Microwave-Assisted Synthesis of KN-93, a Potent and Selective Inhibitor of Ca^{2+}/Calmoduline-Dependent Protein Kinase II

Claudio Bruno,[a] Giovanni Lentini,[a] Alessia Catalano,[a] Alessia Carocci,[a] Angelo Lovece,[a] Antonia Di Mola,[a] Maria Maddalena Cavalluzzi,[a] Paolo Tortorella,[a] Fulvio Loiodice,[a] Guido Iaccarino,[b] Pietro Campiglia,[c] Ettore Novellino,[d] Carlo Franchini*[a]

[a] Dipartimento Farmaco-Chimico, Università di Bari, Via E. Orabona 4, 70126 Bari, Italy
Fax +39(080)5442724; E-mail: cfranc@farmchim.uniba.it

[b] Dipartimento di Medicina Clinica e Scienze Cardiovascolari e Immunologiche, Università degli Studi di Napoli Federico II, Corso Umberto I, 80138 Napoli, Italy

[c] Dipartimento di Scienze Farmaceutiche, Università degli Studi di Salerno, Via Ponte Don Melillo, 84084 Fisciano (SA), Italy

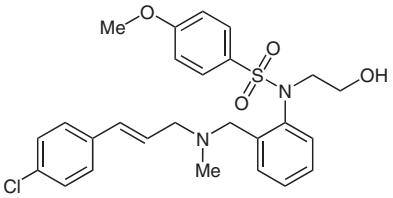
[d] Dipartimento di Chimica Farmaceutica e Tossicologica, Università degli Studi di Napoli Federico II, Via D. Montesano 49, 80131 Napoli, Italy

Received 1 July 2010; revised 16 September 2010

Abstract: Convenient synthetic routes to KN-93, *N*-(2-{[[(2*E*)-3-(4-chlorophenyl)prop-2-enyl](methyl)amino]methyl}phenyl)-*N*-(2-hydroxyethyl)-4-methoxybenzenesulfonamide, a well-known Ca^{2+}/calmoduline-dependent protein kinase II (CaMKII) inhibitor, are described. The methods proposed start from easily available reagents and allow ready preparation of the final compound in moderate overall yields. Most of the synthetic steps proposed were microwave assisted.

Key words: Heck reaction, sulfonamides, enzymes, multicomponent reaction, medicinal chemistry

Calmoduline-dependent protein kinase II (CaMKII) belongs to the superfamily of multifunctional CaM kinases (CaMKs),[1–3] which are involved in inducing or modulating cellular responses to Ca^{2+} signals in cells. CaMKII is a ubiquitous mediator of Ca^{2+}-linked signalling that phosphorylates a wide range of substrates to coordinate and regulate Ca^{2+}-mediated alterations in cellular function. It is an oligomeric serine/threonine kinase that is activated by calcium-bound calmoduline (Ca^{2+}/CaM). Multiple isoforms of human CaMKII are encoded by four closely related yet distinct genes (α, β, χ, and δ) that are spliced differentially to produce more than 20 isoforms of the enzyme.[4,5] CaMKII is expressed throughout the body and it is particularly abundant in brain tissue[4] and in the heart.[6] Given that CaMKII phosphorylates a variety of substrates, this multifunctional serine/threonine kinase has been implicated in regulating many aspects of cellular function in response to Ca^{2+} signalling, including the regulation of carbohydrate, amino acid and lipid metabolism, ion channel/receptors, neurotransmitter synthesis and release, transcription and translation, cytoskeletal organization, and calcium homeostasis.[7–12] Moreover, CaMKII can modulate opioid tolerance via its action on learning and memory.[13] Recently, it has been reported that sufficient inhibition of CaMKII is capable of reversing chronic inflammatory pain,[14] and that CaMKII is involved in cardiac remodelling secondary to pathological stresses such as hypertension or myocardial infarction,[15] as well as endothelial cell pathophysiology.[16] Generally, the studies concerning CaMKII activity are carried out by using a selective CaMKII inhibitor, called KN-93 (Figure 1).[17]



