ACS Medicinal Chemistry Letters

Letter

pubs.acs.org/acsmedchemlett

Low Doses of Allyphenyline and Cyclomethyline, Effective against Morphine Dependence, Elicit an Antidepressant-like Effect

Fabio Del Bello,[†] Eleonora Diamanti,[†] Mario Giannella,[†] Valerio Mammoli,[†] Carla Marchioro,[‡] Laura Mattioli,[§] Federica Titomanlio,[§] Alessandro Piergentili,[†] Wilma Quaglia,[†] Giovanni Benedetti,[∥] Maurizio Varrone,[∥] and Maria Pigini*[,†]

[†]School of Pharmacy, Medicinal Chemistry Unit, University of Camerino, Via S. Agostino 1, 62032 Camerino, Italy

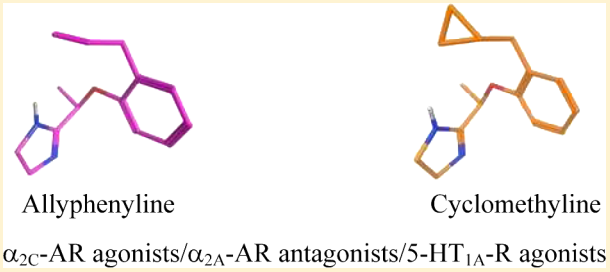
[‡]Aptuit Verona, via Fleming 4, 37135 Verona, Italy

[§]School of Pharmacy, Pharmacognosy Unit, University of Camerino, Via Madonna delle Carceri 9, 62032 Camerino, Italy

[∥]Siena Biotech S.p.A., Strada del Petriccio e Belriguardo 35, 53100 Siena, Italy

Supporting Information

ABSTRACT: This study demonstrated that cyclomethyline (**2**) and the corresponding enantiomers (*R*)-(−)-**2** and (*S*)-(+)-**2**, displaying α_{2C}-adrenoreceptor (AR) agonism/α_{2A}-AR antagonism, similarly to allyphenyline (**1**) and its enantiomers, significantly decreased the naloxone-precipitated withdrawal symptoms in mice at very low doses. It also highlighted that such positive effects on morphine dependence can even be improved by additional serotoninergic 5-HT_{1A} receptor (5-HT_{1A}-R) activation. Indeed, **1** or the single (*S*)-(+)-**1**, **2**, or both its enantiomers, all behaving as α_{2C}-AR agonists/α_{2A}-AR antagonists/5-HT_{1A}-R agonists, alone and at the same low dose, improved morphine withdrawal syndrome and exerted a potent antidepressant-like effect. Therefore, considering the elevated comorbidity between opiate abuse and depressed mood and the benefit of these multifunctional compounds to both disorders, it is possible that they prove more efficacious and less toxic than a cocktail of drugs in managing opioid addiction.



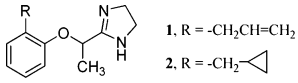
KEYWORDS: *α_2-adrenergic ligands, 5-HT_{1A}-R agonists, morphine withdrawal symptoms reduction, antidepressant-like effect*

Morphine use is the classical approach in the treatment of chronic or cancer-related pain. Nevertheless, even when legally allowed and closely monitored, long-term opioid administration alters central pain-related systems, inducing tolerance and dependence. Opioid addiction, termed a "chronic relapsing disease", is associated with a myriad of health and social problems; hence, its management is an extremely important area of research.[1]

α_2-Adrenoreceptors (α_2-ARs), which have been demonstrated to be extremely sensitive to opioid exposure and to play a key role in opiate withdrawal symptoms,[2] belong to the superfamily of G-protein-coupled receptors. The three α_{2A}-, α_{2B}-, and α_{2C}-AR subtypes are widely distributed in the central nervous system (CNS) and peripheral tissues.[3] Studies with genetically engineered mice have demonstrated that the α_{2A} subtype mediates hypotension, sedation, and analgesia, as well as inhibition of monoamine release and metabolism in the brain. The α_{2B} subtype mediates vasoconstriction, while the α_{2C} subtype appears to be involved in many CNS processes, such as the startle reflex, stress responses, and control of locomotion as well as feedback inhibition of adrenal cathecolamine release. Moreover, in the brain, α_{2A}- and α_{2C}-ARs, as "heteroreceptors", inhibit dopamine and serotonin release. Finally, the α_{2C} subtype can contribute to adrenergic-opioid synergy and spinal α_2-agonist-mediated analgesia.[3−5] In detoxification, α_2-AR agonists such as clonidine and lofexidine are often clinically used alone or in combination with traditional treatments to reduce the intensity of withdrawal symptoms, thus increasing treatment duration. However, the α_{2A}-AR activation triggered by these compounds is responsible for side effects such as sedation and hypotension.[1] It has been reported that in comparison to clonidine, lofexidine showed decreased incidence and severity of adverse events.[1,6] The partial α_{2A}-AR agonism of lofexidine, as recently assessed by us,[7] might justify its behavior (Table 1). Therefore, selective α_{2C}-AR agonists, devoid of the side effects associated with α_{2A}-AR stimulation, alone or in combination with opioids, might afford an improvement over current therapies with clonidine-like drugs. We reported the interesting behavior of allyphenyline (**1**), which displays a significant α_{2C}-AR agonist/α_{2A}-AR antagonist profile[8] ascribed to its preferred *extended* molecular conformation.[7] In in vivo studies, **1** enhanced morphine analgesia (due to its α_{2C}-agonism), was devoid of a sedative effect (due to its α_{2A}-antagonism),[8,9] and contrasted and prevented morphine tolerance and dependence

