Terphenylquinone Inhibitors of the Src Protein Tyrosine Kinase from *Stilbella* sp.

Carsten Puder,*,† Klaus Wagner,† Regine Vettermann,‡ Rudolf Hauptmann,§ and Olivier Potterat†

Boehringer Ingelheim Pharma GmbH & Co. KG, Birkendorfer Strasse 65, D-88397 Biberach/Riss, Germany, CyBio Screening GmbH, Winzerlaer Strasse 2, D-07745 Jena, Germany, and Boehringer Ingelheim Austria GmbH, Dr. Boehringer Gasse 5-11, A-1121 Vienna, Austria

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Three new secondary metabolites, 2,5-dihydroxy-3-(3,4-dihydroxyphenyl)-6-phenyl-1,4-benzoquinone (**3**), 2,5-dihydroxy-3-phenyl-6-(3,4,5-trihydroxyphenyl)-1,4-benzoquinone (**4**), and 2,7,8-trihydroxy-3-phenyl-1,4-dibenzofurandione (**5**), as well as two known natural products were isolated from the fungus *Stilbella* sp. strain 1586. The structures of these compounds were established by spectroscopic and chemical methods. The terphenylquinones **3**−**5** exhibited significant activity against human src protein tyrosine kinase.

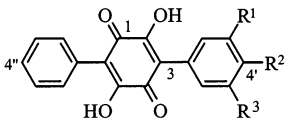
The tyrosine kinase src is the prototype member of the src family of kinases. These enzymes share a common structural organization consisting of a member-specific N-terminal region, an SH3 domain, an SH2 domain, and the kinase domain.

Src itself is expressed in almost any cell type and involved in several signaling pathways. Due to this involvement, inhibition of src activity should be beneficial for the treatment of various diseases including cancer. Enhanced src activity was found in several human tumor types and especially in metastatic tumors.[1−5] Src was also identified to be involved in VEGF signaling, and partial or complete inhibition of src activity in mice provided cerebral protection following stroke.[6] Src activity was also shown to be essential for osteoclastic bone resorption. Therefore, src inhibition should be effective in treatment or prevention of osteoporosis.[7]

To evaluate the consequences of disruption of src-mediated protein−protein interactions, a screening program for new src inhibitors was started. In the course of our HTS-supported lead-finding process more than 80 000 natural product extracts of plant and microbial origin were tested. Among other hits, an extract gained from *Stilbella* sp. strain 1586 showed a remarkable inhibition of src and was subjected to a dereplication process integrating physicochemical data with biological information.[8,9] Comparative analysis of the LC-MS-UV dataset and the bioactivity plot of the fractionated extract indicated that the activity of this extract was based on a group of hydroxyquinone derivatives. In this report we describe the isolation, structure elucidation, and biological activity of these pigments.

Results and Discussion

A defatted and desalted MeOH/acetone extract, obtained from cultures of strain 1586 grown on a solid substrate medium, was fractionated by gel permeation chromatography (Sephadex LH-20, MeOH). Final purification of two pigment-containing fractions by mass spectrometry-controlled preparative HPLC[10] on a Symmetry C-18 column (see Experimental Section for details) led to the isolation of the five terphenylquinones, **1**−**5**.

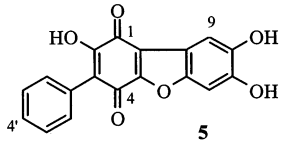


1 $R^1 = R^2 = R^3 = H$

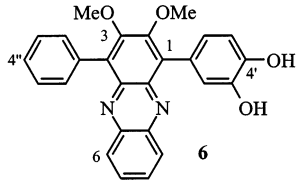
2 $R^1 = R^3 = H$, $R^2 = OH$

3 $R^1 = H$, $R^2 = R^3 = OH$

4 $R^1 = R^2 = R^3 = OH$



5



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According to the information obtained from HRESIMS and from ^1H and ^{13}C NMR spectroscopy, compound **1** was identified as the already known 2,5-dihydroxy-3,6-diphenyl-1,4-benzoquinone.[11,12] Similarly, the identity of compound **2** with the previously described 2,5-dihydroxy-3-(4-hydroxyphenyl)-6-phenyl-1,4-benzoquinone (= ascocorynin)[11,13] was verified by NMR spectroscopic analysis. Interestingly, the ^{13}C NMR spectra of **1** and **2** showed at 303 K in DMSO-d_6 a broadened resonance at δ 168. This signal represents the carbonyl and phenolic carbons C-1, C-2, C-4, and C-5. The observed coalescence is presumably based on the rapid interconversion between two equivalent tautomeric forms of the 2,5-dihydroxy-1,4-benzoquinone ring system.[14]

