = 9.06 Hz, 2H, CH_{Ar}), 6.98 (d, ^3J = 9.06 Hz, 2H, CH_{Ar}), 6.83 (s, 1H, $CH_{Het\text{-}Ar}$), 4.01 (q, ^3J = 6.99 Hz, 2H, CH_2), 1.32 (t, ^3J = 6.96 Hz, 3H, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6) δ 161.01 (C_{Ar}), 154.91 (C_{Ar}), 154.72 (C_{Ar}), 154.35 (C_{Ar}), 147.91 (q, ^2J = 35.21 Hz, *C*-CF_3), 131.99 (C_{Ar}), 120.70 (q, ^1J = 274.70 Hz, CF_3), 120.29 (CH_{Ar}), 115.00 (CH_{Ar}), 106.85 (q, ^3J = 3.29 Hz, $CH_{Het\text{-}Ar}$), 63.23 (OCH_2), 14.63 (CH_3); IR (ATR) υ 3263 (w), 3205 (w), 3075 (w), 3029 (w), 1661 (s), 1586 (m), 1500 (s), 1480 (m), 1430 (w), 1391 (w), 1271 (m), 1187 (m), 1048 (m), 918 (m), 836 (s), 794 (w), 660 (m), 580 (m) cm^{-1}; MS *m*/*z* 356 (M^+, 100), 328(24), 327(20), 196(13), 180(12), 166(6), 134(12), 133(11), 108(6), 93(4); HRMS calcd for $C_{14}H_{11}O_2N_4F_3S$ 356.05493; found 356.05397.

Anal. Calcd for $C_{14}H_{11}O_2N_4F_3S$: C, 47.19; H, 3.11; N, 15.72; found: C, 47.31; H, 2.89; N, 15.44.

6.9. 2-(4-i-Propylphenyl)amino-7-trifluoromethyl-5H-1,3,4-thiadiazolo[3,2-a]pyrimidin-5-one (**4e**)

According to the general procedure, 4-*i*-propylaniline afforded 104 mg of product **4e** (87%) as a brown solid; mp (241–242 °C); ^{1}HNMR (300 MHz, DMSO-d_6) δ 10.76 (s, 1H, NH), 7.52 (d, ^3J = 8.58 Hz, 2H, CH_{Ar}), 7.29 (d, ^3J = 8.49 Hz, 2H, CH_{Ar}), 6.84 (s, 1H, $CH_{Het\text{-}Ar}$), 2.81–2.95 (m, 1H, $CH_{i\text{-}pr}$), 1.20 (d, ^3J = 6,90 Hz, 6H, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6) δ 161.06 (C_{Ar}), 154.75 (C_{Ar}), 154.19 (C_{Ar}), 147.97 (q, ^2J = 34.91 Hz, *C*-CF_3), 143.84 (C_{Ar}), 136.66 (C_{Ar}), 127.07 (CH_{Ar}), 120.70 (q, ^1J = 274.86 Hz, CF_3), 118.63 (CH_{Ar}), 106.89 (q, ^3J = 2.86 Hz, $CH_{Het\text{-}Ar}$), 32.83 ($CH_{i\text{-}pr}$), 23.88 (CH_3); IR (ATR) υ 3263 (w), 3200 (w), 2967 (w), 1663 (s), 1614 (m), 1500 (s), 1418 (m), 1277 (m), 1185 (m), 1137 (s), 1011 (w), 915 (m), 840 (m), 762 (w), 657 (w), 541 (m) cm^{-1}; MS *m*/*z* 354 (M^+, 49), 339(100), 177(5) 159(3), 145(9), 144(7), 119(8), 118(9), 103(4), 91(9); HRMS calcd for $C_{15}H_{13}ON_4F_3S$ 354.07567; found 354.07544. Anal. Calcd for $C_{15}H_{13}ON_4F_3S$: C, 58.84; H, 3.70; N, 15.81; found: C, 58.43; H, 3.56; N, 15.36.

6.10. 2-(4-Aminophenyl)amino-7-trifluoromethyl-5H-1,3,4-thiadiazolo[3,2-a]pyrimidin-5-one (**4f**)

According to the general procedure, 4-aminoaniline afforded 71 mg of product **4f** (65%) as a green solid; mp (314–315 °C); ^{1}HNMR (300 MHz, DMSO-d_6) δ 10.36 (s, 1H, NH), 7.19 (d, ^3J = 8.76 Hz, 2H, CH_{Ar}), 6.77 (s, 1H, $CH_{Het\text{-}Ar}$), 6.57 (d, ^3J = 8.88 Hz, 2H, CH_{Ar}), 5.09 (s, 2H, NH_2); ^{13}C NMR (62 MHz, DMSO-d_6) δ 160.92 (C_{Ar}), 155.35 (C_{Ar}), 154.73 (C_{Ar}), 147.80 (q, ^2J = 34.78 Hz, *C*-CF_3), 145.54 (C_{Ar}), 128.16 (C_{Ar}), 121.31 (CH_{Ar}), 120.65 (q, ^1J = 276.03 Hz, CF_3), 114.48 (CH_{Ar}), 106.71 (q, ^3J = 2.72 Hz, $CH_{Het\text{-}Ar}$); IR (ATR) υ 3476 (w), 3384 (w), 3081 (w), 1662 (s), 1554 (m), 1275 (s), 1262 (s), 1183 (s), 1011 (m), 917 (m), 831 (s), 811 (w), 792 (w), 689 (w), 617 (w), 568 (w) cm^{-1}; MS *m*/*z* 327 (M^+, 100), 180(4), 165(3), 134(5), 133(19), 132(58), 124(10), 118(5), 107(12), 93(20); HRMS (ESI, M + H) calcd for $C_{12}H_8ON_5F_3S$ 328.04744 found 328.04828 (ESI, M + Na) calcd for $C_{12}H_8ON_5F_3S$ 350.02939; found 350.02988. Anal. Calcd for $C_{12}H_8ON_5F_3S$: C, 44.04; H, 2.46; N, 21.40; found: C, 43.86; H, 2.09; N, 21.17.

6.11. 2-Allylamino-7-trifluoromethyl-5H-1,3,4-thiadiazolo[3,2-a]pyrimidin-5-one (**4g**)

According to the general procedure, allylamine afforded 69 mg of product **4g** (75%) as a white solid; mp (249–250 °C); ^{1}HNMR (300 MHz, $CDCl_3$) δ 8.55 (s, 1H, NH), 6.76 (s, 1H, $CH_{Het\text{-}Ar}$), 5.87–6.00 (m, 1H, *CH*=CH_2), 5.17–5.34 (m, 2H, CH=*CH_2*), 3.99 (d, ^3J = 5.07 Hz, 2H, CH_2); ^{13}C NMR (62 MHz, $CDCl_3$) δ 161.16 (C_{Ar}), 160.18 (C_{Ar}), 156.11 (C_{Ar}), 150.04 (q, ^2J = 35.22 Hz, *C*-CF_3), 132.14 (CH_2), 120.49 (q, ^1J = 274.32 Hz, CF_3), 118.83 (CH), 106.94 (q, ^3J = 1.79 Hz, $CH_{Het\text{-}Ar}$), 48.88 (CH_2); IR (ATR) υ 3319 (s), 3105 (w), 2912 (w), 2844 (w), 1658 (s), 1581 (s), 1498 (s), 1421 (s), 1279 (s), 1174 (s), 1134 (s), 1083 (m), 917 (m), 853 (m), 758 (m), 662 (m), 530 (m) cm^{-1}; MS *m*/*z* 276 (M^+, 100), 275(9), 257(13) 196(26), 180(29), 166(19), 163(12), 138(17), 121(9), 100(8), 93(19); HRMS calcd for $C_9H_7ON_4F_3S$ 276.02872; found 276.02868. Anal. Calcd for $C_9H_7ON_4F_3S$: C, 39.13; H, 2.55; N, 20.28; found: C, 38.97; H, 2.42; N, 19.84.

6.12. 2-n-Propylamino-7-trifluoromethyl-5H-1,3,4-thiadiazolo[3,2-a]pyrimidin-5-one (**4h**)

According to the general procedure, *n*-propylamine afforded 53 mg of product **4h** (56%) as a white solid; mp (254–255 °C); ^{1}HNMR (300 MHz, $CDCl_3$) δ 6.88 (s, 1H, NH), 6.73 (s, 1H, $CH_{Het\text{-}Ar}$), 3.40 (q, ^3J = 6.65 Hz, 2H, CH_2), 1.65–1.80 (m, 2H, CH_2), 1.00 (t, ^3J = 7.24 Hz, 3H, CH_3); ^{13}C NMR (62 MHz, $CDCl_3$) δ 162.87 (C_{Ar}), 160.26 (C_{Ar}), 156.03 (C_{Ar}), 149.37 (q, ^2J = 36.16 Hz, *C*-CF_3), 120.56 (q, ^1J = 274.32 Hz, CF_3), 107.19 (q, ^3J = 2.83 Hz, $CH_{Het\text{-}Ar}$), 48.56 (CH_2), 22.82 (CH_2), 11.32 (CH_3); IR (ATR) υ 3280 (m), 3100 (w), 2973 (w), 2870 (w), 1661 (s), 1595 (s), 1504 (s), 1279 (m), 1124 (s), 1083 (m), 1083 (w), 1007 (w), 929 (w), 856 (m), 705 (s), 573 (w) cm^{-1}; MS *m*/*z* 278 (M^+, 50), 259(10), 250(22), 249(39), 236(100), 196(19), 180(14), 166(13), 163(12), 121(5), 108(3), 93(12); HRMS calcd for $C_9H_9ON_4F_3S$ 278.04437; found 278.04424. Anal. Calcd for $C_9H_9ON_4F_3S$: C, 38.85; H, 3.26; N, 20.14; found: C, 38.82; H, 3.12; N, 19.57.

