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Article

Antifungal Activity of Natural and Synthetic Amides from *Piper* species

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Extrato de folhas de *Piper scutifolium* apresentou atividade antifúngica significativa contra *Cladosporium cladosporioides* e *C. sphaerospermum* e seus principais componentes ativos, piperina, piperlonguminina e corcovadina foram isolados por meio de purificação biomonitorada, apresentando limites de detecção de 1 μg. Foi realizado um estudo da relação estrutura-atividade baseado na síntese de doze análogos com variações no número de insaturações, no padrão de substituição no anel aromático e no grupo amídico. As amidas sem substituintes no anel aromático e com apenas uma ligação dupla foram as mais ativas e os derivados *N,N*-dietil-substituídos apresentam maior dose-dependência.

The antifungal leaves extract from *Piper scutifolium* was submitted to bioactivity-guided chromatographic separation against *Cladosporium cladosporioides* and *C. sphaerospermum* yielding piperine, piperlonguminine and corcovadine as the active principles which displayed a detection limit of 1 μg. Structure-activity relationships were investigated with the preparation of twelve analogs having differences in the number of unsaturations, aromatic ring substituents and in the amide moiety. Analogs having a single double-bond and no substituent in the aromatic ring displayed higher activity, while *N,N*,-diethyl analogs displayed higher dose-dependent activity.

Keywords: *Piper*, antifungal, analogs, amides

Introduction

Piper (Piperaceae) are commonly herbs, shrubs or infrequently trees with over 1000 species described so far mostly on tropical regions.[1] In addition to the high economical value of black pepper (*P. nigrum*) as a spice, there are several medicinal uses described for different *Piper* species, including anti-inflammatory, analgesic and treatment of snake-bite.[2,3] Several *Piper* species have been phytochemicaly investigated[4-6] and a plethora of secondary metabolites has been found including kavalactones,[7,8] lignoids,[9-11] chromenes,[12,13] terpenes,[14,15] prenylated benzoic acids[13,16,17] and also amides as the most characteristic classes of compounds.[18,19]

The most prominent example of a *Piper* amide is piperine, the pungent principle of black pepper (*P. nigrum*). It was the first natural product isolated from *Piper* species back in 1819. Piperine and several other amides have shown a variety of biological activity, *e.g.*, antitumoral,[20-22] efflux-pump inhibitor,[23-25] insecticidal[19,26-36] and antifungal activity.[37-39] The importance of insecticidal activity of *Piper* amides is considerable and thus several studies have been addressed to investigate possible structure-activity relationships. For instance, isobutylamides have shown high activity against different insects including *Musca domestica*,[40] *Aedes aegypti*[36] and *A. togoi*[41] and compounds bearing methylenedioxyphenyl substituents can act in some cases as synergists for other compounds by interfering with cytochrome P450-mediated detoxifications of insecticides.[34,42,43] In spite of the occurrence of several antifungal amides in plants,[37,38,44,45] the data concerning the mechanism of action for such compounds is scarce and thus a comprehensive structure-activity relationship studies could not be performed so far.

In the course of bioprospecting studies for antifungal compounds from Piperaceae species,[12,37,46-48] leaf extracts from *P. scutifolium* displayed significant activity against the phytopathogenic fungi *Cladosporium cladosporioides* and *C. sphaerospermum*. The dereplication of extracts by

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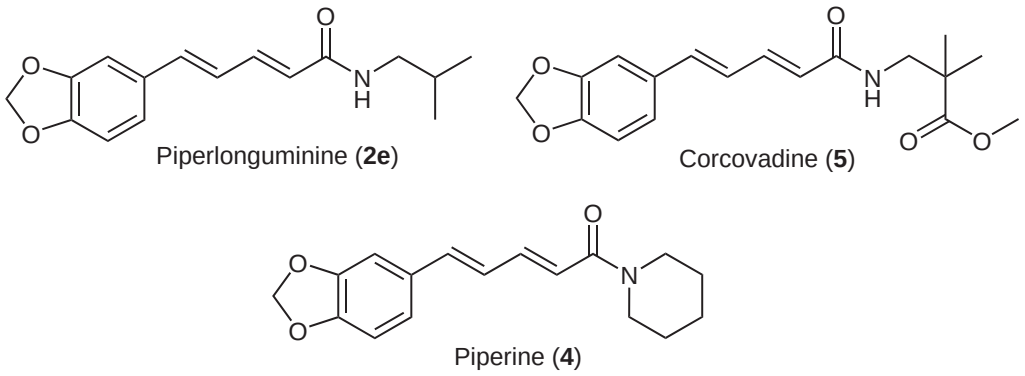