The high-resolution ESI mass spectral analysis of the main metabolite **3** (323.0556, $[M - H]^-$) gave a molecular formula of $C_{18}H_{12}O_6$. Its ^1H NMR spectrum indicated the

* To whom correspondence should be addressed. Present address: Boehringer Ingelheim Pharma GmbH & Co. KG, Binger Strasse 173, D-55216 Ingelheim/Rhein. Tel: (+49)6132-7790341. Fax: (+49)6132-7290341. E-mail: Carsten.Puder@ing.boehringer-ingelheim.com.
† Boehringer Ingelheim Pharma GmbH & Co. KG.
‡ CyBio Screening GmbH.
§ Boehringer Ingelheim Austria GmbH.

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presence of a phenyl group and an oxygenated 1,2,4-trisubstituted benzene ring. The ^{13}C NMR spectrum of **3** displayed resonances for only 13 nonequivalent carbons. This was due to the C_{2v} symmetry of the phenyl group and the presence of a 2,5-dihydroxy-1,4-benzoquinone moiety. The broad signal at δ 167.9 contained the chemical shifts of carbons C-1, C-2, C-4, and C-5. On the basis of a HMBC correlation of C-6 (δ 115.2) with H-2″/H-6″ (δ 7.37) and comparison with the ^{13}C NMR data of **2**, a 2,5-dihydroxyphenyl-1,4-benzoquinone fragment was established. The carbon C-3 (δ 115.8) exhibited a strong HMBC correlation signal to H-2′ (δ 6.84) and a weak one to H-6′ (δ 6.69). These findings did not allow to distinguish clearly between the alternative substructures of a *p*-substituted brenzcatechin and an *o*-substituted hydroquinone. Chemical derivatization of **3** with *o*-phenylenediamine and dimethyl sulfate yielded the phenazine **6**, the ROESY spectrum of which showed correlations of OCH_3-2 (δ 3.71) with H-2′ (δ 6.97) and H-6′ (δ 6.82). Thus, the structure of the parent compound **3** was assigned as 2,5-dihydroxy-3-(3,4-dihydroxyphenyl)-6-phenyl-1,4-benzoquinone.

The molecular formula of the minor compound **4** was established as $C_{18}H_{12}O_7$ by HRESIMS (339.0493, $[M - H]^-$). Its ^1H and ^{13}C NMR data were consistent with the presence of a phenyl group, a 2,5-dihydroxy-1,4-benzoquinone unit, and an oxygenated tetrasubstituted benzene ring. ^{13}C NMR resonances for only 11 nonequivalent carbons were observed, indicating two additional cases of isochrony (C-3′/C-5′ at δ 145.1 and C-2′/C-6′ at δ 109.7) in comparison with **3**. The terphenylquinone core structure of **4** was determined on the basis of HMBC data. Symmetry arguments, biosynthetic considerations,[13] and chemical shift calculations[15] led to the complete location of the substituents in compound **4**. The identified 2,5-dihydroxy-3-phenyl-6-(3,4,5-trihydroxyphenyl)-1,4-benzoquinone (**4**) has been previously obtained by synthesis.[16] To our knowledge, this is the first report on the isolation of **4** from a natural source.

