



Schiff bases of tryptamine as potent inhibitors of nucleoside triphosphate diphosphohydrolases (NTPDases): Structure-activity relationship

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ARTICLE INFO

Keywords:

Schiff bases

Tryptamine

Nucleoside triphosphate diphosphohydrolases

In vitro

Structure-activity relationship

ABSTRACT

Overexpression of NTPDases leads to a number of pathological situations such as thrombosis, and cancer. Thus, effective inhibitors are required to combat these pathological situations. Different classes of NTPDase inhibitors are reported so far including nucleotides and their derivatives, sulfonated dyes such as reactive blue 2, suramin and its derivatives, and polyoxometalates (POMs). Suramin is a well-known and potent NTPDase inhibitor, nonetheless, a range of side effects are also associated with it. Reactive blue 2 also had non-specific side effects that become apparent at high concentrations. In addition, most of the NTPDase inhibitors are high molecular weight compounds, always required tedious chemical steps to synthesize. Hence, there is still need to explore novel, low molecular weight, easy to synthesize, and potent NTPDase inhibitors.

Keeping in mind the known NTPDase inhibitors with imine functionality and nitrogen heterocycles, Schiff bases of tryptamine, **1–26**, were synthesized and characterized by spectroscopic techniques such as EI-MS, HREI-MS, ¹H-, and ¹³C NMR. All the synthetic compounds were evaluated for the inhibitory avidity against activities of three major isoforms of NTPDases: NTPDase-1, NTPDase-3, and NTPDase-8. Cumulatively, eighteen compounds were found to show potent inhibition ($K_i = 0.0200\text{--}0.350\ \mu\text{M}$) of NTPDase-1, twelve ($K_i = 0.071\text{--}1.060\ \mu\text{M}$) of NTPDase-3, and fifteen compounds inhibited ($K_i = 0.0700\text{--}4.03\ \mu\text{M}$) NTPDase-8 activity. As a comparison, the K_i s of the standard inhibitor suramin were 1.260 ± 0.007 , 6.39 ± 0.89 and $1.180 \pm 0.002\ \mu\text{M}$, respectively. Kinetic studies were performed on lead compounds (**6**, **5**, and **21**) with human (*h*-) NTPDase-1, -3, and -8, and Lineweaver-Burk plot analysis showed that they were all competitive inhibitors. *In silico* study was conducted on compound **6** that showed the highest level of inhibition of NTPDase-1 to understand the binding mode in the active site of the enzyme.

1. Introduction

Nucleoside triphosphate diphosphohydrolases (NTPDases) comprises of two families ectoenzymes and endoenzymes located at various sites in the body. They exist in eight different isoforms designated as

NTPDase 1–8, out of which NTPDase-1 to -3 and NTPDase-8 are located on the cell surface. These enzymes can hydrolyze extracellular nucleotides ATP and ADP to AMP [1]. The catalytic activity of these enzymes can be stimulated through milli-molar concentrations of either Ca^{2+} or Mg^{2+} ions. NTPDases may exist in monomeric, dimeric or

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<https://doi.org/10.1016/j.bioorg.2018.10.046>

Received 6 August 2018; Received in revised form 18 October 2018; Accepted 23 October 2018

Available online 30 October 2018

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trimeric forms. NTPDase-1 hydrolyze the ATP and ADP equally, while NTPDases-2, -3, and -8 preferentially hydrolyze ATP. NTPDases express themselves in different tissues such as NTPDase-1 is found to be expressed on natural killer cells, subsets of activated T cells, monocytes, dendritic cells, and on vascular endothelium. NTPDase-1 enzyme controls the cellular immune response by modulating purinergic signaling. It hydrolyzes prothrombotic ADP, maintains vascular fluidity, modulates thrombosis, vascular inflammation, and plays a major role in cerebro- as well as cardio-protections [2]. NTPDase-1 also controls vaso-relaxation that is endothelial P2Y₂ receptor-dependent [3]. NTPDase-2 hydrolyzes nucleoside triphosphates and affords ADP and UDP as the main products [4]. NTPDase-3 is mainly expressed in subsets of neurons in the brain, in various epithelial cells including kidney, airways, digestive, reproductive systems, and in islet of langerhans [5]. The last NTPDase member identified, NTPDase-8, is mainly expressed in the liver, intestine, and kidney. The adenine nucleotides such as ATP and ADP that are released by the cells mainly regulate the vascular response to endothelial injuries. Adenosine acts as an inhibitor of platelet aggregation, however, ADP promotes it and acts on specific receptors. The ectonucleotidases interfere with purinergic signaling pathways and are recognized as pharmaceutical agents. The expression of E-NTPDases at the cell surface is associated with the development of certain pathologies, particularly proliferation of cancer cells. E-NTPDases expression is associated to the virulence of certain parasites and bacteria by protecting these pathological agents from the immune response of the host. The over-expression of E-NTPDases is found to be associated with human differentiated melanoma and myeloid leukocytes cells. The over expression of E-NTPDases at the cell surface reduced the interaction with T-lymphocytes and thus escaping reconnaissance mechanism of the immune system. These results suggest that E-NTPDases could be implicated into a cell-to-cell recognition. Inhibition or modulation of E-NTPDases may prolong the effect of nucleotides at their respective receptors or modulate cell-to-cell interaction [6]. NTPDase inhibitors are found to be important tools as potential therapeutics against pain, cancer, and autoimmune disorders [7–12]. The good NTPDase inhibitors should be specific and should not be P2 receptor antagonist. Some specific inhibitors for NTPDase-1 have been reported *i.e.* ticlopidine an antiplatelet drug.

Schiff bases are condensed products and formed *via* the nucleophilic attack of nitrogen on carbonyl carbon (aldehyde/ketone) thus forming an imine or azomethine bond. They can behave as precursors for the synthesis of various natural products. They are easy to synthesized and due to having special feature C=N group, are considered as good chelating agents [13–15]. Its chelating ability increases if present just adjacent to electron donating substituents like OH and SH on the aryl part. They are amongst the most widely used organic compounds and usually utilized as pigments, dyes, catalysts, polymer stabilizers, and intermediates in organic synthesis [15]. Schiff bases have also been shown a broad range of biological activities including antifungal, antibacterial, anti-malarial, antiproliferative, antiinflammatory, antiviral, and antipyretic activities [16–23]. Our research group had also extensively reported the different biological aspects of Schiff bases *e.g.* antibacterial/anti-biofilm, antioxidant, anticancer, α -glucosidase, β -glucuronidase, and carbonic anhydrase inhibitory activities [24].

Efficacious inhibitors of NTPDases are always required to reduce responses induced by nucleotides in a number of pathological situations such as thrombosis, inflammation, and cancer. Three different classes of NTPDase inhibitors are reported so far including nucleotides and their derivatives (8-Bus ATP), sulfonated dyes such as reactive blue 2, suramin and its derivatives, and polyoxomatalates (POMs) (Fig. 1) [1,7–12,25,26].

Despite being a potent inhibitor, a range of side effects are associated with suramin such as abdominal pain, vomiting, and diarrhoea. Furthermore, various sensations in the skin including tingling sensations, numbness of arm, hands, and legs, tenderness of palms and soles as well as swelling and skin rash are also included. In addition, during

usage, it also make the urine cloudy [27,28]. Furthermore, most commonly known NTPDase inhibitors are high molecular weight compounds, containing multiple acidic groups such as sulfonic acid in suramin. It also requires tedious steps to synthesize these high molecular weight compounds.

Literature revealed that some of the NTPDase inhibitors possess imine (Schiff base) moiety and many inhibitors including nucleotides contain nitrogen heterocycle in the structure [26,29]. These nucleotides specially, purines have fused ring structure with nitrogen atoms. Hence, due to structural resemblance of tryptamine with purine, we intended to install Schiff base functionality on tryptamine to evaluate as inhibitors of NTPDases (Fig. 2). Furthermore, it is worth-mentioning that our compounds are easy to synthesize and requires only a single step transformation of inexpensive chemicals without any harsh conditions, thus can be called as green synthesis. Herein, we report the synthesis, structural characterization, and evaluation of Schiff bases of tryptamine as NTPDase inhibitors.

2. Results and discussion

2.1. Chemistry

Schiff bases 1–26 were synthesized by reacting tryptamine with different benzaldehyde derivatives in methanol. The change in the color of reaction mixture indicated the progress of reaction, however, the completion of reaction was monitored *via* TLC. The solvent was evaporated under vacuum resulting in the formation of a crude product which was purified by crystallization from methanol. The synthetic compounds 1–26 were characterized by different spectroscopic techniques such as EI-MS, HREI-MS, ¹H-, and ¹³C NMR. Compounds 1–3, 6–13, 15–17, 19–20, 22–24, and 26 are new compounds while compounds 5, 14, 18, 21 and 25 have already been reported [30,31] (see Scheme 1.).

3. Inhibition of NTPDase-1, -3, and -8

All synthetic compounds 1–26 were evaluated as inhibitors of three major isoforms of NTPDase enzymes (Table 1). All compounds showed good inhibitory properties. Suramin was used as a known inhibitor (Ki values of 1.260 ± 0.007 , 6.39 ± 0.89 and $1.180 \pm 0.002 \mu\text{M}$) against NTPDase-1, -3, and -8, respectively). A limited SAR suggested that the NTPDase inhibition by this class of compounds mainly depends upon all persistent structural features such as indole ring, imine group and bridging alky moiety, however, the varying features such as the “R” group with different substitution pattern in all compounds were also responsible for the inhibitory avidity of the synthetic compounds (Fig. 3).

4. Structure-activity relationship (SAR)

4.1. NTPDase-1 inhibitory activity

The structure-activity relationship of the synthetic derivatives is established on the basis of Ki values. Amongst the hydroxy substituted derivatives, compound 1 with an *ortho* hydroxy group on aryl part inhibited selectively NTPDase-1 (Ki = $0.03 \pm 0.04 \mu\text{M}$) more potently than suramin (Ki = $1.260 \pm 0.007 \mu\text{M}$). However, the dihydroxy analog 2 with two hydroxyl groups *meta* to each other was found to have less NTPDase-1 inhibitory activity (Ki = $0.04 \pm 0.08 \mu\text{M}$) as compared to compound 1. Furthermore, the selectivity was also lost by the compound 2 with an additional hydroxy group as it inhibited also NTPDase-8. The inhibitory activity and selectivity was further decreased when two hydroxy groups were present at *ortho* to each other, as observed in the case of compound 3 (Ki = $0.06 \pm 0.07 \mu\text{M}$). Inhibitors selectivity is inversely related to the number of hydroxy groups. It means that compounds having dihydroxy substitutions can

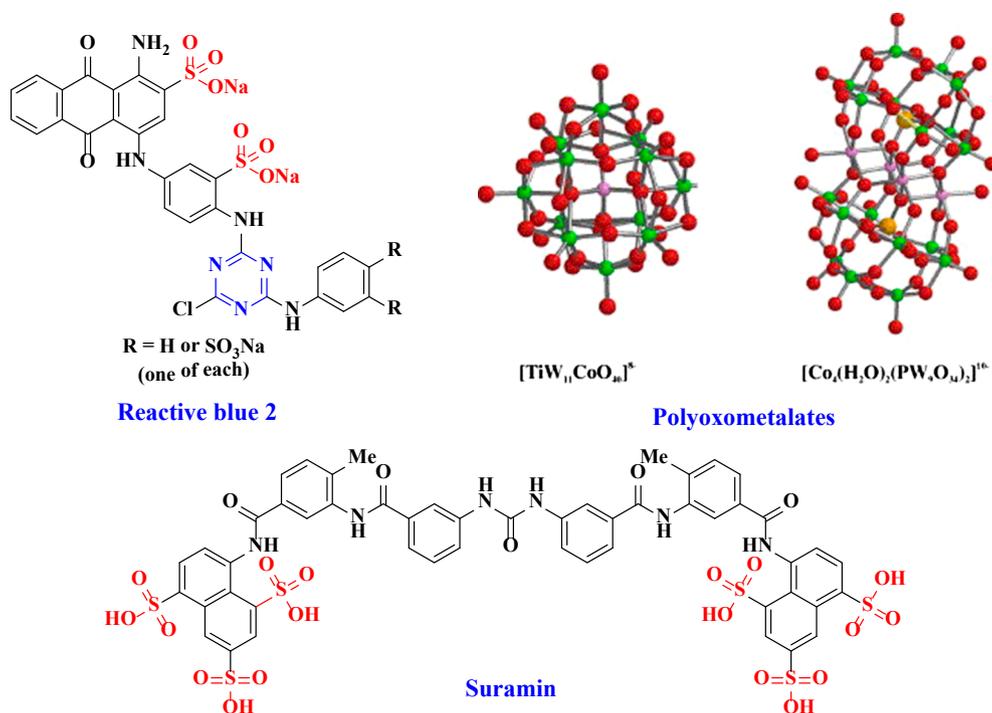


Fig. 1. Inhibitors of NTPDases.

