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Replacement of the lactone moiety on podophyllotoxin and steganacin analogues with a 1,5-disubstituted 1,2,3-triazole via ruthenium-catalyzed click chemistry

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Abstract—Steganacin and podophyllotoxin are two naturally occurring lignans first isolated from plant sources, which share the capability to disrupt tubulin assembly. Although not strictly essential for its activity, the lactone ring on both structures represents Achilles' heel, as it is a potential site of metabolic degradation and epimerization on its C2 carbon brings about a significant loss in potency. In the present manuscript, we have used the ruthenium-catalyzed [3+2] azide–alkyne cycloaddition, a click-chemistry reaction, to replace the lactone ring with a 1,5-disubstituted triazole in few synthetic steps. The compounds were cytotoxic, although to a lesser degree compared to podophyllotoxin, while retaining antitubulin activity. The present structures might therefore represent a good platform for the fast generation of metabolically stable compounds with few stereogenic centers that might be of value from a medicinal chemistry point of view.

1. Introduction

(−)-Steganacin (**1**) and (−)-podophyllotoxin (**2**) (Fig. 1) are two naturally occurring lignans first isolated from plant sources.[1] Both compounds inhibit the assembly of tubulin into microtubules by interacting with the colchicine binding site and have been shown to possess cytotoxic activity against several cancer cell lines.[2] In addition, podophyllotoxin and its congeners have also been shown to possess other activities, for example antiviral and antirheumatic.[3]

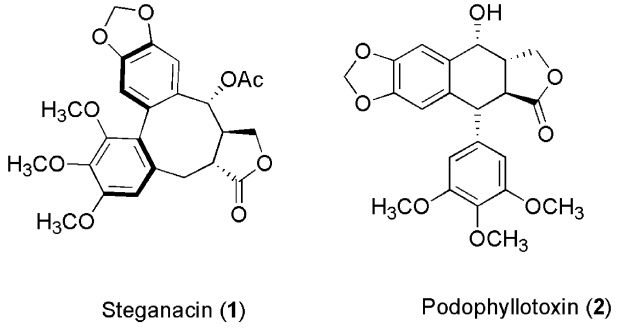

Figure 1. Steganacin and podophyllotoxin.

Over the years, a series of analogues of podophyllotoxin and steganacin have been synthesized and a robust SAR is now available.[4,5] The structure–activity relationships of these two 5-ring compounds share a number of similarities: (i) the dioxolane moiety is fundamental for the cytotoxic activity[6]; (ii) the lactone ring is not essential, although it contributes to potency[7]; (iii) in podophyllotoxin, the hydroxyl at the 4-position is not essential as 4-deoxypodophyllotoxin maintains similar cytotoxic properties in respect to its parent compound[8]; (iv) in steganacin, the acetate is not essential as stegane is equally active[5a]; and (v) the trimethoxyphenyl ring is essential in both compounds for cytotoxic and antitubulic activities.[4,5]

Although the lactone group is not strictly required on podophyllotoxin, when this undergoes epimerization

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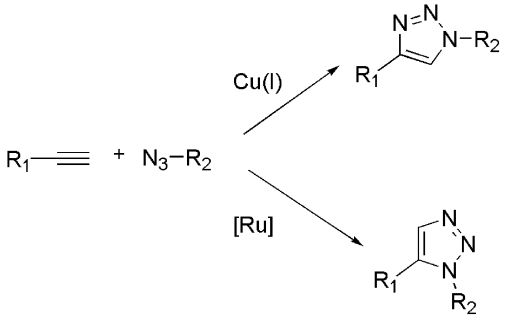


Scheme 1. The copper or ruthenium 1,3-dipolar cycloaddition between azides and alkynes.

(to yield the more thermodynamically stable C2 epimer picropodophyllotoxin),[9] the resulting compound displays a significantly reduced cytotoxicity and antitubulinic activity.[4,10] Indeed, the presence of the trimethoxyphenyl group in a quasi-axial position in respect to the tetracyclic core is fundamental for potency and this is only possible when the lactone ring is in a trans-junction. Epimerization is rather common, as it occurs in slightly basic medium and also under neutral conditions.[11] Furthermore, the lactone ring might be vulnerable to metabolic cleavage and also for this reason it has been replaced by other functional groups.[12]

Among the most recent exciting developments in medicinal chemistry is the use of copper or ruthenium compounds to regioselectively and efficiently catalyze the [3+2] cycloaddition between azides and alkynes (Scheme 1).[13–15] We therefore decided to exploit the ruthenium-catalyzed [3+2] cycloaddition to substitute the lactone moiety in deoxypodophyllotoxin and stegane with a 1,5-disubstituted 1,2,3-triazole. In theory, this heterocyclic ring would be metabolically stable and able to participate as a hydrogen-bond acceptor, further allowing the removal of two stereocenters.

In this manuscript, we report the synthesis and the biological evaluation of these new aza analogues of podophyllotoxin and steganacin which can be readily prepared in a few synthetic steps using the ruthenium 'click' cycloaddition.

2. Chemistry

The retrosynthetic strategy in Scheme 2 was planned to synthesize the desired compounds. Commercially available piperonyl alcohol was brominated using phosphorous tribromide in ether to give piperonyl bromide (**3**) which was subsequently turned into an azide (**4**) using sodium azide in DMF/water. Trimethoxybenzyl azide (**8**) was synthesized using similar conditions starting from 3,4,5-trimethoxybenzyl alcohol via bromide derivative (**7**). Preparation of the desired alkynes was carried out starting from the corresponding bromide derivatives of the piperonyl (**3**) and trimethoxybenzyl alcohol (**7**).

The acetylenic group was inserted using ethynyltrimethylsilane in the presence of ethylmagnesium bromide and freshly purified copper (I) bromide as catalyst[16] (Scheme 3). The ethylnyltrimethylsilyl magnesium bromide was obtained at 0 °C by adding a solution of ethylmagnesium bromide dropwise to ethynyltrimethylsilane in THF. After stirring at room temperature for 30 min, catalytic amounts of copper (I) bromide were added and, after 30 min more, the corresponding bromides **3** or **7** were added and the resulting solution was heated at reflux for 16 h to obtain excellent yields of the desired acetylenic derivatives (**5** and **9**). Reaction times and the reagent amounts (ethynyltrimethylsilane/ethylmagnesium bromide/bromide derivatives in ratio 4:4:1) were fundamental to obtain reproducible results in high yields.

