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Efficient One-pot Synthesis of 5-Perfluoroalkylpyrazoles by Cyclization of Hydrazone Dianions

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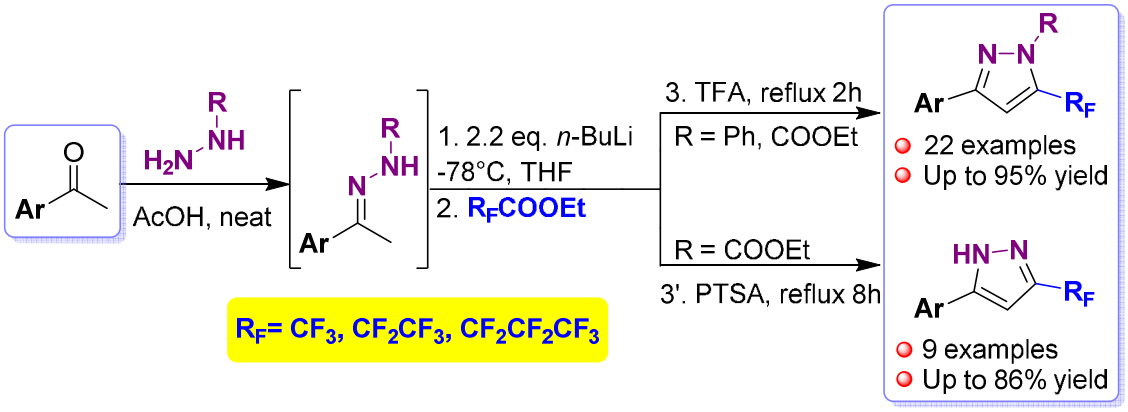

Abstract: A highly selective and efficient method for the synthesis of 5-trifluoromethylated and 5-perfluoroalkylated pyrazoles has been developed which relies on the cyclization of hydrazine dianions with ethyl perfluorocarboxylates. The pyrazoles prepared were evaluated as potential inhibitors of alkaline phosphatases, namely human tissue non-specific alkaline phosphatase (h-TNAP) and tissue specific intestinal alkaline phosphatase (IAP). Most pyrazole derivatives inhibited h-IAP more markedly than h-TNAP and had minor effects on nucleotide

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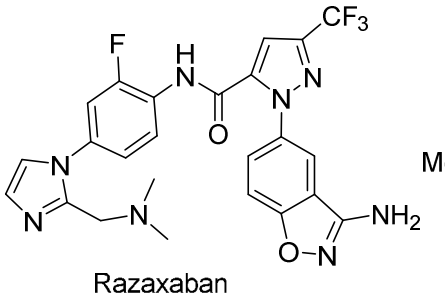
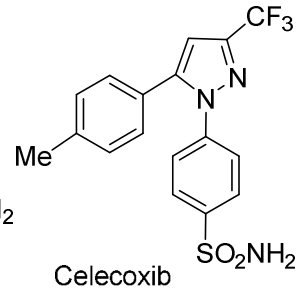
pyrophosphatase/phosphodiesterases. Therefore, the compounds appear as potential selective inhibitors of h-IAP.

Introduction

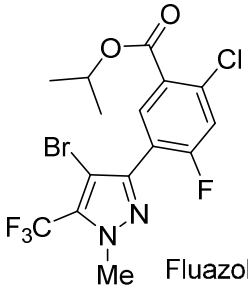
Pyrazole derivatives represent important building blocks in pharmaceutical and agrochemical industry.[1] The pyrazole ring is present in many leading drugs, such as Zometapine[2] and Viagra[3] and in agrochemicals, such as Tolfenpyrad[4] and Fenpyroximate.[5] Recently, the development of efficient methods for the introduction of the trifluoromethyl group into heterocycles allowed the preparation of new fluorinated heterocycles which exhibit significant changes in their chemical, physical and biological properties.[6,7] In fact, most of the recently developed drugs and agrochemicals contain a fluorine atom or a fluorinated side chain.[6,7] Therefore, new methods for the synthesis of organofluorine molecules play an important role for the development of bioactive molecules. Trifluoromethylated and perfluoroalkylated pyrazoles represent pharmacologically relevant core structures[8] which are present in many important drugs and agrochemicals, such as Celecoxib (antiarthritic), Mavacoxib (antiarthritic), Razaxaban (anticoagulant), Fluazolate (herbicide), Penthiopyrad (fungicide).[9,10]



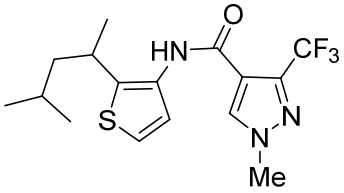
Razaxaban



Celecoxib



Fluazolate



Penthiopyrad

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Figure 1. Drugs and agrochemicals containing trifluoromethylated pyrazoles.

The development of new methods for the synthesis of pyrazole derivatives has become extremely important in recent years.[1] Conventional methods for the synthesis of pyrazoles are based on the cyclocondensation of hydrazines with 1,3-dielectrophiles, such as 1,3-dicarbonyl or *α,β*-unsaturated carbonyl compounds.[1,11] A convenient method is based on the 1,3-dipolar cycloaddition of nitrile imines with alkynes.[12] The disadvantage of these methods is the formation of a mixture of regioisomers of 3- and 5-substituted pyrazoles which need to be separated.[1,11] Another interesting approach is the cyclization of hydrazone dianions with esters.[13] Hauser and co-workers were the first to report the synthesis of 5-substituted pyrazoles by cyclization of hydrazone 1,4-dianions with esters.[14] The same strategy was applied by our group to prepare pyrazole-5-carboxylates and pyrazole-1,5-dicarboxylates.[15]

Trifluoromethylated pyrazoles have been prepared by cyclocondensation of trifluoromethyl-1,3-diketones with phenylhydrazine, however, mixtures of regioisomers were formed.[1,16] 5-Trifluoromethylpyrazoles could be regioselectively synthesized by cyclization of phenylhydrazones with *N*-aryl-trifluoroacetimidoyl iodides. However, this method is limited by the fact that the starting materials have to be prepared in two steps which proceed in unsatisfactory yields.[17] In order to find an atom-economic approach for the synthesis of trifluoromethylated pyrazoles, several research groups have focused on the development of new methods which address the issue of regioselectivity. Frizzo *et al.* reported a useful method for the synthesis of 5-(trifluoromethyl)pyrazoles based on the condensation of 4-alkoxy-1,1,1-trifluoro-3-alken-2-ones with phenylhydrazine using the ionic liquid [BMIM][BF_4] as the solvent.[18] In general, this method gave very good yields of 5-(trifluoromethyl)pyrazoles, but for some derivatives still regioisomeric mixtures were obtained. Recently, some useful methods have been developed to overcome this problem, e.g., the use of fluorinated alcohols (TFE and HFIP)[19] in the cyclocondensation of trifluoromethyl-1,3-diketones with phenylhydrazine or the employment of 4-trifluoromethyl-sydnones as starting materials in the cycloaddition reaction with alkynes.[20] In early 2014, Mykhailiuk and coworkers reported an interesting approach for the synthesis of 3-trifluoromethylpyrazoles in very good yields based on the [3+2] cycloaddition of CF_3CHN_2 with alkynes.[21] Very recently, a new method for the synthesis of 3-trifluoromethylpyrazoles was

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described via trifluoromethylation/cyclization of α,β-alkynic hydrazone with a hypervalent iodine reagent.[22]

Herein, we report a short, convenient and efficient method for the synthesis 5-trifluoromethyl- and 5-perfluoroalkylpyrazoles by one-pot cyclization of hydrazone 1,4-dianions with fluorinated esters. In addition, we report the activity of the pyrazoles prepared as inhibitors of two alkaline phosphatases: human tissue-nonspecific alkaline phosphatase (h-TNAP) and human intestinal alkaline phosphatase (h-IAP). The effects of these molecules were also tested on two other human ectonucleotidases, ecto-nucleotide pyrophosphatase/phosphodiesterase-1 (h-NPP1) and h-NPP3.

Alkaline Phosphatases (APs; EC 3.1.3.1) are homodimeric enzymes that require three metal ions for its catalytic activity, i.e. Ca and two Zn cations. These enzymes catalyze the hydrolysis of the phosphate ester bonds and the transphosphorylation reaction.[23] Different mammalian isozymes are present in the body and they are encoded by four genes. They are classified under two categories; tissue specific alkaline phosphatases and tissue non-specific alkaline phosphatase (TNAP).[24] The tissue specific APs are expressed at restricted areas, such as placenta, germ cells and intestine, whereas the tissue non-specific isozymes are predominant in bone, liver and kidney tissues and are expressed at lower level at other sites. These four isozymes differ from each other in relation to their response to specific inhibitors and their genes of origin. The main physiological role of TNAP is the hydrolysis of extracellular inorganic pyrophosphate (PPi) to inorganic phosphate.[25]

The inorganic pyrophosphate act as a potent inhibitor of calcification. Therefore, TNAP plays an important role in the maintenance of the PPi level in the body and is helpful in the normal process of bone mineralization.[26] The potent inhibitors of TNAP act as a useful therapeutic agent in different disease conditions, such as arterial calcification, ankylosis and osteoarthritis.[27] Because of a high homology between tissue-specific IAP and TNAP, very few selective inhibitors of IAP have been reported.[28] The tissue specific intestinal alkaline phosphatase is enriched in surfactant-like particles (SLP) and is located at the brush boarder of intestine. It plays an important role in the intestinal absorption of lipids and nutrients through its association with SLP. After a fatty meal the IAP level increases significantly. Therefore, IAP plays a major role in the maintenance of intestinal homeostasis.[29]

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E-NPPs-type ectophosphodiesterases are transmembrane bounded and cell surface proteins. NPP2 can also be found extracellularly as a shedded or secreted enzyme. NPPs play an important role in the hydrolysis of pyrophosphate or phosphodiester bonds in a variety of compounds including nucleotides, (lyso) phospholipids and choline phosphate esters and prevent the activation of P2X and P2Y receptors. While NPP1 and NPP3 are mainly involved in the control of purinergic signaling, the other members of the NPP family rather hydrolyze phospholipids. Although NPP1-3 have 50% structural relationship with each other, they show broad substrate specificity for each enzyme.[30]

At a physiological level, NPPs mainly affect a number of processes like bone mineralization, cell proliferation, motility and digestion. The isozymes of NPPs are involved in the pathophysiology of different disease conditions, such as calcification, cancer and insulin resistance. Therefore, efforts are being made to discover selective inhibitors of NPPs. NPP1 plays an important role in bone mineralization as it acts as the main enzyme responsible for the production of PPi for chondrocytes and osteoblast. It also affects the insulin signaling process by inhibiting the tyrosine kinase activity. NPP3 expression is considered as a tumor marker and is associated with metastasis of cancer cells and carcinogenesis.[31]

Results and discussion

Synthesis of perfluoroalkylated pyrazoles:

Hydrazones **2** were prepared by condensation of ketones **1** with hydrazine derivatives. This reaction proceeded under solvent free (‘green’) conditions at room temperature and is catalyzed by acetic acid and provided nearly quantitative yields of hydrazones. Then, **2** were converted to their dianions by treatment with 2 equivalents of *n*-BuLi in THF at -78 ^{o}C. Subsequently, ethyl perfluorocarboxylates **3** were added to the reaction mixture. After warming to ambient temperature, either trifluoroacetic acid (TFA) or *p*-toluenesulfonic acid (PTSA) was added to the reaction mixture to give perfluoroalkylated pyrazoles **4** or **5**, respectively (based on **1**) (Scheme 1). The formation of the product proceeds by attack of the carbon of dianion **A** onto **3** to give intermediate **B**, cyclization by attack of the nitrogen atom onto the carbonyl group to give

