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Identification of novel allosteric nonpeptidergic inhibitors of the human cytomegalovirus-encoded chemokine receptor US28

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ABSTRACT

Human cytomegalovirus (HCMV) is a widespread human pathogen, possessing onco-modulatory properties. Constitutive signaling of the HCMV-encoded chemokine receptor US28 and its ability to bind a broad spectrum of chemokines might facilitate HCMV-associated tumor progression. Novel nonpeptidergic chemotypes were identified as neutral antagonists or inverse agonists on US28, that allosterically inhibit chemokine binding to US28.

1. Introduction

Human cytomegalovirus (HCMV) is an infectious β-herpesvirus that establishes a lifelong latent infection in the majority of the human population and (ab)uses human cells for survival and propagation. Occasional reactivation and shedding of new infectious virions allow viral dissemination to other individuals. In order to establish viral latency and widespread propagation, HCMV has to hijack infected host cells and evade anti-viral immune responses. To this end, HCMV encodes important regulatory proteins that are expressed by infected host cells. One of these virally-encoded genes is the G protein-coupled receptor US28. US28 binds a broad variety of inflammatory chemokines allowing cell migration along chemokine gradients. In addition, recruitment of leukocytes to the microenvironment of HCMV-infected cells is impaired as inflammatory chemokines are depleted by constitutive and rapid US28-mediated internalization.[1–3] Although latent infection is generally asymptomatic, HCMV has been associated with several chronic inflammatory diseases and cancer.[4] Importantly, US28 activates in a ligand-independent manner proliferative and inflammatory signal transduction pathways.[5–7] This US28-mediated constitutive signaling induces a transformed and pro-angiogenic phenotype in NIH-3T3 cells, and promotes tumorigenesis in a nude mice model.[8]

Identification of small nonpeptidergic ligands is essential to investigate the role of US28 in evasion of the immune system and HCMV-associated cancers. Moreover, such molecules might be considered as promising therapeutics for anti-viral intervention. Previously, we identified VUF2274 (**1**) [5-(4-(4-chlorophenyl)-4-hydroxy-piperidin-1-yl)-2,2-diphenylpentanenitrile] as a nonpeptidergic allosteric inverse agonist for US28 (Fig. 1a).[9] Compound **1** inhibited US28-mediated constitutive signaling, chemokine binding, and US28-mediated human immunodeficiency virus (HIV) co-receptor activity with a low μM potency (EC_{50} and IC_{50}, respectively).[9] In subsequent lead optimization programs, approximately 100 new analogues were synthesized and pharmacologically characterized.[10,11] These structure–activity relationship (SAR) studies revealed the importance of the 4-phenylpiperidine moiety. However, the potency of this class of compounds could not be improved beyond the low μM range. In addition to these 4-substituted piperidine derivates (e.g., **1** and **2**), series of piperazinyldibenzothiepine (e.g., **3**, **4**), cinchonidine derivates (e.g., **5**) and benzamides like **6** have been described in the patent literature as inhibitors of chemokine binding to US28 with IC_{50} values in the 0.3–1 μM range (Fig. 1a).[12–14]

In order to identify novel chemotypes that can inhibit constitutive US28-mediated signaling and/or chemokine binding that might be used as new starting point for lead optimization, we screened a selection of compounds from our in-house collection.

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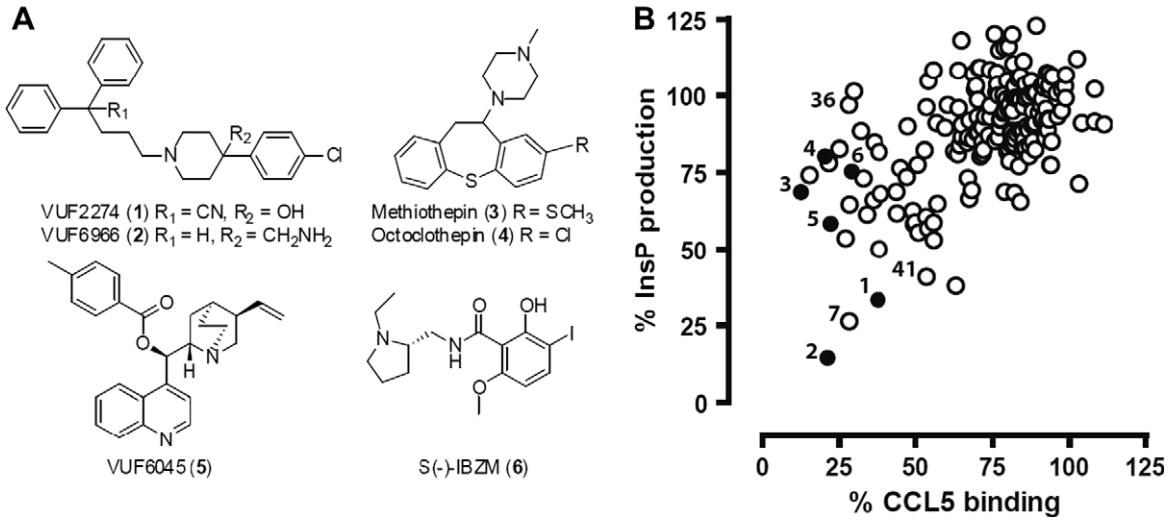
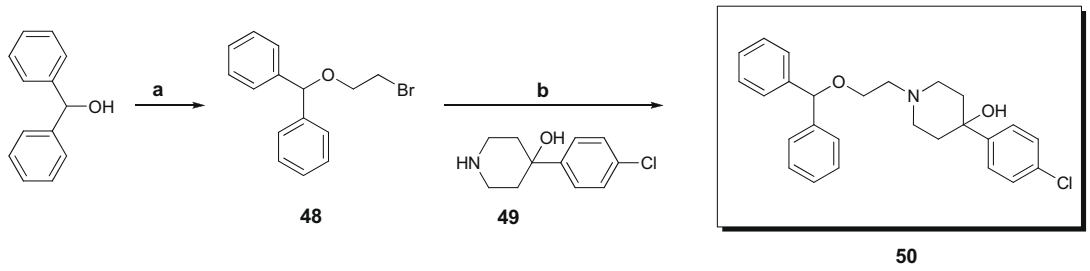


Figure 1. (A) Chemical structures of previously described nonpeptidergic ligands for US28. (B) Screening results using 10 μM nonpeptidergic ligand. Data are represented as percentage CCL5 binding (*X*-axis) and constitutive US28-mediated InsP production (*Y*-axis) in the absence of small ligands. The average of at least three independent experiments are shown. For clarity no error bars (<5%) are shown. Previously described compounds **1**–**6** are indicated and depicted in black, whereas most significant hits of this screen are indicated **7**, **36**, and **41**.



Scheme 1. Synthetic pathway for the synthesis of compound **50**. Reagents and conditions: (a) *p*-TSA, 2-bromoethanol, toluene, reflux; (b) NaI, Na_2CO_3, CH_3CN, microwave (15 min, 160 °C).

Compounds (**7**–**47**) were selected based on (sub)structural similarity to known ligands acting on US28 (Fig. 1a). Subsequently, the structure–activity relationships (SAR) around three promising hits (i.e., (**7**), (**36**), (**41**)) were explored by synthesizing structural analogues and subsequent pharmacological characterization.

2. Compound selection criteria

For this screening campaign, compounds **7**–**47** were selected from our in-house compound collection that share (sub)structural similarity to reported US28 ligands. To this end, substructure similarity searches were performed using ChemFinder Std. 7.0 (CambridgeSoft Corporation). In addition, compounds were selected based on subjective structural similarity criteria.

3. Chemistry

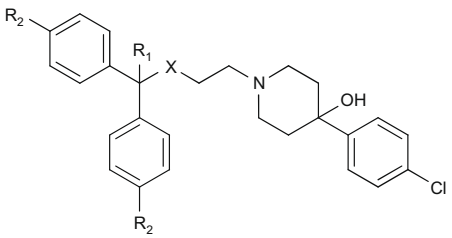
3.1. Synthesis of analogues of hit 7

Bromide intermediate **48**, which was synthesized in a quantitative yield by an acid-catalysed condensation of diphenylmethanol with 2-bromoethanol with azeotropic removal of water,[15] was reacted with 4-(4-chlorophenyl)piperidin-4-ol **49** in the microwave in the presence of NaI, Na_2CO_3 and CH_3CN to give compound **50** (Scheme 1). Compound **51** (Table 1) was synthesized in an analogous manner by the alkylation of the commercially available 4,4′-(4-chlorobutane-1,1-diyl)bis(fluorobenzene) with piperidine **49**.

3.2. Synthesis of analogues of hit 36

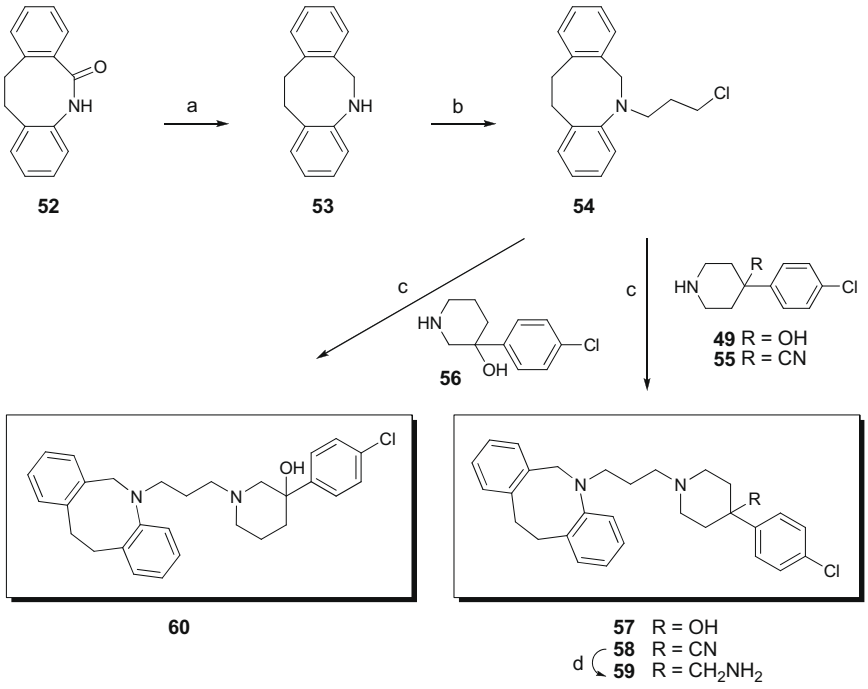
Reduction of 11,12-dihydrodibenzo[*b*,*f*]azocin-6(5*H*)-one **52** with $LiAlH_4$ resulted in 5,6,11,12-tetrahydrodibenzo[*b*,*f*]azocine **53**,[16] which was alkylated with 1-bromo-3-chloropropane in the microwave (15 min, 200 °C) to yield intermediate **54**. N-alkylation of piperidine moieties **55**, **49** and **56** with chloride **54** afforded inter-

Table 1
Chemical structures and pharmacological properties on US28 of compounds **1**, **7** and **50**–**51**



No.	R_1	R_2	X	IC_{50} (μM)	EC_{50} (μM)
1	CN	H	CH_2	4.9 (4.4–5.5)	6.3 (5.9–6.8)
7	H	F	O	2.3 (1.5–3.2)	4.6 (2.5–4.0)
50	H	H	O	2.1 (1.8–2.4)	3.5 (1.7–5.4)
51	H	F	CH_2	3.0 (2.5–3.5)	5.2 (3.2–7.2)

The IC_{50} and EC_{50} values (mean and interval) were generated in radioligand binding experiments using ^{125}I-CCL5 and InsP accumulation experiments, respectively. All experiments were performed in triplicate and repeated at least three times.



Scheme 2. Synthetic pathway for the synthesis of **57**, **59** and **60**. Reagents and conditions: (a) $LiAlH_4$, THF, reflux; (b) 1-bromo-3-chloropropane, Na_2CO_3, microwave (15 min, 200 °C); (c) NaI, Na_2CO_3, CH_3CN, microwave (15 min, 160 °C) or NaI, Na_2CO_3, CH_3CN, reflux; (d) $AlCl_3$, $LiAlH_4$, THF.

mediate **58**, and target compounds **57** and **60**, respectively (Scheme 2). Piperidine moiety **55** was synthesized starting from bis(2-chloroethyl)amine hydrochloride as previously described in the literature.[11,17] The 3-substituted piperidine moiety **56** was synthesized by a Grignard reaction of BOC-protected piperidin-3-one with 4-chlorophenyl magnesium bromide followed by a deprotection under acidic conditions.[18] The nitrile group of intermediate **58** was reduced in the presence of $AlCl_3$ and $LiAlH_4$ to yield target compound **59**.

