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ORIGINAL ARTICLE

# Studies on 1-(2-phenethyl)-4-(*N*-propionylanilino)piperidine (fentanyl) and its related compounds. VI. Structure–analgesic activity relationship for fentanyl, methyl-substituted fentanyls and other analogues

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**Abstract** Eleven chemically modified 1-(2-phenethyl)-4-(*N*-propionylanilino)-piperidine (fentanyl) analogues were synthesized and their analgesic activities were evaluated by the acetic acid writhing method in mice. Their effective dose ($ED_{50}$) and lethal dose ($LD_{50}$) values were compared with those of morphine and fentanyl. The synthesized fentanyl analogues were categorized into three groups: a mono-methylated group, a group in which hydrogen in the *para*-position of the aromatic ring bound to the propionylanilino group was substituted with F, Cl, $CH_3$, or $OCH_3$, and a group in which the propionyl moiety was changed to an acetyl one. 3-Methylfentanyl showed the strongest analgesic activity among these compounds, and the most frequently abused fentanyl derivative, α-methylfentanyl, also showed quite strong activity. The analgesic activities of *p*-fluorofentanyl and acetylfentanyl were also relatively strong and not negligible. The activities of other analogues were significantly lower than that of fentanyl. The ranges between $LD_{50}$ and $ED_{50}$ of these fentanyl analogues were narrower than that of fentanyl. A number of fatal cases in humans caused by the abuse of fentanyl analogues are considered to be due not only to overdosing but also to the narrow ranges between the $ED_{50}$ and $LD_{50}$ values.

**Keywords** Fentanyl · α-Methylfentanyl · 3-Methylfentanyl · *p*-Fluorofentanyl · Analgesic activity · Designer drug

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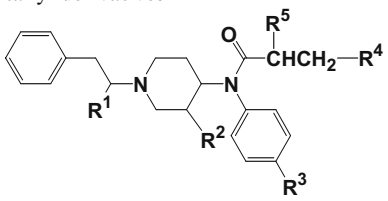
## Introduction

Drug abuse is a serious social problem throughout the world. During the past two or three decades, in addition to classical drugs such as morphine, cocaine, and marijuana, chemically synthesized drugs, so-called designer drugs that are structurally modified from controlled drugs, have become spread in many countries. Among these compounds, a synthesized fentanyl analogue was widely abused in the United States in the early 1980s [1–6]. Initially, this compound was called "bogus China White," because its chemical structure was obscure. After investigation by instrumental analysis, including nuclear magnetic resonance spectroscopy and mass spectrometry, its structure was determined to be 3-methylfentanyl (**3**) [4,5]. In addition, comparison of the reported mass spectra of synthesized 3-methylfentanyl with those of other mono-methylated fentanyl analogues proved that the structure of the most widely abused drug was α-methylfentanyl (**2**) [7]. These compounds had high potential for abuse, because of their ease of synthesis and high analgesic activity. The structures of fentanyl (**1**) and 11 synthesized analogues (**2**–**12**) examined in this study are shown in Tables 1–4.

Although there are a number of reports regarding the analgesic activities of some of these analogues [8–15], there have been only a few reports on the actually abused analogues [8]; there have been no reports on α-methylfentanyl (**2**), the most widely abused analogue. Furthermore, comparison of their analgesic activities with those of morphine and fentanyl was not described. In this report, 11 fentanyl analogues including α-methylfentanyl (**2**) and 3-methylfentanyl (**3**) were synthesized and their effective doses ($ED_{50}$) were measured by the acetic acid writhing method. The lethal doses

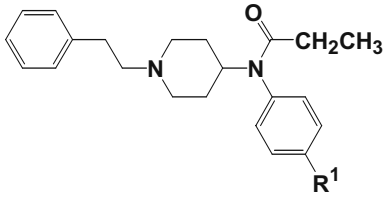
**Table 1** Effective dose ($ED_{50}$), lethal dose ($LD_{50}$) values, and analgesic potency ratios to morphine or fentanyl for mono-methylated fentanyl derivatives




