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Phenolic acid amides: a new type of DNA strand scission agent from *Piper caninum*

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Abstract—In a survey of the active components of crude plant extracts for their ability to cleave DNA, a crude extract prepared from *Piper caninum* was found to induce the relaxation of supercoiled pBR322 plasmid DNA in the presence of Cu^{2+}. Bioassay-guided fractionation was carried out on this extract, guided by an in vitro DNA strand scission assay. Three active principles were isolated and identified as *N-cis*-feruloyl tyramine (**1**),*N-trans*-feruloyl tyramine (**2**), and 1-cinnamoylpyrrolidine (**3**). Compounds **1**–**3** represent a structurally new type of DNA strand scission agent.

1. Introduction

Plants from the genus *Piper* are widely distributed throughout tropical and subtropical regions. *Piper* species have been used in traditional medicinal systems for thousands of years, including the Chinese and Indian systems, as well as in folklore medicines of Latin America and the West Indies.[1] The stem of *Piper futokadsura*, a medicinal plant that grows in Fujian and Taiwan provinces of China, is widely used in traditional Chinese medicine prescriptions for the treatment of asthma and arthritic conditions.[2] *Piper amalago*, distributed geographically from Mexico to Brazil, is used as an antiinflammatory agent that is also capable of alleviating chest pains.[3] *Piper chaba* roots and fruits are described as an Indian medicine used for the treatment of asthma, bronchitis, fever, and abdominal pain. For years, the chemistry of the *Piper* species has been widely investigated, and phytochemical studies from all over the world have resulted in the isolation of numerous biologically active compounds such as alkaloids, propenylphenols, lignans, neolignans, terpenes, steroids, kawapyrones, piperolides, chalcones, dihydrochalcones, flavones, and flavanones.[1,4–7] Piperine, the first amide secondary metabolite to be isolated from a *Piper* species, displayed several interesting activities, including activity as a central nervous system depressant.[8] Aduncamide, a new amide from *P. aduncum* that showed antibacterial activity against *Bacillus subtilis* and *Micrococcus luteus*, was also shown to be cytotoxic toward KB nasopharyngeal carcinoma cells.[9] Kadsurenone, a novel neolignan from *P. futokadsura* was identified as a potent inhibitor in a platelet activating factor (PAF) binding assay.[10] Considering that there are over 700 species belonging to the *Piper* genus, and that *Piper* species have already yielded numerous secondary metabolites of potential therapeutic interest, the genus *Piper* would seem to constitute a rich potential source of additional novel active principles.

Since the discovery of the bleomycin antitumor antibiotics that work at the level of nucleic acid strand scission,[11–17] considerable effort has been made in our laboratory and elsewhere to identify other species that can function analogously in the belief that such molecules may lead to the development of new classes of antitumor agents. Several kinds of natural products have subsequently been reported as DNA strand scission agents, including flavanoid,[18–24] aurone,[22] 5-alkylresorcinol,[18,25,26] pterocarpan,[27] biphenyl,[28] stilbene,[29] anthrapyrone,[30] enediyne,[31] macro-ring lactam,[32] and lignan[23] species that cleave DNA in the absence or presence of certain metal ions. Described herein is the bioassay-guided isolation and structure identification of three aromatic acid amides from *Piper caninum* that induce significant DNA strand scission in the presence of Cu^{2+}.

Keywords: Nucleic acids; Chemotherapy; Natural products.
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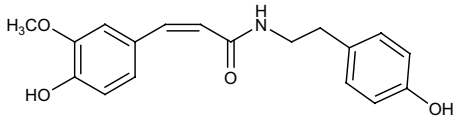
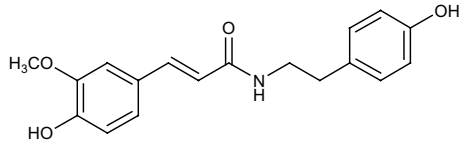
2. Results and discussion

In a survey of crude extracts for DNA cleavage agents using an in vitro assay that monitors the relaxation of supercoiled plasmid DNA, a crude 1:1 CH_2Cl_2–MeOH extract of *P. caninum* exhibited considerable Cu^{2+}-dependent DNA strand scission activity. It was thus selected for bioassay-guided fractionation.