Figure 1. Natural amides.

a combined chromatographic and bioautographic method yielded the amides piperine (**4**), piperlonguminine (**2e**) and corcovadine (**5**) (Figure 1) as the major bioactive compounds. Since the detection limit of 1 μg was comparable to that of positive controls miconazole and nystatin, several analogs were synthesized in order to evaluate preliminary structure-activity relationships.

Experimental

General experimental procedures

UV spectra were recorded in a UV/Visible Shimadzu UV-1601PC spectrophotometer using $CHCl_3$ as solvent. IR spectra were obtained in a Perkin-Elmer model 1750 spectrometer. ^{1}H NMR spectra were recorded at 200 and 300 MHz and ^{13}C NMR at 50 and 75 MHz in Bruker DPX-200 and DRX-300 spectrometers, respectively. $CDCl_3$ (Aldrich) was used as solvent and TMS (Aldrich) as internal standard. Chemical shifts are reported in δ units (ppm) and coupling constants (*J*) in Hz. GCLREIMS were measured in a Shimadzu GC-17A chromatograph interfaced with a MS-QP-5050A mass spectrometer. Temperature programming was performed from 150 to 300 °C at 15 °C min^{-1}, then isothermal at 300 °C for 5 min. The injector and detector temperatures were 150 and 320 °C, respectively, and helium was used as a carrier gas. Analytical HPLC was performed using a Dionex C18 (150 × 5 mm i.d. × 3 μm) column with UVD-DAD 340U as a detector. Silica gel (Merck, 230-400 mesh) and Sephadex LH-20 (Amersham Biosciences) were used for column chromatographic separation, while silica gel 60 PF_{254} (Merck) was used for analytical (0.25 mm) and preparative TLC (1.0 mm).

Plant material

P. scutifolium Jack. leaves were collected in Ubatuba, SP, Brazil, in September 2002 and identified by Dr. Elsie Franklin Guimarães (Instituto de Pesquisas Jardim Botânico do Rio de Janeiro). Voucher specimen (Kato-281) was deposited at the Herbarium of the Jardim Botânico do Rio de Janeiro, RJ, Brazil. Dried fruits of *P. nigrum* were purchased in the local market.

Extraction and isolation

Dried and powdered leaves of *P. scutifolium* (10 g) were exhaustively extracted with CH_2Cl_2. Crude extract was fractionated through successive chromatography as previously described to yield piperlonguminine (4 mg, mp 157-160 °C) and corcovadine (10 mg, mp 143-145 °C).[47] To obtain piperine, dried fruits of *P. nigrum* (1 kg) were crushed, extracted with ethanol and yielded a crystaline solid after concentration under vacuum. Successive recrystallization in EtOH yielded pure piperine (200 mg, mp 129-131 °C) which was identified based on comparison of ^{1}H and ^{13}C NMR data with those reported.[49]

Synthetic procedures

Compounds **1c**, **1d**, **2c**, **2d** e **3c** were synthesized using Wadsworth-Emmons according to Strunz and Finlay's method.[50] The starting aldehydes reacted with triethyl-phosphonocrotonate yielding ethyl 5-phenylpentadienoates. The ester products were then hydrolysed without further purification to the corresponding carboxylic acid, (2*E*,4*E*)-5-phenylpenta-2,4-dienoic acid (**6**) and (2*E*,4*E*)-5-(3-methoxyphenyl)penta-2,4-dienoic acid (**7**). Their corresponding chlorides obtained by reaction with oxalyl chloride reacted with amines yielding the desired amides with an overall yield of *ca*. 80%. Compounds **1d** and **2d** were synthesized according to de Paula and co-workers,[32] with piperic acid obtained from piperine hydrolysis which was then converted into the desired amides similarly as above described. Amides **1a**, **2a** and **3c** were prepared

using commercial cinnamic acid. Amides **1b** and **3b** were synthesized from commercial benzo[d][1,3]dioxole-5-carbaldehyde (**8**) which was converted into (*E*)-3-(benzo[d][1,3]dioxol-5-yl) acrylic acid (**9**), yielding the amides as above described.[51]

Spectroscopic data of **1a**, **1e**, **2a**, **3e**, **6** and **9** were in accordance with those published.[32,51-54] Compounds **1d** and **3c** were not previously described.

