Bioorganic & Medicinal Chemistry Letters 23 (2013) 5519–5522

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and anti-inflammatory activity of three nitro chalcones ☆

Abraham Gómez-Rivera [a], Hidemí Aguilar-Mariscal [b], Nancy Romero-Ceronio [a], Luis F. Roa-de la Fuente [a], Carlos E. Lobato-García [a,*]

[a] División Académica de Ciencias Básicas, Km 1 Carretera Cunduacán-Jalpa de M. Cunduacán, Tabasco 86690, Mexico
[b] División Académica de Ciencias de la Salud, Gregorio Méndez Magaña, Villahermosa 86150, Mexico

ARTICLE INFO

Article history:
Received 7 August 2013
Accepted 13 August 2013
Available online 19 August 2013

Keywords:
Synthesis
Anti-inflammatory
Nitro chalcone
Carrageenan induced edema

ABSTRACT

The aim of this study was to synthesize three nitro substituted chalcones and to evaluate their anti-inflammatory activity in the model of carrageenan induced edema in rats. The nitro chalcone were prepared by aldol condensation using of mechanical agitation and environmentally friendly solvents with 72–73% yields in approximately 2 h. The three structures were evaluated on biological activity at dose of 200 mg/kg and they showed anti-inflammatory protective effect by both oral and intraperitoneal administration, this effect was time dependent.

Chalcones are part of the selected group of chemical compounds associated with diverse pharmacological activities. The chalcone structure has been reported in compounds with anti-inflamatory, anti-ulcerative, antibacterial, antifungal and antimalarial activities among others.[1–5]

Chalcones and their derivatives are polyphenolic compounds of the flavonoids family. They have been found in many plants as metabolic precursors of other flavonoids and isoflavonoids.[6] It is noteworthy to mention that the presence of chalcones have been reported in plants traditionally employed for therapeutical purposes.[7,8]

From a synthetic point of view, there is a great interest for the development of structural analogues of chalcones.[8] The Claisen–Schmidt condensation between acetophenone derivatives with benzaldehyde derivatives, with both acid and basic catalysis has been by far the leading reaction employed for these compounds, as shown in Figure 1.[9]

Furthermore, the synthesis of new chalcone analogues is growing nowadays, because of the potential anti-inflammatory activities they may exhibit, which make them attractive candidates for the treatment of chronic diseases which involve inflammatory processes, for example, *diabetes mellitus*.[10]

On behalf of this, the objectives of this work were to synthesize three nitro-substituted chalcones and to evaluate their anti-inflammatory activity in the carrageenan induced paw edema model in rat.

The synthesis of the three nitro-chalcones was conducted by the Claisen–Schmidt condensation between 2′-nitroacetophenone (**1**), 3′-nitroacetophenone (**2**) and 4′-nitroacetophenone (**3**) and benzaldehyde (4), this reaction was promoted wit sodium hydroxide (NAOH) according to previously reported procedure[11] (Fig. 2).

All the reagents were analytical-reagent grade and were used without further purification. The general procedure started with the preparation of a solution of NaOH (6.7 mmol) in water (6 mL). This solution was cooled at 0 °C with an ice bath, and ethanol (10 mL) was slowly added, the reaction flask was then removed from the ice bath and set at room temperature before the slow addition of the corresponding acetophenone (10 mmol) after this, benzaldehyde (10 mmol) was slowly added to the reaction mixture, which was left at room temperature with mechanical agitation for 2 h, afterwards the reaction mixture was cooled at 0 °C for 24 h. The solid products were filtrated from the crude mixture, washed with cold water and recrystallized with a dichloromethane/ethanol mixture. The crystals thus obtained were dried at 70 °C and properly stored at room temperature prior their physicochemical and spectroscopic characterizations and the determination of their anti-inflammatory activity. The chalcones prepared with this procedure were: (*E*)-1-(2′-nitrophenyl)-3-phenylprop-2-en-1-one (2′-nitrochalcone) (**5**); (*E*)-1-(3′-nitrophenyl)-3-phenylprop-2-en-1-one (3′-nitrochalcone) (**6**) and (*E*)-1-(4′-nitrophenyl)-3-phenylprop-2-en-1-one (4′-nitrochalcone) (**7**).

