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Progress in the proxifan class: heterocyclic congeners as novel potent and selective histamine H_3-receptor antagonists☆

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Abstract

Histamine H_3 receptors are critically involved in the pathophysiology of several disorders of the central nervous system (CNS). Among other families of H_3-receptor ligands, the proxifan class has recently been described to contain numerous potent histamine H_3-receptor antagonists, e.g. ciproxifan or imoproxifan. In the present study, we report on the design of novel heterocyclic proxifan analogues and their antagonist potencies at histamine H_3 receptors. The new compounds were tested for in vitro and in vivo H_3-receptor antagonist potencies in different species as well as for H_3-receptor selectivity vs. H_1 and H_2 receptors. In vitro, all compounds investigated proved to be potent H_3-receptor antagonists in the rat as well as in the guinea-pig. In addition, they showed good to high oral CNS potency in vivo in mice. Especially, oxadiazole derivatives **24**–**26** displayed nanomolar antagonist activity in vitro and high potency in vivo (ED_{50}=0.47–0.57 mg/kg). The results show that the additional heteroaromatic moieties might act as bioisosteres of the ketone or oxime moieties of ciproxifan or imoproxifan, respectively, and might cause divergent pharmacokinetic properties. Thus, these novel H_3-receptor antagonists are interesting leads for further development.

Keywords: Histamine; H_3 receptor; Proxifan; Antagonist; Ciproxifan; Imoproxifan

1. Introduction

Histamine is a neurotransmitter in the brain and until a short time ago, only three histamine receptors (H_1, H_2, and H_3) were known, all of them being G protein-coupled molecules (for a recent review, see Brown et al., 2001). Classic experimental pharmacology (Arrang et al., 1983) demonstrated the existence of histamine H_3 receptors almost two decades before they were cloned. Recently, the long awaited elucidation of the H_3-receptor gene structure in man and other species has been described (Lovenberg et al., 1999, 2000; Tardivel-Lacombe et al., 2000; Drutel et al., 2001). Following these findings another putative heptahelical histamine receptor (H_4) has been discovered (for example, see Oda et al., 2000). In the H_3-receptor field recent molecular studies revealed the existence of multiple mRNA isoforms from a single H_3 gene (Tardivel-Lacombe et al., 2000; Drutel et al., 2001) as well as differential isoform distribution in the brain (Drutel et al., 2001). This heterogeneity is suggested to also occur in humans (Wellendorf et al., 2000). Among this third type of histamine receptor, histamine H_3 receptors were initially characterized as presynaptic autoreceptors playing a key role in the regulation of synthesis (Arrang et al., 1987b) and release (Arrang et al., 1985) of histamine by means of a negative feedback mechanism. On non-histaminergic neurons H_3 heteroreceptors possess the ability to influence several monoaminergic and peptidergic neurotransmitter systems (Schlicker et al., 1994; Hill et al., 1997). The

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predominant localization in the brain (Arrang et al., 1987a), the mediation of important (patho)physiological processes (Stark et al., 1996c), e.g. arousal (Schwartz et al., 1991; Schwartz and Arrang, in press), and the interaction with a variety of different signal transduction mechanisms make the H_3 receptor considerably important. Therefore, the need for highly potent and selective H_3-receptor ligands applicable both for subtype characterization at the receptor level and for establishing new concepts in pharmacotherapy has greatly increased. For antagonists of histamine H_3 receptors, several therapeutic targets have been proposed. Since these ligands increase histaminergic neuron activity by inhibiting the feedback mechanism, their application as cognition enhancers seems to be most promising, e.g. in Alzheimer's disease (Morisset et al., 1996; Panula et al., 1998), attention-deficit hyperactivity disorder (Leurs et al., 1998), and schizophrenia (Schwartz and Arrang, in press).

Major progress in the pharmacology of H_3 receptors was obtained with the development of the first selective and orally active H_3-receptor antagonist thioperamide (Fig. 1; Arrang et al., 1987a). Another reference antagonist is clobenpropit which proved to be a very potent antagonist in vitro, but with only moderate potency in vivo (Fig. 1; van der Goot et al., 1992). Considering potential benefit in affinity, selectivity, pharmacokinetics, and/or toxicology we reported on the successful design of a new series of H_3-receptor antagonists: the proxifan class (Ganellin et al., 1996; Ligneau et al., 1998; Kathmann et al., 1998; Stark et al., 2000a,b; Sasse et al., 2000). Acetoproxifan (FUB 372, Fig. 1) provided the starting point with nanomolar in vitro potency and high antagonist activity in vivo. The compound contains an imidazole heterocycle connected by a three-carbon alkyl chain to a *para*-substituted phenyl ether moiety, features present in all ligands of this novel class. On the basis of the structure–activity relationships in the proxifan series we consequently developed the cyclopropyl ketone derivative ciproxifan (Fig. 1) with even higher in vitro and in vivo potency (Stark et al., 2000b). Continuing efforts to refine and enhance the H_3-receptor potency and selectivity resulted in the recent identification of the oxime derivative imoproxifan (Fig. 1), an antagonist of subnanomolar affinity, high selectivity, and extremely high in vivo potency (Sasse et al., 2000). In this context, another related class of compounds, the proxyfan series, should be mentioned to avoid a possible mixing up with the class cited before (Fig. 1). Compared to the proxifan class, proxyfans possess a similar chemical structure, but with a benzyl ether instead of a phenyl ether group. Proxyfan is the only neutral H_3-receptor antagonist, i.e. a drug that opposes both agonists and inverse agonists, described so far (Morisset et al., 2000). However, the behaviour of this compound is currently a matter of debate depending on both response measured and histamine H_3 receptor expression level (Morisset et al., 2000). Substituted proxyfans possess different pharmacological behaviour depending on the test system (cf. [^{125}I]iodoproxyfan (Ligneau et al., 1994; Stark et al., 1996a)).

Numerous structural modifications of the reference antagonists ciproxifan and imoproxifan have been described which clearly show strong preference for a substituent in the 4-position (Stark et al., 2000a,b; Sasse et al., 2000). Likewise, when the cyclopropyl moiety of ciproxifan was replaced by different aromatic ring systems no further improvement in antagonist activity could be detected (Sasse et al., 2001). However, one interesting compound emerging from this study is the 2-thienyl methanone derivative FUB 478 (Fig. 1) with good antagonist activity in vitro and high oral potency in vivo, one of the few examples of a compound with an additional aromatic heterocycle and promising pharmacological properties. Considering this heteroaromatic compound and the ketone or oxime structure of ciproxifan or imoproxifan, respectively, possible bioisosteric replacements will be investigated.

In this report, we describe in vitro and in vivo H_3-receptor antagonist properties of novel members of the proxifan family with various heteroaromatic moieties. In an effort to reveal principles and applicability of this heterocycle introduction we additionally investigate the new compounds for H_3-receptor selectivity. In order to further characterize these structures we compare all new pharmacological results with data obtained from FUB 478 and already established histamine H_3-receptor antagonists, i.e. thioperamide, clobenpropit, acetoproxifan, ciproxifan, and imoproxifan, respectively.