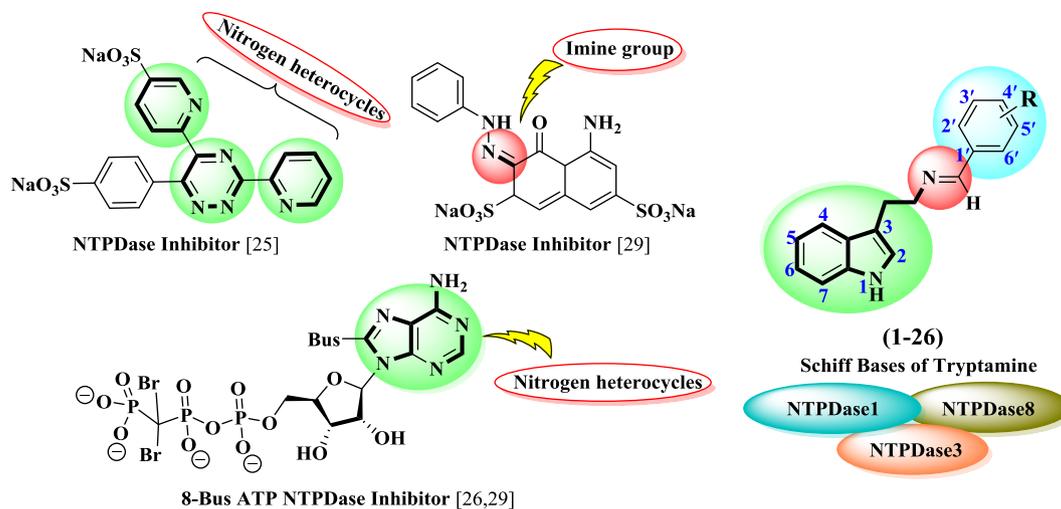
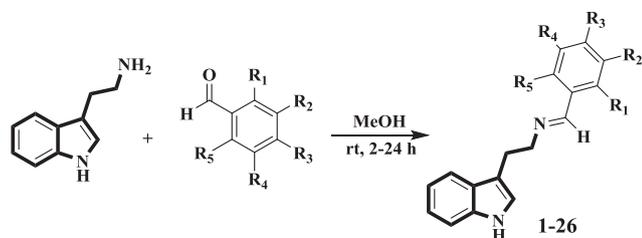


Fig. 2. Rationale of the current study.



interact in various ways to the active site of enzymes and decrease their selectivity (Fig. 4).

Compounds 4–6 with hydroxy and chloro groups are also potent inhibitors of NTPDase-1. Amongst them, compound 4 ($K_i = 0.080 \pm 0.001 \mu\text{M}$) with chloro and hydroxyl groups *ortho* to each other was found to be selective inhibitor for NTPDase-1. However,

in case of its positional isomer 5 ($K_i = 0.13 \pm 0.01 \mu\text{M}$), with chloro and hydroxyl group at *para* each other, lost its selectivity. It showed that compound 4 attained a conformation which only fitted well into the active site of the NTPDase-1 enzyme. Similarly, compound 6 ($K_i = 0.021 \pm 0.001 \mu\text{M}$), which has an extra chloro substitution, displayed stronger inhibitory effect of NTPDase-1 than compounds 4 and 5, however, selectivity was also lost in case of compound 6. It indicates that extra chloro substitution is playing an important role in inhibition of NTPDase-1. It is worth mentioning here that compounds 4–6 were stronger inhibitors than suramin (Fig. 5).

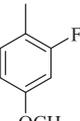
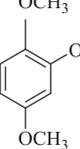
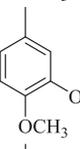
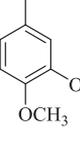
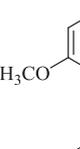
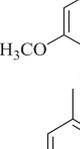
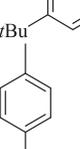
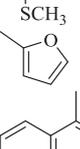
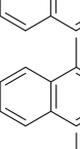
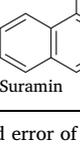
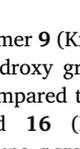
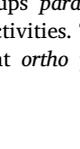
Compounds 8 and 9 with bromo and hydroxy substitutions were potent but non-selective inhibitors. Amongst them, compound 8 ($K_i = 0.050 \pm 0.003 \mu\text{M}$) with bromo and hydroxy substitutions at *ortho* to each other, was potent inhibitor of NTPDase-1. Compound 8 with bromo together with chloro group was more potent than compound 4. It indicated that bromo group adjacent to hydroxy is more efficiently participating in the activity as compared to chloro moiety. Its

Table 1
NTPDase-1, -3 and -8 inhibitory activities of the Schiff bases of tryptamine 1–26.

Comp.	Aryl part	NTPDase-1	NTPDase-3	NTPDase-8	NTPDase-1	NTPDase-3	NTPDase-8
		IC ₅₀ ± SEM ^a (μM)			K _i (μM)		
1		0.43 ± 0.05	↓ ^b	↓ ^b	0.03 ± 0.04	↓ ^b	↓ ^b
2		0.53 ± 0.09	↓ ^b	4.55 ± 0.86	0.04 ± 0.08	↓ ^b	1.31 ± 0.82
3		0.79 ± 0.08	0.51 ± 0.04	14.0 ± 2.01	0.06 ± 0.07	0.13 ± 0.03	4.03 ± 0.02
4		1.01 ± 0.07	↓ ^b	↓ ^b	0.080 ± 0.001	↓ ^b	↓ ^b
5		1.64 ± 0.17	0.26 ± 0.02	0.42 ± 0.03	0.13 ± 0.01	0.071 ± 0.002	0.12 ± 0.01
6		0.27 ± 0.03	0.39 ± 0.04	0.79 ± 0.07	0.021 ± 0.001	0.112 ± 0.002	0.220 ± 0.005
7		0.36 ± 0.01	3.89 ± 0.76	1.57 ± 0.09	0.030 ± 0.003	1.060 ± 0.002	0.450 ± 0.005
8		0.65 ± 0.05	↓ ^b	1.82 ± 0.11	0.050 ± 0.003	↓ ^b	0.520 ± 0.002
9		1.91 ± 0.56	1.01 ± 0.06	8.50 ± 1.01	0.105 ± 0.001	0.270 ± 0.002	2.45 ± 0.01
10		↓ ^b	↓ ^b	↓ ^b	↓ ^b	↓ ^b	↓ ^b
11		0.41 ± 0.03	1.56 ± 0.14	1.06 ± 0.07	0.030 ± 0.001	0.420 ± 0.006	0.301 ± 0.001
12		↓ ^b	↓ ^b	3.31 ± 0.56	↓ ^b	↓ ^b	0.950 ± 0.002
13		↓ ^b	↓ ^b	0.97 ± 0.05	↓ ^b	↓ ^b	0.280 ± 0.004
14		2.38 ± 0.56	0.61 ± 0.03	2.48 ± 0.14	0.190 ± 0.005	0.170 ± 0.002	0.710 ± 0.007

(continued on next page)

Table 1 (continued)

Comp.	Aryl part	NTPDase-1	NTPDase-3	NTPDase-8	NTPDase-1	NTPDase-3	NTPDase-8
		IC ₅₀ ± SEM ^a (μM)			K _i (μM)		
15		↓ ^b	↓ ^b	1.32 ± 0.02	↓ ^b	↓ ^b	0.380 ± 0.002
16		4.51 ± 0.77	↓ ^b	0.71 ± 0.02	0.350 ± 0.001	↓ ^b	0.201 ± 0.001
17		↓ ^b	↓ ^b	7.20 ± 1.01	↓ ^b	↓ ^b	2.070 ± 0.002
18		0.76 ± 0.06	↓ ^b	2.12 ± 0.13	0.060 ± 0.002	↓ ^b	0.610 ± 0.003
19		0.43 ± 0.02	0.75 ± 0.06	7.85 ± 0.97	0.030 ± 0.001	0.201 ± 0.001	2.26 ± 0.01
20		0.28 ± 0.01	0.35 ± 0.03	1.07 ± 0.05	0.0200 ± 0.0005	0.0900 ± 0.0006	0.310 ± 0.002
21		0.43 ± 0.04	3.88 ± 0.99	0.26 ± 0.01	0.0300 ± 0.0001	1.060 ± 0.001	0.0700 ± 0.0001
22		↓ ^b	↓ ^b	1.71 ± 0.04	↓ ^b	↓ ^b	0.490 ± 0.002
23		↓ ^b	↓ ^b	↓ ^b	↓ ^b	↓ ^b	↓ ^b
24		0.64 ± 0.04	0.92 ± 0.06	4.49 ± 0.89	0.0500 ± 0.0003	0.0700 ± 0.0002	1.290 ± 0.003
25		↓ ^b	↓ ^b	↓ ^b	↓ ^b	↓ ^b	↓ ^b
26		4.31 ± 0.98	0.49 ± 0.04	0.69 ± 0.07	0.340 ± 0.003	0.130 ± 0.001	0.190 ± 0.004
Std	Suramin	16.1 ± 1.02	24.1 ± 3.01	4.31 ± 0.41	1.260 ± 0.007	6.39 ± 0.89	1.180 ± 0.002

SEM^a (Standard error of the mean), ↓^b (Not active), Suramin^c (Standard inhibitor for NTPDase activity).

positional isomer **9** (K_i = 0.105 ± 0.001 μM), with bromo substitution at *para* to hydroxy group, displayed decreased NTPDase-1 inhibitory activity as compared to compound **8** (Fig. 6).

Compound **16** (K_i = 0.350 ± 0.001 μM) having hydroxy and methoxy groups *para* to each other showed stronger inhibition of NTPDase-1 activities. This inhibitory pattern showed that the hydroxy substitution at *ortho* position might be actively participating in the

process, however, causes loss of the selectivity. Compound **18** (K_i = 0.060 ± 0.02 μM) bearing two methoxy group reveals that the replacement of OH with OCH₃ leads to increase in inhibitory effect against NTPDase-1. Compound **20** (K_i = 0.0200 ± 0.0005 μM) having an additional methoxy group along with hydroxy and methoxy showed increased inhibition against NTPDase-1 as compared to compound **16**. It indicates that additional methoxy groups are playing important role

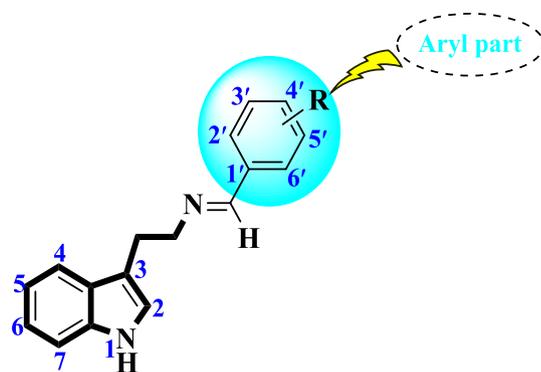


Fig. 3. General structural features of synthetic compounds.

in the inhibition by creating some important interaction with the active site (Fig. 7).

