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Synthesis and pharmacological characterization of new tetrahydrofuran based compounds as conformationally constrained histamine receptor ligands†

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A series of tetrahydrofuran based compounds with a bicyclic core that provides conformational restriction were synthesized and investigated by radioligand displacement studies and functional [^{35}S]GTPγS binding assays at the human histamine receptor (hHR) subtypes. The amines **8a** and **8b** ((1*S*,3*R*,5*S*,6*R*)- and ((1*S*,3*S*,5*S*,6*R*)-3-(1*H*-imidazol-5-yl)-2-oxabicyclo[3.1.0]hexan-6-yl)methanamine), exhibited submicromolar K_i values at the hH_3R with 10-fold higher affinities than their corresponding (6*S*)-epimers and 25- and >34-fold selectivity over the hH_4R, respectively. Both compounds act as neutral antagonists at the hH_3R with K_B values of 181 and 32 nM, respectively. The cyanoguanidines of the imidazole series and the oxazole analogues turned out to be inactive at all hHR subtypes.

Introduction

Histamine is a biogenic amine that mediates its multiple physiological effects by the interaction with four known histamine receptor (HR) subtypes, termed H_1R, H_2R, H_3R and H_4R, all belonging to rhodopsin-like family A of G-protein-coupled receptors (GPCRs).[1]

Activation of the H_1R has long been known to be associated with allergic conditions.[1a,2] Antagonists of this receptor subtype (popularly referred to as antihistamines) are used as anti-allergic drugs since the 1940s.[2] The H_2R plays a pivotal role in gastric acid secretion.[3] H_2R antagonists became blockbuster drugs for the treatment of gastric and duodenal ulcer and gastroesophagal reflux disease. The histamine H_3R is located predominantly in the central nervous system (CNS) and acts both as a presynaptic autoreceptor[4] modulating histamine release and as an inhibitory heteroreceptor[5] regulating the release of multiple neurotransmitters, such as acetylcholine,[6] dopamine,[7] noradrenaline[8] and serotonin.[9] H_3R antagonists are being investigated as potential drugs for therapeutic applications against a variety of CNS disorders such as Alzheimer's disease, attention-deficit/hyperactivity disorder (ADHD), epilepsy, migraine, narcolepsy, obesity, schizophrenia and depression.[10]

Recently, the H_3R antagonist pitolisant (tiprolisant) has been introduced as an orphan drug for the treatment of narcolepsy.[11] In the years 2000 and 2001, the H_4R was identified and cloned independently by several research groups.[12] The H_4R is mainly expressed in blood forming organs and immunocytes such as mast cells, basophils, eosinophils, monocytes, T-lymphocytes and dendritic cells.[13] It is considered as a new therapeutic target for the modulation of various inflammatory and immunological processes and disorders including bronchial asthma, atopic dermatitis, allergic rhinitis, pruritus, colitis, pain, cancer, rheumatoid arthritis and multiple sclerosis.[14]

Due to the significant sequence homology of the H_4R with the H_3R (about 40% overall sequence identity and about 60% within the transmembrane domains),[12] many of the reported H_3R agonists and antagonists showed considerable H_4R activity as well.[15] However, the development of more selective ligands remains indispensable in order to further elucidate the (patho-) physiological roles of H_3R and H_4R, which might offer new opportunities for the therapy of several diseases. Since endogenous ligands such as histamine possess flexible structures owing to rotations around single bonds, a reasonable concept to improve potency and subtype selectivity is to restrict the conformational flexibility.[16] The affinity at the respective receptor subtypes increases if such conformationally restricted analogues superimpose the bioactive conformation of the natural ligand. In the case of membrane-bound proteins where structural information is not known precisely, this

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†Electronic supplementary information (ESI) available: ^{1}H and ^{13}C spectra for all new synthesized compounds, HPLC data. See DOI: 10.1039/c3ob40441b

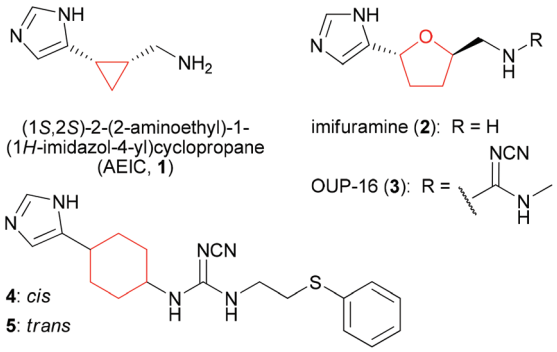
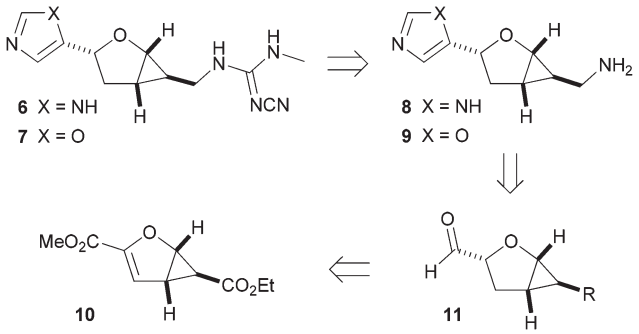


Fig. 1 Conformationally restricted histamine receptor ligands.



Scheme 1 Retrosynthetic analysis of the target compounds.

strategy can be extended by the variation of stereochemistry to explore the right spatial orientation and stereochemical requirements of the pharmacophoric elements in the binding pocket and to refine the models of ligand–receptor interaction.

This approach has been successfully applied for the development of selective H_3R and H_4R ligands. Fig. 1 shows some examples of histamine analogues comprising rigid carbo- and heterocyclic cores. From a series of cyclopropane-based conformationally constrained histamine analogues with diverse stereochemistry, Shuto *et al.* identified the "folded" *cis*-analogue (1*S*,2*S*)-2-(2-aminoethyl)-1-(1*H*-imidazol-4-yl)cyclopropane (**1**, AEIC) to be the most potent agonist at the hH_3R (K_i = 1.3 nM, EC_{50} = 10 nM) which had virtually no effect on the H_4R subtype.[17]

Yamatodani *et al.* synthesized all imifuramine (**2**) stereoisomers and its corresponding cyanoguanidine analogues and examined the binding affinity and functional activity at the human H_3 and H_4 receptors by *in vitro* studies.[18] Replacement of the amino group by the cyanoguanidine moiety, which is uncharged at physiological pH, resulted in a decrease in agonistic activity at the hH_3R. In contrast, the potencies and intrinsic activities increased at the hH_4R for most isomers. As a result, OUP-16 (**3**) was identified as the first selective H_4R agonist.

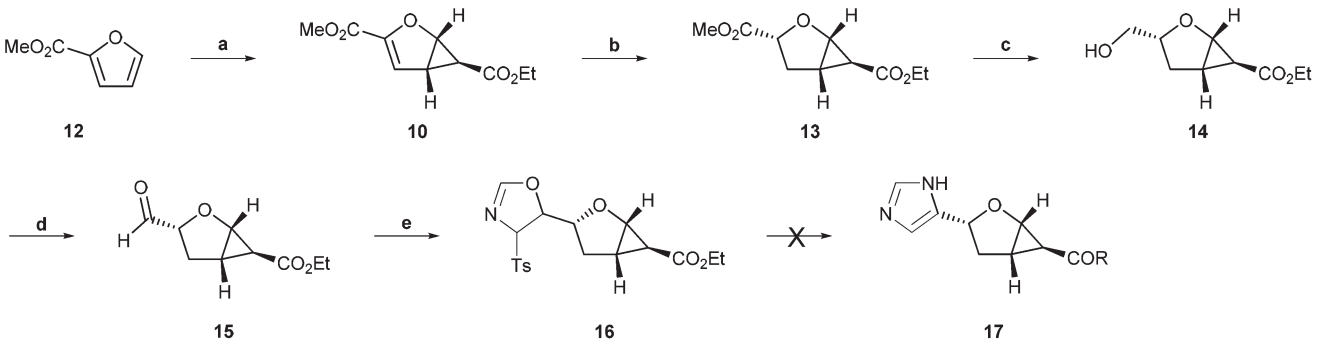
Recently, we explored rigidified cyanoguanidine-type HR ligands having a phenylene or a 1,4-cyclohexylene linker.[19] While the phenylene linker yielded only very weakly active compounds at both hH_3R and hH_4R, the less rigid 1,4-cyclohexylene linker gave *cis*- and *trans*-configured molecules revealing EC_{50} or K_B values ≥110 nM at the hH_3R and hH_4R. The *cis*-configured isomers **4** preferred the hH_4R and were partial agonists, whereas the *trans*-isomers **5** were antagonists at the hH_4R. At the hH_3R the *trans*-diastereomers **5** were superior to the *cis*-isomers **4** by a factor of 10. Thus, previous results suggest that the variation of conformational constraints and stereochemical properties is a promising approach to further explore the structure–activity and structure–selectivity relationships of HR ligands.

As part of our efforts to develop further potent and selective H_3 and H_4 receptor ligands that may serve as pharmacological tools and unravel the interactions within the binding pockets, we herein describe the enantioselective synthesis and pharmacological evaluation of potential HR ligands **6–9** containing a modified tetrahydrofuran spacer with a conformationally constrained scaffold and diverse spatial orientations (Scheme 1). The core structure consists of a fused ring system and is formed by an asymmetric cyclopropanation reaction, which gives rise to the bicyclic building block **10**. The formation of the imidazole moiety represents a key step in the synthetic route and is realized by the conversion of aldehyde **11** *via* the TosMIC strategy. Finally, the amino group of **8** and the cyanoguanidino group of **6** are introduced by further functional group interconversions including a Mitsunobu-type Gabriel reaction. In parallel, analogues **9** and **7** with an oxazole moiety as a potential bioisostere are synthesized and pharmacologically characterized. All target compounds are accessible as both enantiomers depending on the choice of the respective chiral ligand in the asymmetric cyclopropanation step.

Results and discussion

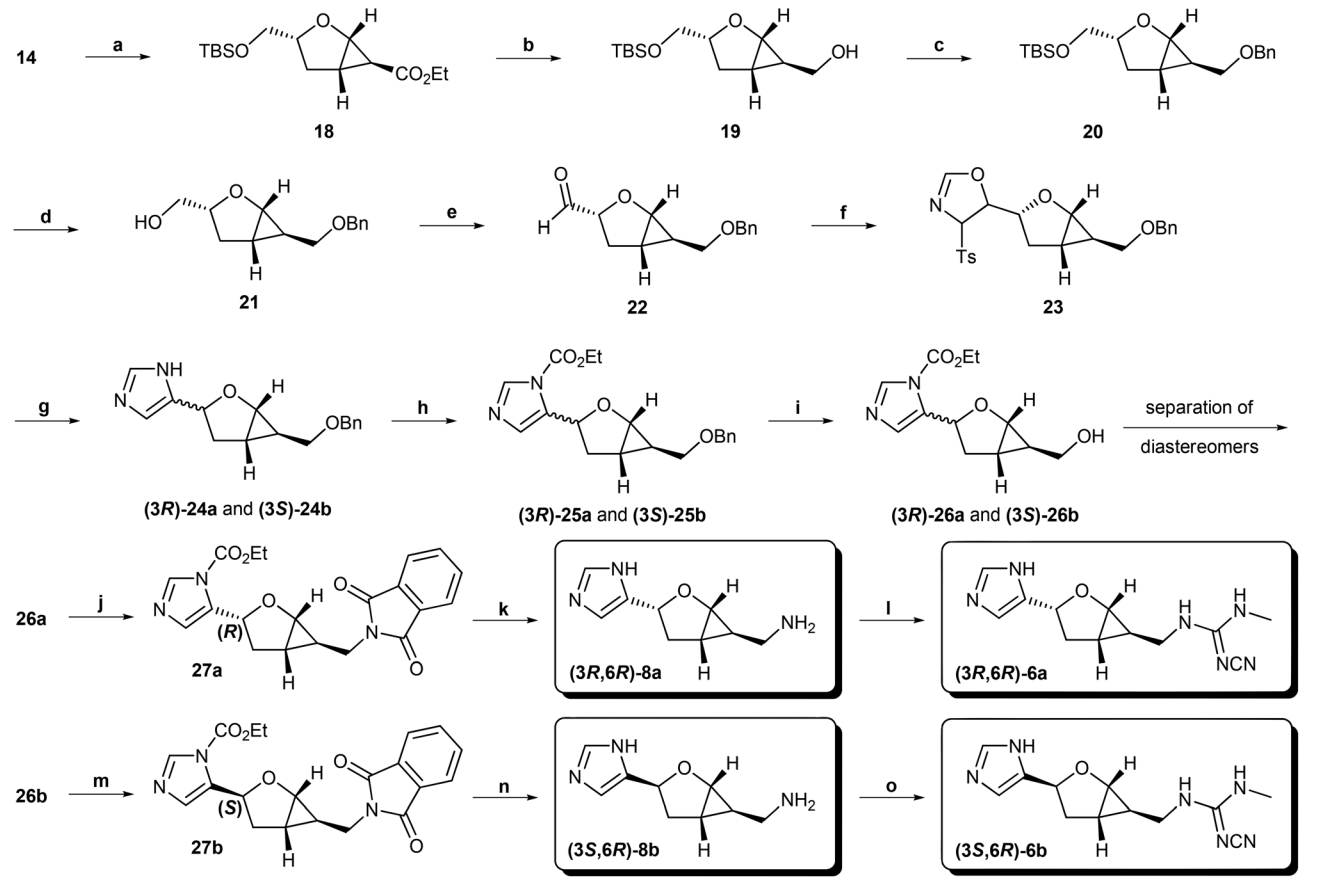
Chemistry

In the course of synthesizing various γ-butyrolactone containing natural products[20] we had previously developed the asymmetric cyclopropanation of furan-2-carboxylic acid ester **12** derived from commercially available 2-furoic acid (Scheme 2).[21] The reaction proceeded with ethyl diazoacetate in the presence of catalytic copper(I)-isopropyl bis(oxazoline) with high enantio- and diastereoselectivity to give bicyclic compound **10**. The double bond in **10** was hydrogenated accordingly using palladium on charcoal in EtOAc.[22] The hydrogenation proceeds *via syn*-addition exclusively from the less hindered convex face of the bicyclic framework to form **13** as a single stereoisomer after recrystallization. A directed thus highly chemoselective reduction of the methyl ester to alcohol **14**, being assisted by the adjacent furan ring oxygen, was followed by a Dess–Martin oxidation to give aldehyde **15**. Subsequent base-induced [3 + 2]cycloaddition with *p*-toluenesulfonylmethyl isocyanide (TosMIC) afforded tosyloxazoline **16** as a mixture of diastereomers.[23] The conversion to the corresponding imidazole **17** by treatment with ammonia according to Horne *et al.* turned out to be not feasible.[24] Also several



Scheme 2 Reagents and conditions: (a) ref. 21; (b) ref. 22; (c) LAH, THF, 0 °C, 45 min, 87%; (d) Dess–Martin periodinane, DCM, rt, 1 h, 88%; (e) TosMIC, NaCN, EtOH, rt, 1 h, 70%; R = OEt, OMe, NH_2.

other TosMIC based methods[25] starting from aldehyde **15** were not successful. Any unexpected ring-opening reaction was ruled out by different test reactions. However, it could not be totally excluded that the ester moiety was interfering with the imidazole forming reaction. To circumvent the difficulties concerning the introduction of the imidazole ring we decided to displace the ethyl ester group by a benzyl ether protecting group which is inert under the prevailing basic conditions. For this reason, the primary alcohol of compound **14** was TBS-protected (Scheme 3). The ester group of intermediate **18** was reduced with $LiAlH_4$ and the resulting primary alcohol **19** was protected with benzyl bromide. After TBAF-mediated cleavage



Scheme 3 Reagents and conditions: (a) NEt_3, TBSCl, DMAP, DCM, rt, 18 h, 95%; (b) LAH, THF, 0 °C, 45 min, 95%; (c) NaH, BnBr, DMF, 0 °C to rt, 2 h, 85%; (d) TBAF, THF, rt, 13 h, 95%; (e) Dess–Martin periodinane, DCM, rt, 2 h, 90%; (f) TosMIC, NaCN, EtOH, rt, 2 h, 77%; (g) NH_3 saturated in MeOH, 95 °C, sealed pressure tube, 16 h, (**24a** : **24b** = 5 : 1); (h) ethyl chloroformate, pyridine, DMAP, benzene, 50 °C, 10 min, 73%; (i) $Pd(OH)_2$–C, cyclohexene, EtOH, reflux, 1 h, 73%; (j) PPh_3, phthalimide, DIAD, THF, rt, 18 h, 29%; (k) hydrazine hydrate, EtOH, reflux, 1 h, 77%; (l) (i) dimethyl *N*-cyanodithioiminocarbonate, MeOH, rt, 18 h; (ii) $MeNH_2$ in EtOH, rt, 18 h, 69% over two steps; (m) PPh_3, phthalimide, DIAD, THF, rt, 18 h, 27%; (n) hydrazine hydrate, EtOH, reflux, 1.5 h, 68%; (o) (i) dimethyl *N*-cyanodithioiminocarbonate, MeOH, rt, 18 h; (ii) $MeNH_2$ in EtOH, rt, 18 h, 64% over two steps.

