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Discovery of potent and selective nonsteroidal indazolyl amide glucocorticoid receptor agonists

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ABSTRACT

Modification of a phenolic lead structure based on lessons learned from increasing the potency of steroidal glucocorticoid agonists lead to the discovery of exceptionally potent, nonsteroidal, indazole GR agonists. SAR was developed to achieve good selectivity against other nuclear hormone receptors with the ultimate goal of achieving a dissociated GR agonist as measured by human in vitro assays. The specific interactions by which this class of compounds inhibits GR was elucidated by solving an X-ray co-crystal structure.

For over 60 years, glucocorticoid receptor (GR) agonists such as hydrocortisone, prednisone, and dexamethasone have been a mainstay in the treatment of inflammatory and allergic diseases. However, their prolonged systemic use has been limited by numerous accompanying side effects such as diabetes, osteoporosis, skin thinning, muscle atrophy, and others.[1] While inhaled glucocorticoids such as fluticasone have avoided these side effects by directly targeting the lung for the treatment of asthma coupled with a very short systemic half-life, there remains a significant clinical need for a GR agonist that could be administered systemically that possesses a safer side effect profile.[2] A number of studies have demonstrated that glucocorticoid-mediated agonism of GR leads to nuclear translocation of the receptor that, in concert with additional cofactors, inhibits the transcription factors NF-κB and activator protein-1 (AP-1) resulting is the suppression of pro-inflammatory genes under their control (transrepression).[3,4] Alternatively, the agonist-activated GR complex can homodimerize and bind to glucocorticoid response elements (GREs) to initiate the transcription of genes directly that are primarily involved in metabolism (transactivation).[5] The report of a GR dimerization-deficient transgenic mouse that remained sensitive to the anti-inflammatory effects of dexamethasone (croton oil-induced ear edema was reduced compared to wild type) but lacked the typical dexamethasone-induced side effects provided pharmacological justification for the hypothesis that transrepression of pro-inflammatory genes could in principle be 'dissociated' from transactivation of genes ultimately associated with the adverse effects of glucocorticoid treatment.[5–8]

In the course of our research towards nonsteroidal GR agonists that might exhibit a dissociated profile, we attempted to improve

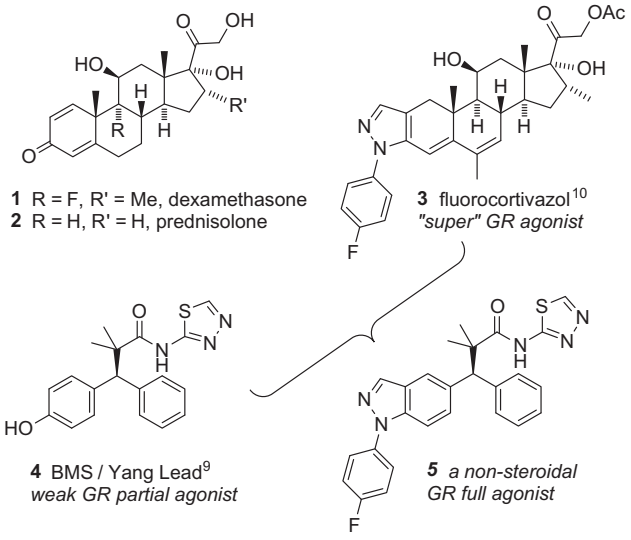

Figure 1. Genesis of the indazolyl class nonsteroidal GR agonist.

