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SAR study of 2,3,4,14b-tetrahydro-1*H*-dibenzo[*b*,*f*]pyrido-[1,2-*d*][1,4]oxazepines as progesterone receptor agonists

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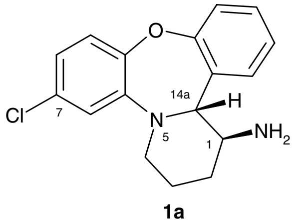
Abstract—We have developed a new class of progesterone receptor agonists having a tetracyclic dibenzo-oxazepine structure **1**. In this paper, the synthesis and structure–activity relationships of this new class are described. This work led to the identification of potent progesterone agonists up to 1 nM activity. Substitution at positions 6, 7 and 1 has proven to be crucial for activity, indicating that probably these positions are involved in important interactions with the receptor.

Over the last 10 years, considerable attention has been given to the identification of non-steroidal ligands for steroidal receptors.[1] Synthetic steroidal ligands designed to interact with these receptors have the downside that they also often bind to other, structurally closely related, steroid receptors as there is a substantial degree of homology in their ligand binding domains. This cross-reactivity is often the reason for side effect profiles. Different chemotypes might have different properties (e.g., PK, physicochemical parameters or ADMET profiles) which might cause different tissue distribution profiles and therefore differences in functionality with respect to steroid ligands. It has also been demonstrated[2] that different chemotypes alter binding modes, which in turn influences the binding of cofactors (functional selectivity).

Progestin ligands find their application mainly in the area of oral contraceptives (OC) and a wide variety of oncological and gynaecological indications. The OC field has undergone evolutionary changes over the years.[3] Four generations of steroidal progestin agents have been developed. The driving force has been to improve desired activity and reduce adverse side effects.[4] An important challenge, however, remains the identification of ligands which show no cross-reactivity to other steroid hormone receptors and therefore have, for example, no androgenic (side effects: acne, hirsutism, fatigue, mood swings/depression, rashes, weight gain) or oestrogenic (side effects: fluid retention, breast tenderness, weight gain, headaches, breakthrough bleeding, hypertension) activity. Non-steroidal progestins have a high potential of having reduced cross-reactivity and could thereby show the desired functional selectivity. Therefore, one potential therapeutic use of non-steroidal progestins is to replace the steroidal progestins in the next generation of OCs.[5]

In a HTS campaign the tetracyclic compound **1a** was identified as a confirmed hit which showed in vitro agonistic PR activity with an EC_{50} in the CHO luciferase assay of 513 nM. The subsequent SAR exploration of substituents on various positions on this tetracyclic core is the topic of the present investigation.



1a

Keywords: Non-steroidal; Progesterone receptor; Agonist.

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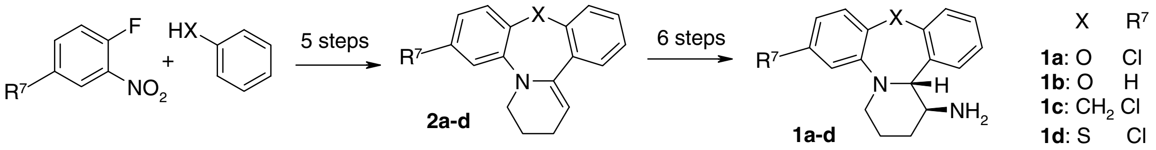
A synthesis of 1-amino substituted tetracyclic core has been described[6] which yielded the key amine intermediate, **1a–d** in 11 steps (Scheme 1). In the first five steps the tetracyclic scaffold was prepared and the last six steps were used to introduce the proper substituents and relative stereochemistry at positions 1 and 14a.

Our first goal was the design of a shorter route to the key amine intermediate. This resulted in a 6-step synthesis (Scheme 2). Central key intermediates in the new synthesis route are the tricyclic imines **5**,[7] which can be prepared via a number of routes. Treatment of **5** with glutaric anhydride yielded tetracycles **6**. A Curtius rearrangement with DPPA and an alcohol resulted in the formation of urethane structures **7**. Selective reduction of the amide functionality was accomplished with borane in THF. Treatment of the resulting urethane structures **8** with HBr in acetic acid afforded the desired amines **1**.