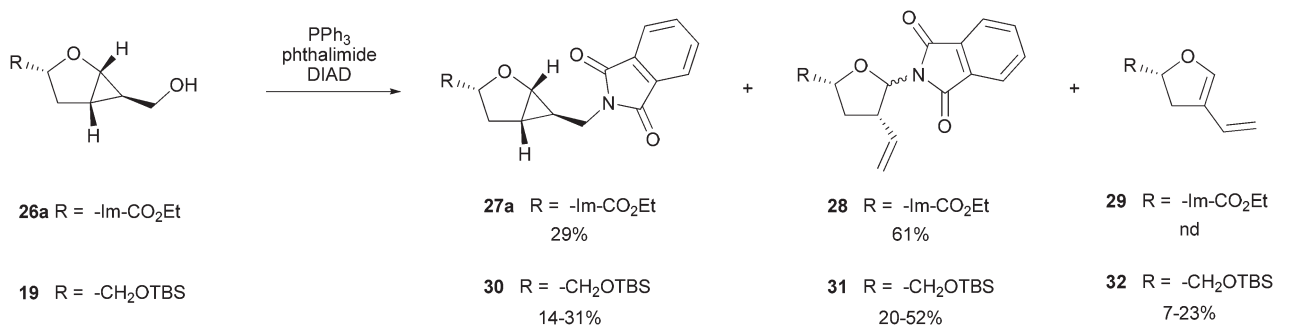
of the silylether, oxidation of alcohol **21** by means of Dess–Martin periodinane afforded aldehyde **22** which underwent cycloaddition with TosMIC.[23] The resulting oxazoline diastereomers **23** were treated with a solution of ammonia in MeOH at elevated temperature in a sealable pressure tube giving rise to the desired imidazole **24** in up to 68% yield.[24] Besides the expected imidazole isomer **24a**, epimer **24b** was identified as well due to the basic and high temperature conditions applied. Best results (**24a** : **24b** = 5 : 1) were obtained by heating at 95 °C, while higher temperatures caused increased epimerization. Following the protocol of Harusawa *et al.*, imidazole **24** was converted to its base-sensitive carbamate-protected derivative **25** using ethyl chloroformate to improve the solubility properties and to facilitate the separation of the epimers at a later stage of the reaction sequence.[26] Cleavage of the benzyl ether to give alcohol **26** was realized by catalytic transfer hydrogenation using palladium hydroxide on carbon and cyclohexene as the hydrogen donor.[27] At this point, separation of the isomers was necessary since the next step provided several side-products, which were otherwise tedious to separate and to characterize. To displace the hydroxyl group of the (3*R*)-isomer **26a** with an amino moiety, a phthaloylimination under Mitsunobu conditions and subsequent hydrazinolysis were performed.[28] By treating **26a** with phthalimide in the presence of PPh_3 and DIAD, the desired phthalimide **27a** was obtained in low yield. Further ring-opening gave phthalimides **28** as a mixture of epimers due to a cyclopropylcarbinyl–homoallylic rearrangement (Scheme 4).[29] Diene **29** was observed as well but was not separable from the triphenylphosphine oxide byproduct. In order to optimize the conditions for the preparation of the desired phthalimide **27a** different conditions were screened using model compound **19**. However, the change of the addition order of the reagents, variation in the relative reagent concentrations and different reaction temperatures and solvents did not lead to a significantly improved ratio of products. Cleavage of the phthalimide moiety of compound **27a** by means of hydrazinolysis proceeded smoothly with simultaneous removal of the base-sensitive carbamate protection group at the imidazole ring to give the desired target compound amine **8a** (Scheme 3). The conversion to the analogous cyanoguanidine-containing compound **6a** required two additional steps. First, amine **8a** was treated with an excess of dimethyl *N*-cyanodithioiminocarbonate in MeOH to furnish an isothiourea compound which was then directly converted without purification to the desired cyanoguanidine **6a** by adding an ethanolic solution of $MeNH_2$.[26]

The respective (3*S*)-configured target compounds, amine **8b** and cyanoguanidine **6b**, were derived from the corresponding (3*S*)-configured alcohol **26b** running through an analogous synthetic pathway *via* phthalimide **27b**. Consequently, the (6*R*)-configured target molecules, amines **6a** and **6b** and the cyanoguanidines **8a** and **8b** could be prepared. Additionally, by employing the (*R*,*R*)-isopropyl bis(oxazoline) ligand in the cyclopropanation step, the respective (6*S*)-enantiomers, amines **8c** and **8d** and cyanoguanidines **6c** and **6d**, were accessible.

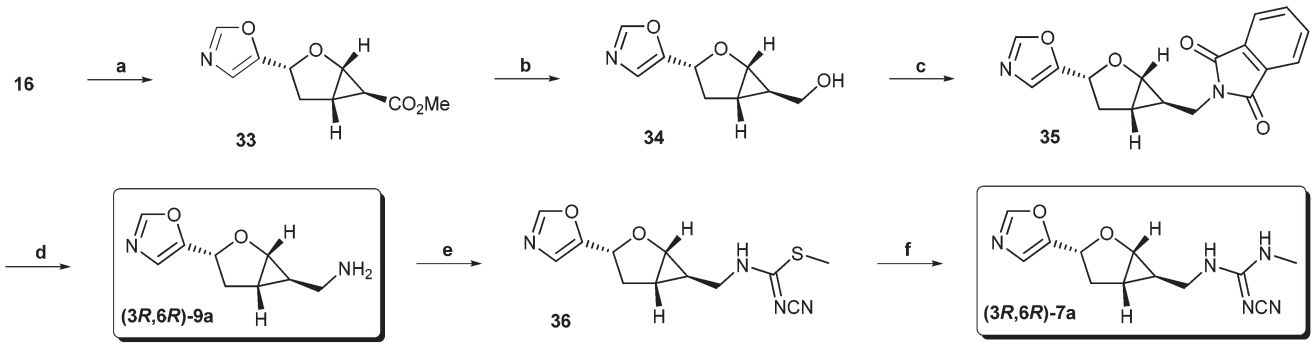
As a putative bioisostere of the imidazole, an oxazole moiety was introduced by elimination of *p*-toluene sulfinic acid from tosyloxazoline **16** accompanied by transesterification of the ethyl ester group to give compound **33** (Scheme 5).[23] Subsequent reduction with $LiAlH_4$ furnished alcohol **34**. The hydroxy group was converted into a phthalimide moiety *via* a Mitsunobu reaction.[28] In comparison to the similar transformation of **26a** to **27a** described above, the reaction proceeded with the formation of smaller amounts of ring opening products and this with higher yield to the desired **35** (55% *vs.* 29% for **27a**) for reasons that are not clear. Hydrazinolysis gave rise to the amine **9a**. The corresponding cyanoguanidine **7a** was readily obtained by converting the amino group with dimethyl *N*-cyanodithioiminocarbonate to isothiourea **36** followed by treatment with $MeNH_2$ in EtOH.[26] In turn, the application of the enantiomeric isopropyl bis(oxazoline) ligand in the asymmetric cyclopropanation reaction provided access to the enantiomeric target compounds, **9b** and **7b**, as well.

Pharmacology

All the synthesized target compounds depicted in Fig. 2 were investigated at the hH_1R, hH_2R, hH_3R and hH_4R in radioligand binding assays using membrane preparations of Sf9 insect cells coexpressing the hH_1R + RGS4, hH_2R–$Gs_{\alpha s}$ fusion protein, hH_3R + $G\alpha_{i2}$ + $G\beta_1\gamma_2$ or hH_4R + $G\alpha_{i2}$ + $G\beta_1\gamma_2$, respectively. Those compounds having submicromolar K_i values were investigated for agonism or antagonism at hH_3R and hH_4R subtypes in functional [^{35}S]GTPγS assays using the above



Scheme 4 Mitsunobu reaction.



Scheme 5 Reagents and conditions: (a) K_2CO_3, MeOH, reflux, 0.5 h, 31%; (b) LAH, THF, 0 °C, 0.5 h, 71%; (c) PPh_3, phthalimide, DIAD, THF, 0 °C, 0.5 h, 55%; (d) hydrazine hydrate, EtOH, reflux, 1.5 h, 72%; (e) dimethyl *N*-cyanodithioiminocarbonate, EtOH, rt, 18 h, quant.; (f) $MeNH_2$ in EtOH, rt, 18 h, 90%.

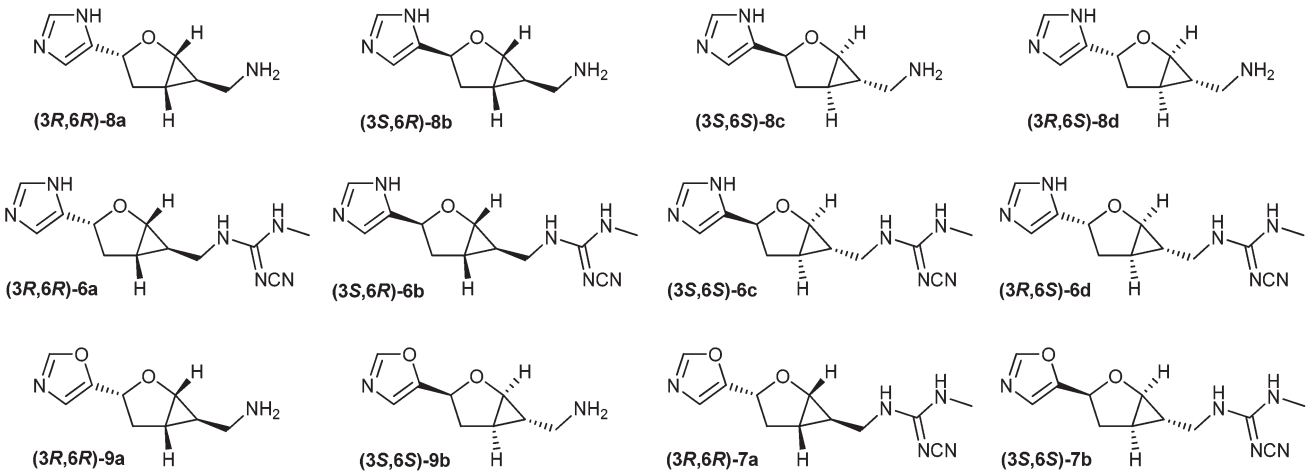


Fig. 2 Synthesized target compounds.

mentioned membrane preparations. Intrinsic activities (α) refer to the maximal response induced by the standard agonist histamine (α = 1.0). Compounds identified to be inactive as agonists (α < 0.13 or negative values, respectively, determined in the agonist mode) were investigated in the antagonist mode. The corresponding K_B values of neutral antagonists were determined from the concentration-dependent inhibition of the histamine-induced increase in [^{35}S]GTPγS binding.

In agreement with the findings of Hashimoto *et al.*, the amines **8a–d** exhibited significantly stronger binding affinities at the hH_3R than at the hH_4R (Table 1). At the hH_3R the (6*R*)-configured eutomers **8a** and **8b** showed submicromolar K_i values. Both compounds were about 10-fold more potent than their (6*S*)-configured distomers **8c** and **8d**. At the hH_4R, compounds **8a** and **8d**, having the (3*R*)-configuration, exhibited weak binding affinities with low micromolar K_i values. In contrast to this, the respective (3*S*)-configured epimers **8b** and **8c** did not show any significant binding (K_i > 10 000 nM) at this receptor subtype. An unambiguous preference for either the folded isomers ((3*R*,6*R*)-*cis*-**8a** and (3*S*,6*S*)-*cis*-**8d**) or the extended analogues ((3*S*,6*R*)-*trans*-**8b** and (3*R*,6*S*)-*trans*-**8c**) was not observed at both receptor subtypes. As a result, binding affinities for the amines **8a**, **8b**, **8c** and **8d** at the hH_3R were 25, >34, >4 and 3-fold higher than at the hH_4R subtype, respectively. Additionally, **8a–d** were devoid of activity at the hH_1R and hH_2R. **8a** and **8b** were investigated for their functional activity at the hH_3R. In contrast to imifuramine and its stereoisomers, which were all reported to act as full agonists at the H_3R,[18] **8a** and **8b** turned out to be almost neutral antagonists with K_B values of 181 and 32 nM, respectively. The elongated spacer and the different spatial arrangement of the pharmacophoric elements were tolerated to a certain extent for the conformationally constrained amines compared to Hashimoto's THF-based ligands. At both HR subtypes comparable K_i values were determined, especially at the hH_3R, but the quality of action was different.

In contrast, the cyanoguanidines **6a–d** turned out to be inactive (K_i > 10 000 nM) at all four HR subtypes, notably at the H_4R. In this case, the orientation of the pharmacophoric elements, provided by the bicyclic core, is detrimental for receptor binding. An increase in hH_4R affinity by replacement of the amino group with a cyanoguanidino moiety – as observed for the imifuramine based compounds[18] – was not achieved.