6.13. 2-n-Butylamino-7-trifluoromethyl-5H-1,3,4-thiadiazolo[3,2-a]pyrimidin-5-one (**4i**)

According to the general procedure, *n*-butylamine afforded 65 mg of product **4i** (66%) as a white solid; mp (231–232 °C); ^{1}HNMR (300 MHz, $CDCl_3$) δ 6.78 (s, 1H, NH), 6.73 (s, 1H, $CH_{Het\text{-}Ar}$), 3.43 (q, ^3J = 6.36 Hz, 2H, CH_2), 1.62–1.73 (m, 2H, CH_2), 1.34–1.49 (m, 2H, CH_2), 0.96 (t, ^3J = 7.27 Hz, 3H, CH_3); ^{13}C NMR (62 MHz, $CDCl_3$) δ 160.17 (C_{Ar}), 160.07 (C_{Ar}), 155.90 (C_{Ar}), 149.79 (q, ^2J = 36.08 Hz, *C*-CF_3), 120.42 (q, ^1J = 274.99 Hz, CF_3), 107.03 (q, ^3J = 3.20 Hz, $CH_{Het\text{-}Ar}$), 44.54 (CH_2), 31.39 (CH_2), 19.89 (CH_2), 13.68 (CH_3); IR (ATR) υ 3307 (m), 2963 (w), 2938 (w), 1660 (s), 1590 (s), 1462 (m), 1421 (m), 1275 (s), 1142 (s), 1083 (m), 1011 (m), 849 (s), 705 (m), 658 (w), 614 (w), 535 (w) cm^{-1}; MS *m*/*z* 292 (M^+, 38), 273(19), 264(60), 236(75), 220(11), 196(100), 180(26), 166(17), 163(25), 162(14), 148(9), 138(24), 220(11), 108(5), 97(23), 93(22); HRMS calcd for $C_{10}H_{11}ON_4F_3S$ 292.06002; found 292.06002. Anal. Calcd for $C_{10}H_{11}ON_4F_3S$: C, 41.09; H, 3.79; N, 19.17; found: C, 40.85; H, 3.44; N, 18.94.

6.14. 2-n-Pentylamino-7-trifluoromethyl-5H-1,3,4-thiadiazolo[3,2-a]pyrimidin-5-one (**4j**)

According to the general procedure, *n*-pentylamine afforded 74 mg of product **4j** (72%) as a white solid; mp (219–220 °C); ^{1}HNMR (300 MHz, $CDCl_3$) δ 6.97 (s, 1H, NH), 6.73 (s, 1H, $CH_{Het\text{-}Ar}$), 3.42 (q, ^3J = 6.51 Hz, 2H, CH_2), 1.65–1.74 (m, 2H, CH_2), 1.33–1.39 (m, 4H, CH_2), 0.91 (t, ^3J = 7.11 Hz, 3H, CH_3); ^{13}C NMR (62 MHz, $CDCl_3$) δ 160.30 (C_{Ar}), 160.11 (C_{Ar}), 155.95 (C_{Ar}), 149.80 (q, ^2J = 36.08 Hz, *C*-CF_3), 120.42 (q, ^1J = 274.83 Hz, CF_3), 106.92 (q, ^3J = 3.02 Hz, $CH_{Het\text{-}Ar}$), 46.94 (CH_2), 29.05 (CH_2), 28.78 (CH_2), 22.28 (CH_2), 13.92 (CH_3); IR (ATR) υ 3311 (m), 2961 (w), 2936 (w), 1660 (s), 1588 (s), 1500 (s), 1421 (m), 1390 (m), 1274 (m), 1142 (s), 1083 (m), 1012 (w), 847 (s), 749 (w), 704 (s), 657 (m), 532 (w) cm^{-1}; MS *m*/*z* 306 (M^+, 27), 291(13), 287(17) 278(46), 273(15), 263(10), 250(40), 249(72), 237(16), 236(100), 220(10), 196(88), 180(22), 149(32), 166(15), 163(20), 162(13), 138(20), 111(20), 93(19); HRMS calcd for $C_{11}H_{13}ON_4F_3S$ 306.07567; found 306.07526. Anal. Calcd for $C_{11}H_{13}ON_4F_3S$: C, 43.13; H, 4.28; N, 18.29; found: C, 43.18; H, 4.04; N, 18.14.

6.15. 2-[2-(Dimethylamino)ethyl]amino-7-trifluoromethyl-5H-1,3,4-thiadiazolo[3,2-a]pyrimidin-5-one (**4k**)

According to the general procedure, dimethylaminoethylamine afforded 47 mg of product **4k** (45%) as a yellow solid; mp (244–245 °C); ^{1}HNMR (300 MHz, $CDCl_3$) δ 8.96 (pt, ^3J = 5.19 Hz, 1H, NH), 6.72 (s, 1H, $CH_{Het\text{-}Ar}$), 4.01 (q, ^3J = 5.35 Hz, 2H, CH_2), 3.47 (t, ^3J = 5.20 Hz, 2H, CH_2), 2.97 (s, 6H, CH_3); ^{13}C NMR (62 MHz, $CDCl_3$) δ 161.36 (C_{Ar}), 158.13 (C_{Ar}), 155.92 (C_{Ar}), 149.44 (q, ^2J = 36.16 Hz, *C*-

CF_3), 120.32 (q, ^1J = 275.14 Hz, CF_3), 107.20 (q, ^3J = 3.20 Hz, $CH_{Het\text{-}Ar}$), 57.10 (CH_2), 43.85 (CH_3), 39.01 (CH_2); IR (ATR) υ 3279 (m), 2982 (w), 2952 (w), 2881 (w), 2862 (w), 2779 (w), 1676 (s), 1570 (s), 1484 (m), 1422 (m), 1340 (w), 1273 (m), 1141 (s), 1011 (w), 917 (w), 845 (s), 703 (s), 658 (w), 679 (w) cm^{-1}; MS *m/z* 307 (M^+, 2), 263(5), 249(2), 196(2), 180(2), 162(4), 93(6); HRMS (ESI, M + H) calcd for $C_{10}H_{12}ON_5F_3S$ 308.07874 found 308.07906, (ESI, M + Na) calcd for $C_{10}H_{12}ON_5F_3S$ 330.06069; found 330.06072. Anal. calcd for $C_{10}H_{12}ON_5F_3S$: C, 39.09; H, 3.94; N, 22.79; found: C, 39.05; H, 3.67; N, 22.36.

6.16. 2-(1-Methylhydrazenyl)-7-trifluoromethyl-5H-1,3,4-thiadiazolo[3,2-a]pyrimidin-5-one (**4l**)

According to the general procedure, methylhydrazine afforded 46 mg of product **4l** (51%) as a yellow solid; mp (271–272 °C); ^{1}HNMR (300 MHz, DMSO-d_6) δ 6.72 (s, 1H, $CH_{Het\text{-}Ar}$), 5.78 (s, 2H, NH_2), 3.25 (s, 3H, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6) δ 165.21 (C_{Ar}), 162.43 (C_{Ar}), 154.79 (C_{Ar}), 147.34 (q, ^2J = 34.67 Hz, *C*-CF_3), 120.65 (q, ^1J = 274.80 Hz, CF_3), 106.25 (q, ^3J = 3.04 Hz, $CH_{Het\text{-}Ar}$), 39.87 (CH_3); IR (ATR) υ 3282 (w), 3235 (w), 3195 (w), 1651 (s), 1504 (s), 1408 (s), 1383 (m), 1291 (m), 1181 (s), 1123 (s), 1059 (m), 1000 (w), 848 (m), 736 (m), 647 (w), 622 (w) cm^{-1}; MS *m/z* 265 (M^+, 100), 246(12), 222(9), 180(24), 163(91), 148(4), 121(7), 93(17); HRMS calcd for $C_7H_6ON_5F_3S$ 265.02397; found 265.2408. Anal. Calcd for $C_7H_6ON_5F_3S$: C, 31.70; H, 2.28; N, 26.41; found: C, 31.64; H, 2.08; N, 25.97.

6.17. 2-Di-n-butylamino-7-trifluoromethyl-5H-1,3,4-thiadiazolo[3,2-a]pyrimidin-5-one (**4m**)

According to the general procedure, di-*n*-butylamine afforded 78 mg of product **4m** (67%) as a yellow solid; mp (94–95 °C); ^{1}HNMR (300 MHz, DMSO-d_6) δ 6.77 (s, 1H, $CH_{Het\text{-}Ar}$), 3.43 (t, ^3J = 7.44 Hz, 4H, CH_2), 1.56–1.66 (m, 4H, CH_2), 1.26–1.38 (m, 4H, CH_2), 0.91 (t, ^3J = 7.30 Hz, 6H, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6) δ 161.31 (C_{Ar}), 159.00 (C_{Ar}), 154.71 (C_{Ar}), 147.46 (q, ^2J = 34.17 Hz, *C*-CF_3), 120.70 (q, ^1J = 274.59 Hz, CF_3), 106.55 (q, ^3J = 3.14 Hz, $CH_{Het\text{-}Ar}$), 50.84 (CH_2), 28.71 (CH_2), 19.36 (CH_2), 13.66 (CH_3); IR (ATR) υ 2962 (w), 2937 (w), 2872 (w), 1699 (s), 1568 (s), 1517 (s), 1467 (w), 1431 (w), 1273 (m), 1174 (m), 1144 (s), 1110 (w), 1004 (w), 846 (m), 702 (m), 529 (m) cm^{-1}; MS *m/z* 348 (M^+, 20), 264(22), 263(33), 250(19), 249(100), 180(13), 153(27), 111(12), 97(8); HRMS calcd for $C_{14}H_{19}ON_4F_3S$ 348.12262; found 348.12239. Anal. Calcd for $C_{14}H_{19}ON_4F_3S$: C, 50.78; H, 6.66; N, 14.80; found: C, 50.93; H, 6.48; N, 14.46.

