Drug Design

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The 1,2,3-Triazole Ring as a Peptido- and Olefinomimetic Element: Discovery of Click Vanilloids and Cannabinoids**

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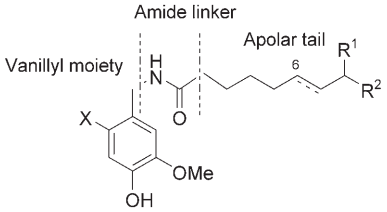
Over the past few years, the 1,3-dipolar cycloaddition of azides and alkynes has emerged as an important "stitching" maneuver to connect structural units through a readily introduced permanent link endowed with unparalleled chemical and biological stability.[1] Although apparently metabolically inert, the 1,2,3-triazole ring could, in principle, be biologically visible, as it features a combination of H-bond donor and acceptor sites capable of mimicking the hydrogen-bonding acidity and basicity of a peptide bond.[2] As the 1,2,3-triazole ring is not susceptible to hydrolytic cleavage or redox modification,[1] potential advantages of this system over other types of peptidomimetics exist, and preliminary evidence to justify the systematic scrutiny of this issue has been reported. Thus, X-ray crystallographic analysis of the HIV protease bound to the amide inhibitor amprenavir and of the same protease bound to two 1,2,3-triazole analogues showed excellent overlap of the binding mode of the amide moiety with that of the 1,4-substituted triazole ring.[3] Similarly, the immunostimulating activity of αGal-Cer, an analogue of the marine natural product α-galactosylceramide, was relatively insensitive to the replacement of the amide moiety with a triazole unit.[4] These observations with respect to the triazole analogues of amprenavir and αGal-Cer suggest that the amide bond and the 1,2,3-triazole ring are potentially bioequivalent. However, the significance of these findings is somewhat undermined by the paucity of information available on the relevance of the amide bond for the bioactivity of both leads, and/or by the additional modifications carried out on their structure. Furthermore, the multiple recognition domains of structurally complex molecules such as amprenavir and αGal-Cer could "dilute" the effect of the isosteric modification in terms of binding to a macromolecular target. Finally, no information has been reported on the ability of the amide-to-triazole isosteric exchange to sustain reversal of activity or modulation of target selectivity, while the effect of the triazole substitution pattern on its amidomimetic properties has not yet been investigated.

To clarify these points, we investigated the effect of amide-to-triazole point mutations in structurally unsophisticated compounds whose peptide bond is critical for activity. Although there is no shortage of candidates to address this issue, few can rival capsaicinoids in terms of the simplicity of the pharmacophore (a vanillyl group linked to an aliphatic chain by an amide bond) and the pleiotropy of the target.[5] Indeed, the relevance of the amide bond for the pungency of capsaicin (**1a**) is one of the oldest observations in the realm of



		X	R^1	R^2
1a	$\Delta^{6,7}$	H	Me	Me
1b	--	H	H	Me
1c	--	I	H	Me
1d	--	H	H	

structure–activity relationships. Only the thiourea group has been identified in modern studies as an equipotent bioisosteric replacement.[6] Furthermore, the discovery that certain fatty-acid-derived capsaicinoids can interact not only with the vanilloid receptor (TRPV1)[7] but also with proteins of the endocannabinoid system (mainly CB_2 and FAAH)[8] has expanded the range of known biomolecules capable of recognizing the key amide linker of these compounds. As the amide linker is a major site of metabolic lability of the capsaicinoids,[9] its replacement with hydrolytically stable groups could both alter its activity towards macromolecules that recognize capsaicinoids and dramatically improve their pharmacokinetic profile.