*(E)-3-(Benzo[d][1,3]dioxol-5-yl)-N,N-diethylacrylamide (**1b**)*[55]

Yield 34%, colorless oil; IR ν_{max}/cm^{-1} 3457, 2932, 2901, 1646, 1598, 1503, 1491, 1038, 929, 811, 523 (KBr); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.18 (t, 3H, *J* 7.0 Hz, H2"a), 1.25 (t, 3H, *J* 7.0 Hz, H2"b), 3.40-3.50 (m, 4H, H1"a and H1"b), 5.98 (s, 2H, OCH_2O), 6.65 (d, 1H, *J* 15.4 Hz, H2), 6.79 (d, 1H , *J* 8.0 Hz, H6'), 7.00 (d, 1H, *J* 8.0 Hz, H5'), 7.03 (s, 1H, H2'), 7.62 (d, 1H, *J* 15.4 Hz, H3); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 13.1 (C2"a), 14.9 (C2"b), 40.9 (C1"a), 42.0 (C1"b), 101.3 (OCH_2O), 106.2 (C2'), 108.3 (C5'), 115.6 (C2), 123.5 (C6'), 129.7 (C1'), 141.8 (C3), 148.0 (C4'), 148.7 (C3'), 165.6 (C=O); EIMS *m/z* 247 (M^+, 24%), 176 (18), 175 (75), 145 (56), 117 (35), 89 (100), 87 (11), 72 (28), 65 (14), 63 (58), 62 (22), 44 (26), 42 (36), 39 (33).

*(2E,4E)-N,N-Diethyl-5-phenylpenta-2,4-dienamide (**1c**)*[56]

Yield 57%, colorless oil; IR ν_{max}/cm^{-1} 3349, 2976, 1711, 1634, 1450, 1268, 1216, 1127 (KBr); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.17 (t, 3H, *J* 6.9 Hz, H2"a), 1.23 (t, 3H, *J* 6.9 Hz, H2"b), 3.42 (q, 2H, *J* 6.9 Hz, H1"a), 3.47 (q, 2H, *J* 6.9 Hz, H1"b), 6.41 (d, 1H, *J* 14.5 Hz, H2), 6.80-7.00 (m, 2H, H4 and H4'), 7.24-7.56 (m, 6H, H2', H8, H5', H6', H5 and H3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 13.2(C2"a), 15.0 (C2"b), 41.0 (C1"a), 42.2 (C1"b), 121.3 (C2), 126.3 (C4), 127.0 (C2' and C6'),128.6 (C4'), 128.7 (C3' and C5'), 136.5 (C1'), 138.7 (C5), 142.4 (C3), 165.8 (C1); EIMS *m/z* 157 (12%), 129 (16), 128 (43), 127 (22), 102 (17), 77 (45), 72 (32), 56 (24), 51 (36), 42 (100), 39 (23).

*(2E,4E)-N,N-Diethyl-5-(3-methoxyphenyl)penta-2,4-dienamide (**1d**)*

Yield 72%, colorless oil; IR ν_{max}/cm^{-1} 3405, 2974, 2937, 2916, 1632, 1605, 1484, 1462, 1264, 1044, 784, 687 (KBr); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.13 (t, 3H, *J* 7.4 Hz, H2"a), 1.23 (t, 3H, *J* 7.4 Hz, H2"b), 3.40 (t, 2H, *J* 7.4 Hz, H1"a), 3.52 (t, 2H, *J* 7.4 Hz, H1"b), 3.74 (s, 3H, OCH_3), 6.41 (d, 1H, *J* 15.0 Hz, H2), 6.69-6.84 (m, 3H , H4', H5 and H4), 6.89 (s, 1H, H2'), 6.97 (d, 1H, *J* 7.9 Hz, H6'), 7.18 (t, 1H, *J* 7.9 Hz, H5'), 7.38 (dd, 1H, *J* 15.0 Hz and 9.1 Hz, H3); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 13.2 (C2"a), 14.9 (C2"b), 41.0 (C1"a), 42.2 (C1"b), 55.2 (OCH_3), 111.9 (C2'), 114.4 (C4'), 119.6 (C6'), 121.0 (C2), 127.2 (C5), 129.7 (C5'), 137.8 (C1'), 138.7 (C4), 142.4 (C3), 159.8 (C3'), 165.8 (C1); EIMS *m/z* 135 (5%), 100 (10), 72 (55), 57 (30), 55 (18), 44 (66), 43 (100), 42 (64), 41 (72).