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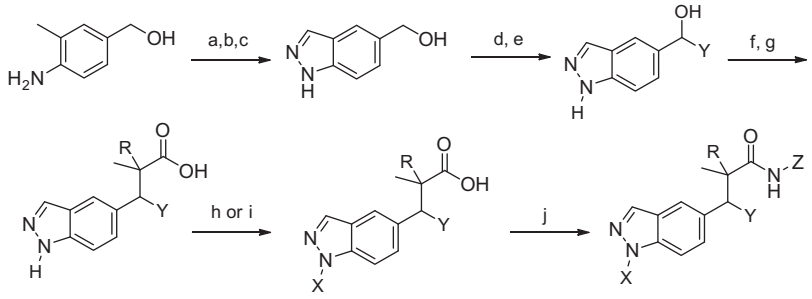
upon the modest GR activity of a previously discovered phenolic series **4**[9] by structurally combining it with fluorocortivazol **3**, one of the most potent glucocorticoids ever reported. (see Fig. 1)[10] We hypothesized that the A-ring of prednisolone was being mimicked by the phenol of **4** which was corroborated by modeling of **4** in the dexamethasone/GR ligand binding domain co-crystal structure (vide infra). Since the glucocorticoid receptor has evolved to bind the steroid ring system (even nonnatural glucocorticoids such as **3**), the fusion of **3** and **4** to create indazole **5** did not appear to be an unreasonable proposition. Furthermore, if only some of the potency of fluorocortivazol was imparted to lead series **4**, it would improve its chances to progress farther in lead optimization. Use of 4-fluorophenyl-substituted indazoles and pyrazoles to enhance the potency of GR agonists was initially reported by the Scanlan group in 2005—a strategy employed by a number of groups since.[8c,d] If successful, the indazole chemotype would dramatically simplify the structural requirements of a GR agonist from a steroidal 4-ring architecture containing 8 contiguous chiral centers to only aromatic rings and a single chiral center.

The indazoles in this and the accompanying Letter were all synthesized from the common synthetic intermediate 5-hydroxymethylindazole (Scheme 1) prepared using the method of Sun et al.[11] Oxidation of the hydroxymethyl moiety using Dess–Martin reagent followed by the addition of a Grignard reagent or alkyl/aryl lithium reagent provided an appropriately substituted secondary alcohol. The alcohol reacted smoothly with silyl ketene acetals in the presence of $TiCl_4$ using a modification of the Mukaiyama aldol reaction,[12] to provide the indazole esters which were hydrolyzed to give the corresponding acids. Substitution at the N1 position of the indazole was accomplished using a Buchwald arylation procedure[13] or by alkylation using sodium hydride and alkyl iodides to give the appropriately substituted indazoles. Finally, coupling with a variety of amines using standard coupling conditions afforded the desired amides.

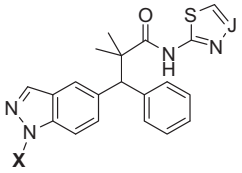
Structure–activity relationships for the indazole GR agonist series are detailed in Tables 1–4 and selectivity against related nuclear hormone receptors is found in Table 5. The in vitro assays used to assess the biological activity of the GR ligands prepared in Scheme 1 consisted of: a GR binding assay, two assays to measure transrepression in an A549 cell line (an AP-1 and an E-selectin (NF-κB-dependent) repression assay), and a previously reported transactivation assay (a NP-1 agonist assay that contains a GR chimera with a GAL4 reporter in a HeLa cell line).[14,15] Based on literature precedence, the desired activity profile of a 'dissociated' GR agonist is a compound which is a partial agonist of the in vitro functional assays for transrepression while showing little to no activity in the transactivation assay.[8,9] Full agonists in the transrepression assays invariably had greater activity in the transactiviation assay. To this end we strove to discover compounds that had good partial agonist activity (EC_{50} <50 nM) whose agonist efficiencies were in the range of 65–90% relative to dexamethasone (100%).

