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Steroidal C-21 heteroaryl thioethers. Part 3: Pregn-4-eno-[3,2-*c*]pyrazole fused A ring modified steroids as selective glucocorticoid receptor modulators (dissociated steroids)

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Dedicated to Professor Gilbert Stork on the occasion of his 90th birthday.

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ABSTRACT

The introduction of A ring pyrazole modification to the hydrocortisone C-21 heteroaryl thioethers generated compounds with excellent transrepression potency (IL-8 inhibition) compared to their hydrocortisone analogs. However, the transcriptional transactivation activity of these compounds were considerably higher than the corresponding hydrocortisone analogs. Among all the compounds evaluated, a quinoxaline thioether modification demonstrated the best overall in vitro separation.

We recently reported[1] compounds of structural types **1**, **3**, and **4** as both inhaled and oral dissociated steroids for the treatment of various inflammatory conditions. The key structural feature critical for the dissociated profile is the C-21 heteroaryl thioether modification. Interestingly the majority of these compounds demonstrated significantly reduced transactivational activity while keeping potent anti-inflammatory transrepressional activity in both in vitro and in vivo assays. Although we were not successful in getting a crystal structure of any of these compounds within the glucocorticoid receptor, molecular modeling was carried out to explore the binding mode of these steroids to the glucocorticoid receptor (GR) and to further understand the mechanisms of the dissociation profile. The protein structure used in this study was retrieved from the PDB (PDB ID: 1P93, GR-LBD in complex with dexamethasone[2]). Due to the large size of the C-21 group, these compounds could not fit into the steroid binding site of the crystal structure. Hence we thought it would be reasonable to use a truncated version of the crystal structure (with residues after

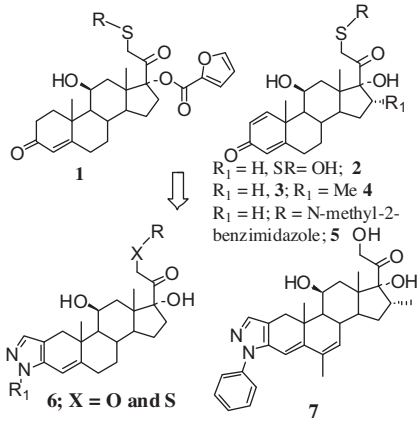

Figure 1. Dissociated steroids: steroidal[3,2-*c*]pyrazole fused steroid A ring modification.

T739 removed) to model these compounds. A previously reported compound[1b] **5** (Fig. 1) was docked to this truncated GR crystal

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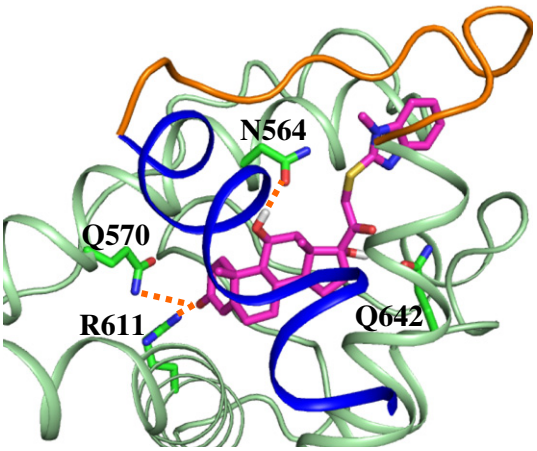


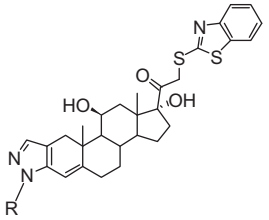
Figure 2. The binding model of compound **5**. GR protein is shown as cartoon with helix 12 colored blue, the loop between helix 11 and helix 12 colored orange and the rest of the protein pale green. The carbon atoms of compound **5** are colored magenta. The C21 group fits under this loop, disturbing its conformation from the agonist bound state. This may induce conformational changes to the helix 12 region and leads to the dissociation profile possibly due to the lack of dimerization of the GR–GC monomer complex, which is a key event required for the transcriptional transactivation.

