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Azines as histamine H_4 receptor antagonists

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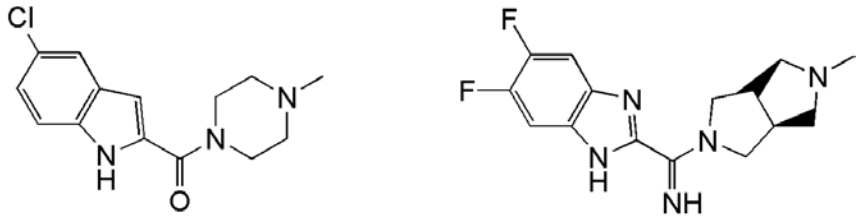
1. ABSTRACT

Since 2000, when the histamine H_4 receptor (H_4R) was cloned, it has constituted an interesting target for drug development. Pharmacological studies suggest the potential utility of histamine H_4R antagonists/inverse agonists in the treatment of inflammatory diseases, e.g. allergic rhinitis, asthma, atopic dermatitis, colitis, or pruritus. The first H_4R ligands were non-selective compounds, but intensive chemical and pharmacological work has led to the discovery of highly potent and selective H_4R antagonists (e.g. JNJ7777120, CZC-13788, PF-2988403, A-940894, A-987306). The first compound (UR-63325) has finally entered into clinical studies for the treatment of allergic respiratory diseases (completing the phase I ascending dose trial) and has been found to be safe and well tolerated. The number of scientific publications and patent applications in the H_4 field is increasing annually. Among the diverse chemical structures of the H_4R antagonists described a 2-aminopyrimidine scaffold is repeatedly found. This review looked at recent advances in the search for H_4R antagonists as reflected in patent applications/patents and peer-reviewed publications over the last two years. The work concerns azines (mono-, di-, triazines) and their fused analogues. The chemistry and pharmacology has been described.

2. INTRODUCTION

Histamine H_4R was identified in 2000 by homology-search strategies used by several research groups independently (e.g. 1-2). Molecular biology analysis showed that $h$$H_4R$ had the highest homology to $h$$H_3R$ (37% protein sequence identity, 58% in transmembrane domains) (3). H_4R is widely expressed in cells and tissues of the immune system (mast cells, dendritic cells, eosinophils, monocytes, basophils and T cells), suggesting its role in the immunological and inflammatory processes (4). Very recently the presence of $h$$H_4R$ in CNS was also described (5).

Histamine H_4R ligands (antagonists/inverse agonists) were evaluated in animal models of some diseases (e.g. allergic rhinitis, airway inflammation, pruritus, itch or pain) and showed positive effects (6,7,8). Soon after the discovery of H_4R, the first potent and selective non-imidazole antagonist (JNJ 7777120; K_i = 4 nM; Figure 1) was identified by Johnson and Johnson (9). JNJ7777120 is used as a pharmacological tool for probing the physiological role of H_4R. For example, JNJ 7777120 has been reported to have anti-inflammatory activity *in vivo* (10), to cause inhibition of pruritus (11), to ameliorate chronic allergic contact dermatitis (12), and to exhibit anti-



K_i = 4 nM
JNJ7777120

K_i = 9.6 nM
PF-2988403

Figure 1. The structures of selected known H_4 receptor antagonists (9,16,17).

nociception in animal models of inflammatory and neuropathic pain (7). Since that time many other potent and selective H_4R antagonists/inverse agonists have been synthesized by pharmaceutical companies and academic researchers. Recent reviews have thoroughly described the chemistry, pharmacology and preclinical evaluations of H_4R antagonists/inverse agonists (6,13,14,15). Preclinical assessment of these compounds in animal models of diseases (e.g. inflammatory disorders) can prove the efficacy of the tested compounds in therapy. Some of the described compounds are very promising and can potentially enter into clinical trials. The preclinical profile of the Pfizer compound PF-2988403 (Figure 1) has recently been demonstrated (16,17). PF-2988403 is a potent and selective H_4R antagonist (hH_4R K_i = 9.6 nM; hH_3R K_i = 3090 nM; hH_2R K_i = 7140 nM; hH_1R K_i = 29 100 nM) which has displayed a full range of functional effects dependent on the species tested (hH_4R, neutral antagonist; *dog* H_4R and *monkey* H_4R, partial agonist; *mouse* H_4R and *rat* H_4R, full agonist). *In vivo* in rats, the compound behaved as a full agonist and showed pro-inflammatory effects (e.g. changes in peripheral blood/bone marrow and spleen). Also very promising is the Palau Pharma compound UR-65318 (structure unknown). UR-65318 displays high affinity and selectivity for H_4R. The compound is in preclinical testing for the treatment of atopic dermatitis and displays the same efficacy profile as gold standard treatments such as Protopic™ (18). Another Palau Pharma ligand, UR-63325, is the first H_4R antagonist to show activity in clinical trials (currently in phase Ib/IIa clinical trials for asthma and allergic rhinitis) (18,19,20). UR-63325 has high binding affinity for hH_4R with IC_{50} of 24 nM and high selectivity compared to other histamine receptors (IC_{50} = 5800 nM for H_3R; % binding inhibition at 10 μM of 46% and 4% for H_1R and H_2R, respectively). UR-63325 behaved as an antagonist in isolated or whole blood eosinophils. In animal asthma models the compound showed good efficacy in both the inflammatory and respiratory parameters. In healthy human volunteers UR-63325 was well tolerated when orally administrated (once a day) and no serious or severe adverse effects were reported.

In the search for H_4R antagonists/inverse agonists various chemical classes of ligands were reported. An important group among them were the pyrimidine-based compounds (differently substituted or fused), which were identified by scientists from many research groups (14). This review was carried out on recent advances in the search for H_4R antagonist/inverse agonists as reflected in publications and patent applications/patents over the last two years (2009 until October 2010). Azine (mono-, di- or triazine) derivatives were surveyed.

3. AZINES AS HISTAMINE H_4 RECEPTOR ANTAGONISTS

A very recent review (14) of H_4R antagonists gave an overview of the literature on azines, and mainly pyrimidines (21-27). The structures of pyrimidine-based H_4R ligands were grouped by the nature of the lipophilic moieties attached at the 6 position of the pyrimidine ring, into aryl (22,23,27), alkyl (24,27) or amino (21,25-27) substituted compounds. This review is a continuation of the previous work (14) and describes the azines from the overview of the literature and patent applications (2009-2010). The compounds were divided into two groups: azines and fused azines (two or three/four rings).

3.1. Monoazines: pyridines, pyrimidines, pyridazines and triazines

Broad ligand-based virtual screening (28,29) has shown that 2,4-diamino pyrimidine is a potent hH_4R affinity scaffold. SARs of this group of compounds made it possible to identify the structures 1, 2, 3 (K_i = 18 nM; K_i = 25 nM; K_i = 12 nM respectively; Figure 2) as the best compounds with hH_4R binding in the nano-concentration range. Extensive investigation discovered that slight structural changes caused great differences in the functional activities and potencies: while 2- and 4-substituted benzyl amines mainly showed partial agonism, 3-substituted and rigidified ones exhibited inverse agonist efficacy. Optimization of the substituents in the benzyl part of the moiety was performed using classical Topliss Tree (30). Electron-releasing hydrophilic groups like 4-methoxy, 4-hydroxy and electron-withdrawing substituents like 3,4-di-Cl or 4-trifluoromethyl decreased binding potency. However, the greatest disadvantage was brought by an acidic moiety at the 4-position. 2-Amino substitution of the pyrimidine scaffold was also crucial for the activity of the exocyclic 4-amino group, as changing the latter group to O or S atom led to a great loss of affinity. Affinities to hH_4R were examined in a (^{3}H)histamine competition binding assay using membrane preparations from S*f*9 cells expressing hH_4R, co-expressed with G protein Galphai2

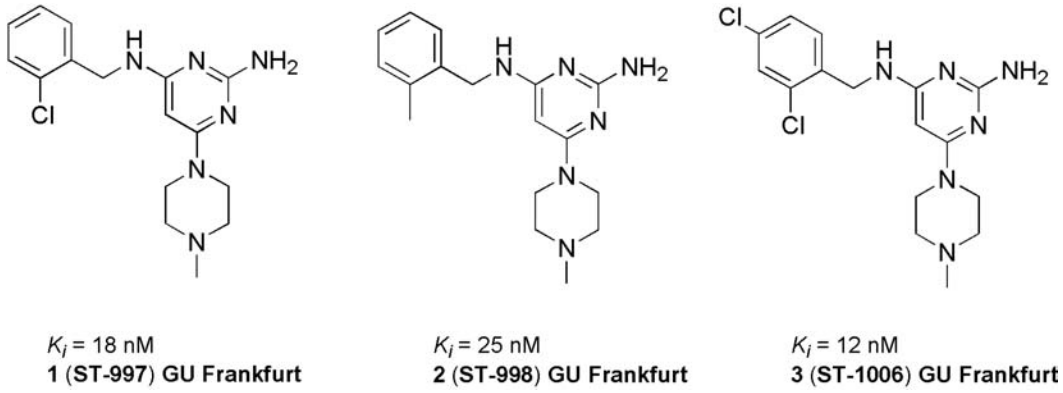


Figure 2. 2,4-Diaminopyrimidines from Goethe University Frankfurt (28,29).

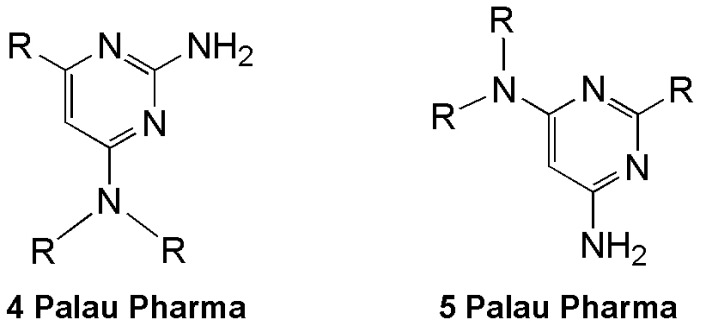


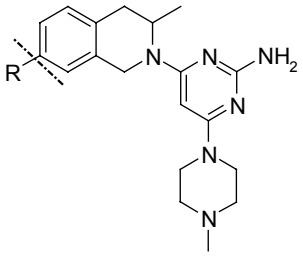
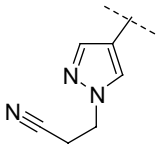
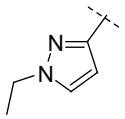
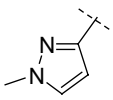
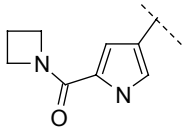
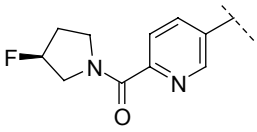
Figure 3. General structures of diamino-pyrimidines from Palau Pharma (31,32).

and Gbeta1gamma2 subunits. Two functional assays were used to determine the efficacy of the examined compounds: the first was based on the exchange of GDP to (^{35}S)GTPgammaS after a ligand has bound to the receptor, while the second determined the steady-state GDP/GTP exchange by measuring hydrolysis of (gamma-^{32}P)GTP by a GTPase enzyme.