Received: March 15, 2012
Accepted: June 8, 2012
Published: June 8, 2012

Table 1. [a]Affinity (pK_i), Antagonist Potency (pK_b), Agonist Potency (pEC_{50}), and Intrinsic Activity (ia) on Human α_2-AR Subtypes[b] and Affinity (pK_i), Agonist Potency (pD_2), and Relative Efficacy ($\%E_{max}$) on Human 5-HT_{1A}-R[c]



1, R = -$CH_2CH{=}CH_2$

2, R = -CH_2-cyclopropyl

	α_{2A}	α_{2B}		α_{2C}		5-HT_{1A}	
compd	pK_b (pK_i)	pEC_{50} (pK_i)	ia	pEC_{50} (pK_i)	ia	pD_2 (pK_i)	$\%E_{max}$
(±)-**1**	7.40 ± 0.06	NA[d]		7.30 ± 0.09	0.90	6.86 ± 0.09	67
	(7.24 ± 0.11)	(6.47 ± 0.20)		(7.07 ± 0.14)		(7.55 ± 0.16)	
(*R*)-(−)-**1**	7.40 ± 0.09	NA[d]		6.73 ± 0.11	0.50	<5	
	(7.00 ± 0.08)	(6.25 ± 0.12)		(6.75 ± 0.11)		(<5)	
(*S*)-(+)-**1**	7.80 ± 0.13	6.00 ± 0.09	0.65	7.60 ± 0.14	0.90	7.19 ± 0.10	96
	(7.28 ± 0.05)	(6.40 ± 0.09)		(7.15 ± 0.09)		(7.45 ± 0.15)	
(±)-**2**	7.70 ± 0.12	5.48 ± 0.15	0.70	8.70 ± 0.08	0.80	7.20 ± 0.13	75
	(7.44 ± 0.09)	(6.39 ± 0.05)		(6.56 ± 0.21)		(7.98 ± 0.07)	
(*R*)-(−)-**2**	7.20 ± 0.11	5.40 ± 0.11	0.65	6.50 ± 0.07	0.75	6.80 ± 0.10	68
	(7.35 ± 0.10)	(6.47 ± 0.07)		(6.63 ± 0.05)		(7.40 ± 0.08)	
(*S*)-(+)-**2**	7.70 ± 0.09	5.80 ± 0.06	0.70	8.70 ± 0.11	0.85	7.40 ± 0.02	97
	(7.38 ± 0.07)	(6.39 ± 0.10)		(6.56 ± 0.13)		(8.24 ± 0.16)	
clonidine[b]	*e*	5.95 ± 0.27	0.45	7.24 ± 0.14	0.60		
						(5.49 ± 0.13)	
lofexidine[f]	*g*	6.90 ± 0.14	0.80	8.86 ± 0.20	0.80		
	(8.36 ± 0.11)	(7.17 ± 0.18)		(7.16 ± 0.07)		(6.90 ± 0.11)	
8-OH-DPAT						7.60 ± 0.06	100
						(8.47 ± 0.13)	