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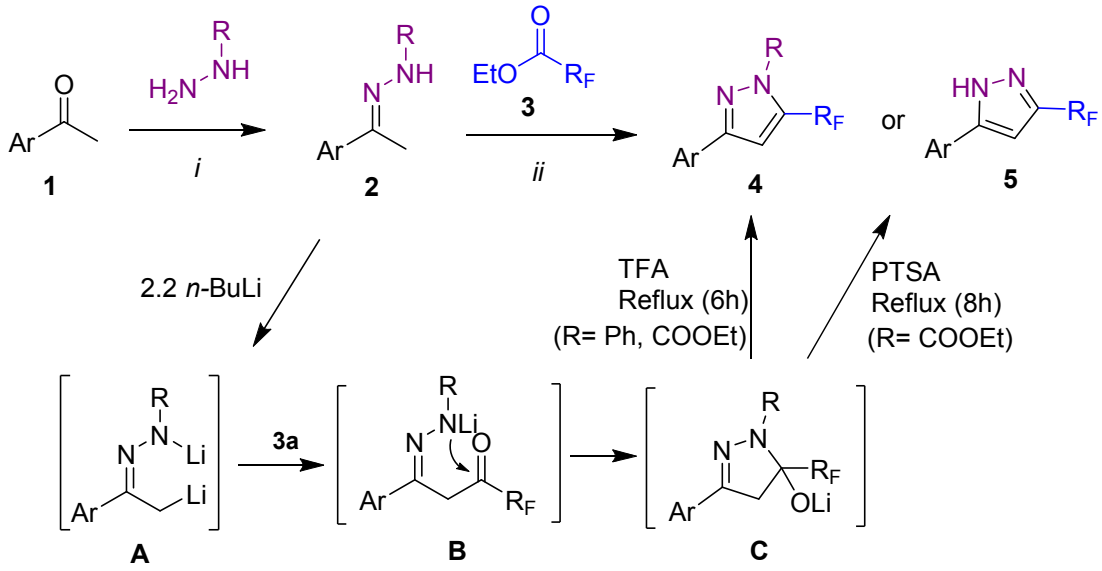
intermediate **C**, and subsequent acid-mediated dehydration. Treatment of intermediate **C** with TFA under reflux in dioxane allowed 5-perfluoromethylated pyrazoles **4** in good to excellent isolated yields. On the other hand, treatment ofintermediate **C** with PTSA (reflux, toluene) gave the *N*-deprotected 3-perfluoromethylpyrazoles **5**. This could be explained by the acidity of TFA and PTSA. PTSA is a stronger acid than TFA. The carbamate protecting group was removed when PTSA was used but remained intact when TFA was used. It is noteworthy that the synthesis of compound **4f** could be scaled up to gram quantities. Starting with 10 mmol of **1f**, product **4f** was isolated in 88% yield (2.67 gram).



Scheme 1. Synthesis of pyrazoles **4** and **5**. *Conditions:i,* neat, acetic acid, 20 ^{o}C; *ii*, 1) 2.2 equiv. *n*-BuLi, THF, -78 ^{o}C to 20 ^{o}C. 2) 1.5 equiv. **3a**, -78 ^{o}C to 20 ^{o}C. 3) TFA, reflux 2h (or PTSA, reflux, 8h).

Table 1. Sythesis of pyrazoles **4** and **5**

No	Ar	R	R_F	Yield (%)[a]
4a	C_6H_5	C_6H_5	CF_3	89
4b	4-(MeO)C_6H_4	C_6H_5	CF_3	79

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4c	3-(MeO)C_6H_4	C_6H_5	CF_3	81
4d	2-(MeO)C_6H_4	C_6H_5	CF_3	61
4e	4-PhC_6H_4	C_6H_5	CF_3	74
4f	4-MeC_6H_4	C_6H_5	CF_3	87
4g	2-FC_6H_4	C_6H_5	CF_3	90
4h	4-FC_6H_4	C_6H_5	CF_3	82
4i	2-Naphthyl	C_6H_5	CF_3	78
4j	4-(F_3C)C_6H_4	C_6H_5	CF_3	64
4k	C_6H_5	C_6H_5	C_2F_5	95
4l	4-(MeO)C_6H_4	C_6H_5	C_2F_5	91
4m	2-Naphthyl	C_6H_5	C_2F_5	93
4n	4-PhC_6H_4	C_6H_5	C_2F_5	85
4o	C_6H_5	C_6H_5	C_3F_7	83
4p	4-(MeO)C_6H_4	C_6H_5	C_3F_7	76
4q	4-(MeO)C_6H_4	CO_2Et	CF_3	57
4r	2-FC_6H_4	CO_2Et	CF_3	65
5a	4-(MeO)C_6H_4	H	CF_3	51
5b	4-(MeO)C_6H_4	H	C_2F_5	56
5c	4-(MeO)C_6H_4	H	C_3F_7	57

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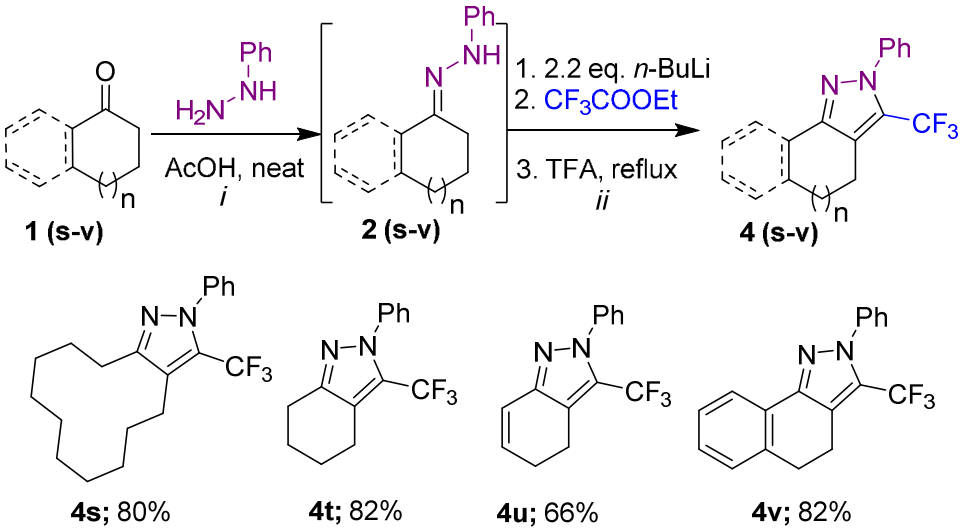
5d	2-Naphthyl	H	CF_3	78
5e	2-Naphthyl	H	C_2F_5	86
5f	2-Naphthyl	H	C_3F_7	64
5g	4-$(F_3C)C_6H_4$	H	CF_3	75
5h	4-$(F_3C)C_6H_4$	H	C_2F_5	74
5i	4-$(F_3C)C_6H_4$	H	C_3F_7	67

[a]Isolated yields

The cyclization of the dianion of the hydrazone of cyclododecanone **2s** with ethyl 2,2,2-trifluoroacetate afforded the annulated trifluoromethylated pyrazole **4s** in 80% yield (Scheme 2). The cyclizations of the hydrazones of cyclohexanone **2t**, cyclohex-2-en-1-one **2u** and tetralone **2v** afforded the corresponding products **4t-v**.



Scheme 2. Synthesis of **4s-v**.*Conditions:i,* neat, acetic acid, 20 °C; *ii*, 1) 2.2 equiv. *n*-BuLi, THF, -78 °C to 20 °C. 2) 1.5 equiv. **3a**, -78 °C to 20 °C. 3) TFA, reflux 2h.

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Recently, it has been reported that trifluoromethylated indazole **D**[32] represents a highly selective ligand for the estrogen receptor *β*. Trifluoromethylated indazole **E**[33] is a useful agent for the treatment of obesity and diabetes (Figure 2). Therefore, we were interested in the preparation of trifluoromethylated indazoles starting from ring-fused pyrazoles.

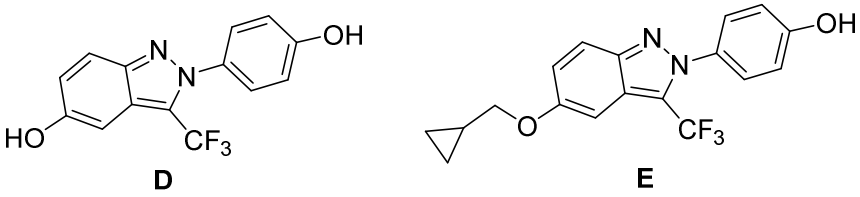
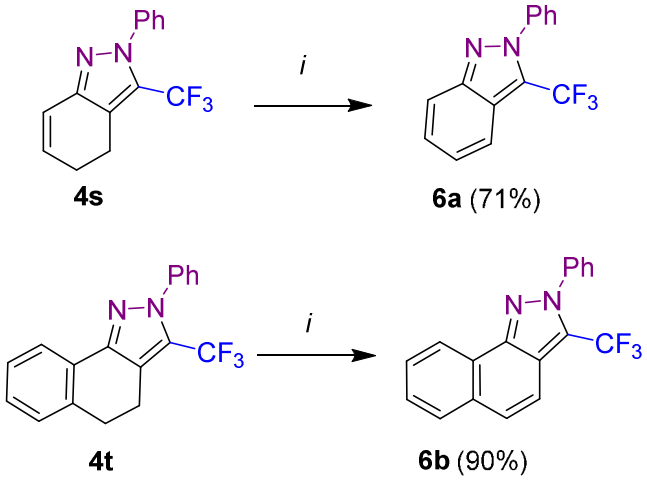


Figure 2. Bioactive trifluoromethylated indazoles **D** and **E**

The dehydrogenation of pyrazoles **4s** and **4t** with DDQ afforded the desired indazoles **6a** and **6b** in high yields, respectively. These experiments show that our methodology can be successfully applied also for the synthesis of trifluorinated indazoles.



Scheme 3. Synthesis of **6a** and **6b**.*Conditions:i*, 2.0 equiv. DDQ, toluene, reflux, 3 h.

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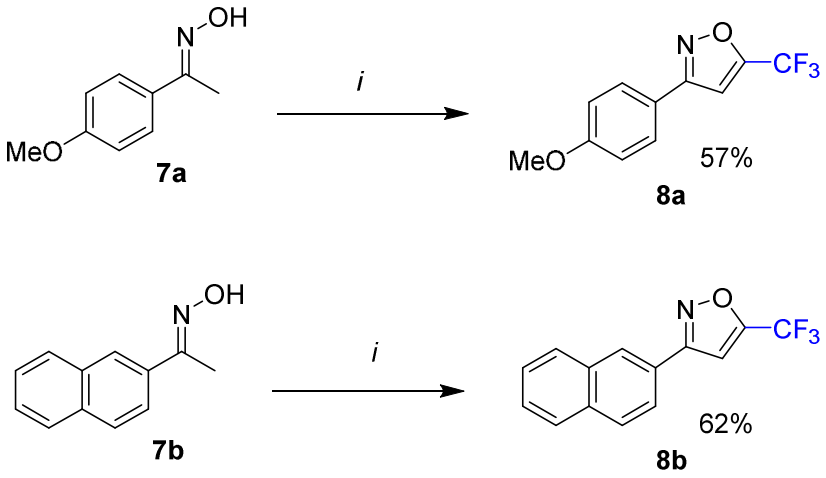
The cyclization of the dianions of oximes **7a, b** with ethyl trifluoroacetate and subsequent treatment with TFA (reflux, 8h) afforded 5-trifluoromethylated isoxazoles **8a** and **8b** in 57% and 62% isolated yields, respectively.



Scheme 4. Synthesis of isoxazoles **8a, b**. *Conditions:i*, 1) 2.2 equiv. *n*-BuLi, THF, -78 °C to 20 °C. 2) 1.5 equiv. CF_3COOEt, -78 °C to 20 °C. 3) TFA, reflux 2h.

Alkaline Phosphatase and Nucleotide Pyrophosphatase Activity and SAR:

All the fluorinated pyrazole derivatives were tested for human recombinant APs and NPPs and they were found that these compounds, in comparison to NPPs, to be selective inhibitors of APs. Against h-NPP1 and h-NPP3, these compounds exhibited low response of inhibition. The data obtained showed that all the values were below 50%. Compound **4i** was found to be the potent inhibitor of h-TNAP having IC_{50} value of IC_{50} ±SEM = 0.45±0.01. It can be suggested that the activity of this compound might be due to the presence of phenyl and a trifluoromethyl group at the parent pyrazole ring. When the activity of this compound was compared with the other derivatives containing naphthalene ring attached to parent pyrazole it was clearly observed that compound having phenyl and side chain with less carbon atom, has more impact on activity against h-TNAP. When the number of carbon and fluorine increased the activity of the compound was decreased as it was reflected in activity values of **4m**, **5d**, **5e** and **5f**. Levamisole was used as a standard inhibitor against h-TNAP. Other compounds inhibited h-TNAP with IC_{50} values in the range of IC_{50} ±SEM = 0.449±0.001 to 50.3±3.28 µM. Compound **4n** exhibited the

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most potent inhibition of h-IAP with an IC_{50}value of IC_{50} ±SEM= 0.65±0.04 μM [sic] which is over 120 folds more efficient than the known standard inhibitor L-phenylalanine. The comprehensive study of the compound structure was justified by comparing with L-phenylalanine (known reference standard) which contain only one phenyl ring. This confirmed that the presence of biphenyl group on the pyrazole ring might be responsible for its high activity against h-IAP. On the other hand, when this compound was compared with **4e** containing biphenyl ring with the different carbon side chain it was observed that with a decreased in the side chain of carbon atom the activity of compound was decreased against h-IAP. Other pyrazole derivatives displayed h-IAP inhibition activity in the range IC_{50} ±SEM = 0.647±0.04 to 7.36±0.25 μM. The above mentioned data showed that most pyrazole derivatives were better inhibitors of h-IAP than of h-TNAP.