Therefore, the vanilloid and cannabinoid profiles of the TRPV1 agonist nonivamide (synthetic capsaicin, **1b**)[10] and the TRPV1 antagonist 6′-iodononivamide (**1c**)[11] were compared with those of the corresponding 1,4- and 1,5-triazole analogues **4a**,**b** and **5a**,**b**. These compounds were prepared by combining a pivaloyl-protected vanillazide (**3a** or **3b**) with

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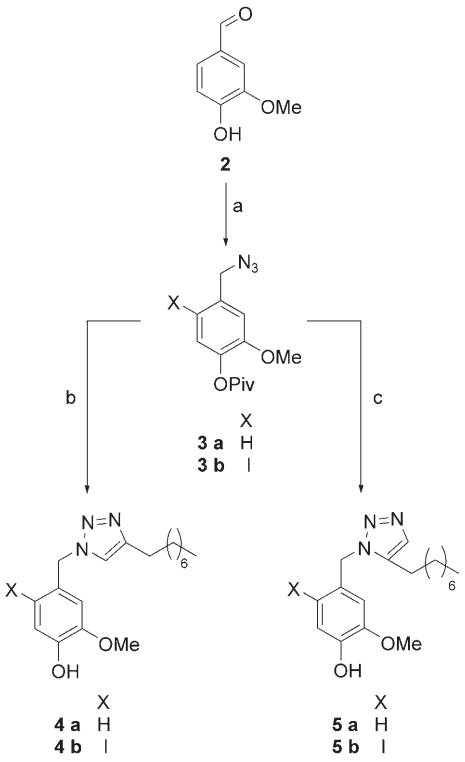
commercial 1-decyne by click chemistry under regiocomplementary copper(I) or ruthenium(II) catalysis[12] followed by deprotection with a base (Scheme 1).[13] Surprisingly, a complete lack of regioselectivity was observed in the ruthenium(II)-catalyzed coupling, but the mixtures of isomeric vanillyl triazoles (**4a**/**5a**) and iodovanillyl triazoles (**4b**/**5b**) could be resolved by preparative HPLC on silica gel, and the final compounds were purified further by crystallization.[13] A remarkable affinity was retained in the agonistic and antagonistic activity of these capsaicinoid analogues towards TRPV1 (Table 1), whereby the *anti* (1,4) adducts (**4a**, **4b**) were found to be more potent than the corresponding *syn* (1,5) regioisomers (**5a**, **5b**), especially in terms of TRPV1 antagonism. The reversal of activity by aromatic iodination and the dependence of the activity on the substitution pattern of the triazole ring suggest that these compounds interact in a specific way with TRPV1 and show convincingly that the triazole ring serves as a good mimic of an amide bond. Furthermore, the recognition of the triazole ring as if it were an amide bond is functional and translated into a biological event.



Scheme 1. Reagents and conditions: a) 1. Et_3N, PivCl, THF, 98%; for **3a**: 2. $NaBH_4$, EtOH/CH_2Cl_2 (10:1), 87%; 3. DBU, DPPA, NaN_3, toluene, 65%; for **3b**: 2. CF_3CO_2Ag, I_2, CH_2Cl_2, 93%; 3. $NaBH_4$, EtOH/CH_2Cl_2 (10:1), 87%; 4. DBU, DPPA, NaN_3, toluene, 65%; b) 1. 1-decyne, CuI, DIPEA, CH_3CN; 2. NH_2NH_2, toluene, 70% for **4a**, 51% for **4b**; c) 1. 1-decyne, [Cp*Ru(PPh_3)$_2$Cl], benzene, Δ; 2. LiOH·H_2O, H_2O/THF (2:1), 34% for **5a**/**4a** (1:1), 25% for **5b**/**4b** (1:1) (see the Supporting Information for their separation). DIPEA = diisopropylethylamine, Piv = 2,2-dimethylpropanoyl, DBU = 1,8-diazabicyclo-[5.4.0]undec-7-ene, DPPA = diphenylphosphoryl azide, Cp* = pentamethylcyclopentadienyl.

Table 1: Vanilloid and cannabinoid activity of capsaicin (**1a**), capsaicinoids **1b**–**1d**, and their triazole analogues **4a**,**b**, **5a**,**b**, **8**, and **9**.

