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Synthesis, chiral resolution, absolute configuration assignment and pharmacological evaluation of a series of melatoninergic ligands

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We report herein the racemic resolution and pharmacological evaluation of naphthalenic ligands analogues of compound **3a**. Propionamide **3b** and fluoroacetamide **3c** have shown good pharmacological profile towards MT_1, MT_2 and 5-HT_{2C}. Hence, their enantiomers were successfully separated from racemates (±)-**3a** and (±)-**3b** and evaluated for their binding affinities and antidepressant activity. Binding results revealed that (-)-*R* were more potent than (+)-*S*-enantiomers. Furthermore, the (-)-*R*-enantiomers exhibited high binding affinities with partial agonist activity at melatonin MT_1 and MT_2 receptor subtypes and antagonist activity at serotonin 5-HT_{2C} receptor subtype. The *R*-fluoroacetamide **3c** demonstrated the most potent binding affinity towards 5-HT_{2C} receptor subtype (pKi = 6.73 ± 0.02).

Key words: Enantiomeric resolution, Melatonin receptors, 5-HT_{2C} antagonist, X-ray analysis.

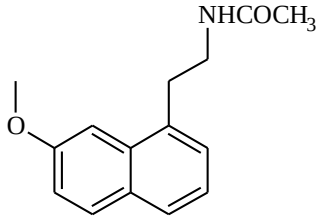
Abbreviations: MT_1, melatonin receptor subtype 1; MT_2, melatonin receptor subtype 2; 5-HT_{2C}, serotonin receptor subtype 2C; [^{35}S]GTPγS, [^{35}S]guanosine-5′-*O*-(3-thio-triphosphate).

Recently, one of the newly emerged therapeutic strategies for treatment of depression is the design and synthesis of multi-targeted ligands (MTLs). It consists of modulating multiple targets and ideally associating therapeutic actions in order to enhance therapeutic efficacy, improve patients' tolerance and their safety. Melatonin, a neurohormone secreted by the pineal gland during the period of darkness and involved in the modulation of circadian rhythms, is one of the most promising targets of MLTs.[1] Its multiple physio/pathological implications will provide the scientific community with the necessary tools to design and develop new efficient medicines. In this context, agomelatine was first designed and synthesized as an analogue of melatonin, then it was shown to act as a non selective agonist at the melatonin MT_1 and MT_2 receptors, and as a selective antagonist at the serotonin 5-HT_{2C} receptor subtype.[2] Agomelatine was then approved by the European Medicines' Agency for the treatment of major depressive disorders. Its efficacy as an antidepressant has been established over clinical trials, extensively reviewed in the literature[3-5] and reported to involve norepinephrine and dopamine release in the frontal cortex resulting from serotoninergic 5-HT_{2C} antagonism.[6] Through its agonist action on melatonin receptors, agomelatine can also resynchronize circadian rhythms[7] and improve sleep quality.[8-9]

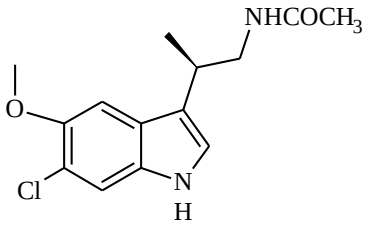
In a synergic effort with pharmacologists, regarding agomelatine successor design, we have been able to develop a library of small-molecules having MT_1/MT_2 agonist and 5-HT_{2C} antagonist activities that exhibit submicromolar binding affinity at these three receptor subtypes. Hence, we recently[10] reported the synthesis of 3-allyl-agomelatine where the metabolic site C-3 of the naphthalene ring is blocked. We also reported the synthesis and biological evaluation of a series of *N*-[3-hydroxy-2-(7-methoxy-naphthalen-1-yl)-propyl] amides issued from the modulation of a known metabolic position, the beta-position of the ethyl amido side chain of agomelatine.[11-12] The actual work is a part of the recently reported results.[11] Hereafter, we

describe the organic synthesis and biological evaluation of compounds **3b-d** issued from the acetamide modulation of a previously described compound (**3a**).[11] We also describe the enantiomeric resolution, X-ray studies and antidepressant effect of the most relevant of their enantiomers in order to establish structure-activity relationships (SARs) and assess their enantiomeric selectivity against the three receptor subtypes. Moreover we confirm the important role of the 5-HT_{2C} antagonist component for the antidepressant properties of these molecules.