Mass spectral analysis of compound **5** by HREIMS (321.0403, $[M - H]^-$) established the molecular formula as $C_{18}H_{10}O_6$. The ^1H NMR spectrum of **5** displayed three exchangeable protons (δ 11.0, 9.91, and 9.64) and seven aromatic protons (δ 7.40−7.17). Its ^{13}C NMR spectrum revealed the presence of seven aromatic methines, two carbonyls, and nine other quaternary sp^2 carbons. Detailed analysis of HSQC, HMBC, and ROESY experiments as well as biosynthetic considerations[13] and spectral comparison with cycloleucomelone[17] enabled the identification of **5** as 2,7,8-trihydroxy-3-phenyl-1,4-dibenzofurandione. Compound **5** can be described as a formal product of the oxidation of **3** to an *o*-quinone followed by an intramolecular Michael addition and aromatization. This was experimentally confirmed by oxidation of **3** to **5** with Ag_2CO_3/Celite, as it was previously described for the analogous reaction of leucomelone to cycloleucomelone.[18]

The isolated terphenylquinones **1**−**5** were tested against human src protein tyrosine kinase using a dissociation-enhanced lanthanide fluoroimmunoassay (DELFIA). The most active compound, **4**, inhibited the tyrosine kinase activity of src with an IC_{50} of 3.9 μmol/L. Of the four other terphenylquinones, **3** (IC_{50} 14.5 μmol/L) and **5** (IC_{50} 23.6 μmol/L) exhibited moderate src inhibitory activity, while **1** (17% inhibition at 35 μmol/L; $IC_{50} \gg 50$ μmol/L) and **2** (IC_{50} 37.5 μmol/L) showed only a weak inhibition in comparison to **4**. The src inhibitory activity of the compounds **1**−**4** decreased in the order **4**−**3**−**2**−**1**, which is accompanied with the stepwise substitution of the phenolic hydroxy groups by hydrogen atoms. Besides the src inhibitory activity reported here, a number of terphenylquinones and related quinols have previously been shown to be potent antioxidants.[16,19,20]

Experimental Section

General Experimental Procedures. Melting points were determined on a Büchi B-545 melting point apparatus and are uncorrected. All homonuclear and heteronuclear 1D and 2D NMR spectra were recorded at 303 K on Bruker DPX400 and DRX600 instruments using the solvent signal as internal reference. EIMS data were collected on a Finnigan MAT 8230 mass spectrometer at 70 eV. IR spectra (KBr) were recorded on a Nicolet Magna IR 550 FT-IR spectrometer and the UV spectra on a Perkin-Elmer Lambda 2S spectrophotometer.

Analytical HPLC-MS-UV experiments were performed on a Waters-Micromass system. Analytical system 1 consists of a Waters 2795 Alliance HPLC, a Waters 996 PDA detector, and a Micromass LCT mass spectrometer equipped with an ESI Z-spray source and a Lockspray device. Analyses were carried out on a Waters Symmetry C-18 precolumn/column setup (5 μm, 3.9 × 20 mm i.d. and 3.9 × 150 mm i.d., respectively) with a linear 1 mmol/L aqueous NH_4OAc/MeCN gradient (95:5 to 0:100 in 30 min; flow rate 1 mL/min).

For the final purification, a mass spectrometry-controlled preparative HPLC system was used.[10] The system consists of a Waters Prep LC 4000 system with Waters 2700 sample manager, Waters 2996 PDA detector, Waters-Micromass ZQ single-quadrupole mass spectrometer, 1:1000 LC Packings ACURATE splitter, Waters reagent manager, and Gilson 215 liquid handler. MS-triggered fractionations were performed with MassLynx software version 3.5. Preparative HPLC was carried out using a Waters Symmetry C-18 precolumn/column setup (5 μm, 19 × 10 mm i.d. and 30 × 100 mm i.d., respectively).

The kinase assay was carried out in Roche StreptaWell plates (streptavidin coated 96-well microtiter plates). The DELFIA reagents and the antibody PY20 were obtained from Wallac. The BACtoBAC baculovirus expression systems were from GibcoBRL Life Technologies. Time-resolved fluorescence was measured in a Wallac Victor V multilabel counter equipped with 1420 mutilabel counter software, and the IC_{50} values were calculated using the standard GraphPad software package (3.03).