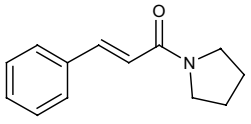
The crude CH_2Cl_2–MeOH extract of *P. caninum* was first fractionated on a polyamide 6S column, which was washed successively with H_2O, 1:1 MeOH–H_2O, 4:1 MeOH–CH_2Cl_2, 1:1 MeOH–CH_2Cl_2, and 9:1 MeOH–NH_4OH. With the removal of most polyphenols, the first two fractions exhibited significant Cu^{2+}-dependent DNA cleavage activity. These two fractions were combined and applied to a C_{18} reversed-phase column, which was washed with MeOH–H_2O mixtures. The fraction eluted by washing with 3:2 MeOH–H_2O possessed the most potent DNA strand scission activity, and was purified further by reversed-phase HPLC fractionation. Three pure compounds (**1**–**3**) were obtained as the active principles responsible for the DNA strand scission activity of the crude extract.



1



2



3

Compound **1** was obtained as a pale yellow oil; the positive ion chemical ionization mass spectrum displayed a quasi-molecular ion at m/z 314 $[M+H]^+$. ^1H NMR data analysis and comparison with literature data suggested that compound 1 was an amide derived from ferulic acid and tyramine.[33] In the ^1H NMR spectrum, the coupling constant for the pair of doublet signals at δ 5.68 and 6.44 corresponding to the olefinic protons was 11.8 Hz, which suggested a *Z*-configuration of the double bond. Accordingly, the structure of 1 was identified as *N-cis*-feruloyl tyramine.[33,34] Compound **2** was obtained as colorless amorphous powder, with the same molecular weight as compound **1** based on the positive ion chemical ionization mass spectrum. The ^1H NMR data for **2** was very similar to that of **1**, suggesting that **2** could be an isomer of **1**. The coupling constant of the olefinic protons was increased from 11.8 Hz in **1** to 15.8 Hz in **2**, indicating an *E*-configuration of the double bond. Therefore, compound **2** was identified as *N-trans*-feruloyl tyramine.[33,34] Compound **3**, a colorless amorphous powder, was identified as 1-cinnamoylpyrrolidine through the comparison of mass spectra and 1D NMR correlation with literature values.[34,35]

The abilities of compounds **1**–**3** to induce DNA cleavage in the absence and presence of Cu^{2+} ion were evaluated using a cell-free DNA strand scission assay involving supercoiled pBR322 DNA. As shown in Figure 1, in the presence of Cu^{2+} compounds **1**–**3** mediated dose-dependent DNA relaxation of the supercoiled pBR322 plasmid DNA. In common with the crude extract from which they were isolated, compounds **1**–**3** showed no DNA strand scission activity in the presence of Fe^{2+}, or in the absence of added metal ion; the limited appearance of nicked pBR322 plasmid DNA in lanes 3, 9 and, 15 is attributed to trace contamination with metal ions in the assay system.

In the presence of 20 μM Cu^{2+}, compounds **1** and **2** exhibited moderately strong DNA strand scission activity; greater than 70% conversion to Form II (nicked) DNA was observed at 40 μM for both compounds **1** and **2** (Fig. 1). Further, in the same experiment, significant single strand DNA breakage was still observed when the ligand concentration was as low as 5 μM (42% and 25% of Form II DNA for **1** and **2**, respectively). Moreover, when **1** and **2** were employed at 160 μM concentration, some Form III (linear duplex) DNA was produced in addition to nicked circular (Form II) DNA.

Previously, we demonstrated the importance of the geometry of the double bond of a coumaroyloxy substituent for the inhibitory activity of some triterpenoids toward DNA polymerase β.[36] In the present case, the configuration of the double bond of the feruloyl moiety

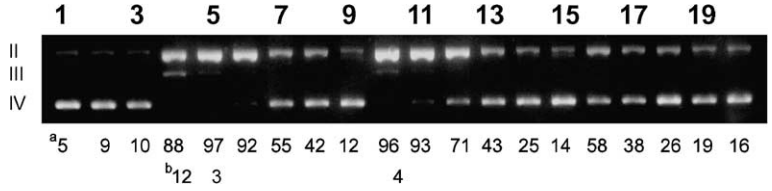


Figure 1. Agarose gel electrophoresis of a reaction mixture illustrating relaxation of supercoiled plasmid DNA by compounds **1**, **2**, and **3**. Lane 1, pBR322 plasmid DNA alone; lane 2, DNA+20 μM Cu^{2+}; lane 3, 160 μM **1**; lanes 4–8, 160, 80, 40, 10, and 5 μM **1**+20 μM Cu^{2+}, respectively; lane 9, 160 μM **2**; lanes 10–14, 160, 80, 40, 10, and 5 μM **2**+20 μM Cu^{2+}, respectively; lane 15, 250 μM **3**; lanes 16–20, 250, 100, 50, 25, and 10 μM **3**+20 μM Cu^{2+}, respectively. The percent Form II[a] and Form III[b] DNA present is shown below each lane.

also influenced the potency of DNA cleavage by compounds **1** and **2**, with the *cis*-isomer exhibiting somewhat greater potency.

