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*Full Length Research Paper*

# Antinociceptive activity of some 1,4-substituted piperidine derivatives using tail flick method in mice

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**We have examined the potential antinociceptive effects of some piperidine derivatives (6a-f), using tail flick method in mice. Morphine was used as positive control drug. The results showed that compound 6b, having a bromine atom at 4 position, was the most active agent tested. The activity of this compound at 50 mg/kg was comparable to morphine (3 mg/kg). Based on the similarities between our compounds and meperidine like structures such as fentanyl, a group of mice was treated with naloxone before administration of 6f. It was concluded that opioid receptors could be the dominant mechanism for the antinociceptive activity of these types of structures. Electrostatic map, distance analysis and superimposition between fentanyl and compound 6b were also studied.**

**Key words:** Piperidine derivatives, antinociceptive activity, tail flick.

## INTRODUCTION

It has been widely accepted that pain can devastate human quality of life and its management is considered as a main challenge in medicine. It has been widely accepted that pain can devastate human quality of life and is considered as a main challenge in medicine (Giovanonni et al., 2007; Nkomo et al., 2010). The two major classes of traditional analgesics for treatment of pain include nonsteroidal anti-inflammatory drugs (NSAIDs) and opioids (Cesari et al., 2006). The clinical use of NSAIDs is associated with side effects including gastrointestinal lesions and nephrotoxicity (Cesari et al., 2006).

Narcotic analgesics in the morphine class are still considered as the main treatment of pain caused by trauma, surgery and cancer (MacDougall et al., 2004). Due to some reasons such as adverse side effects and abuse liability, clinical use of morphine class was also restricted. Therefore, pharmaceutical researches focused on finding new options of analgesic therapy through optimization of the current structures. Identification of compounds able to treat both acute and chronic pain with limited side effects has been also a prominent goal in medicinal chemistry and drug design (Giovanonni et al., 2007; Cesari et al., 2006). In several publications, synthesis and analgesic activity of several substituted piperidines were reported (Kugita et al., 1964, 1965; Saify et al., 2005). Some reports have revealed the CNS related adverse effects of piperidine like structures, such as meperidine (Golder et al., 2010; Guo et al. 2009).

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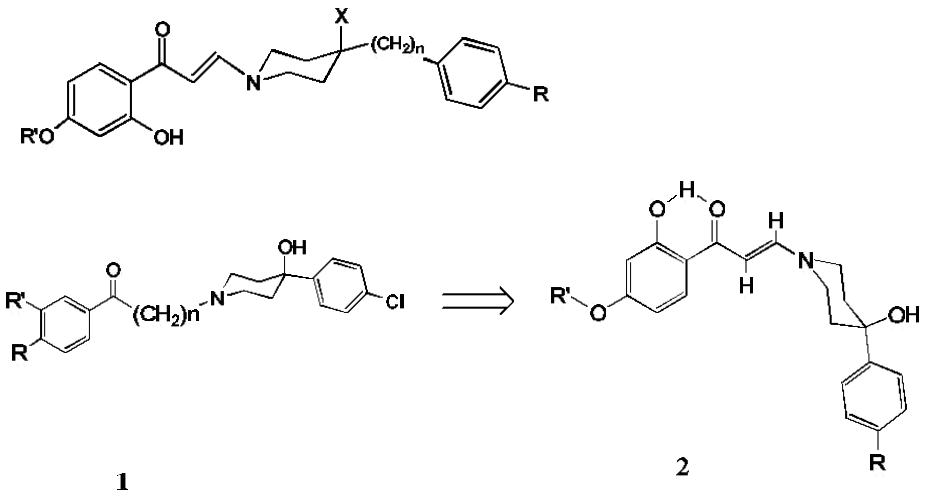


**Scheme 1.** Using , -unsaturated system in compound 2 for conformational restriction of phenacyl moiety in compound 1.

Among the synthesized piperidines, analgesic activity of some 4-phenylpiperidinyl-1-phenylcyclopentane-carboxylates through tail flick studies was described (Saify et al., 2005). Three fragments composed of two phenyl rings on piperidine scaffold, which one of them attached to the nitrogen atom in piperidine ring using an alkyl linker chain with different number of atoms. In continuation of previous studies to find a new CNS active compound (Mahdavi et al., 2010; Foroumadi et al., 2007) and based on the reported scaffold in Scheme 1, our attempt was to investigate the effects of conformational change inside the linker on the activity of the resulted compounds. For this purpose, introduction of a double bond inside the linker was used to induce some conformational restriction in this part of the molecule (compound 1, Scheme 1). Finally, based on the similarities between our synthesized compounds and meperidine like structures such as Fentanyl, analgesic activity of the target compounds were evaluated by an acute pain model (tail flick).