3.3. Synthesis of analogues of compound 57

Unsubstituted amine **65** was commercially available, and amines **66** and **67** were synthesized in a quantitative yield via a reductive amination reaction with the corresponding anilines **61**–**62** and benzaldehydes **63**–**64** in the presence of sodium triacetoxyborohydride and acetic acid in DCE. The amines **65**–**67** were deprotonated with $NaNH_2$ in toluene at reflux temperature followed by an alkylation with 1-bromo-3-chloropropane to give intermediates **68**–**70**, which were reacted with piperidine **49** in the presence of NaI, Na_2CO_3, and CH_3CN (Scheme 3) to give compounds **71**, **72** and **73**.

3.4. Synthesis of compound 80

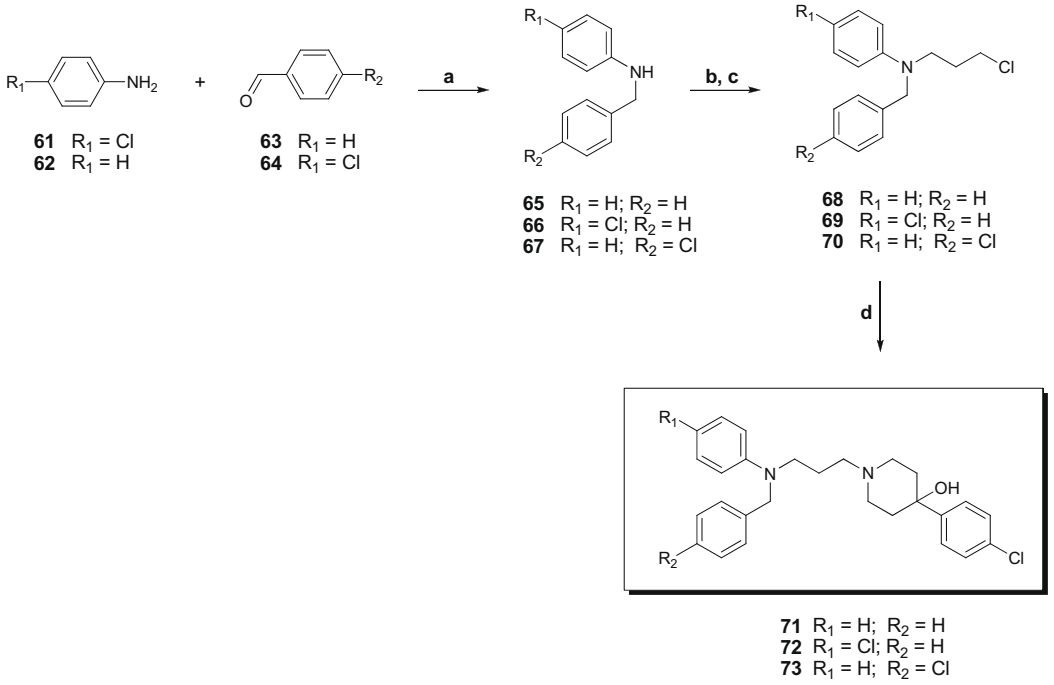
Rigid tricyclic analogue **80** was synthesized as depicted in Scheme 4. The reaction of 2-(2-iodophenyl)acetic acid **74** with 4-(methylthio)benzenethiol **75** in the presence of KOH, Cu and water gave intermediate **76**.[19] A subsequent ring closure to the tricyclic system of **77** was performed by a Friedel–Crafts acylation with PPA in toluene.[20] Reduction of the carbonyl group of **77** with $NaBH_4$ in MeOH resulted in alcohol **78**, which was reacted with $BF_3{\cdot}(Et)_2O$ and 2-bromoethanol in toluene to give bromide **79**.[21] N-Alkylation of piperidine **49** with **79** yielded target compound **80**.

3.5. Synthesis of analogues of hit 41

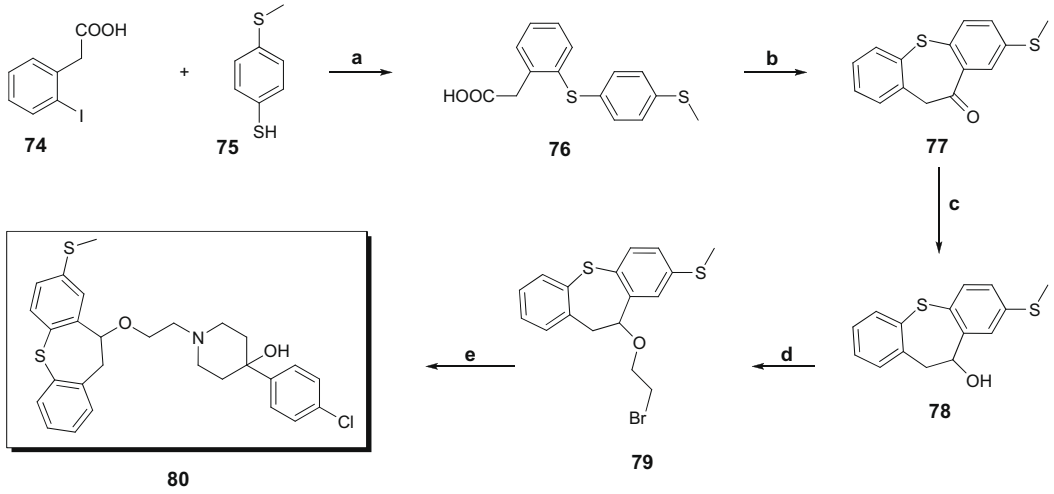
Indanones **81** were treated with the appropriate substituted benzaldehydes in a saturated solution of K_2CO_3 in EtOH (Scheme 5) to give the substituted 2-(phenylmethylene)-2,3-dihydro-1*H*-inden-1-ones **82**.[22] Reduction of both the carbonyl group as well as the double bond in one pot with lithium aluminium hydride was not successful. Therefore, hydrogenation of the double bond and subsequent reduction of the carbonyl group with sodium borohydride afforded the substituted 2-benzyl-2,3-dihydro-1*H*-inden-1-ols **83** as 1:1 mixture of both cis/trans isomers in moderate to high yields. Intermediates **83** were converted to the corresponding 1-azido-2-benzyl-2,3-dihydro-1*H*-indenes **84** in the presence of diphenyl phosphorazidate (DPPA) and 2,3,4,6,7,8,9,10-octahydropyrimidol[1,2-*a*]azepine (DBU) in THF.[23,24] For some of the azide intermediates it was possible to separate the two diastereomers via column purification. Hydrogenation of the azide group with palladium on activated carbon afforded target compounds **41** and **85**–**91**. The separation of the two diastereomers could not be accomplished after the last step of the synthesis route. Treatment of 2-benzyl-2,3-dihydro-1*H*-inden-1-ol derivatives **83** with thionyl chloride gave a 1:1 mixture of the two diastereomers of 1-chloro-2-(2,4-dichlorobenzyl)-2,3-dihydro-1*H*-indenes **92** in a quantitative yield. Alkylation of chloro derivatives **92** with commercially available amines in the presence of NaI, Na_2CO_3, and CH_3CN at reflux temperature or in the microwave, resulted in mainly elimination of HCl.[11] Therefore, the synthesis was performed in a different manner by heating the corresponding amine in CH_3CN to reflux temperature in the presence of Na_2CO_3 and NaI and subsequent addition of the appropriate chloride **92** when reflux temperature was reached. This resulted in the successful synthesis of target compounds **93**–**98** in moderate to low yields. This synthetic route resulted in the formation of only one diastereomer of the desired compounds.

4. Results and discussion

To identify new chemotypes that inhibit chemokine binding to US28 and/or display inverse agonism, 198 compounds were selected on the merit of (sub)structural similarities to known US28 ligands. Efficacy of these compounds (10 μM) was deter-



Scheme 3. Synthetic pathway for the synthesis of **71**–**73**. Reagents and conditions: (a) $NaBH(OAc)_3$, CH_3COOH, DCE; (b) $NaNH_2$, toluene, reflux; (c) 1-bromo-3-chloropropane, reflux; (d) **49**, NaI, Na_2CO_3, CH_3CN, microwave (15 min, 160 °C).

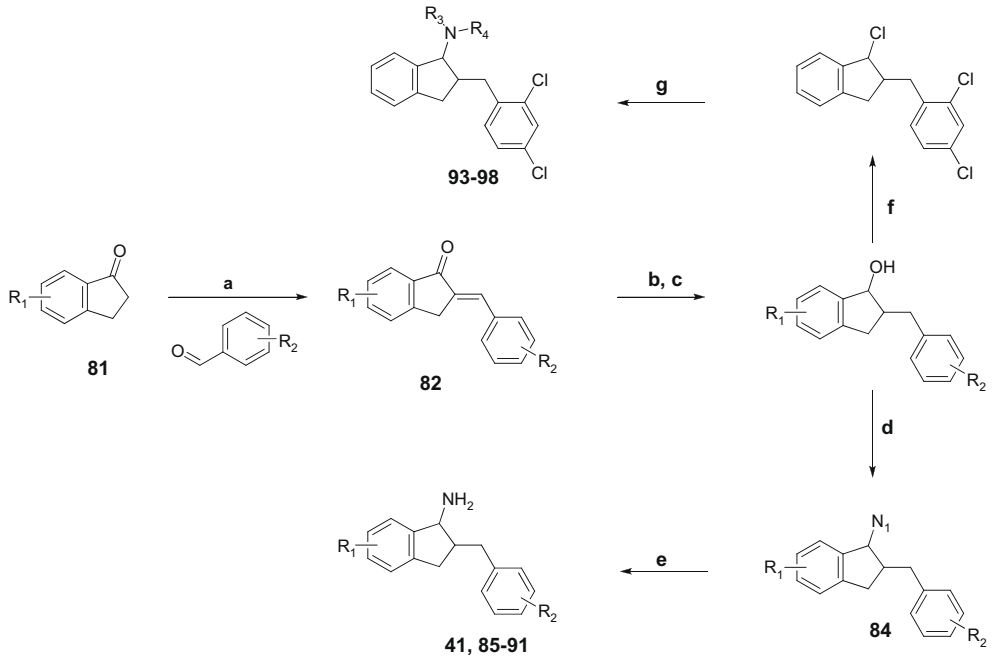


Scheme 4. Synthetic pathway for the synthesis of **80**. Reagents and conditions: (a) Cu, KOH, water, reflux; (b) PPA, toluene, reflux; (c) $NaBH_4$, MeOH; (d) $BF_3{\cdot}(Et)_2O$, 2-bromoethanol, toluene; (e) **49**, NaI, Na_2CO_3, CH_3CN, reflux.

mined by measuring their effect on the US28-mediated constitutive production of inositol phosphates. Their potency to allosterically inhibit chemokine binding to US28 was analyzed by means of ^{125}I-CCL5 radioligand binding assays in the presence of 10 μM of these compounds (Fig. 1b). Compounds that were identified as allosteric inhibitors of ^{125}I-CCL5 binding acted either as antagonists or inverse agonists when considering US28-mediated constitutive signaling. On the other hand, inverse agonistic compounds inhibited ^{125}I-CCL5 binding as well. Typically, no inverse agonists were identified that did not affect ^{125}I-CCL5 binding (i.e., lower left half of Fig. 1b). Several compounds were identified as novel allosteric inhibitors of US28, and those that inhibited CCL5 binding more than 50% were analyzed more extensively by determining their IC_{50} and EC_{50} values from radioligand competition curves and dose–response curves, respectively.

4.1. Structural analogues of VUF2274 (1)

Screening our in-house compound collection yielded the VUF2274 (**1**)-like compound (**7**) that displays a slightly higher potency to inhibit constitutive US28 signaling and CCL5 binding as compared with compound **1** (Fig. 1b and Table 1). To evaluate the importance of the fluoro atoms in the phenyl rings and the ether linkage in the alkyl chain between the diphenyl group and the piperidine moiety of **7**, compounds **50** and **51** were synthesized (Scheme 1). Removal of both fluoro atoms (i.e., **50**) or substitution of the oxygen atom with CH_2 (i.e., **51**) did not significantly affect



Scheme 5. Synthetic pathway for the synthesis of **41**, **85**–**91** and **93**–**98** (R_1–R_4 are listed in Tables 8 and 9). Reagents and conditions: (a) K_2CO_3, EtOH; (b) 10% Pd/C, H_2, MeOH; (c) $NaBH_4$, MeOH; (d) DPPA, DBU, THF; (e) 10% Pd/C, H_2, MeOH; (f) SOCfe, DCM; (g) amine, NaI, CH_3CN, 90 °C.

the potency to inhibit chemokine binding and US28-mediated signaling as compared to **7** (Table 1).

