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Abstract: The ecto-5'-nucleotidase CD73 is involved in the production of immunosuppressive adenosine in the tumoral microenvironment and recently became a validated target in immuno-oncology. To avoid formation of CD73-produced adenosine, several series of potential inhibitors of the target enzyme based on a triazole scaffold were synthetized and evaluated on recombinant purified *h*CD73 and in cell-based assays.

Introduction

Human ecto-5′ -nucleotidase or *h*CD73 (3.1.3.5), a member of the family of 5′-nucleotidases, is an extracellular enzyme attached to the cell membrane via a glycosylphosphatidylinositol (GPI) anchor.[1] In addition, CD73 exists as a soluble and circulating form with enzymatic activity similar to its membrane anchored form. The crystal structure of *h*CD73 has been reported.[2] This enzyme works as a homodimer and is composed of two structural domains, the N-terminal domain and the C-terminal domain, respectively. During its catalytic activity, CD73 undergoes intra and inter domain movements involving these terminal domains that allows switching between an open and a closed conformation.[2][3] This conformational change is critical to the enzymatic activity. The active site is present in the closed conformation, and is situated at the interface between the N- and C-terminal domains. CD73 catalyzes the dephosphorylation of extracellular adenosine 5'-monophosphate (AMP) into adenosine and inorganic phosphate, and as a consequence mediates the formation of immunosuppressive and pro-tumoral adenosine in the tumor microenvironment.[4] Then, the abundance of adenosine exerts immunosuppressive activity on various immune cells such as myeloid-derived suppressor cells, macrophages, dendritic cells, natural killer cells and effector T cells through A2a and A2b receptors recognition.[5] Thus, CD73 appears as an interesting target in oncology owing to its blockade leading to the decrease of tumor growth and metastasis[6] via the use of small molecules[7] and monoclonal antibodies.[8] As a part of our ongoing work targeting CD73, we recently reported ribonucleotide derivatives as substrate analogue inhibitors and evaluated their potential activity against CD73.[9] An alternative approach to inhibit CD73 is to develop small molecules targeting allosteric sites. Thus, by using a bioinformatics approach[3] we identified a binding cavity, distinct from the active site and located at the dimer interface. Such potential inhibitors could block the conformational change and consequently lead to the inhibition of the enzyme. Through molecular docking predictions, we detected suitable scaffolds that could potentially interact with this cavity. Notably, several compounds containing an imidazole ring substituted by aromatics were identified as interesting hits. Some of them being commercially available, they could be evaluated by enzymatic tests and turned out to be strong enzymatic inhibitors. Based on the results from this virtual screening targeting the dimerization site of CD73, we focused on a particular active hit bearing three substitutions on a 1,3-diazole scaffold doubly branched with a biphenyl substituent plus one indole group (hit compound RR3, 93±5% inhibition at 5 µM, Ki = 0.53 µM, Figure 1).[3] Moreover, seven commercially available analogues (AB02, AB03, AB04, AB05, AB06, AB07, AB38) were tested after this hit identification and all analogues showed a significant inhibition of *h*CD73 activity (78%-94% inhibition at 5 µM). However, numerous analogues of RR3 are patented[10] and their synthetic accessibility is less easy than the triazole ring.[11] Consequently, chemical synthesis of new derivatives was envisaged using triazole as more versatile scaffold instead of diazole in order to explore chemical diversity. Herein, we report the synthesis of potential inhibitors of CD73 based on a triazole scaffold (Figure 1) and the evaluation of their effects *in vitro*.

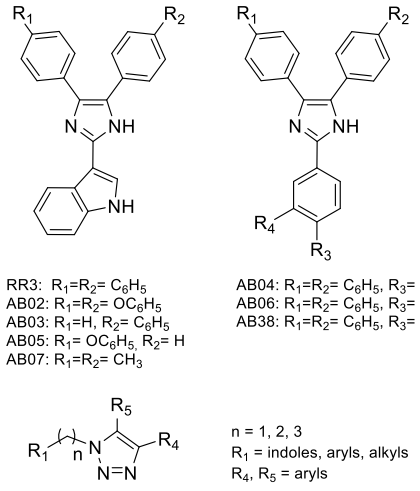


Figure 1. Previously identified 1,3-diazole compounds (RR3 and AB02-38) and proposed analogues based on a triazole scaffold.