KN-93

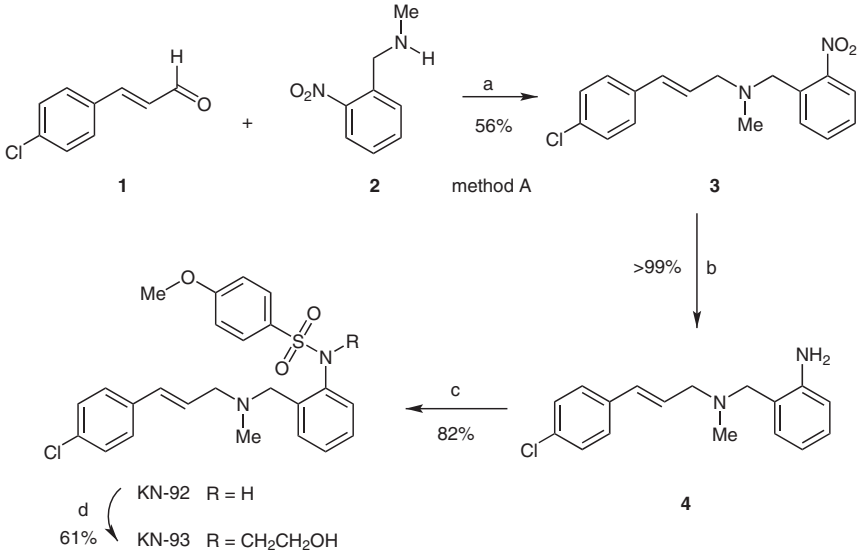
Figure 1 Structure of KN-93

This compound is commercially available in the form of either free amine or its corresponding phosphate salt. In both forms, KN-93 is sold only in small quantities (1 or 5 mg) and it is quite expensive, thus discouraging in vivo pharmacological tests. Therefore, a less expensive access to KN-93 is desirable. To our knowledge, the only synthetic route to KN-93 has been reported in a patent written in Japanese and it consists of six steps which start from 1-(2-nitrophenyl)methanamine and 4-chlorocinnamaldehyde.[18] Here, we present our efforts to develop more convenient and facile synthetic routes to KN-93.

All the synthetic routes herein proposed start from easily available reagents and most of the reactions were carried out under microwave (MW) irradiation.

(*E*)-4-Chlorocinnamaldehyde (**1**) was submitted to direct reductive amination with *N*-methyl-1-(2-nitrophenyl)methanamine (**2**) to give compound **3** in 56% yield (method A; see Scheme 1). This reaction was carried out following a previously reported procedure,[19] shortening the reaction time to 11 minutes by microwave assistance. Reduction of the nitro group with zinc and ammonium chloride[20] gave aniline **4**, which was in turn reacted with 4-methoxybenzenesulfonyl chloride to give KN-92, an

SYNTHESIS 2010, No. 24, pp 4193–4198
Advanced online publication: 14.10.2010
DOI: 10.1055/s-0030-1258298; Art ID: T14310SS

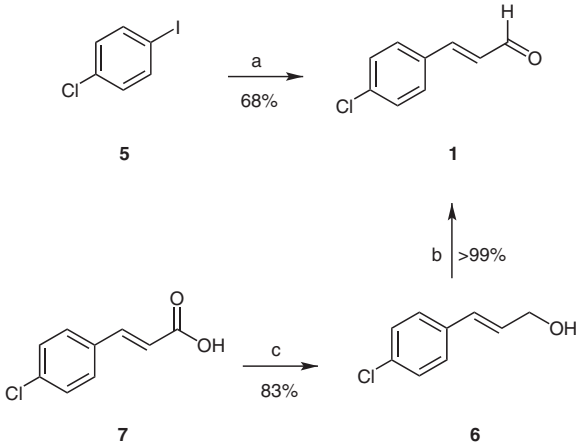


Scheme 1 *Reagents and conditions*: (a) $NaBH_3CN$, MeOH, MW, 110 °C, 11 min; the yield increased to 78% when homemade **2**·HCl was used (see text); (b) Zn, NH_4Cl, H_2O, acetone, reflux, 6 h; (c) 4-$MeOC_6H_4SO_2Cl$, py, anhyd CH_2Cl_2, 0 °C, 3 h; (d) 1,3-dioxolan-2-one, NaOH, DMF, MW, 150 °C, 45 min.

inactive analogue of KN-93.[21] KN-92 was converted into KN-93 by modifying a literature procedure.[22] The ^{1}H NMR aliphatic, cinnamyl, and benzyl signals were unambiguously assigned by COSY experiments. KN-93 was converted into the corresponding phosphate salt by treatment with phosphoric acid.

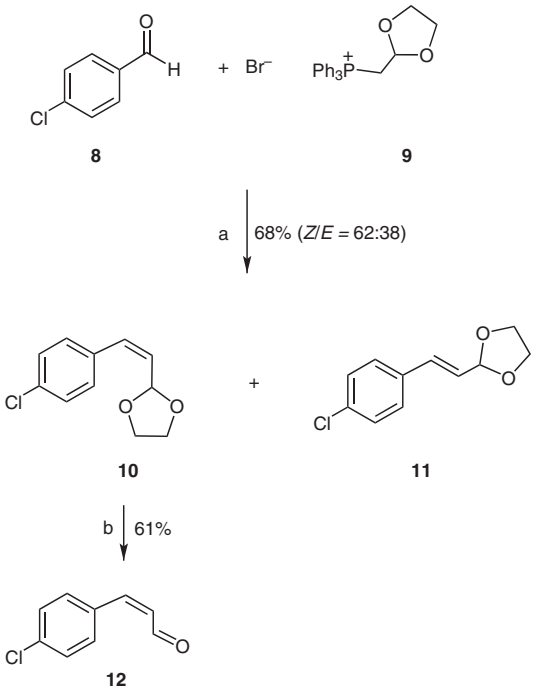
The synthesis of (*E*)-4-chlorocinnamaldehyde (**1**) was achieved from 1-chloro-4-iodobenzene (**5**) modifying a procedure reported in the literature[23] by using microwave irradiation, thus shortening the reaction time (from 3.5 h to 7 min) (Scheme 2).