The synthesized oxazoles **9a**, **9b**, **7a** and **7b** were investigated in radioligand binding assays at the hH_1R and hH_2R

Table 1 Affinities, potencies and efficacies of the synthesized amines and cyanoguanidines at the hHR subtypes determined in radioligand binding studies[a] and functional [^{35}S]GTPγS assays[b]

		hH_1R		hH_2R		hH_3R			hH_4R		
Compound	Config.	K_i (nM)	*N*	K_i (nM)	*N*	K_i (nM) (K_B or EC_{50} [b] (nM))	*α*	*N*	K_i (nM) (K_B or EC_{50} [b] (nM))	*α*	*N*
Histamine[c]		200		5×10^4		10			7.9(13)		
						(5.0)	1.00			1.00	
Imifuramine (**2**)[d]		—		—		229			891		
						(45)	1.04		(1995)	0.70	
OUP-16 (**3**)[d]		—		—		2188			126		
						(3261)	0.79		(78)	0.99	
8a	3*R*,6*R*	Inactive	2	Inactive	2	231 ± 106		3	5787 ± 853		2
						(181 ± 119)	−0.10	2			
8b	3*S*,6*R*	Inactive	2	Inactive	2	295 ± 154		2	>10 000		2
						(32 ± 17)	−0.12	2			
8c	3*S*,6*S*	Inactive[e]	2	Inactive[e]	2	2326 ± 982		2	>10 000		2
8d	3*R*,6*S*	Inactive[e]	2	Inactive[e]	2	2818 ± 1823		2	8415 ± 417		2
6a	3*R*,6*R*	Inactive[e]	2	Inactive[e]	2	Inactive		2	Inactive		2
6b	3*S*,6*R*	Inactive[e]	2	Inactive[e]	2	Inactive		2	Inactive		2
6c	3*S*,6*S*	Inactive[e]	2	Inactive[e]	2	Inactive		2	Inactive		2
6d	3*R*,6*S*	Inactive[e]	2	Inactive[e]	2	Inactive		2	Inactive		2
9a	3*R*,6*R*	Inactive[e]	2	Inactive[e]	2	(Inactive)	−0.08	2	(Inactive)	0.02	2
9b	3*S*,6*S*	Inactive[e]	2	Inactive[e]	2	(Inactive)	−0.06	2	(Inactive)	0.13	2
7a	3*R*,6*R*	Inactive[e]	2	Inactive[e]	2	(Inactive)	0.07	2	(Inactive)	−0.03	2
7b	3*S*,6*S*	Inactive[e]	2	Inactive[e]	2	(Inactive)	−0.07	2	(Inactive)	−0.06	2

[a] Determination of hH_1R binding by displacement of [^{3}H]pyrilamine (5 nM) from Sf9 cell membranes expressing the hH_1R + RGS4, hH_2R binding by displacement of [^{3}H]UR-DE257 (30 nM) from Sf9 cell membranes expressing the hH_2R–$Gs_{\alpha s}$, hH_3R binding by displacement of [^{3}H] N^{α}-methylhistamine (3 nM) or [^{3}H]histamine (15 nM) from Sf9 cell membranes expressing the hH_3R + $G\alpha_{i2}$ + $G\beta_1\gamma_2$ or hH_4R binding by displacement of [^{3}H]histamine (15 nM) from Sf9 cell membranes expressing the hH_4R + $G\alpha_{i2}$ + $G\beta_1\gamma_2$ was determined as described in the Pharmacology section. [b] Functional [^{35}S]GTPγS binding assays with membrane preparations of Sf9 cells expressing the hH_3R + $G\alpha_{i2}$ + $G\beta_1\gamma_2$ or the hH_4R + $G\alpha_{i2}$ + $G\beta_1\gamma_2$ were performed as described in the Pharmacology section. [a,b]Ligands concentrations from 1 nM to 1 mM as appropriate to generate saturated concentration/response curves. Compounds showing no effect in this range were referred to as inactive. *N* gives the number of independent experiments performed in triplicate each. The intrinsic activity (*α*) of histamine was set to 1.00 and *α* values of other compounds were referred to this value. The *α* values of neutral antagonists and inverse agonists were determined at a concentration of 10 μM. The K_B values of neutral antagonists were determined in the antagonist mode *versus* histamine (100 nM) as the agonist. [c] Values taken from Igel *et al.*[30] [d] Values for hH_3R and hH_4R taken from Hashimoto *et al.*[18] [e] Measured at a ligand concentration of 100 μM.

subtypes and in functional [^{35}S]GTPγS assays at the hH_3R and hH_4R subtypes but did not reveal any activity at all receptor subtypes. Even oxazole **9a**, whose imidazole analogue **8a** exhibited submicromolar affinities at the hH_3R, was inactive at the hH_3R. Thus, in this class of HR ligands, independently from the other structural modifications, the oxazole ring proved to be inappropriate as a bioisostere of an imidazole ring. This is presumably due to a different H-bond donor–acceptor interaction pattern and the reduced basicity of the oxazole moiety compared to the imidazole ring.

Conclusion

In conclusion, we have synthesized and pharmacologically characterized a set of bicyclic imifuramine analogues as potential histamine receptor ligands. In the imidazole series, the conformationally constrained amines and cyanoguanidines were obtained in 15 and 17 steps, respectively, starting from commercially available furan-2-carboxylic acid. The oxazole analogues could be realized in 10 and 12 steps, respectively. The synthetic pathways deliver valuable information about the scope and limitations of the rigid bicyclic core in terms of chemical transformations of its substituents. In the case of the imidazole compounds the introduction of the aromatic heterocyles by TosMIC chemistry gave rise to additional epimers. As a result, different isomers with distinct stereochemical orientations could be achieved. Pharmacological investigations at the human HR subtypes revealed affinities of the amines **8** at the hH_3R in a comparable range as reported for the imifuramine derived stereoisomers, but with different qualities of action. Especially **8b** showed high subtype selectivity for the hH_3R with no affinity for the hH_4R. In agreement with these findings and in contrast to the cyanoguanidine **3** bearing the tetrahydrofuran moiety, the cyanoguanidines **6** were devoid of activity at the hH_4R as well. These results suggest that the 2-oxabicyclo[3.1.0]hexane framework used in this study might be a promising scaffold for the hH_3R selective ligands. Replacement of the imidazole ring by an oxazole substituent as a potential bioisostere led to loss of activities at HR subtypes. Apparently, the H-bond donor and acceptor properties and the basicity of the oxazole ring are inappropriate.

These findings contribute to the objective of further elucidating the structural requirements of selective histamine H_3 and H_4 receptor ligands that may help to enable the synthesis of tailored compounds as novel pharmacological tools and potential drugs with the intended quality of action.

Experimental

Chemistry

Analytical HPLC analysis was performed with a system from Merck (Darmstadt, Germany) consisting of a L-5000 controller, a 655A-12 pump, a 655A-40 autosampler and a L-4250 UV-VIS detector on a Eurospher-100 C18 column (250 × 4 mm, 5 μm, Knauer, Berlin, Germany) at a flow rate of 0.8 mL min^{-1}. Mixtures of acetonitrile and 0.05% aq. TFA were used as the mobile phase. Helium degassing was used throughout. Compound purities were calculated as the percentage peak area of the analyzed compound by UV detection at 210 nm. HPLC conditions, retention times (t_R), capacity factors ($k' = (t_R - t_0)/t_0$) and purities of the synthesized compounds are listed in the ESI.†

All reactions were carried out in oven-dried glassware under atmospheric conditions unless otherwise stated. All solvents were dried and distilled prior to use. Thin layer chromatography (TLC) was performed using silica gel 60 F254 aluminium plates (Merck). Eluted plates were visualized using a 254 nm UV lamp and/or by treatment with a suitable stain followed by heating. Column chromatography was performed on silica gel 60 (0.063–0.200 mm, Merck). ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker Avance 300 (300 MHz for ^{1}H, 75 MHz for ^{13}C), Bruker Avance III 400 "Nanobay" (400 MHz for ^{1}H, 101 MHz for ^{13}C) or Avance III 600 (600 MHz for ^{1}H, 151 MHz for ^{13}C) FT-NMR-spectrometer. Chemical shifts are reported in parts per million (ppm). ATR-IR spectroscopy was carried out on a Biorad Excalibur FTS 3000 spectrometer equipped with a Specac Golden Gate Diamond Single Reflection ATR-system. Optical rotations were measured on a P8000T polarimeter (Kruess) at a wavelength of 589 nm in a 5 cm cell of 0.7 mL volume in the specified solvent. Concentrations are indicated in [g/100 mL]. The melting points were measured on a Büchi SMP-20 apparatus in a silicon oil bath. Values thus obtained were not corrected. Mass spectrometry was performed on a Varian MAT 311A, Finnigan MAT 95, Thermoquest Finnigan TSQ 7000 or Agilent Technologies 6540 UHD Accurate-Mass Q-TOF LC/MS. The percentage set in brackets gives the peak intensity related to the basic peak (I = 100%). High resolution mass spectrometry (HRMS): the molecular formula was proven by the calculated precise mass. Elemental analyses (C, H, N, Heraeus Elementar Vario EL III) were performed by the Analytical Department of the University of Regensburg.

Preparative HPLC was performed at room temperature with a system from Knauer (Berlin, Germany) consisting of two K-1800 pumps, a K-2001 detector (UV detection at 220 nm) and a RP-column (VP Nucleodur 100-5 C18 ec, 250 × 21 mm, 5 μm, Macherey Nagel, Düren, Germany) at a flow rate of 15 mL min^{-1} or a RP-column (YMC-Triat C18, 150 × 20.0 m, 5 μm, YMC Europe GmbH, Dinslaken, Germany) at a flow rate of 10 mL min^{-1}. Mixtures of acetonitrile and 0.1% aq. TFA were used as the mobile phase in the case of the Nucleodur column and mixtures of acetonitrile and 0.1% aq. NH_3 were used as the mobile phase in the case of the YMC-Triat column. Acetonitrile was removed from the eluates under reduced pressure at 45 °C prior to lyophilization.

Preparation of 1-(((1*S*,3*R*,5*S*,6*R*)-3-(1*H*-imidazol-5-yl)-2-oxabicyclo[3.1.0]hexan-6-yl)methyl)-2-cyano-3-methylguanidine (6a). A solution of compound **8a** (5.0 mg, 0.028 mmol) and dimethyl *N*-cyanodithioiminocarbonate (9.9 mg, 0.067 mmol, 2.4 equiv.) in anhydrous MeOH (0.55 mL) was stirred at room temperature for 18 h. Then a 33% solution of $MeNH_2$ in EtOH (0.52 mL) was added and stirred for 18 h at room temperature. The solvent was evaporated to give a residual oil that was purified by column chromatography (EtOAc–MeOH 4 : 1) to give compound **6a** (5.0 mg, 0.019 mmol, 69%) as a colorless oil. For pharmacological testing the product was further purified by preparative HPLC (YMC-Triat column, mobile phase: MeCN, 0.1% aq. NH_3).

R_f = 0.19 (EtOAc–MeOH 4 : 1); $[\alpha]_D^{20}$ = +18.2 (MeOH, c = 0.2); ^{1}H-NMR (300 MHz, MeOD): δ_H = 7.62 (d, J = 1.0 Hz, 1H), 6.96 (s, 1H), 5.38 (t, J = 7.9 Hz, 1H), 3.89 (dd, J = 6.4, 1.1 Hz, 1H), 3.05 (dd, J = 14.3, 6.9 Hz, 1H), 2.91 (dd, J = 14.3, 7.9 Hz, 1H), 2.79 (s, 3H), 2.55 (dt, J = 12.8, 7.4 Hz, 1H), 2.06 (ddd, J = 12.8, 8.1, 1.9 Hz, 1H), 1.74–1.63 (m, 1H), 1.46–1.36 (m, 1H); ^{13}C-NMR (75 MHz, MeOD): δ_C = 161.96 (C_q), 140.02 (C_q), 136.79 (+), 120.08 (C_q), 117.42 (+), 83.90 (+), 65.43 (+), 42.58 (−), 36.41 (−), 33.19 (+), 28.67 (+), 24.09 (+); IR (ATR): $\tilde{\nu}$ (cm^{-1}) = 3268 (br), 2928, 2160, 1729, 1575, 1485, 1448, 1404, 1369, 1247, 1174, 1097, 1066, 1030, 988, 838, 752, 716, 618, 570; MS (ESI): m/z (%) = 163.1 [$M^+\Delta C_3H_5N_4$] (60), 261.1 [MH^+] (100); HRMS (ESI): calcd for $C_{12}H_{17}N_6O$ [MH^+] 261.1458, found 261.1458.

Preparation of 1-(((1*S*,3*S*,5*S*,6*R*)-3-(1*H*-imidazol-5-yl)-2-oxabicyclo[3.1.0]hexan-6-yl)methyl)-2-cyano-3-methylguanidine (6b). A solution of compound **8b** (3.0 mg, 0.017 mmol) and dimethyl *N*-cyanodithioiminocarbonate (8.2 mg, 0.05 mmol, 3.0 equiv.) in anhydrous MeOH (0.34 mL) was stirred at room temperature for 18 h. Then a 33% solution of $MeNH_2$ in EtOH (0.31 mL) was added. The resulting mixture was stirred for 18 h at room temperature. The solvent was evaporated to give a residual oil that was purified by column chromatography (EtOAc–MeOH 4 : 1) to give compound **6b** (2.8 mg, 0.011 mmol, 64%) as a colorless oil. For pharmacological testing the product was further purified by preparative HPLC (YMC-Triat column, mobile phase: MeCN, 0.1% aq. NH_3).

R_f = 0.19 (EtOAc–MeOH 4 : 1); $[\alpha]_D^{20}$ = +36.7 (MeOH, c = 0.1); ^{1}H-NMR (600 MHz, MeOD): δ_H = 7.62 (s, 1H), 7.00 (s, 1H), 4.76 (dd, J = 8.7, 7.7 Hz, 1H), 3.94 (dd, J = 5.7, 1.1 Hz, 1H), 3.07 (dd, J = 14.3, 6.9 Hz, 1H), 2.97 (dd, J = 14.3, 7.7 Hz, 1H), 2.81 (s, 3H), 2.29 (dd, J = 12.4, 7.1 Hz, 1H), 2.25–2.19 (m, 1H), 1.62–1.59 (m, 1H), 1.59–1.54 (m, 1H); ^{13}C-NMR (151 MHz, MeOD): δ_C = 162.01 (C_q), 136.88 (+), 120.08 (C_q), 63.74 (+), 49.57 (+), 42.71 (−), 35.51 (−), 28.69 (+), 22.28 (+), 21.94 (+), Im-*C*5 and Im-*C*4 signals too weak to be observed; IR (ATR): $\tilde{\nu}$ (cm^{-1}) = 2934 (br), 2163, 1582, 1486, 1410, 1372, 1322, 1175, 1121, 1100, 922, 892, 833, 689, 617; MS (ESI): m/z (%) = 261.1 [MH^+] (100), 521.2 [$2MH^+$] (15); HRMS (ESI): calcd for $C_{12}H_{17}N_6O$ [MH^+] 261.1458, found 261.1457.

Preparation of 2-cyano-1-methyl-3-(((1*S*,3*R*,5*S*,6*R*)-3-(oxazol-5-yl)-2-oxabicyclo[3.1.0]hexan-6-yl)methyl) guanidine (7a). Compound **36** (26 mg, 0.09 mmol) was dissolved in a 33% solution of $MeNH_2$ in EtOH (2 mL) and stirred for 18 h at room temperature. The solvent was evaporated under reduced pressure. Purification by column chromatography (DCM then DCM–MeOH 9 : 1) afforded compound **7a** (22 mg, 0.08 mmol, 90%) as a colorless oil.

R_f = 0.32 (DCM–MeOH 9 : 1); $[\alpha]_D^{20}$ = +18.9 (DCM, *c* = 1.0); ^{1}H-NMR (400 MHz, $CDCl_3$): δ_H = 7.84 (s, 1H), 6.96 (s, 1H), 5.64 (s, 1H), 5.43 (dd, *J* = 8.3, 7.4 Hz, 1H), 5.20 (s, 1H), 3.95 (dd, *J* = 6.3, 1.0 Hz, 1H), 3.24–3.13 (m, 1H), 2.95–2.86 (m, 1H), 2.85 (d, *J* = 4.9 Hz, 3H), 2.62 (ddd, *J* = 13.1, 8.6, 7.0 Hz, 1H), 2.14 (ddd, *J* = 13.1, 7.0, 1.4 Hz, 1H), 1.73–1.67 (m, 1H), 1.37 (tdd, *J* = 8.0, 4.0, 1.0 Hz, 1H); ^{13}C-NMR (101 MHz, $CDCl_3$): δ_C = 160.65 (C_q), 151.72 (C_q), 151.44 (+), 124.01 (+), 118.53 (C_q), 78.46 (+), 65.08 (+), 42.03 (−), 33.91 (−), 30.74 (+), 28.57 (+), 23.15 (+); IR (ATR): $\tilde{\nu}$ (cm^{-1}) = 3292 (br), 2954, 2929, 2165, 1583, 1507, 1453, 1409, 1370, 1174, 1103, 1028, 963, 838, 717; MS (ESI): *m*/*z* (%) = 262.1 (25) [MH^+], 523.2 (100) [$2MH^+$]; HRMS (EI): calcd for $C_{12}H_{15}N_5O_2$ [$M^{+\cdot}$] 261.1226, found 261.1222.

Preparation of ((1*S*,3*R*,5*S*,6*R*)-3-(1*H*-imidazol-5-yl)-2-oxabicyclo[3.1.0]hexan-6-yl)methanamine (8a). A solution of phthalimide **27a** (30 mg, 0.079 mmol) and hydrazine hydrate (21 μL, 0.43 mmol, 5.4 equiv.) in anhydrous EtOH (1.6 mL) was refluxed for 1 h. The solvent was removed under reduced pressure and the residue was purified by column chromatography (MeOH–saturated NH_3 in MeOH 95 : 5) to afford compound **8a** (11 mg, 0.061 mmol, 77%) as a colorless amorphous solid. For pharmacological testing the product was further purified by preparative HPLC (Nucleodur column, mobile phase: MeCN, 0.1% aq. TFA).

