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Substituted phenyl as a steroid A-ring mimetic: Providing agonist activity to a class of arylsulfonamide nonsteroidal glucocorticoid ligands

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ABSTRACT

A class of arylsulfonamide glucocorticoid receptor agonists that contains a substituted phenyl group as a steroid A-ring mimetic is reported. The structural design and SAR that provide the functional switching of a GR antagonist to an agonist is described. A combination of specific hydrogen bonding and lipophilic elements on the A-ring moiety is required to achieve potent GR agonist activity. This study culminated in the identification of compound **23** as a potent GR agonist with selectivity over the PR and MR nuclear hormone receptors.

Synthetic glucocorticoids such as dexamethasone and prednisolone (Fig. 1) are modulators of the glucocorticoid receptor (GR) and among the most effective agents for the treatment of acute and chronic inflammatory diseases. Their therapeutic application covers a broad range of disorders such as rheumatoid arthritis, asthma, inflammatory bowel disease and chronic obstructive pulmonary disease.[1–3] Despite their clinical efficacy, treatment with acute high and chronic low doses are limited due to a number of severe and sometimes irreversible side effects.[4]

With regard to the functional activity of GR, transrepression is considered to be the key mechanism for beneficial anti-inflammatory activity while transactivation is believed to mediate the mechanisms responsible for many of the glucocorticoid-induced side effects.[5–9] The goal of our non-steroidal glucocorticoid mimetic program has been to provide a potent GR agonist that differentiates between these two functional pathways.

Previously we reported a class of α-methyltryptamine sulfonamides as exemplified by compound **1** (Fig. 2).[10] This compound was identified in a high-throughput chemistry effort where the initial SAR focused mainly on the arylsulfonamide portion of the ligand that was amenable to rapid synthesis. These analogues provided modest GR binding potency (e.g. compound **1** GR IC_{50} = 40 nM) but lacked the desired agonist activity.[9] Previous publications on GR agonists from our laboratories have shown that

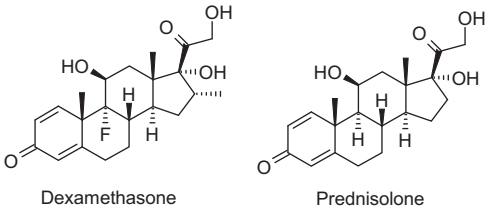

Figure 1. Examples of synthetic glucocorticoids.

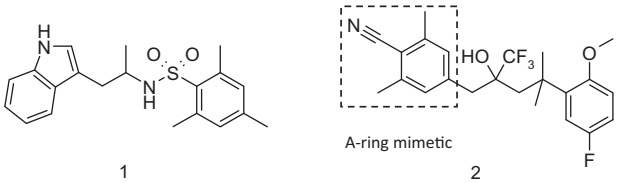

Figure 2. Compound **1** (partial antagonist) and **2** (full agonist).

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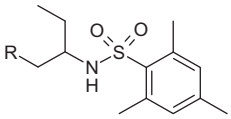
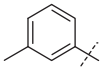
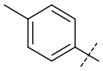
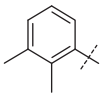
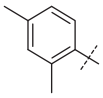
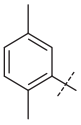
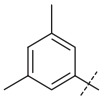
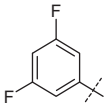
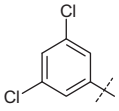
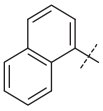
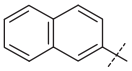
Table 1
SAR of substituted aryl groups as A-ring mimetics