Table 2. Alkaline phosphatase AP (h-TNAP & h-IAP) and NPP (h-NPP1 & h-NPP3) inhibition in presence of the synthesized compounds

No	h-TNAP	h-IAP	h-NPP1	h-NPP3
	IC_{50} [a] (μM)±SEM	IC_{50} [a](μM)±SEM	(% inhibition)[b]	(% inhibition)[b]
4a	9.52±1.53	1.49±0.38	18.9%	12.5%
4b	11.1±1.06	1.22±0.22	23.4%	27.6%
4c	10.46±.65	1.63±0.41	21.2%	22.6%
4d	26.6±2.56	2.31±0.13	1.65%	4.87%
4e	3.23±0.48	1.41±0.05	12.4%	13.5%
4f	1.48±0.72	3.52±0.98	7.98%	19.8%
4g	2.59±0.38	5.78±0.74	13.8%	6.87%
4h	10.1±1.72	1.62±0.23	4.89%	2.76%
4i	0.45±0.01	4.46±0.78	23.2%	2.87%
4j	3.11±0.84	2.37±0.79	6.98%	1.09%
4k	2.11±0.28	5.12±0.84	34.5%	24.8%
4l	5.01±0.79	3.71±0.37	2.89%	4.67%
4m	1.35±0.06	2.19±0.05	38.4%	14.6%
4n	48.6±3.22	0.65±0.04	6.98%	9.87%
4o	50.3±3.28	1.35±0.14	28.2%	12.7%
4p	25.9±1.38	7.11±0.98	11.5%	17.8%
5a	11.6±1.28	7.36±0.25	3.08%	6.08%
5b	13.1±0.63	10.5±1.02	14.8%	18.9%

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5c	4.34±0.03	2.91±0.35	39.1%	34.6%
5d	8.09±1.28	1.95±0.26	22.4%	18.7%
5e	13.9±1.08	4.47±0.97	6.87%	7.98%
5f	12.9±1.38	2.23±0.32	15.9%	10.8%
5g	1.62±0.11	2.39±0.36	19.3%	14.7%
5h	47.1±3.11	2.57±0.77	25.6%	29.8%
5i	2.04±0.17	1.96±0.33	28.2%	31.2%
6b	21.2±1.78	6.03±0.75	27.6%	12.6%
6a	17.2±0.89	5.84±0.37	12.8%	16.7%
Levamisole	19.21±0.001			
L-Phenylalanine	-------	80.21±0.001		

Values are expressed as mean ± SEM of n = 3. [a] The IC_{50} is the concentration at which 50% of the enzyme activity is inhibited. [b] The % inhibition of the enzyme activity caused by 0.1 mM of the tested compound.

Conclusion

In conclusion, we have demonstrated that a series of 5-trifluoromethylated and 5-perfluoroalkylated pyrazoles, including deprotected derivatives, could be efficiently and selectively synthesized by one-pot cyclization of hydrazone dianions with ethyl perfluorocarboxylates. In addition, two trifluoromethylated indazoles were prepared from the corresponding bicyclic hydrazones. The cyclization of oxime dianions afforded trifluoromethyl-substituted isoxazoles. All the compounds were selective inhibitors of APs with little effect on h-NPP1 and h-NPP3. In addition, our data showed that most of the compounds presented here inhibited h-IAP more efficiently than h-TNAP. Therefore these compounds appear as selective inhibitors of h-IAP. The results reported herein are of considerable interest for further applications in medicinal chemistry.

Experimental Section

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General information: All chemicals used are commercially available and were used without further purification. Column chromatography was performed using Merck Silicagel 60 (0.043-0.06 mm). NMR data were recorded on a Bruker AC 250, Bruker ARX 300, Bruker ARX 500 spectrometers. Gas chromatography-mass spectrometric analysis was carried out on an AgilentHP-5890 instrument with an Agilent HP-5973 Mass Selective Detector (EI) and HP-5 capillary column using helium carrier gas. ESI HR-MS measurements were performed on an Agilent 1969A TOF mass-spectrometer. A Finnigan MAT95 XP was used for High Resolution MS (HRMS). Only the measurements with an average deviation from the theoretical mass of ±2mDa were accounted as correct.Infrared Spectra were recorded on a Nicolet 550 FT – IR spectrometer with ATR sampling technique. Signal characterization: w = weak, m = medium, s = strong, vs = very strong.

General procedure for the synthesis of pyrazoles:

Synthesis of hydrazones:

Ketone (0.5 mmol, 1 eq) and phenylhydrazine (0.525 mmol, 1.05 eq) were dissolved in 2 mL of DCM. The solution was stirred for 1 min at room temperature and then the solvent was removed under reduced pressure to obtain a "well-mixed" mixture of starting materials. Then 3 drops of acetic acid was added to the reaction mixture while stirring at room temperature (20 ^{o}C). The mixture was stirred for 5 min to ensure the reaction was complete (in most cases, the product was solid and the reaction mixture became solid as the reaction became complete). The reaction carried out nearly qualitative. Then the crude product was dissolved in 10 mL of DCM (dissolving reaction mixture in DCM helped removing all volatiles easier). After that, all volatiles (DCM, water, and acetic acid) were removed under reduced pressure and hydrazone was transferred to the next step without further purification.

Synthesis of pyrazoles:

To a solution of hyrazone (0.5 mmol) in THF was added *n*-butyllithium (0.44 mL, 2.2 eq, 2.5 M solution in hexane) slowly at -78 ^{o}C under Ar. Then the mixture was allowed to rise to 20 ^{o}C and stirred for 15 min. The reaction was cooled to -78 ^{o}C again and ethyl perfluorocarboxylate (1.5 eq, solution in 1 mL THF) was added. The temperature of the reaction was risen to 20 ^{o}C

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again and stirred for 30 min, subsequently, 1 mL of TFA was added. The mixture was stirred under reflux for 2 h. After cooling to room temperature, a saturated aqueous solution of $NaHCO_3$ was added until no bubble of CO_2 was observed. Then THF was removed and the remained aqueous mixture was extracted with ethyl acetate (10 mLx3). Combined organic layers were dried with $MgSO_4$ and the solvent was removed under reduced pressure. The residue was chromatographed (silica gel, *n*-heptane/DCM) to obtain pure product.

Synthesis of 1,3-diphenyl-5-(trifluoromethyl)-1H-pyrazole (4a)[19]:

Compound **4a** was synthesized following the general procedure using acetophenone for the first step and ethyl trifluoroacetate for the next step. The product was isolated as a white solid (128 mg, 89%). M.p.: 53 – 54 ^{o}C. IR (ATR, cm^{-1}): ν = 549 (s), 615 (m), 685 (vs), 760 (s), 773 (s), 812 (s), 956 (m), 9872 (s), 1028 (s), 1072 (s), 1118 (vs), 1138 (m), 1211 (s), 1232 (s), 1288 (s), 1363 (m), 1444 (s), 1502 (m), 1556 (m), 1593 (m), 3054 (w), 3070 (w), 3139 (w). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.92 – 7.83 (m, 2H), 7.65 – 7.32 (m, 8H), 7.12 (s, 1H). ^{19}F NMR (282 MHz, $CDCl_3$) δ -57.59 (s). ^{13}C NMR (75 MHz, $CDCl_3$) δ 151.8, 139.3, 134.0 (q, J = 39.2 Hz), 131.9, 129.4, 129.26 (2C), 128.9 (2C), 128.8, 126.0 (2C), 125.9, 125.9, 119.9 (q, J = 269.2 Hz), 106.2 (q, J = 2.4 Hz). GC-MS (EI, 70 eV): *m/z* (%) = 288 (100), 267 (37), 219 (9), 77 (20). HRMS (EI): calcd. for $C_{16}H_{11}F_3N_2$ ($[M]^+$): 288.08688, found: 288.08678.

Synthesis of 3-(4-methoxyphenyl)-1-phenyl-5-(trifluoromethyl)-1H-pyrazole (4b):

Compound **4b** was synthesized following the general procedure using 4-methoxyacetophenone for the first step and ethyl trifluoroacetate for the next step. The product was isolated as a yellow solid (127 mg, 79%). M.p.: 77-78 ^{o}C. IR (ATR, cm^{-1}): ν = 548 (m), 617 (m), 685 (s), 767 (vs), 810 (vs), 833 (m), 956 (m), 987 (s), 1031 (s), 1080 (s), 1088 (vs), 1115 (vs), 1151 (s), 1209 (s), 1248 (s), 1290 (s), 1359 (w), 1435 (s), 1450 (s), 1502 (s), 1558 (m), 1595 (m), 1614 (m), 2845 (w), 2943 (w), 2970 (w), 3022 (w), 3068 (w), 3130 (w). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.86 – 7.73 (m, 2H), 7.62 – 7.44 (m, 5H), 7.04 (s, 1H), 7.02 – 6.90 (m, 2H), 3.85 (s, 3H). ^{19}F NMR (282 MHz, $CDCl_3$) δ -57.59 (s). ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.2, 151.6, 139.4, 133.9 (q, J = 39.0 Hz), 129.3, 129.2 (2C), 127.3 (2C), 125.9, 125.9, 124.5, 119.9 (q, J = 269.1 Hz), 114.3 (2C), 105.8 (q, J = 2.4 Hz), 55.47. GC-MS (EI, 70 eV): *m/z* (%) = 318 (100), 303 (26), 275 (12), 77 (12).HRMS (EI): calcd. for $C_{17}H_{13}F_3N_2O$ ($[M]^+$): 318.09745, found: 318.09788.

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Synthesis of 3-(3-methoxyphenyl)-1-phenyl-5-(trifluoromethyl)-1H-pyrazole (4c):

Compound **4c** was synthesized following the general procedure using 3-methoxyacetophenone for the first step and ethyl trifluoroacetate for the next step. The product was isolated as pale yellow oil (129 mg, 81%). IR (ATR, cm^{-1}): ν = 544 (m), 627 (m), 688 (vs), 766 (s), 816 (m), 847 (m), 916 (w), 987 (s), 1041 (s), 1089 (s), 1124 (vs), 1143 (s), 1197 (s), 1224 (s), 1257 (m), 1284 (m), 1354 (m), 1433 (s), 1464 (m), 1500 (s), 1556 (m), 1597 (m), 2835 (w), 2939 (w), 3003 (w), 3063 (w), 3138 (w). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.63 – 7.47 (m, 5H), 7.47 – 7.40 (m, 2H), 7.35 (t, *J* = 8.1 Hz, 1H), 7.10 (s, 1H), 6.93 (ddd, *J* = 8.1, 2.5, 1.2 Hz, 1H), 3.87 (s, 3H). ^{19}F NMR (282 MHz, $CDCl_3$) δ -57.60 (s). ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.2 (s), 151.6 (s), 139.3 (s), 134.0 (q, *J* = 39.2 Hz), 133.2, 130.0, 129.5, 129.3 (2C), 125.9, 125.9, 119.9 (q, *J* = 269.2 Hz), 118.5, 114.8, 111.1, 106.4 (q, *J* = 2.4 Hz), 55.5. GC-MS (EI, 70 eV): *m/z* (%) = 318 (100), 297 (8), 267 (8), 205 (3), 77 (20). HRMS (EI): calcd. for $C_{17}H_{13}F_3N_2O$ ($[M]^+$): 318.09745, found: 318.09718.