6.18. 2-Methylphenylamino-7-trifluoromethyl-5H-1,3,4-thiadiazolo[3,2-a]pyrimidin-5-one (**4n**)

According to the general procedure, *N*-methylaniline afforded 55 mg of product **4n** (50%) as a white solid; mp (166–167 °C); ^{1}HNMR (300 MHz, DMSO-d_6) δ 7.57 (d, ^3J = 4.17 Hz, 4H, CH_{Ar}), 7.44–7.50 (m, 1H, CH_{Ar}), 6.84 (s, 1H, $CH_{Het\text{-}Ar}$), 3.52 (s, 3H, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6) δ 161.06 (C_{Ar}), 159.81 (C_{Ar}), 154.68 (C_{Ar}), 147.87 (q, ^2J = 34.82 Hz, *C*-CF_3), 143.96 (C_{Ar}), 130.47 (CH_{Ar}), 128.66 (CH_{Ar}), 125.51 (CH_{Ar}), 120.61 (q, ^1J = 274.54 Hz, CF_3), 106.94 (q, ^3J = 2.74 Hz, $CH_{Het\text{-}Ar}$), 40.07 (CH_3); IR (ATR) υ 1703 (s), 1598 (w), 1566 (s), 1512(s), 1492 (m), 1398 (m), 1360 (w), 1274 (s), 1150 (s), 1072 (m), 844 (s), 771 (m), 702 (s), 695 (s), 623 (w), 545 (m) cm^{-1}; MS *m/z* 326 (M^+, 100), 150(22), 135(12), 132(14), 109(13), 105(13), 93(6); HRMS calcd for $C_{13}H_9ON_4F_3S$ 326.04437; found 326.04445. Anal. Calcd for $C_{13}H_9ON_4F_3S$: C, 47.85; H, 2.78; N, 17.17; found: C, 47.94; H, 2.60; N, 17.15.

6.19. 2-Phenyl-7-trifluoromethyl-5H-1,3,4-thiadiazolo[3,2-a]pyrimidin-5-one (**5a**)

According to the general procedure, phenylboronic acid afforded 90 mg of product **5a** (90%) as an orange solid; mp (186–188 °C); ^{1}HNMR (300 MHz, $CDCl_3$) δ 7.98 (d, ^3J = 7.85 Hz, 2H, CH_{Ar}), 7.53–7.67 (m, 3H, CH_{Ar}), 6.86 (s, 1H, $CH_{Het\text{-}Ar}$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.42 (C_{Ar}), 160.84 (C_{Ar}), 156.17 (C_{Ar}), 151.10 (q, ^2J = 36.30 Hz, *C*-CF_3), 130.69 (C_{Ar}), 129.75 (CH_{Ar}), 128.04 (CH_{Ar}), 127.91 (CH_{Ar}), 120.41 (q, ^1J = 275.10 Hz, CF_3), 107.79 (q, ^3J = 3.05 Hz, $CH_{Het\text{-}Ar}$); IR (ATR) υ 3087 (w), 3027 (w), 2920 (w), 1701 (s), 1511 (s), 1417 (s), 1278 (s), 1137 (s), 1073 (m), 1018 (m), 998 (w), 844 (s), 761 (s), 715 (w), 691 (m), 605 (s) cm^{-1}; MS *m/z* 297 (M^+, 100), 278(7), 180(12), 166(88), 139(7), 121(53), 105(17), 99(34); HRMS calcd for $C_{12}H_6ON_3F_3S$ 297.01782; found 297.01739. Anal. Calcd for $C_{12}H_6ON_3F_3S$: C, 48.49; H, 2.03; N, 14.14; found: C, 48.28; H, 2.19; N, 14.59.

6.20. 2-(4-Methylphenyl)-7-trifluoromethyl-5H-1,3,4-thiadiazolo[3,2-a]pyrimidin-5-one (**5b**)

According to the general procedure, 4-tolylboronic acid afforded 85 mg of product **5b** (81%) as an orange solid; mp (230–231 °C); ^{1}HNMR (300 MHz, $CDCl_3$) δ 7.86 (d, ^3J = 8.25 Hz, 2H, CH_{Ar}), 7.35 (d, ^3J = 8.01 Hz, 2H, CH_{Ar}), 6.85 (s, 1H, $CH_{Het\text{-}Ar}$), 2.46 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.43 (C_{Ar}), 160.91 (C_{Ar}), 156.22 (C_{Ar}), 151.04 (q, ^2J = 35.62 Hz, *C*-CF_3), 144.81 (C_{Ar}), 130.42 (C_{Ar}), 127.97 (CH_{Ar}), 125.19 (CH_{Ar}), 120.44 (q, ^1J = 275.10 Hz, CF_3), 107.70 (q, ^3J = 3.08 Hz, $CH_{Het\text{-}Ar}$), 21.87 (-CH_3); IR (ATR) υ 3085 (w), 3035 (w), 2959 (w), 1698 (s), 1511 (s), 1495 (s), 1415 (m), 1276 (m), 1139 (m), 1028 (m), 951 (w), 867 (m), 845 (m), 816 (s), 699 (m), 605 (m) cm^{-1}; MS *m/z* 311 (M^+, 100), 292(7), 180(9), 166(81), 119(24), 118(23), 117(14), 91(23); HRMS calcd for $C_{13}H_8ON_3F_3S$ 311.03347; found 311.03312. Anal. Calcd for $C_{13}H_8ON_3F_3S$: C, 50.16; H, 2.59; N, 13.50; found: C, 50.30; H, 2.54; N, 13.30.

6.21. 2-(2-Methylphenyl)-7-trifluoromethyl-5H-1,3,4-thiadiazolo[3,2-a]pyrimidin-5-one (**5c**)

According to the general procedure, 2-tolylboronic acid afforded 78 mg of product **5c** (75%) as a yellow solid; mp (132–133 °C); ^{1}HNMR (300 MHz, $CDCl_3$) δ 7.65 (d, ^3J = 8.49 Hz, 1H, CH_{Ar}), 7.49 (dd, ^3J = 7.36 Hz, ^4J = 1.12 Hz, 1H, CH_{Ar}), 7.37–7.41 (m, 2H, CH_{Ar}), 6.87 (s, 1H, $CH_{Het\text{-}Ar}$), 2.68 (s, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.51 (C_{Ar}), 160.48 (C_{Ar}), 155.78 (C_{Ar}), 150.78 (q, ^2J = 35.64 Hz, *C*-CF_3), 137.95 (C_{Ar}), 132.20 (C_{Ar}), 132.05 (CH_{Ar}), 130.04 (CH_{Ar}), 126.68 (CH_{Ar}), 126.58 (CH_{Ar}), 120.10 (q, ^1J = 275.10 Hz, CF_3), 107.32 (q, ^3J = 3.29 Hz, $CH_{Het\text{-}Ar}$), 21.38 (CH_3); IR (ATR) υ 3082 (w), 2960 (w), 2919 (w), 1701 (s), 1601 (w), 1510 (s), 1408 (m), 1383 (w), 1272 (s), 1139 (m), 1093 (w), 1059 (w), 957 (w), 846 (m), 758 (m), 700 (m), 652 (w), 559 (w) cm^{-1}; MS *m/z* 311 (M^+, 100), 252(10), 180(9), 166(11), 149(45), 148(49), 134(24), 117(17), 116(55), 91(20); HRMS calcd for $C_{13}H_8ON_3F_3S$ 311.03347; found 311.03334. Anal. Calcd for $C_{13}H_8ON_3F_3S$: C, 50.16; H, 2.59; N, 13.50; S, 13.50; found: C, 50.65; H, 2.25; N, 13.96.

6.22. 2-(4-Ethylphenyl)-7-trifluoromethyl-5H-1,3,4-thiadiazolo[3,2-a]pyrimidin-5-one (**5d**)

According to the general procedure, 4-ethylphenylboronic acid afforded 72 mg of product **5d** (66%) as a yellow solid; mp (205–206 °C); ^{1}HNMR (300 MHz, $CDCl_3$) δ 7.88 (d, ^3J = 8.31 Hz, 2H), CH_{Ar}), 7.37 (d, ^3J = 8.31 Hz, 2H, CH_{Ar}), 6.85 (s, 1H, $CH_{Het\text{-}Ar}$), 2.74 (q, ^3J = 7.59 Hz, 2H, -CH_2), 1.28 (t, ^3J = 7.60 Hz, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.63 (C_{Ar}), 161.13 (C_{Ar}), 156.43 (C_{Ar}), 151.23 (q,

^2J = 35.75 Hz, *C*-CF_3), 151.18 (C_{Ar}), 129.46 (C_{Ar}), 128.30 (CH_{Ar}), 125.57 (CH_{Ar}), 120.64 (q, ^1J = 275.01 Hz, CF_3), 107.89 (q, ^3J = 3.17 Hz, $CH_{Het\text{-}Ar}$), 29.32 (CH_2), 15.39 (CH_3); IR (ATR) υ 3032 (w), 2976 (w), 2918 (w), 1697 (s), 1514 (s), 1497 (s), 1412 (m), 1302 (w), 1274 (s), 1185 (s), 1143 (s), 1049 (w), 1027 (m), 1012 (w), 866 (m), 841 (m), 697 (m), 609 (m), 535 (w) cm^{-1}; MS *m/z* 325 (M^+, 100), 166(65), 149(17), 148(9), 134(32), 133(20), 132(23), 116(19), 90(5); HRMS calcd for $C_{14}H_{10}ON_3F_3S$ 325.04912; found 325.04890. Anal. Calcd for $C_{14}H_{10}ON_3F_3S$: C, 51.69; H, 3.10; N, 12.92; found: C, 51.17; H, 3.22; N, 12.94.