Inhibitory activity of compound **20** can be compared with its structurally similar analog **10**, which has bromo group substitution instead of one methoxy group that makes it completely inactive. Compound **10** might attain the conformation, which is not well fitted into the active sites of all three isozymes. Moreover, by changing the positions of the substituent as in compound **11** ($K_i = 0.030 \pm 0.001 \mu\text{M}$), inhibitory effects were restored (Table 1). Inhibitory effect of compound **10** can also be compared with another structurally similar compound **19** ($K_i = 0.030 \pm 0.001 \mu\text{M}$), with iodo instead of bromo substitution, that demonstrates potent inhibition. However, compounds **11** and **19** showed comparable activity to each other (Fig. 8).

Compound **24** ($K_i = 0.0500 \pm 0.0003 \mu\text{M}$) with a hydroxy substituted naphthyl ring showed potent inhibitory effect against NTPDase-1. It can be assumed that the hydroxyl group is actively contributing in the inhibitory effect. Similarly, compound **26** ($K_i = 0.340 \pm 0.003 \mu\text{M}$) with anthracene ring have good inhibitory activity (Fig. 9). The addition of another phenyl group, however, resulted in decreased activity.

4.2. NTPDase-3 inhibitory activity

Twelve compounds showed good inhibitory potential as compared to the standard suramin ($K_i = 6.39 \pm 0.89 \mu\text{M}$). Among them, compound **5** ($K_i = 0.071 \pm 0.002 \mu\text{M}$) having chloro and hydroxy groups at *meta* and *ortho* positions, respectively, showed good inhibitory potential as compared to standard suramin. The addition of another chloro group on the aryl ring resulted in decreased activity in compound **6** ($K_i = 0.112 \pm 0.002 \mu\text{M}$), might be the addition of chloro group resulted in increased steric factor. Compound **24** ($K_i = 0.071 \pm 0.002 \mu\text{M}$) having naphthol ring also showed comparable activity to compound **5**. However, compound **25** having unsubstituted naphthyl ring was inactive, it showed that the presence of hydroxy group in compounds **5** and **24** might be responsible for binding interactions within the active site of enzyme (Fig. 10).

The second most active compound was **20**

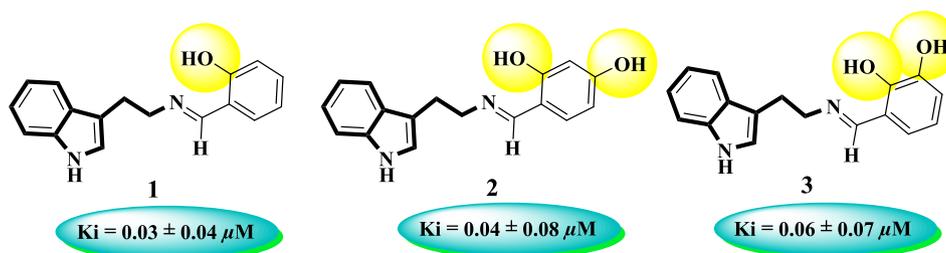


Fig. 4. Structure-activity relationship (SAR) of compounds 1–3.

($K_i = 0.0900 \pm 0.0006 \mu\text{M}$) having two methoxy and one hydroxy group which showed that the addition of methoxy resulted in slight decreased activity. Replacement of one methoxy with iodo resulted in further decreased in activity as in compound **19** ($K_i = 0.201 \pm 0.001 \mu\text{M}$), might be due to increased steric factor of iodo group. Compound **3** ($K_i = 0.13 \pm 0.03 \mu\text{M}$) having two hydroxy groups *ortho* to each other also exhibited good inhibitory activity as compared to standard suramin. Compound **14** ($K_i = 0.0900 \pm 0.0006 \mu\text{M}$) bearing hydroxy and nitro groups showed good inhibition against NTPDase-3, however, this compound was less active as compared to compound **5** which showed that the replacement of nitro group with chloro resulted in decreased activity which might be due to the electron withdrawing effect of nitro group resulting in deactivation of the ring (Fig. 11).

4.3. NTPDase-8 inhibitory activity

Except few derivatives, all synthetic compounds showed good inhibitory potential against NTPDase-8 enzyme. Five compounds **12**, **13**, **15**, **17**, and **22** selectively inhibited NTPDase-8 enzyme. Among them, compound **13** ($K_i = 0.280 \pm 0.004 \mu\text{M}$) with fluoro and hydroxy substituents at *meta* and *ortho* positions, respectively, was most active. The selectivity was lost when fluoro group was replaced with nitro group in compound **14** ($K_i = 0.710 \pm 0.007 \mu\text{M}$), the activity of compound was also decreased. Compound **15** ($K_i = 0.380 \pm 0.002 \mu\text{M}$) having fluoro and methoxy groups *para* to each other was found to have good inhibitory potential and showed selectivity. Interestingly the selectivity of compound was lost and activity was increased when fluoro group was replaced with hydroxy in compound **16** ($K_i = 0.201 \pm 0.001 \mu\text{M}$). The selectivity of compounds **13** and **15** was might be due to the presence of fluoro group which may binds well only with the active site of NTPDase-8 enzyme (Fig. 12).

Compound **22** ($K_i = 0.280 \pm 0.004 \mu\text{M}$) bearing thiomethyl group was also found to be selective inhibitor of NTPDase-8 might be due to good interaction of thiomethyl group with enzyme's active site. Compound **12** ($K_i = 0.950 \pm 0.002 \mu\text{M}$) having one bromo and two methoxy groups was also found to be selective and potential inhibitor of NTPDase-8. Shifting the position of bromo and replacement of one methoxy from hydroxy resulted in loss of activity in compound **11** ($K_i = 0.301 \pm 0.001 \mu\text{M}$), however, the activity was enhanced as compared to compound **12** might be due to better binding interactions of hydroxy group to the active site of enzyme. Compound **17** ($K_i = 2.070 \pm 0.002 \mu\text{M}$) bearing hydroxy and methoxy groups *ortho* to each other selectively inhibited NTPDase-8, nevertheless, the compound was less active as compared to standard suramin ($K_i = 1.180 \pm 0.002 \mu\text{M}$). The enhanced activity was observed when hydroxy was replaced with methoxy in compound **18** ($K_i = 0.610 \pm 0.003 \mu\text{M}$), however, the selectivity was lost (Fig. 13).

5. Mechanism of inhibition

In order to determine the type of inhibition, the inhibitor **6** was selected for human NTPDase-1, **5** was selected for human NTPDase-3 and **21** was selected for human NTPDase-8. The obtained results

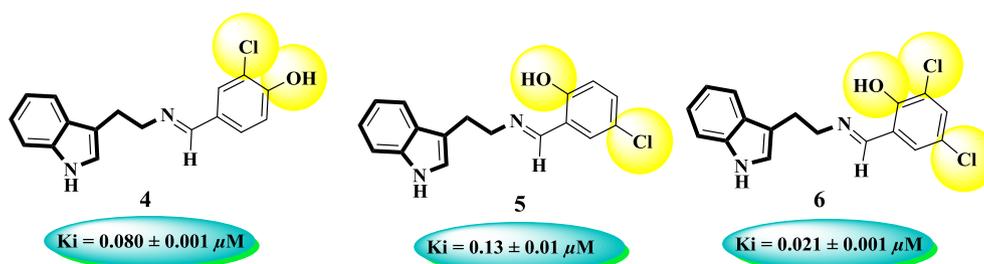


Fig. 5. Structure-activity relationship (SAR) of compounds 4–6.

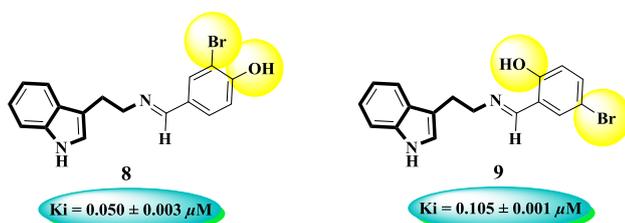


Fig. 6. Structure-activity relationship (SAR) of compounds 7–9.

showed that compounds 6, 5, and 21 exhibited competitive mode of inhibition against human NTPDase-1, -3 and -8 (Fig. 13). Moreover, Michaelis-Menten kinetic parameters (K_m and V_{max}) of human NTPDase-1, -3 and -8 inhibition were also determined in the presence and absence of inhibitor 6, 5 and 21. In these cases, the maximal velocity (V_{max}) found almost constant even by using different concentrations of respective inhibitors, however the Michaelis constant (K_m) was found in increasing order as depicted in Fig. 14 (A–C).

6. Molecular docking

In order to investigate the binding mode of these newly synthesized compounds in the active site of human NTPDase-1, the most active compound 6 was docked in the developed homology model of human NTPDase-1 (Fig. 15A). Nucleotide-binding site (*i.e.* active site), which is comprised of amino acid residues Asp54, Thr131, Ala132, Glu174, Asp213, Gln220, Ser361, Phe360, Phe365 and Trp450, was proposed as the docking area. The lowest energy minimized docked conformation (*i.e.* -43.30 kcal/mol with docking score -6.62) was used to analyze the interactions. It was observed that the compound 6 interacts with the active site of human NTPDase-1 *via* hydrogen bonds and hydrophobic interactions (Fig. 15B). Hydrogen bonds are created with Asp213 (side chain; 2.2 and 2.4 Å), Gly215 and Gly216 (main chain; 2.6 Å and 2.1 Å), whereas, some weak arene-hydrogen interactions were observed with Trp450 and Phe365. Whereas Ca^{++} also shows interaction with the oxygen atom of dichloro methylphenol moiety, which is Schiff bases of the compound. The compound 6 also revealed main hydrophobic interactions with Glu174, Phe360, Ser361 and Leu214. The good interaction of compound 6 may be due to the presence of hydroxyl group and chlorine atoms at *ortho* and *meta* positions of benzene ring. Cumulatively, docking results showed important interactions between

compound 6 and the active site of human NTPDase-1, which correlates with its competitive inhibitory effect.

7. *In-silico* toxicity evaluation

In order to determine toxicity profile of the compounds (1–26), *in-silico* evaluation has been carried out with some physicochemical parameters (OSIRIS) [32]. The results are shown in Table 2. Polar surface area (PSA) is sum of all the polar atoms in compound. It gives us the estimated ability of synthesize derivatives to cross the blood brain barrier (BBB). The value of PSA less than 140 is ideal and our all compounds showing the values less than 100. Another parameter, cLogP shows the octanol water partition coefficient (ratio of compound concentration in octanol to water concentration). The parameter cLogP is responsible to calculate the compound ability to cross the membrane. Similarly, cLogS is the calculation of water solubility at 25 °C. The number of hydrogen bond donors (HD) and hydrogen bond acceptors (HA) must be less than 5 and 10, respectively. All the compounds were estimated to be none mutagenic and tumorigenic.

8. Conclusions

Schiff bases of tryptamine 1–26 were synthesized and evaluated as inhibitors of human NTPDase-1, -3, and -8. Many compounds showed selective and strong inhibitory effects on activities of three major NTPDases, compared with the commonly used inhibitor suramin. Kinetic studies confirm that the strongest compounds competitively inhibit the enzyme activities. Also the binding and interactions between enzyme and the ligand were performed *in-silico* with the most potent inhibitor compound 6. All synthetic compounds were found to be non-cytotoxic. In conclusion, newly identified NTPDase inhibitors may serve as lead candidates for further research in order to get powerful anti-NTPDase agents for various biomedical applications.

9. Materials and methods

^1H - and ^{13}C NMR spectra were recorded in deuterated solvents on Bruker 300 and 400 MHz spectrometers. Mass experiments were carried out on a Finnigan MAT-311A mass spectrometer (Germany). Thin layer chromatography (TLC) was monitored on pre-coated silica gel aluminum plates (Kieselgel 60, 254, E. Merck, Germany). Visualization of TLC chromatograms was performed at wavelengths of 254 and 365 nm

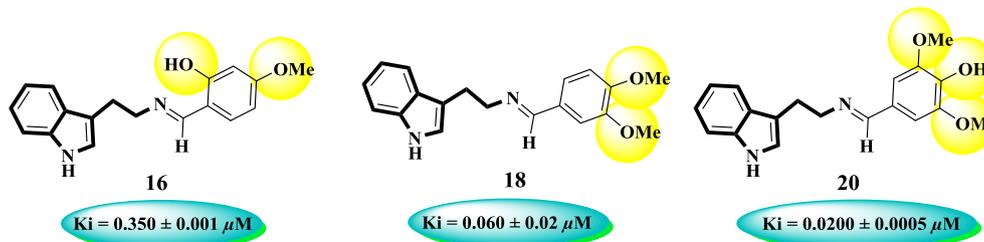


Fig. 7. Structure-activity relationship (SAR) of compounds 16–18 and 20.

