Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 15 (2005) 3483–3486
Bioorganic & Medicinal Chemistry Letters

# Anxiolytic effects of benzalphthalides

Alejandro Zamilpa,[a,b] Maribel Herrera-Ruiz,[b] Esther del Olmo,[a,*] José L. López-Pérez,[a] Jaime Tortoriello[b,*] and Arturo San Feliciano[a]

[a]*Departamento de Química Farmacéutica, Facultad de Farmacia, 37007 Salamanca, Spain*
[b]*Centro de Investigación Biomédica del Sur, IMSS, 62790 Xochitepec, Morelos, Mexico*

Received 18 April 2005; revised 30 May 2005; accepted 8 June 2005

**Abstract**—Anxiolytic effects induced by benzalphthalides on mice have been evaluated. The evaluation was based on the spent time and the number of entries of animals into the open arms in the elevated plus maze test. Single administration of benzalphthalides **1** and **11** induced significant increments in both parameters, thus revealing their potentiality as new leads for the development of non-benzodiazepinic and non-nitrogenated antianxiety agents.


Anxiety is a rather common disorder affecting people in both developed and developing countries.[1] Pathological manifestations of anxiety are prevalent, chronic, and disabling and can include generalized anxiety, obsessive–compulsive disorders, panic, post-traumatic stress disorders, and social and specificphobias.[2] Pharmacological treatment of anxiety is principally based on 1,4-benzodiazepines (BDZs, diazepam and related drugs) or 5-$HT_{1A}$ receptor agonists and selective 5-HT reuptake inhibitors (SSRIs). These approaches have drawbacks because BDZs have a number of unwanted sideeffects, including tolerance, sedation, cognitive impairments, and alcohol interaction and generally, the onset of action of 5-HT receptor ligands is slow.[3] Non-nitrogenated natural compounds such as flavonoids and some related synthetic chromenoids have attracted much attention and have been tested with positive results as anxiolytics [4,5] and their interactions with BDZ (GABA) receptors have also been demonstrated.[6]

The structural analogy between the chromenoids and the phthalide (isobenzofuranone) nuclei (Fig. 1) suggested to us the possibility for the latter of serving as supporting skeleton for anxiolytic compounds. This fact seemed to have not been tested before. Moreover, the phthalide moiety is also present in various natural products[7] displaying a wide range of biological activities, such as antitussive (noscapine),[8] anti-HIV (fuscinarin),[9] antineoplastic cytotoxicity (vermistatin),[10] or antibacterial (cytosporone E).[11] Moreover, various naturally occurring or synthetic 3-alkylidenephthalides[12] showed muscle relaxant and antiasthmatic properties that have been protected by patents,[13] and 3-butylphthalide reduced brain damage in mice, showed anticonvulsant effects, increased the duration of anesthesia, and exhibited cerebral antiischemic properties.[8] The natural benzalphthalide thunberginol F showed inhibitory activity on histamine release from rat peritoneal mast cells.[14] In previous reports, we showed that some benzalphthalides displayed low levels of in vitro leishmanicidal[15] and trypanocidal activities.[16] More recently, we have evaluated their anti-HIV, vasorelaxant, and antihistaminic activities and the results will be published elsewhere. In this article, we present the results of the evaluation of 12 representative benzalphthalides as anxiolytics.

## 1. Chemistry

The simple and general procedure (Scheme 1) used to obtain these benzalphthalides was previously applied and reported by our group.[15,17] Phthalic anhydride derivatives were condensed with differently substituted phenylacetic acids, leading to the corresponding benzalphthalides **1–9**. Oxidation of the sulfane derivative **4** with $KIO_4$[18] or $H_2O_2$/AcOH[19] provided sulfoxide **10** and sulfone **11**, respectively. Transformation of the acid **7** into the amide **12** was carried out through the interme-

*Keywords*: Benzalphthalides; Anxiolytic effect; SAR; Apigenin; Diazepam.
* Corresponding authors. Tel.: +34 923 294528; fax: +34 923 294515; e-mail: olmo@usal.es