As an alternative, aldehyde **1** can be obtained in good yields by Swern oxidation[24] of 4-chlorocinnamyl alcohol (**6**), in turn obtained from 4-chlorocinnamic acid (**7**) following a previously reported procedure (Scheme 2).[25] In agreement with the literature,[23,25] both procedures gave the *E*-isomer, as assessed by ^{1}H NMR spectroscopy



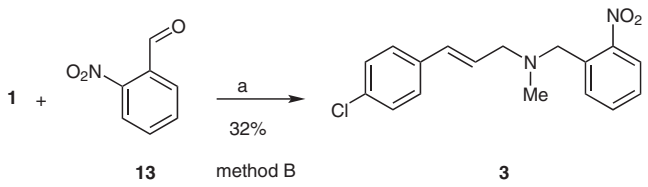
Scheme 2 *Reagents and conditions*: (a) acrolein diethyl acetal, *n*-Bu_4NOAc, K_2CO_3, KCl, $Pd(OAc)_2$, DMF, MW, 140 °C, 7 min; b) $(COCl)_2$, anhyd DMSO, CH_2Cl_2, –60 °C, 30 min; c) $ClCO_2Et$, Et_3N, anhyd THF, $NaBH_4$, –10 °C → r.t.

(J^3 = 16.0 Hz). For comparison, the *Z*-isomer of **1** (compound **12**) was obtained following a previously reported procedure (Scheme 3).[26] Starting from 4-chlorobenzaldehyde (**8**), a mixture of isomeric 1,3-dioxolanes **10** and **11** (*Z*/*E* = 1.6) was obtained by a Wittig-type reaction under phase-transfer conditions using (1,3-dioxolan-2-ylmethyl)triphenylphosphonium bromide (**9**). Dioxolane **10** was isolated by silica gel column chromatography and submitted to mild acidic treatment to give **12** (J^3 = 11.7 Hz). On standing, **12** isomerizes to the more stable isomer **1** ($\Delta E_{Z/E}$ = 13.0 kJ·mol^{-1}, gas phase, 3-21G*//3-21G*).



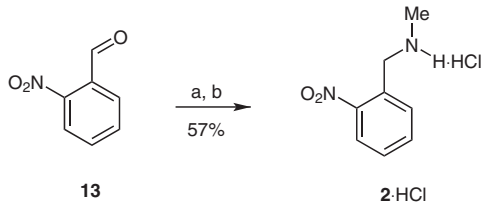
Scheme 3 *Reagents and conditions*: (a) tris(methoxyethoxyethyl)amine, sat. aq K_2CO_3, CH_2Cl_2, 40 °C, 18 h; (b) AcOH, THF, H_2O, r.t., 8 h.

As an alternative for the synthesis of compound **3** (method B; see Scheme 4), a one-pot, microwave, three-component reductive amination of aldehyde **1** and 2-nitrobenzaldehyde (**13**) with methylamine hydrochloride was studied.



Scheme 4 *Reagents and conditions*: (a) $MeNH_2$·HCl, $NaBH_3CN$, MeOH, MW, 90 °C, 8 min.

This route, even though of lower yield, may be preferable to method A described previously (see Scheme 1) because it avoids the use of commercial *N*-methyl-1-(2-nitrophenyl)methanamine (**2**), the purity of which is not high, as assessed by ^{1}H NMR analysis, and also for atom-economy reasons. On the other hand, pure **2**·HCl may be obtained by submitting 2-nitrobenzaldehyde (**13**) to microwave reductive amination with methylamine hydrochloride (Scheme 5).



Scheme 5 *Reagents and conditions*: (a) $MeNH_2$·HCl, $NaBH_3CN$, MeOH, MW, 110 °C, 11 min; (b) aq HCl.

Reaction of **2**·HCl and (*E*)-4-chlorocinnamaldehyde (**1**), as described above, gave compound **3** in 78% yield (see experimental), thus increasing the yield obtained with commercial **2** (56%, see Scheme 1).

In summary, a facile synthesis of KN-93 has been developed. The final product was obtained in moderate yields and short reaction times. With the one exception of a patent written in Japanese,[18] this simple, sustainable synthesis of KN-93 is the first method for the preparation of the target compound. Due to the importance of the product, our approach may contribute to widening the pharmaceutical and biomedical applications of CaMKII inhibitors.

All chemicals were purchased from Sigma-Aldrich or Lancaster and used without further purification. 4-Chlorocinnamyl alcohol (**6**) was obtained as previously reported.[25] The reactions under microwave irradiation were carried out at constant temperature in a CEM Discover BenchMate microwave reactor, with continuous stirring. The temperature was measured and controlled by a built-in infrared detector. Melting points were determined on a Gallenkamp melting point apparatus in open glass capillary tubes and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer Spectrum One FT spectrophotometer; band positions are given in reciprocal centimeters (cm^{-1}). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 300-MHz spectrometer (ASPECT 3000) (or a Bruker AM-500 instrument, where indicated), operating at 300 and 75 MHz (500 and 125 MHz when the Bruker AM-500 instrument was used) for ^{1}H and ^{13}C, respectively, using $CDCl_3$, unless otherwise indicated, as a solvent. Chemical shifts are reported in parts per million (ppm) relative to the residual non-deuterated $CDCl_3$ resonance [δ 7.26 (^{1}H NMR) and δ 77.3 (^{13}C NMR)]. *J* values are given in Hz. EIMS data were recorded on a Hewlett-Packard 6890/5973 MSD gas chromatograph–mass spectrometer at low resolution. ESI$^+$/MS/MS analyses were performed with an Agilent 1100 series LC/MSD trap system VL Workstation. Elemental analyses were performed on a EuroVector Euro EA3000 analyzer. Electrospray ionization (ESI) time-of-flight reflectron experiments were performed on an Agilent ESI-TOF mass spectrometer. Samples were electrosprayed into the TOF reflectron analyzer at an ESI voltage of 4000 V and a flow rate of 200 μL/min. Chromatographic separations were performed on silica gel columns (Kieselgel 60, 0.040–0.063 mm, Merck, Darmstadt, Germany) by flash chromatography using the technique described by Still and co-workers.[27] TLC analyses were performed on precoated silica gel on aluminum sheets (Kieselgel 60 F_{254}, Merck).