Compound	R	GR IC_{50} (nM)	PR IC_{50} (nM)	MR IC_{50} (nM)	IL-6 IC_{50} (nM)	IL-6 % efficacy
3		83	4200	700	>10,000	0
4		23	5500	400	>10,000	0
5		230	3400	1100	>10,000	0
6		110	>10000	>10000	>10,000	0
7		10	2000	350	>10,000	19
8		160	8200	2400	>10,000	0
9		25	>10000	320	>10,000	0
10		11	7400	360	>10,000	36
11		48	3400	400	>10,000	0
12		10	1200	150	>10,000	0
13		10	1500	190	>10,000	0
14		78	720	400	>10,000	0

specific and, at times, seemingly minor substitution changes can have significant effects on functional activity despite similar GR binding affinities.[11–13] The effect that these function-regulating pharmacophores (FRPs) have on the resultant GR-ligand complex might be as drastic as inducing a conformational change similar to that caused by mifepristone, or as subtle as modulating the acidity of a hydrogen bond donor of the ligand.[11]

In particular, we have described the use of a substituted phenyl A-ring mimetic as exemplified by compound **2** (Fig. 2).[12a] The SAR of the phenyl group revealed that moieties providing both a lipophilic and a hydrogen bond interaction with the protein were required in order to achieve potent agonist activity. Similar SAR utilizing a cyano group as a replacement for the A-ring carbonyl has been reported for other nuclear hormone receptors as in the progesterone[12b] and androgen[12c,d] receptors. Based on initial modeling studies and examination of the SAR, we hypothesized that the indole of **1** was likely filling the role of the steroid A-ring. To provide agonist activity to this new series, we envisioned replacing the indole of compound **1** with a phenyl ring and applying a similar strategy as was described for **2**.[14]

As a starting point, replacement of the indole of compound **1** with an unsubstituted phenyl group resulted in an analogue (not shown) with no activity up to a concentration of 10 μM in our GR binding assay.[15] The optimization of substitution on the phenyl ring began by identifying appropriate lipophilic groups to ensure optimal space filling prior to the incorporation of a hydrogen bonding component. This work was conducted utilizing an *N*-α-ethyl group on the sulfonamide core which had previously been described to be a potent alternative to the *N*-α-methyl substituent shown in compound **1**.[10] As shown in Table 1, mono-methyl

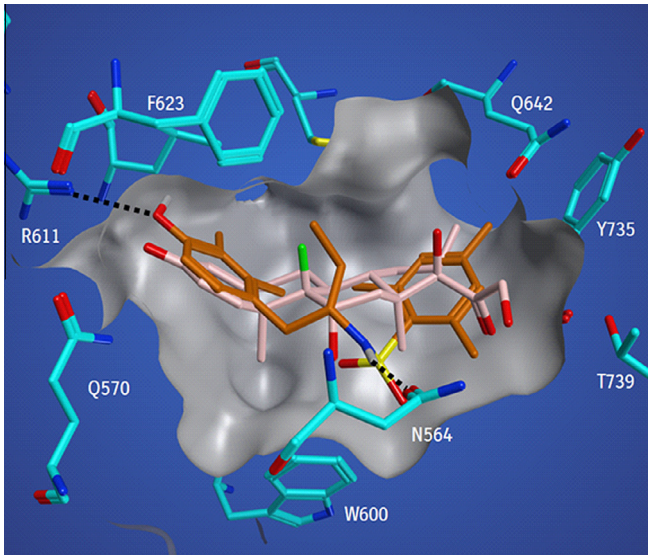
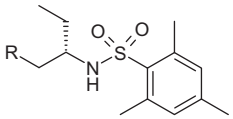


Figure 3. Overlay of the docking pose of compound **16** (orange) with the X-ray complex structure of GR/LBD and dexamethasone (rose).[17] Docking was performed using Glide[18] and the picture was generated with MOE.[19] Dotted lines represent hydrogen bonds.

substitution at both the ortho and meta positions (e.g., compounds **3** and **4**) was preferred over *para* substitution. Interestingly for these compounds, a small increase in lipophilicity, as exemplified by the addition of a single methyl group, provided a significant increase in binding activity for compound **4** (GR IC_{50} = 23 nM) over the unsubstituted phenyl analogue. Despite this increase in binding potency, mono-methyl substitution was not sufficient for agonist activity as determined by the IL-6 assay.[16] For dimethyl-substitution, combining methyl groups in the 2,3-, 2,5-, and 3,5-positions (analogs **7**, **9**, **10**) resulted in binding activity of <25 nM while 2,6- and 2,4-dimethyl analogues **6** and **8** were less active. For compound **10**, potent GR binding potency was also accompanied by weak GR agonist activity as indicated by the IL-6 efficacy.