6.23. 2-(3,5-Di-methylphenyl)-7-trifluoromethyl-5H-1,3,4-thiadiazolo[3,2-a]pyrimidin-5-one (**5e**)

According to the general procedure, 3,5-di-methylphenylboronic acid afforded 66 mg of product **5e** (60%) as a yellow solid; mp (240–241 °C); ^{1}HNMR (300 MHz, $CDCl_3$) δ 7.58 (s, 2H, CH_{Ar}), 7.24 (s, 1H, CH_{Ar}), 6.85 (s, 1H, $CH_{Het\text{-}Ar}$), 2.40 (s, 6H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.43 (C_{Ar}), 161.26 (C_{Ar}), 156.25 (C_{Ar}), 151.05 (q, ^2J = 35.76 Hz, *C*-CF_3), 139.73 (C_{Ar}), 135.49 (C_{Ar}), 127.67 (CH_{Ar}), 125.71 (CH_{Ar}), 120.43 (q, ^1J = 275.10 Hz, CF_3), 107.66 (q, ^3J = 3.16 Hz, $CH_{Het\text{-}Ar}$), 21.21 (CH_3); IR (ATR) υ 3042 (w), 2962 (w), 2920 (w), 1713 (s), 1506 (s), 1480 (w), 1413 (w), 1380 (w), 1278 (s), 1149 (s), 1074 (m), 1001 (w), 884 (m), 846 (m), 763 (m), 707 (m), 684 (s), 623 (w), 558 (w), 531 (m) cm^{-1}; MS *m/z* 325 (M^+, 100), 268(15), 166(50), 149(17), 133(18), 132(21), 131(13), 122(36), 121(14), 107(30), 91(9); HRMS calcd for $C_{14}H_{10}ON_3F_3S$ 325.04912; found 325.04888. Anal. Calcd for $C_{14}H_{10}ON_3F_3S$: C, 51.69; H, 3.10; N, 12.92; found: C, 51.20; H, 3.56; N, 12.64.

6.24. 2-(3,4-Di-methylphenyl)-7-trifluoromethyl-5H-1,3,4-thiadiazolo[3,2-a]pyrimidin-5-one (**5f**)

According to the general procedure, 3,4-di-methylphenylboronic acid afforded 69 mg of product **5f** (63%) as a yellow solid; mp (190–191 °C); ^{1}HNMR (300 MHz, $CDCl_3$) δ 7.79 (s, 1H, CH_{Ar}), 7.65 (d, ^3J = 7.50 Hz, 1H, CH_{Ar}), 7.29 (d, ^3J = 7.89 Hz, 1H, CH_{Ar}), 6.85 (s, 1H, $CH_{Het\text{-}Ar}$), 2.36 (s, 6H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.37 (C_{Ar}), 161.03 (C_{Ar}), 156.19 (C_{Ar}), 150.93 (q, ^2J = 35.53 Hz, *C*-CF_3), 143.52 (C_{Ar}), 138.46 (C_{Ar}), 130.79 (C_{Ar}), 128.63 (CH_{Ar}), 125.62 (CH_{Ar}), 125.35 (CH_{Ar}), 120.36 (q, ^1J = 275.10 Hz, CF_3), 107.56 (q, ^3J = 3.29 Hz, $CH_{Het\text{-}Ar}$), 20.16 (CH_3), 19.68 (CH_3); IR (ATR) υ 3034 (w), 2981 (w), 2849 (w), 1705 (s), 1512 (s), 1493 (m), 1413 (m), 1313 (w), 1273 (s), 1189 (m), 1135 (s), 1074 (m), 1001 (w), 892 (m), 844 (s), 827 (m), 791 (w), 705 (s), 690 (s), 624 (m), 536 (w) cm^{-1}; MS *m/z* 325 (M^+, 100), 166(62), 149(25), 148(10), 134(11), 133(25), 132(29), 116(11), 105(7), 91(3); HRMS calcd for $C_{14}H_{10}ON_3F_3S$ 325.04912; found 325.04891. Anal. Calcd for $C_{14}H_{10}ON_3F_3S$: C, 51.69; H, 3.10; N, 12.92; found: C, 52.01; H, 3.16; N, 12.87.

6.25. 2-(4-Methoxylphenyl)-7-trifluoromethyl-5H-1,3,4-thiadiazolo[3,2-a]pyrimidin-5-one (**5g**)

According to the general procedure, 4-methoxylphenylboronic acid afforded 86 mg of product **5g** (78%) as a yellow solid; mp (210–212 °C); ^{1}HNMR (300 MHz, $CDCl_3$) δ 7.91 (d, ^3J = 8.88 Hz, 2H, CH_{Ar}), 7.82 (d, ^3J = 8.88 Hz, 2H, CH_{Ar}), 6.83 (s, 1H, $CH_{Het\text{-}Ar}$), 3.90 (s, 3H, OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.92 (C_{Ar}), 163.30 (C_{Ar}), 160.34 (C_{Ar}), 156.15 (C_{Ar}), 150.84 (q, ^2J = 36.19 Hz, *C*-CF_3), 129.78 (C_{Ar}), 120.21 (CH_{Ar}), 120.36 (q, ^1J = 275.25 Hz, CF_3), 115.04 (CH_{Ar}), 107.56 (q, ^3J = 3.17 Hz, $CH_{Het\text{-}Ar}$), 55.75 (OCH_3); IR (ATR) υ 3079 (w), 3021 (w), 2957 (w), 2848 (w), 1710 (s), 1601 (s), 1514 (m), 1498 (m), 1437 (m), 1320 (m), 1308 (m), 1276 (m), 1151 (s), 1073 (m), 1019 (m), 963 (w), 858 (m), 838 (s), 782 (m), 695 (m), 624 (w), 604 (s), 529 (w) cm^{-1}; MS *m/z* 327 (M^+, 100), 166(38), 151(26), 136(13), 135(31), 134(22), 133(25), 108(16), 103(3), 93(4); HRMS calcd for $C_{13}H_8O_2N_3F_3S$ 327.02838; found 327.02848. Anal. Calcd for $C_{13}H_8O_2N_3F_3S$: C, 47.71; H, 2.46; N, 12.84; found: C, 47.24; H, 2.10; N, 13.28.

6.26. 2-(3-Methoxyphenyl)-7-trifluoromethyl-5H-1,3,4-thiadiazolo[3,2-a]pyrimidin-5-one (**5h**)

According to the general procedure, 3-methoxyphenylboronic acid afforded 72 mg of product **5h** (65%) as a yellow solid; mp (151–152 °C); ^{1}HNMR (300 MHz, $CDCl_3$) δ 7.44–7.53 (m, 3H, CH_{Ar}), 7.15–7.17 (m, 1H, CH_{Ar}), 6.86 (s, 1H, $CH_{Het\text{-}Ar}$), 3.90 (s, 3H, OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.56 (C_{Ar}), 160.95 (C_{Ar}), 160.66 (C_{Ar}), 156.35 (C_{Ar}), 151.26 (q, ^2J = 36.14 Hz, *C*-CF_3), 130.96 (C_{Ar}), 129.21 (CH_{Ar}), 120.88 (CH_{Ar}), 120.55 (q, ^1J = 275.09 Hz, CF_3), 120.23 (CH_{Ar}), 112.40 (CH_{Ar}), 107.91 (q, ^3J = 3.30 Hz, $CH_{Het\text{-}Ar}$), 56.06 (OCH_3); IR (ATR) υ 3055 (w), 2943 (w), 2917 (w), 1696 (s), 1608 (w), 1506 (s), 1486 (s), 1444 (w), 1417 (s), 1371 (w), 1298(w), 1276 (m), 1227 (m), 1170 (m), 1133 (s), 1076 (m), 1025 (m), 991 (w), 876 (m), 784 (s), 743 (w), 686 (s), 626 (w), 564 (w), 531 (w) cm^{-1}; MS *m/z* 327 (M^+, 100), 166(76), 151(26), 136(11), 135(25), 134(16), 133(27), 108(26), 103(9), 93(5); HRMS calcd for $C_{13}H_8O_2N_3F_3S$ 327.02838; found 327.02805. Anal. Calcd for $C_{13}H_8O_2N_3F_3S$: C, 47.71; H, 2.46; N, 12.84; found: C, 47.41; H, 2.59; N, 12.54.

6.27. 2-(2-Methoxyphenyl)-7- trifluoromethyl-5H-1,3,4-thiadiazolo[3,2-a]pyrimidin-5-one (**5i**)

According to the general procedure, 2-methoxyphenylboronic acid afforded 50 mg of product **5i** (45%) as a yellow solid; mp (247–248 °C); ^{1}HNMR (300 MHz, $CDCl_3$) δ 8.47 (dd, ^3J = 7.95 Hz, ^4J = 1.59 Hz 1H, CH_{Ar}), 7.83 (dd, ^3J = 7.36 Hz, ^4J = 1.69 Hz, 1H, CH_{Ar}), 7.56–7.61 (m, 1H, CH_{Ar}), 7.12–7.18 (m, 1H, CH_{Ar}), 6.82 (s, 1H, $CH_{Het\text{-}Ar}$), 4.06 (s, 3H, OCH_3); ^{13}C NMR (62 MHz, $CDCl_3$) δ 164.36 (C_{Ar}), 157.49 (C_{Ar}), 156.45 (C_{Ar}), 151.21 (q, ^2J = 36.16 Hz, *C*-CF_3), 137.00 (C_{Ar}), 134.88 (C_{Ar}), 133.05 (CH_{Ar}), 128.95 (CH_{Ar}), 121.87 (CH_{Ar}), 120.62 (q, ^1J = 275.46 Hz, CF_3), 111.84 (CH_{Ar}), 106.63 (q, ^3J = 3.02 Hz, $CH_{Het\text{-}Ar}$), 56.24 (OCH_3); IR (ATR) υ 3458 (w), 3351 (w), 3066 (w), 3025 (w), 2981 (w), 1717 (s), 1599 (m), 1520 (m), 1499 (s), 1465 (s), 1434 (m), 1418 (m), 1366 (w), 1312 (w), 1285 (s), 1276 (s), 1204 (m), 1160 (m), 1130 (m), 1078 (m), 1021 (m), 1002 (m), 697 (w), 851 (m), 754 (s), 701 (s), 609 (m), 526 (m) cm^{-1}; MS *m/z* 327 (M^+, 100), 326(16), 308(11), 209(20), 180(19), 166(38), 164(15), 151(10), 150(17), 136(5), 135(13), 134(10), 133(16), 132(28), 122(5), 121(13), 120(10), 119(99), 118(13), 108(26), 107(15), 104(11), 103(6), 93(8), 91(9); HRMS calcd for $C_{13}H_8O_2N_3F_3S$ 327.02838; found 327.02816. Anal. Calcd for $C_{13}H_8O_2N_3F_3S$: C, 47.71; H, 2.46; N, 12.84; found: C, 47.35; H, 2.69; N, 13.04.