0960-894X/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2005.06.031

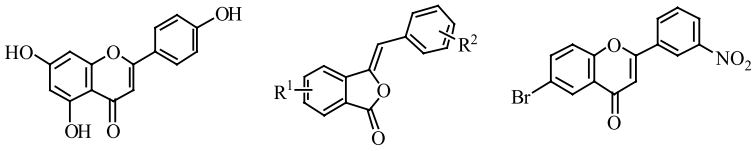
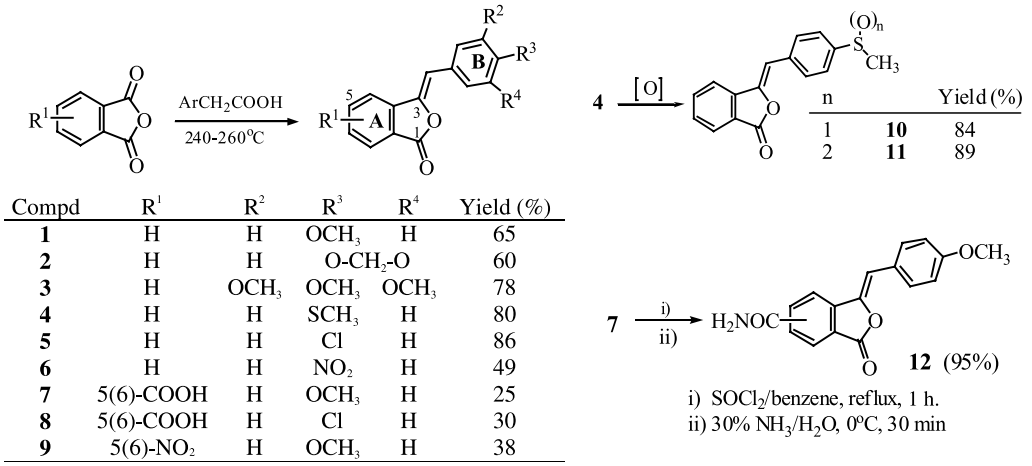



**Figure 1.** Structural relationship between anxiolytic natural and synthetic flavonoids and benzalphthalides.




| Compd | $R^1$ | $R^2$ | $R^3$ | $R^4$ | Yield (%) |
|---|---|---|---|---|---|
| **1** | H | H | $OCH_3$ | H | 65 |
| **2** | H | H | O-$CH_2$-O | | 60 |
| **3** | H | $OCH_3$ | $OCH_3$ | $OCH_3$ | 78 |
| **4** | H | H | $SCH_3$ | H | 80 |
| **5** | H | H | Cl | H | 86 |
| **6** | H | H | $NO_2$ | H | 49 |
| **7** | 5(6)-COOH | H | $OCH_3$ | H | 25 |
| **8** | 5(6)-COOH | H | Cl | H | 30 |
| **9** | 5(6)-$NO_2$ | H | $OCH_3$ | H | 38 |

| n | | Yield (%) |
|---|---|---|
| 1 | **10** | 84 |
| 2 | **11** | 89 |

**Scheme 1.** Benzalphthalides tested as anxiolytics.

diate acid chloride that was prepared by reaction with $SOCl_2$/benzene, by treatment with ammonia solution. Phthalides **7**, **8**, **9**, and **12** were obtained and evaluated as unresolved regioisomeric (approximately 1:1) mixtures.

## 2. Biological assays

The evaluation of anxiolytic effects was performed on albino ICR mice through the elevated plus maze test.[20] In this assay, anxiolytic drugs prolong the time spent and the number of entries into the open arms of the maze, whereas anxiogenic compounds produced a fair reduction of these parameters. The test group was treated with compounds **1**–**12** (15 mg/kg, ip); three other groups were, respectively, treated with the anxiolytic reference drug (diazepam, DZP, 1 mg/kg, ip), the anxiogenic reference drug (picrotoxin, 2 mg/kg, ip), and vehicle (Tween 20, 5%, ip).[21] Tests begun 1 h after administration, and mice evolutions were recorded for 5 min using a video camera. The anxiolytic activity was evaluated through the number of entries and the time spent into the open arms of the maze.[22]

## 3. Results and discussion

The effects of single administration of benzalphthalides **1**–**12**, DZP, PTX, vehicle on the spent time, and the number of entries into the arms of plus maze are shown in Table 1. Figures 2A–D are arranged to provide a graphical comparison related with the influence of substituents attached to ring B on the activity.