4.2. Structural analogues of methiothepin (3) and octoclothepin (4)

The piperazinyldibenzothiepine derivates methiothepin (**3**) and octoclothepin (**4**) inhibit CCL5 binding to US28 with a comparable potency as previously described for the binding of CX3CL1 to US28 (Fig. 1b and Table 2).[13] Compound **3** has a higher potency than the previously described 4-phenylpiperidine scaffold-based compounds (e.g., **1**).[9–11] Replacement of the thiomethylene group of compound **3** by a chloro atom in **4** resulted in a reduced potency to inhibit CCL5 binding which is comparable to that of **1**. On the other hand, both **3** and **4** act as partial inverse agonists on US28 (Fig. 1b) with slightly reduced potency as compared to **1** (Tables 1 and 2). The piperazinyldibenzodiazepine derivate clozapine (**8**), an atypical antipsychotic drug that binds to a variety of bioaminergic receptors, also diminished (~50%) CCL5 binding to US28 at a concentration of 10 μM (Table 2). Elongation of the R_2 group of **8** from methyl into ethyl (**9**), or butyl (**10**) resulted in IC_{50} values in the low micromolar range (Table 2). However, compounds **8**–**10** displayed only weak inverse agonistic activities on US28-mediated constitutive signaling. Substitution of the diazepine with an oxazepine moiety decreases the potency to block CCL5 binding to US28, whereas inverse agonistic activities were similar (**11**) or abolished (**12**) (Table 2). Changing the chloro substituent from the 8- (**11**) to the 2-position (**13**) of the dibenzo[*b*,*f*][1,4]oxazepine moiety attenuates both potency to block CCL5 binding and inverse agonistic activity (Table 2). Substitution of the oxazepine moiety of compounds **13** and **11** with a thiazepine moiety (compounds **14** and **15**, respectively) resulted in neutral antagonists with an increased potency to inhibit CCL5 binding (Table 2). Compounds **16**–**18** have a high flexibility due to their open structure as compared with the tricyclic moieties of compounds **3**–**4** and **8**–**15**. Compound **16** displays a lower potency to block CCL5 binding compared to the distinct tricyclic compounds (i.e., **3**, **4**, **8**, **11**, **13**, **14**, **15**). Interestingly, substitution of the methyl group at R_2 position of **16** with a ω-phenylbutyl group (i.e., compound **17**) restores the potency to inhibit CCL5 binding to US28 (Table 2). Subsequent replacement of the phenyl group of **17** with an alcohol moiety (i.e., **18**) diminishes potency again. Compounds **16** and **17**, but not **18**, display weak inverse agonistic efficacy.

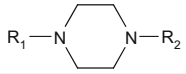
4.3. Tropine derivates

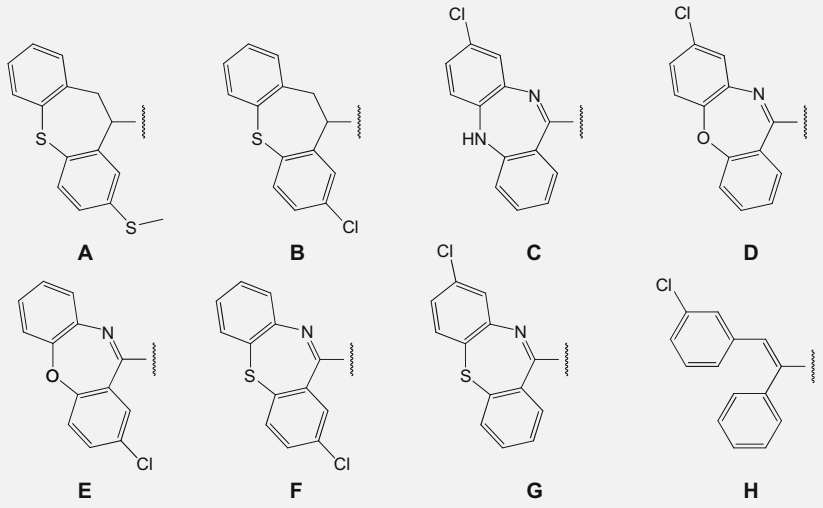
Our screening campaign also identified tropine derivatives as a new hits for US28. Compound B83 (**19**) inhibited CCL5 binding and constitutive US28 activity with IC_{50} and EC_{50} values, respectively, in the low μM range (Table 3). Replacement of the octyl group at the R position of **19** by a phenetyl group (i.e., compound **20**), and subsequent shortening of the alkyl linker between the basic nitrogen atom of the tropine ring and the phenyl ring (i.e., compound **21**) did not significantly affect the inhibitory properties on CCL5 binding or constitutive signaling (Table 3). On the other hand, small methyl and ethyl chains (i.e., compound **22** and **23**, respectively), *N*-hydroxypropionimidamide or ethyl-guanidine groups (i.e., compound **24** and **25**, respectively) are detrimental for their interaction with US28. In compounds **26**–**28** an unsaturated bridge between the two phenyl rings in the tricyclic moiety is introduced, which in combination with short branched alkyl chains at the R position (i.e., compounds **26** and **27**) possess no inhibitory properties on US28. However, placing a cyclohexyl group at this R position yielded an inhibitor (i.e., compound **28**) with IC_{50} and EC_{50} values in the low μM range (Table 3).

4.4. 1-Phenylpropan-2-amine derivates

Various N-substituted analogues of 1-phenylpropan-2-amine (i.e., compounds **29**–**35**) display low μM potencies to inhibit CCL5 binding and constitutive activity, even though their tricyclic moieties are quite different with respect to rigidity and the conformation of the two aromatic phenyl rings (Table 4).The two aromatic rings of the tricyclic moiety of compound **29** are tilted out of plane due to the presence of three carbon atoms between the two phenyl rings, whereas the tricyclic ring system of **30** is nearly planar, as deter-

Table 2
Chemical structures and pharmacological properties on US28 of compounds **3**–**4** and **8**–**18**





No.	R_1	R_2	IC_{50} (μM)	EC_{50} (μM)
3	A	$-CH_3$	0.41 (0.37–0.45)	7.6 (4.5–10.7)
4	B	$-CH_3$	4.9 (1.6–8.1)	12.1 (5.6–18.6)
8	C	$-CH_3$	47%[a]	11%[a]
9	C	$-CH_2CH_3$	3.8 (2.6–5.1)	16%[a]
10	C	$-(CH_2)_3CH_3$	4.7 (3.2–6.2)	19%[a]
11	D	$-CH_3$	19%[a]	22%[a]
12	D	$-CH_2CH_3$	38%[a]	0%[a]
13	E	$-CH_3$	13%[a]	0%[a]
14	F	$-CH_3$	28%[a]	0%[a]
15	G	$-CH_3$	47%[a]	0%[a]
16	H	$-CH_3$	13%[a]	20%[a]
17	H	$-(CH_2)_4Ph$	47%[a]	18%[a]
18	H	$-(CH_2)_4OH$	13%[a]	0%[a]

The IC_{50} and EC_{50} values (mean and interval) were generated in radioligand binding experiments using ^{125}I-CCL5 and InsP accumulation experiments, respectively. All experiments were performed in triplicate and repeated at least three times.
[a] Inhibition of CCL5 binding and constitutive US28 signaling at a 10 μM compound concentration.

mined by conformational analysis using the molecular modeling program MOE (molecular operating environment, version 2004.03). Saturation of the double bond of compound **30** (i.e., compound **31**), or introduction of an unsaturated bridge between the two phenyl rings in compound **32** did not significantly affect their potency to inhibit CCL5 binding or constitutive signaling (Table 4). Interestingly, a thioxanthene tricyclic moiety in combination with a *p*-chloro substituent on the phenethyl group confers low μM potency to block CCL5 binding, but weak inverse agonistic efficacy to compound **33** (Table 4). Similar inhibitory potencies were observed for compound **34** which consists of an acridine tricyclic ring system in combination with a *m*-trifluoromethyl group on the phenethyl group (Table 4). Replacing the tricyclic ring system in **34** with a 4,4′-difluorophenyl group (i.e., compound **35**) reduces the potency to block CCL5 binding to US28 (Table 4).

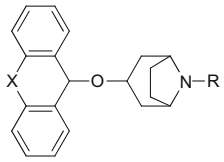
4.5. Identification and conversion of US28 antagonists into inverse agonists

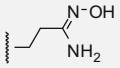
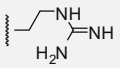
The tricyclic imipramine analogue A194 (**36**) was identified in this screen as the first allosteric neutral antagonist for US28 as it blocks CCL5 binding to US28 with an IC_{50} value of 4.9 μM, but displayed no inverse efficacy (Fig. 1b and Table 5). Replacing the *N*-methyl group in **36** with a 2-ethanol group in compound **37** resulted in a slightly lower IC_{50} value (Table 5). In contrast, converting the amine nitrogen atom in the tricyclic moiety to an amide nitrogen atom (i.e., compounds **38** and **39**) resulted in a significant reduced inhibition of CCL5 binding to US28 (Table 5). Hence, a basic nitrogen in the tricyclic moiety seemed to be essential for interaction of these antagonists with US28.

Previous SAR studies revealed that a 4-phenylpiperidine moiety of lead compound **1** is essential for inverse efficacy at US28.[10] Anticipating that this moiety would confer inverse efficacy to neutral antagonist **36**, we synthesized an hybrid compound **57** in which the *N*-methylpiperazine group of **36** was substituted with 4-(4-chlorophenyl)piperidin-4-ol (Fig. 2 and Scheme 2). Indeed, this hybrid compound **57** displayed inverse efficacy on US28-mediated constitutive signaling with an EC_{50} of 4.7 μM (Table 5). Moreover, compound **57** was sevenfold more potent in inhibiting CCL5 to binding than the two compounds (i.e., **1** and **36**), from which it originated (Table 5). In contrast to the previously described VUF2274-like compound **2**,[11] substitution of the 4-hydroxy group of compound **57** with a methylamine group (i.e., compound **59**), did not yield the anticipated increase in inhibitory potencies (Table 5). Introduction of a chiral center by moving the 4-hydroxy group and the 4-chlorophenyl substituent to the 3-position of the piperidine ring (i.e., compound **60**) did not change the pharmacological properties as compared to compound **57** (Table 5).

Next, flexible analogues of compound **57** were synthesized to explore the significance of rigidity in the tricyclic moiety for the

Table 3
Chemical structures and pharmacological properties on US28 of compounds **19–28**



No.	X	R	IC_{50} (μM)	EC_{50} (μM)
19	CH_2–CH_2	$(CH_2)_7CH_3$	5.6 (3.4–7.8)	4.9 (3.7–6.2)
20	CH_2–CH_2	$(CH_2)_2Ph$	11.9 (8.7–15.1)	4.3 (3.5–5.1)
21	CH_2–CH_2	CH_2Ph	10.5 (8.3–12.6)	8.8 (5.9–11.7)
22	CH_2–CH_2	CH_3	15%[a]	0%[a]
23	CH_2–CH_2	CH_2CH_3	7%[a]	0%[a]
24	CH_2–CH_2		0%[a]	0%[a]
25	CH_2–CH_2		34%[a]	0%[a]
26	CH=CH	$CH(CH_3)_2$	13%[a]	3%[a]
27	CH=CH	$(CH_2)_2CH_3$	18%[a]	0%[a]
28	CH=CH	Cyclohexyl	8.3 (6.8–9.8)	5.8 (3.3–8.3)

The IC_{50} and EC_{50} values (mean and interval) were generated in radioligand binding experiments using ^{125}I-CCL5 and InsP accumulation experiments, respectively. All experiments were performed in triplicate and repeated at least three times.
[a] Inhibition of CCL5 binding and constitutive US28 signaling at a 10 μM compound concentration.

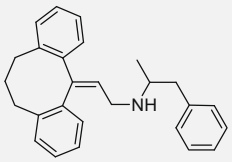
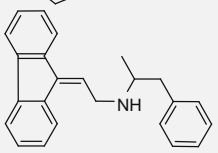
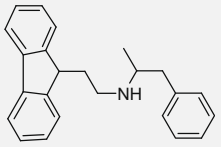
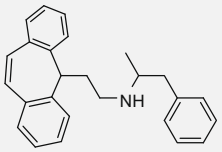
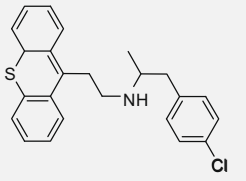
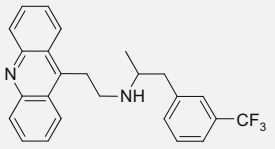
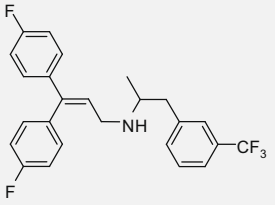
interaction with US28 (Scheme 3). Exclusion of the ethylene bridge between the two phenyl rings as in open analogue **71** decreased the potency to block CCL5 binding (Table 6). Subsequent introduction of a chloro substituent at the 4-position of one of the phenyl rings revealed an interesting regioselectivity with compound **72** having a slightly higher potency to inhibit CCL5 binding than compound **73**. Not surprisingly, these modifications in the tricyclic moiety did not significantly affect the inverse agonistic potencies of compounds **71**, **72**, and **73** as compared to **57** (Table 6).