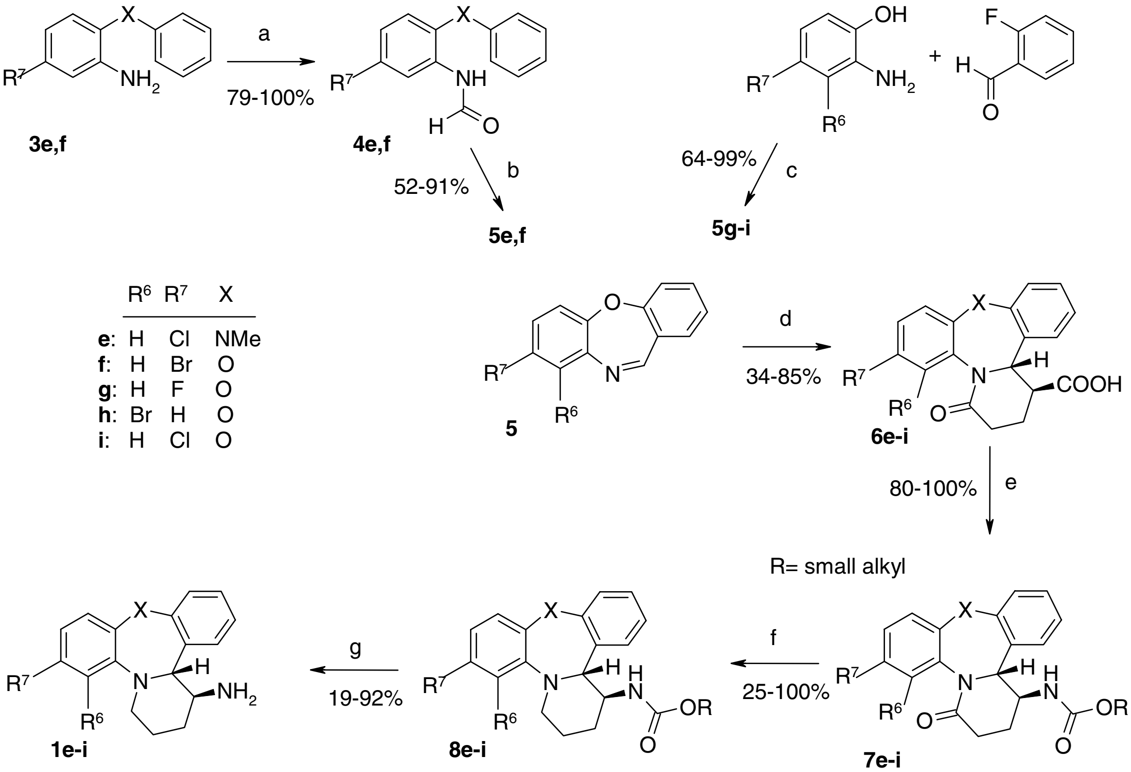
Scheme 3 shows the acylation of the amine functionality of structure **1** via several different routine synthetic methods (e.g., anhydrides, amidation). The resulting amide structures **9** were target of subsequent modification. Treatment of the amide **9a** with phosphorus pentasulfide afforded the thioamide **11**. Alkylation of the amide in **9a** with methyl iodide in the presence of sodium hydride afforded tertiary amide **12**. Reaction of amine **1a** with acetic anhydride gave acetamide **10**. The amidine compound **13** was prepared via amidine formation of the amine functionality of **1a** by treatment with trifluoroacetonitrile. Urethane derivative **14a** was prepared by standard procedure via coupling corresponding chloroformate with **1a**. The thiourea compound **14b** was formed from reaction of isobutyl isothiocyanate with **1a**. Reductive alkylation of corresponding aldehydes with **1a** provided the amines **15**.

The direct chlorination of **9a** or **9b** with NCS in the presence of a catalytic amount of HCl afforded a mixture of 6- and 8-substituted compounds. The two regioisomers (**9i** and **9j** or **9k** and **9l**, respectively) were easily separated (Scheme 4).