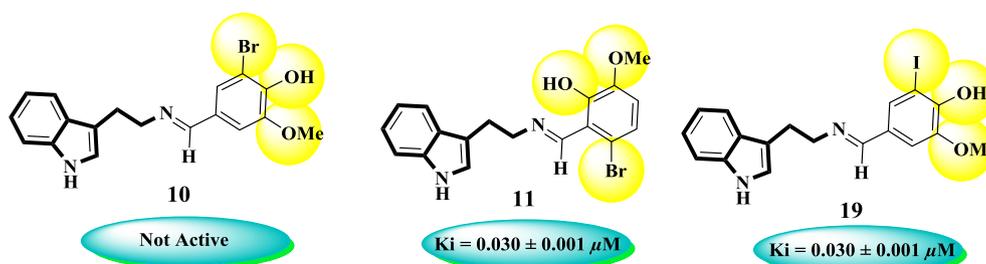


Fig. 8. Structure-activity relationship (SAR) of compounds 10–12, and 19.

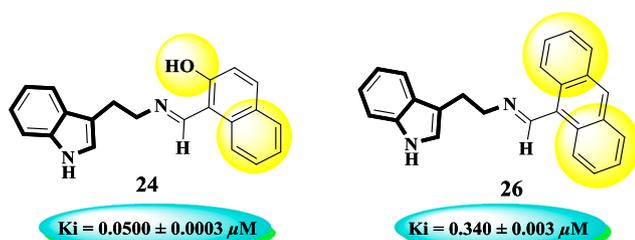


Fig. 9. Structure-activity relationship (SAR) of compounds 23–26.

or iodine vapors. Methanol of analytical grade was used as received from RCI Labscan Limited, Thailand. All benzaldehyde derivatives of analytical grades were used as received from the supplier Wako, Japan, TCI, USA. Tryptamine was purchased from Merck, Germany.

10. General procedure for the synthesis of Schiff bases of tryptamine (1–26)

Tryptamine (0.16 g, 1 mmol) was taken in a 50 mL round-bottom flask in methanol (10 mL). Corresponding benzaldehydes (1 mmol) were added in the reaction mixture and stirred for 24 h. The completion of reaction was monitored via TLC analysis. After completion of the reaction, solvent was dried under vacuum. The residue was then washed with hexane. Pure products were obtained after crystallization using methanol.

11. Spectral data for the synthesized compound

11.1. 2-((2-(1H-Indol-3-yl)ethylimino)methyl)phenol (1)

Yield: 76%, M.p.: 103–105 °C; $^1\text{H NMR}$ (300 MHz, $\text{DMSO-}d_6$): δ 13.65 (s, 1H, OH), 10.79 (s, 1H, NH), 8.48 (s, 1H, N=CH), 7.56 (d, 1H, $J_{7,6} = 7.8$ Hz, H-4), 7.31 (overlapping multiplet, 3H, H-7, H-4', H-6'), 7.13 (d, 1H, $J = 2.1$ Hz, H-2), 7.06 (t, 1H, $J_{5(4,6)} = 7.8$ Hz, H-5), 6.98 (t, 1H, $J_{6(5,7)} = 7.8$ Hz, H-6), 6.84 (overlapping multiplet, 2H, H-3', H-5'), 3.88 (t, 2H, $J = 6.6$ Hz, CH_2), 3.30 (t, 2H, $J = 6.9$ Hz, CH_2); $^{13}\text{C NMR}$ (75 MHz, $\text{DMSO-}d_6$): δ 165.8, 163.4, 136.2, 131.5, 130.8, 125.4, 123.9, 123.4, 121.2, 120.7, 118.6, 118.1, 116.8, 111.2, 110.8, 54.5, 24.9; EI-MS m/z (% rel. abund.): 264 (M^+ , 15), 130 (100.0); HREI-MS m/z : Calcd for $\text{C}_{17}\text{H}_{16}\text{N}_2\text{O}$ [264.1269], Found [264.1263].

11.2. 4-((2-(1H-Indol-3-yl)ethylimino)methyl)benzene-1,3-diol (2)

Yield: 82%, M.p.: 136–138 °C; $^1\text{H NMR}$ (300 MHz, $\text{DMSO-}d_6$): δ 13.93 (s, 1H, OH), 10.79 (s, 1H, NH), 8.26 (s, 1H, N=CH), 7.56 (d, 1H, $J_{7,6} = 7.0$ Hz, H-4), 7.31 (d, 1H, $J_{4,5} = 7.8$ Hz, H-7), 7.10 (overlapping multiplet, 3H, H-6', H-2, H-5), 6.95 (t, 1H, $J_{6(5,7)} = 7.0$ Hz, H-6), 6.20 (dd, 1H, $J_{5,6'} = 8.7$ Hz, $J_{5,3'} = 2.4$ Hz, H-5'), 6.10 (d, 1H, $J_{3,5'} = 2.1$ Hz, H-3'), 3.78 (t, 2H, $J = 6.9$ Hz, CH_2), 3.0 (t, 2H, $J = 6.9$ Hz, CH_2); $^{13}\text{C NMR}$ (75 MHz, $\text{DMSO-}d_6$): δ 164.9, 163.7, 163.4, 135.9, 132.6, 126.8, 122.8, 121.4, 118.4, 118.1, 116.7, 111.1, 110.4, 109.1, 103.4, 52.7, 26.4; EI-MS m/z (% rel. abund.): 280 (M^+ , 11), 151 (23), 130 (100); HREI-MS m/z : Calcd for $\text{C}_{17}\text{H}_{16}\text{N}_2\text{O}_2$ [280.1206], Found [280.1212].

11.3. 3-((2-(1H-Indol-3-yl)ethylimino)methyl)benzene-1,2-diol (3)

Yield: 66%, M.p.: 216–219 °C; $^1\text{H NMR}$ (300 MHz, $\text{DMSO-}d_6$): δ 10.03 (s, 1H, NH), 8.33 (s, 1H, N=CH), 7.63 (d, 1H, $J_{4,5} = 7.8$ Hz, H-4), 7.35 (d, 1H, $J_{7,6} = 8.1$ Hz, H-7), 7.17 (s, 1H, H-2), 7.09 (overlapping multiplet, 1H, H-5), 7.03 (overlapping multiplet, 1H, H-6), 6.81 (dd, 1H, $J_{6,5'} = 7.8$ Hz, $J_{6,4'} = 1.8$ Hz, H-6'), 6.74 (dd, 1H, $J_{4,5'} = 7.8$ Hz, $J_{4,6'} = 1.5$ Hz, H-4'), 6.55 (t, 1H, $J_{5(6,4')} = 7.8$ Hz, H-5'), 3.97 (t, 2H, $J = 6.9$ Hz, CH_2), 3.18 (t, 2H, $J = 7.2$ Hz, CH_2); $^{13}\text{C NMR}$ (75 MHz, $\text{DMSO-}d_6$): δ 160.6, 153.4, 149.7, 135.8, 127.4, 125.6, 123.9, 123.2, 122.5, 121.3, 119.6, 118.6, 118.0, 111.1, 110.6, 54.7, 24.6; EI-MS m/z (% rel. abund.): 280 (M^+ , 62), 184 (20), 130 (100); HREI-MS m/z : Calcd for $\text{C}_{17}\text{H}_{16}\text{N}_2\text{O}_2$ [280.1212], Found [280.1212].

11.4. 4-((2-(1H-Indol-3-yl)ethylimino)methyl)-2-chlorophenol (4)

Yield: 72%, M.p.: 140–142 °C; $^1\text{H NMR}$ (300 MHz, $\text{DMSO-}d_6$): δ 10.77 (s, 1H, NH), 8.11 (s, 1H, N=CH), 7.68 (s, 1H, H-2'), 7.55 (d, 1H, $J_{4,5} = 7.5$ Hz, H-4), 7.50 (overlapping multiplet, 1H, H-6'), 7.32 (d, 1H, $J_{7,6} = 8.1$ Hz, H-7), 7.12 (s, 1H, H-2), 7.06 (t, 1H, $J_{5(4,6)} = 7.5$ Hz, H-5), 6.96 (overlapping multiplet, 2H, H-6, H-5'), 3.80 (t, 2H, $J = 7.2$ Hz, CH_2), 3.00 (t, 2H, $J = 7.2$ Hz, CH_2); $^{13}\text{C NMR}$ (75 MHz, $\text{DMSO-}d_6$): δ 163.6, 158.6, 137.1, 136.4, 132.3, 129.4, 127.0, 125.4, 123.2, 121.4, 118.7, 118.2, 116.8, 111.1, 110.7, 53.6, 24.1; EI MS m/z (% rel. abund.): 298 (M^+ , 10), 300 ($\text{M} + 2$, 3), 130 (100); HREI-MS m/z : Calcd for $\text{C}_{17}\text{H}_{15}\text{ClN}_2\text{O}$ [298.0870], Found [298.0868].

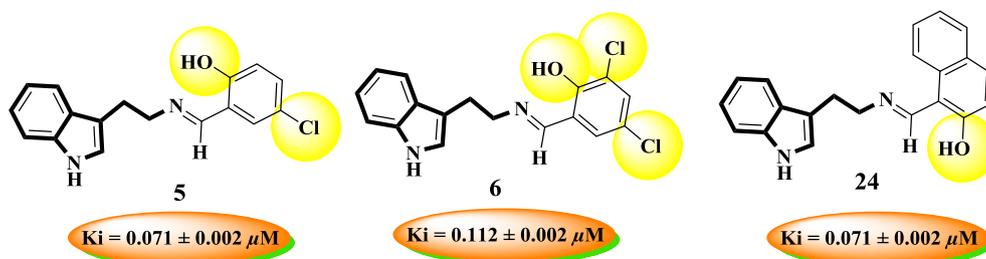


Fig. 10. Structure-activity relationship of compounds 5, 6, and 24.

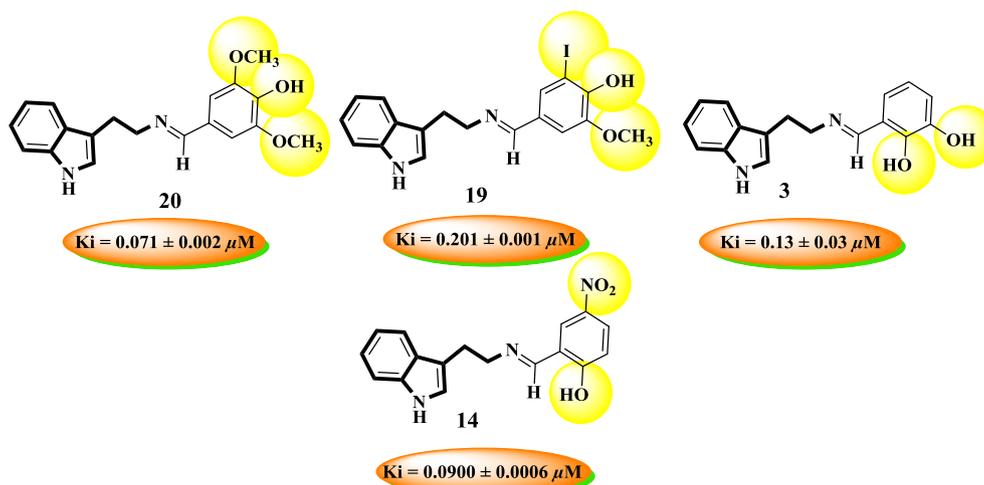


Fig. 11. Structure-activity relationship of compounds 3, 14, 19, and 20.

