Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 4169–4173

# Design, microwave-assisted synthesis, and spasmolytic activity of 2-(alkyloxyaryl)-1*H*-benzimidazole derivatives as constrained stilbene bioisosteres☆

Gabriel Navarrete-Vázquez,[a,*] Hermenegilda Moreno-Diaz,[a] Francisco Aguirre-Crespo,[a] Ismael León-Rivera,[b] Rafael Villalobos-Molina,[c] Omar Muñoz-Muñiz[d] and Samuel Estrada-Soto[a]

[a]*Facultad de Farmacia, Universidad Autónoma del Estado de Morelos, Cuernavaca, Mor. 62210, México*
[b]*Centro de Investigaciones Químicas, Universidad Autónoma del Estado de Morelos, Cuernavaca, Mor. 62210, México*
[c]*Unidad de Biomedicina, FES Iztacala, Universidad Nacional Autónoma de México, Tlalnepantla 54090, México*
[d]*Unidad de Servicios de Apoyo en Resolución Analítica, Universidad Veracruzana, Xalapa, Ver. 91190, México*

Received 7 April 2006; revised 23 May 2006; accepted 30 May 2006
Available online 19 June 2006

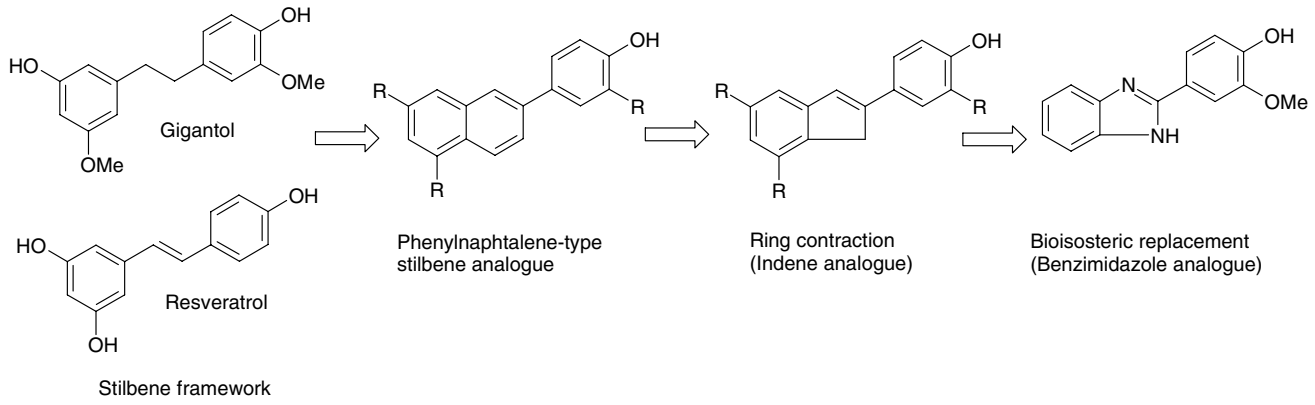
**Abstract**—A simple, fast, and efficient method for the preparation of several 2-(alkyloxyaryl)-1*H*-benzimidazole derivatives is reported. Compounds were synthesized through a rapid one-pot three component reaction via microwave irradiation, starting from commercially available aldehydes and *o*-phenylenediamine, in the presence of $Na_2S_2O_5$ and solvent-free conditions. The design of these compounds explore the hypothesis that the stilbene framework could be mimicked with an appropriate 2-(Alkyloxyphenyl)benzimidazole scaffold. This framework has a similar structural motif as the 6-phenylnaphthalene and behaves like stilbene bioisosteres. The spasmolytic activity of these compounds was recorded using isolated rat ileum test. Compound **12** was the most active of the series, showing an $IC_{50}$ of 1.19 μM.