Challenged by the observed increase in potency of compound **57** to block CCL5 binding to US28 as compared to compounds **1** and **36**, we decided to design and synthesize another hybrid compound by fusing the 4-(4-chlorophenyl)piperidin-4-ol scaffold to the tricyclic moiety of compound **3** (Scheme 4). Compound **3** had the lowest IC_{50} value so far observed and we anticipated to generate a full inverse agonist with an increased potency to inhibit CCL5 binding. Unfortunately, this hybrid compound (**80**) displayed decreased potency to block CCL5 binding to US28 (Table 6).

4.6. Structural analogues of S(−)-IBZM (6)

Automated similarity searches of our in-house compound collection using ChemFinder Std. 7.0 software did not yield any analogues. However, inspired by the substructure of S(−)-IBZM (**6**) several analogues were selected from our library by subjective visual inspection. Pharmacological analysis of these S(−)-IBZM-like analogues revealed some interesting preliminary SAR. Compound **41** [2-(2,4-dichlorobenzyl)-2,3-dihydro-1*H*-inden-1-amine] was identified as a novel scaffold for inverse agonism on US28 (Fig. 1b and Table 7). Conversion of the cyclopentyl group of **41** into cyclohexyl (i.e., compound **42**) or cycloheptyl (i.e., compound **43**) did not affect the potency to inhibit CCL5 binding (Table 7). However, compound **43** displayed significantly less inverse agonistic potency as compared to compounds **41** and **42**. Interestingly, increasing the conformational freedom by replacing the rigid ring structure with a more flexible propyl chain as in compound **44** did not significantly affect the pharmacological properties as compared to compounds **41** and **42** (Table 7). However, the presence of 3,4-dichloro substituents on (at least one of) the phenyl rings

Table 4
Chemical structures and pharmacological properties on US28 of compounds **29–35**

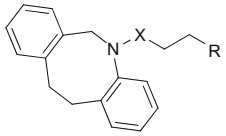
No.	Structure	IC_{50} (μM)	EC_{50} (μM)
29		4.2 (3.6–4.8)	39%[a]
30		7.0 (5.9–8.1)	42%[a]
31		5.2 (4.4–6.0)	10.9 (6.0–15.8)
32		3.7 (3.2–4.2)	5.2 (3.2–7.1)
33		2.6 (2.0–3.2)	21%[a]
34		5.5 (4.5–6.5)	34%[a]
35		39%[a]	38%[a]

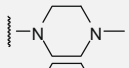
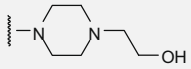
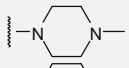
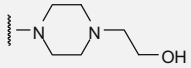
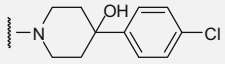
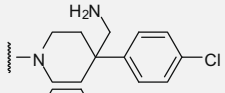
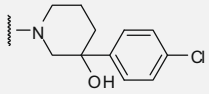
The IC_{50} and EC_{50} values (mean and interval) were generated in radioligand binding experiments using ^{125}I-CCL5 and InsP accumulation experiments, respectively. All experiments were performed in triplicate and repeated at least three times.
[a] Inhibition of CCL5 binding and constitutive US28 signaling at a 10 μM compound concentration.

seems to be essential as indicated by compound **45**. Interestingly, compounds **46** and **47**, in which the amine substituent is replaced with a bulky 2-methoxy-2-phenylethanamine group, displayed similar IC_{50} and EC_{50} values as the smaller analogues **41** and **42** (Table 7). Replacement of the dichloro substituents (**46**) in the phenyl ring with a *t*-butyl group (**47**) at position 4 did not alter the pharmacological properties.

The structure of compound **41** provided some interesting options for further lead optimization. Firstly, compounds **85–91** with various modifications in the 2,4-dichlorobenzyl moiety were designed and synthesized to optimize the substitution pattern in the benzyl ring (Scheme 5 and Table 8). Substitution of the electron withdrawing chloro groups with electron donating methyl groups in compound **85** resulted in a small decrease in the potency to inhibit CCL5 binding to US28, without affecting inverse efficacy. Subsequent introduction of two methoxy groups at the *ortho* and *para*

Table 5
Chemical structures and pharmacological properties on US28 of compounds **36**–**39** and **57**, **59** and **60**



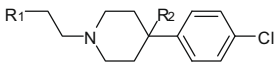
No.	X	R	IC_{50} (μM)	EC_{50} (μM)
36	CH_2		4.9 (2.8–7.1)	3% ± 15%
37	CH_2		1.6 (1.3–1.9)	0%[a]
38	C(=O)		>100	0%[a]
39	C(=O)		>100	0%[a]
57	CH_2		0.7 (0.6–0.9)	4.7 (2.8–6.6)
59	CH_2		1.6 (1.0–2.2)	7.6 (4.8–10.5)
60	CH_2		0.9 (0.7–1.0)	3.9 (3.6–4.2)

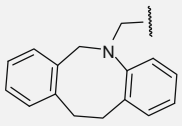
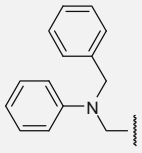
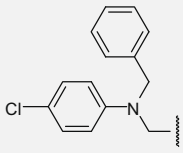
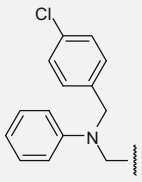
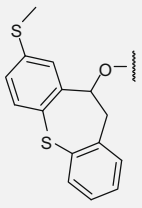
The IC_{50} and EC_{50} values (mean and interval) were generated in radioligand binding experiments using ^{125}I-CCL5 and InsP accumulation experiments, respectively. All experiments were performed in triplicate and repeated at least three times.

[a] Inhibition of CCL5 binding and constitutive US28 signaling at a 10 μM compound concentration.

position of the benzyl ring, significantly attenuated the potency of compound **86** to inhibit CCL5 binding as well as constitutive US28 signaling. Although a 2-methoxy substitution is allowed at the benzyl moiety (compound **87**), the presence of a 4-methoxy group in compounds **86** and **88** seemed to be detrimental for interaction with US28. Interestingly, the potency to inhibit CCL5 binding and inverse agonism was restored in compound **89** by introducing an additional 3-methyl group in 4-methoxyphenyl ring (Table 8).

Table 6
Chemical structures and pharmacological properties on US28 of compounds **57**, **71**–**73** and **80**



No.	R_1	R_2	IC_{50} (μM)	EC_{50} (μM)
57		OH	0.7 (0.6–0.9)	4.7 (2.8–6.6)
71		OH	4.1 (2.8–5.4)	3.0 (2.8–6.0)
72		OH	1.8 (1.4–2.1)	5.0 (3.9–6.2)
73		OH	5.7 (4.3–7.1)	5.5 (5.0–6.0)
80		OH	12.4 (11.2–13.5)	7.1 (4.9–9.3)

The IC_{50} and EC_{50} values (mean and interval) were generated in radioligand binding experiments using ^{125}I-CCL5 and InsP accumulation experiments, respectively. All experiments were performed in triplicate and repeated at least three times.

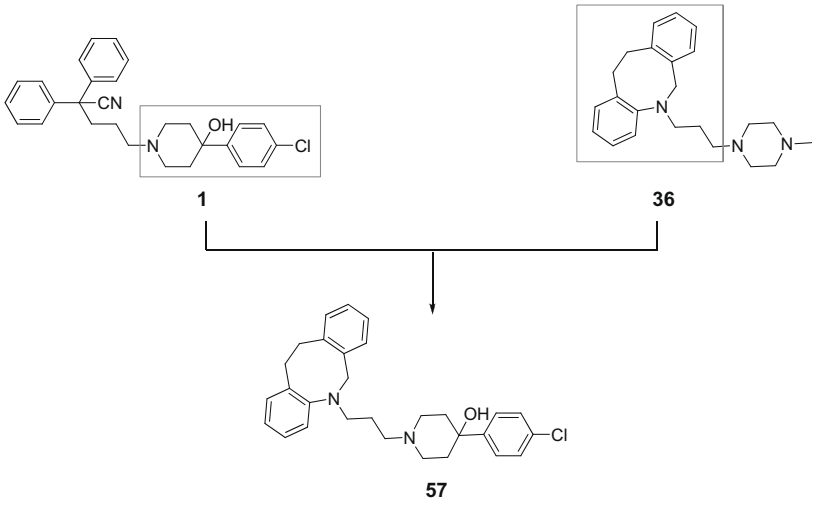
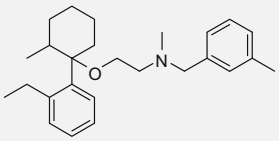
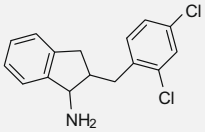
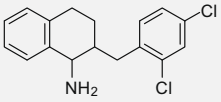
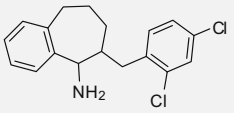
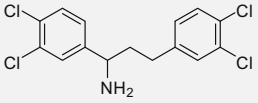
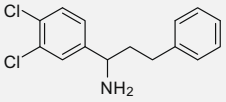
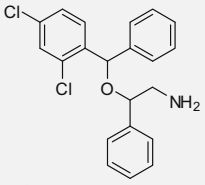
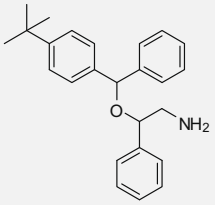


Figure 2. Chemical structures of inverse agonist **1**, neutral antagonist **36** and hybrid compound **57**.

Table 7
Chemical structures and pharmacological properties on US28 of compounds **40**–**47**

No.	Structure	IC_{50} (μM)	EC_{50} (μM)
40		12.9 (5.4–20.4)	10%[a]
41		9.5 (6.8–12.3)	11.1 (8.3–13.8)
42		8.5 (6.8–10.2)	12.6 (10.0–15.1)
43		11.8 (11.5–12.0)	26%[a]
44		7.5 (5.0 -10.0)	11.7 (9.3–14.1)
45		5%[a]	34%[a]
46		9.2 (6.3–12.0)	10.2 (7.2–9.5)
47		8.2 (6.3–10.0)	8.4 (7.2–9.5)

The IC_{50} and EC_{50} values (mean and interval) were generated in radioligand binding experiments using ^{125}I-CCL5 and InsP accumulation experiments, respectively. All experiments were performed in triplicate and repeated at least three times.

[a] Inhibition of CCL5 binding and constitutive US28 signaling at a 10 μM compound concentration.

Introduction of an acetamide substituent at the *para* position of the phenyl ring (i.e., compound **90**) or removal of all substituents (i.e., compound **91**) impaired all inhibitory activities at US28 (Table 8).

To evaluate the importance of the amine group of lead compound **41**, several analogues were designed and synthesized in which the nitrogen atom was incorporated into different ring systems (Scheme 5 and Table 9). Substitution of the amine group with a 4-(4-chlorophenyl)piperidin-4-ol group (i.e., compound **93**), a piperidine ring (i.e., compound **94**), or an *N*-methylpiperazine moiety (i.e., compound **95**) are tolerable and did not have a major impact on the pharmacological properties as compared to lead compound **41** (Table 9). On the other hand, introduction of an *N*-phenylpiperazine group (i.e., compound **96**) or unsaturated tetrahydropyridine moiety (i.e., compound **97**), or attachment of a benzyl group to the nitrogen atom (i.e., compound **98**) are not allowed and impaired the potency to inhibit CCL5 binding to US28, as well as US28-mediated constitutive signaling (Table 9).

5. Conclusions

To identify novel nonpeptidergic ligands acting on the constitutively active HCMV-encoded receptor US28, a selection of chemotypes extracted from our in-house compound collection was screened on their potency to inhibit CCL5 binding to US28 and constitutive receptor signaling. Compounds **7** and **41** were identified as novel inverse agonists acting on US28, whereas compound **36** was identified as a neutral antagonist. Combining the tricyclic group of this neutral antagonist with the 4-(4-chlorophenyl)piperidin-4-ol moiety of lead inverse agonist **1**, resulted in a hybrid compound **57** displaying inverse efficacy and an increased potency to block CCL5 binding. Hence, inverse efficacy can indeed be attributed to the 4-(4-chlorophenyl)piperidin-4-ol moiety as previously indicated.[10,11] SAR of compounds **7**, **57** and **41** were further explored by generating series of structural analogues, however none of the modifications resulted in improved inhibitory activities on US28. The identification of potent nonpeptidergic inverse agonists and antagonists will provide us with a tool to validate the significance of US28 in HCMV-associated pathologies.