The three chalcones were obtained in good yields (>75%). All of them were solids and they were poorly soluble in water and highly

* Corresponding author. Tel.: +52 914 33 609 28; fax: +52 33 609 28.
E-mail address: carlos.lobato@ujat.mx (C.E. Lobato-García).

0960-894X/$ - see front matter © 2013 The Authors. Published by Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2013.08.061

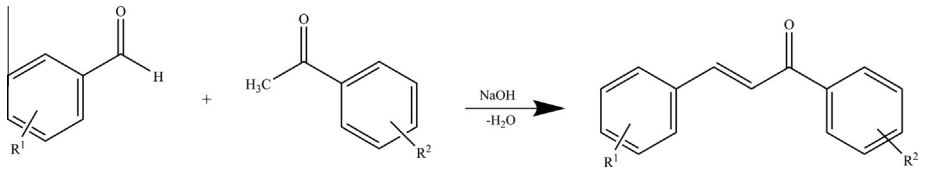

Figure 1. Claisen–Schmidt condensation employed for the synthesis of chalcones.

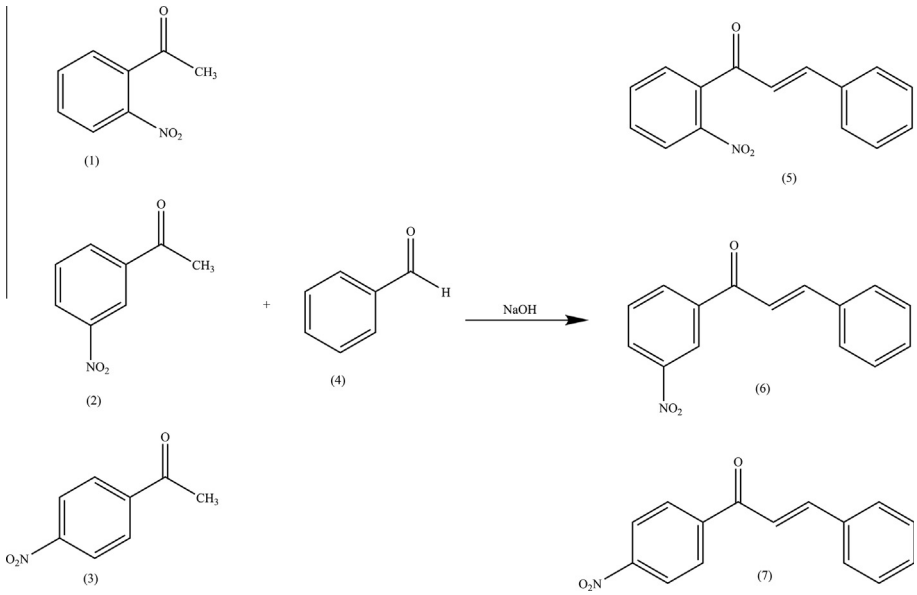

Figure 2. Synthesis of the three nitro-sustituted chalcones. (**1**) 2′-Nitroacetophenone; (**2**) 3′-nitroacetophenone; (**3**) 4′-nitroacetophenone; (**4**) benzaldehyde; (**5**) 2′-nitrochalcone; (**6**) 3′-nitrochalcone; (**7**) 4′-nitrochalcone.

soluble in dimethylsulfoxide (DMSO). The UV–Vis spectra showed the expected maximum absorbance wavelengths, whereas the IR spectra bands were in agreement with the functional groups expected in the structures. The ^{1}H NMR coupling constants for the alquene protons, showed that the stereochemistry around the double bond was (*E*) in the three compounds. The assignment of the ^{13}C NMR spectra confirmed the structures (Supplementary data). The evaluation of the anti-inflammatory effect of the three chalcones was assayed by the carrageenan induced paw edema in rat.[12]