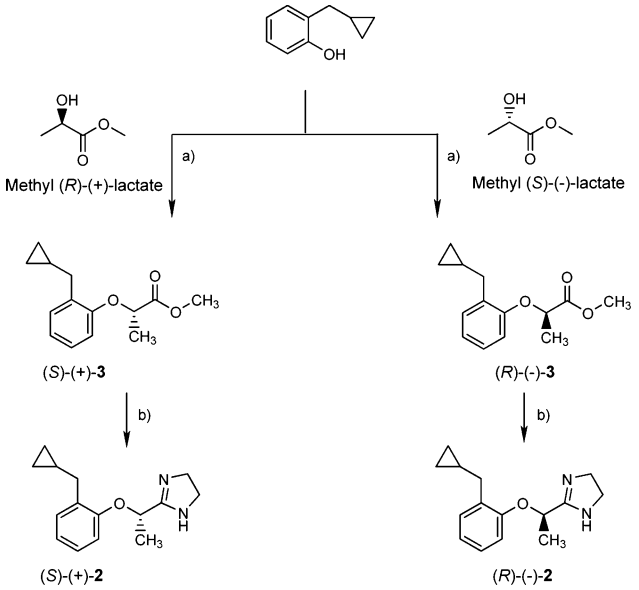
[a]The data were expressed as means ± SEMs of 3−6 separate experiments. [b]According to ref 9. [c]pK_i values were derived from displacement of [^{3}H]-8-OH-DPAT binding on human cloned 5-HT_{1A}-R. pD_2 values are the negative logarithm of the agonist concentration required to obtain 50% of the maximal stimulation of [^{35}S]GTPγS binding. Maximal stimulation ($\%E_{max}$) is expressed as a percentage of the maximal 8-OH-DPAT response. [d]Compounds exhibiting ia of <0.3 were considered not active (NA). [e]pEC_{50} = 8.08 ± 0.12; ia = 0.90. [f]Ref 7. [g]pEC_{50} = 8.20 ± 0.10; ia = 0.60.

at a very low dose (0.05 mg/kg).[9] In contrast, clonidine attenuated the expression of morphine withdrawal symptoms at a higher dose (5 mg/kg) and was ineffective in preventing them.[10] Because α_2-AR antagonists (i.e., yohimbine) also attenuated the intensity of withdrawal symptoms,[11] we attributed the unique profile of **1** to the synergistic combination of its effective dual α_{2C}-AR agonism/α_{2A}-AR antagonism.[9] The positive effects of **1** on dependence were also displayed by (*S*)-(+)-**1** (potent α_{2C}-AR full agonist) and (*R*)-(−)-**1** (α_{2C}-AR partial agonist); they both were endowed with similar α_{2A}-AR antagonism.[9] To confirm the aforementioned results and discover novel tools for the management of opioid withdrawal symptoms that have a major role in relapse to drug-taking behavior after detoxification, we extended the study to the recently reported allyphenyline analogue **2**,[7] now named cyclomethyline, and its enantiomers, prepared in the present investigation. Compound **2** showed interesting α_{2C}-AR agonism/α_{2A}-AR antagonism (Table 1), and according to what reported for **1**,[7] this functional profile might be associated with a preferred *extended* molecular conformation. Therefore, the corresponding enantiomers were evaluated by binding and functional assays on Chinese hamster ovary (CHO) cells expressing recombinant human α_2-AR subtypes according to previous procedures.[9] Moreover, the effects of the racemate **2** at the dose of 0.05 mg/kg and its enantiomers at the dose of 0.025 mg/kg (ip) on the expression and acquisition of morphine dependence were determined. To learn more about the pharmacological properties of this novel class of compounds, expecially in light of the strong comorbidity between opioid addiction and depressive disorders seen in several clinical studies,[12] **1** and **2** and their enantiomers were evaluated in a behavioral model of depression in mice. It is known that the serotoninergic [5-hydroxytryptamine (5-HT)] system is involved in the neural regulation of mood, and abnormalities in 5-HT neurotransmission have been observed in the pathophysiology of depression. Moreover, 5-HT dysfunction is one of the main mechanisms contributing to mood disorders in opiate abstinence.[13] It has also been suggested that α_{1A}-AR agonists might represent a novel treatment for depression.[14] Therefore, the biological profiles of the aforementioned compounds at 5-HT_{1A} receptor (5-HT_{1A}-R), particularly relevant to antidepressant responses in human beings,[15] and at α_{1A}-AR were assessed according to procedures previously reported.[16] Finally, an assessment of the in vitro absorption, distribution, metabolism, excretion (ADME) properties[9] of **2** and of the activity of **1** and **2** on the human ether-à-go-go-related gene (hERG) channel[17] was performed.

The enantiomers of **2** were prepared according to Scheme 1. (*S*)-(+)- and (*R*)-(−)-2-(2-cyclopropylmethyl)propionic acid methyl ester [(*S*)-(+)-**3** and (*R*)-(−)-**3**, respectively] were obtained by Mitsunobu reaction from 2-(cyclopropylmethyl)-phenol[18] and methyl (*R*)-(−)- or (*S*)-(+)-lactate, respectively, with inversion of configuration.[19] The reaction of (*S*)-(+)- or (*R*)-(−)-**3** with ethylenediamine in the presence of $(CH_3)_3Al$ yielded the corresponding imidazolines (*S*)-(+)- and (*R*)-(−)-**2**, whose e.e. determined by ^{1}H NMR spectroscopy was about 85%, due to partial racemization occurring under the conditions used in the reactions. Indeed, their spectra, as well as that of the racemic (±)-**2** on addition of the chiral shift reagent (*S*)-(+)-2,2,2-trifluoro-1-(9-anthryl)-ethanol, showed a double doublet at δ 1.38 ppm for the methyl protons. The resolution of the racemic imidazoline (±)-**2** was performed by HPLC using Chiralcel OD-H (25 cm × 0.46 cm, 5 μm particle size) as the chiral stationary phase and *n*-hexane/2-propanol 90/10 v/v