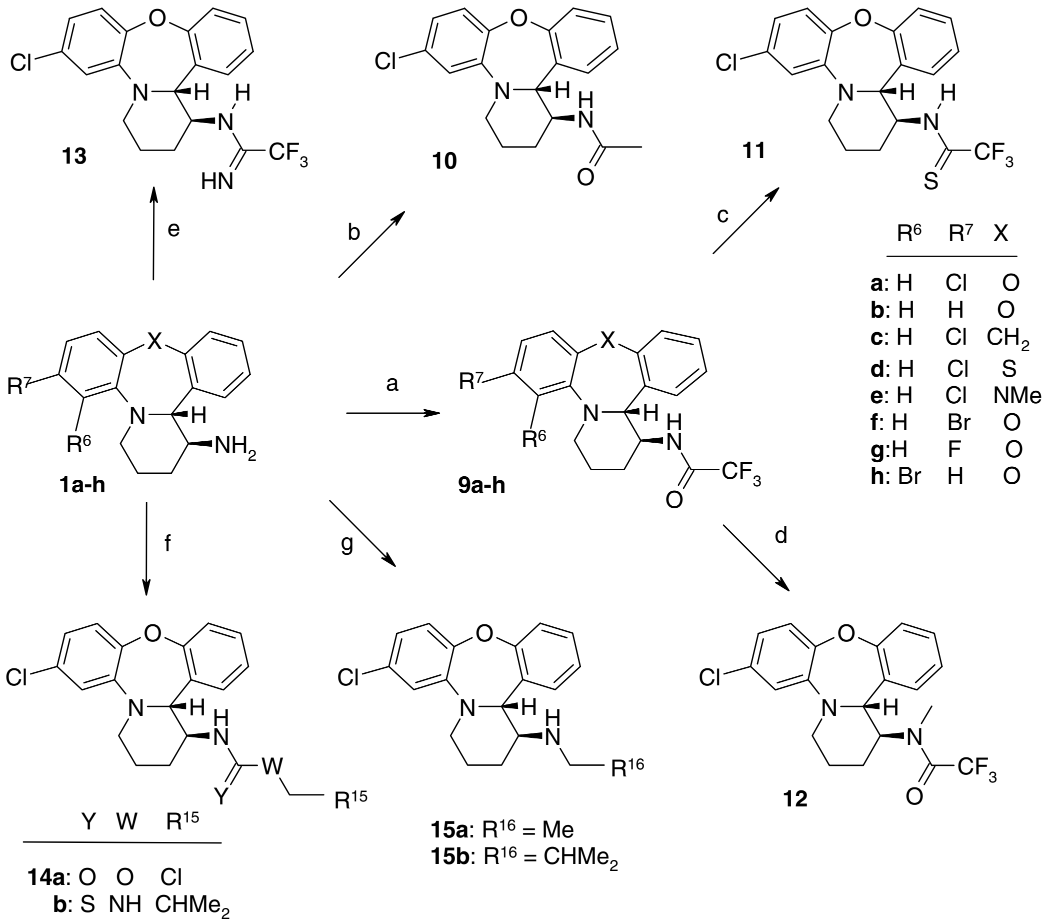
	X	R7
1a:	O	Cl
1b:	O	H
1c:	CH2	Cl
1d:	S	Cl

Scheme 1.

