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Special ergolines are highly selective, potent antagonists of the chemokine receptor CXCR3: Discovery, characterization and preliminary SAR of a promising lead

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ABSTRACT

The special ergoline **1** is a highly potent, selective antagonist of the chemokine receptor CXCR3. The surprising selectivity of this LSD-related compound can be explained by different electronic and steric properties of the ergoline core structure caused by the urea portion of the molecule. Discovery, biopharmaceutical properties and first derivatives of this promising lead compound are discussed.

The chemokine receptor CXCR3 is a G-protein coupled heptahelical cell surface receptor binding the inflammatory chemokines IP-10 (CXCL10), Mig (CXCL9) and I-TAC (CXCL11).[1] The interaction of CXCR3 and its ligands is involved in directing Th1 T cells to sites of inflammation/immune injury.[1] Both, CXCR3 and ligands are upregulated and highly expressed in diseased tissues such as in psoriasis,[2] rheumatoid arthritis,[3] multiple sclerosis,[4] diabetes,[5] and acute transplant rejection[6] suggesting a causal role in pathological processes. Furthermore, the involvement of CXCR3 and its ligands in allograft rejection is claimed by literature reports on transplantation experiments using both KO mice[7] and neutralizing principles such as anti CXCR3 monoclonal antibodies[6a] and antisense peptide nucleic acid.[8] However, these findings could not be confirmed by us and others.[9] Several reports on low molecular weight (LMW) CXCR3 inhibitors have been recently published.[10]

High throughput screening of the Novartis compound collection against CXCR3 led to the discovery of compound **1** (Fig. 1). The original hit **1**[11] was resynthesized starting from the literature known lysergic acid derivative **2**[12] by treatment with phenyl isocyanate (Scheme 1). The promising potency of the hit was confirmed in binding,[13] Ca^{2+}-mobilization,[14] and ligand-induced cell migration assays[15] (Table 1). The compound inhibited murine CXCR3 with similar potencies. Its biopharmaceutical in vitro properties were determined in a variety of profiling assays (Table 1). Compound **1** neither inhibited the cytochrome P450 enzymes nor the hERG channel. Intrinsic clearance and log *P* are acceptable. The expected oral bioavailability (F_m) based on permeability is high, water solubility is low and protein binding is high. Consequently, the overall profile is very promising for a screening hit.

As compound **1** is related to lysergic acid diethylamide (LSD) its receptor selectivity was tested against a broad panel of >50 GPCRs. It did not inhibit other chemokine receptors such as CCR5, CXCR4

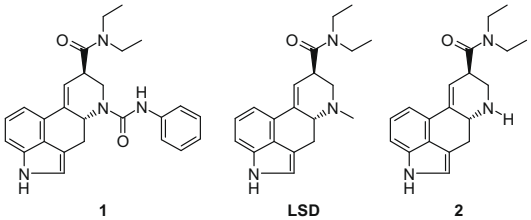

Figure 1. Structures of selected ergolines.