2-Aminopyrimidine and 4-aminopyrimidine derivatives were claimed by Palau Pharma in two patent applications (31,32). The first application described 64 examples, while the second described 28. Compounds 4 and 5 with the general structures presented in Figure 3 were included. Representative compounds were evaluated in two assays: the first one was a competitive binding assay for (^{3}H)-histamine to the *h*H$_4$R receptor stably expressed in recombinant CHO cells. Non-specific binding was defined as binding in the presence of unlabelled histamine. As claimed in the first application - 59 compounds assayed in this test exhibited an inhibition of more than 50% of binding to *h*H$_4$R at 1 μM concentration. In the second application 18 compounds exhibited similar activity. Histamine induced shape change in human eosinophils was used as the second assay (Gated Autofluorescence Forward Scatter assay, GAFS). In this assay the shape change induced by histamine in human eosinophils was determined by flow cytometry, detected as an increase in the size of the cells. 55 compounds from the first application and 13 from the second one assayed in this test produced more than 50% inhibition of histamine-induced human eosinophils shape change at 1 μM concentration. Compounds from both applications possessed basic structural similarities: a 2- (4-)amino group, R^{1}cycloalkylamino substituent and R^2 and R^3 forming with the N atom to which they were bound, a saturated heterocyclic group which can be 4- to 7-membered monocyclic, 7- to 8- membered bridged bicyclic or 8- to 12- membered fused bicyclic. No particular results were disclosed.

2-Amino-4,6-disubstituted pyrimidines with a similar main skeleton were claimed in Incyte Corporation's patent application (33). 158 compounds, with examples of enantiomers of chiral structures, were evaluated in three tests. The first one was an H$_4$R membrane binding assay using recombinant HEK293 SFM cells expressing *h*H$_4$R or mouse H$_4$R with (^{3}H)histamine as the radioligand. The second one was a Ca^{2+} flux assay, and the third one was an H$_4$R eosinophil chemotaxis assay. The IC_{50} values for the example compounds with respect to H$_4$R made it possible to select 5 compounds with IC_{50} values < 20 nM (6, IC_{50} = 12.1 nM, 7, IC_{50} = 17.6 nM; 8, IC_{50} = 8.7 nM; 9, IC_{50} = 8.7 nM; 10, IC_{50} = 15.9 nM; Figure 4). The common features of this group of compounds were 2-amino and 4-methylpiperazine moieties. A substituent (un)substituted with methyl dihydroisoquinoline was placed at the 6 position. The methyl substituent however a chiral center did not decide on activity, although it influenced its degree. Enantiomer activity was compared although the configuration of the enantiomers was not described. The greatest difference between two enantiomers was up to 14

Table 1. Comparison of enantiomers actvities of compounds **11-15** from Incyte Corporation (33)

Enantiomer	Structure	H_4R activity IC_{50} (nM)	B : A
			
		26 352	13.5
		40 231	5.8
		20 104	5.2
		25 127	5.1
		85 355	4.2

fold (see Table 1; compounds 11-15). The nature of the substituents at the 7 position of dihydroisoquinoline had a deciding influence on the activity of the evaluated compounds.

Janssen Pharmaceutica is one of the most active pharmaceutical companies in the field of H_4R research. In the years 2004 to 2008, 11 patent applications were claimed (14), followed by two published in 2009 (34,35). In the first substituted nitrogen-containing heteroaryl derivatives were investigated (34). The activity of ca 150 compounds was evaluated in a binding assay on recombinant hH$_4$R (SK-N-MC cells or COS 7 cells) with (^{3}H)-histamine as the radioligand. The invention was directed to compounds with the following general formula (16; Figure 5) where the A moiety was represented by a group consisting of substituted triazines, pyrimidines or pyridines. From the exemplified structures triazines 17, 18 and 19 showed activity in the range of K_i < 10 nM (17, K_i = 2 nM; 18, K_i = 7 nM, 19, K_i = 8 nM; Figure 5). 4-methyl-piperazine and aryl substituents were generally typical for the investigated structures. Aryl substituents were: (un)substituted phenyl, biphenyl or heteroaryl (pyridyl, thienyl, furyl). For the group of triazines, thienyl derivatives were superior to pyridyl ones. Chlorine substituted thienyls in particular had improved affinity; compare 20 (K_i = 76 nM; Figure 5) with 19 (K_i = 8 nM; Figure 5). More voluminous substituents like 2- or 3-benzothienyl, 1- or 2-naphthyl were not well tolerated, especially in the group of triazines; compare 21 (K_i > 10000 nM; Figure 5) with 22 (K_i > 2000 nM; Figure 5). Among pyridine derivatives aryl substituents were placed mostly at the 2 position. Several compounds showed lower affinity than triazines with the exception of some with biphenyl substituents (e.g. 23 with K_i = 10 nM; Figure 5). Changing the place of the 4-methyl-piperazine and the aryl substituent decreased the affinity. The evaluated pyrimidine derivatives with a (homo)piperazine substituent and without a methyl group were almost devoid of affinity. The place of the amine substituent had little influence on the affinity (4-position was unfavourable for (homo)piperazine).

Further efforts by researchers from Janssen Pharmaceutica (35) were focused on diamino-pyridines, pyrimidines and pyridazines. Very potent H4R ligands were discovered with activity even in the subnanomolar range.

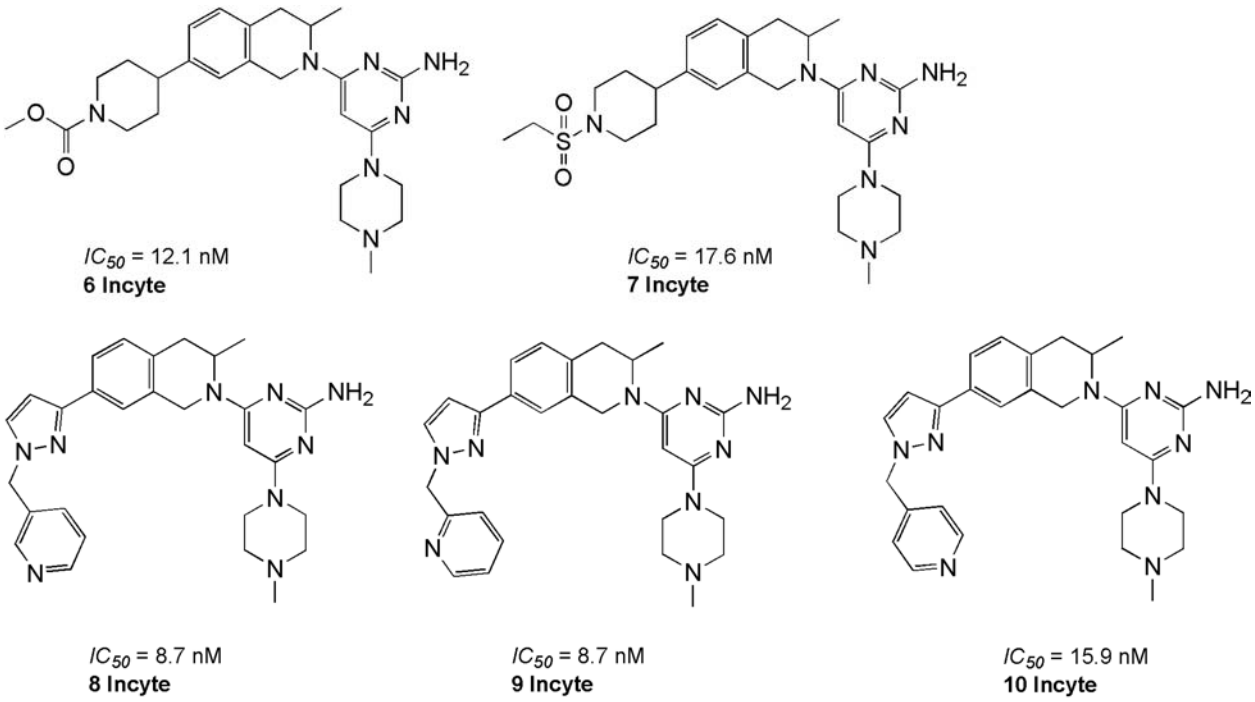


Figure 4. Selected structures from Incyte Corporation (33).

Compounds were evaluated in three types of tests: 1) a binding assay on recombinant *h*H_4R using SK-N-MC cells stably or transiently transfected with *h*H_4R and selected compounds, in a 2) functional cell-based cAMP assay and 3) insulin resistance in the obesity-induced diabetes mouse model. From the ca 400 evaluated compounds with the general structure (24, Figure 6) several pyridine derivatives (25, K_i = 0.5 nM; 26, K_i = 0.4 nM; 27, K_i = 0.5 nM; 28, K_i = 0.9 nM; 29 K_i = 0.9 nM; Figure 6) showed affinity with K_i < 1 nM. It was noticeable that H_4R tolerated bulky substituents (25, 26, 27, 29) and was not sensitive to their different configuration; compare 26 (K_i = 0.4 nM; Figure 6) with 27 (K_i = 0.5 nM; Figure 6). In the functional cAMP assay the most active were 30 (pA_2 =9.24; K_i = 1.5 nM; Figure 6) and 31 (pA_2 =9.24; K_i = 8412 nM; Figure 6). Generally pyridazine derivatives were less potent. Among more than 100 evaluated pyridazines most were less active, though there were two exceptions (32, K_i = 5.5 nM; 33, K_i = 5.6 nM; Figure 6). Among the more potent pyrimidines some were even found with subnanomolar affinity (e.g. 34, K_i = 0.7 nM; Figure 6).

2-Amino triazine derivatives were synthesized by scientists from the Jagiellonian University, Krakow (36,37). 2-Amino-1,3,5-triazines containing a 4-methylpiperazine group at the 4 position were tested as H_4R antagonists. Affinities to *h*H_4R were examined with the method described previously (28) in a (^{3}H)histamine competition binding assay using membrane preparations from S*f*9 cells expressing *h*H_4R, co-expressed with G protein Galphai2 and Gbeta1gamma2 subunits. Until now the tested compounds have been less potent than the corresponding pyrimidine derivatives (e.g. 35 with K_b = 4.8 nM; FLIPR (38) vs 36 with K_i = 203 nM; Figure 7) indicating that *h*H_4R has low tolerance for additional nitrogen in the heterocyclic core. It was stated that the kind and place of substituents in the aryl group at the 6-position had great influence on the affinity. The most potent compound 36 showed moderate potency but it might be a good lead structure for further development.

3.2. Fused azines: pyrimidines, pyridines

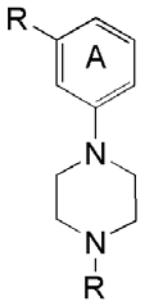
3.2.1. Fused two rings compounds

3.2.1.1. Furopyrimidines

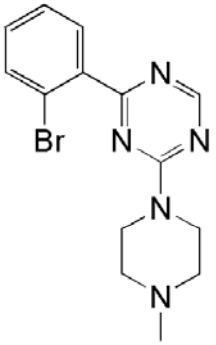
46 Furo (3,2-d)pyrimidine-2-amines were reported by Palau Pharma (39). 40 compounds were substituted at the 4- and 7- position (e.g. 37; Figure 8) whereas 6 compounds were also substituted at the 6 position by chlorine or carbonitrile (e.g. 38; Figure 8). At the 4 position a cycloalkylamine moiety was present, especially 3- (methylamino)-azetidine and (*3R*)-3-(methylamino)-pyrrolidine. At the 7 position mainly (cyclo)alkyl groups were introduced (e.g. cyclopropyl). The compounds were tested in a (^{3}H) histamine binding assay to *h*H_4R (CHO cells) and histamine-induced shape change assay (GAFS) in human eosinophils. The tested compounds showed an inhibition of more than 50% of binding to *h*H_4R at 1 μM and more than 50% inhibition of histamine-induced human eosinophil shape change at 1 μM (no detailed results).