R_f = 0.20 (MeOH–saturated NH_3 in MeOH 95 : 5); $[\alpha]_D^{20}$ = +36.4 (MeOH, *c* = 0.5); ^{1}H-NMR (300 MHz, MeOD): δ_H = 7.61 (d, *J* = 1.0 Hz, 1H), 6.95 (s, 1H), 5.38 (t, *J* = 7.9 Hz, 1H), 3.82 (dd, *J* = 6.4, 1.2 Hz, 1H), 2.63–2.49 (m, 1H), 2.38 (d, *J* = 7.3 Hz, 2H), 2.04 (ddd, *J* = 12.7, 8.0, 1.9 Hz, 1H), 1.61 (tdd, *J* = 6.2, 3.9, 1.9 Hz, 1H), 1.26 (tdd, *J* = 7.4, 4.0, 1.1 Hz, 1H); ^{13}C-NMR (75 MHz, MeOD): δ_C = 136.71 (+), 117.55 (+), 83.87 (+), 65.46 (+), 42.61 (−), 36.63 (−), 36.07 (+), 23.99 (+), Im-C_q-signal too weak to be observed; IR (ATR): $\tilde{\nu}$ (cm^{-1}) = 3094 (br), 2937, 2869, 2625, 1573, 1454, 1414, 1361, 1306, 1177, 1098, 1067, 1028, 980, 912, 841, 632, 540, 497; MS (ESI): *m*/*z* (%) = 163.1 (100) [$MH^+\Delta NH_3$], 180.1 (19) [MH^+], 359.2 (11) [$2MH^+$]; HRMS (ESI): calcd for $C_9H_{14}N_3O$ [MH^+] 180.1131, found 180.1130.

8a·2TFA: ^{1}H-NMR (600 MHz, MeOD): δ_H = 8.83 (d, *J* = 1.0 Hz, 1H), 7.45 (s, 1H), 5.51 (t, *J* = 7.4 Hz, 1H), 4.10 (dd, *J* = 6.3, 0.7 Hz, 1H), 2.90–2.63 (m, 3H), 2.14 (ddd, *J* = 13.1, 7.0, 1.5 Hz, 1H), 1.92–1.87 (m, 1H), 1.31–1.26 (m, 1H); ^{13}C-NMR (151 MHz, MeOD): δ_C = 163.10 (C_q, TFA), 162.87 (C_q, TFA), 136.63 (+), 135.90 (C_q), 119.17 (+, TFA), 117.23 (+, TFA), 116.76 (+), 79.55 (+), 65.65 (+), 40.61 (−), 35.88 (−), 29.34 (+), 24.45 (+).

Preparation of ((1*S*,3*S*,5*S*,6*R*)-3-(1*H*-imidazol-5-yl)-2-oxabicyclo[3.1.0]hexan-6-yl)methanamine (8b). A solution of phthalimide **27b** (16 mg, 0.042 mmol) and hydrazine hydrate (11 μL, 0.23 mmol, 5.4 equiv.) in anhydrous EtOH (0.85 mL) was refluxed for 1.5 h. The solvent was removed under reduced pressure and the residue was purified by column chromatography (MeOH–saturated NH_3 in MeOH 97 : 3) to afford compound **8b** (5.1 mg, 0.028 mmol, 68%) as a colorless amorphous solid. For pharmacological testing the product was further purified by preparative HPLC (Nucleodur column, mobile phase: MeCN, 0.1% aq. TFA).

8b·2TFA: R_f = 0.20 (MeOH–saturated NH_3 in MeOH 95 : 5); $[\alpha]_D^{20}$ = +5.5 (DCM, *c* = 0.2); ^{1}H-NMR (600 MHz, MeOD): δ_H = 8.88 (d, *J* = 1.3 Hz, 1H), 7.50 (d, *J* = 0.9 Hz, 1H), 4.94 (dd, *J* = 8.9, 7.5 Hz, 1H), 4.14 (dd, *J* = 5.8, 1.2 Hz, 1H), 2.82 (dd, *J* = 13.4, 8.0 Hz, 1H), 2.77 (dd, *J* = 13.4, 7.8 Hz, 1H), 2.51 (dd, *J* = 12.7, 7.4 Hz, 1H), 2.22 (ddd, *J* = 12.8, 9.1, 5.6 Hz, 1H), 1.83–1.79 (m, 1H), 1.56 (tdd, *J* = 7.9, 3.9, 1.1 Hz, 1H). ^{13}C-NMR (151 MHz, MeOD): δ_C = 162.80 (C_q, TFA), 162.56 (C_q, TFA), 136.08 (C_q), 134.59 (+), 119.04 (+, TFA), 117.45 (+), 117.11 (+, TFA), 72.81 (+), 64.08 (+), 40.76 (−), 35.51 (−), 22.52 (+), 20.51 (+); IR (ATR): $\tilde{\nu}$ (cm^{-1}) = 3240 (br), 2935, 2873, 2627, 1580, 1492, 1420, 1372, 1312, 1180, 1101, 899, 840, 630, 540; MS (ESI): *m*/*z* (%) = 180.0 (100) [MH^+], 359.2 (20) [$2MH^+$]; HRMS (ESI): calcd for $C_9H_{14}N_3O$ [MH^+] 180.1131, found 180.1133.

Preparation of ((1*S*,3*R*,5*S*,6*R*)-3-(oxazol-5-yl)-2-oxabicyclo-[3.1.0]hexan-6-yl)methanamine (9a). A solution of phthalimide **35** (60 mg, 1.19 mmol) and hydrazine hydrate (48 mg, 0.97 mmol, 5 equiv.) in EtOH (4 mL) was refluxed for 1.5 h and then cooled in an ice bath. The white precipitate was removed by filtration through a Celite pad. The filtrate was concentrated *in vacuo*. Column chromatography (DCM–saturated NH_3 in MeOH 20 : 1) afforded compound **9a** (25 mg, 0.10 mmol, 72%) as a colorless solid.

Mp = 51 °C; R_f = 0.32 (DCM–saturated NH_3 in MeOH 9 : 1); $[\alpha]_D^{20}$ = +36.2 (DCM, *c* = 1.0); ^{1}H-NMR (400 MHz, $CDCl_3$): δ_H = 7.80 (s, 1H), 6.93 (s, 1H), 5.41 (dd, *J* = 8.1, 7.4 Hz, 1H), 3.82 (dd, *J* = 6.3, 1.2 Hz, 1H), 2.58 (ddd, *J* = 12.9, 8.6, 7.0 Hz, 1H), 2.51–2.39 (m, 2H), 2.11 (ddd, *J* = 12.9, 6.9, 1.5 Hz, 1H), 1.59–1.51 (m, 1H), 1.28–1.22 (m, 1H), 1.25 (br s, 2H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ_C = 152.16 (C_q), 151.25 (+), 123.68 (+), 78.37 (+), 65.19 (+), 42.33 (−), 34.78 (−), 34.12 (+), 22.68 (+); IR (ATR): $\tilde{\nu}$ (cm^{-1}) = 3356 (br), 3127, 2949, 1636, 1567, 1508, 1482, 1427, 1377, 1318, 1180, 1103, 1027, 980, 955, 849, 723, 646, 610; MS (ESI): *m*/*z* (%) = 181.0 (7) [MH^+], 222.0 (100) [MH^+MeCN]; HRMS (ESI): calcd for $C_9H_{13}N_2O_2$ [MH^+] 181.0972, found 181.0969.

Preparation of (1*S*,3*R*,5*S*,6*S*)-ethyl 3-(hydroxymethyl)-2-oxabicyclo[3.1.0]hexane-6-carboxylate (14). To a stirred ice-cooled solution of **13** (2.45 g, 11.4 mmol) in anhydrous THF (45 mL) under a nitrogen atmosphere, a suspension of LAH (260 mg, 6.87 mmol, 0.6 equiv.) in anhydrous THF (5 mL) was added dropwise within 10 min. The reaction mixture was stirred for 45 min at 0 °C. After dropwise addition of water (260 μL) the mixture was stirred for another 30 min. Then a 15% aqueous NaOH solution (260 μL) was added, followed by water (780 μL). The mixture was warmed to room temperature, treated with $MgSO_4$ and filtered through a Celite pad. The solvent was evaporated under reduced pressure. The crude product was

purified by chromatography (PE–EtOAc 1 : 1) to obtain compound **14** (1.85 g, 9.94 mmol, 87%) as a colorless oil.

R_f = 0.34 (PE–EtOAc 1 : 1), 0.49 (EtOAc); $[\alpha]_D^{20}$ = +63.7 (DCM, c = 1.0); ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 4.60–4.50 (m, 1H), 4.18 (d, J = 5.9 Hz, 1H), 4.07 (q, J = 7.1 Hz, 2H), 3.58 (ddd, J = 11.9, 6.0, 3.2 Hz, 1H), 3.41–3.31 (m, 1H), 2.37 (ddd, J = 13.1, 8.8, 7.0 Hz, 1H), 2.26–2.11 (m, 1H), 2.14 (br s, 1H), 1.81 (ddd, J = 13.1, 7.7, 1.1 Hz, 1H), 1.72 (dd, J = 3.8, 0.8 Hz, 1H), 1.22 (t, J = 7.1 Hz, 3H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ_C = 170.50 (C_q), 87.00 (+), 67.37 (+), 64.91 (−), 60.59 (−), 33.39 (+), 30.31 (−), 27.56 (+), 14.33 (+); IR (ATR): $\tilde{\nu}$ (cm^{-1}) = 3460 (br), 2978, 2939, 2880, 1713, 1454, 1407, 1386, 1309, 1269, 1175, 1111, 1074, 1048, 980, 878, 851, 808, 712; MS (ESI): m/z (%) = 186.9 (40) [MH^+], 228.0 (100) [MH^+MeCN], 373.1 (40) [$2MH^+$], 390.0 (30) [$2MNH_4^+$]; HRMS (ESI): calcd for $C_9H_{15}O_4$ [MH^+] 187.0965, found 187.0966.

Preparation of (1*S*,3*R*,5*S*,6*S*)-ethyl 3-formyl-2-oxabicyclo-[3.1.0]hexane-6-carboxylate (15). Dess–Martin periodinane (4.24 g, 10.0 mmol, 1.05 equiv.) was added to a solution of alcohol **14** (1.77 g, 9.52 mmol) in DCM (95 mL) at room temperature and stirred for 1 h. After completion the reaction was quenched with a mixture of saturated aqueous $Na_2S_2O_3$ solution (50 mL) and saturated aqueous $NaHCO_3$ solution (50 mL). The mixture was stirred for 15 min; afterwards the organic layer was separated and the aqueous layer was extracted with DCM (2 × 50 mL). The combined organic layers were washed with brine (1 × 50 mL), dried over $MgSO_4$ and evaporated *in vacuo*. The crude product was purified by chromatography (PE–EtOAc 1 : 1) to give compound **15** (1.54 mg, 8.37 mmol, 88%) as a yellowish oil.

R_f = 0.31 (PE–EtOAc 1 : 1); $[\alpha]_D^{20}$ = +44.8 (DCM, c = 0.5); ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 9.59 (s, 1H), 4.64 (dd, J = 10.6, 3.8 Hz, 1H), 4.34 (dd, J = 5.7, 0.8 Hz, 1H), 4.08 (q, J = 7.1 Hz, 2H), 2.51 (ddd, J = 13.4, 10.6, 5.7 Hz, 1H), 2.36 (dd, J = 13.3, 3.9 Hz, 1H), 2.20 (td, J = 5.5, 3.9 Hz, 1H), 1.46 (dd, J = 3.8, 1.0 Hz, 1H), 1.23 (t, J = 7.1 Hz, 3H), 1.26–1.19 (m, 1H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ_C = 203.36 (+), 170.20 (C_q), 85.29 (+), 67.22 (+), 60.85 (−), 30.26 (−), 27.54 (+), 25.21 (+), 14.31 (+); IR (ATR): $\tilde{\nu}$ (cm^{-1}) = 3435 (br), 2984, 1712, 1451, 1411, 1385, 1323, 1298, 1273, 1177, 1107, 1051, 1035, 976, 926, 870, 849, 796, 749, 702; MS (CI): m/z (%) = 185.0 (15) [MH^+], 202.1 (100) [MNH_4^+]; HRMS (ESI): calcd for $C_9H_{13}O_4$ [MH^+] 185.0808, found 185.0808.

Preparation of (1*S*,3*R*,5*S*,6*S*)-ethyl 3-(4-tosyl-4,5-dihydrooxazol-5-yl)-2-oxabicyclo[3.1.0]hexane-6-carboxylate (16). Finely powdered NaCN (70 mg, 1.42 mmol, 0.18 equiv.) was added in one portion to a stirred solution of TosMIC (1.70 g, 8.70 mmol, 1.1 equiv.) and aldehyde **15** (1.46 g, 7.91 mmol) in anhydrous EtOH (80 mL) at room temperature under a nitrogen atmosphere. After 1 h, the solvent was evaporated under reduced pressure. The residue was dissolved in $CHCl_3$ (100 mL) and washed with a saturated aqueous $NaHCO_3$ solution (1 × 100 mL). The aqueous layer was extracted with $CHCl_3$ (1 × 40 mL) and the combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography (PE–EtOAc 1 : 1) afforded a 2 : 1 diastereomeric mixture of compound **16** (2.10 g, 5.54 mmol, 70%) as a yellowish foam.

Major: R_f = 0.29 (PE–EtOAc 1 : 1); ^{1}H-NMR (400 MHz, $CDCl_3$): δ_H = 7.80 (d, J = 8.2 Hz, 2H), 7.37 (d, J = 8.2 Hz, 2H), 7.01 (s, 1H), 4.91 (dd, J = 5.8, 4.4 Hz, 1H), 4.86 (dd, J = 5.9, 1.7 Hz, 1H), 4.60–4.52 (m, 1H), 4.17 (d, J = 6.0 Hz, 1H), 4.09 (q, J = 7.1 Hz, 2H), 2.51–2.41 (m, 1H), 2.44 (s, 3H), 2.27–2.20 (m, 1H), 1.88 (ddd, J = 13.6, 8.2, 1.2 Hz, 1H), 1.73 (d, J = 3.8, 1H), 1.23 (t, J = 7.1, 3H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ_C = 169.87 (C_q), 159.32 (+), 145.83 (C_q), 132.87 (C_q), 129.97 (+), 129.63 (+), 86.60 (+), 85.71 (+), 79.54 (+), 67.06 (+), 60.71 (−), 33.52 (+), 30.01 (−), 26.70 (+), 21.82 (+), 14.29 (+).

Minor: R_f = 0.29 (PE–EtOAc 1 : 1); ^{1}H-NMR (400 MHz, $CDCl_3$): δ_H = 7.79 (d, J = 8.3 Hz, 2H), 7.36 (d, J = 8.3 Hz, 2H), 7.00 (s, 1H), 4.99 (dd, J = 6.4, 1.7 Hz, 1H), 4.88–4.81 (m, 1H), 4.63–4.55 (m, 1H), 4.15 (d, J = 6.0 Hz, 1H), 4.07 (q, J = 7.1 Hz, 2H), 2.57–2.49 (m, 1H), 2.44 (s, 3H), 2.27–2.20 (m, 1H), 2.12 (ddd, J = 13.4, 8.2, 1.0 Hz, 1H), 1.73, (d, J = 3.8, 1H), 1.21 (t, J = 7.1, 3H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ_C = 170.04 (C_q), 159.15 (+), 145.79 (C_q), 133.02 (C_q), 129.97 (+), 129.53 (+), 87.27 (+), 86.65 (+), 68.76 (+), 67.33 (+), 60.64 (−), 33.15 (+), 30.58 (−), 27.11 (+), 21.82 (+), 14.29 (+).

Data for the isomeric mixture: IR (ATR): $\tilde{\nu}$ (cm^{-1}) = 2978, 2936, 1716, 1618, 1453, 1408, 1387, 1306, 1177, 1149, 1108, 1086, 1075, 975, 934, 852, 813, 707, 668; Elemental analysis calcd (%) for $C_{16}H_{21}NO_6S{\cdot}1.2H_2O$: C 53.91, H 5.88, N 3.49, S 8.00, found C 53.83, H 5.93, N 3.34, S 7.92.