Synthesis of 3-(2-methoxyphenyl)-1-phenyl-5-(trifluoromethyl)-1H-pyrazole (4d):

Compound **4d** was synthesized following the general procedure using 2-methoxyacetophenone for the first step and ethyl trifluoroacetate for the next step. The product was isolated as pale yellow solid (109 mg, 61%). M.p.: 70 - 71°C. IR (ATR, cm^{-1}): ν = 542 (m), 692 (s), 750 (s), 777 (s), 814 (s), 989 (s), 1028 (s), 1068 (s), 1084 (s), 1113 (vs), 1157 (s), 1201 (s), 1230 (s), 1246 (s), 1290 (s), 1354 (m), 1421 (m), 1437 (s), 1456 (m), 1473 (s), 1504 (m), 1556 (m), 1585 (m), 1595 (m), 2841 (w), 2943 (w), 3057 (w), 3180 (w). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.04 (dd, *J* = 7.6, 1.7 Hz, 1H), 7.64 – 7.42 (m, 5H), 7.41 – 7.30 (m, 2H), 7.08 – 6.91 (m, 2H), 3.96 (s, 3H). ^{19}F NMR (282 MHz, $CDCl_3$) δ -57.38 (s). ^{13}C NMR (75 MHz, $CDCl_3$) δ 156.9, 148.6, 139.4, 132.9 (q, *J* = 39.0 Hz), 129.8, 129.1, 129.1 (2C), 128.8, 125.8, 125.8, 120.9, 120.6, 120.0 (q, *J* = 269.0 Hz), 111.3, 110.3 (q, *J* = 2.5 Hz), 55.5. GC-MS (EI, 70 eV): *m/z* (%) = 318 (100), 289 (64), 267 (27), 249 (28), 221 (14), 77 (60). HRMS (EI): calcd. for $C_{17}H_{13}F_3N_2O$ ($[M]^+$): 318.09745, found: 318.09699.

Synthesis of 3-([1,1'-biphenyl]-4-yl)-1-phenyl-5-(trifluoromethyl)-1H-pyrazole (4e):

Compound **4e** was synthesized following the general procedure using 4-phenylacetophenone for the first step and ethyl trifluoroacetate for the next step. The product was isolated as pale yellow solid (134 mg, 74%). M.p.: 94 – 95 °C. IR (ATR, cm^{-1}): ν = 546 (m), 685 (vs), 727 (s),

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758 (vs), 820 (s), 845 (m), 987 (s), 1086 (s), 1117 (vs), 1149 (s), 1159 (s), 1201 (m), 1226 (s), 1290 (s), 1358 (m), 1410 (m), 1443 (s), 1502 (s), 1566 (w), 1595 (m), 2850 (w), 2920 (w), 3032 (w), 3053 (w), 3144 (w). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.99 – 7.90 (m, 2H), 7.72 – 7.63 (m, 4H), 7.63 – 7.33 (m, 8H), 7.16 (d, *J* = 0.3 Hz 1H). ^{19}F NMR (282 MHz, $CDCl_3$) δ -57.57 (s). ^{13}C NMR (75 MHz, $CDCl_3$) δ 151.4, 141.5, 140.7, 139.4, 134.1 (q, *J* = 39.2 Hz), 130.8, 129.5, 129.3 (2C), 128.9 (2C), 127.6, 127.6 (2C), 127.2 (2C), 126.4 (2C), 125.9, 125.9, 119.9 (q, *J* = 269.1 Hz), 106.3 (q, *J* = 2.4 Hz). GC-MS (EI, 70 eV): *m/z* (%) = 364 (100), 343 (11), 152 (10), 77(10). HRMS (EI): calcd. for $C_{22}H_{15}F_3N_2$ ($[M]^+$): 364.11818, found: 364.11765.

Synthesis of 1-phenyl-3-(p-tolyl)-5-(trifluoromethyl)-1H-pyrazole (4f)[34]:

Compound **4f** was synthesized following the general procedure using 4-methylacetophenone for the first step and ethyl trifluoroacetate for the next step. M.p: 71 – 72 ^{o}C. IR (ATR, cm^{-1}): ν = 544 (m), 623 (m), 687 (vs), 768 (s), 798 (vs), 827 (m), 989 (s), 1086 (vs), 1122 (vs), 1155 (s), 1203 (m), 1232 (s), 1290 (m), 1440 (s), 1504 (m), 1556 (m), 1595 (w), 2357 (w), 2860 (w), 2922 (w), 3022 (w), 3061 (w). ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.82 – 7.65 (m, 2H), 7.63 – 7.44 (m, 5H), 7.33 – 7.18 (m, 2H), 7.08 (s, 1H), 2.40 (s, 3H). ^{19}F NMR (282 MHz, $CDCl_3$) δ -57.58 (s). ^{13}C NMR (63 MHz, $CDCl_3$) δ 151.8, 139.4, 138.7, 133.9 (q, *J* = 39.2 Hz), 129.6 (2C), 129.4, 129.2 (2C), 129.1, 125.9 (4C), 119.9 (q, *J* = 269.2 Hz), 106.1 (q, *J* = 2.4 Hz), 21.4. GC-MS (EI, 70 eV): *m/z* (%) = 302 (100), 281 (18), 267 (8), 233 (7), 77 (14).HRMS (ESI): calcd. for $C_{17}H_{15}F_3N_2$ ($[M+H]^+$): 303.11036, found: 303.11038.

Synthesis of 3-(2-fluorophenyl)-1-phenyl-5-(trifluoromethyl)-1H-pyrazole (4g):

Compound **4g** was synthesized following the general procedure using 2-fluoroacetophenone for the first step and ethyl trifluoroacetate for the next step. The product was isolated as pale yellow solid (138 mg, 90%). M.p. 73 – 74 ^{o}C. IR (ATR, cm^{-1}): ν = 553 (m), 625 (m), 661 (m), 686 (s), 750 (vs), 771 (s), 820 (s), 833 (s), 947 (m), 960 (m), 989 (s), 1028 (m), 1070 (s), 1115 (vs), 1165 (s), 1201 (s), 1238 (s), 1261 (m), 1290 (s), 1358 (m), 1423 (m), 1444 (s), 1471 (m), 1500 (m), 1554 (m), 1581 (m), 1587 (m), 1913 (w), 3056 (w), 3076 (w), 3165 (w).^{1}H NMR (300 MHz, $CDCl_3$) δ 8.19 – 8.07 (m, 1H), 7.68 – 7.50 (m, 5H), 7.50 – 7.35 (m, 1H), 7.35 – 7.15 (m, 3H). ^{19}F NMR (282 MHz, $CDCl_3$) δ -57.59 (s), -116.00 (s). ^{13}C NMR (126 MHz, $CDCl_3$) δ 160.5 (d, *J* = 250.0 Hz), 146.5, 139.3, 134.3 – 133.3 (m), 130.2 (d, *J* = 8.5 Hz), 129.5, 129.3 (2C), 128.6 (d, *J* = 3.3 Hz), 125.9, 125.9, 124.6 (d, *J* = 3.5 Hz), 119.9 (q, *J* = 269.2 Hz), 119.9 (d, *J* =

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11.8 Hz), 116.3 (d, *J* = 22.0 Hz), 109.6 (m, 1C). GC-MS (EI, 70 eV): *m/z* (%) = 306 (100), 285 (38), 267 (9), 237 (7), 77 (15).

Synthesis of 3-(4-fluorophenyl)-1-phenyl-5-(trifluoromethyl)-1H-pyrazole (4h)[34]:

Compound **4h** was synthesized following the general procedure using 4-fluoroacetophenone for the first step and ethyl trifluoroacetate for the next step. The product was isolated as a yellow solid (125 mg, 82%). M.p.: 64 – 65 ^{o}C. IR (ATR, cm^{-1}): ν = 544 (m), 619 (m), 692 (s), 750 (m), 769 (vs), 808 (vs), 837 (s), 956 (m), 987 (s), 1028 (m), 1066 (s), 1076 (s), 1086 (vs), 1111 (vs), 1155 (s), 1207 (s), 1228 (s), 1290 (s), 1440 (s), 1502 (s), 1525 (m), 1558 (m), 1595 (m), 1606 (m), 1888 (w), 3058 (w), 3141 (w). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.91 – 7.75 (m, 2H), 7.63 – 7.44 (m, 5H), 7.20 – 7.07 (m, 2H), 7.06 (s, 1H). ^{19}F NMR (282 MHz, $CDCl_3$) δ -57.65 (s), -112.97 (s). ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.2 (d, *J* = 247.9 Hz), 150.9, 139.3, 134.2 (q, *J* = 39.2 Hz), 129.5, 129.3 (2C), 128.1 (d, *J* = 3.2 Hz), 127.8 (d, *J* = 8.2 Hz, 2C), 125.8, 125.8, 119.8 (q, *J* = 269.2 Hz), 115.9 (d, *J* = 21.8 Hz, 2C), 106.0 (q, *J* = 2.4 Hz). GC-MS (EI, 70 eV): *m/z* (%) = 306 (100), 285 (40), 237 (9), 77 (17). HRMS (EI): calcd. for C16H10F4N2 ([M]$^{+}$): 306.07746, found: 306.07713.

Synthesis of 3-(naphthalen-2-yl)-1-phenyl-5-(trifluoromethyl)-1H-pyrazole (4i):

Compound **4i** was synthesized following the general procedure using 2-acetonaphthone for the first step and ethyl trifluoroacetate for the next step. The product was isolated as a pale yellow solid (102 mg, 78%). M.p.: 71 – 71 ^{o}C. IR (ATR, cm^{-1}): ν = 542 (m), 687 (s), 742 (s), 767 (s), 798 (s), 804 (s), 819 (m), 856 (s), 885 (m), 945 (m), 989 (s), 1028 (m), 1057 (s), 1074 (s), 1086 (s), 1113 (vs), 1153 (s), 1230 (s), 1246 (s), 1290 (s), 1431 (m), 1485 (m), 1504 (s), 1556 (w), 1595 (m), 3028 (w), 3055 (w). ^{1}H NMR (300 MHz, Acetone) δ 8.50 (s, 1H), 8.13 (d, *J* = 8.3 Hz, 1H), 8.04 – 7.88 (m, 3H), 7.72 – 7.47 (m, 8H). ^{19}F NMR (282 MHz, Acetone) δ 119.44 (s). ^{13}C NMR (75 MHz, Acetone) δ 152.5, 140.4, 134.6, 134.6, 134.5 (q, *J* = 39.0 Hz), 130.6, 130.3, 130.3 (2C), 129.5, 129.3, 128.8, 127.6, 127.4, 126.9, 126.9, 125.7, 124.6, 121.1 (q, *J* = 268.4 Hz), 107.6 (q, *J* = 2.5 Hz). GC-MS (EI, 70 eV): *m/z* (%) = 338 (100), 317 (14), 127 (17), 77 (20). HRMS (EI): calcd. for $C_{20}H_{13}F_3N_2$ ([M]$^{+}$): 338.10253, found: 338.10259.

Synthesis of 1-phenyl-5-(trifluoromethyl)-3-(4-(trifluoromethyl)phenyl)-1H-pyrazole (4j):

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Compound **4j** was synthesized following the general procedure using 4-trifluoromethylacetophenone for the first step and ethyl trifluoroacetate for the next step. The product was isolated as a yellow solid (113 mg, 64%). M.p.: 50 – 51 °C. IR (ATR, cm^{-1}): ν = 552 (m), 592 (s), 625 (m), 687 (vs), 766 (s), 804 (s), 845 (s), 991 (s), 1062 (vs), 1068 (vs), 1089 (s), 1093 (s), 1109 (vs), 1132 (vs), 1232 (s), 1288 (s), 1323 (s), 1419 (w), 1446 (m), 1504 (m), 1531 (w), 1558 (w), 1595 (m), 1622 (m), 3063 (w), 3145 (w). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.98 (d, *J* = 8.1 Hz, 1H), 7.69 (d, *J* = 8.1 Hz, 1H), 7.62 – 7.46 (m, 2H), 7.16 (s, 1H). ^{19}F NMR (282 MHz, $CDCl_3$) δ -57.71 (s), -62.63 (s). ^{13}C NMR (75 MHz, $CDCl_3$) δ 150.3, 139.1, 135.3 (d, *J* = 1.3 Hz), 134.5 (q, *J* = 39.6 Hz), 130.7 (q, *J* = 32.5 Hz), 129.7, 129.4 (2C), 126.17 (2C), 125.9 (q, *J* = 3.8 Hz, 2C), 125.9, 125.8, 124.3 (q, *J* = 272.3 Hz), 119.7 (q, *J* = 269.3 Hz), 106.5 (q, *J* = 2.4 Hz).GC-MS (EI, 70 eV): *m/z* (%) = 356 (100), 335 (51), 287 (10), 267 (10), 77 (27).HRMS (EI): calcd. for $C_{17}H_{10}F_6N_2$ ($[M]^+$): 356.07427, found: 356.07482.