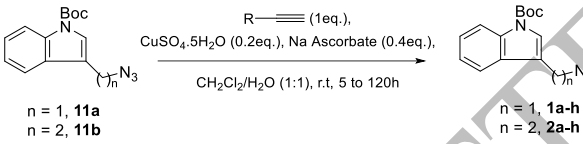
Results and Discussion

Synthesis

The Cu(I)-catalyzed azide alkyne cycloaddition reaction (CuAAc) developed by Sharpless[12] *et al.* allows the synthesis of a huge varieties of 1,4-disubstituted 1,2,3-triazoles from commercially available or synthetic azides and alkynes derivatives. Herein, the synthesis of 1,4-disubstituted 1,2,3-triazoles bearing different aromatic cycles are presented as well as their transformations into more complex structures.

The first series of compounds considered contains an indole framework as RR3. Indeed, the insertion of indoles on heterocyclic compounds have been extensively investigated due to their wide spectrum of pharmacological activity. Numerous natural and synthetic derivatives of indole have shown a wide spectrum of pharmacological properties including antibacterial[13] and anticancer activities.[14] Its preparation was accomplished from common derivatives **11a** and **11b**, obtained following reported and adapted procedures[15] and various alkynes (procedure A). The aromatic amine must be protected to incorporate the azido group when n = 1. Formation of the triazole ring (Table 1, compounds **1a**-**h**, n = 1 or **2a**-**h**, n = 2) was achieved in a mixture of dichloromethane/water with catalytic amount of copper sulfate pentahydrate reduced *in situ* to Cu(I) by sodium ascorbate. Most of the time, the click reaction lasted 5 hours to complete. In this series, we obtained heterocycles with a short linker with good yields (Entry 1-8, n = 1, compounds **1a**-**h,** 35%-97%) and analogues containing an expanded linker (Entry 9 to 16, n=2, compounds **2a**-**h,** 68%-100%) in order to probe the influence of the carbon chain's length against our target.

Table 1. Synthesis of 1,4-disubstituted triazoles bearing N-protected indole



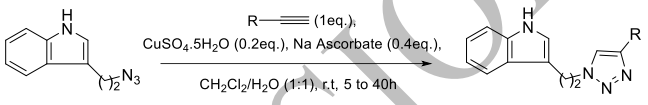
n = 1, **11a**
n = 2, **11b**

n = 1, **1a-h**
n = 2, **2a-h**

Entry	n/R	Cpd	Time [h]	Yield [%]
1	1/benzyl	**1a**	5	92
2	1/4-tolyl	**1b**	5	97
3	1/3-aminophenyl	**1c**	5	85
4	1/4-methoxyphenyl	**1d**	5	92
5	1/hydroxy(phenyl)methyl	**1e**	5	81
6	1/benzoyl	**1f**	5	80
7	1/4-fluorophenyl	**1g**	5	85
8	1/pyridin-2-yl	**1h**	72	85
9	2/benzyl	**2a**	5	90
10	2/4-tolyl	**2b**	5	96
11	2/3-aminophenyl	**2c**	96	68
12	2/4-methoxyphenyl	**2d**	5	100
13	2/hydroxy(phenyl)methyl	**2e**	5	76
14	2/benzoyl	**2f**	5	74
15	2/4-fluorophenyl	**2g**	23	80
16	2/pyridin-2-yl	**2h**	120	98

Because the elimination of the Boc protective group in standard TFA conditions[16] lead to degradation of the indole moiety, milder acidic conditions of deprotection have been attempted leading to the same results.[17] Thus, compounds **1a**-**h** and **2a**-**h** were directly evaluated for their activity on *h*CD73 and on tumor cell lines. Then, we envisaged the synthesis of 1,4-disubstituted 1,2,3-triazoles bearing a free indole. Compound **12** was obtained following reported procedures[18] and reacted with different alkynes using similar conditions presented in Table 1, to afford the corresponding 1,4-disubstituted 1,2,3-triazoles (Table 2, Entry 1-5, compounds **3a-e,** 44%-89%). Despite many attempts, the synthesis of analogue of compound **12**, in which the indole moiety is directly linked to the azido group, as previously reported,[19] did not give any result.

Table 2. Synthesis of 1,4-disubstituted triazoles bearing free indole.

