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Synthesis and evaluation of *N*-alkyl-*S*-[3-(piperidin-1-yl)propyl] isothioureas: High affinity and human/rat species-selective histamine H_3 receptor antagonists

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ABSTRACT

S-Alkyl-*N*-alkylisothiourea compounds containing various cyclic amines were synthesized in the search for novel nonimidazole histamine H_3 receptor (H_3R) antagonists. Among them, four *N*-alkyl *S*-[3-(piperidin-1-yl)propyl]isothioureas **18**, **19**, **22**, and **23** were found to exhibit potent and selective H_3R antagonistic activities against in vitro human H_3R, but were inactive against in vitro human H_4R. Furthermore, three alkyl homologs **18**–**20** showed inactivity for histamine release in in vivo rat brain microdialysis, suggesting differences in antagonist affinities between species. In addition, in silico docking studies of *N*-[4-(4-chlorophenyl)butyl]-*S*-[3-piperidin-1-yl)propyl]isothiourea **19** and a shorter homolog **17** with human/rat H_3Rs revealed that structural differences between the antagonist-docking cavities of rat and human H_3Rs were likely caused by the Ala122/Val122 mutation.

Histamine is an endogenous biogenic amine that has a number of pathophysiological roles. These include not only those in peripheral tissues such as in inflammatory and allergic reactions and gastric acid secretion, but also those in the central nervous system (CNS), which include regulation of the sleep–wake cycle, cognitive function, feeding and drinking behavior and circadian rhythms. These histamine-regulated functions are mediated through four distinct G protein-coupled receptors, referred to histamine H_1, H_2, H_3, and H_4 receptors.[1] The H_3 receptor (H_3R), which is mainly located on the presynaptic membrane of the histaminergic neurons in the CNS, is coupled to the $G_{i/o}$ protein, and acts by decreasing of the intracellular level of cyclic adenosine monophosphate (cAMP) by inhibiting adenylylcyclase.[2] H_3R has high constitutive and spontaneous activity, and several drugs that are classically considered as antagonists have been classified as either inverse agonists or neutral antagonist.[3] Since the first ligand for H_3R was described in 1987,[4] a variety of H_3R antagonists such as thioperamide and clobenpropit have been developed (Fig. 1).[5] However, these molecules contain an imidazole ring, and have been found to additionally inhibit hepatic cytochrome P450 enzymes, as well as having the potential to influence the metabolic pathway of co-administered drugs.[6] In addition, owing to the difficulties in penetrating the blood–brain barrier, they may be unsuccessful in acting on their target CNS disorders.[2] Hence, it is expected that the development of nonimidazole H_3R antagonists/inverse agonists as potential therapeutic agents will be extremely useful. Although a small number of such compounds have already reached clinical trials, including BF2.649 (pitolisant),[7] CEP-26401,[8] and ABT-239,[9] they have had little success (Fig. 1).[10]

There is significant sequence homology between the human (h) and rat (r) H_3Rs; however, sequence analysis and molecular modeling studies suggested that key amino acids at positions 119 and 122 in transmembrane (TM) region 3 play important roles in ligand recognition, and these are known to vary between the two species.[11] The pharmacology of H_3R ligands has been shown to species-dependent, with some compounds showing distinct affinity profiles. Therefore, the identification of novel H_3R antagonists/inverse agonists that are specific to rats or humans is viewed as an important issue.[12]

The H_4 receptor (H_4R), which is mainly expressed in immune and inflammatory cells of the spleen, thymus, and bone marrow, as well as in leukocytes,[13] is considered as a target for the treatment of chronic allergic and inflammatory diseases. H_4R has also been identified as a $G_{i/o}$ protein-coupled receptor, and has constitutive activity like H_3R.[14] The hH_4R shares 37.4% sequence identity at the protein level, and 58% identity in the TM domains with

