

# Antinociceptive Effect of Some Biuret Derivatives on Formalin Test in Mice

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## ABSTRACT

**Purpose:** The current study was designed to investigate the antinociceptive effects of several biuret derivatives with N, N'-diphenyl, N-phenyl-N'-alkylphenyl, N,N'-bis alkylphenyl, 2-methylquinoline-4-yl, benzo[d]thiazol-2-ylthio and (1-phenyl-1H-tetrazol-5-yl)thio substituents on the formalin-evoked pain in mice.

**Methods:** Antinociceptive activity of the nine biurets derivatives were assessed at different doses in mice using formalin test and the results were compared with those of indomethacin (20 mg/kg) and vehicle of the compounds. Area under the pain score curve against time (AUEC) up to 60 minutes was used as the measure of pain behavior.

**Results:** A rather good analgesic effect was seen for most of the tested biuret derivatives. Significant reduction in median AUEC<sub>0-5 minutes</sub> was observed at the doses of 50 and 25 mg/kg for biurets with either benzyl and 2-methylquinoline-4-yl (C8) or phenylethyl and benzo[d]thiazol-2-ylthio (C9) moieties, respectively (p-value < 0.0044). Antinociceptive activities of compound C7 (with bis phenylpropyl substituent), C8 and C9 during the late phase of formaldehyde-induced pain were comparable to that of indomethacin.

**Conclusion:** Unlike indomethacin, the tested biuret compounds are able to induce antinociception in both phases of formalin test and could be considered comparable to indomethacin at the selected doses.

## Introduction

Various pharmacological activities have been reported for biuret derivatives with the general structure shown in Figure 1. McColl reported synthesis of some phenyl biurets and assessed their effects on gastric acid secretion and prevention of peptic ulcer.<sup>1</sup> p-Toluenesulfonyl-biurets and alkyl p-toluenesulfonylthiocarbamates described by Kriesel et al showed hypoglycemic activity.<sup>2</sup> Fouladdel and her colleagues reported synthesis N,N'-diphenyl, N-phenyl-N'-alkylphenyl, and N,N'-bis alkylphenyl biurets and analogous compounds by replacing one phenyl group with 2-methylquinoline-4-yl, benzo[d]thiazol-2-ylthio and (1-phenyl-1H-tetrazol-5-yl)thio moieties and cytotoxicity of them against T47D breast cancer cell line.<sup>3</sup>

During an in silico study by Adibpour et al, it has been shown that biuret derivatives could inhibit pteridine reductase 1 of different strains of leishmania and could be considered as potential antileishmaniasis agents.<sup>4</sup> This effect was further confirmed by Khademvatan and his

coworkers. They found that some of N,N'-diphenyl, N-phenyl-N'-alkylphenyl, and N,N'-bis alkylphenyl biurets were more active against Leishmania major and Leishmania infantum promastigotes in comparison to glucantime.<sup>5</sup>

In an attempt to find new anti-inflammatory and analgesic agents, Kajitani and his co-workers were prepared several arylbiurets and tested them as anti-inflammatory and analgesic agents.<sup>6</sup> Their results showed that the analgesic activities of 1,3-dimethyl-5-phenylbiuret and 5-(4-chlorophenyl)-1, 1, 3, trimethylphenyl biuret were higher than that of aminopyrine. Following their investigation, this study was conducted to assess the antinociceptive activity of several N,N'-diphenyl, N-phenyl-N'-alkylphenyl, and N,N'-bis alkylphenyl biurets and analogous 2-methylquinoline-4-yl, benzo[d]thiazol-2-ylthio and (1-phenyl-1H-tetrazol-5-yl)thio derivatives using pain model of formalin in mice.