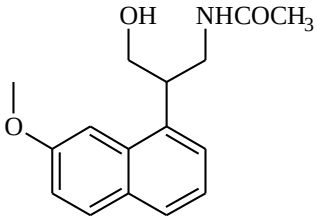
Chart 1. Some of melatonin analogues.



Agomelatine



β-Methyl-6-chloromelatonin



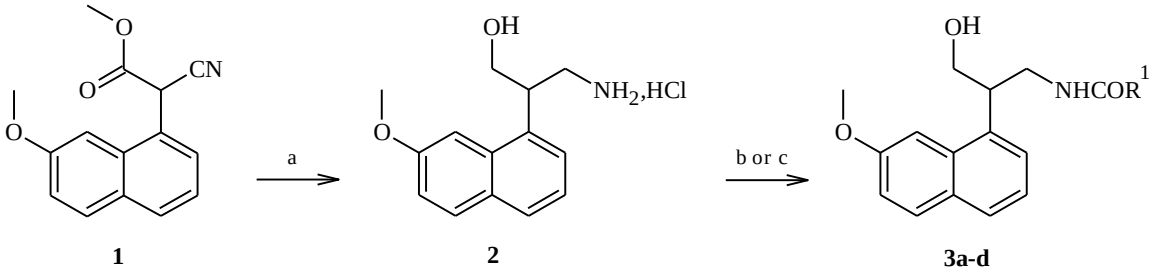
β-Hydroxymethyl-agomelatine

(**3a**)

The insertion of a hydroxymethyl group in the beta position of the ethyl acetamide chain of agomelatine has conducted to the improvement of serotoninergic 5-HT_{2C} binding affinity and the conservation of submicromolar binding affinities at melatonin receptors.[11] The result obtained with compound **3a** led us to extend our pharmacomodulations to the amide function. Hence, in this paper we report the synthesis and pharmacological evaluation of different amides **3a-d**. Among the newly synthesized ligands, two compounds **3b** and **3c** showed an interesting pharmacological profile. Therefore, their corresponding (+) and (-)-enantiomers were separated and their binding affinity and intrinsic activity at MT_1, MT_2 and 5-HT_{2C} tested. Also, their

antidepressant properties were evaluated through tail suspension studies in order to confirm the involvement of the 5-HT_{2C} antagonism in the antidepressant-like properties of these enantiomers.

Scheme 1. Synthesis of compounds **3a-d**[a]



1 **2** **3a-d**

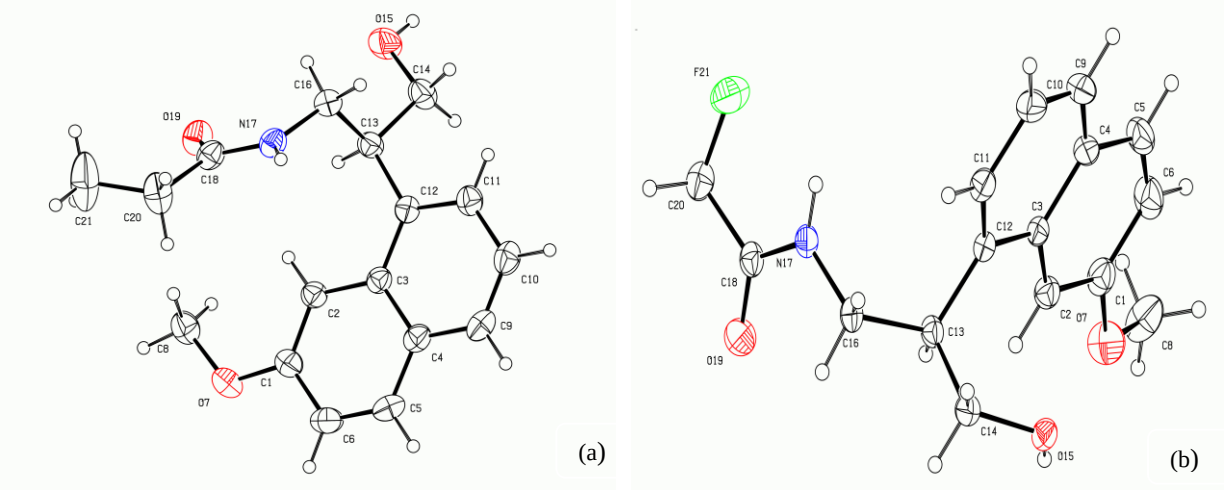
	R^1	Yield (%)
3a	CH_3	80
3b	C_2H_5	80
3c	CH_2F	60
3d	CHF_2	43