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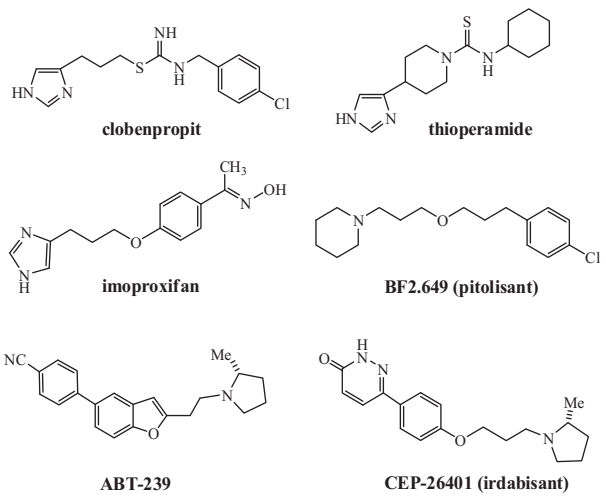


Figure 1. Structures of imidazole or nonimidazole H_3R antagonists.

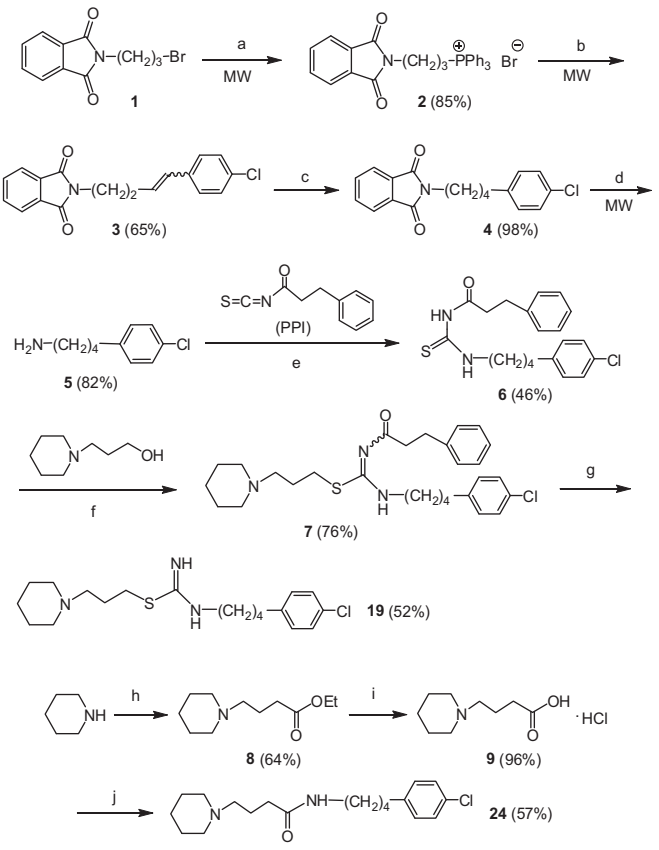
hH_3R.[15] Consequently, most imidazole-containing histamine H_3R ligands also have a high affinity with H_4Rs.[16]

An extremely potent H_3R inverse agonist, clobenpropit is composed of an imidazole ring, a propyl spacer and an isothiourea central core that is connected to a lipophilic 4-chlorobenzyl group (Fig. 1).[5,17] During our investigations into novel and potent H_3/H_4R ligands,[18] we have reported an efficient synthetic method for *S*-alkyl-*N*-alkylisothioureas.[19] In the present paper, using this synthetic method, we first examined whether substitution of the imidazole of clobenpropit with various cyclic amines affected the potency and affinity of hH_3R. We then evaluated the effect of varying the length of the alkyl spacer between the isothioureylene and 4-chlorophenyl group. Further, the effects of *para*-substituents on the phenyl group, and conversion of the isothiourea central core into a carbamoyl group were examined. Among the molecules evaluated, novel isothiourea derivative **19** (OUP-186) and related structures were found to exhibit potent and selective H_3R antagonistic activities, whilst being inactive against hH_4R. However, **19** and its homologs did not have any effect on histamine release when assessed using in vivo rat brain microdialysis. This discrepancy between in vitro and in vivo studies prompted us to investigate the structural differences in the ligand-binding cavities of hH_3R and rH_3R using molecular modeling.

