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Selective naphthalene H_3 receptor inverse agonists with reduced potential to induce phospholipidosis and their quinoline analogs

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ABSTRACT

We reported earlier the refinement of our initial five-point pharmacophore model for the Histamine 3 receptor (H_3R), with a new acceptor feature important for binding and selectivity against the other histamine receptor subtypes 1, 2 and 4. This approach was validated with a new series of H_3R inverse agonists: the naphthalene series. In this Letter, we describe our efforts to overcome the phospholipidosis flag identified with our initial lead compound (**1a**). During the optimization process, we monitored the potency of our molecules toward the H_3 receptor, their selectivity against H_1R, H_2R and H_4R, as well as some key molecular properties that may influence phospholipidosis.

Encouraged by the promising profile of the naphthalene series, we used our deeper understanding of the H_3R pharmacophore model to lead us towards the quinoline series. This series is perceived to have intrinsic advantages with respect to its amphiphilic vector.

Histamine elicits physiological responses mediated by four G-protein coupled receptors (H_1R, H_2R, H_3R and H_4R).[1] The histamine H_1 and H_2 receptors are well established drug targets[2] and the H_4 receptor is currently undergoing rigorous characterization.[3] H_3 receptors are expressed predominantly on the presynaptic termini of CNS neurons, where they function as inhibitory auto- and heteroreceptors.[4,5] H_3 antagonists/inverse agonists can therefore function to increase the release of various neurotransmitters, including histamine, acetylcholine, norepinephrine, serotonin and dopamine.[6–10] These neurotransmitters are known to play important roles in vigilance, attention, cognition and energy homeostasis. In this regard, a remarkable arrays of therapeutic areas in which ligands for the H_3R may prove useful have been identified.

Initial work in the field focused on analogs of the natural ligand, histamine. Due to the presence of the imidazole ring, these compounds have numerous liabilities and poor drug-like properties. Second generation H_3R antagonists are devoid of an imidazole-moiety, but contain one or two basic sp3 nitrogen atoms. Several compounds of this class are currently being developed and recently entered into human clinical trials for the potential treatment of a variety of CNS disorders affecting cognition (e.g., schizophrenia, attention-deficit hyperactivity disorder and Alzheimer's dementia) and sleep (e.g., hypersomnia, narcolepsy).[11] H_3R inverse agonists may also be useful in the control of food intake and obesity.[12]

We identified the naphthalene class of compounds as selective and potent H_3R inverse antagonists from the refinement of our initial five point pharmacophore model[13] by the use of the acceptor functionality in close vicinity to the largest aromatic feature (Fig. 1), important for the binding and the selectivity against H_1R, H_2R and H_4R.[14]

Searching for potent compounds, we identified the (3-piperidin-1-yl-propoxy)-naphthalene derivative **1a**[14] as a lead structure. In fact, compound **1a** has many favorable properties: high solubility,[15] high permeability,[16] and high metabolic stability in rat and human liver microsomes.[17] In terms of safety, this compound did not have any issues with respect to mutagenicity (genotoxicity and clastogenicity)[18] or phototoxicity in vitro (Fig. 2).

However, the combination of the lipophilic naphthalene core with a basic nitrogen tail, protonated at physiological pH, would result in this class of compound being amphiphilic. Cationic amphiphilic drugs are associated with phospholipidosis[19] and indeed compound **1a** was found to induce phospholipidosis in cultured fibroblasts at test concentrations of 2.5–20 μM in a concentration dependent manner.[20]

Phospholipidosis may have adverse physiological consequences and has already been reported in association with some H_3R

