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Trifluoromethyl group as a pharmacophore: Effect of replacing a CF_3 group on binding and agonist activity of a glucocorticoid receptor ligand

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Abstract—Compound **1**, a potent glucocorticoid receptor ligand, contains a quaternary carbon bearing trifluoromethyl and hydroxyl groups. This paper describes the effect of replacing the trifluoromethyl group on binding and agonist activity of the GR ligand **1**. The results illustrate that replacing the CF_3 group with a cyclohexylmethyl or benzyl group maintains the GR binding potency. These substitutions alter the functional behavior of the GR ligands from agonists to antagonists. Docking studies suggest that the benzyl analog **19** binds in a similar fashion as the GR antagonist, RU486. The central benzyl group of **19** and the C-11 dimethylaniline moiety of RU486 overlay. Binding of compound **19** is believed to force helix 12 to adopt an open conformation and this leads to the antagonist properties of the non-CF_3 ligands carrying a large group at the center of the molecule.

The glucocorticoid receptor (GR) belongs to the family of steroid receptors that include the mineralocorticoid (MR), progesterone (PR), estrogen (ER), and androgen (AR) receptors. The anti-inflammatory effects of endogenous steroids have stimulated the development of glucocorticoid (GC) derivatives, such as dexamethasone and prednisolone,[1–4] which have found wide use in the treatment of various inflammatory, immune, and allergic disorders including rheumatoid arthritis, COPD, Crohn's disease, systemic lupus erythematosus, and osteoarthritis.[5] However, use of GCs is associated with a number of side effects that include edema, weight gain, muscle weakness, diabetes mellitus, and osteoporosis.[6,7] Recent investigations have led to an understanding of the molecular mechanisms that mediate GC effects. The anti-inflammatory and immune suppressive properties of GCs have largely been attributed to transrepression, whereas some of the side effects (such as diabetes, glaucoma) have been ascribed to transactivation. In addition, cross-reactivity of GC ligands with other steroid receptors may also lead to a number of side effects.[8,9] GR agonists showing dissociation between transactivation and transrepression activities could provide therapeutic agents with a reduced side-effect profile.[10–23] Furthermore, GCs stimulate the production of glucose in the liver and this can exacerbate Type 2

Keywords: Glucocorticoid receptor; Glucocorticoid receptor ligand; Trifluoromethyl group; Pharmacophore; Glucocorticoid receptor antagonist; RU486; Helix 12.
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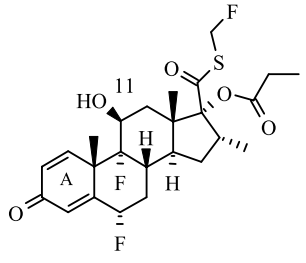
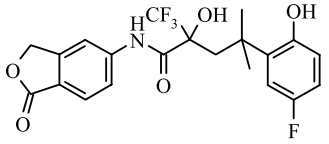
diabetes.[24] Studies with the prototype GR antagonist RU486 have shown that GR antagonism could be an effective way of regulating peripheral glucose metabolism. A liver-selective GR antagonist could decrease the hepatic glucose production without the risk of peripherally driven side effects and may find therapeutic utility in treating Type 2 diabetes.[25] The discovery of dissociated GC agonists, as well as liver-selective GR antagonists, has been the focus of many drug discovery programs in the recent years.[25–33] Studies directed toward understanding the structural basis for the agonist and antagonist activities will aid in the design of GR ligands with the desired functional profile.

Several classes of ligands for the glucocorticoid receptor have been described in the scientific and patent literature.[26–33] For example, compound **1**[31] and more recently, analog ZK 216348[32] (Fig. 1) have been reported as GR agonists demonstrating dissociation between transrepression and transactivation activities.

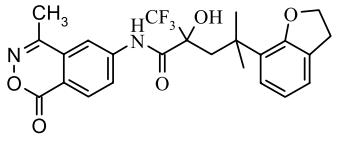
Our attention was drawn to the central quaternary center containing trifluoromethyl and hydroxyl groups, in the GR ligand **1**. A similar structural feature, that is, a quaternary center containing methyl and hydroxyl groups is present in the androgen antagonists, bicalutamide,[34] and hydroxyflutamide[35] (Fig. 2). The quaternary center has shown to be an essential pharmacophore[36] for the biological activity of these ligands. Replacing the central methyl group with a CF_3 affects the functional activity and the CF_3 analogs behave as partial agonists. We were intrigued by the role of the CF_3 group in the GR ligand **1** and asked the following questions: are CF_3 and hydroxyl groups required for the biological activity? How does replacing CF_3 and modifying other



fluticasone propionate

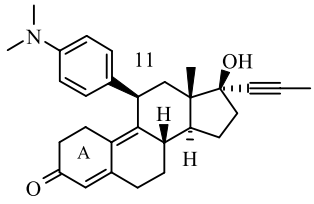


compound **1**

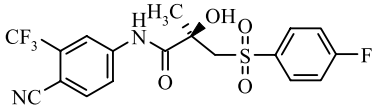


ZK 216348, (+)-enantiomer

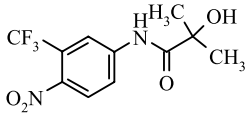
Figure 1. GR agonists reported in the literature.