3.2.1.2. Pyrazolopyridines

An interesting series of pyrazolo (3,4-b)pyridines was described by Kowa CO (40). Amide derivatives (e.g. 39 and 40, Figure 8) tested in an *in vitro* assay showed inhibition of binding at 10 μM (e.g. 95% for 39; 94% for 40).

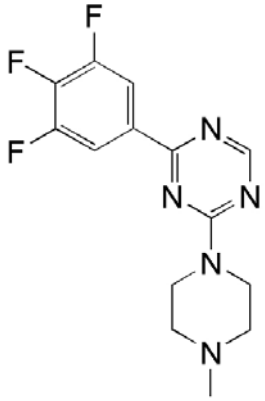


16 General structure



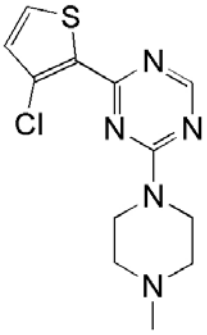
K_i = 2 nM

17 Janssen Pharmaceutica



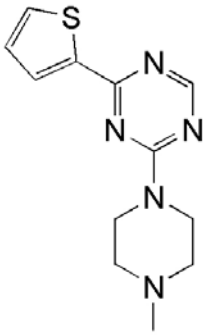
K_i = 7 nM

18 Janssen Pharmaceutica



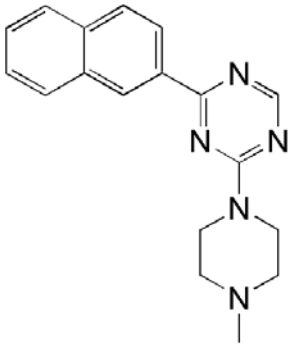
K_i = 8 nM

19 Janssen Pharmaceutica



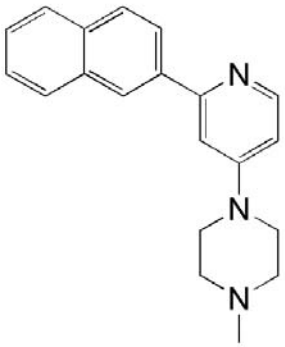
K_i = 76 nM

20 Janssen Pharmaceutica



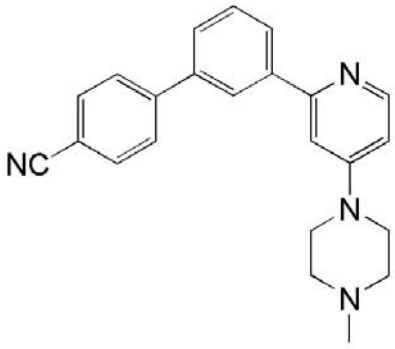
K_i > 10 000 nM

21 Janssen Pharmaceutica



K_i > 2 000 nM

22 Janssen Pharmaceutica



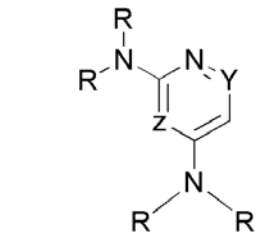
K_i = 10 nM

23 Janssen Pharmaceutica

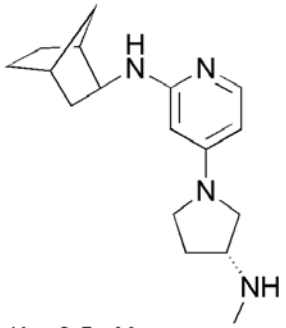
Figure 5. General structure and selected compounds claimed by Janssen Pharmaceutica (34).

3.2.1.3. Pyrazolopyrimidines

Pyrazolopyrimidine derivatives were claimed by Palau Pharma (41). 58 described compounds were tested in two pharmacological assays. In the binding competition assay of (^{3}H) histamine to *h*H$_4$R, the most preferred compounds showed inhibition of more than 50% of binding at 1 μM. The histamine H$_4$R activity of the compounds was also determined in the GAFS assay in human eosinophils.

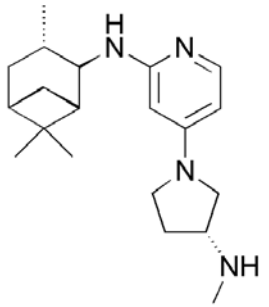


24 General structure



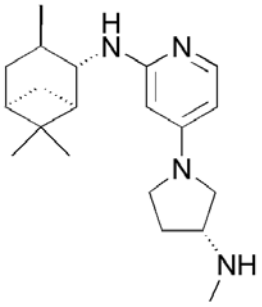
K_i = 0.5 nM

25 Janssen Pharmaceutica



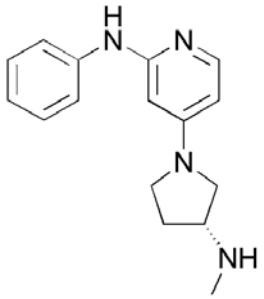
K_i = 0.4 nM

26 Janssen Pharmaceutica



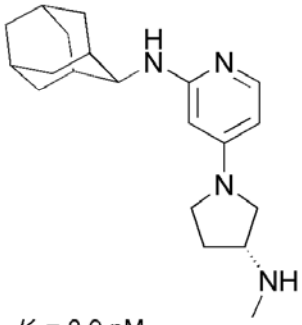
K_i = 0.5 nM

27 Janssen Pharmaceutica



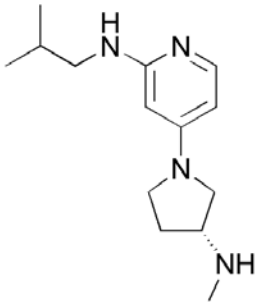
K_i = 0.9 nM

28 Janssen Pharmaceutica



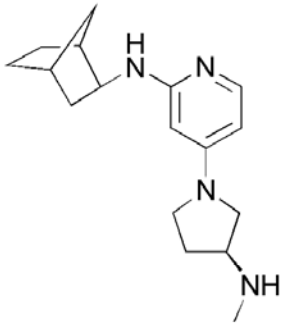
K_i = 0.9 nM

29 Janssen Pharmaceutica



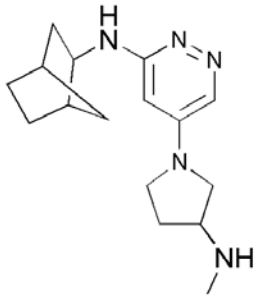
K_i = 1.5 nM

30 Janssen Pharmaceutica



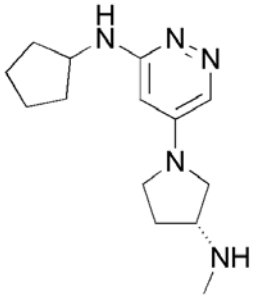
K_i = 8412 nM

31 Janssen Pharmaceutica



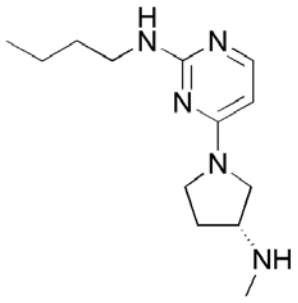
K_i = 5.5 nM

32 Janssen Pharmaceutica



K_i = 5.6 nM

33 Janssen Pharmaceutica



K_i = 0.7 nM

34 Janssen Pharmaceutica

Figure 6. Further selected compounds claimed by Janssen Pharmaceutica (35).

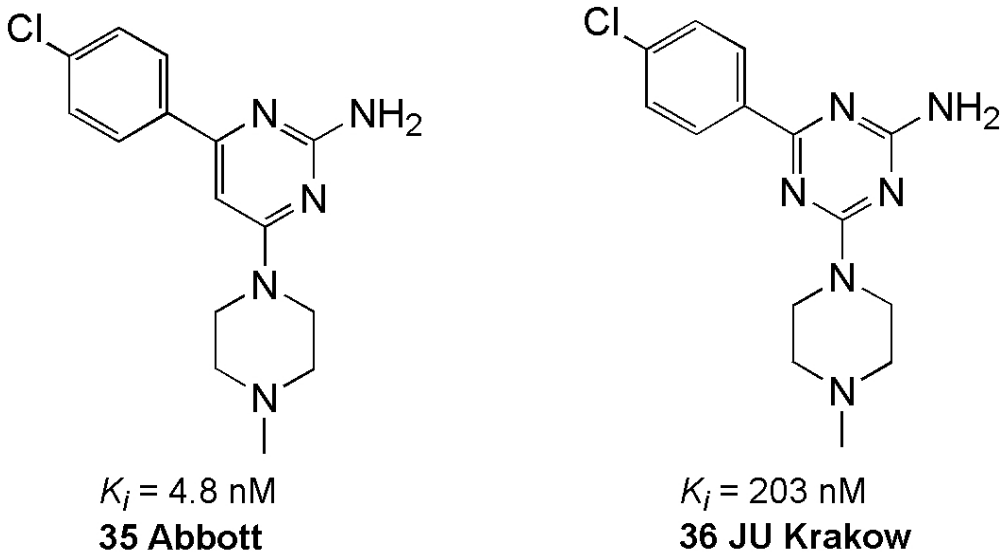


Figure 7. Structures of selected 1,3,5-triazines from Jagiellonian University Krakow and pyrimidines from Abbott (36,37,38).

The tested structures produced more than 50% inhibition of histamine-induced human eosinophil shape change at 1 μM. All the described compounds possessed an amine group at the 5 position and cycloalkylamine moiety at the 7 position (mostly 3- (methylamino)-azetidine and (*3R*)-3-(methylamino)-pyrrolidine). Different alkyl, (cyclo)alkyl, alkyl-aryl or alkyl-cycloalkyl substituents were introduced at the 2- and 3-position. In many compounds 2-methyl and 3-isopropyl groups were present (e.g. 41; Figure 8). Apart from pyrazolopyrimidines, five fused tricyclic derivatives (tetrahydropyridopyrazolopyrimidines) were also described (e.g. 42; Figure 8).

3.2.1.4.Thienopyrimidines

305 Thienopyrimidines and 4 thienopyridines (e.g. 43, Figure 8) were described by Kalypsys Inc and Alcon Research Ltd in their patent application as inhibitors of H_4R and/or H_1R (42). The compounds were tested *in vitro* at hH_4R and hH_1R on cell-based assays and *in vivo* for allergic conjunctivitis in a passively sensitized guinea pig assay, but no detailed results were included. Thienopyrimidines were tri- (4,5,6 position) or di-substituted (4,5 position). In the 4 position a cycloakyl amine moiety (e.g. substituted piperazine 44; Figure 8) or an alkyl amine was present (e.g. 45; Figure 8). In the 5 position the methyl substituent was well tolerated as most compounds possessed this group at that position (e.g. 44, 45; Figure 8). Although a few compounds (9) were 5,6-dimethyl substituted they were all active at H_4R with EC_{50} below 10 μM (e.g. 46, Figure 8). Fifteen compounds showed affinities for both H_1R/H_4R with EC_{50} below 10 μM (e.g. 44, 45, 46, 47, 48; Figure 8) whereas only seven compounds showed higher affinities for H_1R (than H_4R) with EC_{50} below 10 μM (e.g. 49; Figure 8). Four tricyclic derivatives were synthesized with a cyclopentyl (e.g. 50; Figure 8) or cyclohexyl (e.g. 51, 52; Figure 8) ring fused to a thienopyrimidine scaffold. These compounds (without 51) were more active at H_4R ($EC_{50} \leq 10$ μM) than at H_1R ($EC_{50} > 10$ μM).

