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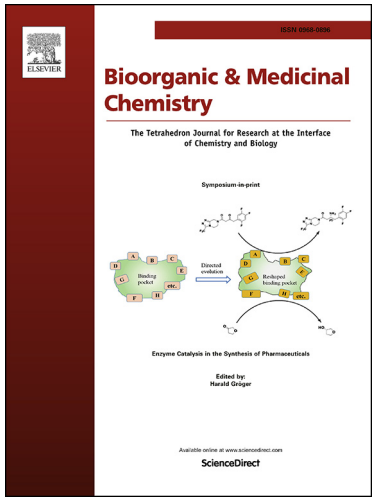

Nature-Inspired Pyrrolo[2,3-*d*]pyrimidines Targeting the Histamine H_3 Receptor

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Nature-Inspired Pyrrolo[2,3-*d*]pyrimidines Targeting the Histamine H_3 Receptor

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dedicated to Prof. Peter Proksch on his 65th birthday

Keywords: Heterocycles, Histamine, G-protein coupled receptor, Natural products, H_3R, Microwave-assisted synthesis

Running title: Nature Inspired Compounds at Histamine H3R

Abstract:

Inspired by marine compounds the derivatization of the natural pyrrolo[2,3-*d*]pyrimidine lead scaffold led to a series of novel compounds targeting the histamine H_3 receptor. The focus was set on improved binding towards the receptor and to establish an initial structure-activity relationship for this compound class based on the lead structure (**compound V**, K_i value of 126 nM). As highest binding affinities were found with 1,4-bipiperidines as basic part of the ligands, further optimization was focused on 4-([1,4'-bipiperidin]-1'-yl)-pyrrolo[2,3-*d*]pyrimidines. Related pyrrolo[2,3-*d*]pyrimidines that were isolated from marine sponges like 4-amino-5-bromopyrrolo[2,3-*d*]pyrimidine (**compound III**), showed variations in halogenation pattern, though in a next step the impact of halogenation at 2-position was evaluated. The chloro variations did not improve the affinity compared to the dehalogenated compounds. However, the simultaneous introduction of lipophilic cores with electron-withdrawing substitution patterns in 7-position and dehalogenation at 2-position (**11b, 12b**) resulted in compounds with significantly higher binding affinities (K_i values of 7 nM and 6 nM, respectively) than the initial lead structure **compound V**. The presented structures allow for a reasonable structure-activity relationship of pyrrolo[2,3-*d*]pyrimidines as histamine H_3 receptor ligands and yielded novel lead structures within the natural compound library against this target.

1 Introduction

Natural products are a highly appreciated source for novel lead compounds, as they are pre-designed for biological activity by natural optimization and provide with large structural heterogeneity high hit rates in pharmacological screenings.[1] A remarkable amount of currently used therapeutics are natural compounds or are at least inspired by nature (e.g. antibiotic or cytostatic agents).[2] The combination of high hit rates and a given drug-likeness motivates researchers to re-investigate compounds, already applied for a certain application field, for the use in other areas as well. One of the most investigated drug targets to date, is the class of G-protein coupled receptors (GPCRs). Among these, the four subtypes of the histamine receptor represent a highly diverse target class as the different receptors are involved in allergy, gastric acid secretion, inflammation or even neurodegenerative diseases.[3] Recent research on the histamine H_3 receptor (H_3R) emphasizes its crucial role in the central nervous system. The presynaptic H_3 autoreceptors modulate histamine release in the central nervous system and due to its heterodimerization with non-histaminergic receptors also a variety of other neurotransmitters such as dopamine, acetylcholine or noradrenaline.[4] Owing to its involvement in the pathophysiology of Alzheimer´s disease, attention deficit hyperactivity disorder and schizophrenia (amongst others), it became apparent that targeting the H_3R may reduce the cognitive impairments of those diseases.[5] The search for agents targeting the receptor led to the investigation of two natural ligands, that were found as novel potential lead structures.[4] **Compound I** (**Figure 1**, K_i value of 30 nM) was isolated from the marine sponge *Aplysina sp.* and inspired the design of a variety of novel non-imidazole-based ligands.[6] Derivatization of the basic moiety and the spacer length of the bromotyrosin derivative led to the discovery of non-imidazole compounds like pitolisant (**Figure 2**) that display high activity and optimized scaffolds towards the H_3R. In a similar manner, various modifications were performed with the natural compound conessine[7] (**compound II**, **Figure 1**) that was also found to be an H_3R antagonist. One attempt aimed for rigidizing the alkaloid, while others attempted to aromatize and simplify the scaffold, resulting in H_3R antagonists like **compound III** (**Figure 2**), displaying affinities in the low nanomolar ranges.[8]

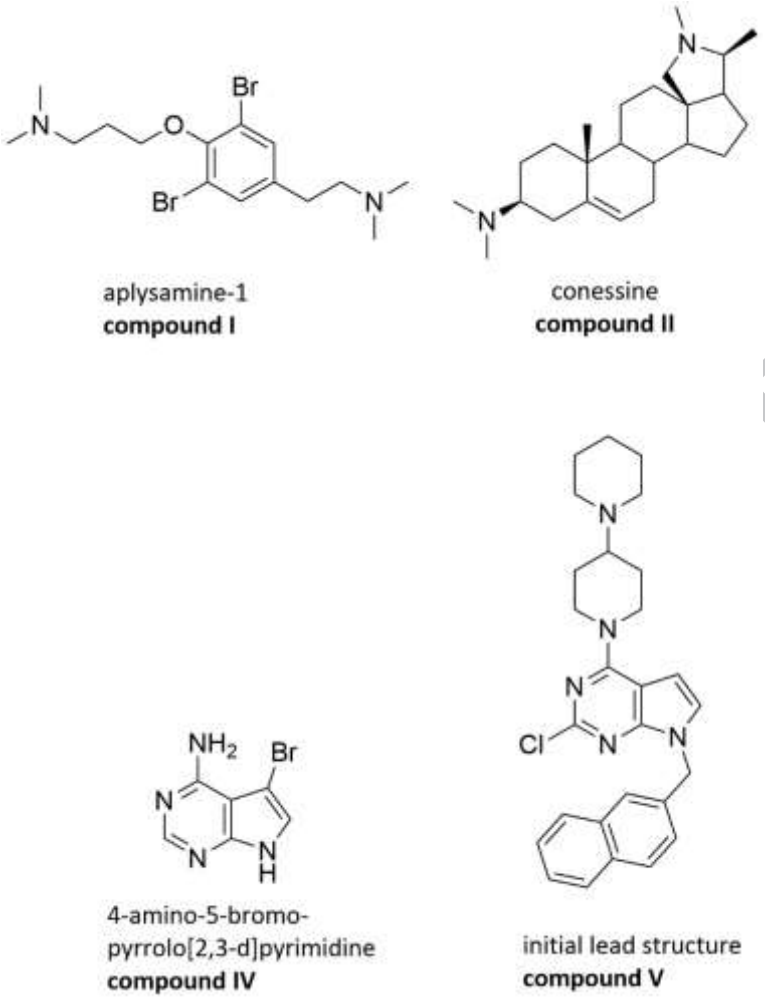


Figure 1: Natural compounds targeting the H_3R and inspired the presented drug design approach.

Although the compound library for targeting the H_3R is increasing rapidly, to date, only one ligand passed clinical evaluation and entered the market. Pitolisant, an inverse H_3R agonist has been approved for the treatment of narcolepsy by the EMA in 2016 and is currently undergoing clinical phase II and III studies for cognitive enhancement in patients with schizophrenia or Parkinson´s diesease.[9,10] The ligand adheres to the typical blueprint of H_3R ligands (**Figure 2**). It consists of a basic aliphatic amine moiety (blue), responsible for binding to the receptor, and a linker, most often an alkyl linker, connecting the amine to the central core (red). Through a second linker the core is connected to an arbitrary, lipophilic region (green), which aids in improving selectivity.[11] As shown with **compounds III** and pitolisant the derivatization of natural products can lead to novel compounds, adhering to this blueprint, with promising activities at the H_3R. Though the drug research community at GPCRs is in constant search for novel scaffolds inspired by Mother Nature.

