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D_1-like receptors distinguishing thieno-azecine regioisomers

Mohamed A. O. Abdel-Fattah,[ab] Ashraf H. Abadi,[c] Jochen Lehmann,[a] Peter M. Schweikert[a] and Christoph Enzensperger*[d]

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Designing ligands with D_1/D_5 subtype selectivity is a challenge because of the high identity within the receptor helices. Based on the lead compounds **1–3**, the thieno-benzazecine regioisomers **4** and **5** were synthesized and biologically evaluated for their affinity towards the five dopamine receptor subtypes utilizing a radioligand binding affinity technique. Within the D_1-like family, compound **4** showed 20 fold selectivity for the D_5 subtype over D_1 subtype (K_i = 3 nM, D_1: 60 nM), while its regioisomer, compound **5** with a reversed thiophene position, prefers the D_1 subtype over the D_5 subtype (K_i = 4 nM, D_5: 15 nM). The benzothieno-benzazecine analog **6** was shown to be one of the few azecine derivatives with high affinity for both the D_1- and the D_2-like family members in the same order of magnitude (K_i = 1.5 nM for D_2 and 1.9 nM for D_5). Thorough analysis of the amino acid residues constituting the binding pockets of the target dopamine receptor subtypes revealed that at the D_5 receptor, either serine S 6.62 and threonine T 7.33 residues or a water network, stabilized by anionic amino acids could contribute to the selectivity pattern of the synthesized compounds.

Introduction

Dopamine mediates its pharmacodynamic actions by stimulating two GPCR families, the D_1-like (D_1 and D_5 subtypes), and the D_2-like (D_2, D_3, and D_4). Among the central dopaminergic pathways, the nigrostriatal and mesolimbic ones, which control motor and cognition functions, respectively, have attracted interest due to their involvement in pathological conditions related to disturbed dopamine neurotransmission.[1–5]

Dopaminergic neurotransmission in the mesolimbic pathway is also responsible for the predominant symptoms of psychosis.[6,7] Antagonizing dopamine on its receptors, mainly those of the D_2-like subfamily, is a powerful tool for treating psychosis; however, D_2-blocking is also associated with undesirable extra-pyramidal symptoms.[8] On the other hand, the selective D_1-like antagonist SCH39166 did not produce an effective antipsychotic action in a clinical study[9] but combined D_1-D_2-like receptor antagonists may act synergistically to treat psychosis.[10] Atypical antipsychotics such as asenapine, olanzapine and clozapine also show considerably high activity at the D_1-like subfamily receptors, Table 1.[11]

Annelated azecines represent a new family of dopaminergic antagonists where their chemistry brings a moderate flexibility and a specific stereochemistry to the bis-arylethylamine pharmacophore. They show high affinity preference towards the D_1-like receptors and only moderate affinity towards the D_2-like receptors.[12,13] Designing a highly selective ligand for either one of the D_1-like receptor subtypes still stands as a challenge because both share a high level of molecular structure identity.[14]

D_1/D_5-selective ligands may contribute to investigate the functions of each receptor. Moreover, they might be of therapeutic interest since recent studies have led to evidence that these subtypes elicit opposite effects in some organs such as kidney.[15] According to recent studies, they also might be useful in treating arthritis or asthma.[16,17]

In this study, we tried to modulate the selectivity/affinity profiles of lead azecine derivatives, namely the indolobenzazecine derivative **1** known as **LE 300** and its dibenzo analogs **2**, **3** towards dopaminergic receptors.

The leads **1–3** exhibit moderate affinity to D_2-like receptor subtypes and higher to the D_1-like ones. Symmetric compound **2** is slightly selective to the D_1 receptor subtype, while hydroxylation of one of the aromatic rings (compound **3**), which not only creates additional hydrogen bonding properties (acceptor and donor) but also increases electron density, enhances affinity and reverses selectivity slightly towards the D_5 receptor subtype.[12]

[a] Institute of Pharmacy, Department of Pharmaceutical and Medicinal Chemistry, Friedrich-Schiller-Universität Jena, Philosophenweg 14, D-07743 Jena, Germany
[b] Department of Chemistry, American University of Middle East, 15453, Kuwait
[c] Department of Pharmaceutical Chemistry, Faculty of Pharmacy and Biotechnology, German University in Cairo, Cairo 11835, Egypt
[d] Institute of Organic Chemistry and Macromolecular Chemistry, Friedrich-Schiller-Universität Jena, Humboldtstrasse 10, D-07743 Jena, Germany. E-mail: ch.enzensperger@uni-jena.de

Table 1 Binding affinities (K_i ± SEM [nM]) for cloned human dopamine receptors

Compound	D_1	D_2	D_3	D_4	D_5
21	>1000	>1000	>1000	>1000	>1000
4	60 ± 4.2	45 ± 2.7	24 ± 1.5	188 ± 17	3 ± 1.7
31	>1000	>1000	>1000	>1000	>1000
5	4 ± 0.4	190 ± 2.7	87 ± 6	99 ± 21	15 ± 3.2
38	>1000	>1000	>1000	>1000	>1000
6	40 ± 1.5	1.5 ± 0.02	18 ± 2	72 ± 7	1.9 ± 0.5
Clozapine[b]	189	431	646	39	235
Olanzapine[b]	70	53	62	19	90
Asenapine[b]	8.9	8.9	9.4	9	N.A.[a]

[a] Not available. [b] From the NIMH-PDSP database.[24]

In order to achieve a similar electronic environment around one of the two aromatic systems without substitution, we prepared the two regioisomers **4** and **5** carrying a thiophene in different orientations, and the benzothiophene derivative **6**, Fig. 1. Thiophene was also selected because olanzapine shows higher affinity for D_1, D_2 and D_5 receptors than clozapine.