dx.doi.org/10.1021/ml300064v | ACS Med. Chem. Lett. 2012, 3, 535−539

Scheme 1. Synthesis of (*S*)-(+)-2 and (*R*)-(−)-2[a]



[a]Reagents and conditions: (a) DIAD, Ph_3P, THF, rt. (b) Ethylenediamine, $Al(CH_3)_3$, toluene, 0−65 °C, 7 h.

as the mobile phase. Detection was monitored at a wavelength of 220 nm, and the retention times were 5.9 and 10.3 min for (+)-**2** and (−)-**2**, respectively; (+)-**2**: $[\alpha]_D^{20}$ = +19.28, e.e. 100%; (−)-**2**: $[\alpha]_D^{20}$ = −19.59, e.e. 100%. In this case, the ^{1}H NMR spectra, similarly performed, showed for the methyl group of (+)-**2** and (−)-**2** only one doublet at δ 1.36 and δ 1.40 ppm, respectively. The absolute configuration of the stereocenter in the bridge of these enantiomers was determined by comparing the sign of their optical rotations with those of the enantiomers obtained by stereoselective synthesis. Consequently, the (*S*) absolute configuration was assigned to the dextrorotatory enantiomer (+)-**2** and (*R*) to the levorotatory (−)-**2**.

The affinity values (pK_i), antagonist properties (pK_b), agonist potencies, and intrinsic activities (pEC_{50} and ia, respectively) of the enantiomers of **2** on α_2-AR subtypes are reported in Table 1. The biological profiles of **2**, **1**, and its enantiomers were also included for useful comparison. This study indicates that (i) both enantiomers of **2** displayed binding affinities for the three α_2-AR subtypes comparable to those of the racemic compound; (ii) (*S*)-(+)-**2** was endowed with the highest α_{2C}-AR agonist activity; (iii) (*R*)-(−)-**2** was characterized by lower but still significant α_{2C}-AR agonism; and (iv) (*R*)-(−)-**2** and (*S*)-(+)-**2** showed weak α_{2B}-AR activation but effective α_{2A}-AR antagonist potencies. Interestingly, **2**, (*R*)-(−)-**2** and (*S*)-(+)-**2** affected expression and acquisition of morphine dependence (Figure 1) as observed previously for **1** and its enantiomers.[9] The frequencies of naloxone-precipitated jumping and other somatic signs (rearing, forepaw tremors, and teeth chatter) decreased significantly. To study possible antidepressant-like activity, the mouse forced swimming test (FST), a behavioral model with good reliability and predictive validity for screening antidepressants,[20] was used. Figure 2A shows that **1** induced significant reduction of immobility time. This effect was maximal at the dose of 0.05 mg/kg and comparable to that obtained with the dose of 20 mg/kg of the selective 5-HT reuptake inhibitor fluoxetine, included as reference compound.[21] Significant antidepressant activity was also observed

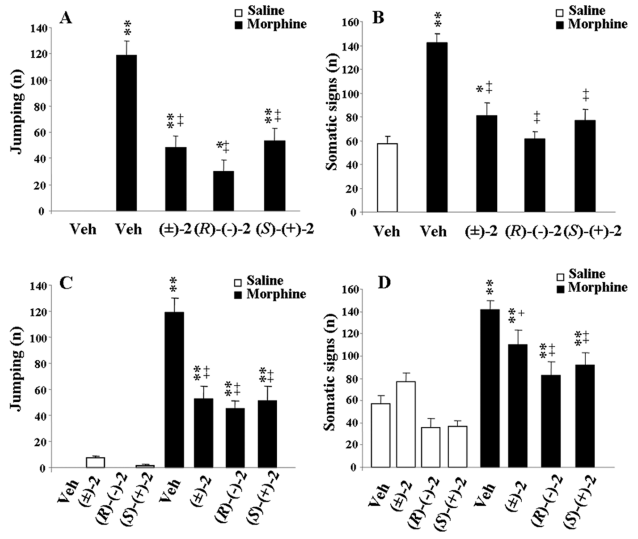


Figure 1. Effects of acute (A, B) or repeated (C, D) ip administration of (±)-**2** (0.05 mg/kg), (*R*)-(−)-**2**, and (*S*)-(+)-**2** (0.025 mg/kg) on expression and acquisition of morphine dependence, respectively. Naloxone-precipitated withdrawal symptoms, in both control and morphine-treated mice, are given as a measure of the frequency of jumping (A, C) and somatic signs (B, D) expressed as a summary of rearing, forepaw tremors, and teeth chatter. Significant differences: $*p < 0.05$, $**p < 0.01$, as compared to vehicle; $^{++}p < 0.01$, as compared to the morphine group.