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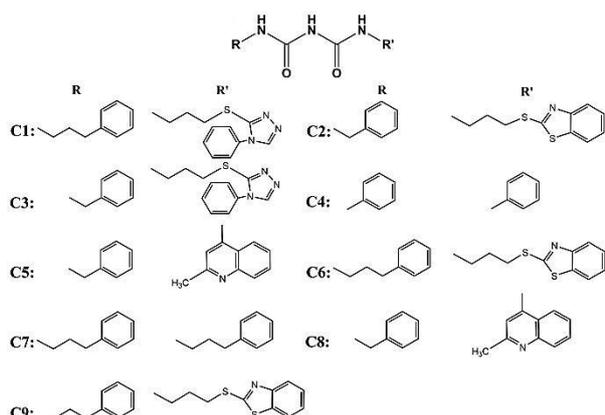


Figure 1. Biuret derivatives

## Materials and Methods

### Chemicals

Biuret derivatives (C1-C9 in Figure 1) were synthesized and purified as previously reported.<sup>3</sup> Indomethacin was a gift from Darou Pakhsh Pharmaceutical Manufacturing Company, Tehran, Iran. Formalin and dimethyl sulfoxide (DMSO) were purchased from Merck, Germany.

### Animal experiments and drug administration

Adult male NMRI mice weighing 24-29 gram were obtained from experimental animal house of Ahvaz Jundishapur University of Medical Sciences and maintained on a 12 hours light/dark cycle with free access to food and water, except during the time of experiments. Mice were randomly divided into groups of 4-5 for test and each animal was used only once. All the ethical issues were considered based on the Ahvaz Medical University Ethical Protocols (AMUEP) on animal experiments. Antinociceptive activity of biuret derivatives were assessed using formalin test and compared with vehicle and indomethacin. Biurets were first dissolved in DMSO and then diluted in saline. Indomethacin was prepared in the minimum amount of alkali solution. Different doses of tested compounds and indomethacin (20 mg/kg) were freshly prepared and injected intraperitoneally in a volume of 10 mL/kg. Fifteen minutes after drug injection, 20  $\mu$ L of 2.5% formalin was injected intraplantar into the left hind paw using a microsyringe with a 29-gauge needle. Mice were immediately returned to the observation box to monitor pain scores. The animals were placed individually in a Plexiglas box and all observations were carried out by a trained investigator blind to the experimental treatment of the animals. A mirror was placed at 45° angle under the observation box to allow the experimenter an unimpeded view of the injected paw. Animal behavior was continuously scored in 15-second intervals for a total of 60 minutes<sup>7-9</sup> and pain behavior was calculated as the area under the pain score curve (AUEC) of pain score-time curve between 0 and 5 minutes and also from 5 up to 60 minutes post drug administration by using of trapezoidal rule.<sup>10</sup>

### Statistical analysis

Comparison of median AUEC<sub>0-5 minutes</sub> and AUEC<sub>5-60 minutes</sub> between different groups of mice were done using Kruskal-Wallis followed by Conover-Inman post hoc tests. Differences between medians were considered statistically significant at p-values of less than 0.05.

### Results

Descriptive statistics summary of AUEC<sub>0-5 minutes</sub> and AUEC<sub>5-60 minutes</sub> for different groups of mice receiving either the tested biuret derivatives, indomethacin or vehicle are presented in Tables 1 and 2. No significant difference was detected between median of AUEC values in mice receiving indomethacin as compared to those of vehicle group during the early phase of formaldehyde-induced pain as could be seen from notched box plot of AUEC<sub>0-5 minutes</sub> in Figure 2. Median AUEC<sub>0-5 minutes</sub> in all groups of mice received different doses of biurets were lower than those of animals in vehicle and indomethacin groups. However, these differences could be only considered statistically significant for compounds C3 and C7-C9 at the dose of 100 mg/kg in comparison to both indomethacin and vehicle (p-value<0.0125). For C9 and C8, significant reduction in median AUEC<sub>0-5 minutes</sub> also observed at lower doses of biurets i.e. 25 and 50 mg/kg, respectively (p-value<0.0044). Figure 3 shows notched box plot of AUEC<sub>0-5 minutes</sub> values for compounds C7-C9 at different levels of administered dose. These compounds showed the greatest antinociceptive activity at early phase of the formaldehyde-induced pain (Table 1). Increasing the dose of compounds C7-C9 lead to increase in antinociceptive activity (decreasing of median AUEC<sub>0-5 minutes</sub>), however, this increase was not always statistically significant.