Results and discussion

In general, the thieno-azecines were synthesized from their corresponding quinolizine precursors[18] by subjecting them to a ring fragmentation procedure (transformation of two 6-membered to one 10-membered heterocycle) by using ethylchloroformate and sodium cyanoborohydride.[13] As shown in Scheme 1, we tried to synthesize thieno[2,3-*a*]quinolizine **11** by reacting carbonitrile **7** and 2-phenylethyl chloride in the presence of tin(IV) chloride yielding isoquinoline **8** which reacted with 2-iodoethanol to give the quaternary iodide salt **9**. After $NaBH_4$ reduction, **10** was subjected to a cyclization reaction with polyphosphoric acid which was successful for the synthesis of **2**.[12] Surprisingly, the product's analytical data revealed the structure of the thiazolo-[2,3-*a*]isoquinoline **12** instead of the expected **11**. For further confirmation, we synthesized **12** independently from 2-(2-bromoethyl)benzaldehyde **13** and 2-aminoethanethiol in the presence of KOH.[19] Analytical data of the compound prepared from the two routes were identical. Trying to understand the formation of **12** by the loss of four carbons, we found that thiophene sulfur can be alkylated followed by ring degradation.[20,21] So it is possible that instead of alkylation at carbon 3, the thiophene sulfur got alkylated and destabilized leading to degradation of the thiophene ring.

We then thought about preparing **11** *via Pictet–Spengler* reaction of 2-thiophene-3-yl-ethylamine **18** and 2-(2-bromoethyl)benzaldehyde **13** in an acidic medium as shown

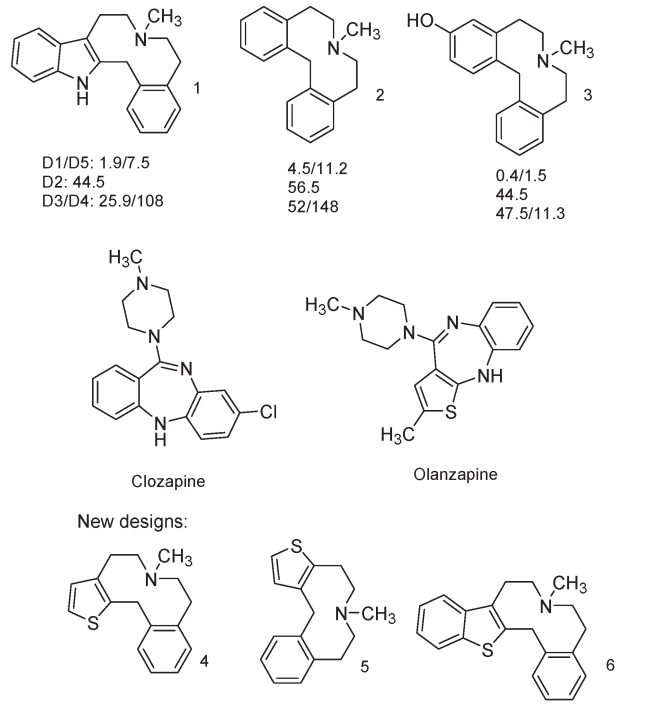
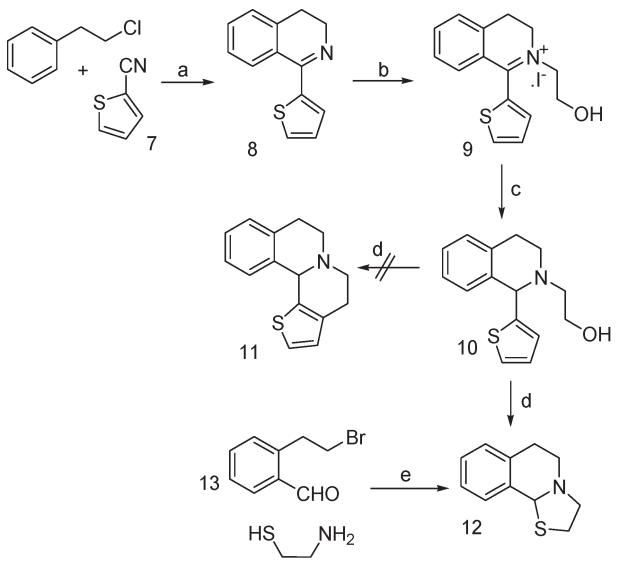
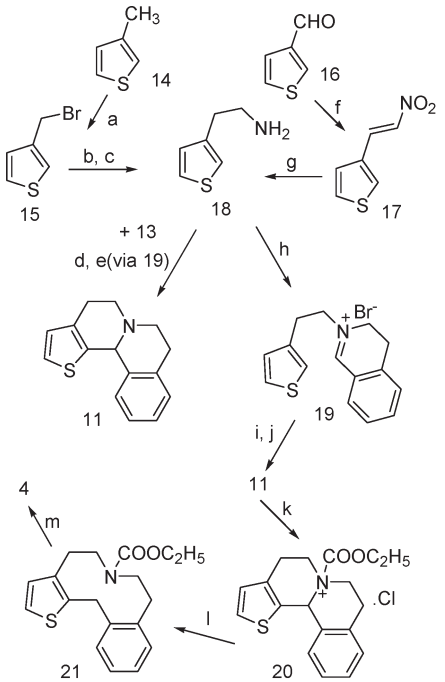