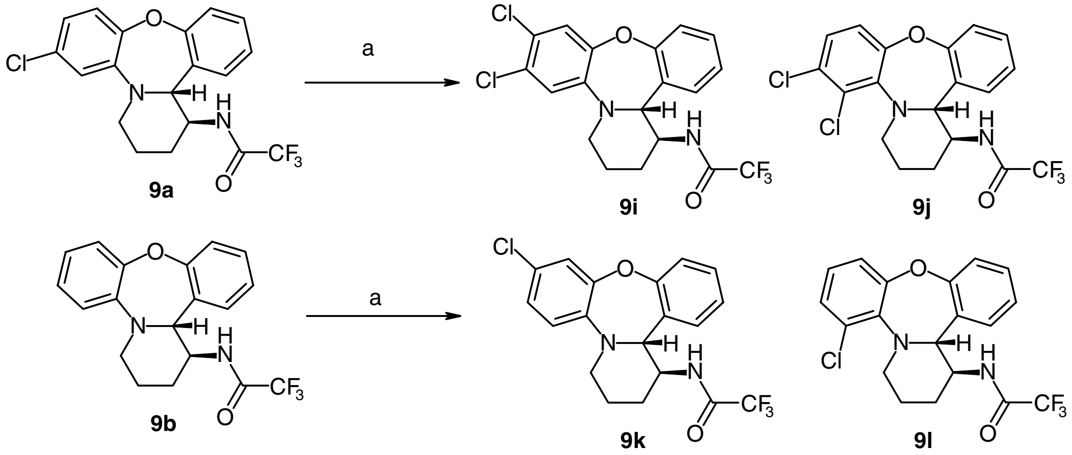


	R6	R7	X
e:	H	Cl	NMe
f:	H	Br	O
g:	H	F	O
h:	Br	H	O
i:	H	Cl	O

Scheme 2. Reagents and conditions: (a) HCOOH, reflux; (b) compound **5e**: PPA, $POCl_3$, compound **5f**: PPA; (c) i—toluene, TsOH or EtOH; ii—K_2CO_3, DMSO, 18-crown-6, 140 °C or Et_3N, DMSO, 160 °C, microwave; (d) glutaric anhydride, xylene, 140 °C; (e) i—toluene, Et_3N, DPPA, reflux; ii—ROH; (f) BH_3, THF; (g) HBr, AcOH, 100 °C.



Scheme 3. Reagents and conditions: (a) $(TFA)_2O$, pyridine, CH_2Cl_2 or CF_3COOEt, Et_3N, MeOH; (b) Ac_2O, pyridine, CH_2Cl_2; (c) P_2S_{10}, dioxane, reflux; (d) NaH, DMF, MeI; (e) F_3CCN, THF; (f) compound **14a**: $ClCOOCH_2Cl$, CH_2Cl_2, satd $NaHCO_3$ (aq); compound **14b**: *i*-BuN=C=S, THF; (g) $NaB(OAc)_3H$, CH_2Cl_2, $R_{16}CHO$.

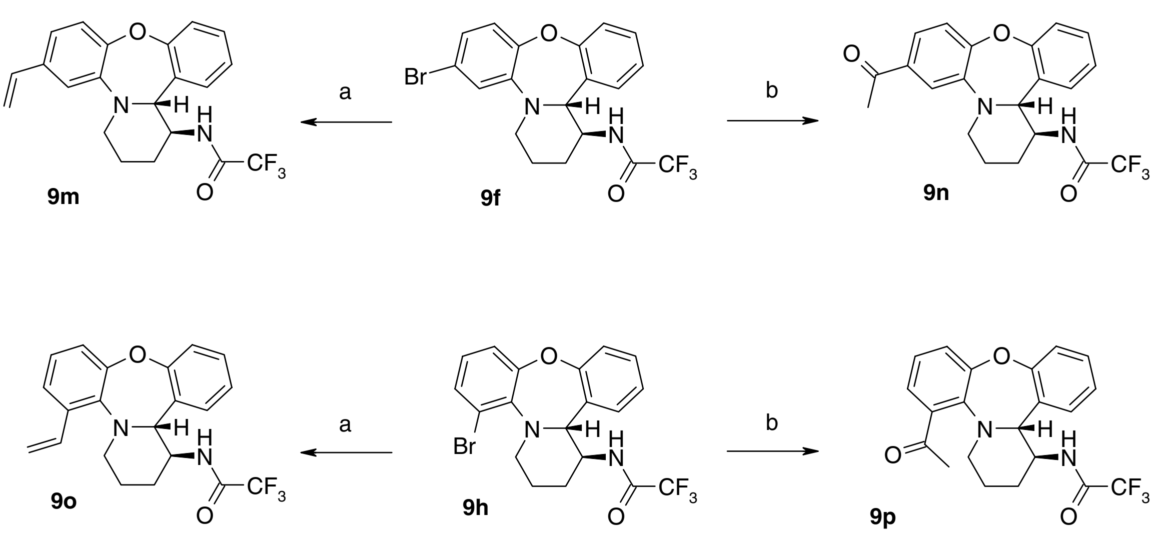


Scheme 4. Reagents and condition: (a) NCS, 1 N hydrochloric acid, acetone.

The bromo derivatives **9f** and **9h** were subjected to the Stille transformation, affording compounds with a vinyl (**9m** and **9o**) or acetyl functionality (**9n** and **9p**) (Scheme 5).

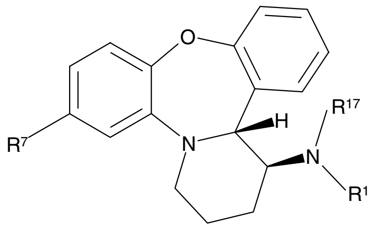
The (anti)-progestin activity of the compounds described in Tables 1–4 was determined in the CHO luciferase assay (see Supplementary material). Of the two possible diastereoisomer pairs formed during the synthesis, only the racemate of the diastereoisomer with the *cis* configuration between the amine at position 1 and the bridgehead hydrogen atom at position 14b demonstrated in vitro potency and the racemate of the *trans* diastereomer turned out to be completely inactive. The compounds described in Tables 1, 3 and 4 are racemates of the *cis*-diastereoisomer.

