DOI: 10.1002/cmdc.200700311

Synthesis and Biological Evaluation of Isosteric Analogues of FK866, an Inhibitor of NAD Salvage

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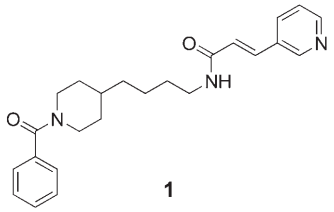
One of the great challenges of medicinal chemistry is to create novel, effective, chemotherapeutic agents that show specificity for cancer cells combined with low systemic toxicity. A novel idea is to target the enzymes of the NAD biosynthesis and recycling pathways given that cancer cells display a higher NAD turnover rate than healthy cells. To this end, the compound FK866 (APO866; (E)-N-[4-(1-benzoylpiperidin-4-yl) butyl]-3-(pyridin-3-yl) acrylamide), which blocks nicotinamide phosphoribosyltransferase (NMPRTase) has entered clinical trials as a potential chemotherapeutic agent. Here we report the synthesis of analogues of FK866 synthesized by click chemistry.

Introduction

The field of antitumoral drugs is in a constant search for molecules which interact with distinct molecular targets. There is a constant need to increase the arsenal of antitumoral drugs, to target cancers which at present have unmet therapeutic needs, to have molecules which will overcome drug resistance, and obviously to increase efficacy while lowering drug side effects and costs to the health system. Among the strategies to achieve these aims is to validate molecular targets distinct from those presently employed by the current generation of antitumoral drugs with small molecules.

Recently, it has been proposed that interfering with NAD levels might lead to cell death of those cells that have a high usage rate of this pyridine nucleotide, that is, tumoral cells with a high division rate.[1] Indeed, lowering NAD levels will not only hamper those enzymatic reactions which require this pyridine nucleotide as a cofactor but will also alter other cell signaling processes which have been shown to be involved in cancer. For example, NAD is the substrate for a specific subclass of histone deacetylases, known as sirtuins, and of mono- and poly-ADP ribosylating enzymes, such as PARP. Indeed, the high PARP activity in tumoral cells should also make these cells more susceptible to interference with NAD levels.[2]

Eukaryotic cells possess several mechanisms to replenish NAD, including a de novo synthesis pathway from the amino acid tryptophan and at least two salvage/recycling pathways.[3] One of these pathways relies on the enzyme nicotinamide phosphoribosyltransferase (NMPRTase) that converts nicotinamide into nicotinamide mononucleotide (NMN) that is subsequently converted to NAD by NMN adenylyltransferase (NMNAT). NMPRTase is the target for the small molecule inhibitor FK866 (**1**) (APO866)[4] that has been shown to induce apoptosis in tumoral cells and in the same context to lower significantly NAD levels.[4,5] Indeed, inhibition of this enzyme alone appears to have an important impact on NAD levels in a number of cell types. Such a mechanism has been reckoned to be promising enough to initiate clinical trials, Phase I clinical trials have been completed and this compound is at present in Phase II.[1,6]



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Given the interest in FK866 and the therapeutic potential of analogues, we have undertaken a strategy to synthesize this compound with fewer steps compared to that previously reported and subsequently to replace the amide bond with a triazole ring via click chemistry reactions,[7] as it has been postulated that this substitution is bioisosteric.[8] The simplicity of this reaction, and its amenability to parallel synthesis would allow the synthesis in a fast and reliable manner of a high number of analogues if the pilot compounds were to maintain a similar biological profile. The compounds were tested both for their ability to deplete intracellular NAD levels and to induce cytotoxicity. Our data show that some 1,4-disubstituted triazole analogues retain activity, albeit losing potency, whereas 1,5-disubstituted triazoles lose virtually all activity. However, in the compound with the highest activity, replacement of the 1,4-disubstituted triazole with an amide resulted in a significant loss of activity suggesting that, at least in the case of FK866, the triazole is not acting as a bioisosteric amide substitution.

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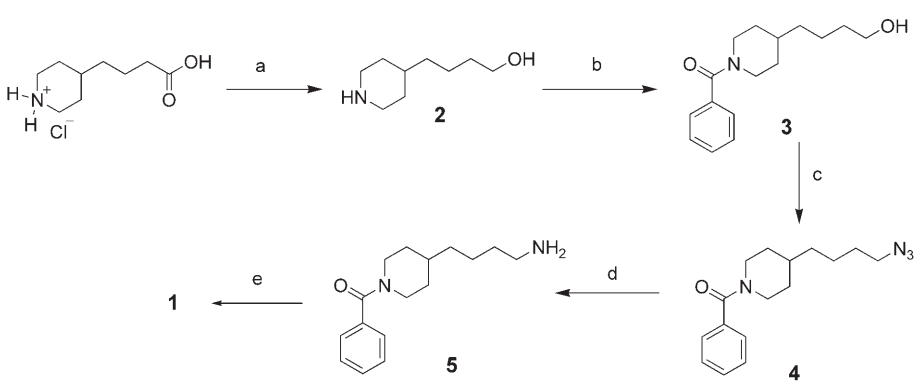
Chemistry

FK866 (**1**) was synthesized using a shorter route respect to that reported previously[9] (Scheme 1) (five synthetic steps versus seven synthetic steps). In brief, the commercially available 4-piperidine butyric acid hydrochloride was reduced to the corresponding alcohol **2** using lithium aluminum hydride and then chemoselectively N-benzoylated to give **3**. Compound **3** was then transformed into an azide (**4**) using diphenylphosphoryl azide (DPPA) and sodium azide. Finally, Staudinger azide-amine reduction gave the amine **5**. Compound **5** was subsequently coupled with (*E*)-3-(3-Pyridinyl)-2-propenylchloride (**6**) to give **1** (overall yield over five steps: 12% compared to 8% for the seven step synthesis).



Scheme 1. Reagents and yields: a) Lithium aluminum hydride, THF, 40%; b) benzoyl chloride, TEA, CH_2Cl_2, 90%; c) DPPA, NaN_3, DBU, DMF, 69%; d) PPh_3, H_2O, THF, 65%; e) **6**, TEA, DMAP, CH_2Cl_2, 75%

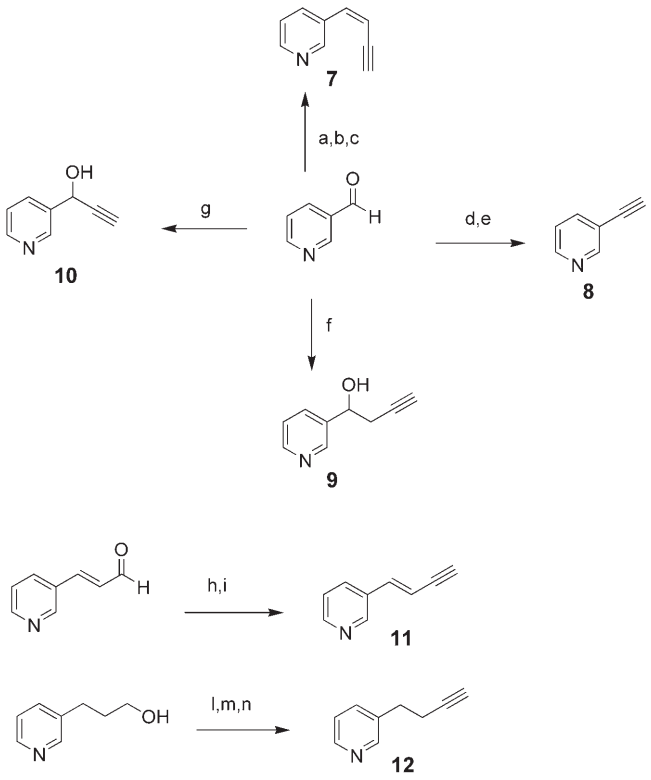
Alkynes were prepared as follows (Scheme 2): the stereoselective Wittig reaction between pyridin-3-carboxaldehyde with the phosphonium salt, (bromomethyl)triphenyl-phosphonium bromide[10] afforded the desired 3-[(*Z*)-2-bromoethenyl]-pyridine in 71% yield which was then subjected to a Sonogashira coupling[11] to give the protected *cis*-enyne. Finally, basic conditions removed the silyl protecting group to give **7** in quantitative yield. 3-ethynyl-pyridine **(8)** was prepared using Corey–Fuchs reaction modified according to Weinreb conditions as reported in the literature.[12]

