

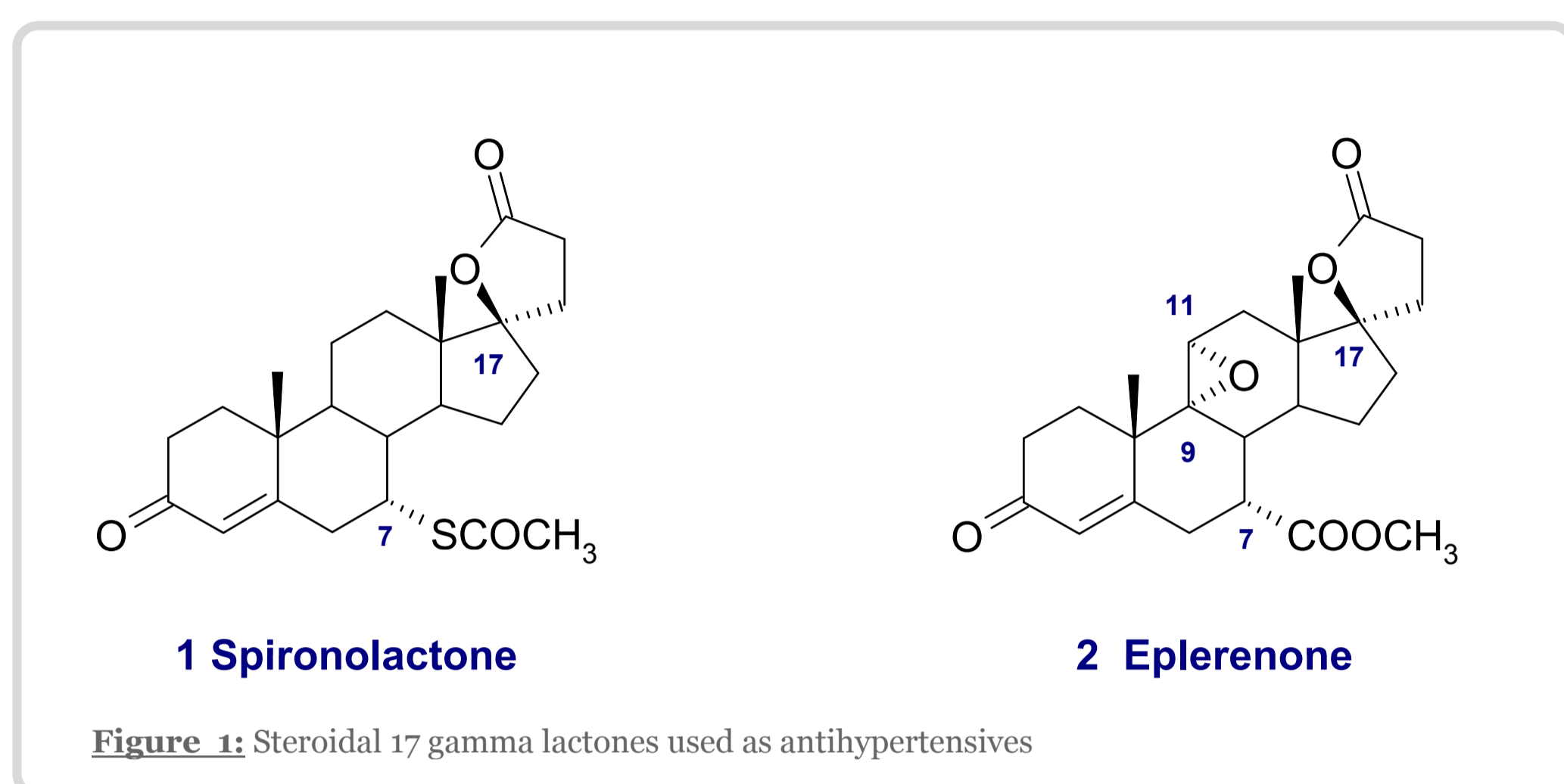
New steroidal mineralocorticoid antagonists