mifepristone (RU486)



bicalutamide



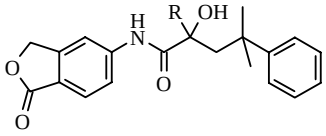
hydroxyflutamide

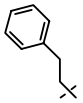
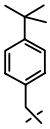
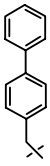
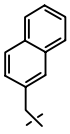
Figure 2. GR and AR antagonists.

parts of the molecule, such as the backbone amide bond and/or the anilide moiety, affect the biological activity? We present here our results on investigating the effect of these modifications on the binding potency, nuclear receptor selectivity, and the agonist activity of compound **1**.

Structure–activity relationships (SAR) were driven by the GR-binding affinities of the ligands. The IC_{50} values for binding to GR, PR, MR, and ER were determined using a fluorescence polarization competitive binding assay.[37] Initially, we focused on exploring lipophilic CF_3 substituents, such as alkyl and cycloalkyl groups, and the SAR are shown in Table 1. Compound **1** has an IC_{50} of 6 nM in the GR-binding assay. To quickly assess the effect of replacing the CF_3 group in **1**, SAR was initiated with the preparation of readily accessible analogs containing an unsubstituted phenyl ring at the right side of the molecule. Toward this goal, we prepared the phenyl analog **2** and the data showed that this compound retains a good binding potency (IC_{50} 79 nM). Replacing CF_3 by a hydrogen atom (**3**) leads to a significant loss in GR binding, whereas a CF_3 to CH_3 substitution (**4**) leads to a 5-fold loss in the GR binding. These results clearly indicate that in compound **3**, the central *secondary*-hydroxyl group alone is not sufficient to confer good GR binding. Isopropyl analog **5** was similarly potent compared to the methyl analog **4**. Increasing the size of the CF_3 substituent from isopropyl (**5**) to cyclohexylmethyl (**6**) leads to a dramatic improvement in the GR binding potency. With an IC_{50} of 9 nM, ligand **6** is 9-fold more potent than the corresponding CF_3 analog **2**. These results clearly demonstrate that the CF_3 group is not essential for potent GR binding and this prompted us to investigate further the CF_3 substitution SAR. The optically active enantiomers (**7** and **8**) of **6**, separated by chiral HPLC,[38] showed a 35-fold difference in the GR binding potencies, indicating a stereochemical preference of the hydroxyl and cyclo-

Table 1. Receptor binding data; alkyl, cycloalkyl, and arylalkyl CF_3 replacements



Compd	R	IC_{50} (nM) mean ± SD	
		GR	PR
1[a]	—	6 ± 2	16 ± 2
2	CF_3	79 ± 8	36 ± 3
3	H	>2000	>2000
4	CH_3	370 ± 10	85 ± 22
5		200 ± 47	210 ± 70
6		9 ± 3	19 ± 12
7[b]		320 ± 60	210 ± 70
8[b]		9 ± 2	35 ± 6
9		460 ± 40	240 ± 40
10		13 ± 1	30 ± 6
11		57 ± 18	59 ± 14
12		72 ± 15	330 ± 70
13		740 ± 310	180 ± 40
14		110 ± 20	48 ± 10

[a] See Figure 1.
[b] Optically active enantiomers of **6**.

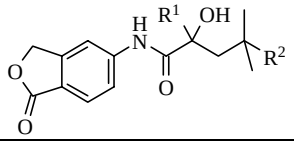
hexylmethyl groups for optimal GR binding. A similar (50-fold) difference in the GR binding affinities has been reported[32] for the optically active enantiomers of the CF_3 compound ZK 216348.