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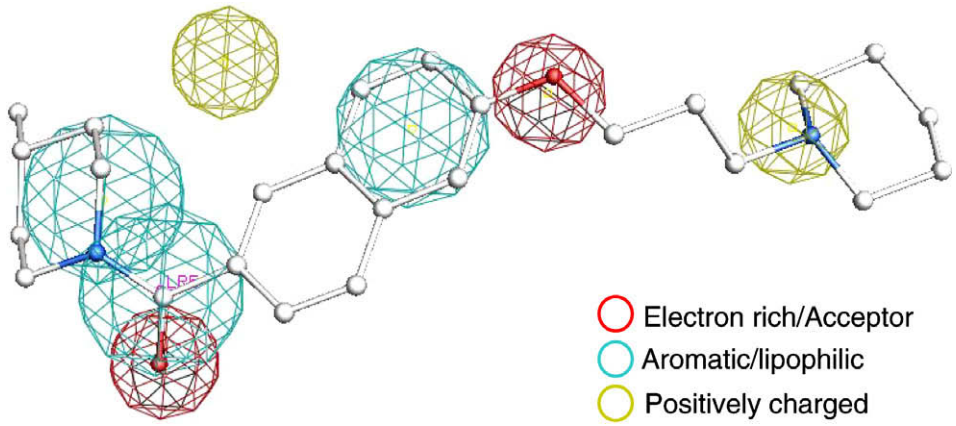
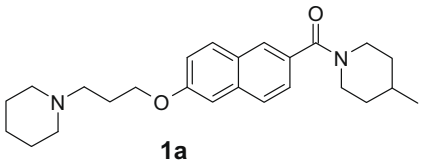


Figure 1. Compound **1a** of the naphthalene class fitting the latest pharmacophore model described previously.[14]



1a

In vitro profile:
hH3 Ki= 7nM [21] , GTPγS: EC_{50}= 200 nM [22]
inverse agonist
Selectivity
H1 / H2 / H4: -14.7 / -0.3 / 0%inhib@3uM
LYSA: >543 ug/mL
PAMPA Peff: 2.6 10^{-6} cm/s (high permeability)
$logD_{7.4}$: 2.47
microsomes:
Clearance
human 32 uL/min/mg protein
rat 33 uL/min/mg protein
IC_{50} CYP3A4, 2D6, 1A2, 2C9, 2C19: all >12μM
not phototoxic in vitro
no liabilities in Ames and MNTtest
Phospholipidosis induction : positive @2.5uM,
strongly positive @5 uM and above

Figure 2. Structure and properties of compound **1a** (See above-mentioned references for further information.)

ligands.[23] The use of the amphiphilicity as a predictor for phospholipidosis liability has already been widely established[19,24] and therefore we decided to closely monitor the amphiphilicity of the naphthalene class, which was calculated by a computational tool (CAFCA).[25]

We investigated the influence of the pK_a[26] on the amphiphilicity by changing the nature of the nitrogen tail (R group on Table 1).

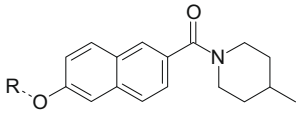
Unfortunately, the calculated free energy of amphiphilicity ($\Delta\Delta G_{AM}$) for the compounds in Table 1 was always below −6 kJ/mol. We aimed for $\Delta\Delta G_{AM}$ values greater than −6 kJ/mol, as it has been found that such a threshold ensures a low probability of being positive on the fibroblast phospholipidosis assay, whereas $\Delta\Delta G_{AM}$ values below −6 kJ/mol indicate a high risk of phospholipidosis.[24]

It was observed within the series that significant modifications of the pK_a (ca. 2 log units) only marginally improved $\Delta\Delta G_{AM}$ (see Table 1, cpds **1a** vs **1f**). When basicity was highly reduced (compound **1f** with the 3-morpholinyl-propoxy has a lower pK_a) amphiphilicity was not further improved and micromolar activity at H_3R was obtained. Compound **1f** with a pK_a of 7.8 is still cationic at physiological conditions and therefore amphiphilic. As a consequence, it can be envisaged that the reduction of pK_a value has no influence on the phospholipidosis hazard, as long as the pK_a is larger than 6.3.[24] However, such a low pK_a value might prevent the compound from binding as the formation of the Asp3.32 salt bridge (i.e., the formation of an ionic bond between the protonated nitrogen of the ligand and the anionic acid Asp3.32) is no longer secured.

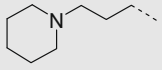
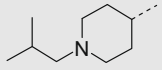
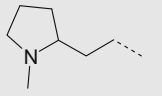
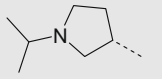
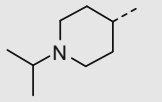
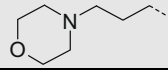
Moreover, as it can be seen in Table 1, the calculated lipophilicity, expressed as KOW_Clog *P*, is not correlated with the amphiphilicity.