To further examine 3,5-substitution, compounds **11** and **12** were prepared. Comparing the data of the di-chloro compound **12** with that of **10** suggested that changing the electronic nature of the phenyl ring in the absence of other substitution has little effect on binding activity, and that the role of the 3,5-dichloro substituents might simply be providing lipophilic interactions with the receptor. The potency of the 2,3-dimethyl analogue **7** led us to envision fusing a second ring at this position. This was accomplished with the 1-napthyl analogue **13**, which resulted in equal GR potency as the corresponding dimethyl analogue **7**. As expected from the methyl SAR, extending the phenyl in the direction of the *para* position, as in the 2-napthyl analogue **14**, resulted in a loss in

Table 3
Receptor activity; evaluation of a lipophilic requirement for agonist activity



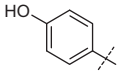
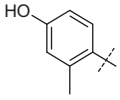
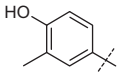
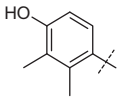
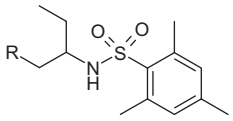
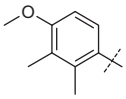
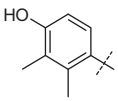
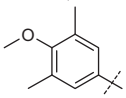
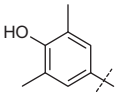
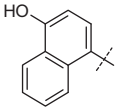
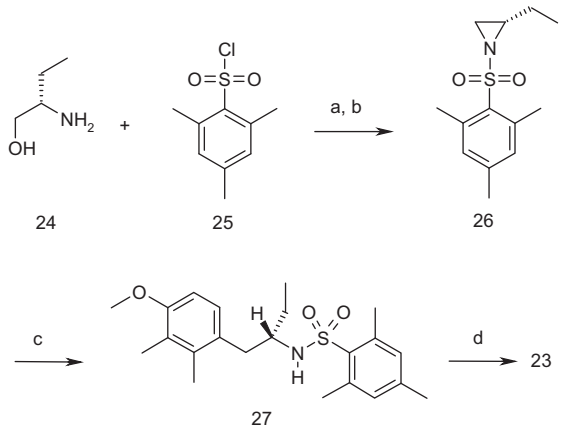
Compound	R	GR IC_{50} (nM)	PR IC_{50} (nM)	MR IC_{50} (nM)	IL-6 IC_{50} (nM)	IL-6 % efficacy
20		120	6400	5200	>2000	0
21		27	2800	1000	>2000	0
22		23	2500	2300	>2000	0
23		2	620	270	22	77

Table 2
SAR of aryl groups containing a hydrogen bonding element as A-ring mimetics



Compound	R	GR IC_{50} (nM)	PR IC_{50} (nM)	MR IC_{50} (nM)	IL-6 IC_{50} (nM)	IL-6 % efficacy
15		62	2100	330	>2000	0
16		5	860	240	15	81
17		250	>10,000	1400	>2000	0
18		12	3500	480	89	58
19		3	210	110	23	80



Scheme 1. Synthesis. Reagents and conditions: (a) Pyr, CH_2Cl_2, rt; (b) KOH, Et_2O, 63%; (c) 4-bromo-2,3-dimethylanisole, Mg, CuI, THF, 53%; (d) BBR_3, CH_2Cl_2, 39%.