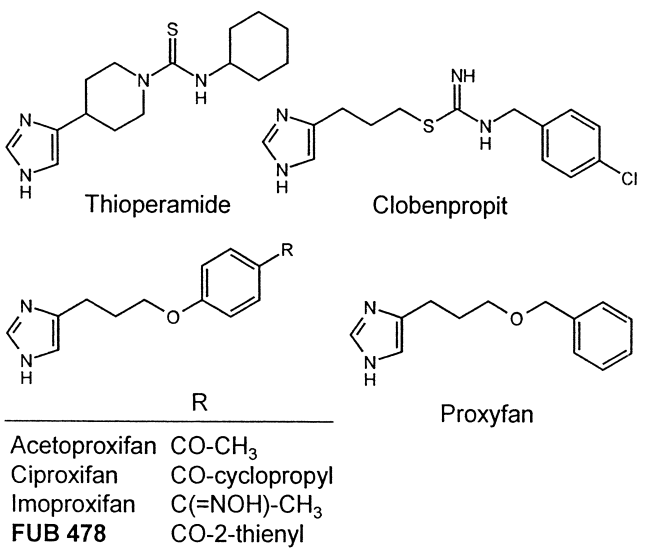


Fig. 1. Different histamine H_3-receptor antagonists.

2. Materials and methods

2.1. Chemistry

2.1.1. General procedures

Melting points (mp) were determined on an Electrother-

mal IA 9000 digital or a Büchi 512 apparatus. ^{1}H NMR spectra were recorded on a Bruker AC 300 (300 MHz) or DPX 400 Avance (400 MHz) spectrometer in Me_2SO-d_6 as standard solvent, unless stated otherwise. ^{13}C NMR recording was performed on a Bruker DPX 400 Avance (100 MHz). Chemical shifts are reported in ppm downfield from internal tetramethylsilane as reference. ^{1}H NMR signals are reported in order: multiplicity (s, singlet; d, doublet; t, triplet; m, multiplet; *, exchangeable by D_2O), number of protons, and approximate coupling constants in Hertz. EI mass spectra were obtained on a Finnigan MAT CH7A (70 eV, 170 °C) and FAB^+ spectra were recorded on a Finnigan MAT CH5DF (Xe, 80 eV, Me_2SO as solvent, glycerol as matrix). Elemental analyses (C, H, N) were measured for final compounds on Perkin-Elmer 240 B or Perkin-Elmer 240 C instruments and are within ±0.4% of the theoretical values. Column chromatography was carried out using silica gel 63–200 μm (Merck). Thin layer chromatography was performed on silica gel F_{254} plates (Merck). The following abbreviations are used: Benz, benzoxathiazolyl, EtOH, ethanol; Et_2O, diethyl ether; Im, imidazolyl; Mal; maleic acid; MeOH, methanol; Me_2SO, dimethyl sulfoxide; Ox, oxadiazolyl; Ph, phenyl; Tet, tetrazolyl; Th, thiazolyl; THF, tetrahydrofuran.

2.1.2. Synthesis

With the exception of **20** and **22**, all novel heterocyclic compounds were synthesized by ring-closing reactions, each derivative via a different synthetic pathway (Fig. 2). Key intermediates were the *para*-substituted phenyl methyl ethers **8**–**12**.

The most common and most versatile procedure for the formation of 1,3-thiazoles is the cyclocondensation of α-haloketones with appropriate thioamide derivatives (Vernim, 1979). In this study, the reaction of commercially available α-bromoketone **4** with thioacetamide under basic conditions resulted in the 4-substituted heterocyclic derivative **8**. The amidoxime **5** provided the starting point for the synthesis of the 3,5-disubstituted 1,2,4-oxadiazole **9**. It was generated by reaction of hydroxylamine, liberated from its hydrochloride using potassium carbonate, with *p*-anisonitrile in refluxing ethanol. Dehydration, cyclization, and aromatization by subsequent treatment with acetic acid methyl ester in the presence of sodium hydride in tetrahydrofuran afforded the corresponding 5-methyl-1,2,4-oxadiazole compound **9** (Swain et al., 1991). The 3-methyl-1,2,4-oxadiazole derivative **10** was prepared adapting the procedure described by Lin et al. (1979). Reaction of the carboxamide **3** with *N*,*N*-dimethylacetamide dimethyl acetal and cyclization of the intermediate acylamidine with hydroxylamine provided **10** in good yield. According to the procedure described by Ainsworth (1955) synthesis of the 5-methyl-1,3,4-oxadiazole derivative **11** was achieved by treatment of the ester **2** with hydrazine resulting in the intermediary hydrazide **6** followed by cyclization with triethyl orthoacetate. In a direct one-pot reaction primary amide **3** was converted with triazidochlorosilane into the 5-substituted tetrazole **7** (El-Ahl et al., 1997). Adapting the protocol of Begtrup and Larsen (1990), **7** was methylated

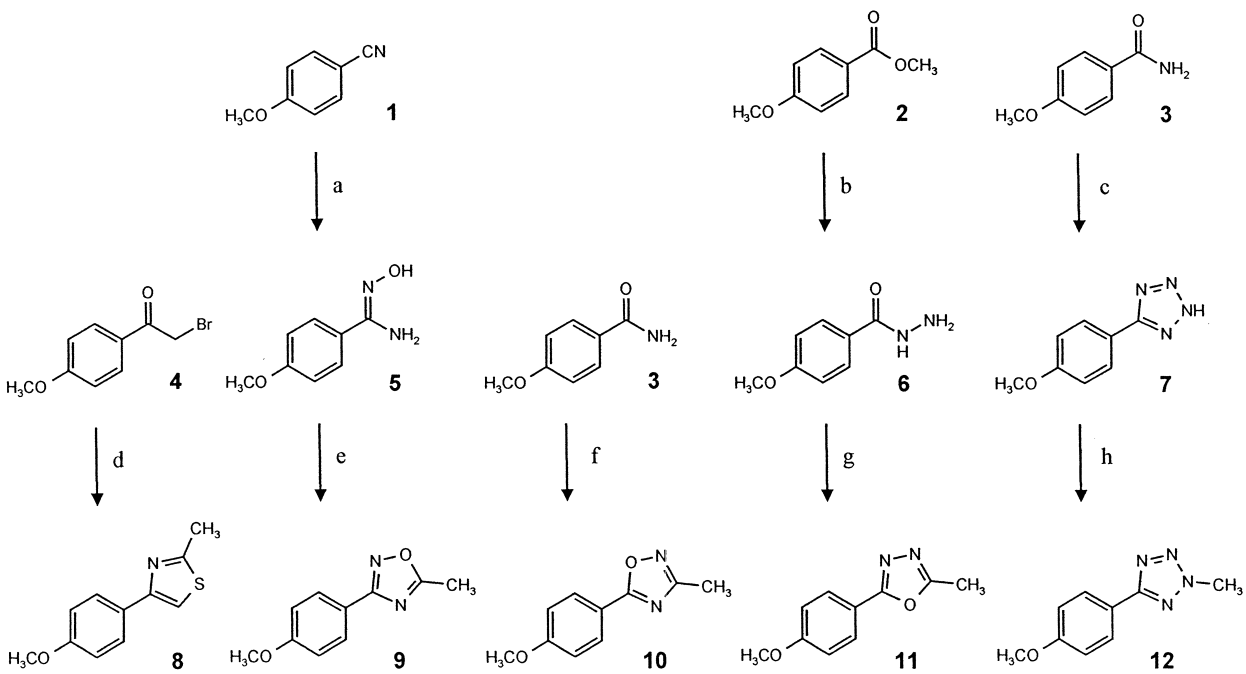


Fig. 2. Synthetic procedures for key intermediates **8**–**12**. [a] $H_2NOH{\cdot}HCl$, K_2CO_3, EtOH, reflux, 18 h; [b] $H_2NNH_2{\cdot}H_2O$, 120 °C, 3 h; [c] $SiCl_4$, NaN_3, H_3CCN, reflux, 3 h; [d] H_3CCSNH_2, K_2CO_3, *N*,*N*-dimethylformamide, 100–120 °C, 2 h; [e] (*i*) molecular sieves (4 Å), THF, 30 min; (*ii*) NaH, $H_3CCOOCH_3$, THF, reflux, 6 h; [f] (*i*) *N*,*N*-dimethylacetamide dimethyl acetal, 120 °C, 2.5 h; (*ii*) $H_2NOH{\cdot}HCl$, 1 N NaOH, dioxane, AcOH, 90 °C, 3 h; [g] (*i*) triethyl orthoacetate, 120 °C, 3 h; (*ii*) 140 °C, 4 h; [h] H_3CI, KOH, MeOH, reflux, 1 h.

with methyl iodide in methanolic potassium hydroxide. The resulting mixture of the isomeric 1-methyl- and 2-methyltetrazole derivatives was separated by column chromatography and showed a product ratio of 4:1 in favour of **12**. The ^{1}H and ^{13}C NMR data for **12** were in full agreement with literature data (Fraser and Haque, 1968; Begtrup, 1973).

