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Synthesis and structure–activity relationships of novel indazolyl glucocorticoid receptor partial agonists

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ABSTRACT

SAR was used to further develop an indazole class of non-steroidal glucocorticoid receptor agonists aided by a GR LBD (ligand-binding domain)-agonist co-crystal structure described in the accompanying paper. Progress towards discovering a dissociated GR agonist guided by human in vitro assays biased the optimization of this compound series towards partial agonists that possessed excellent selectivity against other nuclear hormone receptors.

Glucocortocoids have been used to treat a variety of inflammatory diseases for over 50 years now; however their systemic use is limited due to side effects such as osteoporosis, diabetes, lipodystrophy, and glaucoma.[1] Glucocorticoids exert their anti-inflammatory activity by altering the transcription factor complex and reducing the ability of NFκB and AP-1 to stimulate transcription.[2] This type of inhibition has been termed transrepression (TR).[3] These transrepressive mechanisms are believed to primarily contribute to the anti-inflammatory properties of glucocorticoids.[4] Glucocorticoids can also lead to the induction of some genes which is termed transactivation (TA). Unlike transrepression, transactivation appears to require dimerization of GR and binding glucocorticoid response elements (GREs) to activate genes that contribute to the unwanted side effects of glucocorticoids.[5] A GR agonist which can maintain the anti-inflammatory effects while mitigating the adverse side effect would have a beneficial profile for the treatment of a variety of autoimmune and inflammatory disorders.[6] This type of agonist has been termed a dissociated or selective GR agonist (SEGRA).[7] There are many examples in the literature of new classes of non-steroidal GR modulators which aim to separate these TR and TA activities.[8]

In the accompanying paper, we described the discovery of a class of novel indazole compounds (**I**) that have excellent activities for GR binding and are potent agonists in an in vitro assay of transrepression (AP-1, E-selectin repression) and transactivation (NP-1 agonism).

In an effort to maintain the desired TR activities while reducing or eliminating the TA activity in the NP-1 assay, a new series of compounds was designed and synthesized.

As shown in Figure 1, we sought to explore the effects of reducing the amide bond of the amido indazole series to an amine and converting it to a diverse list of amides, sulfonamides and ureas. This Letter will describe the synthesis, structure–activity relationship as well as the in vitro profiles of these novel non-steroidal compounds.

The synthesis of these 'reversed' amides, ureas, and sulfonamides was easily achieved using intermediates from the previously described indazole series.[9] Starting from compound **1**, the carboxylic acid is transposed to the carboxamide first by treatment with cyanuric fluoride in pyridine/DCM to provide the acid fluoride followed by reaction with ammonia gas in THF at −78 °C to form the intermediate carboxamide. Reduction with $LiAlH_4$ proceeded smoothly to afford intermediate amine **2** that was coupled to a variety of activated carboxylic acids, sulfonyl chlorides and isocyanates to yield the desired products **3** and **4**.

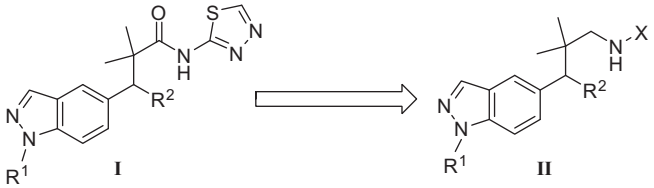

Figure 1. Reversed amide, sulfonamide, and urea series.

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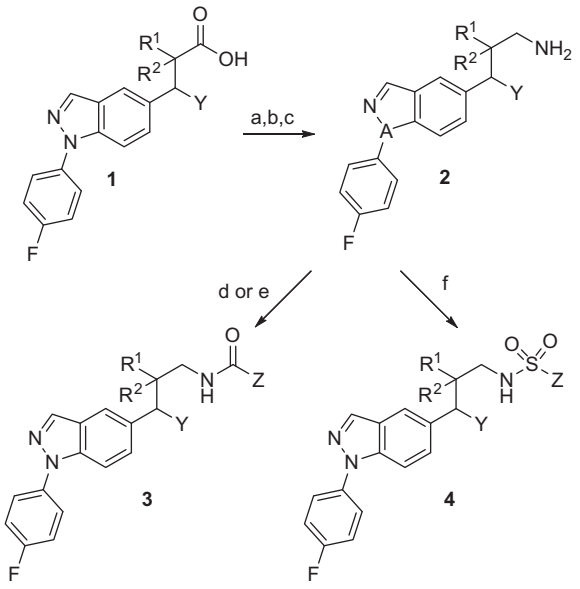