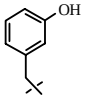
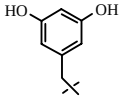
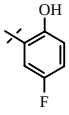
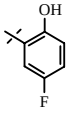
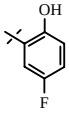
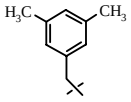
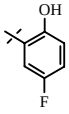
Next, we focused our attention on replacing the CF_3 group in **2** with aryl or arylalkyl groups. Data for the analog **10** show that replacing the CF_3 in **2** with a benzyl group (**10**) (Table 1) results in a 6-fold improvement of the GR binding. However, attaching a phenyl group directly to the central carbon decreases the binding affinity (**9**). A slight loss in GR binding is observed when the central phenyl ring is attached by a two-carbon atom spacer (**11**). Analogs **12** and **14** carrying 4-*tert*-butyl phenyl and 2-naphthyl groups, respectively, bind to GR with IC_{50}s of 70–110 nM. These results suggest that the effect of the bulkier substitutions is not dramatic (6- to 8-fold loss in the GR binding compared to **10**). In contrast, the biphenyl analog **13** is significantly less active than **10**, pointing to a limitation in the size of the hydrophobic group tolerated at this position.

Encouraged by the initial results, we extended the SAR to evaluate analogs of **10** in which various substituents have been introduced on the central phenyl ring. Either 2-hydroxy-5-fluorophenyl, the preferred right side in the CF_3 series (Table 1), or an unsubstituted phenyl group was employed for the right side of the molecule. The SAR on the selected analogs having substituents at the *ortho* or *meta* positions are given in Table 2. We have shown earlier (Table 1) that analog **10**, having an unsubstituted phenyl ring at both the center and the right side of the molecule, is a potent GR ligand. In contrast to the CF_3 analogs **1** and **2**, incorporating 2-hydroxy-5-fluorophenyl at the right side of the molecule decreases the GR binding affinity (**19** and**10**). Small hydrophobic groups, such as CH_3, are tolerated at both *ortho* and *meta* positions (**20**, **21**). However, *meta*-disubstitution leads to an 8-fold loss in GR binding (**22**). Introducing a hydroxyl group at the *ortho* or *meta* positions decreases the GR binding affinity (**15**, **16**). Analogs bearing a polar 3,5-bis-hydroxybenzyl (**17**) or 2-pyridylmethyl side chain (**18**) are significantly less potent than **10**. These results suggest that the central benzyl ring in **10** binds in a lipophilic environment. Data for the methyl substituted analogs reflect the spatial constraints of the binding pocket.

The CF_3 compound **24** containing a benzoxazinone anilide moiety binds to GR with an affinity similar to the corresponding phthalide **1** (Table 3). To expand the scope of CF_3 replacement SAR, we investigated the effect of incorporating the phenolic group, as well as the benzoxazinone anilide moiety, on the affinities of our most potent cyclohexylmethyl and benzyl analogs. The CF_3 analog **2** with an unsubstituted phenyl ring at the right side is 15-fold less potent than the 2-hydroxy-5-fluoro analog **1**. Methylation of the phenolic group in **1** leads to a 40-fold loss in the GR binding (**23**). These results suggest that the phenolic group may be involved in hydrogen bonding interactions, which may account for a higher binding affinity of the CF_3 compound **1** compared to **2** and **23**. In contrast, incorporating the right side phenolic group does not improve the GR binding in the non-CF_3 series as illustrated by the data for the cyclohexylmethyl analogs (**6**, **25**), (**26**, **27**) and benzyl analogs (**10**, **19**), (**28**, **29**). In fact, results with the phthalides (**6**, **25**) and (**10**, **19**) indicate a trend towards a slight decrease in GR affinity when a phenolic group is introduced at the right side of the molecule. A slight improvement in GR binding is observed when

Table 2. Receptor binding data; substituted benzyl analogs

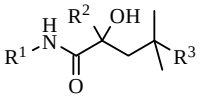


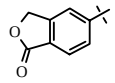
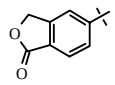
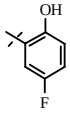
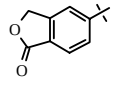
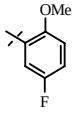
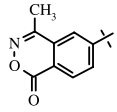
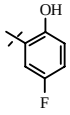
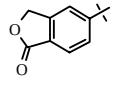
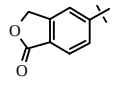
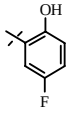
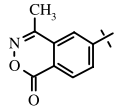
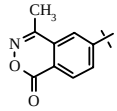
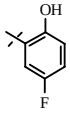
Compd.	R^1	R^2	IC_{50} (nM) mean ± SD	
			GR	PR
10			13 ± 1	30 ± 6
15			61 ± 11	47 ± 6
16			120 ± 10	44 ± 0
17			>2000	160
18			760 ± 230	400 ± 30
19			60 ± 1	35 ± 11
20			48 ± 33	36 ± 16
21			52 ± 5	34 ± 9
22			470 ± 230	51 ± 17

the left side phthalide is replaced with benzoxazinone (analogs **27**, **25** and **29**, **19**). This trend is seen only with the non-CF_3 analogs having a phenolic group at the right side. The SAR shown in Table 3 suggests that the right side phenolic group in the non-CF_3 analogs is not involved in hydrogen bonding interactions, unlike CF_3 compounds **1** and **24**, supported by the docking studies of the compounds **19**, **1**,[33] and **24**[33,39] (*vide modeling section at the end*).