In one-pot reactions with tosylmethyl isocyanide (TosMIC) carbonyl compounds can efficiently be converted into 5-substituted oxazoles (van Leusen et al., 1972). Here, the oxazole compound **21** was obtained in 64% yield by reaction of equimolar quantities of TosMIC and the benzaldehyde derivative FUB 402 (Stark et al., 2000b) with potassium carbonate in refluxing methanol (Fig. 3).

Proceeding from the different heterocyclic derivatives **8**–**12** methyl ether cleavage with BBr_3 in dichloromethane provided the corresponding phenols **13**–**17** in high yields. All final compounds except **26** were synthesized by phenol etherification with trityl-protected 3-(1*H*-imidazol-4-yl)propanol (**18**) under Mitsunobu conditions (Mitsunobu, 1981) and acidic hydrolysis of the protecting group (Stark et al., 1996a,b). When **26** was synthesized the lower stability of the 1,3,4-oxadiazole system did not allow acidolytic or hydrogenolytic deprotection of the trityl group. Hence, **16** was etherified by analogy to Krause et al. (1998) with Boc-protected 3-(1*H*-imidazol-4-yl)propanol (**19**) followed by hydrazinolysis in methanol under mild conditions. Benzoxathiazole **20** and imidazole **22** were readily prepared from commercially available 6-hydroxy-1,3-benzoxathiazol-2-one and 4-(1*H*-imidazol-1-yl)phenol as mentioned above.

*2.1.2.1. Methyl 4-(2-methyl-1,3-thiazol-4-yl)phenyl ether (**8**)*

4-Methoxyphenacyl bromide (**4**, 1.49 g, 6.5 mmol), thioacetamide (0.48 g, 6.5 mmol), and K_2CO_3 (1.20 g, 8.7 mmol) were dissolved in dry *N*,*N*-dimethylformamide (30 ml). The mixture was stirred for 2 h at 100–120 °C, cooled, and the solvent was removed under reduced pressure. The solid residue was recrystallized from EtOH: Yield 79%; mp 59–60 °C (Das and Rout, 1957: 63 °C); ^{1}H NMR δ 2.70 (s, 3H, Th–CH_3), 3.79 (s, 3H, H_3CO), 6.99 (d, $J = 8.7$ Hz, 2H, Ph-3,5H), 7.74 (s, 1H, Th-5H), 7.87 (d, $J = 8.7$ Hz, 2H, Ph-2,6H); EI–MS *m*/*z* (%) 205 (M^+, 100).

*2.1.2.2. 4-Methoxyphenylcarboxamidoxime (**5**)*

p-Anisonitrile (**1**, 6.66 g, 50 mmol), hydroxylamine hydrochloride (6.95 g, 100 mmol), and K_2CO_3 (10.34 g, 75 mmol) in dry EtOH (80 ml) were heated under reflux for 18 h. The mixture was cooled, filtered, and concentrated under reduced pressure. The crystalline residue was suspended in MeOH, and the final product was isolated by filtration: Yield 22%; mp 122–124 °C (Eloy and Lenaers, 1962: 122–123 °C); ^{1}H NMR δ 3.77 (s, 3H, H_3CO), 5.71

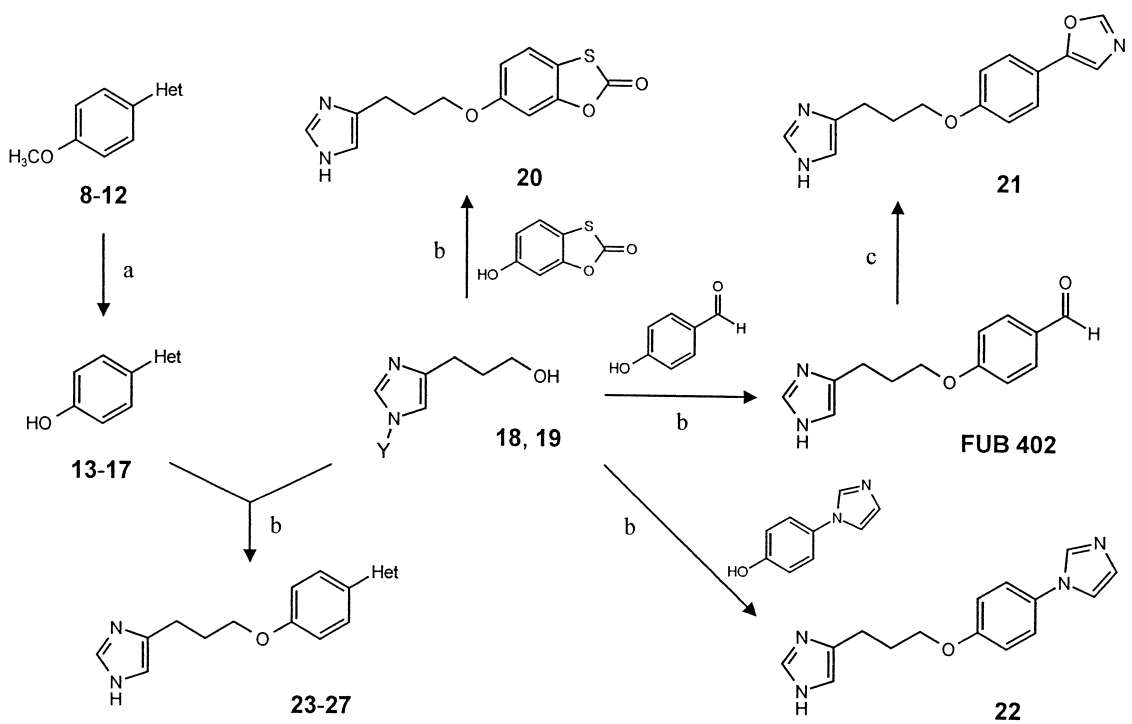


Fig. 3. Synthesis of heterocyclic derivatives **20**–**27**. Het, 2-methyl-1,3-thiazol-4-yl (**8**, **13**, **23**), 5-methyl-1,2,4-oxadiazol-3-yl (**9**, **14**, **24**), 3-methyl-1,2,4-oxadiazol-5-yl (**10**, **15**, **25**), 5-methyl-1,3,4-oxadiazol-2-yl (**11**, **16**, **26**), or 2*H*-2-methyl-1,2,3,4-tetrazol-5-yl (**12**, **17**, **27**); Y, *tert*-butoxycarbonyl (Boc) (**19**, synthesis of **26**) or triphenylmethyl (**18**, all other compounds). [a] BBr_3, CH_2Cl_2, 72 h, −80 °C; [b] (*i*) Ph_3P, DEAD, THF, r.t., 12–72 h; (*ii*) $H_2NNH_2 \cdot H_2O$, MeOH, r.t., 30 min (synthesis of **26**) or 2 N HCl, reflux, 2 h (all other compounds); [c] TosMIC, K_2CO_3, MeOH, reflux, 18 h.