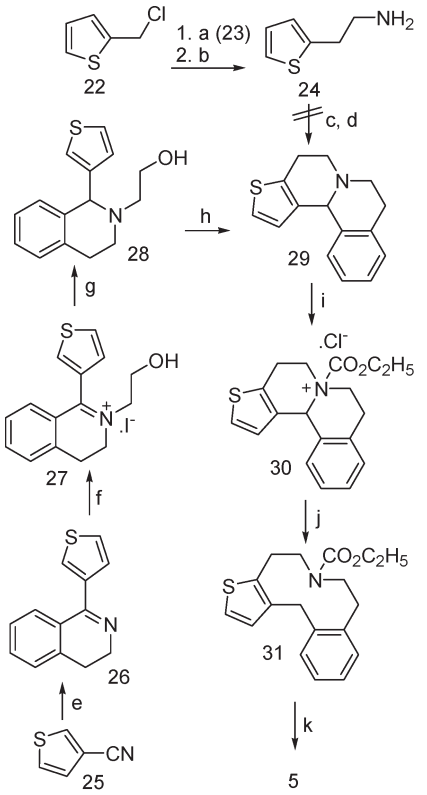
Fig. 1 Novel target compounds **4**, **5**, and **6** based on the lead azecines **1**–**3** (K_i (nM) for D_1/D_5; D_2; D_3/D_4).



Scheme 1 a: $SnCl_4$; yield: 46%. b: 2-iodoethanol, acetone; yield: 55%. c: $NaBH_4$, MeOH; yield: 60%. d: PPA, reflux. e: KOH, EtOH, rt; yield: 77%.



Scheme 2 a: NBS, benzoyl peroxide; yield 9%. b: KCN, TEBA, H_2O; yield: 28%. c: $LiAlH_4$, THF; yield 57%. d: TFA, dioxane e: 33% NH_3; yield: 31%. f: NH_4COOCH_3, CH_3NO_2; yield: 87%. g: $LiAlH_4$, dry THF; yield: 75%. h: 2-(2-bromoethyl)benzaldehyde, dioxane. i: 6 M HCl. j: 30% NH_3; yield: 82%. k: $ClCOOCH_2CH_3$, dry THF. l: $NaBH_3CN$, THF; yield: 62%. m: $LiAlH_4$, THF; yield: 70%.



Scheme 3 a: KCN, TEBA, H_2O; yield: 33%. b: $LiAlH_4$, THF; yield: 71%. c: 2-(2-bromoethyl) benzaldehyde, dioxane, TFA. d: 33% NH_3. e: 2-phenylethyl chloride, $SnCl_4$; yield: 47%. f: 2-Iodoethanol, acetone; yield: 39%. g: $NaBH_4$, MeOH; 85%. h: PPA; yield: 61%. i: $ClCOOCH_2CH_3$, THF. j: $NaBH_3CN$, THF, yield: 59%. k: $LiAlH_4$, THF; yield: 73%.

in Scheme 2. To prepare the amine **18**, we brominated methylthiophene **14** with NBS/benzoyl peroxide and the resulting **15** was reacted with KCN followed by reduction with $LiAlH_4$.[22]

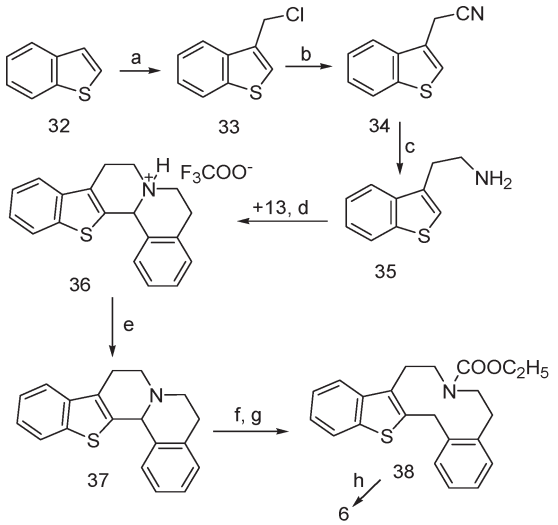
But bromination always ended up in a mixture of poly- and monobrominated analogs, from which **15** was isolated by column chromatography in less than 10% yield. Alternatively, we have reacted aldehyde **16** with nitromethane/ammonium acetate to get nitrovinylthiophene **17** which was then reduced to **18**.[22]

The amine derivative **18** was treated with **13** in dry dioxane/TFA but the yield of quinolizine **11** again was unsatisfactory. Finally, the amine derivative **18** was just heated with **13** in dioxane and the resulting imminum bromide **19** was cyclized by refluxing in 6 M HCl. This gave the HCl salt of **11** and the free base was liberated with NH_3 solution.[18] Ring opening was done through reacting **11** with ethyl chloroformate in dry THF at −80 °C for 4 hours. Intermediate **20** was only generated *in situ* and subjected to $NaBH_3CN$ reduction[13] to yield carbamate **21** which was then reduced to the thienoazecine target candidate **4**.