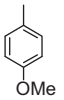
structure. Docking of compound **5** was carried out using glide XP methodology[3] in Schrödinger modeling package. Chain A of 1P93, prepared as described above, was used to generate the energy grids for docking.

In the docking model of compound **5** (Fig. 2), the polar interactions with GR resembled to that of dexamethasone, for example, the C3-carbonyl group with Gln570 and Arg611, the 11β-hydroxyl group with Asn564, and the 17α-hydroxyl group with Gln642. The steroid core maintained tight packing with the hydrophobic residues of the binding pocket. These interactions contributed to the high binding affinity of compound **5** (hGR IC_{50} = 7.3 nM). When compound **5** overlaid on the whole crystal structure, the C-21 group oriented directly under the loop between the helix 11 and helix 12 occupying the space of Ile747. The binding interaction of compound **5** in this region of GR could disrupt the conformation of this loop, which may subsequently induce conformational changes to the downstream region of helix 12. Induced-fit docking was employed to further elucidate protein conformation around the loop region and we observed the proposed conformational changes in the GR structure. Based on these docking studies, we hypothesize that the helix-12 conformational changes, could probably prevent the dimerization of the glucocorticoid receptor–glucocorticoid (steroid) monomer complex, which is a key event required for the transcriptional transactivation.[4]

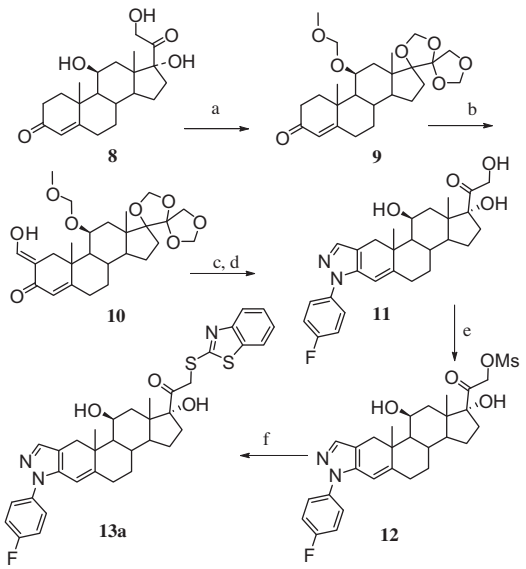
As an ongoing efforts to further optimize the anti-inflammatory potency and dissociation, we were interested in introducing a pyrazole modification in the A ring of compound **1** like deacylcortivazole[5] (DAC) **7**, a well studied glucocorticoid receptor agonist with interesting pharmacological properties. Historically, the androst-4-eno and androstano-[3,2-*c*]pyrazole modification was first introduced by Clinton et al.[6] in 1959 and later by de Ruggieri et al.[7] Later in 1963, Merck researchers[8] Max Tishler and Ralph Hirschman extended this modification to pregn-4-eno-[3,2-*c*]pyrazole and developed a new series of potent anti-inflammatory hybrid steroids. Herein, we report the structure activity studies of a variety of N-substituted steroidal-pyrazoles with various C-21 heteroaryl ether and thioether derivatives towards the optimization of both dissociation and transrepression potency.

Table 1
Transrepression and transactivation screening of C-21 thioether derivatives of various steroidal[3,2-*c*]pyrazoles



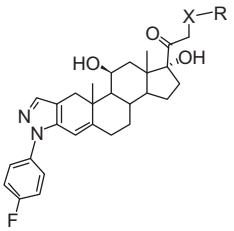
Compds	R	IL-8 inh. IC_{50}, nM[a] efficacy (%)	Rat TAT. IC_{50}, nM[a] efficacy (%)	h-TAT IC_{50}, nM[a] efficacy (%)
11		0.4 (99)	3.2 (107)	40.9 (135)
13a		1.7 (97)	24.1 (83)	101 (110)
14		7.5 (95)	57.3 (67)	165 (45)
15		(20)	NT	NT
16		107 (66)	NT	NT
17		179 (66)	NT	NT
18		86.4 (78)	NT	NT
19		7.1 (98)	54.7 (63)	400 (53)
20		(27)	NT	NT
21		(8)	NT	NT

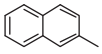
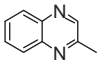
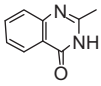
Note: The top concentration for the Emax when there are no IC_{50} available is 1 μM.
[a] Values are means of two experiments.