Preparation of (1*S*,3*R*,5*S*,6*S*)-ethyl 3-((*tert*-butyldimethyl-silyloxy)methyl)-2-oxabicyclo[3.1.0]hexane-6-carboxylate (18). To a stirred solution of alcohol **14** (2.53 g, 13.6 mmol) in DCM (45 mL) under a nitrogen atmosphere, anhydrous NEt_3 (2.8 mL, 20 mmol, 1.5 equiv.), TBSCl (2.48 g, 16.5 mmol, 1.2 equiv.) and DMAP (83 mg, 0.68 mmol, 0.05 equiv.) were added successively. The reaction mixture was stirred for 18 h at room temperature and then quenched with a saturated aqueous NH_4Cl solution (40 mL). The layers were separated and the aqueous layer was extracted with DCM (2 × 20 mL). The combined organic phases were dried over $MgSO_4$, filtered and concentrated *in vacuo.* Purification by column chromatography (PE–EtOAc 5 : 1) afforded compound **18** (3.88 g, 12.9 mmol, 95%) as a colorless oil.

R_f = 0.52 (PE–EtOAc 5 : 1); $[\alpha]_D^{20}$ = +35.0 (DCM, c = 1.0); ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 4.48 (ddt, J = 9.1, 7.0, 4.1 Hz, 1H), 4.13 (d, J = 5.9 Hz, 1H), 4.06 (q, J = 7.1 Hz, 2H), 3.54 (dd, J = 11.0, 4.0 Hz, 1H), 3.45 (dd, J = 11.0, 4.2 Hz, 1H), 2.33 (ddd, J = 13.0, 9.2, 6.9 Hz, 1H), 2.21–2.11 (m, 1H), 1.91 (ddd, J = 13.0, 6.9, 0.8 Hz, 1H), 1.87 (dd, J = 3.9, 0.9 Hz, 1H), 1.21 (t, J = 7.1 Hz, 3H), 0.89 (s, 9H), 0.05 (s, 3H), 0.04 (s, 3H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ_C = 170.74 (+), 86.16 (+), 67.32 (+), 65.31 (−), 60.22 (−), 32.22 (+), 30.13 (−), 27.42 (+), 25.94 (+), 18.38 (C_q), 14.23 (+), −5.35 (+), −5.43 (+); IR (ATR): $\tilde{\nu}$ (cm^{-1}) = 2955, 2931, 2858, 1720, 1463, 1408, 1309, 1256, 1176, 1112, 1096, 1054, 979, 839, 778; MS (ESI): m/z (%) = 301.0 (100) [MH^+]; HRMS (EI): calcd for $C_{15}H_{28}SiO_4$ [$M^{+\cdot}$] 300.1757, found 300.1760.

Preparation of ((1*S*,3*R*,5*S*,6*R*)-3-((*tert*-butyldimethylsilyloxy)-methyl)-2-oxabicyclo[3.1.0]hexan-6-yl)-methanol (19). To a

stirred ice-cooled solution of **18** (3.88 g, 12.9 mmol) in anhydrous THF (50 mL) under a nitrogen atmosphere, a suspension of LAH (412 mg, 10.9 mmol, 0.84 equiv.) in anhydrous THF (5 mL) was added dropwise within 10 min. The reaction mixture was stirred for 45 min at 0 °C. After dropwise addition of water (0.41 mL) the mixture was stirred for another 30 min. Then a 15% aqueous NaOH solution (0.41 mL) was added, followed by water (1.24 mL). The mixture was warmed to room temperature, treated with $MgSO_4$ and filtered through a Celite pad. The solvent was evaporated under reduced pressure. The crude product was purified by column chromatography (PE–EtOAc 3 : 1 then 1 : 1) to obtain compound **19** (3.17 g, 12.3 mmol, 95%) as a colorless oil.

R_f = 0.30 (PE–EtOAc 1 : 1); $[\alpha]_D^{20}$ = +44.6 (DCM, *c* = 1.0); ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 4.43 (tt, *J* = 7.9, 4.9 Hz, 1H), 3.73 (dd, *J* = 6.3, 1.1 Hz, 1H), 3.46 (d, *J* = 4.9 Hz, 2H), 3.37–3.21 (m, 2H), 2.23 (ddd, *J* = 12.8, 8.3, 7.2 Hz, 1H), 2.21 (br s, 1H), 1.68 (ddd, *J* = 12.8, 7.6, 1.5 Hz, 1H), 1.51–1.43 (m, 1H), 1.21–1.13 (m, 1H), 0.85 (s, 9H), 0.01 (s, 6H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ_C = 87.94 (+), 66.01 (−), 64.33 (+), 62.32 (−), 34.78 (+), 31.54 (−), 26.01 (+), 21.98 (+), 18.45 (C_q), −5.25 (+), −5.27 (+); IR (ATR): $\tilde{\nu}$ (cm^{-1}) = 3386 (br), 2953, 2929, 2858, 1463, 1410, 1254, 1130, 1095, 1023, 837, 777, 669; MS (ESI): *m*/*z* (%) = 241.0 (78) [$MH^+\Delta H_2O$], 259.0 (55) [MH^+], 276.1 (20) [MNH_4^+], 300.0 (100) [MH^+MeCN], 481.2 (35) [$2MH^+\Delta 2H_2O$], 499.2 (85) [$2MH^+\Delta H_2O$], 517.2 (50) [$2MH^+$]; HRMS (ESI): calcd for $C_{13}H_{27}O_3Si$ [MH^+] 259.1724, found 259.1731.

Preparation of (((1*S*,3*R*,5*S*,6*R*)-6-(benzyloxymethyl)-2-oxabicyclo[3.1.0]hexan-3-yl)methoxy)(*tert*-butyl)-dimethylsilane (20). To a solution of alcohol **19** (1.00 g, 3.87 mmol) in anhydrous DMF (25 mL), NaH (309 mg, 60 wt% in mineral oil, 7.74 mmol, 2.0 equiv.) was added in one portion at 0 °C under a nitrogen atmosphere. The resulting suspension was stirred at 0 °C for 10 min, and then benzyl bromide (919 μL, 7.74 mmol, 2.0 equiv.) was added dropwise at 0 °C. The mixture was allowed to warm to room temperature and stirred for 2 h. MeOH (5 mL) was added carefully to quench the reaction. The solvent was evaporated under reduced pressure. The residue was diluted in DCM and washed with a saturated aqueous NH_4Cl solution (20 mL). The aqueous phase was extracted with DCM (3 × 20 mL). The organic layers were combined, dried over $MgSO_4$ and concentrated *in vacuo*. The resulting residue was purified by column chromatography (PE–EtOAc 9 : 1) to obtain compound **20** (1.14 g, 3.28 mmol, 85%) as a colorless oil.

R_f = 0.22 (PE–EtOAc 9 : 1), 0.53 (PE–EtOAc 3 : 1); $[\alpha]_D^{20}$ = +22.5 (DCM, *c* = 1.0); ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 7.38–7.22 (m, 5H), 4.46 (ddd, *J* = 9.8, 8.1, 4.9 Hz, 3H), 3.75 (dd, *J* = 6.2, 1.1 Hz, 1H), 3.50 (d, *J* = 5.0 Hz, 2H), 3.35 (dd, *J* = 10.6, 6.6 Hz, 1H), 3.09 (dd, *J* = 10.6, 7.6 Hz, 1H), 2.27 (ddd, *J* = 12.8, 8.3, 7.2 Hz, 1H), 1.74 (ddd, *J* = 12.8, 7.5, 1.5 Hz, 1H), 1.61–1.45 (m, 1H), 1.23 (dddd, *J* = 7.7, 6.7, 4.0, 1.2 Hz, 1H), 0.90 (s, *J* = 2.9 Hz, 9H), 0.06 (d, *J* = 1.0 Hz, 6H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ_C = 138.50 (C_q), 128.49 (+), 127.75 (+), 127.68 (+), 87.80 (+), 72.50 (−), 69.61 (−), 66.20 (−), 64.62 (+), 31.99 (+), 31.66 (−), 26.10 (+), 22.45 (+), 18.54 (C_q), −5.18 (+); IR (ATR): $\tilde{\nu}$ (cm^{-1}) = 3038, 2932, 2858, 1461, 1380, 1254, 1182, 1132, 1091, 1009, 840, 778, 738, 697; MS (ESI): *m*/*z* (%) = 241.1 (100) [$M^+\Delta C_7H_7O$], 349.1 (15) [MH^+], 366.1 (65) [MNH_4^+], 714.5 (20) [$2MNH_4^+$]; HRMS (ESI): calcd for $C_{20}H_{33}O_3Si$ [MH^+] 349.2193, found 349.2197.

Preparation of ((1*S*,3*R*,5*S*,6*R*)-6-(benzyloxymethyl)-2-oxabicyclo[3.1.0]hexan-3-yl)methanol (21). To a solution of compound **20** (3.03 mg, 8.69 mmol) in anhydrous THF (60 mL) a solution of TBAF·$3H_2O$ (4.11 mg, 13.0 mmol, 1.5 equiv.) in anhydrous THF (30 mL) was added and stirred for 13 h at room temperature. After evaporating the solvent the crude product was purified by column chromatography (EtOAc) to give **21** (1.94 mg, 8.28 mmol, 95%) as a colorless oil.

R_f = 0.42 (EtOAc); $[\alpha]_D^{20}$ = +47.2 (DCM, *c* = 1.0); ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 7.39–7.23 (m, 5H), 4.59–4.49 (m, 1H), 4.48 (d, *J* = 2.2 Hz, 2H), 3.78 (dd, *J* = 6.2, 1.1 Hz, 1H), 3.56 (ddd, *J* = 11.4, 5.4, 3.2 Hz, 1H), 3.42–3.31 (m, 1H), 3.26 (dd, *J* = 10.5, 7.0 Hz, 1H), 3.16 (dd, *J* = 10.5, 7.1 Hz, 1H), 2.26 (ddd, *J* = 12.8, 8.1, 7.3 Hz, 1H), 2.07 (br s, 1H), 1.69 (ddd, *J* = 12.8, 8.0, 1.6 Hz, 1H), 1.55 (dddd, *J* = 7.6, 6.0, 4.0, 1.6 Hz, 1H), 1.20 (tdd, *J* = 7.1, 4.0, 1.2 Hz, 1H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ_C = 138.35 (C_q), 128.51 (+), 127.78 (+), 127.74 (+), 88.08 (+), 72.67 (−), 69.47 (−), 65.36 (−), 64.71 (+), 32.57 (+), 31.17 (−), 22.51 (+); IR (ATR): $\tilde{\nu}$ (cm^{-1}) = 3421 (br), 3027, 2924, 2862, 1497, 1454, 1414, 1360, 1180, 1087, 1071, 1028, 987, 844, 810, 739, 698, 614; MS (ESI): *m*/*z* (%) = 235.0 (5) [MH^+], 469.0 (25) [$2MH^+$], 486.1 (75) [$2MH_4^+$], 491.1 (100) [$2MNa^+$]; HRMS (ESI): calcd for $C_{14}H_{18}NaO_3$ [MNa^+] 257.1148, found 257.1153.

Preparation of (1*S*,3*R*,5*S*,6*R*)-6-(benzyloxymethyl)-2-oxabicyclo[3.1.0]hexane-3-carbaldehyde (22). To a stirred solution of alcohol **21** (4.35 g, 18.6 mmol) in DCM (150 mL) was added in one portion Dess–Martin periodinane (8.66 g, 20.4 mmol, 1.1 equiv.) at room temperature. After 2 h, a saturated aqueous $NaHCO_3$ (60 mL) and a saturated aqueous $Na_2S_2O_3$ (60 mL) were added. The mixture was stirred for another 15 min. After completion the reaction was quenched with a mixture of saturated aqueous $Na_2S_2O_3$ solution (60 mL) and saturated aqueous $NaHCO_3$ solution (60 mL). The mixture was stirred for 15 min, then the organic layer was separated and the aqueous layer was extracted with DCM (2 × 50 mL). The combined organic layers were washed with brine (1 × 50 mL), dried over $MgSO_4$ and evaporated *in vacuo*. Purification by column chromatography (PE–EtOAc 1 : 1) afforded compound **22** (3.87 g, 16.7 mmol, 90%) as a colorless oil.

R_f = 0.31 (PE–EtOAc 1 : 1); $[\alpha]_D^{20}$ = +57.5 (DCM, *c* = 1.0); ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 9.57 (d, *J* = 0.8 Hz, 1H), 7.39–7.21 (m, 5H), 4.58 (ddd, *J* = 10.2, 3.9, 0.7 Hz, 1H), 4.47 (d, *J* = 1.1 Hz, 2H), 3.95 (dd, *J* = 5.9, 1.3 Hz, 1H), 3.30 (dd, *J* = 10.5, 6.7 Hz, 1H), 3.17 (dd, *J* = 10.5, 7.1 Hz, 1H), 2.40 (ddd, *J* = 13.0, 10.3, 5.9 Hz, 1H), 2.26 (ddd, *J* = 13.0, 4.0, 0.6 Hz, 1H), 1.53 (tdd, *J* = 5.8, 4.0, 0.6 Hz, 1H), 0.97 (tdd, *J* = 6.9, 3.9, 1.3 Hz, 1H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ_C = 204.38 (+), 138.19 (C_q), 128.54 (+), 127.81 (+), 127.77 (+), 86.02 (+), 72.77 (−), 69.06 (−), 64.86 (+), 31.08 (−), 25.84 (+), 20.30 (+); IR (ATR): $\tilde{\nu}$ (cm^{-1}) = 3031, 2942, 2860, 1730, 1497, 1455, 1422, 1362, 1091, 1076, 1030, 988, 738, 699; MS (EI): *m*/*z* (%) = 91.1 (100) [$C_7H_7^+$], 231.1 (<1)

[M˙ΔH$^+$]; HRMS (ESI): calcd for $C_{14}H_{20}NO_3$ [MNH_4^+] 250.1438, found 250.1439.

Preparation of 5-((1*S*,3*R*,5*S*,6*R*)-6-(benzyloxymethyl)-2-oxabicyclo[3.1.0]hexan-3-yl)-4-tosyl-4,5-dihydro-oxazole (23). Finely powdered NaCN (28 mg, 0.57 mmol, 0.22 equiv.) was added in one portion to a stirred solution of TosMIC (555 mg, 2.84 mmol, 1.1 equiv.) and aldehyde **22** (600 mg, 2.58 mmol) in anhydrous EtOH (25 mL) at room temperature under a nitrogen atmosphere. The reaction mixture was stirred for 2 h. The solvent was evaporated under reduced pressure. The residue was dissolved in $CHCl_3$ (30 mL) and washed with a saturated aqueous $NaHCO_3$ solution (30 mL). The aqueous layer was extracted with $CHCl_3$ (2 × 15 mL) and the combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by column chromatography (PE–EtOAc 1 : 1) afforded a 3 : 2 diastereomeric mixture of compound **23** (854 mg, 2.00 mmol, 77%) as yellowish foam.

Major: R_f = 0.41 (PE–EtOAc 1 : 1); ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 7.81 (d, *J* = 8.3 Hz, 2H), 7.39–7.33 (m, 2H), 7.34–7.20 (m, 5H), 7.00–6.96 (m, 1H), 4.94–4.91 (m, 1H), 4.92–4.89 (m, 1H), 4.62–4.52 (m, 1H), 4.51–4.45 (m, 2H), 3.79 (dd, *J* = 6.2, 0.9 Hz, 1H), 3.31 (dd, *J* = 10.5, 6.5 Hz, 1H), 3.11 (dd, *J* = 10.5, 7.3 Hz, 1H), 2.44 (s, 3H), 2.40–2.20 (m, 1H), 1.81 (ddd, *J* = 13.3, 8.8, 1.3 Hz, 1H), 1.65–1.53 (m, 1H), 1.30–1.18 (m, 1H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ_C = 159.28 (+), 145.62 (C_q), 138.15 (C_q), 133.09 (C_q), 129.89 (+), 129.60 (+), 128.47 (+), 127.75 (+), 127.71 (+), 86.80 (+), 86.36 (+), 79.63 (+), 72.70 (−), 69.00 (−), 64.69 (+), 33.03 (+), 30.85 (−), 22.12 (+), 21.79 (+).