The synthesis of benzo[*d*]thieno[3,2-*g*]azecine **5**, a regioisomer of **4** was described formerly[12] and is illustrated in Scheme 3. We tried to follow the same procedure as described for **4**. It was started with 2-chloromethylthiophene **22** which was converted into amine **24** by reduction of the corresponding nitrile **23**. Reacting **24** with 2-(2-bromoethyl)benzaldehyde **13** and TFA in dry dioxane to prepare the quinolizine **29** was unsuccessful probably due to low reactivity of the thienyl C-3. Therefore, we largely followed the cyclization procedure that was described formerly to synthesize this quinolizine where nitrile derivative **25** was reacted with 2-phenylethyl chloride in the presence of tin(IV) chloride to afford **26** which reacted with 2-iodoethanol to give the isoquinolinium salt **27**. Reduction of **27** yielded **28** which was cyclized with polyphosphoric acid to give the desired quinolizine **29**.[12]

This cyclization reaction did not result in a loss of thiophene due to the availability of the more reactive C2 leaving no chance for degradation by sulfur alkylation. The final compound **5** was synthesized *via* a different ring opening procedure of the quinolizine **29** as described by Höfgen.[12] We applied the same synthetic route including chloroformate ring cleavage for the synthesis of the target compound **6** *via* quinolizine **37**, Scheme 4.

All of the azecines were screened for their binding affinity towards human stably cloned dopamine D_1, D_2, D_3, D_4, and D_5 receptors utilizing radioligand binding experiments. [^{3}H] SCH23390 was used as the radioligand for the D_1-like, and [^{3}H]spiperone for the D_2-like family. Incubations at 27 °C were terminated after 90 minutes by rapid filtration with a Perkin-Elmer Mach III harvester. At least two independent experiments, each in triplicate, were carried out.[23] K_i values are listed in Table 1. All non-carbamate azecines turned out



Scheme 4 a: 37% CH_2O, HCl gas; yield 92%. b: KCN, TEBA, H_2O; yield: 31%. c: $LiAlH_4$, THF; yield: 78%. d: TFA, dioxane; yield: 81%. e: 33% NH_3; yield: 93%. f: $ClCOOCH_2CH_3$, THF. g: $NaBH_3CN$, THF; yield: 88%. h: $LiAlH_4$, THF; yield: 68%.

to be antagonists in a functional fluorescent Calcium assay.[12]

The rationale on which the thiophene scaffold was selected is also based on comparing clozapine to its thiophene congener olanzapine, which has higher affinities in general than clozapine (Table 1). For this couple, not only the affinities are increased by the bioisosteric replacement of benzene to thiophene, but the selectivity profile also changed: clozapine has a slightly higher affinity for D_1 (189 nM) and D_5 (235 nM) than for the D_2 receptor (431 nM), whereas olanzapine has a rather higher affinity for D_2 (53 nM) than for D_1 and D_5 (70 and 90 nM). Selent *et al.* thoroughly analyzed the binding mechanisms of both compounds for different GPCRs including various serotonin receptors, and some dopamine receptors.

Conclusively, they found with the aid of molecular modeling and subsequent site-directed mutagenesis experiments that the H-bond acceptor property of the thiophene S is responsible for olanzapine's special binding profile.[25,26]

It was hypothesized that the SH/N motif (S 5.43 and H or N at 6.55) forms additional polar interactions with the sulfur of the thiophene moiety, which in turn explains the higher affinity of olanzapine.[25] Comparing our two thienoazecine regioisomers to olanzapine, in **4**, the sulfur is in a similar position with that in olanzapine, as shown in the molecular overlay in Fig. 2. Surprisingly, compound **5**, in which the sulfur occupies a different position than in olanzapine, shows for D_1 affinities distinctively higher than the positional isomer **4** where the thiophene has an olanzapine-like orientation. This indicates a different binding mode.

To find out which amino acids are different within the binding pockets of the D_1 and D_5 receptors, we first aligned the D_1 and D_5 sequences with the sequence of the beta 2 receptor which is a closer relative to the D_1 family than the

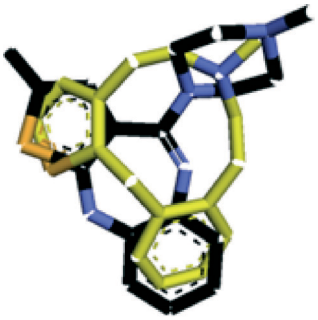


Fig. 2 Overlay of olanzapine and thieno-azecine regioisomer **4**: structures sketched with ACD/ChemSketch, → clean structure, → copy-paste to Accelrys DS 3.1 visualizer, → molecular overlay with 50% steric and 50% electrostatic interactions (default) and a structural consensus between both molecules.

dopamine D_3 receptor[27] and used its architecture as the crystallized template for the D_1 and D_5 receptors. Next, we opened the beta 2 crystal structure (PDB #: 2rh1) in an Accelrys Discovery Studio Visualizer™ v3.50 and selected all amino acids with a 12 Å radius around the co-crystallized ligand carazolol.