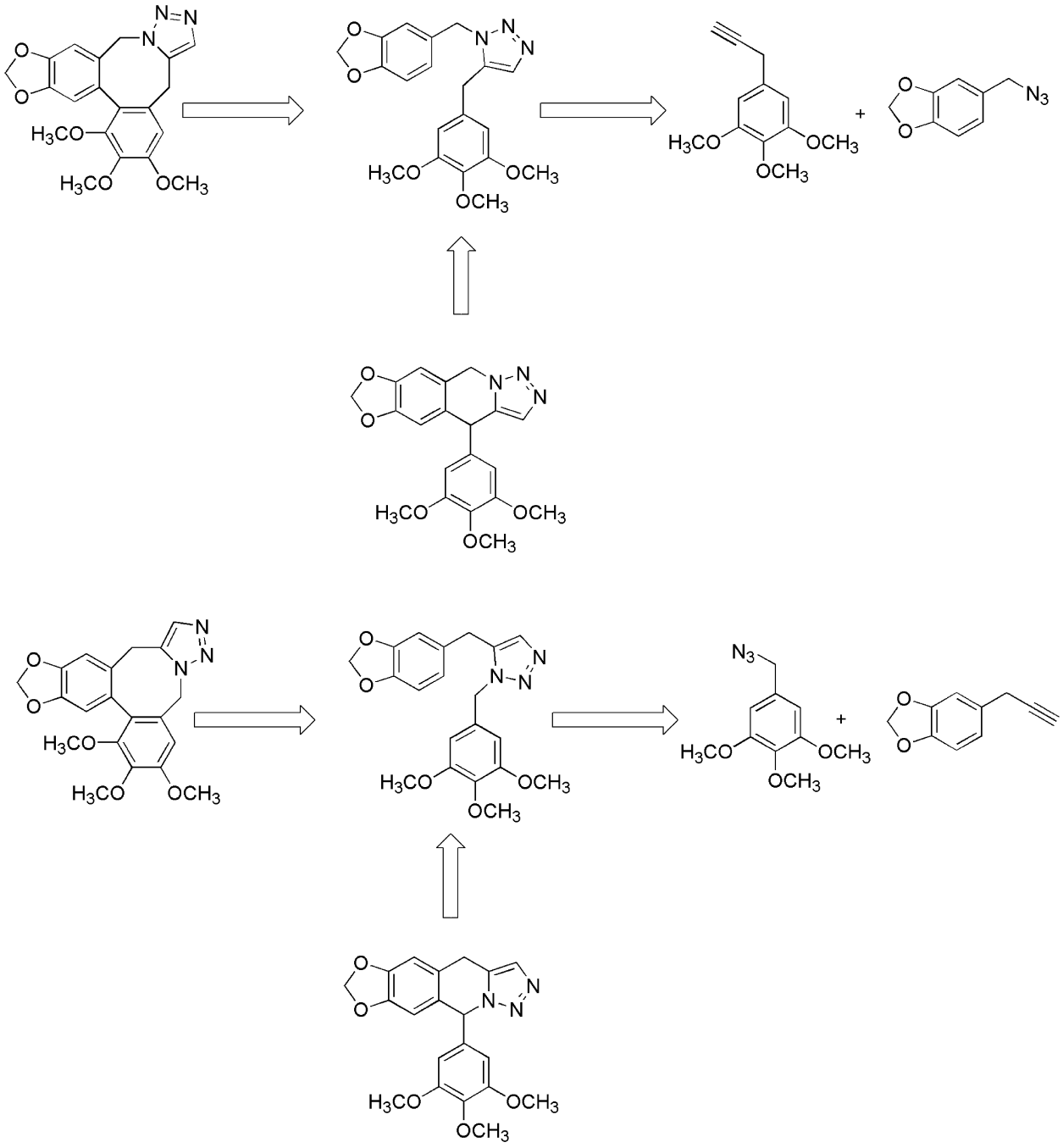
The two alkynes were then deprotected using the classical protocol (TBAF, THF, 0 °C) but, to our surprise, after the cleavage of the silyl group, an alkyne–allene isomerization took place giving the corresponding allenic derivatives. In our opinion, this might have been due to the basicity of the fluoride ion, which might have been enough to catalyze this process.[17] For this reason, we carried out the deprotection using silver nitrate[18] or TBAF in acetic acid to give the desired alkynes **6** and **10**, suppressing the isomerization (Scheme 3).

With these intermediates in our hands, we performed the click-chemistry reaction using $Cp^*Ru(PPh_3)_2Cl$ as catalyst in refluxing benzene to give the 1,5-disubstitued triazoles **11** and **13** in good to excellent yields (Scheme 4). With these substrates, we did not notice the formation of the 1,4-regioisomers (a parallel successful positive control of the 1,4-regioisomer formation was carried out using copper (II) sulphate/sodium ascorbate).[13b]

In our stategy, we sought to generate azasteganacins and azadeoxypodophyllotoxins by using different oxidative coupling protocols[19] from the same reactants (**11** and **13**). To our delight, using thallium (III) oxide/boron trifluoride in trifluoroacetic acid, we were able to obtain the racemic azasteganacin derivatives **12** and **14** in excellent yields (Scheme 4).

Unfortunately, using a number of protocols (e.g., Tl_2O_3 in CF_3COOH or $Mn(OAc)_3$ in CF_3COOH) we were not able to obtain the azadeoxypodophyllotoxin analogues and for this reason a different strategy was used to get these compounds. Indeed, a hydroxyl group at the benzylic position might undergo an intramolecular Friedel–Craft alkylation under acidic condition. For this reason, intermediate **17** was prepared in the following manner: 3,4,5-trimethoxybenzaldehyde was reacted with ethynyltrimethylsilane in the presence of butyl lithium at −78 °C to give the corresponding secondary alcohol **15**. This compound was deprotected using TBAF in THF and the resulting terminal alkyne **16** was reacted with **4** in the presence of $Cp^*Ru(PPh_3)_2Cl$ as catalyst. Also in this case clean formation of the 1,5-disubstituted triazole **17** was observed (Scheme 5).