It can be observed in table and figures that for compounds containing natural oxygenated substituents as *p*-methoxy (**1**), 3,4-methylenedioxy (**2**), or 3,4,5-trimethoxy (**3**) group, the monosubstitution seems to be better for the activity than those di- or tri-substitutions. On the other hand, the replacement of the *p*-methoxy group by other non-natural substituents as methylsulfanyl (**4**), chloro (**5**), or nitro (**6**) leads to lowering the values of anxiolytic parameters, while oxidation of the sulfane group of **4** to the corresponding sulfoxide **10** and sulfone **11** gradually increases the anxiolytic effects to levels even higher than those observed for the methoxy derivative **1**.

With respect to the influence of substituents on ring A, on the light of the results observed for compounds **7**, **8**, **9**, and **12** and within the scarce number of compounds tested, it can be noted that the presence of an electron-withdrawing group linked at position 5 or 6, such as a free carboxyl (compound **7**), a nitro (compound **8**), or an amide (compound **12**) group, provided less active derivatives if compared with the corresponding unsubstituted phthalide **1**.

The most relevant fact within the results observed is that the percentage of entries into the open arms for the sulfone derivative **11** was significantly higher, in comparison with that observed for mice administered with the vehicle only, thus demonstrating for the first time in this type of compounds a certain level anxiolytic activity.

The results observed are in agreement with the close structural proximity between benzalphthalides and flavonoids. This fact can easily be confirmed by simple visual inspection of the superimpositions of the lowest energy calculated structures for the most potent phtha-

**Table 1.** Effects of benzalphthalides **1**–**12** on the relevant anxiolytic parameters in the elevated plus maze test

| Compound | Spent time* into open arms | Number of entries into open arms | Number of entries into closed arms | Total number of entries | % Number of entries into open arms |
|---|---|---|---|---|---|
| **1** | **177.3 ± 33.5** | 13.0 ± 4.2 | 7.1 ± 2.1 | 20.1 ± 5.1 | **64.1 ± 10.8*** |
| **2** | 150.6 ± 27.9 | 11.2 ± 2.8 | 9.0 ± 3.0 | 20.2 ± 4.9 | 54.4 ± 7.3 |
| **3** | 106.6 ± 66.1 | 8.3 ± 3.8 | 9.0 ± 4.8 | 17.3 ± 5.3 | 44.8 ± 19.8 |
| **4** | 130.6 ± 46.5 | 7.9 ± 3.1 | 9.9 ± 3.3 | 17.7 ± 5.1 | 49.5 ± 14.8 |
| **5** | 159.8 ± 82.5 | 8.0 ± 2.8 | 8.5 ± 5.7 | 16.5 ± 7.4 | 55.8 ± 24.8 |
| **6** | 158.7 ± 27.4 | 9.0 ± 3.3 | 7.3 ± 3.1 | 16.3 ± 5.1 | 57.2 ± 11.3 |
| **7** | 137.5 ± 71.1 | 9.8 ± 5.3 | 9.0 ± 4.1 | 18.8 ± 6.7 | 51.2 ± 20.5 |
| **8** | 107.8 ± 50.1 | 9.5 ± 5.2 | 11.7 ± 3.4 | 21.2 ± 6.4 | 42.8 ± 15.7 |
| **9** | 129.1 ± 45.5 | 11.7 ± 7.2 | 10.5 ± 2.1 | 22.2 ± 8.5 | 51.7 ± 10.1 |
| **10** | 153.8 ± 29.4 | 10.8 ± 4.1 | 9.0 ± 3.1 | 19.8 ± 5.5 | 55.6 ± 10.9 |
| **11** | **187.1 ± 44.2** | 10.6 ± 2.8 | 5.6 ± 4.5 | 16.4 ± 7.1 | **69.9 ± 15.2*** |
| **12** | 125.2 ± 43.9 | 13.0 ± 5.1 | 11.5 ± 2.8 | 24.0 ± 6.4 | 50.2 ± 12.1 |
| DZP | **230.4 ± 16.9** | 15.8 ± 4.5 | 3.7 ± 2.5 | 19.5 ± 5.7 | **82.0 ± 11.4** |
| VEH | 122.4 ± 17.4 | 9.6 ± 3.0 | 8.9 ± 1.7 | 18.5 ± 4.0 | 51.2 ± 7.2 |
| PTX | 26.6 ± 17.6 | 2.5 ± 2.0 | 9.4 ± 4 | 11.8 ± 5.6 | 19.0 ± 8 |