In an attempt to identify novel compounds to treat disorders of smooth muscle function, our group initially focused on the modestly selective structure of stilbene and bibenzyl (Scheme 1) as starting points. Stilbenoids are compounds that show a variety of biological activities such as antineoplasic and vasorelaxing properties (e.g., resveratrol).[1–4] Moreover, gigantol (Scheme 1) also belongs to this class of natural products and it induces a concentration-dependent inhibition of the spontaneous contractions of the rat ileum with potency higher than or comparable to that of papaverine.[5] Our initial efforts focused on whether the stilbene framework could be mimicked with an appropriately substituted 2-(alkyloxyaryl) benzimidazole scaffold (Scheme 1). Cyclizing open structures or creating an additional ring system in a given structure represents one of the useful methods in the search for biologically active conformation. The end result is a more constrained molecule, with an imposed conformation. It is well known that the benzimidazole pharmacophore is an important structural core in medicinal chemistry that shows a broad spectrum of pharmacological activities.[6–8] Several compounds containing the benzimidazole scaffold have been used as antiparasitic,[9] antimicrobial,[10] antitumoral,[11] antihistaminic,[12] antifungal,[13] and vasorelaxant agents.[14]

Usually, 2-arylbenzimidazoles have been prepared by classical cyclocondensation of *o*-phenylenediamines with the corresponding carboxylic acids under harsh dehydrating reaction conditions[15,16] or aldehydes under oxidative conditions.[17] The condensation of *o*-phenylenediamines and the aldehydes requires an oxidative reagent to generate the benzimidazole core. Various reagents such as nitrobenzene,[18] benzoquinone,[19] sodium metabisulfite,[10,17,20] $In(OTf)_3$,[21] $I_2$/KI,[22] and even air[23] have been employed for this purpose. Due to the availability of commercial aldehydes, this method has

*Keywords*: Benzimidazole; Spasmolytic; Bioisosteric replacement; Stilbenoids.
☆ Taken in part from the MPharm Thesis of Hermenegilda Moreno-Diaz.
* Corresponding author. Tel./fax: +52 777 3297089; e-mail: gabriel_navarrete@uaem.mx

0960-894X/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2006.05.082




**Scheme 1.** Design and scaffold evolution of 2-(alkyloxyaryl)-1*H*-benzimidazole derivatives as constrained stilbene bioisosteres.

been chosen as a general procedure for their preparation. However, in most of the cases the reaction requires at least 4–48 h, giving yields between 30 and 75%.

Microwave as heating source has been used for the rapid synthesis of a variety of heterocyclic compounds both in solution phase as well as under solvent-free conditions.[24] Using microwave irradiation, the rates of reactions involving polar components are usually very fast. Reactions that require hours or even days by conventional heating may often be accomplished in seconds by microwave heating,[25] and that is the reason why this technology is widely applied to drug discovery.

Taking this into consideration, we plan to apply this methodology in our research projects aimed at the discovery of new spasmolytic agents based on benzimidazole scaffold. As a part of our search for basic information about the structural requirements for smooth muscle relaxant activity, we have synthesized a series of eighteen 2-(alkyloxyaryl)-1*H*-benzimidazole derivatives related to the natural stilbenoid family, reported in Table 1. The in vitro spasmolytic activity of these compounds on the spontaneous contractions of the rat ileum is also reported. A preliminary study concerning the structure–activity relationships in these compounds was also performed.

The design of these benzimidazole derivatives explores the hypothesis that the stilbene framework could be mimicked with an appropriate 6-phenylnaphthalene scaffold. The toxicity related to the naphthalene core caused us to design a constrained indene ring. However, the synthetic inaccessibility of certain functional groups prompted us to investigate the 2-arylbenzimidazole scaffold (Scheme 1), which has a similar structural motif as the 6-phenylnaphthalene. The facile assembly of benzimidazole core allows us to further explore the effects of substitution at positions 2, 3, 4, and 5 of the 2-phenyl ring with oxygenated radicals.

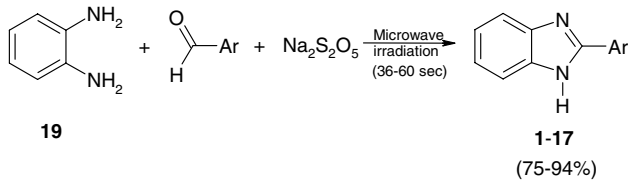
Restriction of conformation is the most commonly used technique in SAR development.[26] The conformational restrictions of a lead structure are often associated with decreased entropy and increased binding affinity and specificity with targets if the elements are oriented toward the correct binding pockets of the targets. Additionally, by systematically reducing the rotatable bonds in the lead compounds, the resulting compounds may have better chance to have oral bioavailability. The concept of transforming a chain structure into a heterocyclic ring moiety is another well-explored method of bioisosteric replacement. Applying a chain-to-ring transformation usually induces some conformational restriction in the lead compound, keeping key binding functionalities in a fixed position. This may improve the selectivity of the lead compound, as a flexible molecule may interact with several closely related molecular targets.[27]