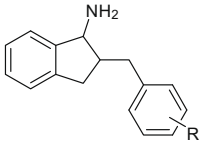
6. Experimental

6.1. General procedures

THF and DCM were freshly distilled from lithium aluminium hydride. All reactions were performed under an atmosphere of dry nitrogen. Microwave reactions were performed in a CEM Explorer single mode MW reactor equipped with auto sampler. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AC-200 (200 MHz) spectrometer unless otherwise stated. J.T. Baker silica gel was used for flash chromatography. Melting points were measured on a MPA100 OptiMelt automated melting point system apparatus and were uncorrected. HRMS mass spectra were recorded on a Finnigan MAT 900 mass spectrometer. Analytical HPLC-MS analyses were conducted using a Shimadzu LC-8A preparative liquid chromatograph pump system with a Shimadzu SPD-10AV UV–vis detector set at 254 nm, with the MS detection performed with a Shimadzu LCMS-2010 liquid chromatograph mass spectrometer. The analyses were performed using the following conditions; condition I: a Xbridge(C18)5um column (100 mm × 4.9 mm) with 70% MeOH–30% H_2O–0.1% formic acid (Method Ia); 60% MeOH–40% H_2O–0.1% formic acid (Method Ib); 50% MeOH–50% H_2O–0.1% formic acid (Method Ic); 40% MeOH–60% H_2O–0.1% formic acid (Method Id) or 30% MeOH–70% H_2O–0.1% formic acid (Method Ie). Flow rate = 1.0 mL/min. Total run time 15 min unless otherwise stated. Condition II: a Xbridge(C18)5um column (100 mm × 4.9 mm) with 50% CH_3CN–50% H_2O–0.1% formic acid (Method IIa); 40% CH_3CN–60% H_2O–0.1% formic acid (Method IIb), 35% CH_3CN–65% H_2O–0.1% formic acid (Method IIc), 30% CH_3CN–70% H_2O–0.1% formic acid (Method IId); 25% CH_3CN–75% H_2O–0.1% formic acid (Method IIe) or 20% CH_3CN–80% H_2O–0.1% formic acid (Method IIf). Flow rate = 1.0 mL/min. Total run time 20 min. Condition III: a Xbridge(C18)5um column (100 mm × 4.9 mm) with 60% CH_3CN–40% H_2O with 10% NH_4HCO_3/NH_4OH buffer pH 8 (Method IIIa); 50% CH_3CN–50% H_2O with 10% NH_4HCO_3/NH_4OH buffer pH 8 (Method IIIb). Flow rate = 1.0 mL/min. Total run time 20 min. Compounds that were isolated as fumaric acid salts all showed an extra peak around 2 min. Fumaric acid blancs were used to determine the *t*R of fumaric acid. Purities calculated are based on RP.

Compounds **1**, **2**, **11** and **12** were previously described.[9,11,25] Compounds **3**, **4**, **6**, **8**, and **13** were purchased from Sigma–Aldrich

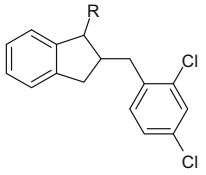
Table 8
Chemical structures and pharmacological properties on US28 of compounds **41**, and **85–91**

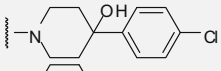
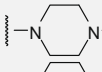
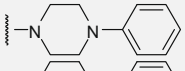
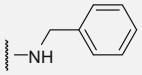


No.	R	IC_{50} (μM)	EC_{50} (μM)
41	2,4-Cl_2	9.5 (6.8–12.3)	11.1 (8.3–13.8)
85	2,4-Me_2	17.7 (14.5–20.9)	13.7 (9.1–18.2)
86	2,4-$(OMe)_2$	>100	>100
87	2-OMe	37.3 (28.8–45.7)	42.7 (41.7–43.7)
88	4-OMe	>100	>100
89	3-Me-4-OMe	59.7 (50.1–69.2)	16.4 (14.1–18.6)
90	4-NH(C=O)CH_3	>100	>100
91	H	80.6 (32.4–128.8)	>100

The IC_{50} and EC_{50} values (mean and interval) were generated in radioligand binding experiments using ^{125}I-CCL5 and InsP accumulation experiments, respectively. All experiments were performed in triplicate and repeated at least three times.

Table 9
Chemical structures and pharmacological properties on US28 of compounds **41**, and **93–98**



No.	R	IC_{50} (μM)	EC_{50} (μM)
41		9.5 (6.8–12.3)	11.1 (8.3–13.8)
93		11.0 (10.0–12.0)	6.0 (4.0–7.9)
94		13.4 (11.2–15.5)	2.6 (2.1–3.0)
95		8.1 (6.6–9.5)	12.1 (9.1–15.1)
96		>100	>100
97		>100	>100
98		>100	>100

The IC_{50} and EC_{50} values (mean and interval) were generated in radioligand binding experiments using ^{125}I-CCL5 and InsP accumulation experiments, respectively. All experiments were performed in triplicate and repeated at least three times.

Co. (USA). Compounds **14** and **15** were kindly donated by Dr. Aebischer from the Sandoz Research Institute in Bern. Compounds **5**, **7**, **9**, **10** and **16**–**47** were selected from our in-house screening library. Purity of all compounds was ⩾95% as determined by LC–MS measurements.

6.1.1. General method A: 1-(2-(benzhydryloxy)ethyl)-4-(4-chlorophenyl)piperidin-4-ol fumarate (50)

Bromide intermediate **48** (0.58 g, 1.99 mmol), which was synthesized following a method previously described,[15] 4-(4-chlorophenyl)piperidin-4-ol **49** (0.51 g, 2.41 mmol), NaI (0.30 g, 2.00 mmol), Na_2CO_3 (0.42 g, 3.96 mmol) and 3 mL CH_3CN were added in a 10 mL microwave vessel and this was reacted during 15 min in the microwave at a temperature of 160 °C (settings: ramp time 5 min, hold time 15 min, power 200 watt, pressure 17.2 bar). The solvent was removed in vacuo and the residue was diluted with water (20 mL), followed by an extraction with DCM (3 × 15 mL). The combined organic layers were washed with water (3 × 40 mL) and brine (40 mL), dried over anhydrous $MgSO_4$, filtered, and evaporated in vacuo. Purification by flash chromatography (0–50% EtOAc in DCM) gave 480 mg (57%) of the free base as an oil. This was dissolved in EtOAc and acidified by the addition of a saturated solution of fumaric acid in Et_2O. The fumaric salt was isolated by filtration and recrystallized from MeOH/Et_2O to give 281 mg (52%) of **50** as a white solid. ^{1}H NMR ($CDCl_3$): δ 1.60–1.81 (m, 2H), 2.08–2.43 (m, 2H), 2.84–3.25 (m, 6H), 3.65–3.78 (m, 2H), 5.28 (s, 1H), 6.63 (s, 2H), 7.11–7.36 (m, 14H). ^{13}C ($CDCl_3$): δ 36.29, 49.42, 56.91, 64.68, 69.54, 84.64, 126.57, 127.30, 128.09, 128.89, 134.06, 135.30, 141.78, 148.74, 169.53. Anal. RP-HPLC *Ib*: t_R = 8.60 min (purity 100%), *IId*: t_R = 12.13 min (purity 100%). HRMS (ESI) *m*/*z* calcd for $C_{26}H_{28}ClNO_2$: 421.1808; found: 421.1803.

6.1.2. General method B: 1-(4,4-bis(4-fluorophenyl)butyl)-4-(4-chlorophenyl)piperidin-4-ol (51)

A solution of 4,4′-(4-chlorobutane-1,1-diyl)bis(fluorobenzene) (0.56 g, 1.99 mmol), 4-(4-chlorophenyl)piperidin-4-ol **49** (0.51 g, 2.41 mmol), NaI (0.30 g, 2.00 mmol) and Na_2CO_3 (0.43 g, 4.06 mmol) in CH_3CN (20 mL) was refluxed overnight. The solvent was removed in vacuo, the residue was diluted with water (50 mL) and extracted with DCM (3 × 30 mL). The combined organic layers were washed with water (3 × 50 mL) and brine (50 mL), dried over anhydrous $MgSO_4$, filtered and evaporated in vacuo. Purification by flash chromatography (0–100% EtOAc in DCM) and recrystallization from EtOAc gave 853 mg (94%) of **51** as a white solid. ^{1}H NMR ($CDCl_3$): δ 1.48–1.72 (m, 5H), 1.92–2.24 (m, 4H), 2.30–2.55 (m, 4H), 2.69–2.85 (m, 2H), 3.86 (t, *J* = 7.8 Hz, 1H), 6.90–7.43 (m, 12H). ^{13}C ($CDCl_3$): δ 25.76, 34.27, 38.71, 49.83, 50.14, 58.92, 71.41, 115.57, 115.78, 126.47, 128.82, 129.43, 129.51, 133.22, 140.86, 140.89, 147.16, 160.54, 162.97. Anal. RP-HPLC *Ib*: t_R = 8.78 min (purity 100%), *IIb*: t_R = 13.99 min (purity 100%). HRMS (EI) *m*/*z* calcd for $C_{27}H_{28}ClF_2NO$: 455.1827; found: 455.1826.

6.1.3. General method C: 1-(3-((4-chlorobenzyl)(phenyl)amino)-propyl)-4-(4-chlorophenyl)-piperidin-4-ol fumarate (73)

(i) A solution of aniline **62** (1.82 mL, 20.0 mmol), 4-chlorobenzaldehyde **64** (2.35 mL, 20.0 mmol) and CH_3COOH (1.14 mL, 19.9 mmol) in DCE (100 mL) was stirred for 24 h at room temperature and $NaBH(OAc)_3$ (6.36 g, 30.0 mmol) was added. The reaction mixture was stirred for another 18 h, quenched with 5% Na_2CO_3 (100 mL) and the water layer was extracted with DCM (3 × 100 mL). The combined organic layers were washed with brine (150 mL), dried over anhydrous Na_2SO_4, filtered and evaporated in vacuo to give 4.42 g (100%) of **67** as a brown solid. ^{1}H NMR ($CDCl_3$): δ 4.06 (br s, 1H), 4.29 (s. 2H), 6.51–6.58 (m, 2H), 7.07–7.14 (m, 2H), 7.24–7.36 (m, 5H).

(ii) $NaNH_2$ (0.39 g, 10.0 mmol) was added to a solution of **67** (1.09 g, 5.01 mmol) in toluene (15 mL) and this was refluxed for 18 h. 1-Bromo-3-chloropropane (2.5 mL, 25.3 mmol) was added and the reaction mixture was refluxed for another 18 h. Water (25 mL) was added and the water layer was extracted with EtOAc (3 × 20 mL). The combined organic layers were washed with water (3 × 30 mL) and brine (40 mL), dried over anhydrous Na_2SO and filtered. After evaporation under reduced pressure, the residue was purified by flash chromatography (5% DCM in hexane) to give 142 mg (10%) of **70** as a light yellow oil. ^{1}H NMR ($CDCl_3$): δ 1.98–2.18 (m, 2H), 3.45–3.68 (m, 4H), 4.50 (s, 2H), 6.51–6.75 (m, 3H), 7.03–7.28 (m, 6H).

(iii) Following method B using **70** gave 142 mg of the free base as an oil. This was converted to the fumaric salt as described for **50**

to give 178 mg (63%) of **73** as a white solid. ^{1}H NMR (MeOH-d_4): δ 1.81–1.93 (m, 2H), 1.97–2.17 (m, 2H), 2.19–2.39 (m, 2H), 2.98–3.39 (m, 6H), 3.50 (t, J = 7.2 Hz, 2H), 4.54 (s, 2H), 6.59–6.30 (m, 4H), 7.02–7.51 (m, 11H).^{13}C (MeOH-d_4): δ 23.81, 36.88, 49.57, 50.09, 55.21, 55.84, 69.75, 114.39, 118.29, 127.48, 129.44, 129.58, 129.61, 130.30, 133.52, 134.07, 137.00, 139.26, 147.47, 149.37, 173.62. Anal. RP-HPLC *Ib*: t_R = 4.87 min (purity 100%), *IIb*: t_R = 3.89 min (purity 100%). HRMS (EI) m/z calcd for $C_{27}H_{30}Cl_2N_2O$: 468.1735; found: 468.1721.

6.1.4. 5-(3-Chloropropyl)-5,6,11,12-tetrahydrodibenzo[*b*,*f*]azocine (54)

5,6,11,12-Tetrahydrodibenzo[*b*,*f*]azocine **53** (0.42 g, 2.01 mmol), which was synthesized as previously described in the literature,[16] and Na_2CO_3 (0.42 g, 3.96 mmol) were added in a 10 mL microwave vessel and 1-bromo-3-chloropropane (3 mL) was added. This solution was reacted in the microwave for 15 min at a temperature of 200 °C (settings: ramp time 5 min, hold time 15 min, power 200 watt, pressure 17.2 bar) and filtered. The solvent was evaporated in vacuo and the residue was purified by flash chromatography (15% DCM in hexane) to give 228 mg (40%) of **54** as a colorless oil. ^{1}H NMR ($CDCl_3$): δ 1.85–1.98 (m, 2H), 3.04–3.48 (m, 8H), 4.15 (s, 2H), 6.80–7.24 (m, 8H).