## MATERIALS AND METHODS

### Synthesis

3-(Piperidin-1-yl) -1-(4-substitutedphenyl)prop-2- en-1-onederivatives (6a-f) has been synthesized according to the reported procedure (Scheme 1, compound 2) (Sakhteman et al., 2009).

### Tail flick test

White male albino mice, weighing 20 to 30 g, were used for thermal tail flick test. The animals were kept under conditions of 12 h light and 12 h darkness and temperature: 21 ± 2°C. All animals had access to food and water except during the experiment. Six animals were used for each dose. The synthesized compounds were dissolved in a solvent system containing of Tween 80 (5%), ethanol (5%), Propylene glycol (10%) and water (80%) (Kugita et al., 1964). The administered dose of each compound included 25, 50 and 100 mg/kg (ip). The doses were selected according to the results obtained in pilot studies. Morphine (3 mg/kg) was administered to the positive control group, and for the negative control group, equivalent volumes of the vehicle was injected (Nikfar et al., 1997). Subsequently, the mice were subjected to thermal stimuli of the tail flick apparatus at the time intervals of 45, 90 and 180 min after administration of the compounds and the readings were recorded for each group. The intensity of the apparatus was set to 50 and a cut off value of 10 s was considered to lessen the animals suffer. In one group of mice, Naloxone (4 mg/kg, sc) was used as morphine antagonist 15 min before administration of the test compound, 6f (50 mg/kg, ip) (Heidari et al., 2009; Sharifzadeh et al., 2006). The whole procedure was in accord with the ethics rules of Tehran University of Medical Sciences.

## RESULTS AND DISCUSSION

### Statistical analysis

The results of tail flick test in terms of mean response time (sec) ± SE at the time intervals 45, 90 and 180 min after administration of the target compounds and controls are listed in Table 1. Statistical analysis on the raw data was performed by means of ANOVA followed by a post Tukey test. The P-value < 0.05 was used to represent a significant difference (confidence interval > 95%) between the two groups.

It was observed that most of the compounds were showing significant activity at 50 and 100 mg/kg doses in respect to vehicle. The activity of compounds 6b and c at 50 and 100 mg/kg was comparable to morphine at 3 mg/kg during all time intervals (P>0.05). The order of

**Table 1.** Tail flick response time (sec) ± SE for compounds 6a-f.

| Time Intervals | | | | | 45 min | | | 90 min | | | 180 min | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Code** | **R** | **R'** | **x** | **n** | **25** | **50 (mg/Kg)** | **100** | **25** | **50 (mg/Kg)** | **100** | **25** | **50 (mg/Kg)** | **100** |
| 6a | H | Me | H | 1 | 4.93±1.4$^{\wedge}$ | 5.51±0.47$^{\wedge}$ | 4.87±0.36$^{**}$ | 5.10± 0.48$^{\wedge}$ | 5.00 ± 0.62$^{\wedge}$ | 4.54 ± 0.36$^{\wedge}$ | 5.50 ±0.37$^{**}$ | 5.68± 0.41$^{**}$ | 5.34± 0.47$^{**}$ |
| 6b | Br | Me | OH | 0 | 4.23±0.85 | 5.80±0.67$^{**}$ | 5.86±0.54$^{**}$ | 4.58 ± 0.82 | 6.63± 0.92$^{*}$ | 6.92 ± 0.43$^{**}$ | 3.8 ± 0.54 | 6.65± 0.52$^{*}$ | 6.32 ± 0.4$^{*}$ |
| 6c | H | Me | OH | 0 | 4.30 ± 0.59 | 6.00±0.73$^{**}$ | 6.32±0.48$^{**}$ | 4.77 ± 0.79 | 4.86 ± 0.64$^{*}$ | 6.02 ± 0.56$^{**}$ | 4.93 ± 0.54 | 5.37 ± 0.24$^{*}$ | 4.77± 0.19$^{*}$ |
| 6d | OH | Me | H | 0 | 4.52 ± 0.56 | 5.18± 0.39$^{*}$ | 6.10±0.75$^{**}$ | 5.20 ± 0.87$^{*}$ | 4.72 ± 0.25 | 5.65 ± 0.36$^{**}$ | 5.05 ± 0.71$^{*}$ | 3.18 ± 0.62$^{*}$ | 4.43 ± 0.79$^{*}$ |
| 6e | Cl | Me | OH | 0 | 5.17 ± 0.33$^{*}$ | 4.68 ± 0.15 | 5.45±0.54$^{*}$ | 4.90 ± 0.73$^{*}$ | 5.10 ± 0.65$^{*}$ | 5.10 ± 0.57$^{*}$ | 5.18 ± 0.53$^{*}$ | 4.60 ± 0.38$^{*}$ | 4.57 ± 0.35$^{*}$ |
| 6f | Br | H | OH | 0 | 4.23 ±0.95 | 5.30±0.38$^{**}$ | 5.18 ±0.70$^{**}$ | 4.05±0.54 | 5.6 ±0.25$^{**}$ | 5.34 ±0.21$^{**}$ | 4.20 ±1.60 | 3.62 ±1.44 | 3.80 ±0.40 |
| Morphine (3 mg/Kg) | | | | | | 5.99 ± 0.23$^{**}$ | | | 5.34 ± 0.21$^{**}$ | | | 6.06 ± 0.22$^{**}$ | |
| Vehicle | | | | | | 3.13 ± 0.12 | | | 3.19 ± 0.11 | | | 2.67 ± 0.12 | |