Compound **9** was then prepared using indium chemistry as reported.[13] In brief, pyridin-3-carboxaldehyde was reacted with propargyl bromide in the presence of a molar excess of metallic indium to give a mixture of two products; the desired homopropargylic alcohol along with the allenic alcohol in a ratio of 7:3. This mixture was not further purified but directly used for the [3+2] cycloaddition reaction. Finally, pyridin-3-carboxaldehyde was reacted with ethynyltrimethylsilane in the presence of LDA at −78 °C to give the corresponding secondary alcohol. This compound was then deprotected using potassium carbonate in methanol to give the desired terminal alkyne **10.**

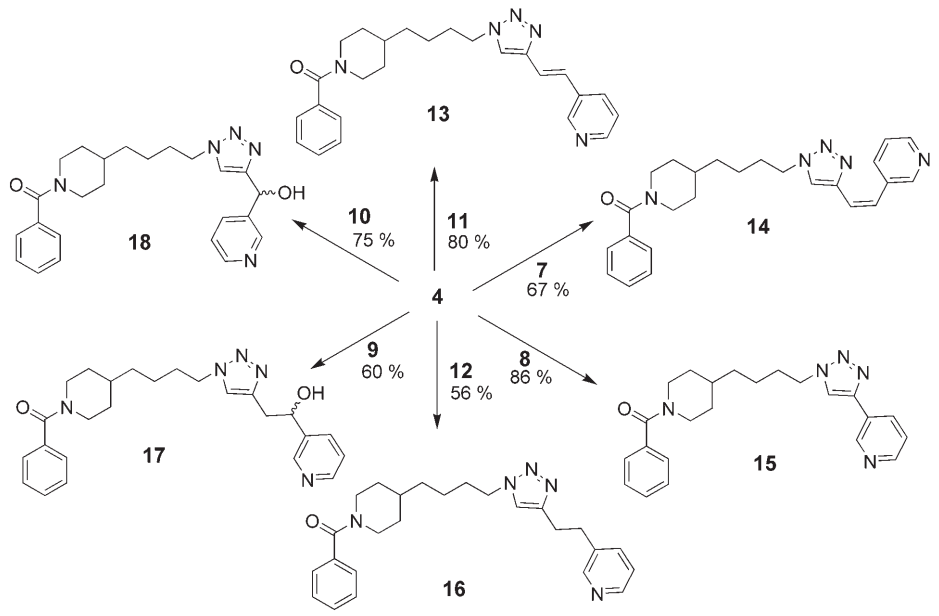
The *trans*-enyne **11** was prepared starting from commercially available (*E*)-pyridine-3-acrylaldehyde using again the Corey–Fuchs-Weinreb protocol. Finally, alkyne **12** was prepared starting from 3-pyridinepropanol which was first oxidized to the corresponding aldehyde using IBX reagent. Then, the aldehyde was transformed into the alkyne using the Bestmann-Ohira reagent.[14]

With these alkynes, we carried out the [3+2] cycloaddition reaction with **4** using the Fokin-Sharpless protocol[7b] ($CuSO_4$; sodium ascorbate; *tert*-butanol-water). 1,4-disubtituted triazoles (**13**–**18**) were obtained in good to excellent yields after column chromatography purification. (Scheme 3)

Attempts to generate pure 1,5-disubtituted triazoles using ruthenium catalysis[15] failed as we observed the formation of an inseparable mixture of 1,5 and 1,4 disubstituted triazoles in very poor yields (Table 1). In this case, however, as a preliminary



Scheme 2. Reagents and yields: a) (bromomethyl)triphenyl-phosphonium bromide, *t*BuOK, THF, 71%; b) ethinyltrimethylsilane, CuI, $PdCl_2(PPh_3)_2$, TEA, 69%; c) K_2CO_3, MeOH, 99% (see ref. [10] and [11]); d) CBr_4, PPh_3, CH_2Cl_2, TEA, 77%; e) LDA, THF, 70%; f) Propargyl bromide, Indium, THF/H_2O, 90% (see ref. [13]); g) ethinyltrimethylsilane, LDA, THF, 67%; h) K_2CO_3, MeOH, 60%; i) CBr_4, PPh_3, CH_2Cl_2, TEA, 82%; j) LDA, THF, 94%; k) IBX, DMSO, 50%; l) Bestmann reagent, K_2CO_3, MeOH, 86%.



Scheme 3. 1,4-disubstituted triazoles derivatives synthesized. General reaction conditions: $CuSO_4 \cdot 5H_2O$, sodium ascorbate, H_2O/*t*BuOH. The second reagents and yields for each reaction are reported alongside the arrows.

Table 1. Experimental conditions for the ruthenium catalyzed azide-alkyne cycloaddition.[a]

Reagents	1,4-disubstituted triazole	1,5-disubstituted triazole	Yield	Reaction time	Eluants
4 + **11**	56% **13**	44%	12%	3 days	PE/EtOAc 2:8 then EtOAc
4 + **7**	50% **14**	50%	10%	1 week	PE/EtOAc 2:8 then EtOAc/MeOH 9:1
4 + **8**	6% **15**	94%	14%	3 days	PE/EtOAc 2:8 then EtOAc
4 + **12**	67% **16**	33%	6%	1 week	PE/EtOAc 2:8 then EtOAc/MeOH 9:1
4 + **9**	34% **17**	66%	8%	1 week	PE/EtOAc 2:8 then EtOAc/MeOH 9:1
4 + **10**	56% **18**	44%	64%	1 week	PE/EtOAc 2:8 then EtOAc/MeOH 9:1

[a] 1,4-, 1,5-disubstituted triazole ratios were determined by ^{1}H NMR.

Table 2. Effects of compounds (left) or mixtures (right) on SH-SY5Y cell viability.[a]

1,4-triazole analogues (10 μM)	Cell Viability (% of control)	Mixture (10 μM)	Cell viability (% of control)
13	19.2 ± 8.5	**4** + **11**	28.7 ± 12.6
14	91.6 ± 5.7	**4** + **7**	81.0 ± 2.8
15	26.8 + 14.2	**4** + **8**	26.4 + 13.8
16	103.0 ± 7.0	**4** + **12**	76.8 ± 4.8
17	73.6 ± 7.1	**4** + **9**	84.4 ± 5.9
18	92.9 ± 4.2	**4** + **10**	126.5 ± 8.4

[a] The 1,4- and 1,5-triazole mixtures are reported as the reagents from which they were generated. The relative amounts of the two regioisomers are reported in Table 1. Values are expressed as mean ± S.E.M. (n = 8–16 from 2–4 separate experiments).

biological screening we decided to test the mixtures directly.