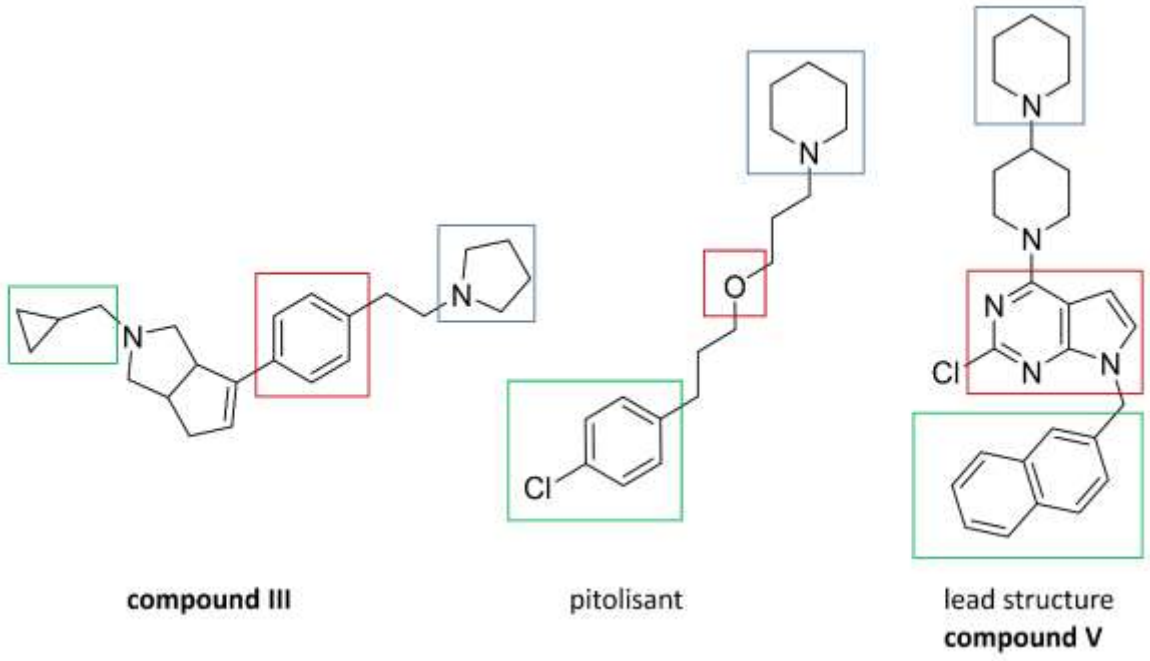


Figure 2: General blueprint for H_3R ligands and structures of pitolisant and the lead structure. Blue: basic amine, red: central core, green: arbitrary region.

Pyrrolo[2,3-*d*]pyrimidines are a class of natural compounds that were found in several marine organisms, like sponges or algae. The brominated analogue **compound IV** (**Figure 1**) for example, was isolated from *Echinodictyum sp.* and is a promising inhibitor of the adenosine kinase.[12] The compound class is known to provide promising results in anti-inflammatory and anti-infectious assays in various studies.[13,14] As the pathophysiology of neurodegenerative diseases involves inflammatory events too, multiple treatment strategies focus on the anti-inflammatory properties of novel substances.[15] H_3R antagonists are reported to improve cognitive functions in neurodegenerative diseases,[5] though the synergistic effect of targeting the H_3R and the known anti-inflammatory properties of pyrrolo[2,3-*d*]pyrimidines[13] may aid current drug development of neurological diseases. Though we recently conducted the combination of the natural pyrrolo[2,3-*d*]pyrimidines with the general pharmacophore of H_3R ligands to expand possible application fields towards neurodegenerative diseases.[16] The novel ligands showed selectivity for the H_3R compared to the H_4R and displayed promising structure-activity relationships (SARs). Among the tested derivatives, a 1,4-bipiperidine warhead as the basic moiety of the molecule was superior to morpholines or piperazines regarding its affinity towards the H_3R, with the most active compound (**compound V**, **Figure 1**) displaying a K_i value of 126 nM. Based on these results we went for a rationalized drug optimization approach to improve pharmacological activity of the novel scaffold. As morpholines are frequently used in the design of H_3R ligands,[17] the first derivatization introduced morpholines as basic group (**Y**, **Figure 3**) and into the lipophilic core at 7-position of the pyrrolo[2,3-*d*]pyrimidine (**R**, **Figure 3**). A second focus was set on the impact of halogenation at 2-position (**X**, **Figure 3**), as pyrrolo[2,3-*d*]pyrimidines isolated from marine organisms

are typically halogenated, as seen with **compound IV**. To complete the SAR investigation **R** was derivatized by introducing varying lipophilic residues. All compounds were tested for their H_3R binding affinities and screened for selectivity towards the H_4R. The goal of this study was to optimize the existing pharmacophore of pyrrolo[2,3-*d*]pyrimidines and to increase H_3R binding affinity compared to **compound V**. In doing so, the class of H_3R ligands can be expanded by natural inspired compounds that may facilitate the jump from bench to bedside.

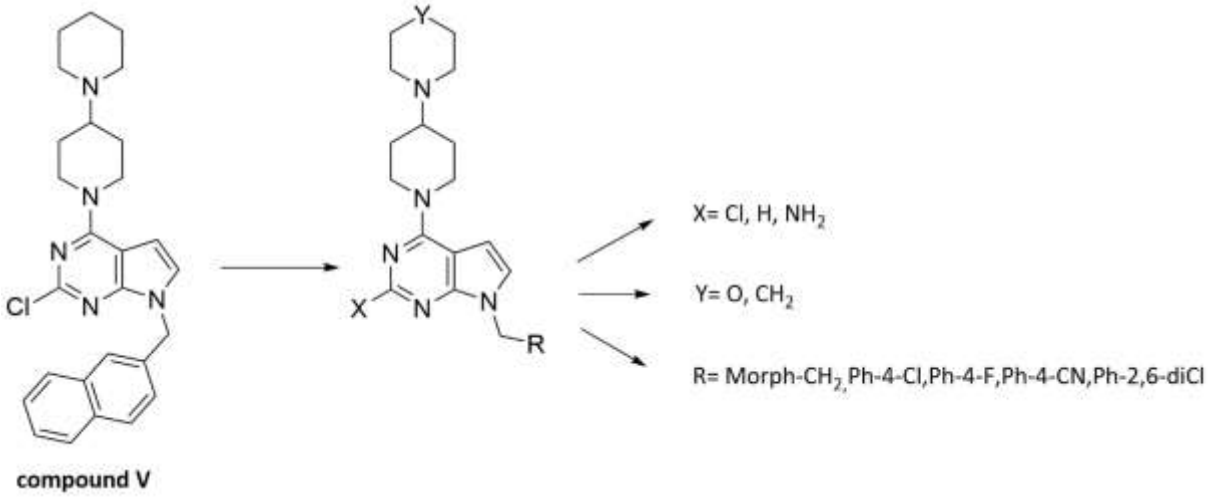


*Figure 3: Possible derivatization strategies of the initial **lead structure**.*

2 Material and Methods

2.1 Materials

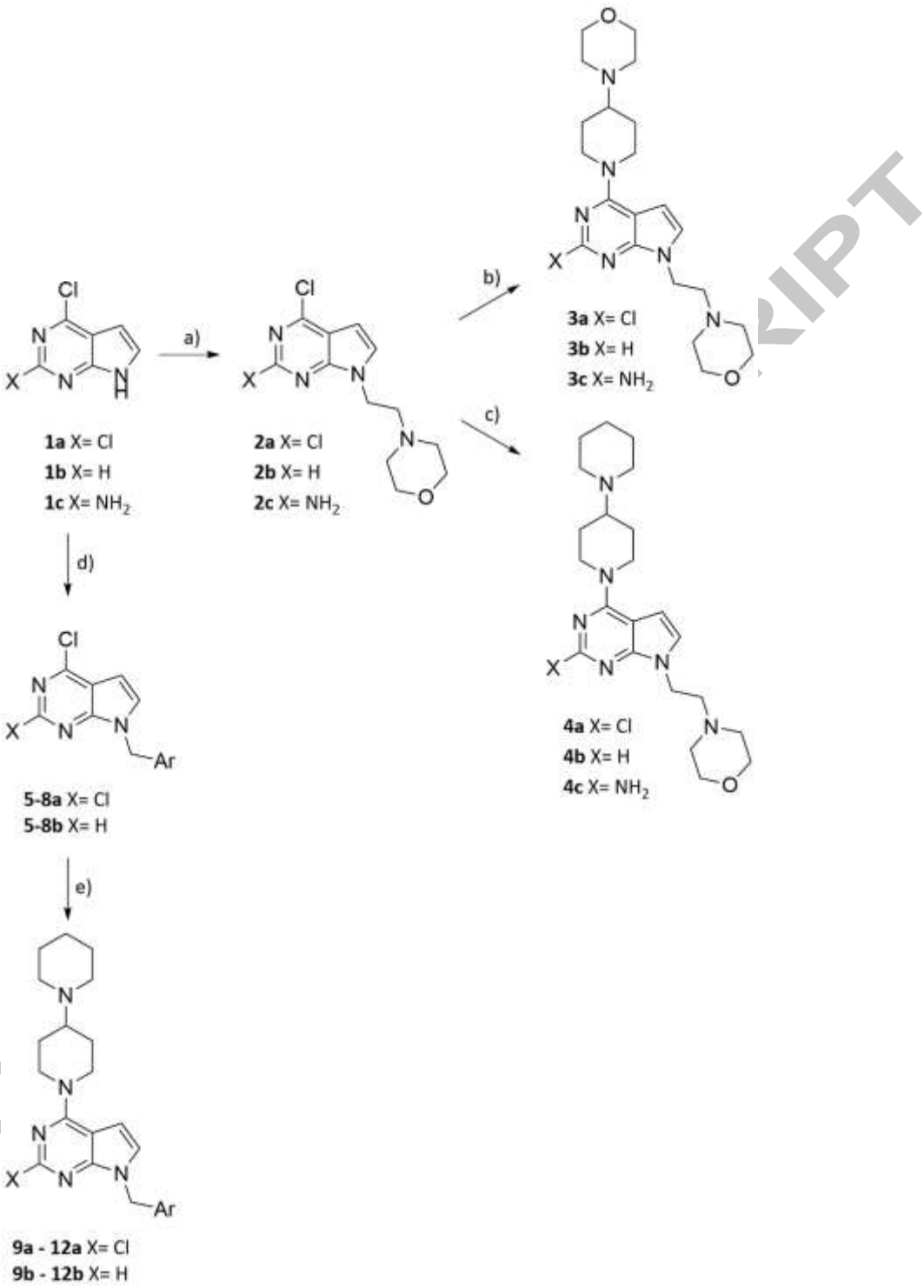
Melting points were determined on a Kofler Thermogerate apparatus and were uncorrected. All reagents were purchased from Sigma-Aldrich, unless otherwise specified. The NMR spectra were recorded on NMR Bruker AV 400. Chemical shifts were given in parts per million relative to TMS [^{1}H and ^{13}C, δ(SiMe4) = 0]. Most NMR assignments were supported by additional 2D experiments. HRMS-ESI-MS experiments were carried out using a Thermo Scientific Exactive Plus Orbitrap Spectrometer. Thin layer chromatography (TLC) was performed using Merck GF-254 type 60 silica gel. Column chromatography was carried out using Merck silica gel 60 (70–230 mesh). Radioligands [^{3}H]N^{α}-methylhistamine and [^{3}H]histamine were purchased at PerkinElmer. HEK-293 cells stably expressing the human H_3 receptor were kindly gifted by Prof. Dr. Jean-Charles Schwartz (Bioprojet, France). Sf9-hH_4-$G\alpha i_2$-$G\beta_1\gamma_2$ cells were a kind donation by Prof. Dr. Seifert.

