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Potential utility of histamine H_3 receptor antagonist pharmacophore in antipsychotics

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ABSTRACT

Histamine H_3 receptor (H_3R) antagonists have some antipsychotic properties although the clear molecular mechanism is still unknown. As actually the most effective and less side effective antipsychotics are drugs with multiple targets we have designed typical and atypical neuroleptics with an additional histamine H_3 pharmacophore. The 4-(3-piperidinopropoxy)phenyl pharmacophore moiety has been linked to amitriptyline, maprotiline, chlorpromazine, chlorprothixene, fluphenazine, and clozapine. Amide, amine and ester elements have been used generally to maintain or slightly shift affinity at dopamine D_2-like receptors (D_2 and D_3), to decrease affinity at histamine H_1 receptors, and to obtain H_3R ligands with low nanomolar or subnanomolar affinity. Change of effects at D_1-like receptors (D_1 and D_5) were heterogeneous. With these newly profiled compounds different antipsychotic properties might be achieved.

Schizophrenia and related mood disorders are diseases of the central nervous system (CNS), regarded to be neurodevelopmental with epigenetic and genetic factors.[1–3] In general, dysregulations in different neurotransmitter systems mainly dopamine, serotonin, GABA, and glutamate are hypothesized.[4] Moreover, there is indirect evidence that histaminergic pathways may play a role in schizophrenia and that histamine H_3 receptor (H_3R) antagonists could lead to therapeutic effects, namely on cognitive deficits.[5–7] Today, typical and atypical neuroleptics are used for treatment of schizophrenia. Their effects are mainly mediated by inhibition of dopamine D_2-like receptors (D_2, D_3 (and may be D_4)) and some other aminergic receptors.

Extrapyramidal side effects and weight gain problems are some of the most important side effects which are related to their antagonist properties at dopamine D_2-like and histamine H_1 receptors, respectively.[8–11]

The H_3R is acting as presynaptic auto- and heteroreceptor mainly in the CNS[12] controlling the synthesis and the release of histamine, but also modulating several other neurotransmitter systems e.g., dopamine, serotonin, GABA, noradrenalin, or ACh. H_3R antagonists have shown distinct pharmacological actions in preclinical and clinical trials revealing the importance for diverse CNS-related therapeutic applications like schizophrenia, depression, sleep–wake disorders, dementia, or epilepsy.[13–15] In fact, H_3R inverse agonists/antagonists displayed significant inhibitory activity in several rodent models of schizophrenia, a disease in which N^{τ}-methylhistamine level is usually significantly high in cerebral cerebrospinal fluid of patients.[16,17] Reduction of undesirable side effects connected to antipsychotic therapy like weight gain, somnolence, and cognitive impairment have been demonstrated.[17]

The data mentioned above indicate that a drug combination of H_3R antagonist and neuroleptic properties may be beneficial in therapy. Instead of a combination of two drugs, we used the multiple target approach designing one new drug by combination of two related pharmacophore elements in one structure.[18,19] This approach has been realized by linking the known antagonist H_3R pharmacophore 4-(3-piperidinopropoxy)phenyl to known neuroleptics like amitriptyline (**1**), maprotiline (**2**), chlorpromazine (**3**), chlorprothixene (**4**), clozapine (**5**), and fluphenazine (**6**) (Figs. 1 and 2).

For introduction of H_3R affinity all novel antipsychotic derivatives need to follow a broad general construction pattern shown in Figure 2[20,21], bearing a relatively flexible part at the 'eastern' part of the molecule. The lead structure of 4-(3-piperidinopropoxy)phenyl was taken for further derivatization by linking the neuroleptic compounds **1**–**6** by amide, amine or ester functionalities. Related hybrid structure in the class of H_3R antagonists have been described with H_1, ACh, BuCh, NO, and SSRI (for reviews see Refs. 12–14).

The objective of our study was the development of novel multiacting antipsychotic drugs showing an optimized target profile with potent action, fast onset and reduced side effects by maintaining D_2/D_3 affinity, reducing H_1 affinity and introducing high H_3R affinity (Fig. 2). The novel compounds were tested in vitro for several aminergic G-protein-coupled receptors (GPCRs) to examine their enlarged pharmacological profiles. One selected compound was chosen for further H_3R in vivo screening.