To assess the influence of the central hydroxyl group on GR binding potency, the hydroxyl group in the benzyl analog **10** was replaced with a hydrogen atom. This substitution leads to a poor GR ligand (**30**) (Table 4). We have shown earlier that the presence of a central hydroxyl group alone will not lead to potent GR binding (**3**, Table 4). Taken together, binding data for **3**, **10**, and **30** clearly indicate that the central quaternary center in **10** (and the other non-CF_3 analogs) is essential for potent GR binding. Both the hydroxyl group and the

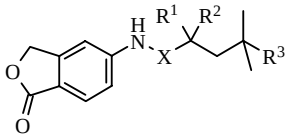
Table 3. Receptor binding data; R^1, R^2, and R^3 modifications

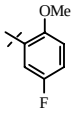
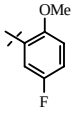


Compd.	R^1	R^2	R^3	IC_{50} (nM) mean ± SD	
				GR	PR
2		CF_3		79 ± 8	36 ± 3
1		CF_3		6 ± 2	16 ± 2
23		CF_3		230 ± 50	85 ± 48
24		CF_3		4 ± 1	22 ± 15
6				9 ± 3	19 ± 12
25				20 ± 8	42 ± 8
26				9 ± 6	40 ± 22
27				7 ± 1	35 ± 9
10				13 ± 1	30 ± 6
19				60 ± 1	35 ± 11
28				7 ± 3	30 ± 5
29				8 ± 2	32 ± 13

CF_3 substituent (benzyl or cycloalkyl group) are involved in important interactions with GR and function as essential pharmacophores. Comparison of the binding data for the analogs **31** and **32** suggests that the backbone carbonyl is not involved in critical interactions with the GR.

Table 4. Receptor binding data; backbone modifications and CF_3 replacements



Compd	R^1	R^2	R^3	X	IC_{50} (nM) mean ± SD	
					GR	PR
3	H	OH		CO	>2000	2000
10		OH		CO	13 ± 1	30 ± 6
30		H		CO	>2000	2000
31		OH		CH_2	81 ± 6	80 ± 20
32		OH		CO	52 ± 3	35 ± 1

The following observations were made on the GR/PR selectivity. Binding data show that the CF_3 analogs **1** and **24** have similar affinities toward GR and PR (Tables 1 and 3). Replacing CF_3 with a cyclohexylmethyl or benzyl group (**6**, **10**) leads to a slight improvement in the GR/PR selectivity (Table 1). Introducing a bulky or polar group on the central phenyl ring decreases the GR binding affinity. However, the PR binding is not significantly altered (Tables 1 and 2). Both the right side phenolic group and the left side anilide moieties do not influence the GR/PR selectivity (Table 3). Overall, replacing the CF_3 group and modifying other parts of the molecule do not improve the binding selectivity over PR. Similarly, selectivity over MR and ER is not affected by the CF_3 replacements and other modifications described in the present work. Nuclear receptor selectivity data for selected compounds are shown in Table 5.

Non-CF_3 compounds with the GR binding affinities less than 2 μM were tested for their agonist and antagonist activities. The agonist activities were determined by measuring the transcriptional repression of IL-6 production in IL-1-stimulated human foreskin fibroblasts.[40] Mifepristone (RU486) is shown to compete with dexamethasone and inhibit the dexamethasone-induced GR transactivation. This assay[33,41,42] was employed to evaluate antagonist activities of GR ligands by measuring the inhibition of the dexamethasone-induced GR transactivation of the mouse mammary tumor virus (MMTV) luciferase gene in HeLa cells.[43] Dexamethasone and RU486 were used as standards for GR agonism and antagonism, and have EC_{50}s of 1 and 2 nM, respectively. A dissociated GR ligand is expected to block the dexamethasone-induced transactivation in the MMTV assay, while maintaining the transrepression activity. CF_3 compounds **1** and **24** do exhibit such a profile in these functional assays. Both compounds **1** and **24** show potent agonist activities with EC_{50}s of 45 nM (efficacy 59% of dexamethasone) and 10 nM (efficacy 87% of dexamethasone), respectively (Table 6). In the antagonist assay, while compound **1** has an EC_{50} of 150 nM and was able to completely antagonize the MMTV transactivation, **24** gave a maximum inhibition of 60% at 5 μM. Interestingly, replacing the 2-hydroxy-5-fluorophenyl group in the agonist **1** with an unsubstituted phenyl ring leads to the full antagonist **2** with an EC_{50} of 150 nM. Thus, compound **2** showed no IL-6 agonism at 2 μM, while completely inhibiting the transactivation. Non-CF_3 compounds behaved similarly, showing no agonist activity at a similar concentration (Table 6). Potent antagonist activities with EC_{50}s of 120–760 nM and 100% inhibition at 5 μM were observed for most of the non-CF_3 compounds tested