11.5. 2-((2-(1H-Indol-3-yl)ethylimino)methyl)-4-chlorophenol (5)

Yield: 77%, M.p.: 136–138 °C; ^1H NMR (300 MHz, DMSO- d_6): δ 13.79 (s, 1H, OH), 10.81 (s, 1H, NH), 8.46 (s, 1H, N=CH), 7.56 (d, 1H, $J_{4,5} = 7.8$ Hz, H-4), 7.45 (d, 1H, $J_{6',3'} = 2.7$ Hz, H-6'), 7.31 (overlapping multiplet, 2H, H-7, H-4'), 7.13 (d, 1H, $J = 1.8$ Hz, H-2), 7.06 (t, 1H, $J_{5(4,6)} = 7.8$ Hz, H-5), 6.95 (t, 1H, $J_{6(5,7)} = 7.8$ Hz, H-6), 6.86 (d, 1H, $J_{3',4'} = 8.7$ Hz, H-3'), 3.88 (t, 2H, $J = 6.6$ Hz, CH₂), 3.05 (t, 2H, $J = 6.9$ Hz, CH₂); ^{13}C NMR (75 MHz, DMSO- d_6): δ 164.6, 160.2, 136.1, 131.8, 130.4, 127.0, 122.9, 121.3, 120.9, 119.4, 118.7, 118.2, 118.2, 111.4, 111.3, 58.5, 26.3; EI-MS m/z (% rel. abund.): 298 (M^+ , 44), 300 ($\text{M} + 2$, 28), 143 (34), 130 (100); HREI-MS m/z : Calcd for $\text{C}_{17}\text{H}_{15}\text{ClN}_2\text{O}$ [298.0866], Found [298.0873].

11.6. 2-((2-(1H-Indol-3-yl)ethylimino)methyl)-4,6-dichlorophenol (6)

Yield: 75%, M.p.: 160–162 °C; ^1H NMR (300 MHz, DMSO- d_6): δ 14.61 (s, 1H, OH), 10.87 (s, 1H, NH), 8.45 (s, 1H, N=CH), 7.58 (d, 1H, $J_{4,5} = 7.8$ Hz, H-4), 7.54 (d, 1H, $J_{4',6'} = 2.7$ Hz, H-4'), 7.31 (overlapping multiplet, 2H, H-7, H-2), 7.16 (br. s, 1H, H-6'), 7.06 (t, 1H, $J_{5(4,6)} = 7.8$ Hz, H-5), 6.96 (t, 1H, $J_{6(5,7)} = 7.8$ Hz, H-6), 3.91 (t, 2H, $J = 6.6$ Hz, CH₂), 3.09 (t, 2H, $J = 6.9$ Hz, CH₂); ^{13}C NMR (75 MHz, DMSO- d_6): δ 169.1, 155.0, 136.0, 132.5, 127.2, 123.7, 120.9, 120.5,

118.3, 118.2, 111.3, 108.6, 55.1, 33.6; EI-MS m/z (% rel. abund.): 332 (M^+ , 16), 334 ($\text{M} + 2$, 9), 130 (100); HREI-MS m/z : Calcd for $\text{C}_{17}\text{H}_{14}\text{Cl}_2\text{N}_2\text{O}$ [332.0474], Found [332.0483].

11.7. 2-((2-(1H-Indol-3-yl)ethylimino)methyl)-6-bromo-4-chlorophenol (7)

Yield: 66%, M.p.: 94–96 °C; ^1H NMR (300 MHz, DMSO- d_6): δ 14.65 (s, 1H, OH), 10.86 (s, 1H, NH), 8.43 (s, 1H, N=CH), 7.66 (d, 1H, $J_{4',6'} = 2.7$ Hz, H-6'), 7.58 (d, 1H, $J_{4,5} = 7.3$ Hz, H-4), 7.34 (overlapping multiplet, 2H, H-7, H-2), 7.15 (d, 1H, $J_{6',4'} = 2.7$ Hz, H-6'), 7.06 (t, 1H, $J_{5(4,6)} = 7.3$ Hz, H-5), 6.96 (t, 1H, $J_{6(5,7)} = 7.3$ Hz, H-6), 3.91 (t, 2H, $J = 6.6$ Hz, CH₂), 3.0 (t, 2H, $J = 6.9$ Hz, CH₂); ^{13}C NMR (100 MHz, DMSO- d_6): δ 165.2, 164.6, 136.2, 135.8, 130.9, 126.9, 123.2, 121.0, 118.3, 118.3, 117.0, 116.2, 115.4, 111.4, 110.5, 55.6, 26.3; EI-MS m/z (% rel. abund.): 378 (M^+ , 19), 380 ($\text{M} + 2$, 4), 130 (100); HREI-MS m/z : Calcd for $\text{C}_{17}\text{H}_{14}\text{BrClN}_2\text{O}$ [377.9881], Found [377.9896].

11.8. 4-((2-(1H-Indol-3-yl)ethylimino)methyl)-2-bromophenol (8)

Yield: 68%, M.p.: 118–120 °C; ^1H NMR (300 MHz, DMSO- d_6): δ 10.77 (s, 1H, NH), 8.10 (s, 1H, N=CH), 7.84 (s, 1H, H-2'), 7.54 (d, 2H, $J_{4,5/6',5'} = 8.1$ Hz, H-4, H-6'), 7.30 (d, 1H, $J_{7,6} = 8.1$ Hz, H-7), 7.12 (s,

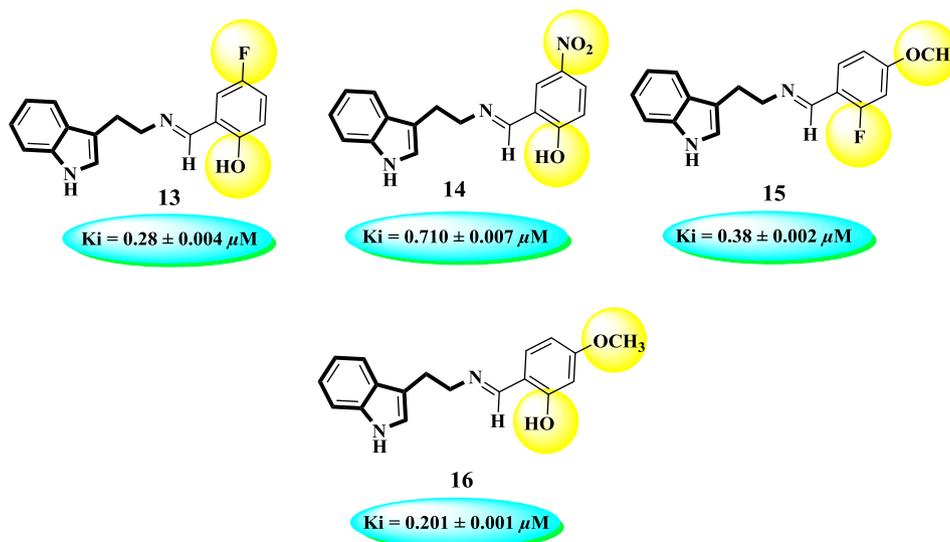


Fig. 12. Structure-activity relationship of compounds 13–16.

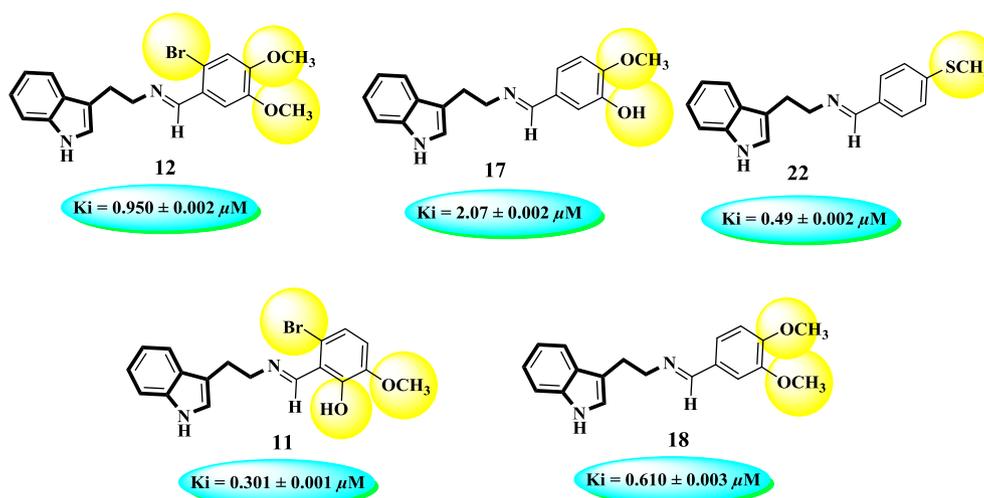


Fig. 13. Structure-activity relationship of compounds 11, 12, 17, 18, and 22.

1H, H-2), 7.01 (t, 2H $J_{5(4,7)/5,6/6,5} = 8.1$ Hz, H-5, H-6), 6.97 (d, 1H, $J_{5',6'} = 7.2$ Hz, H-5'), 3.75 (t, 2H, $J = 7.2$ Hz, CH₂), 3.01 (m, 2H, CH₂); ¹³C NMR (75 MHz, DMSO-*d*₆): δ 164.6, 160.7, 136.7, 135.3, 131.4, 130.6, 126.9, 123.1, 120.8, 118.8, 118.1, 117.5, 114.5, 111.1, 110.9, 54.2, 23.9; EI-MS *m/z* (% rel. abund.): 342 (M⁺, 27), 344 (M + 2, 26), 130 (1 0 0); HREI-MS *m/z*: Calcd for C₁₇H₁₅BrN₂O [342.0361], Found [342.0368].

11.9. 2-((2-(1H-Indol-3-yl)ethylimino)methyl)-4-bromophenol (9)

Yield: 73%, M.p.: 144–146 °C; ¹H NMR (400 MHz, DMSO-*d*₆): δ 13.79 (s, 1H, OH), 10.80 (s, 1H, NH), 8.45 (s, 1H, N=CH), 7.57 (overlapping multiplet, 2H, H-4, H-6'), 7.43 (dd, 1H, $J_{4',3'} = 6.4$ Hz, $J_{3',6'} = 2.8$ Hz, H-4'), 7.33 (d, 1H, $J_{7,6} = 7.6$ Hz, H-7), 7.12 (d, 1H, $J = 1.2$ Hz, H-2), 7.07 (t, 1H, $J_{5(4,6)} = 7.6$ Hz, H-5), 6.97 (t, 1H, $J_{6(5,7)} = 7.6$ Hz, H-6), 6.81 (d, 1H, $J_{3',4'} = 8.8$ Hz, H-3'), 3.90 (t, 2H, $J = 7.2$ Hz, CH₂), 3.07 (t, 2H, $J = 7.2$ Hz, CH₂); ¹³C NMR (75 MHz, DMSO-*d*₆): δ 164.5, 160.7, 136.1, 134.6, 133.3, 127.0, 122.9, 120.8, 120.0, 119.2, 118.2, 118.2, 111.4, 111.3, 108.5, 58.4, 26.3; EI-MS *m/z* (% rel. abund.): 342 (M⁺, 33), 344 (M + 2, 35), 130 (1 0 0); HREI-MS *m/z*: Calcd for C₁₇H₁₅BrN₂O [342.0367], Found [342.0368].

11.10. 4-((2-(1H-Indol-3-yl)ethylimino)methyl)-2-bromo-6-methoxyphenol (10)

Yield: 69%, M.p.: 234–237 °C; ¹H NMR (300 MHz, DMSO-*d*₆): δ 10.78 (s, 1H, OH), 9.90 (s, 1H, NH), 8.10 (s, 1H, N=CH), 7.56 (overlapping multiplet, 2H, H-2', H-6'), 7.32 (d, 2H, $J_{7(6,5)} = 7.8$ Hz, H-4), 7.13 (s, 1H, H-2), 7.04 (t, 1H, $J_{5(4,6)} = 7.8$ Hz, H-5), 6.96 (t, 1H, $J_{6(5,7)} = 7.8$ Hz, H-6), 3.77 (s, 5H, CH₂, OCH₃), 3.0 (s, 2H, CH₂); ¹³C NMR (75 MHz, DMSO-*d*₆): δ 164.9, 162.4, 155.7, 135.8, 134.6, 126.7, 123.1, 121.3, 118.9, 118.2, 115.2, 111.2, 110.5, 109.7, 55.8, 53.7, 25.2; EI-MS *m/z* (% rel. abund.): 372 (M⁺, 7), 374 (M + 2, 7), 163 (34), 130 (1 0 0); HREI-MS *m/z*: Calcd for C₁₈H₁₇BrN₂O₂ [372.0457], Found [372.0473].

