

## Evaluation of Analgesic Activity of Newly Synthesized Phthalyl- tyrosyl-glycin Sodium

Muthanna S. AL-Tae\* , Kawkab Y. Saour\*, Haider M. Mohammed\*\*<sup>1</sup>

\* Department of Pharmaceutical Chemistry, College of Pharmacy, University of Baghdad – Iraq.

\*\* Department of Pharmacology and Toxicology, College of Pharmacy, University of Baghdad –Iraq .

### Abstract

Alteration in the backbone structure of the endogenously released opioid peptides Leu<sup>5</sup>/Met<sup>5</sup> enkephalins may result in compounds having comparable profile of pharmacological activity but with different physicochemical properties and side effects. Phthalyl amino acid and phthalyl esters are among the derivatives that have been synthesized and evaluated for their antibacterial and antifungal activities. This study was conducted to evaluate the possible analgesic activity of phthalyl-tyrosyl-glycin sodium that has been recently synthesized by our team. The study was carried out on 24 albino mice using hot plate method. The animals were allocated in to three groups; the first group received saline and represent a control group; the second group received morphine HCl as a standard drug; and the third group received phthalyl-tyrosyl-glycin sodium. The onset with which the animal lift his forearm and the number of jumps per 25 seconds were recorded for each group.

The results of this study showed that phthalyl-tyrosyl-glycin sodium resulted in significant improvement ( $P < 0.05$ ) in analgesia score as well as significant delay in the onset of induced hyperalgesia in comparison to saline-treated group, and in comparison to morphine HCl, no significant difference ( $P > 0.05$ ) was observed in analgesia score but with significant delay in induced hyperalgesia. The results obtained in this study provide experimental evidences for the effectiveness of the prepared compound as analgesic with comparable effect to that of morphine.

**Key words:** Phthalyl-tyrosyl-glycin sodium, phthalyl group, analgesia .

### الخلاصة

ان بعض التعديلات الكيميائية على الببتيدات الحياتية المورفينية الداخلية (ليوسين ومثيونين انكفالين) قد تنتج مركبات لها فعاليات دوائية مقارنة كمسكنات الم قوية ولكن بخواص كيميائية وفيزيائية مختلفة مع اختلاف في بعض الأعراض الجانبية الناتجة عن استخدامها. تعتبر المركبات الحاوية على الفثاليل كحامض أميني واسترات الفثاليل من المركبات المصنعة والمقيمة فعاليتها ضد الالتهابات الجرثومية والفطرية وعليه تم تصميم هذه الدراسة لتقييم الفعالية الدوائية لببتيد يحتوي على مجموعة الفثاليل كمركب مسكن للألم. ان هذا المركب وهو (Phthalyl-tyrosyl glycin sodium) هو مركب جديد قد تم تصنيعه من قبل فرع الكيمياء الصيدلانية في كلية الصيدلة جامعة بغداد. لقد تمت الدراسة باستخدام 24 فأراً ذكراً أيضاً لتقييم الفعالية الدوائية للمركب الجديد كمسكن للألم باستخدام طريقة الصفيحة الحارة (hot plate) وتم توزيع الحيوانات على ثلاثة مجاميع، حققت المجموعة الأولى بالماء المالح (saline) وتمثل مجموعة السيطرة والمجموعة الثانية بالمورفين كعقار قياسي والثالثة بمركب (Phthalyl-tyrosyl glycin sodium) والذي هو موضوع البحث وتم احتساب الوقت المستغرق لیسحب الحيوان قدمه عن الصفيحة الحارة وعدد القفزات خلال 25 ثانية أظهرت النتائج أن هناك تحسن واضح ومعنوي في الوقت المستغرق لیسحب الحيوان قدمه عن الصفيحة وكذلك في عدد القفزات خلال 25 ثانية في الحيوانات المعالجة بالمركب الجديد عن مجموعة السيطرة. لا فرق معنوي قد لوحظ في معدل قفز الحيوانات المعالجة بالمركب الجديد بالمقارنة مع المجموعة المعالجة بالمورفين إلا أن سرعة سحب الحيوان لقدمه عن الصفيحة الحارة كان أبطأ. تعطي النتائج المستخلصة من هذه الدراسة أدلة تجريبية على الفعالية الدوائية لمركب (glycin-Phthalyl-tyrosyl) كمركب مسكن للألم قوي وفعال مقارنة للمورفين.