Table 1 summarizes the GR binding and functional activity for a series of indazole-based GR agonists possessing a diverse set of indazole N-linked substituents using dexamethasone and compound **4** as positive controls. Our initial SAR effort was directed at establishing whether the phenol of compound **4** could indeed be replaced by an indazole. The enantiomeric[16] indazoles **5a** and **5b** both exhibited good binding to the GR (as did essentially all of the analogs in Table 1) but even similar activity than the phenol **4** in the AP-1 and E-selectin assays with the (**3*S***) stereoisomer **5a** being the more potent of the two. Nevertheless, the efficacy of **5a** relative to dexamethasone showed it to be a partial agonist just like the phenol **4**. The indazole nitrogen was substituted with an phenyl group to give **5b** which showed a dramatic increase in AP-1 activity. The 4-fluorophenyl analog **5c** which is the same moeity as in fluorocortivasol also showed good activity as a racemic mixture. This compound was separated into its enantiomers (**3*S***)-**5c** and (**3*R***)-**5c**. The (**3*S***) stereoisomer showed exceptional activity both in the AP-1 and E-selectin assays (2.4 and 4 nM, respectively) and was 40× more potent than the (**3*R***) enantiomer. Though close in activity to dexamethasone, compound (**3*S***)-**5c** showed strong NP-1 agonism as well, implying no dissociation between transrepression and transactivation like dexamethasone itself. Changing from a thiadiazole amide to a thiazole amide (**5d**) gave compounds with similar GR binding as well as functional activities including the undesired potent activity in the NP-1 assay. The thiazole amide derivatives also showed much greater potency in the (**3*S***) stereoisomer. Next, a series of changes to the phenyl ring were studied (**5e**–**5l**). Moving from a 4-F to a 4-Cl gave a compound with similar activities in the binding and functional assays, however changing to a 4-Br group led to a loss of binding and functional activity. The activity profile of the three pyridyl isomers are shown in compounds **5g**–**5i**. The 3-pyridyl compound (**5h**) was the most potent of these isomers and was further analyzed as the homochiral analogs.[16] Compound (**3*S***)-**5h** shows strong GR binding and functional activities but again exhibited significant activity in the transactivation assay though it was less active in this assay than dex. Compounds **5m**–**5t** show a series of changes in which the N-linked indazole substituent was changed from aryl to alkyl. Most of the *N*-alkyl analogs gave significantly less active compounds in the functional assays with the exception of the isopentyl (**5o**) and cycloheptyl (**5t**) substitutions. Unfortunately, the latter two more active compounds also showed increased potency in the NP-1 transactivation assay.



Scheme 1. Reagents and conditions: (a) Ac_2O (3 equiv), KOAc (2 equiv), $CHCl_3$ reflux, 2 h; (b) isoamyl nitrite (2.2 equiv), 18-crown-6 (0.05 equiv), reflux 24 h; (c)1 M NaOH (1 equiv), MeOH, 1 h; (d) Dess–Martin reagent (1.2 equiv), DCM, 2 h; (e) Y-MgBr or Y-Li (1.1 equiv), THF, 0 °C or −78 °C, 3 h; (f) (1-methoxy-2-methylprop-1-enyloxy)trimethylsilane [R = CH_3] or (*E*)-(1-methoxyprop-1-enyloxy)trimethylsilane [R = H] (1.2 equiv), $TiCl_4$, (1.1 equiv), DCM, 0 °C, 12 h; (g) 1 M NaOH, DMSO, MeOH, 100 °C, 12 h; (h) CuI (0.05 equiv), K_3PO_4 (1.8 equiv), *t*-cyclohexanediamine (0.5 equiv), X-I (1 equiv), dioxane, 110 °C, 24 h; or (i) NaH (2 equiv), X-I (1.5 equiv), THF, 3 h; (j) Z-NH_2 (1 equiv), EDC (1.3 equiv), HOBt (1.3 equiv), TEA (2 equiv), DCM, 12 h or cyanuric fluoride (1.1 equiv), pyridine (1.1 equiv), DCM, 2 h, then Z-NH_2 (1 equiv), DMAP (cat), THF, 80 °C, 12 h.