(s*, 2H, NH_2), 6.92 (d, $J=8.8$ Hz, 2H, Ph-2,6H), 7.59–7.62 (m, 2H, Ph-3,5H), 9.45 (s*, 1H, OH); EI–MS m/z (%) 166 (M^+, 100).

*2.1.2.3. Methyl 4-(5-methyl-1,2,4-oxadiazol-3-yl)phenyl ether (**9**)*

Powdery molecular sieves (4 Å, 4 g) were added to a solution of **5** (1.66 g, 10 mmol) in dry THF (60 ml). After stirring for 30 min NaH (suspended in mineral oil, $\omega=$ 60%, 0.22 g, 5.5 mmol) was added, and the mixture was heated for 45 min at 60 °C. After cooling acetic acid methyl ester (1.48 g, 20 mmol) dissolved in dry THF was added dropwise. Subsequently, the mixture was refluxed for 6 h under anhydrous conditions. Cooling, filtration, and removal of the solvent under reduced pressure resulted in a solid residue which was recrystallized from EtOH: Yield 83%; mp 61–62 °C (Bianchi et al., 1974: 58–60 °C); ^{1}H NMR δ 2.64 (s, 3H, Ox–CH_3), 3.83 (s, 3H, H_3CO), 7.11 (d, $J=8.8$ Hz, 2H, Ph-3,5H), 7.92–7.95 (m, 2H, Ph-2,6H); FAB$^+$–MS m/z (%) 191 ($M^+ + H^+$, 100).

*2.1.2.4. Methyl 4-(3-methyl-1,2,4-oxadiazol-5-yl)phenyl ether (**10**)*

A solution of 4-methoxybenzamide (**3**, 1.01 g, 6.7 mmol) in *N,N*-dimethylacetamide dimethyl acetal (15 ml) was stirred for 2.5 h at 120 °C under N_2. The solvent was removed under reduced pressure and hydroxylamine hydrochloride (0.65 g, 9.4 mmol) dissolved in an aqueous NaOH solution (9.14 ml, $c=1$ mol/l) was added. After addition of dioxane (10 ml) and acetic acid (12.5 ml) the mixture was stirred for 1.5 h at room temperature. Subsequently, the mixture was heated 3 h at 90 °C, cooled, and a saturated solution of K_2CO_3 in H_2O was added. Then, the mixture was concentrated under vacuum and the residual aqueous layer was extracted with CH_2Cl_2. The organic extracts were combined, dried, and concentrated under reduced pressure. The oily product was purified by column chromatography (eluent: CH_2Cl_2) and crystallized at 4 °C: Yield 66%; mp 59–61 °C; ^{1}H NMR δ 2.39 (s, 3H, Ox–CH_3), 3.87 (s, 3H, H_3CO), 7.15–7.18 (m, 2H, Ph-3,5H), 8.03 (d, $J=8.8$ Hz, 2H, Ph-2,6H); EI–MS m/z (%) 190 (M^+, 92).

*2.1.2.5. 4-Methoxybenzoic acid hydrazide (**6**)*

A mixture of 4-methoxybenzoic acid methyl ester (**2**, 4.99 g, 30 mmol) and hydrazine monohydrate (3.00 g, 60 mmol) was heated at 120 °C for 3 h. Cooling and dilution with H_2O (10 ml) resulted in a precipitate which was filtered, washed with H_2O, and dried in vacuo: Yield 89%; mp 128–130 °C (Horner and Fernekess, 1961: 136 °C); ^{1}H NMR δ 3.80 (s, 3H, H_3CO), 4.42 (s*, 2H, NH_2), 6.96–7.01 (m, 2H, Ph-3,5H), 7.79–7.84 (m, 2H, Ph-2,6H), 9.63 (s*, 1H, NH); EI–MS m/z (%) 166 (M^+, 11).

*2.1.2.6. Methyl 4-(5-methyl-1,3,4-oxadiazol-2-yl)phenyl ether (**11**)*

Triethyl orthoacetate (10 ml) and **6** (1.66 g, 10 mmol) were heated at 120 °C for 3 h. Excess triethyl orthoacetate was evaporated, and the residue heated for further 2 h at 140 °C. The reaction was diluted with H_2O (10 ml), saturated with K_2CO_3, and extracted with Et_2O. Evaporation of the dried extracts afforded **11** as an oil: Yield 66%; mp 88 °C (Huisgen et al., 1960: 91–92 °C); ^{1}H NMR δ 2.56 (s, 3H, Ox–CH_3), 3.85 (s, 3H, H_3CO), 7.13 (d, $J=8.8$ Hz, 2H, Ph-3,5H), 7.91 (d, $J=8.7$ Hz, 2H, Ph-2,6H); EI–MS m/z (%) 190 (M^+, 93).

*2.1.2.7. Methyl 4-(2H-tetrazol-5-yl)phenyl ether (**7**)*

A suspension of silicon(IV) chloride (9.35 g, 55 mmol) and sodium azide (7.15 g, 110 mmol) in dry acetonitrile (20 ml) was stirred for 1 h at ambient temperature. 4-Methoxybenzamide (**3**, 4.00 g, 27 mmol) was added dropwise. After stirring under reflux for 3 h the reaction mixture was poured into an ice-cooled solution of K_2CO_3 in H_2O, filtered, and acidified with aqueous HCl. The precipitated product was filtered, washed with H_2O, and dried in vacuo: Yield 80%; mp 237–238 °C (Herbst and Wilson, 1957: 238–239 °C); ^{1}H NMR δ 3.85 (s, 3H, H_3CO), 7.15–7.18 (m, 2H, Ph-3,5H), 7.97–8.00 (m, 2H, Ph-2,6H); EI–MS m/z (%) 176 (M^+, 43).

*2.1.2.8. Methyl 4-(2-methyl-2H-tetrazol-5-yl)phenyl ether (**12**)*

A solution of **7** (1.86 g, 10.6 mmol) and KOH (2.81 g, 50 mmol) in dry MeOH (30 ml) was mixed at −25 °C with iodomethane (4.56 g, 32.1 mmol) dissolved in dry MeOH (5 ml). The temperature was raised to 20 °C during 1 h and the mixture refluxed for an additional hour. The solvent was removed and the crude product purified by column chromatography (eluent: CH_2Cl_2): Yield 60%; mp 56–57 °C (Butler et al., 1984: 85–86 °C); ^{1}H NMR δ 3.83 (s, 3H, H_3CO), 4.40 (s, 3H, NCH_3), 7.10–7.13 (m, 2H, Ph-3,5H), 7.97–8.00 (m, 2H, Ph-2,6H); ^{13}C NMR ($CDCl_3$) δ 39.7 (NCH_3), 55.8 (H_3CO), 114.7 (Ph-2,6C), 120.4 (Ph-4C), 128.7 (Ph-3,5C), 161.7 (Ph-1C), 165.6 (Tet-5C); EI–MS m/z (%) 190 (M^+, 21).

2.1.2.9. General procedure for ether cleavage

A solution of the corresponding ether (4 mmol) in dry CH_2Cl_2 (20 ml) was cooled to −80 °C under N_2. BBr_3 (4 mmol, $c=1.0$ mol/l, solution in CH_2Cl_2), was added dropwise with the temperature not exceeding −60 °C. The reaction mixture was then allowed to warm to room temperature and stirred for additional 72 h. Subsequently, the mixture was cooled to −80 °C and MeOH (25 ml) was added dropwise. The organic layer was removed from the mixture under reduced pressure. After addition of a saturated K_2CO_3 solution in H_2O to the aqueous layer the crude product precipitated. It was isolated by filtration and recrystallized from EtOH.