After identification of **1a** the compounds **1b** and **15a**, published in 1996,[6] were also tested. Both compounds proved to be not active, thereby indicating that $R^7 = Cl$ is involved in a relevant interaction with the receptor. The only compound with $R^7 = H$ which showed substantial activity is compound **9b**. This result suggests that the trifluoro group might also be involved in an important interaction with the progesterone receptor. Combination of the observations that Cl as R_7 and $COCF_3$ as R^1 were both potential interaction points with the receptor resulted in the very potent agonist **9a**. However, this compound has a mixed profile. It was decided to investigate first the SAR on the amino functionality (R^1 and R^{17}) and for this purpose R^7 was kept constant as a chlorine. The acetamide **10** was also a compound with a mixed profile showing weak agonistic and reasonable antagonistic activity. The diminished potency of secondary amide compound **12** indicated the importance of a free NH. Shifting from the trifluoroamide to the more



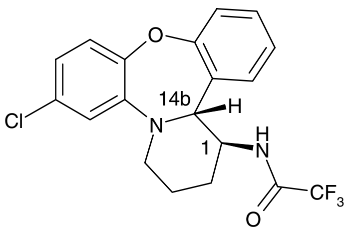
Scheme 5. Reagents and conditions: (a) $PdCl_2(PPh_3)_2$, vinyltributyltin, toluene, 110 °C; (b) i—$PdCl_2(PPh_3)_2$, (1-ethoxyvinyl)tributyltin, toluene, reflux; ii—3 N hydrochloric acid, THF.

Table 1.



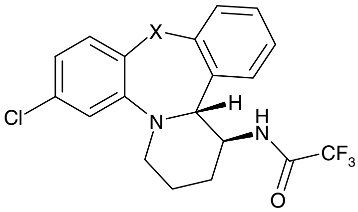
Compound	R^7	R^1	R^{17}	PRB luciferase			
				EC_{50} (nM)	i.a.	IC_{50} (nM)	i.a.
1a	Cl	H	H	513	0.79	NA	
1b[6]	H	H	H	NA		NA	
9a	Cl	$COCF_3$	H	10.6	0.63	43	0.27
9b	H	$COCF_3$	H	222	0.67		
10	Cl	$COCH_3$	H	4450	0.36	193	0.62
11	Cl	$CSCF_3$	H	1.3	0.97		
12	Cl	$COCF_3$	Me	450	0.56		
13	Cl	$C(=NH)CF_3$	H	10.3	0.76	133	0.14
14a	Cl	$COOCH_2Cl$	H	598	0.79		
14b	Cl	$CSNHCH_2CHMe_2$	H	NA		314	0.97
15a[6]	H	Et	H	NA		NA	
15b	Cl	CH_2CHMe_2	H	1340	0.74		

Table 2.



Compound	Config. C1	Config. C14b	PRB luciferase			
			EC_{50} (nM)	i.a.	IC_{50} (nM)	i.a.
9a	Racemate		10.6	0.63	43	0.27
9a(SR)	S	R	3.0	0.75	26	0.19
9a(RS)	R	S	54	0.65		

Table 3.



Compound	X	PRB luciferase			
		EC_{50} (nM)	i.a.	IC_{50} (nM)	i.a.
9a	O	10.6	0.63	43	0.27
9c	CH_2	2.0	0.74	26	0.20
9d	S	2.8	0.70		
9e	NMe	9.8	0.66		

polarizable trifluorothioamide **11** resulted in a 10-fold increase of the potency while the trifluoroamidine compound **13** proved to be equipotent to the amide **9a**. The carbamate analogue **14a** and the amine derivative **15b** turned out to be micromolar active agonists, while the thiourea derivative **14b** is a full antagonist, albeit a rather weak one.

It can be concluded that R^1 is an important substituent by which potency and agonism versus antagonism can be controlled. An electron-withdrawing group like $CXCF_3$ (X = O, S, NH) appears to be especially favourable. Taken together, the effects of the substitutions at the 1-amine and at position 7 suggest that these two functionalities mimic the two carbonyl groups of progesterone. The distance between the 3- and 20-keto groups in progesterone is 11.9 Å, whereas that between the 7-Cl substituent and the fluoroacetyl group in compound **17d** is 11.5 Å. If this conjecture were true, additional (especially hydrophilic) substituents in positions 2–4 and 11–14 should hardly increase progestin activity, because of the predominantly lipophilic nature of the progesterone receptor active site.