6.28. 2-(3,5-Di-methoxyphenyl)-7-trifluoromethyl-5H-1,3,4-thiadiazolo[3,2-a]pyrimidin-5-one (**5j**)

According to the general procedure, 3,5-di-methoxyphenylboronic acid afforded 106 mg of product **5j** (88%) as a yellow solid; mp (205–206 °C); ^{1}HNMR (300 MHz, $CDCl_3$) δ 7.05 (d, ^4J = 2.16 Hz, 2H, CH_{Ar}), 6.85 (s, 1H, $CH_{Het\text{-}Ar}$),6.67 (pt, ^4J = 2.16 Hz, 1H, CH_{Ar}), 3.87 (s, 6H, OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.40 (C_{Ar}), 161.65 (C_{Ar}), 160.86 (C_{Ar}), 156.21 (C_{Ar}), 151.11 (q, ^2J = 36.30 Hz, *C*-CF_3), 129.49 (C_{Ar}), 120.41 (q, ^1J = 275.20 Hz, CF_3), 107.75 (q, ^3J = 3.30 Hz, $CH_{Het\text{-}Ar}$), 105.96 (CH_{Ar}), 105.62 (CH_{Ar}), 56.00 (OCH_3); IR (ATR) υ 3076 (w), 2963 (w), 2917 (w), 2844 (w), 1711 (s), 1591 (s), 1513 (s), 1456 (m), 1427 (m), 1411 (w), 1390 (m), 1350 (m), 1316 (w), 1279 (s), 1207 (s), 1140 (s), 1074 (w), 1061 (m), 1029 (m), 988 (w), 928 (w), 886 (w), 859 (m), 768 (m), 708 (m), 676 (m), 537 (w) cm^{-1}; MS *m/z* 357 (M^+, 100), 274(28), 181(17), 166(57), 165(34), 164(17), 163(63), 138(11),

123(10), 103(5), 91(2); HRMS calcd for $C_{14}H_{10}O_3N_3F_3S$ 357.03895; found 357.03863. Anal. Calcd for $C_{14}H_{10}O_3N_3F_3S$: C, 47.06; H, 2.82; N, 11.76; found: C, 47.35; H, 2.59; N, 11.54.

6.29. 2-(2,5-Di-methoxyphenyl)-7-trifluoromethyl-5H-1,3,4-thiadiazolo[3,2-a]pyrimidin-5-one (**5k**)

According to the general procedure, 2,5-di-methoxyphenylboronic acid afforded 42 mg of product **5k** (35%) as a yellow solid; mp (72–73 °C); ^{1}HNMR (300 MHz, $CDCl_3$) δ 8.16 (d, ^3J = 3.19 Hz, 1H, CH_{Ar}), 7.40 (dd, ^3J = 9.13 Hz, ^4J = 3.10 Hz, 1H, CH_{Ar}), 7.29 (s, 1H, CH_{Ar}), 7.08 (s, 1H, $CH_{Het\text{-}Ar}$), 4.27 (s, 3H, OCH_3), 4.12 (s, 3H, OCH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.36 (C_{Ar}), 156.37 (C_{Ar}), 154.24 (C_{Ar}), 151.13 (q, ^2J = 35.99 Hz, *C*-CF_3), 122.36 (C_{Ar}), 120.74 (C_{Ar}), 119.05 (q, ^1J = 275.64 Hz, CF_3), 118.57 (C_{Ar}), 116.98 (CH_{Ar}), 113.41 (CH_{Ar}), 111.21 (CH_{Ar}), 106.52 (q, ^3J = 3.07 Hz, $CH_{Het\text{-}Ar}$), 56.61 (OCH_3), 56.27 (OCH_3); IR (ATR) υ 3447 (w), 3353 (w), 3067 (w), 2983 (w), 2946 (w), 2844 (w), 1714 (s), 1612 (w), 1581 (w), 1504 (s), 1478 (m), 1417 (m), 1337 (w), 1278 (s), 1239 (w), 1222 (w), 1205 (m), 1180 (w), 1166 (w), 1155 (w), 1133 (w), 1079 (m), 1006 (w), 868 (w), 809 (m), 701 (m) cm^{-1}; MS *m*/*z* 357 (M^+, 100), 342(16), 338(8), 194(10), 180(15), 166(18), 163(21), 162(24), 150(11), 149(16), 148(36), 123(14), 120(11), 109(3), 108(3), 93(5); HRMS calcd for $C_{14}H_{10}O_3N_3F_3S$ 357.03895; found 357.03868. Anal. Calcd for $C_{14}H_{10}O_3N_3F_3S$: C, 47.06; H, 2.82; N, 11.76 found: C, 46.84; H, 3.10; N, 11.84.

6.30. 2-(4-Ethoxyphenyl)-7-trifluoromethyl-5H-1,3,4-thiadiazolo[3,2-a]pyrimidin-5-one (**5l**)

According to the general procedure, 4-ethoxyphenylboronic acid afforded 102 mg of product **5l** (90%) as a yellow solid; mp (198–199 °C); ^{1}HNMR (300 MHz, $CDCl_3$) δ 7.89 (d, ^3J = 8.87 Hz, 2H, CH_{Ar}), 7.00 (d, ^3J = 8.68 Hz, 2H, CH_{Ar}), 6.83 (s, 1H, $CH_{Het\text{-}Ar}$), 4.12 (q, ^3J = 6.99 Hz, 2H, OCH_2), 1.46 (t, ^3J = 7.06 Hz, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.44 (C_{Ar}), 163.39 (C_{Ar}), 160.47 (C_{Ar}), 156.23 (C_{Ar}), 150.90 (q, ^2J = 36.05 Hz, *C*-CF_3), 129.85 (C_{Ar}), 120.45 (q, ^1J = 275.10 Hz, CF_3), 120.05 (C_{Ar}), 116.15 (C_{Ar}), 115.69 (CH_{Ar}), 115.52 (CH_{Ar}), 107.62 (q, ^3J = 3.18 Hz, $CH_{Het\text{-}Ar}$), 64.23 (OCH_2), 14.72 (CH_3); IR (ATR) υ 2978 (w), 2920 (w), 2849 (w), 1714 (s), 1602 (s), 1574 (w), 1512 (m), 1493 (s), 1472 (m), 1415 (m), 1394 (w), 1315 (w), 1304 (w), 1276 (m), 1260 (s), 1203 (w), 1175 (m), 1143 (s), 1119 (w), 1075 (m), 1027 (m), 921 (m), 842 (m), 812 (w), 699 (s), 605 (s) cm^{-1}; MS *m*/*z* 341 (M^+, 100), 313(24), 194(5), 180(15), 166(63), 148(11), 137(35), 121(22), 120(21), 119(25), 108(10), 93(3); HRMS (ESI, M + H) calcd for $C_{14}H_{10}O_2N_3F_3S$ 342.05186 found 342.05235, (ESI, M + Na) calcd for $C_{14}H_{10}O_2N_3F_3S$ 364.0338; found 364.03432. Anal. Calcd for $C_{14}H_{10}O_2N_3F_3S$: C, 49.27; H, 2.95; N, 12.31 found: C, 49.81; H, 3.02; N, 11.88.

6.31. 2-(2-Ethoxyphenyl)-7-trifluoromethyl-5H-1,3,4-thiadiazolo[3,2-a]pyrimidin-5-one (**5m**)

According to the general procedure, 4-ethoxyphenylboronic acid afforded 46 mg of product **5m** (40%) as a yellow solid; mp (87–88 °C); ^{1}HNMR (300 MHz, $CDCl_3$) δ 7.84 (dd, ^3J = 7.29 Hz, ^4J = 1.65 Hz, 1H, CH_{Ar}), 7.39–7.45 (m, 1H, CH_{Ar}), 7.02 (pt, ^3J = 7.34 Hz, 1H, CH_{Ar}), 6.89 (d, ^3J = 8.34 Hz, 1H, CH_{Ar}), 6.09 (s, 1H, $CH_{Het\text{-}Ar}$), 4.15 (q, ^3J = 6.99 Hz, 2H, OCH_2), 1.49 (t, ^3J = 6.99 Hz, 3H, CH_3); ^{13}C NMR (62 MHz, $CDCl_3$) δ 163.98 (C_{Ar}), 160.98 (C_{Ar}), 160.26 (C_{Ar}), 156.32 (C_{Ar}), 150.97 (q, ^2J = 36.61 Hz, *C*-CF_3), 136.81 (CH_{Ar}), 134.75 (C_{Ar}), 132.85 (CH_{Ar}), 121.16 (CH_{Ar}), 119.37 (q, ^1J = 274.66 Hz, CF_3), 110.90 (CH_{Ar}), 106.48 (q, ^3J = 3.66 Hz, $CH_{Het\text{-}Ar}$), 63.99 (OCH_2), 14.90 (CH_3); IR (ATR) υ 3443 (w), 3354 (s), 3068 (w), 2987 (w), 2918 (w), 2849 (w), 1708 (m), 1598 (s), 1573 (m), 1504 (w), 1473 (m), 1448 (s), 1394 (s), 1343 (s), 1292 (m), 1279 (m), 1229 (s), 1164 (m), 1155 (m), 1107 (s), 1035 (s), 976 (w), 925 (m), 776 (m), 756 (s), 674 (s), 609 (m), 542 (w), cm^{-1}; MS *m*/*z* 341 (M^+, 79), 327(11), 326(71), 322(13), 313(100), 267(16), 244(18), 180(34), 166(99), 148(14), 147(11) 146(56), 145(11), 137(32), 131(15), 121(37), 120(29), 109(17), 108(39), 103(6), 91(20); HRMS (ESI, M + H) calcd for $C_{14}H_{10}O_2N_3F_3S$ 342.05186 found 342.05207, (ESI, M + Na) calcd for $C_{14}H_{10}O_2N_3F_3S$ 364.0338; found 364.03397. Anal. Calcd for $C_{14}H_{10}O_2N_3F_3S$: C, 49.27; H, 2.95; N, 12.31 found: C, 49.61; H, 2.71; N, 12.71.