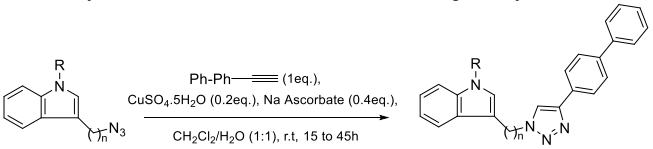


12 **3a - e**

Entry	R	Cpd	Time [h]	Yield [%]
1	4-tolyl	**3a**	5	80
2	4-methoxyphenyl	**3b**	6	65
3	4-biphenyl	**3c**	15	44
4	4-phenoxyphenyl	**3d**	5	85
5	4-cyanophenyl	**3e**	40	89

Owing to the efficiency of the CuAAc reaction, we also applied it upon alkylated indoles containing azide with good yields (Table 3, Entry 1-2, compounds **4a** and **4b**, 73%-79%). Nevertheless, starting compounds **13a** (see supporting information) and **13b** [20] were obtained with low yields, due to low reactivity and stability of their respective alkylated intermediates. Thus, another synthetic strategy was considered.

Table 3. Synthesis of 1,4-disubstituted triazoles bearing N- alkylated indole.



n = 1, R = $(CH_2)_2CN$, **13a**
n = 2, R = Me, **13b**

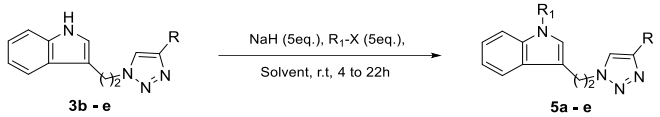
n = 1, **4a**
n = 2, **4b**

Entry	n/R	Cpd	Time [h]	Yield [%]
1	1/propanenitrile	**4a**	45	79
2	2/methyl	**4b**	15	73

Amine alkylation upon free indole was more effective once the triazole moiety was formed. This allowed us to easily prepare new derivatives with N-alkylated indoles starting from previous compounds **3b-e**, that are represented in Table 4 (Entry 1-5, compounds **5a-e**, 47%-92%). The amine alkylation was achievable with a large excess of sodium hydride and alkylating

agent in anhydrous THF. Low solubility of starting materials **3b-c** in THF forced us to use DMF as co-solvent (Entry 2-4).

Table 4. Amine alkylation upon free indole-containing 1,4-disubstituted triazole.

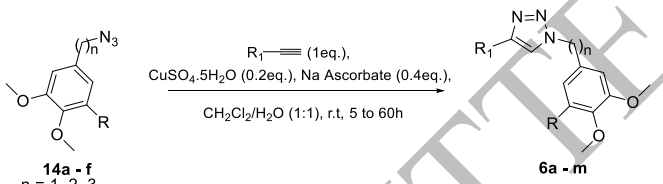


3b - e **5a - e**

Entry	R/R_1	Solvent	Cpd	Time [h]	Yield [%]
1	4-cyanophenyl/Me	THF	**5a**	14	47
2	4-biphenyl/propanenitrile	THF/DMF (5:1)	**5b**	4	84
3	4-methoxyphenyl/TEG	THF/DMF (17:1)	**5c**	16	85
4	4-biphenyl/TEG	THF/DMF (9:1)	**5d**	4	92
5	4-phenoxyphenyl/TEG	THF	**5e**	22	84

As we faced many difficulties during the synthesis of azido-derivatives containing an indole moiety (vide supra), we decided to replace it by aromatic residues analogous to AB06 and AB38 derivatives. Thus, we prepared 1,4-disubstituted 1,2,3-triazoles bearing di- or tri-methoxybenzene substituent instead of the indole moiety (Table 5, compounds **6a**-**m**), using similar conditions as presented above (procedure A).

Table 5. Synthesis of 1,4 disubstituted triazole bearing aromatic derivatives.



14a - f
n = 1, 2, 3
R = H, OMe

6a - m

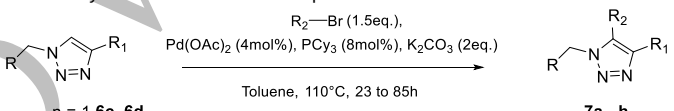
Entry	n/R/R_1	Cpd	Time [h]	Yield [%]
1	1/H/4-biphenyl	**6a**	60	96
2	1/H/4-phenoxyphenyl	**6b**	60	80
3	1/OMe/4-biphenyl	**6c**	4	84
4	1/OMe/4-phenoxyphenyl	**6d**	15	94
5	2/H/4-biphenyl	**6e**	4	80
6	2/H/4-phenoxyphenyl	**6f**	14	88
7	2/H/4-cyanophenyl	**6g**	22	71
8	2/OMe / 4-biphenyl	**6h**	60	87
9	2/OMe/4-phenoxyphenyl	**6i**	14	64
10	3/H/4-biphenyl	**6j**	13	49 [a]
11	3/H/4-phenoxyphenyl	**6k**	15	55 [a]
12	3/OMe/4-biphenyl	**6l**	15	33 [a]
13	3/OMe/4-phenoxyphenyl	**6m**	15	64 [a]