These 112 amino acids within the 12 Å radius were compared in the alignment with the respective amino acids of the D_1 and the D_5 receptor. From these 112 amino acids in the pocket, only 16 were found to be different within the D_1 and D_5 receptors, Fig. 3.

Amino acids with different physicochemical properties are highlighted in red.

Fig. 3 shows all the amino acids that are different in D_1 and D_5 in the liberally selected binding area. Therefore, the amino acids that contribute to the observed selectivity must be part of Fig. 3.

Among the red marked amino acids, the positions S 6.62 and T 7.33 could probably form at the D_5 receptor an H-bond to the ligand's thiophene moiety similar to the polar interaction observed by Selent *et al.*[26] However, it is also evident that the D_5 receptor contains four negatively charged amino acids (D 3.26, D 4.68, D 5.24 and E 7.32) and the D_1 receptor not a single one. Since the binding pocket is actually flooded with water molecules, it might also be the case that the polar interaction is mediated indirectly by the anionic amino acids by forming a water network. Masson and coworkers found for choline esterases that glutamate and aspartate form an important water network in the binding pocket.[28]

	2.52	3.27	3.26	3.23	4.54	4.67	4.68	5.23	5.24	5.35	5.44	6.57	6.58	6.62	7.32	7.33
B2	V	N	E	F	G	R	A	C	Y	N	I	V	H	D	K	E
D1	L	I	**N**	**S**	V	K	**A**	**L**	**A**	**S**	V	I	L	**G**	**S**	**N**
D5	F	V	**D**	**A**	I	R	**D**	**P**	**D**	**N**	L	M	V	**S**	**E**	**T**

Fig. 3 Aligned sequences of B_2, D_1 and the D_5 receptor; presented amino acids are within 12 Å radius around the ligand carazolol and different in D_1 and D_5. Red indicates different physicochemical properties.

The key protein–ligand interaction among all aminergic receptors is the salt bridge between aspartate D 3.36 and the protonated amine.[29] This is in line with our observation that none of the azecine-carbamates (**21**, **31** and **38**) show receptor affinity for dopamine receptors.

The use of the thiophene ring was also suggested in order to generally make the best use of the hydrophobic region of the studied binding pockets, where the large atomic polarizability of the sulfur atom and the electron-rich thiophene system would provide higher dispersion forces compared to benzene which may lead to better π–π stacking and/or Van der Waals interaction with the hydrophobic residues lining the hydrophobic pocket of the target receptors. This suggestion was further supported by a quantum mechanics-based study which has proven that benzene–thiophene heterodimers are more stable π–π complexes than benzene–benzene homodimers with interaction energies of −2.774 and −0.38 Kcal mol^{-1}, respectively.[30]

Examining the new test compounds *versus* the leads, we have observed that compound **4** was shown to have the best selectivity towards the D_5 receptor subtype with D_1/D_5 selectivity index of 20 *versus* 0.4 for compound **2** and 0.26 for compound **3**. Shifting the position of the sulfur atom of the thiophene ring to regioisomer **5** completely reverses the selectivity, showing better selectivity to D_1 receptors with a D_5/D_1 selectivity index of 3.3 *versus* 2.5 for compound **2** revealing the importance of the position of the thiophene sulfur atom for binding to the D_5 receptor.

In terms of affinity, compounds **4** and **5** exhibit lower affinities to D_1 and D_5 receptors relative to the lead compounds **1**–**3** pointing out the importance of the molar volume and the electron density of the ring adjacent to the azecine. Thus, establishing other aromatic systems with a similar electronic effect but larger molar volume seemed attractive. Accordingly, we synthesized the benzothieno-benzazecine **6** which shows a comparable selectivity profile to compound **4** towards the D_5 receptor subtype over D_1 receptors with a similar D_1/D_5 selectivity index of 20. This confirmed the importance of the sulfur atom's position and emphasized the value of enlarging the size of the aromatic system at this area of the compounds' scaffold. Regarding the D_2-like receptors, compounds **4** and **6** showed better affinity to D_2 and D_3 receptors than the leads **2** and **3**. Compound **4** showed K_i values of 45 and 24 nM for D_2 and D_3, respectively *versus* 60 nM for D1. The most interesting compound, **6**, was found to be more selective to D_2 and D_3 receptors with K_i values of 1.5 and 18 nM respectively *versus* 40 nM for D_1 receptor and showed a unique high affinity pattern towards D_2 and D_5 receptors.

This could be rationalized based on the molecular structure of the recently co-crystallized human D_3 receptors where it was approved that S 5.46 residue is involved in hydrogen bond interactions (acting as hydrogen bond donor) with the antagonist eticlopride in the D_3 binding pockets.[31] Also, the developed homology model by Kalani *et al.* for the D_2 receptor subtypes with the antagonist clozapine showed that S 5.42 residue contributed similarly to the interaction between the ligand and the target protein.[32] Thus, it could be a similar binding fashion for the thieno- and benzothieno-benzazecine derivatives **4** and **6**. Reversing the position of the sulfur atom in compound **5** again led to lower affinity to the D_2-like members with more or less similar binding affinity data to the lead compounds.