11.11. 2-((2-(1H-Indol-3-yl)ethylimino)methyl)-3-bromo-6-methoxyphenol (11)

Yield: 69%, M.p.: 138–140 °C; ¹H NMR (300 MHz, DMSO-*d*₆): δ 14.90 (s, 1H, OH), 10.85 (s, 1H, NH), 8.49 (d, 1H, $J = 5.7$ Hz, N=CH), 7.59 (d, 1H, $J_{4,5} = 7.5$ Hz, H-4), 7.32 (d, 1H, $J_{7,6} = 7.5$ Hz, H-7), 7.15 (s, 1H, H-2), 7.06 (t, 1H, $J_{5(4,6)} = 7.5$ Hz, H-5), 6.96 (t, 1H, $J_{6(5,7)} = 7.5$ Hz, H-6), 6.72 (overlapping multiplet, 2H, H-4', H-5'), 3.96 (overlapping multiplet, 2H, CH₂), 3.15 (s, 3H, OCH₃), 3.07 (t, 2H,

$J = 6.9$ Hz, CH₂); ¹³C NMR (75 MHz, DMSO-*d*₆): δ 165.4, 157.9, 152.3, 136.2, 131.6, 129.7, 127.5, 126.1, 123.0, 121.2, 118.7, 118.0, 116.4, 111.3, 110.6, 56.7, 54.5, 25.7; EI-MS *m/z* (% rel. abund.): 372 (M⁺, 2), 374 (M + 2, 2), 130 (1 0 0); HREI-MS *m/z*: Calcd for C₁₈H₁₇BrN₂O₂ [372.0497], Found [372.0473].

11.12. N-(2-Bromo-4,5-dimethoxybenzylidene)-2-(1H-indol-3-yl)ethanamine (12)

Yield: 71%, M.p.: 220–222 °C, ¹H NMR (300 MHz, DMSO-*d*₆): δ 7.89 (d, 2H, $J_{4(5,6)} = 7.6$ Hz, H-4, H-7), 7.48 (s, 2H, H-2, H-3'), 7.33 (s, 1H, H-6'), 7.27 (overlapping multiplet, 2H, H-5, H-6), 3.85 (d, 10H, $J = 6.9$ Hz, CH₂, CH₂, OCH₃, OCH₃); ¹³C NMR (75 MHz, DMSO-*d*₆): δ 164.5, 153.7, 150.2, 136.0, 135.1, 126.3, 123.4, 121.5, 119.6, 118.9, 118.3, 117.1, 115.8, 111.1, 110.4, 54.6, 26.2; EI-MS *m/z* (% rel. abund.): 387 (M⁺, 4), 389 (M + 2, 4), 130 (9); HREI-MS *m/z*: Calcd for C₁₉H₁₉BrN₂O₂ [386.1604], Found [387.2704].

11.13. 2-((2-(1H-Indol-3-yl)ethylimino)methyl)-4-fluorophenol (13)

Yield: 76%, M.p.: 154–157 °C; ¹H NMR (300 MHz, DMSO-*d*₆): δ 13.38 (s, 1H, OH), 10.80 (s, 1H, NH), 8.45 (s, 1H, N=CH), 7.56 (d, 1H, $J_{4,5} = 7.0$ Hz, H-4), 7.33 (d, 1H, $J_{7,6} = 7.0$ Hz, H-7), 7.26 (overlapping multiplet, 1H, H-6'), 7.18 (overlapping multiplet, 2H, H-4', H-2), 7.05 (t, 1H, $J_{5(4,6)} = 7.0$ Hz, H-5), 6.95 (t, 1H, $J_{6(5,7)} = 7.0$ Hz, H-6), 6.88 (overlapping multiplet, 1H, H-3'), 3.88 (t, 2H, $J = 6.9$ Hz, CH₂), 3.05 (t, 2H, $J = 6.9$ Hz, CH₂); ¹³C NMR (75 MHz, DMSO-*d*₆): δ 164.9, 158.7, 156.2, 136.3, 127.3, 126.8, 122.7, 121.4, 118.9, 118.1, 117.8, 116.1, 111.3, 110.6, 53.7, 25.2; EI-MS *m/z* (% rel. abund.): 282 (M⁺, 24), 130 (1 0 0); HREI-MS *m/z*: Calcd for C₁₇H₁₅FN₂O [282.1156], Found [282.1168].

11.14. 2-((2-(1H-Indol-3-yl)ethylimino)methyl)-4-nitrophenol (14)

Yield: 65%, M.p.: 183–185 °C; ¹H NMR (300 MHz, DMSO-*d*₆): δ 14.13 (s, 1H, OH), 10.88 (s, 1H, NH), 8.63 (s, 1H, N=CH), 8.32 (d, 1H, $J_{6',4'} = 2.4$ Hz, H-6'), 8.01 (dd, 1H, $J_{4',3'} = 5.1$ Hz, $J_{4',6'} = 2.4$ Hz, H-4'), 7.60 (d, 1H, $J_{4,5} = 6.0$ Hz, H-4), 7.34 (d, 1H, $J_{7,6} = 6.0$ Hz, H-7), 7.17 (d, 1H, $J = 1.5$ Hz, H-2), 7.08 (t, 1H, $J_{5(4,6)} = 6.0$ Hz, H-5), 6.98 (t, 1H, $J_{6(5,7)} = 6.0$ Hz, H-6), 6.57 (d, 1H, $J_{3',4'} = 7.2$ Hz, H-3'), 3.94 (t, 2H, $J = 5.1$ Hz, CH₂), 3.14 (t, 2H, $J = 5.1$ Hz, CH₂); ¹³C NMR (75 MHz, DMSO-*d*₆): δ 177.8, 166.9, 136.2, 133.5, 132.5, 129.2, 126.8, 123.3, 122.7, 121.1, 118.3, 118.2, 113.3, 111.4, 110.0; EI-MS *m/z* (% rel. abund.): 309 (M⁺, 13), 130 (1 0 0); HREI-MS *m/z*: Calcd for C₁₇H₁₅N₃O₃ [309.1111], Found [309.1113].

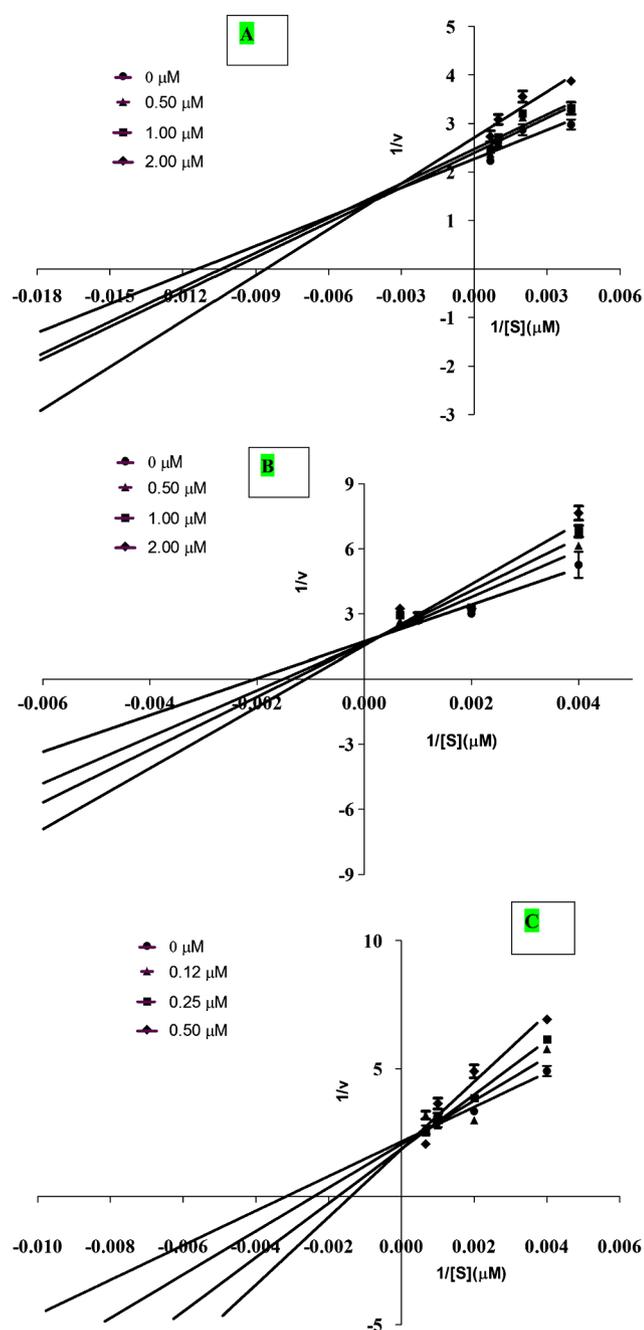


Fig. 14. Lineweaver-Burk Plot of lead compounds (A) compound 6, (B) compound 5, and (C) compound 21, against NTPDase-1, -3 and -8, respectively. S: is the concentration of substrate used (0, 250, 500, 1000 and 1500 μM); concentration of compounds (A) Compound 6, (B) Compound 5, & (C) Compound 21 black circle (0 μM); black triangle (0.5 μM for NTPDase-1 and -3, while 0.12 μM for NTPDase-8); black square (1 μM for NTPDase-1 and -3, while 0.25 μM for NTPDase-8); and black diamond (2 μM for NTPDase-1 and -3, while 0.50 μM for NTPDase-8).

11.15. N-(2-Fluoro-4-methoxybenzylidene)-2-(1H-indol-3-yl)ethanamine (15)

Yield: 70%, M.p.: 118–120 $^{\circ}\text{C}$; ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ 10.77 (s, 1H, NH), 8.36 (s, 1H, N=CH), 7.87 (d, 1H, $J_{6',5'} = 8.4$ Hz, H-6'), 7.56 (d, 1H, $J_{4,5} = 7.5$ Hz, H-4), 7.32 (d, 1H, $J_{7,6} = 7.5$ Hz, H-7), 7.12 (s, 1H, H-2), 7.06 (t, 1H, $J_{5(4,6)} = 7.5$ Hz, H-5), 6.96 (t, 1H, $J_{6(5,7)} = 7.5$ Hz, H-6), 6.85 (overlapping multiplet, 2H, H-3', H-5'), 3.84 (overlapping multiplet, 5H, CH_2 , OCH_3), 3.06 (t, 2H, $J = 7.2$ Hz, CH_2);

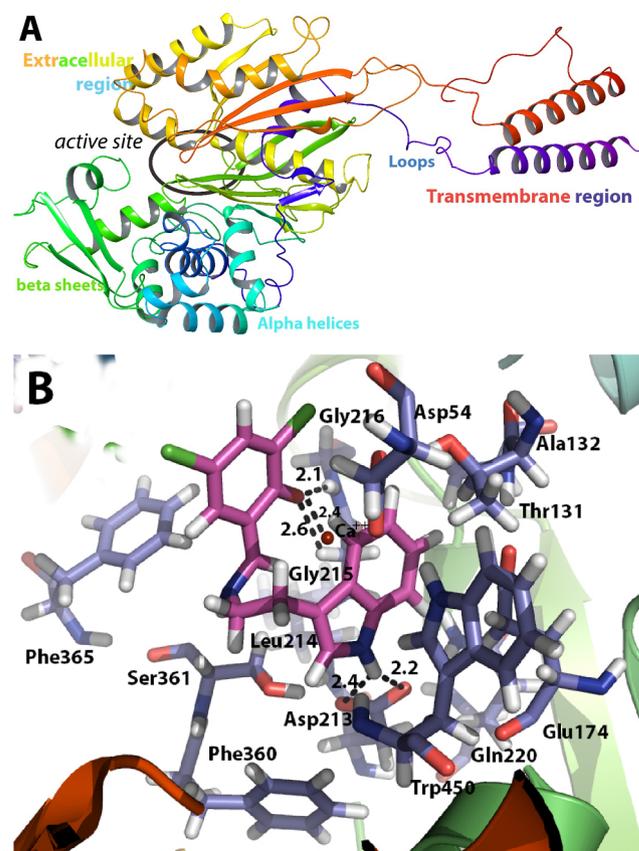


Fig. 15. (A) The 3D structure homology model and active site of human NTPDase-1. Note: The enzyme has two extracellular domains (EC) and two transmembrane (TM) helices. (B) Schematic illustration of the compound 6 docking in the active site of human NTPDase-1. The dashed lines show the hydrogen bonds between the active site residues (blue) and compound 6 (pink).

