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Highly selective and potent μ opioid ligands by unexpected substituent on morphine skeleton

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ABSTRACT

Unexpected substituent on the well-known morphine skeleton is described to be account for highly selective and potent μ opioid ligands, which is strongly connected to substituted aromatic groups on this omitted 8α-position.

Thebaine (Fig. 1) is a naturally occurred alkaloid isolated from opium. Though it bears much similar structure with morphine, thebaine is inactive as analgesic with very low affinities to opioid receptors.[1] However, it could be converted to a variety of potent narcotics[2] (e.g., Buprenorphine, Oxycodone) widely used in clinic through several organic transformations. And the Diels–Alder reaction of thebaine with dienophiles is obviously the most valuable one among these reactions, which leads to the discovery of extremely highly active analgesics than morphine.[3] And due to less selective characters[1] of these so-called oripavines yielded from the transformations of thebaine, the tritiated form of Diprenorphine and Etorphine has been used universally for opioid receptors.

It is widely accepted that subtle differences in critical structural loci of bioactive compounds may result in great changes in biological responses. And these may provide useful information on drug-receptor interactions and on receptor characteristics.[4] Since the greatly enhanced biological activities were suggested strongly to be linked with the substituent on C7 position which may be accommodated at the postulated lipophilic domain on the opioid receptor,[5] it was desirable to investigate other cycloadducts of thebaine with dienophiles besides the well investigated 7α-adducts experimentally and theoretically.[6] However, the 8α-adducts have been not considered seriously because not only are they not accessible through the cycloaddition of thebaine and dienophiles to date, but also significant decrease in analgesic potency[7] and reduced affinities[8] to opioid receptors have been observed in the known analogue in comparison to their main 7α-counterparts.

In this Letter we reported unexpected 8α-adducts yielded from thebaine and styrenes which bear obviously different SARs (Structure–Activity Relationships) with other oripavines. And the substituent on the C8-position is strongly linked with μ opioid agonistic activity and selectivity which has been omitted before.

A straightforward synthetic strategy was developed (Scheme 1), and thebaine was preferably attacked by styrenes from the less bulky β face[9] to afford 6α, 14α-endo-ethenotetrahydrothebaine. In addition to known 7α-adducts yielded as main products, unexpected by-products were separated in case of styrene, *m*-nitrostyrene and *p*-nitrostyrene. Follow-up works examined compound-5 by X-ray crystallography (Fig. 2, compound-5, CCDC 696689) and found it was a regioisomeric 8α-adduct in comparison to its main 7α-adduct (compound-**4**, CCDC 696688). Similar large deshielding effect to the proximity of the tertiary amine is observed for H-8β in ^{1}H NMR spectra of all regioisomeric adducts, as well as characteristic resonances of H7β upfield due to absence of substitution (Table 1). And normal 7β-adducts as were absent (Table 1).

In the absence of the polarizing methoxy functionality at C-6 of thebaine, Diels–Alder addition to 3-buten-2-one can give a regioisomeric adduct in which the acetyl group is attached at C-8.[7] And the Diels–Alder addition to styrenes may afford similar

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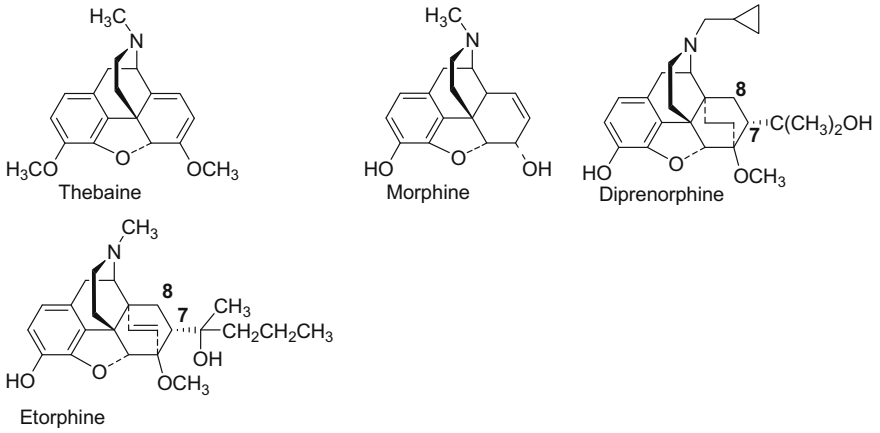
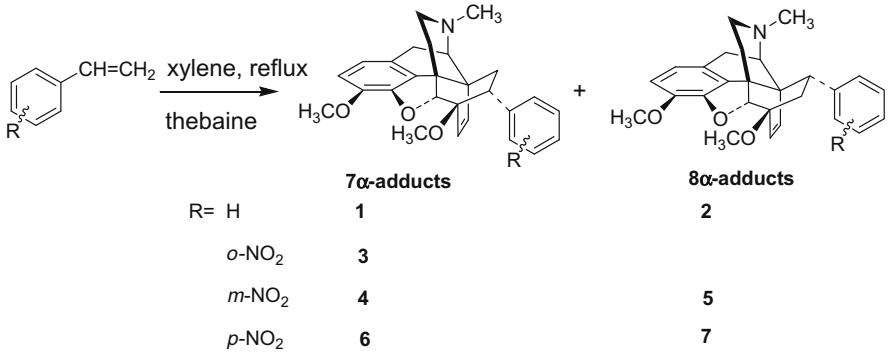