Biological evaluation and conclusions

To screen the FK866 analogues, we first performed a cell viability assay on a neuroblastoma cell line (SH-SY5Y). Briefly, cells were incubated for 72 h with a fixed concentration of compound and viability was assayed by a MTT assay. In this protocol, viability in cells incubated with 10 nM FK866 was reduced to 16.6 ± 0.2% (n = 6). Of the six compounds screened, two 1,4-disubstituted triazole analogues (**13**, **15**) displayed a cytotoxic activity similar to FK866 although the analogues were less potent. Similar results were obtained with the respective 1,4-/1,5-disbstituted triazole mixtures (Table 2). We then proceeded to perform concentration-response curves for **1**, **13**, and **15** with an identical protocol. FK866 displayed an IC_{50} value for toxicity of 1.1 ± 0.1 nM. Compound **15**, also showed significant activity, with an IC_{50} value of approximately 92.9 ± 25.8 nM, whereas compound **13**, the closest analogue to FK866, which displays the triazole instead of the amide group, displayed a micromolar IC_{50} value (3.0 ± 0.2 μM; Figure 1). Both mixtures displayed IC_{50} values above 1 μM and their residual activity can be ascribed to the 1,4-triazole component (not shown). For example, the IC_{50} value displayed by the ruthenium catalyzed reaction of **4** + **8** is about tenfold higher than that of **15**, and the 1,4:1,5 ratio is 6:94. An absence of activity was also observed in those compounds (**17** and **18**) where a hydroxyl group was inserted into the molecule to mimic the retention of a water molecule in the active site.[5b]

To evaluate whether the cytotoxic nature of compound **15** could be ascribed to the depletion of NAD levels, we incubated cells with increasing concentrations of compounds for 24 h (a length of time which does not yield significant cell death) and measured intracellular NAD levels with a cycling assay. In 24 h, FK866 displayed an IC_{50} value for NAD depletion of approximately 1.1 ± 0.13 nM, a concentration consistent with the effects on cell death, and a reduction up to the detection limit

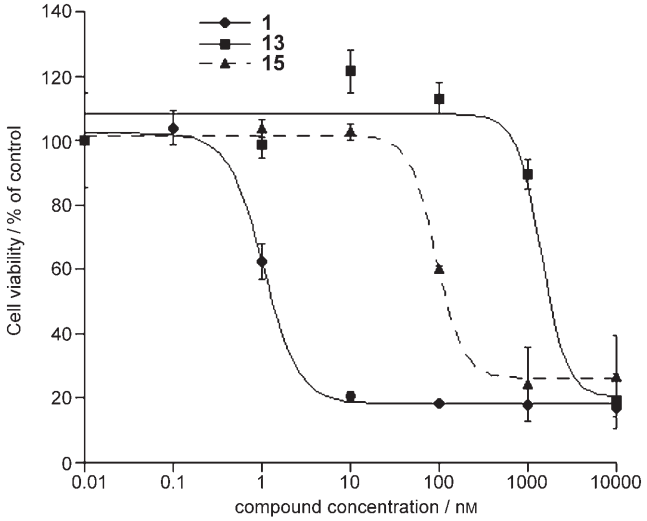


Figure 1. SH-SY5Y cell viability after 72 h in the presence of increasing concentrations of compounds **1**, **13** and **15**. Results are expressed as mean ± SEM in percent of control cell viability in the presence of DMSO alone. (n = 8–16 from 2–4 separate experiments)

of the assay was achieved by concentrations over 3 nM. The effect of compound **15** on NAD levels was also consistent with a cytotoxic effect, with an IC_{50} value of approximately 5.7 ± 1.3 nM and a reduction down to the detection limit at concentrations over 100 nM. To investigate whether the rank order of potency was maintained, we also investigated the effect of **13**, a low potency analogue, which, as expected displayed a higher IC_{50} value compared to **15** or FK866. (Figure 2)

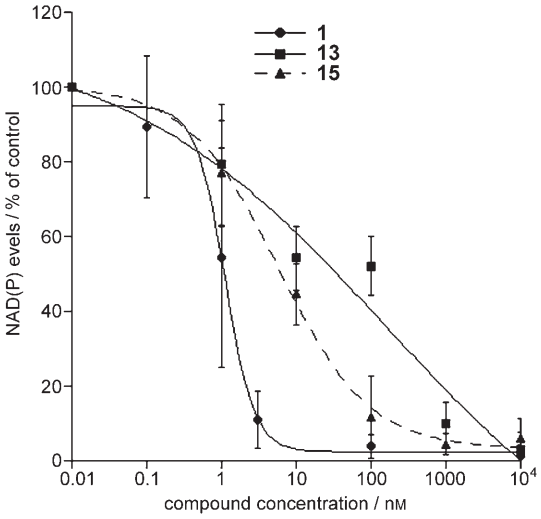


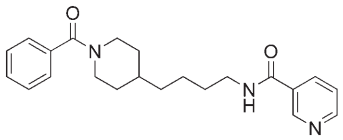
Figure 2. Total NAD(P) levels in SH-SY5Y cells after treatment for 24 h with compounds **1**, **13**, and **15**. Results expressed as mean ± SEM (n = 8–20).

Furthermore, **18** and the mixture obtained by the ruthenium catalyzed reaction of **4** + **10** (an analogue and a mixture without significant effect on cell viability; not shown), at all concentrations tested (up to 1 μM) were unable to reduce NAD levels.

In conclusion, this study was designed to assess: 1) the feasibility of click chemistry reactions to generate analogues of FK866; 2) the amenability of replacement of the amide group with a nonclassical bioisostere (1,4-disubstituted triazole); and 3) the possibility to apply this isosteric replacement to generate analogues.

Our data show that the copper-catalyzed reaction between azides and alkynes can be applied to FK866, and that the products give good yields. On the contrary, the ruthenium-catalyzed reaction, gave low yields of the 1,5-disubstituted triazole and was not regiospecific in most reactions. Although ruthenium has reported previously as a good regioselective catalyzer,[15] it would appear that its use is highly dependent on the reactants,[16] and that its efficiency cannot be given for granted in any given reaction.

The most successful compound generated, **15**, suggests that the triazole was acting as a bioisostere of the amide group. Yet, we found it surprising that **15**, and not **13**, which is a true analogue of FK866, was the most potent compound. Indeed, the triazole-analogue **15** where the olefinic moiety was removed and the distance between the triazole and the pyridine ring was shortened displayed nanomolar potency. To further our understanding on this, we replaced the triazole ring with an amide, reasoning that, as these two groups might have been bioisosteres, the activity of this new compound should have been identical or improved. This was not the case, as compound **19** appeared inactive both in cytotoxicity and in



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lowering NAD levels. This suggests that in this circumstance the double bond plays a pivotal role in determining the potency of FK866 to place the amide group at the right distance to interact with a retained molecule of water in the active site.[5b] As the 1,4-disubstituted triazole is 1.1 Å longer, it is likely that compound **15** does not require the double bond to maintain this distance. One possible explanation is that the N3–C4 bond of the triazole might replace the olefinic moiety whereas the slightly acidic proton on C5 might mimic the N-H group of the amide.

In conclusion, we have successfully synthesized, with fewer passages, the antitumoral drug FK866 and have constructed a small library of triazole analogues. Among these, one displayed nanomolar potency suggesting that click chemistry might represent a suitable synthetic strategy for the rapid generation of future FK866 analogues.

Experimental Section

Commercially available reagents and solvents were used without further purification and were purchased from Fluka-Aldrich or Lan-

caster. Dichloromethane was dried by distillation from P_2O_5 and stored on activated molecular sieves (4 Å). Tetrahydrofuran (THF) and diethyl ether were distilled immediately before use from Na/benzophenone under a slight positive atmosphere of N_2. Dimethylformamide (DMF) was purified by distillation at reduced pressure collecting the fraction having b.p. 76 °C at 39 mmHg and stored on activated molecular sieves (4 Å). Dimethyl sulfoxide (DMSO) was purified by distillation over calcium hydride at reduced pressure and stored on activated molecular sieves (4 Å).

When needed, the reactions were performed in flame- or oven-dried glassware under a positive pressure of dry N_2.