In this study, 17 benzimidazole derivatives (**1**–**17**, Table 1) have been synthesized by the reaction of 1,2-phenylenediamine with adequate aromatic substituted aldehydes, utilizing sodium metabisulfite under microwave irradiation (Scheme 2).[28] All reactions were performed without solvent in only 60 s as a maximum time, confirming that the focused microwave irradiation is a very effective technique for accelerating thermal organic reactions in solvent-free conditions. Solid compounds were purified by recrystallization and the structure of the pure compounds was established by spectroscopic and spectrometric data.[29] All prepared compounds showed blue emissions under UV irradiation in methanol solutions. The reaction between *o*-phenylenediamine and the corresponding aromatic aldehyde was carried out in 36–60 s under microwave irradiation and afforded the corresponding products **1**–**17** in good yields (Table 1). After the first irradiation for 10 s, the reaction mixture was taken out, mixed again, and then heated at the same power level for an additional 10 s. This step was repeated until the starting materials were consumed, monitored by TLC analysis. Compound **18** was obtained with low yield from **1**, using an excess of alkylating agent iodoethane (Scheme 3). When microwave irradiation time was extended, it was possible to observe a decrease of the yield due to formation of several by-products. For comparison, a classical method for the preparation of the benzimidazoles **1**–**17** was also done by refluxing the adequate *o*-phenylenediamine, the aldehyde and sodium metabisulfite in DMF for 3–4.5 h. It was demonstrated that the classical heating afforded low yields of almost all compounds, in addition to other

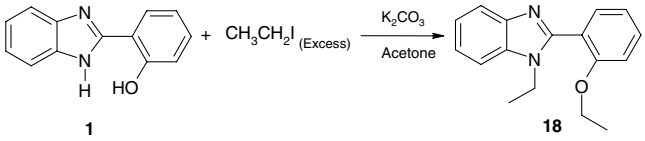
**Table 1.** Physicochemical properties and pharmacological activity of synthesized 2-(alkyloxyaryl)-1*H*-benzimidazole derivatives **1**–**17**

| Compound | $R_1$ | Ar | Mp (°C) | *C* log *P* | Reaction time | Yield[a] (%) | Spasmolytic activity | | Potency index[b] |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | $IC_{50}$ (μM) | $E_{max}$ (%) | |
| **1** | –H | 2-Hydroxyphenyl | 242.1–243.5 | 3.97 ± 0.34 | 36 s | 81 | 5.15 ± 0.58 | 42.4 ± 1.93 | 1.13 |
| **2** | –H | 2-Methoxyphenyl | 171.7–173.9 | 3.43 ± 0.30 | 49 s | 89 | 7.7 ± 1.17 | 52.8 ± 1.8 | 0.75 |
| **3** | –H | 2-Ethoxyphenyl | 149.4–150.3 | 3.97 ± 0.30 | 48 s | 88 | 1.94 ± 0.20 | 50 ± 2.46 | 3.0 |
| **4** | –H | 2-Propoxyphenyl | 219.6–220.2 | 4.50 ± 0.30 | 50 s | 87 | 1.9 ± 0.20 | 80 ± 7.11 | 3.06 |
| **5** | –H | 2-(Benzyloxy)phenyl | 141.2–143.3 | 5.09 ± 0.32 | 47 s | 75 | 28.2 ± 9.4 | 51.4 ± 2.03 | 0.21 |
| **6** | –H | 2-(4-Chlorobenzyloxy)phenyl | 194.0–196.0 | 5.68 ± 0.34 | 48 s | 78 | 59 ± 9.14 | 38.2 ± 2.18 | 0.09 |
| **7** | –H | 2-(4-Methylbenzyloxy)phenyl | 229.2–231.1 | 5.55 ± 0.32 | 56 s | 68 | 15 ± 0.29 | 47.7 ± 0.98 | 0.38 |
| **8** | –H | 2-Nitrophenyl | 168.0–170.0 | 3.20 ± 0.30 | 48 s | 88 | 78.6 ± 0.16 | 11.1 ± 0.99 | 0.07 |
| **9** | –H | 4-Hydroxyphenyl | 254.1–256.6 | 3.34 ± 0.29 | 50 s | 92 | 5.25 ± 0.39 | 61.2 ± 1.32 | 1.11 |
| **10** | –H | 4-Methoxyphenyl | 229.9–231.4 | 3.87 ± 0.30 | 60 s | 90 | 10.6 ± 0.22 | 54.9 ± 3.06 | 0.55 |
| **11** | –H | 4-(*N*,*N*-Dimethylamino)phenyl | 294.2–296.3 | 4.12 ± 0.31 | 60 s | 87 | 30 ± 5.6 | 34.9 ± 5.28 | 0.19 |
| **12** | –H | 4-Hydroxy-3-methoxyphenyl | 224.7–225.4 | 3.29 ± 0.33 | 60 s | 83 | 1.19 ± 0.20 | 70 ± 1.66 | 4.90 |
| **13** | –H | 3,4-(Dimethoxy)phenyl | 235.5–236.7 | 3.94 ± 0.33 | 60 s | 85 | 5.6 ± 0.32 | 55 ± 3.33 | 1.04 |
| **14** | –H | 2,3,4-(Trimethoxy)phenyl | 259.9–262.1 | 3.89 ± 0.39 | 50 s | 94 | 1.8 ± 0.39 | 65 ± 5.89 | 3.23 |
| **15** | –H | 2,4,5-(Trimethoxy)phenyl | 258.7–259.7 | 3.64 ± 0.39 | 40 s | 86 | 4.0 ± 0.86 | 90 ± 1.45 | 1.45 |
| **16** | –H | 3,4-(Methylenedioxy)phenyl | 251.6–253.0 | 3.12 ± 0.37 | 40 s | 88 | 3.4 ± 0.83 | 59 ± 3.34 | 1.71 |
| **17** | –H | 4-Pyridyl | 153.0–155.0 | 2.44 ± 0.29 | 50 s | 75 | 190 ± 19.8 | 50 ± 1.94 | 0.03 |
| **18** | $CH_2CH_3$ | 2-Ethoxyphenyl | 232.1–233.0 | 4.68 ± 0.59 | 8 h | 46 | 1.72 ± 0.23 | 60 ± 1.23 | 3.39 |
| Gigantol | — | — | — | 2.96 ± 0.25 | — | — | 5.83 ± 0.55 | 93.5 ± 3.02 | 1 |
| Papaverine | — | — | — | 3.42 ± 1.03 | — | — | 1.55 ± 0.12 | 96.7 ± 5.02 | 3.76 |