Minor: R_f = 0.41 (PE–EtOAc 1 : 1); ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 7.80 (d, *J* = 8.3 Hz, 2H), 7.39–7.33 (m, 2H), 7.34–7.20 (m, 5H), 7.00–6.96 (m, 1H), 4.98 (dd, *J* = 6.2, 1.7 Hz, 1H), 4.84 (dd, *J* = 6.2, 3.4 Hz, 1H), 4.58–4.50 (m, 1H), 4.47–4.42 (m, 2H), 3.76 (dd, *J* = 6.3, 0.8 Hz, 1H), 3.26 (dd, *J* = 10.5, 6.8 Hz, 1H), 3.14 (dd, *J* = 10.5, 7.2 Hz, 1H), 2.44 (s, 3H), 2.43–2.33 (m, 1H), 2.01 (ddd, *J* = 13.0, 8.4, 1.6 Hz, 1H), 1.65–1.53 (m, 1H), 1.30–1.18 (m, 1H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ_C = 159.35 (+), 145.62 (C_q), 138.19 (C_q), 133.09 (C_q), 129.89 (+), 129.52 (+), 128.45 (+), 127.75 (+), 127.71 (+), 87.66 (+), 87.31 (+), 79.13 (+), 72.63 (−), 69.04 (−), 64.92 (+), 32.83 (+), 31.19 (−), 22.12 (+), 21.79 (+).

Data for the isomeric mixture: IR (ATR): $\tilde{\nu}$ (cm^{-1}) = 3033, 2948, 2861, 1616, 1597, 1486, 1455, 1362, 1319, 1304, 1292, 1148, 1108, 1086, 1071, 1028, 939, 848, 813, 739, 700, 664, 651, 587, 533.

Preparation of 5-((1*S*,3*R*,5*S*,6*R*)-6-(benzyloxymethyl)-2-oxabicyclo[3.1.0]hexan-3-yl)-1*H*-imidazole (24a) and 5-((1*S*,3*S* ,5*S*,6*R*)-6-(benzyloxymethyl)-2-oxabicyclo[3.1.0]hexan-3-yl)-1*H*-imidazole (24b). In a sealable pressure tube, oxazoline **23** (1.70 g, 3.98 mmol) and a saturated solution of NH_3 in anhydrous MeOH (40 mL, 70 equiv.) were heated at 95 °C for 16 h. Within this time the solution turned red. After cooling, the solvent was removed under reduced pressure. The residue was purified by column chromatography (DCM–saturated NH_3 in MeOH 9 : 1) to give a 5 : 1 epimeric mixture of compounds **24a** and **24b** (726 mg, 2.69 mmol, 68%) as a colorless oil.

24a: R_f = 0.22 (DCM–saturated NH_3 in MeOH 9 : 1); ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 8.28 (br s, 1H), 7.48 (s, 1H), 7.38–7.22 (m, 5H), 6.83 (s, 1H), 5.42 (t, *J* = 7.5 Hz, 1H), 4.47 (d, *J* = 1.7 Hz, 2H), 3.86 (dd, *J* = 6.2, 1.2 Hz, 1H), 3.29–3.15 (m, 2H), 2.64–2.51 (m, 1H), 2.15 (ddd, *J* = 12.8, 7.0, 1.4 Hz, 1H), 1.66–1.57 (m, 1H), 1.45–1.34 (m, 1H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ_C = 139.57 (C_q), 138.27 (C_q), 135.36 (+), 128.54 (+), 127.87 (+), 127.80 (+), 116.10 (+), 81.53 (+), 72.69 (−), 69.71 (−), 64.45 (+), 35.21 (−), 30.89 (+), 22.67 (+).

24b: R_f = 0.22 (DCM–saturated NH_3 in MeOH 9 : 1); ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 8.28 (br s, 1H), 7.55 (s, 1H), 7.38–7.22 (m, 5H), 6.90 (s, 1H), 4.76 (t, *J* = 8.2 Hz, 1H), 4.50 (d, *J* = 2.6 Hz, 2H), 3.90–3.78 (m, 1H), 3.42–3.31 (m, 1H), 3.23–3.11 (m, 1H), 2.38–2.23 (m, 2H), 1.59–1.48 (m, 1H), 1.19–1.05 (m, 1H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ_C = 138.22 (C_q), 137.81 (C_q), 135.62 (+), 128.54 (+), 127.86 (+), 127.80 (+), 115.90 (+), 74.14 (+), 72.72 (−), 69.90 (−), 62.56 (+), 34.54 (−), 21.40 (+), 20.80 (+).

Data for the isomeric mixture: IR (ATR): $\tilde{\nu}$ (cm^{-1}) = 3090 (br), 2936, 2858, 1716, 1670, 1496, 1453, 1362, 1313, 1273, 1087, 1071, 1027, 839, 738, 698, 626; MS (ESI): *m*/*z* (%) = 271.0 (100) [MH^+], 312.1 (30) [MH^+MeCN], 541.2 (40) [$2MH^+$]; HRMS (ESI): calcd for $C_{16}H_{19}N_2O_2$ [MH^+] 271.1441, found 271.1446.

Preparation of ethyl 5-((1*S*,3*R*,5*S*,6*R*)-6-(benzyloxymethyl)-2-oxabicyclo[3.1.0]hexan-3-yl)-1*H*-imidazole-1-carboxylate (25a) and ethyl 5-((1*S*,3*S*,5*S*,6*R*)-6-(benzyloxymethyl)-2-oxabicyclo-[3.1.0]hexan-3-yl)-1*H*-imidazole-1-carboxylate (25b). A solution of a 3 : 1 epimeric mixture of imidazole **24a** and **24b** (1.10 g, 4.06 mmol), ethyl chloroformate (733 μL, 7.72 mmol, 1.9 equiv.), anhydrous pyridine (623 μL, 7.72 mmol, 1.9 equiv.) and DMAP (79 mg, 0.65 mmol, 0.16 equiv.) in benzene (80 mL) was stirred for 10 min at 50 °C. After the addition of water (5 mL), the solvent was evaporated. A saturated aqueous NH_4Cl solution (50 mL) was added and extracted with DCM (3 × 25 mL). The extract was washed with brine, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residual oil was purified by column chromatography (PE–EtOAc 1 : 1) to give a 3 : 1 epimeric mixture of compounds **25a** and **25b** (1.01 g, 2.95 mmol, 73%) as a colorless oil.

25a: R_f = 0.26 (PE–EtOAc 1 : 1); ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 8.06 (d, *J* = 1.3 Hz, 1H), 7.37–7.27 (m, 5H), 7.28 (t, *J* = 1.2 Hz, 1H), 5.38 (ddd, *J* = 8.4, 6.7, 0.9 Hz, 1H), 4.48 (d, *J* = 3.8 Hz, 2H), 4.45 (q, *J* = 7.1 Hz, 2H), 3.88 (dd, *J* = 6.1, 1.2 Hz, 1H), 3.33 (dd, *J* = 10.5, 6.7 Hz, 1H), 3.13 (dd, *J* = 10.6, 7.4 Hz, 1H), 2.61 (ddd, *J* = 12.8, 8.6, 6.9 Hz, 1H), 2.15 (ddd, *J* = 12.8, 6.7, 1.4 Hz, 1H), 1.67–1.57 (m, 1H), 1.47–1.39 (m, 1H), 1.42 (t, *J* = 7.1 Hz, 3H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ_C = 148.63 (C_q), 145.81 (C_q), 138.32 (C_q), 137.17 (+), 128.41 (+), 127.70 (+), 127.26 (+), 113.26 (+), 81.75 (+), 72.52 (−), 69.50 (−), 64.68 (+), 64.45 (−), 43.88 (−), 30.63 (+), 22.66 (+), 14.21 (+).

25b: R_f = 0.24 (PE–EtOAc 1 : 1), 0.54 (EtOAc); ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 8.07 (d, *J* = 1.3 Hz, 1H), 7.35–7.26 (m, 5H), 7.33–7.31 (m, 1H), 4.72 (dd, *J* = 8.8, 7.4 Hz, 1H), 4.49 (d, *J* = 3.1 Hz, 2H), 4.43 (q, *J* = 7.1 Hz, 2H), 3.91 (dd, *J* = 5.5, 1.6 Hz, 1H), 3.38 (dd, *J* = 10.5, 6.2 Hz, 1H), 3.13 (dd, *J* = 10.5, 7.5 Hz, 2H), 2.35 (dd, *J* = 12.3, 7.3 Hz, 1H), 2.24 (ddd, *J* = 12.4,

9.0, 5.0 Hz, 1H), 1.59–1.50 (m, 2H), 1.40 (t, J = 7.1 Hz, 3H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ_C = 148.58 (C_q), 143.45 (C_q), 138.34 (C_q), 137.26 (+), 128.44 (+), 127.72 (+), 127.67 (+), 113.85 (+), 74.88 (+), 72.58 (−), 69.71 (−), 64.49 (−), 62.91 (+), 34.52 (−), 22.14 (+), 20.86 (+), 14.21 (+).

Data for isomeric mixture: IR (ATR): $\tilde{\nu}$ (cm^{-1}) = 3032, 2937, 2859, 1759, 1482, 1454, 1409, 1388, 1336, 1252, 1207, 1093, 1069, 1019, 843, 769, 740, 699, 607; MS (ESI): m/z (%) = 342.9 (100) [MH^+]; HRMS (ESI): calcd for $C_{19}H_{23}N_2O_4$ [MH^+] 343.1652, found 343.1656.

Preparation of ethyl 5-((1*S*,3*R*,5*S*,6*R*)-6-(hydroxymethyl)-2-oxabicyclo[3.1.0]hexan-3-yl)-1*H*-imidazole-1-carboxylate (26a) and ethyl 5-((1*S*,3*S*,5*S*,6*R*)-6-(hydroxymethyl)-2-oxabicyclo-[3.1.0]hexan-3-yl)-1*H*-imidazole-1-carboxylate (26b). A 3 : 1 epimeric mixture of compounds **25a** and **25b** (134 mg, 0.39 mmol), $Pd(OH)_2$–C (20%, 95 mg) and cyclohexene (1.6 mL, 16 mmol, 40 equiv.) in anhydrous EtOH (15 mL) was refluxed for 1 h. After filtration through a Celite pad the solvent was evaporated. The residue was purified by column chromatography (EtOAc, then EtOAc–MeOH 19 : 1) to afford a 3 : 1 epimeric mixture of alcohol **26a** and **26b** (72 mg, 0.29 mmol, 73%) as a colorless foam. Separation of the epimers by iterated chromatography.

26a: R_f = 0.38 (EtOAc–MeOH 19 : 1); $[\alpha]_D^{20}$ = −4.6 (DCM, c = 1.0); ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 8.06 (d, J = 1.3 Hz, 1H), 7.29 (m, 1H), 5.38 (ddd, J = 8.5, 6.6, 0.9 Hz, 1H), 4.45 (q, J = 7.1 Hz, 2H), 3.90 (dd, J = 6.2, 1.3 Hz, 1H), 3.40 (dd, J = 11.6, 7.4 Hz, 1H), 3.33 (dd, J = 11.6, 7.3 Hz, 1H), 2.61 (ddd, J = 12.8, 8.6, 6.9 Hz, 1H), 2.16 (ddd, J = 12.8, 6.7, 1.5 Hz, 1H), 1.75 (br s, 1H), 1.62 (tdd, J = 6.8, 4.0, 1.5 Hz, 1H), 1.50–1.43 (m, 1H), 1.42 (t, J = 7.1 Hz, 3H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ_C = 148.69 (C_q), 145.65 (C_q), 137.31 (+), 113.54 (+), 81.81 (+), 64.60 (−), 64.51 (+), 62.61 (−), 34.83 (−), 33.45 (+), 22.44 (+), 14.30 (+); IR (ATR): $\tilde{\nu}$ (cm^{-1}) = 3373 (br), 2982, 2943, 2876, 1758, 1489, 1409, 1336, 1253, 1176, 1103, 1068, 1018, 847, 768, 606; MS (ESI): m/z (%) = 252.9 (40) [MH^+], 294.0 (15) [MH^+MeCN], 505.1 (100) [$2MH^+$]; HRMS (ESI): calcd for $C_{12}H_{17}N_2O_4$ [MH^+] 253.1183, found 253.1190.

26b: R_f = 0.36 (EtOAc–MeOH 19 : 1); $[\alpha]_D^{20}$ = +10.5 (DCM, c = 1.0); ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 8.05 (d, J = 1.2 Hz, 1H), 7.32–7.29 (m, 1H), 4.70 (dd, J = 8.6, 7.5 Hz, 1H), 4.42 (q, J = 7.1 Hz, 2H), 3.91 (dd, J = 5.5, 1.6 Hz, 1H), 3.41 (dd, J = 11.5, 6.8 Hz, 1H), 3.32 (dd, J = 11.5, 7.1 Hz, 1H), 2.32 (dd, J = 12.4, 7.2 Hz, 1H), 2.21 (ddd, J = 12.4, 9.0, 5.0 Hz, 1H), 1.58–1.47 (m, 2H), 1.38 (t, J = 7.1 Hz, 3H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ_C = 148.66 (C_q), 143.43 (C_q), 137.38 (+), 113.98 (+), 75.10 (+), 64.64 (−), 62.86 (−), 62.42 (+), 34.59 (−), 24.90 (+), 20.54 (+), 14.30 (+); IR (ATR): $\tilde{\nu}$ (cm^{-1}) = 3349 (br), 2939, 2869, 1760, 1487, 1409, 1342, 1254, 1123, 1018, 852, 768, 607; MS (ESI): m/z (%) = 252.8 (100) [MH^+], 505.1 (30) [$2MH^+$]; HRMS (ESI): calcd for $C_{12}H_{17}N_2O_4$ [MH^+] 253.1183, found 253.1188.

Preparation of ethyl 5-((1*S*,3*R*,5*S*,6*R*)-6-((1,3-dioxoisoindolin-2-yl)methyl)-2-oxabicyclo[3.1.0]hexan-3-yl)-1*H*-imidazole-1-carboxylate (27a), ethyl 5-((2*R*,4*S*,5*R*)-5-(1,3-dioxoisoindolin-2-yl)-4-vinyltetrahydrofuran-2-yl)-1*H*-imidazole-1-carboxylate (28a) and ethyl 5-((2*R*,4*S*,5*S*)-5-(1,3-dioxoisoindolin-2-yl)-4-vinyltetrahydrofuran-2-yl)-1*H*-imidazole-1-carboxylate (28b). DIAD (211 mg, 0.98 mmol, 1.5 equiv.) was added to a solution of PPh_3 (257 mg, 0.98 mmol, 1.5 equiv.) in anhydrous THF (7 mL) at room temperature under a nitrogen atmosphere. After stirring for 10 min phthalimide (144 mg, 0.98 mmol, 1.5 equiv.) was added and stirred for another 10 min. After addition of alcohol **26a** (165 mg, 0.65 mmol) in THF the reaction mixture was stirred overnight. The solvent was evaporated under reduced pressure. The residue was purified by column chromatography (PE–EtOAc 5 : 1 to EtOAc) to obtain **28a** (126 mg, 0.33 mmol, 51%), **28b** (25 mg, 0.07 mmol, 10%) and **27a** (72 mg, 0.19 mmol, 29%) as colorless oils.