(*E*)-4-Chlorocinnamaldehyde (1)
Via a Microwave-Assisted Heck Reaction

A mixture of 1-chloro-4-iodobenzene (**5**; 0.50 g, 2.10 mmol), acrolein diethyl acetal (0.96 mL, 6.30 mmol), *n*-Bu_4NOAc (1.27 g, 4.20 mmol), K_2CO_3 (0.44 g, 3.14 mmol), KCl (0.16 g, 2.10 mmol), and $Pd(OAc)_2$ (0.014 g, 0.063 mmol) in DMF (10 mL) was stirred for 7 min at 140 °C in a microwave reactor under continuous stirring. When the reaction was completed, the mixture was cooled, 2 N HCl (3 mL) was slowly added, and the reaction mixture was stirred at r.t. for 10 min. Then, the mixture was diluted with Et_2O (50 mL) and washed with H_2O (3 × 20 mL). The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure. Purification of the residue by flash chromatography (EtOAc–petroleum ether, 1:9) gave **1** as a white solid; yield: 0.24 g (68%); mp 62–63 °C (Lit.[23] 59–60 °C, Lit.[28] 61–63 °C).

Spectroscopic and spectrometric data were in agreement with the literature.[23]

Via Swern Oxidation[24]

A soln of oxalyl chloride (2.0 mL, 22 mmol) in CH_2Cl_2 (50 mL) was stirred under N_2 atmosphere for 5 min. Then, DMSO (1.7 mL, 22 mmol) dissolved in CH_2Cl_2 (5 mL) was added dropwise to the solution at –60 °C. The reaction mixture was stirred for 3 min and alcohol **6** (3.3 g, 20 mmol) in CH_2Cl_2 (20 mL) was added within 5 min using a syringe; stirring was continued for an additional 15 min. Et_3N (14.0 mL, 100 mmol) was then added and the reaction mixture was allowed to warm to r.t. H_2O (100 mL) was added and the aqueous layer was extracted with additional CH_2Cl_2 (100 mL). The combined organic layers were washed with sat. NaCl soln and dried (anhyd $MgSO_4$). Evaporation of the solvent gave an oil, which was purified on silica gel (EtOAc–hexane, 3:7) to afford **1** as a white solid; yield: 3.28 g (quant).

2-[(*Z*)-2-(4-Chlorophenyl)ethenyl]-1,3-dioxolane (10) and 2-[(*E*)-2-(4-Chlorophenyl)ethenyl]-1,3-dioxolane (11)

To a soln of 4-chlorobenzaldehyde (**8**; 0.14 g, 1.0 mmol) and tris(methoxyethoxyethyl)amine (0.32 g, 1.0 mmol) in CH_2Cl_2 (15 mL), sat. aq K_2CO_3 soln (15 mL) and phosphonium bromide **9** (0.43 g, 1.0 mmol) were added. The mixture was heated at 40 °C for 18 h. The products were extracted with CH_2Cl_2 (3 × 20 mL), and the extracts were washed with H_2O (3 × 10 mL) and then dried (Na_2SO_4). Dioxolanes **10** and **11** were separated by silica gel chromatography (EtOAc–petroleum ether, 0.5:9.5) to give **10** (0.090 g) as a colorless

oil and **11** (0.054 g) as a white solid; combined yield: 68%; *Z*/*E* = 62:38.

^{1}H NMR (compound **10**): δ = 3.8–4.2 (m, 4 H, 2 CH_2O), 5.45 (d, *J* = 7.4 Hz, 1 H, OCHO), 5.73 (dd, *J* = 11.5, 7.4 Hz, 1 H, CHC*H*=CH), 6.75 (d, *J* = 11.5 Hz, 1 H, ArC*H*), 7.31 (s, 4 H, Ar).

^{1}H NMR (compound **11**): δ = 3.9–4.1 (m, 4 H, 2 CH_2O), 5.42 (d, *J* = 5.8 Hz, 1 H, OCHO), 6.14 (dd, *J* = 16.0, 6.0 Hz, 1 H, CHC*H*=CH), 6.73 (d, *J* = 16.0 Hz, 1 H, ArC*H*), 7.26–7.38 (m, 4 H, Ar).

(2*Z*)-3-(4-Chlorophenyl)prop-2-enal (12, see Scheme 3)

To a mixture of THF (15 mL), H_2O (15 mL), and AcOH (7.5 mL), dioxolane **10** (0.29 g, 1.4 mmol) was added. The mixture was stirred at r.t. for 8 h and then concentrated under reduced pressure. The product was extracted with CH_2Cl_2 (3 × 20 mL), and the extracts were washed with sat. aq $NaHCO_3$ soln (2 × 10 mL) and H_2O (10 mL), then dried (Na_2SO_4) and purified by chromatography (EtOAc–petroleum ether, 0.5:9.5) to give **12** as a white solid; yield: 0.14 g (61%); mp 43–44 °C.