2.2 Methods

2.2.1 Chemical Part

For the synthesis of pyrrolo[2,3-*d*]pyrimidin-7-yl-ethylmorpholine derivatives **3a-c** and **4a-c**, a simple synthetic strategy was used (**Scheme 1**). Pyrrolo[2,3-*d*]pyrimidines **1a-c** (1 eq.), 2-chloroethylmorpholine (2 eq.) and potassium carbonate (3 eq.) were mixed in acetonitrile and the reaction mixture was stirred at 90 °C for 6 h, obtaining the corresponding **2a-c** derivatives. Subsequently, the target compounds were obtained through a microwave-assisted aromatic nucleophilic substitution reaction. The bipiperidine and morpholino-piperidine scaffolds were incorporated in the 4-position of the heterocycle using triethylamine and ethanol as the solvent.

The derivatives **9a-12a** and **9b-12b** were synthesized from the pyrrolopyrimidines **1a-b** according to **scheme 1**. In a first synthetic step, an *N*-alkylation reaction was performed using various benzyl halides in acetonitrile under reflux conditions and then mediated by microwave-assisted conditions the bipiperidine fragment was added.

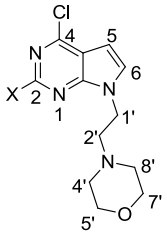


Scheme 1: Reagents and conditions. a) 2-(Chloroethyl)morpholine, K_2CO_3, MeCN, reflux, 6 h. b) Bipiperidine, EtOH, NEt_3, 80°C, mw, 15 min. c) 4-(Piperidin-4-yl)morpholine, EtOH, NEt_3, 80°C, mw, 15 min. d) Benzyl halides, K_2CO_3, CH_3CN, reflux, 3 h. e) Bipiperidine, EtOH, NEt_3, 80°C, mw, 15 min.

2.2.2 Chemistry

2.2.2.1 General Synthetic Procedure to Obtain 4-(2-(4-Chloro-7H-pyrrolo[2,3-*d*]pyrimidin-7-yl)ethyl)morpholine Derivatives **2a-c**.

A mixture of the corresponding pyrrolopyrimidine (1.0 mmol), 4-(2-chloroethyl)morpholine (2.0 mmol) and potassium carbonate (3.0 mmol) in acetonitrile (5 mL) was stirred for 6 h, then the mixture was filtered and evaporated under vacuum. The products were separated by flash chromatography on silica gel eluting with methanol/methylene chloride 1:20.



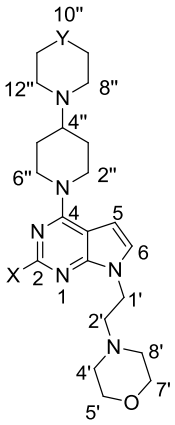
4-(2-(2,4-Dichloro-7H-pyrrolo[2,3-*d*]pyrimidin-7-yl)ethyl)morpholine 2a. Light brown solid, yield 76 %, Mp 79-80 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.91 (d, *J* = 3.6 Hz, 1H, H6), 6.33 (d, *J* = 3.6 Hz, 1H, H5), 4.13 (t, *J* = 6.5 Hz, 2H, 2 x H1'), 3.67 – 3.57 (m, 4H, 2 x H5' and 2 x H7'), 2.67 (t, *J* = 6.5 Hz, 2H, 2 x H2'), 2.53 – 2.43 (m, 4H, 2 x H4' and 2 x H8'). ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.42, 153.41, 152.52, 126.24, 110.66, 99.49, 66.94 (2C), 57.94, 53.66 (2C), 41.67.

4-(2-(4-Chloro-7H-pyrrolo[2,3-*d*]pyrimidin-7-yl)ethyl)morpholine 2b. Brown solid, yield 77 %, Mp 126-128 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.57 (s, 1H, H2), 7.32 (d, *J* = 3.6 Hz, 1H, H6), 6.54 (d, *J* = 3.6 Hz, 1H, H5), 4.34 (t, *J* = 6.3 Hz, 2H, 2 x H1'), 3.65 – 3.56 (m, 4H, 2 x H5' and 2 x H7'), 2.72 (t, *J* = 6.3 Hz, 2H, 2 x H2'), 2.51 – 2.39 (m, 4H, 2 x H4' and 2 x H8'). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.48, 151.96, 151.03, 150.43, 129.73, 117.42, 99.25, 66.88 (2C), 58.04, 53.57 (2C), 42.02.

4-Chloro-7-(2-morpholinoethyl)-7H-pyrrolo[2,3-*d*]pyrimidin-2-amine 2c. White solid, yield 75 %, Mp 133-135 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.91 (d, *J* = 3.6 Hz, 1H, H6), 6.33 (d, *J* = 3.6 Hz, 1H, H5), 5.04 (s, 2H, NH_2), 4.13 (t, *J* = 6.5 Hz, 2H, 2 x H1'), 3.69 – 3.50 (m, 4H, 2 x H5' and 2 x H7'), 2.67 (t, *J* = 6.5 Hz, 2H, 2 x H2'), 2.51 – 2.40 (m, 4H, 2 x H4' and 2 x H8'). ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.45, 153.41, 152.51, 126.23, 110.63, 99.48, 66.94, 57.94, 53.66, 41.67.

2.2.2.2 General Synthetic Procedure to Obtain 4-(2-(4-([1,4'-Bipiperidin]-1'-yl)-7H-pyrrolo[2,3-*d*]pyrimidin-7-yl)ethyl)morpholine Derivatives **3a-c** and **4a-c**

Compounds **2a-c** and (1.0 mmol), 4-piperidinopiperidine or 4-(piperidin-4-yl)morpholine (3.0 mmol), triethylamine (4.5 mmol) and ethanol (5 mL) were added to a microwave reaction flask and the reaction mixture was irradiated for 15 min at 80 °C. Then the solvent was evaporated under vacuum and the crude product was purified by column chromatography on silica gel using chloroform/methanol (10:1) mixture.