Scheme 1. Reagents and conditions: (a) formaldehyde, $CHCl_3$, concd HCl (b) sodium methoxide, THF, 60 °C, 45 min, then HCOOEt, 24 h (c) *p*-fluorophenylhydrazine, NaOAc, CH_3COOH, rt, 60% (d) 50% aq. HCOOH, 70 °C, 65% (e) CH_3SO_2Cl, Hunig's base, CH_2Cl_2, 0 °C–rt, 90% (f) 2-mercaptobenzothiazole, K_2CO_3, acetone, reflux, 92%.

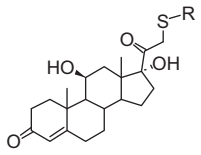
Table 2
Transrepression and transactivation screening of C-21 ether and thioether derivatives in 4-fluorophenyl pyrazole series

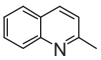
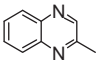


Compds	R	X	IL-8 inh. IC_{50}, nM[a] efficacy (%)	r. TAT. IC_{50}, nM[a] efficacy (%)	h-TAT IC_{50}, nM[a] efficacy (%)
11	R=H	O	0.4 (99)	3.2 (107)	40.9 (135)
13a		S	1.7 (97)	24.1 (83)	101 (110)
13b		O	2.1 (96)	84 (71)	(31)
22a		S	1.1 (99)	174 (89)	88.7 (115)
22b		O	2.9 (97)	78 (74)	(34)
23a		S	0.72 (99)	52.5 (98)	30.5 (68)
23b		O	1.06 (99)	58 (106)	29.2 (67)
24a		S	5.3 (97)	24.3 (115)	18.3 (116)
24b		O	1.8 (97)	44.7 (59)	(38)
25a		S	1.99 (98)	(25)	(2)
25b		O	1.89 (97)	(41)	98.7 (52)
26		S	1.6 (99)	65.4 (54)	37.3 (63)
27		S	0.32 (99)	13.3 (80)	38.6 (63)
28		S	0.5 (99)	4.5 (111)	4.7 (116)
29		S	0.6 (99)	14.3 (66)	37.7 (72)
30		S	3.0 (99)	86.5 (59)	93.5 (51)

Note: The top concentration for the Emax when there are no IC_{50} available is 1 μM.
[a] Values are means of two experiments.

Table 3
Transrepression and transactivation screening of C-21 tether derivatives in hydrocortisone series



Compds	R	IL-8 inh. IC_{50}, nM[a] efficacy (%)	hGRE. IC_{50}, nM[a] efficacy (%)	h-GR IC_{50}, nM
31		36 (97)	(30)	4.8
32		56.1 (84)	(8)	8.9
33		26.7 (98)	(35)	3.9
34		21.9 (95)	(57)	4.3
35		89.3 (83)	(0)	6.6
36		22.1 (83)	(1)	NT
37		61.3 (87)	(37)	NT
38		59.1 (75)	(16)	4.7
39		29.2 (80)	58.5 (54)	NT
40		23.8 (65)	(14)	14.9
41		127 (59)	(18)	NT
42		27.2 (90)	(28)	NT

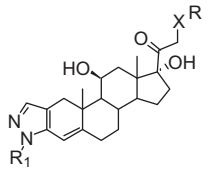
Note: The top concentration for the Emax when there are no IC_{50} available is 1 μM.
[a] Values are means of two experiments.