3.2.1.5. Quinazolines

Researchers at VU University in Amsterdam reported a series of quinazoline-containing compounds as H_4R inverse agonists (43). Now, as a continuation of this work, a series of 6-chloro-2- (4-methylpiperazin-1-yl) quinazoline sulfonamides has been developed and reported (44). Found using parallel synthesis, diethyl sulfonamide 53 (Figure 9) showed high affinity in H_4R screening (K_i = 7.6 nM) and was chosen for further optimization and SAR studies. Replacing diethyl sulfonamide with methyl sulfonamide (54, K_i = 4.3 nM; Figure 9), methylphenyl sulfonamide (55, K_i = 5.4 nM; Figure 9), phenyl sulfonamide (56, K_i = 4.9 nM; Figure 9) or it remaining unsubstituted (57, K_i = 4.5 nM; Figure 9) led to an increase in potency. Although the incorporation of an amine group into a cyclic system (e.g. 2-methylpiperidine or morpholine 58; K_i = 9.3 nM; Figure 9) was well tolerated by H_4R, the replacement of this group by a suitable isostere (e.g. carboxamide or thiazolidinedione 59; K_i = 178 nM; Figure 9) decreased the affinity more than 30-fold.

Spiro cyclic derivatives of quinazolines or cyclohepta (*d*)pyrimidines were described by Abbott (45,46). The presence at the 8 position of a spiro cycloalkyl ring (cyclopentyl, cyclohexyl or indenyl) was tolerated by hH_4R but the potency depended on the size of the ring. Compounds with a spiro cyclopentyl ring (e.g. 60, K_b = 2.7 nM; Figure 10) were more potent than corresponding compounds with a spiro cyclohexyl (e.g. 61, K_b/EC_{50} = 47nM; Figure 10) or a spiro indenyl group (e.g. 62, K_b = 501 nM; Figure 10). The most potent in the spiro cyclopentyl series were compounds with a (*R*)-3-aminopyrrolidine (60; Figure 10), (*R*)-3- (methylamino)pyrrolidine (63, K_b = 2.9 nM; Figure 10) or 3- (methylamino)azetidine moiety (64: K_b = 4.6 nM; Figure 10). These compounds were also tested in binding assays for both human H_4R (63: K_i = 2.6 nM; 64: K_i = 1.4 nM; Figure 10) and rat (63: K_i = 2.6 nM; 64: K_i = 1.4 nM; Figure 10). In order to check the selectivity of compounds they were tested in binding assays for human and rat H_3Rs respectively and showed moderate antagonistic activity

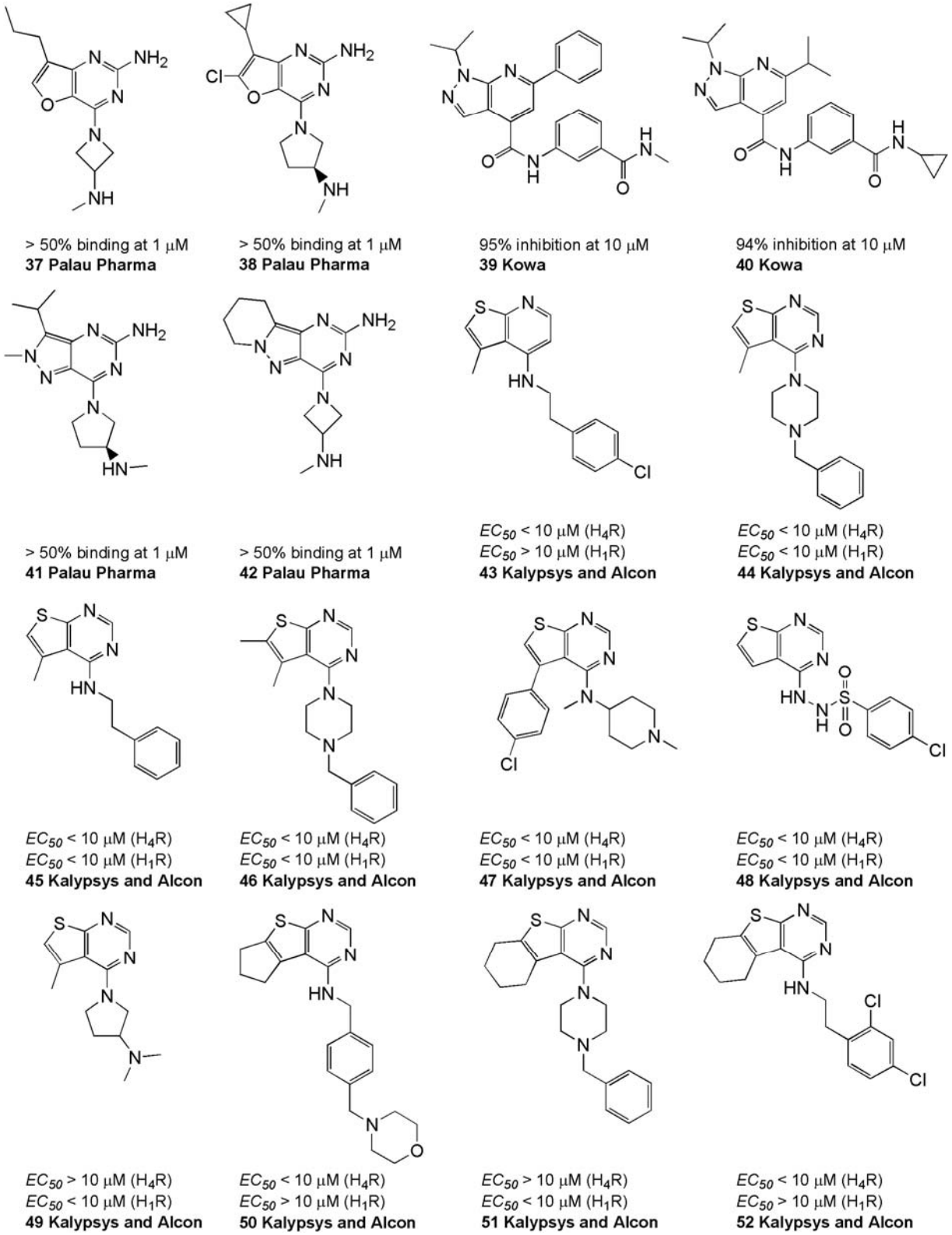


Figure 8. Selected structures of fused two rings compounds from Palau Pharma, Kowa, Kalypsys and Alcon (39-42).

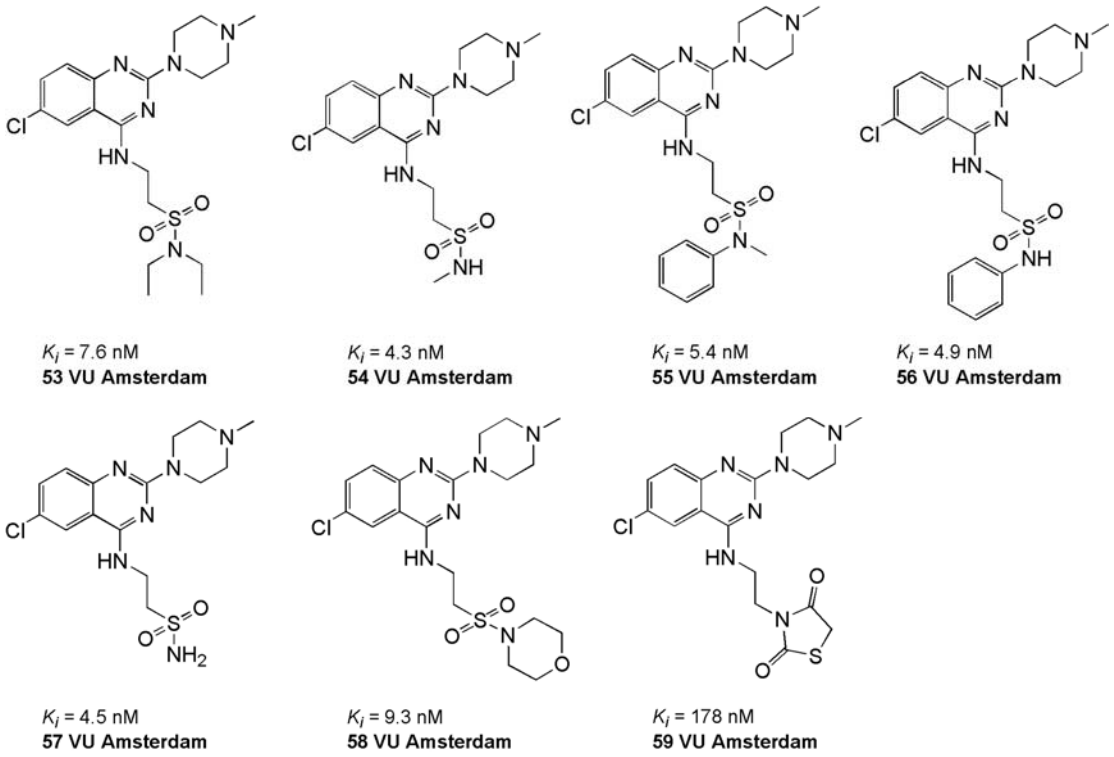


Figure 9. Selected structures of compounds from Vrije University Amsterdam (44).

(H_3R 60: K_i = 295nM; K_i = 79 nM; 63: K_i = 105 nM; K_i = 72 nM; 64: K_i = 63 nM; K_i = 6.8 nM). In addition, compound 60 was tested in a mouse model of H_4R agonist (clobenpropit)-induced scratching and was able to completely block itch response after i.p. administration (ED_{50} of 1 μmol/kg).

3.2.1.6. Cycloheptylpyrimidines

Researchers from Abbott, continuing their previous work in the H_4R field (14), developed rigidified 2-aminopyrimidines (45,47). The rigidifying ring was six- or seven-membered. The size of the ring did not have much influence on the potency (e.g. 65 with K_b of 30 nM vs 66 with K_b of 58 nM; Figure 11). In application (47) mostly seven-membered derivatives, mono- or di-substituted at the 8 or 9 position, were described. Alpha (9 position) compounds substituted with phenyl were more potent than beta (8 position) substituted analogues, e.g. 66 (K_b of 58 nM; Figure 11) vs 67 (K_b of 246 nM; Figure 11). The introduction of the pyridyl moiety at the 9 position instead of the phenyl one was unfavorable, if you compare 66 (K_b of 58 nM; Figure 11) with 68 (K_b of 2354 nM; Figure 11). Disubstitution in the cycloalkyl ring caused decreased activity at the *h*H_4R as the substituent grew larger. Compare e.g. the dimethyl derivative 69 (K_b of 35 nM; Figure 11) with the diethyl one 70 (K_b of 246 nM; Figure 11) or dibenzyl 71 (K_b of 26779 nM; Figure 11).

