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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

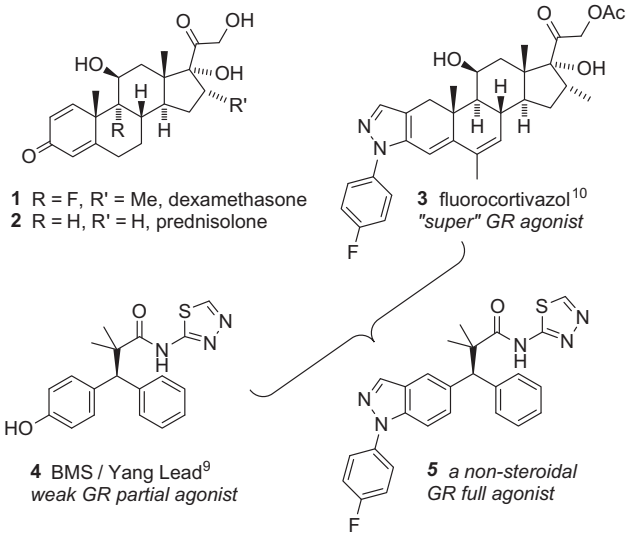

Figure 1. Genesis of the indazolyl class nonsteroidal GR agonist.

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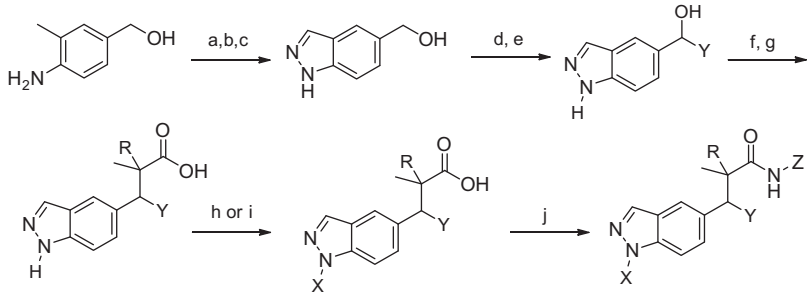
upon the modest GR activity of a previously discovered phenolic series **4**[9] by structurally combining it with fluorocortivazol **3**, one of the most potent glucocorticoids ever reported. (see Fig. 1)[10] We hypothesized that the A-ring of prednisolone was being mimicked by the phenol of **4** which 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.