2.1.2.9.1. 4-(2-*Methyl*-1,3-*thiazol*-4-*yl*)*phenol* (**13**) From **8**; yield 70%; mp 151 °C; ^{1}H NMR δ 2.69 (s, 3H, Th–CH_3), 6.81 (d, J = 8.6 Hz, 2H, Ph-3,5H), 7.64 (s, 1H, Th-5H), 7.75 (d, J = 8.6 Hz, 2H, Ph-2,6H), 9.55 (s*, 1H, OH); EI–MS m/z (%) 191 (M^+, 100).

2.1.2.9.2. 4-(5-*Methyl*-1,2,4-*oxadiazol*-3-*yl*)*phenol* (**14**) From **9**; yield 84%; mp 189–191 °C (Behr, 1962: 185 °C); ^{1}H NMR δ 2.62 (s, 3H, CH_3), 6.89–6.92 (m, 2H, Ph-3,5H), 7.81–7.83 (m, 2H, Ph-2,6H); EI–MS m/z (%) 176 (M^+, 100).

2.1.2.9.3. 4-(3-*Methyl*-1,2,4-*oxadiazol*-5-*yl*)*phenol* (**15**) From **10**; yield 83%; mp 186 °C; ^{1}H NMR δ 2.37 (s, 3H, CH_3), 6.94–6.98 (m, 2H, Ph-3,5H), 7.92 (d, J = 8.7 Hz, 2H, Ph-2,6H), 10.49 (s*, 1H, OH); EI–MS m/z (%) 176 (M^+, 69).

2.1.2.9.4. 4-(5-*Methyl*-1,3,4-*oxadiazol*-2-*yl*)*phenol* (**16**) From **11**; yield 55%; mp 236 °C (El Masri et al., 1958: 239 °C); ^{1}H NMR δ 2.56 (s, 3H, CH_3), 6.98 (d, J = 8.6 Hz, 2H, Ph-3,5H), 7.85 (d, J = 8.6 Hz, 2H, Ph-2,6H), 10.26 (s*, 1H, OH); EI–MS m/z (%) 176 (M^+, 100).

2.1.2.9.5. 4-(2-*Methyl*-2*H*-*tetrazol*-5-*yl*)*phenol* (**17**) From **12**; yield 95%; mp 210–212 °C; ^{1}H NMR δ 4.38 (s, 3H, NCH_3), 6.92 (d, J = 8.6 Hz, 2H, Ph-3,5H), 7.87 (d, J = 8.5 Hz, 2H, Ph-2,6H), 9.96 (s*, 1H, OH); EI–MS m/z (%) 176 (M^+, 22).

*2.1.2.10. General procedure for Mitsunobu-type etherification (compounds **20**–**25, 27**)*

3-(1-Triphenylmethyl-1*H*-imidazol-4-yl)propanol (**18**, 1.84 g, 5 mmol) (Stark et al., 1996b), triphenylphosphine (1.57 g, 6 mmol), and the corresponding phenol (6 mmol) were dissolved in freshly distilled THF (15 ml) under Ar atmosphere and ice-cooling. Then, diethyl azodicarboxylate (0.95 ml, 6 mmol) was slowly added followed by additional stirring for 12–72 h at room temperature. After removal of the solvent in vacuo the crude product was purified by column chromatography (eluent: EtOAc). The oily residue was dissolved in THF (40 ml), aqueous HCl (c = 2 mol/l, 20 ml) was added and the mixture was refluxed for 2 h. After removal of the organic solvent, filtration, and extraction with Et_2O the aqueous phase was basified (K_2CO_3) and extracted with Et_2O. The latter organic extracts were combined, washed (H_2O), dried (Na_2SO_4), and concentrated under reduced pressure. The residue was crystallized and recrystallized as free base, hydrogen oxalate or hydrogen maleate from EtOH/Et_2O.

*2.1.2.11. Modifications of Mitsunobu-type etherification (compound **26**)*

4-(3-Hydroxypropyl)-1*H*-imidazol-1-yl carboxylic acid *tert*-butyl ester (**19**, 1.13 g, 5 mmol) (Krause et al., 1998) was used. Column chromatography was performed with a different eluent (CH_2Cl_2/MeOH, NH_3-sat.; 95:5, v/v). The Boc-protected product was deprotected by stirring in methanolic hydrazine monohydrate solution (ω = 10%, 25 ml) at ambient temperature for 30 min.

*2.1.2.12. 6-[3-(1H-Imidazol-4-yl)propoxy]-1,3-benzoxathiazol-2-one hydrogen maleate (**20**)*

From **18** and 6-hydroxy-1,3-benzoxathiazol-2-one; ^{1}H NMR δ 2.07 (m, 2H, Im–CH_2CH_2), 2.79 (t, J = 7.5 Hz, 2H, Im–CH_2), 4.04 (t, J = 6.2 Hz, 2H, CH_2O), 6.03 (s, 2H, Mal), 6.93 (d, J = 8.8 Hz, 1H, Benz-5H), 7.16 (s, 1H, Im-5H), 7.63 (d, J = 8.8 Hz, 2H, Benz-4H), 8.87 (s, 1H, Im-2H); EI–MS m/z (%) 276 (M^+, 9). Anal. ($C_{13}H_{12}N_2O_3S \cdot C_4H_4O_4 \cdot 1.75H_2O$) C, H, N.

*2.1.2.13. 3-(1H-Imidazol-4-yl)propyl 4-(1,3-oxazol-5-yl)phenyl ether hydrogen maleate (**21**)*

p-Toluenesulfonylmethyl isocyanide (0.27 g, 1.4 mmol) and FUB 402 (0.32 g, 1.4 mmol), prepared according to Stark et al. (2000b), were dissolved in dry MeOH (30 ml). During 30 min K_2CO_3 (0.19 g, 1.4 mmol) was added slowly. After refluxing for 18 h the solvent was removed. The oily residue was purified by column chromatography (eluent: CH_2Cl_2/MeOH, NH_3-sat.; 95:5, v/v) and crystallized with maleic acid from EtOH/Et_2O. ^{1}H NMR δ 2.04–2.09 (m, 2H, Im–CH_2CH_2), 2.80 (t, J = 7.4 Hz, 2H, Im–CH_2), 4.05 (t, J = 6.0 Hz, 2H, CH_2O), 6.03 (s, 2H, Mal), 7.02 (d, J = 8.6 Hz, 2H, Ph-3,5H), 7.40 (s, 1H, Im-5H), 7.52 (s, 1H, Ox-4H), 7.64 (d, J = 8.6 Hz, 2H, Ph-2,6H), 8.36 (s, 1H, Ox-2H), 8.83 (s, 1H, Im-2H); EI–MS m/z (%) 269 (M^+, 9). Anal. ($C_{15}H_{15}N_3O_2 \cdot C_4H_4O_4 \cdot 0.25H_2O$) C, H, N.

*2.1.2.14. 4-(1H-Imidazol-1-yl)phenyl 3-(1H-imidazol-4-yl)propyl ether dihydrogen maleate (**22**)*

From **18** and 4-(1*H*-imidazol-1-yl)phenol; ^{1}H NMR δ 2.08–2.13 (m, 2H, Im–CH_2CH_2), 2.83 (t, J = 7.6 Hz, 2H, Im–CH_2), 4.08 (t, J = 6.1 Hz, 2H, CH_2O), 6.13 (s, 4H, Mal), 7.11 (d, J = 9.0 Hz, 2H, Ph-3,5H), 7.47 (s, 1H, Im-5H), 7.49 (m, 1H, Im-4′H), 7.63 (d, J = 9.0 Hz, 2H, Ph-2,6H), 7.90 (m, 1H, Im-5′H), 8.81 (s, 1H, Im-2H), 9.00 (s, 1H, Im-2′H); FAB$^+$–MS m/z (%) 269 ($M^+ + H^+$, 100). Anal. ($C_{15}H_{16}N_4O \cdot 2C_4H_4O_4 \cdot 0.75H_2O$) C, H, N.