| Compound (no.) | R[a] | $ED_{50}$[b] (mg/kg) | $LD_{50}$[b] (mg/kg) | Potency ratio to morphine | Potency ratio to fentanyl |
|---|---|---|---|---|---|
| Morphine | — | 0.33 | 470 | 1 | 0.02 |
| Fentanyl (**1**) | All R groups = H | 0.0061 | 62 | 54.1 | 1 |
| α-Methylfentanyl (**2**) | $R^1 = CH_3$ | 0.0058 | 8.6 | 56.9 | 1.1 |
| 3-Methylfentanyl (**3**) | $R^2 = CH_3$ | 0.00058–0.0068[c] | — | 48.5–569 | 0.9–10.5 |
| *p*-Methylfentanyl (**4**) | $R^3 = CH_3$ | 0.220 | — | 1.5 | 0.03 |
| *n*-Butyrylfentanyl (**5**) | $R^4 = CH_3$ | 0.047 | — | 7.0 | 0.13 |
| Isobutyrylfentanyl (**6**) | $R^5 = CH_3$ | 0.048 | — | 6.9 | 0.13 |

[a] R groups = H when not specified
[b] Perorally administered
[c] From Van Bever et al.[8]

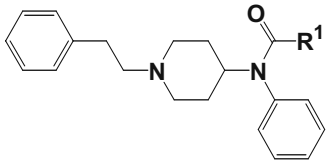
**Table 2** $ED_{50}$, $LD_{50}$ values, and analgesic potency ratios to morphine or fentanyl for derivatives in which the *para*-position of the aromatic ring bound to the propionylanilino group is substituted




| Compound (no.) | $R^1$ | $ED_{50}$[a] (mg/kg) | $LD_{50}$[a] (mg/kg) | Potentcy ratio to morphine | Potency ratio to fentanyl |
|---|---|---|---|---|---|
| Morphine | — | 0.33 | 470 | 1 | 0.02 |
| Fentanyl (**1**) | H | 0.0061 | 62 | 54.1 | 1 |
| *p*-Fluorofentanyl (**7**) | F | 0.021 | 9.3 | 15.7 | 0.29 |
| *p*-Chlorofentanyl (**8**) | Cl | 0.220 | — | 1.5 | 0.03 |
| *p*-Methylfentanyl (**4**) | $CH_3$ | 0.261 | — | 1.3 | 0.02 |
| *p*-Methoxyfentanyl (**9**) | $OCH_3$ | 0.940 | — | 0.4 | 0.006 |

[a] Perorally administered

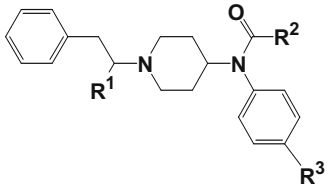
**Table 3** $ED_{50}$, $LD_{50}$ values, and analgesic potency ratios to morphine or fentanyl for propionyl-substituted analogues




| Compound (no.) | $R^1$ | $ED_{50}$[a] (mg/kg) | $LD_{50}$[a] (mg/kg) | Potency ratio to morphine | Potency ratio to fentanyl |
|---|---|---|---|---|---|
| Morphine | — | 0.33 | 470 | 1 | 0.02 |
| Acetylfentanyl (**12**) | $CH_3$ | 0.021 | 9.3 | 15.7 | 0.29 |
| Fentanyl (**1**) | $CH_2CH_3$ | 0.0061 | 62 | 54.1 | 1 |
| *n*-Butyrylfentanyl (**5**) | $CH_2CH_2CH_3$ | 0.220 | — | 1.5 | 0.03 |
| Isobutyrylfentanyl (**6**) | $CH(CH_3)_2$ | 0.261 | — | 1.3 | 0.02 |

[a] Perorally administered

**Table 4** $ED_{50}$, $LD_{50}$ values, and analgesic potency ratios to morphine or fentanyl for other analogues




| Compound (no.) | $R^1$ | $R^2$ | $R^3$ | $ED_{50}$[a] (mg/kg) | $LD_{50}$[a] (mg/kg) | Potency ratio to morphine | Potency ratio to fentanyl |
|---|---|---|---|---|---|---|---|
| Morphine | — | — | — | 0.33 | 470 | 1 | 0.02 |
| Fentanyl (**1**) | H | $CH_2CH_3$ | H | 0.0061 | 62 | 54.1 | 1 |
| α-Methylfentanyl (**2**) | $CH_3$ | $CH_2CH_3$ | H | 0.0058 | 8.6 | 56.9 | 1.1 |
| α-Methylacetylfentanyl (**11**) | $CH_3$ | $CH_3$ | H | 0.106 | 125 | 3.1 | 0.06 |
| *p*-Fluorofentanyl (**7**) | H | $CH_2CH_3$ | F | 0.021 | 9.3 | 15.7 | 0.29 |
| *p*-Fluoroacetylfentanyl (**10**) | H | $CH_3$ | F | 0.045 | 70.0 | 7.3 | 0.14 |