IR (KBr): 1678 (CO) cm^{-1}.

^{1}H NMR: δ = 6.21 (dd, *J* = 11.7, 8.1 Hz, 1 H, C*H*CHO), 7.3–7.4 (m, 4 H, Ar), 7.55 (d, *J* = 11.7 Hz, 1 H, ArC*H*), 9.94 (d, *J* = 8.1 Hz, 1 H, CHO).

^{13}C NMR: δ = 129.1 (2 C), 131.1 (2 C), 131.3 (1 C), 132.8 (1 C), 136.2 (1 C), 147.2 (1 C), 192.1 (1 C).

MS (70 eV): *m*/*z* (%) = 166 (25) [M$^+$], 131 (100).

***N*-Methyl-1-(2-nitrophenyl)methanamine Hydrochloride (2·HCl)**

A soln of 2-nitrobenzaldehyde (**13**; 0.30 g, 1.99 mmol), $MeNH_2$·HCl (0.37 g, 5.49 mmol), and $NaBH_3CN$ (0.088 g, 1.39 mmol) in MeOH (4 mL) was stirred for 11 min at 110 °C in a microwave reactor under continuous stirring. When the reaction was completed, 6 N HCl was added dropwise to destroy the excess cyanohydride. The aqueous solution was made alkaline and extracted with EtOAc (2 × 10 mL). The combined extracts were dried (Na_2SO_4) and concentrated under reduced pressure to give 0.33 g of a slightly yellowish oil, which was treated with 2 N HCl, with azeotropic removal of H_2O (abs EtOH–toluene), to give a yellow solid (**2**·HCl). **2**·HCl was recrystallized (EtOH–Et_2O) to give yellow crystals; yield: 0.23 g (57%); mp 141–142 °C.

IR (KBr): 3392 (NH_2^+), 1530 (NO_2) cm^{-1}.

^{1}H NMR [500 MHz, DMSO-d_6 (δ 2.50)]: δ = 2.63 (t, *J* = 5.0 Hz, 3 H, CH_3), 4.41 (t, *J* = 5.5 Hz, 2 H, CH_2), 7.70–7.75 (m, 1 H, Ar), 7.85–7.90 (m, 2 H, Ar), 8.20 (d, *J* = 8.3 Hz, 1 H, Ar), 9.50 (br s, 2 H, NH_2^+).

^{13}C NMR [125 MHz, DMSO-d_6 (δ 39.5)]: δ = 32.6 (1 C), 48.3 (1 C), 125.2 (1 C), 127.2 (1 C), 130.6 (1 C), 133.0 (1 C), 134.2 (1 C), 148.2 (1 C).

ESI-MS: *m*/*z* = 167 [M + H]$^+$; ESI$^+$/MS/MS: *m*/*z* (%) = 136 (100).

(2*E*)-3-(4-Chlorophenyl)-*N*-methyl-*N*-(2-nitrobenzyl)prop-2-en-1-amine (3)

Method A (Scheme 1)

A soln of (*E*)-4-chlorocinnamaldehyde (**1**; 0.23 g, 1.38 mmol), *N*-methyl-1-(2-nitrophenyl)methanamine (**2**; 0.69 g, 4.14 mmol), and $NaBH_3CN$ (0.061 g, 0.97 mmol) in MeOH (5 mL) was stirred for 11 min at 110 °C in a microwave reactor under continuous stirring. When the reaction was completed, 6 N HCl was added dropwise to destroy the excess cyanohydride. The aqueous solution was made alkaline and extracted with Et_2O (2 × 10 mL). The combined extracts were dried (Na_2SO_4) and concentrated under reduced pressure. Purification of the residue by flash chromatography (EtOAc–petroleum ether, 1:9) gave 0.28 g of a yellow solid consisting of a mixture of amine **3** (56%) and its analogue reduced at the vinylic bond (8%), as assessed by ^{1}H NMR spectroscopy. Yields increased to 78% and 11% for the desired product **3** and its reduced analogue, respectively, when homemade **2**·HCl was used.

Method B (Scheme 4)

A soln of (*E*)-4-chlorocinnamaldehyde (**1**; 0.33 g, 1.99 mmol), 2-nitrobenzaldehyde (**13**; 0.68 g, 4.48 mmol), $MeNH_2$·HCl (0.23 g, 3.48 mmol), and $NaBH_3CN$ (0.16 g, 2.49 mmol) in MeOH (15 mL) was stirred for 8 min at 90 °C in a microwave reactor under continuous stirring. When the reaction was completed, 6 N HCl was added dropwise to destroy the excess cyanohydride. The aqueous solution was made alkaline and extracted with Et_2O (2 × 20 mL). The combined extracts were dried (Na_2SO_4) and concentrated under reduced pressure. Purification of the residue by flash chromatography (EtOAc–petroleum ether, 1:9) gave 0.26 g of a yellow solid consisting of a mixture of amine **3** (32%) and its analogue reduced at the vinylic bond (9%), as assessed by ^{1}H NMR spectroscopy. Amine **3** was recrystallized ($CHCl_3$–hexane) to give pale yellow crystals; yield: 0.18 g (29%); mp 66–67 °C.

IR (KBr): 1522 (NO_2) cm^{-1}.