We carried on with the exploration of the amide side chain, combined with the two most potent basic tails: the piperidinyl propoxy and the isopropyl piperidinoxy (R groups in Table 2).

Table 1
Exploration of in vitro potency, selectivity, lipophilicity, basicity and in silico $\Delta\Delta G_{AM}$ for the naphthalene class

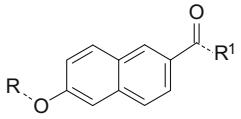


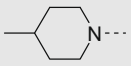
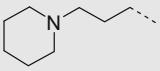
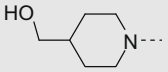
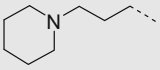
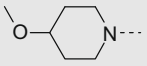
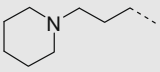
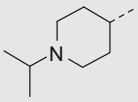
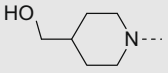
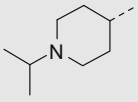
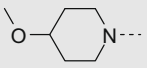
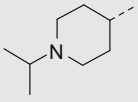
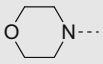
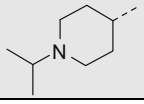
1

Compound	hH_3 K_i (nM)	R	$hH_1/hH_2/hH_4$ inhibition[a] (%)	KOW_Clog *P*	pK_a [b]	$\Delta\Delta G_{AM}$ [c] (kJ/mol)
1a	7		−14.7/0.3/0	5.3	9.86	−10.4
1b	36		−4.5/4.1/0	5.1	9.8	−9.7
1c	571		—	4.6	9.9	−10.1
1d	234		—	4.5	9.4	−9.4
1e	34		0.9/−8/0	4.3	9.6	−9.5
1f	2000		—	4	7.8	−10

[a] Percentage measured at 10 μM.
[b] Measured pK_a.
[c] Calculated free energy of amphiphilicity as measure of phospholipidosis potential.

Table 2
Second round on the exploration of in vitro potency and amphiphilicity. In silico $\Delta\Delta G_{AM}$



Compound	hH$_3$ K_i (nM)	R^1	R	KOW_Clog*P*	pK_a	$\Delta\Delta G_{AM}$ (kJ/mol)
1a	7			5.28	9.86	−10.4
2	103			3.29	9.6	−5.9
3	30			3.24	9.57	−6.7
4	15			3.98	9.71	−9. 07
5	154			2.55	9.6	−6.1
6	62			2.33	9.4	−6.5
7	40			2.10	9.3	−4.27

These two R groups have different conformational restrictions, and they also introduce slightly different distances from the cationic charge to the hydrophobic center.

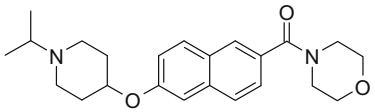
For all the 3-piperidinyl propoxy side chains, amphiphilicity was highly reduced with the use of more hydrophilic amides (**2** and **3** compared to **1a**). Unfortunately, the potency was lost when $\Delta\Delta G_{AM}$ was greater than −6 kJ/mol (compound **2**, Table 2). Compound **3** is still sufficiently active and has an acceptable and improved amphiphilicity.

The use of an isopropyl piperidinyloxy as the basic nitrogen tail introduced a slightly different distance from the cationic charge to the hydrophobic center and marginally reduced the amphiphilic vector (compounds **5** vs **2**; compounds **6** vs **3** in Table 2; compounds **1e** vs **1a** in Table 1). This effect becomes slightly synergistic when combined with the introduction of more hydrophilic amides at the terminal side of the molecule (compound **4** compared with **1a** in Table 2). Compound **4** has a slightly shorter nitrogen tail (thus a slightly reduced amphiphilic vector) but it is still quite lipophilic, even if much less than **1a,** so that the absolute value for $\Delta\Delta G_{AM}$ is significantly reduced but still very high. Unfortunately, less potent compounds are obtained when the lipophilicity is further reduced (from the methyl pyrrole compound **4** to the hydroxyl methyl piperidinyloxy analog **5** and the 3-methoxy piperidinyl analog **6**).