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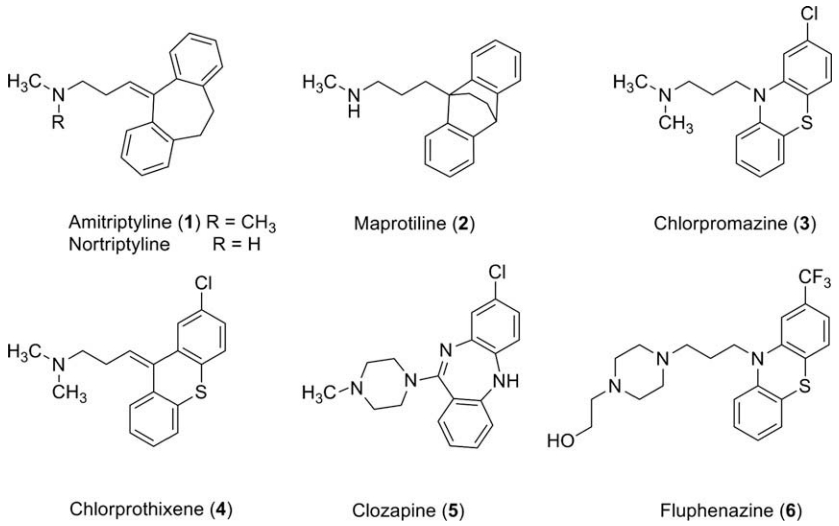

Figure 1. Structures of antipsychotics used in this study.

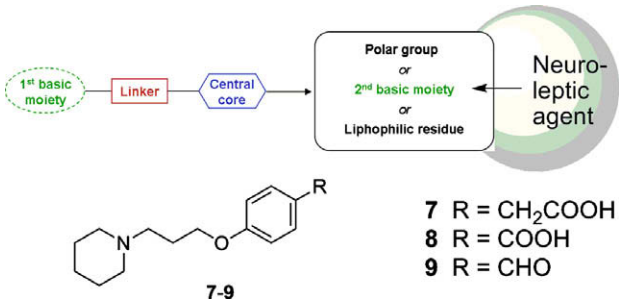

Figure 2. General construction pattern of histamine H_3 receptor antagonists with neuroleptics and H_3 pharmacophore starting structures.

For the modular approach with the final combination of different antipsychotic structures to H_3R pharmacophores functionalized benzoic acid, phenyl acetic acid and benzaldehyde derivatives **7**–**9** were prepared according to literature methods (Fig. 2).[22]

The acid derivates **7**×HCl and **8**×HCl were transformed into reactive carboxylic chlorides for convenient synthesis using the commercially available desmethylated amitriptyline derivative, nortriptyline (Fig. 1), resulting in amide derivatives **10** and **11**, respectively (Fig. 3).

For comparison of amide and amine linker H_3R hybrids with a second basic amine functionality were prepared by reductive amination of **9** with nortryptiline or **2** receiving the corresponding amitriptyline or maprotiline derivatives, respectively (Fig. 4).[23]

The tertiary amine functionality of chlorpromazine, chlorprothixene and clozapine was conveniently demethylated by reaction of α-chloroethyl chloroformate (ACE-Cl) and following stirring and heating in methanol. The intermediate carbamates are cleaved leaving the secondary amines as well as gaseous by-products, which can easily be removed under reduced pressure.[24,25] Then the resulting secondary amines could be used for reductive amination with **9** as described before (Fig. 5).

In addition to amines and amides as nitrogen connection, we extended these linking elements to oxygen ports. Therefore, we decided that an ester group may be a suitable functionality. Fluphenazine hybrid **17** was synthesized by simple esterification of activated **8** (Fig. 6).