Finally, acidic intramolecular Friedel–Craft reaction using trifluoroacetic acid gave the desired azadeoxypodophyllotoxin analogue **18** (Scheme 6). All attempts to

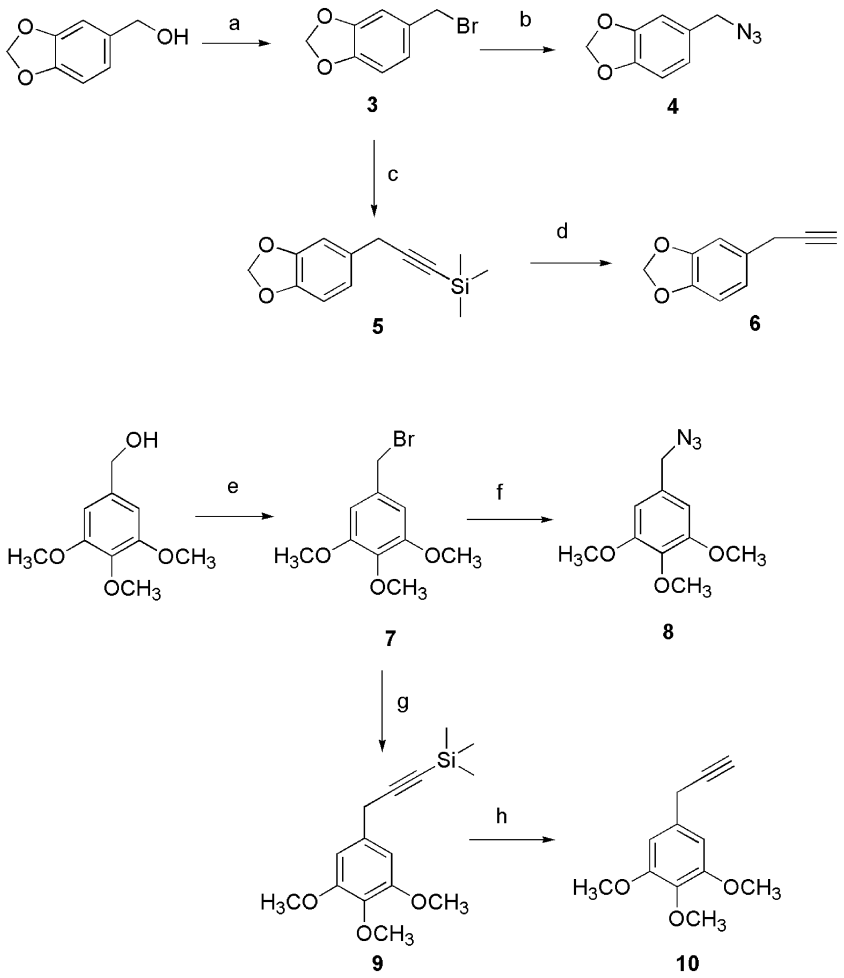


Scheme 2. Retrosynthetic analysis for the synthesis of the target compounds.

generate the other azapodophyllotoxin analogue failed (see Supplementary materials for the synthetic attempts).

3. Biological results and conclusion

To evaluate the biological potential of the synthesized compounds, we first screened their cytotoxicity in comparison to podophyllotoxin (**2**). Compounds were incubated for 48 h with a neuroblastoma cell line (SH-SY5Y) and viability was evaluated via the MTT method. Under these conditions, **2** displayed an IC_{50} of approx. 7.0 ± 0.9 nM, a concentration comparable to those reported previously by others.[4] The synthetic intermediates where the triazole had not been closed (**11**, **13**, **17**), were devoid of any activity (data not shown and Fig. 2), since no cytotoxicity was observable up to 100 μM. The steganacin analogue **12** displayed some cytotoxic potential at concentrations over 10 μM, but this was not further studied. On the contrary, **14** displayed cytotoxic activity which was comparable to **1** as far as maximal effect, but with a significantly lower potency (IC_{50} of approx. 1.1 ± 0.4 μM). Strikingly, the cytotoxic potential of this compound is similar, if not higher compared to that reported by others for

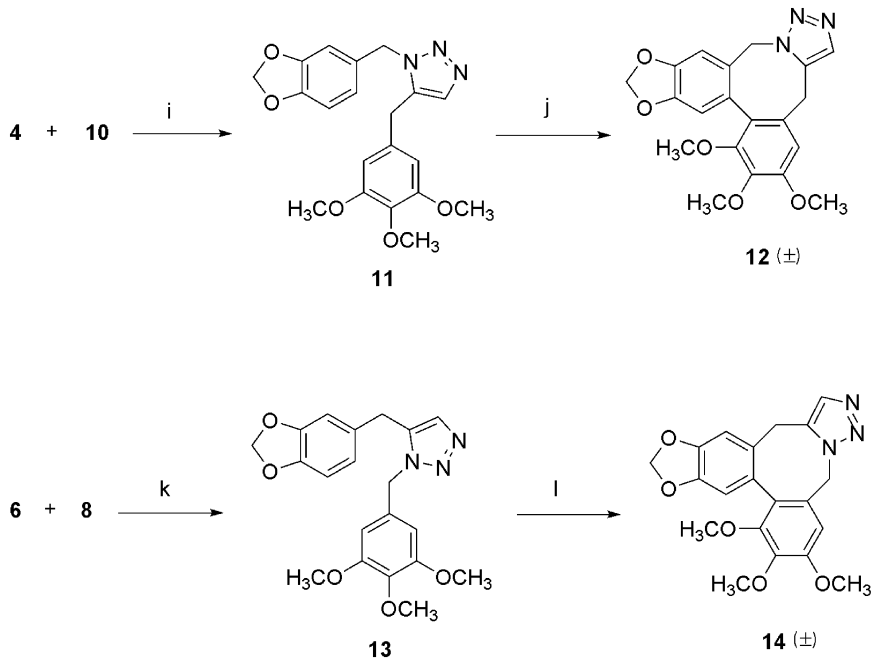


Scheme 3. Reagents and conditions: (a) PBr_3, Et_2O, 0 °C, 70%; (b) NaN_3, DMF, 40 °C, 65%; (c) ethynyltrimethylsilane, EtMgBr, CuBr, THF, reflux, 90%; (d) CH_3COOH, TBAF, 0 °C, 90%; (e) PBr_3, CH_2Cl_2, 0 °C, 81%; (f) NaN_3, DMF, 40 °C, 72%; (g) ethynyltrimethylsilane, EtMgBr, CuBr, THF, reflux, 69%; (h) $AgNO_3$, THF/H_2O/2,3-lutidine, 0 °C, 71%.