[a]*Reagents:* a) i) $LiAlH_4$, $AlCl_3$, Et_2O, rt; ii) HCl_g, Et_2O; b) K_2CO_3, EtOAc/H_2O, R^1COCl, 0°C for **3a-b**; c) FCH_2CO_2Et or F_2CHCO_2Et, CF_3CH_2OH, reflux for **3c-d**.

Compounds **3a-d** were prepared using the synthetic route depicted in Scheme 1 and starting from the previously described cyanoester **1**.[13-14] A one-pot chemical reduction of both nitrile and ester was performed in dry ethyl ether using a mixture of $LiAlH_4$ and $AlCl_3$ and led to the corresponding aminoalcohol **2**.[15] Then a *N*-acylation of aminoalcohol **2** according to the Schotten–Baumann reaction conditions[16] afforded the desired compounds **3a** and **3b**. Finally, the fluoroacetamides **3c-d** were prepared according to a previously described procedure using ethyl fluoroacetate or methyl difluoroacetate in 2,2,2-trifluoroethanol (Scheme 1).

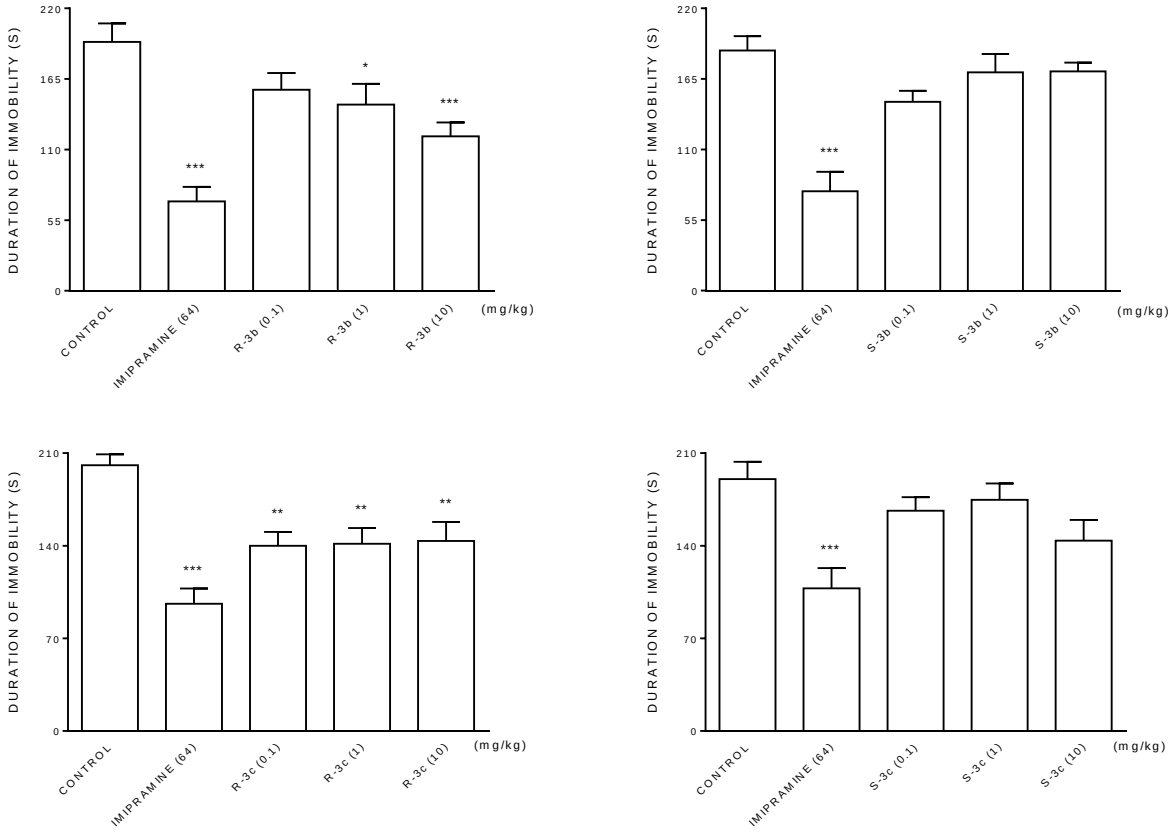
Compounds **3b** and **3c** were synthesized as racemates and their enantiomers were separated using a semi-preparative chiral high performance liquid chromatography, carried out on a Chiralpak AS column (96.8 x 10 mm; id), (Daicel Chemical Industries, Baker France) in the normal phase mode; eluted isocratically with heptane/isopropanol (9:1), and characterized using a photodiode array (PDA) detector at 254 nm. Eluted fractions were collected on the visual basis of the chromatograms. From synthesized racemates **3b** and **3c** were separated two pairs of pure enantiomers (*R*,*S*-**3b**) and (*R*,*S*-**3c**), and later characterized by their NMR and mass spectra. Two compounds; one enantiomer of each pair; were then submitted to X-ray analysis and their configurations was established as (13*S*)-**3b** and (13*R*)-**3c** (Figure 1). The absolute configuration on the two cristal samples was established by anomalous-dispersion effects in diffraction measurements by full-matrix least squares on F^2.[17-18]