Previous studies of the mechanism of the DNA strand scission by 5-alkylresorcinols (in the presence of Cu^{2+} ion) indicated an involvement of oxygenation of the aromatic nucleus, affording catecholic moieties that were proposed to coordinate Cu^{2+} and subsequently effect the reduction of dioxygen to reactive species, followed by the oxidation of the catecholic moiety via the coordinated Cu^{2+} ion.[37,38] This mechanism may also involved in the DNA strand scission observed for a number of other natural products containing catecholic moieties or analogous functionalities.[19–22,27–29] Further supporting this mechanism is the observed *lack* of DNA strand scission activity of some closely related structural analogues not having catecholic functionalities.[22,27]

In this context, it is interesting to consider the mechanism of DNA strand scission by compounds **1** and **2** since neither contains a catechol moiety, which would seem to be an essential functionality for potent Cu^{2+}-dependent DNA cleavage activity by the aforementioned compounds. Similar natural products that lack a catechol functionality but which contain phenolic hydroxyl groups have also been reported to be Cu^{2+}-dependent DNA strand scission agents.[22–24,27,28] A comparison of the structures of these compounds argues that the potency of Cu^{2+}-dependent DNA cleavage for these species is closely related to the numbers and positions of substitution of phenolic hydroxyl groups; activity increases in direct proportion to the number of phenolic OH groups. It must be noted, however that the actual role of Cu^{2+} ion in the DNA strand scission activity of these natural products is not understood at present.

As reflected in Figure 1, in the presence of Cu^{2+} compound **3** mediated DNA strand scission, but did so only weakly; about 58% conversion of Form I to Form II DNA was apparent when **3** was employed at 250 μM concentration. However, it is the first time that an aromatic amide lacking any phenolic OH group has been identified as a Cu^{2+}-dependent DNA strand scission agent. It seems reasonable to suggest that compound **3** may function by a novel mechanism.

3. Experimental

3.1. General methods

Polyamide 6S was purchased from Serva Electrophoresis GmbH. Silica C_{18} (40 μm) was obtained from J. T. Baker Chemicals. A Higgins Kromasil 100 C_{18} column (250 × 10 mm, 5 μm) was used for reversed-phase HPLC. ^1H and ^{13}C NMR spectroscopic experiments were performed on Varian Unity Inova 300 and 500 spectrometers. Low-resolution chemical ionization mass spectra (LRCIMS) were recorded on a Finnigan MAT 4600 mass spectrometer. Ethidium bromide, bromophenol blue, and Trizma were purchased from Sigma Chemicals. Boric acid was purchased from EM Sciences. (Ethylenedinitrilo)tetraacetic acid (EDTA), disodium salt was obtained from J. T. Baker. Cupric chloride and glycerol were from Mallinckrodt, Inc. Ultrapure agarose was from Bethesda Research Laboratories. pBR322 plasmid DNA was purchased from New England Biolabs. Pierce microdialysis cassette (New England Biolabs) was used to remove EDTA from the pBR322 plasmid DNA.

3.2. Plant material

The twigs of *P. caninum* were collected in Celebes, Sulawesi on March 10, 1990. Voucher specimen Q66O8931 is preserved at the US National Arboretum Herbarium, Washington, DC.