*2.1.2.15. 3-(1H-Imidazol-4-yl)propyl 4-(2-methyl-1,3-thiazol-4-yl)phenyl ether hydrogen maleate (**23**)*

From **18** and **13**; ^{1}H NMR δ 2.05–2.12 (m, 2H, Im–CH_2CH_2), 2.70 (s, 3H, CH_3), 2.84 (t, J = 8.1 Hz, 2H, Im–CH_2), 4.05 (t, J = 6.2 Hz, 2H, CH_2O), 6.05 (s, 2H, Mal), 6.97 (d, J = 8.7 Hz, 2H, Ph-3,5H), 7.43 (s, 1H, Im-5H), 7.74 (s, 1H, Th-5H), 7.85 (d, J = 8.7 Hz, 2H, Ph-2,6H), 8.87 (s, 1H, Im-2H); EI–MS m/z (%) 299 (M^+, 3). Anal. ($C_{16}H_{17}N_3OS \cdot C_4H_4O_4$) C, H, N.

*2.1.2.16. 3-(1H-Imidazol-4-yl)propyl 4-(5-methyl-1,2,4-oxadiazol-3-yl)phenyl ether (**24**)*

From **18** and **14**; ^{1}H NMR ($CDCl_3$) δ 2.12–2.21 (m, 2H, Im–CH_2CH_2), 2.63 (s, 3H, CH_3), 2.83 (t, J = 7.4 Hz, 2H, Im–CH_2), 4.05 (t, J = 6.2 Hz, 2H, CH_2O), 6.81 (s, 1H, Im-5H), 6.96 (d, J = 8.7 Hz, 2H, Ph-3,5H), 7.58 (s, 1H,

Im-2H), 7.97 (d, $J=8.7$ Hz, 2H, Ph-2,6H); EI–MS m/z (%) 284 (M^+, 6). Anal. ($C_{15}H_{16}N_4O_2 \cdot H_2O$) C, H, N.

*2.1.2.17. 3-(1H-Imidazol-4-yl)propyl 4-(3-methyl-1,2,4-oxadiazol-5-yl)phenyl ether hydrogen maleate (**25**)*

From **18** and **15**; ^{1}H NMR δ 2.08–2.15 (m, 2H, Im–CH_2CH_2), 2.39 (s, 3H, CH_3), 2.82 (t, $J=7.5$ Hz, 2H, Im–CH_2), 4.14 (t, $J=6.2$ Hz, 2H, CH_2O), 6.05 (s, 2H, Mal), 7.14 (d, $J=8.9$ Hz, 2H, Ph-3,5H), 7.43 (s, 1H, Im-5H), 8.03 (d, $J=8.8$ Hz, 2H, Ph-2,6H), 8.88 (s, 1H, Im-2H); EI–MS m/z (%) 284 (M^+, 2). Anal. ($C_{15}H_{16}N_4O_2 \cdot C_4H_4O_4 \cdot 0.25H_2O$) C, H, N.

*2.1.2.18. 3-(1H-imidazol-4-yl)propyl 4-(5-methyl-1,3,4-oxadiazol-2-yl)phenyl ether hydrogen maleate (**26**)*

From **19** and **16**; ^{1}H NMR δ 2.08–2.11 (m, 2H, Im–CH_2CH_2), 2.55 (s, 3H, CH_3), 2.81 (t, $J=7.4$ Hz, 2H, Im–CH_2), 4.10 (t, $J=6.1$ Hz, 2H, CH_2O), 6.03 (s, 2H, Mal), 7.10 (d, $J=8.7$ Hz, 2H, Ph-3,5H), 7.42 (s, 1H, Im-5H), 7.89 (d, $J=8.7$ Hz, 2H, Ph-2,6H), 8.85 (s, 1H, Im-2H); EI–MS m/z (%) 284 (M^+, 1). Anal. ($C_{15}H_{16}N_4O_2 \cdot C_4H_4O_4 \cdot 0.25H_2O$) C, H, N.

*2.1.2.19. 3-(1H-Imidazol-4-yl)propyl 4-(2-methyl-2H-tetrazol-5-yl)phenyl ether hydrogen oxalate (**27**)*

From **18** and **17**; ^{1}H NMR δ 2.08–2.11 (m, 2H, Im–CH_2CH_2), 2.79 (t, $J=7.5$ Hz, 2H, Im–CH_2), 4.09 (t, $J=6.2$ Hz, 2H, CH_2O), 4.40 (s, 3H, NCH_3), 7.10 (d, $J=8.8$ Hz, 2H, Ph-3,5H), 7.27 (s, 1H, Im-5H), 7.98 (d, $J=8.8$ Hz, 2H, Ph-2,6H), 8.53 (s, 1H, Im-2H); EI–MS m/z (%) 284 (M^+, 2). Anal. ($C_{14}H_{16}N_6O \cdot C_2H_2O_4 \cdot 0.5H_2O$) C, H, N.

2.2. Pharmacology

2.2.1. General methods

2.2.1.1. Histamine H_3-receptor antagonist potency in vitro on synaptosomes of rat cerebral cortex

Antagonist potency of the novel compounds was investigated using an in vitro protocol where K^+-evoked depolarization induces [^{3}H]histamine release from rat synaptosomes (Garbarg et al., 1992). The synaptosomal fraction was prepared according to Whittaker (1966), preincubated with L-[^{3}H]histidine (0.4 μM) at 37 °C for 30 min in a modified Krebs–Ringer solution, washed extensively, and transferred into a fresh Krebs–Ringer buffer containing 2 mM K^+. Compounds and 1 μM histamine were added 5 min before the depolarization stimulus (30 mM K^+ final concentration). Incubation was terminated by rapid centrifugation. [^{3}H]Histamine levels were determined by liquid scintillation spectrometry (Garbarg et al., 1992). K_i values were calculated according to Cheng and Prusoff (1973). Data are presented as mean±standard error of the mean (S.E.M.) of experiments performed at least in triplicate.

2.2.1.2. Histamine H_3-receptor antagonist potency in vivo in the mouse

Compounds were examined for their antagonist potencies in vivo after oral administration to male Swiss mice (for details, see Garbarg et al., 1992). Brain histaminergic neuronal activity was assessed by determination of the main metabolite of histamine, N^τ-methylhistamine. Mice were fasted for 24 h before p.o. treatment. Animals were decapitated 90 min after treatment. The brain was isolated and homogenized in 10 vol of ice-cold perchloric acid (0.4 M). The N^τ-methylhistamine level was measured by radioimmunoassay (Garbarg et al., 1989). Treatment with 3 mg/kg ciproxifan gave the maximal increase in N^τ-methylhistamine level (Ligneau et al., 1998) which was related to the level reached with the administered drug. Each experiment was performed at least in triplicate. The ED_{50} value is expressed as mean±S.E.M.