3.2.2. Fused three/four rings compounds

3.2.2.1. Benzofuro- or Benzothienopyrimidines

Several rotationally constrained analogs of the aminopyrimidines (e.g. benzofuropyrimidines or benzothienopyrimidines) were synthesized by researchers from a few companies. Some years ago the first such structures were investigated by scientists from Argenta and Cellzome (14,15). Recently, more results and SARs were reported (48). Found using ligand-based virtual screening, compound 72 (Figure 12), with IC50 of 19 nM, was chosen as a lead structure. Three main modifications were introduced: (I) different cycloalkyl amines at the 4 position, different substituents at the 8 position (II) and at the 2 position (III). The aim of these changes was to enhance the metabolic stability while retaining potency. The approaches adopted led to the discovery of potent and selective H4R inverse agonists. The most potent were compounds with the (3R)-methylamino pyrrolidine ring (e.g. 73, IC50 = 30 nM; Figure 12). At the 8 position a small lipophilic substituent (e.g. CF3: 74, IC50 = 30 nM; Figure 12) was well tolerated by H4R but did not cause changes in stability. The presence of an amino group at the 2 position resulted in a great increase in potency (e.g. 75, IC50 = 1 nM; Figure 12) but also without an improvement in stability. Compound 75, when investigated further, was shown to be an inverse agonist (GTPgammaS assay: IC50 = 3 nM) with excellent selectivity compared to other histamine receptors (H1R, IC50 > 30 μM; H2R, IC50 > 30 μM; H3R, IC50 = 5.8 μM) and activity against CYP1A2 with 45% inhibition at 1 μM. PK studies showed an acceptable oral profile in dogs and monkeys. Following this project Cellzome claimed sulphur containing benzofuropyrimidines (49). The preferred compounds from this invention were tested in the radioligand binding assay and had IC50 values below 100 μM. Mentioned in particular were compounds 76, 77 and 78 (Figure 12).

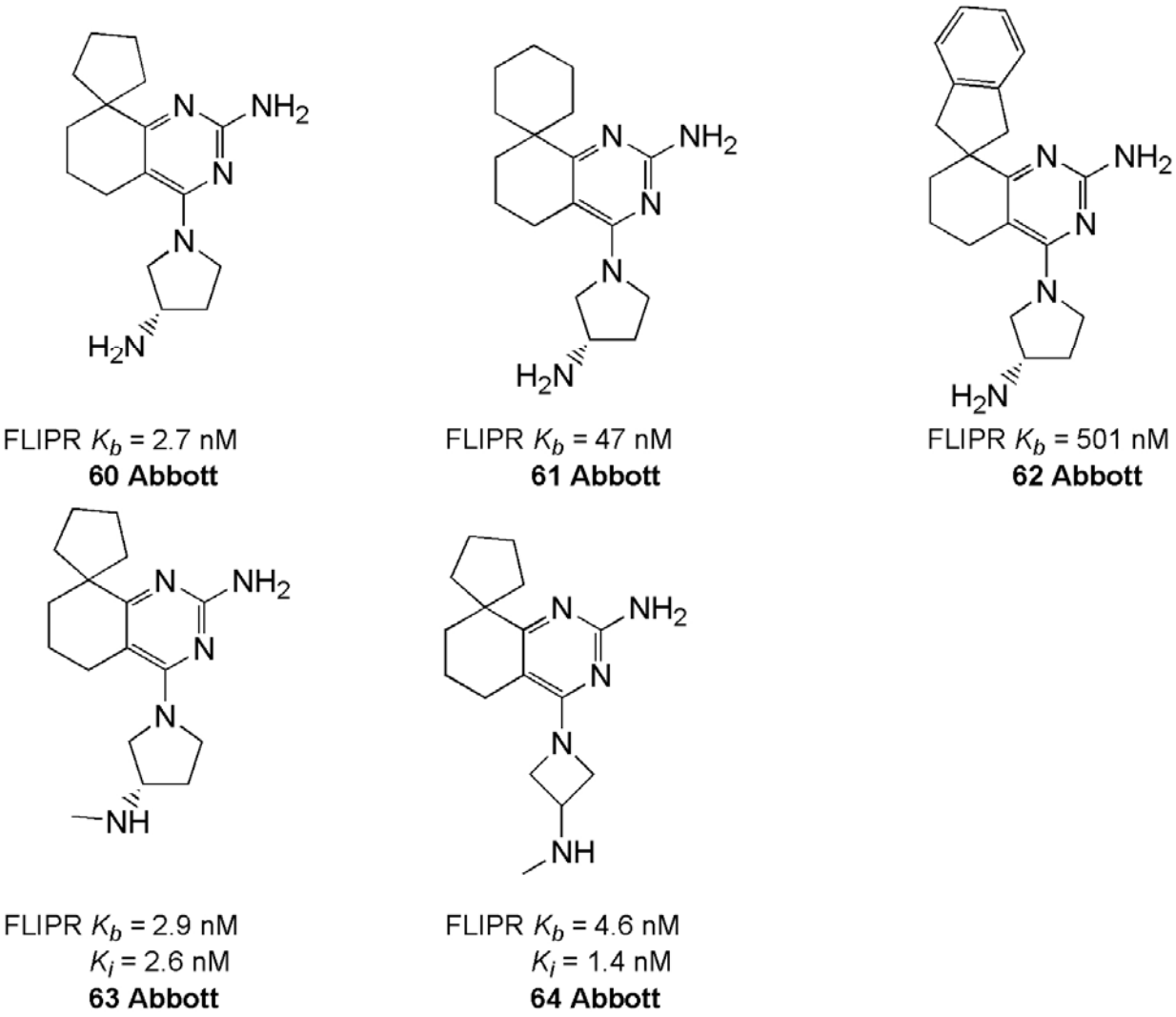


Figure 10. Selected structures of spiro derivatives of quinazolines from Abbott (45,46).

Janssen Pharmaceutica, continuing previous work on benzofuro/benzo-thienopyrimidines (14,15), described a series of new (benzo)thieno- and benzofuropyrimidine derivatives (50). Among 150 synthesized compounds 86 were benzothienopyrimidines. Binding assays were performed on recombinant *h*H_4R (SK-N-MC cells or COS7 cells) using (^{3}H)-histamine as a radioligand. Results from these studies were presented for 83 selected compounds. At the 2 position the presence of the amine group improved *h*H_4R affinity from 6-fold to 20-fold; compare 79 (K_i = 53 nM; Figure 13) with 80 (K_i = 8 nM; Figure 13) and 81 (K_i = 110 nM; Figure 11) with 82 (K_i = 5 nM; Figure 13). At the 4 position different amine moieties (cycloalkylamines or alkylamines) were introduced. Compounds which were very potent were those with (3*R*)-methylaminopyrrolidine, (3*R*)-aminopyrrolidine, (4*aR*, 7*aR*)-octahydropyrrolo (3,4-b)pyridine or 4-methylpiperazine moieties. In most cases benzofuropyrimidines were more potent (~5 fold or more, e.g. 83 vs 84; Figure 13) or just equipotent (e.g. 85 vs 86; Figure 13) to benzothienopyrimidines. Different substituents (methyl, dimethyl, difluoro, trifluoromethyl, tert-butyl or methoxy) were introduced at the 8 position of the benzothieno- or benzofuropyrimidine ring. Introduction to the benzothienopyrimidine ring (at the 8 position) of methyl (87; Figure 13), dimethyl (88; Figure 13) and difluoro (84; Figure 13) substituent (s) was well tolerated by hH4R, but did not cause an improvement in potency (compare 89 with 87, 88 or 84; Figure 13). No data for trifluoromethyl, methoxy and *tert*-butyl substituents were reported. Increasing the ring size of the fused cyclohexane moiety to a cycloheptane one also failed to improve *h*H_4R activity (compare 89 with 90; Figure 13).

Benzofuropyrimidine derivatives were also reported by scientists from Palau Pharma and Kowa (51,52). The first group described 2-amine substituted compounds (e.g. 91; Figure 14), whereas the second described 2-phenyl derivatives (e.g. 92; Figure 14). Compounds from Palau Pharma also had a cycloalkylamine moiety in the pyrimidine ring and when

tested in the

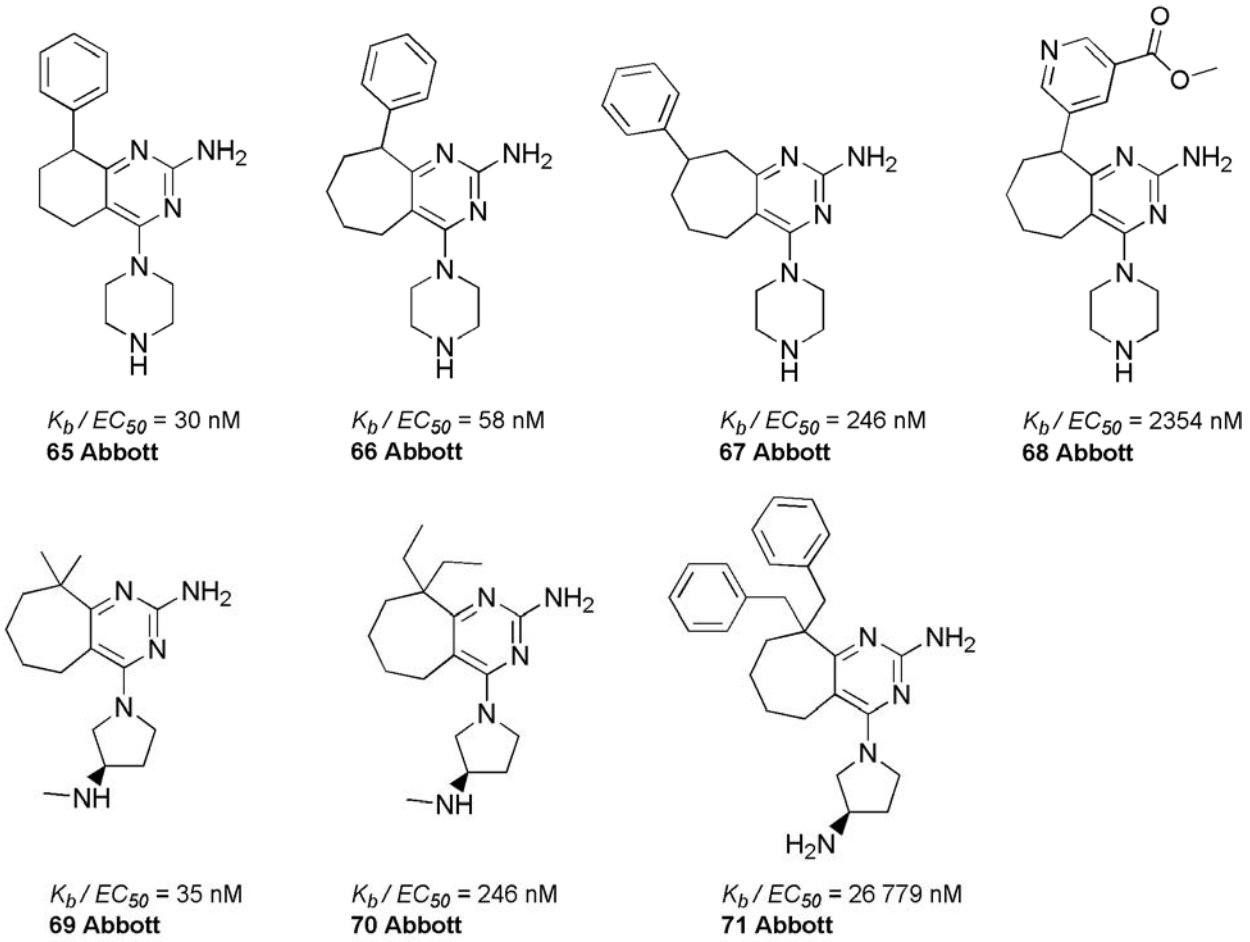


Figure 11. Structures of selected fused (cyclohexyl)cycloheptylpyrimidines from Abbott (45,47).

competition binding assay of (^{3}H) histamine to hH$_4$R showed more than 50% inhibition at 1 μM (no detailed results). Compounds from Kowa had an alkylamine substituent (e.g. 3-morpholinopropylamine) and when tested in an *in vitro* assay showed inhibition of binding at 10 μM (e.g. 69% for 87).