Synthesis of 5-(perfluoroethyl)-1,3-diphenyl-1H-pyrazole (4k):

Compound **4k** was synthesized following the general procedure using acetophenone for the first step and ethyl pentafluoropropionate for the next step. The product was isolated as a pale yellow solid (95%). M.p.: 140 – 142 °C. IR (ATR, cm^{-1}): ν = 544 (w), 580 (w), 602 (m), 619 (m), 630 (m), 688 (vs), 711 (m), 750 (s), 765 (vs), 777 (s), 814 (m), 914 (m), 937 (s), 958 (s), 1020 (m), 1041 (s), 1076 (m), 1092 (s), 1134 (s), 1190 (s), 1203 (s), 1223 (s), 1331 (m), 1444 (m), 1498 (m), 1595 (w), 3076 (w), 3151 (w). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.90 – 7.82 (m, 2H), 7.55 – 7.48 (m, 5H), 7.48 – 7.32 (m, 3H), 7.10 (d, *J* = 0.9 Hz, 1H). ^{19}F NMR (282 MHz, $CDCl_3$) δ -83.38 (t, *J* = 2.9 Hz), -106.28 (q, *J* = 2.9 Hz). ^{13}C NMR (63 MHz, $CDCl_3$) δ 152.1, 139.9, 132.0 (t, *J* = 27.6 Hz), 131.7, 129.7, 129.1 (2C), 128.9 (2C), 128.9, 126.9 (2C), 126.0 (2C), 122.8 – 108.2 (CF_2CF_3), 107.4 – 107.0 (m, 1C). GC-MS (EI, 70 eV): *m/z* (%) = 338 (100), 319 (6), 269 (14), 219 (8), 77 (24). HRMS (EI): calcd. for $C_{17}H_{11}F_5N_2$ ($[M]^+$): 388.08369, found: 388.08322.

Synthesis of 3-(4-methoxyphenyl)-5-(perfluoroethyl)-1-phenyl-1H-pyrazole (4l):

Compound **4l** was synthesized following the general procedure using 4-methoxyacetophenone for the first step and ethyl pentafluoropropionate for the next step. The product was isolated as a pale yellow solid (168 mg, 91%). M.p.: 82 – 83 °C. IR (ATR, cm^{-1}): ν = 532 (s), 576 (m), 602 (m), 617 (m), 634 (m), 694 (s), 748 (s), 771 (vs), 804 (s), 835 (s), 935 (s), 958 (s), 1026 (s), 1036 (s), 1092 (vs), 1134 (s), 1176 (s), 1190 (vs), 1215 (vs), 1250 (s), 1292 (m), 1331 (m), 1443 (m),

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1502 (m), 1552 (w), 1596 (m), 1614 (m), 2839 (w), 2943 (w), 3010 (w), 3139 (w). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.91 – 7.68 (m, 2H), 7.57 – 7.44 (m, 5H), 7.02 (s, 1H), 7.00 – 6.92 (m, 2H), 3.85 (s, 3H). ^{19}F NMR (282 MHz, $CDCl_3$) δ -83.39 (t, *J* = 2.7 Hz), -106.26 (q, *J* = 2.6 Hz). ^{13}C NMR (63 MHz, $CDCl_3$) δ 160.2, 151.9, 139.9, 131.9 (t, *J* = 27.6 Hz), 129.6, 129.0 (2C), 127.3 (2C), 126.9 (2C), 124.5, 114.3 (2C), 121.8 - 109.6 (CF_2CF_3), 106.9 – 106.5 (m, 1C), 55.5. GC-MS (EI, 70 eV): *m/z* (%) = 368 (100), 353 (20), 77 (14). HRMS (EI): calcd. for $C_{18}H_{13}F_5N_2O$ ($[M]^+$): 368.09426, found: 368.09390.

Synthesis of 3-(naphthalen-2-yl)-5-(perfluoroethyl)-1-phenyl-1H-pyrazole (4m):

Compound **4m** was synthesized following the general procedure using 2-acetonaphthone for the first step and ethyl pentafluoropropionate for the next step. The product was isolated as a pale brown solid (181 mg, 93%). M.p.: 104 – 106 ^{o}C. IR (ATR, cm^{-1}): ν = 544 (w), 575 (m), 617 (m), 628 (m), 642 (m), 685 (s), 748 (vs), 771 (s), 804 (vs), 860 (s), 887 (m), 941 (vs), 980 (m), 1020 (s), 1043 (s), 1095 (s), 1134 (s), 1190 (vs), 1329 (m), 1431 (m), 1487 (m), 1504 (m), 1594.92 (m), 3043 (w), 3058 (w). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.33 (s, 1H), 8.01 (dd, *J* = 8.6, 1.7 Hz, 1H), 7.95 – 7.79 (m, 3H), 7.64 – 7.41 (m, 7H), 7.23 (d, *J* = 0.7 Hz, 1H). ^{19}F NMR (282 MHz, $CDCl_3$) δ -83.31 (t, *J* = 2.9 Hz), -106.28 (q, *J* = 2.9 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 152.1, 139.9, 133.6 (2C), 132.2 (t, *J* = 27.9 Hz), 129.8, 129.1 (3C), 128.7, 128.4, 127.9, 126.9 (2C), 126.6, 126.5, 125.0, 123.9, 120.6 – 106.3 (CF_2CF_3), 107.6 – 107.3 (m, 1C). GC-MS (EI, 70 eV): *m/z* (%) = 388 (100), 269 (7), 194 (6), 127 (16), 77 (18). HRMS (EI): calcd. for $C_{21}H_{13}F_5N_2$ ($[M]^+$): 388.09934, found: 388.09878.

Synthesis of 3-([1,1'-biphenyl]-4-yl)-5-(perfluoroethyl)-1-phenyl-1H-pyrazole (4n):

Compound **4n** was synthesized following the general procedure using 4-phenylacetophenone for the first step and ethyl pentafluoropropionate for the next step. The product was isolated as pale yellow solid (178 mg, 85%). M.p.: 140 -142 ^{o}C. IR (ATR, cm^{-1}): ν = 694 (vs), 727 (s), 746 (s), 765 (vs), 819 (s), 846 (m), 937 (s), 958 (s), 1020 (m), 1041 (s), 1072 (m), 1093 (s), 1134 (s), 1186 (vs), 1219 (vs), 1278 (w), 1331 (m), 1409 (w), 1441 (m), 1502 (m), 1597 (m), 3061 (w), 3132 (w). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.86 (d, *J* = 8.0 Hz, 2H), 7.73 – 7.51 (m, 4H), 7.51 – 7.21 (m, 8H), 7.04 (s, 1H). ^{19}F NMR (282 MHz, $CDCl_3$) δ -83.33 (t, *J* = 2.5 Hz), -106.24 (q, *J* = 2.5 Hz). ^{13}C NMR (63 MHz, $CDCl_3$) δ 151.7, 141.6, 140.7, 139.9, 132.1 (t, *J* = 27.4 Hz), 130.7, 129.7, 129.1 (2C), 129.0 (2C), 127.7, 127.6 (2C), 127.2 (2C), 126.9 (2C), 126.4 (2C), 125.4 –

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110.9 (CF2CF3), 107.4 – 107.1 (m, 1C). GC-MS (EI, 70 eV): *m/z* (%) = 414 (100), 395 (3), 345 (5), 295 (4), 207 (3), 152 (8), 116 (1), 77 (8). HRMS (EI): calcd. for $C_{23}H_{15}F_5N_2$ ($[M]^+$): 414.11499, found: 414.11460.

Synthesis of 5-(perfluoropropyl)-1,3-diphenyl-1H-pyrazole (4o):

Compound **4o** was synthesized following the general procedure using acetophenone for the first step and ethyl heptafluorobutyrate for the next step. The product was isolated as a white solid (161 mg, 83%). M.p.: 90 – 91 °C. IR (ATR, cm^{-1}): ν = 532 (m), 590 (m), 648 (s), 692 (vs), 746 (s), 765 (vs), 777 (s), 814 (s), 874 (vs), 957 (m), 1003 (m), 1026 (m), 1078 (m), 1109 (vs), 1138 (s), 1182 (s), 1223 (vs), 1344 (s), 1443 (m), 1500 (s), 1595 (w), 3053 (w), 3157 (w). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.91 – 7.82 (m, 2H), 7.50 (s, 5H), 7.48 – 7.32 (m, 3H), 7.12 (s, 1H). ^{19}F NMR (282 MHz, $CDCl_3$) δ -80.04 (t, *J* = 10.1 Hz), -103.84 – -104.15 (m), -124.86 – -125.05 (m). ^{13}C NMR (63 MHz, $CDCl_3$) δ 152.1, 139.9, 132.3 (t, *J* = 28.7 Hz), 131.7, 130.0, 129.3 (2C), 129.2 (2C), 129.1, 127.4 (2C), 126.3 (2C), 125.8 – 92.5 ($CF_2CF_2CF_3$), 108.0 – 107.6 (m, 1C). GC-MS (EI, 70 eV): *m/z* (%) = 388 (100), 269 (39), 77 (18). HRMS (EI): calcd. for $C_{18}H_{11}F_7N_2$ ($[M]^+$): 388.08050, found: 388.07991.

Synthesis of 3-(4-methoxyphenyl)-5-(perfluoropropyl)-1-phenyl-1H-pyrazole (4p):

Compound **4p** was synthesized following the general procedure using 4-methoxyacetophenone for the first step and ethyl heptafluorobutyrate for the next step. The product was isolated as a pale solid (160 mg, 76%). M.p.: 97 – 98 °C. IR (ATR, cm^{-1}): ν = 644 (m), 692 (s), 744 (s), 773 (s), 800 (vs), 872 (vs), 904 (m), 958 (m), 1006 (s), 1030 (s), 1070 (m), 1078 (m), 1109 (vs), 1138 (s), 1178 (s), 1184 (s), 1223 (vs), 1251 (s), 1344 (m), 1435 (s), 1446 (s), 1502 (s), 1523 (m), 1552 (w), 1596 (w), 1614 (m), 2835 (w), 2939 (w), 2964 (w), 3001 (w), 3066 (w). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.83 – 7.79 (m, 1H), 7.79 – 7.76 (m, 1H), 7.49 (s, 3H), 7.03 (s, 1H), 7.01 – 6.96 (m, 1H), 6.96 – 6.92 (m, 1H), 3.85 (s, 2H). ^{19}F NMR (282 MHz, $CDCl_3$) δ -80.05 (t, *J* = 10.1 Hz), -103.83 – -104.18 (m), -124.90 – -125.02 (m). ^{13}C NMR (63 MHz, $CDCl_3$) δ 160.2, 151.9, 139.9, 132.9 – 131.6 (m, 1C), 129.6, 128.9 (2C), 127.3 (2C), 127.1 (2C), 124.5, 114.3 (2C), 107.2 – 106.9 (m, 1C), 55.5 (signals of $CF_2CF_2CF_3$ could not be detected). GC-MS (EI, 70 eV): *m/z* (%) = 418 (100), 375 (4), 299 (12), 77 (9). HRMS (EI): calcd. for $C_{19}H_{13}F_7N_2O$ ($[M]^+$): 418.09106, found: 418.09036.

Ethyl 3-(4-methoxyphenyl)-5-(trifluoromethyl)-1H-pyrazole-1-carboxylate (4q):

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Compound **4q** was synthesized following the general procedure using 4-methoxyacetophenone, ethyl carbazatefor the first step and ethyl trifluoroacetate for the next step. The product was isolated as a white (90mg, 57%). M.p.: 73 – 74 ^{o}C. IR (ATR, cm^{-1}): ν = 3519.0 (w), 3117.7 (w), 2966.8 (w), 2842.0 (w), 1766.3 (s), 1613.6 (m), 1587.0 (m), 1464.0 (m), 1441.8 (m), 1293.7 (s), 1143.8 (s), 1006.6 (m), 946.1 (m), 834.4 (s), 746.3 (m), 680.4 (m), 554.2 (m). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.87 – 7.71 (m, 2H), 7.10 (s, 1H), 7.01 – 6.89 (m, 2H), 4.57 (q, *J* = 7.1 Hz, 2H), 3.85 (s, 3H), 1.49 (t, *J* = 7.1 Hz, 3H). ^{19}F NMR (282 MHz, $CDCl_3$) δ -60.03. ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.1, 153.5, 148.3, 135.8 (q, *J* = 41.3 Hz), 128.0 (2C), 123.1, 119.3 (q, *J* = 269.1 Hz), 114.4 (2C), 110.8 (q, *J* = 3.2 Hz), 65.5, 55.5, 14.1. GC-MS (EI, 70 eV): *m/z* (%) = 314 (47), 270 (12), 255 (17), 242 (100), 227 (58), 213 (17), 199 (36), 170 (12), 151 (22), 120 (5), 101 (5), 75 (6), 63 (5). HRMS (+EI): calcd. for $C_{14}H_{13}O_3F_3N_2$ ($[M]^+$): 314.08728, found: 314.08732.