[a] Perorally administered

($LD_{50}$) of widely abused fentanyl analogues were also measured by the same method. To our knowledge, such a comprehensive study on fentanyl analogues has never been reported.

## Materials and methods

### Synthesis of fentanyl analogues

Fentanyl was purchased from Sankyo (Tokyo, Japan). Fentanyl analogues with a methyl group (e.g., α-methyl, 3-methyl, *p*-methyl, *n*-butyryl, and isobutyryl) were synthesized in accordance with previous reports [6,7,10–15]. The *para*-substituted fentanyls (e.g., *p*-fluoro, *p*-chloro, *p*-methyl, and *p*-methoxy) were synthesized by using *p*-fluoroaniline, *p*-chloroaniline, *p*-methylaniline, and *p*-methoxyaniline, respectively, instead of aniline. Acetylfentanyl analogues were synthesized by using acetic acid instead of propionic acid. The *para*-substituted acetylfentanyl analogues were synthesized by a combination of the above two methods. The reagents for the syntheses were purchased from Aldrich (Milwaukee, WI, USA). The water used in the experiments was prepared by the Milli-Q system (Nihon Millipore, Tokyo, Japan).

### Determination of $ED_{50}$ and $LD_{50}$ values of fentanyl analogues in mice

For determination of $ED_{50}$ and $LD_{50}$ values, ddy-mice (weight 23–26 g) were orally administered a dilute sample solution of a fentanyl analogue and 0.1 ml of acetic acid per 10 g body weight was given intraperitoneally [10–15]. The number of writhing episodes after 5 min up to 15 min was counted, and the results were compared with those from control animals that received 0.9% NaCl instead of the dilute fentanyl analogue. The Litchfield-Wilcoxon method was utilized to statistically evaluate the data and the $ED_{50}$ values of the analogues were calculated. The $LD_{50}$ values were estimated only for the analogues that showed higher or similar analgesic activities comparable with that of morphine, and $LD_{50}$ values were measured by the same method as that for $ED_{50}$, using several concentrations of each analogue. For each concentration of the injected solution, five mice were used. Furthermore, the dead mice were autopsied to clarify the cause of death. These experiments were conducted with the permission of the Animal Experiment Committee of Banyu Pharmaceutical (Tokyo, Japan).

## Results and discussion

### Analgesic activities of methyl-substituted fentanyl analogues

The first fentanyl analogues being widely abused are the mono-methylated fentanyl analogues, such as α-methylfentanyl (**2**), 3-methylfentanyl (**3**), *p*-methylfentanyl (**4**), *n*-butyrylfentanyl (**5**), and isobutyrylfentanyl (**6**) shown in Table 1. Their $ED_{50}$ and $LD_{50}$ values and potency ratios were compared with those of morphine and fentanyl. The $ED_{50}$ of 3-methylfentanyl (**3**) is quoted from the previous report [8], because it could not be separated from its diastereomers. The configurations of these diastereomers are shown in Fig. 1.

The $ED_{50}$ values of these analogues were at a similar level or higher than that of fentanyl, and all these analogues had stronger analgesic activities than morphine.