GR binding activity. With respect to nuclear receptor selectivity, cross reactivity with the mineralocorticoid receptor (MR) and progesterone receptor (PR) were monitored to evaluate the potential for off-target pharmacology. It should be noted that each improvement in GR binding was generally accompanied by increases in MR binding, with the exception of compound **6** which is selective over both the PR and MR.

To assess whether the R group of analogues in Table 1 could potentially fill space similar to that occupied by the A-ring of dexamethasone when bound to GR, a select group of substituted phenyl analogues were docked into the GR ligand binding domain (GR-LBD) using the GR-LBD/dexamethasone X-ray co-crystal structure.[17] Docking of these compounds did suggest that these phenyl groups can be overlaid with the A-ring of dexamethasone. Figure 3 shows the docking pose of compound **16** ((*S*)-enantiomer) in the GR-LBD X-ray co-crystal structure of dexamethasone. The 2,4,6-trimethylphenyl moiety of the sulfonamide group extends into an area typically occupied by the steroid D-ring while the sulfonamide replaces the function of the C-11 hydroxyl by engaging N564. The para-position of the phenyl group which occupies the A-ring pocket was identified as a position where a hydrogen bond acceptor could be within binding distance of residues R611/Q570. These results suggested that a hydrogen bonding element could be combined with optimal lipophilic substitution as an A-ring mimetic. To this end, methoxy and hydroxyl groups were incorporated into the 4-position of compounds **7**, **10** and **13** to provide the analogues shown in Table 2. The hydroxyl group in combination with specific lipophilic substitution has been reported to be an effective FRP, demonstrating efficacious agonist activity in a non-steroidal glucocorticoid A-ring mimetic.[20] A clear preference for 4-hydroxyl substitution as compared to the corresponding 4-methoxy derivative was apparent as shown by the potent GR binding affinities (IC_{50} <15 nM) of compounds **16**, **18** and **19**. More importantly, the addition of a hydrogen bond interaction provided modest to very potent agonist activity. Although not clearly apparent by the GR binding IC_{50} alone, the 2,3-dimethyl substitution appeared to be preferred over the 3,5-dimethyl pattern based on GR agonist data (**16**: IL-6 IC_{50} = 15 nM, 81% maximal efficacy vs. **18**: IL-6 IC_{50} = 89 nM, 58% maximal efficacy). The corresponding 1-napthyl analogue **19** also displayed binding potency and agonist activity similar to **16**. It is interesting to note that for each of these examples the addition of the hydroxyl group is essential for potent agonist activity despite having little to no effect on GR binding affinity, suggesting the importance of this hydrogen bonding interaction as an essential component in function regulation.

To further understand the essential components required for agonist activity, the importance of the lipophilic substitution on the A-ring mimetic was evaluated by systematically removing the methyl groups in the presence of the 4-hydroxyl group. This SAR was examined with the *(S)*-enantiomer, as shown in Table 3. A chiral preference for substitution at this position has been previously reported.[10] As seen in the results shown in Table 3, the hydrogen bond interaction provided by the hydroxyl in compound **20** was not sufficient to provide agonist activity in the absence of lipophilic groups on the phenyl ring. Interestingly, the deletion of a single methyl group from racemic analogue **16** to provide compounds **21** and **22** (single enantiomers) rendered them inactive with respect to agonist activity, despite modest GR binding potency. Comparing data for the racemate **16** with the chiral compound **23**, we find no significant enhancement of IL-6 agonist activity. Examining the binding potencies for the various nuclear receptors shows that exceptional GR activity is displayed for **23** and selectivity for GR is maintained with 310-fold selectivity over PR and a 135-fold selectivity over MR.

The synthesis of compound **23** is described in Scheme 1. Central to the synthesis was the preparation of the aziridine sulfonamide **26**, which was synthesized from the readily available amino alcohol **24** and two equivalents of the aryl sulfonylchloride **25** in the presence of pyridine followed by treatment with 2 N KOH.[21] Next, the aziridine **26** was reacted with 4-bromo-2,3-dimethylanisole in the presence of magnesium and copper iodide at 85 °C in a microwave reactor to provide **27** which was subsequently demethylated using boron tribromide to afford **23**.