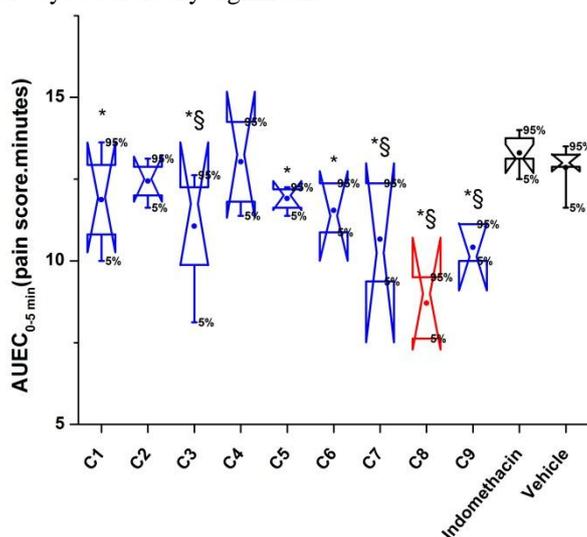


Figure 2. Notched box plot of area under the pain score-time profile up to 5 minutes post administration (AUEC<sub>0-5 minutes</sub>) at the biuret dose of 100 mg/kg and 20 mg/kg of indomethacin. Solid circles represent the mean of the values in each group. Boxes and whiskers show the interquartile range and 5 and 95 percentiles, respectively. 95% confidence intervals around the medians are illustrated by the notches. Significant difference of median AUEC<sub>0-5 minutes</sub> with indomethacin and vehicle groups are depicted by (\*) and (\$), respectively. Plots of the groups that their median AUECs were not statistically significant are shown in the same color.

**Table 1.** Descriptive statistics summary of area under the pain score-time curve between 0 and 5 minutes (AUEC<sub>0-5 minutes</sub>) in different groups of mice receiving either biuret derivatives at various doses, indomethacin(5 mg/kg) or vehicle.

-	N <sup>a</sup>	Mean	SD <sup>b</sup>	1st Quartile	Median	3rd Quartile	5th Percentile	5th Percentile	Rank Sum <sup>c</sup>
C1-100 mg/Kg	4	11.9	1.5	10.8	11.9	12.9	10.0	13.6	205
C1-200 mg/Kg	4	10.6	2.9	9.1	11.9	12.1	6.3	12.4	164.5
C2-100 mg/Kg	4	12.4	0.6	12.0	12.5	12.9	11.6	13.1	262.5
C2-200 mg/Kg	4	10.3	1.8	8.3	11.3	11.5	8.3	11.5	74.5
C2-50 mg/Kg	4	12.3	1.4	11.3	12.1	13.3	10.9	14.0	230
C3-100 mg/Kg	4	11.1	2.0	9.9	11.8	12.3	8.1	12.6	169
C3-200 mg/Kg	4	11.5	1.0	10.7	11.6	12.3	10.3	12.4	172
C4-100 mg/Kg	4	13.0	1.5	11.8	13.3	14.3	11.4	14.3	279.5
C4-200 mg/Kg	4	10.6	2.5	9.2	11.4	12.0	7.0	12.6	145.5
C5-100 mg/Kg	4	11.9	0.4	11.6	12.0	12.2	11.4	12.3	201
C5-200 mg/Kg	4	9.8	2.2	8.0	10.2	11.5	7.0	11.6	86
C6-100 mg/Kg	4	11.5	0.8	10.9	11.4	12.4	10.9	12.4	126.5
C6-200 mg/Kg	4	10.3	1.1	9.7	9.9	10.9	9.5	12.0	94
C7-100 mg/Kg	4	10.7	1.5	9.4	10.3	12.4	9.4	12.4	96
C7-25 mg/Kg	4	12.6	1.1	11.9	12.2	13.3	11.9	14.3	260.5
C7-50 mg/Kg	4	12.7	0.9	12.3	12.4	13.2	12.1	14.0	279
C8-100 mg/Kg	4	8.7	1.0	7.6	9.0	9.5	7.6	9.5	22
C8-25 mg/Kg	4	12.4	1.0	11.9	12.0	13.0	11.9	13.9	241
C8-50 mg/Kg	4	10.7	1.0	10.1	10.5	11.4	9.8	12.1	115.5
C9-100 mg/Kg	4	10.4	0.6	10.0	10.1	11.1	10.0	11.1	64.5
C9-10 mg/Kg	4	11.6	1.6	10.3	11.6	12.9	9.9	13.1	193
C9-25 mg/Kg	4	11.0	1.0	10.4	11.2	11.6	9.6	11.9	119
Indomethacin	5	13.3	0.6	13.1	13.1	13.8	12.5	14.0	407
Vehicle	5	12.9	0.7	12.9	13.0	13.3	11.6	13.5	363.5

