Bioorganic & Medicinal Chemistry Letters 20 (2010) 6237–6241

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Optimisation of 2-cyano-pyrimidine inhibitors of cathepsin K: Improving selectivity over hERG

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ARTICLE INFO

Article history:
Received 6 August 2010
Revised 18 August 2010
Accepted 19 August 2010
Available online 24 August 2010

Keywords:
Cathepsin K
Cysteine protease
Osteoporosis

ABSTRACT

Several structure-guided optimisation strategies were explored in order to improve the hERG selectivity profile of cathepsin K inhibitor **1**, whilst maintaining its otherwise excellent in vitro and in vivo profile. Ultimately, attenuation of *c* log *P* and pK_a properties proved a successful approach and led to the discovery of a potent analogue **23**, which, in addition to the desired selectivity over hERG (>1000-fold), displayed a highly attractive overall profile.

Osteoporosis is the most common bone disorder which predominately affects postmenopausal women, but also occurs in premenopausal women as well as in men. It is a skeletal disorder which is characterised by low bone mass and an accompanying micro-architectural deterioration that results in skeletal fragility and an increased risk of fracture.[1] Bone tissue is renewed every 5–7 years throughout the skeleton by a dynamic process involving balanced bone resorption and formation in which osteoclasts and bone-forming osteoblasts play a critical role. The bone loss associated with osteoporosis is attributed to an imbalance between these two processes. Cathepsin K (catK) is one of 11 lysosomal cysteine proteases from the papain family expressed in the human genome, and is highly expressed in osteoclasts, which suggests an important role in bone remodelling.[2]

A large body of evidence accumulated over the last decade indicates that inhibition of catK is an important therapeutic approach in the treatment of osteoporosis.[3]

We recently disclosed a novel series of 2-cyano-pyrimidines as potent inhibitors of cathepsin K, typified by compound **1** (Fig. 1).[4]

Despite attractive in vitro and in vivo profiles of several compounds in this series, their further progression was precluded by a prohibitively high hERG potency (e.g., **1** hERG IC_{50} = 160 nM). Blockade of the hERG channel is associated with QT interval prolongation which, in turn, can cause a life threatening cardiac arrhythmia *torsades de points* (TdP).[5] Therefore, we endeavoured to improve hERG selectivity, whilst retaining the desirable in vitro and in vivo properties of compounds in this series.

Our aim was to achieve a minimum 100-fold separation between activity in the functional bone resorption (rabbit osteoclast, ROC[6]) and hERG ([^{3}H]-Dofetilide binding) assays. This margin translated into a >1000-fold catK/hERG selectivity requirement in order to take into account up to a 10-fold lower potency of our compounds in the ROC assay when compared to the catK

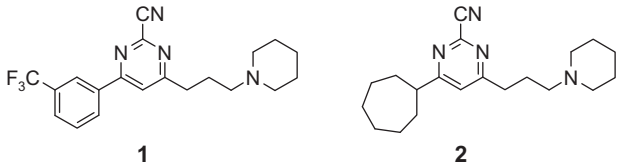

Figure 1. Lead compound **1** and its P2-aliphatic analogue **2**.

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doi:10.1016/j.bmcl.2010.08.101

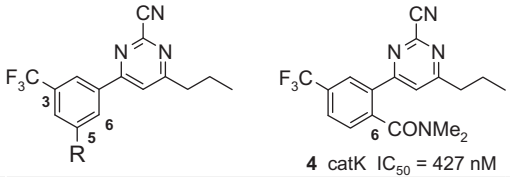
enzymatic assay. This selectivity profile was expected to ensure at least a 30-fold safety window in vivo between the plasma free fraction C_{max} values for a therapeutic dose, and maximum dose devoid of any changes in the QT interval.[7]

Basing our rationale on reported mutation studies and homology models, we postulated that the CF_3-phenyl moiety of **1** participates in hydrophobic/π-stacking interactions with Phe656, whilst the amine group may form a cation-π interaction with Tyr652, another aromatic residue in the hERG S6 domain that forms the channel's large intracellular cavity.[8] Discrete modification of substitution around the aromatic ring or its replacement by a non-aromatic group, which aims to disrupt the interaction of a ligand with the above mentioned aromatic residues in the hERG S6 domain, are reportedly successful medicinal chemistry strategies for removing hERG block.[9] Unfortunately, in this series, non-aromatic P2 derivatives such as the cycloheptyl **2** retained high hERG binding whilst displaying markedly reduced catK potency (**2** catK IC_{50} = 100 nM, hERG = 0.7 μM).

