Communication

Efficient TBD-Catalyzed Synthesis of Capsaicin-like Molecules

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Abstract

Capsaicin, the natural compound responsible for the spicy flavor in pepper fruits, has shown antioxidant and weight-reductive properties, cardiovascular benefits, anti-carcinogenic activity and recently pain relief effects. Studies on a series of synthetic capsacinoids indicated that the length of alkyl chain affected piquancy, with the possibility of obtaining non-pungent capsaicin analogues of potential interest for clinical application perspectives. We report here on a new efficient procedure for the synthesis of capsaicin analogues, based on the aminolysis of vanillylamine with methyl esters showing structural changes in alkyl chain, catalyzed by the inexpensive and non-toxic triazabicyclo[4.4.0]dec-5-ene (TBD). It is a valid alternative to the known synthesis of this class of molecules, based on chemical reactions involving acyl chloride or lipase–catalyzed procedure. The TBD-method is improved by microwave irradiation, which allows to carry out the reaction under solvent-free conditions and to obtain high yields of products. This is an effective and eco-friendly approach for the access to a wide library of capsaicinoids for further biological evaluations, as well as for a large-scale production.

Keywords: aminolysis, capsaicinoids, microwave-assisted synthesis, solvent-free, TBD catalyst, vanillylamine.

Introduction

Capsaicin is a natural compound structurally characterized by the presence of an aromatic unit, an amide group and a lipophilic alkyl chain (Figure 1). The molecule is responsible for the spicy flavor in chili peppers. In pure form it has a very high value of piquancy, corresponding to a value of about 15,000,000-16,000,000 on the Scoville scale, which measures the pungency of chili peppers by an organoleptic test. It is commonly used as an animal repellent and as well as a pesticide and insecticide. Recently this natural molecule has attracted interest for its biological properties and its potential in some medical applications has been evaluated.

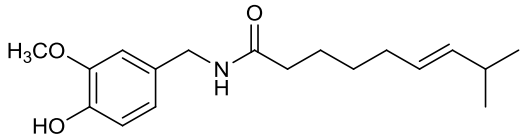


Figure1. Molecular structure of capsaicin (=(*E*)-N-(4- hydroxy-3-methoxybenzyl)-8-methyl-6-nonenamide).

The receptor for capsaicin was found to be the transient receptor potential vanilloid subfamily member 1 (TRPV1). It is found on key sensory afferents and is involved in the modulation of nociceptive inputs to spinal cord and brain stem centers, as well as the integration of diverse painful stimuli. For this reason capsaicin is unique among naturally occurring irritant compounds because the initial neuronal excitation evoked is followed by a long-lasting refractory period, during which the previously excited neurons are no longer responsive to a broad range of stimuli. This process known as defunctionalisation has been exploited for therapeutic use of capsaicin in various painful conditions.[1-3]

Capsaicin was observed to alter the expression of several genes involved in cancer cell survival, growth arrest, angiogenesis and metastasis. Recently it has been found that capsaicin targets multiple signaling pathways, oncogenes and tumor-suppressor genes in various types of cancer models, including colon adenocarcinoma, pancreatic cancer, hepatocellular carcinoma, prostate cancer, breast cancer, and many others. [4] The exact mechanism whereby capsaicin causes cell death in cancer cells is not completely elucidated, but it involves intracellular calcium increase, reactive oxygen species generation, disruption of mitochondrial membrane transition potential and activation of transcription factors such as NFκB (Nuclear Factor kappa-light-chain-enhancer of activated B cells) and STATS (Signal Transducers and Activators of Transcription). Recently, a role for the AMP-dependent kinase (AMPK) and autophagy pathways in capsaicin-triggered cell death has been proposed.[5] It was observed that capsaicin is able to induce apoptosis and inhibit adipogenesis. Epidemiological data showed that consumption of foods containing capsaicin is

associated with a lower prevalence of obesity. This evidence supports a role of capsaicin as an anti-obesity agent.[6]

It has been demonstrated that the bioactivity of natural capsaicin depends greatly on the presence of the OH phenolic group in *para* position which is capable of acting as hydrogen bond donor and the length of the alkyl chain, able to affect the pungent property.[7] It is really interesting that non-pungent capsaicin analogues are also able to activate TRPV1,[8] without producing irritant responses when applied to the skin surface. Due to the limited use of capsaicin because of the irritation caused by its pungency, the production of non-pungent capsacinoids is of particular interest for clinical application perspectives.