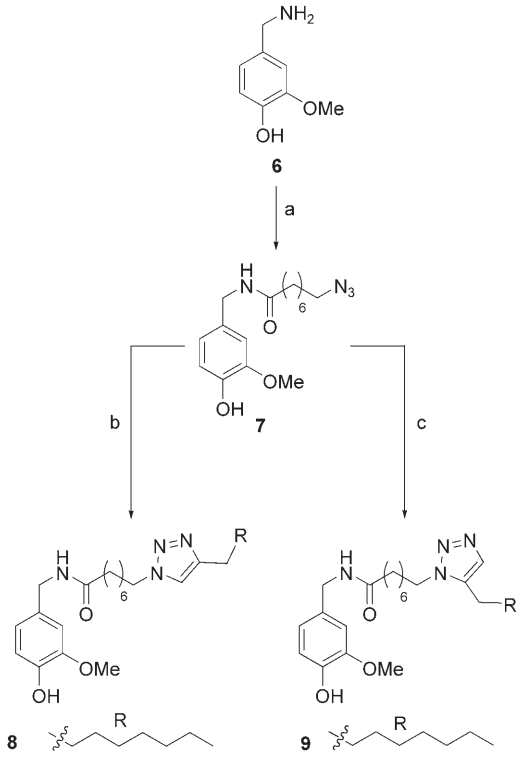
Cmpd.	hTRPV1 $EC_{50} \pm$ SE [nM]	hTRPV1 $IC_{50} \pm$ SE [μM][a]	hCB_1 K_i [μM]	hCB_2 K_i [μM]
1a	30.2 ± 3.9		> 5.6	> 7.9
1b	63.1 ± 4.2		> 5.6	> 7.9
1c	> 10000	0.01 ± 0.002	> 5.6	> 7.9
1d	0.7 ± 0.3		> 5.6	> 7.9
4a	170 ± 38		4.0	7.9
4b	> 10000	0.69 ± 0.16	0.44	7.9
5a	661 ± 167		> 5.6	7.9
5b	> 10000	3.7 ± 0.5	5.6	7.9
8	67.6 ± 9.0		5.6	> 7.9
9	42.7 ± 5.7		5.6	> 7.9

[a] Only compounds inactive as TRPV1 agonists (EC_{50} > 10000 nM) were tested as TRPV1 antagonists. SE = standard error.

The relevance of the 1,2,3-triazole ring as a pharmacophore was further substantiated by the results obtained when the triazole adducts were assayed against the cannabinoid receptors CB_1 and CB_2. Although capsaicin (**1a**), nonivamide (**1b**), and 6′-iodononivamide (**1c**) show no cannabinomimetic activity, the two 1,4-adducts, **4a**,**b**, showed CB_1-selective cannabinoid affinity (Table 1), whereas the corresponding 1,5-adducts, **5a**,**b**, were essentially inactive. Cannabinoid affinity is not unprecedented for capsaicinoids, but is essentially limited to affinity for CB_2.[8a,b] A significant (submicromolar) affinity for CB_1, the psychotropic cannabinoid receptor, is unprecedented for TRPV1 ligands.[14] Compound **4b** was tested in a functional assay for activity towards the CB_1 receptor, namely, for its effect on the forskolin-induced formation of cAMP in intact mouse N18TG2 neuroblastoma cells that selectively express CB_1 receptors. It was inactive at a low concentration of 0.1 μM and acted as an inverse agonist at 1 and 10 μM; that is, it elevated forskolin-induced cAMP formation by 113.2 ± 5.2 and 138.0 ± 8.1% (5.2 and 8.1 are the standard deviation (SD), $n = 3$), respectively, when forskolin was present at a concentration of 1 μM. When assayed at 1 μM, compound **4b** also antagonized the effect of the CB_1/CB_2 agonist WIN 55,212-2 by attenuating the reduction of cAMP levels that is induced by WIN 55,212-2 (0.1 μM) from 25.3 ± 3.2 to 1.1 ± 1.0% (that is, ± SD, $n = 3$). In view of its dual and unique TRPV1/CB_1 antagonism, **4b** can be used as a new template for drug development.[15]