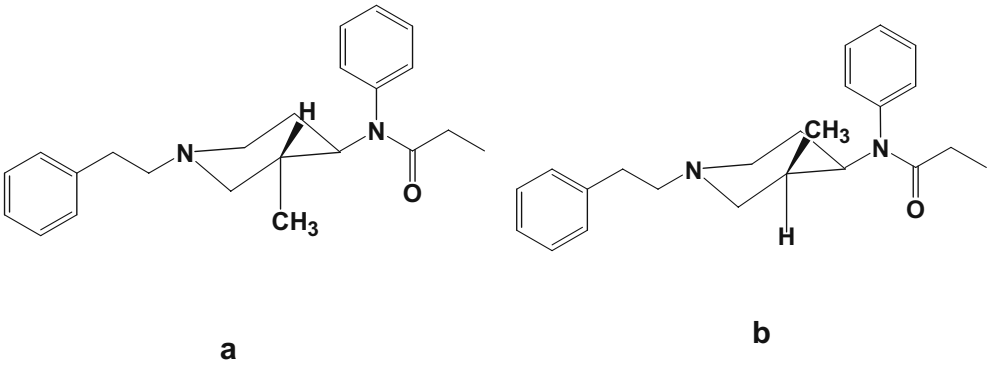



**Fig. 1a, b** Diastereomers of 3-methylfentanyl. **a** *Trans*-3-methylfentanyl; **b** *cis*-3-methylfentanyl

In particular, α-methylfentanyl (**2**) and 3-methylfentanyl (**3**) showed 56.9 and 48.5 to 569 times stronger analgesic activities than morphine, respectively, and 1.1 and 0.9 to 10.5 times stronger analgesic activities than fentanyl, respectively. Among these analogues, most fatal cases were caused by α-methylfentanyl.

As shown in Table 1, the range between $ED_{50}$ and $LD_{50}$ of α-methylfentanyl is narrower than that of fentanyl. Overdose of α-methylfentanyl may be the main reason for a number of the deaths, but the narrow range between $ED_{50}$ and $LD_{50}$ may also be a contributing factor.

### Activities of compounds substituted in the *para*-position of the aromatic ring bound to the propionylanilino group

The analogues with substitution in the *para*-position of the aromatic ring bound to the propionylanilino group, such as *p*-fluorofentanyl (**7**), *p*-chlorofentanyl (**8**), *p*-methylfentanyl (**4**), and *p*-methoxyfentanyl (**9**), were tested for their analgesic activities (Table 2). The two former compounds changed the atomic diameters of the *para*-position atoms while retaining similar electronic character; the *p*-methyl substituent in **4** has a slight electron-accepting character, while the *p*-methoxy group in **9** can be electron donating through resonance or electron accepting through induction. According to the $ED_{50}$ values, these analogues, except *p*-methoxyfentanyl, had analgesic activities higher than that of morphine. In particular, *p*-fluorofentanyl showed an activity about 16 times higher than that of morphine; however, its activity was lower than that of fentanyl. The $LD_{50}$ value was measured only for *p*-fluorofentanyl, and it showed a much lower value than fentanyl or morphine (Table 2).

### Activities of propionyl-substituted analogues

To evaluate the effect of substitution of the propionyl side chain on analgesic activities, acetylfentanyl (**12**), *n*-butyrylfentanyl (**5**), and isobutyrylfentanyl (**6**) were tested (Table 3). The $ED_{50}$ values were measured in all analogues and the $LD_{50}$ value only for acetylfentanyl (**12**). The analgesic activities of all of these compounds were lower than fentanyl, but higher than morphine. The range between $ED_{50}$ and $LD_{50}$ of acetylfentanyl was ten times narrower than that of morphine.

### Activities of α-methylacetylfentanyl and *p*-fluoroacetylfentanyl

The analgesic activities of α-methylacetylfentanyl (**11**) and *p*-fluoroacetylfentanyl (**10**) were measured and compared with those of each nonacetyl precursor (Table 4). In each case, when the propionyl group was changed to the acetyl group, a significant decrease of activity and a much higher $LD_{50}$ value was observed.

### Mouse autopsies

Autopsies of dead mice were conducted to clarify the cause of death. In all mice, significant bleeding in the small intestine was observed. This bleeding was presumed to be one of the causes of death.

## Conclusions

Numerous fatalities have occurred by the abuse of α-methylfentanyl. It is believed that overdose may be the major cause of death, and its narrow safety range may also be a critical factor. Apart from α-methylfentanyl, attention should be paid to 3-methylfentanyl, *p*-fluorofentanyl, and acetylfentanyl during analysis when abuse or poisoning by fentanyl-related designer drugs is suspected, because their effects are more potent than those of morphine.

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