The combination of the morpholine amide with the isopropyl piperidinyloxy as the nitrogen tail leads to compound **7** with reduced lipophilicity and an appropriate $\Delta\Delta G_{AM}$ greater than −6 kJ/mol, indicating a low risk for phospholipidosis. This compound has an acceptable potency on binding and behaves as an H_3R inverse agonist.

It is obvious that the substituents on the amide side chain in the naphthalene series play a key role on the lipophilicity, and even more on the amphiphilicity of the compound (compounds **1a**, **2**, **3** and also **4**, **5**, **6**, **7**).

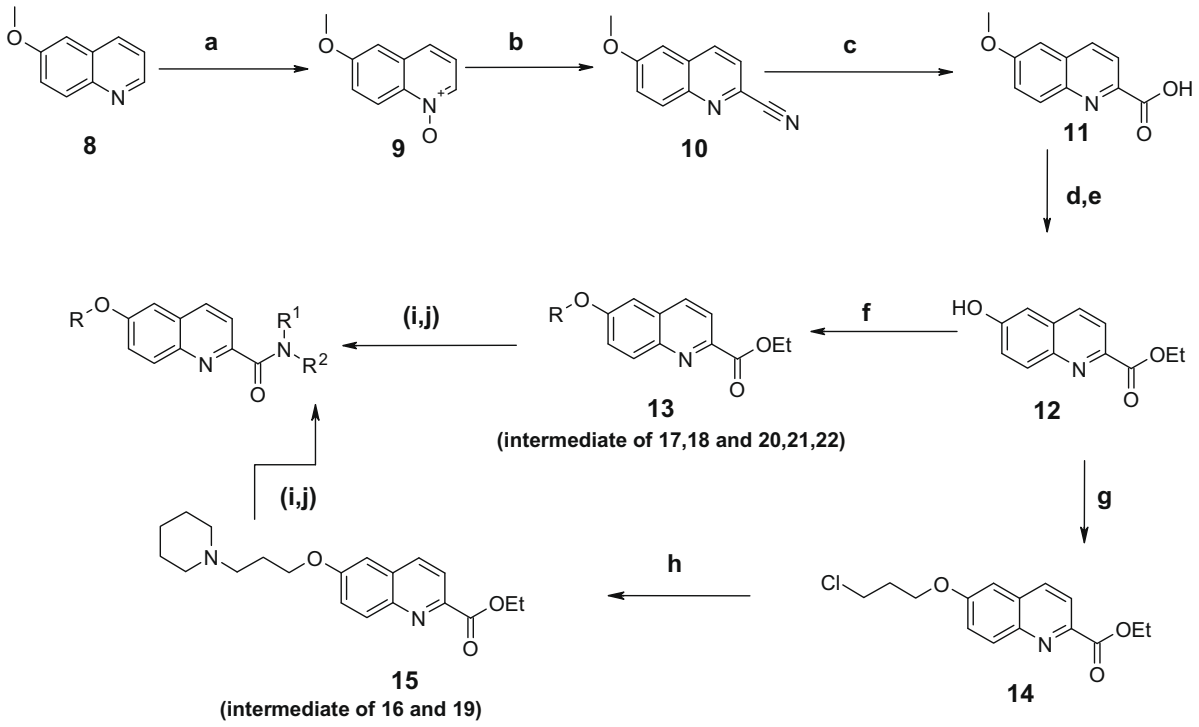
Compound **7** is potent in vitro at the H_3R, and selective against H_1R, H_2R and H_4R. It has many favorable properties such as high solubility, high permeability and high metabolic stability in rat and human liver microsomes, and a clean safety profile, like the initial lead compound **1a**. However, in contrast to **1a**, **7** has a much reduced amphiphilicity, and no flag in phospholipidosis (Fig. 3). Despite this achievement, we decided to investigate other central cores, to potentially overcome some of the limitations from the naphthalene scaffold, particularly with regard to phospholipidosis.