As a flat bivalent element, a triazole ring can also mimic the conformational constraint imposed on alkyl chains by a double bond.[16] Capsaicinoids offer opportunities to investigate the olefin bioisostery of the 1,2,3-triazole ring in a complex biological process: the translocation of biomolecules through membranes. Indeed, fatty-acid vanillamides provide some of the most spectacular examples of the biological relevance of a double bond, as the presence of at least one unit of unsaturation (in either the *cis* or the *trans* configuration)[17] is apparently necessary for the translocation of these highly lipophilic compounds across the cell membrane and for their interaction with the intracellular vanilloid-recognition site of TRPV1.[18] Thus, whereas olvanil (**1d**), the

vanillamide derivative of oleic acid,[17] shows subnanomolar affinity for hTRPV1, vanilloid activity was not detected for its saturated analogue at concentrations at least five orders of magnitude higher.[19] To evaluate the double-bond mimicry of the triazole ring in this molecular setting, the two isomeric triazole analogues **8** and **9** of olvanil (**1d**) were prepared by combining the ω-azidovanillamide **7** with 1-decyne by click chemistry under complementary Cu^{I} or Ru^{II} catalysis (Scheme 2). The vanilloid activity of **8** and **9** was weaker



Scheme 2. Reagents and conditions: a) 8-azidooctanoic acid, Et_3N, PPAA, 53%; b) 1-decyne, *tert*-butanol/water, $CuSO_4{\cdot}5H_2O$, sodium ascorbate, 50%; c) 1-decyne, $[Cp^*Ru(PPh_3)_2Cl]$, benzene, 6%. PPAA = propanephosphonic acid anhydride.

than that of **1d** by approximately two orders of magnitude, presumably as a result of the greater polarity of the triazole ring relative to that of a carbon–carbon double bond.[2] Interestingly, the *syn* adduct **9** was more potent than the *anti* adduct **8**. This difference in activity parallels the higher activity of the *cis* isomer of olvanil relative to that of its *trans* counterpart.[16] Polarity, although an asset for mimicking an amide functionality, may be detrimental in the case of olefin isostery in biological processes in which the double bond is involved directly.[20] Cannabinoid activity was marginal for both triazole analogues.

In summary, we have identified an interesting new class of potential pharmacological agents: mixed CB_1/TRPV1-antagonists. Our results highlight the possibility of developing the 1,2,3-triazole group as a peptidomimetic element capable of both sustaining and modulating amide-related bioactivity. On the other hand, this highly polar group can have a detrimental effect on bioactivity when implanted in a lipophilic moiety as an isosteric surrogate for a double bond. A certain degree of caution should therefore be exerted in the use of the triazole element in bioconjugation experiments.

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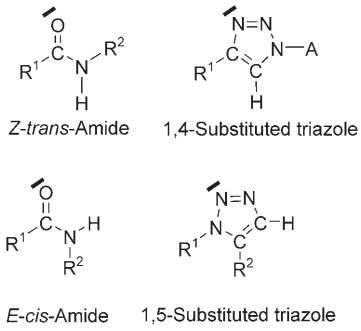
[1] For recent reviews, see: a) J. E. Moses, A. D. Moorhouse, *Chem. Soc. Rev.* **2007**, *36*, 1249–1262; b) P. Wu, V. V. Fokin, *Aldrichimica Acta* **2007**, *40*, 7–17; c) M. V. Gil, M. J. Arévalo, Ó. López, *Synthesis* **2007**, 1589–1620; d) J. F. Lutz, *Angew. Chem.* **2007**, *119*, 1036–1043; *Angew. Chem. Int. Ed.* **2007**, *46*, 1018–1025; e) V. D. Bock, H. Hiemstra, J. H. van Maarseveen, *Eur. J. Org. Chem.* **2006**, 51–68.