28a: R_f = 0.37 (PE–EtOAc 1 : 1); $[\alpha]_D^{20}$ = −23.6 (DCM, c = 1.0); ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 8.10 (d, J = 1.2 Hz, 1H), 7.85 (dd, J = 5.5, 3.0 Hz, 2H), 7.72 (dd, J = 5.5, 3.0 Hz, 2H), 7.40 (dd, J = 1.3, 0.7 Hz, 1H), 5.93 (d, J = 7.5 Hz, 1H), 5.86 (ddd, J = 17.1, 10.3, 8.0 Hz, 1H), 5.50 (dd, J = 10.6, 4.9 Hz, 1H), 5.13 (dt, J = 17.1, 1.2 Hz, 1H), 5.10–5.04 (m, 1H), 4.44 (q, J = 7.1 Hz, 2H), 4.01–3.85 (m, 1H), 2.65 (ddd, J = 12.2, 7.2, 5.0 Hz, 1H), 2.24 (dt, J = 12.2, 11.3 Hz, 1H), 1.41 (t, J = 7.1 Hz, 3H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ_C = 167.92 (C_q), 148.63 (C_q), 143.33 (C_q), 137.37 (+), 136.39 (+), 134.37 (+), 132.02 (C_q), 123.60 (+), 117.58 (−), 114.40 (+), 85.06 (+), 76.33 (+), 64.59 (−), 46.68 (+), 39.34 (−), 14.27 (+); IR (ATR): $\tilde{\nu}$ (cm^{-1}) = 2985, 2927, 2853, 1760, 1716, 1468, 1410, 1367, 1332, 1252, 1210, 1084, 1019 977, 919, 891, 845, 769, 736, 721, 655, 611, 531; MS (ESI): m/z (%) = 381.9 (100) [MH^+], 422.9 (45) [MH^+MeCN], 763.2 (90) [$2MH^+$]; HRMS (ESI): calcd for $C_{20}H_{20}N_3O_5$ [MH^+] 382.1375, found 382.1365.

28b: R_f = 0.33 (PE–EtOAc 1 : 1); $[\alpha]_D^{20}$ = +73.6 (DCM, c = 0.5); ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 8.09 (d, J = 1.3 Hz, 1H), 7.83 (dd, J = 5.5, 3.0 Hz, 2H), 7.72 (dd, J = 5.5, 3.0 Hz, 2H), 7.61–7.53 (m, 1H), 6.21 (d, J = 8.6 Hz, 1H), 5.69 (ddd, J = 17.5, 10.2, 7.5 Hz, 1H), 5.20 (dt, J = 17.2, 1.3 Hz, 1H), 5.11–5.04 (m, 1H), 5.06–5.00 (m, 1H), 4.46 (q, J = 7.1 Hz, 2H), 3.56–3.41 (m, 1H), 2.93–2.77 (m, 1H), 2.51–2.40 (m, 1H), 1.42 (t, J = 7.1 Hz, 3H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ_C = 167.77 (C_q), 148.78 (C_q), 143.44 (C_q), 136.78 (+), 134.30 (+), 133.84 (+), 131.98 (C_q), 123.61 (+), 118.82 (−), 114.31 (+), 82.69 (+), 77.63 (+), 64.53 (−), 47.93 (+), 36.93 (−), 14.33 (+); IR (ATR): $\tilde{\nu}$ (cm^{-1}) = 2985, 2955, 2925, 1762, 1720, 1468, 1410, 1364, 1326, 1258, 1228, 1113, 1090, 1018, 901, 838, 792, 770, 722, 604, 530; MS (ESI): m/z (%) = 381.9 (100) [MH^+], 763.2 (10) [$2MH^+$]; HRMS (ESI): calcd for $C_{20}H_{20}N_3O_5$ [MH^+] 382.1397, found 382.1403.

27a: R_f = 0.27 (PE–EtOAc 1 : 1); $[\alpha]_D^{20}$ = −12.4 (DCM, c = 1.0); ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 8.03 (d, J = 1.2 Hz, 1H), 7.83 (dd, J = 5.5, 3.0 Hz, 2H), 7.70 (dd, J = 5.5, 3.0 Hz, 2H), 7.24 (t, J = 1.0 Hz, 1H), 5.36 (t, J = 7.7 Hz, 1H), 4.43 (q, J = 7.1 Hz, 2H), 4.05 (dd, J = 6.2, 0.9 Hz, 1H), 3.51 (dd, J = 14.3, 7.1 Hz, 1H), 3.38 (dd, J = 14.3, 8.2 Hz, 1H), 2.59 (ddd, J = 12.9, 8.4, 7.1 Hz, 1H), 2.07 (ddd, J = 12.7, 7.2, 1.6 Hz, 1H), 1.76 (ddd, J = 7.0, 3.9, 1.5 Hz, 1H), 1.57–1.48 (m, 1H), 1.40 (t, J = 7.1 Hz, 3H), 1.30–1.20 (m, 1H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ_C = 168.45 (C_q), 148.65 (C_q), 145.26 (C_q), 137.27 (+), 134.06 (C_q), 132.31 (+), 123.37 (+), 113.43 (+), 82.36 (+), 65.09 (+), 64.54 (−), 38.00 (−), 35.03 (−), 30.67 (+), 23.63 (+), 14.28 (+); IR (ATR): $\tilde{\nu}$ (cm^{-1}) =

2977, 2931, 1760, 1711, 1467, 1433, 1409, 1391, 1357, 1336, 1253, 1211, 1137, 1102, 1019, 950, 846, 769, 721, 614, 530; MS (ESI): *m*/*z* (%) = 381.9 (100) [MH^+], 763.3 (75) [$2MH^+$]; HRMS (ESI): calcd for $C_{20}H_{20}N_3O_5$ [MH^+] 382.1397, found 382.1396.

Preparation of ethyl 5-((1*S*,3*S*,5*S*,6*R*)-6-((1,3-dioxoisoindolin-2-yl)methyl)-2-oxabicyclo[3.1.0]hexan-3-yl)-1*H*-imidazole-1-carboxylate (27b). DIAD (54 mg, 0.25 mmol, 1.5 equiv.) was added to a solution of PPh_3 (65.5 mg, 0.25 mmol, 1.5 equiv.) in anhydrous THF (1.3 mL) at room temperature under a nitrogen atmosphere. After stirring for 10 min phthalimide (37 mg, 0.25 mmol, 1.5 equiv.) was added and stirred for another 10 min. After addition of alcohol **26b** (42 mg, 0.17 mmol) the reaction mixture was stirred for 18 h. The solvent was evaporated under reduced pressure. The residue was purified by column chromatography (PE–EtOAc 3 : 1 to 1 : 1) to obtain compound **27b** (17 mg, 0.04 mmol, 27%) as a colorless oil.

R_f = 0.43 (PE–EtOAc 1 : 3); $[\alpha]_D^{20}$ = +17.1 (DCM, *c* = 0.2); ^{1}H-NMR (400 MHz, $CDCl_3$): δ_H = 8.07 (d, *J* = 1.3 Hz, 1H), 7.90–7.82 (m, 2H), 7.76–7.67 (m, 2H), 7.30 (s, 1H), 4.68 (t, *J* = 8.0 Hz, 1H), 4.45 (q, *J* = 7.1 Hz, 2H), 4.11 (dd, *J* = 5.7, 1.2 Hz, 1H), 3.54 (dd, *J* = 14.2, 6.9 Hz, 1H), 3.42 (dd, *J* = 14.3, 7.8 Hz, 1H), 2.36–2.19 (m, 2H), 1.73–1.62 (m, 1H), 1.48–1.38 (m, 1H), 1.41 (t, *J* = 7.1 Hz, 3H); ^{13}C-NMR (101 MHz, $CDCl_3$): δ_C = 168.50 (C_q), 148.68 (C_q), 143.44 (C_q), 137.38 (+), 134.12 (+), 132.33 (C_q), 123.43 (+), 113.93 (+), 75.15 (+), 64.59 (−), 63.38 (+), 38.09 (−), 34.48 (−), 21.89 (+), 21.56 (+), 14.30 (+); IR (ATR): $\tilde{\nu}$ (cm^{-1}) = 2978, 2936, 2873, 1758, 1707, 1467, 1391, 1336, 1251, 1139, 1087, 1017, 944, 850, 769, 721, 611, 541, 501; MS (ESI): *m*/*z* (%) = 381.9 (100) [MH^+], 763.3 (10) [$2MH^+$]; HRMS (ESI): calcd for $C_{20}H_{20}N_3O_5$ [MH^+] 382.1397, found 382.1405.

Preparation of 2-(((1*S*,3*R*,5*S*,6*R*)-3-((*tert*-butyldimethylsilyloxy)methyl)-2-oxabicyclo[3.1.0]hexan-6-yl)-methyl)isoindoline-1,3-dione (30), 2-((2*R*,3*S*,5*R*)-5-((*tert*-butyldimethylsilyloxy)-methyl)-3-vinyltetra-hydrofuran-2-yl)isoindoline-1,3-dione (31a), 2-((2*S*,3*S*,5*R*)-5-((*tert*-butyldimethylsilyloxy)-methyl)-3-vinyltetra-hydrofuran-2-yl)isoindoline-1,3-dione (31b), and (*R*)-*tert*-butyl-dimethyl((4-vinyl-2,3-dihydrofuran-2-yl)methoxy)silane (32). DIAD (1.58 g, 8.72 mmol, 1.5 equiv.) was added dropwise to a solution of alcohol **19** (1.50 g, 5.82 mmol), PPh_3 (2.29 g, 8.72 mmol, 1.5 equiv.) and phthalimide (1.28 mg, 8.72 mmol, 1.5 equiv.) in anhydrous THF (116 mL) at 50 °C. After stirring at 50 °C for 1 h the mixture was cooled to room temperature quenched with water (50 mL). The phases were separated and the organic layer was extracted with DCM (3 × 25 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by column chromatography (PE–EtOAc 9 : 1, then 5 : 1) to obtain compound **32** (100 mg, 0.42 mmol, 7%) as a colorless oil and compound **31b** (115 mg, 0.30 mmol, 5%), compound **31a** (1.04 g, 2.67 mmol, 46%) and compound **30** (665 mg, 1.72 mmol, 29%) as colorless solids.

32: R_f = 0.59 (PE–EtOAc 9 : 1); $[\alpha]_D^{20}$ = −125.8 (DCM, *c* = 1.0); ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 6.46 (ddd, *J* = 18.0, 11.0, 0.6 Hz, 1H), 6.41 (m, 1H), 4.85 (dd, *J* = 10.7, 1.1 Hz, 1H), 4.85–4.76 (m, 1H), 4.70 (dddd, *J* = 10.5, 7.3, 5.9, 4.9 Hz, 1H), 3.73 (dd, *J* = 10.9, 5.9 Hz, 1H), 3.65 (dd, *J* = 10.9, 4.8 Hz, 1H), 2.73 (dddd, *J* = 14.4, 10.4, 1.8, 0.7 Hz, 1H), 2.48 (dddd, *J* = 14.6, 7.3, 1.7, 0.6 Hz, 1H), 0.89 (s, 9H), 0.07 (s, 3H), 0.07 (s, 3H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ_C = 144.97 (+), 129.08 (+), 116.31 (C_q), 109.70 (−), 83.00 (+), 65.64 (−), 30.71 (−), 26.01 (+), 18.50 (C_q), −5.14 (+), −5.19 (+); IR (ATR): $\tilde{\nu}$ (cm^{-1}) = 2955, 2929, 2857, 1641, 1472, 1463, 1253, 1105, 1006, 980, 834, 776, 667.

31b: R_f = 0.40 (PE–EtOAc 5 : 1); $[\alpha]_D^{20}$ = +95.5 (DCM, *c* = 0.5); ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 7.83 (dd, *J* = 5.6, 3.0 Hz, 2H), 7.72 (dd, *J* = 5.6, 3.0 Hz, 2H), 6.13 (d, *J* = 8.4 Hz, 1H), 5.62 (ddd, *J* = 17.5, 10.2, 7.5 Hz, 1H), 5.15 (dt, *J* = 17.1, 1.4 Hz, 1H), 4.99 (ddd, *J* = 10.2, 1.5, 1.0 Hz, 1H), 4.18 (ddt, *J* = 10.7, 6.5, 5.2 Hz, 1H), 3.97 (dd, *J* = 10.5, 6.6 Hz, 1H), 3.82 (dd, *J* = 10.5, 4.9 Hz, 1H), 3.41–3.26 (m, 1H), 2.39 (dt, *J* = 12.1, 11.2 Hz, 1H), 2.13 (ddd, *J* = 11.7, 7.6, 5.5 Hz, 1H), 0.89 (s, 9H), 0.07 (s, 3H), 0.06 (s, 3H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ_C = 167.89 (C_q), 134.26 (+), 134.12 (+), 131.99 (C_q), 123.50 (+), 118.46 (−), 83.27 (+), 82.56 (+), 65.93 (−), 47.39 (+), 34.02 (−), 26.14 (+), 18.62 (C_q), −5.04 (+), −5.11 (+); IR (ATR): $\tilde{\nu}$ (cm^{-1}) = 2956, 2928, 2857, 1787, 1772, 1720, 1470, 1416, 1370, 1351, 1327, 1255, 1117, 1101, 1059, 1005, 924, 891, 838, 777, 720; MS (ESI): *m*/*z* (%) = 388.0 (100) [MH^+], 729.4 (15) [$2MH^+$]; HRMS (ESI): calcd for $C_{21}H_{30}NO_4Si$ [MH^+] 388.1939, found 388.1941.

31a: R_f = 0.38 (PE–EtOAc 5 : 1); $[\alpha]_D^{20}$ = −41.5 (DCM, *c* = 1.0); ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 7.84 (dd, *J* = 5.6, 3.0 Hz, 2H), 7.72 (dd, *J* = 5.6, 3.0 Hz, 2H), 5.79 (ddd, *J* = 17.1, 10.2, 8.0 Hz, 1H), 5.74 (d, *J* = 7.6 Hz, 1H), 5.09 (dt, *J* = 17.7, 1.4 Hz, 1H), 5.04 (ddd, *J* = 7.9, 1.4, 0.9 Hz, 1H), 4.53 (dq, *J* = 5.2, 4.1 Hz, 6H), 3.87–3.73 (m, 7H), 3.73 (dd, *J* = 11.0, 4.2 Hz, 10H), 3.67 (dd, *J* = 11.0, 4.4 Hz, 1H), 2.35 (ddd, *J* = 12.6, 7.6, 5.3 Hz, 1H), 1.89 (ddd, *J* = 12.2, 11.1, 10.1 Hz, 1H), 0.90 (s, 9H), 0.07 (s, 3H), 0.06 (s, 3H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ_C = 167.95 (C_q), 136.90 (+), 134.31 (+), 132.06 (C_q), 123.57 (+), 117.25 (−), 85.23 (+), 80.85 (+), 65.07 (−), 46.15 (+), 35.26 (−), 26.08 (+), 18.51 (C_q), −5.10 (+), −5.21 (+); IR (ATR): $\tilde{\nu}$ (cm^{-1}) = 2954, 2929, 2857, 1775, 1717, 1470, 1405, 1368, 1328, 1253, 1084, 996, 921, 872, 836, 777, 718, 665, 530; MS (ESI): *m*/*z* (%) = 388.0 (70) [MH^+], 405.0 (70) [MNH_4^+], 775.4 (20) [$2MH^+$], 792.4 (100) [$2MNH_4^+$]; HRMS (ESI): calcd for $C_{21}H_{30}NO_4Si$ [MH^+] 388.1939, found 388.1942.

30: mp = 63–65 °C; R_f = 0.25 (PE–EtOAc 5 : 1); $[\alpha]_D^{20}$ = +28.9 (DCM, *c* = 1.0); ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 7.79 (dd, *J* = 5.5, 3.0 Hz, 2H), 7.67 (dd, *J* = 5.4, 3.1 Hz, 2H), 4.46–4.34 (m, 1H), 3.89 (dd, *J* = 6.2, 0.6 Hz, 1H), 3.47 (dd, *J* = 14.3, 6.9 Hz, 1H), 3.41 (d, *J* = 4.9 Hz, 2H), 3.28 (dd, *J* = 14.3, 8.4 Hz, 1H), 2.20 (ddd, *J* = 12.7, 8.1, 7.3 Hz, 1H), 1.70–1.53 (m, 2H), 1.36–1.25 (m, 1H), 0.82 (s, 9H), −0.02 (s, 6H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ_C = 168.28 (C_q), 133.94 (+), 132.23 (C_q), 123.22 (+), 87.79 (+), 65.97 (−), 64.86 (+), 37.87 (−), 31.45 (−), 31.13 (+), 25.97 (+), 23.09 (+), 18.42 (C_q), −5.30 (+), −5.31 (+); IR (ATR): $\tilde{\nu}$ (cm^{-1}) = 2955, 2929, 2856, 1772, 1712, 1468, 1433, 1391, 1356, 1330, 1253, 1188, 1137, 1088, 1007, 990, 950, 836, 777, 720, 529; MS (ESI): *m*/*z* (%) = 388.1 (50) [MH^+], 405.0 (100) [MNH_4^+]; HRMS (ESI): calcd for $C_{21}H_{30}NO_4Si$ [MH^+] 388.1939, found 388.1940.