An alternative strand of the above strategy, which was adopted to further explore SAR around this series as well as to improve selectivity over hERG, was born out of the biostructural information derived from the crystal structure of **1** bound to cathepsin K.[4] The structure indicated the possibility of transferring the amine moiety from the solvent-exposed prime side into the S3 pocket, where it may form favourable interactions with residues such as Asp61 or Tyr67 (Fig. 2). It was hypothesised that the new structural arrangement may improve selectivity by favoring catK over hERG binding. The solvent-exposed portion of the CF_3-phenyl group buried into the S2 pocket was considered to be the most suitable position for linking such an amino group (i.e., from C-5 or C-6). Since the phenyl ring was in a perpendicular orientation with respect to the S3 pocket, an appropriate 'bend' had to be incorporated into the linker in order to provide optimal directionality. As such, we opted to utilise an amide linker as the point of attachment to the phenyl ring; a tertiary amide would also provide an out of plane 'twist' which would be required to direct the P3-amine group into the pocket (Fig. 2).

In order to establish if such changes around the P2 group were tolerated, 6- and 5-dimethylcarbamide analogues **4** and **5** were initially synthesized (Table 1).[10] 6-Dimethylcarbamide **4** displayed a 10-fold lower catK potency (427 nM) compared to the parent compound **3**, making it an unsuitable starting point for extension into the S3 pocket. This was attributed to a greater torsion angle between the two aromatic moieties induced by the *ortho*-substitution in **4** (Table 1), compared to the 20°–30° angle required for positioning of the nitrile warhead and P2 group for optimal interaction with the Cys25 and S2 pocket in the cathepsin K active site.

Table 1
Optimisation of the P3 group



4 catK IC_{50} = 427 nM

Compds	R	catK[a] IC_{50} (nM)	hERG[b] K_i (μM)
3	H	33	ND
5	$CONMe_2$	12	>10
6	$CONMeCH_2CH_2NMe_2$	4	0.71
7	$CONBnCH_2CH_2NMe_2$	<1	0.12
8	CONMeBn	9	0.37

[a] Inhibition of recombinant human cathepsin K in a fluorescence assay, employing Z-Phe-Arg-MCA as synthetic substrates. Data represent means of two experiments performed in duplicate.

[b] Inhibition of [^{3}H]-Dofetilide binding to hERG K^+ channel in HEK293 cells following a 10 μM dose of test compound. Data represent means of two experiments performed in duplicate.

Gratifyingly, attachment of the dimethylcarbamide group onto the adjacent C5 position proved more successful. The resulting 5-dimethylcarbamide analogue **5** displayed a threefold improvement in catK potency compared to **3** (Table 1). The crystal structure of **5** co-crystallized with catK suggested a 'face-to-face' interaction between the amide group and Tyr67 at the entrance to the S3 pocket, which may account for the observed improvement in catK potency (Fig. 3).

Encouraged by this result, amide **6**, with a dimethyl amine group linked through an ethyl spacer, was prepared.[10] As initially hypothesised, the P3-amine-containing analogue **6** displayed a low nanomolar catK potency, similar to that of the lead compound **1** which featured the amine group in the prime side of the molecule. Unfortunately, this compound also proved to be a potent hERG blocker (0.71 μM). Further attempts to optimise interactions within the S3 pocket were successful only when lipophilic aromatic groups were incorporated, such as the benzyl group in compound **7**. The co-crystal structure of **7** with catK provided a rationale for this observation (Fig. 4). In this case, the benzyl group