3.3. Extraction and isolation

Dried twigs of *P. caninum* were steeped in 1:1 methylene chloride–methanol overnight at room temperature, then drained, and washed with methanol. The solvent was concentrated under diminished pressure to afford the crude extract, which displayed potent Cu^{2+}-dependent DNA cleavage activity. In a typical fractionation experiment, the crude extract (1050 mg) was applied to a polyamide 6S column, which was washed successively with H_2O, 1:1 MeOH–H_2O, 4:1 MeOH–CH_2Cl_2, 1:1 MeOH–CH_2Cl_2, and 9:1 MeOH–NH_4OH. The H_2O and MeOH–H_2O fractions (284 and 202 mg, respectively), exhibited the most potent DNA strand scission activity at concentrations of 100 and 50 μg mL^{-1}. These two fractions were combined and subjected to further fractionation on a C_{18} column using MeOH–H_2O for elution. The 3:2 MeOH–H_2O fraction (62 mg) showed the greatest potency in DNA strand scission assay, and was applied to a C_{18} reversed-phase HPLC column (250 × 10 mm, 5 μm), and washed with a linear gradient of 1:19 → 13:7 CH_3CN–H_2O over a period of 60 min at 3.0 mL min^{-1} (UV monitoring at 254 nm). Three active fractions (5.8, 4.9, and 6.2 mg, respectively), were obtained in order of elution from the reversed-phase HPLC fractionation. Purification of the 5.8-mg fraction, employing the same reversed-phase C_{18} HPLC column and eluting with 6:19 CH_3CN–H_2O at a flow rate of 3.0 mL min^{-1} (monitoring at 254 nm), afforded active compound **1** (2.6 mg). The 4.9-mg fraction was also purified by the same HPLC system with a linear gradient solvent system (3:17 → 3:1 CH_3CN–H_2O over a period of 60 min at a flow rate of 3.0 mL min^{-1}), and 2.4 mg of pure compound **2** was obtained. Finally, purification of the 6.2-mg fraction using a linear gradient of 1:3 → 3:1 CH_3CN–H_2O over a period of 50 min at a flow rate of 3.0 mL min^{-1} on the same HPLC column provided active compound **3** (1.9 mg).

N-cis-Feruloyl tyramine (**1**): $C_{18}H_{19}NO_4$; pale-yellow oil; positive ion CI-MS *m/z* (rel int.) 315 $[M+2H]^+$ (33), 314 $[M+H]^+$ (100), 177 (35) and 145 (10); ^1H NMR

(CD_3OD): δ 2.70 (2H, t, $J = 7.0$ Hz, H-2″), 3.39 (2H, t, $J = 7.0$ Hz, H-1″), 3.76 (3H, s, 3′-OCH_3), 5.68 (1H, d, $J = 11.8$ Hz, H-2), 6.44 (1H, d, $J = 11.8$ Hz, H-3), 6.69 (2H, d, $J = 8.4$ Hz, H-3‴, H-5‴), 6.74 (1H, d, J =8.1 Hz, H-5′), 6.93 (1H, dd, $J = 8.1$, 2.0 Hz, H-6′), 7.00 (2H, d, $J = 8.4$ Hz, H-2‴, H-6‴), and 7.36 (1H, d, $J = 2.0$ Hz, H-2′).

N-trans-Feruloyl tyramine (**2**): $C_{18}H_{19}NO_4$; colorless amorphous powder; positive ion. CI-MS m/z (rel int.) 315 $[M+2H]^+$ (33), 314 $[M+H]^+$ (100), 177 (35), 145 (10); ^{1}H NMR (CD_3OD): δ 2.76 (2H, t, $J = 7.5$ Hz, H-2″), 3.48 (2H, t, $J = 7.5$ Hz, H-1″), 3.89 (3H, s, 3′-OCH_3), 6.40 (1H, d, $J = 15.8$ Hz, H-2), 6.72 (2H, d, $J = 8.4$ Hz, H-3‴, H-5″), 6.80 (1H, d, $J = 8.2$ Hz, H-5′), 7.03 (1H, dd, $J = 8.2$, 1.8 Hz, H-6′), 7.05 (2H, d, $J = 8.4$ Hz, H-2″, H-6‴), 7.12 (1H, d, $J = 1.8$ Hz, H-2′), and 7.43 (1H, d, $J = 15.8$ Hz, H-3); ^{13}C NMR (CD_3OD): δ 35.8 (C-2″), 42.5 (C-1″), 56.4 (3′-OCH_3), 111.5 (C-2′), 116.2 (C-3‴, C-5″), 116.6 (C-5′), 118.8 (C-2), 126.5 (C-6′), 128.2 (C-3′), 130.7 (C-2‴, C-6″), 131.3 (C-1‴), 142.0 (C-3), 149.9 (C-4′), 149.3 (C-1′), 156.9 (C-4‴), and 169.5 (C-1).

