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Utilization of click chemistry in drug discovery. Applications to the synthesis of new bioactive triarylmethanes

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Abstract: The process of drug discovery or lead optimization involves the efficient synthesis of molecules and the creation of chemical libraries. For this reason, the rapid generation of new molecules is essential. Originally defined by Professors Barry K. Sharpless and M. G. Finn in 2001, click chemistry is a very powerful tool to develop a set of original, selective, and modular building blocks such as azide and alkyne in small and large scales. It is a new type of chemistry that generates complex molecules in an efficient way. The applications of this modular approach concern several domains of drug discovery, extending from lead finding through combinatorial chemistry, bionanoparticles, target-template to proteomics and DNA research using bioconjugation reactions. This article summarizes some progress and applications of click chemistry in drug discovery. We also describe the synthesis and characterization of a new triarylmethane prepared in our laboratory using this chemical strategy.

Keywords: click chemistry, drug discovery, cycloaddition reaction, 1,2,3 triazole.

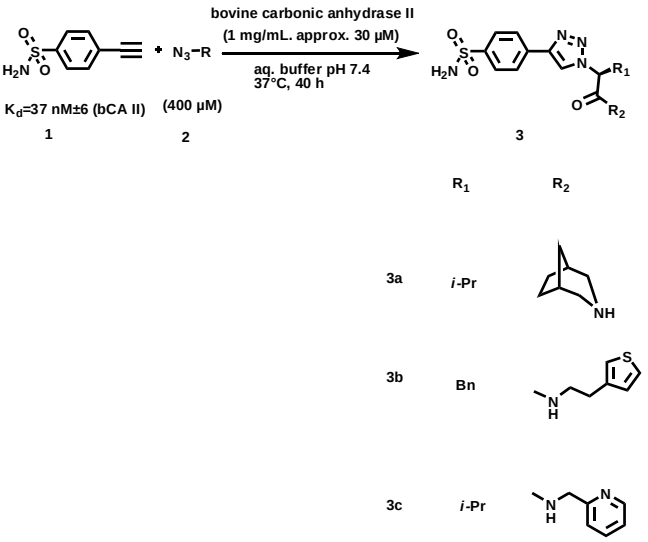
1. Introduction

In the recent years, there has been an ever-increasing need for powerful, straightforward and rapid strategies for drug discovery. Despite many successes, drug discovery approaches that are often hampered by slow and complex syntheses. Thus, click chemistry has recently emerged to become one of the most powerful tools in drug discovery, chemical biology, and proteomic applications. Using the most facile and selective chemical transformations, click chemistry simplifies compound synthesis, providing the

faster lead discovery and optimization. Furthermore, the ease of purification of product, the simplicity of this reaction has opened new pathways in generating new series of compounds with a therapeutic interest. Click reaction promotes essential criteria of a good synthesis process efficiency, versatility and selectivity. It includes simple conditions without any insensitivity to oxygen or water, readily available starting materials and reagents, no solvents or green and ecofriendly solvents such as water, successful performance at room temperature and simple purification without using chromatography. The present article highlights some applications of click chemistry in medicinal chemistry with a particular insight into the Cu(I) catalyzed Huisgen cycloaddition, the most studied reaction between an azide and a terminal alkyne affording the 1,2,3-triazole moiety. This reaction allows the introduction of a wide range of substituents. Moreover, triazoles are a privileged structures in medicinal chemistry present in numerous bioactive compounds such as anticancer[1-3] , antifungal, antibacterial[4-6], antituberculosis [7-9] and antiviral compounds [10-12]. Triazoles have also the interesting physicochemical properties. They are stable to acid and basic hydrolysis and reductive and oxidative conditions, indicative of a high aromatic stabilization. Its high dipole moment allows to participate actively in hydrogen bond formation as well as in dipole-dipole and π stacking interactions. In some cases, triazole moiety improve pharmacokinetic properties.
We also present an example developed in our laboratory concerning the functionalization by click chemistry of a new bioactive triarylmethane.

1.1 In situ Click Chemistry

Kolb and co-workers have successfully employed the *in situ* click-chemistry to identify a novel carbonic anhydrase (CA) inhibitors. Carbonic anhydrases are zinc-containing enzymes involved in respiration processes and the transport of CO_2/HCO_3^-, acid secretion and pH control, calcification acetylene/azide and tumorigenicity. Carbonic anhydrase inhibitors have long been used to control the elevated intraocular pressure related with glaucoma. Furthermost inhibitors are aromatic or heteroaromatic sulfonamides whose anion coordinates to the Zn^{2+} ion in the active site. Authors chosen the acetylenic benzenesulfonamide **(1)** as a reactive scaffold for capturing complementary azide reagents to form "divalent" CA inhibitors *in situ*. Compound **1** binds to bovine carbonic anhydrase II with nanomolar affinity (K_d=37 nM±6) (Scheme 1).