[a] Yield over 3 steps from the corresponding alcohol precursor

Azido derivatives **14a-f** were synthesized from their commercially available precursors (hydroxyl or carboxylic acid form) following reported procedures.[21] Briefly, using diphenylphosphoryl azide (DPPA) and DBU, benzyl derivatives **14a-b** (n = 1, R=H or OMe) were obtained in one step. Compounds **14c-f**, with longer chain (n = 2 or 3, R = H or OMe), were obtained in a two steps procedure: formation of *O*-tosylated derivative and nucleophilic substitution using NaN_3 as incoming nucleophile. Combination between those azides and two commercial alkynes containing aromatic cores, such as 4-biphenyl and 4-phenoxyphenyl, led to the expected derivatives (Table 5). Incorporation of a 4-cyanophenyl group was also accomplished (Entry 7, **6g**, 71 %) and can be used as starting material for the subsequent synthesis of more complex structures. In this series, we could afford 1,4-disubstituted 1,2,3-triazoles with good yields for compounds **6a-i** (Table 5, entry 1-9, 64%-96%) and modest yields for compounds **6j-m** (Table 5, entry 10-13, 33%-64%, over 3 steps). Indeed, when n = 3, an impurity was recovered with the tosyle intermediate and could not be separated from the azide, and thus finally removed after the triazole formation.

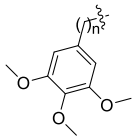
The third series corresponds to more functionalized entities such as 1,4,5-trisubstituted 1,2,3-triazoles based on **6c**-**d**, **2b**, **4b**, **5d** and **5e** as starting materials. The modified Heck coupling developed by Ackermann *et al*.[22] and catalyzed by palladium, allowed us to obtain derivatives presented in Table 6.

Table 6. Synthesis of tetrazole core upon indole scaffold.



n = 1 **6c, 6d**
n = 2 **2b, 4b**
n = 2 **5d, 5e**

7a - h

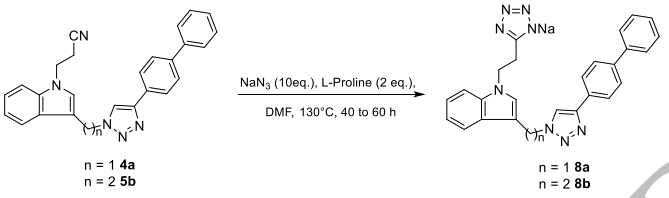
Entry	R	R_1	R_2	Cpd	Yield [%]
1		4-biphenyl	4-biphenyl	**7a**	71
2		4-biphenyl	4-phenoxy phenyl	**7b**	76
3		4-phenoxy phenyl	4-biphenyl	**7c**	80
4		4-phenoxy phenyl	4-phenoxy phenyl	**7d**	74
5	1-*N*-Boc-3-CH_2-indole	4-methyl phenyl	4-biphenyl	**7e**	36
6	1-*N*-methyl-3-CH_2-indole	4-biphenyl	4-biphenyl	**7f**	51
7	1-*N*-TEG-3-CH_2-indole	4-biphenyl	4-biphenyl	**7g**	21
8		4-phenoxy phenyl	4-biphenyl	**7h**	29

With an excess of brominated agent, the Heck coupling was achievable upon 1,4-disubstituted 1,2,3-triazoles with a catalytic amount of commercially available palladium acetate reduced *in situ* by tricyclohexylphosphine. The reaction was carried out in refluxing toluene for several hours under basic conditions (procedure B). To investigate the influence of the 4-biphenyl and 4-phenoxyphenyl moieties toward our target, we accomplished the synthesis of **7a-d** from **6c-d** with good yields (71%-80%). This

reaction was also applied to 1,4-disubstituted 1,2,3-triazoles bearing *N*-substituted indole and gave rise to compounds **7e** and **7f** with modest yields (36%-51%). The presence of the indole moiety (**2b**, **4b**, **5d** and **5e**) gave rise to the formation of side products and purification of compounds **7e-h** was tedious. Unfortunately, synthesis of 1,4,5-trisubstituted 1,2,3-triazoles could not be done using free indole derivative. We did not observe any reactivity, probably due to the irreversible chelation of palladium by the amine function.