Melting points were determined in open glass capillary with a Stuart scientific SMP3 apparatus and are uncorrected. All the compounds were checked by IR (FT-IR THERMO-NICOLET AVATAR); ^{1}H and ^{13}C APT (JEOL ECP 300 MHz) and mass spectrometry (Thermo Finningan LCQ-deca XP-plus) equipped with an ESI source and an ion trap detector. Chemical shifts are reported in parts per million (ppm). Column chromatography was performed on silica gel (Merck Kieselgel 70–230 mesh ASTM) or neutral aluminum oxide Brockmann grade III using the indicated eluants. Thin layer chromatography (TLC) was carried out on 5×20 cm plates with a layer thickness of 0.25 mm (Merck Silica gel 60 F_{254}) or (Merck Aluminumoxid 60 F_{254} neutral). When necessary they were developed with $KMnO_4$, Dragendorff reagent, and Phosphomolibdic reagent. Elemental Analysis (C, H, N) of the target compounds **1**, **13**, **14**, **15**, **16**, **17**, **18**, and **19** are within ±0.4% of the calculated values unless otherwise noted.

Compounds **7**,[11] **9**,[13] and ruthenium catalyst Cp*Ru(PPh_3)$_2$Cl were synthesized as described previously.[17]

4-piperidinebutanol (2). Under nitrogen, 1.10 g of lithium aluminum hydride was added portionwise (28.9 mmol; 3 equiv) to a cooled (0 °C) suspension of 4-piperidine butyric acid hydrochloride (2 g; 19.63 mmol) in dry THF (40 mL). The reaction was warmed to room temperature and stirred for 24 h. The reaction was cooled to 0 °C and worked up by careful addition of EtOAc and water. The suspension was filtered on a celite bed and the cake was washed with MeOH. After evaporation of the solvent, the crude was purified by column chromatography using MeOH/NH_4OH 98:2 to give 600 mg of **2** as a yellow oil (40%). IR (neat): $\tilde{\nu}$=3440, 3288, 2926, 2853, 1495 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ=3.62 (t, J=6.6 Hz, 2-H), 3.07 (m, 2-H), 2.57 (m, 2-H), 2.33 (br s, –OH + –NH), 1.67 (m, 2-H), 1.54 (m, 2-H), 1.35 (m, 3-H), 1.24 (m; 2-H), 1.13 ppm (m; 2-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=62.8, 46.6, 36.9, 36.1, 33.3, 33.0, 22.8 ppm; MS (ESI) m/z 158 $[M+H]^+$.

***N*-benzoyl-4-piperidinebutanol (3).** Under nitrogen, TEA (3.60 mL; 25.74 mmol; 2 equiv) and benzoyl chloride (1.50 mL; 12.87 mmol; 1 equiv) were subsequently added to a cooled (0 °C) solution of **2** (2.02 g; 12.87 mmol) in dry CH_2Cl_2 (20 mL). After 1 h, the reaction was worked up by dilution with sat. aq. NH_4Cl and extracted with CH_2Cl_2 (×3). The combined organic extracts were washed with NaOH 2 M (×1) and brine (×1). After drying over sodium sulfate, filtration, and evaporation of the solvent, the crude was purified by column chromatography using PE/EtOAc 3:7 as eluant to give 3.02 g of **3** as a colorless oil (90%). IR (neat): $\tilde{\nu}$=3357, 2930, 2856, 1612, 1446 1280, 709 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ=7.35–7.30 (m, 5-H), 4.62 (br d, 1-H), 3.66 (br d, 1-H), 3.52 (t, J=6.7 Hz, 2-H), 3.16 (br s, -OH), 2.88 (br t, 1-H), 2.66 (br t, 1-H), 1.72 (br d, 1-H), 1.58 (br d, 1-H), 1.47 (m, 3-H), 1.36–1.05 ppm (m, 6-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=170.4, 136.3, 129.5, 128.5, 126.8, 62.4, 48.2, 42.6, 36.2, 36.1, 32.8 (2C), 32.0, 22.9 ppm; MS (ESI) m/z 262 $[M+H]^+$.

4-(4-azidobutyl)-*N*-benzoyl-piperidine (4). Under nitrogen, DPPA (6.76 mL; 31.38 mmol; 3 equiv) and 1,8-Diazabicyclo[5.4.0]undec-7-en (DBU) (4.69 mL; 31.38 mmol; 3 equiv) were subsequently added dropwise to a cooled (0 °C) solution of **3** (2.73 g; 10.46 mmol) in dry DMF (30 mL). After 30 min, NaN_3 was added (2.00 g; 31.38 mmol; 3 equiv). After the addition, the cooling bath was removed and the resulting solution was heated at 100 °C for 4 h. After cooling to room temperature, the reaction was worked up by dilution with diethyl ether. The organic layer was washed with water (×2). The aqueous layer was further washed with diethyl ether (×1), and the combined organic extracts were washed with brine (×1). After drying over sodium sulfate, filtration, and evaporation of the solvent, the crude was purified by column chromatography using PE/EtOAc 8:2 to give 2.06 g of **4** as a colorless oil (69%). IR (neat): $\tilde{\nu}$=2930, 2856, 2094, 1631, 1434, 1280, 708 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ=7.40–7.23 (m, 5-H), 4.66 (br s, 1-H), 3.70 (br s, 1-H), 3.24 (t, J=6.72 Hz, 2-H), 2.94 (br s, 1-H), 2.75 (br s, 1-H), 1.76 (br s, 1-H), 1.56 (m, 3-H), 1.40–1.00 ppm (m, 7-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=170.4, 136.5, 129.5, 128.5, 126.9, 51.4, 48.0, 42.6, 36.2, 36.0, 32.8, 32.0, 29.1, 23.8 ppm; MS (ESI) m/z 287 $[M+H]^+$.

***N*-benzoyl-4-piperidinebutanamine (5).** Water (0.151 mL; 8.39 mmol; 6 equiv) and triphenylphosphine (0.55 g; 2.10 mmol; 1.5 equiv) were added to a solution of **4** (0.40 g; 1.40 mmol) in THF (5 mL). The resulting mixture was heated at reflux for 3 h under magnetic stirring. The solvent was evaporated and the residue was purified by column chromatography using EtOAc and MeOH/NH_4OH 98:2 as eluants to give 0.24 g of **5** as a yellowish oil (65%). IR (neat): $\tilde{\nu}$=3436 (broad), 2923, 2853, 1601, 1447, 1124, 711 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ=7.33–7.20 (m, 5-H), 4.62 (br s, 1-H), 3.65 (br s, 1-H), 2.89 (br t, 1-H), 2.62 (br s, 3-H), 2.00 (br s, -NH_2), 1.72 (br s, 1-H), 1.58 (br s, 1-H), 1.46–1.00 ppm (m; 9-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=170.3, 136.5, 129.4, 128.4, 126.8, 48.2, 42.6, 42.0, 36.2, 36.1, 33.5, 33.0, 32.0, 23.9 ppm; MS (ESI) m/z 261 $[M+H]^+$.