Recently Palau Pharma presented results for tricyclic aminofuropyrimidine derivatives (53). Three series of compounds with a fused cycloalkyl ring were prepared: I (5-membered ring), II (6-membered ring) and III (7-membered ring). Compounds were tested in binding and cellular assays (GAFS) and showed high *in vitro* potency. The reduced affinity for the hERG channel (in comparison to 2-aminobenzofuropyrimidines) was achieved by decreasing the size of the fused ring (the best results were for 5-carbon ring analogs). One of the most interesting compounds, 93 (Figure 14), showed high binding (IC_{50} = 12 nM) and functional activity (IC_{50} = 10 nM; GAFS), good selectivity vs hH$_3$R (7% inhibition at 1 μM) and reduced the hERG channel inhibition (IC_{50} = 76 μM).

3.2.2.2. Cyclohexylpyrimidines/cycloheptylpyrimidines

Another series of condensed 2-amino-cycloalkylpyrimidines (tri- or four cyclic) was published by Abbott (54). In this patent three series of derivatives were noted (with general structures presented in Figure 15): I (benzofuro (2,3-h)quinazolines), II (octahydrobenzo (h)quinazolines) and III (ethanobenzo (6,7)cyclohepta (1,2-d)pyrimidines). Among the 28 compounds described 11 were from series I, 12 from series II and 5 from series III. D the fused pyrimidine scaffolds. Results from the hH4R FLIPR assays for all the compounds were presented. In all series the most potent were compounds with piperazine, a (3R)-3- (methylamino)pyrrolidine or a (3R)-3-aminopyrrolidine moiety whereas 4-methylpiperazine and 1,4-diazepane (or 4-isopropyl-1,4-diazepane or 4-cyclobutyl-1,4-diazepane) scaffolds led to a decrease in affinity. Generally compounds from the II series were more potent with Kb/EC50 below 10 nM (compare 94 with 95 and 96; Figure 15). In the second series (II) two kinds of isomers were possible, cis or trans, however this structural change did not have a significant influence on affinity; the potency is comparable: 94 vs 97; different cycloalkylamines were introduced at the 4 position of Figure 15). In series III, hexahydro- or octahydro-derivatives were synthesized but this structural change did not influence potency (compare 96 with 98; Figure 15). Some of the selected compounds were also tested in the

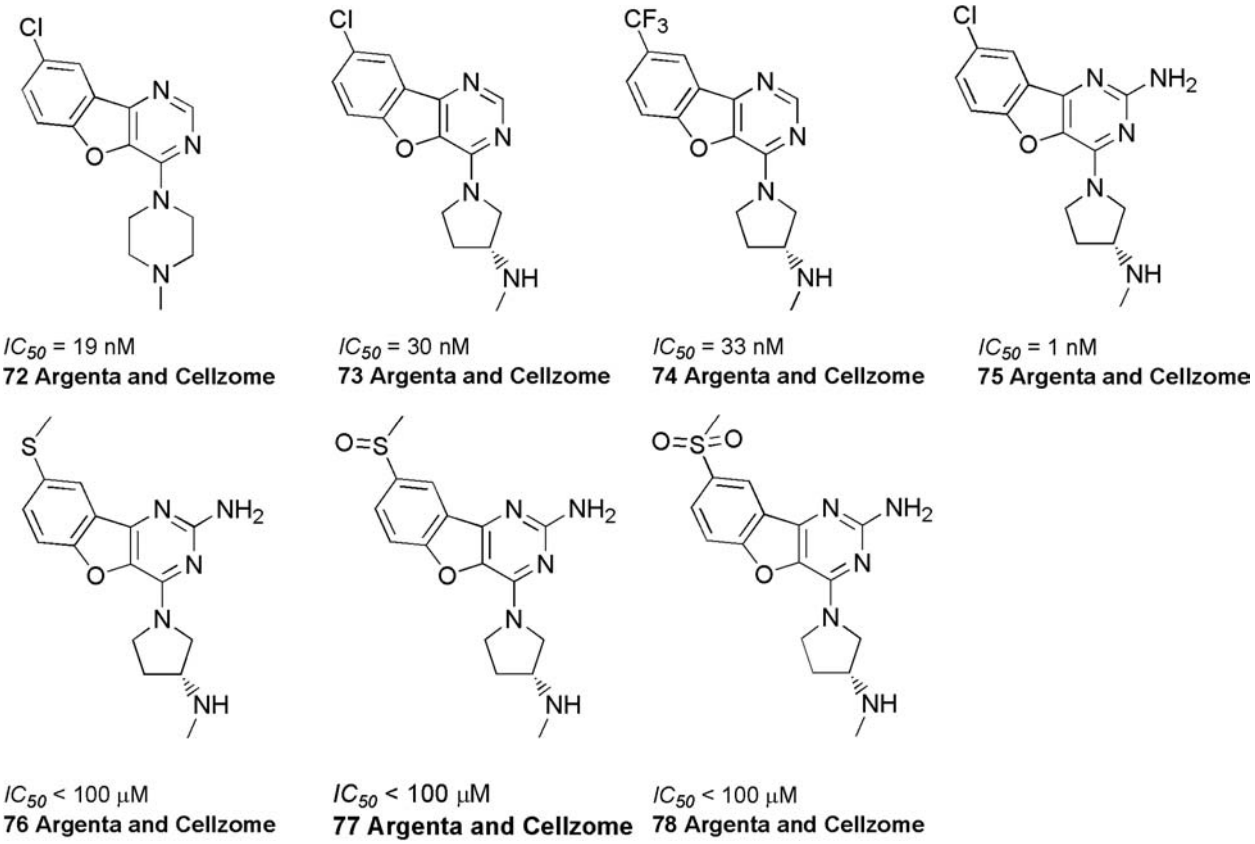


Figure 12. Selected compounds described by Argenta and Cellzome (48).

binding assay (hH4R,) and showed affinity with Ki below 15 nM (e.g. 99 with Ki of 0.9 nM; Figure 15). Generally compounds tested in this assay showed lower affinity than in the FLIPR assay (about twice or more).

3.2.2.3. Fused Quinazolines and Related Structures

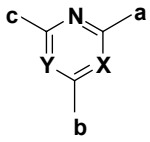
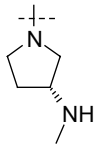
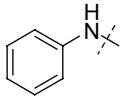
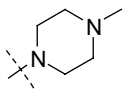
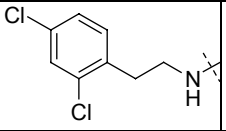
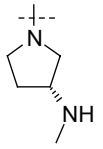
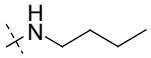
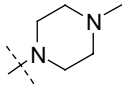
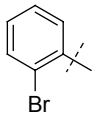
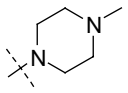
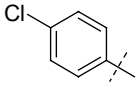
A series of fused quinolines (49 compounds), quinazolines (56 compounds) and quinoxalines (145 compounds) were reported by Kalypsys and Alcon Research (55). A fused ring was an unsaturated heterocyclic 5-membered ring e.g. imidazole, pyrrole, oxazole, triazole or tetrazole. Common features of these structures were a cycloalkylamine moiety (at the 4 position; piperazine or 4-methylpiperazine) and a mono- or disubstituted (with e.g. -Cl, -F, -CF3, -CH3, -OCF3) benzene ring. Compounds were tested in two in vitro cell-based assays at hH1R and hH4R but no detailed results were included (Ki ≤ 10 μM or Ki > 10 μM). Seventeen compounds (mostly quinoline derivatives) showed affinities both for hH1R and hH4R with Ki ≤ 10 μM (e.g. 100 , 101 and 102; Figure 16).

A patent application from Kowa C.O. described a fused quinazoline ring (phthalazinoquinazoline) (56). Compound 103 (Figure 16) was tested in a cell-based Ca2+-flux functional assay (FLIPR) at hH4R (CHO-K1 cells) and showed 24% inhibition at 10 μM.

4. CONCLUSIONS

Azines, especially pyrimidine derivatives, are a promising class of H4R antagonists/inverse agonists. Some of the interesting structures have been intensively evaluated in preclinical studies e.g. CZC-13788 (probably a benzofuropyrimidine derivative), A-943931 or A-987306 (14). Analysis of the structural features common to the series of compounds described in this review resulted in the construction of a general pattern for monoazines (Figure 17) and fused azines (Figure 18). These general construction patterns contain: a central core (an unsaturated heterocycle with at least one nitrogen, mostly pyrimidine moiety), a basic center (saturated nitrogen heterocycle or N-alkyl amine) and a lipophilic center (diverse straight or branched substituents are possible e.g. alkyl, aryl, substituted amine or fused cycloalkyl, cyclo (hetero)aryl ring). An additional basic moiety (e.g. -NH2, or saturated nitrogen heterocycle or substituted amine) in most cases increased the H4R affinity (especially –NH2 substituent in fused azines). Tested as the basic center were many different alkylamines (e.g. 2-phenyloethylamine, 2-morpholinoethylamine), saturated nitrogen heterocycles (e.g. pyrrolidines, cyclobutylamines, piperazines) and fused unsaturated nitrogen heterocycles (e.g. fused pyrrolidinopiperazines, fused piperazines). However, the most potent compounds were those with a 4-methylpiperazine, 3- (methylamino)-azetidine, (3R)-3-

Table 2. Summarizing data of selected monoazines

No	Company	Structure					H_4R affinities (nM)
							
		X	**Y**	**a (additional basic moiety)**	**b (basic moiety)**	**c (lipophilic moiety)**	
28	Janssen Pharmaceutica	C	C	H			K_i = 0.9
3	Goethe University Frankfurt	N	C	NH_2			K_i = 12
34	Janssen Pharmaceutica	N	C	H			K_i = 0.7
12	Janssen Pharmaceutica	N	N	H			K_i = 2
36	Jagiellonian University Krakow	N	N	NH_2			K_i = 203

methylamino)-pyrrolidine or (*3R*)-3-amino-pyrrolidine ring.