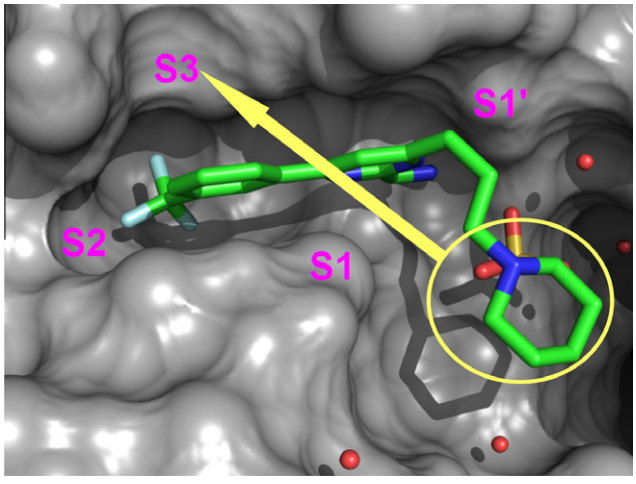


Figure 2. Co-crystal structure of pyrimidine **1** with cathepsin K (1.9 Å resolution; PDB 3KWZ). The sulphate molecule in the prime side is an artefact of the crystallization.

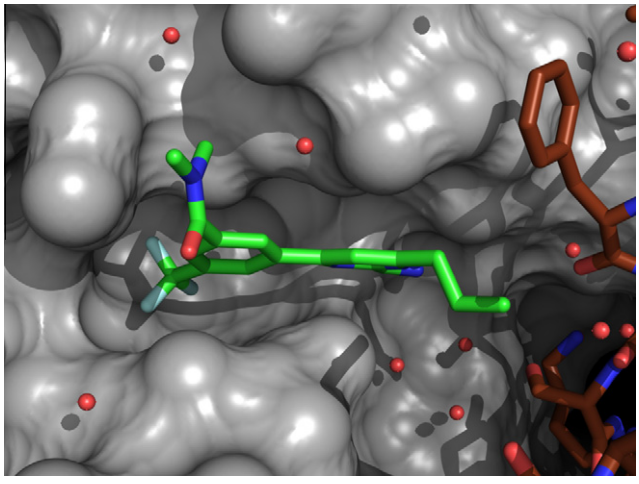


Figure 3. Co-crystal structure of **5** with cathepsin K (1.8 Å resolution; PDB 3O0U). The amide group is at an angle of approx. 90° with respect to the phenyl ring, positioned in parallel to Tyr67, and pointing into the S3 pocket.

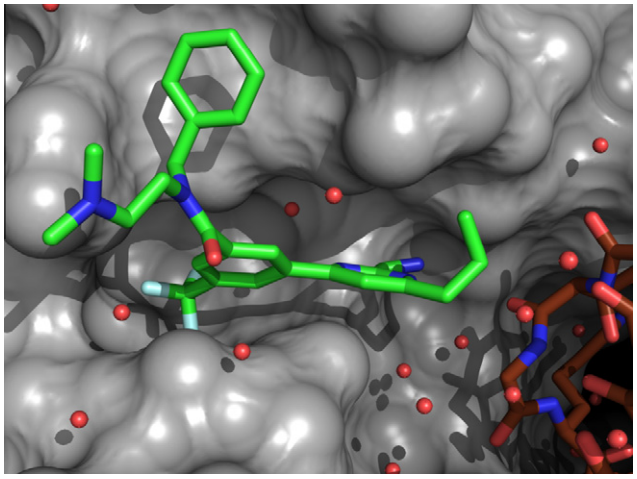
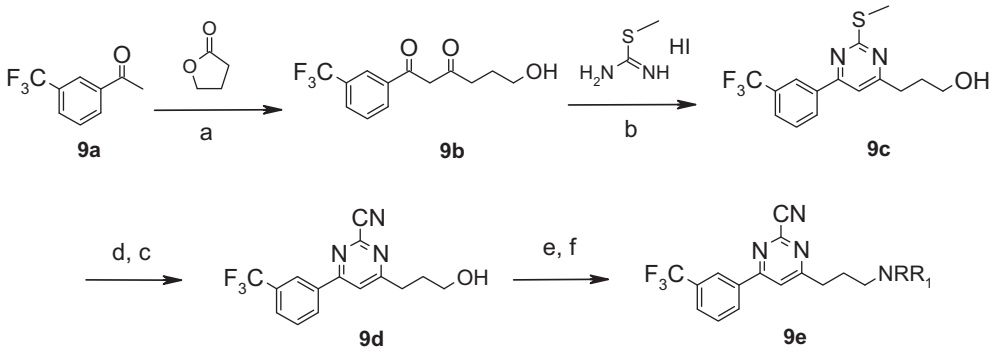