[2] In 1,4-substituted triazoles, the carbon atom at the 4-position and the C–H bond can act as an electrophilic site and a hydrogen-bond donor, respectively, whereas the pyridine-type lone pair of electrons on the nitrogen atom at the 3-position serves as a hydrogen-bond-acceptor element. The overall dipolar moment of the triazole system is larger than that of the amide bond, and its hydrogen-bond donor and acceptor properties are therefore more marked that those of an amide bond. The larger dipole moment may compensate for the increased distance between the substituents relative to the distance between substituents on a *Z* amide bond (3.9 versus 5.0 Å).[1d] Although this geometrical mismatch does not exist in 1,5-substituted derivatives, the degree to which they mimic an *E* amide bond is undermined somewhat by the poor electrophilicity of the pyrrole-like nitrogen atom relative to that of a carbonyl carbon atom. For a discussion, see: H. C. Kolb, K. B. Sharpless, *Drug Discovery Today* **2003**, *24*, 1128–1137.



[3] A. Brik, J. Alexandratos, Y.-C. Lin, J. H. Elder, A. J. Olson, A. Wlodawer, D. S. Goodsell, C.-H. Wong, *ChemBioChem* **2005**, *6*, 1167–1169.

[4] T. Lee, M. Cho, S. Y. Ko, H.-J. Youn, D. J. Baek, W.-J. Cho, C.-Y. Kang, S. Kim, *J. Med. Chem.* **2007**, *50*, 585–589; for a related study involving a cellular endpoint (induction of apoptosis), see: S. Kim, M. Cho, T. Lee, S. Lee, H.-Y. Min, S. Kook, *Bioorg. Med. Chem. Lett.* **2007**, *17*, 4584–4587.

[5] For a recent review on the structure–activity relationships of capsaicinoids, see: G. Appendino in *Modern Alkaloids: Structure, Isolation, Synthesis and Biology* (Eds.: E. Fattorusso, O. Taglialatela-Scafati), Wiley-VCH, Weinheim, **2007**, pp. 75–112.

[6] C. S. J. Walpole, R. Wrigglesworth, S. Bevan, E. A. Campbell, A. Dray, I. F. James, K. J. Masdin, M. N. Perkins, J. Winter, *J. Med. Chem.* **1993**, *36*, 2373–2380.