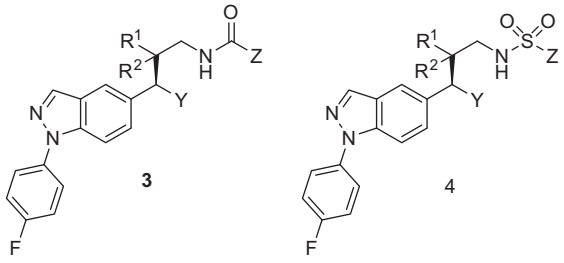
Scheme 1. Reagents and conditions: (a) cyanuric fluoride (1.1 equiv), pyridine (1.1 equiv), DCM, 3 h; (b) NH_3, THF, −78 °C to rt, 3 h; (c) $LiAlH_4$ (1.3 equiv), THF, 50 °C for 3 h; (d) various acids (1.1 equiv), EDC (1.1 equiv), HOBt (1.1 equiv), TEA (1.5 equiv), THF or DCM, 12 h; (e) isocyanates (1.1 equiv), TEA (1.5 equiv), DCM, 12 h: (f) various sulfonyl chlorides (1.1 equiv), TEA (1.5 equiv), DCM, 12 h.

The in vitro assays used to assess the biological activity of the GR ligands prepared in Scheme 1 are a GR binding assay, two measures of transrepression (AP-1 and a NF-κB dependent E-selectin repression assay), and a transactivation assay (NP-1 agonism which uses a GAL4 reporter in a HeLa cell line), all of which have been previously reported.[10,11] Based on literature precedents, the desired activity profile of a dissociated GR agonist is a compound that is a partial agonist of the in vitro functional assays for transrepression while showing little to no activity in the transactivation assay.[8] Full agonists in the transrepression assays invariably had significant activity in the transactivation assay. To this end we strove to discover compounds that had good partial agonist activity (EC_{50} <50 nM) whose agonist efficiencies (Y_{max}) were in the range of 65–90% when normalized to dexamethasone (100%). Structure–activity relationships for the compounds prepared in Scheme 1 are shown in Table 1 and selectivity data for other nuclear hormone receptors is shown in Table 2.

For the compounds described in this Letter, the substituent on the nitrogen of the indazole moiety was limited to a 4-fluorophenyl group as this gave the best results in the previous forward amide indazole series. As described in the previous Letter,[9] the optimal substituent at the benzyl position (Y) was either a phenyl or isobutyl group which held true in this series as well. The R^1 and R^2 groups were limited to monomethyl and dimethyl substitutions.

In the amide series (**3a**–**m**), SAR began by incorporating small groups that would increase the p*K*a of the amide NH to strengthen

Table 1
In vitro GR binding and functional activity for indazoles **3a**–**m** and **4a**–**m**