*(2E,4E)-N-Isobutyl-5-phenylpenta-2,4-dienamide (**2c**)*[57]

Yield 79%, white crystals, mp 154.2-154.9 °C; IR ν_{max}/cm^{-1} 3292, 2961, 1646, 1612, 1546, 1446, 1346, 1160, 989, 508 (KBr); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.94 (d, 6H, *J* 6.8 Hz, H3"), 1.82 (hept, 1H, *J* 6.8 Hz, H2"), 3.19 (t, 2H, *J* 6.6 Hz, H1"), 5.71 (sl, 1H, NH), 5.98 (d, 1H, *J* 14.9 Hz, H2), 6.83-6.88 (m, 2H, H4 and H4'), 7.24-7.50 (m, 6H, H2', H3', H5', H6', H5 and H3); ^{13}C NMR ($CDCl_3$, 75 MHz,) δ 20.1 (C3"), 28.6 (C2"), 47.0 (C1"), 124.1 (C2), 126.3 (C4), 126.9 (C2' and C6'), 128.6 (C4'), 128.7 (C3' and C5'), 136.3 (C4'), 139.0 (C5), 140.8 (C3), 166.1 (C1); EIMS *m/z* 229 (M^+, 34%), 172 (13), 158 (12), 157 (100), 129 (35), 128 (98), 96 (42), 41 (24).

*(2E,4E)-N-Isobutyl-5-(3-methoxyphenyl)penta-2,4-dienamide (**2d**)*[58]

Yield 82%, white crystals, mp 99.5-101.0 °C, IR ν_{max}/cm^{-1} 3308, 2950, 1644, 1609, 1578, 1432, 1161, 1052, 990, 863, 572 (KBr); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.94 (d, 6H, *J* 6.6, H3"), 1.83 (m, 1H, *J* 6.6 Hz, H2"), 3.19 (t, 2H, *J* 6.6 Hz, H1"), 3.81 (s, 3H, OCH_3), 5.99 (d, 1H, *J* 14.5 Hz, H2), 6.75-6.90 (m, 3H , H4', H5 and H4), 6.95 (t, 1H, *J* 2.0 Hz, H2'), 7.03 (d, 1H, *J* 7.9 Hz, H6'), 7.24 (t, 1H, *J* 7.9 Hz, H5'), 7.53 (m, 1H, H3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 20.1 (C3"), 28.6 (C2"), 47.0 (C1"), 55.2 (OCH_3), 112.1 (C4'), 114.3 (C2'), 119.6 (C6'), 124.2 (C2), 126.6 (C4), 129.7 (C5'), 137.7 (C1'), 138.9 (C5), 140.7 (C3), 159.8 (C3'), 166.0 (C1); EIMS *m/z* 259 (M^+, 4%), 187 (35), 160 (11), 158 (71), 145 (12), 144 (52), 115 (88), 77 (11), 64 (10), 43 (100), 41 (90), 39 (41).

*N-Pentylcinnamamide (**3a**)*[59]

Yield 95%, white crystals, mp 87.6-88.2 °C; IR ν_{max}/cm^{-1} 3276, 2955, 2933, 1653, 1607, 1553, 1475, 764, 489 (KBr); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.86 (brm, 3H, H5"), 1.29 (brm, 4H, H3" and H4"), 1.59 (brm, 2H, H2"), 3.38 (q, 2H, *J* 6.6 Hz, H1"), 6.68 (d, 1H, *J* 15.8 Hz, H2), 7.20-7.35 (m, 4H , H3', H4', H5' and NH), 7.41-7.46 (m, 2H, H2' and H6'), 7.64 (d, 1H, *J* 15.8 Hz, H3); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 13.7 (C5"), 22.1 (C4"), 28.9 (C3"), 29.0 (C2"), 39.6 (C1"), 121.3 (C2), 127.4 (C2' and C4'), 128.4 (C3' and C5'), 129.1 (C4'), 134.7 (C1'), 139.9 (C3), 166.3 (C=O); EIMS *m/z* 217 (M^+, 4%), 160 (13), 146 (25), 131 (100), 103 (49), 102 (16), 84 (18), 77 (58), 51 (24), 41 (28), 39 (15).