[a] Isolated yield, characterized by HR-MS, $^1$H NMR, $^{13}$C NMR.
[b] Potency related to gigantol.




**Scheme 2.** Preparation of 2-(alkyloxyaryl)-1*H*-benzimidazole derivatives **1**–**17**.




**Scheme 3.** Synthesis of 2-(2-ethoxyphenyl)-1-ethyl-1*H*-benzimidazole (**18**).

by-products, in which separation was difficult compared with products obtained by microwave heating. The reaction was found to proceed smoothly under microwave irradiation within 36–60 s. whereas under reflux conditions in 3–4.5 h. In previous reports[10,16] benzimidazole derivatives were obtained by the reflux of 1,2-phenylenediamines with the $Na_2S_2O_5$ adduct of appropriated aldehyde, which was previously prepared and kept in a refrigerator for several hours. The most important result of our approach is the optimization of yields and reaction times using microwave irradiation.

The isolated rat ileum test[30] allowed us to evaluate the spasmolytic activity of all the synthesized compounds (Table 1). Pharmacological assay results indicate that compounds **3**, **4**, **12**, **14**, and **18** produced a significant antispasmodic effect in a concentration-dependent manner. These compounds were three to five times more active than gigantol. In order to establish a preliminary structure–activity relationship, the contribution of oxygenated radical substituting the C-2 (compounds **3**, **4**, and **18**) or C-4 (compounds **12**, **14**, and **16**) of the phenyl ring was analyzed for their selective and potent relaxant smooth muscle activity. On the other hand, the presence of the $-N(CH_3)_2$ and $-NO_2$ groups in C-4 or C-2 (compounds **8** and **11**, respectively) and those bearing a hindered substituent (**5**–**7**) proved detrimental to spasmolytic activity. Finally, the absence of oxygenated substituent groups, as in compound **17**, with a pyridyl instead of alkyloxyphenyl substituent, did not show activity. This suggests that bioactivity of compounds depends on the presence of oxygenated radicals attached in positions 2 and/or 4 of the phenyl ring. Further experiments are in progress to determine the mechanisms underlying spasmolytic activity of these compounds.