All of the drugs **1**–**6** and new multiple target compounds **10**–**17** were first tested for their histamine H_3 receptor activity obtained by [^{125}I]iodoproxyfan binding assay on CHO/HEK293 cells stably expressing the hH_3 receptor (Table 1).[26] To enlarge their pharmacological profile, we also investigated their binding affinities in competition binding experiments with seven-point measurements in at least duplicates ($n \geqslant 2$). Displacement assay were carried out using membrane suspension of cell lines stably expressing the human histamine hH_1 receptor (CHO) with [^{3}H]pyrilamine, dopamine hD_1 and hD_5 receptors (HEK) against [^{3}H]SCH23390 and hD_{2S}, hD_3 receptors (CHO) using [^{3}H]spiperone, respectively (Table 1).[27–29]

All new compounds revealed high H_3R binding affinities in low nanomolar to even picomolar concentration range (**17**) whereas

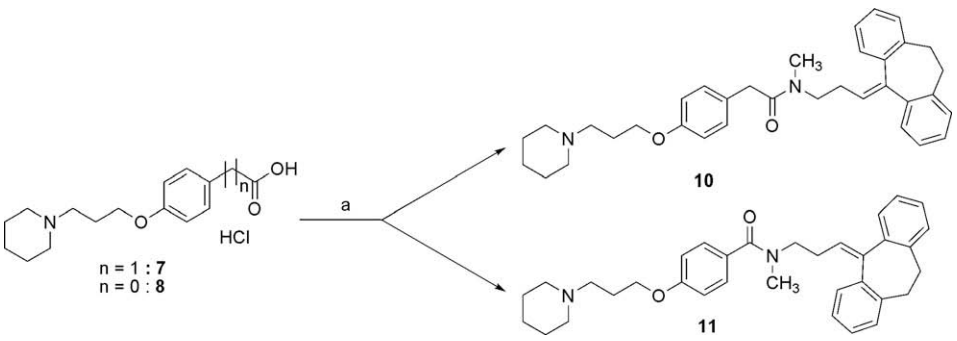

Figure 3. Synthesis of amide derivatives **10** and **11**. Reagents and conditions: (a) i—$SOCl_2$, toluene, 70 °C, 3 h; ii—**1**, CH_2Cl_2, $N(Et)_3$, rt, 2 h.

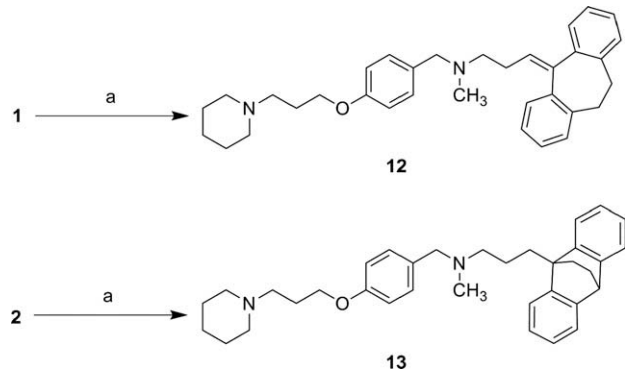

Figure 4. Synthesis of tertiary amine amitriptyline hybrid **12** and maprotiline hybrid **13**. Reagents and conditions: (a) **9**, $NaBH(OAc)_3$, 1,2-dichloroethane, rt, 18 h.

the marketed antipsychotics do not have any H_3R affinity (Table 1). For clozapine some moderate affinity for rodent H_3R has been reported whereas for hH_3R affinity is in the micromolar concentration range.

The easily prepared amide hybrids of nortriptyline, **10** and **11**, were first screened as an early proof-of-concept. Although decrease in D_2 receptor affinities was observed, D_3 receptor affinities were maintained, H_1 receptor affinities decreased and H_3R affinities introduced. Whereas the data for D_2 were more or less expected we were pleasantly surprised by the relative high affinity of these amides for D_3R.

Comparing the results of the amides **10** and **11** with those of the related amine hybrid **12**, which can be seen as amitriptyline hybrid as well as nortriptyline hybrid, a tenfold increase to subnanomolar H_3R affinity could be observed simultaneously with increased affinities at D_2, D_3, and H_1 receptors.

Encouraged by these outcomes, further amine derivatives **13**–**16** have been synthesized and tested, resulting in comparable binding affinities at H_3R. The ester hybrid of fluphenazine, **17**, even showed another 100-fold increase compared to that of the amides, to picomolar affinity (K_i = 42 pM). An additional amine group can increase binding affinity.[30] In contrast to the other amine hybrids **12**–**16**, the distances on all of the basic moieties of compound **17** from the phenyl group as central core are roughly comparable to each other suggesting another pharmacophore imitation with additional lipophilic binding areas. This may lead to the assumption that compound **17** could have more interaction possibilities with additional H_3R binding pockets than the other hybrids.