Figure 1. Perspective view of the crystal structures of compounds (+)-*S*-**3b** (a) and (-)-*R*-**3c** (b).



To evaluate binding affinity and functional activity of the synthesized compounds, they were subjected to competition binding studies against human MT_1, MT_2 and 5-HT_{2C} receptors subtypes stably transfected in Chinese Hamster Ovarian (CHO) cells and the obtained results are shown hereafter.[19-20] As we can notice from Table 1, the acetamide **3a** demonstrated good binding affinities at melatonin (MT_1, MT_2) and serotonin 5-HT_{2C} receptor subtypes. The substitution of the methyl of its acetamide with an ethyl group (**3b**) improved its binding affinity at melatonin MT_1 and MT_2 receptor subtypes. Also, the same increasing effect in the melatoninergic binding affinities, in comparison with **3a**, was observed by replacing the acetamide with a fluoro or difluoroacetamide (**3c, 3d**). Furthermore, all these compounds showed binding affinities at $5HT_{2C}$ in the same order of magnitude as the parent compound **3a**. Subsequently, compounds **3b** and **3c** were identified to be the most potent of this series exhibiting good melatoninergic and serotoninergic (5-HT_{2C}) binding affinities.

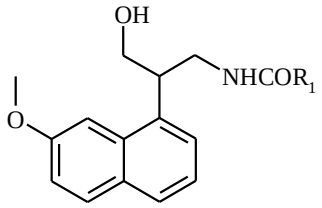
Figure 2. Mean duration of immobility (± SEM) in the tail suspension test for OF1-mice treated either with the *R*- and *S*-**3b** compound or the *R*- and *S*-**3c** compound at 0.1,1 and 10 mg/kg. An imipramine group was used for test validation. **P*<0.05, ***P*<0.01, ****P*<0.001 pairwise comparisons with the vehicle treated group using Dunnett's t test.



In order to investigate the effect of their stereochemistry, the (+), (-)-enantiomers of **3b** and **3c** were then separated, their cristallographic analysis and biological results reported herein. The pharmacological results of their separated enantiomers showed significant differences confirming the favorable influence of the *R*-configuration as was reported for β-methyl-6-chloromelatonin ((*R*)-*N*-[2-(6-chloro-5-methoxy-1*H*-indol-3-yl)propyl]acetamide, chart 1), a melatonin analogue with high affinity for melatonin receptors (MT_1: pKi = 10.1, MT_2: pKi = 10.4).[21] Indeed, the (-)-*R*-**3b** and (-)-*R*-**3c** isomers showed more potent binding affinities at MT_1, MT_2 and 5-HT_{2C} than the *S*-enantiomers. Meanwhile, the (+)-*S*-**3b** and (+)-*S*-**3c** enantiomers, which had no affinity for 5-HT_{2C}, showed a decrease in binding affinities in particular at MT_1 leading to a weak MT_2-selectivity (53 and 7-folds respectively, Table 1). Interestingly, we also

noticed a slight decrease in the binding affinities of (-)-*R* and (+)-*S* separated enantiomers in comparison with the racemic mixtures (**3b**, **3c**) especially for MT_1 and MT_2 receptor subtypes (Table 1).