In conclusion, we have described a new class of nonsteroidal GR agonists. Through incorporation of substituted phenyl A-ring mimetics, we have identified specific function regulating pharmacophores that provide potent GR agonist activity to a unique structural class. This SAR has confirmed the importance of both a lipophilic and a hydrogen bonding component which work synergistically to provide agonist activity. In particular, identification of 2,3-dimethyl substitution as requisite lipophilic components in combination with a 4-hydroxyl group as the hydrogen bonding element has provided compounds with potent GR agonist activity as exemplified by compound **23** which displayed potency and efficacy against GR as well as selectivity over PR and MR. The synthesis of this class of compounds via a key aziridine intermediate allowed for rapid access to chiral material and provided for a point of diversification to gain access to a broad range of structural variants.

References and notes

1. Evans, R. M. *Science* **1988**, *240*, 889.
2. Beato, M.; Herrlich, P.; Schutz, G. *Cell* **1995**, *83*, 851.
3. Lowenberg, M.; Stahn, C.; Hommes, D. W.; Buttgereit, F. *Steroids* **2008**, *73*, 1025.
4. Schacke, H.; Docke, W. D.; Asadullah, K. *Pharmacol. Ther.* **2002**, *96*, 23.
5. Eggert, M.; Schulz, M.; Neeck, G. *J. Steroid Biochem. Mol. Biol.* **2001**, *77*, 185.
6. Belvisi, M. G.; Thomas, J. B.; Wicks, S.; Foster, M. L. *Pulm. Pharmacol. Ther.* **2001**, *14*, 221.
7. Belvisi, M. G.; Wicks, S.; Battram, C. H.; Bottoms, S. E. W.; Redford, J. E.; Woodman, P.; Brown, T. J.; Webber, S. E.; Foster, M. L. *J. Immunol.* **1975**, *2001*, 166.
8. Zhou, J.; Cidlowski, J. A. *Steroids* **2005**, *70*, 407.
9. De Bosscher, K.; Van Craenenbroek, K.; Meijer, O. C.; Haegeman, G. *Eur. J. Pharm.* **2008**, *583*, 290.
10. (a) Marshall, D. R.; Rodriguez, G.; Nelson, R.; Thomson, D. Abstracts of Papers, 227th ACS National Meeting, Anaheim, CA, United States, March 28-April 1, 2004, MEDI-226.; (b) Marshall, D. R.; Rodriguez, G.; Thomson, D. S.; Nelson, R.; Capolina, A. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 315.
11. Betageri, R.; Zhang, Y.; Zindell, R.; Kuzmich, D.; Kirrane, T. M.; Bentzien, J.; Cardozo, M.; Capolino, A. J.; Fadra, T. N.; Nelson, R.; Paw, Z.; Shih, C. K.; Shih, D. T.; Reilly, P.; Zuvela-Jelaska, L.; Nabozny, G.; Thomson, D. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4761.
12. (a) Kuzmich, D.; Kirrane, T.; Proudfoot, J.; Bekkali, Y.; Zindell, R.; Beck, L.; Nelson, R.; Shih, C. K.; Kukulka, A. J.; Paw, Z.; Reilly, P.; Deleon, R.; Cardozo, M.; Nabozny, G.; Thomson, D. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 5025; (b) Zhang, P.;