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Introduction

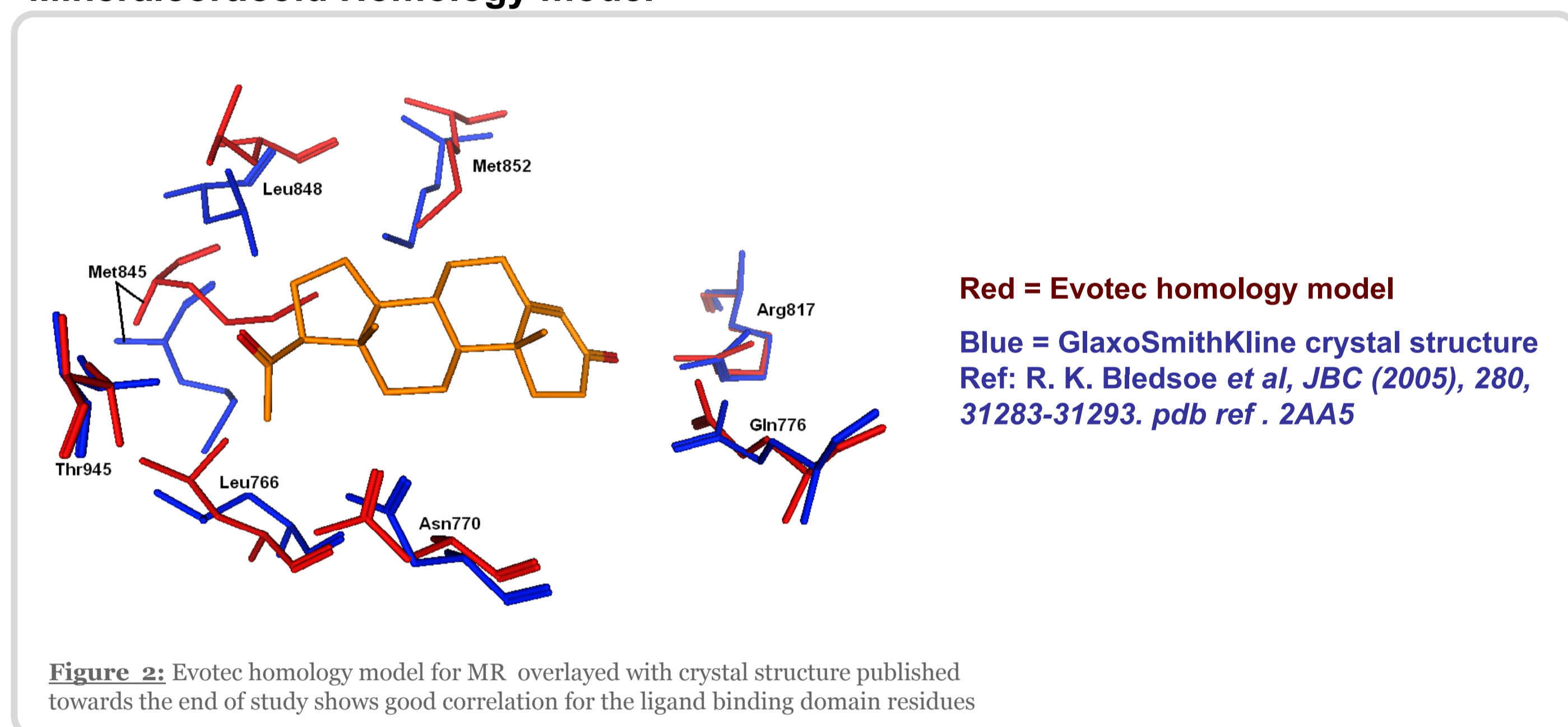
The most widely used aldosterone (Mineralocorticoid Receptor) antagonists are spiro lactones, which have steroidal structures characterised at carbon 17 by a gamma lactone. **Spirolactone 1** and **Eplerenone 2** have both been used in the clinic for the treatment of hypertension. Spirolactone's use is however limited due to adverse clinical effects associated with lack of selectivity over other steroid receptors such as androgen (AR), estrogen (ER), glucocorticoid (GR) and progesterone (PR). Eplerenone shows increased selectivity over spiro lactone due to key structural changes alleviating undesired side effects however it provides a 20 to 30-fold lower affinity for human MR and is only 50-75% as potent as spiro lactone *in vivo*.



Objectives

The goal of our collaboration is the identification of selective, potent, novel aldosterone antagonists related to the 17-spirolactam framework for the treatment of hypertension and indications such as cardiovascular disease. The novel analogues should have clinical advantages over 17 gamma lactones already in the clinic.