Table 2

In silico calculation of some ADME parameters /toxicity profile for compounds (1–26).

Compounds	PSA	cLogP	cLogS	HD	HA	Mutagenic	Tumorigenic
1	48.38	3.651	-3.2431	6	3	None	None
2	68.61	3.380	-2.8811	4	2	None	None
3	68.61	3.341	-2.8811	4	2	None	None
4	48.38	4.280	-3.9774	4	2	None	None
5	48.38	4.280	-3.9774	4	2	None	None
6	48.38	4.907	-4.7117	4	2	None	None
7	48.38	5.113	-5.0678	4	2	None	None
8	48.38	4.486	-4.3335	4	2	None	None
9	48.38	4.486	-4.3335	4	2	None	None
10	57.61	4.477	-4.3839	5	3	None	None
11	57.61	4.401	-4.3839	5	3	None	None
12	46.61	4.492	-4.7962	4	3	None	None
13	48.38	3.841	-3.5381	4	2	None	None
14	94.20	3.623	-4.0333	4	2	None	None
15	37.38	4.105	-3.9504	3	2	None	None
16	57.61	3.644	-3.2935	5	3	None	None
17	57.61	3.644	-3.2935	5	3	None	None
18	46.61	3.659	-3.7058	4	3	None	None
19	57.61	4.869	-4.2850	5	3	None	None
20	66.84	3.635	-3.3438	6	4	None	None
21	48.38	6.721	-6.9688	4	2	None	None
22	28.15	4.553	-4.6264	2	1	None	None
23	41.29	2.676	-3.3566	2	1	None	None
24	48.38	4.871	-5.1210	4	2	None	None
25	28.15	5.181	-5.4829	2	1	None	None
26	28.15	6.438	-7.3608	2	1	None	None

^{13}C NMR (75 MHz, DMSO- d_6): δ 164.9, 163.7, 161.3, 135.7, 132.3, 126.4, 122.8, 120.9, 118.7, 118.3, 114.3, 111.4, 110.7, 109.4, 103.6, 56.3, 52.7, 24.9; EI-MS m/z (% rel. abund.): 296 (M^+ , 22), 166 (44), 130 (1 0 0); HREI-MS m/z : Calcd for $\text{C}_{18}\text{H}_{17}\text{FN}_2\text{O}$ [296.1333], Found [296.1313].

11.16. 2-((2-(1H-Indol-3-yl)ethylimino)methyl)-5-methoxyphenol (16)

Yield: 71%, M.p.: 109–111 °C; ^1H NMR (300 MHz, DMSO- d_6): δ 12.97 (s, 1H, OH), 10.79 (s, 1H, NH), 8.45 (s, 1H, N=CH), 7.57 (d, 1H, $J_{4,5} = 7.8$ Hz, H-4), 7.33 (d, 1H, $J_{7,6} = 7.8$ Hz, H-7), 7.12 (d, 1H, $J = 2.1$ Hz, H-2), 7.07 (t, 1H, $J_{5(4,6)} = 7.8$ Hz, H-5), 6.97 (overlapping multiplet, 3H, H-6, H-6', H-3'), 6.79 (d, 1H, $J_{5',6'} = 8.7$ Hz, H-5'), 3.89 (t, 2H, $J = 7.2$ Hz, CH_2), 3.68 (s, 3H, OCH_3), 3.07 (t, 2H, $J = 7.2$ Hz, CH_2); ^{13}C NMR (75 MHz, DMSO- d_6): δ 165.7, 162.2, 160.6, 136.1, 134.3, 127.1, 123.1, 121.5, 118.7, 118.2, 116.3, 111.1, 110.7, 106.8, 101.7, 53.6, 24.1; EI-MS m/z (% rel. abund.): 294 (M^+ , 54), 130 (1 0 0); HREI-MS m/z : Calcd for $\text{C}_{18}\text{H}_{18}\text{N}_2\text{O}_2$ [294.1369], Found [294.1368].

11.17. 5-((2-(1H-Indol-3-yl)ethylimino)methyl)-2-methoxyphenol (17)

Yield: 67%, M.p.: 147–149 °C; ^1H NMR (300 MHz, DMSO- d_6): δ 10.75 (s, 1H, NH), 8.10 (s, 1H, N=CH), 7.54 (d, 1H, $J_{4,5} = 7.8$ Hz, H-4), 7.31 (d, 1H, $J_{7,6} = 7.8$ Hz, H-7), 7.22 (d, 1H, $J = 1.8$ Hz, H-2'), 7.10 (s, 1H, H-2), 7.03 (overlapping multiplet, 2H, H-5, H-6'), 6.94 (overlapping multiplet, 2H, H-6, H-5'), 3.78 (overlapping multiplet, 5H, CH_2 , OCH_3), 2.97 (t, 2H, $J = 7.5$ Hz, CH_2); ^{13}C NMR (75 MHz, DMSO- d_6): δ 164.7, 159.8, 151.6, 135.6, 134.6, 126.9, 123.4, 122.6, 121.1, 118.5, 118.0, 116.4, 114.3, 111.2, 109.6, 56.7, 53.8, 23.8; EI-MS m/z (% rel. abund.): 294 (M^+ , 31), 164 (44), 130 (1 0 0); HREI-MS m/z : Calcd for $\text{C}_{18}\text{H}_{18}\text{N}_2\text{O}_2$ [294.1379], Found [294.1368].

11.18. N-(3,4-Dimethoxybenzylidene)-2-(1H-indol-3-yl)ethanamine (18)

Yield: 73%, M.p.: 100–102 °C; ^1H NMR (300 MHz, DMSO- d_6): δ 10.76 (s, 1H, NH), 8.19 (s, 1H, N=CH), 7.56 (d, 1H, $J_{4,5} = 7.5$ Hz, H-4), 7.36 (s, 1H, H-2'), 7.30 (d, 1H, $J_{7,6} = 8.1$ Hz, H-7), 7.19 (d, 1H, $J_{6',5'} = 6.9$ Hz, H-6'), 7.13 (s, 1H, H-2), 7.01 (overlapping multiplet, 3H, H-5, H-6, H-5'), 3.78 (overlapping multiplet, 8H, CH_2 , OCH_3 , OCH_3), 3.00 (t, 2H, $J = 7.2$ Hz, CH_2); ^{13}C NMR (75 MHz, DMSO- d_6): δ 164.4, 153.7, 150.6, 135.8, 132.8, 127.2, 123.6, 123.2, 121.2, 118.9, 118.5, 112.3, 111.4, 110.1, 108.7, 55.8, 52.4, 26.2; EI-MS m/z (% rel. abund.): 308 (M^+ , 88), 179 (95), 130 (1 0 0); HREI-MS m/z : Calcd for $\text{C}_{19}\text{H}_{20}\text{N}_2\text{O}_2$ [308.1507], Found [308.1525].

11.19. 4-((2-(1H-Indol-3-yl)ethylimino)methyl)-2-iodo-6-methoxyphenol (19)

Yield: 70%, M.p.: 225–228 °C; ^1H NMR (300 MHz, DMSO- d_6): δ 10.79 (s, 1H, NH), 8.13 (s, 1H, N=CH), 7.55 (d, 1H, $J_{4,5} = 7.8$ Hz, H-4), 7.31 (d, 1H, $J_{7,6} = 8.1$ Hz, H-7), 7.13 (d, 1H, $J = 1.8$ Hz, H-2), 7.04 (m, 1H, H-5), 6.99 (overlapping multiplet, 2H, H-2', H-6'), 6.95 (m, 1H, H-6), 3.81 (overlapping multiplet, 5H, CH_2 , OCH_3), 2.99 (t, 2H, $J = 6.9$ Hz, CH_2); ^{13}C NMR (75 MHz, DMSO- d_6): δ 164.6, 160.2, 158.7, 137.2, 136.4, 131.3, 126.8, 123.1, 121.1, 118.6, 118.0, 111.4, 110.6, 109.8, 89.6, 56.4, 54.2, 26.3; EI-MS m/z (% rel. abund.): 420 (M^+ , 41), 163 (30), 130 (1 0 0); HREI-MS m/z : Calcd for $\text{C}_{18}\text{H}_{17}\text{IN}_2\text{O}_2$ [420.0342], Found [420.0335].

11.20. 4-((2-(1H-Indol-3-yl)ethylimino)methyl)-2,6-dimethoxyphenol (20)

Yield: 68%, M.p.: 119–121 °C; ^1H NMR (300 MHz, DMSO- d_6): δ 10.77 (s, 1H, NH), 8.14 (s, 1H, N=CH), 7.57 (d, 1H, $J_{4,5} = 7.5$ Hz, H-4), 7.31 (d, 1H, $J_{7,6} = 7.2$ Hz, H-7), 7.12 (s, 1H, H-2), 7.06 (overlapping multiplet, 4H, H-5, H-2', H-6', H-6), 3.77 (overlapping multiplet, 8H,

CH_2 , OCH_3 , OCH_3), 3.01 (t, 2H, $J = 7.2$ Hz, CH_2); ^{13}C NMR (75 MHz, DMSO- d_6): δ 164.7, 150.2, 138.7, 136.5, 133.6, 127.1, 122.8, 120.7, 118.6, 118.0, 111.4, 110.7, 105.2, 56.8, 53.9, 24.7; EI-MS m/z (% rel. abund.): 324 (M^+ , 25), 130 (1 0 0); HREI-MS m/z : Calcd for $\text{C}_{19}\text{H}_{20}\text{N}_2\text{O}_3$ [324.1482], Found [324.1474].

11.21. 2-((2-(1H-Indol-3-yl)ethylimino)methyl)-4,6-di-tert-butylphenol (21)

Yield: 66%, M.p.: 113–115 °C; ^1H NMR (300 MHz, DMSO- d_6): δ 14.22 (s, 1H, OH), 10.82 (s, 1H, NH), 8.51 (s, 1H, N=CH), 7.58 (d, 1H, $J_{7,6} = 7.8$ Hz, H-4), 7.32 (d, 1H, $J_{4,5} = 7.8$ Hz, H-7), 7.28 (d, 1H, $J_{6',4'} = 2.4$ Hz, H-6'), 7.20 (d, 1H, $J_{4',6'} = 2.1$ Hz, H-4'), 7.15 (d, 1H, $J = 2.1$ Hz, H-2), 7.06 (t, 1H, $J_{5(4,6)} = 7.8$ Hz, H-5), 6.96 (t, 1H, $J_{6(5,7)} = 7.8$ Hz, H-6), 3.86 (t, 2H, $J = 7.2$ Hz, CH_2), 3.06 (t, 2H, $J = 7.2$ Hz, CH_2), 1.37 (s, 9H, $t\text{Bu}$), 1.24 (s, 9H, $t\text{Bu}$); ^{13}C NMR (75 MHz, DMSO- d_6): δ 165.2, 157.6, 139.7, 138.4, 136.5, 129.6, 126.9, 124.7, 124.1, 123.2, 120.6, 118.7, 118.4, 111.0, 110.4, 54.5, 34.6, 33.9, 31.7, 31.2, 26.3; EI-MS m/z (% rel. abund.): 376 (M^+ , 89), 361 (41), 130 (1 0 0); HREI-MS m/z : Calcd for $\text{C}_{25}\text{H}_{32}\text{N}_2\text{O}$ [376.2509], Found [376.2515].