<sup>1</sup>corresponding author email [r74th@yahoo.com](mailto:r74th@yahoo.com)

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## Introduction

Opioid peptides, defined as peptides with opiate-like pharmacological effects, are the oldest pharmacological substances known for their analgesic, euphoric and addictive effects<sup>(1,2)</sup>. Morphine is the first narcotic analgesic alkaloid isolated from plant source in 1806<sup>(3)</sup>. In 1975, Hughes and Kosterlitz succeeded in isolating two pentapeptides, Leucine- and Methionine-enkephalin (Leu/Met enkephalin) from pig brain, which compete strongly with morphine-like drugs for binding to receptors in the brain with pharmacological actions resembling those of morphine itself<sup>(2)</sup>. However, to date, morphine-like compounds remain the only class known to act by mimicking these peptides<sup>(4)</sup>. Several families of peptides were discovered with multiple categories of opioid receptors.

Binding studies by Synder and colleagues (1973), demonstrated that opioids are recognized by specific receptors<sup>(5)</sup>. Various pharmacological observations like analgesia, sedation, antitussive, antidiarrheal, pupillary constriction, and bradycardia produced by different drugs implied the mediation of more than one type of receptors.

Receptor cloning studies revealed the existence of six different opiate receptors named as  $\mu$  (mu),  $\delta$  (delta),  $\kappa$  (kappa),  $\sigma$  (sigma),  $\epsilon$  (epsilon) and NFQ (nociceptin FQ)<sup>(6)</sup>. These receptors mediate several pharmacological and side effects of opiates including: analgesia (supraspinal, spinal, peripheral), respiratory depression, pupillary constriction, reduced GI motility, euphoria, dysphoria, sedation and physical dependence<sup>(3)</sup>. Concerning their molecular pharmacology, these opioid receptors belong to the family of inhibitory G-protein coupled receptor, mediate the reduction of intracellular cAMP level as a main event beyond receptor binding<sup>(7)</sup>. Other mechanisms involve the opening of  $K^+$  and block of  $Ca^{2+}$ -channels thereby reducing both neuronal excitability and transmitter release<sup>(8)</sup>.

Understanding the powerful molecular, biological and physiological terms of opioids was used to develop analgesic compounds with significant advantages over morphine<sup>(9)</sup>. In this respect, incorporation of a new group in the main backbone of naturally occurring enkephalin was aimed in synthesizing compounds with possible analgesic effects. On the other hand, one of the earliest types of structural modifications applied to the enkephalin was shortening of the peptide chain by removal of residues from the essential sequence or by removal of residues from C-terminal. These experiments showed that significant potency remained in producing

analgesia *in vivo* as in Tyr-D-ala-phe-met amide<sup>(10,11)</sup>.

In previous work, Muthanna, *et al.* (2005) were succeed in designing and synthesis of enkephalin analogues having even shortest chain by removing the C-terminal residue gly-phe-leu/met; keeping N-tyrosine residue protected by phthalyl group. Accordingly, the newly synthesized phthalyl-tyrosyl-glycin and its sodium salt (Fig. 1 and 2) are novel compounds produced in our laboratories with possible analgesic activity<sup>(12)</sup>. The present study was conducted to investigate the analgesic effect of the sodium salt of this compound on experimental animals.