Terefenko, E.; Kern, J.; Fensome, A.; Trybulski, E.; Unwalla, R.; Wrobel, J.; Lockhead, S.; Zhu, Y.; Cohen, J.; LaCava, M.; Winneker, R. C.; Zhang, Z. *Bioorg. Med. Chem.* **2007**, *15*, 6556; (c) Li, J. J.; Iula, D. M.; Nguyen, M. N.; Hu, L.-Y.; Dettling, D.; Johnson, T. R.; Du, D. Y.; Shanmugasundaram, V.; Van Camp, J. A.; Wang, Z.; Harter, W. G.; Yue, W.-S.; Boys, M. L.; Wade, K. J.; Drummond, E. M.; Samas, B. M.; Lefker, B. A.; Hoge, G. S.; Lovdahl, M. J.; Asbill, J.; Carroll, M.; Meade, M. A.; Ciotti, S. M.; Krieger-Burke, T. *J. Med. Chem.* **2008**, *51*, 7010; (d) Ostrowski, J.; Kuhns, J. E.; Lupisella, J. A.; Manfredi, M. C.; Beehler, B. C.; Krystek, S. R., Jr.; Bi, Y.; Sun, C.; Seethala, R.; Golla, R.; Sleph, P. G.; Fura, A.; An, Y.; Kish, K. F.; Sack, J. S.; Mookhtiar, K. A.; Grover, G. J.; Hamann, L. G. *Endocrinology* **2007**, *148*, 4.

13. Regan, J.; Lee, T. W.; Zindell, R. M.; Bekkali, Y.; Bentzien, J.; Gilmore, T.; Hammach, A.; Kirrane, T. M.; Kulkulka, A. J.; Kuzmich, D.; Nelson, R. M.; Proudfoot, J. R.; Ralph, M.; Pelletier, J.; Souza, D.; Zuvela-Jelaska, L.; Nabozny, G.; Thomson, D. S. *J. Med. Chem.* **2006**, *49*, 7887.
14. This work was previously disclosed in part: DiSalvo, D.; Kuzmich, D.; Betageri, R.; Bentzien, J.; Kukulka, A.; Marshall, D. R.; Nabozny, G. H.; Nelson, R.; Thomson, D. S. Abstract of Papers, 235th ACS National Meeting of the American Chemical Society, New Orleans, LA; American Chemical Society: Washington, DC, 2008; Abstract MEDI-322.
15. GR, PR and MR binding assays were performed in a fluorescence polarization format that measures competition for binding to the nuclear receptor, present in lysates of baculovirus-infected insect cells, between a test compound and a fluorescently labeled receptor ligand, or probe. IC_{50} values were determined by fitting the fluorescence polarization signal data to a 4-parameter logistic equation. All IC_{50} values shown represent the mean of at least two independent determinations. Repeated testing of reference compounds in these assays demonstrate typical IC_{50} standard deviations of 20–40% about the mean.
16. In the IL-6 functional assay, potency and% efficacy (percent of the maximal response observed for dexamethasone) are reported. Human foreskin fibroblasts are stimulated with 1 ng/mL recombinant human IL-1 in the presence of test compound. After 24 h, the degree of GR agonist activity (transrepression) is determined by measuring IL-6 in the tissue culture media and calculating EC_{50} values. Top concentration in the assay was 2 mM.
17. Bledsoe, R. K.; Montana, V. G.; Stanley, T. B.; Delves, D. J.; Apolito, C. J.; McKee, D. D.; Consler, T. G.; Parks, D. J.; Stewart, E. L.; Willson, T. M.; Lambert, M. H.; Moore, J. T.; Pearce, K. H.; Xu, E. H. *Cell* **2002**, *110*, 93.
18. Glide, version 5.8, Schrödinger LLC., New York, NY, 2012.
19. Molecular Operating Environment (MOE), 2012.10; Chemical ComputingGroup Inc., 1010 Sherbrooke St. West, Suite #910, Montreal, H3A 2R7, QC, Canada, 2012.
20. Roach, S. L.; Higuchi, R. I.; Hudson, A. R.; Adams, M. E.; Syka, P. M.; Mais, D. E.; Miner, J. N.; Marschke, K. B.; Zhi, L. *Bioorg. Med. Chem.* **2011**, *21*, 168.
21. McCoull, W.; Davis, F. A. *Synthesis* **2000**, *10*, 1347.