The known methods for the synthesis of capsaicin analogues are essentially two: i) a chemical method,[7] where the amide bond is built by a nucleophilic substitution carried out using an acyl chloride.[9] This 'classical' method for the access to capsacinoids presents some disadvantages, given by the use of a very reactive species like the acyl chloride which provides no selectivity, by the reaction conditions which involve oil bath heating for overnight and the production of hydrochloride acid acting as an environment pollutant if the process is applied on industrial scale, and by quite low yields (about 53%);[9] ii) an enzymatic method based on a lipase-catalyzed reaction between an amine and an acyl electrophile,[7] ,able to work at 45°C in 2-methyl-2-butanol with conversions higher than 83% ,[10] in oleic acid to produce vanillyloleamide. [11]

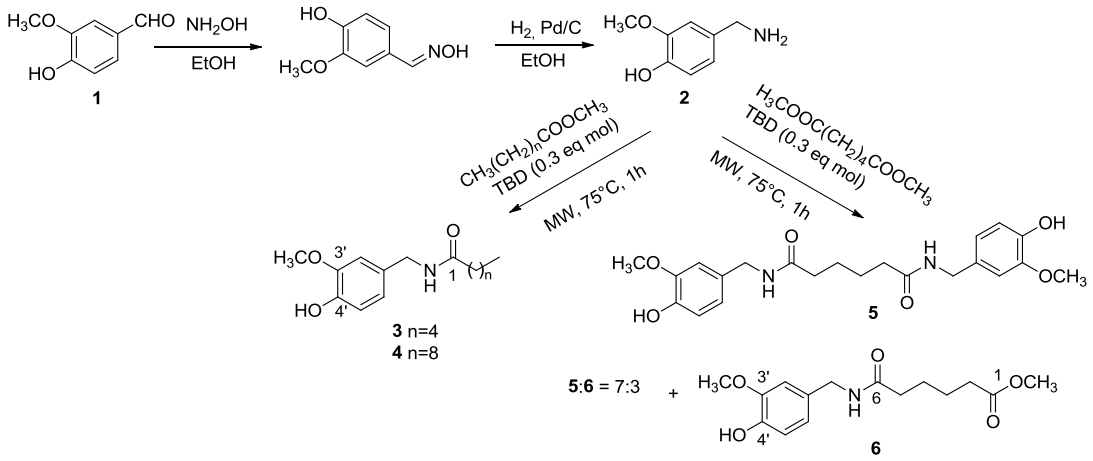
The guanidine base 1,5,7-triazabicyclo[4.4.0]dec-5-ene (TBD) is a commercially available, inexpensive and non-toxic compound. An efficient method for the TBD-catalyzed synthesis of an amide bond by direct aminolysis of esters was reported. The reaction of esters with primary or secondary amine occurs in high yields by heating at 75 °C in solvent-free conditions for twelve hours. [12] The mechanism proposed for TBD catalyst was investigated by a theoretical approach.[13]

We report here on the first method for obtaining capsaicin analogues based on TBD-catalyzed aminolysis of ester, which also represents a new example of microwave-assisted synthesis of this class of molecules.