Figure 1. The structures of thebaine, morphine, diprenorphine and etorphine.



Scheme 1. The Diels–Alder reactions of thebaine and styrenes.

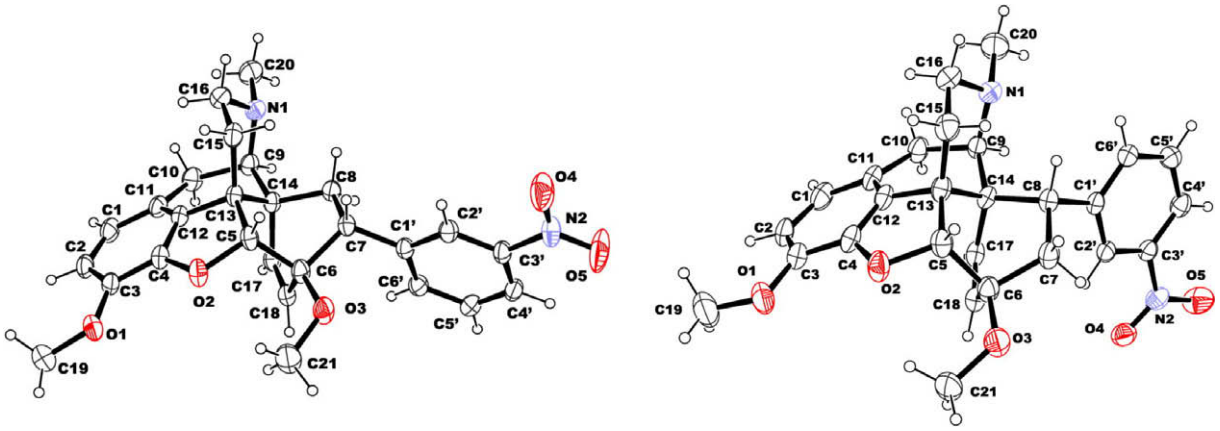
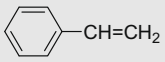
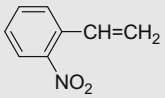
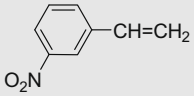
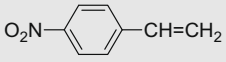


Figure 2. X-ray crystal structures of compound-**4**(left) and -**5**(right).

regioisomeric 8α-adducts, since these dienophiles are also devoid of polarizing functionality in their structures which are different to other dienophiles such as 3-buten-2-one and acrylonitrile. Although other factors such as solvent, harsh conditions may take effects, the trend of Diels–Alder reactions forwarded to main 7α-adducts has not changed in all of these cases.

Affinities of these compounds to opioid receptors were determined by competitive inhibition of [^{3}H] diprenorphine binding (Table 2). Ligand regulation of the binding of [^{35}S]GTPγS is one of the most widely used methods to measure receptor activation of heterotrimeric G protein, and has been used as a functional measure for determination of potencies and efficacies of agonists. Thus, the agonistic activities of these compounds were determined by regulation of the binding of [^{35}S]GTPγS (Table 3). Since most compounds have no undetectable affinities for δ- and κ-opioid receptors at the concentrations up to 10 μM, only μ opioid receptor was employed in the functional assays. The binding profiles of these compounds for three types of opioid receptors (μ, δ, κ) have been shown in Table 2 and the agonistic activity of these compounds for μ opioid receptors has been shown in Table 3.

Table 1
Isolated yields and characteristic resonances of H7β upfield and H8β downfield of 8α-adducts in comparison to 7α-adducts

Compound entry	Dienophile[a]	7α-Adduct[b]	8α-Adduct[b]	$\delta_{H7\beta}$ ppm	$\delta_{H8\beta}$ ppm
1		40.9		3.04	3.32–3.27
2			1.3	2.16	4.28
3		83.6		3.71	3.46
4		75.6		3.18–3.14	3.40–3.34
5			2.7	2.25–2.20	4.44
6		71.7		3.16	3.38
7			3.0	2.24–2.17	4.41

[a] The ratio of dienophile versus diene (thebaine) was 3:1.
[b] (Isolated yield)%.