Organism and Fermentation. The fungal strain 1586 was obtained from P. J. Fisher (University of Exeter, UK). It was isolated from a stem sample of *Miconia tetranda* collected in Luquillo (Puerto Rico). A voucher specimen of the fungal strain is stored above liquid N_2 at the CyBio Screening GmbH in Jena. Strain 1586 was identified by the DSMZ (Germany) as *Stilbella* sp. The strain is characterized by the formation of compact undifferentiated synnemata. Ellipsoid conidia are born on short conidiophores with terminal located verticillate phialides or directly at the surface of the synnemata. The colonies are reddish-brownish with a sparely developed aerial mycelium on oat agar.

Strain 1586 was maintained as a stock culture on agar plates containing malt extract 4%, yeast extract 0.4%, and agar 1.5%. Seed cultures were carried out in 300 mL Erlenmeyer flasks with three baffles. Caps of foamed plastic material were used as closures. Each flask was filled with 100 mL of seed medium (glucose 2%, soya protein 0.75%, malt extract 0.1%, yeast extract 0.1%, KH_2PO_4 0.05%, $MgSO_4 \times 7\ H_2O$ 0.005%, $CaCl_2 \times 2\ H_2O$ 0.002%, NaCl 0.001%, bacto agar 0.1%, and trace elements solution 0.1%[21] in deionized water; adjusted to pH 6.0 before sterilization), sterilized at 121 °C for 20 min, and then inoculated at room temperature with a ca. 3 cm^2 piece of a well-grown agar culture. The seed cultures were incubated at 23 °C on a rotary shaker (110 rpm) for 3 days prior to inoculation of production flasks. Production cultures were performed on a solid substrate medium in 500 mL Erlenmeyer flasks. Each production flask was filled with 40 g of rice and 100 mL of nutrient solution (maltose 2%, potato starch 0.4%,

and soya peptone 0.1% in deionized water; adjusted to pH 5.5) and capped with a cotton plug. The rice was soaked for 2 h at room temperature, and then the production flasks were autoclaved at 121 °C for 20 min. After inoculation of each production flask with 4 mL of seed culture, the production cultures were incubated without agitation at 23 °C for 21 days.

Extraction and Isolation. Each of the harvested cultures (60 flasks) was extracted with 100 mL of MeOH/acetone (1:1) overnight. The combined extracts were filtered and then concentrated under reduced pressure. The aqueous residue was reextracted twice with EtOAc (2 × 500 mL). Evaporation of the organic solvent under reduced pressure yielded 17.4 g of a brown viscous gum. The crude product was dissolved in MeOH (100 mL) and defatted twice with cylohexane (2 × 100 mL). The methanolic layer was evaporated to dryness under reduced pressure, and the residue was dissolved in THF (10 mL). Aqueous HCl (10 mmol/L, 100 mL) was added slowly under stirring. The resultant precipitate was collected by filtration and chromatographed on a Sephadex LH-20 column (5 × 85 cm i.d.). Elution with MeOH at 2 mL/min afforded two pigment-containing fractions. The final purification of the two fractions was achieved by mass spectrometry-controlled preparative HPLC using ESIMS (negative ion mode) as detection method. The first fraction was chromatographed in four portions on a Symmetry C-18 column and eluted with a linear 0.02% aqueous HOAc/MeCN gradient (0 min 5% MeCN with 4 mL/min, 1 min 5% MeCN with 4 mL/min, 1.5 min 5% MeCN with 28 mL/min, 31.5 min 100% MeCN with 28 mL/min). Fraction collection was triggered by detection of *m/z* 291.1. The automated fractionation process furnished 16 mg of **1** (t_R 20 min). The second fraction was purified on Symmetry C-18 by means of isocratic elution with 0.02% aqueous HOAc/MeCN (0 min 28% MeCN with 4 mL/min, 1 min 28% MeCN with 4 mL/min, 1.5 min 28% MeCN with 28 mL/min, 30 min 28% MeCN with 28 mL/min, 35 min 100% MeCN with 28 mL/min). Collecting of fractions was carried out with *m/z* 307.1, 321.1, 323.1, and 339.1 as fractionation criteria. Eighteen chromatographic runs led to 7 mg of **4** (t_R 8 min), 246 mg of **3** (t_R 15 min), 35 mg of **2** (t_R 24 min), and 73 mg of **5** (t_R 28 min).