Data represent the mean ± SEM; $n = 8$. DZP, diazepam; VEH, Tween 20 (5%); PTX, picrotoxin.
* s/5 min.

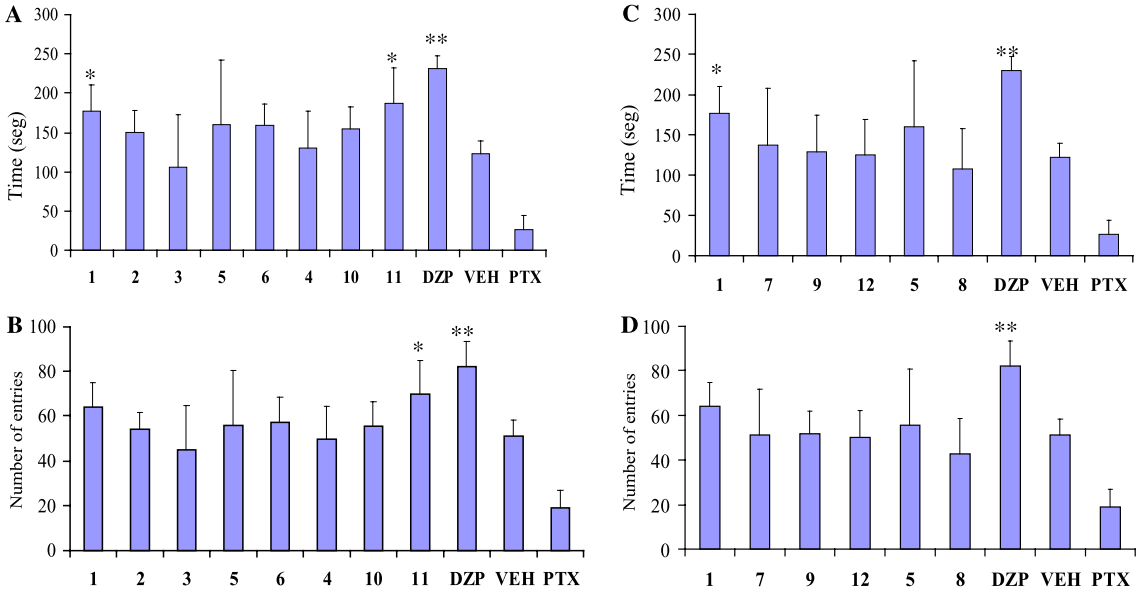



**Figure 2.** Effect produced by benzalphthalides, without (A,B) or with (C,D) substituents on ring A, upon the spent time (A,C) and the percentage of entries into the open arms (B,D) in elevated plus maze test. DZP, diazepam; VEH, Tween 20 (5%); PTX, picrotoxin. $^{*}p < 0.05$, $^{**}p < 0.001$.

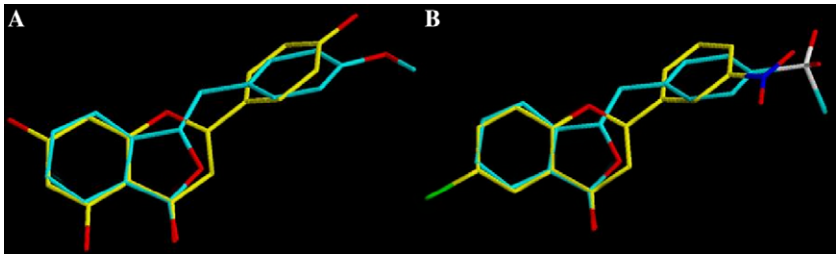



**Figure 3.** Superimposition of calculated structures: (A) apigenin (yellow) with compound **1** (blue); (B) 6-bromo-3′-nitroflavone (yellow) with compound **11** (blue).

lides found in this work and the flavonoids apigenin and bromonitroflavone mentioned above (Fig. 3).