In conclusion, we have developed a simple, rapid, and efficient method for the preparation of 2-(alkyloxyaryl)-1*H*-benzimidazole compounds under solvent-free conditions using readily available and inexpensive reagents utilizing microwave irradiation. These compounds behave like stilbene bioisosteres, retaining spasmolytic activity, and could be considered leads for the development of new therapeutic agents, including drugs to treat disorders of smooth muscle function.

## Acknowledgments

This work was supported in part by grants from PROMEP-SEP, UAEMOR-PTC-131 (GNV), and CONACyT, 43440 (SES). We are grateful to Gumersindo Mirón-López from CIQ, UAEM, for the determination of all

spectra. Hermenegilda Moreno-Diaz acknowledges the fellowship awarded by CONACyT to carry out graduate studies.

## References and notes


1. Amorati, R.; Lucarini, M.; Mugnaini, V.; Pedulli, G. F.; Roberti, M.; Pizzirani, D. *J. Org. Chem.* **2004**, *69*, 7101.
2. El-Mowafy, A. M. *Biochem. Biophys. Res. Commun.* **2002**, *291*, 1218.
3. Estrada-Soto, S.; López-Guerrero, J. J.; Villalobos-Molina, R.; Mata, R. *Fitoterapia* **2006**, *77*, 236.
4. Orallo, F.; Álvarez, E.; Camina, M.; Leiro, J. M.; Gómez, E.; Fernández, P. *Mol. Pharmacol.* **2002**, *61*, 294.
5. Hernández-Roméro, Y.; Rojas, J. I.; Castillo, R.; Rojas, A.; Mata, R. *J. Nat. Prod.* **2004**, *67*, 160.
6. Bali, A.; Bansal, Y.; Sugumaran, M.; Singh-Saggu, J.; Balakumar, P.; Kaur, G.; Bansal, G.; Sharma, A.; Singh, M. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3962.
7. Cheng, J.; Xie, J.; Luo, X. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 267.
8. Ueno, H.; Katoh, S.; Yokota, K.; Hoshi, J.; Hayashi, M.; Uchida, I.; Aisaka, K.; Hase, Y.; Cho, H. *Bioorg. Med. Chem. Lett.* **2004**, *15*, 4281.
9. Navarrete-Vázquez, G.; Yepez-Mulia, L.; Hernández-Campos, A.; Tapia, A.; Hernández-Luis, F.; Cedillo, R.; González, J.; Martínez-Fernández, M.; Martínez-Grueiro, M.; Castillo, R. *Bioorg. Med. Chem.* **2003**, *11*, 4615.
10. Özden, S.; Atabey, D.; Yıldız, S.; Göker, H. *Bioorg. Med. Chem.* **2005**, *13*, 1587.
11. Andrzejewska, M.; Yépez-Mulia, L.; Cedillo-Rivera, R.; Tapia, A.; Vilpo, L.; Vilpo, J.; Kazimierczuk, Z. *Eur. J. Med. Chem.* **2002**, *37*, 973.
12. Terzioglu, N.; van Rijn, R.; Bakker, R. A.; De Esch, I. J. P.; Leurs, R. . *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5251.
13. Küçükbay, H.; Durmaz, R.; Orhan, E.; Günal, S. *Farmaco* **2003**, *58*, 431.
14. Estrada-Soto, S.; Villalobos-Molina, R.; Aguirre-Crespo F.; Vergara-Galicia, J.; Moreno-Díaz, H.; Torres-Piedra, M.; Navarrete-Vázquez, G. *Life Sci.*, in press. doi:10.1016/j.lfs.2006.01.019.
15. Navarrete-Vázquez, G.; Cedillo, R.; Hernández-Campos, A.; Yepez-Mulia, L.; Hernández-Luís, F.; Valdez, J.; Morales, R.; Cortes, R.; Hernandez, M.; Castillo, R. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 187.
16. Thompson, L. K.; Ramaswamy, B. S.; Seymour, E. A. *Can. J. Chem.* **1977**, *55*, 878.
17. Göker, H.; Ku, C.; Boykin, D. W.; Yildiz, S.; Altanar, N. *Bioorg. Med. Chem.* **2002**, *10*, 2589.