Figure 4. Co-crystal structure of **7** with cathepsin K (1.65 Å resolution; PDB 3O1G). A conserved water is in contact with Gly66 and the ligand pyrimidine ring, and has been modelled in the unprimed side.

was found to form extensive interactions with the floor of the S3 pocket (made up of the Gly65-Gly66-Tyr67 backbone), and engaged in an edge-to-face interaction with Tyr67. The dimethylamino group was also close to Tyr67, either interacting with the hydroxyl group from Tyr67 or simply exposed to solvent (this part of the molecule was too disordered to allow for more precise insight). Unfortunately, the improved catK potency of **7** was accompanied with a similar increase in hERG block and, indeed, this proved to be an unbreakable pattern as more analogues were synthesized in the P3-amine series. High lipophilicity of these compounds was considered to be the main contributing factor to their potent hERG block, as well as the presence of the basic amine functionality. However, it is important to note that basic amine group presence is not an absolute requirement for hERG block, especially for highly lipophilic compounds.[11] For example, compound **8** ($c\log P = 4.8$) retained the high hERG potency ($K_i = 370$ nM) exhibited by its amine-containing analogue **7** ($K_i = 120$ nM).

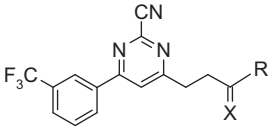
In summary, despite the success achieved in making significant structural changes whilst maintaining high catK potency, this approach ultimately failed to deliver the desired hERG selectivity profile, providing further evidence of the channel's binding promiscuity.[9]

Consequently, we focused our optimisation efforts on an alternative approach which, up to this point, had been pursued in parallel to the above: since lead compound **1** was lipophilic and contained a relatively basic amine ($c\log P = 4$, $pK_a = 9.2$), we aimed to attenuate these properties. Such an approach has been employed successfully in a number of hERG optimisation



Scheme 1. Reagents and conditions: (a) NaH/EtOH (cat), Et_2O, 0 °C; (b) *i*PrOH, 100 °C, 4 h, Et_3N, MeOH, reflux, 6 h; (c) oxone, CH_3CN/H_2O, 20 °C, 18 h; (d) NaCN, DMSO, 20 °C, 3 h; (e) Dess–Martin, CH_2Cl_2, 20 °C, 3 h; (f) amine, $NaBH(OAc)_3$, CH_2Cl_2, 20 °C, 18 h.

Table 2
Analogues of **1** containing alternative solubilising groups



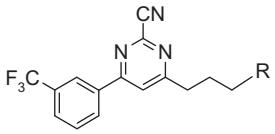
Compds	X	R	catK[a] IC_{50} (nM)	hERG[b] K_i (μM)	$c\log P$[c]	pK_a[d]
1	H_2	–Piperidine	4	0.16	4	9.2
9d	H_2	–OH	8	31	2	NA
10	O	–OH	166	>10	2.2	4.1[e]
11	O		389	3.16	3.2	NA

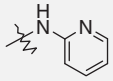
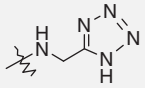
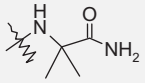
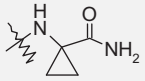
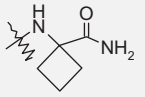
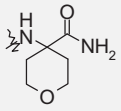
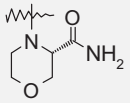
[a] Inhibition of recombinant human cathepsin K in a fluorescence assay, employing Z-Phe-Arg-MCA as synthetic substrates. Data represent means of two experiments performed in duplicate.
[b] Inhibition of [^{3}H]-Dofetilide binding to hERG K^+ channel in HEK293 cells following a 10 μM dose of test compound. Data represent means of two experiments performed in duplicate.
[c] Calculated log *P*.[17]
[d] Calculated pK_a[18] of conjugated base.
[e] Calculated pK_a[18] of acid.

campaigns reported in the literature.[11] Furthermore, reduction in log *P* and pK_a is undoubtedly a sensible strategy to adopt as it often confers beneficial effects on wider selectivity, pharmacokinetic and safety properties.[12] Inspection of the available SAR and crystal structure of **1** bound to catK (Fig. 2), led to the conclusion that the region around the solvent-exposed propyl-piperidine was most suitable for modulation of the compound's physicochemical properties. Incorporation of polar and electron withdrawing groups in this region of the molecule was not expected to perturb the key interactions believed to be providing the main contribution to the inhibitor's overall binding energy, such as the reversible covalent bond formation between the nitrile warhead and Cys25, and the CF_3-phenyl group interaction with the S2 pocket. To explore the above hypothesis, a range of analogues of **1** were synthesized according to the general route depicted in Scheme 1.