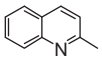
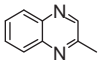
A general procedure for the synthesis of fused pyrazole compounds is outlined in Scheme 1. The commercially available hydrocortisone **8**, was converted to the ketal **9** by treating with formaldehyde in chloroform. The formylation of the A ring was carried out with sodium methoxide and ethyl formate in anhydrous THF to afford the keto-aldehyde **10**. Subsequent treatment of the keto-aldehyde **10** with 4-fluorophenyl hydrazine and sodium acetate in glacial acetic acid generated the corresponding pyrazole, which was deprotected to the pyrazole-steroid **11** by the treatment with 50% aqueous formic acid at 70 °C in moderate yield. The alcohol **11** was converted to the corresponding mesylate **12** and subsequent treatment with 2-mercaptobenzothiazole and potassium carbonate in acetone generated the target compound **13** in excellent yield.

By following the sequence of reactions outlined in Scheme 1, the keto-aldehyde **10** was treated with a number of hydrazines and generated the corresponding 2-mercaptobenzothiazole compounds (Table 1). The dissociated profile of these compounds were studied in two different cell based assays. Transrepression was measured[9] in a TNF stimulated IL-8 cytokine synthesis assay in human bronchial epithelial cells (H292 cells). Functional transactivation was measured by induction of tyrosineaminotransferase (TAT), an enzyme with well characterized endogenous glucocorticoid response elements that catalyzes the degradation of tyrosine to *p*-hydroxy phenyl pyruvate. Human HepG2 liver cells and rat H4II-E cells were used for the TAT functional assay. A recombinant hGRE luciferase assay was also used for measuring the transcriptional transactivation.

The parent hydroxy compound **11** exhibited a classical steroid like profile with a very potent IL-8 inhibition and a robust transcriptional transactivation in both rat and human TAT induction assays. A limited SAR was generated (Table 1) with N-substituted pyrazoles ranging from aromatic and aliphatic hydrophobic groups (e.g., phenyl, 4-fluorophenyl, cyclohexyl and *t*-butyl) to aromatic and aliphatic polar groups (e.g., pyridyl, methoxy-phenyl, tetrahydropyranyl, and propionitrile). Pyrazoles with 4-fluorophenyl **13a**, phenyl **14** and cyclohexyl **19** demonstrated promising anti-inflammatory profile, with a relatively weaker TAT induction compared to the parent steroid **11**. The pyrazole substitution with polar groups such as methoxy-phenyl, pyridyl, tetrahydropyran, and

Table 4
Transrepression and transactivation screening of C-21 ether and thioether derivatives in phenyl and cyclohexyl pyrazole series



Compds	R	X	R_1	IL-8 inh.	r. TAT.	h-TAT	hGRE luciferase
				IC_{50}, nM[a] efficacy (%)	IC_{50}, nM[a] efficacy (%)	IC_{50}, nM[a] efficacy (%)	
43a	R=H	O	Ph	0.63 (100)	3.23 (96)	143 (70)	
43b	R=H	O	Cyclohexyl	2.37 (99)	NT	NT	5.57 (96)
14		S	Ph	7.5 (95)	57.3 (67)	165 (45)	36.9 (89)
19			Cyclohexyl	7.1 (98)	54.7 (63)	400 (53)	130 (78)
44a		S	Ph	4.7 (97)	78.0 (75)	66.7 (49)	19.1 (64)
44b			Cyclohexyl	6.6 (96)	1000 (44)	(19)	108 (75)
45		S	Cyclohexyl	3.12 (99)	31.6 (65)	82.1 (66)	30.1 (72)
46		S	Ph	0.89 (99)	33.8 (81)	37.7 (64)	6.9 (83)
47a		S	Ph	1.5 (100)	225 (82)	596 (64)	8.4 (102)
47b			Cyclohexyl	4.4 (96)			43.3 (87)
48		S	Cyclohexyl	7.3 (98)	(36)	1000 (46)	137 (76)
49a		S	Ph	14.8 (95)	82.3 (95)	60.1 (43)	101 (69)
49b			Cyclohexyl	44.7 (92)			262 (71)
50a		S	Ph	5.36 (97)	113 (67)	226 (41)	28.8 (68)
50b		S	Cyclohexyl	12.4 (97)	(40)	(27)	158.0 (84)
50c		O	Ph	3.7 (95)	(33)	(43)	31.0 (56)
51		S	Cyclohexyl	2.8 (99)	135 (96)	168 (59)	31.8 (88)
52		S	Ph	3.43 (97)	258 (62)	278 (50)	28.6 (86)