18. Ben-Alloum, A.; Bakkas, S.; Soufiaoui, M. *Tetrahedron Lett.* **1998**, *39*, 4481.
19. Verner, E.; Katz, B. A.; Spencer, J. R.; Allen, D.; Hataye, J.; Hruzewicz, W.; Hui, H. C.; Kolesnikov, A.; Li, Y.; Luong, C.; Martelli, A.; Radika, K.; Rai, R.; She, M.; Shrader, W.; Sprengeler, P. A.; Trapp, S.; Wang, J.; Young, W. B.; Mackman, R. L. *J. Med. Chem.* **2001**, *44*, 2753.
20. Lombardy, R. L.; Tanious, F. A.; Ramachandran, K.; Tidwell, R. R.; Wilson, W. D. *J. Med. Chem.* **1996**, *39*, 1452.
21. Trivedi, R.; De, S. K.; Gibbs, R. A. *J. Mol. Catal. A: Chem.* **2006**, *245*, 8.
22. Gogoi, P.; Konwar, D. . *Tetrahedron Lett.* **2006**, *47*, 79.
23. Lin, S.; Yang, L. *Tetrahedron Lett.* **2005**, *46*, 4315.
24. Mavandadi, F.; Lidström, P. *Curr. Top. Med. Chem.* **2004**, *4*, 773.
25. (a) Yu, H.; Kawanishi, H.; Koshima, H. *Heterocycles* **2003**, *60*, 1457; (b) Su, Y. S.; Lin, M. J.; Sun, M. C. *Tetrahedron Lett.* **2005**, *46*, 177; (c) Boufatah, N.; Gellis, A.; Maldonado, J.; Vanelle, P. *Tetrahedron* **2004**, *60*, 9131; (d) Bendale, P. M.; Sun, C. M. *J. Comb. Chem.* **2002**, *4*, 359.
26. Chen, X.; Wang, W. *Annu. Rep. Med. Chem.* **2003**, *38*, 333.
27. Olesen, P. H. *Curr. Opin. Drug Discovery Dev.* **2001**, *4*, 4711.
28. General method of synthesis of 2-(Alkyloxyaryl)-1*H*-benzimidazoles **1**–**17**. *Microwave irradiation conditions:* A mixture of 1,2-phenylenediamine (0.0313 mol), 1.01 equivalents of appropriate aldehyde, and 1.01 equivalents of sodium metabisulfite was mixed and introduced in an open Erlenmeyer flask. The mixture was irradiated in a household microwave oven (Samsung MW1446WC, 1000 W) for 24–60 s. After irradiation, the mixture was poured onto cold water. The precipitate was collected by filtration, washed with water, dried, and recrystallized. *Classical conditions:* A mixture of 1,2-phenylenediamine (0.0313 mol), 1.01 equivalents of appropriate aldehyde, and 1.01 equivalents of sodium metabisulfite in 10 mL DMF was heated to reflux for 3–4.5 h. After cooling, water (20 mL) was added and the mixture was extracted with AcOEt ($3 \times 15$ mL). The organic layer was dried over magnesium sulfate and removed under vacuum. Purification was done by chromatography on silica gel eluting with chloroform and recrystallization from adequate solvent.
29. *Selected data for compounds*. 2-(1*H*-Benzimidazol-2-yl)phenol (**1**): White solid, mp 242.1–243.5 °C (methanol). $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 6.73 (dd, 1H, H-3′, $J$ = 7.91, $J$ = 1.3 Hz), 7.11 (td, 1H, H-5′, $J$ = 7.7, $J$ = 7.7, $J$ = 1.3 Hz), 7.18 (td, 1H, H-4′, $J$ = 7.7, $J$ = 7.9, $J$ = 1.7 Hz), 7.21–7.26 (m, 2H, H-5, H-6, $J$ = 8.5, $J$ = 1.3 Hz), 7.59–7.63 (m, 2H, H-4, H-7, $J$ = 8.5, $J$ = 6.8, $J$ = 1.4 Hz), 7.76 (dd, 1H, H-6′, $J$ = 7.7, $J$ = 1.7 Hz), 11.76 (br s, 2H, N–H, O–H) ppm; $^{13}$C NMR (75.5 MHz, DMSO-$d_6$) $\delta$ 113.93 (C-1), 116.05 (C-4, C-7), 117.56 (C-3′), 120.65 (C-5′), 123.48 (C-5, C-6), 128.77 (C-6′), 132.72 (C-4′), 141.11 (C-3a, C-7a), 156.39 (C-2), 157.60 (C-2′) ppm; EIMS: *m*/*z* (% rel. int.) 210 ($M^+$, 100), 192 (2), 181 (25); HR-MS: calcd for $C_{13}H_{10}N_2O$: 210.0793. Found: 210.0795. 