Table 2
Affinity values for the binding of compounds to μ-, δ- and κ-opioid receptors[a]

	K_i (nM)		
	μ	δ	κ
1	>5000	>10,000	>10,000
2	>5000	>10,000	>10,000
4	>10,000	>10,000	>10,000
5	22.0 ± 8.6	>10,000	>10,000
6	>10,000	>10,000	>10,000
7	674.0 ± 117.1	>10,000	>10,000
Morphine	6.9 ± 1.3	116.8 ± 15.4	76.9 ± 16.3

[a] Membrane from CHO cell expressing μ-, δ- and κ-opioid receptors were incubated with varying concentrations of compounds in the presence of 0.45 nM [^{3}H]diprenorphine as described under 'Experimental section' in Supplementary data. Data are expressed as Mean ± SEM for at least three determinations performed in triplicate.

Table 3
Stimulation of [^{35}S]GTPγS binding to membrane receptors by compounds[a]

	[^{35}S]GTPγS	
	EC50 (nM)	Maximum (% of basal)
1	>10,000	175 ± 6.0
2	>10,000	174 ± 5.2
4	ND[b]	ND[b]
5	302.3 ± 7.5	221 ± 2.8
6	ND[b]	ND[b]
7	751.0 ± 95.0	248 ± 8.6
Morphine	123.5 ± 30.5	209 ± 15.9

[a] Assays were performed in membranes prepared from the cell expressing μ opioid receptor with varying concentrations of compounds in the presence of 0.1 nM [^{35}S]GTPγS as described under 'Material and methods'. Data are expressed as the Mean ± SEM for at least three experiments performed in triplicate.
[b] ND: not determinable, which means that the compounds nearly have no ability to stimulate [^{35}S]GTPγS binding to membrane receptors (inactive).

Unlike typical oripavines (e.g., diprenorphine and etorphine) with flexible acyclic hydrophobic substituent on the 7α-position, more compact and rigid aromatic groups attached directly to 7α-adducts result in reduced affinities to all opioid receptors. Furthermore, more bulky nitro-group substituted analogues (e.g., compound-**3**, **4**) almost abandoned activities against all opioid receptors. However, same substituents on 8α-position show different pharmacological profiles on μ opioid receptor with enhanced affinities and selectivity which becomes even more significant when a nitro-group is introduced. And the K_i values of compound-**5** and compound-**7** are 22.0 ± 8.6 and 674.0 ± 117.1 nM, respectively. Although compound-**5** bears similar affinities with morphine,[10] it is highly selective to μ opioid receptor while morphine reserves certain affinities to μ and κ-opioid receptors. Follow-up functional assays demonstrated that both compounds bear similar potencies and efficacies with morphine with the EC_{50} values to be 302.3 ± 7.5 (compound-**5**) and 751.0 ± 95.0 nM (compound-**7**) and Maximal stimulation values of 221 ± 2.8 (compound-**5**) and 248 ± 8.6 (compound-**7**), respectively.

Compound-**5** is potent and highly selective μ opioid agonist based on these pharmacological assays. The substituent on 8α-position accounting for μ opioid selectivity and activity of this compound is different from that on 7α-position producing non-selective opioids although these two positions are sterically close to each other. And this difference in SAR results seemed to be connected to the substituted aromatic substituent on 8α-position which may selectively increase the agonistic activities against μ opioid receptor, but not δ- or κ-opioid receptors. Further investigations on this specific position will be reported later.

Morphine is a standard therapeutic agent of the treatment of moderate-to-severe pain. However, its clinical use is greatly limited due to adverse side effects such as tolerance and dependence. Lack of exclusive selectivity for individual receptor subtype might be linked to its high potential to develop tolerance and dependence.[11] Thus, the findings of the present study may have potential importance for the development of new opioid analgesics with less liability to develop tolerance and dependence.

In conclusion, unexpected 8α-adducts from thebaine and dienophiles were reported, and it was proposed that the appearance of these by-products may be connected to the structural features of dienophiles. Furthermore, compound-**5** is potent and highly selective μ opioid agonist, which is connected to the substituent on the 8α-position on morphine skeleton. Further investigation on this omitted position may be valuable to provide useful information for drug-receptor interactions since it is still in darkness for detailed interactions between opioid receptors and opioids, and

opioids with novel pharmacological profiles from the classical structure of analgesics.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.07.119.

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