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Refinement of histamine H3 ligands pharmacophore model leads to a new class of potent and selective naphthalene inverse agonists

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ABSTRACT

The refinement of our original five point pharmacophore model for the H_3 receptor with the addition of a new acceptor feature is presented. The importance of this new acceptor feature for the binding and the selectivity against H_1, H_2 and H_4 has been validated using a newly synthesized naphthalene series. With the SAR deduced from several hundred naphthalene derivatives in various sub-classes the specific role of each pharmacophoric feature, by varying the geometry, size and charge of the molecules, was elucidated. This led to the discovery of a highly potent and selective new compounds series.

To date four distinct histamine receptors are known.[1] They belong to the family A of G-protein coupled receptors and are involved in the regulation of various pharmacological processes. The H_1 and H_2 receptors are clinically validated targets. H_1 receptor antagonists are used for the treatment of allergies and sleep disorders,[2] whereas H_2 receptor antagonists serve as agents for the treatment of the peptic ulcer disease.[3] More recently, H_3 ligands have advanced to clinical phase trials for the treatment of CNS disorders like migraine, narcolepsy, attention-deficit hyperactivity disorder and Alzheimer's dementia.[4] However, H_3 ligands might also be useful to control food-intake and obesity.[5] H_4 receptor agonists and antagonists are less well studied. Nevertheless, due to their preferred expression in T-cells, dendritic cells, monocytes and mast cells, it is likely that H_4 receptors are involved in the regulation of the immune responses.[6]

Many of the first generation H_3 receptor antagonists contained an imidazole moiety which is structurally close to the natural ligand histamine but carry a potential liability towards cytochrome-P450 interaction.[7] The second generation H_3 receptor antagonists are devoid of an imidazole moiety, but contain one or even two basic nitrogens, and several compounds of this class are currently being developed and recently entered human clinical trials.[4]

As part of our strategy to develop new and potent H_3 receptor inverse agonists, we refine and apply the five features pharmacophore model that has been validated with the results of the de novo initiative described previously.[8] At the time, the model consisted of two aromatics, two positively charged features and one central electron rich features (Fig. 1a). The observation that many ligands (Fig. 2) displayed an acceptor functionality in close vicinity to the largest aromatic feature led to the refinement of our existing model (Fig. 1b).

In parallel to this ligand-based approach, a homology model has been built based on the bovine rhodopsin template using the software MOE in order to discover the potential amino acids interacting with our features (data not shown).[11] From the spatial distribution of our pharmacophore, we identified the same residues than the ones described in the work of B. Schlegel. et al., meaning an interaction between the distal basic nitrogen and the conserved aspartate in helix 3 (ASP3.32),[12] but more interestingly, we also identified an interaction between the new acceptor position and the Threonine 6.52, which is specific to H_3.[13]

This newly in-house established pharmacophore model served for the prioritization of all proposals for new chemical series. From the many ideas filtered by the pharmacophore model, naphthalene derivatives were investigated first. Isomeric naphthalenes with an oxygen-atom linker **3** and **4** were pursued. The pharmacophore model predicted a much better fit for the 2-naphthoic amide derivatives **3** than the respective 1-naphthoic amide derivatives **4**. To verify this hypothesis both compound series were synthesized. Also, naphthalene derivatives with a nitrogen linker-atom **7** (of which only the 4-amino-piperidine derivatives are shown as example) have been synthesized to support the pharmacophore model validation. For each naphthalene derivative **3**, **4** and **7**, a straight-

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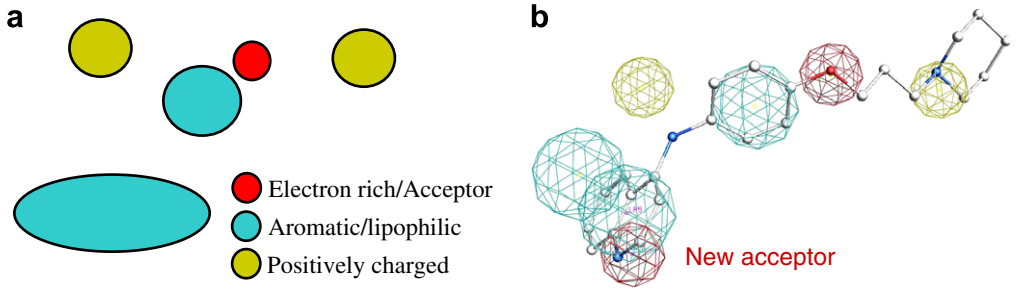


Figure 1. (a) Pharmacophore model used with the de novo approach (b) New pharmacophore model used in this study with FUB 836 fitted.[9]

