Aryl aminopyrazole benzamides as oral non-steroidal selective glucocorticoid receptor agonists

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ABSTRACT

Aryl aminopyrazole amides capped with *N*-alkylbenzamides 13–16 are selective glucocorticoid receptor agonists. 2,6-Disubstituted benzamides have prednisolone-like potency or better in vitro. Good oral exposure was demonstrated in the rat, with compounds with lower lipophilicity, for example *N*-hydroxy-ethyl benzamides (e.g., 16e).

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Glucocorticoid receptor (GR) agonists such as prednisolone 1 (Fig. 1) or fluticasone esters 2 have potent anti-inflammatory and immunosuppressive properties. There has been considerable effort in recent years to try to produce selective anti-inflammatory drugs which act as GR ligands that suppress the expression of inflammatory cytokines (transrepression activity) but do not affect genes associated with typical steroid side effects (transactivation activity) such as glucose intolerance, muscle wasting, skin thinning and osteoporosis.[1–3] Boehringer–Ingelheim have demonstrated that a molecule with such dissociated pharmacology BI-115 3, had an improved side effect profile over 1 in a mouse model.[3] Another cause of off-target pharmacology with some GR agonists is cross-reactivity with other members of the nuclear receptor (NR) family, such as the progesterone receptor (PR) androgen receptor (AR) and mineralocorticoid receptor (MR). Selectivity in this respect has also been achieved with some non-steroidal compounds.[4,5]

We have previously reported a series of GR agonists containing a tetrahydronaphthalene (THN) group, for example, 5a,b, in which both NR and transrepression (TR) selectivity can be achieved.[4,5] The angular alkyl group R in 5a,b is crucial for agonist activity and it was designated an agonist trigger. Compound 5a, in which R is ethyl, is a full agonist in both TR and transactivation (TA) assays, whereas 5b, in which R is cyclopentyl, is selective for TR. In this series, we found that the aryl aminopyrazole group is a good replacement for the steroidal cyclohexadienone A-ring. We[5] and other groups[6–8] report the use of an aryl indazole, reminiscent of that present in cortivazol 4a. The X-ray crystal structure of deacylcortivazol 4b bound into the GR ligand binding domain (LBD)[9] showed that the space occupied by the steroidal A-ring in the active site, can open up to accommodate the arylpyrazole structure.

Although the THN series had attractive pharmacology, it was structurally complex and more hydrophobic than typical oral drugs. We thus envisaged replacing the THN portion by an aryl group linked to the remainder of the molecule by a hydrophilic linker such as an amide, to give molecules 13–16 (Scheme 1) with physicochemical properties more appropriate for oral drugs. The aryl group was designed to occupy the hydrophobic pocket populated by the 17α ester group in FP and FF 2, and R^1 the position occupied by the trigger group R in the THN series.

Molecular modelling of a typical example, the 2,6-dichlorobenzamide 13h, supported this concept. The structural information about the GR LBD from our recently published fluticasone furoate structure[10] was used to provide the starting point for the docking experiments used to help guide this work. The docking models were created using FLO+ which allows flexibility of side chains during docking.[5] The model created for this series is compared in Figure 2a–c with the previous model developed for arylpyrazoles[5]

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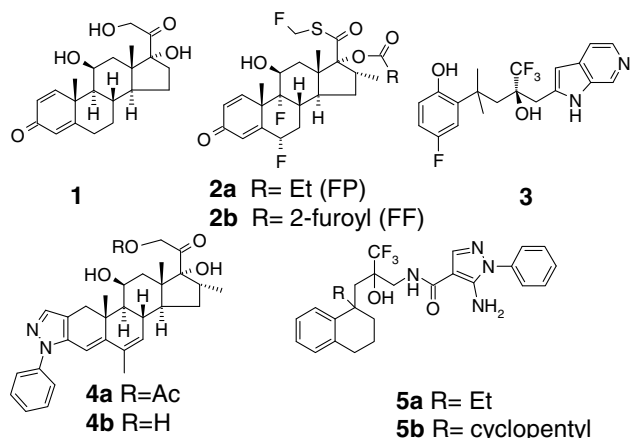 *E-mail address:* ggw1517@yahoo.com (G.G. Weingarten).