Table 1
In vitro GR binding and functional activity for indazole N-linked substituents



Compd	X	J	GR binding[a] K_i (nM)	AP-1 repression[b]		E-selectin repression[b]		NP-1 agonism[c]	
				EC_{50}[a] (nM)	%Dex[d]	EC_{50}[a] (nM)	%Dex[d]	EC_{50}[a] (nM)	% dex[d]
Dex			1.2	2.5	100	1.1	100	4.5	100
4			1.1	21	71	21	71	526	28
(**3*S***)-**5a**	H	N	2.7	24	79	15	77	208	41
(**3*R***)-**5a**	H	N	13	617	35	>5000	x	>10,000	x
5b	Phenyl	N	5.0	20	73	ND	ND	ND	ND
5c	4-F-phenyl	N	18	23	81	ND	ND	ND	ND
(**3*S***)-**5c**	4-F-phenyl	N	5.1	2.4	93	4	86	5.8	106
(**3*R***)-**5c**	4-F-phenyl	N	6.7	92	50	ND	ND	ND	ND
5d	4-F-phenyl	C	15	4.1	79	ND	ND	ND	ND
(**3*S***)-**5d**	4-F-phenyl	C	6.0	1.4	92	0.5	97	7.6	94
(**3*S***)-**5d**	4-F-phenyl	C	7.9	400	56	58	81	435	59
5e	4-Cl-phenyl	N	18	23	96	10	84	256	55
5f	4-Br-phenyl	N	29	956	*x*	ND	ND	ND	ND
5g	2-Pyridyl	N	8.1	127	62	ND	ND	ND	ND
(**3*S***)-**5h**	3-Pyridyl	N	0.9	19	74	19	77	132	57
(**3*R***)-**5h**	3-Pyridyl	N	4.0	349	58	185	35	>10,000	
5i	4-Pyridyl	N	1.9	106	56	78	56	>10,000	*x*
5j	3-MeOPh	N	36	1200	*x*	ND	ND	>10,000	*x*
5k	4-MeOPh	N	8.8	56	76	143	62	>10,000	*x*
5l	4-HOPh	N	7.2	271	69	>5000	*x*	>10,000	*x*
5m	Isopropyl	N	10	301	82	195	44	>10,000	*x*
5n	Isobutyl	N	19	114	76	1823	77	>10,000	*x*
5o	Isopentyl	N	5.7	45	96	49	87	132	66
5p	2-Hydroxyethyl	N	47	550	32	1047	27	ND	ND
5q	3-Hydroxypropyl	N	17	1020	45	ND	ND	ND	ND
5r	Benzyl	N	14	1932	62	ND	ND	ND	ND
5s	$PhCH_2CH_2$-	N	46	>10,000	*x*	ND	ND	ND	ND
5t	cycloheptyl	N	12	29	85	21	86	188	60

Dex is dexamethasone, *x* means <30%, and ND means not determined.
[a] Values are means of two or more experiments performed in triplicate. All data is on racemates unless specified otherwise.
[b] Activator protein-1 (AP-1) and E-selectin assays were performed in an A549 lung epithelial cell line.
[c] GR transactivation NP-1 assay (run in agonist mode) was performed in the HeLa cell line.
[d] efficacy is represented as a percentage of the maximal response of dexamethasone (100%).

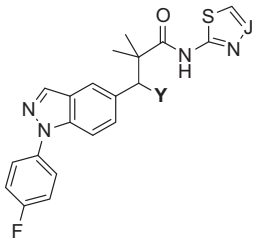
Table 2 summarizes the GR binding and AP-1 activity for a series of indazole-based GR agonists possessing a diverse set of indazole side chains (**Y**) using dexamethasone and compounds **5c** and **5d** as positive controls. The compound lacking any substituent at the Y position (**6a**) had much lower GR binding and was devoid of AP-1 activity. Substitution at this position with a variety of alkyl groups (**6a**–**6h**) led to increased activity in both the GR binding and AP-1 assay underscoring the requirement for a hydrophobic but not necessarily an aromatic group at this position of the ligand. Of the alkyl substituents tested, the most potent compounds were the propyl and isobutyl substitutions (**6c** and **6e**) which have a K_i's of 1.7 and 7.5 nM in the GR binding assay and an EC_{50} of 18 and 29 nM respectively in the AP-1 assay. Replacing the phenyl group with benzyl or pyridyl led to compounds with significantly less potent activity in the AP-1 assay.