2,5-Dihydroxy-3,6-diphenyl-1,4-benzoquinone (1): brown solid; HPLC t_R 15.6 min (analytical system 1); HRESIMS [M − H]$^-$ obsd 291.0638, calcd for $C_{18}H_{11}O_4$ 291.0657. Compound **1** exhibited spectral data (UV, IR, ^{1}H NMR, ^{13}C NMR, and EIMS) comparable to published values.[11,12]

2,5-Dihydroxy-3-(4-hydroxyphenyl)-6-phenyl-1,4-benzoquinone (2): green solid; HPLC (analytical system 1) t_R 12.8 min; ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 168 br (C, C-1, C-2, C-4, C-5), 156.7 (C, C-4′), 131.6 (CH, C-2′, C-6′), 130.9 (C, C-1″), 130.4 (CH, C-2″, C-6″), 127.5 (CH, C-3″, C-5″), 127.3 (CH, C-4″), 121.0 (C, C-1′), 115.5 (C, C-3), 115.2 (C, C-6), 114.4 (CH, C-3′, C-5′); HRESIMS [M − H]$^-$ obsd 307.0597, calcd for $C_{18}H_{11}O_5$ 307.0606. Compound **2** exhibited spectral data (UV, IR, ^{1}H NMR, and EIMS) comparable to published values.[11,13]

2,5-Dihydroxy-3-(3,4-dihydroxyphenyl)-6-phenyl-1,4-benzoquinone (3): brown solid, mp 271−274 °C (dec); HPLC (analytical system 1) t_R 11.6 min; UV (EtOH) λ_{max} (log ϵ) 267 (4.35), 355 sh (3.47), 505 sh (2.70) nm; IR (KBr) ν_{max} 3396, 3273, 1630, 1608, 1518, 1346, 1325, 1292, 1279, 1001 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.8 (2H, s br, OH-2, OH-5), 8.95 (1H, s br, OH-4′), 8.87 (1H, s br, OH-3′), 7.40 (2H, m, H-3″, H-5″), 7.37 (2H, m, H-2″, H-6″), 7.32 (1H, m, H-4″), 6.84 (1H, d, J = 2.0 Hz, H-2′), 6.75 (1H, d, J = 8.2 Hz, H-5′), 6.69 (1H, dd, J = 8.2, 2.0 Hz, H-6′); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 167.9 br (C, C-1, C-2, C-4, C-5), 144.9 (C, C-4′), 144.2 (C, C-3′), 130.8 (C, C-1″), 130.4 (CH, C-2″, C-6″), 127.5 (CH, C-3″, C-5″), 127.3 (CH, C-4″), 121.9 (CH, C-6′), 121.3 (C, C-1′), 118.0 (CH, C-2′), 115.8 (C, C-3), 115.2 (C, C-6), 114.8 (CH, C-5′); EIMS *m/z* 324 [M]$^+$ (100), 296 [M − CO]$^+$ (12), 278 (5); HRESIMS [M − H]$^-$ obsd 323.0556, calcd for $C_{18}H_{11}O_6$ 323.0556.

2,5-Dihydroxy-3-phenyl-6-(3,4,5-trihydroxyphenyl)-1,4-benzoquinone (4): brown-violet solid; HPLC (analytical system 1) t_R 10.4 min; UV (EtOH) λ_{max} (log ϵ) 271 (4.39), 375 (3.43), 510 sh (2.77) nm; IR (KBr) ν_{max} 3309, 1622, 1539, 1323, 1261, 1198, 1030, 997 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.8 (2H, s br, OH-2, OH-5), 8.76 (2H, s br, OH-3′, OH-5′), 8.13 (1H, s br, OH-4′), 7.39 (2H, m, H-3″, H-5″), 7.37 (2H, m, H-2″, H-6″), 7.31 (1H, m, H-4″), 6.36 (2H, s, H-2′, H-6′); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 168 br (C, C-1, C-2, C-4, C-5), 145.1 (C, C-3′, C-5′), 132.8 (C, C-4′), 131.1 (C, C-1″), 130.3 (CH, C-2″, C-6″), 127.4 (CH, C-3″, C-5″), 127.1 (CH, C-4″), 120.5 (C, C-1′), 115.8 (C, C-3), 114.8 (C, C-6), 109.7 (CH, C-2′, C-6′); EIMS *m/z* 340 [M]$^+$ (100), 312 [M − CO]$^+$ (40), 294 (6); HRESIMS [M − H]$^-$ obsd 339.0493, calcd for $C_{18}H_{11}O_7$ 339.0505.