^{1}H NMR: δ = 2.20 (s, 3 H, CH_3), 3.16 (d, *J* = 6.3 Hz, 2 H, CH_2CH), 3.82 (s, 2 H, ArCH_2), 6.20 (dt, *J* = 16.0, 6.5 Hz, 1 H, C*H*CH_2), 6.46 (d, *J* = 16.0 Hz, 1 H, ArC*H*), 7.18–7.33 (m, 4 H, Ar), 7.38 (t, *J* = 7.7 Hz, 1 H, Ar), 7.53 (t, *J* = 7.4 Hz, 1 H, Ar), 7.63 (d, *J* = 7.4 Hz, 1 H, Ar), 7.81 (d, *J* = 8.0 Hz, 1 H, Ar).

^{13}C NMR: δ = 42.7 (1 C), 58.4 (1 C), 60.3 (1 C), 124.6 (1 C), 127.8 (2 C), 128.1 (1 C), 128.3 (1 C), 128.9 (2 C), 131.1 (1 C), 131.5 (1 C), 132.6 (1 C), 133.2 (1 C), 134.9 (1 C), 135.8 (1 C), 150.1 (1 C).

MS (70 eV): *m*/*z* (%) = 316 (3) [M$^+$], 151 (100).

2-{[[(2*E*)-3-(4-Chlorophenyl)prop-2-enyl](methyl)amino]methyl}aniline (4)

To a soln of **3** (0.40 g, 1.26 mmol) in acetone (4 mL), a soln of NH_4Cl (0.16 g, 3.02 mmol) in H_2O (0.6 mL) was added. The mixture was brought to reflux; then, Zn dust (0.41 g) was added in small portions to maintain a moderate reaction. After 6 h, the solution was filtered while hot and the solid was washed with CH_2Cl_2 (2 ×). The combined washings and filtrate were concentrated and the residue was taken up with CH_2Cl_2. The solution was dried (Na_2SO_4) and the solvent was removed under reduced pressure to obtain amine **4** as a yellow solid; yield: 0.36 g (>99%); mp 69–70 °C.

IR (KBr): 3361 (NH_2) cm^{-1}.

^{1}H NMR: δ = 2.23 (s, 3 H, CH_3), 3.16 (d, *J* = 6.6 Hz, 2 H, CH_2CH), 3.57 (s, 2 H, ArCH_2), 4.74 (br s, 2 H, NH_2), 6.25 (dt, *J* = 15.7, 6.6 Hz, 1 H, C*H*CH_2), 6.48 (d, *J* = 16.0 Hz, 1 H, ArC*H*), 6.70 (dd, *J* = 12.6, 7.4 Hz, 2 H, Ar), 7.03 (d, *J* = 6.9 Hz, 1 H, Ar), 7.13 (t, *J* = 7.7 Hz, 1 H, Ar), 7.30 (s, 4 H, Ar).

^{13}C NMR: δ = 42.1 (1 C), 59.5 (1 C), 61.6 (1 C), 115.7 (1 C), 117.8 (1 C), 123.2 (1 C), 127.7 (2 C), 128.3 (1 C), 128.6 (1 C), 128.9 (2 C), 130.7 (1 C), 131.7 (1 C), 133.3 (1 C), 135.8 (1 C), 147.3 (1 C).

MS (70 eV): *m*/*z* (%) = 286 (<1) [M$^+$], 180 (100).

***N*-(2-{[[(2*E*)-3-(4-Chlorophenyl)prop-2-enyl](methyl)amino]methyl}phenyl)-4-methoxybenzenesulfonamide (KN-92)**

To a stirred soln of amine **4** (0.20 g, 0.70 mmol) in anhyd CH_2Cl_2 (2 mL) under N_2 atmosphere, pyridine (0.17 mL, 2.10 mmol) was added at 0 °C. Then, a soln of 4-$MeOC_6H_4SO_2Cl$ (0.17 g, 0.84 mmol) in anhyd CH_2Cl_2 (2 mL) was added. After 3 h, the reaction mixture was concentrated and the residue was taken up with H_2O, made alkaline with $NaHCO_3$, and extracted with CH_2Cl_2 (3 × 10 mL). The combined organic layers were dried (Na_2SO_4) and the solvent was removed under reduced pressure. Purification of the residue by

flash chromatography (EtOAc–petroleum ether, 2:8) gave KN-92 as a colorless oil; yield: 0.26 g (82%).

IR (neat): 1337 (SO_2) cm^{-1}.

^1H NMR: δ = 2.16 (s, 3 H, NCH_3), 3.14 (d, *J* = 6.9 Hz, 2 H, CH_2CH), 3.27 (s, 2 H, $ArCH_2$), 3.74 (s, 3 H, OCH_3), 6.31 (dt, *J* = 15.7, 6.6 Hz, 1 H, $CHCH_2$), 6.50 (d, *J* = 16.0 Hz, 1 H, ArC*H*), 6.63–6.72 (m, 2 H, Ar), 6.92–7.02 (m, 2 H, Ar), 7.16–7.27 (m, 2 H, Ar + NH), 7.28–7.42 (m, 4 H, Ar), 7.53 (d, *J* = 7.7 Hz, 1 H, Ar), 7.58–7.66 (m, 2 H, Ar).