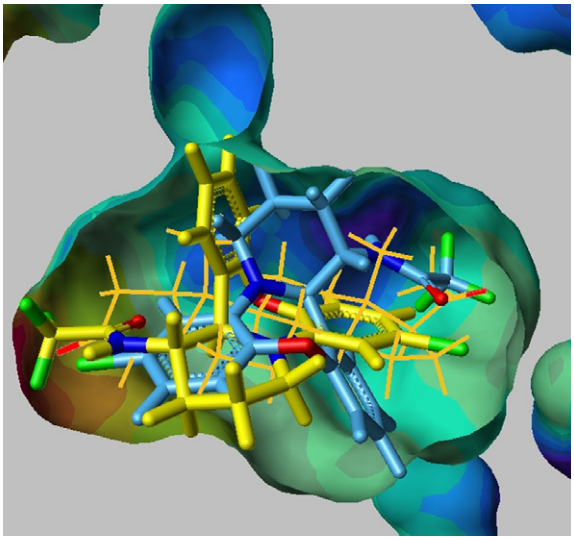
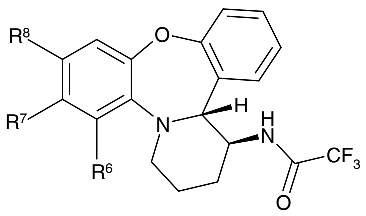


Figure 1. Two poses: assumed eutomer (1*S*,14b*R*) on progesterone (orange) in the active site of PR. Yellow: orientation 1; blue: orientation 2.

From the activities of the separated enantiomers[8] we learned that the eutomer has the (1*S*,14b*R*) configura-

Table 4.



Compound	R^6	R^7	R^8	PRB luciferase			
				EC_{50} (nM)	i.a.	IC_{50} (nM)	i.a.
9a	H	Cl	H	10.6	0.63	43	0.27
9g	H	F	H	198	0.73	730	0.20
9i	H	Cl	Cl	38	0.47	318	0.28
9j	Cl	Cl	H	1.6	0.96		
9k	H	H	Cl	993	0.17	2220	0.62
9l	Cl	H	H	19	0.87		
9m	H	Vinyl	H	>1000			
9n	H	COMe	H	258	0.47		
9o	Vinyl	H	H	0.81	1.26		
9p	COMe	H	H	0.96	0.93		

tion. The other enantiomer (1*R*,14*bS*) showed a more than 15-fold reduction in potency (Table 2).

To date, we have not been able to obtain an X-ray diffraction structure of any of the ligands described above bound to PR. In order to assess the positioning of the tetracyclic scaffold in the progesterone receptor, a docking experiment was performed (see Supplementary material). Compound **9a** was selected as the model compound. Figure 1 shows these minimal-energy poses for the two orientations. When the two eutomers are compared, orientation 2 ($COCF_3$ group coinciding with the C20 carbonyl) has a slightly lower interaction energy. This would seem to make orientation 2 more likely. However, if we compare the resulting space occupied within PR between the two orientations, we observe that in orientation 1 ($COCF_3$ group coinciding with the C3 carbonyl), aromatic ring C occupies the space above C10 and C11 in steroids. Indeed, active progestagens with rather large 11β substituents are known.[9] On the contrary, in orientation 2, ring C occupies a space below and beyond C7 and C8, and only slightly touches the pocket frequently postulated[10] below ring D of steroids. Based on these data, an unequivocal choice between the two orientations is hardly possible.

Investigation of the bridgehead atom by compounds **9c–e** demonstrated that C, S, O and N are all well tolerated but that CH_2 and S are more potent than O and NMe. Although X = O was not the most potent option in the bridgehead position, the difference with either methylene or sulfur is not substantial. Therefore, we decided to keep oxygen as the most preferred bridgehead, both for reasons of synthetic feasibility as well as because of the fact it is expected to be less prone to metabolic conversions or instability as is known by potential oxidation of S and N and radical formation of CH_2.