*(E)-3-(Benzo[d][1,3]dioxol-5-yl)-N-pentylacrylamide (**3b**)*[55]

Yield 47%, orange crystals, mp 99.8-100.2 °C; IR ν_{max}/cm^{-1} 3298, 2934, 1655, 1621, 1504, 1491, 97, 927 (KBr); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.79 (brm, 3H, H5"), 1.24 (brm, 4H, H3" and H4"), 1.48 (brm, 2H, H2"), 3.28 (q, 2H, *J* 6.7 Hz, H1"), 5.86 (s, 2H, OCH_2O), 6.25 (d, 1H, *J* 15.4 Hz, H2), 6.36 (sl, 1H, NH), 6.66 (d, 1H, *J* 8.0 Hz, H6'), 6.85 (d, 1H, *J* 8.0 Hz, H5'), 6.88 (s, 1H, H2'), 7.43 (d, 1H, *J* 15.4 Hz, H3); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 13.8 (C5"), 22.3 (C4"), 29.0 (C3"), 29.2 (C2"), 39.7 (C1"), 101.2 (OCH_2O), 106.2 (C2'), 108.3 (C5'), 119.1 (C2), 123.6 (C6'), 129.2 (C1'), 140.1 (C3), 148.0 (C4'), 148.8 (C3'), 166.2 (C=O); EIMS *m/z* 261 (M^+, 19%), 190 (53), 176 (35), 175 (89), 145 (56), 135 (40), 117 (29), 89 (100), 41 (58), 39 (43).

*(2E,4E)-N-Pentyl-5-phenylpenta-2,4-dienamide (**3c**)*

Yield 80%, yellowish crystals, mp 103.9-104.3 °C; IRν_{max}/cm^{-1} 3287, 2958, 1644, 1615, 1547, 1443, 1146, 998 (KBr); ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.90 (t, 3H, *J* 6.6 Hz, H5"), 1.25-1.36 (m, 4H, H3" and H4"), 1.55 (t, 2H, *J* 6.6 Hz, H2"), 3.29 (q, 2H, *J* 6.6 Hz, H1"), 5.77 (sl, 1H, NH), 5.98 (d, 1H, *J* 14.9 Hz, H2), 6.80-6.89 (m, 2H, H4 and H5), 7.24-7.50 (m, 6H, H3, H2', H3', H4', H5' and H6'); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 13.9 (C5"), 22.3 (C4"), 29.0 (C3"), 29.2 (C2"), 39.6 (C1"), 124.3 (C4), 126.3 (C2), 126.9 (C2' and C6'),128.5 (C4'), 128.6 (C3' and C3''), 136.2 (C1'), 138.8 (C5), 140.4 (C3), 166.1 (C1); EIMS *m/z* 243 (M^+, 28%), 186 (17), 157 (90), 130 (23), 129 (58), 128 (100), 127 (45), 115 (16), 96 (41), 91 (11), 77 (22), 64 (22), 51 (17), 43 (20), 41 (35), 39 (18).

*(2E,4E)-5-(3-Methoxyphenyl)penta-2,4-dienoic acid (**7**)*[60]

Yield 88%, colorless needles, mp 135.9-136.6 °C; IR ν_{max}/cm^{-1} 3025, 3007, 1687, 1612, 1579, 1426, 1158, 1044, 995, 873 (KBr); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.84 (s, 3H, OCH_3), 5.99 (d, 1H, *J* 15.2 Hz, H2), 6.85-6.95 (m, 2H , H4' and H4), 6.93 (d, 1H, *J* 15.7 Hz, H5), 6.99 (t, 1H, *J* 2.5 Hz, H2'), 7.07 (d, 1H, *J* 7. 8 Hz, H6'), 7.28 (t, 1H, *J* 7.8 Hz, H5'), 7.53 (ddd, *J* 15.2, 7.5 and 2.3 Hz, 1H, H3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 55.3 (OCH_3), 112.3 (C4'), 115.1 (C2'), 120.1 (C6'), 120.4 (C2), 126.2 (C4), 129.8 (C5'), 137.2 (C1'), 141.54 (C5), 146.9 (C3), 159.9 (C3'), 172.3 (C1); EIMS *m/z* 204 (M^+, 65%), 159 (98), 144 (100), 127 (40), 115 (75), 89 (12), 77 (14), 63 (20), 51 (17), 39 (19).