Finally, and in order to solve solubility issues encountered during biological tests, we decided to introduce polar moieties such as a tetrazole ring or the sulfonamide group. We firstly optimized the tetrazole synthesis with a large excess of sodium azide and L-proline in DMF at 130° C. L-Proline was initially used in catalytic amounts[23] but the reaction was not complete after 3 days. Adding up to 2 equivalents of amino-acid and 10 equivalents of sodium azide allowed us to obtain a rapid conversion. These conditions were applied to **4a** and **5b** and provided compound **8a-b** with low to modest yields (Table 7, entry 1-2, 27%-60%). As previously observed the presence of the indole moiety leads to incomplete reactions and partial degradation of the starting materials.

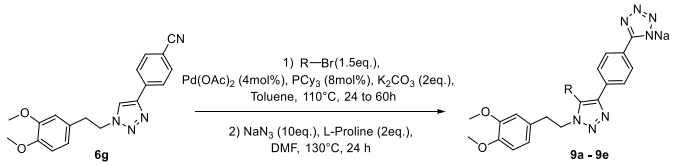
Table 7. Synthesis of tetrazole core upon indole scaffold.



Entry	n	Cpd	Time [h]	Yield [%]
1	1	**8a**	62	27
2	2	**8b**	39	60

Incorporation of the tetrazole ring was also achieved upon 1,4,5-trisubstituted 1,2,3-triazoles, previously obtained from compound **6g** as shown in Table 8 (procedure C). Except for compound **9a** (Entry 1, 85%) for which the tetrazole synthesis was directly achieved, the formation of the latter was subsequently done after the Heck coupling (compounds **9b**-**e**, 2 steps, 42%-47%). In spite of the difficulty to form two tetrazole rings instead of one, we could obtain compound **9b** (Entry 2, 42% over 2 steps). We have also synthesized compounds **9c-e** (Entry 3-5, 47%-77% over two steps) despite the formation of light sensitive intermediates after the Heck coupling. As in the case of compound **9b**, the synthesis of the two tetrazole rings of compound **9e** (Entry 5) was delicate and lead to incomplete conversion in both cases.

Table 8. Synthesis of 1,4-disubstituted and 1,4,5-trisubstituted triazoles bearing tetrazole group.



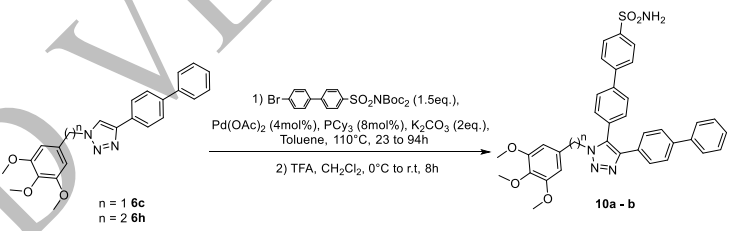
[a] Yield over 2 steps

Entry	R	Cpd	Time [h]	Yield [%]
1	H	**9a**	48h	85
2	(tetrazolemethyl)phenyl	**9b**	17h	42
3	4-biphenyl	**9c**	20h	77[a]
4	4-phenoxyphenyl	**9d**	20h	74[a]
5	(tetrazole)phenyl	**9e**	20h	47[a]

To access the sulfonamide form of 1,4,5-trisubstituted 1,2,3-triazoles starting from **6c** and **6h,** the sulfonamide moiety has to be protected by a Boc group. Then, cleavage of the carbamate bond was accomplished with an excess of trifluoroacetic acid in anhydrous dichloromethane to afford compounds **10a-b** (Table 9, entry 1-2, 25%-35% over two steps).

Due to low reactivity and solubility of the starting materials in toluene, both reactions were not complete, and led to the formation of the desired compounds as well as their mono-deprotected forms that were pooled for the final cleavage step.

Table 9. Synthesis of sulfonamide derivatives containing 1,4,5-trisubstituted triazole.



[a] Yield over 2 steps

Enzymatic inhibition assays

Entry	n	Solvent [step 1]	Cpd	Time [h] [step: 1 / 2]	Yield [%]
1	1	Toluene	**10a**	23 / 8	35[a]
2	2	Toluene/ DMF (3:1)	**10b**	94 / 8	25[a]

The compounds were evaluated by kinetics inhibition assays using the recombinant purified enzyme. At high concentration (100 µM) many compounds were able to inhibit CD73 activity (23 derivatives inducing more than 80% of enzyme inhibition, see Table in SI) while a more limited number of derivatives was still active at 10 µM. Compounds **4b**, **5b**, **6a**, **7c** and **10a** were the most efficient inhibitors at this lower concentration with 68 to 87% of enzyme inhibition.