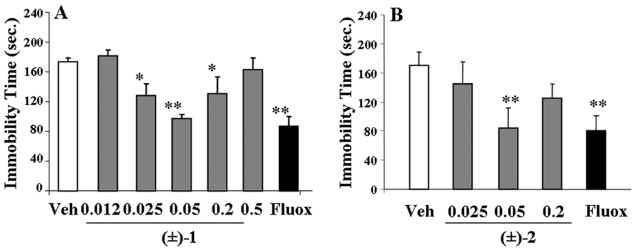


Figure 2. Immobility time in the FST in mice following ip administration of (±)-**1** at 0.012, 0.025, 0.05, 0.2, and 0.5 mg/kg (A), (±)-**2** at 0.025, 0.05, and 0.2 mg/kg (B), and fluoxetine (Fluox; 20 mg/kg) as a reference drug. Data represent means (±SEMs) of 8−9 animals. Differences from vehicle-treated: $*p < 0.05$, $**p < 0.01$; where not indicated, the differences are not statistically significant.

at 0.025 and 0.2 mg/kg doses, whereas no activity was observed at lower and higher doses. Similarly, the maximal effect of **2** was displayed at the dose of 0.05 mg/kg (Figure 2B). Nevertheless, the antidepressant response of **1** was only associated with the (*S*)-(+)-**1** enantiomer, whereas the effect of **2** was similarly induced by both its enantiomers (Figure 3). In Table 1, affinities and functional values of all compounds at 5-HT_{1A}-R are also reported. The 5-HT_{1A}-R agonist 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) was included for useful comparison.[16] From the data, it emerged that **1** displayed good 5-HT_{1A}-R affinity and behaved as an agonist. These properties were maintained and even enhanced only by the (*S*)-(+)-**1** enantiomer, whereas (*R*)-(−)-**1** showed negligible affinity ($pK_i < 5$).

In contrast, **2**, (*R*)-(−)-**2** and (*S*)-(+)-**2** displayed similar and significant 5-HT_{1A}-R affinities and agonist potencies. None of the compounds studied showed significant α_{1A}-AR agonism (ia < 0.35). Altogether, the data indicated that dual α_{2C}-AR/5-HT_{1A}-R activation was requested for the antidepressant-like

dx.doi.org/10.1021/ml300064v | ACS Med. Chem. Lett. 2012, 3, 535−539

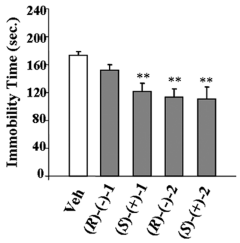


Figure 3. Immobility time in the FST in mice following ip administration of (*R*)-(−)-**1**, (*S*)-(+)-**1**, (*R*)-(−)-**2**, and (*S*)-(+)-**2** (0.025 mg/kg). Data represent means (±SEMs) of eight animals. Differences from vehicle-treated: $^{**}p < 0.01$; where not indicated, the differences are not statistically significant.

effect induced by low doses of **1** and the single (*S*)-(+)-**1** enantiomer, **2**, and both its enantiomers. This consideration does not contrast with the results of previous studies.[22,23] Indeed, they indicated that low doses of clonidine (0.01−0.06 mg/kg) increased 5-HT neurotransmission by attenuating the release of endogenous noradrenaline via activation of α_2-AR autoreceptors on adrenergic neurones[22] and, hence, induced anti-immobility effects with subactive doses of 5-HT$_{1A}$-R agonists (i.e., 8-OH-DPAT) in mouse FST.[23] On the contrary, higher doses (0.1−0.4 mg/kg) decreased 5-HT neurotransmission through the direct activation of the α_2-AR heteroreceptors on the 5-HT terminals.[22] The peculiar relationship between α_2-AR activation and 5-HT function, highlighted by such studies, might justify the dose-dependent U-shaped trend exhibited by the anti-immobility effect of our α_{2C}-AR/5-HT$_{1A}$-R agonists. To support our observations, experiments in the presence of the 5-HT$_{1A}$-R antagonist WAY 100135[24] and the α_2-AR antagonist yohimbine[25] were carried out. Both antagonists, ineffective when given alone, significantly and similarly contrasted the actions evoked by 0.025 mg/kg dose of (*S*)-(+)-**1**, (*R*)-(−)-**2**, and (*S*)-(+)-**2** (Figure 4). Therefore, these results confirmed that dual α_{2C}-