After this preliminary prospection of anxiolytic activity, it can be concluded that, as expected, the benzalphthalide skeleton supporting adequate functionalities could provide new potential candidates for developing new anxiolytic drugs. Nevertheless, with the model of **1** and **11** more compounds with electron donating or withdrawing groups attached to the same or other positions

on ring A or B need to be prepared and evaluated. It is also interesting to note that the absence of nitrogen in the structure could prevent some of those secondary adverse effects displayed by the currently most used DZP and its related drugs.

On the basis of the results of this research, apart from the progression of studies on the benzalphthalide group, several other structurally related heterocycles are being evaluated in vivo for their anxiolytic activity.

## Acknowledgments

Financial support came from Spanish MCYT (Grant: AGL2000-0039-P4-02). A.Z. thanks the CIS-IMSS (Mexico) for a Post-doctoral fellowship. Collaborative work performed under the auspices of 'Programa Iberoamericano de Ciencia y Tecnología para el Desarrollo (CYTED), SubPrograma X'.

## References and notes

1. Airaksinen, E.; Larsson, M.; Forsell, Y. *J. Psych. Res.* **2005**, *39*, 207.
2. Barkow, K.; Heun, R.; Üstün, T. B.; Berger, M.; Bermejo, I.; Gaebel, W.; Härter, M.; Schneider, F.; Stieglitz, R.; Maier, W. *Eur. Psych.* **2004**, *19*, 250.
3. Cryan, J. F.; Kaupmann, K. *Trends Pharmacol. Sci.* **2005**, *26*, 1.
4. Medina, J. H.; Viola, H.; Wolfman, C.; Marder, M.; Wasowski, C.; Calvo, D.; Paladini, A. C. *Neurochem. Res.* **1997**, *22*, 419.
5. Halla, B. J.; Chebibb, M.; Hanrahanb, J. R.; Johnston, G. A. R. *Eur. J. Pharmacol.* **2004**, *491*, 1.
6. Marder, M.; Estiú, G.; Blanch, L. B.; Viola, H.; Wasowski, C.; Medina, J. H.; Paladini, A. C. *Bioorg. Med. Chem.* **2001**, *9*, 323.
7. Raghao, S. M.; Kantipudi, N. B.; Prakash, G. J. *J. Chem. Soc.* **2001**, *1*, 3017.
8. Karlsson, M. O.; Dahlström, B.; Neil, A. *Eur. J. Pharmacol.* **1988**, *145*, 195.
9. Yoganathan, K.; Rossant, C.; Siewbee, N.; Huang, Y.; Mark, S.; Butler, M. S.; Buss, A. D. *J. Nat. Prod.* **2003**, *66*, 1116.
10. Arai, M.; Tomoda, H.; Okuda, T.; Wang, H.; Tabata, N.; Masuma, R.; Yamaguchi, Y.; Omura, S. *J. Antibiot.* **2002**, 55.
11. Hall, J. D.; Duncan-Gould, N. W.; Siddiqi, N. A.; Kelly, J. N.; Hoeferlin, A.; Morrison, S. J.; Wyatt, J. K. *Bioorg. Med. Chem.* **2005**, *13*, 1409.
12. Bellina, F.; Ciucci, D.; Vergamini, P.; Rossi, R. *Tetrahedron* **2000**, *56*, 2533.
13. Fukuyama, Y.; Hiroyoshi, O.; Yoshio, O. *Jpn. Kokai Tokkyo Koho JP* 60,155,175 [85,155,175]. Fukuyama, Y.; Fuzen, O.; Sensuke, K.; Yoshio, O. *Jpn. Kokai Tokkyo Koho JP* 6107,276 [8607,267]. Kimura, M.; Harada, M.; Sekida, S.; Yuda, M. *Jpn. Kokai Tokkyo Koho JP* 01,207,233 [89,207,233].
14. Matsuda, H.; Shimoda, H.; Yoshikawa, M. *Bioorg. Med. Chem.* **1999**, *7*, 1445.
15. Del Olmo, E.; García, M.; López-Pérez, J. L.; Muñoz, V.; Deharo, E.; San Feliciano, A. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2123.