Investigation of substitution on the aromatic A ring indicated that small polar and non-polar groups (e.g., chlorine (**9l**), vinyl (**9o**), acetyl (**9p**)) are permitted in the 6 position (R^6), resulting in potent agonists. 6,7-Disubstitution resulted in the potent agonist **9j**. For the 7 position (R^7) we clearly see that chlorine (**9a**) is still the most active substituent, followed by fluorine (**9g**). Substitution at this position by an acetyl (**9n**) or vinyl (**9m**) moiety diminished the potency. Substitution of the 8 position (R^8) with chlorine (**9k**, **9i**) is not favoured, which might be an indication for restricted space at this area of the receptor site.

In conclusion, we have identified a novel series of potent progesterone receptor agonists, the 2,3,4,14b-tetrahydro-1*H*-dibenzo[*b*,*f*]pyrido[1,2-*d*][1,4]oxazepines. SAR studies demonstrated the importance of a 6- and/or 7-substituent for activity. Incorporation of a trifluoroacetamide or a trifluorothioamide substituent at the N-1 position significantly improved the potency in this series, producing the very potent agonists **9a**, **9o**, **9p** and **11**. Probably these two functionalities, 6- or 7-substituent and trifluoroacetamide or trifluorothioamide, mimic the two carbonyl groups of progesterone. Further developments of this series will be published in due course.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.12.065.

References and notes

1. Reviews on non-steroidal ligands (a) Coghlan, M. J.; Kort, M. E. *Expert Opin. Ther. Patents* **1999**, 1523; (b) Lin, X.; Huebner, V. *Curr. Opin. Drug Discov. Devel.* **2000**, *3*, 383; (c) Buijsman, R. C.; Hermkens, P. H. H.; van Rijn, R. D.; Stock, T.; Teerhuis, M. M. *Curr. Med. Chem.* **2005**, *12*, 1017; (d) Hermkens, P. H. H.; Kamp, S.; Lusher, S.; Veeneman, G. H. *Idrugs* **2006**, *7*, 488.
2. Veeneman, G. H. *Curr. Med. Chem.* **2005**, *12*, 1077.
3. Madauss, K. P.; Stewart, E. L.; Williams, S. P. *Med. Res. Rev.* **2007**, *27*, 374.
4. (a) Schindler, A. E.; Campagnoli, C.; Druckman, R.; Huber, J.; Pasqualini, J. R.; Schweppe, K. W.; Thijssen, J. H. H. *Maturitas* **2003**, *46S1*, S7; (b) Edwards, L. A. *Formulary* **2004**, *39*, 104.
5. (a) Zhi, L.; Marschke, K. B. *Expert Opin. Ther. Patents* **1999**, *9*, 695; (b) Allan, G.; Macielag, M. *Expert Opin. Ther. Patents* **1999**, *9*, 955; (c) Woolfrey, J. R.; Avery, M. A. *Chemtracts-Org. Chem.* **2000**, *13*, 20; (d) Zhang, P.; Fensome, A.; Wrobel, J.; Winneker, R.; Zhang, Z. *Expert Opin. Ther. Patents* **2003**, *13*, 1839.
6. Caulfield, W. L.; Gibson, S.; Rae, D. R. *J. Chem. Soc., Perkin Trans. 1* **1996**, 545.
7. Note of caution: tricyclic imines with the general structure **5** are reported to be potentially strongly irritant and sensitising compounds. See the aquatic toxicity of the sensory irritant and riot control agent dibenz(*b*,*f*)-1,4-oxazepine (CR). Johnson, Dennis W.; Haley, Mark V.; Landis, Wayne G. ASTM Special Technical Publication 1990, 1096 (Aquat. Toxicol. Risk Assess.: 13th Vol.), 176.
8. Racemate **1a** was separated into its enantiomers by chiral HPLC (Chiracel OJ column with heptanes/ethanol 85:15 as eluent). Both enantiomers were converted to the corresponding trifluoroamides **9a**. The absolute stereochemistry has been determined by means of VCD (see Supplementary material). It was established with many compounds that the activities of the eutomers correlate well with those of the racemates (data not shown).
9. Schoonen, W. G. E. J.; Deckers, G.; de Gooijer, M. E.; de Ries, R.; Mathijssen-Mommers, G.; Hamersma, H.; Kloosterboer, H. J. *J. Steroid Biochem. Mol. Biol.* **2000**, *74*, 109.
10. (a) van der Sijde, D.; Kooreman, H. J.; Jaitly, K. D.; Marx, A. F. *J. Med. Chem.* **1972**, *15*, 909; (b) Solo, A. J.; Kapoor, J. N. *J. Med. Chem.* **1973**, *16*, 270.