4-(2-(2-Chloro-4-(4-morpholinopiperidin-1-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)ethyl)morpholine 3a. Brown oil, yield 95 %. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.96 (d, *J* = 3.6 Hz, 1H, H6), 6.40 (d, *J* = 3.7 Hz, 1H, H5), 4.72 (d, *J* = 13.3 Hz, 2H, H2'' and H6''), 4.21 (t, *J* = 6.3 Hz, 2H, 2 x H1'), 3.71 – 3.67 (m, 4H, 2 x H9'' and 2 x H11''), 3.66 – 3.62 (m, 4H, 2 x H5' and 2 x H7'), 3.14 – 3.00 (m, 2H, H2'' and H6''), 2.68 (t, *J* = 6.3 Hz, 2H, 2 x H2'), 2.56 – 2.52 (m, 4H, 2 x H8'' and 2 x H12''), 2.51 – 2.43 (m, 5H, 2 x H4', 2 x H8' and H4''), 1.95 (d, *J* = 12.8 Hz, 2H, H3'' and H5''), 1.54 (ddd, *J* = 15.9, 12.5, 4.1 Hz, 2H, H3'' and H5''). ^{13}C NMR (101 MHz, $CDCl_3$) δ 157.13, 153.17, 152.35, 124.13, 101.24, 100.92, 67.25 (2C), 66.98 (2C), 61.88, 58.19, 53.65 (2C), 49.78 (2C), 45.13 (2C), 41.68, 28.24 (2C). HRMS m/z 435.2270 [M+H$^+$] (calcd. for $C_{21}H_{31}ClN_6O_2$, 435.2201).

4-(1-(7-(2-Morpholinoethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)piperidin-4-yl)morpholine 3b. Light brown solid, yield 65 %, Mp 114-115 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.29 (s, 1H, H2), 6.99 (d, *J* = 3.6 Hz, 1H, H6), 6.43 (d, *J* = 3.6 Hz, 1H, H5), 4.75 (d, *J* = 13.3 Hz, 2H, H2'' and H6''), 4.27 (t, *J* = 6.5 Hz, 2H, 2 x H1'), 3.71 – 3.66 (m, 4H, 2 x H9'' and 2 x H11''), 3.66 – 3.61 (m, 4H, 2 x H5' and 2 x H7'), 3.12 – 3.01 (m, 2H, H2'' and H6''), 2.71 (t, *J* = 6.5 Hz, 2H, 2 x H2'), 2.57 – 2.52 (m, 4H, 2 x H8'' and 2 x H12''), 2.52 – 2.43 (m, 5H, 2 x H4', 2 x H8' and H4''), 1.94 (d, *J* = 11.4 Hz, 2H, H3'' and H5''), 1.55 (ddd, *J* = 24.1, 12.3, 4.1 Hz, 2H, H3'' and H5''). ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.87, 151.19, 151.14, 123.87, 103.08, 100.59, 67.29 (2C), 66.98 (2C), 62.16, 58.22, 53.68 (2C), 49.78 (2C), 45.24 (2C), 41.80, 28.29 (2C). HRMS m/z 401.2660 [M+H$^+$] (calcd. for $C_{21}H_{32}N_6O_2$, 401.2599).

7-(2-Morpholinoethyl)-4-(4-morpholinopiperidin-1-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-amine 3c. Light brown solid, yield 22 %, Mp 153-155 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.70 (d, *J* = 3.6 Hz, 1H, H6), 6.29 (d, *J* = 3.6 Hz, 1H, H5), 4.70 (d, *J* = 13.2 Hz, 2H, H2'' and H6''), 4.52 (s, 2H, NH_2), 4.11 (t, *J* = 6.7 Hz, 2H, 2 x H1'), 3.71 – 3.65 (m, 8H, 2 x H5', 2 x H7', 2 x H9'' and 2 x H11''), 2.97 (t, *J* = 12.0 Hz, 2H, H2'' and H6''), 2.67 (t, *J* = 6.7 Hz, 2H, 2 x H2'), 2.56 – 2.49 (m, 4H, 2 x H8'' and 2 x H12''), 2.48 – 2.41 (m, 5H, 2 x H4', 2 x H8' and H4''), 1.89 (d, *J* = 11.7 Hz, 2H, H3'' and H5''), 1.51 (ddd, *J* = 24.2, 12.2, 3.9 Hz, 2H, H3'' and H5''). ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.72, 157.62, 153.62, 121.03, 100.97, 97.13, 67.30 (2C), 66.98

(2C), 62.29, 58.13, 53.71 (2C), 49.74 (2C), 45.06 (2C), 41.46, 28.23 (2C). HRMS m/z 416.2768 [M+H$^+$] (calcd. for $C_{21}H_{33}N_7O_2$, 416.2708).

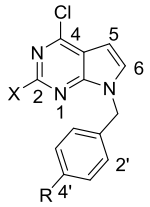
4-(2-(4-([1,4'-Bipiperidin]-1'-yl)-2-chloro-7H-pyrrolo[2,3-*d*]pyrimidin-7-yl)ethyl)morpholine 4a. Yellow oil, yield 87 %. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.96 (d, *J* = 3.6 Hz, 1H, H6), 6.40 (d, *J* = 3.7 Hz, 1H, H5), 4.76 (d, *J* = 13.3 Hz, 2H, H2'' and H6''), 4.21 (t, *J* = 6.3 Hz, 2H, 2 x H1'), 3.65 – 3.61 (m, 4H, 2 x H5' and 2 x H7'), 3.08 – 2.98 (m, 2H, H2'' and H6''), 2.67 (t, *J* = 6.3 Hz, 2H, 2 x H2'), 2.64 – 2.58 (m, 1H, H4''), 2.57 – 2.51 (m, 4H, 2 x H8'' and 2 x H12''), 2.48 – 2.43 (m, 4H, 2 x H4', 2 x H8'), 1.96 (d, *J* = 12.0 Hz, 2H, H9'' and H11''), 1.65 – 1.52 (m, 6H, 2 x H3'', 2 x H5'', H9'' and H11''), 1.46 – 1.38 (m, 2H, 2 x H10''). ^{13}C NMR (101 MHz, $CDCl_3$) δ 157.09, 153.16, 152.34, 124.11, 101.22, 100.95, 66.98 (2C), 62.67, 58.19, 53.65 (2C), 50.17 (2C), 45.51 (2C), 41.68, 27.79 (2C), 26.00 (2C), 24.52. HRMS m/z 433.2477 [M+H$^+$] (calcd. for $C_{22}H_{33}ClN_6O$, 433.2418).

4-(2-(4-([1,4'-Bipiperidin]-1'-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)ethyl)morpholine 4b. Brown solid, yield 72 %, Mp 110-112 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.31 (s, 1H, H2), 7.02 (d, *J* = 3.6 Hz, 1H, H6), 6.44 (d, *J* = 3.6 Hz, 1H, H5), 4.84 (d, *J* = 13.3 Hz, 2H, 2 x H2''), 4.29 (t, *J* = 6.5 Hz, 2H, 2 x H1'), 3.71 – 3.60 (m, 4H, 2 x H5' and 2 x H7'), 3.06 (t, *J* = 12.4 Hz, 2H, H2'' and H6''), 2.84 (t, *J* = 10.9 Hz, 1H, H4''), 2.76 – 2.61 (m, 6H, 2 x H2', 2 x H8'' and 2 x H12''), 2.52 – 2.46 (m, 4H, 2 x H4', 2 x H8'), 2.09 (d, *J* = 11.9 Hz, 2H, H9'' and H11''), 1.81 – 1.60 (m, 6H, 2 x H3'', 2 x H5'', H9'' and H11''), 1.49 (br, 2H, 2 x H10''). ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.72, 151.22, 151.06, 124.08, 103.10, 100.48, 66.97 (2C), 63.31, 58.20, 53.67 (2C), 50.05 (2C), 45.35 (2C), 41.80, 27.33 (2C), 25.17 (2C), 24.01. HRMS m/z 399.2867 [M+H$^+$] (calcd. for $C_{22}H_{34}N_6O$, 399.2811).

4-([1,4'-Bipiperidin]-1'-yl)-7-(2-morpholinoethyl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-amine 4c. White solid, yield 39 %, Mp 146-148 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.68 (d, *J* = 3.6 Hz, 1H, H6), 6.27 (d, *J* = 3.6 Hz, 1H, H5), 4.73 (d, *J* = 13.3 Hz, 2H, H2'' and H6''), 4.57 (s, 2H, NH_2), 4.09 (t, *J* = 6.7 Hz, 2H, 2 x H1'), 3.68 – 3.61 (m, 4H, 2 x H5' and 2 x H7'), 2.92 (t, *J* = 12.1 Hz, 2H, 2 x H2'), 2.71 – 2.61 (m, 3H, H2'', H4'' and H6''), 2.60 – 2.54 (m, 4H, 2 x H8'' and 2 x H12''), 2.48 – 2.43 (m, 4H, 2 x H4', 2 x H8'), 1.94 (d, *J* = 12.0 Hz, 2H, H9'' and H11''), 1.68 – 1.50 (m, 6H, 2 x H3'', 2 x H5'', H9'' and H11''), 1.50 – 1.33 (m, 2H, 2 x H10''). ^{13}C NMR (101 MHz, $CDCl_3$) δ 158.72, 157.52, 153.55, 121.09, 100.96, 97.10, 66.95 (2C), 63.20, 58.09, 53.69 (2C), 50.06 (2C), 45.30 (2C), 41.51, 27.53 (2C), 25.62 (2C), 24.31. HRMS m/z 414.2976 [M+H$^+$] (calcd. for $C_{22}H_{35}N_7O$, 414.2914).