Table 5. Nuclear receptor selectivity; GR, PR, MR, and ER binding data for selected compounds

Compd.	IC_{50} (nM) mean ± SD			
	GR	PR	MR	ER
1	6 ± 2	16 ± 2	30 ± 6	n.b.[a]
2	79 ± 8	36 ± 3	215 ± 35	n.b.
24	4 ± 1	22 ± 15	41 ± 7	n.b.
4	370 ± 10	85 ± 22	60 ± 16	n.b.
5	200 ± 47	210 ± 70	315 ± 50	n.b.
6	9 ± 3	19 ± 12	170 ± 30	n.b.
10	13 ± 1	30 ± 6	160 ± 14	n.b.
12	72 ± 15	330 ± 70	175 ± 55	n.b.
19	60 ± 1	35 ± 11	190 ± 42	n.b.
25	20 ± 8	42 ± 8	140 ± 26	n.b.
27	7 ± 1	35 ± 9	104 ± 14	n.b.
29	8 ± 2	32 ± 13	104 ± 9	n.b.
31	81 ± 6	80 ± 20	195 ± 29	n.b.

[a] n.b.: no detectable binding at 2 μM.

Table 6. IL-6 agonism and MMTV antagonsim data for selected compounds

Compd	IL-6		MMTV	
	EC_{50} (nM) mean ± SD	% efficacy at 2 μM	EC_{50} (nM) mean ± SD	% inhibition at 5 μM
Dex.	1	100		
RU486	—	44	2 ± 1	100% at 100 nM
1	45 ± 9	59	150 ± 35	100
2	—	n.d.[a]	150 ± 90	100
24	10 ± 3	87	—	60
4	—	n.d.	2370 ± 580	70
5	—	n.d.	620 ± 200	95
6	—	n.d.	140 ± 65	100
10	—	n.d.	205 ± 110	100
12	—	n.d.	760 ± 270	90
19	—	n.d.	420 ± 170	100
25	—	n.d.	130 ± 90	100
29	—	n.d.	130 ± 90	100
27	—	n.d.	120 ± 40	100
31	—	n.d.	610 ± 160	100

[a] n.d.: no detectable inhibition at 2 μM.

(Table 6). Lack of agonist activity of the methyl analog **4** could be due to the poor GR binding affinity. In contrast, the non-CF_3 compounds with a large group at the center of the molecule are potent GR ligands and the lack of agonist activity of these compounds cannot be attributed to poor GR binding affinities. For example, analogs **6**, **25**, **27**, and **29** bind to GR with affinities comparable to that of dexamethasone (IC_{50} 6 nM) and yet have no agonist activities. Similar to RU486, these compounds **6**, **25**, **27**, and **29** behave as potent GR antagonists by completely inhibiting the dexamethasone-induced MMTV transactivation with EC_{50}s in the range of 120–140 nM. The benzyl analogs **10** and **19** also showed 100% inhibition at 5 μM and had EC_{50}s of 205 and 420 nM, respectively. These results clearly demonstrate that replacing the CF_3 group in **1** and **24** with a large lipophilic moiety affects the functional profile and imparts antagonist properties to these ligands.

Our results on the non-CF_3 analogs of compound **1** and **24** indicate that: (i) the CF_3 group can be replaced with a cyclohexylmethyl or benzyl group and potent GR binding can be retained; (ii) the central hydroxyl group is essential; (iii) the central quaternary center is required for binding to GR; (iv) the backbone carbonyl can be replaced with a methylene group and good GR affinity can be maintained; (v) the right side phenolic group does not improve the GR binding; (vi) the selectivity over PR, MR, and ER is not affected dramatically by the CF_3 substitutions. The CF_3 group, by increasing the acidity of the central hydroxyl group, could enhance its hydrogen bond donor ability. Replacing the CF_3 with a cycloalkyl or arylalkyl group weakens the ability of the central hydroxyl moiety to be involved in hydrogen bonding interactions.[35,36,44] Consequent loss in GR binding could be compensated by a gain in the binding energy due to the hydrophobic interactions of the CF_3 substituents (benzyl or cyclohexylmethyl groups) with the receptor. In addition, replacing the CF_3 group with a cyclohexylmethyl or benzyl moiety changes the functional behavior of these compounds from agonists to antagonists.