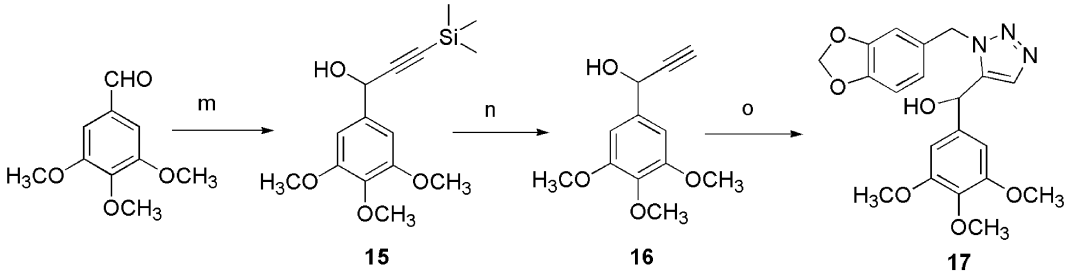
steganacin, its reference analogue.[5] Last, the podophyllotoxin analogue (**18**) was the most efficacious compound tested, and displayed an IC_{50} of approx. 1.5 ± 0.7 μM.

To evaluate whether the active compounds synthesized still retained the antitubulin activity of their parent compounds, we decided to investigate the effect of (**18**) in an in vivo tubulin assay, comparing its effects to podophyllotoxin, combretastatin A-4, and paclitaxel (Fig. 3). In brief, cells were incubated with tubulin disrupting agents for 16 h and then proteins were harvested in the presence of paclitaxel to freeze the polymerized and unpolymerized forms of tubulin. Proteins were then run on SDS–PAGE and an antitubulin antibody was used to detect differences in polymerization. In this assay, combretastatin A-4 and **2** both displayed, as expected, a depolymerizing effect, since soluble protein was more than that found in the pellet. As expected, paclitaxel displayed the opposite behavior. As shown in Figure 3, compound **18** still retained the capacity to disrupt tubulin polymerization, as it displayed a pattern similar to **2** and combretastatin A-4.

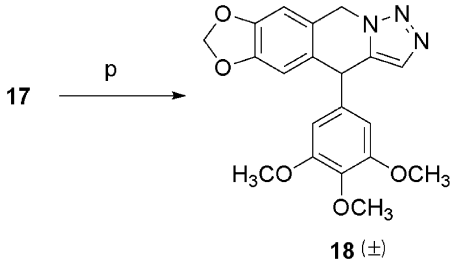
In conclusion, we report the synthesis of steganacin and podophyllotoxin analogues that present a triazole moiety in place of the lactone ring. The synthetic strategy used is composed of few steps and makes use of a click-chemistry reaction that capitalizes on the capacity of ruthenium to catalyze [3+2] Huisgen's cycloaddition between azides and alkynes to yield the 1,5-disubstituted triazole selectively. Indeed, the number of steps used for the synthesis is considerably fewer than those utilized to synthesize similar compounds,[20] once again strengthening the claim that click chemistry is a valid aid to the synthetic medicinal chemist. The synthesized compounds appear to be active as cytotoxic agents and retain antitubulin activity. Indeed, in our opinion they could form the basis for novel analogues, as the trans lactone presents low thermodynamic stability (compared to the biologically inactive cis-form) and might undergo metabolic cleavage. The aza analogues also present a reduced number of stereogenic centers allowing the possibility to generate a small library of compounds in a relatively short period of time. Although podophyllotoxin itself has not reached the market as antitumoral agent, due to its low therapeutic index, it was the molecular platform for the discovery of two



Scheme 4. Reagents and conditions: (i) Cp*Ru(PPh$_3$)$_2$Cl, benzene, reflux, 85%; (j) Tl_2O_3, BF_3 etherate, CF_3COOH, rt, 62%; (k) Cp*Ru(PPh$_3$)$_2$Cl, benzene, reflux, 94%; (l) Tl_2O_3, BF_3 etherate, CF_3COOH, rt, 90%.



Scheme 5. Reagents and conditions: (m) ethynyltrimethylsilane, BuLi, THF, −78 °C, 92%; (n) TBAF, THF, 0 °C, 96%; (o) Cp*Ru(PPh$_3$)$_2$Cl, benzene, reflux, 56%.



Scheme 6. Reagents and conditions: (p) CF_3COOH, molecular sieves 4 Å, reflux, 22%.

blockbuster topoisomerase II inhibitors, such as etoposide and teniposide,[21] and therefore such libraries could be of use for multiple targets.

4. Experimental

Commercially available reagents and solvents were used without further purification and were purchased from Fluka–Aldrich or Lancaster. Dichloromethane was

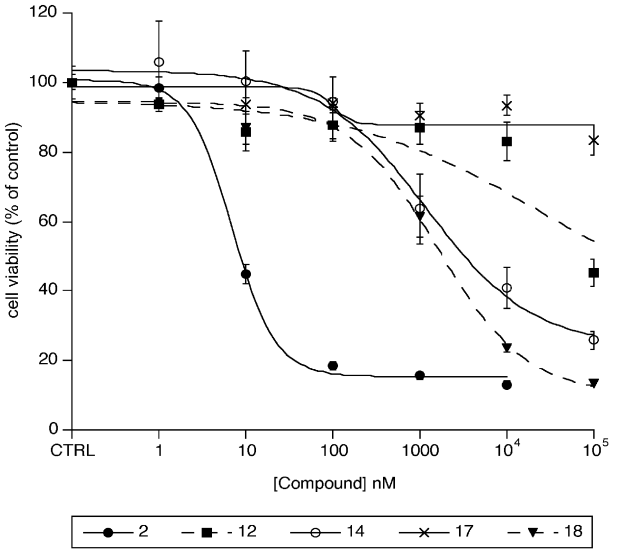


Figure 2. Dose–response curve of cytotoxicity for the active compounds synthesized. Compounds **11** and **13** were devoid of any activity at the highest concentration tested (100 μM).