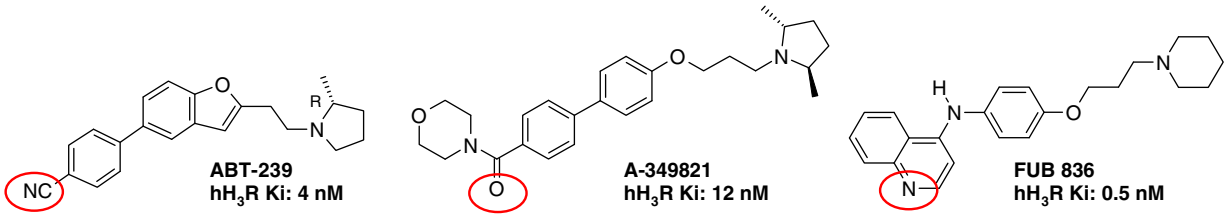
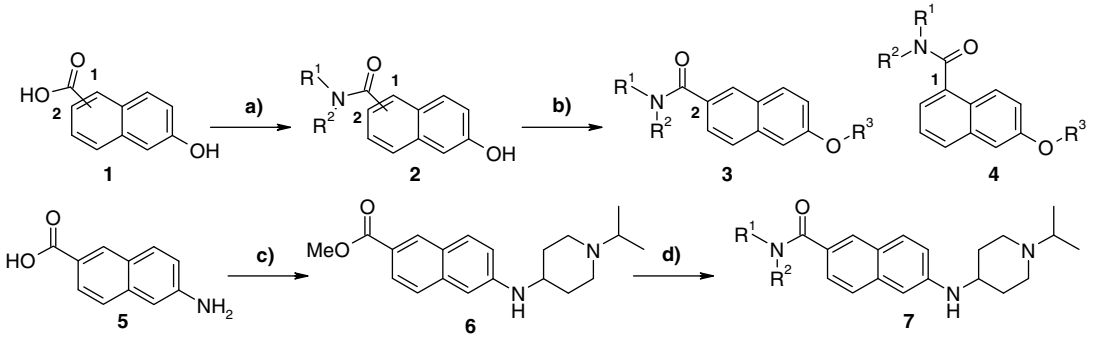


Figure 2. Representative set of potent H_3 receptor antagonists displaying the new acceptor feature (red circle).[10]

forward two-step chemistry route was devised giving access to a variety of compounds (Scheme 1).

The commercially available isomeric 6-hydroxy-naphthoic acids **1** (6-hydroxy-1-naphthoic and 6-hydroxy-2-naphthoic) were converted to their respective amides **2** by coupling with the appropriate amine and TBTU as the coupling reagent. Final conversion to naphthalene derivatives **3** and **4** was achieved using a Mitsunobu coupling protocol employing polymer-bound tri-phenyl phosphine which allowed, after filtration, for easy purification by preparative HPLC on reversed phase. Conversely, 6-amino-naphthoic acid **5** was first converted by reductive amination with 1-isopropyl-piperidin-4-one and sodium tri-acetoxyborohydride to 6-(1-isopropyl-piperidin-4-ylamino)-naphthalene-2-carboxylic acid methyl ester **6**, which was subsequently converted to naphthalene derivatives **7** by saponification and coupling of the resulting acid derivative with the appropriate amines under TBTU conditions. Several hundred compounds in various sub-series have been synthesized and tested for their binding activity towards the H_3 receptor.[14] To illustrate the general structure activity trend, a few compounds and their human binding affinity data are shown in Figure 3.

Naphthalenes **3a–c** showed the highest potency in binding towards the H_3 receptor (functionally characterized as inverse agonists by GTPγS assay, exemplified with **3c**; EC_{50} = 21 nM) in the nano-molar range, whereas in comparison the respective isomers **4a–4c** were in general markedly less active. This was in agreement with the prediction from the pharmacophore model and validated our ligand-based approach. Also in comparison to compounds **3a–3c**, nitrogen-atom linked naphthalenes **7a–7c** were generally found to be only active in the micro-molar range. We could observe here an important shift in activity between the oxygen-atom linked naphthalene **3b** and the nitrogen-atom linked naphthalene **7a**. These two types of linkers have a small influence on the conformation of the molecule and a moderate influence on the electronic properties, but they interact quite differently with the receptor. Indeed, knowing that the ether-oxygen is electron rich but a poor acceptor, whereas the amine-nitrogen is a strong donor, and additionally looking at the homology model, it is very likely that this ether oxygen has a positive interaction with the sulfur of CYS3.36, which the nitrogen cannot have.[15] As can be seen from Figure 4, naphthalene derivative **3b**, optimized with AM1 in MOE, fits perfectly the pharmacophore model using the new acceptor position. Since the binding is strongly driven by the interaction between the basic amine and ASP3.32, it restrains the number of possible fits. Distance-wise, the carbonyl function of the naphthalene can only match the new acceptor feature (THR6.52). Moreover, compound **3c** shows a very nice selectivity against all the other histamine subtypes by displaying less than 25% inhibition



Scheme 1. Reagents and conditions: (a) 1.2 equiv TBTU, 1.2 equiv HNR^1R^2, DMF, DIPEA. (b) 1.2 equiv R^3-OH, 2 equiv di-*tert*-butyl azodicarboxylate, polymer bound TPP, THF. (c) 1.2 equiv 1-isopropyl-piperidin-4-one, $NaBH(OAc)_3$, THF. (d) 1—LiOH·H_2O, THF, H_2O, 2—1.2 equiv TBTU, 1.2 equiv HNR^1R^2, DMF, DIPEA.