***N*-[4-(1-benzoyl-4-piperidinyl)butyl]-3-(3-pyridinyl)-(*E*)- 2-propenamide (1).** Under nitrogen, oxalyl chloride (0.14 mL; 1.61 mmol; 1.8 equiv) dissolved in dry CH_2Cl_2 (3 mL) was slowly added to a cooled (0 °C) suspension of 3-(3-pyridinyl)-(*E*)- 2-propenoic acid (0.20 g; 1.34 mmol; 1.5 equiv) in dry CH_2Cl_2 (3 mL). After the addition was completed, a catalytic amount of dry DMF (1 mol%) was added. The reaction was stirred at room temperature overnight and then evaporated to give **6** as a yellow oil. The residue was dissolved in dry CH_2Cl_2, cooled at 0 °C. To this solution, a mixture of **5** (0.23 g; 0.89 mmol; 1 equiv), TEA (0,19 mL; 1.34 mmol; 1.5 equiv), and a catalytic amount of DMAP (5 mol%) was added dropwise. After stirring at room temperature for 24 h, the reaction was worked up by dilution with CH_2Cl_2 and washed with NaOH 2 M (×1). The basic aqueous layer was further washed with CH_2Cl_2 (×2), and the combined organic extracts were washed with brine (×1). After drying over sodium sulfate, filtration, and evaporation of the solvent, the crude was purified by column chromatography using EtOAc and EtOAc/NH_4OH 9:1 as eluants to give 0.26 g of **1** as a yellow oil (75%) which solidify on standing. IR (neat): $\tilde{\nu}$=3282, 2929, 2855, 1669, 1618, 1445, 980, 710 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ=8.72 (br s, 1-H), 8.56 (br s, 1-H), 7.76 (d, J=7.9 Hz, 1-H), 7.59 (d, J=15.9 Hz, 1-H), 7.40–7.35 (m, 5-H), 7.32–7.28 (m, 1-H), 6.46 (d, J=15.6 Hz, 1-H), 5.98 (br t, 1-H), 4.69 (br d, 1-H), 3.72 (br d, 1-H), 3.37 (q, J=6.7 Hz, 2-H), 2.95 (br t, 1-H), 2.72 (br t, 1-H), 1.81 (br s, 3-H), 1.63–1.51 (m, 4-H), 1.40–1.25 ppm (m, 4-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=170.4, 165.2, 149.6, 148.4, 136.8, 136.4, 135.1, 131.2, 129.6, 128.5, 126.8, 124.0, 123.6, 48.2, 42.6, 39.8, 36.1, 36.0, 33.0, 32.0, 29.9, 24.0 ppm; MS (ESI) m/z 392 $[M+H]^+$.

Anal. Calcd for $C_{24}H_{29}N_3O_2$: C, 80.18; H, 8.13; N, 11.69. Found: C, 80.12; H, 7.98; N, 11.50.

3-ethynyl- pyridine (8). Triphenylposphine (4.90 g; 18.67 mmol; 4 equiv) was added portionwise to a cooled (0 °C) and under a nitrogen atmosphere solution of CBr_4 (3.09 g; 9.33 mmol; 2 equiv) in dry CH_2Cl_2 (40 mL). After 15 min, TEA (0.65 mL; 4.67 mmol; 1 equiv) was added. The resulting solution was then cooled at −78 °C and a solution of 3-pyridinecarboxaldehyde (0.44 mL; 4.67 mmol; 1 equiv) in dry CH_2Cl_2 (3 mL) was added dropwise. After the addition the cooling bath was removed and the resulting brown solution was stirred at room temperature for 30 min and quenched adding water. The aqueous layer was washed with CH_2Cl_2 (×6) and the combined organic extracts were washed with brine (×1). After drying over sodium sulfate, filtration, and evaporation of the solvent (temperature of the heating bath <40 °C because of the instability of the compound), the crude was taken up in diethyl ether and filtered on a neutral alumina (Brockmann grade III) pad. The filtrate was evaporated and the crude was purified by column chromatography on neutral alumina (Brockmann grade III) using PE/EtOAc 9:1 as eluant to give 0.94 g of the corresponding dibromoethene derivative as a yellow solid (77%). This compound is unstable and must be stored at −20 °C. m.p. 57–59 °C; IR (neat): $\tilde{\nu}$ = 3010, 2962, 2920, 1567, 1409, 1023, 802 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 8.66 (s, 1-H), 8.55 (d, J = 4.9 Hz, 1-H), 7.96 (dt, J = 8.0/1.8) 8.72 (br s, 1-H), 7.45 (s, 1-H), 7.30 ppm (dd, J = 7.9/4.9 Hz, 1-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 149.8, 149.4, 135.0, 133.6, 131.5, 123.3, 92.4 ppm; MS (ESI) m/z 264 $[M+H]^+$.

LDA 1.8 M in THF (8.60 mL; 15.42 mmol; 3.2 equiv) was added dropwise to a solution containing the dibromoethene derivative (1.27 g; 4.82 mmol; 1 equiv) in dry THF cooled at −78 °C. After the addition the cooling bath was removed and the resulting solution was stirred at room temperature for 1.5 h. The reaction was quenched by addition of sat. aq. NH_4Cl and the mixture extracted with EtOAc (×3). The combined organic extracts were washed with brine (×1). After drying over sodium sulfate and evaporation of the solvent, the crude was purified by column chromatography using PE/EtOAc 95: 5 as eluant to give 0.35 g of **8** as an orange oil (70%). The corresponding alkyne is not stable and must be stored at −20 °C. IR (neat): $\tilde{\nu}$ = 3270, 2120 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 8.72 (d, J = 1.53 Hz, 1-H), 8.57 (dd, J = 4.92/1.53 Hz, 1-H), 7.77 (dt, J = 7.65/2.16 Hz, 1-H), 7.26 (m, 1-H), 3.21 ppm (s, 1-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 152.9, 149.2, 139.2, 123.1, 119.4, 80.8, 80.4 ppm; MS (ESI) m/z 104 $[M+H]^+$.

α-ethynyl-3-pyridinemethanol (10). To a cooled (−78 °C) solution of ethinyltrimethylsilane (1.38 g; 14.02 mmol; 1.5 equiv) in dry THF (14 mL), 7.79 mL of LDA 1.8 M solution in THF (14.02 mmol; 1.5 equiv) were added dropwise. The resulting mixture was stirred for 30 min, then 0.88 mL of 3-pyridinecarboxaldehyde (9.35 mmol; 1 equiv) dissolved in 10 mL of dry THF was added dropwise. After 3 h the reaction was worked up by slow addition of sat. aq. NH_4Cl. When the resulting mixture reached room temperature, the aqueous layer was washed with EtOAc (×3) and the combined organic extracts were washed with brine (×1). After drying over sodium sulfate, filtration, and evaporation of the solvent, the crude was purified by column chromatography using PE/EtOAc 5.5 as eluant to give 1.27 g of the protected alkyne as a yellowish oil (67%). IR (neat): $\tilde{\nu}$ = 3112, 2959, 2827, 2170, 1251, 985, 841 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 8.63 (d, J = 2.1 Hz, 1-H), 8.42 (dd, J = 4.6/1.5 Hz, 1-H), 7.88 (dt, J = 7.9/1.5 Hz, 1-H), 7.26 (m, 1-H), 5.48 (s, 1-H), 0.15 ppm (s, 9-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 148.6, 147.9, 137.1, 135.0, 123.6, 104.7, 91.8, 62.3, −0.14 ppm; MS (ESI) m/z 206 $[M+H]^+$.

Potassium carbonate (0.81 g; 5.85 mmol; 1.2 equiv) was added dropwise to a solution of the protected alkyne (1.0 g, 4.88 mmol) in MeOH (10 mL). After the addition, the resulting solution was stirred at room temperature for 30 min. Methanol was evaporated and the residue was taken up in EtOAc and washed with brine (×1). After drying over sodium sulfate, filtration, and evaporation of the solvent, the crude was purified by column chromatography using PE/EtOAc 4:6 as eluant to give 0.39 g of **10** as a pale yellow oil (60%). IR (neat): $\tilde{\nu}$ = 3290, 2832, 2113, 1426, 1028, 711 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 8.62 (d, J = 1.5 Hz, 1-H), 8.39 (br d, J = 4.9 Hz, 1-H), 7.88 (dt, J = 7.9/1.5 Hz, 1-H), 7.26 (dd, J = 7.9/4.9, 1-H), 6.24 (br s, -OH), 5.48 (d, J = 1.8 Hz, 1-H), 2.63 ppm (d, J = 1.8 Hz, 1-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 148.6, 147.7, 136.9, 135.1, 123.7, 83.3, 75.0, 61.6 ppm; MS (ESI) m/z 134 $[M+H]^+$.