First, it was assayed the temporal evolution of the inflammatory activity of carrageenan: 2 groups of 6 male Wistar rats (180–220 g) were employed. At $t = 0$, the volume of the right hind paw was measured. After this, one group was applied via intraplantar (i.pl.) in the right hind paw with a single dose (50 μL) of carrageenan at 0.3% in saline solution 0.9%. The second group served as control and was applied i.pl. in the right hind paw with a single dose (50 μL) of saline solution 0.9%. The volume of the right hind paw of the 12 specimens was measured every hour for 7 h. Each measurement was made in triplicate with a pletismometer (Ugo Basile 7140). The i.pl. application of carrageenan in the right hind paw of the rats derived into an edema development from a basal value of 1.1 ± 0.01 mL to a maximum of 1.9 ± 0.04 mL which was presented 3 h after the administration of carrageenan. The control group showed a basal volume of 1.1 ± 0.01 mL and there were no statistical significance differences (p <0.05) in the volume of the control group along the 7 h of the experiment (Fig. 3). When the volumes of the carrageenan and control groups were compared, statistical significance differences (p <0.05) were found.

The anti-inflammatory protective effect of the three chalcones (**5**–**7**) was evaluated at a single dose of 200 mg kg^{-1}, the vehicle employed was DMSO. To perform this test, an arrangement of 7

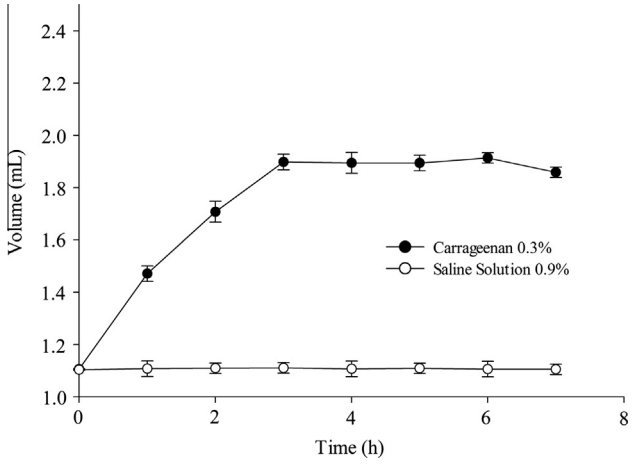

Figure 3. Time course of the volume of the right rear paw of the rat: a rise of the volume after the i.pl. application of carrageenan 0.3% is shown. Each point represents average ± standard error (n = 6).

groups, each of them with 6 rats, was made. Three of these groups received their corresponding chalcone by oral administration (p.o.), while other three received their corresponding chalcone by intraperitoneal injection (ip). The seventh group was the control and only received the vehicle p.o. Meloxicam (**8**) was employed as a reference drug: a group of 6 rats received p.o. a single dose (10 mg kg^{-1}) of the reference.[13] After this administrations, a single dose of carrageenan 0.3% vas applied i.pl. in the right hind paw of each specimen, in a similar way as described above. The volume of

the right hind paw of the specimens was measured every hour for 7 h, employing the pletismometer technique.

For each animal, the Maximum Anti-inflammatory Protective Effect (MAPE) was calculated every hour during the length of the experiment. MAPE was calculated as a percentage, according to the following equation:MAPE(%) = $[(V_{carr}-V_{treat})/(V_{carr}-V_o)]$-$*$[100]Where:$V_{carr}$ = volume of the rear paw of the rat which received carrageenan i.pl.V_{treat} = volume of the rear paw of the rat who received either meloxicam or chalcone prior the application of carrageenan.V_o = volume of the rear paw of the rat at zero time.[14]

The results for the MAPE are presented as the average ± standard deviation. In order to compare the differences between the experimental groups, an ANOVA a Tukey Test and a Contrast Analysis were performed. Results were considered statistically significant at p <0.05.