To gain insight into the structural reasons for the observed agonistic and antagonistic behavior, we have docked the CF_3 compound **1** and the benzyl analog **19** into the GR-ligand binding domain (GR-LBD) binding pocket.[39,45–49] Compound **1** can be docked into the binding pocket observed in the co-complex X-ray structure of GR-LBD/fluticasone.[33] The docking study shows that compound **1** occupies a similar space as the known GR agonist, fluticasone propionate. The binding mode is highlighted by the position of helix 12 (H12) that sits on the binding pocket (Fig. 3A). This position of helix 12 has shown to be a salient feature of the agonist conformation. Benzyl analog **19** cannot bind in the same fashion, as the large benzyl moiety requires the relocation of helix 12. Compound **19** was docked into the binding pocket using the co-complex X-ray structure of GR-LBD/RU486.[46] The docking study shows that **19** and RU486 occupy similar space and the benzyl moiety of **19** overlays with the C-11 dimethylaniline group of RU486 (Fig. 3B). The benzyl group, like the C-11 dimethylaniline group of RU486, forces helix 12 to adopt an open conformation. In the case of RU486, the helix 12 is displaced into a coactivator recognition surface known as activation function-2 (AF-2) domain. The displaced helix 12 blocks the recruitment of coactivators and provides a molecular basis for RU486 antagonsim of GR.[41] Our docking studies indicate that binding of compound **19** to GR induces similar conformational changes, which may account for the antagonist activity of the benzyl analog **19** and other non-CF_3 analogs that contain a large group at the quaternary center.

A more detailed analysis of the binding interactions of the agonist **1** and the antagonist **19** shows similarities, as well as differences (Figs. 4A and B). The left side heterocyclic group of the CF_3 compound **1** overlays with the A-ring of fluticasone and is likely to interact with Gln570 and Arg611. The central hydroxyl of **1** overlays with the C-11 hydroxyl of the steroid and similarly forms a hydrogen bond with the amide side chain of Asn564. The ligand amide nitrogen is suggested to form an additional hydrogen bond with the backbone

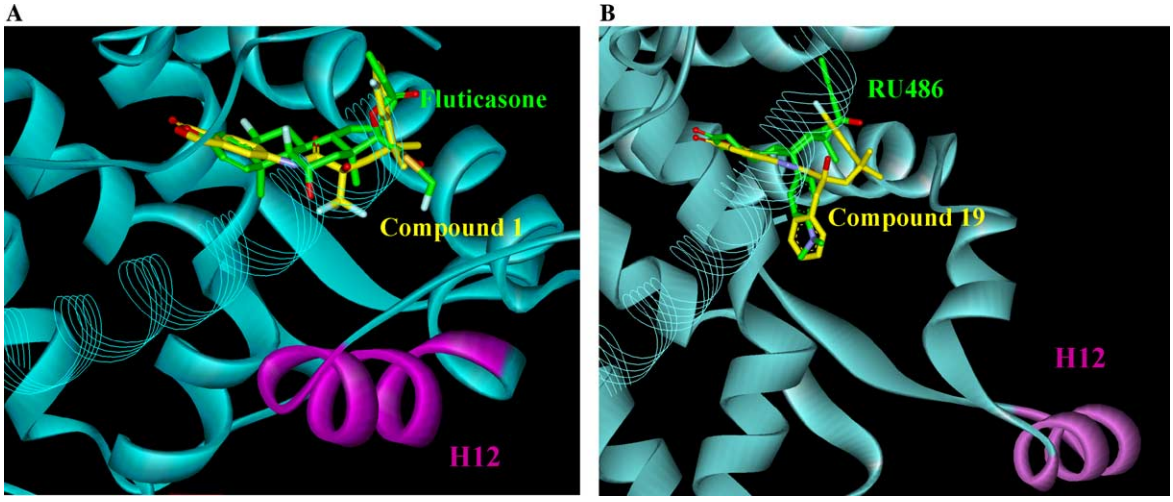


Figure 3. (A) Docking of compound **1** into the GR-LBD using the GR-LBD/fluticasone co-complex X-ray structure. (B) Docking of compound **19** into the GR-LBD using the GR-LBD/RU486 co-complex X-ray structure.