Compds[a]	Y	Z	R^1	R^2	GR binding[b] K_i (nM)	AP-1 repression[c] EC_{50}, nM[b] (Y_{max}, %dex[e])	E-selectin repression[c] EC_{50}, nM[b] (Y_{max}, %dex[e])	NP-1 agonism[d] EC_{50}, nM[b] (Y_{max}, %dex[e])
Dex					1.2	2.5 (100)	1.1 (100)	4.5 (100)
3a	Phenyl	CH_3	Me	Me	2.4	>5000	1152 (58)	nt[f]
3b	Phenyl	CF_3	Me	Me	1.74	725 (44)	124 (39)	>10,000
3c	Phenyl	CH_2CF_3	Me	Me	0.81	12.4 (91)	6.85 (90)	129 (65)
3d	Phenyl	$C(CH_3)_3$	Me	Me	2.0	78 (100)	23 (80)	394(25
3e	Phenyl	$CH_2(CH_3)_3$	Me	Me	1.9	215 (62)	138 (46)	>10,000
3f	Phenyl	CH_2Ph	Me	Me	nt[f]	2520 (33)	nt[f]	977 (100)
3g	Isobutyl	CH_2CF_3	Me	Me	1.3	61 (101)	38 (96)	262 (81)
3h	Phenyl	CH_2CF_3	Me	H	2.3	52 (84)	nt[f]	374 (52)
3i	Phenyl	CH_2CF_3	Me	H	2.4	7.5 (88)	nt[f]	20.7 (102)
3j	Phenyl	$NHCH_2CF_3$	Me	Me	10.2	252 (66)	393 (55)	>10,000
3k	Isobutyl	$NHCH_2CF_3$	Me	Me	1.42	118 (86)	87 (85)	501 (58)
3l	Phenyl	$NH(CH_3)_3$	Me	Me	5.14	57 (74)	58 (65)	429 (12)
3m	Isobutyl	$NH(CH_3)_3$	Me	Me	3.6	25 (100)	11.3 (94)	126 (86)
4a	Phenyl	CH_3	Me	Me	0.53	20.5 (86)	19.1 (97)	148 (32)
4b	Phenyl	CH_2CH_3	Me	Me	0.72	28.8 (84)	15.6 (87)	214 (19)
4c	Phenyl	$CH_2CH_2CH_3$	Me	Me	1.51	143 (66)	86 (63)	>10000
4d	Phenyl	Cyclopropyl	Me	Me	1.15	26.8 (76)	29.3 (82)	261 (19)
4e	Phenyl	CH_2CF_3	Me	Me	0.64	20.4 (74)	7.6 (86)	202 (24)
4f	Phenyl	CH_2CF_3	Me	H	1.34	5.2 (97)	0.66 (105)	26 (112)
4g	Phenyl	CH_3	Me	H	0.93	8.4 (100)	1.4 (105)	24 (101)
4h	Phenyl	CH_2CH_3	Me	H	0.87	4.3 (96)	1.8 (100)	12.8 (111)

[a] All data is on homochiral compounds. Absolute stereochemistry of **3h**, **3i**, and **4f**–**h** is unknown at the R^1/R^2 center.
[b] Values are means of two or more experiments performed in triplicate.
[c] Activator protein-1 (AP-1) and E-selectin assays were performed in an A549 lung epithelial cell line.
[d] GR transactivation NP-1 assay (run in agonist mode) was performed in the HeLa cell line.
[e] Efficacy is represented as a percentage of the maximal response of dex (100%). Dexamethasone is abbreviated as dex.
[f] nt means not tested.

Table 2
In vitro NHR selectivity profiles of selected indazoles

Compds	GR binding K_i (nM)	AR binding K_i (nM)	PR binding K_i (nM)	MR agonism IC_{50} (nM)
3b	0.81	>5000	>5000	>5000
3j	5.14	>5000	>5000	>5000
4e	0.64	>5000	406	>5000