Experimental

All chemicals were obtained from Sigma-Aldrich and Alfa Aesar and were used without further purification unless otherwise indicated. THF was distilled from sodium immediately prior to use. Reactions were monitored by TLC performed on silica gel F254 plates (Merck) and visualization was done by UV light. Column chromatography was performed with silica gel 60 63–200 μm (Baker). The melting points were uncorrected and were measured in open capillary tubes using a Gallenkamp melting point apparatus. ^{1}H-NMR and ^{13}C-NMR spectral data were obtained from a Bruker Avance 250 spectrometer (250 MHz); the *J* values are given in Hz. MS data were determined by GC/MS, using a Hewlett Packard GCD-Plus (G1800C) apparatus (HP-5MS column; J&W Scientific). Elemental analyses were performed on a Hereaus Vario EL apparatus; Obtained values were within ±0.4 of the theoretical ones. For compound **4**, the elemental analysis did not match the ±0.4 for hydrogen (calcd: 7.44, found: 6.98). Therefore, additionally, HPLC and LC/MS data were recorded. The HPLC system consisted of a Shimadzu 10ATVP (binary pump, degaser and autosampler) with a diode array detector. LC separation was performed on a RP18 column (Macherey Nagel, Düren, Germany 125 × 4 mm i.d., 5 μm Nucleodur c18 Gravity) at 25 °C using mobile phase A (water + 0.1% TFA) and phase B (MeCN + 0.1% TFA) in a gradient program with a flow of 1 mL min^{-1}; 0–11 min 10% B: 12–11 min 95% B. For quantitative analysis, we used the wavelengths 254 and 220 nm respectively. At 254 nm, the purity is 96.5%, at 220 nm the purity is 99.3%. The LC/MS system consisted of a LCQ (Thermo-Finnigan with C18 Nucleodur Isis, Macherey Nagel, Düren, Germany 125 × 4 mm i.d., 3 μm) and was recorded under the same conditions as above in a positive mode, with a capillary voltage of 4.5 kV, which proved the identity of compound **4** by mass (*m*/*z* = 258.4 (M + H)). All intermediates have been synthesized and had matched spectral data according to the literature.[12,18,19,22]

Procedure for the preparation of 2,3,5,6-tetrahydro-10b*H*-thiazolo[2,3-*a*] isoquinoline (12)

To a solution of potassium hydroxide (0.03 g, 0.53 mmol) in ethanol (50 mL), 6.80 g (88 mmol) of 2-aminoethanthiol and 18.74 g (88 mmol) of 2-(2-bromoethyl)benzaldehyde were added. The reaction mixture was stirred at room temperature for 24 hours. The precipitated compound was filtered and dried under vacuum to give compound **12** as white solid (12.9 g, 77%); m.p. 90–91 °C; ^{1}H NMR (250 MHz; $CDCl_3$) δ (ppm) 2.60–2.95 (3H, m, C(5, 6) H), 3.01–3.33 (4H, m, C(2, 3) H),

3.57–3.60 (1H, m, C(5) H), 5.79 (1H, s, (C10b) H), 7.00–7.25 (4H, m, Ph); *m*/*z* 191 (M^+, 20%), 115 (60), 104 (100); anal. found: C, 69.07; H, 6.80; N, 6.91. Calc. for $C_{11}H_{13}NS$: C, 69.11; H 6.49; N 7.30%.

General procedure for the synthesis of carbamate derivatives 21, 31, and 38

A solution of the given amounts of the respective quinolizine derivative (2 mmol) in dry THF (30 mL) was cooled in methanol/dry ice at −80 °C. To the solution, 1 g (10 mmol) of ethylchloroformate was added under inert atmosphere. The reaction mixture was stirred for 4 h and then a solution of 0.37 g (6 mmol) of sodium cyanoborohydride in dry THF (20 mL) was added after cooling again to −80 °C. The reaction mixture was allowed to reach to room temperature and stirred for 48 h. It was then treated with 2 N NaOH (100 mL), and the organic layer was separated, washed with a brine solution (2 × 30 mL), and the organic solvent was then evaporated under reduced pressure. The residue obtained was purified by silica gel column chromatography using hexane : EtOAc 3 : 1.

Ethyl 4,5,6,7,8,13-hexahydrobenzo[*d*]thieno[2,3-*g*]azecine-6-carboxylate (21)

Yellow oil (0.4 g, 62%); ^{1}H NMR (250 MHz; $CDCl_3$) δ (ppm) 0.92 (3H, t, *J* 7, CH_2CH_3), 2.67–3.57 (8H, m, C(4, 5, 7, 8) H), 3.74 (2H, q, *J* 7, CH_2CH_3), 4.14 (2H, d, *J* 10, C(13) H), 6.70 (1H, d, *J* 5, C(3) H), 6.80 (1H, d, *J* 5, C(2) H), 7.01–7.20 (4H, m, Ph); ^{13}C NMR (62.5 MHz; $CDCl_3$) δ (ppm) 14.20 (CH_2CH_3), 28.47 (C4), 32.50 (C13), 32.83 (C7), 34.05 (CH_2CH_3), 53.92 (C5), 61.00 (C7), 122.54 (C3a), 126.73 (C10), 126.98 (C11), 130.19 (C9), 130.36 (C12), 131.14 (C3), 135.87 (C2), 138.45 (C13a), 138.59 (C8a), 139.27 (C12a), 156.48 (CO); *m*/*z* 315 (M^+, 20%), 211 (30), 184 (60), 115 (80), 104 (100); anal. found: C, 68.31; H, 6.41; N, 4.42. Calc. for $C_{18}H_{21}NO_2S$: C, 68.54; H 6.71; N 4.44%.