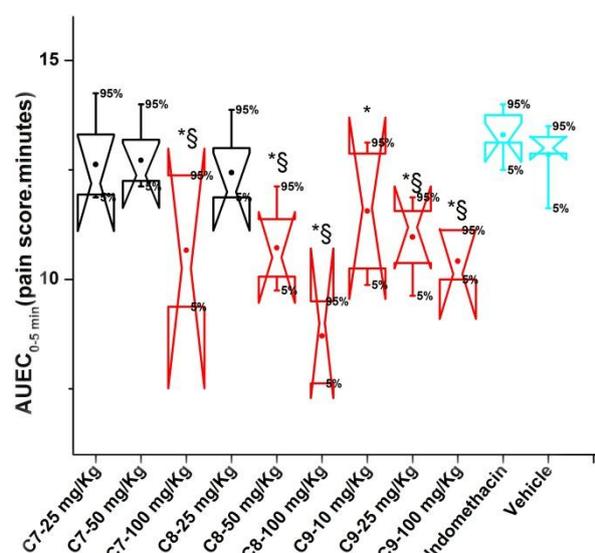
<sup>a</sup> Number of mice in the treatment group, <sup>b</sup> Standard deviation, <sup>c</sup> Kruskal-Wallis sum of the ranks in each groups of mice

**Table 2.** Descriptive statistics summary of area under the pain score-time curve between 5 and 60 minutes (AUEC<sub>5-60minutes</sub>) in different groups of mice receiving either biuret derivatives at various doses, indomethacin(5 mg/kg) or vehicle.

-	N <sup>a</sup>	Mean	SD <sup>b</sup>	1st Quartile	Median	3 <sup>rd</sup> Quartile	5 <sup>th</sup> Percentile	95 <sup>th</sup> Percentile	Rank Sum <sup>c</sup>
C1-100 mg/Kg	4	84.4	15.1	73.0	84.6	95.8	66.4	102.1	337.0
C1-200 mg/Kg	4	25.8	9.2	18.3	26.4	33.3	15.1	35.3	116.0
C2-100 mg/Kg	4	22.6	6.9	16.9	22.0	28.3	15.8	30.5	105.0
C2-200 mg/Kg	3	16.6	6.2	11.1	15.4	23.3	11.1	23.3	58.5
C2-50 mg/Kg	4	79.0	15.0	67.6	75.5	90.4	65.9	99.0	320.0
C3-100 mg/Kg	4	66.4	7.3	60.9	64.3	71.8	60.8	76.3	279.0
C3-200 mg/Kg	4	23.4	22.2	4.6	23.0	42.3	1.1	46.5	103.0
C4-100 mg/Kg	4	82.1	8.3	76.4	82.8	87.7	71.3	91.4	345.0
C4-200 mg/Kg	4	21.7	15.2	12.1	19.0	31.3	6.1	42.5	100.5
C5-100 mg/Kg	4	66.2	5.9	61.3	67.1	71.1	59.0	71.5	278.0
C5-200 mg/Kg	4	39.0	14.2	29.1	35.9	48.9	25.6	58.5	166.5
C6-100 mg/Kg	3	66.4	0.6	66.0	66.1	67.1	66.0	67.1	206.5
C6-200 mg/Kg	4	31.1	18.3	17.6	36.2	44.6	6.0	46.0	134.0
C7-100 mg/Kg	3	7.9	7.4	1.1	6.8	15.8	1.1	15.8	28.0
C7-25 mg/Kg	4	52.2	16.0	39.4	49.3	65.0	38.0	72.3	225.5
C7-50 mg/Kg	4	27.6	9.0	20.9	26.3	34.3	18.5	39.3	128.0
C8-100 mg/Kg	3	6.3	5.6	0.0	8.4	10.6	0.0	10.6	25.0
C8-25 mg/Kg	4	58.8	13.5	48.9	62.3	68.6	40.3	70.1	248.5
C8-50 mg/Kg	4	19.3	13.2	8.0	18.0	30.5	7.8	33.3	86.0
C9-100 mg/Kg	3	14.4	4.7	9.0	16.6	17.6	9.0	17.6	53.0
C9-10 mg/Kg	4	65.3	1.1	64.4	65.2	66.1	64.0	66.6	262.0
C9-25 mg/Kg	4	22.1	16.3	8.1	23.0	36.0	5.0	37.3	97.0
Indomethacin	5	52.7	3.4	50.9	54.5	54.5	47.6	55.9	265.0
Vehicle	5	73.8	5.7	70.8	73.6	75.0	67.3	82.5	404.0