Cell-based assays

Compounds were also evaluated on their capacity to inhibit the membrane-bound CD73, using CD73-expressing breast cancer cells (MDA-MB-231) as earlier reported.[9a] Many compounds precipitated in the experimental conditions and were not evaluated at 100 µM. Some compounds showed decent inhibitory activity (25-48%) at 100 µM (**1d-h**, **2a-c**, **2e-h**, **3c-d**, **6c-d**, **8b** and **9d**), whereas none reached 25% when evaluated at 10 µM. The three days survival of cancer cells exposed to these compounds was rather low at 100 µM for the majority of the compounds,

whereas only low (<35%) for **3c** and **5b** at 10 µM (see Table in SI).

Molecular modelling and docking

Molecular docking was carried out in order to better understand the structural rules leading to weak or strong activity of newly synthesized compounds. As shown in Fig. 2, the docking pose obtained for original hit **RR3** was compared with commercially available structural analogues. The common diazole scaffold was found to be perfectly superimposed for all of them and in most cased the phenyl groups as well.

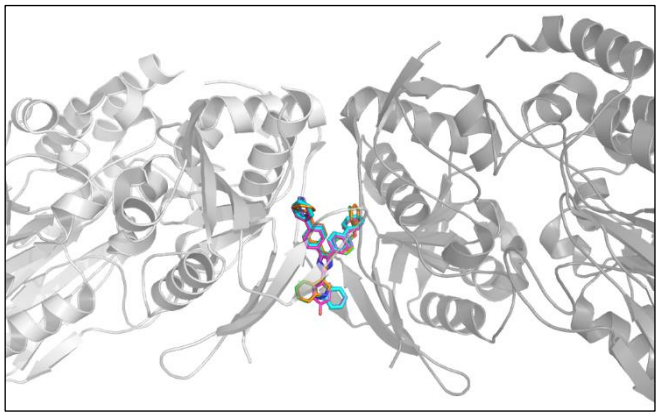


Fig 2: Binding pose obtained by docking at dimerization interface of CD73 dimeric enzyme (grey cartoon representation) for original hit **RR3** (cyan thick sticks), AB02 (orange), AB03 (blue), AB05 (green) and AB06 (pink).

The analysis of the binding mode of compound **5b** showed that the indole group was trapped between three tyrosine residues (Tyr-531 from both chains and Tyr-539, Fig. 3B). Biphenyl group is interacting with CD73 by making hydrophobic contacts with Glu-543 (both chains) and one hydrogen bond with Gly-454. Interestingly, two π-cation interactions were observed between Arg-545 and triazole moiety and between His-456 and the second phenyl group. The cyano group contributes to the binding through hydrogen bonding and hydrophobic contact with Tyr-531. A very similar binding mode was observed for derivative **4b** (Fig. 3A, highly superimposable to that of **5b**) but without the contacts induced by the cyano group.

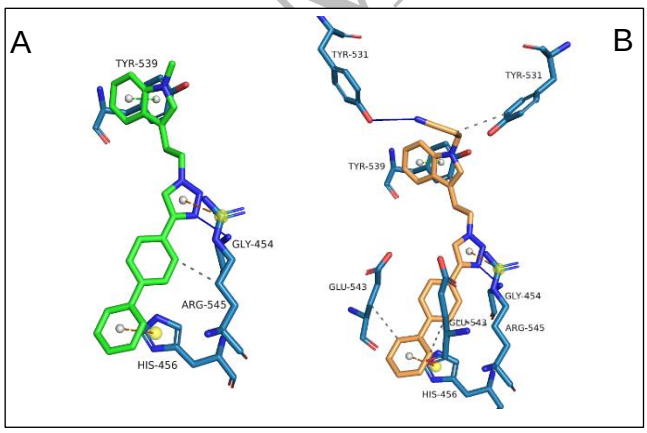


Fig. 3: Similar binding mode for compounds **4b** (panel A, green sticks) and **5b** (panel B, orange sticks). CD73 residues are depicted as blue sticks (yellow and white spheres indicate π-cation and π-stacking interactions, respectively).

Subtle modifications such as a methyl group introduced on indole (**4b**) seemed to strengthen the stacking with Tyr-539 when compared to the structure of derivative **3c** even if binding modes were found identical (Fig. 4A). Also, the replacement of the biphenyl group by a phenoxyphenyl in series 6 induced a shift in the binding mode in addition to additional contacts. The resulting orientation did not improve the inhibitory activity for derivative **6b** in comparison to that of **6a** (Fig. 4B).