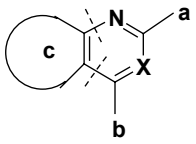
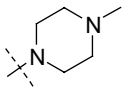
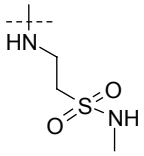
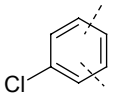
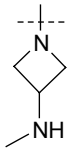
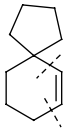
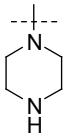
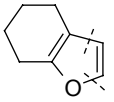
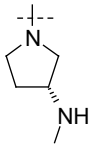
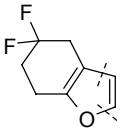
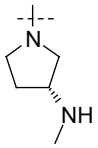
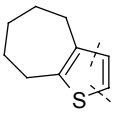
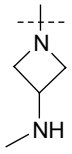
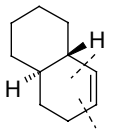
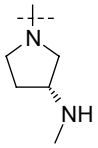
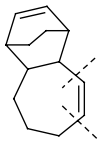
Different pharmacological tests were used to evaluate the H_4R activity of the compounds: radioligand binding assays and/or functional assays. The radioligand binding assays were performed on membranes prepared from cells expressing any H_4R sequence, human or non-human (e.g. rats, mice). Cloned human (rat, mouse) H_4R was stably expressed in SK-N-MC, CHO-K1, HEK293, COS7 or S*f*9 cells (co-expressed with G protein Galphai2 and Gbeta1gamma2 subunits). (^{3}H)-histamine or (^{3}H)JNJ7777120 were used as radioligands. Moreover, different functional assays were used to determine the efficacy of the compounds on H_4R: (1) histamine-induced shape change assay (GAFS) in human eosinophils, (2) functional cell-based cAMP assay, (3) Ca^{2+}-flux functional assay, (4) GTPase assay or (5) (^{35}S)GTPgammaS assay.

The diversity of the applied tests and sometimes the lack of detailed data (even for a single compound) made it difficult to compare the activity of the compounds and to draw conclusions. To summarize the relationship between structure and activity the most potent compounds, tested in binding assays on *h*H_4R (with given K_i values), were chosen. The selected structures are collected in Table 2 (monoazines) and Table 3 (fused azines).

5. PERSPECTIVES

Since 2000, when H_4R was discovered, the number of articles and patent applications in the H_4R field is growing annually. HTS, as well as virtual screening based on homology models of *h*H_4R, are useful tools in the search for new H_4R ligands. Many groups of scientists have developed their own pharmacophore models which have helped them to find potent compounds (e.g. 43,57). The potential therapeutic utility of H_4R antagonists/inverse agonists constitutes an attractive target in the search for new drugs. It is suggested that H_4R antagonists/inverse agonists can be useful for the treatment of allergic rhinitis, asthma, rheumatoid arthritis, atopic dermatitis, idiopathic chronic urticaria, inflammatory pain, neuropathic pain or osteoarthritic pain. However, the close homology of *h*H_4R to *h*H_3R and pharmacological heterogeneity among species and splice variants ($H_4R_{(302)}$ and $H_4R_{(67)}$), makes the preclinical evaluation of compounds difficult. The verification of pharmacological activity with potential

Table 3. Summarizing data of selected fused azines

No	Company	X	Structure			H_4R affinities (nM)
						
			a (additional basic moiety)	**b (basic moiety)**	**c (lipophilic moiety)**	
54	Vrije University Amsterdam	N				K_i = 4.3
64	Abbott	N	NH_2			K_i = 1.4
82	Janssen Pharmaceutica	N	NH_2			K_i = 5.0
83	Janssen Pharmaceutica	N	NH_2			K_i = 0.48
90	Janssen Pharmaceutica	N	NH_2			K_i = 5.0
99	Abbott	N	NH_2			K_i = 0.9
96	Abbott	N	NH_2			K_i = 5.6

utility is also not easy. Moreover, the choice of the appropriate animal models to predict human pharmacology and dose selection is complicated. However, the last two years have brought important progress in the assessment of the pharmacological utility of H_4R ligands. The first H_4R antagonist/inverse agonist UR-63325 (Palau Pharma) has entered into clinical trials (finishing phase I with promising interim data) and the results from studies are eagerly awaited. As the largest pharmaceutical companies (e.g. in alphabetic order: Abbott, Bayer Healthcare, Johnson and Johnson or Pfizer) are engaged in the search for active and selective H_4R antagonists/inverse agonists, it is also expected that more promising compounds from preclinical evaluations will undergo investigation in clinical developments.

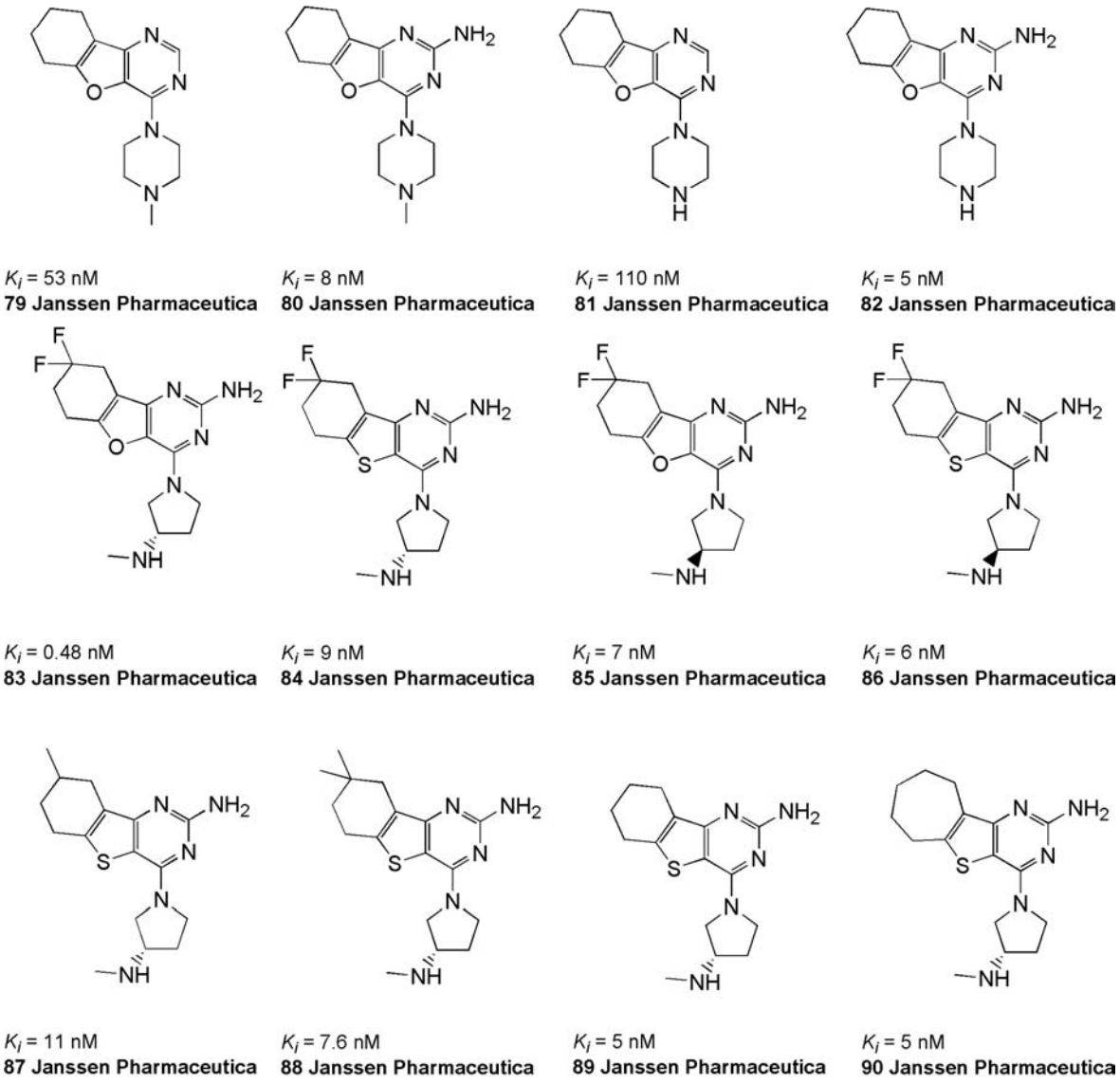


Figure 13. Selected benzofuro-/benzothienopyrimidines from Janssen Pharmaceutica (50).

Finally, European Cooperation in Science and Technology (COST) Action BM0806, which focuses on Recent Advances in Histamine Receptor H_4R Research, can greatly aid the progress of H_4R ligands on their way to market. This international cooperation, bringing together both scientists from academia and industry (more than 20 teams), is expected to result in a better understanding of the biochemistry and pharmacology of H_4R, as well as the development of new instrumentation, reliable experimental models, and potent and selective H_4R ligands.