*- Tukey post ANOVA test, P < 0.05 in respect to vehicle. **- Tukey post ANOVA test, P <0.001 in respect to vehicle.

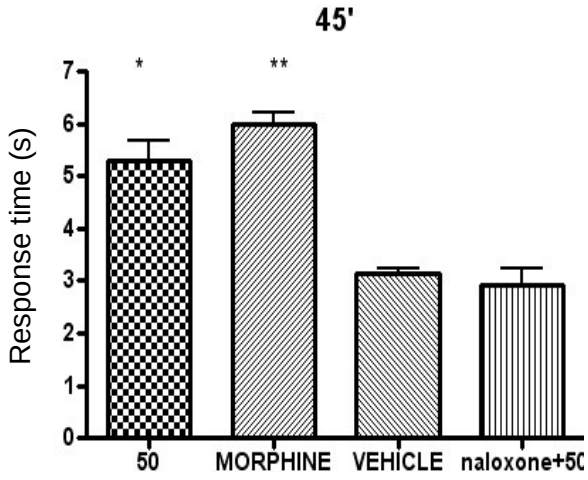


**Figure 1.** Tail flick response time in compound 6f (50 mg/kg) at the time interval of 45'; in one group naloxone was injected before 6f. *P<0.01 in respect to vehicle, **P<0.01 in respect to vehicle.

activity for 50 mg/kg dose of compounds 6a-f was: 6b > 6f > 6a > 6d > 6c > 6e. It was also demonstrated that the highest activity at the time intervals of 90 and 180 min was pertained to compound 6b (100 mg/kg). A simple modification in one phenyl ring was done through replacing $OCH_3$ in 6b with OH in 6f. The result of this modification was a slight decrease in the activity and duration of action of compound 6f in respect to 6b. To ensure whether the mechanism of activity for these compounds is through opioid mechanism, a group of mice was treated with naloxone, 15 min before administration of compound 6f. Results of mean response time ± SE for compound 6f in comparison with its positive and negative controls are depicted in Figure 1 for the time interval of 45'. It was observed that the mean response time for compound 6f is significantly more than that observed for vehicle. The activity of this compound at 50 mg/kg was comparable to morphine (3 mg/kg). It was also demonstrated that the activity of compound 6f is significantly decreased in the presence of naloxone. This result was verifying that opioid receptor might be responsible for antinociceptive activity of the target compounds. In an attempt to compare the activities with the previously reported structures depicted in Scheme 1, tail flick latency difference (TFLD) was calculated for the synthesized compounds (Time interval 90', 50 mg/kg doses). The plot of calculated TFLDs for the synthesized structures together with the reference compounds (Scheme 1, compound 1, time interval 90', 50 mg/kg doses) is shown in Figure 2. It was observed that 6b is the most potent compound in both groups. The second order of activity was attributed to the reference compound (Kugita et al., 1964) with chlorine and bromine at para position of first and second phenyl rings, respectively. The most inactive compound was attributed to compound with no aromatic phenyl attached to piperidine ring (Cesari et al., 2006). This pattern suggests that presence of both aromartic phenyl rings is necessary for the activity of these structures. The activity of our synthesized compound 6e, with chlorine at para position of phenyl ring and a two atom linker with double bond, is also more than its counterpart with no double bond (Saify et al., 2005). The result of activity for these compounds is also suggesting that introducing a double bond in the linker could