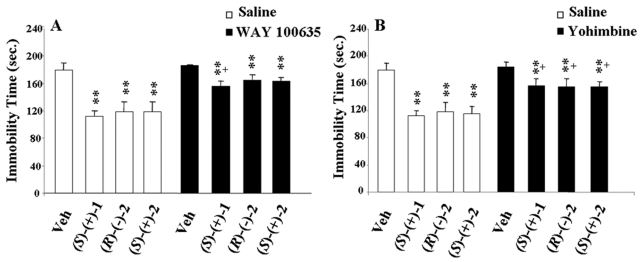


Figure 4. Influence of the 5-HT$_{1A}$-R antagonist WAY 100635 (0.1 mg/kg, sc) (A) and the α_2-AR antagonist yohimbine (1.250 mg/kg, ip) (B) on the antidepressant-like effect of the enantiomers (*S*)-(+)-**1**, (*R*)-(−)-**2**, and (*S*)-(+)-**2** (0.025 mg/kg, ip) on the FST. Data represent means (±SEMs) of 7−8 animals. Significant differences: $^{**}p < 0.01$, as compared with vehicle group; $^{++}p < 0.01$, as compared with enantiomers related-treated mice; and $^{\circ}p < 0.05$, as compared with antagonist-treated mice; where not indicated, the differences are not statistically significant.

AR/5-HT$_{1A}$-R activation, favorably triggered by the same molecule, took part in the anti-immobility effect. It has been reported that selective α_{2C}-AR antagonists, allowing α_{2A}-AR stimulation, produced important antidepressant effects in the FST.[26] Because α_{2A}- and α_{2C}-AR subtypes complement each other to integrate CNS function and behavior,[5] it is possible that also the α_{2C}-AR agonism/α_{2A}-AR antagonism combination, associated with 5-HT$_{1A}$ activation, as verified for the examined compounds, is equally compatible with an antidepressant-like effect. The advantage of this peculiar biological profile was also supported by the observation that yohimbine, a 5-HT$_{1A}$-R agonist[27] and antagonist at all three α_2-AR subtypes,[25] did not display antidepressant activity. The physicochemical and in vitro ADME[9] profile of **2** proved to be similar to that of **1**. Indeed, **2** was highly soluble in water at physiological pH. The permeation rate in a parallel artificial membrane permeability assay (PAMPA) assay and the percentage remaining after incubation for 1 h with recombinant hCYP3A4 were very high. From an automatic patch clamp assay on HEK293 cells overexpressing the hERG channel,[17] compounds **1** and **2** showed to be weak inhibitors, with IC_{50} values of 16.9 and 8.9 μM, respectively, suggesting a low cardiotoxicity risk associated with their potential therapeutic use.

In summary, the present study demonstrated that cyclomethyline (**2**) and its enantiomers, characterized by α_{2C}-AR agonist/α_{2A}-AR antagonist profile, significantly decreased the naloxone-precipitated withdrawal symptoms in mice at very low doses. These properties are in agreement with those shown by the lead **1** and its enantiomers and confirm that dual α_{2C}-AR agonism/α_{2A}-AR antagonism represents a favorable condition for inducing positive effects on morphine dependence.[9] Interestingly, such effects can even be improved by additional 5-HT$_{1A}$-R activation. Indeed, unlike clonidine, **1** or the single (*S*)-(+)-**1** enantiomer, **2**, or both its enantiomers, all behaving as α_{2C}-AR agonists/α_{2A}-AR antagonists/5-HT$_{1A}$-R agonists, alone and at the same low dose, improved morphine withdrawal syndrome and exerted a potent antidepressant-like effect. It has been suggested that opiate abuse may induce depressed mood, or conversely, depression can drive to drug abuse in a self-medication hypothesis. Therefore, such multifunctional compounds might be beneficial to both disorders.[13] In addition, their therapeutic potential is strongly enhanced by the lack of sedative side effects (due to their α_{2A}-antagonism) and alteration of motor activity (data not shown), as well as by their favorable in vitro ADME profiles and limited activity on the hERG channel.

■ ASSOCIATED CONTENT

Supporting Information

Synthetic procedure, hERG activity assay, detail of biological assay, and elemental analysis of the final compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel: +39-0737-402257. Fax: +39-0737-637345. E-mail: maria.pigini@unicam.it.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank the MIUR (Rome), the University of Camerino, and the Fondazione Monte dei Paschi (Siena) for financial support.