1-Cinnamoylpyrrolidine (**3**): $C_{13}H_{15}NO$; white amorphous powder; positive ion CI-MS m/z (rel int.) 203 $[M+2H]^+$ (28),202 $[M+H]^+$ (100),201 $[M]^+$ (4); ^{1}H NMR (CD_3OD): δ 1.94 (2H, quintet, $J = 7.0$ Hz, H-3″), 2.04 (2H, quintet, $J = 7.0$ Hz, H-4″), 3.54 (2H, quintet, $J = 7.0$ Hz, H-5″), 3.72 (2H, quintet, $J = 7.0$ Hz, H-2″), 6.94 (1H, d, $J = 15.5$ Hz, H-2), 7.39 (3H, m, H-3′, H-4′ and H-5′), 7.59 (1H, d, $J = 15.5$ Hz, H-1), 7.62 (2H, $J = 7.7$ Hz, 1.7 Hz, H-2′, H-6′); ^{13}C NMR (CD_3OD): δ 25.3 (C-3″), 27.0 (C-4″), 47.3 (C-5″), 48.0 (C-2″), 119.8 (C-2), 129.1 (C-3′, C-5′), 129.9 (C-2, C-6′), 131.0 (C-4′), 136.4 (C-1′), 143.3 (C-3), 167.0 (C-1).

3.4. DNA strand scission assay

A DNA strand scission assay utilized supercoiled DNA (pBR322 plasmid DNA, 500 ng) in the absence or presence of 20 μM Cu^{2+} in 25 μL (total volume) of 10 mM Tris–HCl buffer, pH 8.0, which was treated with crude extracts or fractions (dissolved in DMSO, with the final DMSO concentration being no more than 5% in the 25-μL reaction solution). Each set of experiments included one blank control (DNA alone) and one metal ion control (DNA+Cu^{2+}). After incubation at 37 °C for 60 min, the incubation mixture was treated with 5 μL of 30% glycerol–0.01% bromophenol blue and was analyzed by electrophoresis in a 1.0% agarose gel containing 0.7 μg mL^{-1} ethidium bromide. The electrophoresis was carried out in TBE buffer (89 mM Tris, 89 mM boric acid containing 2 mM EDTA, pH 8.3) at 110–120 V for 2–3 h. Following electrophoresis, the gel was photographed under ultraviolet light.