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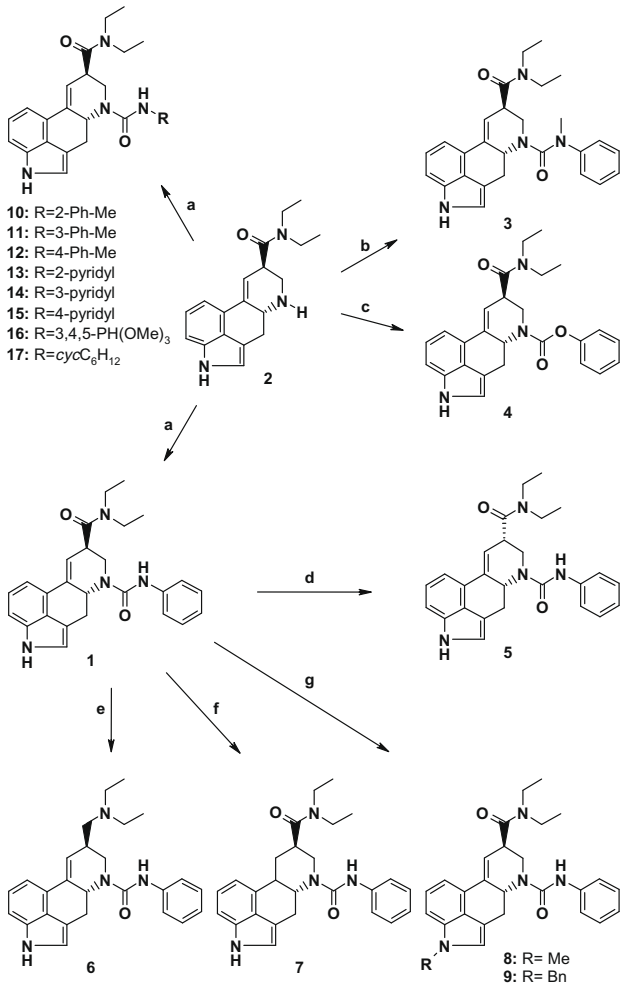

Scheme 1. Reagents and conditions: (a) Ar–NCO or Alkyl–NCO, NEt_3, acetone, 3 h, 25 °C, 25–70%; (b) PhN(Me)COCl, pyridine, THF, 16 h, 25 °C, 60%; (c) PhOCOCl, pyridine, THF, 16 h, 25°C, 65%; d) NaOH, MeOH, equilibration to ~1:1 mixture of epimers, separation by chromatography; (e) $LiAlH_4$, THF, 0.3 h, 25 °C, 29%; (f) H_2, Pd(10%)/C, MeOH, 2 h, 25°, 28%; (g) MeI or BnBr; NaOH, Et_3BnNCl, CH_2Cl_2, 3 h, 25 °C, **8**: 56%. **9**: 32%.

Table 1
Properties of compound **1**

Assay	Result
hu CXCR3 (binding)	IC_{50} = 54 nM*
m CXCR3 (binding)	IC_{50} = 200 nM*
hu CXCR3 (Ca^{2+})	IC_{50} = 18 nM*
m CXCR3 (Ca^{2+})	IC_{50} = 30 nM*
hu CXCR3 (I-TAC-induced migration)	IC_{50} = 74 nM*
CYP1A2	IC_{50} >10,000 nM
CYP2C9	IC_{50} >10,000 nM
CYP2C19	IC_{50} >10,000 nM
CYP2D6	IC_{50} >10,000 nM
CYP3A4	IC_{50} >10,000 nM
hERG	IC_{50} >30,000 nM
Log *P*	4.2
Intrinsic clearance (*hu*)	60 μL min^{-1} mg^{-1}
Intrinsic clearance (*rat*)	120 μL min^{-1} mg^{-1}
Solubility (pH 1.0)	5 mg/L
Solubility (pH 6.8)	5 mg/L
Permeability (PAMPA)	Log *Pe* = −4
Protein binding (hu)	Free fraction: 1%
Protein binding (rat)	Free fraction: 2%

* Mean values of at least two independent measurements.

and CXCR2. Contrary to LSD and its close derivative **2** which efficiently bind to various Serotonin, Dopamine and Adrenergic receptors, compound **1** inhibits only the 5HT2A receptor with an IC_{50} value below 1 μM (Table 2). Interestingly, compound **2** did not inhibit CXCR3.