carbonyl of Leu563. No hydrogen bond interaction is observed between the ligand amide carbonyl group and the GR-LBD. The right side phenol is engaged in potential hydrogen bonding interactions with the side chain of Thr739. This is in line with the SAR reported in Table 3 where substitution of the 2-hydroxy-5-fluorophenyl right side by phenyl (**2**) or 2-methoxy-5-fluorophenyl (**23**) leads to a loss in GR binding. We believe that compounds **1**, **2**, and **24** bind in a similar fashion with helix 12 capping the binding pocket. Absence of the key interaction between Thr739 and compound **2** stabilizes an inactive GR conformation, which accounts for the lack of the agonist activity of **2**. But the non-CF_3 compounds discussed in this paper carry a bulky group at the center of the molecule, which pushes the helix 12 out of the agonist position, leading to a classical RU486-like mode of GR antagonism.

The antagonist binding mode of **19** shows potential interactions between the left side heterocyclic ring and the side chains of Gln570 and Arg611, similar to the agonist **1**. The central hydroxyl of the benzyl analog **19** could be involved in forming a hydrogen bond with the amide side chain of Asn564, although at 4 Å this distance is large for a normal hydrogen bond. The amide group of the antagonist **19** shows similar interactions with the GR-LBD as the agonist **1**. The SAR shown in Table 4 is supported by the presence of a potential hydrogen bond between the amide nitrogen and the backbone carbonyl of Leu563, and a lack of hydrogen bonding interaction between the ligand amide carbonyl and the GR-LBD. Different interactions were observed between the GR-LBD and the right sides of the agonist **1** and the antagonist **19**. Repositioning of helix 12 in the antagonist binding mode alters the location of Thr739, which is no longer able to form a hydrogen bond with the ligand. This could explain why no increase in the GR binding affinity and no agonist activity were observed for **19** when a phenolic group was introduced on the right side phenyl ring in **10** (Table 3). This is in contrast to analogs **1** and **2** where the presence of the phenolic group at the right side phenyl ring has an effect on the functional profile (Table 6). These results further lend support to our suggestion of different binding modes for the CF_3 and non-CF_3 analogs presented in the paper. The central phenyl ring of the antagonist **19** is surrounded by the side chains of the hydrophobic amino acids Val571, Trp600, Met604, and Phe737. The spatial constraints of this pocket could explain the effect of the methyl substitutions on the central phenyl ring (Table 2). The model suggests that groups extending out from the *para* position of the central phenyl ring are pointing toward the protein surface. The poor binding affinity of the biphenyl analog **13** could be due to steric interactions of the *para* phenyl group with the nearby amino acid side chains. Perhaps, introducing a spacer group between the two phenyl rings will extend the *para* phenyl group and may improve the GR binding of **13**. In this context, it is interesting to note that large groups have been introduced in RU486 using the amine group on the C-11 phenyl ring as the point of attachment without significantly affecting the GR binding.[25] In conclusion, these modeling studies suggest that replacing the CF_3 group in **1** and **24** by large substituents changes the position of helix 12, leading to the antagonistic activity of these ligands. The observed binding interactions between **19** and GR-LBD also offer explanations for the SAR of other regions in these non-CF_3 compounds.

The syntheses of the compounds described in the present work have been published elsewhere.[50] Analogs with an unsubstituted phenyl ring at the right side of the molecule were synthesized using commercially available 2-phenyl-2,2-dimethylethylmagnesium bromide. The reaction of this Grignard reagent with diethyl oxalate and hydrolysis of the resulting ester gave the desired keto-acid. The keto-acid (**35**) was synthesized, according to a modified literature procedure[31] (Scheme 1). Organolithium reagent generated from 2-bromo-4-fluoroanisole (**33**) was quenched with acetone to give the *tert*-alcohol (**34**). Tin chloride-mediated coupling of this alcohol (**34**) with the trimethylsilyl enol ether of ethyl pyruvate and hydrolysis of the resulting ester led to the keto-acid (**35**). The keto-acids were converted into the corresponding acid chlorides and reaction with the appropriate anilines gave the keto-amides. Treatment of the keto-amides with a corresponding Grignard (or organolithium) reagent, followed by demethylation of the anisole intermediates, led to the desired *para*-fluorophenols (**36**).