11.22. 2-(1H-Indol-3-yl)-N-(4-(methylthio)benzylidene)ethanamine (22)

Yield: 72%, M.p.: 167–169 °C; ^1H NMR (300 MHz, DMSO- d_6): δ 10.76 (s, 1H, NH), 8.22 (s, 1H, N=CH), 7.65 (d, 1H, $J_{4,5} = 7.5$ Hz, H-4), 7.56 (d, 1H, $J_{2',3'/6',5'} = 7.8$ Hz, H-2', H-6'), 7.29 (overlapping multiplet, 3H, H-3', H-5', H-7), 7.12 (d, 1H, $J = 1.5$ Hz, H-2), 7.06 (t, 1H, $J_{5(4,6)} = 7.5$ Hz, H-5), 6.96 (t, 1H, $J_{6(5,7)} = 7.5$ Hz, H-6), 3.82 (t, 2H, $J = 7.2$ Hz, CH_2), 3.02 (t, 2H, $J = 7.2$ Hz, CH_2); ^{13}C NMR (75 MHz, DMSO- d_6): δ 164.7, 145.4, 136.2, 133.6, 131.2, 127.6, 127.4, 123.3, 121.5, 118.8, 118.1, 111.4, 110.1, 53.7, 24.9, 15.3; EI-MS m/z (% rel. abund.): 294 (M^+ , 36), 164 (73), 130 (1 0 0); HREI-MS m/z : Calcd for $\text{C}_{18}\text{H}_{18}\text{N}_2\text{S}$ [294.1199], Found [294.1191].

11.23. N-(Furan-2-ylmethylene)-2-(1H-indol-3-yl)ethanamine (23)

Yield: 63%, M.p.: 135–137 °C; ^1H NMR (300 MHz, DMSO- d_6): δ 10.76 (s, 1H, NH), 8.09 (s, 1H, N=CH), 7.78 (s, 1H, H-5'), 7.55 (d, 1H, $J_{4,5} = 7.8$ Hz, H-4), 7.30 (d, 1H, $J_{7,6} = 7.8$ Hz, H-7), 7.12 (d, 1H, $J = 1.8$ Hz, H-2), 7.04 (t, 1H, $J_{5(4,6)} = 7.8$ Hz, H-5), 6.94 (overlapping multiplet, 1H, H-6), 6.86 (d, 1H, $J = 3.3$ Hz, H-3'), 6.58 (overlapping multiplet, 1H, H-4'), 3.80 (t, 2H, $J = 7.2$ Hz, 2H, CH_2), 2.98 (t, 2H, $J = 7.2$ Hz, CH_2); ^{13}C NMR (75 MHz, DMSO- d_6): δ 151.5, 149.8, 145.0, 136.1, 127.2, 122.7, 120.8, 118.4, 118.1, 113.7, 112.1, 111.8, 111.2, 61.3, 26.6; EI-MS m/z (% rel. abund.): 238 (M^+ , 73), 130 (1 0 0); HREI-MS m/z : Calcd for $\text{C}_{15}\text{H}_{14}\text{N}_2\text{O}$ [238.1114], Found [238.1106].

11.24. 1-((2-(1H-Indol-3-yl)ethylimino)methyl)naphthalen-2-ol (24)

Yield: 73%, M.p.: 178–180 °C; ^1H NMR (300 MHz, DMSO- d_6): δ 14.00 (s, 1H, OH), 10.87 (s, 1H, NH), 9.0 (s, 1H, N=CH), 7.92 (d, 1H, $J_{4',3'} = 8.4$ Hz, H-4'), 7.68 (overlapping multiplet, 2H, H-5', H-8'), 7.59 (d, 1H, $J_{4,5} = 7.8$ Hz, H-4), 7.36 (overlapping multiplet, 2H, H-7, H-7'), 7.20 (s, 1H, H-2), 7.16 (t, 1H, $J_{5(4,6)} = 7.8$ Hz, H-5), 7.09 (t, 1H, $J_{6(7,5)} = 7.8$ Hz, H-6), 7.01 (t, 1H, $J_{6'(5',7')} = 7.0$ Hz, H-6'), 6.67 (d, 1H, $J_{3',4'} = 9.3$ Hz, H-3'), 3.94 (m, 2H, CH_2), 3.12 (t, 2H, $J = 6.6$ Hz, CH_2); ^{13}C NMR (75 MHz, DMSO- d_6): δ 167.8, 164.3, 136.8, 134.2, 133.0, 130.1, 129.3, 128.1, 127.3, 124.7, 123.0, 121.9, 119.8, 118.7, 118.4, 118.0, 115.2, 111.2, 110.5, 54.7, 23.9; EI-MS m/z (% rel. abund.): 314 (M^+ , 44), 157 (42), 130 (1 0 0); HREI-MS m/z : Calcd for $\text{C}_{21}\text{H}_{18}\text{N}_2\text{O}$ [314.1425], Found [314.1419].

11.25. 2-(1H-Indol-3-yl)-N-(naphthalen-1-ylmethylene)ethanamine (25)

Yield: 65%, M.p.: 146–148 °C; ^1H NMR (300 MHz, DMSO- d_6): δ

10.78 (s, 1H, NH), 8.88 (s, 1H, N=CH), 7.98 (overlapping multiplet, 3H, H-2', H-4', H-5'), 7.87 (d, 1H, $J_{4,5} = 7.0$ Hz, H-4), 7.58 (overlapping multiplet, 4H, H-3', H-6', H-7', H-8'), 7.32 (d, 1H, $J_{7,6} = 7.0$ Hz, H-7), 7.17 (d, 1H, $J = 2.1$ Hz, H-2), 7.05 (t, 1H, $J_{5(4,6)} = 7.0$ Hz, H-5), 6.96 (t, 1H, $J_{6(5,7)} = 7.0$ Hz, H-6), 3.86 (t, 2H, $J = 7.2$ Hz, CH₂), 3.06 (t, 2H, $J = 7.2$ Hz, CH₂); ¹³C NMR (75 MHz, DMSO-*d*₆): δ 164.8, 136.8, 134.2, 131.4, 131.0, 130.1, 129.1, 127.7, 127.2, 126.3, 125.7, 124.2, 123.0, 121.6, 118.4, 118.0, 111.2, 110.5, 54.7, 23.9; EI-MS *m/z* (% rel. abund.): 298 (M⁺, 26), 181 (54), 130 (1 0 0); HREI-MS *m/z*: Calcd for C₂₁H₁₈N₂ [298.1456], Found [298.1470].

11.26. *N*-(Anthracen-9-ylmethylene)-2-(1*H*-indol-3-yl)ethanamine (26)

Yield: 79%, M.p.: 164–167 °C; ¹H NMR (300 MHz, DMSO-*d*₆): δ 10.84 (s, 1H, NH), 9.23 (s, 1H, N=CH), 8.64 (s, 1H, H-10'), 8.24 (d, 2H, $J_{1,2'/4',3'} = 8.4$ Hz, H-1', H-4'), 8.09 (d, 2H, $J_{5',6'/8',7'} = 8.1$ Hz, H-5', H-8'), 7.70 (d, 1H, $J_{4,5} = 7.3$ Hz, H-4), 7.53 (overlapping multiplet, 4H, H-2', H-3', H-6', H-7'), 7.39 (d, 1H, $J_{7,6} = 7.3$ Hz, H-7), 7.22 (s, 1H, H2), 7.11 (t, 1H, $J_{5(4,6)} = 7.3$ Hz, H-5), 7.02 (t, 1H, $J_{6(5,7)} = 7.3$ Hz, H-6), 4.26 (t, 2H, $J = 6.6$ Hz, CH₂), 3.26 (t, 2H, $J = 6.9$ Hz, CH₂); ¹³C NMR (75 MHz, DMSO-*d*₆): δ 164.8, 136.4, 134.8, 132.3, 129.5, 127.9, 127.3, 126.1, 124.4, 122.9, 121.1, 118.7, 118.2, 111.1, 110.7, 53.6, 24.1; EI-MS *m/z* (% rel. abund.): 348 (M⁺, 54), 218 (1 0 0), 130 (64); HREI-MS *m/z*: Calcd for C₂₅H₂₀N₂ [348.1641], Found [348.1626].

12. Biological protocols

12.1. Cell transfection with human NTPDase

The plasmids expressing human NTPDases *i.e.* *h*-NTPDase 1 [33], *h*-NTPDase 3 [34] and *h*-NTPDase 8 [35] were transfected with COS-7 in 10 cm plates using lipofectamine, as previously reported [36]. The confluent cells (80–90%) were incubated with 6 μg of plasmid DNA and 24 μL of lipofectamine reagent for 5 h at 37 °C in Dulbecco's modified Eagle's medium (DMEM) without fetal bovine serum (FBS). Finally, transfection was stopped by adding same volume of DMEM/F-12 containing 20% FBS and cells were further harvested for 40–72 h.

12.2. Preparation of membrane fractions

To prepare protein extracts, the transfected cells were washed twice with Tris-saline buffer (4 °C). Afterwards, cells were collected by scraping in the harvesting buffer (95 mM NaCl, 0.1 mM PMSF and 45 mM Tris at pH 7.5) and further washed twice by 5 min centrifugation (300 × *g*) at 4 °C [35]. The obtained cells were resuspended in the same harvesting buffer additionally containing 10 μg/μL aprotinin and sonicated. Cellular debris and nucleus were separated out by centrifugation at 300 × *g* for 10 min at 4 °C. The resulting supernatant *i.e.* crude protein extract was aliquoted and kept at –80 °C until used for activity assay. Protein concentration was measured by using modified Bradford microplate assay using bovine serum albumin as a reference standard [36].

12.3. Nucleoside triphosphate diphosphohydrolase inhibition assay

The inhibitory effect of synthetic compounds on nucleoside triphosphate diphosphohydrolase (*h*-NTPDase-1, -3 and -8) activities were determined by adopting slight modifications in previously reported method [37]. Briefly, Tris-HCl (50 mM, pH 7.4, supplemented with 5 mM CaCl₂) was used as reaction buffer. Initial screenings of all compounds were conducted at a final concentration of 0.2 mM (with final DMSO 1% (v/v)). The total assay volume of 100 μL contained 45 μL of the *tris*-buffer, 10 μL of tested compound and 10 μL of *h*-NTPDase-1 (58 ng of protein/well) or 10 μL *h*-NTPDase-3 (163 ng of protein/well) or 10 μL *h*-NTPDase-8 (66 ng of protein/well). The reaction mixture was pre-incubated for 10 min at 37 °C and absorbance was

measured at 630 nm using microplate reader (BioTek ELx800 Instruments, Inc. USA). The reaction was initiated by the addition of 10 μL of adenosine triphosphate (ATP) substrate at final concentration of 0.2 mM. The reaction mixture was incubated at 37 °C for 20 min and then 25 μL of malachite green reagent was added. Change in the absorbance was measured after 6–8 min. Compounds which exhibited over 50% inhibition of any isoform of *h*-NTPDase activity were further evaluated for determination of IC₅₀ values. All experiments were carried out in triplicate. The IC₅₀ values were determined by using non-linear curve fitting program PRISM 5.0 (GraphPad, San Diego, California, USA).

13. Molecular modeling and docking

The 3D structural model of human NTPDase-1 (UniProt Sequence ID: P49961) was constructed by homology modeling, based on crystallographic structure (PDB ID: 3ZX3) of the ATPDase-1 from *Rattus norvegicus* revealed the highest sequence identity of 74%, using the program Modeler-8, as described earlier by Nunes et al. [38]. The structure derived from homology modeling was submitted to the validation process, using ERRAT analysis and the program PROCHECK [39]. The molecular docking program MOE was used to dock most active compound 6 to the nucleotide-binding site of human NTPDase-1. Before molecular docking, the 3D structures of both protein and compound 6 were energy minimized and prepared for docking study using MOE [40].

Acknowledgement

This work was financially supported by the Higher Education Commission (HEC) Pakistan (Project No. 20-1910), under the National Research Program for Universities. J. Sévigny received support from the and he was also the recipient of a “Chercheur National” research award from the Fonds de recherche du Québec – Santé (FRQS). J. Iqbal is grateful to the Organization for the Prohibition of Chemical Weapons (OPCW), The Hague, The Netherlands and Higher Education Commission of Pakistan for the financial support through Project No. 20-3733/NRPU/R&D/ 14/520.

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