6.32. 2-(2-Fluorophenyl)-7-trifluoromethyl-5H-1,3,4-thiadiazolo[3,2-a]pyrimidin-5-one (**5n**)

According to the general procedure, 2-fluorophenylboronic acid afforded 54 mg of product **5n** (51%) as a yellow solid; mp (148–149 °C); ^{1}HNMR (300 MHz, $CDCl_3$) δ 8.38–8.43 (m, 1H, CH_{Ar}), 7.60–7.68 (m, 1H, CH_{Ar}), 7.35–7.41 (m, 1H, CH_{Ar}), 7.27–7.33 (m, 1H, CH_{Ar}), 6.86 (s, 1H, $CH_{Het\text{-}Ar}$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.77 (C_{Ar}), 160.80 (d, ^1J = 254.74 Hz, C-F), 156.06 (C_{Ar}), 154.55 (d, ^3J = 4.89 Hz, CH_{Ar}), 151.25 (q, ^2J = 35.96 Hz, *C*-CF_3), 135.28 (d, ^3J = 9.35 Hz, CH_{Ar}), 129.10 (d, ^5J = 0.95 Hz, C_{Ar}), 125.49 (d, ^4J = 3.29 Hz, CH_{Ar}), 124.15 (d, ^2J = 24.76 Hz, C_{Ar}), 120.34 (q, ^1J = 275.21 Hz, CF_3), 116.67 (d, ^2J = 21.45 Hz, CH_{Ar}), 107.32 (q, ^3J = 3.10 Hz, $CH_{Het\text{-}Ar}$); IR (ATR) υ 3067 (w), 2917 (w), 2848 (w), 1707 (s), 1613 (m), 1581 (m), 1507 (s), 1464 (m), 1413 (m), 1314 (w), 1258 (s), 1212 (m), 1180 (m), 1154 (s), 1102 (m), 1074 (m), 1017 (m), 968 (w), 889 (m), 866 (s), 827 (m), 793 (s), 699 (m), 608 (m) cm^{-1}; MS *m*/*z* 315 (M^+, 100), 296(8), 194(4), 180(12), 166(83), 139(56), 123(11), 121(14), 95(15); HRMS calcd for $C_{12}H_5ON_3F_4S$ 315.00840; found 315.00807. Anal. Calcd for $C_{12}H_5ON_3F_4S$: C, 45.72; H, 1.60; N, 13.33 found: C, 45.93; H, 2.01; N, 13.71.

6.33. 2-(3-Fluorophenyl)-7-trifluoromethyl-5H-1,3,4-thiadiazolo[3,2-a]pyrimidin-5-one (**5o**)

According to the general procedure, 3-fluorophenylboronic acid afforded 54 mg of product **5o** (65%) as a yellow solid; mp (164–166 °C); ^{1}HNMR (300 MHz, $CDCl_3$) δ 7.70–7.76 (m, 2H, CH_{Ar}), 7.51–7.58 (m, 1H, CH_{Ar}), 7.31–7.37 (m, 1H, CH_{Ar}), 6.86 (s, 1H, $CH_{Het\text{-}Ar}$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.23 (C_{Ar}), 163.13 (d, ^1J = 250.43 Hz, C-F), 159.44 (d, ^4J = 3.22 Hz, C_{Ar}), 156.00 (C_{Ar}), 151.17 (q, ^2J = 36.31 Hz, *C*-CF_3), 131.60 (d, ^3J = 8.24 Hz, C_{Ar}), 129.78 (d, ^3J = 8.25 Hz, CH_{Ar}), 123.95 (d, ^4J = 3.23 Hz, CH_{Ar}), 120.78 (d, ^2J = 21.45 Hz, CH_{Ar}), 120.34 (q, ^1J = 275.05 Hz, CF_3), 114.84 (d, ^2J = 24.75 Hz, CH_{Ar}), 107.96 (q, ^3J = 3.13 Hz, $CH_{Het\text{-}Ar}$); IR (ATR) υ 3075 (w), 2965 (w), 2918 (w), 1706 (s), 1610 (w) 1506 (s), 1486 (s), 1414 (m), 1371 (w), 1316 (w), 1279 (s), 1216 (m), 1176 (m), 1144 (s), 1077 (m), 1034 (m), 983 (w), 910 (w), 887 (m), 746 (s), 685 (m), 545 (w) cm^{-1}; MS *m*/*z* 315 (M^+, 100), 296(8), 194(4), 180(14), 166(93), 139(68), 123(13), 121(18), 95(27); HRMS calcd for $C_{12}H_5ON_3F_4S$ 315.00840; found 315.00748. Anal. Calcd for $C_{12}H_5ON_3F_4S$: C, 45.72; H, 1.60; N, 13.33 found: C, 45.23; H, 1.88; N, 13.21.

6.34. 2-(3-Fluoromethylphenyl)-7-trifluoromethyl-5H-1,3,4-thiadiazolo[3,2-a]pyrimidin-5-one (**5p**)

According to the general procedure, 3-fluoromethylphenylboronic acid afforded 68 mg of product **5p** (55%) as an orange solid; mp (152–153 °C); ^{1}HNMR (300 MHz, $CDCl_3$) δ 8.25 (s, 1H, CH_{Ar}), 8.15 (d, ^3J = 8.27 Hz, 1H, CH_{Ar}), 7.91 (d, ^3J = 7.89 Hz, 1H, CH_{Ar}), 7.73 (pt, ^3J = 7.87 Hz, 1H, CH_{Ar}), 6.89 (s, 1H, $CH_{Het\text{-}Ar}$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.11 (C_{Ar}),159.25 (C_{Ar}), 156.01 (C_{Ar}), 151.26 (q, ^2J = 36.08 Hz, *C*-CF_3), 132.62 (q, ^2J = 33.56 Hz, *C*-CF_3), 131.18 (C_{Ar}), 130.51 (CH_{Ar}), 130.12 (q, ^3J = 3.69 Hz, CH_{Ar}), 128.81 (CH_{Ar}), 124.75 (q, ^3J = 3.66 Hz, CH_{Ar}), 123.29 (q, ^1J = 272.49 Hz, CF_3), 120.32 (q, ^1J = 275.64 Hz, CF_3), 108.11 (q,

^{3}J = 3.09 Hz, $CH_{Het\text{-}Ar}$); IR (ATR) υ 3044 (w), 2961(w), 2918 (w), 1709 (s), 1617 (w), 1588 (w), 1620 (s), 1509 (s), 1488 (m), 1418 (m), 1341 (s), 1277 (s), 1194 (m), 1143 (m), 1122 (m), 1074 (m), 1030 (s), 974 (w), 912 (w), 873 (m), 810 (s), 744 (m), 694 (s), 585 (m) cm^{-1}; MS *m/z* 365 (M^{+}, 100), 346(19), 189(47), 180(13), 173(12), 166(90), 145(16), 139(15), 125(7), 120(8), 93(5); HRMS calcd for $C_{13}H_5ON_3F_6S$ 365.00520; found 365.00401. Anal. Calcd for $C_{13}H_5ON_3F_6S$: C, 42.75; H, 1.38; N, 11.50 found: C, 42.85; H, 1.80; N, 11.43.

6.35. 2-(4-Trifluoromethoxylphenyl)-7-trifluoromethyl-5H-1,3,4-thiadiazolo[3,2-a]pyrimidin-5-one (**5q**)

According to the general, using 4-trifluoromethoxylphenylboronic acid afforded 77 mg of product **5q** (60%) as a yellow solid; mp (167–168 °C); ^{1}HNMR (300 MHz, $CDCl_3$) δ 8.02–8.07 (m, 2H, CH_{Ar}), 7.40 (dd, ^{3}J = 8.94 Hz, ^{4}J = 0.95 Hz, 2H, CH_{Ar}), 6.88 (s, 1H, $CH_{Het\text{-}Ar}$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.26 (C_{Ar}), 159.22 (C_{Ar}), 156.06 (C_{Ar}), 153.00 (C_{Ar}), 151.19 (q, ^{2}J = 36.82 Hz, *C*-CF_3), 129.87 (C_{Ar}), 126.26 (CH_{Ar}), 124.01 (q, ^{1}J = 275.50 Hz, CF_3), 121.67 (CH_{Ar}), 120.37 (q, ^{1}J = 259.51 Hz, OCF_3), 108.03 (q, ^{3}J = 3.06 Hz, $CH_{Het\text{-}Ar}$); IR (ATR) υ 3104 (w), 3067 (w), 2962 (w), 1716 (s), 1606 (w), 1589 (w), 1518 (s), 1496 (s), 1413 (m), 1303 (w), 1258 (m), 1246 (m), 1173 (s), 1138 (s), 1072 (m), 1027 (m), 966 (w), 894 (m), 853 (s), 844 (s), 745 (w), 703 (s), 603 (m) cm^{-1}; MS *m/z* 381 (M^{+}, 100), 205(34), 189(18), 180(14), 166(87), 148(5), 139(14), 108(17), 93(4); HRMS calcd for $C_{13}H_5O_2N_3F_6S$ 381.00012; found 380.99940. Anal. Calcd for $C_{13}H_5O_2N_3F_6S$: C, 40.95; H, 1.32; N, 11.02 found: C, 40.77; H, 1.56; N, 10.76.