Figure 1. Examples of some steroidal and non-steroidal GR agonists.

and with the structure for fluticasone furoate **2b**. In Figure 2a, showing the docking model for **13h**, N2 of the arylpyrazole moiety shows H-bonding to Gln570, and the central hydroxyl group is seen H-bonding to Asn564; S configuration is predicted at this centre. Figure 2b shows a superimposition of the model for **13h** and the FF crystal structure. The similar positioning of the furoate group and the dichlorophenyl group can be seen. Both the model and crystal structure have Gln642 and Tyr735 positioned similarly allowing access to the steroid 17α pocket. In addition, the opening of the receptor to accommodate the arylpyrazole moiety is apparent, through shifts in Arg611 and Gln570 relative to their positions in the steroidal structure in which Gln570 H-bonds to the 3-keto group. In Figure 2c the models for **13h** and the THN **5a** are superimposed. The ethyl trigger groups are seen to be very similarly placed. Gln642 and Tyr735 can be seen to adopt a different position for **13h** compared to **5a**, in order to accommodate the dichlorophenyl group. In contrast to FF **2b** and the THN **5a**, the model shifts the Arg611 side chain out of H-bond contact with Gln570 for **13h**, apparently caused by interaction with the *para*-fluoro substituent.
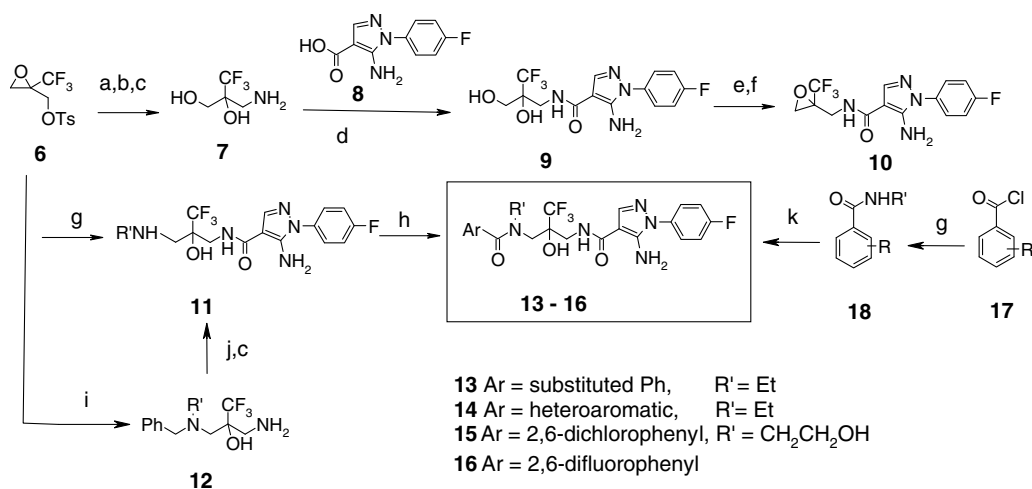
The synthesis of compounds **13–16** is shown in Scheme 1. We recently reported the synthesis of a versatile doubly electrophilic α-trifluoromethyl-α-tosyloxymethyl epoxide synthon **6**.[11] Compound **6** was treated with a slight excess of benzylamine in dioxan

to open the epoxide ring and then with hot aqueous sodium hydroxide to give a benzylaminodiol from which the benzyl group was removed by hydrogenolysis to furnish the aminodiol **7**. The aminopyrazole acid **8** was coupled to **7** using HATU to give **9**. The terminal hydroxyl was tosylated, following which, treatment with polymer-bound carbonate in THF gave the epoxide **10**. The trigger group was installed by opening the epoxide **10** with an alkylamine to give **11**. In order to prepare compounds incorporating a hydroxyethyl trigger group, the hydroxyl group of ethanolamine was protected either as a TBDMS ether (by treatment with TDMSCl and imidazole in dichloromethane) or as a *t*Bu ether, and the protecting group was removed by conventional means as a final step. Amine **11** was capped with a benzoic or heteroaromatic acid to give compounds **13–16**. HATU was used as the coupling reagent unless the benzoic acid was 2,6-disubstituted, in which case it was usually necessary to use an acid chloride.[12]

Alternatively, a benzoyl chloride **17** could first be reacted with the trigger amine to give an *N*-alkylbenzamide **18** which after deprotonation with sodium hydride, added to the aminopyrazole epoxide **10** to give compounds **13–16**. The synthesis could be shortened by treating the epoxytosylate **6** in dioxan with an *N*-alkylbenzylamine followed by ammonia to give the diamine **12**. This was then coupled to the aminopyrazole acid **8** and the benzyl group was removed by hydrogenolysis to yield the last stage intermediate **11**.