Table 3 summarizes the GR binding and AP-1 activity for a series of indazole-based GR agonists possessing a diverse set of amide groups (**Z**) again using dexamethasone and compounds **5c** and **5d** as positive controls. In general, the amide groups presented in this table exhibited good GR binding. Changing the amide to a 3-thienyl led to some loss of AP-1 activity and the tetrazolyl led to complete loss of AP-1 activity. The 2-triazolyl compound had comparable activity to the 2-thiadiazolyl. Methylation on the various positions of either the thiazole or thiadiazole was then explored. Both 5-methyl derivatives led to some loss of AP-1 activity while the 4-methyl derivative resulted in a compound with comparable AP-1 activity and agonist efficiency. Larger groups on the thiazole and thiadiazole led to compounds with decreased activity (data not shown).

Table 4 shows the activity profiles of the compounds in which the gem dimethyl substitution at the C2-position is replaced with a single methyl group. Each of the four stereoisomers of the monomethyl product was isolated using chiral HPLC and tested discretely.[16] One of the isomers, **8a**, gave modest GR binding activity and poor activity in the AP-1 assay. The other three isomers were all very potent in both the AP-1 and NP-1 assays with compound **8c** showing exceptional activity. This compound was in fact more potent than dexamethasone with a GR binding K_i of 0.48 nM and EC_{50} of 0.30 nM in the AP-1 assay. Yet again, potent AP-1 agonism appeared to be accompanied by potent NP-1 agonism with an EC_{50} of 0.10 nM in the NP-1 assay.

To monitor selectivity against other nuclear hormone receptors, a subset of compounds was screened in binding or activation assays for the androgen receptor (AR), the progesterone receptor (PR), and the mineralocorticoid receptor (MR).[17] Compounds (**3*S***)-**5c**, (**3*R***)-**5c**, **6c** and **8c** all exhibited over 1000 fold selectivities between GR and AR. Compound **8b** showed the lowest selectivity between GR and AR at around 400 fold. A modest selectivity was observed between GR and PR with a 10–50 fold separation of binding affinities for all the compounds except **8b** which has a 350 fold

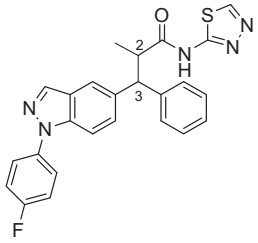
Table 2
Structure–activity relationship of indazole side chain



Compd	Y	J	GR binding[a] K_i (nM)	AP-1 repression[b]	
				EC_{50}[a] (nM)	%Dex[c]
Dex			1.2	2.5	100
5c	Phenyl	N	18	23	81
5d	Phenyl	C	15	4.1	79
6a	H	N	84	4322	36
6b	Ethyl	C	9.8	87	62
6c	Propyl	N	1.7	18	60
6d	Isopropyl	N	2.4	81	29
6e	Isobutyl	N	7.5	29	60
6f	Benzyl	N	1.1	705	37
6g	Cyclopentyl	C	21	82	47
6h	Cyclohexyl	C	18	66	55
6i	4-Pyridyl	C	12	389	60

[a] Values are means of two or more experiments performed in triplicate. All data is on racemates.
[b] Activator protein-1 (AP-1) was performed in an A549 lung epithelial cell line.
[c] Efficacy is represented as a percentage of the maximal response of dex (100%).