3-[(*E*)-1-buten-3-ynyl]-pyridine (11). Triphenylposphine (11.83 g; 45.11 mmol; 4 equiv) was added portionwise to a cooled (0 °C) and under a nitrogen atmosphere solution of CBr_4 (7.48 g; 22.55 mmol; 2 equiv) in dry CH_2Cl_2 (86 mL). After 15 min, TEA (1.57 mL; 11.28 mmol; 1 equiv) was added. The resulting solution was then cooled at −78 °C and a solution of (*E*)-pyridine-3-acryaldehyde (1.50 g; 11.28 mmol; 1 equiv) in dry CH_2Cl_2 (8 mL) was added dropwise. After the addition the cooling bath was removed and the resulting brown solution was stirred at room temperature for 30 min and quenched adding water. The aqueous layer was washed with CH_2Cl_2 (×3) and the combined organic extracts were washed with brine (×1). After drying over sodium sulfate, filtration and evaporation of the solvent (temperature of the heating bath <40 °C because of the instability of the compound), the crude was taken up in diethyl ether and filtered on a neutral alumina (Brockmann grade III) pad. The filtrate was evaporated and the crude was purified by column chromatography on neutral alumina (Brockmann grade III) using PE/EtOAc 9:1 as eluant to give 2.67 g of the corresponding dibromoethene derivative (82%). The compound was recrystallized with PE. The compound is not stable and must be stored at −20 °C. m.p. = 86.5–88 °C IR (neat): $\tilde{\nu}$ = 3080, 3021, 1568, 1473, 961, 804, 699 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 8.62 (br s, 1-H), 8.50 (d, J = 4.6 Hz, 1-H), 7.76 (d, J = 7.9 Hz, 1-H), 7.25 (dd, J = 7.9/4.6 Hz, 1-H), 7.09 (d, J = 9.7 Hz, 1-H), 6.82 (dd J = 15.6/9.7 Hz, 1-H), 6.66 ppm (d, J = 15.6 Hz, 1-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 149.4, 148.8, 136.7, 133.0, 131.7, 131.5, 127.3, 123.7, 93.3 ppm; MS (ESI) m/z 290 $[M+H]^+$.

LDA 1.8 M in THF (9.23 mL; 16.61 mmol; 3.2 equiv) was added dropwise to a solution containing the dibromoethene derivative (1.50 g; 5.19 mmol; 1 equiv) in dry THF cooled at −78 °C. After the addition, the cooling bath was removed and the resulting solution was stirred at room temperature for 2.5 h. The reaction was quenched by addition of sat. aq. NH_4Cl and the mixture extracted with EtOAc (×3). The combined organic extracts were washed with brine (×1). After drying over sodium sulfate and evaporation of the solvent, the crude was purified by column chromatography using PE/EtOAc 9:1 as eluant to give 0.63 g of **11** as a brown oil (94%). IR (neat): $\tilde{\nu}$ = 3291, 3026, 2957, 2096, 1568, 1414, 959, 704 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ = 8.60 (d, J = 1.9 Hz, 1-H), 8.51 (dd, J = 4.9/1.5 Hz, 1-H), 7.69 (dt, J = 8.0/1.5 Hz, 1-H), 7.27 (m, 1-H), 7.00 (d, J = 16.2 Hz, 1-H), 6.19 (dd, J = 16.2/2.1 Hz, 1-H), 3.11 ppm (d, J = 2.1 Hz, 1-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 149.9, 148.3, 139.4, 132.6, 131.6, 123.7, 109.5, 82.2, 80.5 ppm; MS (ESI) m/z 130 $[M+H]^+$.

3-(3-butynyl)- pyridine (12). IBX (5.57 g; 13.14 mmol; 3 equiv) was added portionwise to a solution of 3-pyridinepropanol (0.60 g; 4.38 mmol; 1 equiv) in dry DMSO (8 mL). After the addition the resulting suspension was stirred at room temperature for 12 h. The suspension was filtered and the solid was washed with EtOAc. The organic layer was washed with NaOH 2 M (×4) and the combined basic aqueous layers were further washed EtOAc (×2). The combined organic layers were washed with brine (×1). After drying over sodium sulfate and evaporation of the solvent, the crude was purified by column chromatography using PE/EtOAc 1:9 as eluant to give 0.29 g of the desired aldehyde as a yellowish oil (50%).

(Note: If the reaction is stirred for 72 h, the main product isolated was the corresponding α,β unsaturated aldehyde 3-(3-pyridinyl)-(*E*)-2-propenal, (80%)[18] along with traces of the saturated aldehyde (7%). IR (neat): $\tilde{\nu}=3190$, 2924, 2852, 1719, 1424, 1028, 714 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): $\delta=9.82$ (br s, 1-H), 8.49 (br s, 2-H), 7.52 (d, $J=7.6$ Hz, 1-H), 7.25 (br s, 1-H), 2.95 (t, $J=7.3$ Hz, 2-H), 2.81 ppm (t, $J=7.1$ Hz, 2-H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta=200.2$, 149.6, 147.6, 136.3, 135.7, 123.4, 44.9, 25.5 ppm; MS (ESI) *m/z* 136 $[M+H]^+$.

Bestmann reagent (0.35 g; 1.84 mmol; 1.2 equiv) and potassium carbonate (0.42 g; 3.07 mmol; 2 equiv) were subsequently added to a solution of 3-pyridinepropanal (0.27 g; 1.53 mmol; 1 equiv) in dry methanol (2 mL). After the addition the resulting solution was stirred at room temperature for 12 h. The reaction was quenched by addition of water and the mixture extracted with diethyl ether (×4). The combined organic extracts were washed with NaOH 2 M (×1) and brine (×1). After drying over sodium sulfate and evaporation of the solvent, the crude was purified by column chromatography using PE/EtOAc 5:5 to give 0.17 g of **12** as a yellowish oil (86%). IR (neat): $\tilde{\nu}=3296$, 3031, 2931, 2117, 1576, 1029, 713 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): $\delta=8.43$ (d, $J=2.1$ Hz, 1-H), 8.40 (dd, $J=4.9/1.2$ Hz, 1-H), 7.25 (dt, $J=7.9/1.8$ Hz, 1-H), 7.15 (dd, $J=7.9/4.8$ Hz, 1-H), 2.75 (t, $J=7.3$ Hz, 2-H), 2.42 (td, $J=7.3/2.7$ Hz, 2-H), 1.94 ppm (t, $J=2.4$ Hz, 1-H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta=150.0$, 147.9, 136.1, 135.7, 123.4, 82.9, 69.8, 31.9, 20.4 ppm; MS (ESI) *m/z* 132 $[M+H]^+$.

General procedure for the synthesis of 1,4-disubstituted triazoles : Alkyne (1 equiv) and azide (1 equiv) were dissolved in a mixture of water/*t*BuOH (1:1). Sodium ascorbate (0.1 equiv) of a freshly prepared 1 M solution in water was added, followed by the addition of copper (II) sulfate pentahydrate (0.01 equiv). The resulting reaction mixture was vigorously stirred at room temperature until the completion of the reaction. The reaction mixture was then diluted with water and extracted with EtOAc (×3). The combined organic extracts were washed with brine (×1). After drying over sodium sulfate and evaporation of the solvent, the crude was purified by column chromatography to give the desired triazole derivative.

