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Letter

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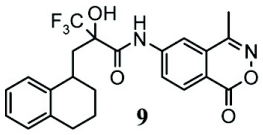

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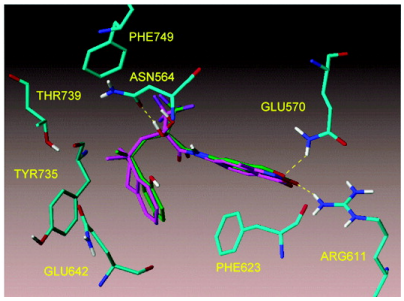

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Abstract: Structurally related glucocorticoid receptor (GR) binders were docked into the GR active site to select the binding mode closest to the true docking mode. This process, termed an "agreement docking method", led to the design of tetrahydronaphthalene **9**. The method was validated by the syntheses of **9** and related analogues, which are potent binders of GR. **15a** is a partial agonist while **9e** and **15a** are micromolar antagonists in a mouse mammary tumor virus transactivation assay.

Steroidal glucocorticoid agonists such as fluticasone propionate (FP) **1**, dexamethasone (dex) **2**, and prednisolone **3** are antiinflammatory agents used widely against a broad spectrum of inflammatory diseases (Figure 1).[1] There has recently been considerable interest in a hypothesis of selective glucocorticoid agonism where the beneficial antiinflammatory effects are postulated to be derived from transrepression (TR) pathways and may be separated from the often severe side effects derived from transactivation (TA) pathways.[2] Recent publications have described new glucocorticoid receptor (GR) agonists that feature nonsteroidal structures that demonstrate TR/TA selectivity.[3−7] There is also considerable interest in GR antagonists as potential therapies for diabetes, depression, and as antiobesity agents.[8,9] We describe here the design of novel nonsteroidal glucocorticoid modulators using an "agreement docking" method and its validation by the synthesis of modulators possessing micromolar levels of agonism and antagonism.

The GR crystal structure was first published by Bledsoe et al.[10] as a complex with dexamethasone. Subsequently the complex with FP was published revealing, in this case, two differing forms of the protein in the asymmetric unit: FP-GR form A and form B.[11] Key H-bond interactions were revealed between the 11β hydroxyl group of FP and the Asn564 and also between the 3-keto group of FP and both Arg611 and Gln570. In addition, the fluoromethyl group of FP was found to show a favorable electrostatic interaction with the Asn564 while the 17α propionate group projects into a hydrophobic pocket bordered by Met639, Met636, Phe623, and Ile629 (Figure 2).

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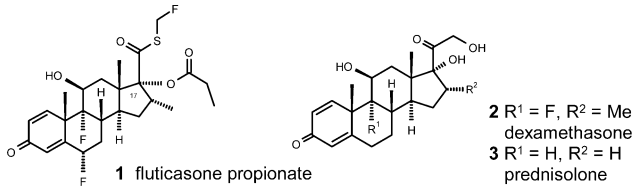

Figure 1. Structures of fluticasone propionate, dexamethasone, and prednisolone.

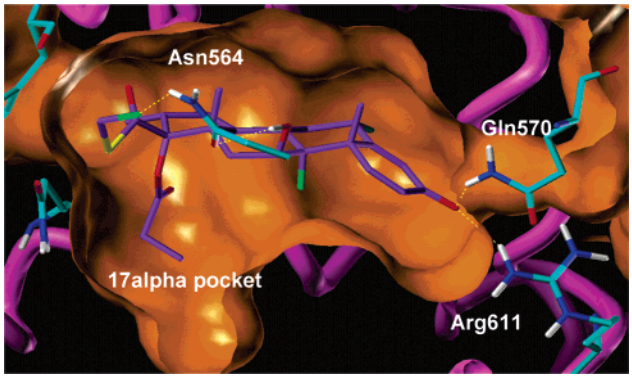

Figure 2. GR-FP form A. Dotted yellow lines represent key H-bonds (see text).