Scheme 1: *In situ* screening protocol and reagents used to develop carbonic anhydrase inhibitors by *in situ* click chemistry.

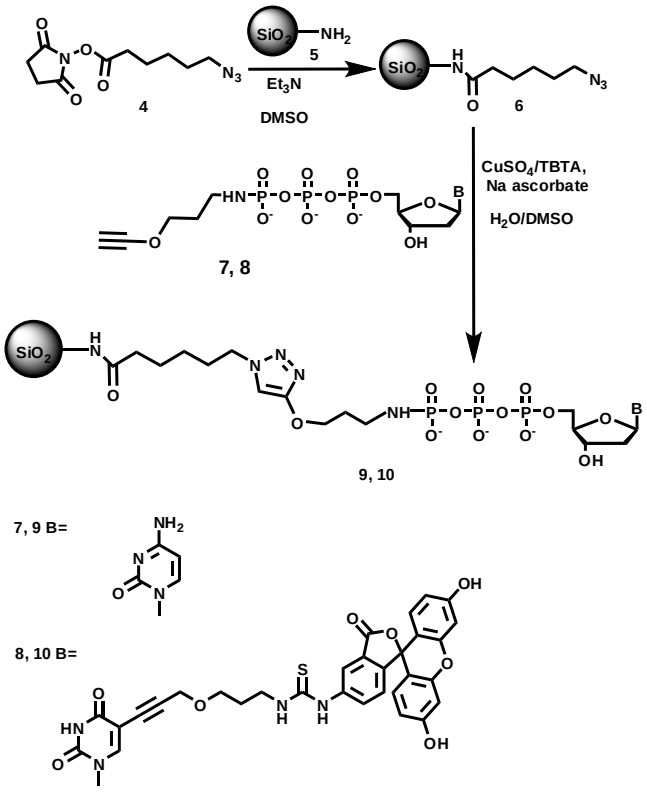
In situ click experiments were performed in a 96-well microtiter plates with each well containing a mixture of bovine CA II (bCA II), an acetylenic benzenesulfonamide at a concentration enough to saturate the enzyme active site, and a corresponding azide reagent in phosphate buffer solution (pH 7.4). The formation of the product **(3)** was monitored by HPLC analysis and mass spectrometry by electrospray ionization. Each acetylene/azide combination was incubated with mixtures of enzyme and the known active-site inhibitors ethoxazolamide and with bovine serum albumin in place of bCAII.
Analysis of the crude reaction mixture with LCMS-SIM revealed that 12 out of the 24 reagent combinations acetylene/azide led to triazole formation in the presence of the enzyme. Most of the *in situ* hits are derived from α-substituted azido acid amides. The results obtained by the authors revealed that enzyme reaction was highly anti-selective compared with the thermal cycloaddition.

This suggest that the formation of the product is enzyme controlled [13].

1.2 Click chemistry for functionalizing nanoparticles to drug delivery application

Vasilyeva and co-workers explored click chemistry to develop a delivery system for 2'-deoxyribonucleoside triphosphates. They designed nanocomposites containing analogues of 2'-deoxyribonucleoside triphosphate (dNTP) immobilized into SiO_2 and to study their substrate properties in reactions catalyzed by DNA polymerases and their ability to penetrate into eukaryotic cells.

They suggested a simple and versatile method of their covalent attachment to nanoparticle: the click reaction between premodified nanoparticles **(6)** bearing the azido groups and dNTP bearing the alkyne-modified gamma-phosphate group **(7,8)**. Scheme 2 illustrates the cooper(I)-catalyzed azide-alkyne cycloaddition (CuAAC) reaction that resulted in the formation of the wanted nanocomposites **(9,10)** [14].



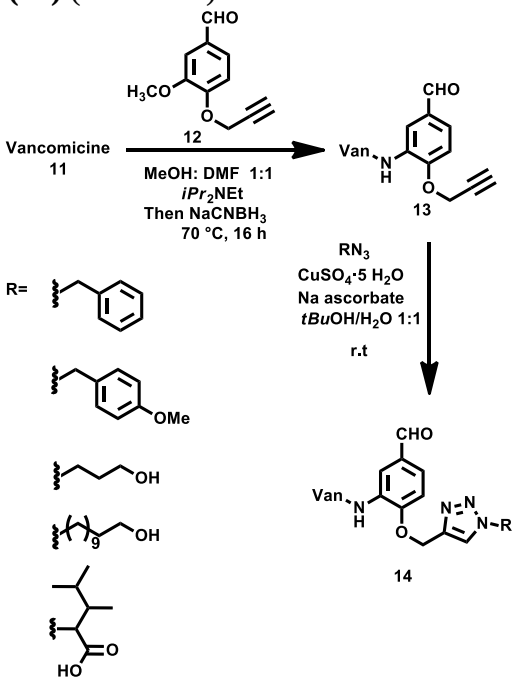
Scheme 2: Functionalizing of SiO_2 nanoparticles by click chemistry

In 2015, the same authors successfully applied CuAAC click chemistry in order to develop a similar delivery system for analogues of AZT-triphosphates (AZT*TP) based on SiO_2 nanoparticles.