In comparison to the antipsychotic binding patterns, the amine compounds (**12**–**17**) in general show different but still moderate binding profile for the dopamine D_2-like receptor subtypes (K_i values (D_2): 285 to 41 nM; K_i values (D_3): 223 to 15 nM). The amine hybrids of amitriptyline (or nortriptyline), **12**, and of maprotiline, **13**, even showed slight enhancements at D_2-like receptor affinities (factors of about 1.5–4.5).

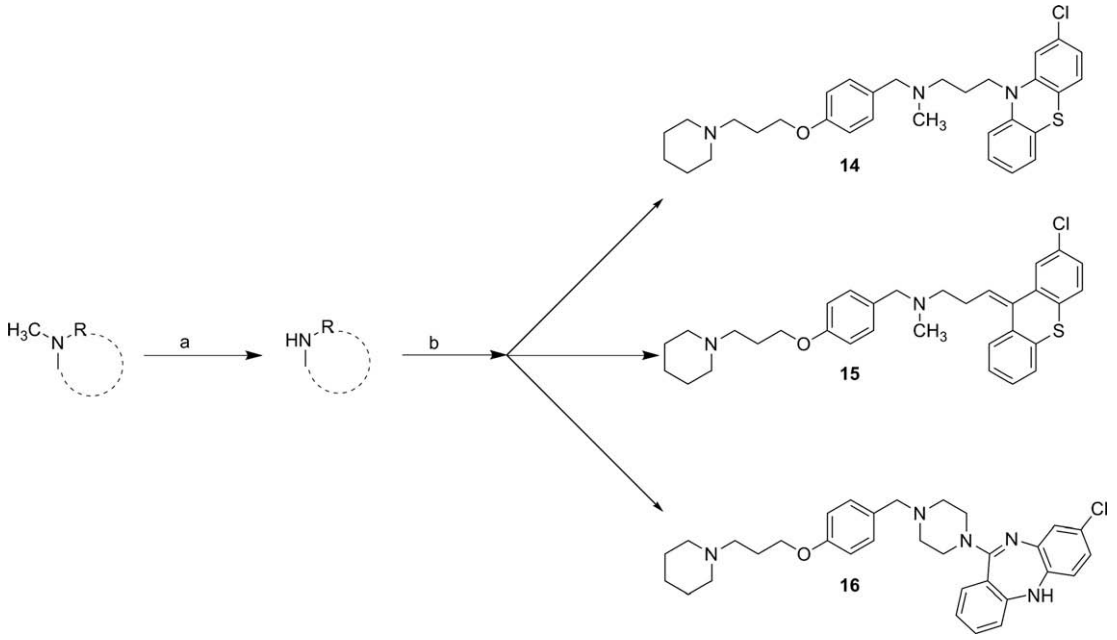

Figure 5. Synthesis of tertiary amine derivatives after previous demethylation (**14**–**16**). Reagents and conditions: (a) i—**3**, **4**, or **5**, ACE-Cl, 1,2-dichloroethane, reflux, 24 h; ii—MeOH, 50 °C, 2 h; (b) **9**, $NaBH(OAc)_3$, 1,2-dichloro ethane, rt, 18 h.

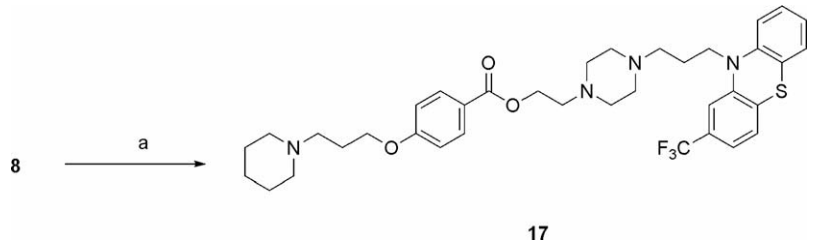

Figure 6. Synthesis of ester fluphenazine hybrid (**17**). Reagents and conditions: (a) i—(**8**×HCl), $SOCl_2$, toluene, 70 °C, 3 h; ii—**6**, CH_2Cl_2, $N(Et)_3$, rt, 2 h.