Results and discussion

In the synthesis of capsaicin analogues with structural changes in the alkyl chain, the building of amide bond involved the reaction of methyl ester and vanillylamine **2** (Scheme I). Starting from the commercial hydrochloride salt, vanillylamine can be obtained by the reaction with one molar equivalent of aqueous sodium hydroxide or saturated aq. $NaHCO_3$ solution monitoring pH values till neutrality and next extraction with dichloromethane, however the recovery was low. Free amine

2 was obtained in poor yield also by treatment with solid aluminium trioxide in ethanol and followed by filtration and evaporation. Even the procedure involving the use of a strong excess of *N*,*N*-diisopropylethylamine[11] was not effective in recovering vanillylamine in high yield. The best procedure in terms of final yield involved the cheap vanilline (=4-hydroxy-3-methoxybenzaldehyde, **1**), which was converted into the corresponding oxime by reaction with hydroxylamine and subsequently reduced by Pd/C-catalyzed hydrogenation giving the desired product **2** by a simple filtration.



Scheme I. Synthesis of capsacinoids **3**-**6**. Numbering is for convenience.

As model compounds in the homologous series of capsacinoids, products **3** and **4** were synthesized by reaction of amine **2** with methyl hexanoate and methyl decanoate, respectively under MW irradiation at 75°C for one hour, in the presence of TBD used in 0.3 molar equivalent as previously reported, [12] which resulted the best catalytic amount also in this procedure (Scheme I). The products were purified by preparative TLC and structurally characterized by NMR and ESI-MS analysis.

Product **3** was previously reported as a component of a series of capsaicinoids with increasing lateral chain lengths (C2-C16) by the reaction of **2** with hexanoyl chloride in a 61% yield,[14] with a yield increasing to 93% using a water/ chloroform system [15] and in a modest yield (23%) by a solid supported-lipase catalyzed amidation starting from methyl hexanoate at pH 9, heating at 70°C for 72 hours.[11]

Dimethyl adipate was selected as a difunctionalized reagent in the presence of two molar equivalent of vanillylamine. Under MW irradiation at 75°C for one hour, **5** and **6** were obtained in 7:3 ratio as established by ^{1}HNMR spectrum of the crude mixture and a global yield of about 88%. A better selectivity in favor of product **5** was observed by using a higher amount of TBD under the same reaction conditions.

We have efficiently recovered TBD through chromatographic purification of the crude reaction mixtures by preparative TLC to obtain pure products. After identification of the structure by NMR analysis, it could be used again as catalyst of further synthesis of the desired products.

In conclusion, capsaicin analogues different in alkyl chain moiety were first obtained by a TBD-catalyzed aminolysis of esters, reducing time reactions and providing high yields by the replacement of conventional heating with microwave irradiation. Reduced time reactions, solvent-free conditions and catalyst recycling make the procedure efficient and eco-friendly. The method here reported can be successfully exploited for the access to wide libraries of capsaicinoids for further biological evaluations, as well as for a large-scale production. In the latter application, a further improvement can come by the use of a commercially available polymer supported-TBD form.

Materials and Methods

General Methods

The reagents (Sigma Aldrich) and solvents were used in chemical reactions. Microwave-assisted reactions were carried out in sealed vessels using the mono-mode CEM Discover apparatus. All evaporations were carried out at room temperature at reduced pressure. The reaction yields were calculated on the products after chromatographic purification. Thin layer chromatography (TLC) was carried out on Merck Kieselgel 60 PF_{254} and flash-chromatography (FC) on Merck silica gel 60 (15÷25 μm), preparative thin layer chromatography was realized on 20 x 20 cm Merck Kieselgel 60 F_{254} 0.5 mm plates. Infrared spectra were recorded by using a FT-IR Tensor 27 Bruker spectrometer (Attenuated Transmitter Reflection, ATR configuration) at 1 cm^{-1} resolution in the absorption region 4,000– 1,000 cm^{-1}. A thin solid layer is obtained by evaporation of methanol solution of the sample. The instrument was purged with a constant dry air flux and clean ATR crystal as background was used. Spectra processing was made using Opus software package. NMR spectra were recorded by a Bruker-Avance 400 spectrometer by using a 5 mm BBI probe with 90° proton pulse length of 8 μs at a transmission power of 0 db; ^{1}H at 400 MHz and ^{13}C at 100 MHz in $CDCl_3$, δ values in ppm, in $CDCl_3$ relative to the solvent residual signals δ_H = 7.25 and δ_C = 77.00 ppm, *J* values in Hz. Structural assignments are from heteronuclear single quantum correlation (HSQC) and heteronuclear multiple bond correlation (HMBC) experiments. Electrospray ionization (ESI)-MS mass spectra were recorded using a Bruker Esquire-LC spectrometer equipped with an electrospray ion source used in positive or negative ion mode by direct infusion of a methanolic solution of the sample, under the following conditions: source temperature 300°C, drying gas N_2, 4 L/min, scan range 100–1,000 *m/z*.