■ ABBREVIATIONS

AR, adrenoreceptor; CNS, central nervous system; CHO, Chinese hamster ovary; 5-HT, 5-hydroxytryptamine; 5-HT$_{1A}$-R, 5-HT$_{1A}$ receptor; ee, enantiomeric excess; ip, intraperitoneally;

sc, subcutaneously; ADME, absorption, distribution, metabolism, excretion; PAMPA, parallel artificial membrane permeability assay; DIAD, diisopropyl azodicarboxylate; CYP, cytochrome P450; hERG, human ether-à-go-go-related gene

REFERENCES

(1) Veilleux, J. C.; Colvin, P. J.; Anderson, J.; York, C.; Heinz, A. J. A review of opioid dependence treatment: pharmacological and psychological interventions to treat opioid addiction. *Clin. Psychol. Rev.* **2010**, *30*, 155−166 and references therein.

(2) Streel, E.; Dan, B.; Campanella, S.; Meyvaert, A.; Hanak, C.; Pelc, I.; Verbanck, P. A pharmacological modulation of opiate withdrawal using an up-/down-regulation of the noradrenergic system in opiate-dependent rats. *J. Neuropsychoparmacol.* **2006**, *9*, 621−626.

(3) Tan, C. M.; Limbird, L. E. The α_2-adrenergic receptors. In *The Receptors: The Adrenergic Receptors in the 21st Century*; Perez, D., Ed.; Humana Press Inc.: Totowa, NJ, 2006; pp 241−265.

(4) Fairbanks, C. A.; Stone, L. S.; Wilcox, G. L. Pharmacological profile of alpha 2 adrenergic receptor agonists identified using genetically altered mice and isobolographic analysis. *Pharmacol. Ther.* **2009**, *123*, 224−238 and references therein.

(5) Hein, L. Adrenoceptors and signal transduction in neurons. *Cell Tissue Res.* **2006**, *326*, 541−551 and references therein.

(6) Gish, E. C.; Miller, J. L.; Honey, B. L.; Johnson, P. N. Lofexidine, an alpha2-receptor agonist for opioid detoxification. *Ann. Pharmacother.* **2010**, *44*, 343−351.

(7) Diamanti, E.; Del Bello, F.; Carbonara, G.; Carrieri, A.; Fracchiolla, G.; Giannella, M.; Mammoli, V.; Piergentili, A.; Pohjanoksa, K.; Quaglia, W.; Scheinin, M.; Pigini, M. Might the observed α_{2A}-adrenoreceptor agonism or antagonism of allyphenyline analogues be ascribed to different molecular conformations? *Bioorg. Med. Chem.* **2012**, *20*, 2082−2090.

(8) Cardinaletti, C.; Mattioli, L.; Ghelfi, F.; Del Bello, F.; Giannella, M.; Bruzzone, A.; Paris, H.; Perfumi, M.; Piergentili, A.; Quaglia, W.; Pigini, M. Might adrenergic α_{2C}-agonists/α_{2A}antagonists become novel therapeutic tools for pain treatment with morphine? *J. Med. Chem.* **2009**, *52*, 7319−7322 and references therein.

(9) Del Bello, F.; Mattioli, L.; Ghelfi, F.; Giannella, M.; Piergentili, A.; Quaglia, W.; Cardinaletti, C.; Perfumi, M.; Thomas, R. J.; Zanelli, U.; Marchioro, C.; Dal Cin, M.; Pigini, M. Fruitful adrenergic α_{2C}-agonism/α_{2A}-antagonism combination to prevent and contrast morphine tolerance and dependence. *J. Med. Chem.* **2010**, *53*, 7825−7835 and references therein.

(10) Özdoğan, Ü. K.; Lähdesmäki, J.; Scheinin, M. Influence of prazosin and clonidine on morphine analgesia, tolerance and withdrawal in mice. *Eur. J. Pharmacol.* **2003**, *460*, 127−134.

(11) Özdogan, Ü, K.; Lähdesmäki, J.; Hakala, K.; Scheinin, M. The involvement of α_{2A}-adrenoceptors in morphine analgesia, tolerance and withdrawal in mice. *Eur. J. Pharmacol.* **2004**, *497*, 161−171.

(12) Cerdá, M.; Sagdeo, A.; Galea, S. Comorbid forms of psychopathology: key patterns and future research directions. *Epidemiol. Rev.* **2008**, *30*, 155−177.

(13) Goeldner, C.; Lutz, P.-E.; Darcq, E.; Halter, T.; Clesse, D.; Ouagazzal, A.-M.; Kieffer, B. L. Impaired emozional-like behavior and serotonergic function during protracted abstinence from chronic morphine. *Biol. Psychiatry* **2010**, *69*, 236−244 and references therein.