The more active compounds were separated into their enantiomers by preparative chiral HPLC.[13] The two enantiomers are designated E1 and E2, indicative of the order in which they eluted. Circular dichroism showed that there was no consistency in the order in which the two enantiomers eluted.

The compounds were assayed for their ability to bind to GR using competition experiments with fluorescent-labelled dexamethasone. A functional GR agonist assay was carried out using human A549 lung epithelial cells engineered to contain a secreted placental alkaline phosphatase gene under the control of the NFκB dependent ELAM promoter. This assay allows determination of the ability of compounds to repress transcription.[14] Transactivation activity was assayed using human A549 lung epithelial cells engineered to contain a renialla luciferase gene under the control of the distal region of the LTR from the mouse mammary tumor virus (MMTV).[5] Compounds were tested for nuclear receptor selectivity with respect to PR and AR by similar binding assays and functional assays using an MMTV-luciferase reporter in monkey kidney CV1 cells.[14,15]

Scheme 1. Synthesis of **13–16**. Reagents and conditions: (a) PhCH₂NH₂, dioxan, rt; (b) 2 M NaOH, dioxan, reflux; (c) H₂/Pd(OH)₂/C, ethanol; (d) HATU, DIPEA, DMF, rt; (e) TsCl, pyridine; (f) MP-CO₃ or PS-CO₃, THF; (g) R¹NH₂, THF; (h) ArCO₂H, HATU, DIPEA, DMF or ArCOCl (sometimes prepared in situ from ArCO₂H, oxalyl chloride and cat DMF), DIPEA, THF; (i) *N*-alkylbenzylamine, dioxan followed by ammonia in dioxan; (j) **8**, HATU, DIPEA, DMF; (k) NaH, DMF then **10**.
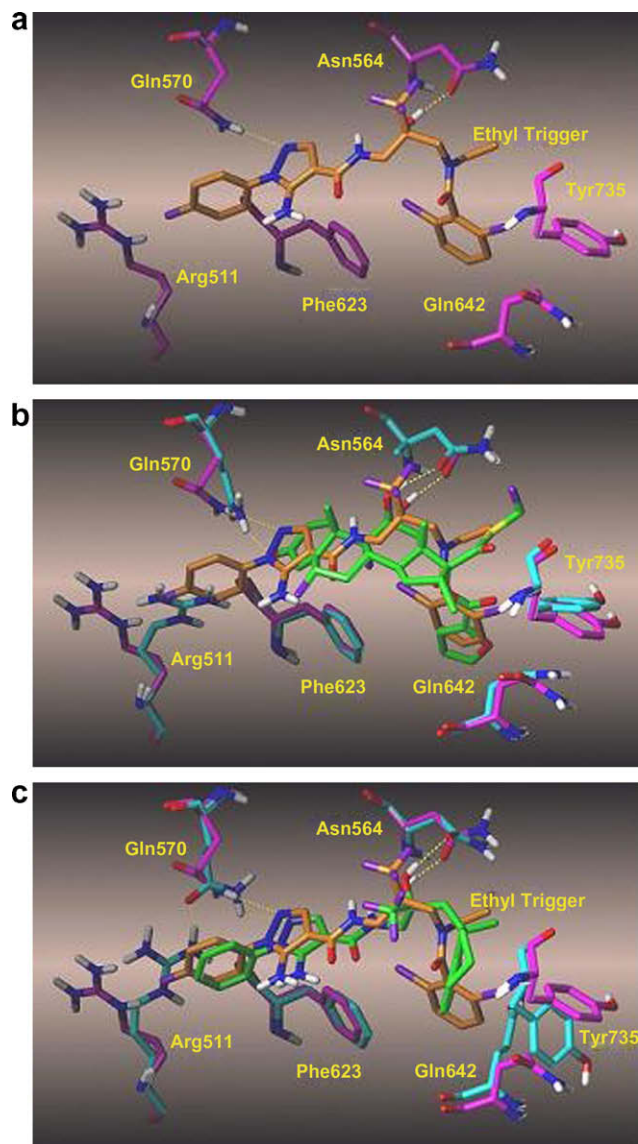
Figure 2. (a) The receptor docking model for the benzamide **13h**. (b) Superimposition of the model for **13h** (gold) and the FF **2b** (green) crystal structure. GR residues in magenta for **13h** and cyan for FF **2b**. (c) Superimposition of the models for **13h** (gold) and **5a** (green). GR residues in magenta for **13h** and cyan for **5a**.