Note: The top concentration for the Emax when there are no IC_{50} available is 1 μM.
[a] Values are means of two experiments

propionitrile produced compounds with significantly weaker inhibitory activity towards IL-8.

Owing to the interesting anti-inflammatory profile of 4-fluorophenyl, phenyl and cyclohexyl substituted pyrazoles, we decided to investigate the C-21 oxo and thio SAR in detail (Table 2) to optimize the pharmacological properties. As evident from the Table 2, the compounds with thioether modification showed very potent IL-8 inhibition. Compounds with azabenzoxazole **27**, pyridyl **28**, and pyrimidinyl **29** demonstrated excellent potency towards IL-8 inhibition, a several fold improvement compared to their hydrocortisone analogs (Table 3). However, their transactivation potency and efficacy were significantly higher than the corresponding hydrocortisone analogs (Table 3). Generally most of the hydrocortisone analogs demonstrated potent binding affinity to GR and a highly desirable dissociation profile with minimal transactivational activity. The quinazoline compound **25a** notably showed a very potent IL-8 inhibition, well separated from TAT activation in both rat and human cell types. At this stage we prepared some of the C-21 oxygen analogs. Interestingly most of them displayed a parallel SAR with that of the thioethers with a lesser hTAT activation. Again a noticeable difference in the dissociation profile was observed for the quinazoline compound **25b** like the corresponding thioether analog **25a**. Table 4 lists some of the C-21 modifications in the phenyl and cyclohexyl pyrazole series. These compounds showed a similar overall profile to fluorophenyl series. Interestingly, the best compounds identified in the phenyl series were the thio and oxo quinoxaline compounds **50a** and **50c**. The benzimidazole compound **44b** and the quinoxaline compound **50b** were identified as the best compounds in the cyclohexyl series.

In order to evaluate some of these compounds anti-inflammatory activity in vivo, the blood levels in rats following oral administration were determined (Table 5). Compounds **11**, **27**, **43a**, 46 showed good blood levels, with a comparable pharmacokinetic profile to that of prednisolone. We were interested in the quinoxaline compound **25a** and **50a** due to the negligible blood levels as a possible inhaled steroid. However, these two compounds did not demonstrate significant inhibition of total cells when dosed intratracheally in Brown-Norway rat allergic lung inflammation model. The compounds with better blood levels were evaluated in a three day Brown-Norway rat oral allergic lung inflammation model. The

Table 5
Rat PK and in vivo transrepression and transactivation screening

Compds	BNR lung inflammation (3 d, 10 mg/kg po) % total cell inhibition	BNR thymolysis (3 d, 10 mg/kg po) % thymus wt decrease	Rat PK AUC (hr ng/mL)/ Cmax (ng/mL)
2	56	53	1252/682
11	NT	NT	1044/240
25a	9*	NT	0/0
27	48	70	1631/348
32	39	5	150/115
36	39	0	274/255
43a	NT	NT	2484/628
46	62	67	2034/467
50a	28*	NT	64/21

* Meaning compound is tested in intratracheal assay (it) at 2 mg/kg and not po.

anti-inflammatory activity was measured as the percent inhibition of the total number of cells, while transactivation was measured by the percentage decrease of the thymus[10] weight after dosing the compound at 10 mg/kg orally for three days. Although compounds **27** and **46** showed significant inhibition of total cells, they showed significant thymolysis compared to the hydrocortisone analogs **32** and **36** and the previously reported[1b] prednisolone compounds.