2.2.2.3 General Synthetic Procedure to Obtain *N*-Benzyl-pyrrolo[2,3-*d*]pyrimidine Derivatives **5-8a** and **5-8b.**

A mixture of 4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidine or 2,4-dichloro-7*H*-pyrrolo[2,3-*d*]pyrimidine (1.0 mmol), the respective benzyl halide (1.2 mmol) and potassium carbonate (3.0 mmol) in acetonitrile (5 mL) was stirred for 3 h, then the mixture was filtered and evaporated under vacuum. The products were separated by flash chromatography on silica gel eluting with methylene chloride.



2,4-Dichloro-7-(4-chlorobenzyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine 5a. White solid, yield 72 %, Mp 129-131 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.24 (d, *J* = 8.4 Hz, 2H, H3' and H5'), 7.12 – 7.07 (m, 3H, H6, H2' and H6'), 6.55 (d, *J* = 3.6 Hz, 1H, H5), 5.31 (s, 2H, N-CH_2). ^{13}C NMR (101 MHz, $CDCl_3$) δ 152.84, 152.21, 152.11, 134.41, 134.18, 129.40, 129.24 (2C), 129.15 (2C), 116.30, 100.73, 47.93. HRMS m/z 311.9857 [M+H$^+$] (calcd. for $C_{13}H_8Cl_3N_3$, 311.9814).

2,4-Dichloro-7-(4-fluorobenzyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine 6a. White solid, yield 67 %, Mp 96-98 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 (dd, *J* = 8.4, 5.3 Hz, 2H, H3' and H5'), 7.37 (d, *J* = 3.6 Hz, 1H, H6), 7.23 (t, *J* = 8.6 Hz, 2H, H2' and H6'), 6.81 (d, *J* = 3.6 Hz, 1H, H5), 5.58 (s, 2H, N-CH_2). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.65 (d, J_{CF} = 247.6 Hz), 152.78, 152.14, 152.06, 131.53 (d, J_{CF} = 3.3 Hz), 129.68 (d, J_{CF} = 8.3 Hz, 2C), 129.41, 116.31, 116.01 (d, J_{CF} = 21.7 Hz, 2C), 100.63, 47.91. HRMS m/z 296.0152 [M+H$^+$] (calcd. for $C_{13}H_8Cl_2FN_3$, 296.0111).

4-((2,4-Dichloro-7H-pyrrolo[2,3-*d*]pyrimidin-7-yl)methyl)benzonitrile 7a. White solid, yield 56 %. Mp 175-177 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 (d, *J* = 8.2 Hz, 2H, H3' and H5'), 7.22 (d, *J* = 8.1 Hz, 2H, H2' and H6'), 7.13 (d, *J* = 3.6 Hz, 1H, H6), 6.58 (d, *J* = 3.6 Hz, 1H, H5), 5.41 (s, 2H, N-CH_2). ^{13}C NMR (101 MHz, $CDCl_3$) δ 153.04, 152.37, 152.18, 140.98, 132.82 (2C), 129.46, 128.15 (2C), 118.22, 116.31, 112.36, 101.14, 48.08. HRMS m/z 303.0199 [M+H$^+$] (calcd. for $C_{14}H_8Cl_2N_4$, 303.0155).

2,4-Dichloro-7-(2,6-dichlorobenzyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine 8a. White solid, yield 64 %, Mp 140-142 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.24 (d, *J* = 8.0 Hz, 2H, H3' and H5'), 7.14 (dd, *J* = 8.7, 7.3 Hz, 1H, H4'), 6.83 (d, *J* = 3.7 Hz, 1H, H6), 6.38 (d, *J* = 3.7 Hz, 1H, H5), 5.52 (s, 2H, N-CH_2). ^{13}C NMR (101 MHz, $CDCl_3$) δ 152.56, 152.10, 151.94, 136.90, 130.93 (2C), 130.70, 128.90 (2C), 128.45, 116.18, 100.44, 43.67. HRMS m/z 345.9467 [M+H$^+$] (calcd. for $C_{13}H_7Cl_4N_3$, 345.9421).

4-Chloro-7-(4-chlorobenzyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine 5b. White solid, yield 46 %, Mp 144-145 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.73 (s, 1H, H2), 7.38 – 7.33 (m, 2H, H3' and H5'), 7.26 (d, *J* = 3.6 Hz, 1H, H6), 7.22 (d, *J* = 7.6 Hz, 2H, H2' and H6'), 6.70 (d, *J* = 3.6 Hz, 1H, H5), 5.49 (s, 2H, N-CH_2). ^{13}C NMR (101

MHz, $CDCl_3$) δ 152.37, 151.14, 151.00, 134.78, 134.19, 129.16 (2C), 128.98 (2C), 128.83, 117.51, 100.26, 47.84. HRMS m/z 278.0246 [M+H$^+$] (calcd. for $C_{13}H_9Cl_2N_3$, 278.0210).

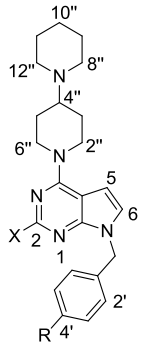
4-Chloro-7-(4-fluorobenzyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine 6b. White solid, yield 56 %, Mp 103-105 °C ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.69 (s, 1H, H2), 7.27 – 7.22 (m, 3H, H6, H3' and H5'), 7.07 – 7.00 (m, 2H, H2' and H6'), 6.65 (d, *J* = 3.6 Hz, 1H, H5), 5.45 (s, 2H, N-CH_2). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.53 (d, J_{CF} = 247.2 Hz), 152.28, 151.08, 150.92, 132.11 (d, J_{CF} = 3.3 Hz), 129.46 (d, J_{CF} = 8.3 Hz, 2C), 128.84, 117.50, 115.89 (d, J_{CF} = 21.7 Hz, 2C), 100.13, 47.80. HRMS m/z 262.0542 [M+H$^+$] (calcd. for $C_{13}H_9ClFN_3$, 262.0507).

4-((4-Chloro-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)methyl)benzonitrile 7b. White solid, yield 59 %, Mp 157-159 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.59 (s, 1H, H2), 7.55 (d, *J* = 8.2 Hz, 2H, H3' and H5'), 7.22 (d, *J* = 8.1 Hz, 2H, H2' and H6'), 7.16 (d, *J* = 3.6 Hz, 1H, H6), 6.61 (d, *J* = 3.6 Hz, 1H, H5), 5.46 (s, 2H, N-CH_2). ^{13}C NMR (101 MHz, $CDCl_3$) δ 152.60, 151.18 (2C), 141.55, 132.77 (2C), 128.80, 127.99 (2C), 118.28, 117.53, 112.23, 100.69, 48.04. HRMS m/z 269.0589 [M+H$^+$] (calcd. for $C_{14}H_9ClN_4$, 269.0552).

4-Chloro-7-(2,6-dichlorobenzyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine 8b. White solid, yield 51 %, Mp 151-153 °C ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.59 (s, 1H, H2), 7.24 (d, *J* = 8.1 Hz, 2H, H3' and H5'), 7.18 – 7.06 (m, 1H, H4'), 6.87 (d, *J* = 3.7 Hz, 1H, H6), 6.40 (d, *J* = 3.7 Hz, 1H, H5), 5.59 (s, 2H, N-CH_2). ^{13}C NMR (101 MHz, $CDCl_3$) δ 152.09, 151.15, 150.76 (2C), 136.89, 131.25, 130.71, 128.85 (2C), 127.81, 117.32, 100.02, 43.42. HRMS m/z 311.9857 [M+H$^+$] (calcd. for $C_{13}H_8Cl_3N_3$, 311.9815).

2.2.2.4 General Synthetic Procedure to Obtain Substituted Pyrrolo[2,3-*d*]pyrimidines **9a-12a** and **9b-12b.**

The *N*-benzyl-pyrrolo[2,3-*d*]pyrimidine **5-8a or 5-8b** (1.0 mmol), 4-piperidinopiperidine (3.0 mmol), triethylamine (4.5 mmol) and ethanol (5 mL) were added to a microwave reaction flask and the reaction mixture was irradiated for 15 minutes at 80 °C. Then the solvent was evaporated under vacuum and the crude product was purified by column chromatographic on silica gel using chloroform/methanol (10:1) mixture.