DMPK profiles were also obtained routinely. CYP450 inhibition potential was assayed in a fluorimetric assay with Cypex cDNA expressed isoforms.[16] Human plasma protein binding was determined by an ultrafiltration method[17] and human serum albumin (HSA) binding was determined by a fast gradient HPLC method on an immobilised HSA HPLC column.[18] PK studies were carried out on selected compounds using adult male Sprague–Dawley rats.[19]

The prototype molecule, the *N*-ethylbenzamide **13a**, was a full agonist, about a quarter as potent as prednisolone **1**. In the TR NFκB assay it had pIC$_{50}$ 7.3 and in the TA MMTV assay it had pEC$_{50}$ 7.3. On testing the individual enantiomers, we found that one enantiomer bound more strongly than the other, consistent with modelling predictions, and hence the potency resided largely in this enantiomer. Thus the more slowly eluting enantiomer **13c** (E2) had GR binding pIC$_{50}$ 7.9 and NFκB pIC$_{50}$ 8 (which is equivalent to prednisolone **1**), while the faster eluting enantiomer **13b** (E1) had GR binding pIC$_{50}$ 6.9 and NFκB pIC$_{50}$ < 5 (Table 1).

It became apparent from the first arrays of structural analogues of the prototype molecule, that the lipophilicity needed to be relatively low in order to avoid high plasma protein binding, CYP450 inhibition and poor aqueous solubility. The strategy used to achieve this was to introduce heteroatoms into the phenyl substituents, the phenyl core and the trigger group.

Potency was enhanced by *ortho* substituents, but at the cost of increased lipophilicity. Analogues with polar substituents, for example, SO$_2$Me **13g** were less potent. A second *ortho* substituent enhanced potency further, so that 2,6-dichlorobenzamide **13h** had NFκB pIC$_{50}$ 9.3, and 2,6-difluorobenzamide **13i**, with a less severe increase in *c*log*P*, was 5 times as potent as prednisolone. Where the second *ortho* substituent reduced *c*log*P* as in the sulphone **13j**, potency enhancement was compromised. In **13j**, containing two dissimilar *ortho* substituents, restricted rotation in the tertiary amide group caused atropisomerism. Because of this complication such molecules were not progressed further. In contrast to the unsubstituted benzamide, the less active enantiomers of *ortho*-substituted benzamides had some measurable agonism, albeit typically partial (compare **13d** and **13e**). The more active 2,6-dichlorobenzamide enantiomer **13h** had the *S* configuration. This was determined from our recently reported X-ray crystal structure of **13h** in the GR LBD.[20] Comparison of CD spectra of active enantiomers of other examples with that of **13h**, indicated that they have the same *S* stereochemistry. All compounds showed excellent nuclear receptor selectivity with PR and AR binding pIC$_{50}$ < 5 and pEC$_{50}$ < 6 in cellular agonism assays.

No potency enhancement was seen with *meta* substituents on the phenyl ring. *Para* substitution as in **13m**, significantly reduced binding and potency, nullifying gains made by 2,6-disubstitution in a 2,4,6-trisubstituted benzamide (compare **13o** with **13h**). Heteroaromatic amides were active either with an *ortho* substituent in molecules such as **14a,b**, or where in a bicyclic amide, for example, indole **14c**, the heterocycle forms the *ortho* substituent. Potency was highly dependent on the position of the heteroatom as seen in pyridines **14a,b**. Two heteroatoms were generally not tolerated.