Ethyl 3-(2-fluorophenyl)-5-(trifluoromethyl)-1H-pyrazole-1-carboxylate (4r):

Compound **4r** was synthesized following the general procedure using 2-fluoroacetophenone, ethyl carbazatefor the first step and ethyl trifluoroacetate for the next step. The product was isolated as a white (98mg, 57%). M.p.: 84 – 85 ^{o}C. IR (ATR, cm^{-1}): ν = 3505.3 (w), 3171.4 (w), 3076.4 (w), 2996.6 (w), 2918.6 (w), 1761.2 (s), 1620.7 (w), 1591.2 (w), 1446.9 (s), 1304.1 (m), 1229.2 (m), 1141.8 (s), 1026.1 (m), 1032.2 (m), 949.0 (m), 840.6 (m), 747.9 (m), 676.2 (m), 552.1 (w). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.10 (td, *J* = 7.7, 1.8 Hz, 1H), 7.47 – 7.34 (m, 1H), 7.30 (d, *J* = 3.5 Hz, 1H), 7.27 – 7.10 (m, 2H), 4.59 (q, *J* = 7.1 Hz, 2H), 1.50 (t, *J* = 7.1 Hz, 3H). ^{19}F NMR (282 MHz, $CDCl_3$) δ -60.00, -115.67. ^{13}C NMR (63 MHz, $CDCl_3$) δ 160.8 (d, *J* = 251.0 Hz), 148.8, 148.1, 135.5 (q, *J* = 42.0 Hz), 131.5 (d, *J* = 8.6 Hz), 129.2 (d, *J* = 2.8 Hz), 124.7 (d, *J* = 3.5 Hz), 119.3 (d, *J* = 269.2 Hz), 118.5 (d, *J* = 11.5 Hz), 116.4 (d, *J* = 21.9 Hz), 114.5 – 113.4 (m), 65.7, 14.1.

Synthesis of 2-phenyl-3-(trifluoromethyl)-4,5,6,7,8,9,10,11,12,13-decahydro-2H-cyclododeca[*c*]pyrazole (4s):

Compound **4s** was synthesized following the general procedure using cyclododecanone for the first step and ethyl trifluoroacetate for the next step. The product was isolated as a white solid (140 mg, 80%). M.p.: 56 – 57 ^{o}C. IR (ATR, cm^{-1}): ν = 546 (m), 692 (s), 771 (s), 993 (s), 1086 (s), 1111 (vs), 1168 (s), 1242 (m), 1309 (m), 1329 (m), 1350 (m), 1452 (m), 1504 (s), 1556 (w),

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1595 (m), 2856 (m), 2904 (m), 2933 (m). ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.44 (s, 5H), 2.73 – 2.47 (m, 4H), 1.90 – 1.62 (m, 4H), 1.59 – 1.30 (m, 12H). ^{19}F NMR (282 MHz, $CDCl_3$) δ -55.61 (s). ^{13}C NMR (63 MHz, $CDCl_3$) δ 153.2, 140.1, 129.0 (2C), 129.0 (q, J = 37.2 Hz), 128.9, 126.2, 126.1, 123.2 – 123.0 (m), 120.9(q, J = 269.4 Hz), 28.9, 28.3, 26.0, 25.8, 25.4, 25.2, 23.0 (2C), 22.9, 20.8. GC-MS (EI, 70 eV): *m/z* (%) = 350 (100), 331 (9), 307 (50), 293 (41), 281 (71), 267 (36), 253 (43), 240 (79), 77 (40). HRMS (EI): calcd. for $C_{20}H_{25}F_3N_2$ ($[M]^+$): 350.19643, found: 350.19626.

Synthesis of 2-phenyl-3-(trifluoromethyl)-4,5,6,7-tetrahydro-2H-indazole (4t)[17]:

Compound **4t** was synthesized following the general procedure using cyclohexanone for the first step and ethyl trifluoroacetate for the next step. The product was isolated as a yellow oil (109 mg, 82%). IR (ATR, cm^{-1}): ν = 546 (m), 681 (m), 692 (s), 744 (m), 766 (s), 914 (m), 964 (m), 993 (s), 1028 (m), 1074 (s), 1097 (vs), 1116 (vs), 1149 (s), 1170 (s), 1213 (m), 1296 (m), 1352 (m), 1377 (m), 1464 (m), 1504 (s), 1574 (w), 1598 (m), 2854 (w), 2939 (m). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.49 – 7.37 (m, 5H), 3.07 – 2.53 (m, 4H), 2.13 – 1.61 (m, 4H). ^{19}F NMR (282 MHz, $CDCl_3$) δ -55.92 (s). ^{13}C NMR (75 MHz, $CDCl_3$) δ 150.2, 139.8, 129.0 (2C), 128.9, 128.1 (q, J = 38.2 Hz), 125.9, 125.9, 120.9 (q, J = 269.4 Hz), 119.8 (q, J = 1.7 Hz), 23.4, 22.8, 22.8, 20.9 (d, J= 1.3 Hz). GC-MS (EI, 70 eV): *m/z* (%) = 266 (100), 247 (6), 238 (48), 217 (4), 197 (52), 77(39). HRMS (EI): calcd. For $C_{14}H_{13}F_3N_2$ ($[M]^+$): 266.10253, found: 266.10212.

Synthesis of 2-phenyl-3-(trifluoromethyl)-4,5-dihydro-2H-indazole (4u):

Compound **4u** was synthesized following the general procedure using 2-cyclohexen-1-one for the first step and ethyl trifluoroacetate for the next step. The product was isolated as a brown oil (87 mg, 66%). IR (ATR, cm^{-1}): ν = 536 (m), 596 (m), 661 (m), 690 (s), 765 (s), 993 (s), 1095 (vs), 1117 (vs), 1163 (s), 1207 (m), 1304 (m), 1325 (m), 1377 (m), 1462 (m), 1502 (s), 1577 (w), 1597 (m), 1705 (w), 2837 (w), 2898 (w), 2937 (w), 3060 (w). ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.53 – 7.32 (m, 5H), 6.60 (dt, J = 9.9, 2.0 Hz, 1H), 6.14 (dt, J = 9.9, 4.3 Hz, 1H), 3.08 – 2.78 (m, 2H), 2.55 – 2.36 (m, 2H). ^{19}F NMR (282 MHz, $CDCl_3$) δ -56.16 (s). ^{13}C NMR (63 MHz, $CDCl_3$) δ 148.6, 139.7, 131.5, 129.1 (2C), 128.9, 127.7 (q, J = 38.4 Hz), 125.9, 125.8, 120.8 (q, J = 269.6 Hz), 119.7, 118.7 (q, J = 1.8 Hz), 23.4, 18.5 (d, J = 1.3 Hz). GC-MS (EI, 70 eV): *m/z* (%) = 314 (100), 245 (33), 218 (10), 77 (27), 51 (12). HRMS (EI): calcd. for $C_{18}H_{13}F_3N_2$ ($[M]^+$): 314.10253, found: 314.10234.

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Synthesis of 2-phenyl-3-(trifluoromethyl)-4,5-dihydro-2H-benzo[g]indazole (4v):

Compound **4v** was synthesized following the general procedure using α-tetralones for the first step and ethyl trifluoroacetate for the next step. The product was isolated as a brown solid (130 mg, 82%). M.p.: 78 – 79 ^{o}C. IR (ATR, cm^{-1}): ν = 549 (m), 648 (m), 696 (s), 731 (s), 781 (s), 897 (m), 945 (m), 987 (s), 1026 (m), 1047 (s), 1103 (vs), 1157 (s), 1174 (s), 1226 (s), 1267 (m), 1307 (m), 1331 (m), 1352 (m), 1377 (m), 1446 (s), 1500 (s), 1595 (m), 2848 (w), 2901 (w), 2964 (w). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.07 – 7.75 (m, 1H), 7.67 – 7.35 (m, 5H), 7.36 – 7.26 (m, 3H), 3.16 – 2.77 (m, 4H). ^{19}F NMR (282 MHz, $CDCl_3$) δ -56.04 (s). ^{13}C NMR (75 MHz, $CDCl_3$) δ 148.9, 139.8, 136.5, 129.2, 129.2(2C), 128.6, 128.5, 128.5, 128.3 (q, *J* = 38.3 Hz), 127.2, 126.2, 126.2, 122.8, 120.7 (q, *J* = 269.6 Hz), 120.1 (q, *J* = 1.8 Hz), 28.9, 19.4 (d, *J* = 1.3 Hz). GC-MS (EI, 70 eV): *m/z* (%) = 314 (100), 245 (33), 218 (10), 142 (13), 115 (10), 77 (27). HRMS (EI): calcd. For $C_{18}H_{13}F_3N_2$ ([M]$^{+}$): 314.10253, found: 314.10234.

5-(4-methoxyphenyl)-3-(trifluoromethyl)-1H-pyrazole (5a)[35]:

Compound **5a** was synthesized following the general procedure using 4-methoxyacetophenone, ethyl carbazate for the first step and ethyl trifluoroacetate for the next step. After adding ethyl trifluoroacetate, the reaction was allowed to rise to 20 ^{o}C and stirred for 30 min. Then the solvent was removed under reduced pressure. After that, the residue was dissolved in toluene and 2 mmol (4 eq) of PTSA was added. The reaction mixture was stirred under reflux for 8 h. After cooling, a saturated aqueous solution of $NaHCO_3$ was added until no bubble of CO_2 was observed. Then THF was removed and the remained water was extracted with ethyl acetate (10 mLx3). Combined organic layers were dried with $MgSO_4$ and the solvent was removed under reduced pressure. The residue was purified by chromatography (silica gel, *n*-heptane/DCM). The product was isolated as a white solid (62 mg, 51%). M.p.: 147 – 148 ^{o}C. IR (ATR, cm^{-1}): ν = 3225.7 (w), 2974.4 (w), 2845.7 (w), 1614.5 (m), 1574.2 (m), 1516.7 (m), 1490.1 (m), 1458.8 (s), 1440.5 (m), 1274.9 (s), 1243.7 (s), 1109.9 (s), 1056.2 (s), 980.9 (m), 836.3 (s), 795.3 (s), 742.4 (m). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.95 – 7.31 (m, 2H), 7.11 – 6.77 (m, 2H), 6.66 – 6.62 (m, 1H), 3.85 (s, 3H).^{19}F NMR (282 MHz, $CDCl_3$) δ -62.17 (s). ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.7, 145.2, 143.7 (q, *J* = 38.1 Hz), 127.3 (2C), 121.3 (q, *J* = 268.6 Hz), 120.7, 114.8 (2C), 100.5, 55.5. GC-MS (EI, 70 eV): *m/z* (%) = 242 (100), 227 (41), 223 (10), 199 (41), 169 (3), 151 (24). HRMS (+EI): calcd. for $C_{11}H_9O_1F_3N_2$ ([M]$^{+}$): 242.06615, found: 242.0660.

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5-(4-methoxyphenyl)-3-(perfluoroethyl)-1H-pyrazole (5b):

Compound **5b** was synthesized following the procedure for compound **5a** using 4-methoxyacetophenone for the first step and ethyl pentafluoropropionate for the next step. The product was isolated as pale yellow solid (82 mg, 56%). M.p.: 137 – 138 °C. IR (ATR, cm^{-1}): ν = 3185.5 (w), 3142.1 (w), 3033.4 (w), 1619.0 (m), 1513.2 (s), 1330.6 (m), 1258.5 (s), 1185.6 (s), 1029.1 (s), 933.0 (s), 830.5 (m), 747.8 (m), 614.4 (m). ^{1}H NMR (300 MHz, MeOD) δ 7.90 – 7.49 (m, 2H), 7.16 – 6.94 (m, 2H), 6.86 (s, 1H), 3.86 (s, 3H). ^{19}F NMR (282 MHz, MeOD) δ -86.12, -114.10. ^{13}C NMR (63 MHz, MeOD) δ 161.9, 128.3 (2C), 115.6 (2C), 102.2, 55.8 (signals of CF_2CF_3 could not be detected). GC-MS (EI, 70 eV): *m/z* (%) = 292 (199), 277 (30), 249 (39), 223 (12), 151 (20), 111 (6). HRMS (EI): calcd. for $C_{12}H_9O_1N_2F_5$ ($[M]^+$): 292.06296, found: 292.06263.