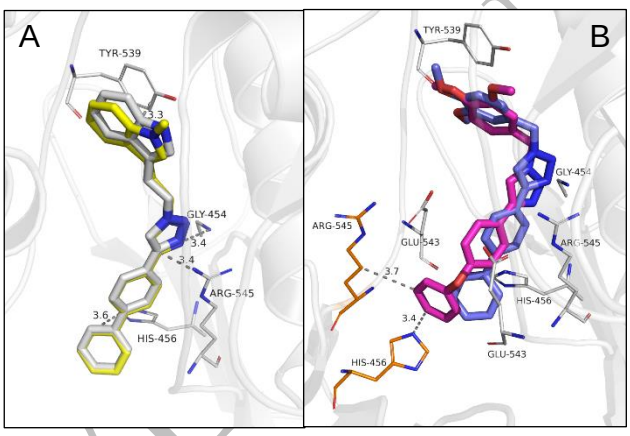


Fig 4: A) Overlay of binding poses obtained by docking for derivative **3c** (white sticks) and **4b** (yellow sticks). B) Changes in binding orientation between derivatives **6a** (blue) and **6b** (pink). The additional residues interacting with **6b** are shown as thin orange stick).

The length of the linker (n = 1 or 2) between triazole and indole or di/tri-methoxyphenyl was found to play a role for enzymatic inhibition. Indeed, for compounds **8a** (n = 1) and **8b** (n = 2), a stronger inhibition was observed for **8a** and these compounds were found to bind the cavity similarly but with a notable shift (Fig. 5A) leading to weaker interactions between biphenyl group and His-456 residues. Another example is given by dimethoxyphenyl-based derivatives **6a** (n = 1, 68% of inhibition at 10 µM) and **6e** (n = 2, 0% of inhibition at 10 µM) therefore not equivalent in terms of inhibition but exhibiting the same positional shift (Fig. 5B). In addition, the same observation was made in series 3 for derivatives **10a** and **10b** (Fig. 5C). Here again, the impact of the linker length is confirmed by a shift in the binding mode between these two compounds, complemented by the loss of the interaction with Asp-366 (interaction with the sulfonamide group) for **10b**. This feature may represent a general rule for series 2 and 3 with a shorter linker being more advantageous for enzymatic inhibition. However, the impact seems to be different according to the series and the substituting groups used as seen by the exception with compounds **4a** and **5b** (linker n = 2 promoting a stronger inhibition than n = 1).

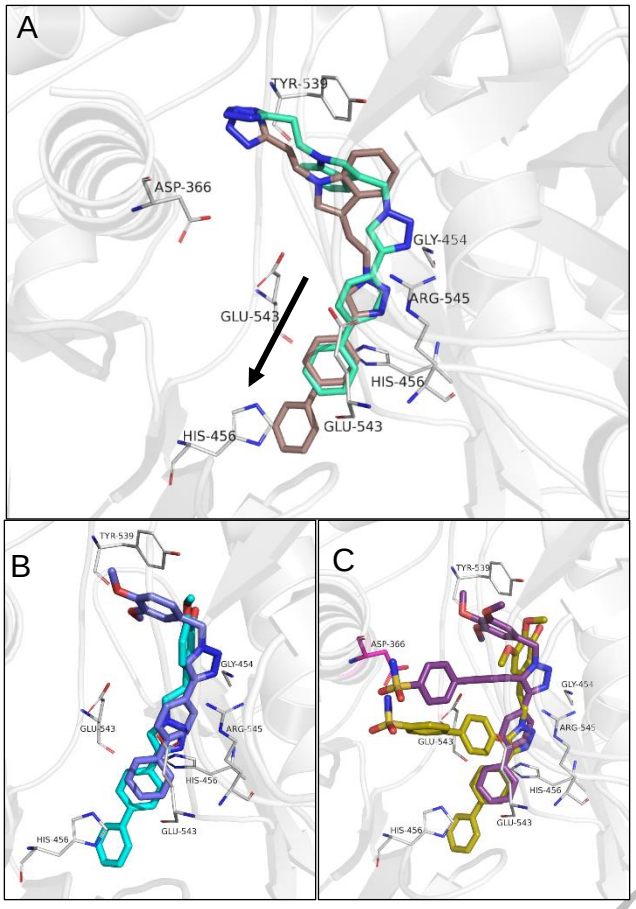


Fig. 5: Impact of the linker chain on binding and efficiency of derivatives. A) Binding modes obtained for indole-derived compounds **8a** (depicted in light green sticks) and **8b** (brown sticks) highlighting the positional offset (indicated by the arrow). B) Comparison of derivatives **6a** (blue) and **6e** (cyan). C) Comparison of docking poses between derivatives **10a** (purple) and **10b** (dark yellow) and positional shifting with loss of interaction with Asp-366 (pink).