Table 4
Conformational effects of 2-methyl groups



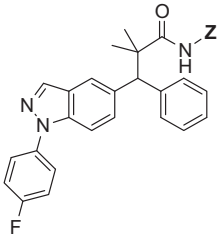
Compd	GR binding[a] K_i (nM)	AP-1 repression[b]		NP-1 agonism[c]	
		EC_{50}[a] (nM)	%Dex[d]	EC_{50}[a] (nM)	%Dex[d]
Dex	1.2	2.5	100	4.5	100
(**3*S***)-**5c**	5.1	2.4	93	5.8	106
(**3*R***)-**5c**	6.7	92	50	—	—
8a	44	453	42	>100	—
8b	0.59	13	103	27	76
8c	0.48	0.30	106	0.10	122
8d	2.4	20	89	20	100

[a] Values are means of two or more experiments performed in triplicate. **8b** and **8c** are diastereomeric at C2 and assumed to be (**3*S***).
[b] Activator protein-1 (AP-1) and E-selectin assays were performed in an A549 lung epithelial cell line.
[c] GR transactivation NP-1 assay (run in agonist mode) was performed in the HeLa cell line.
[d] efficacy is represented as a percentage of the maximal response of dex (100%).

selectivity over PR. Finally, neither compound (**3*S***)-**5c** nor (**3*R***)-**5c** showed agonist activity in a cellular MR activation assay.

During the course of our lead optimization effort on the indazole series, we were able to successfully solve the crystal structure of compound (**3*S***)-**5a** co-crystallized with the GR ligand binding domain (GR LBD).[18] In Figure 2 above, the left panel shows the 2.8 Å resolution co-crystal structure of compound (**3*S***)-**5a** with the GR ligand binding domain. The indazole forms two hydrogen bonds to Arg611 and Gln570 which have been observed crystallographically to engage the enone of dexamethasone.[19] The far right panel shows a 2.5 Å resolution co-crystal structure of fluorocortivazol with the GR LBD from Suino-Powell et al[20] for comparison. In this structure, only Gln570 is engaged by the pyrazole of cortivasol and the large phenyl group forces an expansion of the binding pocket to accommodate it. Interestingly, the unsubstituted indazole of (**3*S***)-**5a** is rotated 180 degrees relative to the orientation of cortivazol and hydrogen bonds to Gln570 as a hydrogen bond donor and an acceptor to Arg611. This indazole orientation may be an artifact, however, since Met604 has been mutated to a Leu to achieve diffraction-quality crystals and does contact the ligand. The 2-amidothiadiazole forms a bidentate hydrogen bond to Asn564 on helix 3 but also accepts a hydrogen bond from Gln642 on helix 11—the same residues engaged by cortivazol but

Table 3
Structure–activity relationship of the amide group



Compd	Z	GR binding[a] K_i (nM)	AP-1 repression[b]	
			EC_{50}[a] (nM)	%Dex[c]
Dex		1.2	2.5	100
5c	2-Thiadiazolyl	18	23	81
5d	2-Thiazolyl	15	4.1	79
7a	3-Thienyl	11	73	68
7b	2-Triazolyl	11	17	73
7c	2-Tetrazolyl	17	>10,000	—
7d	2-(5-Me-thiazolyl)	11	49	48
7e	2-(4-Me-thiazolyl)	14	29	74
7f	2-(5-Me-1,3,4-thiadiazolyl)	21	141	56

[a] Values are means of two or more experiments performed in triplicate. All data for on racemates.
[b] Activator protein-1 (AP-1) was performed in an A549 lung epithelial cell line.
[c] Efficacy is represented as a percentage of the maximal response of dex (100%).

Table 5
In vitro NHR selectivity of selected examples

Compd	GR binding[a] K_i (nM)	AR binding K_i (nM)	PR binding K_i (nM)	MR activation
(**3*S***)-**5c**	6.7	>25000	107	>5000
(**3*R***)-**5c**	5.1	>25000	56	>5000
6c	1.7	2513	109	—
8b	0.59	243	214	—
8c	0.48	>4,166	22	—

[a] Values are means of two or more experiments performed in triplicate.