5-(4-methoxyphenyl)-3-(perfluoropropyl)-1H-pyrazole (5c):

Compound **5c** was synthesized following the procedure for compound **5a** using 4-methoxyacetophenone for the first step and ethyl heptafluorobutyrate for the next step. The product was isolated as pale yellow solid (98 mg, 57%). M.p.: 127 – 128 °C. IR (ATR, cm^{-1}): ν = 3144.1 (w), 3031.2 (w), 2947.1 (w), 1890.6 (w), 1617.6 (m), 1512.5 (s), 1427.8 (m), 1348.6 (m), 1311.7 (m), 1255.9 (m), 1229.3 (s), 1178.2 (s), 1106.6 (s), 1029.6 (m), 1000.1 (m), 874.2 (s), 829.8 (m), 746.3 (s), 652.1 (m), 620.4 (m). ^{1}H NMR (300 MHz, MeOD) δ 7.78 – 7.47 (m, 2H), 7.22 – 6.91 (m, 2H), 6.85 (s, 1H), 3.85 (s, 3H). ^{19}F NMR (282 MHz, MeOD) δ -81.15 – -82.64 (m), -111.79 (d, J = 9.5 Hz), -127.00 – -130.06 (m). ^{13}C NMR (63 MHz, MeOD) δ 161.9, 146.4, 143.0, 128.3, 122.2, 115.6, 102.4, 55.8 (signals of $CF_2CF_2CF_3$ could not be detected). GC-MS (EI, 70 eV): *m/z* (%) = 342 (100), 327 (18), 299 (25), 223 (29), 208 (4), 180 (5), 151 (15), 111 (6). HRMS (EI): calcd. for $C_{13}H_9O_1N_2F_7$ ($[M]^+$): 342.05976, found: 342.05959.

5-(naphthalen-2-yl)-3-(trifluoromethyl)-1H-pyrazole (5d)[35]:

Compound **5d** was synthesized following the procedure for compound **5a** using 2-acetonaphthone for the first step and ethyl trifluoroacetate for the next step. The product was isolated as white solid (102 mg, 78%). M.p.: 180 – 181 °C. IR (ATR, cm^{-1}): ν = 3227.5 (w), 3043.6 (w), 2923.5 (w), 1631.9 (w), 1608.5 (w), 1582.5 (w), 1567.3 (w), 1500.0 (m), 1474.0 (w), 1255.6 (m), 1150.1 (s), 1120.6 (s), 1103.6 (s), 992.0 (m), 860.5 (m), 803.6 (s), 741.1 (s), 713.1 (m), 679.4 (m), 601.7 (m). ^{1}H NMR (300 MHz, MeOD) δ 8.26 (s, 1H), 8.11 – 7.72 (m, 4H), 7.56

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(dd, J = 6.3, 3.0 Hz, 2H), 7.10 (s, 1H). ^{19}F NMR (282 MHz, MeOD) δ -63.58. ^{13}C NMR (75 MHz, Acetone) δ 145.5, 144.3 (q, J = 37.5 Hz), 134.4, 134.4, 129.9, 129.1, 128.7, 127.8, 127.8, 126.7,125.6, 124.3, 122.9 (q, J = 267.5 Hz), 102.1. GC-MS (EI, 70 eV): *m/z* (%) = 262 (100), 243 (7), 214 (10), 183 (6), 165 (22), 152 (1), 139 (4), 127 (7), 69 (3). HRMS (+ESI): calcd. for $C_{14}H_{10}F_3N_2$ ($[M+H]^+$): 263.07906, found: 263.07925.

5-(naphthalen-2-yl)-3-(perfluoroethyl)-1H-pyrazole (5e):

Compound **5e** was synthesized following the procedure for compound **5a** using 2-acetonaphthone for the first step and ethyl pentafluoropropionate for the next step. The product was isolated as pale yellow solid (134 mg, 86%). M.p.: 124 – 126 ^{o}C. IR (ATR, cm^{-1}): ν = 3147.0 (w), 3114.4 (w), 2933.1 (w), 1955.6 (w), 1906.3 (w), 1785.8 (w), 1679.0 (w), 1583.7 (w), 1566.5 (w), 1513.6 (w), 1431.1 (w), 1332.5 (s), 1214.1 (s), 1183.3 (s), 1131.7 (s), 1069.4 (m), 1028.9 (s), 940.5 (s), 801.5 (m), 745.4 (s), 619.4 (m). ^{1}H NMR (300 MHz, MeOD) δ 8.21 (d, J = 4.6 Hz, 1H), 8.08 – 7.70 (m, 4H), 7.62 – 7.42 (m, 2H), 7.07 (s, 1H). ^{19}F NMR (282 MHz, MeOD) δ -86.02, -114.00. ^{13}C NMR (63 MHz, MeOD) δ 146.4, 134.8, 130.1, 129.3, 128.8, 127.9, 126.9, 125.8, 124.4, 103.3 (some signals could not be detected). GC-MS (EI, 70 eV): *m/z* (%) = 312 (100), 293 (5), 243 (18), 214 (13), 194 (4), 165 (17), 121 (11), 82 (3), 69 (2). HRMS (+ESI): calcd. for $C_{15}H_{10}F_5N_2$ ($[M+H]^+$): 313.07587, found: 313.07617.

3-(3,3,3,3,3,3,3-heptafluoro-3l8-prop-1-yn-1-yl)-5-(naphthalen-2-yl)-1H-pyrazole (5f):

Compound **5f** was synthesized following the procedure for compound **5a** using 2-acetonaphthone for the first step and ethyl heptafluorobutyrate for the next step. The product was isolated as pale yellow solid (116 mg, 64%). M.p.: 181 – 182 ^{o}C. IR (ATR, cm^{-1}): ν = 3183.6 (w), 3067.3 (w), 2877.8 (w), 1565.2 (w), 1512.8 (w), 1415.8 (w), 1349.1 (m), 1224.1 (m), 1176.5 (s), 1103.7 (m), 1000.7 (m), 874.8 (m), 791.3 (m), 744.3 (m), 651.2 (m). ^{1}H NMR (300 MHz, MeOD) δ 8.26 (s, 1H), 8.12 – 7.72 (m, 4H), 7.68 – 7.44 (m, 2H), 7.11 (s, 1H).^{19}F NMR (282 MHz, MeOD) δ -81.78 (t, J = 9.5 Hz), -111.71 (d, J = 8.6 Hz), -128.28 (s). ^{13}C NMR (75 MHz, Acetone) δ 145.7, 134.4, 134.4, 129.9, 129.2, 128.7, 127.8, 127.8, 125.7, 124.3, 103.6 (some signals could not be detected). GC-MS (EI, 70 eV): *m/z* (%) = 362 (100), 343 (9), 243 (34), 214 (17), 194 (4), 165 (14), 122 (13), 83 (4), 69 (4). HRMS (+ESI): calcd. for $C_{16}H_{10}F_7N_2$ ($[M+H]^+$): 363.007267, found: 363.07305.

3-(trifluoromethyl)-5-(4-(trifluoromethyl)phenyl)-1H-pyrazole (5g)[35]:

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Compound **5g** was synthesized following the procedure for compound **5a** using 4-trifluoromethylacetophenone for the first step and ethyl trifluoroacetate for the next step. The product was isolated as white solid (105 mg, 75%). M.p.: 135 – 136 °C. IR (ATR, cm^{-1}): ν = 3239.9 (w), 3161.7 (w), 3066.3 (w), 2987.4 (w), 1914.8 (w), 1790.5 (w), 1738.4 (w), 1671.9 (w), 1622.3 (w), 1587.6 (w), 1494.7 (w), 1325.4 (m), 1253.0 (m), 1172.6 (m), 1109.0 (s), 1068.0 (m), 982.9 (m), 916.7 (m), 813.6 (m), 747.3 (m), 661.5 (m), 592.3 (m). ^{1}H NMR (300 MHz, MeOD) δ 7.95 (d, *J* = 8.2 Hz, 1H), 7.80 (d, *J* = 8.3 Hz, 1H), 7.12 (s, 1H). ^{19}F NMR (282 MHz, MeOD) δ -63.66, -64.34. ^{13}C NMR (75 MHz, $CDCl_3$) δ 144.2, 143.5 (q, *J* = 38.0 Hz), 131.5 (q, *J* = 33.0 Hz), 131.2, 126.4 (2C, q, *J* = 3.8 Hz), 125.8 (2C), 123.7 (q, *J* = 272.3 Hz), 120.7 (q, *J* = 269.0 Hz), 102.1. GC-MS (EI, 70 eV): *m/z* (%) = 280 (100), 261 (23), 231 (7), 211 (20), 201 (5), 182 (15), 164 (4), 145 (8), 133 (49, 87 (2), 69 (6). HRMS (+ESI): calcd. for $C_{11}H_7F_6N_2$ ($[M+H]^+$): 281.05579, found: 281.05099.

5-(perfluoroethyl)-3-(4-(trifluoromethyl)phenyl)-1H-pyrazole (5h):

Compound **5h** was synthesized following the procedure for compound **5a** using 4-trifluoromethylacetophenone for the first step and ethyl pentafluoropropionatefor the next step. The product was isolated as white solid (122 mg, 74%). M.p.: 142 – 143 °C. IR (ATR, cm^{-1}): ν = 3191.2 (w), 3032.7 (w), 2887.0 (w), 1623.7 (w), 1590.6 (w), 1465.6 (w), 1429.3 (w), 1325.2 (s), 1225.8 (s), 1194.1 (s), 1125.4 (s), 1063.8 (m), 1030.5 (m), 973,8 (m), 939.2 (m), 841.6 (m), 807.8 (m), 750.1 (m), 693.5 (m), 622.0 (w), 591.5 (w). ^{1}H NMR (300 MHz, MeOD) δ 7.96 (d, *J* = 8.2 Hz, 1H), 7.80 (d, *J* = 8.3 Hz, 1H), 7.14 (s, 1H). ^{19}F NMR (282 MHz, MeOD) δ -64.34, -86.12, -114.07. ^{13}C NMR (75 MHz, $CDCl_3$) δ 144.6, 131.7 (q, *J* = 32.9 Hz), 126.4 (2C, q, *J* = 3.8 Hz), 123.8 (q, *J* = 272.2 Hz), 103.9 (some signals could not be detected). GC-MS (EI, 70 eV): *m/z* (%) = 330 (81), 311 (21), 261 (100), 232 (4), 213 (9), 182 (6), 164 (18), 145 (5), 121 (5), 105 (5), 69 (6). HRMS (+ESI): calcd. for $C_{12}H_7F_8N_2$ ($[M+H]^+$): 331.04760, found: 331.04806.

3-(3,3,3,3,3,3,3-heptafluoro-3l8-prop-1-yn-1-yl)-5-(4-(trifluoromethyl)phenyl)-1H-pyrazole (5i):

Compound **5i** was synthesized following the procedure for compound **5a** using 4-trifluoromethylacetophenone for the first step and ethyl heptafluorobutyrate for the next step. The product was isolated as white solid (127 mg, 67%). M.p. 109 – 110 °C. IR (ATR, cm^{-1}): ν = 3171.2 (w), 3034.4 (w), 2950.7 (w), 2887.4 (w), 1623.5 (w), 1591.8 (w), 1467.1 (w), 1424.8 (w),

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1326.4 (s), 1276.0 (w), 1232.3 (s), 1171.5 (m), 1109.2 (s), 1063.0 (s), 1001.4 (m), 876.1 (m), 842.4 (m), 810.1 (m), 747.9 (m), 652.6 (m), 592.2 (m). ^{1}H NMR (300 MHz, MeOD) δ 7.96 (d, *J* = 8.2 Hz, 1H), 7.80 (d, *J* = 8.3 Hz, 1H), 7.14 (s, 1H). ^{19}F NMR (282 MHz, MeOD) δ -64.35 (s), -81.84 (t, *J* = 9.6 Hz), -111.76 (s), -128.29 – -128.38 (m). GC-MS (EI, 70 eV): *m/z* (%) = 380 (64), 361 (23), 261 (100), 213 (8), 182 (5), 164 (17), 69 (6). HRMS (+ESI): calcd. for $C_{13}H_7F_{10}N_2$ ($[M+H]^+$): 381.04441, found: 381.04428.