7

In vitro profile:
hH3 Ki= 40nM [21]; GTPγS: EC_{50}= 17nM [22]
inverse agonist
Selectivity
H1 / H2 / H4: 1.6 / -9.9 / 0%inhib@3uM
LYSA: >558ug/mL
PAMPA Peff: 2.5 (high permeability)
$logD_{7.4}$: 0.6
microsomes:
Clearance
human: 3 uL/min/mg protein
rat : 23 uL/min/mg protein
CYP3A4, 2D6, 1A2, 2C9, 2C19: all > 41 uM
not phototoxic in silico
no liabilities in Ames and MNT tests
Phospholipidosis: No flag for induction

Figure 3. Structure and properties of compound **7**.



Scheme 1. Reagents and conditions: (a) CH_3COOH, H_2O_2, reflux, 2 h, 81%;(b) benzoyl chloride, AgCN, rt 4 h–refluxing overnight, 81% or alternative Me_3SiCN, Me_2NCOCl, CH_2Cl_2, rt, 87% (c) (i) NaOH, MeOH, 90 °C, 90%; (ii) HCl (25%), H_2O; (d) HBr, reflux, 48 h, (alternative microway, 160 °C, 1 h), 95%; (e) H_2SO_4, EtOH, reflux, 83%; (f) ROH, PPh_3, diethyl azodicarboxylate, THF, 60–80%; or two step procedure with (g) $Br(CH_2)_3Cl$, K_2CO_3, butanone, rt 90%; (h) piperidine, K_2CO_3, 70%; (i) HCl dioxane, reflux; (j) CDI, $R^1R^2NH_2$, DMF (60–85%yields).

The H_3R pharmacophore model indicated that the modification of the central core should be possible, and we hypothesized that quinoline analogs could be of interest. Replacement of the naphthalene core by a quinoline should have a positive impact on the phospholipidosis profile of our compounds by reducing the hydrophobicity of the central core, thus reducing the amphiphilic vector. General synthesis for these new analogs is indicated in Scheme 1. Initially the conversion of 6-methoxy-quinoline **8** to 2-cyano-6-methoxyquinoline **10** was performed without isolation of the intermediate Reissert compound. This involved treating 6-methoxyquinoline with tosyl chloride, potassium cyanide and water in dichloromethane for one week at room temperature.[27] This reported reaction gave in our hands only a 31% yield. Moreover, the procedure was not suitable for scaling up.

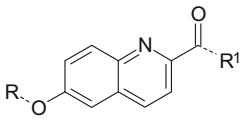
An improved synthesis of the quinoline analogs started from the commercially available 6-methoxy-quinoline **8**, which was converted to the N-oxide **9** by treatment with hydrogen peroxide in acetic acid. Modification of the *ortho*-alkylation procedure by using benzoyl chloride as activating agent and silver cyanide in chloroform afforded the desired 6-methoxy-quinoline-2-carbonitrile **10**.[28] Later on with the use of TMSCN and diisopropycarbamoyl chloride compound **10** was obtained in 87% yield. Hydrolysis of the cyanide to the carboxylic acid **11** was performed by treatment with sodium hydroxide in methanol. The phenolic function was liberated in refluxing hydrobromic acid in 48 h. This reaction was highly accelerated with microwave irradiation at 160 °C. Esterification under usual conditions to make the compound easier to manipulate yielded 6-hydroxy-quinoline-2-carboxylic acid ethyl ester **12** in high yield. The basic side chain was introduced by the use of Mitsunobu conditions with the corresponding alcohol, or in a two step procedure by alkylation with 1-bromo-3-chloropropane using potassium carbonate to the *O*-alkylchloride **14,** followed by heating with the corresponding amine to prepare intermediate **15**. The amide was formed after saponification or acidic hydrolysis of the ester to the 6-alkoxy-quinoline-2-carboxylic acid; in our hands the use of carbonyl diimidazole as coupling reagent gave high yields of the final compounds.