[7] M. J. Caterina, M. A. Schumacher, M. Tominaga, F. T. A. Rosen, J. D. Levine, D. Julius, *Nature* **1997**, *389*, 816 – 824.
[8] a) G. Appendino, L. De Petrocellis, M. Trevisani, A. Minassi, N. Daddario, A. Schiano Moriello, D. Mazzieri, A. Ligresti, B. Campi, G. Fontana, C. Pinna, P. Geppetti, V. Di Marzo, *J. Pharmacol. Exp. Ther.* **2004**, *312*, 561 – 570; b) G. Appendino, M. G. Cascio, S. Bacchiega, A. Schiano Moriello, A. Minassi, A. Thomas, R. Ross, R. Pertwee, L. De Petrocellis, V. Di Marzo, *FEBS Lett.* **2006**, *580*, 568 – 574.
[9] C. A. Reilly, W. J. Ehlardt, D. A. Jackson, P. Kulanthaivel, A. E. Mutlib, R. Espina, D. E. Moody, D. J. Crouch, G. S. Yost, *Chem. Res. Toxicol.* **2003**, *16*, 336 – 349.
[10] Nonivamide is actually a minor constituent of the mixture of capsaicinoids present in hot pepper (H. Constant, G. A. Cordell, D. P. West, J. H. Johnson, *J. Nat. Prod.* **1995**, *58*, 1925 – 1928). Its biological profile is very similar to that of capsaicin, whose acyl chain contains unsaturation and branching that are redundant for its activity.[5]
[11] G. Appendino, N. Daddario, A. Minassi, A. Schiano Morello, L. De Petrocellis, V. Di Marzo, *J. Med. Chem.* **2005**, *48*, 4663 – 4669.
[12] L. Zhang, X. Chen, P. Xue, H. H. Y. Sun, I. D. Williams, K. B. Sharpless, V. V. Fokin, G. Jia, *J. Am. Chem. Soc.* **2005**, *127*, 15998 – 15999.
[13] TRP channels are sensitive to metal inhibition, and the dye ruthenium red is a general inhibitor of this type of channel (A. Szallasi, G. Appendino, *J. Med. Chem.* **2004**, *47*, 2717 – 2723). In previous studies involving metathesis reactions, we observed that capsaicinoids strongly retain metal impurities.[19] The final products were therefore purified carefully by crystallization and preparative HPLC, and examined for inorganic contaminants by atomic spectroscopy. A copper or ruthenium content of less than 3 ppm was found. Furthermore, **4a** showed the same activity against the three endpoints investigated (TRPV1, CB_1, and CB_2) regardless of whether it had been obtained under Cu^{I} or Ru^{II} catalysis. Despite the presence of the same potential copper impurity, iodine-induced reversal of activity was observed, as expected, for **4b**. Taken together, these observations make it unlikely that transition-metal contaminants have affected the bioactivity data. For recent examples of the mediation of the activity of proteins within the endocannabinoid system by spurious metal impurities, see: S. Vandevoorde, K. O. Jonsson, G. Labar, E. Persson, D. M. Lambert, C. J. Fowler, *Br. J. Pharmacol.* **2007**, *150*, 186 – 191; J. K. Makara, M. Mor, D. Fegley, S. I. Szabó, S. Kathuria, G. Astarita, A. Duranti, A. Tontini, G. Tarzia, S. Rivara, T. T. Freund, D. Piomelli, *Nat. Neurosci.* **2007**, *10*, 134.
[14] The most potent capsaicinoid ligand developed so far for the CB_1 receptor is the arachidonic acid derivative arvanil, which has a K_i value of approximately 1–2 μM for CB_1: D. Melck, T. Bisogno, L. De Petrocellis, H. Chuang, D. Julius, M. Bifulco, V. Di Marzo, *Biochem. Biophys. Res. Commun.* **1999**, *262*, 275 – 284.
[15] V. Di Marzo, T. Bisogno, L. De Petrocellis, *Chem. Biol.* **2007**, *14*, 741 – 756.
[16] F. Pagliai, T. Pirali, E. Del Grosso, R. Di Brisco, G. C. Tron, G. Sorba, A. A. Genazzani, *J. Med. Chem.* **2006**, *49*, 467 – 470.
[17] J. M. Janusz, B. L. Buckwalter, P. A. Young, T. R. LaHann, R. W. Farmer, G. B. Kasting, M. E. Loomans, G. A. Kerkaert, C. S. Maddin, E. F. Berman, R. L. Bohne, T. L. Cupps, J. R. Milstein, *J. Med. Chem.* **1993**, *36*, 2595 – 2604.
[18] J. Lazar, D. C. Braun, A. Tóth, Y. Wang, L. V. Pearce, V. A. Pavlyukovets, P. M. Blumberg, S. H. Garfield, S. Wincovitch, H. K. Choi, J. Lee, *Mol. Pharmacol.* **2006**, *69*, 1166 – 1173.
[19] G. Appendino, A. Minassi, A. Schiano Moriello, L. DePetrocellis, V. Di Marzo, *J. Med. Chem.* **2002**, *45*, 3739 – 3745.
[20] A similar observation was made for a series of phosphatidylcholine-derived bolaamphiphiles, whereby the presence of a 1,4-triazole moiety in the lipid linker was detrimental to vesicle formation: E. J. O'Neil, K. M. DiVittorio, B. D. Smith, *Org. Lett.* **2007**, *9*, 199 – 202.