6. ACKNOWLEDGEMENT

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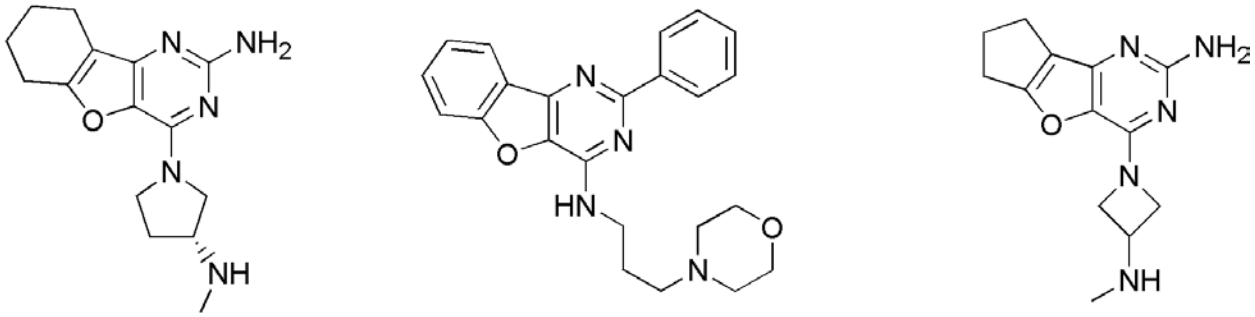
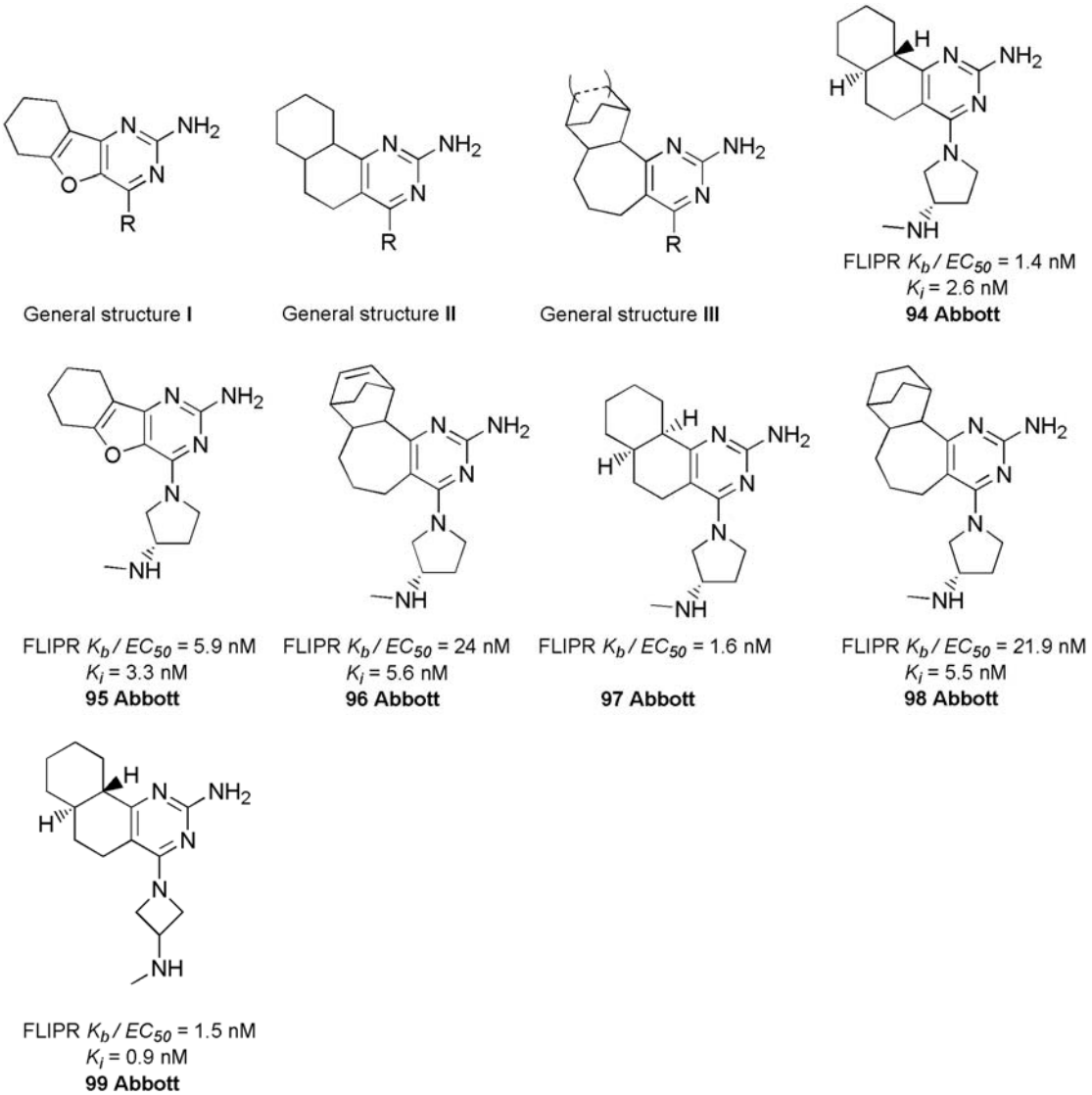


> 50% binding at 1 μM
91 Palau Pharma

69% inhibition at 10 μM
92 Kowa

IC_{50} = *12* nM
93 Palau Pharma

Figure 14. Selected benzofuropyrimidines and related structures from Palau Pharma and Kowa (51-53).



General structure **I**

General structure **II**

General structure **III**

FLIPR K_b / EC_{50} = 1.4 nM
K_i = 2.6 nM
94 Abbott

FLIPR K_b / EC_{50} = 5.9 nM
K_i = 3.3 nM
95 Abbott

FLIPR K_b / EC_{50} = 24 nM
K_i = 5.6 nM
96 Abbott

FLIPR K_b / EC_{50} = 1.6 nM
97 Abbott

FLIPR K_b / EC_{50} = 21.9 nM
K_i = 5.5 nM
98 Abbott

FLIPR K_b / EC_{50} = 1.5 nM
K_i = 0.9 nM
99 Abbott

Figure 15. General structures and selected cyclohexyl-/cycloheptyl-pyrimidines from Abbott (54).

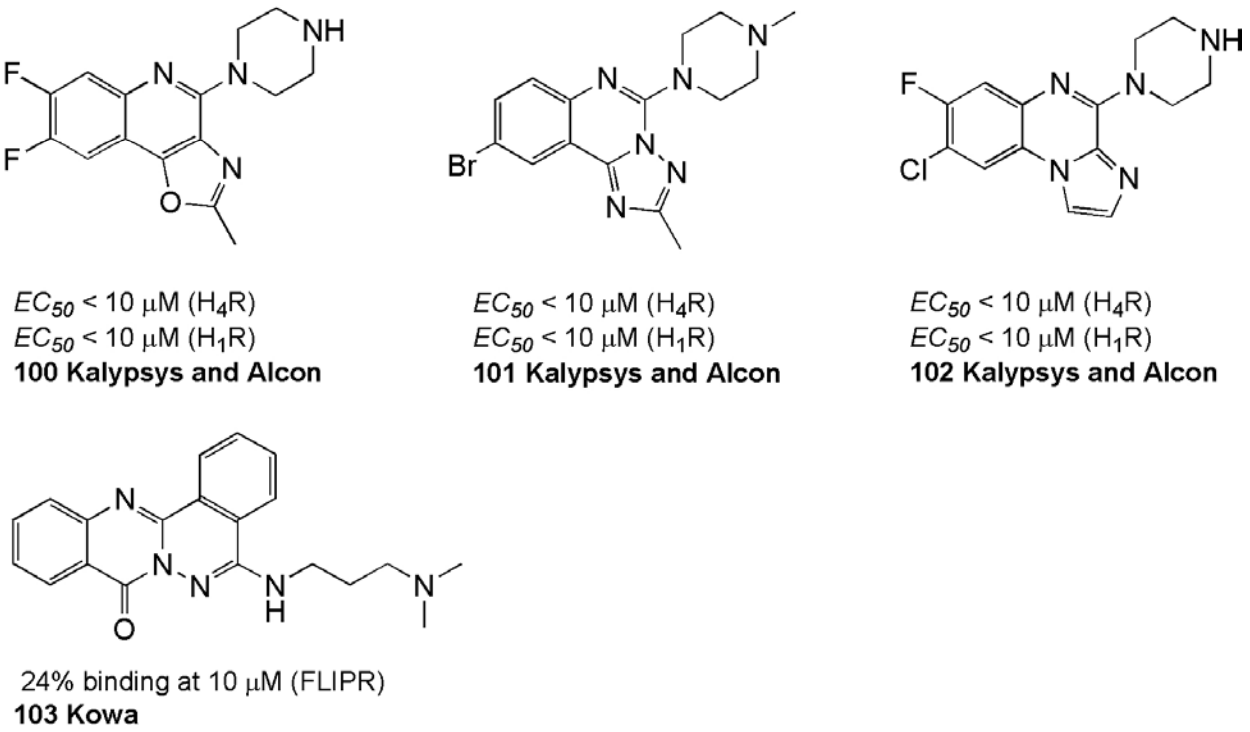


Figure 16. Selected structures from Kalypsys and Alcon, and Kowa (55,56).

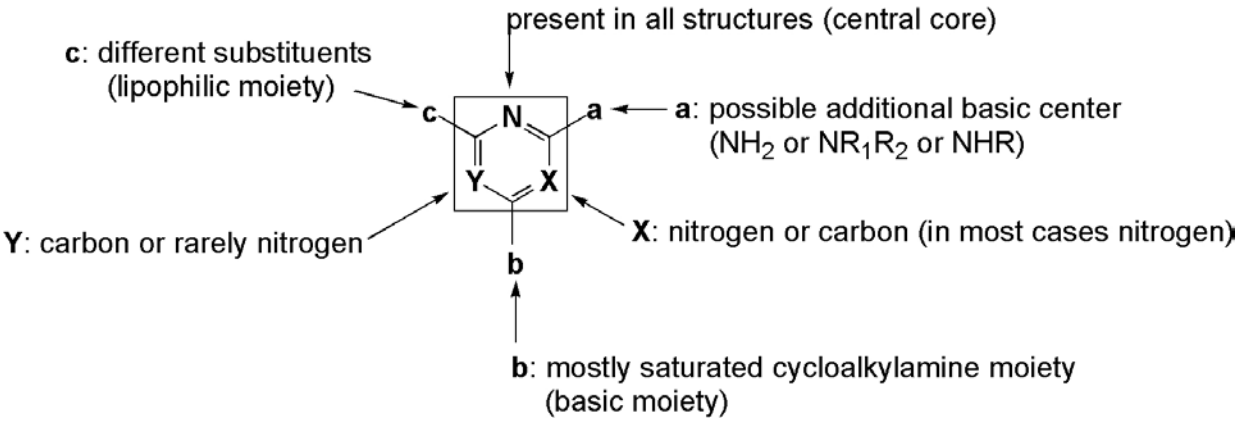


Figure 17. General structure of monoazines.

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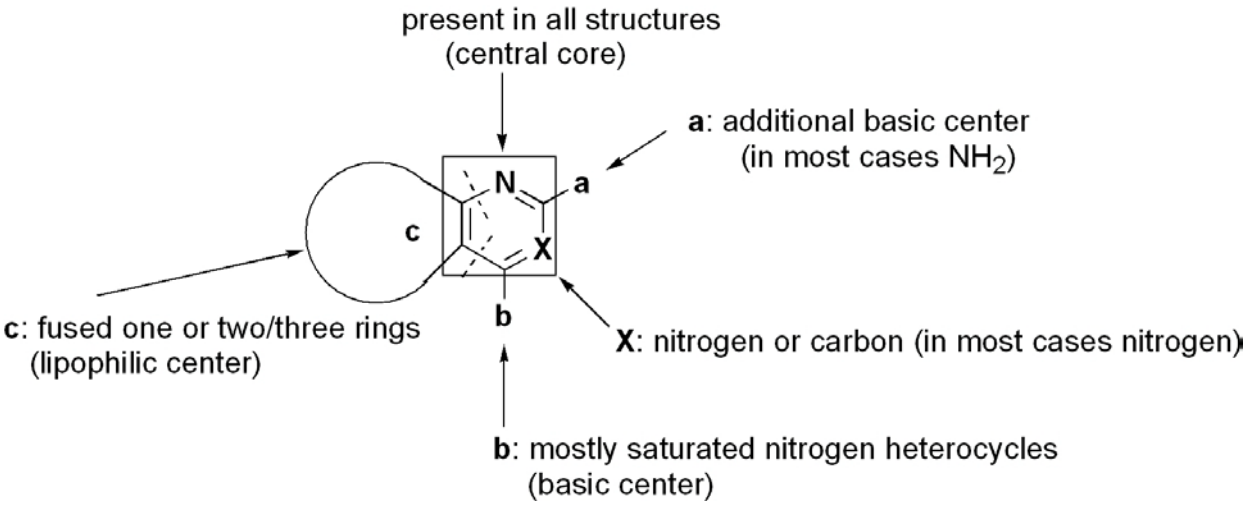

Figure 18. General structure of fused azines.

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Abbreviations: H_4R: histamine H_4 receptor; *h*H_4R: human histamine H_4 receptor; CNS: central nervous system; GAFS: gated autofluoresence forward scatter; HTS: high-throughput screening; FLIPR: Fluorometric Imaging Plate Reader; i.p.: intraperitoneal route of drug administration.

Key words: GPCR**,** Histamine H_4 Receptor, H_4 Antagonists, H_4 Inverse Agonists, Azines, Pyrimidines, Pyridines, Triazines, Fused Pyrimidines, Review

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