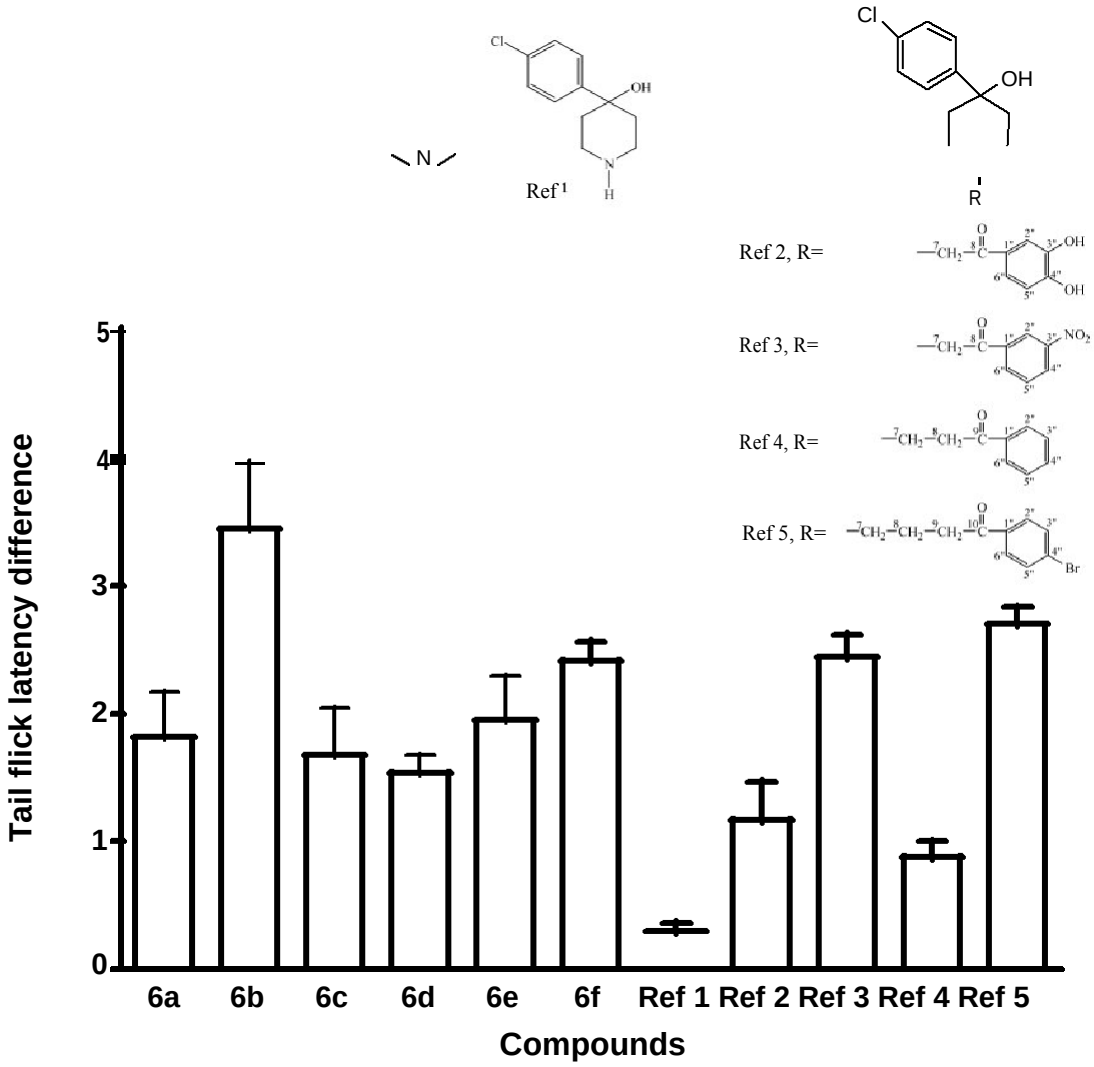


**Figure 2.** A comparison between antinociceptive activity of the synthesized compounds (6a-f) and previously reported derivatives (Ref 1- Ref 5).