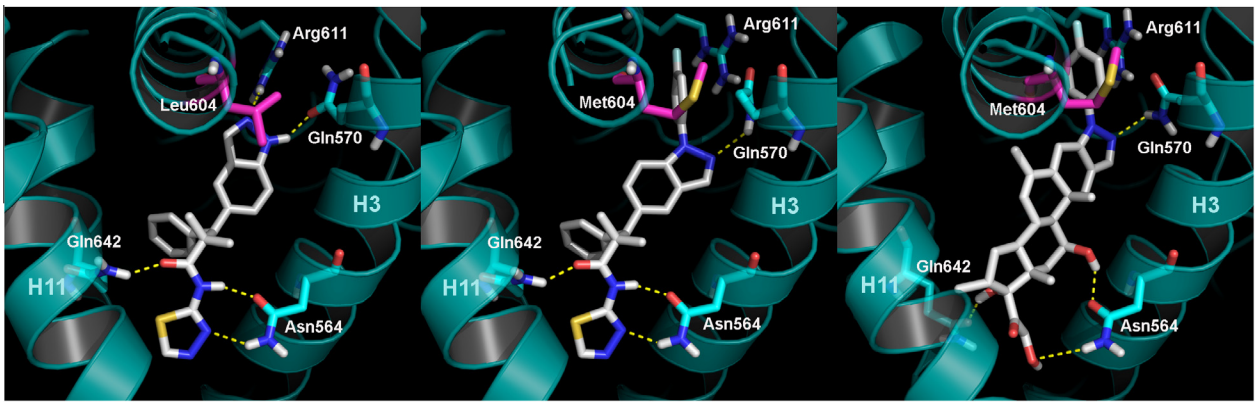


Figure 2. (Left) 2.8 Å resolution co-crystal structure of compound (**3*S***)-**5a** with the GR ligand binding domain. The M604L point mutation shown in purple was necessary to achieve diffraction-quality crystals. (Center) molecular model of compound (**3*S***)-**5c** docked into the GR LBD-dex co-crystal structure (1m2z) after removal of dex. (Right) 2.5 Å resolution co-crystal structure of deacylcortivazol in the GR LBD.[20]

in a fundamentally different way. Asn564 has been identified as a critical amino acid that acts as a conformational trigger by closing the distance between helix 11 and 3 which results in a protein conformational change of helix 12 (not shown), the position of which determines cofactor recruitment to the GR LBD-ligand complex and receptor agonism or antagonism.[21] The decrease in the functional activity of **5c** and **5d** relative to **7a** (replacement of the 2-thiazolyl or 2-thiadiazolyl rings with an isosteric 2-thienyl group) is readily explained in light of the crystal structure since the latter can only hydrogen bond to Asn564 via a monodentate interaction.

The center panel contains a docked model of compound (**3*S***)-**5c** in the GR LBS crystal structure wherein an interaction of the fluorophenyl-substituted indazole with Gln570 is seen to mimic that of cortivazol which cannot rotate the indazole to the conformation observed in the crystal structure of (**3*S***)-**5a** (left panel).[22] The amidothiadiazole of (**3*S***)-**5c** was found to hydrogen bond to Asn564 and Gln642 as observed for (**3*S***)-**5a**. The improved potency of (**3*S***)-**5c** over (**3*S***)-**5a** observed by adding the 4-fluorophenyl group to the indazole is thought to be due to the forced accommodation of the 4-fluorophenyl group between helix 1 and 3—an analogous structural change and potency enhancement observed in changing the enone of dexamethasone to deacylcortivazol.

In summary, we have described a novel series of indazoyl amides which are potent and selective ligands of the glucocorticoid receptor. These compounds have much simpler structures than glucocorticoids while maintaining in vitro potencies comparable and in some cases better than dexamethasone and fluorocortivasol. Crystallographic evidence established that these indazoyl amides bind the GR ligand binding domain using the same residues as engaged by glucocorticoids but in a unique manner. While compounds in this series were extremely effective at inhibiting transrepression as measured in an AP-1 and ELAM assay, they also show concomitant potency increases in the NP-1 transactivation assay. An accompanying Letter describes further work on this scaffold in search of 'dissociated' glucocorticoid inhibitors.