6.36. 2-(3-Cyanophenyl)-7-trifluoromethyl-5H-1,3,4-thiadiazolo[3,2-a]pyrimidin-5-one (**5r**)

According to the general procedure, 3-cyanophenylboronic acid afforded 72 mg of product **5r** (55%) as a yellow solid; mp (228–229 °C); ^{1}HNMR (300 MHz, $CDCl_3$) δ 8.28 (pt, ^{4}J = 1.41 Hz,1H, CH_{Ar}), 8.02–8.23 (m, 1H, CH_{Ar}), 7.91–7.94 (m, 1H, CH_{Ar}), 7.73 (pt, ^{3}J = 7.75 Hz,1H, CH_{Ar}), 6.90 (s, 1H, $CH_{Het\text{-}Ar}$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 162.97 (C_{Ar}), 158.38 (C_{Ar}), 155.88 (C_{Ar}), 151.30 (q, ^{2}J = 36.73 Hz, *C*-CF_3), 136.46 (CN), 131.76 (C_{Ar}), 131.21 (C_{Ar}), 130.81 (CH_{Ar}), 129.30 (CH_{Ar}), 120.29 (q, ^{1}J = 275.10 Hz, CF_3), 117.05 (CH_{Ar}), 114.62 (CH_{Ar}), 108.23 (q, ^{3}J = 2.78 Hz, $CH_{Het\text{-}Ar}$); IR (ATR) υ 3073 (m), 3035 (w), 2959 (w), 2918 (m), 2849 (m), 2234 (m), 2230 (m), 1702 (s), 1577 (w), 1523 (s), 1508 (m), 1415 (m), 1281 (s), 1194 (s), 1143 (s), 1079 (m), 1038 (m), 995 (w), 894 (w), 870 (s), 809 (s), 746 (m), 683 (s), 560 (s) cm^{-1}; MS *m/z* 322 (M^{+}, 100), 294(9), 180(13), 166(99), 146(48), 139(13), 128(15), 102(22), 93(5); HRMS calcd for $C_{13}H_5ON_4F_3S$ 322.01307; found 322.01214. Anal. Calcd for $C_{13}H_5ON_4F_3S$: C, 48.45; H, 1.56; N, 17.69 found: C, 48.62; H, 2.16; N, 17.94.

6.37. 2-(3-Nitrophenyl)-7-trifluoromethyl-5H-1,3,4-thiadiazolo[3,2-a]pyrimidin-5-one (**5s**)

According to the general procedure, 3-nitrophenylboronic acid afforded 75 mg of product **5s** (65%) as a yellow solid; mp (247–249 °C); ^{1}HNMR (300 MHz, $CDCl_3$) δ 8.75 (pt, ^{4}J = 1.84 Hz, 1H, CH_{Ar}), 8.48–8.52 (m, 1H, CH_{Ar}), 8.35–8.36 (m, 1H, CH_{Ar}), 7.81 (pt, ^{3}J = 8.07 Hz,1H, CH_{Ar}), 6.91 (s, 1H, $CH_{Het\text{-}Ar}$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.00 (C_{Ar}), 158.29 (C_{Ar}), 155.87 (C_{Ar}), 151.31 (q, ^{2}J = 36.31 Hz, *C*-CF_3), 148.96 (C_{Ar}), 133.23 (C_{Ar}), 131.10 (CH_{Ar}), 129.56 (CH_{Ar}), 127.83 (CH_{Ar}), 122.87 (CH_{Ar}), 120.25 (q, ^{1}J = 275.10 Hz, CF_3), 108.23 (q, ^{3}J = 3.28 Hz, $CH_{Het\text{-}Ar}$); IR (ATR) υ 3087 (w), 3033 (w), 2850 (w), 1693 (s), 1615 (w), 1580 (w), 1508 (s), 1434 (w), 1345 (s), 1275 (s), 1259 (m), 1189 (m), 1150 (s), 1076 (m), 1040 (w), 997 (w), 910 (m), 874 (s), 811 (m), 710 (m), 563 (w) cm^{-1}; MS *m/z* 342 (M^{+}, 98), 296(3), 180(15), 166(100), 148(7), 134(11), 120(45), 102(6), 94(7); HRMS calcd for $C_{12}H_5O_3N_4F_3S$ 342.00290; found 342.98217. Anal. Calcd for $C_{12}H_5O_3N_4F_3S$: C, 42.11; H, 1.47; N, 16.37 found: C, 42.66; H, 1.44; N, 16.94.

6.38. 2-(3-Phenylphenyl)-7-trifluoromethyl-5H-1,3,4-thiadiazolo[3,2-a]pyrimidin-5-one (**5t**)

According to the general procedure, 3-phenylphenylboronic acid afforded 88 mg of product **5t** (70%) as an orange solid; mp (202–203 °C); ^{1}HNMR (300 MHz, $CDCl_3$) δ 8.15 (pt, ^{4}J = 1.69 Hz,1H, CH_{Ar}), 7.82–7.92 (m, 2H, CH_{Ar}), 7.59–7.64 (m, 3H, CH_{Ar}), 7.41–7.52 (m, 3H, CH_{Ar}), 6.87 (s, 1H, $CH_{Het\text{-}Ar}$); ^{13}C NMR (62 MHz, $CDCl_3$) δ 163.39 (C_{Ar}), 160.82 (C_{Ar}), 156.17 (C_{Ar}), 151.09 (q, ^{2}J = 36.13 Hz, *C*-CF_3), 143.15 (C_{Ar}), 139.22 (C_{Ar}), 132.37 (C_{Ar}), 130.18 (CH_{Ar}), 129.20 (CH_{Ar}), 128.51 (CH_{Ar}), 128.42 (CH_{Ar}), 127.37 (CH_{Ar}), 126.78 (CH_{Ar}), 126.42 (CH_{Ar}), 120.41 (q, ^{1}J = 275.11 Hz, CF_3), 107.82 (q, ^{3}J = 3.11 Hz, $CH_{Het\text{-}Ar}$); IR (ATR) υ 3070 (w), 3035 (w), 2917 (w), 2848 (w), 1700 (s), 1599 (w), 1584 (w), 1502 (s), 1470 (m), 1416 (s), 1367 (w), 1312 (w), 1277 (s), 1238 (w), 1184 (m), 1160 (m), 1146 (s), 1075 (m), 1017 (w), 918 (w), 873 (m), 850 (w), 754 (s), 698 (s), 624 (m), 559 (w) cm^{-1}; MS *m/z* 373 (M^{+}, 100), 345(3), 197(22), 181(18), 180(20), 179(35), 166(46), 152(24), 151(10), 139(5), 93(3); HRMS calcd for $C_{18}H_{10}ON_3F_3S$ 373.04912; found 373.04855. Anal. Calcd for $C_{18}H_{10}ON_3F_3S$: C, 57.91; H, 2.70; N, 11.25 found: C, 57.77; H, 2.85; N, 11.56.

7. Biochemical assays

7.1. Cell transfection and membrane fractions of APs

The transfection of human alkaline phosphatase expressing plasmids was carried out into the COS-7 cells according to the previously reported method of Kukulski et al., 2005, [31]. The whole procedure was performed in lipofectamine reagent as discussed earlier [31]. After getting confluent cells *i.e.*, upto 90%, the cells were transferred to Dulbecco's modified Eagle's medium (DMEM) (without fetal bovine serum (FBS)), along with 24 μL of Lipofectamine and 6 μg of plasmid DNA. The cells were kept at 37 °C for 5 h (in the presence of 5% CO_2). The transfection was stopped after 24 h of incubation by adding the same medium containing 20% FBS [31]. The transfected cells were further processed to get the membrane fractions as reported by Bravo et al., 2014 [32].The total protein estimation was done by Bradford microplate assay [33] and the aliquots were prepared with the addition of 7.5% glycerol and kept at −80 °C.

7.2. Alkaline phosphatase inhibition assay (h-TNAP & h-IAP)

The alkaline phosphatase (*h*-TNAP and *h*-IAP) inhibition studies of compounds were determined by using the same protocol [34] as that of our previously reported data [35]. Initially, 10 μL of the tested compound was pre-incubated for 5–7 min at 37 °C with 20 μL of the enzymes, either *h*-TNAP (47 ng protein/well) or of *h*-IAP (56 ng protein/well). The luminescence signals were observed as pre-read values using microplate reader (BioTek FLx800, Instruments, Inc. USA). Then 20 μL of CDP-star® substrate was added to each reaction mixture and again signals were observed after 15–20 min of incubation. The results were interpreted by PRISM 5.0 (GraphPad, San Diego, California, USA) software and the inhibitory concentration values were also obtained as discussed by our group [35].

8. Molecular docking methodology

Our previously reported models of *h*-TNAP and *h*-IAP were used for the docking studies [35]. The chemical structures of potent compounds were sketched in ACD/ChemSketch tool and energies minimization of both target enzymes as well as ligands were optimized in Molecular Operating Environment (MOE 2014.009) [36]. After preparation of targets and ligands, the docking calculations were carried out by using LeadIT (BioSolveIT GmbH, Germany) [37]. Prior to docking calculation the active site within the receptor was defined as discussed by our group previously [35]. Molecular docking of the selected compounds was then carried out by using default docking parameters. For each ligand 50 top ranking poses were selected and further analyzed by HYDE assessment. Pose having lowest binding free energy were selected and further analyzed by Discovery Studio Visualizer [38].