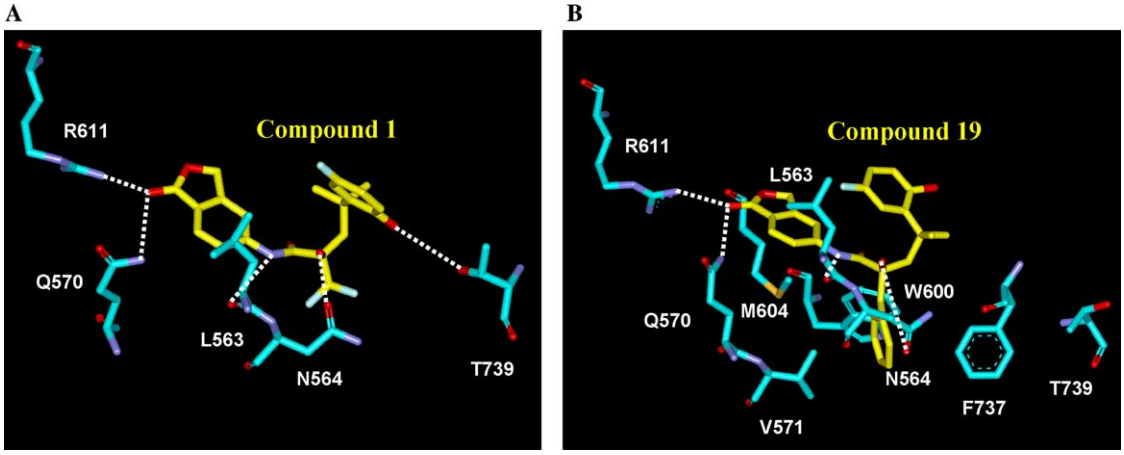
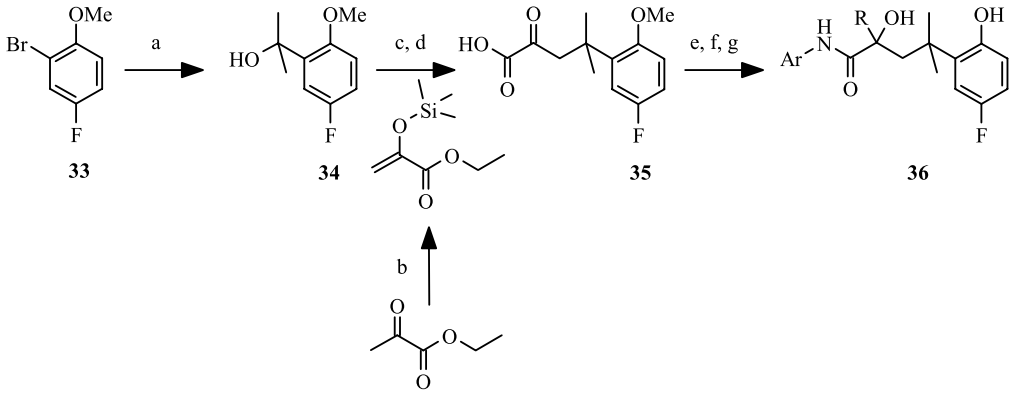


Figure 4. (A) Binding mode of compound **1**. (B) Binding mode of compound **19**. Solid lines indicate potential hydrogen bonds.



Scheme 1. Reagents and conditions: (a) *n*-BuLi, THF, −78 °C, acetone, 85%; (b) trimethylsilyl trifluoromethanesulfonate, di isopropylethylamine, CH_2Cl_2, 0 °C, 90%; (c) $SnCl_4$, CH_2Cl_2, −78 to −50 °C, 35%; (d) KOH, aq. ethanol, reflux, 93%; (e) thionyl chloride, dimethylacetamide, 0 °C, $ArNH_2$, 0 °C to rt, 60%; (f) Grignard reagent, 0 °C to rt, 60%, (g) BBr_3, CH_2Cl_2, 0 °C to rt, 75%.

In conclusion, we have discussed our efforts in probing the pharmacophoric elements required for binding to GR with a focus on the role of CF_3 group in the ligands **1** and **24**. Although, further work is needed to fully understand the role of the CF_3 group and its effect on the agonist activity we believe our work sheds light on the binding modes of agonists and antagonists based on the compounds **1** and **24**. Docking studies suggest a binding mode similar to that of RU486, wherein the conformational changes involving the repositioning of helix 12 explain the observed GR antagonism. We have shown that by introducing a large group in a non-steroidal GR ligand, functional activity can be altered from agonism to antagonism. These observations may be applicable to other potential GR scaffolds wherein appropriate modifications of the size and shape, while maintaining the key interactions, could be an effective way of modulating the functional activity.

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