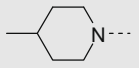
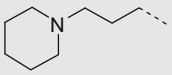
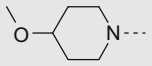
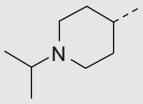
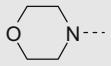
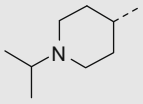
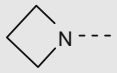
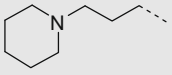
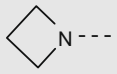
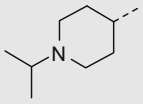
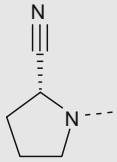
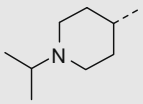
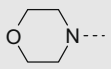
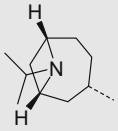
The direct comparison between the naphthalene and the quinoline series with the same substitution pattern at R and R^1 (**1a** vs **16**; **6** vs **17**; **7** vs **18**) indicates that our initial hypothesis about the reduction of the lipophilicity for the central core was correct and accompanied by a reduction of the amphiphilicity, which improved the phospholipidosis profile. Unfortunately, the potency at the H_3R was also reduced for the quinoline series compared to its naphthalene analog.

The Structure–Activity Relationship (S.A.R.) of the in vitro potency for this new class of compounds is slightly different to the naphthalene class, and a new series of derivatives were prepared in order to study and to improve the potency at the H_3R, as indicated in Table 3. The replacement of the basic isopropyl piperidinyloxy for a bicyclic analog improved the potency at the H_3R (compound **18** is less potent than **22**) and had a similar effect on the calculated free energy of amphiphilicity $\Delta\Delta G_{AM}$ (kJ/mol).

In conclusion, we presented the delicate balance existing between amphiphilicity and H_3R affinity in these series. Extensive S.A.R. investigations around the naphthalene core were rewarded by the identification of compound **7** as a potent H_3R inverse agonist, with no phospholipidosis flag and a promising overall profile. The encouraging profile of the naphthalene series and the use of our deeper understanding of the pharmacophore model for the H_3R led us towards another series of compounds with interesting activity as H_3R inverse agonists: the quinoline series. This series showed an improvement in the phospholipidosis profile, as was predicted from the observation of the general effect of the lipophilicity of the central core on the amphiphilicity. However, this series seems to have a lower potency in direct comparison of analogs from the naphthalene class.

Table 3
Quinoline series. Lipophilicity and amphiphilicity calculated by $\Delta\Delta G_{AM}$



Compound	hH_3 K_i (nM)	R^1	R	KOW_Clog *P*	pK_a	$\Delta\Delta G_{AM}$ (kJ/mol)
16	300			4.47	9.7	−9.4
17	125			1.53	9.5	−5.06
18	238			2.18	9.5	−3.2
19	119			2.84	9.4	−5.4
20	62			2.09	9.5	−4.7
21	87			2.24	9.5	−3.21
22	30			2.5	8.97	−3.4

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parameter is the increase in the number of micronucleated cells (%) of treated compared to untreated control in cultivated mammalian cells.
19. Phospholipidosis describes the intracellular accumulation of various phospholipids reflecting a disorder in phospholipid storage, that is, in the lysosomes. Most of the agents that induce phospholipidosis are so-called cationic amphiphilic drugs (CAD) like amiodarone, clomipramine, perhexilline, tamoxifen. CAD's can be described by two fundamental physico-chemical properties, the basic pK_a value reflecting the cationic nature of the molecule and it's amphiphilicity that is defined as the distance between the charged residue and the more remote hydrophobic residues. For a more detailed description see: Muster, W.; Breidenbach, A.; Fischer, H.; Kirchner, S.; Mueller, L.; Paehler, A. *Drug Discovery Today* **2008**, *13*, 303.
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25. A program called CAFCA (**CA**lculated **F**ree energy of **C**harged **A**mphiphiles) was developed and used for the calculation of the amphiphilic properties of molecules. The amphiphilicity is the vector sum calculated from the charged group to each atom/residue within a molecule and weighted with respect to its hydrophobic/hydrophilic property on the basis of an atom/fragment based contribution method. The sum of the calculated vectors is calibrated by means of measured amphiphilicities taking into account the conformational effects of the individual molecules. Finally, the amphiphilicity of a molecule is expressed in terms of free energy ($\Delta\Delta G_{AM}$). For a more detail description, see: Fischer, H.; Kansy, M.; Bur, D. *Chimia* **2000**, *54*, 640.
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