2,7,8-Trihydroxy-3-phenyl-1,4-dibenzofurandione (5): brown solid, mp 303−305 °C (dec); HPLC (analytical system 1) t_R 11.9 min; UV (EtOH) λ_{max} (log ϵ) 259 (4.53), 295 (4.25), 355 (3.74), 424 (3.67), 525 sh (3.33) nm; IR (KBr) ν_{max} 3369, 1674, 1633, 1566, 1485, 1363, 1338, 1327, 1292, 1086, 1026 cm^{-1}; H NMR (400 MHz, DMSO-d_6) δ 11.0 (1H, s br, OH-2), 9.91 (1H, s, OH-7), 9.64 (1H, s, OH-8), 7.40 (2H, m, H-3′, H-5′), 7.34 (2H, m, H-2′, H-6′), 7.33 (2H, m, H-9, H-4′), 7.17 (1H, s, H-6); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 179.3 (C, C-1 or C-4), 176.0 (C, C-1 or C-4), 154.3 (C, C-2 or C-4a), 151.3 (C, C-2 or C-4a), 150.5 (C, C-5a), 148.5 (C, C-7), 146.1 (C, C-8), 131.0 (C, C-1′), 130.7 (CH, C-2′, C-6′), 127.4 (CH, C-3′, C-5′), 127.3 (CH, C-4′), 118.6 (C, C-9b), 117.8 (C, C-3), 113.1 (C, C-9a), 105.0 (CH, C-9), 98.8 (CH, C-6); EIMS *m/z* 322 [M]$^+$ (100), 294 [M − CO]$^+$ (9), 266 (5), 237 (7); HRESIMS [M − H]$^-$ obsd 321.0403, calcd for $C_{18}H_9O_6$ 321.0399.

Preparation of 1-(3,4-Dihydroxyphenyl)-2,3-dimethoxy-4-phenylphenazine (6). An excess of *o*-phenylenediamine (45 mg) was added to a solution of **3** (33 mg, 0.10 mmol) in HOAc (9 mL). After stirring for 5 h at 115 °C, the reaction mixture was allowed to cool to room temperature and then poured into a mixture of CH_2Cl_2 (100 mL) and H_2O (100 mL). The resultant red precipitate was collected by filtration and dried under vacuum. The precipitate (17 mg), K_2CO_3 (30 mg), and an excess of dimethyl sulfate (0.10 mL) were dissolved in DMF (3 mL) and stirred at room temperature for 5 h. Then the reaction mixture was diluted with EtOAc (100 mL) and washed with H_2O (2 × 50 mL). After removal of the solvent under reduced pressure, the crude product was purified by gel permeation chromatography (Sephadex LH-20, MeOH) to afford 12 mg of **6** (0.028 mmol, 28%) as a yellow solid: mp 242 °C; HPLC (analytical system 1) t_R 19.1 min; UV (MeOH) λ_{max} (log ϵ) 265 (4.54), 373 (3.96), 420 sh (3.44) nm; IR (KBr) ν_{max} 3250 br, 1601, 1514, 1456, 1441, 1385, 1308, 1279, 1053, 1024, 995 cm^{-1}; ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.01 (1H, s, OH-4′), 8.95 (1H, s, OH-3′), 8.03 (1H, m, H-9), 7.97 (1H, m, H-6), 7.81 (2H, m, H-7, H-8), 7.54 (4H, m, H-2″, H-3″, H-5″, H-6″), 7.47 (1H, m, H-4″), 6.97 (1H, d, J = 2.0 Hz, H-2′), 6.90 (1H, d, J = 8.0 Hz, H-5′), 6.82 (1H, dd, J = 8.0, 2.0 Hz, H-6′), 3.73 (3H, s, OCH_3-3), 3.71 (3H, s, OCH_3-2); ^{13}C NMR (150.9 MHz, DMSO-d_6) δ 153.7 (C, C-3), 153.5 (C, C-2), 144.8 (C, C-4′), 144.4 (C, C-3′), 141.2 (C, C-5a or C-9a), 141.1 (C, C-5a or C-9a), 140.8 (C, C-4a or C-10a), 140.5 (C, C-4a or C-10a), 134.1 (C, C-1″), 131.3 (CH, C-2″, C-6″), 130.8 (C, C-1), 130.1 (CH, C-7), 130.0 (CH, C-8), 129.9 (C, C-4), 129.2 (CH, C-9), 129.1 (CH, C-6), 127.5 (CH, C-3″, C-5″), 127.2 (CH, C-4″), 124.6 (C, C-1′), 122.6 (CH, C-6′), 118.9 (CH, C-2′), 115.0 (CH, C-5′), 61.1 (CH_3, OCH_3-3), 60.8 (CH_3, OCH_3-2); EIMS *m/z* 424 [M]$^+$ (100), 407 (28), 393 [M − OCH_3]$^+$ (68); HRESIMS [M − H]$^-$ obsd 423.1323, calcd for $C_{26}H_{19}N_2O_4$ 423.1345.