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References and notes

1. Parmar, V. S.; Jain, S. C.; Bisht, K. S.; Jain, R.; Taneja, P.; Jha, A.; Tyagi, O. D.; Prasad, A. K.; Wengel, J.; Olsen, C. E.; Boll, P. M. *Phytochemistry* **1997**, *46*, 597.
2. Matsui, K.; Munakata, K. *Tetrahedron Lett.* **1975**, 1905.
3. Dominguez, X. A.; Alcorn, J. B. *J. Ethnopharmacol.* **1985**, *13*, 139.
4. Khanom, F.; Kayahara, H.; Tadasa, K. *Biosci. Biotech. Biochem.* **2000**, *64*, 1967.
5. Setzer, W. N.; Setzer, M. C.; Bates, R. B.; Nakkiew, P.; Jackes, B. R.; Chen, L.; McFerrin, M. B.; Meehan, E. J. *Planta Med.* **1999**, *65*, 747.
6. Parmar, V. S.; Jain, S. C.; Gupta, S.; Talwar, S.; Rajwanshi, V. K.; Kumar, R.; Azim, A.; Malhotra, S.; Kumar, N.; Jain, R.; Sharma, N. K.; Tyagi, O. D.; Lawrie, S. J.; Errington, W.; Howarth, O. W.; Olsen, C. E.; Singh, S. K.; Wengel, J. *Phytochemistry* **1998**, *49*, 1069.
7. Orjala, J.; Mian, P.; Rali, T.; Sticher, O. *J. Nat. Prod.* **1998**, *61*, 939.
8. Lee, E. B.; Shin, K. H.; Woo, W. S. *Arch. Pharmacol. Res.* **1984**, *7*, 127.
9. Orjala, J.; Wright, A. D.; Rali, T.; Sticher, O. *Nat. Prod. Lett.* **1993**, *2*, 231.
10. Chang, M. N.; Han, G.-Q.; Arison, B. H.; Springer, J. P.; Hwang, S.-B.; Shen, T. Y. *Phytochemistry* **1985**, *24*, 2079.
11. Umezawa, H. *Lloydia* **1977**, *40*, 67.
12. Crooke, S. T. In *Bleomycin: Current Status and New Developments*; Carter, S. K., Crooke, S. T., Umezawa, H., Eds.; Academic: New York, 1978; pp 1–8.
13. Umezawa, H. In *Bleomycin: Current Status and New Developments*; Carter, S. K., Crooke, S. T., Umezawa, H., Eds.; Academic: New York, 1978; pp 15–19.
14. *Bleomycin Chemotherapy*; Sikic, B. I., Rozencwieg, M., Carter, S. K., Eds.; Academic: Orlando, 1985.
15. Sugiyama, H.; Ehrenfeld, G. M.; Shipley, J. B.; Kilkuskie, R. E.; Chang, L.-H.; Hecht, S. M. *J. Nat. Prod.* **1985**, *48*, 869.
16. Hecht, S. M. *Bioconjugate Chem.* **1994**, *5*, 513.
17. Hecht, S. M. *J. Nat. Prod.* **2000**, *63*, 158.
18. Hecht, S. M. *Pure Appl. Chem.* **1989**, *61*, 577.
19. Chrisey, L. A.; Bonjar, G. H. S.; Hecht, S. M. *J. Am. Chem. Soc.* **1988**, *110*, 644.
20. Huang, L.; Fullas, F.; McGivney, R. J.; Brown, D. M.; Wani, M. C.; Wall, M. E. *J. Nat. Prod.* **1996**, *59*, 290.
21. Hayakawa, F.; Kimura, T.; Maeda, T.; Fujita, M.; Sohmiya, H.; Fujii, M.; Ando, T. *Biochim. Biophys. Acta* **1997**, *1336*, 123.
22. Huang, L.; Wall, M. E.; Wani, M. C.; Navarro, H.; Santisuk, T.; Reutrakul, V.; Seo, E.-K.; Farnsworth, N. R.; Kinghorn, A. D. *J. Nat. Prod.* **1998**, *61*, 446.
23. Deng, J.-Z.; Newman, D. J.; Hecht, S. M. *J. Nat. Prod.* **2000**, *63*, 1269.
24. Fullas, F.; Kornberg, L. J.; Wani, M. C.; Wall, M. E. *J. Nat. Prod.* **1996**, *59*, 190.
25. Scannell, R. T.; Barr, J. R.; Murty, V. S.; Reddy, K. S.; Hecht, S. M. *J. Am. Chem. Soc.* **1988**, *110*, 3650.
26. Barr, J. R.; Murty, V. S.; Yamaguchi, K.; Singh, S.; Smith, D. H.; Hecht, S. M. *Chem. Res. Toxicol.* **1988**, *27*, 204.
27. Chaudhuri, S. K.; Huang, L.; Fullas, F.; Brown, D. M.; Wani, M. C.; Wall, M. E.; Tucker, J. C.; Beecher, C. W. W.; Kinghorn, A. D. *J. Nat. Prod.* **1995**, *58*, 1966.
28. Seo, E.-K.; Huang, L.; Wall, M. E.; Wani, M. C.; Navarro, H.; Mukherjee, R.; Farnsworth, N. R.; Kinghorn, A. D. *J. Nat. Prod.* **1999**, *62*, 1484.
29. Fukuhara, K.; Miyata, N. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3187.

30. Hara, M.; Yoshida, M.; Nakano, H. *Biochemistry* **1990**, *29*, 10449.
31. Sugiura, Y.; Matsumoto, T. *Biochemistry* **1993**, *32*, 5548.
32. Hara, M.; Saitoh, Y.; Nakano, H. *Biochemistry* **1990**, *29*, 5676.
33. Fukuda, N.; Yonemitsu, M.; Kimura, T. *Chem. Pharm. Bull.* **1983**, *31*, 156.
34. Singh, S. K.; Prasad, A. K.; Olsen, C. E.; Jha, A.; Jain, S. C.; Parmar, V. S.; Wengel, J. *Phytochemistry* **1996**, *43*, 1355.
35. Tyagi, O. D.; Prasad, A. K.; Wengel, J.; Boll, P. M.; Olsen, C. E.; Parmar, V. S.; Sharma, N. K.; Jha, A.; Bisht, K. S. *Acta Chem. Scand.* **1995**, *49*, 142.
36. Ma, J.; Starck, S.; Hecht, S. M. *J. Nat. Prod.* **1999**, *62*, 1660.
37. Lytollis, W.; Scannell, R. T.; An, H.; Murty, V. S.; Reddy, K. S.; Barr, J. R.; Hecht, S. M. *J. Am. Chem. Soc.* **1995**, *117*, 12683.
38. Singh, U. S.; Scannell, R. T.; An, H.; Carter, B. J.; Hecht, S. M. *J. Am. Chem. Soc.* **1995**, *117*, 12691.