In summary, the A ring pyrazole modification of hydrocortisone combined with various C-21 thio and oxo heteroaryl modification generated compounds with potent anti-inflammatory activity and moderate dissociation profile in in vitro functional assays. Although the key compounds failed to differentiate efficacy and thymolysis, we have continued interest in these types of compounds. The discovery of a selective glucocorticoid receptor modulator may offer potential therapeutic benefits over the existing oral anti-inflammatory steroids for treatment of various inflammatory conditions.

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References and notes

1. (a) Biju, P.; McCormick, K.; Aslanian, R.; Berlin, R.; Solomon, D.; Chapman, R.; McLeod, R.; Prelusky, D.; Eckel, S.; Kelly, G.; Natiello, M.; House, A.; Fernandez, X.; Bitar, R.; Phillips, J.; Anthes, J. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 6343; (b) Biju, P.; McCormick, K.; Aslanian, R.; Berlin, M.; Solomon, D.; Wang, H.; Lee, Y. J.; Bitar, R.; Prelusky, D.; McLeod, R.; Jia, Y.; Fernandez, X.; Eckel, S.; House, A.; Lieber, G.; Jimenez, J.; Kelly, G.; Chapman, R.; Phillips, J.; Anthes, J. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 1086.
2. Kauppi, B.; Jakob, C.; Farnegardh, M.; Yang, J.; Ahola, H.; Alarcon, M.; Calles, K.; Engstrom, O.; Harlan, J.; Muchmore, S.; Ramqvist, A. K.; Thorell, S.; Ohman, L.; Greer, J.; Gustafsson, J. A.; Carlstedt-Duke, J.; Carlquist, M. *J. Biol. Chem.* **2003**, *278*, 22748.
3. Friesner, R. A.; Banks, J. L.; Murphy, R. B.; Halgren, T. A.; Klicic, J. J.; Mainz, D. T.; Repasky, M. P.; Knoll, E. H.; Shelley, M.; Perry, J. K.; Shaw, D. E.; Francis, P.; Shenkin, P. S. *J. Med. Chem.* **2004**, *47*, 1739.
4. (a) Barnes, P. J. *Clin. Sci. (London, England: 1979)* **1998**, *94*, 557; (b) Rhen, T.; Cidlowski, J. A. *N. Engl. J. Med.* **2005**, *353*, 1711.
5. (a) Harmon, J. M.; Schmidt, T. J.; Thompson, E. B. *J. Steroid Biochem.* **1981**, *14*, 273; (b) Madauss, K. P.; Bledsoe, R. K.; Mclay, I.; Stewart, E. L.; Uings, I. J.; Weingarten, G.; Williams, S. P. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 6097.
6. (a) Clinton, R. O.; Manson, A. J.; Stonner, F. W.; Neumann, H. C.; Christiansen, R. G.; Clarke, R. L.; Ackerman, J. H.; Page, D. F.; Dean, J. W.; Dickinson, W. B.; Carabateas, Clarissa *J. Am. Chem. Soc.* **1961**, *83*, 1478; (b) Clinton, R. O.; Manson, A. J.; Stonner, F. W.; Beyler, A. L.; Potts, G. O.; Arnold, Aaron *J. Am. Chem. Soc.* **1959**, *81*, 1513.
7. DeRuggieri, P.; Gandolfi, C.; Chiaramonti, D. *Gazz. Chim. Ital.* **1962**, *92*, 768.
8. (a) Tishler, M.; Steinberg, N. G.; Hirschmann, R. F. **1962**, 24 pp US 3067193.; (b) Hirschmann, R.; Buchschacher, P.; Steinberg, N. G.; Fried, J. H.; Ellis, R.; Kent, G. J.; Tishler, M. *J. Am. Chem. Soc.* **1964**, *86*, 1520.
9. For biological assay details, refer WO 2009085879.
10. Reichardt, H. M.; Kaestner, K. H.; Tuckermann, J.; Kretz, O.; Wessely, O.; Bock, R.; Gass, P.; Schmid, W.; Herrlich, P.; Angel, P.; Schutz, G. *Cell* **1998**, *93*, 531.