2.2.1.3. Histamine H_3-receptor antagonist potency on guinea-pig ileum

For selected compounds H_3-receptor potency in vitro was measured by inhibition of concentration-dependent relaxation of field-stimulated isolated guinea-pig ileum segments (longitudinal muscle with adhering plexus myentericus) induced by (*R*)-α-methylhistamine according to Ligneau et al. (1994). Four to seven experiments were performed for each compound. Longitudinal muscle strips were prepared from the small intestine, 20–50 cm proximal to the ileocecal valve. The muscle strips were mounted between two platinum electrodes (4 mm apart) in 20 ml of Krebs buffer, containing 1 μM of mepyramine, connected to an isometric transducer, continuously gassed with oxygen containing 5% CO_2 at 37 °C. After equilibration of the muscle segments for 1 h accompanied by washing every 10 min, they were stimulated continuously with rectangular pulses of 15 V and 0.5 ms at a frequency of 0.1 Hz. After 30 min of stimulation, a cumulative concentration–response curve to (*R*)-α-methylhistamine was recorded. Subsequently the preparations were washed three times every 10 min without stimulation. The antagonist was incubated 20–30 min before redetermination of the concentration–response curve of (*R*)-α-methylhistamine (Schlicker et al., 1994). The new antagonists were tested at concentrations that did not block ileal muscarine M_3 receptors (data not shown). In the absence of H_3-receptor antagonist, two successive concentration–relaxation curves for (*R*)-α-methylhistamine were superimposable (data not shown).

2.2.1.4. In vitro screening at other histamine receptors

Selected compounds were screened for histamine H_2-receptor activity on the isolated spontaneously beating guinea-pig right atrium as well as for H_1 receptor activity

on the isolated guinea-pig ileum by standard methods described by Hirschfeld et al. (1992). With the exception of **20** (single experiment) H_1-receptor potency was investigated at least in triplicate for each compound, while for H_2 receptors each experiment was performed at least in duplicate.

3. Results and discussion

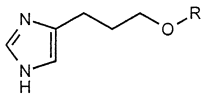
The novel heterocyclic compounds **20**–**26** were initially evaluated for their abilities to effect the release of [^{3}H]histamine on synaptosomes of rat cerebral cortex (Table 1), a functional assay for in vitro determination of histamine H_3-receptor potency (Garbarg et al., 1992). Compounds **20**–**27** were further tested in an in vivo model for their effect at histamine H_3 receptors on mice brain after oral administration measuring the increase in N^{τ}-methylhistamine level, the main histamine metabolite in the brain (Garbarg et al., 1992). The results of the pharmacological screening are summarized in Table 1. From these data, it is obvious that all investigated compounds clearly display antagonist properties at H_3 receptors in vitro with affinities in the low to high nanomolar concentration range (K_i = 4.7–137 nM). Antagonist potency can also be detected in vivo for all derivatives. Depending on the structure of the heterocycle, some of the novel compounds showed high antagonist potency in vivo, while others were less potent.

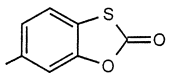
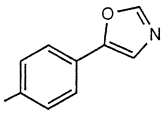
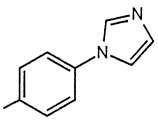
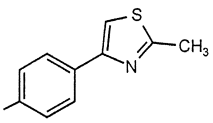
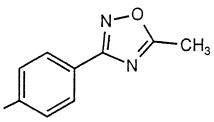
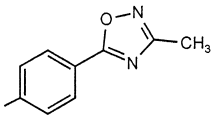
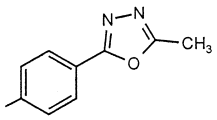
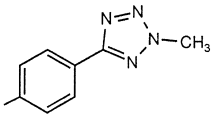
The starting point for this study was the heterocyclic derivative FUB 478, an antagonist of high in vitro and in vivo potency (Sasse et al., 2001). Since this heteroaromatic compound and the structurally related compounds ciproxifan and imoproxifan display high H_3-receptor affinity, we tried to bioisosterically replace the ketone or oxime moiety by different heteroaromatic structures. For this purpose, we chose different heterocyclic moieties containing the corresponding CO or NO element of the prototypes. To further elaborate the structure-activity relationship, we extended our investigation to derivatives with higher structural diversity but still with substantial resemblance to ciproxifan or imoproxifan. Since the derivatives selected differ much in their chemical and/or physical properties, they are ideal candidates for evaluating the scope and limitations of bioisosteric replacement within this structural class. Bicyclic derivative **20**, containing a 1,3-benzoxathiazol-2-one moiety, clearly showed antagonist activity in vitro and in vivo, but at a lower level when compared to the parent compound acetoproxifan (Stark et al., 2000b). This result strengthens the importance of a *para*-substituted phenyl ether functionality as seen in many other related potent H_3-receptor antagonists (e.g. Stark et al., 2000b; Sasse et al., 2000). This led us to change the line and to move on to compounds again possessing the *para*-substituted phenyl spacer like FUB 478, but with various aromatic heterocycles instead of the thienyl methanone group. The first two compounds are the azole derivatives **21** and **22**. Not surprisingly, their physicochemical divergences reoccurred in the in vitro pharmacology of the compounds, but the in vivo data in both cases paralleled the result obtained with **20**. With the introduction of a thiazole moiety (**23**) no enhancement in in vitro potency, but still a favourable oral in vivo potency could be detected. Major improvement in biological activity was obtained with the next three compounds, the methyloxadiazole derivatives **24**–**26**. Within this homogeneous series, the oxadiazoles differ from each other solely in the position of the three heteroatoms of the five-membered aromatic ring with regard to the methyl or phenyl substituent. Whereas potencies in vitro are significantly different from each other, in vivo, the three compounds are equipotent at a high level of oral potency (ED_{50} values of 0.47–0.57 mg/kg p.o.). Although the oxadiazoles showed reduced potencies compared to those of ciproxifan and especially imoproxifan, their high in vivo potencies are particularly noteworthy as in vivo they exceed the antagonist activities of the reference antagonists thioperamide and clobenpropit (Table 1). The comparatively lower potencies of **24**–**26** in vitro, but high potencies in vivo might be caused by potential benefits in pharmacokinetics, e.g. metabolism or distribution. Since a carboxylic acid group can bioisosterically be replaced by a tetrazole, the next compound (**27**) can be interpreted as a potential bioisoster of the corresponding benzoic acid methyl ester derivative FUB 567 (R = 4-H_3CCOO–C_6H_4) (Sasse et al., 2000). The equipotency of FUB 567 (ED_{50} = 4.1 mg/kg p.o.) with tetrazole **27** (ED_{50} = 3.7 mg/kg p.o.) in the same assay is in good accordance with the anticipation detailed above.

The discovery that all of the novel heterocyclic derivatives described here possess significant antagonist potencies at histamine H_3 receptors in vitro and in vivo led us to investigate selected compounds in another in vitro H_3-receptor assay (Table 2). The compounds were screened on isolated segments of the guinea-pig ileum (Ligneau et al., 1994; Schlicker and Marr, 1996). The results obtained on rat cerebral cortex and those from the guinea-pig were generally in good agreement. Minor inconsistencies for some compounds may be attributed to species differences between the rat and the guinea-pig amino acid sequences (Tardivel-Lacombe et al., 2000) and/or assay conditions. It is interesting to note that the affinities obtained from the guinea-pig ileum as a rule are lower than those derived from rat cerebral cortex by up to 1.5-orders of magnitude. Similar results have repeatedly been reported (e.g. Sasse et al., 2000), but the reasons thereof have yet to be clarified. In summary, all compounds investigated proved to be relatively potent histamine H_3-receptor antagonists on guinea-pig ileum (pA_2 = 6.18–7.35).