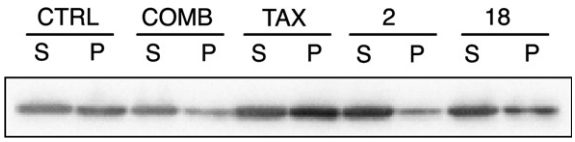


Figure 3. In vitro tubulin polymerization assay. S stands for soluble (non-polymerized) and P stands for pellet (polymerized). Tubulin was visualized with a monoclonal mouse antibody.

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 bp 76 °C at 39 mmHg 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) 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 $60F_{254}$). When necessary they were developed with $KMnO_4$., Dragendorff reagent, and Phosphomolibdic reagent. Elemental Analysis (C, H, N) of the target compounds **11**, **12**, **13**, **14**, **17**, and **18** are within ±0.4% of the calculated values unless otherwise noted.

Ruthenium catalyst Cp*Ru(PPh_3)$_2$Cl was synthesized as described previously.[22]

Compounds were evaluated via the MTT method and via the in vitro tubulin assay as described previously.[23]

4.1. 5-(Bromomethyl)-1,3-benzodioxole (3)

To a cooled (0 °C) solution of piperonyl alcohol (5 g; 32.86 mmol) in dry diethyl ether (50 mL), 3.1 mL of phosphorous tribromide (32.86 mmol; 1 equiv) dissolved in 40 mL of diethyl ether was added dropwise. After 10 min, the reaction was worked up by dilution with water. The organic layer was then washed with brine (1×). After drying over sodium sulphate, filtration, and evaporation of the solvent, the crude was recrystallized with petroleum ether to give 5 g of the bromine **3** as white solid (70%). mp 97.0–98.0 °C; IR (KBr) 2904, 1500, 1420, 1035, 776 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.86 (m, 2-H), 6.75 (d, $J = 8.2$ Hz, 1-H), 5.97 (s, 2-H), 4.45 (s, 2-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 148.0, 147.9, 131.6, 122.9, 109.6, 108.4, 101.4, 34.4.

4.2. 1,3-Benzodioxol-5-ylmethyl azide (4)

To a solution of **3** (1.1 g; 5.1 mmol) in DMF (14 mL) and water (3 mL), 500 mg of sodium azide (7.7 mmol; 1.5 equiv) were added. The resulting mixture was heated at 40 °C for 2 h. The reaction was worked up by dilution with diethyl ether and washed with water (2×). The aqueous layer was further washed with EtOAc (2×), and the combined organic extracts were washed with brine (1×). After drying over sodium sulphate, filtration, and evaporation of the solvent, the crude was purified by column chromatography using PE/EtOAc 95:5 as eluant to give 600 mg of the corresponding azide **4** as colorless oil (65%). IR (neat) 2094, 1489, 1444, 1243, 1037, 927 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.79 (m, 3-H), 5.98 (s, 2-H), 4.23 (s, 2-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 148.2, 147.8, 129.3, 122.0, 108.8, 108.4, 101.4, 54.7.

4.3. [3-(1,3-Benzodioxol-5-yl)-1-propynyl](trimethyl)silane (5)

In a three-necked round bottom flask in dry conditions ethynyltrimethylsilyl magnesium bromide was prepared in situ in the following manner: to a cooled (0 °C) solution of ethynyltrimethylsilane (10.2 mL; 73.4 mmol; 4 equiv) in dry THF (60 mL), 73.4 mL of ethyl magnesium bromide (1 M solution in THF; 73.4 mmol; 4 equiv) was added dropwise. After the addition, the cooling bath was removed and the resulting solution was stirred at room temperature for 30 min. Then, 395 mg of copper (I) bromide (2,75 mmol; 0.2 equiv) was added and after 30 min 3.9 g of **3** (18.35 mmol; 1 equiv) was added. The resulting mixture was heated at reflux for 16 h. The reaction was then cooled at 0 °C and satd aq NH_4Cl was added dropwise. The resulting solution was then diluted with EtOAc and the organic layer was washed with water (1×) and brine (1×). After drying over sodium sulphate, filtration, and evaporation of the solvent, the crude was purified by column chromatography using PE/EtOAc 9:1 as eluant to give 3.85 g of **5** as pale yellow oil (90%). IR (neat) 1502, 1488, 1245, 1039, 838 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.76 (m, 3-H), 5.94 (s, 2-H), 3.56 (s, 2-H), 0.17 (s, 9-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 147.8, 146.3, 130.2, 120.8, 108.6, 108.2, 104.5, 101.0, 86.9, 25.9, 0.18.

4.4. 5-(2-Propynyl)-1,3-benzodioxole (6)

To a solution of **5** (3.8 g; 16.37 mmol) in THF (38 mL), 1.1 mL of acetic acid was added (19.55 mmol; 1.2 equiv). The resulting mixture was cooled at 0 °C and 19.55 ml of TBAF sol. 1 M in THF was added dropwise (19.55 mmol; 1.2 equiv). After 4 h, the reaction was worked up by evaporation of the solvent, and the residue was dissolved in EtOAc and washed with water (1×) and brine (1×). After drying over sodium sulphate, filtration, and evaporation of the solvent, the crude was purified by column chromatography using PE/EtOAc 9:1 as eluant to give 2.3 g of **6** as pale yellow oil (90%). IR (neat) 3292, 1501, 1487,1243, 1037, 803 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.85 (d, $J = 1.1$ Hz, 1-H), 6.78 (m, 2-H), 5.93 (s, 2-H), 3.51 (d,

J = 2.7 Hz, 2-H), 2.18 (t, J = 2.7 Hz, 1-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 147.8, 146.4, 129.8, 121.0, 108.4, 108.1, 101.0, 82.0, 70.5, 24.3.