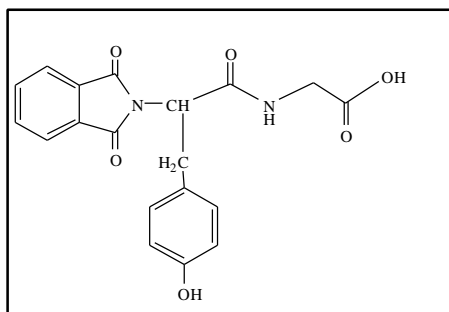


Figure (1) Phthalyl-tyrosyl-glycin<sup>(12)</sup>

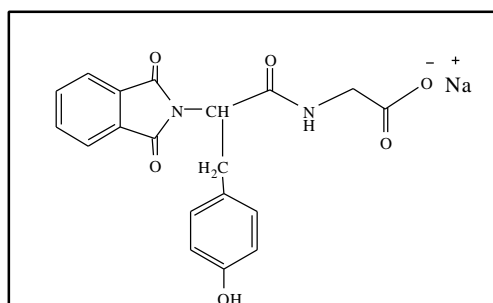


Figure (2) Phthalyl-tyrosyl-glycin sodium salt

## Material and Methods

**Animals:** Twenty-four adult male albino mice weighing  $25.23 \pm 3.1$  gm were used in this study. They were obtained from Iraqi Sera and Vaccine Institute and were housed under standard conditions in the animal house of the College of Pharmacy-University of Baghdad. Animals were fed commercial pellet and tap water in free access *ad libitum*.

**Materials:** Morphine HCl was supplied by May and Baker Ltd, England. Phthalyl-tyrosyl-glycin has been obtained from reference<sup>(13)</sup> and its corresponding sodium salt has been synthesized according to reference<sup>(14)</sup> to increase its water solubility to be suitable for intraperitoneal administration. All compounds

were dissolved in normal saline and were administered intraperitoneally.

**Methods:** Hot plate method as described by Woolfe and MacDonald <sup>(13)</sup> was used for evaluation of the analgesic effect of the tested compound compared with morphine as a reference. Animals were allocated into three groups: first group received normal saline, second group received morphine HCl (May and Baker Ltd, England), and third group received phthalyl-N-tyrosyl-glycin sodium. The plate was heated to 55 °C and the animal was put on the plate. The onset with which the animal lift his forearm and the number of jumps per 25 seconds were recorded for each group. The results were expressed as mean  $\pm$  standard error and were analyzed using ANOVA and unpaired Student *t*-test.

### Results and Discussion

The data presented in table (1) clearly showed that animals in control group lift their forearms in about (1.2  $\pm$  0.66) seconds which represents the normal onset of heat-induced hyperalgesia. The animal jumps 24.8  $\pm$  2.4 times/25 seconds which represents the analgesia score. Table (1) also showed that morphine significantly ( $P < 0.05$ ) delayed the onset of heat-induced hyperalgesia (2.6  $\pm$  0.74) in comparison to control group and significantly ( $P < 0.05$ ) improved the analgesia score (11.0  $\pm$  2.4) in comparison to control group (24.8  $\pm$  2.4) (Fig. 3 and 4). Interestingly, phthalyl-tyrosyl-glycin sodium resulted in significant ( $P < 0.05$ ) improvement in analgesia score (10.8  $\pm$  1.43), an effect seems comparable to that of morphine (11.0  $\pm$  2.4,  $P > 0.05$ ). However, phthalyl-tyrosyl-glycin sodium showed slightly more significant delay in the onset of heat-induced hyperalgesia over that of morphine (Fig. 3 and 4).