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15. *Cellular assays*: AP-1 activity is measured using an AP-1 response element (containing 5 copies) cloned into a luciferase reporter vector. This reporter is stably transfected into the human A549 lung epithelial cell line. AP-1 activity is induced by PMA (15 ng/ml), and inhibition of the induction by compounds is quantitated by measuring decreased luciferase activity. NFκB is measured using a truncated, NFκB-dependent, E-selectin promoter (300 bp) cloned into a luciferase reporter vector. This reporter is stably transfected into the human A549 lung epithelial cell line. NFκB activity is induced using IL-1β (0.5 ng/ml) and inhibition of the induction by compounds is quantitated by measuring decreased luciferase activity. NP-1 GRE activation activity is measured using a GR ligand binding domain (GR-LBD) chimera cloned into a GAL4 luciferase reporter system. This reporter system is stably transfected into a HeLa cell line (NP-1). Response to ligand induced binding is quantitated by measuring luciferase activity. Direct activation of the GR-LBD by compounds (agonist) can be measured as increased luciferase activity.
16. Respective enantiomers of compounds **5a**, **5c**, **5d**, and **5h** were prepared by separating the precursor 3-(1*H*-indazol-5-yl)-2,2-dimethyl-3-phenylpropanoic acid on a preparative Chiralcel OJ column using an SFC method with a mobile phase of 20% EtOH/MeOH (1:1)/80% CO_2 and 0.1% TFA. Retention times of the enantiomers were 12.3 and 17.1 min. Absolute stereochemistry was unambiguously determined by X-ray crystallography of 3-(1*H*-indazol-5-yl)-2,2-dimethyl-3-phenylpropanoic acid (deposited into the Cambridge Crystallography Center, CCDC 922816) and it (and in parallel, its enantiomer) was used to make the (**3*S***) and (**3*R***) enantiomers of **5a**, **5c**, **5d**, and **5h**. (b) Respective enantiomers of compounds **8a**, **8b**, **8c**, and **8d** were prepared by separating the precursor methyl-3-phenylpropanoic acid on a preparative Chiralcel OJ column using an SFC method with a mobile phase of 27% MeOH/73% CO_2. Absolute stereochemistry was not determined for these compounds.
17. GR, PR, and AR ligand binding assays were conducted in fluorescence polarization format which measures the competition between a test compound and a fluorescently labeled ligand for binding to the full length or ligand binding domain of the nuclear hormone receptor. IC_{50} values were determined by fitting the fluorescence polarization signal data using the four parameter logistic equation. The K_i values were determined by application of the Cheng–Prusoff equation to the IC_{50} values, where $K_i = IC_{50}/(1 + \text{ligand concentration}/K_d)$. Data shown represent the means of duplicate experiments. The MR agonist assay was determined in an A549 cell line. EC_{50} was determined as (mM)/(% maximal efficacy) using aldosterone as a positive control. Data shown represent the means of duplicate experiments.
18. >60 GR mutants, multiple co-activator peptides, and numerous compounds were screened. Manuscript in preparation.
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22. Illustrated in Figure 2, panel 2 are the major H-bonding contacts surrounding ligand (**3*S***)-**5c**. These were arrived at by substituting the dexamethasone molecule in the published Bledsoe et al. X-ray structure (1M2Z.PDB), positioning the compound in the conformation that was observed crystallographically for compound (**3*S***)-**5a** in panel 1, and allowing contact residues to relax in place. Crystallographic waters at the Arg611/Gln570 end were retained. Gln642 was repositioned to allow an H-bond to form with the pendant thiadiazole. The molecular mechanics minimization was conducted using the Amber force field as implemented in Flo (Thistlesoft, CT) software. Accommodation of the 4-fluorophenyl group as shown required some manual repositioning of contacting residues.