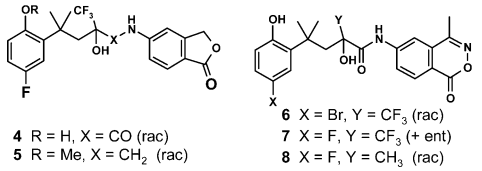

Figure 3. Structures of analogues used in the "agreement docking method".

Automated docking of nonsteroidal ligands to these GR sites invariably provide multiple docking solutions, with each solution being a valid one when judged by the internal scoring function. The "agreement docking method" described here was developed to allow one single docking mode to be selected as the one closest to the true binding mode. The simple technique requires the docking of not one compound but a series of structurally related compounds. The dockings are then evaluated to identify the mode common to all members of the series. The method relies on the concept that the true binding mode will be adopted at least once by each member of the series, while spurious modes will be present for only a minority of compounds.

Five structurally related, nonsteroidal compounds **4**−**8** were utilized in the study (Figure 3).[12] Each is a GR agonist or has reasonably good GR ligand binding affinity (IC_{50} < 100 nM). The stereochemistry of the active isomer was unknown. Therefore, unrestrained dockings, using the FLO+ method,[13] were carried out for each form of the FP-GR (see above) utilizing a set of *R* isomers and a separate set of *S* isomers. For each protein/isomer set, five dockings for each compound were generated. In each case the variety of docking poses was very large (Figure 4). Four docking combinations were produced: forms A and B each in combination with *R* and *S* isomers. The four sets of dockings were analyzed through visual inspection[14] to identify

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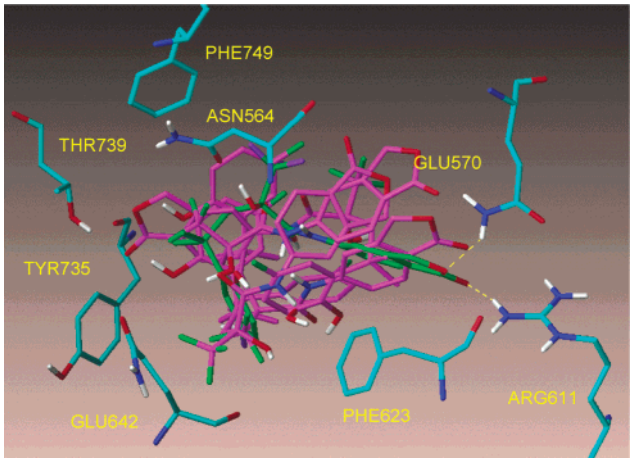


Figure 4. Docking results for **4**, *R* isomer with form A of the GR-FP. Docked ligands are largely in magenta with the pose identified through "agreement docking" in green.

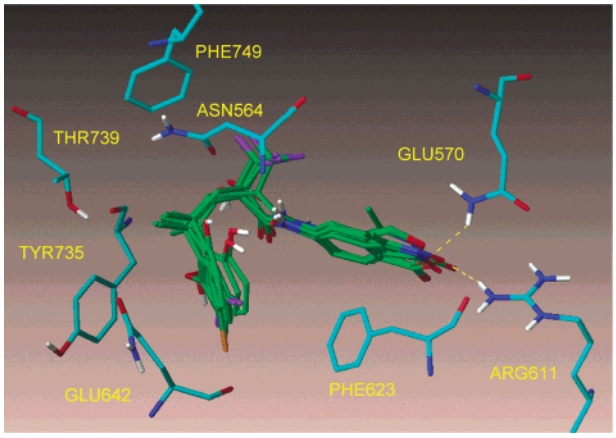


Figure 5. *S*-Configuration, GR form A, agreement docking mode. Note that the tertiary alcohols of **4**–**8** are unable to H-bond to Asn564.