We designed the new nonimidazole H_3R antagonists by modification of clobenpropit, substituting the imidazole with typical secondary cyclic amines: pyrrolidine, morpholine, piperidine, methylpiperadine, and piperazine (Scheme 1). The synthesis of an important intermediate 4-chlorophenylbutyl amine **5**, commenced from commercially available bromopropylphthalimide **1**. Using microwave (MW) irradiation in the four synthetic steps, 4-(4-chlorophenyl)butan-1-amine **5** was synthesized in rapid and straightforward manner through formation of phosphonium salt **2** (85%), subsequent Wittig olefination (65%), catalytic reduction of alkene **3** (98%), and deprotection of phthalimide **4** with hydrazine (82%). The H_3R antagonist target, *N*-[(4-chlorophenyl)butyl]-*S*-[3-(piperidin-1-yl)propyl]isothiourea **19**, was synthesized from amine **5** by using the protocol that we previously reported for the preparation of *S*-alkyl-*N*-alkylisothioureas.[19] Reaction of **5** with 3-phenylpropionyl isothiocyanate (PPI)[19] as an intercalating agent of SCN atoms afforded thiourea **6** (46%). This thiourea **6** was subsequently converted into isothiourea **7** (76%) via Mitsunobu S-alkylation with 1-piperidinepropanol using *N*,*N*,*N*′,*N*′-tetramethylazodicarboxamide (TMAD) and tributylphosphine. Selective cleavage of the N–CO bond of **7** with hydrazine hydrate, with retention of the fissile *S*-alkyl moieties, produced target compound **19** (52%),[20a] which was subsequently treated with hydrochloric acid to give a dihydrochloride.

In addition, amide compound **24** was easily prepared via three steps starting from piperidine, as outlined in Scheme 1. The other *S*-alkyl-*N*-alkylisothioureas (**10**–**18** and **20**–**23**) and amide analogs **25** and **26** were successfully synthesized in good yields using the reaction conditions detailed for **19** and **24**, respectively. All final compounds (**10**–**26**) were provided as di- or monohydrochlorides, and their structures were confirmed using standard spectral techniques (^1H and ^{13}C NMR, IR, and HRMS).[20b]

The seventeen synthesized compounds were tested consisting of an in vitro functional assay using LANCE kit (PerkinElmer) with ES-392-C and ES-393-C cells that stably express hH_3R and hH_4R, respectively (PerkinElmer). All tested compounds showed antagonistic activities for hH_3R. Potencies (pIC_{50}) were determined by analyzing concentration–response curves obtained for the test compounds in the presence of agonist *R*-α-methylhistamine at EC_{80} concentration (see Supplementary data, Section 2). As 3-piperidino- or pyrrolidinopropyl groups were identified as being the key pharmacophores in the nonimidazole antagonists that have reached clinical trials (Fig. 1),[10] the spacer between the cyclic amines and central isothiourea was set as three methylene–carbons in the first step (Table 1). The respective pIC_{50} values for pyrrolidine and piperidine derivatives **10** and **12** were 7.7 and 7.1 (entries 1 and 3), but those of morpholine, methylpiperazine, and piperazine derivatives **11**, **13**, and **14** gave lower values of 5.2–5.8 (entries 2, 4, and 5). Among these compounds, piperidine analog **12** was previously reported as FUB661 ($pA_2 = 7.4$) by Stark



Scheme 1. Synthesis of isothiourea **19** and amide **24**. Reagents and conditions: (a) Ph_3P, CH_3CN, MW, 200 °C, 15 min; (b) 4-chlorobenzaldehyde, NaOMe, DMF, MW, 200 °C, 15 min; (c) H_2/Pd–C, THF–MeOH, 50 min; (d) $NH_2NH_2{\cdot}H_2O$, EtOH, MW, 120 °C, 15 min; (e) PPI, toluene, 60 °C, 0.5 h; (f) 1-piperidinepropanol, TMAD, Bu_3P, THF, rt, 16 h; (g) $NH_2NH_2{\cdot}H_2O$, EtOH, rt, 16 h; (h) ethyl 4-bromobutyrate, MeCN, reflux, 2 h; (i) 6NHCl, reflux, 1.5 h; (j) **5**, $(EtO)_2P(O)CN$, Et_3N, DMF, 14 h.