Preparation of 2,7,8-Trihydroxy-3-phenyl-1,4-dibenzofurandione (5). To a solution of **3** (21 mg, 0.065 mmol) in THF (5 mL) was added Ag_2CO_3/Celite (50%, 43 mg), and the mixture was stirred at room temperature. After 22 h, aqueous HCl (1 mol/L, 0.3 mL) was added and the suspension was filtered. The filtrate was evaporated under reduced pressure, and the residue was purified by gel permeation chromatography (Sephadex LH-20, MeOH) to give 14 mg of **5** (0.043 mmol, 66%) as a brown solid. The spectroscopic data of the synthesized sample of 2,7,8-trihydroxy-3-phenyl-1,4-dibenzofurandione were completely identical to those of the isolated sample.

Src Kinase Inhibition Assay. The src enzyme used in the assay was expressed as the full length human protein tyrosine kinase with the first 9 amino acid residues replaced by a His tag in a baculovirus system.[22] After harvesting the infected Sf9 insect cells, the src enzyme was purified from the biomass by Ni^{2+}-agarose affinity column chromatography.[23] The enzyme

concentration used in the assay was 0.5 μg/mL. The enzymatic reaction was performed in the presence of 1 μg/mL biotinylated poly EY [poly(L-glutamic acid-L-tyrosine) sodium salt, Glu/Tyr (4:1)], 500 μg/mL unbiotinylated poly EY, 250 μmol/L ATP, 15 mmol/L $Mg(OAc)_2$, 0.2 mmol/L Na_3VO_4, 26 mmol/L NaCl, 0.2 mmol/L DTT, 0.01% Triton X-100, 44 mmol/L HEPES (pH 7.4), 2% glycerol, and various concentrations of the natural products **1**−**5** at room temperature for 30 min in 100 μL total volume. ATP and substrate concentrations were well beyond their respective Km values, and the lysate containing the active kinase was diluted to make sure that the reaction was in the linear range. The reaction was stopped after 30 min by adding 50 μL of stop mix (250 mmol/L EDTA and 20 mmol/L HEPES, pH 7.4). A 100 μL portion of the solution was transferred to a streptavidin-coated 96-well microtiter plate and incubated at ambient temperature for 60 min. Each well was washed extensively with PBS (3 × 200 μL, pH 7.0), and 200 μL of a 250 ng/mL dilution of Eu^{3+}-N1 labeled anti-phosphotyrosine antibody (PY20) in DELFIA assay dilution buffer was added. After 30 min, the excess antibody was washed away with DELFIA wash concentrate (3 × 200 μL), and the reaction was developed for 10 min by adding 200 μL of DELFIA enhancement solution. The kinase activity was measured using a time-resolved fluorescency europium program.

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