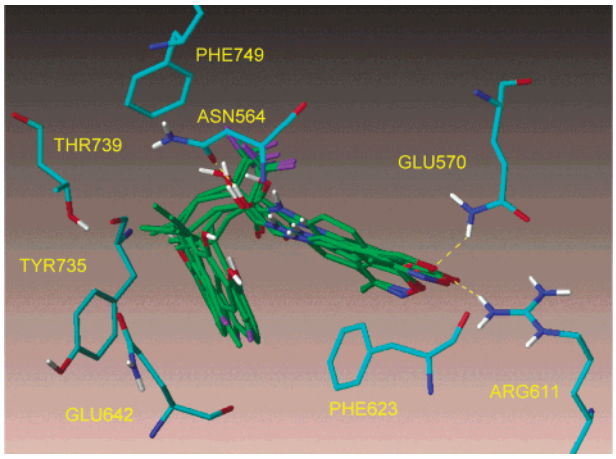


Figure 6. *R*-Configuration, GR form A, agreement docking mode. Note that the tertiary alcohols of **4**–**8** are able to H-bond to Asn564.

whether an "agreement mode" could be seen. Form B of the protein failed to produce any agreement dockings, with no consistent docking modes seen for *S* or *R* isomers. Form A produced agreement docking modes for the set of *R* isomers and the set *S* isomers (Figures 5 and 6).

Separation of compounds into pure enantiomers demonstrated that activity lay very largely in one isomer,

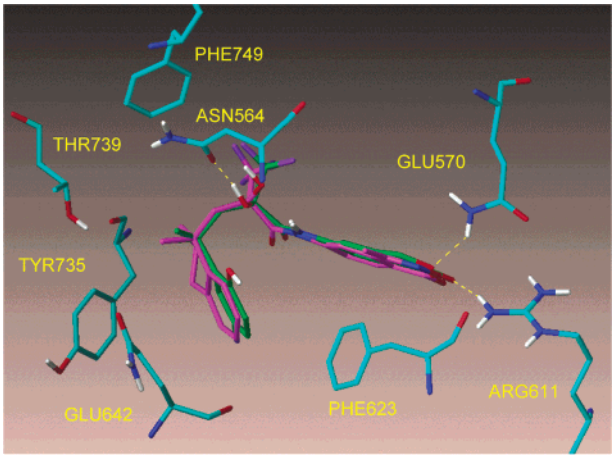


Figure 7. Docking of *RR*-tetrahydronaphthalene **9** (magenta) with **4** (green) in the "agreement mode".

but the absolute configuration of this was unknown. The *R* isomers were seen, in the agreement mode, to interact through H-bonding with residue Asn564 (Figure 6), believed to be a key residue for agonist binding,[10] while the *S* isomers did not (Figure 5). On this basis, the *R* configuration was selected as the likely active form. Therefore, from these docking studies it was concluded that the form A of the protein was the closest to the true binding form (for this nonsteroidal series) and that the active configuration was *R*.

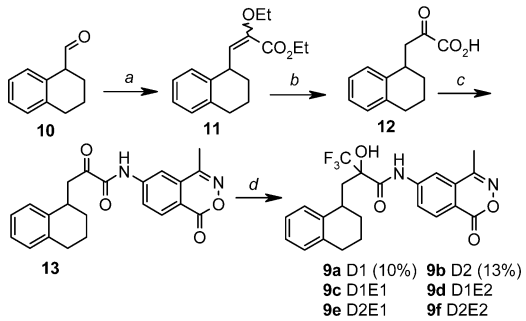
Detailed examination of the "agreement mode" identified a set of key H-bond interactions between the benzoxazinone and Arg611 and Gln570 and the adoption of a folded conformation in which the aryl ring can be seen to dip into the pocket for the 17α substituent of fluticasone propionate but does not extend deeply into this pocket (Figure 2). Acceptance of this docking mode as the most likely led to a design phase in which novel compounds were proposed and docked to form A to establish whether the "agreement docking mode" was found. One such candidate was the tetrahydronaphthalene (THN) **9**.

The introduction of a second chiral center requires that the *RR* and *SR* forms be docked. Both were found to reproduce the "agreement mode" very well (see Figure 7 for docking of the *RR* form). On the basis of these favorable docking results, the THNs were identified as high-priority targets for synthesis together with the smaller indane and larger suberan analogues.