The pharmacologic results may indicate that tyrosine is the essential amino acid residue within the peptide chain of leu / met enkephalin <sup>(15)</sup>; and the enzymatic resistance is doubtless an important factor in the high potency of this analgesic <sup>(16)</sup> because, chemically, the presence of phthalyl group may enhance the availability and hence the binding of the compound to opioid receptors and this in turn may potentiate the pharmacological effect of the tested compound, this may explain why the compound showed a comparable analgesic effect to that of morphine. Furthermore, the bulky phthalyl group may increase the lipophilicity and thus enhance a sufficient bioavailability and tissue penetration, an effect which may explain why the compound has slightly faster onset of action than morphine. On the other hand, the presence of bulky

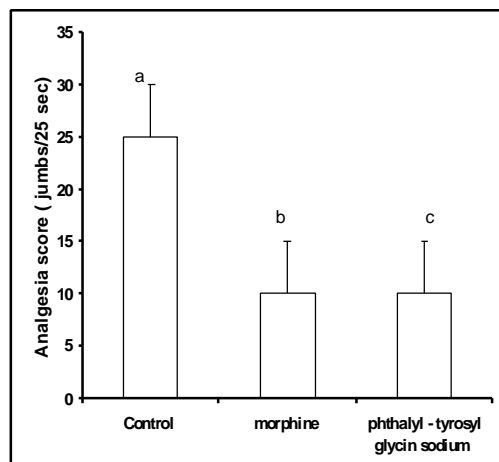
phthalyl group is suspected to reduce enzyme degradation of phthalyl-tyrosyl-glycin inside the body by adding a steric hindrance <sup>(17)</sup>; however, this expectation requires further pharmacokinetic studies.

In conclusion, phthalyl-tyrosyl-glycin sodium showed promising analgesic activity approximately equal to that of morphine with reliable onset of action, making it a good candidate for further development in the field of morphine-replacement therapy based on the idea that enkephalins are compounds devoid of addictive liability.

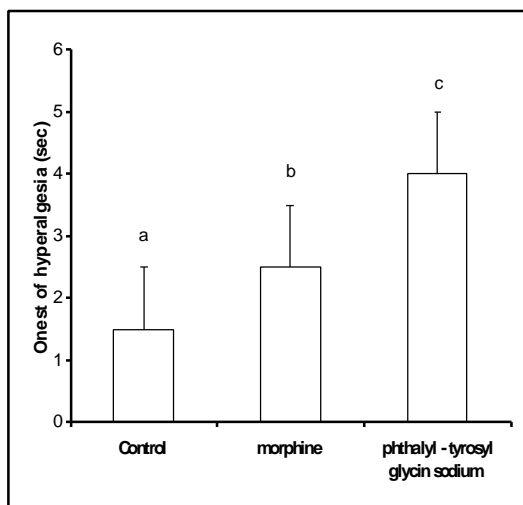
**Table (1) Effect of Phthalyl-tyrosyl-glycin sodium and morphine on the analgesia score and heat-induced hyperalgesia in mice**

Groups	Jumps/25 seconds	Onset of hyperalgesia (sec.)
Control group (n=6)	24.8 $\pm$ 2.4 <sup>a</sup>	1.2 $\pm$ 0.66 <sup>a</sup>
Morphine-treated group (n=6)	11.0 $\pm$ 2.4 <sup>b</sup>	2.6 $\pm$ 0.74 <sup>b</sup>
Phthalyl-tyrosyl-glycin sodium-treated group (n=6)	10.8 $\pm$ 1.43 <sup>b</sup>	4.2 $\pm$ 1.04 <sup>c</sup>

Values are presented as mean  $\pm$  SEM. Values with non-identical superscripts (a, b, c) among different groups are considered significantly different ( $P < 0.05$ ).



**Figure (3) :The effect of phthalyl-tyrosyl-glycin sodium and morphine on analgesia score in mice recorded 25 second. Results are expressed as the mean (jumps)  $\pm$  SEM;  $n = 8$ ; non-identical superscripts (a, b) represent significant differences among groups by ANOVA ( $P < 0.05$ )**



**Figure (4) :Comparison of the effect of phthalyl-tyrosyl-glycin sodium and morphine on the onset of heat-induced hyperalgesia in mice. Results are expressed as the mean  $\pm$  SEM;  $n=8$ ; non-identical superscripts (a, b, c) represent significant differences among groups by ANOVA**

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