Figure 4 show that the oral administration of every tested chalcone, at a single oral dose of 200 mg kg^{-1}, produced an anti-inflammatory protective effect which is time-dependent. For compound **2**, the maximum anti-inflammatory effect (34.8 ± 2.5%) was obtained 3 h after the administration and the anti-inflammatory effect was maintained in around 30% during the rest of the experiment. For compound **3** the maximum effect (31.0 ± 5.6%) was reached 2 h after the administration, but it began to diminish one hour later to reach values near zero 7 h after the administration. For compound **4**, the highest protective effect (38.0 ± 6.3%) was reached one hour after de administration, it diminished at around 20% 2 h after the administration and remained steady during the next 6 h. For the reference group (compound **8**) the maximum effect (59.3 ± 3.8%) was reached within 2 h after the administration, and remained steady (around 50–60%) during the 7 h of the experiment.

The area under the curve (AUC) for the temporal evolution of the anti-inflammatory protective effect of each chalcone and the reference drug administered p.o. were also calculated and the results are presented in Figure 5. The highest AUC corresponds to compound **5** (303.71 ± 34.32), whereas compound **6** has the lowest AUC (137.26 ± 16.82). The Tukey Test applied for the AUC values, showed that the differences between the AUC's and therefore the differences in the anti-inflammatory protective effect of each tested chalcone, are statistically significant at p <0.05. Compound **5** (with the nitro group at the *ortho* position), developed the highest protective anti-inflammatory effect, while compound **6** (with the nitro group at the *meta* position), showed the lowest protective anti-inflammatory effect. When compared the AUC values of the three tested chalcones versus the reference drug **8**, it was found a statistically significant difference at p <0.05, being meloxicam the compound with the best anti-inflammatory protective effect.

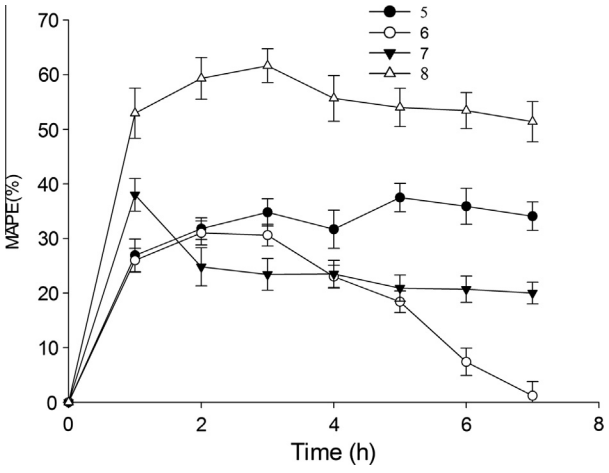

Figure 4. Time course of the MAPE of chalcones **5**, **6** and **7** (200 mg kg^{-1}, p.o.) and the reference drug **8** (10 mg kg^{-1}, p.o.) on the carregeenan induced paw edema in rats. Each point represents the average ± standard deviation (n = 6).

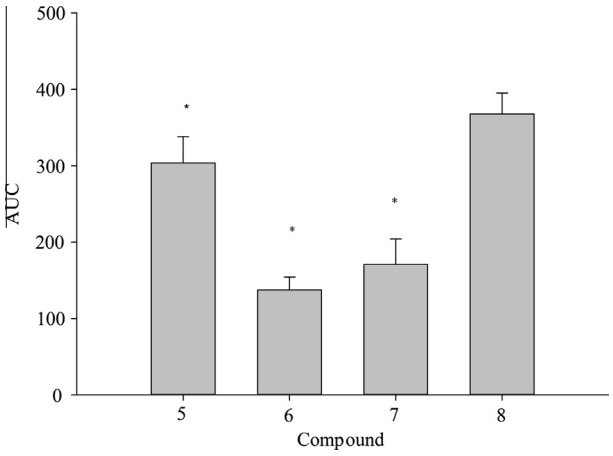

Figure 5. AUC for the MAPE of chalcones **5**, **6** and **7** (200 mg kg^{-1}, p.o.) and the reference drug **8** (10 mg kg^{-1}, p.o.) Each bar represents the average ± standard deviation (n = 6). *Statistically significant difference (p <0.05).