4-([1,4'-Bipiperidin]-1'-yl)-2-chloro-7-(4-chlorobenzyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine 9a. White solid, yield 53 %, Mp 97-99 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.24 (d, *J* = 7.9 Hz, 2H, H3' and H5'), 7.09 (d, *J* = 8.2 Hz, 2H, H2' and H6'), 6.77 (d, *J* = 3.5 Hz, 1H, H6), 6.43 (d, *J* = 3.6 Hz, 1H, H5), 5.26 (s, 2H, CH_2), 4.78 (d, *J* = 13.2 Hz, 2H, H2'' and H6''), 3.05 (t, *J* = 12.5 Hz, 2H, H2'' and H6''), 2.72 (t, *J* = 11.3 Hz, 1H, H4''), 2.60 (br, 4H, 2 x H8'' and 2 x H12''), 2.02 (d, *J* = 12.1 Hz, 2H. H9'' and H11''), 1.73 – 1.53 (m, 6H, 2 x H3'', 2 x H5'', H9'' and H11''), 1.45 (d, *J* = 4.6 Hz, 2H, 2 x H10''). ^{13}C NMR (101 MHz, $CDCl_3$) δ 157.09, 153.56, 152.54, 135.47, 133.76, 129.05 (2C), 128.94 (2C), 123.28, 101.91, 101.22, 62.80, 50.14 (2C), 47.34, 45.41 (2C), 27.56 (2C), 25.62 (2C), 24.27. HRMS m/z 444.1716 [M+H$^+$] (calcd. for $C_{23}H_{28}Cl_2N_5$, 444.1657).

4-([1,4'-Bipiperidin]-1'-yl)-2-chloro-7-(4-fluorobenzyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine 10a. White solid, yield 57 %, Mp 129-130 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.17 (dd, *J* = 8.3, 5.4 Hz, 2H, H3' and H5'), 6.98 (t, *J* = 8.6 Hz, 2H, H2' and H6'), 6.79 (d, *J* = 3.6 Hz, 1H, H6), 6.44 (d, *J* = 3.7 Hz, 1H, H5), 5.28 (s, 2H, CH_2), 4.79 (d, *J* = 13.3 Hz, 2H, H2'' and H6''), 3.06 (t, *J* = 12.1 Hz, 2H, H2'' and H6''), 2.73 – 2.64 (m, 1H, H4''), 2.61 – 2.53 (m, 4H, 2 x H8'' and 2 x H12''), 2.00 (d, *J* = 12.2 Hz, 2H, H9'' and H11''), 1.70 – 1.55 (m, 6H, 2 x H3'', 2 x H5'', H9'' and H11''), 1.50 – 1.37 (m, 2H, 2 x H10''). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.37 (d, J_{CF} = 246.5 Hz), 157.11, 153.53, 152.48, 132.77 (d, J_{CF} = 3.3 Hz), 129.47 (d, J_{CF} = 8.2 Hz, 2C), 123.22, 115.65 (d, J_{CF} = 21.6 Hz, 2C), 101.85, 101.21, 62.70, 50.16 (2C), 47.29, 45.47 (2C), 27.70 (2C), 25.86 (2C), 24.42. ^{19}F NMR (376 MHz, $CDCl_3$) δ -114.37. HRMS m/z 428.2012 [M+H$^+$] (calcd. for $C_{23}H_{27}ClFN_5$, 428.1961).

4-((4-([1,4'-Bipiperidin]-1'-yl)-2-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)methyl)benzonitrile 11a. White solid, yield 77 %, Mp 158-160 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56 (d, *J* = 8.2 Hz, 2H, H3' and H5'), 7.22 (d, *J* = 8.1 Hz, 2H, H2' and H6'), 6.82 (d, *J* = 3.7 Hz, 1H, H6), 6.49 (d, *J* = 3.7 Hz, 1H, H5), 5.37 (s, 2H, CH_2), 4.79 (d, *J* = 13.3 Hz, 2H, H2'' and H6''), 3.08 (t, *J* = 12.0 Hz, 2H, H2'' and H6''), 2.77 – 2.66 (m, 1H, H4''), 2.66 – 2.53 (m, 4H, 2 x H8'' and 2 x H12''), 2.03 (d, *J* = 12.1 Hz, 2H, H9'' and H11''), 1.72 – 1.61 (m, 6H, 2 x H3'', 2 x H5'', H9'' and H11''), 1.46 (d, *J* = 5.0 Hz, 2H, 2 x H10''). ^{13}C NMR (101 MHz, $CDCl_3$) δ 157.08, 153.71, 152.59, 142.41, 132.56 (2C), 127.97 (2C), 123.22, 118.50, 111.71, 102.38, 101.18, 62.66, 50.16 (2C), 47.51, 45.41 (2C), 27.65 (2C), 25.71 (2C), 24.31. HRMS m/z 435.2058 [M+H$^+$] (calcd. for $C_{24}H_{27}ClN_6$, 435.1999).

4-([1,4'-Bipiperidin]-1'-yl)-2-chloro-7-(2,6-dichlorobenzyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine 12a. White solid, yield 65 %, Mp 94-95 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.24 (d, *J* = 8.0 Hz, 2H, H3' and H5'), 7.15 – 7.11 (m, 1H, H4'), 6.42 (d, *J* = 3.7 Hz, 1H, H6), 6.23 (d, *J* = 3.7 Hz, 1H, H5), 5.46 (s, 2H, CH_2), 4.66 (d, *J* = 13.3 Hz, 2H, H2'' and H6''), 2.92 (t, *J* = 12.1 Hz, 2H, H2'' and H6''), 2.58 (t, *J* = 10.9 Hz, 1H, H4''), 2.47 (br, 4H, 2 x H8'' and 2 x H12''), 1.88 (d, *J* = 12.2 Hz, 2H, H9'' and H11''), 1.63 – 1.43 (m, 6H, 2 x H3'', 2 x H5'', H9'' and H11''), 1.36 – 1.22 (m, 2H, 2 x H10''). ^{13}C NMR (101 MHz, $CDCl_3$) δ 157.06, 153.31, 152.52 (2C), 137.04, 131.58, 130.44, 128.72 (2C), 121.99, 101.48, 101.20, 62.82, 50.12 (2C), 45.42, 43.39 (2C), 27.56 (2C), 25.69 (2C), 24.32. HRMS m/z 478.1327 [M+H$^+$] (calcd. for $C_{23}H_{26}Cl_3N_5$, 478.1265).

4-([1,4'-Bipiperidin]-1'-yl)-7-(4-chlorobenzyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine 9b. Brown solid, yield 47 %, Mp 103-104 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.45 (s, 1H, H2), 7.36 (d, *J* = 8.1 Hz, 2H, H3' and H5'), 7.22 (d, *J* = 8.0 Hz, 2H, H2' and H6'), 7.00 (d, *J* = 3.1 Hz, 1H, H6), 6.60 (d, *J* = 3.2 Hz, 1H, H5), 5.45 (s, 2H, CH_2), 4.97 (d, *J* = 13.0 Hz, 2H, H2'' and H6''), 3.19 (t, *J* = 12.5 Hz, 2H, H2'' and H6''), 3.00 (t, *J* = 11.1 Hz, 1H, H4''), 2.83 (br, 4H, 2 x H8'' and 2 x H12''), 2.22 (d, *J* = 11.6 Hz, 2H, H9'' and H11''), 1.94 – 1.77 (m, 6H, 2 x H3'', 2 x H5'', H9'' and H11''), 1.61 (s, 2H, 2 x H10''). ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.74, 151.44 (2C), 135.87, 133.56, 128.88 (2C), 128.79 (2C), 123.38, 103.05, 101.41, 63.28, 50.02 (2C), 47.30, 45.30 (2C), 27.23 (2C), 25.01 (2C), 23.90. HRMS m/z 410.2106 [M+H$^+$] (calcd. for $C_{23}H_{28}ClN_5$, 410.2048).

4-([1,4'-Bipiperidin]-1'-yl)-7-(4-fluorobenzyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine 10b. Brown solid, yield 58 %, Mp 118-120 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.61 (s, 1H, H2), 7.42 (dd, *J* = 8.2, 5.5 Hz, 2H, H3' and H5'), 7.23 (t, *J* = 8.6 Hz, 2H, H2' and H6'), 7.15 (d, *J* = 3.6 Hz, 1H, H6), 6.74 (d, *J* = 3.6 Hz, 1H, H5), 5.60 (s, 2H, CH_2), 5.11 (d, *J* = 13.3 Hz, 2H, H2'' and H6''), 3.33 (t, *J* = 12.2 Hz, 2H, H2'' and H6''), 3.07 (t, *J* = 11.5 Hz, 1H, H4''), 2.93 (br, 4H, 2 x H8'' and 2 x H12''), 2.34 (d, *J* = 12.0 Hz, 2H, H9'' and H11''), 2.06 – 1.84 (m, 6H, 2 x H3'', 2 x H5'', H9'' and H11''), 1.75 (d, *J* = 4.9 Hz, 2H, 2 x H10''). ^{13}C NMR (101 MHz, $CDCl_3$) δ 162.30 (d, J_{CF} = 246.1 Hz), 156.79, 151.46, 151.42, 133.16 (d, J_{CF} = 3.2 Hz), 129.19 (d, J_{CF} = 8.2 Hz, 2C), 123.27, 115.61 (d, J_{CF} = 21.6 Hz, 2C), 103.05, 101.36, 63.20, 50.08 (2C), 47.24, 45.40 (2C), 27.43 (2C), 25.34 (2C), 24.11. ^{19}F NMR (376 MHz, $CDCl_3$) δ -114.67. HRMS m/z 394.2402 [M+H$^+$] (calcd. for $C_{23}H_{28}FN_5$, 394.2346).