^{13}C NMR: δ = 41.9 (1 C), 55.7 (1 C), 59.7 (1 C), 60.6 (1 C), 114.1 (2 C), 121.1 (2 C), 124.2 (1 C), 126.5 (1 C), 127.5 (1 C), 128.0 (1 C), 128.8 (2 C), 129.0 (1 C), 129.1 (2 C), 130.0 (1 C), 132.5 (1 C), 133.0 (1 C), 133.7 (1 C), 135.3 (1 C), 138.1 (1 C), 163.0 (1 C).

ESI-MS: *m*/*z* = 479 $[M + Na]^+$; ESI+/MS/MS: *m*/*z* (%) = 151 (100).

HRMS (ESI-TOF): *m*/*z* calcd for $C_{24}H_{25}ClN_2O_3S$: 457.1347 ($[M + H]^+$); found: 457.1345.

_N_-(2-{[[(2_E_)-3-(4-Chlorophenyl)prop-2-enyl](methyl)amino]methyl}phenyl)-_N_-(2-hydroxyethyl)-4-methoxybenzenesulfonamide (KN-93)

A soln of KN-92 (0.30 g, 0.66 mmol), 1,3-dioxolan-2-one (0.26 g, 2.96 mmol), and NaOH (0.024 g, 0.59 mmol) in DMF (2 mL) was stirred for 45 min at 150 °C in a microwave reactor under continuous stirring. When the reaction was completed, the reaction mixture was diluted with H_2O (7 mL) and extracted with Et_2O (2 × 10 mL). The combined extracts were washed with brine (3 × 5 mL), dried (Na_2SO_4), and concentrated under reduced pressure. Purification of the residue by flash chromatography (EtOAc–petroleum ether, gradient 1:9 to 1:1) gave KN-93 as a white solid; yield: 0.20 g (61%); mp 59–60 °C.

IR (KBr): 3430 (OH), 1344 (SO_2) cm^{-1}.

^1H NMR (COSY): δ = 2.22 (s, 3 H, NCH_3), 2.88 (d, *J* = 12.2 Hz, 1 H, ArC*H*H), 3.05–3.15 (m, 1 H), 3.22 (dd overlapping br m at 3.25–3.30, *J* = 13.5, 7.4 Hz, 1 H, CH=CHC*H*H), 3.25–3.30 (br m overlapping dd at 3.22, 1 H), 3.43 (dd, *J* = 13.5, 5.8 Hz, 1 H, CH=CHCH*H*), 3.60–3.75 (m, 1 H), 3.88 (s, 3 H, OCH_3), 4.05–4.15 (m, 1 H), 4.99 (d, *J* = 12.2 Hz, 1 H, ArCH*H*), 6.35–6.48 (m, 2 H, Ar + CH=C*H*CH_2), 6.54 (d, *J* = 16.0 Hz, 1 H, C*H*=$CHCH_2$), 6.90–7.00 (m, 2 H, Ar), 7.17 (td, *J* = 7.6, 1.7 Hz, 1 H, Ar), 7.20–7.35 (m, 4 H, Ar), 7.35–7.45 (m, 2 H, Ar), 7.50–7.60 (m overlapping br s at 7.58, 2 H, Ar), 7.58 (br s overlapping m at 7.50–7.60, 1 H, OH).

^{13}C NMR: δ = 41.9 (1 C), 55.8 (1 C), 56.0 (1 C), 59.3 (1 C), 59.5 (1 C), 60.8 (1 C), 114.1 (2 C), 126.4 (1 C), 127.6 (1 C), 128.1 (2 C), 128.2 (1 C), 128.8 (4 C), 130.8 (2 C), 133.1 (1 C), 133.4 (1 C), 133.7 (1 C), 135.5 (1 C), 139.7 (1 C), 140.3 (1 C), 164.4 (1 C).

ESI-MS: *m*/*z* = 523 $[M + Na]^+$; ESI+/MS/MS: *m*/*z* (%) = 151 (100).

_N_-(2-{[[(2_E_)-3-(4-Chlorophenyl)prop-2-enyl](methyl)amino]methyl}phenyl)-_N_-(2-hydroxyethyl)-4-methoxybenzenesulfonamide Phosphate (KN-93·H_3PO_4)

To a soln of KN-93 (0.13 g, 0.26 mmol) in MeOH (0.5 mL), a soln of 85% H_3PO_4 (56 μL) in MeOH (0.5 mL) was added at r.t. Then, the reaction mixture was concentrated and the oil obtained was crystallized (MeOH–Et_2O) to give white crystals; yield: 0.12 g (77%); mp 113–114 °C.

IR (KBr): 3402 (OH), 1346 (SO_2) cm^{-1}.

^1H NMR [COSY, CD_3OD (δ 3.30)]: δ = 2.74 (s, 3 H, NCH_3), 3.15–3.30 (m, 1 H), 3.30–3.35 (m, 1 H), 3.83 (dt, *J* = 12.3, 4.0 Hz, 1 H), 3.89 (s, 3 H, OCH_3), 3.90–4.00 (m, 2 H, ArC*H*H + 1 H), 4.00–4.15 (m, 2 H), 5.37 (d, *J* = 12.9 Hz, 1 H, ArCH*H*), 6.48 (dt, *J* = 15.8, 7.5 Hz, 1 H, CH=C*H*CH_2), 6.58 (m, 1 H, Ar), 6.93 (d, *J* = 15.8 Hz, 1 H, C*H*=$CHCH_2$), 7.05–7.15 (m, 2 H, Ar), 7.30–7.70 (m, 9 H, Ar).