the putative hydrogen bond to Asn 564 observed in the GR ligand binding domain crystal structure (vide infra). As shown in Table 1, the acetamide **3a** (Z=Me) shows good GR binding but very little activity in any of the functional assays. This led us to investigate other groups that could lower the p*K*a of the NH even further. Compound **3b**, which has a CF_3 substitution has good GR binding and modest functional activity in the AP-1 and E-selectin assays. Placing a methylene spacer to give a trifluoroethyl derivative (**3c**) led to a compound with good activity in both the AP-1 (12.4 nM, 91% efficiency) and E-selectin assays (6.85 nM, 90% efficiency). This trifluoroethyl derivative also shows modest activity in the NP-1 assay (129 nM, 65% efficiency). Adding another methylene spacer leads to a loss of activity in the AP-1 and E-selectin assays (data not shown). These data suggest that the GR binding and functional activity in the transrepression assays is independent of the p*K*a of the amide nitrogen. Other larger groups such as pivaloyl (**3d**) and benzyl (**3f**) also lead to diminished activity especially in the case of benzyl. When the CF_3 group of the trifluoroethyl moiety is replaced with a *t*-butyl group (**3e**), the resulting compound has modest functional activity in the AP-1 (251 nM, 62% efficiency) and E-selectin (138 nM, 46% efficiency) assays and no activity in the NP-1 assay (>10,000 nM). The trifluoroethyl series was further explored by exchanging the Y group from phenyl to isobutyl (**3g**) which resulted in diminished functional activity. Next, the effect of monomethyl substitution at the R^1/R^2 positions was examined—a change in the forward amide series[9] that conferred significant increase in potency. The two more active stereoisomers from the forward amide series were prepared in the reversed amide series (**3h** and **3i**) and are shown in Table 1. The monomethyl substitution led to a modest boost in potency in the AP-1 assay for one stereoisomer while the other was found to be less potent. Unfortunately, the more active stereoisomer **3i** also exhibited a significant increase in the NP-1 activity (20.7 nM, 102% efficiency).

For the urea analogs (**3j**–**m**), two sets of compounds are disclosed in Table 1. One set is a trifluoroethyl urea derivative with phenyl and isobutyl groups at the Y position and the other is a *t*-butyl urea derivative with the phenyl and isobutyl groups at the Y position. The trifluoroethyl urea series has moderate activity in the AP-1 and ELAM assays whereas the *t*-butylurea series has much better activity in these functional assays. The most active compound in this series is **3m** which has the isobutyl group at the Y position. This compound has an AP-1 EC_{50} of 25 nM with 100% efficiency and an ELAM EC_{50} of 11.3 nM with 94% efficiency but also shows increased transactivation as measured in the NP-1 assay with an EC_{50} of 126 nM (86% of dex). The most promising compound in this series is **3l** which has moderate functional activity in AP-1 (57 nM, 74% efficiency) and E-selectin (58 nM; 65% efficiency) and poor activity in the NP-1 assay (429 nM; 12% efficiency).

For the sulfonamide derivatives (**4a**–**h**), we examined a set of small alkyl groups ranging from methyl to propyl in length since larger alkyl substituents showed significantly lower activity (data not shown). These compounds all had good GR binding activity. The methyl (**4a**) and ethyl (**4b**) sulfonamide derivatives had very similar activities in both the transrepression and transactivation functional assays with activities of 20.5 nM (86% efficiency) and 28.8 nM (84% efficiency) in the AP-1 assay, 19.1 nM (97% efficiency) and 15.6 nM (87% efficiency) in the E-selectin assay, and 148 nM (32% efficiency) and 214 nM (19% efficiency) in the NP-1 assay, respectively. The increased size of the propyl compound **4c** led to the some loss of the transrepression functional activities, however the cyclopropyl derivative restored both the AP-1 and E-selectin activity to that of the methyl and ethyl analogs. The trifluoroethyl sulfonamide **4e** gave a compound with similar AP-1 activity (20.4 nM; 74% efficiency and slightly better E-selectin activity (7.6 nM; 86% efficiency) while retaining poor activity in the NP-1 assay (202 nM; 24% efficiency). When the R^1/R^2 groups in the sulfonamide series are changed from dimethyl to monomethyl (**4f**–**h**), activity in the AP-1 and E-selectin assays increased for all the compounds tested analogous to the regular amides described in the previous Letter. However, these compounds also showed a significant increase in NP-1 activity ranging from 12 to 26 nM with >100% efficiencies.

The selectivities against other nuclear hormone receptors for one representative compound from each series are shown in Table 2.[12,13] The compounds from the amide and urea series show excellent selectivity's against progesterone receptor (PR), androgen receptor (AR) and mineralcorticoid receptor (MR) whereas compounds from the sulfonamide series show excellent selectivies over AR and MR but only modest selectivity versus PR.