General procedure for attempted synthesis of 1,5-disubstituted triazoles using ruthenium catalyst: 1 equiv of the alkyne and 0.02 equiv if $Cp^*RuCl(PPh_3)_2$ were added to a solution of the azide (1 equiv) in benzene. The resulting mixture was heated at reflux (see Table 1 for the time reaction) and worked up by evaporation of the solvent. The resulting crude was purified by column chromatography using a mixture of PE/EtOAc and EtOAc/MeOH. (see Table 1)

1-benzoyl-4-[4-[4-[(*E*)-2-(3-pyridinyl)ethenyl]-1*H*-1,2,3-triazol-1-yl]butyl]- piperidine (13). Reaction time: 1.5 h; Eluant: EtOAc; White solid recrystallized with PE/EtOAc; M.p. 124–126 °C. Yield: 80% IR (neat): $\tilde{\nu}=1618$, 1434, 1277, 988, 733 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): $\delta=8.69$ (br s , 1-H), 8.47 (d, $J=4.3$ Hz, 1-H), 7.78 (dt, $J=7.9/1,8$ Hz, 1-H), 7.59 (s, 1-H), 7,36 (m, 5-H), 7.28 (d, $J=16.2$ Hz, 1-H), 7.27 (m, 1-H), 7.12 (d, $J=16.5$ Hz, 1-H), 4.67 (br s, 1-H), 4.36 (t, $J=7.0$ Hz, 2-H), 3.72 (br s, 1-H), 2.92 (br s, 1-H), 2.71 (br s, 1-H), 1.90 (m; 3-H), 1.74 (br s, 1-H), 1.60 (br s, 1-H), 1.49 (m, 1-H), 1.31 ppm (m, 5-H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta=170.4$, 148.9, 148.5, 145.7, 136.4, 132.8, 132.6, 129.5, 128.5, 126.9, 126.8, 123.7, 120.6, 118.9, 50.3, 48.1, 42.5, 36.1, 35.8, 32.9, 32.0, 30.5, 23.6 ppm; MS (ESI) *m/z* 416 $[M+H]^+$.

Anal. Calcd for $C_{25}H_{29}N_5O$: C, 75.15; H, 7.31; N, 17.52. Found: C, 75.54; H, 7.81; N, 17.10.

1-benzoyl-4-[4-[4-[(*Z*)-2-(3-pyridinyl)ethenyl]-1*H*-1,2,3-triazol-1-yl]butyl]-piperidine (14).

Reaction time: 6 h; Eluant: EtOAc; Colorless oil; Yield: 67% IR (neat): $\tilde{\nu}=2928$, 1627,1437, 1276, 974, 710 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): $\delta=8.57$ (br s , 1-H), 8.49 (br s, 1-H), 7.75 (d, $J=7.6$ Hz, 1-H), 7.34 (m, 5-H), 7,26 (t, $J=5.2$ Hz, 1-H), 7.05 (s,1-H), 6.75 (d, $J=12.2$ Hz, 1-H), 6.59 (d, $J=12.2$ Hz, 1-H), 4.65 (br s, 1-H), 4.20 (t, $J=7.0$ Hz, 2-H), 3.68 (br s, 1-H), 2.90 (br s, 1-H), 2.68 (br s, 1-H), 1.76 (m; 3-H), 1.56 (m, 1-H), 1.43 (m, 1-H), 1.33 (m, 5-H), 1.05 ppm (m, 1-H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta=170.3$, 149.6, 148.7, 143.9, 136.4, 135.9, 133.5, 129.5, 128.5, 127.3, 126.9, 123.5, 122.0, 121.4, 50.2, 48.1, 42.4, 36.0, 35.7, 32.8, 31.9, 30.4, 23.5 ppm; MS (ESI) *m/z* 416 $[M+H]^+$.

Anal. Calcd for $C_{25}H_{29}N_5O$: C, 75.15; H, 7.31; N, 17.52. Found: C, 75.25; H, 7.50; N, 17.24.

1-benzoyl-4-[4-[4-(3-pyridinyl)-1*H*-1,2,3-triazol-1-yl]butyl]- piperidine (15).

Reaction time: 24 h; Eluant: EtOAc; Colorless oil; Yield: 86% IR (neat): $\tilde{\nu}=2925$, 2853, 1624, 1446, 1282, 710 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): $\delta=8.95$ (s, 1-H), 8.51 (d, $J=4.0$ Hz, 1-H), 8.14 (dt, $J=7.9/1,8$ Hz, 1-H), 7.84 (s, 1-H), 7,32 (m, 6-H), 4.63 (br s, 1-H), 4.37 (t, $J=7.0$ Hz, 2-H), 4.06 (br s, 1-H), 2.89 (br t, 1-H), 2.67 (br t, 1-H), 1.90 (m, 2-H), 1.71 (m; 1-H), 1.57 (br s, 1-H), 1.46 (m, 1-H), 1.29 (m, 4-H); 1.12 (br s, 1-H), 1.05 ppm (br s, 1-H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta=170.3$, 149.3, 147.1, 144.8, 136.4, 133.0, 132.8, 129.5, 128.5, 126.9, 123.9, 119.8, 50.5, 48.0, 42.5, 36.1, 35.8, 32.8, 32.0, 30.5, 23.6 ppm; MS (ESI) *m/z* 390 $[M+H]^+$.

Anal. Calcd for $C_{23}H_{27}N_5O$: C, 73.96; H, 7.28; N, 18.75. Found: C, 73.65; H, 7.10; N, 19.10.

1-benzoyl-4-[4-[4-[2-(3-pyridinyl)ethyl]-1*H*-1,2,3-triazol-1-yl]butyl]-piperidine (16).

Reaction time: 1 h; Eluant: EtOAc/MeOH 9:1; Colorless oil; Yield: 56%

IR (neat): $\tilde{\nu}=2929$, 1627, 1445, 1278 711 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): $\delta=8.36$ (br s , 2-H), 7.42 (d, $J=7.6$ Hz, 1-H), 7.31 (m, 5-H), 7,12 (dd, $J=7.4/4.6$ Hz, 1-H), 7.08 (s, 1-H), 4.60 (m, 1-H), 4.21 (t, $J=7.0$ Hz, 2-H), 3.63 (m, 1-H), 2.97 (m, 4-H), 2.90 (m, 1-H), 2.66 (m, 1-H), 1.76 (m; 2-H), 1.68 (m, 1-H), 1.54 (m, 1-H), 1.42 (m, 1-H), 1.22 (m, 5-H), 1.02 ppm (m, 1-H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta=170.3$, 150.0, 147.6, 146.5, 136.5, 136.4, 136.0, 129.5, 128.4, 126.8, 123.4, 120.9, 50.0, 48.0, 42.4, 36.0, 35.7, 32.7 (2C), 32.1, 30.5, 27.1, 23.5 ppm; MS (ESI) *m/z* 418 $[M+H]^+$.

Anal. Calcd for $C_{25}H_{31}N_5O$: C, 74.77; H, 7.78; N, 17.44. Found: C, 74.54; H, 7.45; N, 17.60.

α-[[1-[4-(1-benzoyl-4-piperidinyl)butyl]-1*H*-1,2,3-triazol-4-yl]-methyl]-3-pyridinemethanol (17).

Reaction time: 24 h; Eluant: EtOAc/MeOH 9:1; Yellowish oil; Yield: 60%

IR (neat): $\tilde{\nu}=3290$, 2931, 1625, 1446, 1276, 712 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): $\delta=8.50$ (br s , 1-H), 8.42 (br s, 1-H), 7.70 (d, $J=7.9$ Hz, 1-H), 7.35 (m, 5-H), 7,28 (s, 1-H), 7.23 (m, 1-H), 5.05 (t, $J=6.4$ Hz, 1-H), 4.62 (br s, 1-H), 4.27 (t, $J=7.0$ Hz, 2-H), 3.68 (m, 1-H), 3.08 (d, $J=6.1$ Hz, 2-H), 2.92 (m, 1-H), 2.69 (m, 1-H), 1.83 (m; 2-H), 1.71 (m, 1-H), 1.59 (m, 1-H), 1.47 (m, 1-H);1.26 (m, 5-H), 1.09 ppm (m, 1-H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta=170.4$, 148.6, 147.7, 144.4, 139.5, 136.4, 133.8, 129.6, 128.5, 126.9, 123.5, 122.1, 70.9, 50.2, 48.1, 42.5, 36.0, 35.7, 35.3, 32.9, 31.9, 30.4, 23.6 ppm; MS (ESI) *m/z* 434 $[M+H]^+$.