The syntheses of the THNs (Scheme 1) started from the aldehyde **10**. This was converted in two steps to the α-ketoacid **12**, which was coupled to 6-aminobenzoxazinone.[15] Trifluoromethylation of the resultant pyruvamide **13** using Ruppert's reagent gave a separable 1:1 mixture of the two diastereomers[16] **9a** and **9b**, which in turn were separated into their component enantiomers **9c**–**f**.[16]

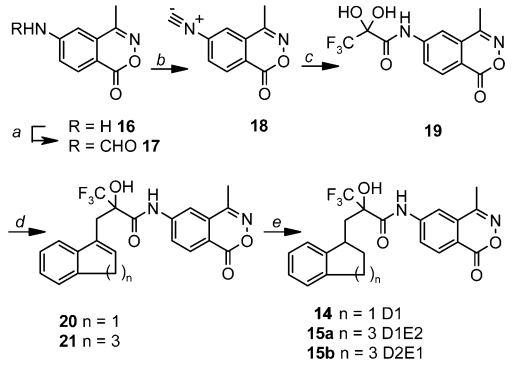
The corresponding indane **14** and benzosuberan **15** were prepared (Scheme 2) using a different method. Starting from the known 6-aminobenzoxazine,[15] formylation and dehydration gave the corresponding isonitrile **18**. In a Passerini reaction, this was converted with trifluoromethylacetic anhydride into the trifluoromethylpyruvamide hydrate **19**. Thermal ene reactions between α-methyleneindane/suberan and **19** gave the coupled products **20** and **21** in good yield. Reduction of

Scheme 1. Synthesis of **9a**−**f**[a]



[a] Conditions and notes: (a) $(EtO)_2POCH(OEt)CO_2Et$, LDA, THF, −10 °C to room temp, 18 h, 78%; (b) TFA/H_2O (3:4), room temp, 3 h, used crude; (c) 6-amino-4-methyl-2,3-benzoxazin-1-one, $SOCl_2$, DMA, −8 to 0 °C, 3 h, 26%; (d) CF_3TMS, Cs_2CO_3, DMF, 18 h and then TBAF (1 M in THF), 30 min. D = diastereomer, E = enantiomer.

Scheme 2. Synthesis of **14** and **15**[a]



[a] Conditions and notes: (a) HCO_2H, Ac_2O, 55 °C, 2 h and then **16**, THF, room temp, 2 days, used crude; (b) $POCl_3$, Et_3N, THF/DMPU (2.5:1), room temp, 2 days, 67%; (c) TFAA, room temp, 2 days and then MeOH, 87%; (d) 1-methyleneindane or 6,7,8,9-tetrahydro-5-methylene-5*H*-benzocycloheptene, 200 °C, 10 min, 90−97%; (e) $TsNHNH_2$, NMP, 150 °C, microwave, 30 min, 11−20%. D = diastereomer, E = enantiomer.

the trisubstituted olefins without simultaneous reduction of the benzoxazinone proved to be very difficult but was eventually achieved with diimide in a melt of the olefin and tosyl hydrazide. These gave approximately a 1:1 mixture of diastereomers of **14** and **15**.[16] The diastereomers and enantiomers are separable using conventional techniques.

The compounds were tested for their ability to bind to GR using competition experiments with fluorescent-labeled dexamethasone. A functional GR agonist assay was carried out using human A549 lung epithelial cells engineered to contain a secreted placental alkaline phosphatase gene under the control of the distal region of the NFkB dependent ELAM promoter.[17] This assay allows determination of the ability of compounds to *repress* transcription (i.e., transrepression). The GR antagonist assay also used human A549 lung epithelial cells stably transfected with the mouse mammary tumor virus (MMTV) luciferase reporter gene. Compounds were tested for their ability to antagonize dexamethasone-induced activation[18] (i.e., transactivation). In one scenario, a TR/TA selective compound would be a full agonist in the NFkB transrepression assay and an antagonist in the MMTV transactivation assay. Data for target compounds and standards (dexamethasone for agonism and mifepristone (RU486) for antagonism) in these assays are reported (Table 1).