2-(4-Methoxyphenyl)-1*H*-benzimidazole (**10**): White solid, mp 229.9–231.4 °C (ethanol). $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 3.82 (s, 3H, $CH_3$–O–), 7.05–7.09 (m, 2H, H-3′, H-5′, $J$ = 8.5, $J$ = 2.2 Hz), 7.21–7.25 (m, 2H, H-5, H-6, $J$ = 8.5, $J$ = 7.0, $J$ = 1.4 Hz), 7.57–7.61 (m, 2H, H-4, H-7, $J$ = 8.5, $J$ = 1.4 Hz), 8.03–8.06 (m, 2H, H-2′, H-6′, $J$ = 8.5, $J$ = 1.4 Hz), 10.88 (br s, 1H, N–H) ppm; $^{13}$C NMR (75.5 MHz, DMSO-$d_6$) $\delta$ 55.43 ($CH_3$–O–), 114.59 (C-3′, C-5′), 116.08 (C-4, C-7), 123.48 (C-5, C-6), 125.28 (C-2′, C-6′), 125.22 (C-1′), 139.85 (C-3a, C-7a), 152.12 (C-2), 161.06 (C-4′) ppm; EIMS: *m*/*z* (% rel. int.) 224 ($M^+$, 100), 209 (35), 181 (25); HR-MS: calcd for $C_{14}H_{12}N_2O$: 224.0949. Found: 224.0952. 4-(1*H*-Benzimidazol-2-yl)-2-methoxyphenol (**12**): pale yellow solid, mp 224.7–225.4 °C (methanol). $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 3.97 (s, 3H, $CH_3$O–), 6.91(d, 1H, H-5′, $J$ = 8.4 Hz), 7.11–7.16 (m, 2H, H-5, H-6, $J$ = 9.6, $J$ = 3 Hz), 7.53 (br s, 1H, H-6′, $J$ = 0.9 Hz), 7.61 (dd, 2H, H-4, H-7, $J$ = 8.1, $J$ = 1.8 Hz), 7.74 (d, 1H, H-2, $J$ = 1.5 Hz), 9.57 (s, 2H, N–H, O–H) ppm; $^{13}$C NMR (75.5 MHz, DMSO-$d_6$) $\delta$ 55.67 ($CH_3$), 110.24 (C-2′), 116.07 (C-4, C-7), 117.28 (C-5′),123.08 (C-1′), 123.48 (C-5, C-6), 124.72 (C-6′), 139.73 (C-3a, C-7a), 146.81 (C-4′), 150.22 (C-3′), 151.67 (C-2) ppm; EIMS: *m*/*z* (% rel. int.) 240 ($M^+$, 100), 225 (20), 210 (60), 197 (25); HR-MS: calcd for $C_{14}H_{12}N_2O_2$: 240.0898. Found: 240.0900.
30. Rat ileum test. Determination of spasmolytic activity: male Wistar rats (200–250 g) were used. The animals were

killed by ether exposure. The ileum was dissected and placed in Krebs solution, pH 7.4, with the following composition (in mM): NaCl, 118; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$, 1.2 mM; $KH_2PO_4$, 1.2; $NaHCO_3$, 25.0; EDTA, 0.026 and glucose, 11.1. Ileum strips (1–1.5 cm long) were dissected and mounted in organ baths containing Krebs solution gassed with a mixture of $O_2/CO_2$ (19:1) and under a constant tension of 1 g. After 30 min stabilization time, a 10 min control period was recorded. The test compounds dissolved in DMSO were added to the bath in a volume of 100 μL at different concentrations. Cumulative concentration–response curves were obtained for each ileum, from 0.01 to 500 μM. The effect of the compounds and positive control was determined by comparing the muscular tone, inscribed by the frequency and amplitude of the ileum contractions before and after the application of the test materials. Muscular tone was calculated from the tracings, using Acknowledge software (BIOPAC® Systems Inc.). All the results are expressed as means of six experiments ± SEM. Concentration–response curves (CRC) for compounds were plotted and the experimental data from the CRC were adjusted by the nonlinear, curve fitting program (ORIGIN® 6.0). The statistical significance ($p < 0.05$) of differences between means was assessed by an analysis of variance (ANOVA).