This remarkable selectivity of compound **1** can be explained by different electronic and steric properties compared to LSD and compound **2**. Under physiological conditions (pH 7.4) compound **1** is neutral whereas LSD (pK_a = 7.8) is highly protonated which is believed to be required for its hallucinogenic activity.[16] Comparison of the X-ray crystal structures of compound **1**[17] and LSD[18] revealed distinct conformations of the tetracyclic core structure (Fig. 2). The D ring adopts a chair-like conformation in LSD and related ergolines[18] but a boat-like conformation in compound **1**. This causes different positioning of the groups attached to N-6 and C-8. NMR analyses in DMSO pointed towards similar conformations of compound **1** in solution and solid state. A strong NOE observed between H-4_{ax} and H-7_{ax} (but no NOE between H-5_{ax} and H-7_{ax} which would be required in case of a chair-like conformation of the D-ring) indicated a boat-like conformation of the D-ring in solution. Furthermore, the coupling constant (J_{8-9} = 5.5 Hz) indicated a torsion angle between H-8 and H-9 of 40–45° which is in good agreement with the solid state (50°).[19] In contrast, for amines such as LSD and compound **2** a strong NOE between H-5_{ax} and H-7_{ax} was observed confirming a chair-like conformation of the D-ring as found in the solid state.[18] Compound **2** showed a very small coupling constant (J_{8-9} <2 Hz) indicating a torsion angle close to 90° which is in line with the solid state structure of LSD. In addition, the bulky urea group at the 6 position might disturb high affinity binding of compound **1** to most Serotonin, Dopamine and Adrenergic receptors.[12a]

The metabolic stability of compound **1** after incubation with human, rat, mouse and dog liver microsomes was assessed by capillary HPLC/MS–MS. The compound proved to be very stable with a similar metabolite pattern in all species. The major metabolic pathways were N-de-alkylation of the amide, hydroxylation of the urea phenyl and hydroxylation of the ergoline core. No evidence was found for the cleavage of the urea leading to compound **2**-like structures which could cause undesirable side effects in the nervous system.

A limited number of derivatives were prepared (Scheme 1 for structures and Table 3 for potencies). The methylated urea **3** and the carbamate **4** were found to be inactive or significantly less potent than **1** pointing towards a key interaction of the urea hydrogen with the receptor. The C-8 epimer **5** and the amine **6** were inactive indicating close contacts between amide group and protein. Hydrogenation of the double bond between C-9 and C-10 led to the considerably less potent compound **7**. Thus, even relatively moderate changes of the core modification strongly affect

Table 2
Selectivity profiles of compounds **1** and **2**

Receptor	Compound **1** IC_{50} (nM)	Compound **2** IC_{50} (nM)
CXCR3	54	>10,000
Serotonin 5HT1A	3600	3
Serotonin 5HT2A	750	6
Serotonin 5HT2B	2470	54
Serotonin 5HT2C	>10,000	1290
Serotonin 5HT6	9580	16
Serotonin 5HT7	>10,000	143
Dopamine D1	>10,000	4900
Dopamine D2	>10,000	1590
Dopamine D3	>10,000	660
Dopamine D4.4	>10,000	2650
Adrenergic α1	>10,000	4030
Adrenergic α2A	>10,000	1930
Adrenergic α2C	>10,000	215
Adrenergic β1	>10,000	5760