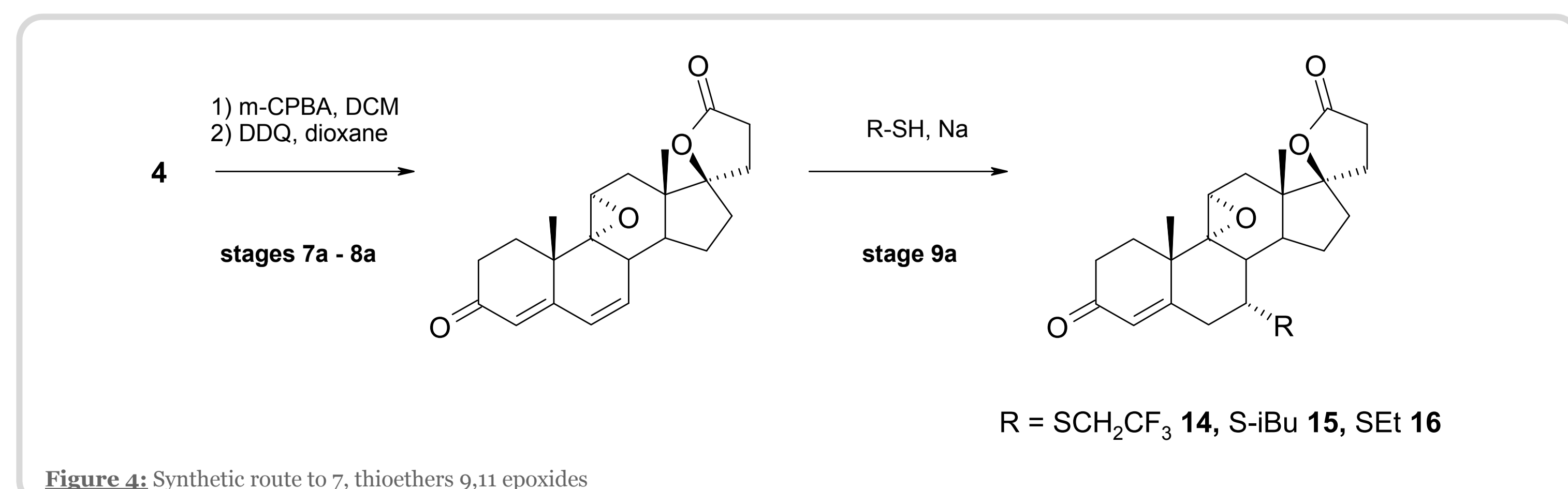
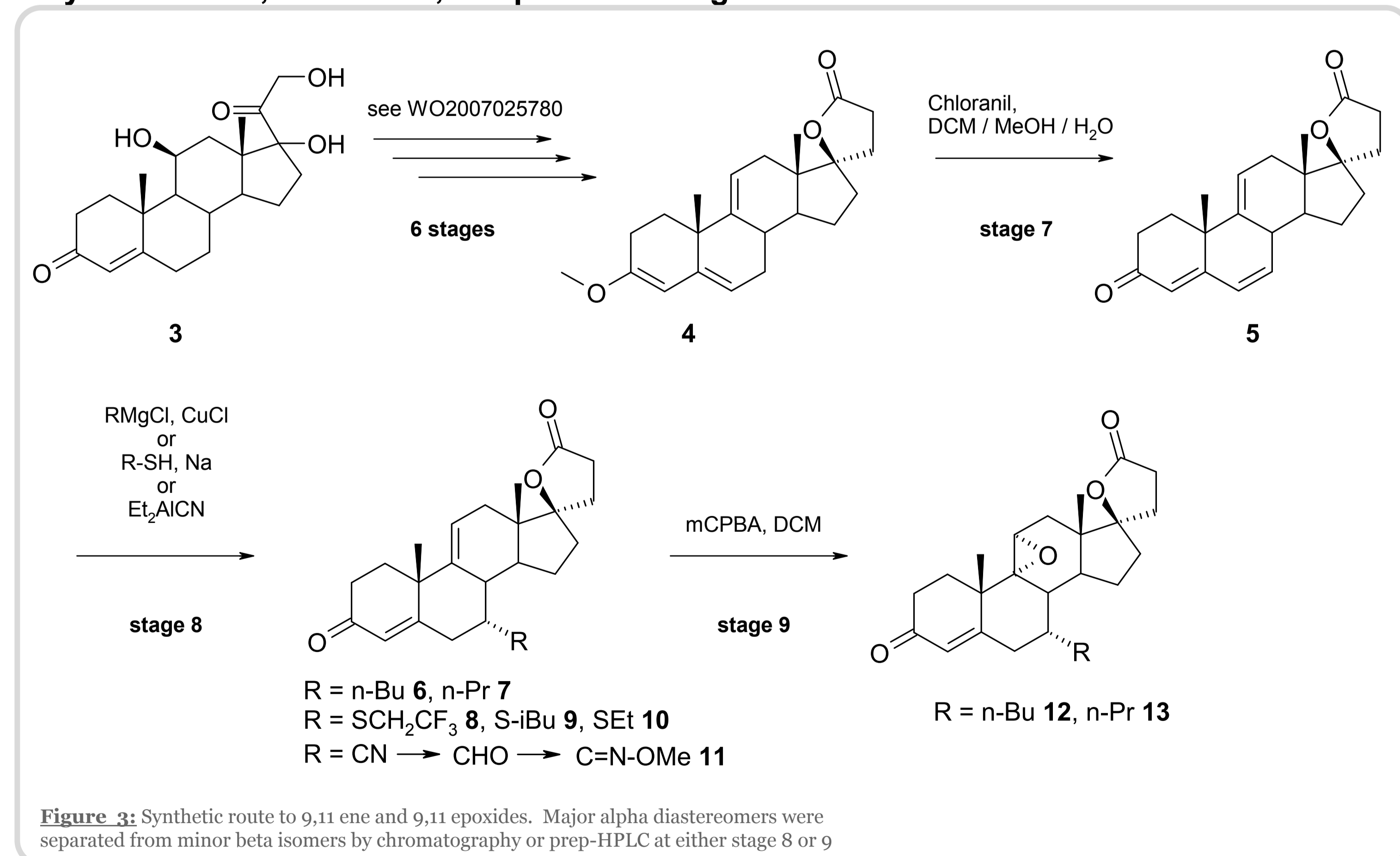
Mineralocorticoid Homology model