4.5. 5-(Bromomethyl)-1,2,3-trimethoxybenzene (7)

To a cooled (0 °C) solution of trimethoxybenzyl alchool (3 g; 15.13 mmol) in dry dichloromethane (30 mL), 1.4 mL of phosphorous tribromide, (15.13 mmol; 1 equiv) dissolved in 40 mL of diethyl ether, was added dropwise. After 10 min, the reaction was worked up by dilution with water. The organic layer was then washed with brine (1×). After drying over sodium sulphate, filtration, and evaporation of the solvent, the crude was recrystallized with petroleum ether to give 3.2 g of the bromine **7** as white solid (81%). mp 86–87 °C; IR (KBr) 2942, 1589, 1465, 1245, 993 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6,59 (s, 2-H), 4.43 (s, 2-H), 3.83 (s, 6-H), 3.81 (s,3-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.1, 138.2, 133.3, 106.2, 60.9, 56.2, 34.4; MS (ESI) m/z 261–263 $(M+H)^+$.

4.6. 5-(Azidomethyl)-1,2,3-trimethoxybenzene (8)

To a solution of **7** (3 g; 11.49 mmol) in DMF (36 mL) and water (8 mL), 1.1 g of sodium azide (17.23 mmol; 1.5 equiv) was added. The resulting mixture was heated at 40 °C for 2 h. The reaction was worked up by dilution with diethyl ether and washed with water (2×). The aqueous layer was further washed with EtOAc (2×), and the combined organic extracts were washed with brine (1×). After drying over sodium sulphate, filtration, and evaporation of the solvent, the crude was purified by column chromatography using PE/EtOAc 9:1 and PE/EtOAc 8:2 as eluants to give 1.8 g of the corresponding azide **8** as colorless oil (72%). IR (neat) 2943, 2100, 1593, 1460, 1128 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.48 (s, 2-H), 4.22 (s, 2-H), 3.81 (s, 6-H), 3.78 (s, 3-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.5, 137.7, 131.1, 105.1, 60.1, 56.1, 55.1; MS (ESI) m/z 224 $(M+H)^+$.

4.7. Trimethyl[3-(3,4,5-trimethoxyphenyl)-1-propynyl] silane (9)

In a three-necked round bottom flask in dry condition, ethynyltrimethylsilyl magnesium bromide was prepared in situ in the following manner: to a cooled (0 °C) solution of ethynyltrimethylsilane (1.06 mL; 7.66 mmol; 4 equiv) in dry THF (4 mL), 7.66 mL of ethyl magnesium bromide (1 M solution in THF; 7.66 mmol; 4 equiv) was added dropwise. After the addition the cooling bath was removed and the resulting solution was stirred at room temperature for 30 min. Then, 41 mg of copper (I) bromide (0.29 mmol; 0.2 equiv) was added and after 30 min 500 mg of **7** (1.9 mmol; 1 equiv) was added. The resulting mixture was heated at reflux for 16 h. The reaction was then cooled at 0 °C and satd aq NH_4Cl was added dropwise. The resulting solution was then diluted with EtOAc and the organic layer was washed with water (1×) and brine (1×). After drying over sodium sulphate, filtration, and evaporation of the solvent, the crude was purified by column chromatography using PE/EtOAc 9:1 as eluant to give 1.9 g of **9** as pale yellow oil (69%). IR (neat) 1590, 1505, 1420, 1236, 1125, 840 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.56 (s, 2-H), 3.83 (s, 6-H), 3.80 (s, 3-H), 3.57 (s, 2-H), 0.17 (s, 9-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.2, 136.7, 132.0, 104.9, 104.3, 87.4, 60.9, 56.0, 26.4, 0.14. MS (ESI) m/z 301 $(M+Na)^+$.

4.8. 1,2,3-Trimethoxy-5-(2-propynyl)benzene (10)

To a cooled (0 °C) solution of **7** (700 mg; 2.69 mmol) in THF (12 mL), water (12 mL), ethanol (12 mL), and 2,3-lutidine (1.2 mL), 4.6 g of silver nitrate was added (26.9 mmol; 10 equiv). After 30 min the reaction was worked up by acidification with H_2SO_4 2 M. The resulting precipitate was filtered off and the solution was diluted with EtOAc and washed with brine (1×). After drying over sodium sulphate, filtration, and evaporation of the solvent, the crude was purified by column chromatography using PE/EtOAc 9:1 as eluant to give 1.9 g of **10** as pale yellow oil (71%). IR (neat) 3283, 1590, 1504, 1420, 1234, 1121, 1004, 816 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.57 (s, 2-H), 3.86 (s, 6-H), 3.82 (s, 3-H), 3.56 (d, J = 2.7 Hz, 2-H), 2.20 (t, J = 2.7 Hz, 1-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.4, 136.8, 131.8, 104.9, 81.9, 70.7, 60.9, 56.2, 25.1; MS (ESI) m/z 207 $(M+H)^+$.

4.9. 1-(1,3-Benzodioxol-5-ylmethyl)-5-(3,4,5-trimethoxybenzyl)-1*H*-1,2,3-triazole (11)

To a solution of the azide **4** (650 mg; 3.67 mmol; 1 equiv) in benzene (40 mL), 1.3 g of the alkyne **10** (6.24 mmol; 1.7 equiv) and 52 mg of Cp*RuCl$(PPh_3)_2$ (0.073 mmol; 0.02 equiv) were added. The resulting mixture was heated at reflux for 24 h and worked up by evaporation of the solvent. The resulting crude was purified by column chromatography using PE/EtOAc 7:3 and PE/EtOAc 4:6 as eluants to give 1.2 g of **11** as pale yellow solid (85%). mp 101–102 °C; IR (KBr) 2940, 1591, 1335, 1127, 923 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.55 (s, 1-H), 6.73 (d, J = 8.0 Hz, 1-H), 5.57 (br d, J = 8.0 Hz, 2-H), 6.18 (s, 2-H), 5.95 (s, 2-H), 5.34 (s, 2-H), 3.84 (s, 2-H), 3.82 (s, 3-H), 3.76 (s, 6-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.5, 148.3, 147.7, 137.1, 135.6, 134.4, 131.5, 128.4, 120.9, 108.4, 107.9, 105.4, 101.4, 61.0, 56.2, 51.9, 29.6; MS (ESI) m/z 406 $(M+Na)^+$.