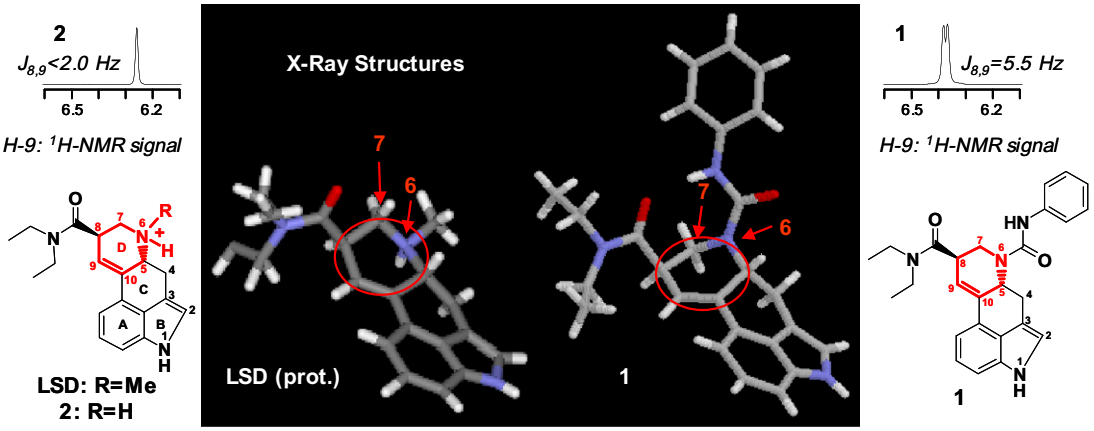

Figure 2. Molecular structures of protonated LSD and compound **1** and ^{1}H NMR signals of H-8 of close LSD derivative **2** and compound **1**.

Table 3
Potencies of derivatives of compound **1**

Compd	Binding IC_{50}* (nM)	Ca^{2+}-mobilization IC_{50}* (nM)
1	54	18
2	>10,000	n.t.
3	>10,000	>10,000
4	803	809
5	>10,000	n.t.
6	>10,000	>10,000
7	5300	1930
8	220	60
9	590	510
10	146	28
11	231	45
12	313	100
13	428	62
14	285	67
15	2000	6800
16	3050	564
17	179	78

* Mean values of at least two independent measurements.

potency. Methylation of the indole nitrogen gave the slightly less potent derivative **8**. The sterically more demanding benzyl group of compound **9** led to a more pronounced drop of potency. We also prepared a small series of compounds with modified urea groups. Methyl-substitution of the phenyl ring was tolerated resulting in slightly less potent derivatives **10–12**. Introduction of a pyridyl instead of the phenyl ring also led to reduced potency (**13–15**) particularly if the pyridine nitrogen is in the 4-position. The trimethoxy-substituted compound **16** showed modest potency whereas the cyclohexyl urea **17** was only slightly less active than compound **1**. Thus, the unsubstituted phenylurea seems to be optimal for potency to CXCR3.

In conclusion, we discovered the special ergoline **1** which is a highly selective, potent antagonist of the chemokine receptor CXCR3. A preliminary structure activity relationship has been established. Compound **1** was thoroughly profiled and is considered to be a very promising lead structure.