Strategy

The initial strategy involved the preparation of a series of 9,11 enes and epoxides modified at the 7 position. The MR homology model was employed to aid analogue selection, specifically avoiding 7 substituent clashes with the receptor. The synthesis and biological results for selected compounds are detailed.

Synthesis of 9, 11 ene & 9, 11 epoxide analogues



Scaleable synthetic routes were developed to support *in vitro* and *in vivo* studies.

Potency and selectivity data for analogues modified at positions 7,9 & 11

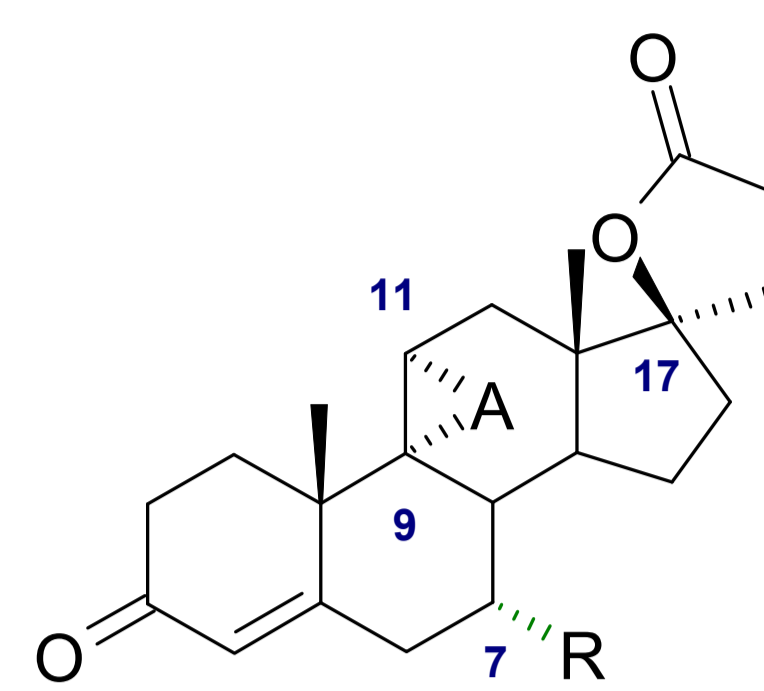


Table 1 highlights compound potencies in MR binding, agonist & antagonist assays. Additionally fold selectivities over AR, ER, GR and PR receptors are detailed. Compounds **10** & **16** highlighted in green demonstrated favourable profiles compared with spiro lactone & eplerenone.