Antifungal assay

The microorganisms used in the antifungal assay, *Cladosporium cladosporioides* (Fresen) de Vries SPC 140 and *C. sphaerospermum* (Perzig) SPC 491, have been maintained at the Instituto de Botânica, São Paulo, SP, Brazil. The assay was carried out for all amides and their activities determined as previously described (Table 3).[47] Nystatin and miconazole were used as positive controls whereas ampicillin and chloramphenicol were used as negative controls.[61]

Results and Discussion

Natural amides were isolated through successive chromatographic procedures as previously described.[47] Analogs of (2*E*, 4*E*)-5-phenylpenta-2,4-dienamides and (*E*)-cinnamamides were synthesized (Figure 2) aiming at determination of overall effect of aromatic ring substitution and nitrogen substituent in antifungal activity.[62,63] The importance of the amide moiety for the antifungal activity was investigated by replacing the natural piperidyl, isobutyl or its acetylated derivative by *N*,*N*-diethyl or *N*-pentyl analogs.

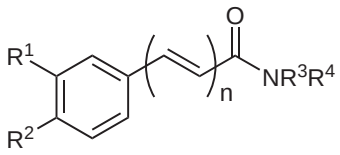
The replacement of isobutyl or piperidyl moieties by diethyl groups in (2*E*, 4*E*)-5-phenylpenta-2,4-dienamides resulted in a noticeable increase in the dose-response activity, as observed for amides **1a**, **1b**, **1c**, **1d** and **1e** against *C. cladosporioides* and *C. sphaerospermum*. Some selectivity was detected between the two strains in which *C. sphaerospermum* was more sensible to **1e** than *C. cladosporioides* (Table 1).

Analysis of aromatic substitution pattern indicated that amides having methylenedioxy or methoxyl groups displayed lower antifungal potency when compared to those having no substituents. The amides **2a**, **2c**, **3a** and **3c** displayed higher activities at 1 μg against both strains while **1d**, **3b** and **3e** were the least active among all amides assayed against *C. cladosporioides*. At lower concentrations *N*-isobutyl and *N*-pentyl derivatives (**2a**-**2e**, **3a**-**3c** and **3e**) showed higher activity. In this case, there is an apparent positive correlation with the lipophylicity and the amides having a α,β-conjugated carbonyl were more active than those having an extended α,β,γ,δ-conjugated system.

Conclusions

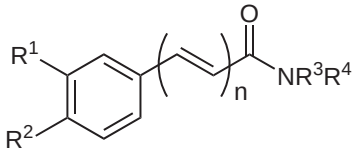
Investigation of natural and synthetic amides as antifungal compounds have shown preliminary structure-activity relationship in which *N*,*N*-diethyl showed higher dose dependent activity while *N*-pentyl and *N*-isobutyl derivatives showed lower detection limit. In general, substituents in the aromatic ring such as methoxyl and methylenedioxy decreased antifungal activity and the shorter aliphatic chain, the higher activity was observed.