^{13}C NMR [CD_3OD (δ 47.8)]: δ = 39.5 (1 C), 54.2 (1 C), 55.2 (1 C), 56.4 (1 C), 58.7 (1 C), 59.2 (1 C), 114.3 (2 C), 118.7 (1 C), 126.8 (1 C), 127.9 (1 C), 128.6 (4 C), 129.2 (1 C), 130.8 (2 C), 131.0 (1 C), 133.0 (1 C), 133.7 (1 C), 134.3 (1 C), 134.5 (1 C), 138.7 (1 C), 140.1 (1 C), 164.3 (1 C).

HRMS (ESI-TOF): *m*/*z* calcd for $C_{26}H_{29}ClN_2O_4S$: 501.1609 ($[M + H]^+$); found: 501.1610.

Acknowledgment

This work was accomplished thanks to financial support from Progetto Strategico Regione Puglia (PS131) entitled 'Realizzazione di molecole a basso peso molecolare attive sulle CAM chinasi da utilizzare nelle procedure diagnostiche riguardanti le strategie di terapia innovativa nelle patologie umane associate ad iperproliferazione cellulare'.

References

(1) Soderling, T. R. *Trends Biochem. Sci.* **1999**, *24*, 232.
(2) Hook, S. S.; Means, A. R. *Annu. Rev. Pharmacol. Toxicol.* **2001**, *41*, 471.
(3) Soderling, T. R.; Stull, J. T. *Chem. Rev.* **2001**, *101*, 2341.
(4) Hudmon, A.; Schulman, H. *Biochem. J.* **2002**, *364*, 593.
(5) Hudmon, A.; Schulman, H. *Annu. Rev. Biochem.* **2002**, *71*, 473.
(6) Griffith, L. C. *Cell* **2008**, *133*, 397.
(7) Hanson, P. I.; Schulman, H. *Annu. Rev. Biochem.* **1992**, *61*, 559.
(8) Braun, A. P.; Schulman, H. *Annu. Rev. Physiol.* **1995**, *57*, 417.
(9) Soderling, T. R. *Adv. Second Messenger Phosphoprotein Res.* **1995**, *30*, 17.
(10) Schulman, H.; Braun, A. In *Calcium as a Cellular Regulator*; Crafoli, E.; Klee, C., Eds.; Oxford University Press: New York, **1999**, 311–343.
(11) Heist, E. K.; Schulman, H. *Cell Calcium* **1998**, *23*, 103.
(12) Soderling, T. R.; Chang, B.; Brickey, D. *J. Biol. Chem.* **2001**, *276*, 3719.
(13) Wang, Z. J.; Tang, L.; Xin, L. *Eur. J. Pharmacol.* **2003**, *465*, 199.
(14) Luo, F.; Yang, C.; Chen, Y.; Shukla, P.; Tang, L.; Wang, L. X.; Wang, Z. J. *J. Pharmacol. Exp. Ther.* **2008**, *325*, 267.
(15) McKinsey, T. A. *Cardiovasc. Res.* **2007**, *73*, 667.
(16) Cai, H.; Liu, D.; Garcia, J. G. N. *Cardiovasc. Res.* **2008**, *77*, 30.
(17) Sumi, M.; Kiuchi, K.; Ishikawa, T.; Ishii, A.; Hagiwara, M.; Nagatsu, T.; Hidaka, H. *Biochem. Biophys. Res. Commun.* **1991**, *181*, 968.
(18) Hidaka, H.; Ishikawa, T.; Inoe, T.; Morita, T.; Nakano, H.; Yuasa, M.; Naito, K.; Sakuma, O.; Kidokoro, S.; Tachikawa, M. Jpn. Kokai Tokkyo Koho JP 06293730, **1994**; *Chem. Abstr.* **1995**, *122*, 314463.
(19) Bruno, C.; Carocci, A.; Catalano, A.; Cavalluzzi, M. M.; Corbo, F.; Franchini, C.; Lentini, G.; Tortorella, V. *Chirality* **2006**, *18*, 227.
(20) Boyer, J. H.; Alul, H. *J. Am. Chem. Soc.* **1959**, *81*, 2136.
(21) Hendrickson, J. B.; Bergeron, R. *Tetrahedron Lett.* **1970**, 345.
(22) Bogdal, D. In *Microwave-Assisted Organic Synthesis*, Vol. 25; Bäckvall, J.-E.; Baldwin, J. E.; Williams, R. M., Eds.; Elsevier: Oxford, **2005**, 55–56.
(23) Battistuzzi, G.; Cacchi, S.; Fabrizi, G. *Org. Lett.* **2003**, *5*, 777.
(24) Mancuso, A. J.; Huang, S.-L.; Swern, D. *J. Org. Chem.* **1978**, *43*, 2480.

(25) Penjiševic, J.; Šukalovic, V.; Andrić, D.; Kostić-Rajačić, S.; Šoškic, V.; Roglić, G. *Arch. Pharm. Chem. Life Sci.* **2007**, *340*, 456.
(26) Daubresse, N.; Francesch, C.; Rolando, C. *Tetrahedron* **1998**, *54*, 10761.
(27) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.
(28) Olsen, D. K.; Torian, B. E.; Morgan, C. D.; Braun, L. L. *J. Org. Chem.* **1980**, *45*, 4049.