Compd. No.	9,11 moiety	R	MR IC ₅₀ nM	AR fold selectivity (IC ₅₀ nM)	ER fold selectivity (IC ₅₀ nM)	GR fold selectivity (IC ₅₀ nM)	PR fold selectivity (IC ₅₀ nM)	Agonism @ 10 μM	Antagonism EC ₅₀ nM (Aldosterone 10 nM)
6	ene	CH ₂ CH ₂ CH ₃	6.5	>20	>100	<20	>100	11.6%	19.4
7	ene	CH ₂ CH ₂ CH ₃	31	>20	>100	<20	>100	12%	37.9
8	ene	S-CH ₂ CF ₃	51	>20	>100	>20	>20	12%	131
9	ene	S-CH ₂ CH ₂ CH ₃	8.7	>100	>100	>100	20	12%	16.9
10	ene	S-CH ₂ CH ₂ CH ₃	43	20 (780)	>100 (>100K)	>20 (1428)	>100 (>100K)	8.6%	56
11	ene	N-O	57.9	<20	>100	>20	>100	8.1%	91.6
12	epoxide	CH ₂ CH ₂ CH ₃	18	>100	>100	>20	>100	12.8%	110
13	epoxide	CH ₂ CH ₂ CH ₃	26	>100	>100	20	>20	22%	28
14	epoxide	S-CH ₂ CF ₃	695	>20	>100	>100	20	11%	568
15	epoxide	S-CH ₂ CH ₂ CH ₃	738	>100	>100	>100	>100	1.1%	684
16	epoxide	S-CH ₂ CH ₂ CH ₃	252	>100 (>100K)	>100 (>100K)	>20 (16,560)	>100 (>100K)	16.4%	85
17	epoxide	N-O	130	>20	>100	<20	<20	5.6%	139
1	9, 11 saturated	Spirolactone	126	<20 (338)	>100 (>50K)	<20 (1116)	<20 (1908)	4.8%	150
2	epoxide	Eplerenone	3569	>20 (>100K)	>20 (>100K)	<20 (59K)	>20 (200K)	4.8%	3688

Table 1: *In vitro* data for steroidal 17 gamma lactone analogues

ADME data for leading analogues

Comparison of 7- α thioethyl compounds **10** and **16** with spiro lactone & eplerenone. **16** demonstrated the most favourable profile to the standards.

Compound Number	R	Aq. Solubility (mg/ml)	PAMPA (pH 6.4)	SPB % unbound	Human micros t _{1/2}	Rat micros t _{1/2}	%inhib CYP450 2C19, 2D6, 3A4 10 μM
10	S-CH ₂ CH ₂ CH ₃	0.02	13	16	15.8	2.6	12 / 2 / 1
16	S-CH ₂ CH ₂ CH ₃	0.06	24	58	46.2	21.6	5 / -0.4 / 1
1	Spirolactone	0.07	46	22	24.4	5.1	20 / 18 / 0
2	Eplerenone	0.43	22	46	39	26	2 / 22 / 0

Table 2: Selected ADME data for leading compounds

In Vivo Results – Kagawa rat model

Ene **10** and epoxide **16** provided a statistically significant increase in urinary Na⁺/K⁺ ratio relative to animals only receiving aldosterone (no test compound). The results of the assay show that **10** and **16** effectively antagonise the effects of aldosterone *in vivo* (subcutaneous administration).

Conclusions

- Synthetic routes to analogues established using homology modeling to prioritise targets
- Good correlation between binding and functional activity for the MR was identified
- Compounds with a 7 alkyl chain are very potent as either 9,11 ene or 9, 11 epoxide
- Several compounds resulted in high selectivity for MR against GR
- Compounds did not significantly activate MR (weak to very weak MR agonists)
- Acceptable ADME profiles were identified for leading analogues
- Compounds were identified which are selective MR antagonists with some analogues more potent and selective than eplerenone and spiro lactone
- Compounds **10** and **16** showed significant *in vivo* activity in the Kagawa model
- Analogues of interest were selected for further investigation towards meeting clinical goals