*Preparation of vanillylamine (**2**)*

A solution containing vanilline (0.76 g, 5 mmol), hydroxylamine hydrochloride (0.7 g, 10 mmol) sodium acetate trihydrate (1.36 g, 10 mmol) methanol (27 mL) and water (3 mL) was stirred at room temperature for 3 h. After the completion of reaction, the solvent was removed under reduced pressure and the resulting residue was dissolved in ethyl acetate (30 mL), and then washed with water. The organic phase was dried over anhydrous Na_2SO_4 and the solvent was evaporated *in vacuo* to obtain oxime as a grey powder (0.8 g, 96%). It was dissolved in ethanol (50 mL), added of a catalytic amount of 10% Pd/C and treated with a H_2 flow at 1 atm at room temperature for 4 h. The mixture was filtered and evaporated under reduced pressure to obtain **2** in quantitative yield (0.73 g,). The structure was confirmed by ^{1}HNMR analysis.

*General Synthesis of capsaicinoids **3** and **4**.*

Vanillylamine (**2**, 24 mg, 0.14 mmol)) was added to methyl hexanoate (0.020 mL, 0.12 mmol) and TBD (6 mg, 0.042 mmol). The mixture was MW-irradiated at 75 °C for 1h, then subjected to preparative TLC (CH_2Cl_2/MeOH 9:1), recovering the UV-detected band at R_f 0.8. Pure product **3** obtained by CH_2Cl_2 washing and filtration (27 mg). Similar procedure using methyl decanoate gave product **4**

*Data of N-(4-hydroxy-3-methoxybenzyl)hexanamide (**3**)* .Yield: 91%. IR (neat) ν_{max} 3300w, 2960s, 1640s cm^{-1}. ^{1}HNMR (400MHz, $CDCl_3$): δ=6.84 (d, *J*=8Hz, 1H, H-5'), 6.79 (brs, 1H, H-2'), 6.74 (brd, *J*=8Hz, 1H, H-6'), 5.69 (brs, 1H, NH), 4.34 (d, *J*=5.5Hz, 2H, CH_2-N), 3.85 (s, 3H, OCH_3), 2.18 (t, *J*=6.7 Hz, 2H, CH_2-CO), 1.63 (quint, *J*=6.7Hz, 2H, 2H-3), 1.29 (m, 4H, 2H-4 and 2H-5), 0.87 (t, *J*=6.8Hz, 3H, 3H-10). ESI(-)MS: *m/z* 250 $[M-H]^-$; ESIMS/MS (250) : *m/z* 114.

*Data of N-(4-hydroxy-3-methoxybenzyl)decanamide (**4**).* Yield: 90%. ^{1}HNMR (400MHz, $CDCl_3$): δ=6.84 (d, *J*=8Hz, 1H, H-5'), 6.79 (brs, 1H, H-2'), 6.75 (brd, *J*=8Hz, 1H, H-6'), 5.64 (brs, 1H, NH), 4.34 (d, *J*=5.4Hz, 2H, CH_2-N), 3.86 (s, 3H, OCH_3), 2.18 (t, *J*=6.7 Hz, 2H, CH_2-CO), 1.63 (m, 2H, 2H-3), 1.29 (m, 12H, 2H-4 ÷2H-9), 0.87 (t, *J*=6.8Hz, 3H, 3H-10). ^{13}CNMR ($CDCl_3$): δ= 172.9 (CONH), 120.3 (C-6'), 114.0 (C-5'), 108.4 (C-2'), 55.6 (OCH_3), 43.7 (CH_2N), 34.8 (C-2), 29.6 (C-4÷ C-9), 25.1 (C-3), 15.7 (C-10). ESI(-)MS: *m/z* 306 $[M-H]^-$; ESIMS/MS (250) : *m/z* 170.