Acknowledgment

We are grateful to the DAAD (Programms Deutsch- Tadschikistan and Deutsch-Pakistanische Hochschulzusammenarbeit) for financial support. J. Iqbal is thankful to the Organization for the Prohibition of Chemical Weapons (OPCW), The Hague, The Netherlands and Higher Education Commission of Pakistan for the financial support through Project No. 20-3733/NRPU/R&D/14/520. J.S. was supported by grants from the Canadian Institutes of Health Research. J.S. is also a recipient of a “Chercheur National” Scholarship award from the “Fonds de recherche du Québec-Santé” (FRQS).

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ejmech.2017.11.068.

References

[1] U. Sharma, D. Pal, R. Prasad, Indian J. Clin. Biochem. 29 (2014) 269.
[2] H. Zimmermann, Arch. Pharmacol. 362 (2000) 299.
[3] M. Bortolato, F. Besson, B. Roux, Proteins Struct. Funct. Bioinformat. 37 (1999) 310.
[4] M. Đokić-Lišanin, V. Pantović, Z. Jovanović, G. Samardžić, V. Jurišić, Arch. Oncol. 21 (2013) 14.
[5] A. Kozlenkov, M.H. Le Du, P. Cuniasse, T. Ny, M.F. Hoylaerts, J.L. Millán, J. Bone Miner. Res. 19 (2004) 1862.
[6] R. Sapir-Koren, G. Livshits, IBMS BoneKEy 8 (2011) 286.
[7] H. Takinami, M. Goseki-Sone, H. Watanabe, H. Orimo, R. Hamatani, M. Fukushi-Irie, I. Ishikawa, J. Med. Dent. Sci. 51 (2014) 67.
[8] C.R. Sheen, P. Kuss, S. Narisawa, M.C. Yadav, J. Nigro, W. Wang, T.N. Chhea, E.A. Sergienko, K. Kapoor, M.R. Jackson, J. Bone Miner. Res. 30 (2015) 824.
[9] H. Bentala, W.R. Verweij, A. Huizinga-Van der Vlag, A.M. van Loenen-Weemaes, D.K. Meijer, K. Poelstra, Shock 18 (2002) 561.
[10] J.P. Lallès, Nutr. Rev. 68 (2010) 323.
[11] B. Nageshwari, M. Ramchander, Int. J. Res. Pharm. Sci. 3 (2013) 371.
[12] K.A. Lomashvili, P. Garg, S. Narisawa, J.L. Millan, W.C. O'Neill, Kid. Int. 73 (2008) 1024.
[13] a) B. Jafari, N. Yelibayeva, M. Ospanov, S.A. Ejaz, A. Afzal, S.U. Khan, Z.A. Abilov, M.Z. Turmukhanova, S.N. Kalugin, S. Safarov, J. Lecka, J. Sévigny, Q. Rahman, P. Ehlers, J. Iqbal, P. Langer, RSC Adv. 6 (2016), 107556 and references cited therein;
b) K.R. Sathisha, S.A. Khanum, J.N. Narendra Sharath Chandra, F. Ayisha, S. Balaji, G.K. Marathe, S. Gopal, K.S. Rangappa, Bioorg. Med. Chem. 19 (2011) 211.
[14] B.S. Coller, T. Craig, M. Filizola, J. McCoy, W. Huang, M. Shen, Patent: US2015/374697 A1, 2015.
[15] J.K. Jiang, J.G. McCoy, M. Shen, C.A. LeClair, W. Huang, A. Negri, J. Li, R. Blue, A.W. Harrington, S. Naini, G. David III, W.S. Choi, E. Volpi, J. Fernandez, M. Babayeva, M.A. Nedelman, M. Filizola, B.S. Coller, C.J. Thomas, Bioorg. Med. Chem. Lett. 24 (2014) 1148.
[16] K.C. Ravindra, H.M. Vagdevi, V.P. Vaidya, Arkivoc Xi (2008) 1.
[17] D.B. Harper, D. O'Hagan, C.D. Murphy, HDBK. Environ. Chem. 3 (2003) 141.
[18] a) J.P. Bégué, D. Bonnet-Delpon, J. Fluorine Chem. 127 (2006) 992;
b) K. Müller, C. Faeh, F. Diederich, Science 31 (2007) 1881.
[19] J.P. Bégué, D. Bonnet-Delpon, J. Fluorine Chem. 127 (2006) 992.
[20] C. Isanbor, D. O'Hagan, J. Fluorine Chem. 127 (2006) 303.
[21] K.L. Kirk, J. Fluorine Chem. 127 (2006) 1013.
[22] D.T. Wong, F.P. Bymaster, E.A. Engleman, Life Sci. 57 (1995) 411.
[23] B.D. Roth, Prog. Med. Chem. 40 (2002) 1.
[24] F. Russo, A. Sanagati, M. Sanagati, J. Het. Chem. 22 (1985) 297.
[25] a) T. Okabe, K. Maekawa, E. Taniguchi, Agr. Biol. Chem. 35 (1973) 1197;
b) S. Isoda, S. Aibara, T. Miwa, H. Fujiwara, S. Yokohama, H. Matsumoto, Patent: US1990/4898943, 1990;
c) E.G.E. Devries, J.A. Gietma, P. Workman, J.E. Scott, A. Crawshaw, Dobbis, H.J.I. Dennis, N. Mulder, D. Th. Sleijfer, P.H.B. Willemse, Br. J. Cancer 68 (1993) 661;
d) S. Sh. Shukurov, M.A. Kukaniev, M.A. Nasyrov, K.S. Zakharov, R.A. Karakhanov, Russ. Chem. Bull. 42 (1993) 1874;
e) S. Sh. Shukurov, M.A. Kukaniev, Khim Geterotsikl 8 (1992) 1148.
[26] a) Z.G. Sangov, Z.J. Ashurova, Z.D. Khalikova, M.A. Kukaniev, T.M. Salimov, J.N. Dzhamshedov, Pharm. Chem. J. 45 (2012) 721;
b) M.A. Kukaniev, C. Párkányi, J. Heterocycl. Chem. 48 (2011) 1308;
c) M.A. Kukaniev, M.M. Akbarova, Z.G. Sangov, D.M. Osimov, S.S. Safarov, Chem. Het. Comp. 46 (2010) 605;
d) M.A. Kukaniev, M.D. Osimov, Z.G. Sangov, S.S. Safarov, M.B. Karimov, T.R. Radjabov, Chem. Het. Comp. 44 (2008) 882;
e) T.M. Salimov, M.A. Kukaniev, I.T. Sattorov, D.M. Osimov, Pharm. Chem. J. 39 (2005) 311.
[27] S.E. Dine, Act. Chim. Aca. Sci. Hung. 84 (1975) 85.
[28] a) T. Tadakazu, T. Keiko, Bull. Chem. Soc. Jpn. 55 (1982) 637;
b) S. Sh. Shukurov, M.A. Kukaniev, B.M. Bobogaribov, S.S. Sabirov, Russ. Chem. Bull. 44 (1995) 1955.
[29] a) C. Copin, N. Henry, F. Buron, S. Routier, Eur. J. Org. Chem. (2012) 3079;
b) M. Suiko, K. Maekawa, Agri. Biol. Chem. 41 (1977) 2047.
[30] a) S. Sh. Shukurov, M.A. Kukaniev, Khim Geterotsikl 8 (1992) 1148;
b) S. Sh. Shukurov, M.A. Kukaniev, M.A. Nasyrov, K.S. Zakharov, R.A. Karakhanov, Russ. Chem. Bull. 42 (1993) 1874;
c) S. Sh. Shukurov, M.A. Kukaniev, M.A. Nasyrov, K.S. Zakharov, R.A. Karakhanov, Russ. Chem. Bull. 43 (1994) 854;
d) S. Sh. Shukurov, M.A. Kukaniev, B.M. Bobogafirov, S.S. Sabirov, Chem. Het. Comp. 30 (1994) 127;
e) M.A. Kukaniev, S. Sh. Shukurov, M.A. Nasyrov, Chem. Het. Comp. 32 (1996) 479;
f) M.A. Kukaniev, U. Nurov, S. Sh. Shukurov, Yu. Khodzhibaev, Russ. Chem. Bull. 48 (1999) 1143;
g) T.M. Salimov, M.A. Kukaniev, I.T. Sattorov, D.M. Osimov, Pharm. Chem. J. 39 (2005) 311;
h) M.A. Kukaniev, T.M. Salimov, M.S. Murvatulloeva, I.K. Imatshoev, Pharm. Chem. J. 40 (2006) 421;
i) M.A. Kukaniev, M.D. Osimov, Z.G. Sangov, S.S. Safarov, M.B. Karimov, T.R. Radjabov, Chem. Het. Comp. 44 (2008) 882.
[31] F. Kukulski, S.A. Lévesque, E.G. Lavoie, J. Lecka, F. Bigonnesse, A.F. Knowles, S.C. Robson, T.L. Kirley, J. Sévigny, Purinergic Signal. 1 (2005) 193.
[32] Y. Bravo, P. Teriete, R.P. Dhanya, R. Dahl, P. San Lee, T. Kiffer-Moreira, S.R. Ganji, E. Sergienko, L.H. Smith, C. Farquharson, Bioorg. Med. Chem. Lett. 24 (2014) 4308.
[33] M.M. Bradford, Anal. Biochem. 72 (1976) 248.
[34] E.A. Sergienko, J.L. Millán, Nat. Protoc. 5 (2010) 1431.
[35] I. Khan, A. Ibrar, S.A. Ejaz, S.U. Khan, S.J. Shah, S. Hameed, J. Simpson, J. Lecka, J. Sévigny, J. Iqbal, RSC Adv. 5 (2015) 90806.
[36] Chemical Computing Group (CCG), Montreal, Canada, http://www.chemcomp.com/MOEMolecular_Operating_Environment.htm.
[37] Lead IT, http://www.biosolveit.de/LeadIT/.
[38] A. Inc, Discovery Studio Modeling Environment, Release 4.0, Accelrys Discovery Studio. Accelrys Software Inc, San Diego, 2013.