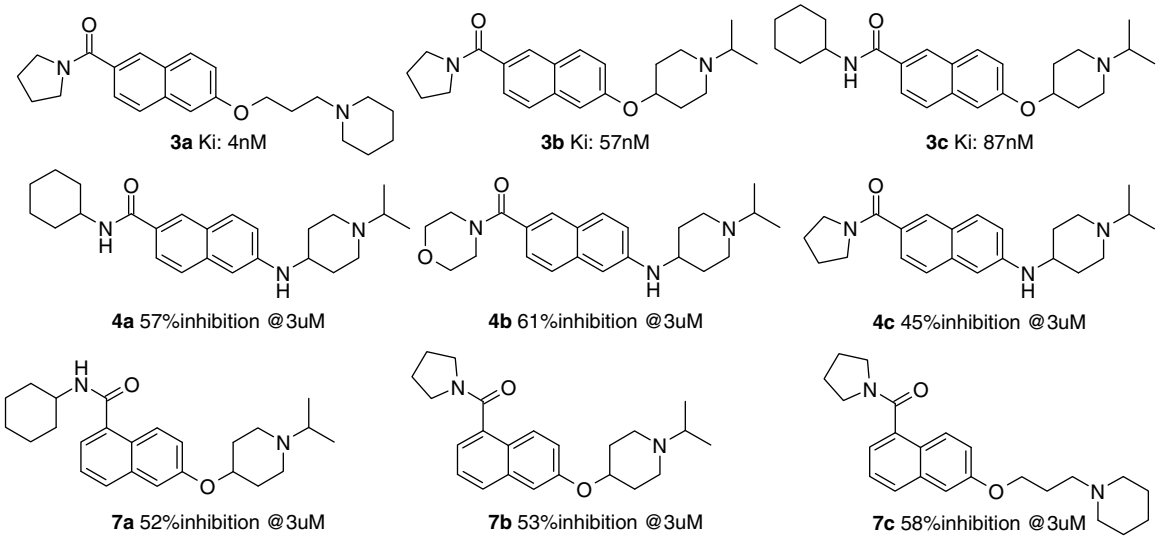


Figure 3. Selected compounds and their binding activity towards the human histamine H_3 receptor.[14]

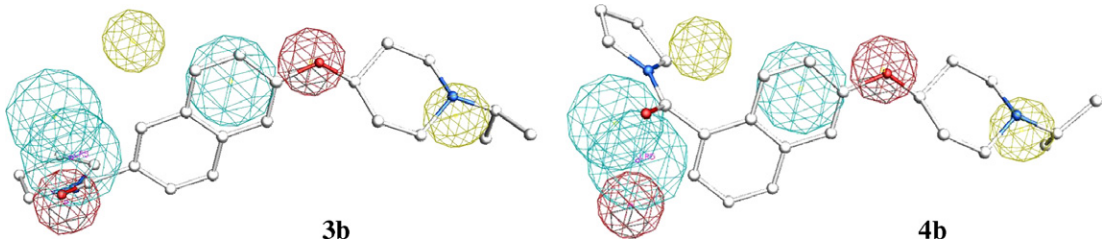


Figure 4. Molecules **3b** and **4b** in the pharmacophore model.

at 3 μM against H_1, H_2 and H_4.[16] This selectivity was expected from the model since THR6.52 is only present in the H_3 receptor subtype. On the other hand, the fit of the isomeric naphthalene derivative **4b** in Figure 4 is far less good, and that was confirmed by the experimental values (**3b**: Ki: 57 nM, **4b**: 61% inhibition at 3 μM), and more broadly by the SAR of both sub-series.

In conclusion, we have refined our original five point pharmacophore model for the H_3 receptor with the addition of a new acceptor. The importance of this new acceptor feature for the binding and the selectivity against H_1, H_2 and H_4 has been validated using a newly synthesized naphthalene series. With the SAR deduced from several hundred naphthalene derivatives in various sub-classes, we have been able to elucidate the specific role of each pharmacophoric feature by varying the geometry, size and charge of the molecules. This led to the discovery of a highly potent and selective new compound series.

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