4-((4-([1,4'-Bipiperidin]-1'-yl)-7*H*-pyrrolo[2,3-*d*]pyrimidin-7-yl)methyl)benzonitrile 11b. Brown solid, yield 52 %, Mp 101-103 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.32 (s, 1H, H2), 7.57 (d, *J* = 8.1 Hz, 2H, H3' and H5'), 7.22 (d, *J* = 8.0 Hz, 2H, H2' and H6'), 6.90 (d, *J* = 3.5 Hz, 1H, H6), 6.53 (d, *J* = 3.5 Hz, 1H, H5), 5.43 (s, 2H, CH_2), 4.84 (d, *J* = 13.2 Hz, 2H, H2'' and H6''), 3.08 (t, *J* = 12.4 Hz, 2H, H2'' and H6''), 2.77 (t, *J* = 11.3 Hz, 1H, H4''), 2.68 – 2.57 (br, 4H, 2 x H8'' and 2 x H12''), 2.06 (d, *J* = 11.9 Hz, 2H, H9'' and H11''), 1.77 – 1.56 (m, 6H, 2 x H3'', 2 x H5'', H9'' and H11''), 1.47 (s, 2H, 2 x H10''). ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.83, 151.69, 151.52, 142.84, 132.56 (2C), 127.77 (2C), 123.15, 118.55, 111.61, 102.99, 101.94,

63.05, 50.14 (2C), 47.53, 45.43 (2C), 27.59 (2C), 25.54 (2C), 24.23. HRMS m/z 401.2448 [M+H$^+$] (calcd. for $C_{24}H_{28}N_6$, 401.2392).

4-([1,4'-Bipiperidin]-1'-yl)-7-(2,6-dichlorobenzyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine 12b. White solid, yield 51 %, Mp 122-124 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.27 (s, 1H, H2), 7.24 (d, *J* = 8.0 Hz, 2H, H3' and H5'), 7.12 (t, *J* = 8.0 Hz, 1H, H4'), 6.52 (d, *J* = 3.6 Hz, 1H, H6), 6.25 (d, *J* = 3.7 Hz, 1H, H5), 5.52 (s, 2H, CH_2), 4.73 (d, *J* = 13.4 Hz, 2H, H2'' and H6''), 3.01 – 2.77 (m, 3H, H2'', H6'' and H4''), 2.67 (br, 4H, 2 x H8'' and 2 x H12''), 2.04 (d, *J* = 11.7 Hz, 2H, H9'' and H11''), 1.72 (br, 4H, 2 x H3'' and 2 x H5''), 1.57 (qd, *J* = 12.1, 3.6 Hz, 2H,), 1.40 (s, 2H, 2 x H10''). ^{13}C NMR (101 MHz, $CDCl_3$) δ 156.63, 151.51, 151.17 (2C), 136.98, 131.93, 130.36, 128.72 (2C), 122.18, 103.01, 100.97, 63.65, 49.93 (2C), 45.14 (2C), 43.10, 26.83 (2C), 24.41 (2C), 23.54. HRMS m/z 444.1716 [M+H$^+$] (calcd. for $C_{23}H_{27}Cl_2N_5$, 444.1655).

2.2.3 Pharmacological Part

2.2.3.1 Radioligand Displacement Assays at the H_3R

Radioligand displacement assays at the human histamine H_3R were performed as published previously.[18] Briefly, membrane preparations of HEK-293 cells stably expressing the human histamine H_3R (20 µg/well) were incubated with the compound and [^{3}H]N^α-methylhistamine (2 nM) in 200 µL of binding buffer (10 mM $MgCl_2$, 100 mM NaCl and 75 mM Tris/HCl, pH 7.4) for 90 min. Compounds were tested in duplicates with 11 concentrations in at least three independent experiments. Data was analyzed with GraphPad Prism 7, using non-linear least squares fit and equation "one site competition" (representative figures given in the SI). K_i values were calculated according to the Cheng-Prusoff equation.[19] Values are reported as means with the 95% confidence interval. All statistical operations were performed on the pK_i values and converted afterwards to mean K_i values and the 95% confidence interval. Differences are considered significant if 95% confidence intervals are not overlapping.

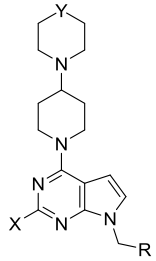
2.2.3.2 Radioligand Displacement Assays at the H_4R

H_4R radioligand displacement assays were performed as described previously.[20] Briefly, Sf9 cell membrane preparations, expressing the human histamine H_4R (40 µg/well), were co-incubated with the compound and [^{3}H]histamine (10 nM) in 200 µL for 60 min. Compounds were tested in duplicates with at least five concentrations in two independent experiments. Data was analyzed with GraphPad Prism 7.

3 Results and Discussion

The first synthesized set (**compounds 3a-3c** and **4a-4c**) consisted of compounds bearing a morpholine group instead of the lipophilic core (**R**) that was connected to the pyrrolo[2,3-*d*]pyrimidine via an ethyl linker. All compounds displayed marked affinity at the H_3R without H_4R affinity (K_i value > 10 µM). The general introduction of the morpholine group instead of a lipophilic core improved binding affinity slightly, when compared to **compound V** (K_i value 126 nM), with K_i values below 50 nM for derivatives **4a** and **4b**, although results were not significant. By comparing binding affinities of series **3** to **4** (**Table 1**) the pharmacodynamic superiority of 1,4-bipiperidine moieties (**series 4**) as basic warhead (**Y**), compared to 4-(piperidin-4-yl)morpholines (**series 3**), was once more verified.[16] The exchange towards the 1,4-bipiperidine resulted in a significant increase of binding affinity. Derivatization at position 2 (**X**) gave no significant difference between chlorinated compounds or the unsubstituted central core but revealed a pronounced loss of affinity for the aminated compounds (**3c**, **4c**). Due to these findings, only compounds **4a** and **4b** were further derivatized.

*Table 1: Affinities at the H_3R of **compounds 3a-3c, 4a-4c, 9a-12a** and **9b-12b**.*



Compound	Substitution pattern			K_i (nM)[a] [95% CI nM]
	X	Y	R	
3a	Cl	O	Morph-CH_2[b]	317 [135;743]
3b	H	O	Morph-CH_2	374 [162;865]
3c	NH_2	O	Morph-CH_2	848 [386;1866]
4a	Cl	CH_2	Morph-CH_2	22 [11;45]
4b	H	CH_2	Morph-CH_2	31 [16;62]
4c	NH_2	CH_2	Morph-CH_2	121 [49;301]
9a	Cl	CH_2	Ph-4-Cl[c]	42 [14;127]
9b	H	CH_2	Ph-4-Cl	26 [15;46]
10a	Cl	CH_2	Ph-4-F[d]	30 [11;81]
10b	H	CH_2	Ph-4-F	35 [10;120]
11a	Cl	CH_2	Ph-4-CN[e]	18 [5;66]
11b	H	CH_2	Ph-4-CN	7 [4;11]
12a	Cl	CH_2	Ph-2,6-diCl[f]	54 [25;116]
12b	H	CH_2	Ph-2,6-diCl	6 [2;19]

[a] K_i values were measured at the human histamine H_3R. Membrane preparations of HEK-293T cells expressing the H_3R (20 µg/well) were incubated with the compound of interest and [^{3}H]*N*$^\alpha$-methylhistamine (2 nM) in 200 µL for 90 min. [b] Morph-CH_2, morpholinomethyl. [c] Ph-4-Cl, 4-chlorophenyl. [d] Ph-4-F, 4-fluorophenyl. [e] Ph-4-CN, 4-cyanophenyl. [f] Ph-2,6-diCl, 2,6-dichlorophenyl.

In **compounds 9a-12a** the 4-([1,4'-bipiperidin]-1'-yl)-2-chloro-7H-pyrrolo[2,3-*d*]pyrimidine moiety was connected to differently substituted benzyl moieties. Inspired by the substitution pattern

of pitolisant, the first ligand displayed a 4-chlorophenyl residue, which was exchanged by several other electron-withdrawing substituted benzyl derivatives. The compounds showed high affinity at the H_3R, without affinity at the H_4R (K_i value > 10 µM) and no significant changes in affinity, based on the derivatization pattern of the benzyl (**Table 1**).