16. Del Olmo, E.; García, M.; López-Pérez, J. L.; Grace, R.; Vargaz, F.; Giménez, A.; Deharo, E.; San Feliciano, A. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2755.
17. Phthalic anhydride (3.0 g, 24.0 mmol) and phenyl acetic acid (3.0 g, 22.0 mmol) in addition to sodium acetate (60 mg, 1.2 mmol) and 5 mL of benzene were placed into a flask provided of a Dean-Stark system and the mixture was maintained at 230–240 °C during 6 h. The reaction was controlled by TLC. After completion of the reaction, the mixture was cooled to room temperature, poured into water (100 mL), and extracted with 2× with EtOAc. The organic layer was washed with aqueous 10% $Na_2CO_3$ and water, dried, filtered and evaporated off. The crude material crystalised from methanol afforded compound **1** as a white powder (4.3 g, 70%); mp = 79–80 °C. IR $\lambda_{max}$: 2974, 2844, 1786, 1603, 1514, 1260, 1029, 978, 859, 823, and 762 $cm^{-1}$. $^1H$ NMR (200 MHz, $CDCl_3$): $\delta$ 3.84 (3H, s, $OCH_3$), 6.37 (1H, s, H-8), 6.93 (2H, d, $J = 8.8$ Hz, H-11 + H-13), 7.50 (1H, m, H-6), 7.68 (1H, m, H-5), 7.73 (1H, m, H-4), 7.79 (2H, d, $J = 8.8$ Hz, H-10 + H-14) and 7.91 ppm (1H, d, $J = 7.7$ Hz, H-7). $^{13}C$ NMR (50.3 MHz, $CDCl_3$): $\delta$ 55.3, 106.9, 114.2, 119.5, 123.0, 125.5, 125.8, 129.2, 131.7, 134.3, 140.7, 143.0, 159.7, and 167.2 ppm. MS (FAB) *m/z* 253 $(M+H)^+$. Anal. Calcd for $C_{16}H_{12}O_3$: C, 76.18; H, 4.79. Found: C, 76.07; H, 4.55.
18. Leonard, N. J.; Johnson, C. R. *J. Org. Chem.* **1962**, *27*, 282.
19. Jenner, E. L. *Org. Syn.* **1960**, *40*, 90.
20. Elevated plus maze apparatus, made of Plexiglas, consisted of two open arms (30 × 5 cm) and two closed arms (30 × 5 cm) with 25 cm walls. The arms extended from a central platform (5 × 5 cm). The maze was mounted on a base raising 38.5 cm above the room floor. Test begun with the mice placed singly in the center of plus-maze facing a closed arm. The number of entries and the time spent in closed and open arms was recorded for 5 min. Entry into an arm was defined as the animal placing all four paws onto the arm. Total exploratory activity (number of entries) and other ethologically derived measures (grooming, rearing, stretched attend postures, and head dipping) were also registered. All tests were taped by using a video camera. After each test, the maze was carefully cleaned up with a wet tissue paper (10% ethanol solution). Groups of eight male albino ICR mice (32–38 g) were conditioned to laboratory environment (12 h light and 12 h dark), with free access to water and food. Data obtained in the test were compared against the control group by using the ANOVA method and followed by a post post Dunnett test. Probability values <5% ($p < 0.05$) were considered significant. Statistical analysis was performed using the SPSS software package.
21. Pellow, S.; Chopin, P.; File, S.; Briley, M. *J. Neurosci. Methods* **1985**, *14*, 149.
22. Sienkiewicz-Jarosz, E.; Szyndler, J.; Członkowska, A. I.; Atkowski, M. S.; Maciejak, P.; Wisłowska, A.; Zienowicz, M.; Lehner, M.; Turzynska, D.; Bidzinski, A.; Płaznik, A. *Behav. Brain Res.* **2003**, *145*, 17.