Conclusion

Three series of 1,4-disubstituted 1,2,3-triazoles bearing different aromatic cycles as well as complex structures were successfully obtained. All synthesized compounds were evaluated for their ability to inhibit 5'-ectonucleotidase CD73. Among them, compounds **4b**, **5b**, **6a**, **7c** and **10a** were the most potent inhibitors at 10 µM while more than 20 derivatives producing inhibition superior at 80% of *h*CD73 at 100 µM. However, these compounds were much weaker inhibitor than the original hit RR3. Several factors could be responsible for the weaker activity such as changing imidazole scaffold into triazole one as well as the nature of substituents/length of the linker which could promote orientation more or less favourable for the interactions with the protein target. When tested in the cell based assay, all the compounds showed marginal inhibition of CD73. We assume that these differences may arise from solubility issues in the culture media and/or the use of the soluble form of the protein, whereas in the cell-based assay the protein is anchored to the membrane. In addition, molecular modeling has helped to identify the interactions between the targeted protein and the compounds exhibiting the best inhibition. Considering the literature data related to the design of inhibitors of CD73, these results will certainly contribute to the establishment of a SAR study and further development of novel derivatives.

Supplementary Information

Experimental procedures, compound characterizations, NMR spectra, data for enzymatic inhibition, cell-based assays and molecular docking methods are provided in supporting information.

General procedure A: 1,4-disubstituted 1H-1,2,3-triazole synthesis. To a stirred solution of appropriate azide (0.26 mmol) and alkyne (0.26 mmol) in CH_2Cl_2 (2 mL) was added 2 mL of an aqueous solution of a mixture of copper sulfate pentahydrate (0.05 mmol) and sodium ascorbate (0.10 mmol). The mixture was stirred under an argon atmosphere at room temperature for the indicated time, then diluted with CH_2Cl_2 (60 mL) and water (30 mL). The aqueous layer was extracted with CH_2Cl_2 (3 x 60 mL). The organic layers were combined, washed with 100 mL of an aqueous solution of EDTA (1%) and ammonia (28%) in a 1:1 ratio, brine (100 mL), dried (Na_2SO_4), filtered and concentrated in vacuo. Purification was achieved by flash chromatography on silica gel to provide the desired product.

General procedure B: 1,4,5-trisubstituted 1H-1,2,3-triazole synthesis. Appropriate 1,4 disubstituted 1H-1,2,3 triazole (0.074 mmol), aryl bromide (0.111 mmol), $Pd(OAc)_2$ (0.003 mmol), PCy_3 (0.006 mmol) and K_2CO_3 (0.149 mmol) were suspended, under an argon atmosphere, in dry toluene (1 mL) then heated at 110 °C for the indicated time. The reaction mixture was diluted with CH_2Cl_2 (20 mL) and water (10 mL). The aqueous layer was extracted with CH_2Cl_2 (3 x 20 mL). The organic layers were combined, washed with a saturated solution of $NaHCO_3$ (30 mL), water (30 mL), brine (30 mL), dried (Na_2SO_4), filtered and concentrated *in vacuo*. Purification was achieved by flash chromatography on silica gel to provide the desired product.

General procedure C: tetrazole synthesis. Appropriate nitrile (0.156 mmol), NaN_3 (1.56 mmol), and L-Proline (0.312 mmol) were suspended, under an argon atmosphere, in dry DMF (2 mL) then heated at 130 °C for the indicated time. Solvent was removed *in vacuo*. Purification was achieved by reverse-phase column chromatography (H_20/MeOH, 0 to 100%) followed by ion exchange on Dowex 50WX2 (Na^+ form) and freeze-drying to provide the desired product.

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Conflict of Interest
The authors declare no conflict of interest.

Keywords: 5'-ecto-nucleotidase • CD73 • enzymatic inhibitors • triazole derivatives • immuno-oncology

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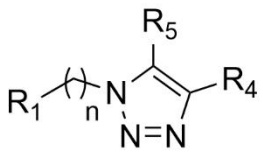

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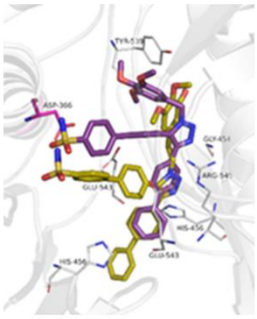

Several series of substituted 1,4,5-triazole derivatives, as inhibitors of 5'-ectonucleotidase CD73, were synthetized and evaluated for their potential inhibitory effect on purified recombinant *h*CD73 and in cell-based assays.