Ethyl 4,5,6,7,8,13-hexahydrobenzo[*d*]thieno[3,2-*g*]azecine-6-carboxylate (31)

Yellow oil (0.37 g, 59%); ^{1}H NMR (250 MHz; $CDCl_3$) δ (ppm) 0.95 (3H, t, *J* 7, CH_2CH_3), 2.66–3.56 (8H, m, C(4, 5, 7, 8) H), 3.75 (2H, q, *J* 7, CH_2CH_3), 3.96 (2H, d, *J* 7, C(13) H), 6.82–7.28 (6H, m, C(1, 2, 9, 10, 11, 12) H); ^{13}C NMR (62.5 MHz; $CDCl_3$) δ (ppm) 14.20 (CH_2CH_3), 27.83 (C8), 31.57 (C13), 32.72 (C4), 33.99 (CH_2CH_3), 54.23 (C7), 60.98 (C5), 121.58 (C13a), 126.60 (C10), 126.94 (C11), 130.23 (C9), 130.58 (C12), 131.28 (C1), 136.65 (C2), 137.14 (C3a), 139.04 (C8a), 139.19 (C12a), 156.50 (CO); *m*/*z* 315 (M^+, 15%), 211 (25), 184 (70), 115 (85), 104 (100); anal. found: C, 68.21; H, 6.53; N, 4.35. Calc. for $C_{18}H_{21}NO_2S$: C, 68.54; H, 6.71; N 4.44%.

Ethyl 5,6,7,8,9,15-hexahydrobenzo[*d*]benzothieno [2,3-*g*]-azecine-7-carboxylate (38)

Yellow oil (0.5 g, 69%); ^{1}H NMR (250 MHz; $CDCl_3$) δ (ppm) 0.93 (3H, t, CH_2CH_3), 2.06 (2H, brs, CH_2CH_3), 2.75–3.73 (8H, m, C(5, 6, 8, 9) H), 4.26 (2H, d, *J* 8.8, C(15) H), 7.14–7.78 (8H, m, Ph); ^{13}C NMR (62.5 MHz; $CDCl_3$) δ (ppm) 13.16 (CH_2CH_3), 26.13 (C9), 32.81 (C15), 33.07 (C5), 34.34 (CH_2CH_3), 54.17 (C8), 60.91 (C6), 120.53 (C13), 121.96 (C9a), 123.68 (C10), 123.79 (C11), 126.71 (C12), 127.03 (C3), 127.32 (C2), 129.02 (C9b), 130.29 (C4), 130.66 (C1), 138.28 (C13a), 138.96 (C14a), 140.00 (C4a), 141.32 (C15a), 156.40 (CO); *m*/*z* 365 (M^+, 35%), 249 (70), 234 (80), 115 (75), 104 (100); anal. found: C, 71.93; H, 6.36; N, 3.72. Calc. for $C_{22}H_{23}NO_2S$: C, 72.30; H, 6.34; N, 3.83%.

General procedure for the synthesis of the azecine derivatives 4, 5, and 6

To an ice-cooled suspension of $LiAlH_4$ (1 g, 2.6 mmol) in dry THF (15 mL), a solution of the respective carbamate derivative (6 mmol) in dry THF (10 mL) was added while stirring under inert atmosphere. The ice bath was then removed and the reaction mixture was heated to reflux for 3 h. It was then allowed to cool to room temperature and the excess unreacted $LiAlH_4$ was quenched with the careful addition of saturated Rochelle solution under inert atmosphere and with cooling in an ice bath until no H_2 evolves. The reaction mixture was then filtered, washed with dry THF, and the filtrate was evaporated under reduced pressure. The obtained residue was subjected to a purification process by silica gel chromatography using hexane : EtOAc 3 : 2.

6-Methyl-4,5,6,7,8,13-hexahydrobenzo[*d*]thieno[2,3-*g*]azecine (4)

Yellow oil (1.10 g, 70%); ^{1}H NMR (250 MHz; $CDCl_3$) δ (ppm) 2.22 (3H, s, N-Me), 2.59–2.87 (8H, m, C(4, 5, 7, 8) H), 4.35 (2H, s, C(13) H), 6.72 (1H, d, *J* 5, C(3), H), 7.03(1H, d, *J* 5, C(2), H), 7.06–7.18 (4H, m, Ph); ^{13}C NMR (62.5 MHz; $CDCl_3$) δ (ppm) 29.42 (C4), 32.84 (C13), 35.00 (C8), 46.20 (N-Me), 59.51 (C5), 59.59 (C7), 121.63 (C3a), 126.35 (C10), 126.50 (C11), 129.93 (C9), 130.21 (C12), 130.30 (C3), 137.37 (C2), 139.66 (C13a), 139.78 (C8a), 140.28 (C12a); *m*/*z* 257 (M^+, 25%), 115 (95), 199 (60), 184 (100), 165 (70), 152 (90); anal. found: C, 74.59; H, 6.98; N, 5.38. Calc. for $C_{16}H_{19}NS$: C, 74.66; H, 7.44; N, 5.44%.