Different trigger groups were examined in an array of 2-chlorobenzamides, and the better ones as 2,6-difluorobenzamides (Table 2). The agonist potency rank order was Et > 2-fluoroethyl > *n*-Pr > 2-hydroxyethyl > Me ≫ CH$_2$CHMe$_2$, cyclopropyl > *n*Bu, H. Compounds with larger and branched alkyl trigger groups were less potent (and had higher *c*log*P*). In contrast to the THN series, none of the trigger groups gave potent compounds with TR selectivity. The power of the 2,6-disubstitution potency enhancement was such that even molecules which lacked a trigger group, for example, **16a** were active, but these were *partial* agonists. The hydroxyethyl group had a profound effect on lowering *c*log*P* but this came at the cost of reduced potency. However the 2,6-difluorobenzamide **16e** struck a good balance with *c*log*P* of 2.64 and prednisolone-like potency.

Table 3 shows the P450 inhibition and plasma protein binding profiles of selected examples. In general, it was found that molecules with *c*log*P* < 3 were likely to have P450 inhibition IC$_{50}$ > 3 μM for all the five isoforms tested. The P450 inhibition profiles were different for a pair of enantiomers, so that for example, **13e** had IC$_{50}$ > 4 μM for all isoforms while its enantiomer **13d** was 2–3 times more potent. The hydroxyethyl trigger was particularly effective at lowering *c*log*P*, but even molecules with *c*log*P* > 3 with this trigger group, for example, **15** had a satisfactory P450 inhibition profile. Plasma protein binding was also *c*log*P* dependent and the hydroxyethyl trigger group was particularly effective in reducing plasma protein binding. Thus **16e** with both the advantage of a low lipophilicity and a hydroxyethyl trigger was only 68% bound to human plasma protein.

Table 4 shows rat PK data for selected examples. This series typically had moderate clearance and volume of distribution and moderate or good oral bioavailability in rats. The strategies employed for lowering lipophilicity by adding heteroatoms raised

Table 1
Biological data for *N*-ethyl arylamides (**13**, **14**).

Compound	(13) Ph substituents (14) Ar =	Enant[a]	$clogP$[b]	GR binding pIC$_{50}$[c]	NFκB pIC$_{50}$[d]	NFκB max[e] (%)	MMTV pEC$_{50}$[f]	MMTV max[g] (%)
1	Prednisolone			7.9	8	100	7.3	81
13a	–	rac	3.08	7.3	7.3	88	7.3	104
13b	–	E1	3.08	6.9	<5	–	<5.2	–
13c	–	E2	3.08	7.9	8	94	7.6	108
13d	2-Cl	E1	3.87	nd	8.5	95	8.2	113
13e	2-Cl	E2	3.87	nd	7	75	6.5	70
13f	2-F	E2	3.3	7.9	7.9	98	7.7	130
13g	2-SO$_2$Me	E1	1.86	7.2	7.7	99	6.8	120
13h	2,6-DiCl	E2	4.61	8.5	9.3	101	8.7	120
13i	2,6-DiF	E2	3.46	8.1	8.6	104	8.1	122
13j	2-Cl,6-SO$_2$Me	rac	2.62	7.3	8.0	103	7.6	128
13k	3-Cl	rac	3.87	7.8	7.5	96	6.6	125
13l	2-Cl,3-F	rac	4.04	8.1	7.7	92	7.3	106
13m	4-Cl	rac	3.87	6.5	<5	–	<5.2	–
13n	2-Cl,4-F	rac	4.04	7.7	7	76	6.5	56
13o	2,4,6-TriCl	rac	5.33	8.2	7.6	100	7.3	86
14a	2-Cl-pyrid-3-yl	rac	2.73	7.4	6.6	75	6.1	74
14b	3-Cl-pyrid-2-yl	E1	2.72	nd	8	99	7.4	93
14c	5-Indolyl	E1	3.37	8.2	9	100	8.5	112

[a] Data shown for more active enantiomer where this was available or otherwise for racemate.
[b] Calculated using daylight.
[c] Generally *n* = 1.
[d] Mean potency for $n \geqslant 2$ SD ± 0.34 using the pooled variance from the tested compounds.
[e] Mean asymptotic maximum relative to dexamethasone SD ± 8.5% using the pooled variance from the tested compounds.
[f] Mean potency for $n \geqslant 2$; SD ± 0.23 using the pooled variance from the tested compounds.
[g] Mean asymptotic maximum relative to dexamethasone SD ± 13.6% using the pooled variance from the tested compounds.