<sup>a</sup> Number of mice in the treatment group, <sup>b</sup> Standard deviation, <sup>c</sup> Kruskal-Wallis sum of the ranks in each groups of mice

As it could be seen from Figure 3 and Table 2, in the late phase of formalin-induced pain (up to 60 minutes post drug administration), difference of median  $AUEC_{5-60 \text{ minutes}}$  values between indomethacin and vehicle groups was significant ( $p$ -value=0.0006). A rather good analgesic activity was seen by compounds C2 and C7-C9 at the dose of 100 mg/kg in comparison to indomethacin (Figure 4). However, these compounds did not show any statistically significant difference of median  $AUEC_{5-60 \text{ minutes}}$  at this level of dose. Compounds C7-C9 had comparable analgesic activity to indomethacin at the dose of 25 mg/kg (Figure 5). Increasing the dose of compounds C7-C9 to the values greater than 25 mg/kg did not result in significant reduction of median  $AUEC_{5-60 \text{ minutes}}$ .

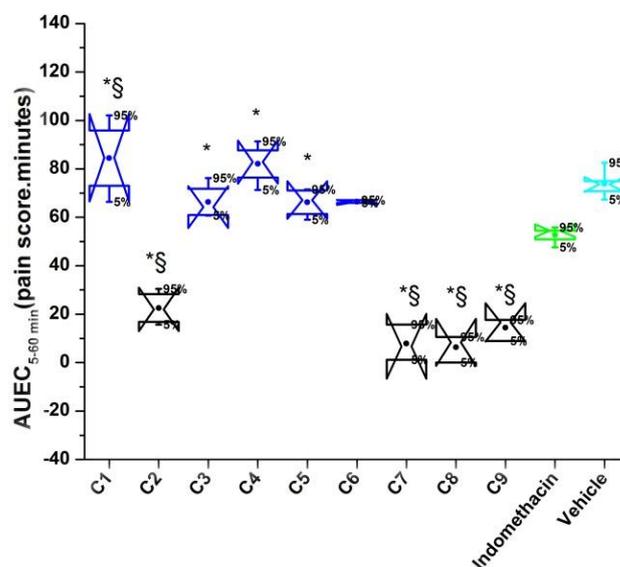


**Figure 3.** Notched box plot of area under the pain score-time profile up to 5 minutes post administration ( $AUEC_{0-5 \text{ minutes}}$ ) at different doses of compounds C7-C9 and 20 mg/kg of indomethacin. Solid circles represent the mean of the values in each group. Boxes and whiskers show the interquartile range and 5 and 95 percentiles, respectively. 95% confidence intervals around the medians are illustrated by the notches. Significant difference of median  $AUEC_{0-5 \text{ minutes}}$  with indomethacin and vehicle groups are depicted by (\*) and (\$), respectively. Plots of the groups that their median AUECs were not statistically significant are shown in the same color.