In an effort to better understand the binding and functional activity of this series of indazoles, we used molecular modeling to dock selected compounds into the GR X-ray crystal structure with dexamethasone.[14] Figure 2 shows the docking and minimization studies done with compound **3c** (amide series), compound **3l** (urea series), and compound **4e** (sulfonamide series). All three compounds show a similar binding poses in the GR active site. Their respective carbonyl or sulfonyl groups form a hydrogen bond to Gln 642 on helix 11 and the NH of these moieties is hydrogen bonded to Asn 564 on helix 3. Additionally, the N-1 nitrogen of the indazole forms a hydrogen bond to Gln 570 in all three poses. The 4-fluorophenyl moiety on the indazole is positioned in a hydrophobic pocket that appears to mimic the same group in the 2.5A co-crystal structure of fluorocortivazol with the GR LBD from Suino-Powell et al.[15]

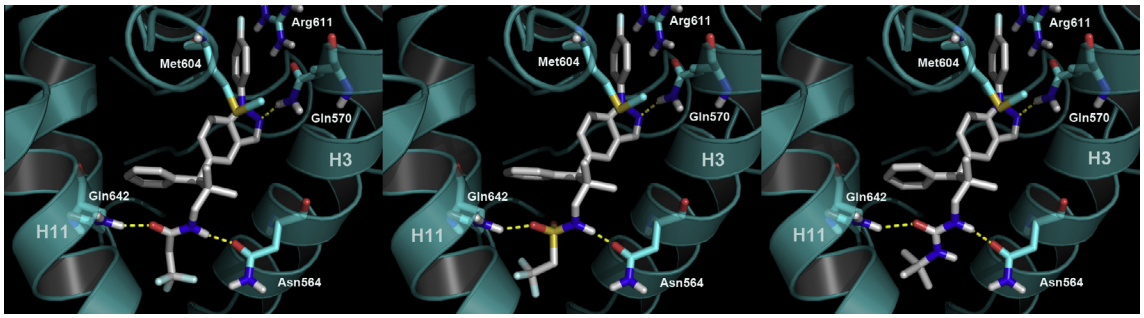


Figure 2. Molecular models of compound **3c** (amide series), compound **3l** (urea series), and compound **4e** (sulfonamide series) docked into the GR LBD.

In summary, we have discovered a novel series of indazole 'reversed' amides, ureas, and sulfonamides which are potent and selective non-steroidal ligands of the glucocorticoid receptor. Compounds have been identified which exhibit an improved dissociated profile relative to their 'forward' amide analogs and show superior selectivity over the other nuclear hormone receptors. Compound **3d** from the amide series, **3l** from the urea series, and **4e** from the sulfonamide series all show partial agonism in a transrepression assay (70–80% of the activity of dexamethasone) with very little activity in an NP-1 transactivation assay indicating that they are dissociated in vitro. While NP-1 activity appears to be more sensitive to structural changes, compounds that give complete repression in the AP-1 and E-selectin assays similar to that seen with dexamethasone with no NP-1 activity remain an elusive profile in this series.

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11. *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 dependant, 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 1b (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.
12. 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 $K_i = IC_{50}/(1 + \text{ligand concentration}/K_d)$. MR agonist assay was determined in an A549 cell line. EC_{50} was determined as (μM)/(% maximal efficacy) using aldosterone as a positive control. Data shown represent the means of duplicate experiments.
13. Other compounds from each series were also tested for their selectivities against PR, AR, and MR and gave similar results to the representative compounds shown in Table 2.
14. In Figure 2, the ligands **3c**, **3l**, and **4e** were generated by replacing them for the dexamethasone molecule in the published Bledsoe et al. X-ray structure (1M2Z.pdb) starting from a conformation analogous to that observed for the amido indazole in our own GR LBD co-crystal structure (see accompanying Letter). Crystallographic waters at the Arg611/Gln570 end were retained. Gln642 was repositioned to allow an H-bond to form with the pendant thiadiazole. Molecular mechanics minimization was conducted using the Amber force field as implemented in Flo (Thistlesoft, CT) software. Accomodation of the 4-fluorophenyl group as shown required some manual repositioning of contacting residues. Major H-bonding contacts are shown as yellow dashed lines.
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