6.1.5. 4-(4-Chlorophenyl)-1-(3-(11,12-dihydrodibenzo[*b*,*f*]azocin-5(6*H*)-yl)propyl)piperidine-4-ol fumarate (57)

Following method A using **54** (0.29 g, 1.01 mmol) gave 289 mg of the free base as an oil. This was converted to the fumaric salt as described for **50** and recrystallized from MeOH/Et_2O to give 199 mg (38%) of **57** as white crystals. ^{1}H NMR ($CDCl_3$): δ 1.50–1.92 (m, 5H), 2.09–2.36 (m, 2H), 2.41–2.70 (m, 4H), 2.78–3.34 (m, 8H), 4.11 (s, 2H), 6.69 (s, 2H), 6.72–7.35 (m, 12H). ^{13}C ($CDCl_3$): δ 23.15, 33.28, 34.22, 35.92, 48.11, 51.14, 55.08, 60.96, 69.57, 119.32, 122.17, 125.91, 126.82, 127.15, 128.39, 128.79, 129.73, 131.08, 132.94, 135.45, 136.06, 137.65, 141.83, 145.37, 150.02, 171.33. Anal. RP-HPLC *Ib* (total run time 20 min): t_R = 14.43 min (purity 100%), *IIc*: t_R = 11.35 min (purity 97%). HRMS (EI) m/z calcd for $C_{29}H_{33}ClN_2O$: 460.2281; found: 460.2284.

6.1.6. (4-(4-Chlorophenyl)-1-(3-(11,12-dihydrodibenzo[*b*,*f*]azocin-5(6*H*)-yl)propyl)piperidin-4-yl)methanamine fumarate (59)

(i) Following method B using **54** and 4-(4-chlorophenyl)-piperidine-4-carbonitrile hydrochloride **55**, which was synthesized as previously described in the literature,[11] afforded 675 mg of **58** as a light yellow oil. The crude product was used without further purification.

(ii) $AlCl_3$ (0.37 g, 2.78 mmol) was added portion wise to a suspension of $LiAlH_4$ (0.11 g, 2.90 mmol) in THF (10 mL) at 0 °C and this was stirred for 5 min followed by the drop wise addition of a solution of **58** (675 mg, 1.44 mmol) in THF (5 mL). The reaction mixture was allowed to warm to room temperature, stirred overnight, cooled with an ice bath and quenched with a saturated solution of Na_2CO_3 in water until the foaming stopped. Subsequently, the suspension was filtered; the filtrate was dried over anhydrous $MgSO_4$, filtered again and evaporated in vacuo. Purification by flash chromatography (5% Et_3N in EtOAc) afforded 183 mg (39%) of the free base as an oil. This was converted to the fumaric salt as described for **50** to give 199 mg (24%) of **59** as a light brown solid. ^{1}H NMR ($CDCl_3$/DMSO-d_6): δ 1.54–1.75 (m, 2H), 1.82–1.93 (m, 2H), 2.05–2.18 (m, 4H), 2.27–2.39 (m, 2H), 2.59–2.68 (m, 2H), 2.86 (s, 2H),2.92–2.99 (m, 2H), 3.03–3.19 (m, 6H), 4.11 (s, 2H),6.60 (s, 2H), 6.73 (t, J = 7.3 Hz, 1H), 6.89–7.04 (m, 6H), 7.11–7.39 (m, 5H). ^{13}C NMR ($CDCl_3$/DMSO-d_6): δ 24.06, 31.36, 32.79, 33.75, 38.67, 48.42, 50.68, 54.91, 60.22, 119.50, 121.68, 125.47, 126.33, 126.68, 128.51, 128.59, 128.79, 129.20, 130.57, 131.95, 134.13, 135.82, 137.06, 141.06, 146.64, 149.77. Anal. RP-HPLC *Ib*: t_R = 4.43 min (purity 99%), *Ic* (total run time 20 min): t_R = 14.35 min (purity 100%), *IIe* (total runtime 30 min): t_R = 21.45 min (purity 96%). HRMS (EI) m/z calcd for $C_{30}H_{36}ClN_3$: 473.2598; found: 473.2579.

6.1.7. 3-(4-Chlorophenyl)-1-(3-(11,12-dihydrodibenzo[*b*,*f*]azocin-5(6*H*)-yl)propyl)piperidin-3-ol fumarate (60)

Following method A using **54** (0.18 g, 0.63 mmol) and 3-(4-chlorophenyl)piperidin-3-ol **56** (0.11 g, 0.52 mmol), which was synthesized as described in the literature,[18] gave 77 mg of the free base as an oil. This was converted to the fumaric salt as described for **50** to give 50 mg (17%) of **60** as a white solid. ^{1}H NMR (MeOH-d_4): δ 1.76–2.29 (m, 6H), 2.79–3.10 (m, 5H), 2.91 (s, 2H), 3.13–3.39 (m, 6H), 4.13 (s, 2H), 6.69 (s, 2H), 6.79–7.12 (m, 8H), 7.37–7.49 (m, 4H). ^{13}C NMR (MeOH-d_4): δ 20.44, 23.57, 34.56, 34.75, 35.80, 52.18, 53.72, 56.81, 61.36, 62.93, 71.47, 121.23, 124.28, 127.22, 127.77, 128.10, 128.49, 129.67, 129.94, 130.90, 132.24, 134.91, 136.16, 138.21, 139.02, 143.20, 144.30, 151.23, 171.08. Anal. RP-HPLC *Ib* (total run time 20 min): t_R = 6.78 min (purity 99%), *IIa* (total run time 30 min): t_R = 14.64 min (purity 98%). HRMS (EI) m/z calcd for $C_{29}H_{33}ClN_2O$: 460.2281; found: 460.2283.

6.1.8. 1-(3-(Benzyl(phenyl)amino)propyl)-4-(4-chlorophenyl)piperidin-4-ol hemifumarate (71)

Following method C starting with commercially available *N*-benzylaniline gave 331 mg of the free base as an oil. The final product was converted to the fumaric salt as described for **50** and recrystallized from MeOH/Et_2O to give 261 mg (24% over two steps) of **71** as white crystals. ^{1}H NMR ($CDCl_3$/DMSO-d_6): δ 1.53–1.72 (m, 2H), 1.74–1.97 (m, 2H), 2.00–2.21 (m, 2H), 2.41–2.70 (m, 4H), 2.82–3.01 (m, 2H), 3.26–3.48 (t, J = 7.4 Hz, 2H), 4.42 (s, 2H), 6.49–6.88 (m, 5H), 6.98–7.42 (m, 11H). ^{13}C NMR ($CDCl_3$/DMSO-d_6): δ 22.90, 36,45, 48.43, 54.32, 54.76, 69.23, 112.30, 116.32, 126.13, 126.43, 126.61, 128.02, 128.34, 129.04, 135.15, 138.49, 146.62, 148.14, 170.21. Anal. RP-HPLC *Ib*: t_R = 10.01 min (purity 100%), *IIb* (total run time 30 min): t_R = 8.05 min (purity 100%), *IIb* (total run time 30 min): t_R = 18.22 min (purity 100%). HRMS (EI) m/z calcd for $C_{27}H_{31}ClN_2O$: 434.2125; found: 434.2113.

6.1.9. 1-(3-(Benzyl(4-chlorophenyl)amino)propyl)-4-(4-chlorophenyl)piperidin-4-ol hemifumarate (72)

Following the analogous route (including the reductive amination and alkylation of the piperidine nitrogen atom) as described in method C starting with benzaldehyde and 4-chloroaniline. Conversion of the final product to the fumaric salt as described for **50** and recrystallized from Et_2O/MeOH resulted in the isolation of 112 mg of **72** as white crystals (6% over three steps) ^{1}H NMR ($CDCl_3$/DMSO-d_6): δ 1.61–2.05 (m, 4H), 2.13–2.37 (m, 2H), 2.53–2.86 (m, 2H), 2.99–3.12 (m, 2H), 3.37 (t, J = 7.1 Hz, 2H), 4.44 (s, 2H), 6.46–6.68 (m, 2H), 6.72 (s, 2H), 7.02–7.39 (m, 11H). ^{13}C NMR ($CDCl_3$/DMSO-d_6): δ 22.64, 36.16, 48.38, 48.70, 54.54, 69.37, 113.62, 121.22, 126.02, 126.40, 126.86, 128.26, 128.49, 128.88, 132.73, 135.35, 137.92, 145.84, 146.72, 170.93. Anal. RP-HPLC *Ib* : t_R = 5.03 min (purity 100%), *IIa* (total run time 30 min): t_R = 14.46 min (purity 98%). HRMS (EI) m/z calcd for $C_{27}H_{30}Cl_2N_2O$: 468.1735; found: 468.1718.

6.1.10. 4-(4-Chlorophenyl)-1-(2-(8-(methylthio)-10,11-dihydrodibenzo[*b*,*f*]thiepin-10-yloxy)-ethyl)piperidin-4-ol fumarate (80)

(i) 4-(Methylthio)benzenethiol **75** (5.24 g, 20.0 mmol) was added to a solution of 2-(2-iodophenyl)acetic acid **74** (3.12 g, 20.0 mmol) and KOH (4.49 g, 80.0 mmol) in water (60 mL). After the addition of Cu (205 mg), the reaction mixture was refluxed for 48 h, cooled to room temperature and filtered. The filtrate was acidified with 1.0 M HCl, filtered and the residue was dried under vacuum to yield 4.83 g (83%) of 2-(2-(4-(methylthio)phenyl-

thio)phenyl)acetic acid **76** as a pink solid. ^{1}H NMR ($CDCl_3$): δ 2.43 (s, 3H), 7.12–7.35 (m, 8H).

(ii) PPA (50.0 g) was added to a solution of **76** (4.83 g, 16.6 mmol) in toluene (30 mL) and the reaction mixture was heated and refluxed for 18 h. Water (50 mL) was added, the organic layer was separated and the water layer was extracted with EtOAc (3 × 30 mL). The combined organic extracts were washed with 5% NaOH (50 mL) and brine (50 mL), dried over anhydrous Na_2SO_4, filtered and evaporated in vacuo to give 3.50 g (77%) of 8-(methylthio)dibenzo[*b*,*f*]thiepin-10(11*H*)-one **77** as a brown oil. ^{1}H NMR ($CDCl_3$): δ 2.46 (s, 3H), 4.36 (s, 2H), 7.13–7.64 (m, 6H), 8.02 (d, *J* = 2.3 Hz, 1H).

(iii) A solution of **77** (3.50 g, 12.8 mmol) and $NaBH_4$ (1.01 g, 26.7 mmol) in MeOH (100 mL) was stirred overnight at room temperature. The reaction mixture was evaporated in vacuo, water was added (50 mL) and the water layer was extracted with Et_2O (3 × 25 mL). The combined organic extracts were washed with water (3 × 50 mL) and brine (50 mL), dried over anhydrous Na_2SO_4, filtered and evaporated in vacuo to give 3.14 g (89%) of 8-(methylthio)-10,11-dihydrodibenzo[*b*,*f*]thiepin-10-ol **78** as a brown solid. ^{1}H NMR ($CDCl_3$): δ 2.44 (s, 3H), 3.31–3.49 (m, 1H), 3.69–3.78 (m, 1H), 5.49 (br s, 1H), 6.95–7.49 (m, 7H).

(iv) $BF_3{\cdot}(Et)_2O$ (1.60 mL, 12.6 mmol) was added drop wise to a solution of **78** (3.14 g, 11.4 mmol) and 2-bromoethanol (1.22 mL, 17.2 mmol) in toluene (30 mL) and the reaction mixture was stirred for 1 h. Water (25 mL) was added and the water layer was extracted with EtOAc (3 × 20 mL). The combined organic extracts were washed with water (25 mL), 5% NaOH (2 × 25 mL) and brine (25 mL), dried over anhydrous Na_2SO_4, filtered and evaporated in vacuo to give 3.37 g of 11-(2-bromoethoxy)-2-(methylthio)-10,11-dihydrodibenzo[*b*,*f*]thiepine **79** as a brown oil. The crude product was used without further purification.

(v) Following method B using crude **79** (0.57 g, 1.49 mmol) and 4-(4-chlorophenyl)piperidin-4-ol **49** (0.21 g, 1.00 mmol) gave 88 mg of a light yellow oil. This was converted to the fumaric salt as described for **50** to give 101 mg (16%) of **80** as a white solid. ^{1}H NMR ($CDCl_3$/DMSO-d_6): δ 1.67–1.80 (m, 2H), 2.21–2.57 (m, 5H), 2.78–3.31 (m, 6H), 3.42–3.62 (m, 2H), 3.78–3.99 (m, 2H), 5.22–5.33 (m, 1H), 6.73 (s, 2H), 7.00–7.41 (m, 12H). ^{13}C NMR ($CDCl_3$/DMSO-d_6): δ 15.53, 35.91, 38.84, 48.92, 49.19, 56.61, 64.57, 68.79, 77.22, 125.02, 125.61, 126.28, 127.43, 128.16, 129.64, 130.97, 131.39, 131.82, 132.58, 133.66, 134.94, 137.86, 138.80, 142.22, 146.10, 169.42. Anal. RP-HPLC *Ia*: t_R = 7.76 min (purity 99%), *IIa* (total run time 30 min): t_R = 19.48 min (purity 97%). HRMS (EI) *m*/*z* calcd for $C_{28}H_{30}ClNO_2S_2$: 511.1406; found: 511.1405.