6-Methyl-4,5,6,7,8,13-hexahydrobenzo[*d*]thieno[3,2-*g*]azecine (5)[12]

Yellow oil (1.13 g, 73%); ^{1}H NMR (250 MHz; $CDCl_3$) δ (ppm) 2.26 (3H, s, N-Me), 2.65–3.09 (8H, m, C(4, 5, 7, 8) H), 4.34 (2H, s, C(13) H), 6.92 (1H, d, *J* 5.8, C(1) H), 7.01–7.26 (4H, m, Ph) 7.31 (1H, d, *J* 5.8, C(2) H); ^{13}C NMR (62.5 MHz; $CDCl_3$) δ (ppm) 29.91 (C8), 33.71 (C13), 34.88 (C4), 46.29 (N-Me), 59.41 (C7), 60.67 (C5), 121.45 (C13a), 126.28 (C10), 129.93 (C11), 130.37 (C9), 130.49 (C12), 131.36 (C1), 137.77 (C2), 138.32 (C3a), 139.94 (C8a), 140.35 (C12a); *m*/*z* 257 (M^+, 25%), 199 (40), 184 (100), 165 (60), 152 (80), 115 (90); anal. found: C, 74.38; H, 7.05; N, 5.48. Calc. for $C_{16}H_{19}NS$: C, 74.66; H, 7.44; N, 5.44%.

7-Methyl-5,6,7,8,9,15-hexahydrobenzo[*d*]benzothieno[2,3-*g*]-azecine (6)

Pale yellow solid (1.2 g, 68%); m.p. 96–98 °C; ^{1}H NMR (250 MHz; $CDCl_3$) δ (ppm) 2.18 (3H, s, N-Me), 2.71–2.94 (8H, m, C(5, 6, 8, 9) H), 4.47 (2H, s, C(15) H), 7.09–7.45 (6H, m, Ph), 7.58 (1H, dd, *J* 1.5, 7, C(10) H), 7.77 (1 H, dd, *J* 1.5, 7, C(13) H); ^{13}C NMR (62.5 MHz; $CDCl_3$) δ (ppm) 25.63 (C9), 33.80 (C15), 34.93 (C5), 46.38 (N-Me), 59.22 (C8), 59.50 (C6), 121.01 (C13), 122.26 (C9a), 123.55 (C10), 123.75 (C11), 126.47 (C12), 126.84 (C3), 127.30 (C2), 130.39 (C9b), 130.55 (C4), 130.83 (C1), 138.80 (C13a), 138.92 (C14a), 140.25 (C4a), 140.36 (C15a); *m*/*z* 307 (M^+, 40%), 249 (40), 234 (100), 115 (90); anal. found: C, 78.48; H, 7.10; N, 4.13. Calc. for $C_{20}H_{21}NS \times 1/12$ hexane: C, 78.27; H, 7.12; N, 4.44%.

Conclusions

We have synthesized and evaluated two thieno-benzazecine regioisomeric compounds **4** and **5** possessing a reversed orientation for the thiophene sulfur atom. Within D_1-like family receptor subtypes, compound **4** showed high selectivity towards D_5 over D_1 receptors with a D_1/D_5 selectivity index of 20. On the other hand, compound **5** showed high selectivity towards D_1 over D_5 receptors with a D_5/D_1 selectivity index of 3.3. Analyzing the amino acids constituting the binding pockets of the D_1 and D_5 receptor subtypes, we identified the amino acids that are different within the binding pockets of the two target receptors. Similar to the findings for olanzapine,[25] we hypothesize that the thiophene *S* of **4** must be able to undergo more favorable protein–ligand interactions with the D_5 receptor. The amino acids S 6.62 and T 7.33 might act as direct H donors at the D_5 receptor. Alternatively, a water network that actually forms the polar interaction to the thiophene *S* is responsible for the D_5 selectivity. Such a water network might be formed more easily at the D_5 receptor, because it contains within the binding area four negatively charged aspartate or glutamate residues (D 3.26, D 4.68, D 5.34 and E 7.32).

Interestingly, reversing the position of the sulfur atom in compound **5** has led to shifting the selectivity towards D_1 receptors. To enhance the affinity towards the target receptors, the bulkier benzothieno-benzazecine analog **6** was synthesized keeping the same orientation of the sulfur atom as in compound **4**. Compound **6** showed better affinity to the target receptors (K_i for D_1 is 40 nM and for D_5 is 1.9 nM) and similar with compound **4**, it exhibited higher selectivity to D_5 over D_1 subtypes with a D_1/D_5 selectivity index of 20. Moreover, compound **6** also showed high affinity to D_2 receptor subtypes (K_i 1.5 nM) being one of the few azecine ligands with high affinity for both the D_1 and the D_2-like family members in the same order of magnitude.

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