Diketone **9b**, obtained in one step from commercially available acetophenone **9a** and γ-butyrolactone in the presence of sodium hydride, was heated with *S*-methyl-isothiourea hydroiodide[13] in iso-propanol at 100 °C for 4 h. After cooling to room temperature, triethylamine and methanol were added, and the resulting reaction mixture was refluxed for another 6 h to yield pyrimidine **9c**. The methylsulfide of **9c** was oxidised using ozone to the corresponding sulphone, which was then displaced by cyanide to afford the desired 6-cyanopyrimidine **9d**. A high yielding oxidation of **9d** with Dess–Martin reagent afforded the corresponding aldehyde, which was then subjected to a standard reductive amination procedure using sodium triacetoxyborohydride and the relevant amine to obtain desired product **9e**. The synthetic route was designed to allow the final step to be carried out in a high throughput fashion, which enabled a rapid and efficient exploration of the SAR in this region.

Table 3
Attenuation of *c* log *P* and pK_a to improve catK/hERG profile



Compds	R	catK[a] IC_{50} (nM)	hERG[b] K_i (μM)	*c* log *P*[c]	pK_a[d]
12		4	0.32	4.1	10.4
13	–NHMe	6	0.63	2.2	10.1
14	$-NHCH_2CF_3$	30	0.5	3	4.9
15	–*N*-Methyl piperazine	10	1.0	2	7.6
16	–Morpholine	9	0.32	2.9	7.0
17	$-NHCH_2CH_2OMe$	10	0.56	3	9.3
18	4-Hydroxy piperidine	9	1.12	1.9	8.4
19		3	0.4	3.8	6.4
20	$-NH_2CH_2COOH$	9	>10	–0.4	9.6 (2.3)[e]
21		11	>10	2.1	9.4 (3.5)[e]
22	$-NH_2CH_2CONH_2$	8	3.98	1.2	7.4
23		3	3.16	1.8	7.4
24		12	1.58	1.5	6.1
25		4	2.51	2.0	7.7
26		5	>10	0.7	6.6
27		13	>10	1.9	5.3

[a] Inhibition of recombinant human cathepsin K in a fluorescence assay, employing Z-Phe-Arg-MCA as synthetic substrates. Data represent means of two experiments performed in duplicate.
[b] Inhibition of [^{3}H]-Dofetilide binding to hERG K^+ channel in HEK293 cells following a 10 μM dose of test compound. Data represent means of two experiments performed in duplicate.
[c] Calculated log *P*.[17]
[d] Calculated pK_a[18] of conjugated base.
[e] Calculated pK_a[18] of acid.

We started by replacing the amine group in **1** with a range of alternative solubilising groups, such as the OH in **9d**. This compound displayed catK potency similar to that of lead compound **1**, and acceptable solubility (60 mg/L). Importantly, the less lipophilic **9d** (Table 2) was found to be practically devoid of hERG binding (K_i = 31 μM). Disappointingly, however, the compound displayed poor inhibition in the ROC assay (IC_{50} = 686 nM), probably due to poor permeability (Caco-2, AB <20 nm/s), as well as poor pharmacokinetic properties, all of which proved difficult to optimise. Alternative replacements such as the carboxylic and amide groups in **10** and **11** (Table 2), led to reduction in both catK and hERG potency. Interestingly, amide **11** retained a moderate hERG block (*c* log *P* = 3.2). Its direct analogue **12** further demonstrated the importance of the basic amine for binding to both hERG and catK (Table 3).