6.1.11. General method for the synthesis of target compounds 41, 85–91

(i) 2,4-Dimethylbenzaldehyde (1.67 g, 10.0 mmol) and 1-indanone (**77**, R1 = H); 1.33 g, 10.0 mmol) were added to a saturated solution of anhydrous K_2CO_3 in EtOH (25 mL).The resulting reaction mixture was stirred vigorously for 24 h, 37% HCl (2 mL) was added and the solvent was evaporated in vacuo. Water (50 mL) was added and the water layer was extracted with DCM (3 × 25 mL). The combined organic extracts were washed with brine (50 mL), dried over anhydrous Na_2SO_4, filtered and evaporated in vacuo. The residue was recrystallized from EtOH to give 1.95 g (79%) of 2-(2,4-dimethylbenzylidene)-2,3-dihydro-1*H*-inden-1-one as white crystals. ^{1}H NMR ($CDCl_3$): δ 2.33 (s, 3H), 2.43 (s, 3H), 3.95 (s, 2H), 6.98–7.11 (m, 2H), 7.31–7.60 (m, 3H), 7.80–7.95 (m, 2H).

(ii) A suspension of 2-(2,4-dimethylbenzylidene)-2,3-dihydro-1*H*-inden-1-one (1.14 g, 5.49 mmol) and 10% Pd/C (5 mg) in MeOH (10 mL) was hydrogenated with H_2 (atmospheric pressure) for 18 h. The reaction mixture was filtrated over Hyflo and the residue was washed with EtOH. The filtrate was evaporated in vacuo to give 2-(2,4-dimethylbenzyl)-2,3-dihydro-1*H*-inden-1-one in a quantitative yield. ^{1}H NMR ($CDCl_3$): δ 2.28 (s, 3H), 2.30 (s, 3H), 2.41–2.59 (m, 1H), 2,72–3.05 (m, 2H), 3.10–3.22 (m, 1H), 3.37 (dd, *J* = 10.4 Hz and 3.9 Hz, 1H), 6.81–7.01 (m, 3H), 7.25–7.41 (m, 2H), 7.54 (d, *J* = 7.5 Hz, 1H), 7.77 (d, *J* = 7.6 Hz, 1H).

(iii) A solution of 2-(2,4-dimethylbenzyl)-2,3-dihydro-1*H*-inden-1-one (1.15 g, 4.59 mmol) and $NaBH_4$ (0.17 g, 4.59 mmol) in MeOH (10 mL) was stirred overnight at room temperature. The reaction mixture was evaporated in vacuo, water was added (25 mL) and the water layer was extracted with EtOAc (3 × 20 mL). The combined organic extracts were washed with water (3 × 25 mL) and brine (25 mL), dried over anhydrous Na_2SO_4, filtered and evaporated in vacuo to give a quantitative yield of 2-(2,4-dimethylbenzyl)-2,3-dihydro-1*H*-inden-1-ol as a 1:1 mixture of two diastereomers. ^{1}H NMR ($CDCl_3$): δ 1.70 (br s, 1H), 2.30 (s, 6H), 2.35–2.88 (m, 3H), 2.98–3.12 (m, 2H), 4.83–5.01 (m, 1H), 6.89–7.01 (m, 2H), 7.04–7.41 (m, 6H).

(iv) A solution of 2-(2,4-dimethylbenzyl)-2,3-dihydro-1*H*-inden-1-ol (1.15 g, 4.59 mmol) and DPPA (1.39 mL, 6.41 mmol) in THF (10 mL) was stirred for 10 min, cooled to 0 °C and DBU (0.98 mL, 6.42 mmol) was added in a drop wise manner. The reaction mixture was allowed to warm to room temperature and stirred for 18 h. The solvent was evaporated in vacuo, water (25 mL) was added and the water layer was extracted with Et_2O (3 × 15 mL). The combined organic extracts were washed with brine (25 mL), dried over anhydrous Na_2SO_4, filtered and evaporated in vacuo. Purification by column chromatography (hexane) afforded 763 mg (60%) of 1-azido-2-(2,4-dimethylbenzyl)-2,3-dihydro-1*H*-indene as a 1:1 mixture of two diastereomers. ^{1}H NMR ($CDCl_3$): δ 2.29 (s, 3H), 2.30 (s, 3H), 2.51–2.84 (m, 4H), 2.90–3.11 (m, 1H), 4.52 and 4.70 (d, *J* = 4.7 and 5.0 Hz, 1H), 6.87–7.03 (m, 3H), 7.11–7.39 (m, 4H).

(v) A solution of 1-azido-2-(2,4-dimethylbenzyl)-2,3-dihydro-1*H*-indene (0.76 g, 2.74 mmol) and 10% Pd/C (3 mg) in MeOH (10 mL) was hydrogenated with H_2 (atmospheric pressure) for 3 days. The reaction mixture was filtrated over Hyflo, the residue was washed with EtOH and the filtrate was evaporated in vacuo. Purification by flash chromatography (2% Et_3N in hexane) gave 646 mg of the free base. This was dissolved in a solution of HCl gas in EtOH and the solvent was evaporated in vacuo. The hydrochloride salt was isolated by filtration and recrystallized from Et_2O/MeOH.

6.1.12. 2-(2,4-Dimethylbenzyl)-2,3-dihydro-1*H*-inden-1-amine hydrochloride (85)

Yield: 665 mg (84%) of **85** as a 1:1 mixture of two diastereomers. ^{1}H NMR (MeOH-d_4): δ 2.30 (s, 3H), 2.33 (s, 3H), 2.61–3.18 (m, 5H), 4.51 and 4.83 (d, *J* = 3.5 and 6.0 Hz, 1H), 6.92–7.15 (m, 3H), 7.21–7.40 (m, 3H), 7.49–7.63 (m, 1H). ^{13}C NMR (MeOH-d_4): δ 18.64, 20.02, 31.51, 35.25, 35.87, 43.08, 58.19, 125.44, 125.73, 126.78, 127.33, 129.29, 129.96, 131.25, 134.43, 136.07, 136.21, 138.73, 143.83 (one diastereomer). ^{13}C NMR (MeOH-d_4): δ 17.38, 18.59, 31.51, 35.25, 35.87, 45.21, 60.52, 125.18, 125.31, 127.48, 129.66, 131.27, 134.49, 136.27, 136.30, 137.94, 143.58 (other diastereomer). Anal. RP-HPLC *Ib* (total run time 30 min): t_R = 12.89 min (purity 100%), *IIb*: t_R = 11.79, 12.79 min (purity 100%). HRMS (EI) *m*/*z* calcd for $C_{18}H_{21}N$: 251.1674; found: 251.1665.

6.1.13. 2-(2,4-Dimethoxybenzyl)-2,3-dihydro-1*H*-inden-1-amine hydrochloride (86)

Yield: 539 g (17% yield over five steps) of **86** as a 1:1 mixture of two diastereomers. ^{1}H NMR (MeOH-d_4): δ 2.58–3.14 (m, 5H), 3.78 and 3.79 (s, 3H), 3.81 and 3.82 (s, 3H), 4.43 and 4.69 (d, *J* = 4.1 and 5.8 Hz, 1H), 6.45–6.57 (m, 2H), 7.02–7.52 (m, 5H). ^{13}C NMR (MeOH-d_4): δ 35.44, 35.79, 45.35, 54.82, 54.84, 54.91, 57.98, 98.55, 104.89, 119.62, 125.36, 125.41, 127.24, 129.95, 131.31, 138.83, 144.09, 158.89, 160.45 (one diastereomer). ^{13}C NMR

(MeOH-d_4): δ 28.70, 32.75, 43.45, 54.82, 54.84, 54.91, 60.26, 98.50, 104.61, 119.77, 124.92, 125.59, 127.32, 129.80, 130.80, 138.12, 143.62, 158.54, 160.52 (other diastereomer). Anal. RP-HPLC *Ia* : t_R = 8.47 and 9.81 min (purity 100%), *IIIb*: t_R = 5.29 and 5.61 min (purity 100%). HRMS (EI) *m*/*z* calcd for $C_{18}H_{21}NO_2$: 283.1572; found: 283.1582.

6.1.14. 2-(2-Methoxybenzyl)-2,3-dihydro-1*H*-inden-1-amine hydrochloride (87)

Yield: 439 mg (17% yield over five steps) of one diastereomer of **87** as a white solid. ^{1}H NMR (MeOH-d_4): δ 2.52–2.80 (m, 2H), 2.85–3.15 (m, 3H), 3.85 (s, 3H), 4.71 (d, *J* = 5.9 Hz, 1H), 6.89–6.97 (m, 2H), 7.01–7.36 (m, 5H), 7.50 (d, *J* = 6.6 Hz, 1H). ^{13}C NMR (MeOH-d_4): δ 30.37, 36.41, 44.21, 55.82, 59.00, 111.76, 121.82, 126.30, 126.43, 128.28, 128.54, 129.17, 131.01, 131.43, 139.80, 145.96, 158.71. RP-HPLC *Ic*: t_R = 4.19 min (purity 100%), *IIb*: t_R = 3.95 min (purity 100%). HRMS (EI) *m*/*z* calcd for $C_{17}H_{19}NO$: 253.1467; found: 253.1476.

6.1.15. 2-(4-Methoxybenzyl)-2,3-dihydro-1*H*-inden-1-amine hydrochloride (88)

Yield: 439 mg (19% yield over five steps) of **88** as a 1:1 mixture of two diastereomers. ^{1}H NMR (MeOH-d_4): δ 2.51–3.29 (m, 5H), 3.77 (s, 3H), 3.79 (s, 3H), 4.49 and 4.77 (d, *J* = 3.9 and 6.2 Hz, 1H), 6.80–6.94 (m, 2H), 7.08–7.52 (m, 6H). ^{13}C NMR (MeOH-d_4): δ 35.89, 38.28, 46.58, 54.73, 60.39, 114.14, 125.03, 125.67, 127.44, 129.75, 130.06, 131.55, 137.93, 143.47, 158.92 (one diastereomer). ^{13}C NMR (MeOH-d_4): δ 34.12, 35.33, 44.47, 54.73, 58.08, 114.11, 125.35, 125.46, 127.34, 129.92, 129.98, 131.49, 138.73, 143.82, 158.92 (other diastereomer). RP-HPLC *Ic*: t_R = 5.73 min (purity 100%), *IId*: t_R = 5.48 min (purity 96%). HRMS (EI) *m*/*z* calcd for $C_{17}H_{19}NO$: 253.1467; found: 253.1459.

6.1.16. 2-(4-Methoxy-3-methylbenzyl)-2,3-dihydro-1*H*-inden-1-amine hydrochloride (89)

Yield: 470 mg (20% yield over five steps) of one diastereomer of **89** as a white solid. ^{1}H NMR (MeOH-d_4): δ 2.42 (s, 3H), 2.42–3.09 (m, 5H), 3.80 (s, 3H), 4.77 (d, *J* = 6.0 Hz, 1H) 6.82–7.54 (m, 7H). ^{13}C NMR (MeOH-d_4): δ 15.35, 34.14, 35.37, 44.48, 54.84, 58.09, 110.21, 125.30, 125.46, 126.72, 131.04, 127.05, 127.31, 129.96, 130.89, 138.78, 143.86, 156.94. Anal. RP-HPLC *Ib*: t_R = 4.56 min (purity 100%), *IIa*: t_R = 4.33 min (purity 99%). HRMS (EI) *m*/*z* calcd for $C_{18}H_{21}NO$: 267.1623; found: 267.1623.

6.1.17. *N*-(4-((1-Amino-2,3-dihydro-1*H*-inden-2-yl)methyl)phenyl)acetamide (90)

Yield: 581 mg (23% yield over five steps) of **90** as a 1:5 mixture of two diastereomers. (MeOH-d_4): δ 2.10 (s, 3H), 2.20–2.34 (m, 1H), 2.41–77 (m, 2H), 2.81–2.92 (m, 1H), 3.00–3.15 (m, 1H), 3.98 and 4.28 (d, *J* = 7.6 and 7.8 Hz, 1H), 6.98–7.51 (m, 8H). ^{13}C NMR (MeOH-d_4): δ 22.73, 36.38, 38.92, 52.76, 61.76, 120.45, 123.50, 124.48, 126.56, 127.41, 129.28, 136.94, 137.22, 141.91, 146.11, 170.55 (major diastereomer). ^{13}C NMR (MeOH-d_4): δ 22.73, 34.10, 35.19, 46.63, 58.46, 120.32, 124.23, 124.65, 124.77, 127.63, 129.17, 136.82, 137.34, 141.91, 146.11, 170.55 (minor diastereomer). RP-HPLC *Ie* (total run time 30 min): t_R = 10.42 min (minor diastereomer), 13.62 min (major diastereomer) (purity 100%), *IIf*: t_R = 5.21 min (minor diastereomer), 6.22 min (major diastereomer) (purity 100%). HRMS (EI) *m*/*z* calcd for $C_{18}H_{20}N_2O$: 280.1576; found: 280.1571.