Synthesis of 2-phenyl-3-(trifluoromethyl)-2H-indazole (6a):

To a solution of **4s** (100 mg) in toluene (7 mL), DDQ (2 equiv.) was added. The reaction mixture was stirred under reflux for 3h. Then the reaction mixture was cooled to room temperature and ethyl acetate (10 mL) and water (10 mL) were added. The organic layer was separated and washed with water three times. After drying and removal of solvent, the residue was purified by chromatography (silica gel, *n*-heptane/DCM). The product was isolated as a yellow oil (71 mg, 71%). IR (ATR, cm^{-1}): ν = 534 (m), 567 (m), 627 (m), 640 (m), 692 (s), 743 (vs), 768 (s), 829 (m), 914 (m), 933 (m), 989 (s), 1001 (s), 1030 (m), 1074 (s), 1103 (vs), 1147 (s), 1174 (s), 1223 (s), 1298 (s), 1381 (w), 1429 (s), 1469 (m), 1500 (s), 1522 (m), 1551 (w), 1597 (m), 2361 (w), 2858 (w), 2929 (w), 3066 (w). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.90 – 7.76 (m, 2H), 7.64 – 7.49 (m, 5H), 7.49 – 7.37 (m, 1H), 7.37 – 7.27 (m, 1H). ^{19}F NMR (282 MHz, $CDCl_3$) δ -54.47 (s). ^{13}C NMR (75 MHz, $CDCl_3$) δ 148.3, 139.7, 130.1, 129.2 (2C), 127.4, 126.3, 126.3, 125.2, 123.8 (q, *J* = 39.5 Hz), 121.7, 121.1 (q, *J* = 269.0 Hz), 119.5 (q, *J* = 1.7 Hz), 118.6. GC-MS (EI, 70 eV): *m/z* (%) = 262 (100), 236 (7), 193 (34), 166 (11), 77 (15), 51 (12). HRMS (EI): calcd. for $C_{14}H_9F_3N_2$ ($[M]^+$): 262.07123, found: 262.07106.

Synthesis of 2-phenyl-3-(trifluoromethyl)-2H-benzo[g]indazole (6b):

To a solution of **4t** (100 mg) in toluene (7 mL), DDQ (2 equiv.) was added. The reaction mixture was stirred under reflux for 3h. Then the reaction mixture was cooled to room temperature and ethyl acetate (10 mL) and water (10 mL) were added. The organic layer was separated and washed with water three times. After drying and removal of solvent, the residue was purified by chromatography (silica gel, *n*-heptane/DCM). The product was isolated as a pale yellow solid (90 mg, 90%). M.p.: 100 – 102 °C. IR (ATR, cm^{-1}): ν = 549 (s), 681 (s), 746 (s), 767 (s), 804 (s), 885 (m), 982 (s), 1045 (s), 1099 (vs), 1176 (s), 1217 (s), 1238 (m), 1269 (m), 1309 (m), 1385 (w), 1441 (s), 1473 (m), 1504 (s), 1558 (w), 1597 (m), 3024 (w), 3049 (w). ^{1}H

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NMR (300 MHz, $CDCl_3$) δ 8.66 (dd, *J* = 5.7, 3.5 Hz, 1H), 7.93 – 7.80 (m, 1H), 7.76 – 7.52 (m, 9H). ^{19}F NMR (282 MHz, $CDCl_3$) δ -54.74 (s). ^{13}C NMR (63 MHz, $CDCl_3$) δ 146.1, 139.7, 132.3, 129.8, 129.1 (2C), 128.5, 127.7, 127.5, 127.3, 126.4, 126.3, 125.0, 124.7 (q, *J* = 39.8 Hz), 122.5, 120.9 (q, *J* = 269.2 Hz), 119.3, 116.9 (q, *J* = 1.9 Hz). GC-MS (EI, 70 eV): *m/z* (%) = 312 (100), 242 (30), 77 (10). HRMS (EI): calcd. for $C_{18}H_{11}F_3N_2$([M]$^+$): 312.08688, found: 312.08667.

3-(4-methoxyphenyl)-5-(trifluoromethyl)isoxazole (8a)[36]:

Compound **8a** was synthesized following the general procedure using 4-methoxyacetophenone, hydroxylamine for the first step and ethyl trifluoroacetate for the next step. The product was isolated as a white (69 mg, 57%). M.p. 76 – 78 ^oC. IR (ATR, cm^{-1}): ν = 3114.5 (w), 2963.7 (w), 2844.2 (w), 1611.5 (m), 1532.2 (w), 1459.8 (m), 1432.7 (m), 1319.7 (m), 1242.4 (m), 1174.7 (m), 1114.0 (s), 1023.3 (m), 966.8 (m), 915.7 (m), 837.2 (m), 821.7 (m), 747.2 (m), 680.0 (w).^1H NMR (300 MHz, $CDCl_3$) δ 7.82 – 7.68 (m, 2H), 7.04 – 6.96 (m, 2H), 6.94 (d, *J* = 0.9 Hz, 1H), 3.87 (s, 3H). ^{19}F NMR (282 MHz, $CDCl_3$) δ -64.24. ^{13}C NMR (75 MHz, $CDCl_3$) δ 162.3, 161.8, 159.1 (q, *J* = 42.4 Hz), 128.6 (2C), 119.9, 118.1 (q, *J* = 270.3 Hz), 114.7 (2C), 103.3 (d, *J* = 2.1 Hz), 55.5. GC-MS (EI, 70 eV): *m/z* (%) = 243 (82), 174 (82), 146 (100), 131 (14), 119 (7), 103 (9), 92 (11), 76 (18), 63 (15), 50 (9). HRMS (+EI): calcd. for $C_{11}H_8O_2F_3N_1$ ([M]$^+$): 243.05016, found: 243.05028.

3-(naphthalen-2-yl)-5-(trifluoromethyl)isoxazole (8b):

Compound **8b** was synthesized following the general procedure using 2-acetonaphthone, hydroxylamine for the first step and ethyl trifluoroacetate for the next step. The product was isolated as a white (82 mg, 62%). M.p. 103 – 104 ^oC. IR (ATR, cm^{-1}): ν = 3232.3 (w), 3126.3 (w), 2921.0 (w), 1631.3 (w), 1486.7 (m), 1438.6 (m), 1303.8 (m), 1143.6 (s), 1104.9 (s), 1057.6 (m), 964.1 (m), 901.5 (m), 827.7 (s), 745.5 (m), 633.1 (w).^1H NMR (300 MHz, $CDCl_3$) δ 8.26 (d, *J* = 0.7 Hz, 1H), 8.00 – 7.84 (m, 4H), 7.64 – 7.48 (m, 2H), 7.14 (d, *J* = 0.9 Hz, 1H).^{19}F NMR (282 MHz, $CDCl_3$) δ -64.15. ^{13}C NMR (75 MHz, $CDCl_3$) δ 162.8, 159.4 (q, *J* = 42.7 Hz), 134.5, 133.2, 129.3, 128.7, 128.1, 127.7, 127.3, 127.2, 124.8, 123.7, 118.1 (d, *J* = 270.5 Hz), 103.7 (d, *J* = 2.1 Hz). GC-MS (EI, 70 eV): *m/z* (%) = 263 (100), 194 (72), 166 (41), 139 (21), 127 (68), 115 (12), 97 (8), 69 (10). HRMS (+ESI): calcd. for $C_{14}H_9O_1F_3N_1$ ([M+H]$^+$): 264.06307, found: 264.06321.

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Biological protocols

Cell Transfection with Human APs and NPPs

COS-7 cells were transfected with plasmids expressing human APs (TNAP & IAP)[37] or human NPPs ((NPP-1)[38] or (NPP-3)[39]) in 10-cm plates, by using Lipofectamine. The confluent cells were incubated for 5 hr at 37°C in DMEM/F-12 in the absence of fetal bovine serum and with 6 µg of plasmid DNA and 24 µL of Lipofectamine reagent. The same volume of DMEM/F-12 containing 20% FBS was added to stop the transfection and cells were harvested 48–72 h later.

Preparation of membrane fractions

The transfected cells were washed three times at 4°C, with Tris–saline buffer, collected by scraping in the harvesting buffer (95 mM NaCl, 0.1 mM PMSF, and 45 mM Tris buffer, pH 7.5) and washed twice by centrifugation at 300×*g* for 5 min at 4°C[37]. Subsequently, cells were resuspended in the harvesting buffer containing 10 µg/mL aprotinin and sonicated. Nuclear and cellular debris were discarded by 10 min centrifugation (300×*g* at 4°C). Glycerol was added to the resulting supernatant at a final concentration of 7.5%.

Samples were kept at -80°C until used. Protein concentration was estimated using Bradford microplate assay and bovine serum albumin was used as a standard.

Protocol of Alkaline Phosphatase Assay (h-TNAP & h-IAP)

A chemiluminescent substrate, CDP-star, was used for the determination of activity of h-TNAP and h-IAP. The conditions for the assay were optimized with the slight modifications in previously used spectrophotometric method.[40] The assay buffer was composed of 2.5 mM $MgCl_2$, 0.05 mM $ZnCl_2$ and 8 M DEA (pH 9.8). Initial screening was performed at a concentration of 0.2 mM of the tested compounds. The total volume of 50 µL contained 10 µL of tested compound (0.2 mM with final DMSO 1% (v/v)), 20 µL of h-TNAP (46 ng of protein from COS cell lysate in assay buffer) or of h-IAP (57 ng protein in assay buffer). The mixture was pre-incubated for 5-7 minutes at 37 ^{o}C and luminescence was measured as pre-read using microplate reader (BioTek FLx800, Instruments, Inc. USA). Then, 20 µL of CDP-star (final concentration of 110 µM) was added to initiate the reaction and the assay mixture was incubated

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for 15 min more at 37 ^{o}C. The change in the luminescence was measured as after-read. The activity of each compound was compared with total activity control (without any inhibitor). Levamisole (2 mM per well) and L-phenylalanine (4 mM per well) were used as a positive control for the inhibition of h-TNAP and h-IAP, respectively. For the compounds which exhibited over 50% inhibition of either h-TNAP activity or h-IAP activity, full concentration inhibition curves were produced to evaluate IC_{50} values. For this purpose 6 to 8 serial dilutions of each compound were prepared in assay buffer and their dose response curves were obtained by assaying each inhibitor concentration against both ALPs using the above mentioned reaction conditions. All experiments were repeated three times in triplicate. The Cheng Prusoff equation was used to calculate the IC_{50} values, determined by the non-linear curve fitting program PRISM 5.0 (GraphPad, San Diego, California, USA).

Protocol of Nucleotide pyrophosphatase (h-NPP-1 & h-NPP-3) activity

The conditions for the assay were optimized with the slight modifications in previously used spectrophotometric method.[41]The reaction was carried out in the assay buffer which contained 5 mM $MgCl_2$, 0.1 mM $ZnCl_2$, 50% glycerol and 50 mMtris-hydrochloride (pH: 9.5). Initial screening was performed at a concentration of 0.1 mM of the tested compounds. The total volume of 100 µL contained 70 µL of the assay buffer, 10 µL of tested compound (0.1 mM with final DMSO 1% (v/v)) and 10 µL of h-NPP-1 (27 ng of protein from COS cell lysate in assay buffer) or 10 µL of h-NPP-3 (25 ng of protein from COS cell lysate in assay buffer). The mixture was pre-incubated for 10 minutes at 37 ˚C and absorbance was measured at 405 nm as pre-read using microplate reader (BioTek FLx800, Instruments, Inc. USA). The reaction was then initiated by the addition of 10 µL of p-Nph-5-TMP substrate at a final concentration of 0.5 mM and the reaction mixture was incubated for 30 more min at 37°C. The change in the absorbance was measured as after-read. The activity of each compound was compared with the reaction in absence of synthesized compounds/inhibitors. The compounds which exhibited over 50% inhibition of either the h-NPP-1 activity or h-NPP-3 activity were further evaluated for determination of IC_{50} values. For this purpose their dose response curves were obtained by assaying each inhibitor concentration against both NPPs using the above mentioned reaction conditions. All experiments were repeated three times in triplicate. The Cheng Prusoff equation

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was used to calculate the IC_{50} values, determined by the non-linear curve fitting program PRISM 5.0 (GraphPad, San Diego, California, USA).

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