The results obtained demonstrated a possibility of the utilization of SiO_2 nanoparticles as vehicles for the delivery of nucleoside triphosphates analogues into cells. It was shown that the proposed SiO_2 dNTP nanocomposites **(9,10)** penetrated into eukaryotic cells. Preliminary result also displayed that these nanocomposites at low concentrations can inhibit the reproduction of Herpes viruses. All this justify the use of nanobiocomposites bearing nucleoside triphosphate analogues as promising therapeutic drugs [15].

1.3 Click chemistry for reengineering drugs

Click chemistry has been used for reengineering drugs. Thus, Silverman and co-workers reported a click chemistry approach towards reengineered vancomycin derivatives with high potency against methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin resistant *Enterococci* (VRE; VanB). They synthesized a series of click vancomycin derivatives starting from vancomycin itself **(11)** (Scheme 3).



Scheme 3: Synthesis of vancosamine modified triazole derivative

This strategy facilitated the rapid discovery of potent dimeric vancomycin derived antibiotics. Some compounds were active against vancomycin-susceptible and vancomycin-resistant bacteria. The enhanced biological activities seen against numerous bacterial strains and the ease of preparation of the compounds, makes the used click chemistry reaction an interesting tool in drug discovery [16].

2.3 Click chemistry to develop clickable photoprobes

Photoaffinity labeling is a well-known biochemical technique in development due to its combination with biorthogonal/click chemistry. One of the major challenges in medicinal chemistry is the identification of the biologically relevant targets of hits that rise via phenotypic (organismal or cell) screening. In that sense, clickable photoprobes have simplified the target discovery over the past decade, particularly for hit compounds originating from screening campaigns. Lapinsky and co-workers have designed and synthesized some clickable photoprobes bearing an aryl azide photoreactive group **(15, 16, 17, 18)** to facilitate target identification of a hit compound discovered from screening (Figure 1) [17].

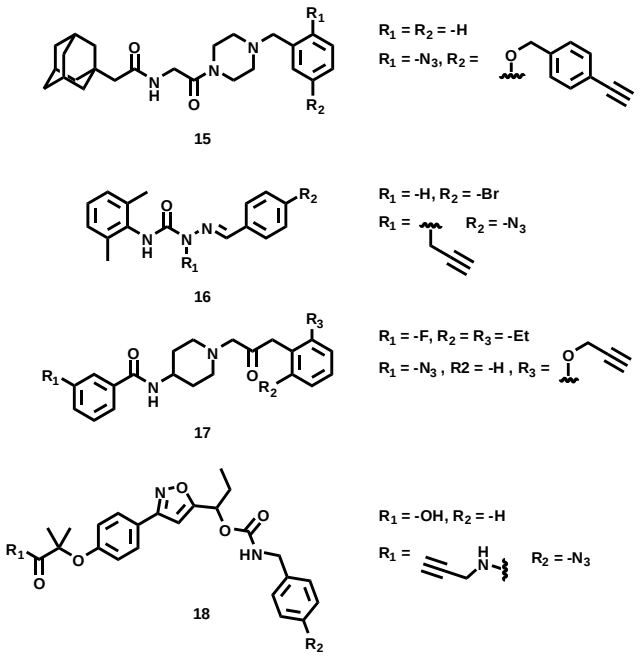
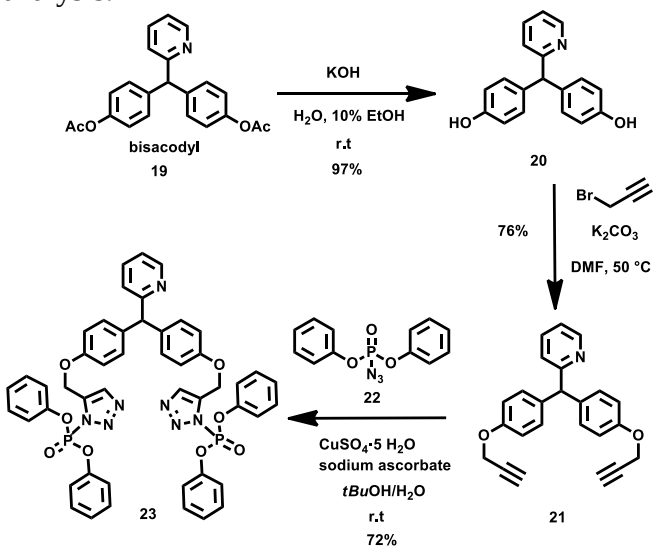