Table 1. Binding affinity results on MT_1, MT_2 and 5-HT_{2C} of compounds **3a-d**



3a-d

Compound	h-MT_1	h-MT_2	h-5-HT_{2C}
	pK_i ± SEM (n)[a]	*pK_i ± SEM (n)*	*pK_i ± SEM (n)*
Agomelatine	9.92 ± 0.00 (2)	9.42 ± 0.06 (3)	6.15 ± 0.04 (3)
3a	7.96 ± 0.10 (2)	7.86 ± 0.03 (2)	6.64 ± 0.14 (2)
(rac)[b] (±)-3b	8.87 ± 0.15 (2)	8.53 ± 0.17 (2)	6.15 ± 0.10 (3)
***S*-3b**	6.74 ± 0.02 (3)	8.26 ± 0.05 (3)	< 5 (2)
***R*-3b**	8.47 ± 0.02 (3)	9.50 ± 0.08 (4)	6.38 ± 0.02 (2)
(rac) (±)-3c	8.33 ± 0.02 (2)	9.27 ± 0.12 (2)	6.78 ± 0.16 (3)
***S*-3c**	6.46 ± 0.03 (2)	7.28 ± 0.06 (2)	< 5 (2)
***R*-3c**	8.23 ± 0.02 (2)	9.01 ± 0.05 (2)	6.73 ± 0.02 (2)
3d	8.72 ± 0.02 (2)	9.46 ± 0.07 (2)	6.16 ± 0.15 (2)

[a]*Mean from (n) experiments,* [b]*rac: racemic*

The synthesized compounds and their separated enantiomers were then tested in functional assays for their agonist activities at melatonin (MT_1, MT_2) receptors and their pEC_{50} values are reported in Table 2. Most of the compounds showed partial agonist activity at MT_1 and/or MT_2 receptors subtypes with compounds **3b** and **3c** being the most interesting at melatonin MT_1 and

MT_2 receptors. Only separated enantiomers were then evaluated for their antagonist activities on 5-HT_{2C} and the pK_b values are reported hereafter (Table 3). Both *R*-**3b** and *R*-**3c** exhibited full antagonist activity at 5-HT_{2C}. Finally, enantiomers (-)-*R*-**3b**, (-)-*R*-**3c**, (+)-*S*-**3b** and (+)-*S*-**3c** were assayed *in vivo* in the tail suspension test, an animal model of depression (Figure 2) using imipramine as a reference antidepressant. The results showed that imipramine induced a significant decrease in immobility time as compared to controls (Dunnett's test, $p<0.001$) in the four studies. The *R*-**3b** enantiomer given at 0.1, 1 and 10 mg/kg had a significant group effect [$F(4,74)=13.75$, $p<0.0001$] whereas the enantiomer *S*-**3b** given at 0.1, 1 and 10 mg/kg had no significant effects in mice in the tail suspension test. *R*-**3b** decreased immobility time in a dose dependent manner, the doses 1 and 10 mg/kg induced a significant decrease of immobility (Dunnett's test, $p<0.05$) whereas the dose 0.1 mg/kg was inactive. The animal experiments were performed in accordance with the guidelines of the European Communities Council Directive 86/6609/EEC and approved by the ethics committee of Lille Nord de France University.