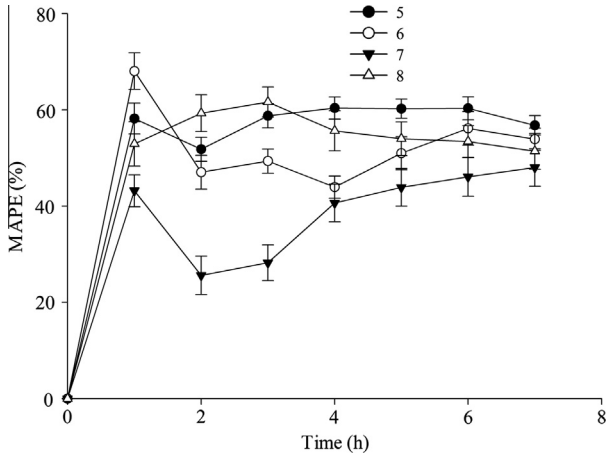

Figure 6. Time course of the MAPE of chalcones **5**, **6** and **7** (200 mg kg^{-1}, ip) and the reference drug **8** (10 mg kg^{-1}, p.o.) on the carregeenan induced paw edema in rats. Each point represents the average ± standard deviation (n = 6).

On the other hand, the intraperitoneal administration of the three nitro-substituted chalcones showed anti-inflammatory protective effects which were all time-dependent (Fig. 6). For compound **5** the maximum effect (58.2 ± 3.2%) was reached 1 h after the administration. The MAPE for **5** was maintained between 50% and 60% during the 7 h of the experiment. Compound **6** recorded the highest MAPE for the three chalcones (68.0 ± 3.8%) and it was reached within the first hour of the experiment, although 5 h after the administration. Compound **7** reached its highest MAPE 2 h after the administration (43.2 ± 3.3%), this effect was maintained steady along the experiment. The highest MAPE for the reference drug **8** (59.3 ± 3.8%) was reached 2 h after the administration and it was maintained steady during the 7 h of the experiment.

The AUC for the temporal evolution of the anti-inflammatory protective effect of each chalcone and the reference drug administered ip were also calculated and the results are presented in Figure 7. As occurred in the oral administration, compound **5** also showed the highest AUC (378.08 ± 32.34), but unlike the former procedure, it was compound **7** and not **6** the one with the smallest AUC (246.78 ± 36.8). The application of the Tukey test over the AUC values showed statistically significant differences when the AUC of

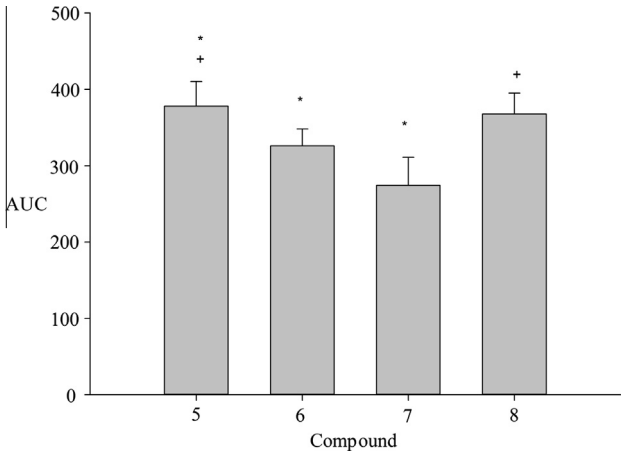

Figure 7. AUC for the MAPE of chalcones **5**, **6** and **7** (200 mg kg^{-1}, ip) and the reference drug **8** (10 mg kg^{-1}, p.o.) Each bar represents the average ± standard deviation (n = 6). *Statistically significant difference (p <0.05). (+) No statistically significant difference was found.

the three chalcones were compared to one another. Thus, it was found that the chalcone **5**, whith the nitro group at the *ortho* position, develops the highest MAPE and the chalcone 7, with the nitro group at the *para* position, showed the smallest MAPE effect. These results, combined with those obtained for the oral administration lead to state that the nitro group located at the ortho position plays an important role in both the anti-inflammatory protective effect and the bioavailability of chalcone **5**.