Anal. Calcd for $C_{25}H_{31}N_5O_2$: C, 69.26; H, 7.20; N, 16.15. Found: C, 69.60; H, 7.10; N, 16.45.

α-[1-[4-(1-benzoyl-4-piperidinyl)butyl]-1*H*-1,2,3-triazol-4-yl]-3-pyridinemethanol (18).

Reaction time: 1 h; Eluant: EtOAc/MeOH 9:1; Yellowish oil; Yield: 75%

IR (neat): $\tilde{\nu}=3291$, 2932, 1623, 1446, 1284, 711 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): $\delta=8.48$ (s, 1-H), 8.28 (dd, $J=4.9/1.5$ Hz, 1-H),

7.67 (dt, J=7.6/1.5 Hz, 1-H), 7.34 (s, 1-H), 7.25 (m, 5-H), 7,12 (dd, J=7.6/4.9 Hz, 1-H), 5.90 (s, 1-H), 4.49 (br s, 1-H), 4.17 (t, J=7.0 Hz, 2-H), 3.56 (br s, 1-H), 2.81 (br s, 1-H), 2.58 (br s, 1-H), 1.72 (m, 2-H), 1.60 (br s; 1-H), 1.46 (br s, 1-H), 1.34 (br s, 1-H), 1.14 (m, 5-H), 1.01 ppm (br s, 1-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=170.3, 151,1, 148.4, 147.8, 138.8, 136.2, 134.6, 129.5, 128.4, 126.8, 123.5, 121.3, 66.7, 50.2, 48.0, 42.4, 35.8, 35.6, 32.6, 31.8, 30.3, 23.5 ppm; MS (ESI) m/z 420 $[M+H]^+$.

Anal. Calcd for $C_{24}H_{29}N_5O_2$: C, 68.71; H, 6.96; N, 16.69. Found: C, 69.10; H, 7.25; N, 16.32.

***N*-[4-(1-benzoyl-4-piperidinyl)butyl]nicotinamide (19).** EDC (39 mg, 0.19 mmol, 1 equiv), DMAP (25 mg, 0.19 mmol, 1 equiv), and nicotinic acid (25 mg; 0.19 mmol, 1 equiv) were sequentially added to a cooled (0 °C) solution of **5** (50 mg; 0.19 mmol) in dry CH_2Cl_2 (1 mL). The reaction mixture was stirred at room temperature for 12 h, and an additional 0.5 equivalents of EDC, DMAP, and nicotinic acid were added. After 24 h, the reaction was worked up by dilution with CH_2Cl_2 and washing with $NaHCO_3$ sol. sat. (×2) and brine (×1). After drying over sodium sulfate and removal of the solvent, the residue was purified by column chromatography using EtOAc/MeOH 9:1 as eluants to give 50 mg of **19** as a yellowish oil (71%).

IR (neat): $\tilde{\nu}$=3304, 2935, 1617, 1447, 1283, 708 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ=8.95 (s, 1-H), 8.61 (br s, 1-H), 8.06 (d, J= 7.7 Hz, 1-H), 7.33–7.20 (br s, 6-H), 7.15 (br t, NH), 4.59 (br s, 1-H), 3.66 (br s, 1-H), 3.36 (m, 2-H), 2,89 (br s, 1-H), 2.67 (br s, 1-H), 1.70–1.15 ppm (m, 11-H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=170.4, 165.6, 151.8, 147.9, 136.4, 135.4, 130.6, 129.5, 128.5, 126.8, 123.6, 48.1, 42.6, 40.1, 36.1, 36.0, 32.9, 32.0, 29.8, 24.0 ppm; MS (ESI) m/z 388 $[M+Na]^+$.

Anal. Calcd for $C_{22}H_{27}N_3O_2$: C, 72.30; H, 7.44; N, 11.49. Found: C, 72.60; H, 7.20; N, 11.55.

Cell Culture

SH-SY 5Y (neuroblastoma) cells were cultured in DMEM (Sigma) supplemented with 10% FBS, 2 mM glutamine, 10 U mL^{-1} penicillin, and 100 μg mL^{-1} streptomycin. Cells were maintained in a humidified incubator supplied with 5% CO_2/95% air at 37 °C. Cells were subcultured as needed by detaching the cells with 0.25% trypsin and 5 mM EDTA.

Cell viability assay

To analyze cell viability, the colorimetric MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-dipehenyltetrazolium bromide) assay was used. Briefly, cells were plated in 24-well plates and treated as indicated for the appropriate time. FK866 (dissolved in DMSO) and the vehicle control were added to the cells to give a final DMSO concentration no greater than 0.5%. Cells were washed once in Locke Buffer and 300 μL of MTT (250 μg mL^{-1} in Locke Buffer) was added before returning the cells to the incubator for one hour to allow the formation of the purple formazan crystals. After one hour, 600 μL of isopropanol/0.1 M HCl was added to each well and the absorbance was read at 570 nm in a plate reader (Victor^{3}V, Perkin–Elmer).

Alternatively cell counts were performed. Briefly, cells were detached from the wells by trypsinization, stained with Trypan Blue to exclude dead cells, and counted in a Burker Chamber.

NAD(P) cycling assay

Total cellular NAD(P) was measured using an adaptation of the method described by Gasser.[19] Cells, grown and treated in 24-well plates, were scraped with the piston of a 1 mL syringe in 100 μL ddH_20 on ice, and were extracted with 1 volume of $HClO_4$ (2 M) for 45 min on ice. The samples were then centrifuged for 1 min at 13,000 g and the supernatant was diluted with an equal volume of K_2CO_3 (1 M). After a further 45 min incubation on ice, the insoluble potassium perchlorate was removed by centrifugation for 1 min at 13000 g. The final pH of the supernatant was 8–8.5. In a black 96-well plate (OptiPlate™-96 F: PerkinElmer) 60 μL of cycling mix was added to 100 μL of the extracted NAD(P). The cycling mix (60 μL) consisted of: 20 μL 500 mM NaH_2PO_4, pH 8.0, 2 μL 5 mg mL^{-1} BSA, 0.1 μL 10 mM resazurin (prepared fresh), 5 μL 100 mM glucose-6-phosphate (G8404, Sigma), 15 μL 1 mg mL^{-1} glucose-6-phosphate dehydrogenase, 15 μL of purified diaphorase (see below). Fluorescence (excitation 544 nm, emission 590 nm) was measured for each well using a plate reader (Victor^{3}V, PerkinElmer). Standard curves were generated with authentic NAD subjected to mock extractions used to generate calibration curves.

The diaphorase was purified as follows: 100 μL of enzyme solution (diaphorase 12 mg mL^{-1} in 50 mM NaH_2PO_4, pH 8.0 adjusted with NaOH) was mixed with 200 μL BSA (5 mg mL^{-1} in water) and 1.2 mL of a suspension (2% w/v) of activated charcoal in 50 mM NaCl, 20 mM NaH_2PO_4, pH 8.0. After incubation at 37 °C for 30 min, the charcoal was removed by centrifugation (11,000 g, 10 min, 4 °C), and the supernatant used directly for the cycling assay. FK866 did not interfere with the cycling assay.

Acknowledgements

Financial support from Università del Piemonte Orientale and the Regione Piemonte (Progetto Ricerca Sanitaria Finalizzata 2007) to RAB is gratefully acknowledged.

Keywords: bioisostere · chemotherapeutic agent · click chemistry · FK866 · NAD · triazole

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Received: November 2, 2007
Revised: December 14, 2007
Published online on February 5, 2008