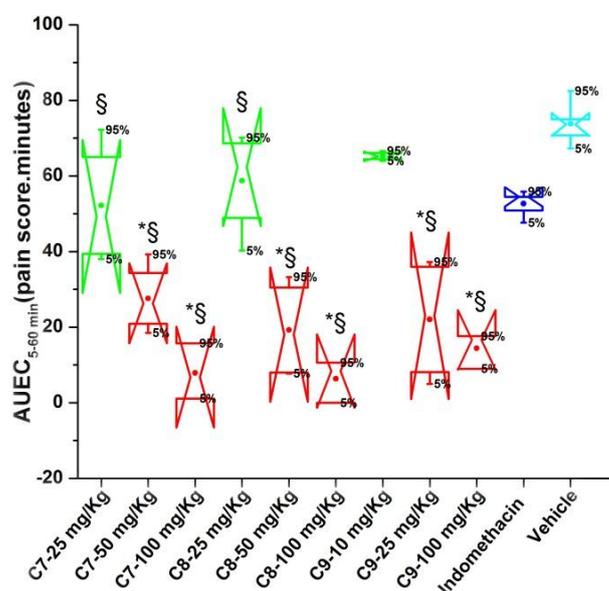
## Discussion

Among the several models of persistent nociception, formalin test has been well established as a valid model for screening of anti-inflammatory and antinociceptive agents.<sup>11,12</sup> A number of analogies have been specified between formalin-evoked pain and human persistent clinical pain.<sup>13</sup> Intraplantar injection of formalin evokes signs of nociception (flinching and licking of the injected paw) with early (phase 1), followed by a quiescent period characterized by fewer pain behaviours, and late-hyperalgesic (phase 2) components that last for approximately 1 hr.<sup>7,8</sup> The early phase or neurogenic nociception results direct activation of peripheral nociceptors whereas the late phase due to inflammatory nociception that reflect induction of a spinal state of facilitation, central sensitization, development of

inflammation and enlargement of receptive fields and also the concurrent presence of low-level input from both large and small afferents.<sup>11,13</sup>



**Figure 4.** Notched box plot of area under the pain score-time profile between 5 and 60 minutes post administration ( $AUEC_{5-60 \text{ minutes}}$ ) at the biuret dose of 100 mg/kg and 20 mg/kg of indomethacin. Solid circles represent the mean of the values in each group. Boxes and whiskers show the interquartile range and 5 and 95 percentiles, respectively. 95% confidence intervals around the medians are illustrated by the notches. Significant difference of median  $AUEC_{5-60 \text{ minutes}}$  with indomethacin and vehicle groups are depicted by (\*) and (\$), respectively. Plots of the groups that their median AUECs were not statistically significant are shown in the same color.



**Figure 5.** Notched box plot of area under the pain score-time profile between 5 and 60 minutes post administration ( $AUEC_{5-60 \text{ minutes}}$ ) at different doses of compounds C7-C9 and 20 mg/kg of indomethacin. Solid circles represent the mean of the values in each group. Boxes and whiskers show the interquartile range and 5 and 95 percentiles, respectively. 95% confidence intervals around the medians are illustrated by the notches. Significant difference of median  $AUEC_{5-60 \text{ minutes}}$  with indomethacin and vehicle groups are depicted by (\*) and (\$), respectively. Plots of the groups that their median AUECs were not statistically significant are shown in the same color.

Several aryl biurets like 1,3-dimethyl-5-phenylbiuret, 1-ethyl-3-methyl-5-phenylbiuret and 1,1,3-trimethyl-5-phenyl biuret were found to have more potent anti-inflammatory activity than phenylbutazone using carrageenan-induced paw edema in rat. It has also been shown that 1,3-dimethyl-5-phenylbiuret and 5-(4-chlorophenyl) 1,1,3-trimethylphenyl biuret showed higher analgesic activity than that of aminopyrine in acetic acid stretching test.<sup>6</sup>

Unlike indomethacin, the biuret derivatives under investigation in the current study showed antinociceptive activity in both phases of formaldehyde-induced pain in mice. The highest activity was seen by biuret derivative with phenylethyl and benzo[d]thiazol-2-ylthio moieties (C9). Compounds C7 and C8 with phenylpropyl, benzyl and 2-methylquinoline-4-yl substituents also showed good activities in comparison to indomethacin. However, difference between median AUECs were not significant at doses higher than 25 mg/kg in both phases of pain. Almost all the biuret compounds, showed a rather good analgesic activities at the dose of 100 mg/kg. It seems that the more lipophilic show higher analgesic activity.

### Conclusion

In conclusion, our findings may suggest that biuret compounds, unlike indomethacin are able to induce antinociception in both phases of formalin test and could be considered comparable to indomethacin at the selected doses. However, further investigations are necessary to elucidate their safety and efficacy.

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### Conflict of Interest

There is no conflict of interest to be reported.

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