Chalcones **6** and **7**, also showed statistically significant differences when compared with the reference drug, nevertheless it was not found a statistically significant difference between the AUC of the MAPE for chalcone **5** and the corresponding value for the reference drug.

The statistical contrast of the anti-inflammatory protective effect of **5**, **6** and **7** administered at the same dose either orally or by the intraperitoneal route, showed that the effect is different and it depends on the route of administration, the latter being the most suitable in order to generate a protective effect against inflammation in the model employed.

In conclusion, three nitro-chalcones (**5**, **6** and **7**) were synthesized, employing shorter reaction times than the previously reported in the literature. The spectroscopic characterizations of the three products are in agreement with the structures expected. These chalcones showed an anti-inflammatory protective effect when administered orally or by the intraperitoneal route. The chalcone with the nitro group at the *ortho* position was found to be the most effective, with no statistically significant difference when compared to a reference drug (meloxicam).

Acknowledgments

The authors wish to thank FOMIX and PFICA-UJAT for the financial support via the Projects FOMIX-TAB 2009-C18-122141, PFICA-UJAT-2009-CO5-03 and PFICA-UJAT-2011-CO7-22.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.08.061.

References and notes

1. Peng, F.; Wang, G.; Li, X.; Cao, D.; Yang, Z.; Ma, L.; Ye, H.; Liang, X.; Ran, Y.; Chen, J.; Qiu, J.; Xie, C.; Deng, C.; Xiang, M.; Peng, A.; Wei, Y.; Chen, L. *Eur. J. Med. Chem.* **2012**, *54*, 272.
2. Shibuya, A.; Onda, K.; Kawahara, H.; Uchiyama, Y.; Nakayama, H.; Omia, T.; Nagaoka, M.; Matsui, H.; Hirano, T. *Biochem. Biophys. Res. Commun.* **2010**, *398*, 581.
3. Konduru, N. K.; Dey, S.; Sajid, M.; Owais, M.; Ahmed, N. *Eur. J. Med. Chem.* **2013**, *59*, 23.
4. Yu-Ting, L.; Xiao-Ming, S.; Da-Wei, Y.; Fang, Y. *Res. Chem. Intermed.* **2013**, *39*(3), 1037.
5. Yadav, N.; Dixit, S. K.; Bhattacharya, A.; Mishra, L. C.; Sharma, M.; Awasthi, S. K.; Bhasin, V. K. *Chem. Biol. Drug Des.* **2012**, *80*, 340.
6. Maria, K.; Dimitra, H. L.; Maria, G. *Med. Chem.* **2008**, *4*, 586.
7. Viana, G. S.; Bandeira, M. A.; Matos, F. *J. Phytomed.* **2003**, *10*, 189.
8. Fontenele, J. B.; Leal, L. K.; Felix, F. H.; Silveira, E. R.; Viana, G. S. *Nat. Prod. Res.* **2009**, *23*, 1677.
9. Perozo, E.; Martín, R. M.; Casal, B.; Durán, C. J.; Lau, W. N.; Zhang, X. F.; Yeung, K. L. *Cat. Today* **2006**, *4413*, 1.
10. Najafian, M.; Ebrahim-Habibi, A.; Yaghmaei, P.; Parivar, K.; Larijani, B. *Acta Biochim. Pol.* **2010**, *57*, 553.
11. Cocconcelli, G.; Diodato, E.; Caricasole, A.; Gaviraghi, G.; Genesio, E.; Ghiron, C.; Magnoni, L.; Pecchioli, E.; Plazzi, P. V.; Terstappen, G. C. *Bioorg. Med. Chem.* **2008**, *16*, 2043.
12. Winter, C. A.; Risley, G. A.; Nuss, G. W. *Proc. Soc. Exp. Biol. Med.* **1962**, *111*, 544.
13. Aguilar, H.; Rodríguez, J.; Torres, J. E.; Flores, F. J. *Proc. West Pharmacol. Soc.* **2006**, *49*, 45.
14. Aguilar, H. Ph. D. Dissertation, IPN, (2008).