Preparation of (1*S*,3*R*,5*S*,6*S*)-methyl 3-(oxazol-5-yl)-2-oxabicyclo[3.1.0]hexane-6-carboxylate (33). To a solution of oxazoline **16** (396 mg, 1.04 mmol) in anhydrous MeOH (10 mL), K_2CO_3

(289 mg, 2.09 mmol, 2 equiv.) was added under a nitrogen atmosphere. The reaction mixture was refluxed for 30 min, quenched with water (15 mL) and extracted with DCM (3 × 15 mL). The combined organic layers were dried over $MgSO_4$, filtrated and concentrated *in vacuo*. Purification by column chromatography (PE–EtOAc 1 : 1) afforded compound **33** (67 mg, 0.32 mmol, 31%) as a colorless solid.

Mp = 61 °C; R_f = 0.36 (PE–EtOAc 1 : 3); $[\alpha]_D^{20}$ = +30.6 (DCM, c = 1.0); ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 7.84 (s, 1H), 6.97 (s, 1H), 5.47 (dd, J = 9.4, 6.1 Hz, 1H), 4.30 (dd, J = 5.9, 0.6 Hz, 1H), 3.65 (s, 3H), 2.72 (ddd, J = 13.4, 9.5, 6.6 Hz, 1H), 2.37–2.30 (m, 1H), 2.26 (ddd, J = 13.5, 6.1, 0.7 Hz, 1H), 1.98 (dd, J = 3.9, 0.9 Hz, 1H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ_C = 170.80 (C_q), 151.63 (C_q), 151.51 (+), 124.18 (+), 77.16 (+), 67.36 (+), 51.89 (+), 33.00 (−), 31.18 (+), 27.25 (+); IR (ATR): $\tilde{\nu}$ (cm^{-1}) = 3435 (br), 3129, 2954, 1716, 1507, 1440, 1394, 1311, 1274, 1198, 1171, 1107, 1070, 963, 860, 715; MS (EI): m/z (%) = 95.0 (100), 180.1 (39) [M^+ΔCHO], 209.1 (<1) [$M^{+\cdot}$]; HRMS (EI): calcd for $C_{10}H_{11}NO_4$ [$M^{+\cdot}$] 209.0688, found 209.0694.

Preparation of (1*S*,3*R*,5*S*,6*R*)-3-(oxazol-5-yl)-2-oxabicyclo-[3.1.0]hexan-6-yl)methanol (34). To a stirred ice-cooled solution of oxazole **33** (65 mg, 0.31 mmol) in anhydrous THF (3 mL) under a nitrogen atmosphere, LAH (9.3 mg, 0.25 mmol, 0.8 equiv.) was added in small portions within 5 min. The reaction mixture was stirred for 30 min at 0 °C. After addition of water (10 μL) the mixture was stirred for another 30 min. Then a 15% aqueous NaOH solution (10 μL) was added followed by water (30 μL). The mixture was warmed to room temperature, treated with $MgSO_4$ and filtered through a Celite pad. The solvent was evaporated under reduced pressure. The crude product was purified by column chromatography (EtOAc) to obtain compound **34** (40 mg, 0.22 mmol, 71%) as a colorless solid.

Mp = 95 °C; R_f = 0.19 (EtOAc); $[\alpha]_D^{20}$ = +25.8 (DCM, c = 1.0); ^{1}H-NMR (400 MHz, $CDCl_3$): δ_H = 7.81 (s, 1H), 6.93 (s, 1H), 5.41 (dd, J = 8.4, 6.9 Hz, 1H), 3.90 (dd, J = 6.2, 1.2 Hz, 1H), 3.39 (dd, J = 11.6, 7.2 Hz, 1H), 3.33 (dd, J = 11.5, 7.1 Hz, 1H), 2.59 (ddd, J = 13.0, 8.7, 6.9 Hz, 1H), 2.13 (ddd, J = 13.0, 6.7, 1.4 Hz, 1H), 2.11 (br s, 1H), 1.69–1.62 (m, 1H), 1.40 (tdd, J = 7.1, 4.0, 1.2 Hz, 1H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ_C = 152.18 (C_q), 151.31 (+), 123.62 (+), 78.11 (+), 64.70 (+), 62.12 (−), 33.85 (−), 33.45 (+), 22.08 (+); IR (ATR): $\tilde{\nu}$ (cm^{-1}) = 3369 (br), 3125, 2945, 2881, 1508, 1461, 1414, 1353, 1262, 1176, 1106, 1026, 990, 966, 910, 885, 846, 645; MS (CI): m/z (%) = 182.1 (99) [MH^+], 199.1 (100) [MNH_4^+]; HRMS (LSI): calcd for $C_9H_{12}NO_3$ [MH^+] 182.0817, found 182.0816.

Preparation of 2-(((1*S*,3*R*,5*S*,6*R*)-3-(oxazol-5-yl)-2-oxabicyclo-[3.1.0]hexan-6-yl)methyl)isoindoline-1,3-dione (35). DIAD (96 mg, 0.45 mmol, 1.5 equiv.) was added dropwise to a solution of oxazole **34** (54 mg, 0.30 mmol), PPh_3 (117 mg, 0.45 mmol, 1.5 equiv.) and phthalimide (66 mg, 0.45 mmol, 1.5 equiv.) in anhydrous THF (6 mL) at 0 °C under a nitrogen atmosphere. After stirring at 0 °C for 30 min the mixture was allowed to warm to room temperature and the solvent was evaporated under reduced pressure. The residue was purified by column chromatography (PE–EtOAc 3 : 1 to EtOAc) to obtain compound **35** (51 mg, 0.17 mmol, 55%) as a colorless solid.

Mp = 83 °C; R_f = 0.51 (EtOAc); $[\alpha]_D^{20}$ = +18.9 (DCM, c = 1.0); ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 7.84 (dd, J = 5.5, 3.1 Hz, 2H), 7.79 (s, 1H), 7.72 (dd, J = 5.5, 3.1 Hz, 2H), 6.92 (s, 1H), 5.45–5.37 (m, 1H), 4.08 (dd, J = 6.3, 1.0 Hz, 1H), 3.56 (dd, J = 14.4, 6.9 Hz, 1H), 3.36 (dd, J = 14.4, 8.4 Hz, 1H), 2.59 (ddd, J = 13.1, 8.6, 7.1 Hz, 1H), 2.09 (ddd, J = 13.1, 7.2, 1.4 Hz, 1H), 1.84–1.75 (m, 1H), 1.59–1.51 (m, 1H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ_C = 168.33 (C_q), 151.59 (C_q), 151.30 (+), 134.08 (+), 132.18 (C_q), 123.87 (+), 123.36 (+), 78.48 (+), 65.23 (+), 37.73 (−), 33.92 (−), 30.69 (+), 23.24 (+); IR (ATR): $\tilde{\nu}$ (cm^{-1}) = 3141, 3938, 1769, 1708, 1509, 1467, 1433, 1392, 1357, 1329, 1260, 1225, 1197, 1136, 1101, 1071, 1026, 950, 851, 798, 720, 645, 531; MS (EI): m/z (%) = 77.1 (8), 95.1 (100), 104.1 (6), 130.1 (6), 160.1 (18), 310.1 (1) [$M^{+\cdot}$]; HRMS (EI): calcd for $C_{17}H_{14}N_2O_4$ [$M^{+\cdot}$] 310.0954, found 310.0956.

Preparation of methyl-*N*′-cyano-*N*-(((1*S*,3*R*,5*S*,6*R*)-3-(oxazol-5-yl)-2-oxabicyclo[3.1.0]hexan-6-yl)methyl)carbamimido-thioate (36). A solution of aminooxazole **9a** (19 mg, 0.11 mmol) and dimethyl *N*-cyanodithioiminocarbonate (34 mg, 0.22 mmol, 2 equiv.) in EtOH was stirred at room temperature for 18 h. The solvent was evaporated under reduced pressure. The crude product was purified by column chromatography (DCM then DCM–MeOH 9 : 1) to afford compound **36** (29 mg, 0.11 mmol, quantitative) as a colorless oil.

R_f = 0.51 (PE–EtOAc 9 : 1); $[\alpha]_D^{20}$ = +14.3 (DCM, c = 1.0); ^{1}H-NMR (300 MHz, $CDCl_3$): δ_H = 7.84 (s, 1H), 7.16 (s, 0.5H), 6.96 (s, 1H), 6.53 (s, 0.5H), 5.43 (dd, J = 8.4, 7.2 Hz, 1H), 3.96 (dd, J = 6.3, 0.9 Hz, 1H), 3.45–2.86 (m, signal broadening due to rotamers, 2H), 2.73–2.32 (m, signal broadening due to rotamers, 3H), 2.62 (ddd, J = 13.0, 8.6, 7.0 Hz, 1H), 2.15 (dd, J = 13.0, 6.9 Hz, 1H), 1.77–1.68 (m, 1H), 1.50–1.37 (m, 1H); ^{13}C-NMR (75 MHz, $CDCl_3$): δ_C = 151.67 (C_q), 151.44 (+), 123.99 (+), 78.34 (+), 64.99 (+), 44.01 (signal broadening due to rotamers, −), 33.82 (−), 30.09 (+), 23.30 (+), 14.64 (signal broadening due to rotamers, +), **C**═N and **C**≡N signals too weak to be observed; IR (ATR): $\tilde{\nu}$ (cm^{-1}) = 3263 (br), 3126, 3011, 2939, 2174, 1716, 1554, 1511, 1430, 1357, 1285, 1182, 1104, 938, 846, 645; MS (ESI): m/z (%) = 279.0 (30) [MH^+], 296.0 (40) [MNH_4^+], 557.1 (100) [$2MH^+$]; HRMS (EI): calcd for $C_{12}H_{14}N_4O_2S$ [$M^{+\cdot}$] 278.0837, found 278.0833.

Pharmacology

Histamine dihydrochloride was purchased from Alfa Aesar GmbH & Co. KG (Karlsruhe, Germany). [^{3}H]N^α-methylhistamine and [^{3}H]histamine were from Perkin Elmer Life Sciences (Boston, MA). Guanosine diphosphate (GDP) was from Sigma-Aldrich Chemie GmbH (Munich, Germany), and unlabeled GTPγS was from Roche (Mannheim, Germany). [^{3}H]pyrilamine was purchased from Hartmann Analytic (Braunschweig, Germany). [^{35}S]GTPγS was from PerkinElmer Life Sciences (Boston, MA) or Hartmann Analytic GmbH (Braunschweig, Germany). [^{3}H]UR-DE257 (*N*-(6-(3,4-dioxo-2-(3-(3-(piperidin-1-ylmethyl) phenoxy)propylamino)-cyclobut-1-enylamino)hexyl)-[2,3-^{3}H]-propionamide) was synthesized in our laboratory.[31]

GF/C filters were from Whatman (Gaithersburg, USA). For liquid scintillation counting was used: PerkinElmer MicroBeta^2 2450 MicroplateCounter (Massachusetts, USA), Brandel Harvester MWXRT-96TI, Brandel (Gaithersburg, USA). Scintillation cocktail Rotiszint™ eco plus was from Carl Roth GmbH & Co KG (Karlsruhe, Germany).

Radioligand binding experiments[32] were performed on the hH_1R, hH_2R, hH_3R and hH_4R as follows. H_1R assays: Sf9 insect cell membranes expressing the hH_1R + RGS4 were employed; H_2R assays: Sf9 insect cell membranes expressing the hH_2R $-Gs_{\alpha s}$ fusion protein were employed; H_3R assays: Sf9 insect cell membranes coexpressing the hH_3R, mammalian $G\alpha_{i2}$ and $G\beta_1\gamma_2$ were employed, H_4R assays: Sf9 insect cell membranes coexpressing the hH_4R, mammalian $G\alpha_{i2}$ and $G\beta_1\gamma_2$ were employed. The respective membranes were thawed and sedimented by centrifugation at 4 °C and 13 000*g* for 10 min. Membranes were resuspended in binding buffer (12.5 mM $MgCl_2$, 1 mM EDTA and 75 mM Tris/HCl, pH 7.4). Each well (total volume 250 μL) contained 20 μg (hH_1R), 28 μg (hH_2R), 50 μg (hH_3R), or 120 μg (hH_4R) of membrane protein. Competition binding experiments were performed in the presence of 5 nM [^{3}H]pyrilamine (hH_1R), 30 nM [^{3}H]UR-DE257 (hH_2R), 3 nM [^{3}H]N^α-methylhistamine (hH_3R) or 15 nM [^{3}H]histamine (hH_3R and hH_4R) and increasing concentrations of unlabeled ligands. Incubations were conducted for 60 min at 25 °C and shaking at 250 rpm. Bound radioligand was separated from free radioligand by filtration through 0.3% polyethyleneimine-pretreated (PEI) GF/C filters, followed by three washes with 2 mL of cold binding buffer (4 °C) using a Brandel Harvester. Filter-bound radioactivity was determined after an equilibration phase of at least 12 h by liquid scintillation counting.

Functional [^{35}S]GTPγS assays[33] were performed as previously described for the H_3R[34] and H_4R.[35] H_3R assays: Sf9 insect cell membranes coexpressing the hH_3R, mammalian $G\alpha_{i2}$ and $G\beta_1\gamma_2$ were employed. H_4R assays: Sf9 insect cell membranes coexpressing the hH_4R, mammalian $G\alpha_{i2}$ and $G\beta_1\gamma_2$ were employed. The respective membranes were thawed, sedimented by a 10 min centrifugation at 4 °C and 13 000*g*. Membranes were resuspended in binding buffer (12.5 mM $MgCl_2$, 1 mM EDTA, and 75 mM Tris/HCl, pH 7.4). Each assay tube contained Sf9 membranes expressing the respective HR subtype (15–30 μg protein per tube), 1 μM GDP, 0.05% (w/v) bovine serum albumin, 0.2 nM [^{35}S]GTPγS and the investigated ligands (dissolved in millipore water or in a mixture (v/v) of 80% millipore water and 20% DMSO) at various concentrations in binding buffer (total volume 250 μL). All H_4R assays additionally contained 100 mM NaCl. For the determination of K_B values (antagonist mode of the functional [^{35}S]GTPγS assay) histamine was added to the reaction mixtures (final concentration for $H_{3/4}R$: 100 nM). Incubations were conducted for 90 min at 25 °C and shaking at 250 rpm. Bound [^{35}S]GTPγS was separated from free [^{35}S]GTPγS by filtration through GF/C filters, followed by three washes with 2 mL of binding buffer (4 °C) using a Brandel Harvester. Filter-bound radioactivity was determined after an equilibration phase of at least 12 h by liquid scintillation counting. The experimental conditions chosen ensured that not more than 10% of the total amount of [^{35}S]GTPγS added was bound to filters. Non-specific binding was determined in the presence of 10 μM unlabeled GTPγS.

All data are presented as mean ± SEM of *N* independent experiments performed in triplicate. Maximal responses (intrinsic activities) were expressed as α-values. The α-value of histamine was set to 1.00; α-values of other compounds were referred to this value. IC_{50} values were converted to K_i and K_B values using the Cheng–Prussoff equation.[36] K_i values were analyzed by nonlinear regression and best fit to one-site (monophasic) competition isotherms. pK_B values from the functional [^{35}S]GTPγS were analyzed by nonlinear regression and best fit to sigmoidal dose-response curves (GraphPad Prism 5.0 software, San Diego, CA).

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