Table 2. pEC_{50} and Emax data of agomelatine and compounds **3a-d**

Compound	h-MT_1		h-MT_2	
	pEC50 ± SEM	*Emax (%) ± SEM*	*pEC50 ± SEM*	*Emax (%) ± SEM*
Agomelatine	9.70 ± 0.01 (2)	96 ± 11 (2)	10.16 ± 0.06 (2)	96 ± 1 (2)
3a	7.14 ± 0.05 (2)	69 ± 5 (2)	6.84 ± 0.16 (2)	48 ± 6 (2)
(rac) (±)-3b	7.31 ± 0.13 (2)	76 ± 1 (2)	8.56 ± 0.06 (2)	77 ± 9 (2)
***S*-3b**	6.41 ± 0.22 (3)	70 ± 11 (3)	8.13 ± 0.07 (5)	115 ± 9 (5)
***R*-3b**	7.71 ± 0.08 (5)	59 ± 9 (5)	8.90 ± 0.13 (5)	107 ± 14 (5)
(rac) (±)-3c	7.60 ± 0.08 (2)	94 ± 1 (2)	9.20 ± 0.39 (3)	96 ± 20 (3)
***R*-3c**	7.10 ± 0.09 (2)	62 ± 13 (2)	9.10 ± 0.17 (2)	99 ± 10 (2)
***S*-3c**	5.98 (1)	37 (1)	7.60 ± 0.06 (2)	93 ± 5 (2)
3d	7.99 ± 0.16 (3)	112 ± 7 (3)	9.43 ± 0.15 (3)	132 ± 30 (3)

[a]*Mean from (n) experiments*

In the *R*-**3c** experiment, imipramine and the 3 doses of *R*-**3c** elicited a significant decrease in immobility time (group effect, F(4,74)=10.48, $p<0.0001$; Dunnett's tests, imipramine vs control, $p<0.001$; each doses of *R*-**3c** vs control, $p<0.01$). In the opposite, in the *S*-**3c** experiment only imipramine significantly reduced immobility time (group effect, F(1,74=5.65, $p<0.001$, Dunnett's test; $p<0.001$). Hence, *S*-**3c** did not induce any significant change in immobility time when given at 0.1, 1 and 10 mg/kg. The comparison of the *R* and *S* enantiomers which have the same pharmacokinetic profile (data not shown) in animal models of depression confirmed the requirement of a 5-HT_{2C} antagonist activity to have antidepressant like effect. Indeed only the *R* enantiomers which are 5-HT_{2C} antagonists have antidepressant effects. The most potent was, *R*-**3c,** wich was found active even at the dose of 0.1 mg/kg. The *S* enantiomers, devoid of 5-HT_{2C} activity, have no effects.

The flexibility of the amide side chain of compound **3a** and its analogues provides these ligands with different spatial arrangements during binding to MT_1, MT_2 and 5-HT_{2C} receptors. Moreover, the presence of a stereogenic centre should add another effect to this spatial disposition of the molecule inside the binding site. These two aspects were separately studied by some researchers wherein constrained and flexible structures of melatonin analogues were reported.[22] However, nowadays any similar study on both melatonin and serotonin receptors was performed and more work is needed to understand the effects of both flexibility and stereochemistry upon binding to these receptors.

Table 3. pK_B and I_{max} on 5-HT_{2C} receptor of Agomelatine and *R*-**3b** and *R*-**3c**

Compound	*5-HT_{2C}* $pK_B \pm SEM\ (n)$	*5-HT_{2C}* $I_{max}\ (\%) \pm SEM\ (n)$
Agomelatine	6.06 ± 0.15 (3)	98.00 ± 2.00 (3)
***R*-3b**	6.00 ± 0.02 (2)	79.00 ± 1.00 (2)
***R*-3c**	6.3 ± 0.25 (2)	97.00 ± 5.00 (2)

[a]*Mean from (n) experiments*

In conclusion, enantiomers of newly synthesized analogues of acetamide **3a** were successfully separated using HPLC and their absolute configurations established using X-ray crystallography. Among the synthesized compounds, enantiomers of analogues **3b** and **3c** showed an interesting pharmacological profile. In fact, the analysis of their biological results had shown that (-)-*R*-fluoroacetamide (**3c**) possesses the most potent binding affinity towards the serotoninergic 5-HT_{2C} receptor subtype. (-)-*R*-**3b** and (-)-*R*-**3c** enantiomers were found to be MT_1/MT_2 partial agonists and 5-HT_{2C} full antagonists in the functional assay studies. Finally, the *in vivo* comparison of the *R* and *S* isomers in animal models of depression revealed that only the *R* enantiomers which acts as 5-HT_{2C} antagonists, have antidepressant effects. The most potent was, *R*-**3c,** wich was found active even at the dose of 0.1 mg/kg. The *S* enantiomers, devoid of 5-HT_{2C} activity have no effects confirming hence the necessity of a 5-HT_{2C} antagonist activity in order to have antidepressant like effect.

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