Anal. Calcd for $C_{20}H_{21}N_3O_5$: C, 62.65; H, 5.52; N, 10.95. Found: C, 62.64; H, 5.53; N, 10.96.

4.10. 6,7,8-Trimethoxy-4,14-dihydrobenzo[*d*][1,3]benzodioxolo[5,6-*f*][1,2,3]triazolo[1,5-*a*]azocine (12)

To a solution of **11** (200 mg; 0.52 mmol; 1 equiv) in trifluoroacetic acid (2 mL), 0.2 mL of boron trifluoride diethyl ether (1.6 mmol; 3 equiv) was added. Then a suspension containing 123 mg of thallium oxide (0.27 mmol; 0.52 equiv) in trifluoroacetic acid (0.5 mL) was added. After 15 min, the reaction was worked up by dilution with EtOAc and the organic layer was washed with NaOH 1 M (2×) and brine (1×). After drying over sodium sulphate, filtration, and evaporation of the solvent, the crude was purified by column chroma-

tography using PE/EtOAc 5:5 to give 124 mg of **12** as white powder (62%). mp 203–204 °C; IR (KBr) 2941, 1596, 1486, 1238, 1038 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.47 (s, 1-H), 6.94 (s, 1-H), 6.83 (s, 1-H), 6.58 (s, 1-H), 6.03 (s, 1-H), 5.98 (s, 1-H), 5.53 (d, J = 14.1 Hz; 1-H), 4.59 (d, J = 14.1 Hz, 1-H), 3.88 (s, 3-H), 3.84 (s, 3-H), 3.83 (d, J = 15.3 Hz, 1-H), 3.70 (s, 3-H), 3.30 (d, J = 15.3 Hz, 1-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 154.0, 150.9, 148.2, 148.1, 141.7, 134.2, 132.4, 129.8, 126.6, 124.4, 110.1, 110.0, 108.5, 101.7, 61.1, 61.0, 56.1, 52.2, 28.9; MS (ESI) m/z 382 $(M+H)^+$.

Anal. Calcd for $C_{20}H_{19}N_3O_5$: C, 62.98; H, 5.02; N, 11.01. Found: C, 62.73; H, 5.43; N, 11.30.

4.11. 5-(1,3-Benzodioxol-5-ylmethyl)-1-(3,4,5-trimethoxybenzyl)-1*H*-1,2,3-triazole (13)

To a solution of the azide **8** (1.5 g; 6.62 mmol; 1 equiv) in benzene (62 mL), 1.8 g of the alkyne **6** (11.25 mmol; 1.7 equiv) and 96 mg of Cp*RuCl(PPh_3)$_2$ (0.13 mmol; 0.02 equiv) was added. The resulting mixture was heated at reflux for 24 h and worked up by evaporation of the solvent. The resulting crude was purified by column chromatography using PE/EtOAc 7:3 and PE/EtOAc 4:6 as eluants to give 2.4 g of **13** as pale yellow solid (94%). mp 98–99 °C; IR (KBr) 2941, 1592, 1461, 1242, 1037 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.53 (s, 1-H), 6.69 (d, J = 8.0 Hz, 1-H), 6.46 (m, 2-H), 6.26 (s, 2-H), 5.93 (s, 2-H), 5.32 (s, 2-H), 3.84 (br s, 5-H), 3.81 (s, 3-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.6, 148.6, 146.7, 137.8, 136.0, 134.1, 130.3, 129.5, 121.4, 108.7, 108.4, 104.3, 101.2, 60.8, 56.1, 52.0, 28.9; MS (ESI) m/z 384 $(M+H)^+$.

Anal. Calcd for $C_{20}H_{21}N_3O_5$: C, 62.65; H, 5.52; N, 10.95. Found: C, 63.05; H, 5.65; N, 11.10.

4.12. 7,8,9-Trimethoxy-5,15-dihydro[1,3]benzodioxolo [5,6-*d*]benzo[*f*][1,2,3]triazolo[1,5-*a*]azocine (14)

To a solution of **13** (200 mg; 0.52 mmol; 1 equiv) in trifluoroacetic acid (2 mL), 0.2 mL of boron trifluoride diethyl ether (1.6 mmol; 3 equiv) was added. Then a suspension containing 123 mg of thallium oxide (0.27 mmol; 0.52 equiv) in trifluoroacetic acid (0.5 mL) was added. After 15 min the reaction was worked up by dilution with EtOAc and the organic layer was washed with NaOH 1 M (2×) and brine (1×). After drying over sodium sulphate, filtration and evaporation of the solvent, the crude was purified by column chromatography using PE/EtOAc 5:5 to give 183 mg of **14** as white powder (90%). mp 199–200 °C; IR (KBr) 2942, 1598, 1483, 1352, 1141, 995 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.55 (s, 1-H), 6.81 (br s, 1-H), 6.04 (s, 1-H), 5.99 (s, 1-H), 5.55 (d, J = 14.1 Hz, 1-H), 4.59 (d, J = 14.1 Hz, 1-H), 3.92 (s, 3-H), 3.87 (s, 3-H), 3.79 (d, J = 15.0 Hz, 1-H), 3.71 (s, 3-H), 3.36 (d, J = 15.0 Hz, 1-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.9, 150.9, 148.1, 141.7, 134.0, 132.4, 129.9, 126.7, 124.4, 110.2, 110.0, 108.5, 101.7, 61.1, 61.0, 56.1, 52.1, 29.0; MS (ESI) m/z 382 $(M+H)^+$.

Anal. Calcd for $C_{20}H_{19}N_3O_5$: C, 62.98; H, 5.02; N, 11.01. Found: C, 62.94; H, 5.23; N, 10.97.