led to a slight increase in the activity of the synthesized compounds. In order to compare the structure of these compounds with a meperidine like structure, fentanyl was drawn in Hyperchem 7 (HyperChem. ,HyperCube Inc, 2010) and its structure was minimized with the semiempirical method, PM3 (HyperChem. ,HyperCube Inc, 2010). The same procedure was also taken for the most potent compound 6b. A superposition of both minimized structures was done using Hyperchem and the result is depicted in Figure 3a. Electrostatic map of the two compounds (Fentanyl and 6b) is displayed in Figure 3b. The distance between the two aromatic rings was calculated for each compound. The calculated distances in fentanyl and compound 6b were 9.83 Å and 9.87 Å, respectively. This implies that most graphical features present in fentanyl derivatives could be also observed in this series of compounds.

## Conclusion

Some meperidines like structures were synthesized and evaluated for acute antinociceptive activity in mice model. These structures were similar to some reported structures in such a way that a double bond was introduced in the linker to investigate the role of conformational restriction on the antinociceptive activity of the resulted compounds. The most active compound in these series was compound 6b with a bromine atom at para position of phenyl ring. The moderate antinociceptive activity of the synthesized compounds is suggesting a favored conformation similar to meperidine like analogues. Further pharmacological studies are needed for elucidation of the mechanisms of action, peripheral vs. central mechanisms and the effectiveness of our compounds in chronic model of hypernociception

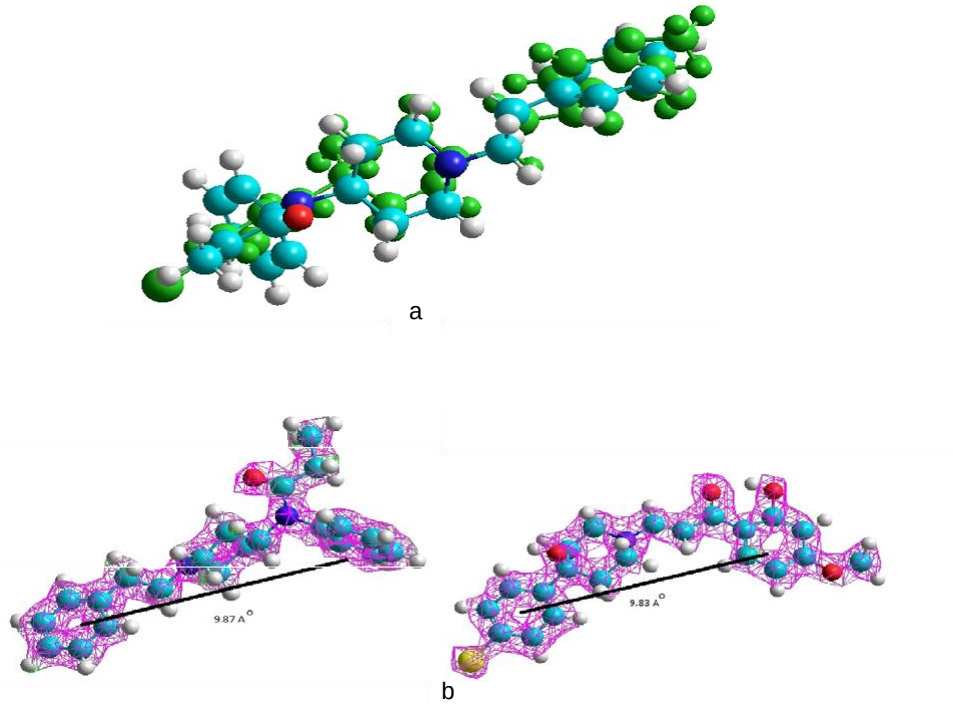


**Figure 3.** (a) Superimposition of one meperidine like structure fentanyl (colored) with the synthesized structure 6b , (b) Electrostatic map and distance analysis between fentanyl (Left) and 6b (Right).

(Nguelefack et al., 2010).

## ACKNOWLEDGEMENTS

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