References and notes

1. (a) Murphy, P. M.; Baggiolini, M.; Charo, I. F.; Hebert, C. A.; Horuk, R.; Matsushima, K.; Miller, L. H.; Oppenheim, J. J.; Power, C. A. *Pharmacol. Rev.* **2000**, *52*, 145; (b) Moser, B.; Loetscher, P. *Nat. Immunol.* **2001**, *2*, 123.
2. Flier, J.; Boorsma, D. M.; van Beek, P. J.; Nieboer, C.; Stoof, T. J.; Willemze, R.; Tensen, C. P. *J. Pathol.* **2001**, *194*, 398.
3. Motoki, Y.; Tani, K.; Shimizu, T.; Tamiya, H.; Hase, K.; Ohmoto, Y.; Matsushima, K.; Sone, S. *Mod. Rheumatol.* **2003**, *13*, 114.
4. Xanthou, G.; Williams, T. J.; Pease, J. E. *Eur. J. Immunol.* **2003**, *33*, 2927.
5. (a) Tornwall, J.; Lane, T. E.; Fox, R. I.; Fox, H. S. *Lab. Invest.* **1999**, *79*, 1719; (b) Christen, U.; McGavern, D. B.; Luster, A. D.; v. Herrath, M. G.; Oldstone, M. B. A. *J. Immunol.* **2003**, *171*, 6838.
6. (a) Hy, H.; Aizenstein, B. D.; Puchalski, A.; Burmania, J. A.; Hamawy, M. M.; Knechtle, S. J. *Am. J. Transplant.* **2004**, *4*, 432; (b) Loetscher, M.; Gerber, B.; Loetscher, P.; Jones, S. A.; Piali, L.; Clark-Lewis, I.; Baggiolini, M.; Moser, B. *J. Exp. Med.* **1996**, *184*, 963; (c) Cole, K. E.; Strick, C. A.; Paradis, T. J.; Ogborne, K. T.; Loetscher, M.; Gladue, R. P.; Lin, W.; Boyd, J. G.; Moser, B.; Wood, D. E.; Sahagan, B. G.; Neote, K. *J. Exp. Med.* **1998**, *187*, 2009; (d) Luster, A. D.; Ravetch, J. V. *J. Exp. Med.* **1987**, *166*, 1084; (e) Farber, J. M. *Biochem. Biophys. Res. Commun.* **1993**, *192*, 223; (f) Liao, F.; Rabin, R. L.; Yannelli, J. R.; Koniaris, L. G.; Vanguri, P.; Farber, J. M. *J. Exp. Med.* **1995**, *182*, 1301.
7. (a) Hancock, W. W.; Lu, B.; Gao, W.; Csizmadia, V.; Faia, K.; King, J. A.; Smiley, S. T.; Ling, M.; Gerard, N. P.; Gerard, C. *J. Exp. Med.* **2000**, *192*, 1515; (b) Hancock, W. W.; Gao, W.; Csizmadia, V.; Faia, K. L.; Shemmeri, N.; Luster, A. D. *J. Exp. Med.* **2001**, *193*, 975.
8. Zhang, Z.; Kaptanoglu, L.; Haddad, W.; Ivancic, D.; Alnadjim, Z.; Hurst, S.; Tishler, D.; Luster, A. D.; Barrett, T. A.; Fryer, J. *J. Immunol.* **2002**, *168*, 3205.
9. (a) Zerwes, H.-G.; Li, J.; Kovarik, J.; Streiff, M.; Hofmann, M.; Roth, L.; Luyten, M.; Pally, C.; Loewe, R. P.; Wieczorek, G.; Banteli, R.; Thoma, G.; Luckow, B. *Am. J. Trans.* **2008**, *8*, 1604; (b) Halloran, P. F.; Fairchild, R. L. *Am. J. Trans.* **2008**, *8*, 1578; (c) Kwun, J.; Hazinedaroglu, S. M.; Schadde, E.; Kayaoglu, H. A.; Fechner, J.; Hu, H. Z.; Roenneburg, D.; Torrealba, J.; Shiao, L.; Hong, X.; Peng, R.; Szewczyk, J. W.; Sullivan, K. A.; DeMartino, J.; Knechtle, S. J. *Am. J. Trans.* **2008**, *8*, 1593.