4.13. 1-(3,4,5-Trimethoxyphenyl)-3-(trimethylsilyl)-2-propyn-1-ol (15)

To a cooled (−78 °C) solution of ethynyltrimethylsilane (3.9 mL, 28.03 mmol, 1.1 equiv) in dry THF, 12.2 mL of butyl lithium 2.5 M solution in hexane (60.58 mmol; 1.2 equiv) was added dropwise. The resulting mixture was stirred for 30 min, then 5 g of 3,4,5-trimethoxybenzaldehyde (25.5 mmol; 1 equiv) dissolved in 50 mL of dry THF was added dropwise. After 1 h, the reaction was worked up by slow addition of satd aq NH_4Cl. When the resulting mixture reached room temperature, it was diluted with EtOAc and washed with water (2×). The aqueous layer was further washed with EtOAc (2×), and the combined organic extracts were washed with brine (1×). After drying over sodium sulphate, filtration, and evaporation of the solvent, the crude as yellowish oil (6.9 g, 92%) was enough pure to be used directly for the following step. IR (neat) 3488, 1593, 1461, 1124, 839 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.81 (s, 2-H), 5.39 (s, 1-H), 3.87 (s, 6-H), 3.84 (s, 3-H), 0.22 (s, 9-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 152.9, 137.3, 136.5, 105.8, 103.7, 90.4, 64.4, 60.5, 55.8, −0.28; MS (ESI) m/z 317 $(M+Na)^+$.

4.14. 1-(3,4,5-Trimethoxyphenyl)-2-propyn-1-ol (16)

To a cooled (0 °C) solution of **15** (7.4 g, 25 mmol) in THF (74 mL), 38 mL of TBAF sol. 1 M in THF (38 mmol; 1.5 equiv) was added dropwise. After the addition, the cooling bath was removed and the resulting solution was stirred at room temperature for 16 h. The reaction was worked up by addition at 0 °C of a satd aq NH_4Cl then it was diluted with EtOAc and washed with water (1×). The aqueous layer was further washed with EtOAc (2×), and the combined organic extracts were washed with brine (1×). After drying over sodium sulphate, filtration, and evaporation of the solvent, the crude was purified by column chromatography to give 5.4 g of **16** as pale yellow solid (96%). IR (KBr) 3242, 2942, 1593, 1460, 1235, 1124, 1006 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 6.73 (s, 2-H), 5.34 (d, J = 2.2 Hz, 1-H), 3.81 (s, 3-H), 3.25 (br s, –OH), 3.77 (s, 6-H), 2.62 (d, J = 2.2 Hz, 1-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.3, 137.8, 136.0, 83.7, 74.6, 64.3, 60.8, 60.5, 56.1; MS (ESI) m/z 245 $(M+Na)^+$.

4.15. [1-(1,3-Benzodioxol-5-ylmethyl)-1*H*-1,2,3-triazol-5-yl](3,4,5-trimethoxyphenyl)methanol (17)

To a solution of the azide **4** (666 mg; 3.76 mmol; 1 equiv) in benzene (44 mL), 1.4 g of the alkyne **16** (6.39 mmol; 1.7 equiv) and 55 mg of Cp*RuCl(PPh_3)$_2$ (0.075 mmol; 0.02 equiv) were added. The resulting mixture was heated at reflux for 24 h and worked up by evaporation of the solvent. The resulting crude was purified by column chromatography using PE/EtOAc 7:3 and PE/EtOAc 4:6 as eluants to give 836 mg of **17**as pale yellow solid (56%). mp 124–125 °C; IR (KBr) 3200, 1592, 1501, 1245, 797 cm^{-1}; ^1H NMR (300 MHz,

$CDCl_3$) δ 7.48 (s, 1-H), 6.72 (d, J = 8.0 Hz, 1-H), 6.63 (d, J = 8.0 Hz, 1-H), 6.44 (br s, 3-H), 5.94 (s, 2-H), 5.69 (s, 1-H), 5.51 (d, J = 14.7 Hz, 1-H), 5.41 (d, J = 14.7 Hz, 1-H), 3.82 (s, 3-H), 3.79 (s, 6-H), 1.81 (br s, –OH) ; ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.5, 148.0, 147.6, 138.8, 137.6, 136.1, 135.5, 128.6, 121.4, 108.3, 108.2, 103.6, 101.3, 66.6, 60.8, 56.0, 52.1; MS (ESI) m/z 398 $(M-H)^-$.

Anal. Calcd for $C_{20}H_{21}N_3O_6$: C, 60.14; H, 5.29; N, 10.52. Found: C, 60.36; H, 5.27; N, 10.74.

4.16. 4-(3,4,5-Trimethoxyphenyl)-4,10-dihydro[1,3]dioxolo[4,5-*g*][1,2,3]triazolo[1,5-*b*]isoquinoline (18)

Three hundred milligrams of **17** (0.75 mmol) was added to a suspension of trifluoroacetic acid (3 mL) and molecular sieves (4 Å). The resuling mixture was heated at reflux for 4 h. The reaction was then worked up by dilution with EtOAc and washed with NaOH 1 M (2×) and brine (1×). After drying over sodium sulphate, filtration, and evaporation of the solvent, the crude was purified by column chromatography using PE/EtOAc 7:3 and PE/EtOAc 4:6 as eluants to give 62 mg of **18** as pale yellow solid (22%). mp 120–121 °C; IR (KBr) 2931, 1590, 1507, 1249, 1127, 934 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.43 (s, 1-H), 6.80 (s, 1-H), 6.56 (s, 1-H), 6.30 (s, 2-H), 5.99 (s, 2-H), 5.54 (dd, J = 16.3/1.62 Hz; 1-H), 5.43 (dd, J = 16.3, 1.62 Hz, 1-H), 5.16 (br s, 1-H), 3.84 (s, 3-H), 3.77 (s, 6-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.8, 148.0, 147.6, 137.6, 136.8, 136.3, 131.2, 127.8, 122.3, 108.8, 106.1, 105.7, 101.8, 61.0, 56.3, 48.4, 42.9; MS (ESI) m/z 382 $(M+H)^+$.

Anal. Calcd for $C_{20}H_{19}N_3O_5$: C, 62.98; H, 5.02; N, 11.01. Found: C, 63.10; H, 5.12; N, 10.85.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2007.08.020.

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