Table 1. Antifungal activity of natural and synthetic compounds



	n	R^1	R^2	R^3	R^4	*Cladosporium cladosporioides*#						*Cladosporium sphaerospermum*#					
						100	50	25	10	5	1	100	50	25	10	5	1
1a	1	H	H	Et	Et	***	***	***	**	**	*	***	***	***	**	**	-
1b	1	O_2CH_2		Et	Et	***	***	***	**	**	*	***	***	***	***	**	*
1c	2	H	H	Et	Et	***	***	***	***	**	*	***	***	***	**	*	-
1d	2	OMe	H	Et	Et	***	***	**	*	*	-	***	***	**	*	*	-
1e	2	O_2CH_2		Et	Et	**	**	**	*	*	-	***	***	**	*	*	*
2a	1	H	H	^{i}Bu	H	**	**	**	**	**	**	**	**	**	**	**	*
2c	2	H	H	^{i}Bu	H	**	**	**	**	**	*	**	**	**	**	**	**
2d	2	OMe	H	^{i}Bu	H	**	**	**	**	*	*	**	**	**	**	*	*
2e	2	O_2CH_2		^{i}Bu	H	**	**	**	**	**	*	**	**	**	**	**	*
3a	1	H	H	pentyl	H	**	**	**	**	**	**	**	**	**	**	**	**
3b	1	O_2CH_2		pentyl	H	*	*	*	*	*	*	**	**	**	*	*	*
3c	2	H	H	pentyl	H	**	**	**	**	**	**	**	**	**	**	**	**
3e	2	O_2CH_2		pentyl	H	*	*	*	*	*	-	*	*	*	*	*	*
4	2	O_2CH_2		piperidyl	**	**	**	**	*	*	**	**	**	**	**	**	
5	2	O_2CH_2		$CH_2C(OCOCH_3)(CH_3)_2$	H	**	**	**	**	**	*	**	**	**	**	**	*

Numbers in *italic* and underlined are natural amides; # µg; *** : high activity; ** : medium; * : low; - : not active.



1a: n = 1; $R^1 = R^2 = H$; $R^3 = R^4 = Et$
1b: n = 1; $R^1+R^2 = O_2CH_2$; $R^3 = R^4 = Et$
1c: n = 2; $R^1 = R^2 = H$; $R^3 = R^4 = Et$
1d: n = 2; $R^1 = OMe$; $R^2 = H$;$R^3 = R^4 = Et$
1e: n = 2; $R^1 + R^2 = O_2CH_2$; $R^3 = R^4 = Et$
2a: n = 1; $R^1 = R^2 = H$; R^3 = i-Bu; $R^4 = H$
2c: n = 2; $R^1 = R^2 = H$; R^3 = i-Bu; $R^4 = H$
2d: n = 2; $R^1 = OMe$; $R^2 = H$; R^3 = i-Bu; $R^4 = H$
2e: n = 2; $R^1 + R^2 = O_2CH_2$; R^3 = i-Bu; $R^4 = H$

3a: n = 1; $R^1 = R^2 = H$; R^3 = pentyl; $R^4 = H$
3b: n = 1; $R^1 + R^2 = O_2CH_2$; R^3 = pentyl; $R^4 = H$
3c: n = 2; $R^1 = R^2 = H$; R^3 = pentyl; $R^4 = H$
3e: n = 2; $R^1 + R^2 = O_2CH_2$; R^3 = pentyl; $R^4 = H$
4: n = 2; $R^1 + R^2 = OCH_2O$; $R^3 + R^4$ = piperidyl
5: n = 2; $R^1 + R^2 = O_2CH_2$; $R^3 = CH_2C(OCOCH_3)(CH_3)_2$; $R^4 = H$

Figure 2. Synthetic and natural amides evaluated as antifungal.

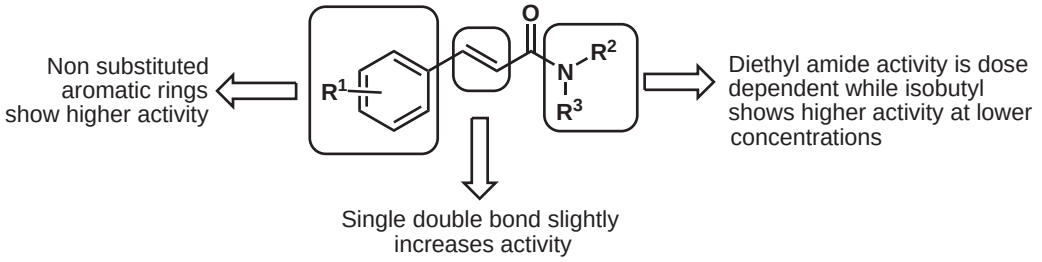


Figure 3. Preliminary structure-activity relationship for antifungal amide.

Further investigations including quantitative biological activity assessment and determination of physicochemical descriptors are required for a thorough understanding of the observed antifungal activities and in the development of effective antifungal agents.

Supplementary Information

The spectral data are available free of charge at http://jbcs.sbq.org.br, as pdf file.

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