For the **compounds 9b-12b** without chlorination on the heterocyclic moiety affinity at the H_3R increased by increasing electron-withdrawing properties of the aromatic core (**Table 1**), while no compound showed considerable affinity at the H_4R (K_i values > 10 µM). For **compound 11b** the increase of binding affinity at the H_3R was significant when compared to those of **4b** (morpholinomethyl as **R**) or **9b**, the compound with the weakest electron-withdrawing properties. Furthermore, **compound 12b** is the only compound where dechlorination led to a significant increase of binding affinity compared to the chlorinated compound (**12a**). When compared to **compound V** the introduction of a cyano moiety (**11b**) or a 2,6-dichlorobenzyl (**12b**) increased affinity significantly with K_i values of 7 and 6 nM, respectively. Although functional studies were not performed on the scaffold of pyrrolo[2,3-*d*]pyrimidines, the evidence of many non-imidazole ligands being H_3R antagonist or inverse agonists (aplysamine-1, conessine and pitolisant[6,7,11]) could rationalize an *in vivo* evaluation of these novel compounds as H_3R antagonists/inverse agonists.

Based on the **lead structure** the presented compounds allow for an initial structure-activity relationship evaluation for the class of pyrrolo[2,3-*d*]pyrimidine as novel H_3R scaffolds.
The 1,4-bipiperidine motif in position 4 seems to be crucial for high affinity towards the H_3R (compare **series 3** and **4**). The introduction of a 2-morpholinoethyl instead of a 2-naphthylmethyl moiety in position 7 (**compounds 3a-3c, 4a-4c**) improved binding affinity not significantly compared to **compound V**. The introduction of an amine group at position 2 leads to a loss in affinity (**series c**, significant for **4a** to **4c**). For a given structure, with or without chloro substitution in 2-position did not result in any significant affinity changes (compare **3a** to **3b**, **4a** to **4b**, etc.), except for the most active **compound 12b**. The introduction of substituted benzyl moieties at 7-position did not further increase binding affinity, if chlorinated at 2-position (**compounds 9a-12a**) and derivatization pattern of the benzyl did not influence affinity. For compounds without chloro or amino substitution at 2-position of the heterocycle (**9b-12b**) affinity was increased by increasing electron-withdrawing abilities of the substitution pattern, with the most active compounds (**11b** and **12b**) displaying a significantly higher affinities than that of **compound V**.

4 Conclusion

Within this study we were able to synthesize 14 novel pyrrolo[2,3-*d*]pyrimidines that adhere to the general pharmacophore of H_3R ligands. The 1,4-bipiperidine motif as basic centre has proven itself highly effective for designing H_3R ligands with superior binding affinity. The arbitrary, lipophilic region was best modulated by benzyl residues with electron-withdrawing groups. *In vivo* evaluation of these nature inspired synthetic pyrrolo[2,3-*d*]pyrimidines will be highly interesting, as the potential anti-inflammatory properties of this class may have synergistic effects with the human H_3R modulation[21] on cognitive impairments in neurodegenerative diseases. By that approach drug development of CNS disorders may be complemented by a new class of active agents and natural compounds have once more proven their versatility within the drug research and development community.

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Additional analytic information can be found in the Supplemental Material at www…..

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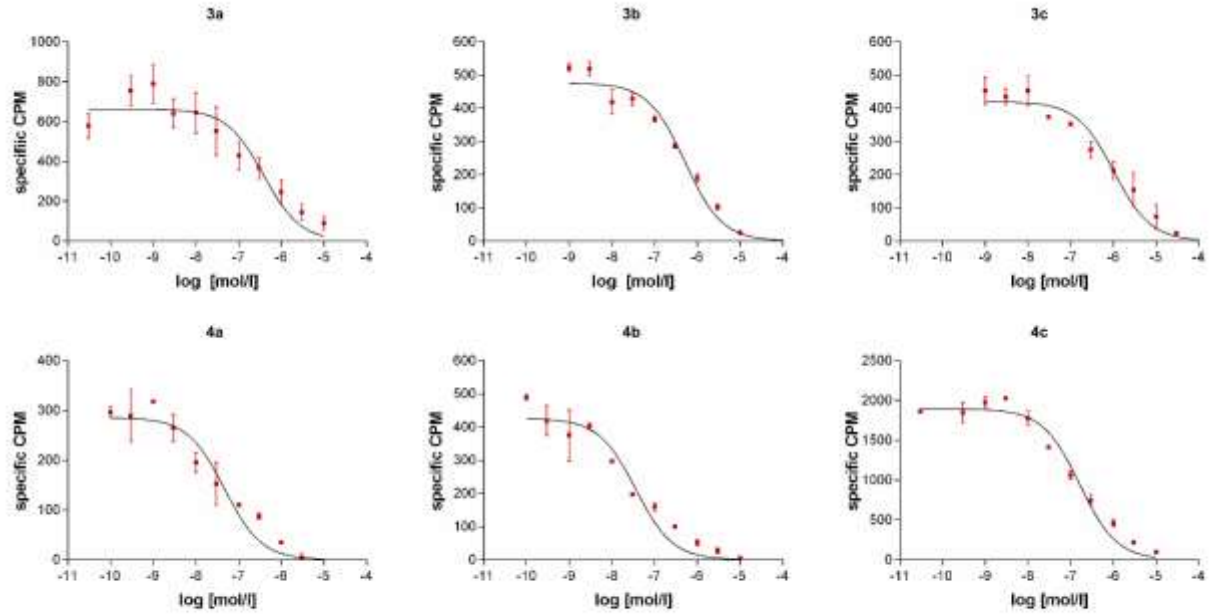


*Figure 4: Representative figures of the radioligand displacement assays at the human H_3R for compounds **3a-3c** and **4a-4c**. Figure displays specific CPM as means with respective standard error of the mean (SEM), non-normalized.*

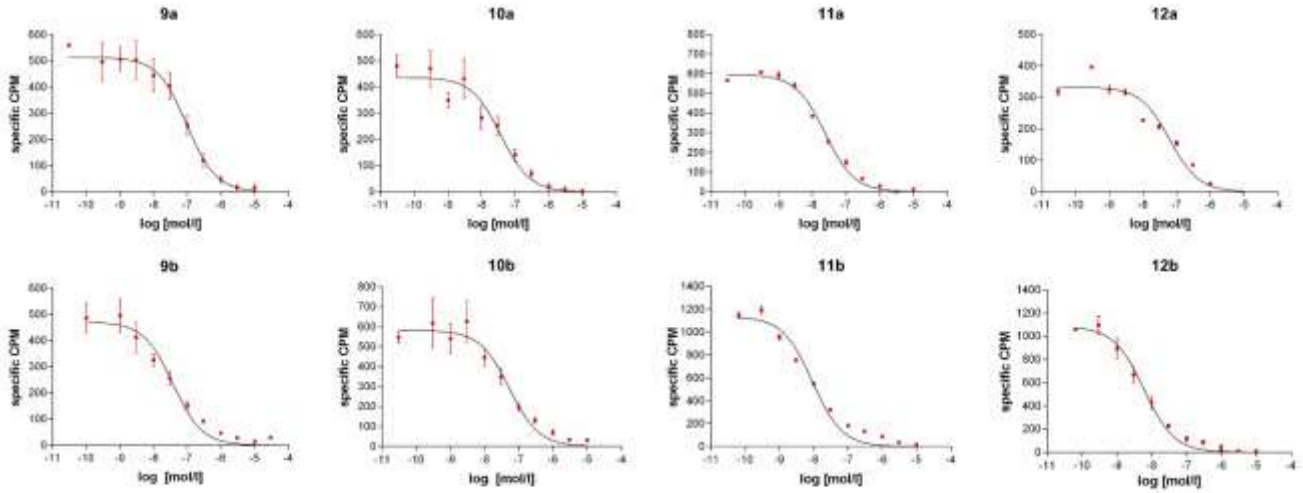
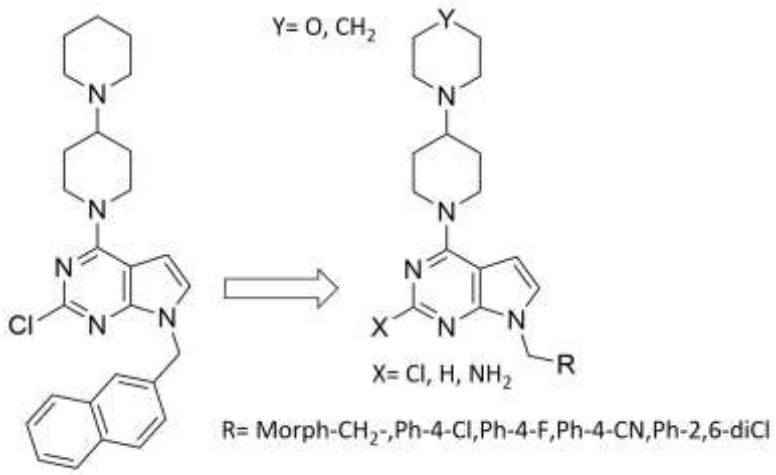


*Figure 5: Representative figures of the radioligand displacement assays at the human H_3R for compounds **9a-12a** and **9b-12b**. Figure displays specific CPM as means with respective standard error of the mean (SEM), non-normalized.*

Graphical abstract



compound V **histamine H_3 receptor K_i values 6 - 848 nM**