10. (a) Storelli, S.; Verdijk, P.; Verzijl, D.; Timmerman, H.; Van de Stolpe, A. C.; Tensen, C. P.; Smit, M. J.; De Esch, I. J. P.; Leurs, R. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2910; (b) Heise, C. E.; Pahuja, A.; Hudson, S. C.; Mistry, M. S.; Putnam, A. L.; Gross, M. M.; Gottlieb, P. A.; Wade, W. S.; Kiankarimi, M.; Schwarz, D.; Crowe, P.; Zlotnik, A.; Alleva, D. G. *J. Pharm. Exp. Ther.* **2005**, *313*, 1263; (c) Cole, A. G.; Stroke, I. L.; Brescia, M.-R.; Simhadri, S.; Zhang, J. J.; Hussain, Z.; Snider, M.; Haskell, C.; Ribeiro, S.; Appell, K. C.; Henderson, I.; Webb, M. L. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 200; (d) Allen, D. R.; Bolt, A.; Chapman, G. A.; Knight, R. L.; Meissner, J. W. G.; Owen, D. A.; Watson, R. J. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 697; (e) Watson, R. J.; Allen, D. R.; Birch, H. L.; Chapman, G. A.; Hannah, D. R.; Knight, R. L.; Meissner, J. W. G.; Owen, D. A.; Thomas, E. J. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 6806; (f) Watson, R. J.; Allen, D. R.; Birch, H. L.; Chapman, G. A.; Galvin, F. C.; Jopling, L. A.; Knight, R. L.; Meier, D.; Oliver, K.; Meissner, J. W. G.; Owen, D. A.; Thomas, E. J.; Tremayne, N.; Williams, S. C. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 147; (g) Du, X.; Chen, X.; Mihalic, J. T.; Deignan, J.; Duquette, J.; Li, A.-R.; Lemon, B.; Ma, J.; Miao, S.; Ebsworth, K.; Sullivan, T. J.; Tonn, G.; Collins, T. L.; Medina, J. C. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 608; (h) Knight, R. L.; Allen, D. R.; Birch, H. L.; Chapman, G. A.; Galvin, F. C.; Jopling, L. A.; Lock, C. J.; Meissner, J. W. G.; Owen, D. A.; Raphy, G.; Watson, R. J.; Williams, S. C. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 629; (i) Li, A.-R.; Johnson, M. G.; Liu, J.; Chen, X.; Du, X.; Mihalic, J. T.; Deignan, J.; Darin, J. G.; Duquette, J.; Fu, Z.; Zhu, L.; Marcus, A. P.; Bergeron, P.; McGee, L. R.; Danao, J.; Lemon, B.; Carabeo, T.; Sullivan, T.; Ma, J.; Tang, L.; Tonn, G.; Collins, T. L.; Medina, J. C. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 688; (j) Hayes, M. E.; Wallace, G. A.; Grongsaard, P.; Bischoff, A.; George, M. G.; Miao, W.; McPherson, M. J.; Robert, H. S.; Green, D. W.; Roth, G. P. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1573; (k) Hayes, M. E.; Breinlinger, E. C.; Wallace, G. A.; Grongsaard, P.; Miao, W.; McPherson, M. J.; Robert, H. S.; Green, D. W.; Roth, G. P. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 2414; (l) Bongartz, J.-. P.; Buntinx, M.; Coesemans, E.; Hermans, B.; Van Lommen, G.; Van Wauwe, J. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5819; (m) Wang, Y.; Busch-Petersen, J.; Wang, F.; Kiesow, T. J.; Graybill, T. L.; Jin, J.; Yang, Z.; Foley, J. J.; Hunsberger, G. E.; Schmidt, D. B.; Sarau, H. M.; Capper-Spudich, E. A.; Wu, Z.; Fisher, L. S.; McQueney, M. S.; Rivero, R. A.; Widdowson, K. L. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 114; (n) Wijtmans, M.; Verzijl, D.; van Dam, C. M. E.; Bosch, L.; Smit, M. J.; Leurs, R.; de Esch, I. J. P. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2252.