(14) Doze, V. A.; Handel, E. M.; Jensen, K. A.; Darsie, B.; Luger, E. J.; Haselton, J. R.; Talbot, J. N.; Rorabaugh, B. R. α_{1A}- and α_{1B}-adrenergic receptors differentially modulate antidepressant-like behaviour in the mouse. *Brain Res.* **2009**, *1285*, 148−157.

(15) Blier, P.; Ward, N. M. Is there a role for 5-HT_{1A} agonists in the treatment of depression? *Biol. Psychiatry* **2003**, *53*, 193−203.

(16) Quaglia, W.; Piergentili, A.; Del Bello, F.; Farande, Y.; Giannella, M.; Pigini, M.; Rafaiani, G.; Carrieri, A.; Amantini, C.; Lucciarini, R.; Santoni, G.; Poggesi, E.; Leonardi, A. Structure-activity relationships in 1,4-benzodioxan-related compounds. From 1,4-benzodioxan to 1,4-dioxane ring as a promising template of novel α_{1D}-adrenoreceptor antagonists, 5-HT_{1A} full agonists, and cytotoxic agents. *J. Med. Chem.* **2008**, *51*, 6359−6370.

(17) Sanguinetti, M. C.; Tristani-Firouzi, M. hERG potassium channels and cardiac arrhythmia. *Nature* **2006**, *440*, 463−469.

(18) He, Z.; Yudin, A. K. Palladium-catalyzed oxidative activation of arylcyclopropanes. *Org. Lett.* **2006**, *25*, 5829−5832.

(19) Tottie, L.; Baeckstrom, P.; Moberg, C.; Tegenfeldt, J.; Heumann, A. Molecular sieve controlled diastereoselectivity: Effect in the palladium-catalyzed cyclization of *cis*-1,2-divinylcyclohexane with R-oxygen-substituted acids as chiral nucleophiles. *J. Org. Chem.* **1992**, *57*, 6579−6587.

(20) Petit-Demouliere, B.; Chenu, F.; Bourin, M. Forced swimming test in mice: A review of antidepressant activity. *Psychopharmacology* **2005**, *177*, 245−255.

(21) Page, M. E.; Detke, M. J.; Dalvi, A.; Kirby, L. G.; Lucki, I. Serotonergic mediation of the effects of fluoxetine, but not desipramine, in the rat forced swimming test. *Psychopharmacology* **1999**, *147*, 162−167.

(22) Mongeau, R.; Blier, P.; de Montigny, C. In vivo electrophysiological evidence for tonic activation by endogenous noradrenaline of alpha 2-adrenoceptors on 5-hydroxytryptamine terminals in the rat hippocampus. *Naunyn-Schmiedebergs Arch. Pharmacol.* **1993**, *347*, 266−272.

(23) Redrobe, J. P.; Bourin, M. Clonidine potentiates the effects of 5-HT_{1A}, 5-HT_{1B} and 5-$HT_{2A/2C}$ antagonists and 8-OH-DPAT in the mouse forced swimming test. *Eur. J. Neuropsychofarmacol.* **1998**, *8*, 169−173.

(24) Cliffe, I. A.; Brightwell, C. I.; Fletcher, A.; Forster, E. A.; Mansell, H. L.; Reilly, Y.; Routledge, C.; White, A. C. (S)-N-tert-Butyl-3-(4-(2-methoxyphenyl)piperazin-1-yl)-2-phenylpropanamide [(S)-WAY-100135]: A selective antagonist at presynaptic and postsynaptic 5-HT_{1A} receptors. *J. Med. Chem.* **1993**, *36*, 1509−1510.

(25) Audinot, V.; Fabry, N.; Nicolas, J.-P.; Beauverger, P.; Newman-Tancredi, A.; Millan, M. J.; Try, A.; Bornancin, F.; Canet, E.; Boutin, J. A. Ligand modulation of [^{35}S]GTPγS binding at human α_{2A}, α_{2B} and α_{2C} adrenoceptors. *Cell Signalling* **2002**, *14*, 829−837.

(26) Sallinen, J.; Höglund, I.; Engström, M.; Lehtimäki, J.; Virtanen, R; Sirviö, J.; Wurster, S.; Savola, J.-M.; Haapalinna, A. Pharmacological characterization and CNS effects of a novel highly selective α_{2C}-adrenoceptor antagonist JP-1302. *Br. J. Pharmacol.* **2007**, *150*, 391−402.

(27) Holmes, A.; Quirk, G. J. Pharmacological facilitation of fear extinction and the search for adjunct treatments for anxiety disorders—The case of yohimbine. *Trends Pharmacol. Sci.* **2009**, *31*, 2−7.