6.1.18. 2-Benzyl-2,3-dihydro-1*H*-inden-1-amine hydrochloride (91)

Yield: 350 mg (14% yield over five steps) of one diastereomer of **91** as a white solid. ^{1}H NMR (MeOH-d_4): δ 2.52–2.66 (m, 1H), 2.70–3.05 (m, 3H), 3.09–3.20 (m, 1H), 4.79 (d, *J* = 6.4 Hz, 1H), 7.25–7.55 (m, 9H). ^{13}C NMR (MeOH-d_4): δ 35.03, 35.29, 44.29, 58.13, 125.29, 125.47, 125.61, 127.37, 128.73, 128.80, 130.02, 138.68, 139.59, 143.77. RP-HPLC *Ic*: t_R = 5.52 min (purity 100%), *IId*: t_R = 5.94 min (purity 97%), *IIIa*: t_R = 3.37 min (purity 99%). HRMS (EI) *m*/*z* calcd for $C_{16}H_{17}N$: 223.1361; found: 223.1363.

6.1.19. General method for the synthesis of target compounds 93–98

(i) 2,4-Dichlorobenzaldehyde (17.5 g, 100 mmol) and 1-indanone **81** (13.2 g, 100 mmol) were added to a saturated solution of anhydrous K_2CO_3 in EtOH (250 mL). The resulting reaction mixture was stirred vigorously for 18 h and 37% HCl (20 mL) was added. The precipitate was isolated by filtration, washed with water and dried under vacuum to give 25.8 g (89%) of 2-(2,4-dichlorobenzylidene)-2,3-dihydro-1*H*-inden-1-one as white crystals. ^{1}H NMR ($CDCl_3$): δ 3.92 (s, 2H), 7.12–7.66 (m, 5H), 7.80–7.99 (m, 2H).

(ii) A suspension of 2-(2,4-dichlorobenzylidene)-2,3-dihydro-1*H*-inden-1-one (11.6 g, 40.1 mmol) and 10% Pd/C (40 mg) in MeOH (100 mL) was hydrogenated with H_2 (atmospheric pressure) for 6 h at a temperature of 50 °C. The reaction mixture was filtrated over Hyflo and the filtrate was evaporated in vacuo. Recrystallization from EtOH afforded 4.6 g (39%) of 2-(2,4-dichlorobenzyl)-2,3-dihydro-1*H*-inden-1-one as white crystals. ^{1}H NMR ($CDCl_3$): δ 2.65–2.88 (m, 2H), 2.95–3.20 (m, 2H), 3.27–3.40 (m, 1H), 7.06–7.20 (m, 2H), 7.26–7.40 (m, 3H), 7.56 (t, *J* = 7.4 Hz, 1H), 7.75 (d, *J* = 7.6 Hz, 1H).

(iii) A solution of 2-(2,4-dichorolbenzyl)-2,3-dihydro-1*H*-inden-1-one (1.86 g, 6.40 mmol) and $NaBH_4$ (0.17 g, 4.59 mmol) in MeOH (40 mL) was stirred for 3 h at room temperature. The reaction mixture was evaporated in vacuo, water was added (20 mL) and the water layer was extracted with EtOAc (3 × 15 mL). The combined organic extracts were washed with water (3 × 25 mL) and brine (25 mL), dried over anhydrous Na_2SO_4, filtered and evaporated in vacuo to give 1.84 (98%) of 2-(2,4-dichlorobenzyl)-2,3-dihydro-1*H*-inden-1-ol as a 1:1 mixture of two diastereomers. ^{1}H NMR ($CDCl_3$): δ 1.72 (br s, 1H), 2.41–3.02 (m, 5H), 4.92 (br s, 1H), 7.16–7.38 (m, 7H).

(iv) Thionyl chloride (0.75 mL, 10.3 mmol) was added to a solution of 2-(2,4-dichlorobenzyl)-2,3-dihydro-1*H*-inden-1-ol (1.84 g, 6.28 mmol) in toluene (30 mL) at 0 °C. The reaction mixture was stirred at room temperature for 30 min, heated to 55 °C and stirred for 1 h. The solution was cooled to room temperature, washed with ice-water (2 × 15 mL), dried over anhydrous Na_2SO_4, filtered and evaporated in vacuo to give 1.88 g (96%) of 1-chloro-2-(2,4-dichlorobenzyl)-2,3-dihydro-1*H*-indene **92** as a 1:1 mixture of two diastereomers. ^{1}H NMR ($CDCl_3$): δ 2.55–3.29 (m, 5H), 5.10 and 5.27 (d, *J* = 5.3 and 4.8 Hz, 1H), 7.08–7.41 (m, 7H).

(v) A solution of piperidine (70 μL, 0.71 mmol), NaI (75 mg, 0.50 mmol), and Na_2CO_3 (106 mg, 1.0 mmol) in CH_3CN (10 mL) was heated to reflux temperature and 1-chloro-2-(2,4-dichlorobenzyl)-2,3-dihydro-1*H*-indene **92** (0.16 g, 0.51 mmol) in CH_3CN (1 mL) was added. The reaction mixture was refluxed overnight, the solvent was removed in vacuo and the residue was diluted with water (15 mL) followed by an extraction with DCM (3 × 10 mL). The combined organic layers were dried over anhydrous $NaSO_4$, filtered, evaporated in vacuo, and subsequently purified by flash chromatography (25% EtOAc in DCM) and recrystallized from EtOAc.

6.1.20. 1-(2-(2,4-Dichlorobenzyl)-2,3-dihydro-1*H*-inden-1-yl)piperidine (94)

Yield: 90 mg (49%) of **94** as white crystals. ^{1}H NMR ($CDCl_3$): δ 1.29–1.51 (m, 6H), 2.29–2.70 (m, 6H), 2.76–2.99 (m, 3H), 3.95 (d, *J* = 3.4 Hz, 1H), 7.03–7.22 (m, 5H), 7.29–7.41 (m, 3H). ^{13}C ($CDCl_3$): δ 24.61, 26.41, 37.00, 38.18, 38.80, 50.19, 76.04, 124.50, 125.88, 126.09, 126.65, 127.38, 129.12, 131.78, 132.15, 134.78, 137.23, 142.58. Anal. RP-HPLC *Ic* (total run time 30 min): t_R = 19.88 min (purity 100%), *IIb* (total run time 30 min): t_R = 13.77 min (purity 100%). HRMS (EI) *m*/*z* calcd for $C_{21}H_{23}Cl_2N$: 359.1208; found: 359.1199.

6.1.21. 4-(4-Chlorophenyl)-1-(2-(2,4-dichlorobenzyl)-2,3-dihydro-1*H*-inden-1-yl)-1,2,3,6-tetrahydropyridine hydrochloride (97)

Yield: 50 mg (19%) of one diastereomer of **97** as a light brown solid. ^{1}H NMR (MeOH-d_4): δ 2.61–2.92 (m, 6H), 3.15–3.39 (m, 3H), 3.51–3.81 (m, 2H), 4.61–4.69 (m, 1H), 6.01–6.07 (m, 1H), 7.31–7.54 (m, 10H), 7.67 (d, J = 7.1 Hz, 1H). ^{13}C (MeOH-d_4): δ 26.22, 37.74, 38.29, 40.69, 47.72, 47.83, 75.74, 118.08, 127.30, 127.70, 128.68, 128.96, 129.74, 130.48, 132.14, 134.00, 134.59, 135.07, 135.83, 136.27, 136.78, 138.51, 146.52. Anal. RP-HPLC *Ib* (total run time 20 min): t_R = 13.04 min (purity 99%), *IIb*: t_R = 6.78 min (purity 96%). HRMS (EI) m/z calcd for $C_{27}H_{24}Cl_3N$: 469.0945 (second isotope); found: 469.0931.

6.1.22. 4-(4-Chlorophenyl)-1-(2-(2,4-dichlorobenzyl)-2,3-dihydro-1*H*-inden-1-yl)piperidin-4-ol hydrochloride (93)

Yield: 61 mg (23%) of one diastereomer of **93** as a white solid ^{1}H NMR (MeOH-d_4): δ 1.81–1.92 (m, 4H), 2.50 (br s, 1H), 2.70–3.12 (m, 5H), 3.15–3.36 (4H), 4.41–4.47 (m, 1H), 7.15–7.48 (m, 10H), 8.00 (s, 1H), 12.08 (br s, 1H). ^{13}C (MeOH-d_4): δ 35.03, 35.26, 37.25, 37.94, 38.73, 44.31, 45.84, 46.37, 69.58, 75.31, 125.81, 126.00, 127.64, 127.97, 128.67, 128.76, 129.60, 131.01, 132.58, 132.80, 133.54, 133.63, 134.80, 134.83, 144.42, 144.51. Anal. RP-HPLC *Ib* : t_R = 6.19 min (purity 100%), *IIa* (total run time 30 min): t_R = 13.45 min (purity 100%). HRMS (EI) m/z calcd for $C_{27}H_{26}Cl_3NO$: 485.1080; found: 485.1073.

6.1.23. 1-(2-(2,4-Dichlorobenzyl)-2,3-dihydro-1*H*-inden-1-yl)-4-phenylpiperazine dihydrochloride (96)

Yield: 48 mg (22%) of one diastereomer of **96** as a white solid. ^{1}H NMR (MeOH-d_4): δ 2.72–2.97 (m, 3H), 3.05–3.55 (m, 8H), 3.69–3.88 (m 2H), 4.64–4.72 (m, 1H), 6.88–7.08 (m, 2H), 7.19–7.54 9 m, 9H), 7.74 (d, J = 7.2 Hz, 1H). ^{13}C (MeOH-d_4): δ 37.56, 38.05, 40.90, 50.58, 76.79, 118.22, 122.92, 127.46, 128.73, 128.78, 129.44, 130.41, 130.51, 132.52, 133.79, 134.00, 134.66, 136.28, 136.55, 146.84, 150.63. Anal. RP-HPLC *Ib*: t_R = 7.07 min (purity 100%), *IIa*: t_R = 4.13 min (purity 98%), *IIb* (total run time 30 min): t_R = 19.11 min (purity 98%). HRMS (EI) m/z calcd for $C_{26}H_{26}Cl_2N_2$: 436.1473; found: 436.1471.

6.1.24. 1-(2-(2,4-Dichlorobenzyl)-2,3-dihydro-1*H*-inden-1-yl)-4-methylpiperazine dihydrochloride (95)

Yield: 105 mg (46%) of one diastereomer of **95** as a light yellow solid. ^{1}H NMR (MeOH-d_4): δ 2.61–3.03 (m, 4H), 2.93 (s, 5H), 3.12–3.87 (m, 8H), 4.55–4.64 (m, 1H), 7.15–7.49 (m, 6H), 7.72 (d, J = 7.1 Hz, 1H). ^{13}C (MeOH-d_4): δ 37.59, 38.05, 40.99, 43.36, 47.18, 51.93, 77.02, 127.37, 128.72, 129.35, 130.46, 132.33, 133.98, 134.55, 136.27, 136.76, 146.61. Anal. RP-HPLC *Ib* (total run time 20 min): t_R = 12.99 min (purity 95%), *IId*: t_R = 12.70 min (purity 96%). HRMS (EI) m/z calcd for $C_{21}H_{24}Cl_2N_2$: 374.1317; found: 374.1311.

6.1.25. *N*-Benzyl-2-(2,4-dichlorobenzyl)-2,3-dihydro-1*H*-inden-1-amine fumarate (98)

Yield: 20 mg (8%) of one diastereomer of **98** as a white solid. ^{1}H NMR (MeOH-d_4): δ 2.69–2.79 (m, 2H), 2.89–2.95 (m, 1H), 3.02–3.08 (m, 1H), 3.17–3.25 (m, 1H), 4.14–4.21 (m, 2H), 4.62 (d, J = 6.1 Hz, 1H), 6.68 (s, 2H), 7.27–7.53 (m, 12H). ^{13}C NMR (MeOH-d_4): δ 32.41, 36.00, 44.64, 51.56, 65.52, 126.53, 126.60, 127.91, 128.49, 129.71, 130.01, 130.30, 130.33, 130.51, 133.42, 134.08, 135.48, 135.89, 136.00, 137.77, 144.58, 170.70. Anal. RP-HPLC *Ib*: t_R = 8.22 min (purity 100%), *IIa*: t_R = 12.37 min (purity 97%). HRMS (ESI) m/z calcd for $C_{27}H_{25}Cl_2NO_4$: 381.1051; found: 381.1046.

6.2. Pharmacology

Pharmacological characterization of nonpeptidergic ligands was performed as previously described.[11]

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