In addition to the H_3-receptor studies specified above the selectivity of the compounds for the H_3 receptor versus the other two brain histamine receptors is of interest (Table 2). Determination of the H_2-receptor potency was per-

Table 1
Structures, physical data, and results of the pharmacological screening of aromatic 3-(1*H*-imidazol-4-yl)propyl ethers for histamine H_3-receptor antagonist potency in vitro and in vivo in rodents



No.	R	Formula	M_r	Yield (%)	mp (°C)	K_i±S.E.M.[a] (nM)	ED_{50}±S.E.M.[b] (mg/kg)
20		$C_{13}H_{12}N_2O_3S \cdot C_4H_4O_4 \cdot 1.75H_2O$	423.9	70	147	15±4	4.6±1.5
21		$C_{15}H_{15}N_3O_2 \cdot C_4H_4O_4 \cdot 0.25H_2O$	389.9	64	126–128	4.7±0.8	6.3±2.0
22		$C_{15}H_{16}N_4O \cdot 2C_4H_4O_4 \cdot 0.75H_2O$	514.0	61	112	67±15	6.0±1.5
23		$C_{16}H_{17}N_3OS \cdot C_4H_4O_4$	415.4	60	149–151	137±59	7.9±2.2
24		$C_{15}H_{16}N_4O_2 \cdot H_2O$	302.3	56	145	44±7	0.47±0.16
25		$C_{15}H_{16}N_4O_2 \cdot C_4H_4O_4 \cdot 0.25H_2O$	404.9	57	160–162	118±21	0.56±0.18
26		$C_{15}H_{16}N_4O_2 \cdot C_4H_4O_4 \cdot 0.25H_2O$	404.9	78	161	13±3	0.57±0.21
27		$C_{14}H_{16}N_6O \cdot C_2H_2O_4 \cdot 0.5H_2O$	383.4	80	194–196	n.d.[c]	3.7±1.5
Acetoproxifan[d]	4-H_3CCO–C_6H_4–					0.8±0.2	0.24±0.06
Ciproxifan[d]	4-(cyclopropyl–CO)–C_6H_4–					0.49±0.09	0.14±0.03
Imoproxifan[e]	4-$H_3CC(NOH)$–C_6H_4–					0.26±0.03	0.034±0.009
FUB 478[f]	4-(2-thienyl–CO)–C_6H_4–					4.3±1.4	1.0±0.6
Thioperamide[d]						4±1	1.0±0.5
Clobenpropit[d]						0.6±0.1	26±7

[a] Functional H_3-receptor assay in vitro on synaptosomes of rat cerebral cortex (Garbarg et al., 1992).
[b] Central H_3-receptor screening in vivo after oral administration to mice (Garbarg et al., 1992).
[c] n.d., not determined.
[d] Data from Stark et al. (2000b) and literature therein.
[e] Data from Sasse et al. (2000).
[f] Data from Sasse et al. (2001).

Table 2
Potency of selected compounds at histamine receptor subtypes[a]

No.	H_3		H_2	H_1
	pK_i[b]	$pA_2 \pm$S.E.M.[c]	$pD_2' \pm$S.E.M.[d]	$pA_2 \pm$S.E.M.[e]
20	7.8[f]	7.35±0.04	<4.3	5.0[g]
21	8.3[f]	6.77±0.05	4.35±0.05[e]	4.47±0.06
23	6.9[f]	<6.3[h]	4.50±0.21	4.81±0.23[i]
24	7.4[f]	6.43±0.12	4.61±0.02	4.32±0.14[i]
25	6.9[f]	6.79±0.14	4.65±0.25	4.80±0.08[i]
26	7.9[f]	6.70±0.20	<3.8	<4.5
27	n.d.[j]	6.18±0.10	4.44±0.05	4.33±0.04[i]

[a] For structures of compounds, see Table 1.
[b] Functional H_3-receptor assay in vitro on synaptosomes of rat cerebral cortex (Garbarg et al., 1992).
[c] Functional H_3-receptor assay on guinea-pig ileum (Ligneau et al., 1994; Schlicker et al., 1994).
[d] Functional H_2-receptor assay on guinea-pig atrium (Hirschfeld et al., 1992).
[e] Functional H_1-receptor assay on guinea-pig ileum (Hirschfeld et al., 1992; Ligneau et al., 1994; Schlicker et al., 1994).
[f] For corresponding S.E.M., see Table 1.
[g] Single experiment.
[h] Exact value could not be determined with regard to the M_3-receptor blocking properties of **23** at higher concentrations.
[i] pD_2' value.
[j] n.d., not determined.

formed on the spontaneously beating guinea-pig atrium and H_1-receptor affinity was investigated on the isolated guinea-pig ileum (Hirschfeld et al., 1992). All compounds tested displayed no or low potency at H_1 or H_2 receptors (pD_2' or $pA_2 \leq 5$), thus proving their pronounced selectivity for the histamine H_3 receptor. When data for the rat H_3 receptor were compared with those for the other histamine subreceptors investigated, H_3 receptor affinities in all cases were more than 100 times higher. Since potencies of the ligands in the guinea-pig test model were generally slightly lower, the differences were subsequently smaller, but still differ by more than 1.5 log units.

4. Conclusion

In this study we report on novel members of the proxifan family. The new proxifans are non-symmetrical aliphatic aromatic ethers containing a 3-(1*H*-imidazol-4-yl)propyl moiety and a bicyclic structure (**20**) or a phenyl group with different heteroaromatic substituents in *para*-position (**21**–**27**). Except **20** and **22**, each derivative was prepared by ring formation via different multi-step synthetic pathways. In vitro investigation in two different species (rat and guinea-pig) proved their H_3-receptor antagonist potencies. Studies on histamine H_1 and H_2 receptors showed pronounced selectivity for H_3 receptors. Moreover, all of the new compounds displayed high CNS activity when orally administered. The new compounds presented here clearly differ in their corresponding biological potencies in vitro and in vivo depending on the type of the heterocycle.

In the course of this study, particularly the derivatives possessing methyloxadiazole moieties (**24**–**26**) were identified as highly potent histamine H_3-receptor antagonists. In vivo, they proved to be more potent than the reference antagonists clobenpropit or thioperamide with ED_{50} values clearly below 1 mg/kg p.o. These results, combined with potential benefits or at least differences in pharmacokinetics make the oxadiazoles not only interesting leads for the further optimization of the proxifan class but also potentially interesting candidates for further improvement in the development of H_3-receptor antagonists for CNS disorders.

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Appendix A

Results of elemental analysis (C, H, N) for final compounds

No.	Formula		C	H	N
20	$C_{13}H_{12}N_2O_3S \cdot C_4H_4O_4 \cdot 1.75H_2O$	Calc.	48.2	4.64	6.61
		Found	48.0	4.48	6.38
21	$C_{15}H_{15}N_3O_2 \cdot C_4H_4O_4 \cdot 0.25H_2O$	Calc.	58.5	5.04	10.8
		Found	58.3	4.95	10.5
22	$C_{15}H_{16}N_4O \cdot 2C_4H_4O_4 \cdot 0.75H_2O$	Calc.	53.8	5.00	10.9
		Found	53.6	5.05	10.7
23	$C_{16}H_{17}N_3OS \cdot C_4H_4O_4$	Calc.	57.8	5.10	10.1
		Found	57.7	5.18	9.91
24	$C_{15}H_{16}N_4O_2 \cdot H_2O$	Calc.	59.6	6.00	18.5
		Found	59.8	5.84	18.2
25	$C_{15}H_{16}N_4O_2 \cdot C_4H_4O_4 \cdot 0.25H_2O$	Calc.	56.4	5.10	13.8
		Found	56.4	5.09	13.7
26	$C_{15}H_{16}N_4O_2 \cdot C_4H_4O_4 \cdot 0.25H_2O$	Calc.	56.4	5.10	13.8
		Found	56.4	5.05	13.6
27	$C_{14}H_{16}N_6O \cdot C_2H_2O_4 \cdot 0.5H_2O$	Calc.	50.1	5.00	21.9
		Found	49.9	4.68	21.5

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