11. *Analytical data of compound* **1**: ^{1}H NMR (400 MHz, DMSO) δ 1.03 (3H, t, J = 7.0 Hz, NCH_aH_b–CH_3), 1.27 (3H, t, J = 7.0 Hz, NCH_aH_b–CH'_3), 2.93 (1H, t (br s), J = 12.5 Hz, H-4_{ax}), 3.18–3.27 (3H, m, H-7_{ax}, NCH_aH_b–CH_3, H-4_{eq}), 3.33–3.42 (1H, m, NCH_aH_b–CH_3), 3.48 (1H, dq, J = 14.0/7.0 Hz, NCH'_aH_b–CH_3), 3.59 (1H, dq, J = 14.0/7.0 Hz, $NCH_aH'_b$–CH_3), 3.71 (1H, dd, J = 5.5/4.0 Hz, H-8), 4.42 (1H, d, J = 14.0 Hz, H-7_{eq}), 4.83 (1H, dd, 12.0/4.0 Hz, H-5), 6.40 (1H, d, J = 5.5 Hz, H-9), 6.91 (1H, t, J = 7.5 Hz, Ar–H), 7.02–7.11 (3H, m, Ar–H), 7.22 (3H, m, Ar–H), 7.39 (1H, d, J = 8.0 Hz), 8.95 (1H, s, NH_{urea}), 10.76 (1H, s, NH_{indole}). MS/HR HRMS: *m/z* calcd for $C_{26}H_{28}N_4O_2$ $[M+H]^+$: 429.2285; found: 429.2285.
12. (a) Hoffman, A. J.; Nichols, D. E. *J. Med. Chem.* **1985**, *28*, 1252; (b) Nakahara, Y.; Niwaguchi, T. *Chem. Pharm. Bull.* **1971**, *19*, 2337.
13. *Ligand binding assay*: cell membranes were prepared from CHO cells transfected with human CXCR3. The binding of the ^{125}I labeled CXCR3 ligand I-TAC (CXCL11) to CXCR3 was assessed using the Scintillation Proximity Assay (SPA) technology (Amersham Pharmacia Biotech). Buffer or serial dilutions of compound were incubated for 2 h at room temperature with labeled CXCR3 ligand (e.g., I-TAC), CXCR3 expressing membranes and WGA coated PVT beads. The plates were then centrifuged and counted in a Topcount (Packard) instrument. The data are reported as the concentration of compound required to achieve 50% inhibition of ^{125}I ligand binding. This assay has been adapted to high throughput screening.
14. *Ca^{2+}-mobilization assay*: CXCR3 ligand-induced Ca^{2+} mobilization was assessed in CXCR3 transfected L1.2 cells (a mouse pre B cell line). For this, cells were loaded with the Ca^{2+}-sensitive fluorochrome Fluo-4 (Molecular Probes). After washing, the cells were pre-incubated with low molecular weight inhibitors for 2 h at room temperature. The transient increase in intracellular Ca^{2+} after the addition of the CXCR3 ligand (I-TAC) was monitored in a fluorescence image plate reader (FLIPR) instrument. The inhibition of CXCR3 ligand induced Ca^{2+} mobilization in the presence of CXCR3 antagonists is reported as IC_{50} values i.e. the concentration of compound which reduced the maximal Ca^{2+} response to 50%.
15. *Migration assay*: the directed cell migration induced by CXCR3 ligands, for example, I-TAC was assessed using 96-well disposable chemotaxis chambers (Multiscreen MIC, Costar) with polycarbonate membranes containing pores of 5 μM diameter. Chemokine (I-TAC) was placed in the bottom well of the chamber and cells (e.g., CXCR3 transfected L-1.2 cells) were placed in the top compartment of the chemotaxis chamber. Cell migration across the porous membrane was allowed for 4 h at 37 °C. Cells migrated from the top compartment to the bottom compartment were quantified by flow cytometry. When LMW inhibitors were tested, compounds were added to both compartments at identical concentrations; Serial dilutions of compounds were tested to assess their inhibitory effect on CXCR3 dependent cell migration. The concentration of LMW CXCR3 inhibitors which led to a reduction of migrated cells by 50% was reported as IC_{50}.
16. Baker, R. W.; Chothia, C.; Pauling, P.; Weber, H. P. *Science* **1972**, *178*, 614.
17. Figure created with PLATON: Spek, A.L. *Acta Crystallogr., Sect. C* **1990**, 34. Crystallographic data for compound **1** have been deposited with the Cambridge Crystallographic Data center as supplementary publication number CCDC 746865.
18. It has been shown by X-ray structure analysis of LSD and a variety of related ergoline derivatives that the conformation of their rigid fused ring system is nearly identical in the solid state. Thus, it can be assumed that LSD and compound **2** have very similar core structures in the solid state. Zhu, N.; Johnson, L.; White, J.; Klein-Stevens, C. L.; *Struct. Chem.* **2002**, *13*, 491.
19. Karplus, M. *J. Am. Chem. Soc.* **1963**, *85*, 2870.