*Synthesis of **5** and **6**.*

Vanillylamine (**2**, 44 mg, 0.28 mmol) was added to dimethyl adipate (0.022 mL, 0.14 mmol) and TBD (11.7 mg, 0.084 mmol). The mixture was MW-irradiated at 75 °C for 1h, then subjected to preparative TLC (CH_2Cl_2/MeOH 9:1).The UV-detected band at Rf 0.7 was recovered by extraction with CH_2Cl_2, the solvent evaporated *in vacuo*, obtaining a residue (47mg), which showed the presence of **5** and **6** in 7:3 ratio, respectively as deduced by ^{1}HNMR spectrum.

*Data of N^1,N^6-bis(4-hydroxy-3-methoxybenzyl)adipamide (**5**).* ^{1}HNMR (400MHz, $CDCl_3$):δ= 6.87 (d, J=8Hz, 2H, H-5'), 6.79 (s, 2H, H-C-2'), 6.75(d, *J*=8Hz, 2H, H-C-6'), 4.32 (d, *J*=5.5Hz, 4H, CH_2-N), 3.87 (s, 6H, OCH_3), 2.15 (t, *J*=6.7Hz, 4H, CH_2-CO), 1.58 (quint, *J*=6.7 Hz, 4H, H_2C-3). ESI(+)-MS:*m/z* 439 $[M+Na]^+$; ESIMS/MS (439) : *m/z* 424, 303.

*Data of methyl 6-((4-hydroxy-3-methoxybenzyl)amino)-6-oxohexanoate (**6**).*^{1}HNMR (400MHz, $CDCl_3$):δ= 6.87 (d, *J*=8Hz, 1H, H-5'), 6.80 (brs, 1H, H-2'), 6.75 (brd, *J*=8Hz, 1H, H-6'), 5.60 (brs, 1H, NH), 4.34 (d, *J*=5.6 Hz, 2H, CH_2-N), 3.87 (s, 3H, OCH_3), 3.65 (s, 3H, $COOCH_3$), 2.33 (t, *J*=6.7 Hz, 2H, 2H-5), 2.21 (t, *J*=6.7 Hz, 2H, 2H-2), 1.67 (m, 4H, 2H-3 and 2H-4). ^{13}CNMR ($CDCl_3$, from HMBC correlations): δ=173.8 (COO), 172.0 (CON), 146.0(C-3'), 145.8(C-4'), 131.5(C-1'), 120.4 (C-6'), 110.7 (C-2'), 57.1 (OCH_3), 24.9 and 24.1 (C-3 and C-4).

Recovery of TBD catalyst.

TBD was efficiently recovered by the same preparative TLC used for the purification of the product **5/6**; it was easily separated due to its different polarity (at R_f 0.2 from a CH_2Cl_2/MeOH 9:1 elution). ^{1}HNMR analysis in $CDCl_3$ confirmed the TBD structure for the collected fraction, obtaining a completely overlapping spectrum with the commercial sample. The recovered TBD was used as a catalyst in further production of **3** or **4**.

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Author Contributions: A.Defant designed the synthesis, M.Demozzi and A.Defant carried out the reactions and products purification, I.Mancini performed NMR analysis and analyzed spectroscopic and MS data, A.Defant, M.Demozzi and I.Mancini wrote the paper.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

The following abbreviations are used in this manuscript:

ESI-MS	Electrospray-Mass spectrometry
MW	Microwave
PLC	Preparative liquid chromatography
TBD	1,5,7-triazabicyclo[4.4.0]dec-5-ene

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