Table 2
Biological data for *N*-alkyl 2,6-difluorobenzamides (**16**).

Compound	Trigger R^1	Enant[a]	$clogP$[b]	GR binding pIC$_{50}$[c]	NFκB pIC$_{50}$[d]	NFκB max[e] (%)	MMTV pEC$_{50}$[f]	MMTV max[g] (%)
16a	H	rac	2.28	6.4	6.5	80	<5.2	
16b	Me	rac	2.94	7.1	7.3	95	6.6	112
13i	Et	E2	3.46	8.1	8.6	104	8.1	122
16c	*n*Pr	rac	3.99	7.8	7.8	96	7.6	128
16d	CH$_2$CH$_2$F	rac	3.19	7.7	8.0	94	7.3	105
16e	CH$_2$CH$_2$OH	E2	2.64	7.8	7.7	94	7.3	119

[a–g] Notes as for Table 1.

Table 3
P450 inhibition[a] and binding to human plasma protein[b] (PPB) or human serum albumin[c] (HSA) for selected compounds.

Compound	R^1	Substituents	$clogP$	1A2 (μM)	2C19 (μM)	2C9 (μM)	2D6 (μM)	3A4 (μM)	HSA (%)	PPB (%)
13g	Et	2-SO$_2$Me	1.86	$\geqslant$40	87	23	96	3	81	nd
16e	CH$_2$CH$_2$OH	2,6-DiF	2.64	100	40	13	100	76	81	68
14b	Et	3-Cl-pyrid-2-yl*	2.73	100	100	14	100	4	83	85
16b	Me	2,6-DiF	2.94	100	49	10	36	100	nd	nd
16d	CH$_2$CH$_2$F	2,6-DiF	3.19	100	8	4	20	0.5	93	93
13i	Et	2,6-DiF	3.46	100	18	3	74	2	93	97
15	CH$_2$CH$_2$OH	2,6-DiCl	3.78	100	20	3	54	100	89	93
13d	Et	2-Cl	3.87	100	8	4	27	2	nd	nd
13e	Et	2-Cl	3.87	100	20	10	34	4	nd	nd
16c	Pr	2,6-DiF	3.99	$\geqslant$40	5	3	44	0.5	94	nd
13h	Et	2,6-DiCl	4.61	100	7	0.8	89	0.7	95	99

[a] $n \geqslant 1$.
[b] $n \geqslant 2$.
[c] $n = 1$.
[*] Ar=.

the polar surface area; nevertheless such molecules showed improved oral exposure. Examples of this are molecules with a hydroxyethyl trigger **16e** or an *ortho*-sulphone substituent **13g,j**, or where phenyl was replaced by pyridine **14b**. However, compounds with higher *clogP*, for example, **16c** had higher clearance and volume of distribution resulting in lower oral exposure as well as having a low free fraction.

In summary, we have shown that the aryl aminopyrazole pharmacophore discovered in the context of our THNs can be linked to a benzamide bearing a trigger group to give potent agonists, selective for GR over other NRs, which have oral-drug-like lipophilicity and good oral exposure of free drug. These molecules are full agonists and do not display dissociated TR/TA pharmacology. The molecule that emerged with the best overall profile is **16e**. A full

Table 4
Pharmacokinetics of selected compounds in rat[19].

Compound	R^1	Substituents	Cl (mL/min/kg)	V_{ss} (L/kg)	%F
13a	Et	–	21	2.5	12
13i	Et	2,6-DiF	27	2.8	36
16c	nPr	2,6-DiF	36	5.3	16
16d	CH_2CH_2F	2,6-DiF	21	2.6	39
16e	CH_2CH_2OH	2,6-DiF	8	0.9	44
13d[*]	Et	2-Cl	32	4	25
14b[*]	Et	3-Cl-pyrid-2-yl[**]	23	2.8	48
13g[*]	Et	2-SO_2Me	17	3.6	77
13j	Et	2-Cl,6-SO_2Me	9	2.6	62

[*] Data for racemate.
[**] Ar=.

in vitro and in vivo study in which it is compared with a steroid will be reported.

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