Table 1. GR Binding, NFkB Agonism, and MMTV Antagonism Data for Standards and Selected Compounds[a]

	stereochem	GR binding[b] pIC_{50}	NFkB agonism[b] pIC_{50} (% max[c])	MMTV antagonism[b] pIC_{50}
2[d]		8.10 ± 0.04	8.93 ± 0.07 (100 ± 5)	<5
RU486[d]		8.24 ± 0.09	21% ± 6 @ 10 μM[e]	8.33 ± 0.36
9d	D2E2	7.15 ± 0.06	23% ± 6 @ 10 μM[e]	5.41 ± 0.14
9e	D1E1	8.08 ± 0.12	51% ± 22 @ 10 μM[e]	6.25 ± 0.06
14	D1 (rac)	7.81 ± 0.04	73% ± 16 @ 10 μM[e]	5.32 ± 0.02
15a	D1E2	8.57 ± 0.02	6.84 ± 0.26 (60% ± 11)	6.15 ± 0.09
15b	D2E1	7.75 ± 0.04	24% ± 4 @ 10 μM[e]	6.00 ± 0.05

[a] Only the data for the active isomers are shown. The other isomers are inactive. [b] pIC_{50} values are from duplicate wells with at least $n = 3$ from 11 point dose−response curves with a top concentration of 10 μM. Standard errors are shown. [c] % maxima are quoted with reference to the maximum from dexamethasone. [d] Dexamethasone **2** and RU486 (mifepristone) were used as the standards for GR agonism and antagonism, respectively. [e] With a top concentration of 10 μM being tested, pIC_{50} values are not quoted for values less than 6.

Initially all the compounds were tested in the assays as racemic diastereomers.[19] The more potent analogues were then separated into their enantiomers for further testing. Compounds **9d**, **9e**, **14**, **15a**, and **15b** show activity in the GR binding assay, with **9e**, **15a**, and **15b** having binding values similar to that of dexamethasone **2**. Most of the activity for a diastereomeric mixture resides in one enantiomer. However, one enantiomer of the *other* diastereomer also has binding activity (compare **9d** with **9e** or **15a** with **15b**), as was predicted by the agreement docking. We hypothesize that the chiral center bearing the alcohol is the conserved chiral center for activity because the alcohol forms a hydrogen bond with Asn564 (Figures 6 and 7).

In the functional assays for agonism (NFkB) and antagonism (MMTV), the indane derivative **14** is a weak agonist with a percentage maximum of 73% compared with dex. Increasing the size of the saturated ring of the bicycle to the THN analogue **9e** provides some evidence of agonism with a 50% maximum compared with dex. However, **9e** also possesses MMTV antagonism with $pIC_{50} = 6.25$, suggesting that the compound may possess selectivity for transrepression (TR) over transactivation (TA). Increasing the size of the saturated ring of the bicycle further from the THN to the suberan leads to an increase in agonist efficacy and potency. Thus, **15a** has a $pIC_{50} = 6.84$ with a 60% maximum compared with dex in the NFkB assay and MMTV antagonism of $pIC_{50} = 6.15$, also suggesting TR/TA selectivity. One enantiomer of the other diastereomer, **15b**, is a micromolar antagonist. None of the compounds tested displayed any agonist activity in an MMTV agonist assay (data not shown). A fuller description of the TR/TA selectivity of this series will be described elsewhere.

These compounds demonstrate a feature typical of this series, namely, that relatively small changes in structure can cause relatively large changes in the biological profile (compare indane **14** with suberan **15a**). The observation of agonism and antagonism, and particularly the suggestions of TR/TA selectivity, made these compounds highly attractive for a full lead optimization program that has led to potent and selective compounds.[20] These studies will be described in due course.

In summary, the agreement docking method is a technique that can be applied simply and effectively using any docking program. In the case reported here it enabled the identification of a novel series of GR receptor agonists. It was instrumental in identifying the most suitable protein for docking studies and in the assignment of the *R* configuration to the active enantiomer. This series has been explored very thoroughly to provide potent and highly selective compounds.[20] The prediction of *R* as the active configuration was subsequently confirmed through small-molecule crystallography. The observation that docking of *SR* and *RR* isomers for the tetrahydronaphthalenes provided solutions in the "agreement mode" has since been validated with the finding that *SR* and *RR* isomers show similar binding potencies.

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Supporting Information Available: Experimental procedures and spectroscopic data. This material is available free of charge via the Internet at http://pubs.acs.org.

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