Table 1
Pharmacological binding profile of compounds **1–17** at selected human dopamine and histamine receptor subtypes

Compound	Histamine/dopamine receptor subtypes binding affinities, K_i (nM)					
	hH_3[a]	hH_1[b]	hD_2[c]	hD_3[c]	hD_1[d]	hD_5[d]
Amitriptyline (**1**)	>1000	1.12 ± 0.21	196 ± 40	206	89 ± 31	170 ± 49
10	4.90 ± 2.6	559 ± 40	904 ± 185	326 ± 84	879 ± 69	>1000
11	3.50 ± 1.9	686 ± 144	>1000	243 ± 70	>1000	>1000
12	0.250	79 ± 9	101 ± 49	67 ± 9	305 ± 49	273
Maprotiline (**2**)	>1000	1.67	665 ± 197	504 ± 282	402 ± 62	429 ± 199
13	0.358	40 ± 16	146 ± 33	149 ± 27	203 ± 68	265 ± 28
Chlorpromazine (**3**)	>1000	4.25 ± 0.23	4.06 ± 0.16	6.90 ± 1.18	96 ± 7	172 ± 43
14	1.21 ± 0.04	205 ± 4	41 ± 17	50 ± 19	232 ± 74	153 ± 36
Chlorprothixene (**4**)	>1000	3.75 ± 0.16	2.96 ± 1.73	4.56 ± 0.77	18 ± 12	9 ± 2.68
15	1.54	295	52 ± 5	50 ± 13	248 ± 101	297 ± 6
Clozapine (**5**)	>1000	2.38 ± 0.40	83 ± 6	295 ± 159	89 ± 67	198 ± 41
16	3.27	190 ± 17	285	223	866 ± 470	921 ± 171
Fluphenazine (**6**)	>1000	40	1.44 ± 0.52	3.21 ± 1.81	179	21 ± 6
17	0.042	390 ± 57	47	15	203 ± 68	265 ± 28

[a] Ref. 26.
[b] Ref. 27.
[c] Ref. 28.
[d] Ref. 29.

Concerning H_1 receptor affinity which may be responsible for weight gain and sleep-inducing effects a reduction in affinity was observed for the hybrid structures by a factor of 10–600.

The effects at D_1-like receptor were heterogeneous, with the trend that a decrease in affinity was observed with the hybrids (with the exception of **13** (D_1 and D_5) and **14** (D_5)). The potential effects in therapy of these changes are unclear.

One amine derivative, hybrid **12**, with high H_3R receptor affinity and a good profile was selected for early in vivo screening for central H_3R antagonist potency. Potency was determined 90 min after oral application of the compound to male swiss mice by measurement of the increase in N^{τ}-methylhistamine level in brain.[31] Unfortunately, this compound seems to be inactive (ED_{50} > 10 mg/kg po). It is unclear if pharmacokinetic reasons like absorption, distribution or metabolism or pharmacodynamic reasons like cross reactivity are responsible for this lack of in vivo potency.

On this hybrid approach, we have developed novel hybrid compounds, bearing an antagonistic histamine H_3R pharmacophore element linked to an antipsychotic molecule. We were able to explore preliminary structure–affinity relationships (SAR) on histamine hH_1 and hH_3 as well as on human dopamine receptor subtypes with this small number of derivatives.

While amine hybrids **12–17** maintained or slightly shifted affinity profile for dopamine D_2-like receptor subtypes, there is a marked decrease in histamine H_1 receptor binding profile as compared to that of the antipsychotic reference drugs **1–6**.

In conclusion, in agreement with published results antagonist H_3R pharmacophore proved to be a highly robust element introducing H_3R affinity into other pharmacophoric elements. H_3R affinity in low nanomolar to subnanomolar concentration range could be obtained. D_2 and D_3 receptor affinity was maintained in most cases in nanomolar concentration range. H_1 receptor affinity could in most cases be reduced by a factor of 10–80 potentially improving the therapeutic window for weight gain side effects. The effect of H_3R antagonism on weight gain is actually a point of discussion.

As psychosis is a highly complex disease with the involvement of several neurotransmitter systems and adaptive processes the therapeutic effects of these new multiple target compounds cannot be foreseen. It would be interesting to see the effects in behavioral studies with this novel multiple hybrids giving comprehensive details of the enlarged pharmacological profile and the concept of H_3R antagonism in schizophrenia.

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Supplementary data

Supplemental material with analytical data of target compounds will be freely available, in the online version, at doi:10.1016/j.bmcl.2008.09.012.

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