Compounds **13**–**19** indicated that significant attenuation of only one of the two parameters, *c* log *P* or pK_a, is unlikely to be sufficient to exert the desired effect on hERG binding. In fact, an analysis of all hERG data within this series suggested that the targeted hERG potency (K_i >3 μM) is most likely to be achieved for compounds with *c* log *P* <2 and pK_a <7.5. This afforded a very narrow window of opportunity, particularly since pushing these parameters a little further below these levels would start impacting on cellular permeability and, consequently, on potency in the functional assay and, in addition, on pharmacokinetic properties. For example, glycine analogue **20** (*c* log *P* = −0.4) displayed a good catK/hERG selectivity margin, but showed poor permeability (P_{app} <22 nm/s) and, inevitably, it proved inactive in the ROC assay. Still, encouraged by the selectivity profile of this compound, we synthesized a number of closely related analogues designed to yield improved permeability. Bioisosteric replacements such as the tetrazole in **21** maintained the favourable selectivity profile of **20**, but showed little improvement in the ROC assay (IC_{50} = 1640 nM). A breakthrough in our efforts came with the synthesis of primary amide **22**, which demonstrated a fitting *c* log *P*/pK_a profile (1.2 and 7.4, respectively). This compound yielded not only a catK/hERG selectivity margin of 500-fold, but also showed a high potency in the ROC assay (IC_{50} = 49 nM). Further progression of **22** was, however, prevented by its poor plasma stability—a consequence of rapid hydrolysis of the amide group by plasma peptidases ($t_{1/2}$ = 65 min). Introduction of alpha substitution in compounds **23**–**27** markedly improved plasma stability without affecting the desired catK/hERG/ROC profile (plasma $t_{1/2} \gg 150$ min). The most promising overall profile was displayed by *gem*-dimethyl analogue **23** (Table 4).

This compound showed high selectivity over closely related cathepsins B and L, and no significant interactions at a concentration of 10 μM in a broader selectivity panel of 65 receptors, ion channels, enzymes and nuclear receptors. The cathepsin S component (IC_{50} = 16 nM) was not considered detrimental, but potentially beneficial for this programme. We hypothesised that inhibition of cathepsin S, recently linked to the reversal of neuropathic pain[15], may prove beneficial for the management of chronic pain associated with osteoporosis.[16]

In cardiovascular safety studies in conscious pig **23** produced no significant electrocardiographic or hemodynamic effect when dosed orally up to 40 mg/kg (C_{max} = 2650 nM). The compound was clean in the Ames test at concentrations of up to and including 100 μM in both the absence and presence of rat liver S9 fraction, and showed no significant adverse reaction in a 2-week safety study in rats (po up to 100 mg/kg).

In summary, several crystal structure-guided optimisation strategies were explored in order to improve the hERG selectivity profile of lead compound **1** (~40-fold). Ultimately, attenuation of *c* log *P* and pK_a properties proved a successful approach and led to the discovery of a potent catK inhibitor **23**, which, in addition to the desired selectivity over hERG (>1000-fold), displayed an attractive overall in vitro and in vivo profile. More detailed preclinical data discussing the efficacy of this compound on bone resorption markers in primates will be reported elsewhere.

Table 4
Potency, selectivity and pharmacokinetic data for **23**

Parameter	Value[a]
catK; catS; catB; catL IC_{50} (nM)	3; 16; 2512; >10,000
ROC; HOC[c] IC_{50} (nM)	30; 39
hERG[b] K_i (μM)	3.0
Papp (Caco-2) AB; BA (nm/s)	90; 140
Solubility of the HCl salt in PBS, pH 7.4 (mg/L)	393
CYP inhibition: 1A2; 2C9; 2C19; 3A4; IC_{50} (μM)	>35
PPB: human, rat, dog, pig, cyno (% bound)	75–87
Rat *F* (%); wistar, iv 2 mg/kg; po 10 mg/kg	29
Dog *F* (%); beagle, iv 0.78 mg/kg; po 1.96 mg/kg	29
Cyno *F* (%); iv 2 mg/kg; po 4.63 mg/kg	57

[a] Values represent means of at least two experiments performed in duplicate.
[b] Manual patch clamp recordings in HEK293 cells stably expressing hERG channel.
[c] Human osteoclasts assay (HOC).[14]

Acknowledgement

We thank Han Kok and Wim Koot for running fermentors to produce cathepsin K protein.

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