Figure 1: Lead compounds and their clickable aryl azide-based photoprobe derivatives for target identification**.**

2. Results and Discussion

We have explored Huisgen cycloaddition to prepare functionalized triarylmethane scaffolds **(23)** derived from bisacodyl **(19)**. This drug is used in therapeutics as a laxative and it have also been described for its antibacterial [18] and anticancer activities [19].

The synthesis of the functionalized triarylmethane (**23)** was carried out following the synthetic pathways represented in Scheme 4. First the preparation of 4,4'-(pyridin-2-ylmethylene) diphenol **(20)** has been performed by saponification reaction of bisacodyl in aqueous solution of KOH containing 10% of ethanol. The deacetylated bisacodyl **(20)** was obtained in excellent yield (97%) and clean enough without supplementary purification as suggested ^{1}H NMR analysis.



Scheme 4: Application of click chemistry to the synthesis of triarylmethane derivatives.

The 2-(bis (4-(prop-2-ynyloxy)phenyl) methyl) pyridine **(21)** was obtained in a good yield (76%) *via* an *O*-alkylation reaction between compound **(20)** and propargyl bromide in anhydrous DMF at 50 °C following the procedure of Berscheid et co-workers [20].

The desired compound, 2-(bis (4-(prop-2-ynyloxy) phenyl)methyl)pyridine **(21)** was prepared by copper catalyzed 1,3 cycloaddition reaction with diphenylphosphorylazide **(22)** in *tBu*OH/H_2O at room temperature. The desired product **(23)** was isolated in 72% using only a filtration and washing.

3. Materials and Methods

Experimental

Materials:

All reagents were obtained from commercial sources unless otherwise noted and used as received. All reactions were monitored by analytical thin layer chromatography (TLC). TLC was performed on aluminium sheets precoated silica gel plates (60 F254, Merck). TLC plates were visualized using irradiation with light at 254 nm or in an iodine chamber as appropriate.

Physical measurements:

The structure of the products was checked by comparison of their NMR, IR and by the TLC behaviour. ^{1}H NMR spectra was acquired on a Bruker BioSpin GmbH spectrometer 400 MHz, at room temperature. Chemical shifts are reported in δ units, parts per million (ppm). Coupling constants (J) are measured in hertz (Hz). Various 2D techniques and DEPT experiments were used to establish the structures and to assign the signals. For the assignments of the NMR signals, we use the convention presented in Figure 2. Infrared spectra were recorded over the 400-4000 cm^{-1} range with an Agilent Technologies Cary 630 ATR spectrometer.

Tetraphenyl 5,5'-(4,4'-(pyridin-2-ylmethylene) bis (4,1 phenylene)) bis (oxy) bis (methylene) bis (1H-1,2,3-triazole-5,1-diyl)diphosphonate

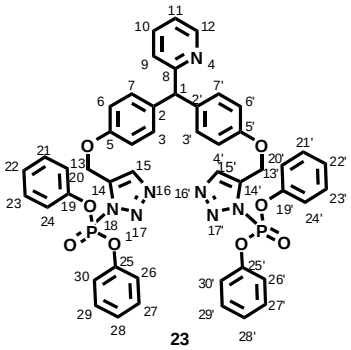


Figure 2

To a solution of $CuSO_4.5H_2O$ and Na ascorbate in *tBu*OH/H_2O (1:1) was added 2-(bis (4-(prop-2-ynyloxy)phenyl)methyl)pyridine **(21)** and diphenyl phosphorylazide **(22)**. The mixture was stirred at room temperature. The product was then precipitated, collected by filtration after 5 h, washed with *tBu*OH/H_2O then with MeOH.

A green coloured solid was isolated in 72% yield. **^{1}H NMR (400 MHz, DMSO-*d6*+TFA-*d*): *δ*** 8.12 (m, 4H, H10, H12, H15, H15'), 7.48-727 (m, 3H, H9, H11), 6.91-6.60 (m, 27 H, H3, H7, H4, H6, H3' H7', H4'H6', H20-24, H26-30, H20'-24', H26-30'), 5.51 (s, 1H, H1), 4.98 (s, 4H, H13, H13'). **IR (ATR): ν** 3080, 3040 (ν_{Csp2-H}); 2929 (ν_{Csp3-H}); 1640, 1600, 1500 and 1470($\nu_{C=C}$); 1200 ($\nu_{asym\ C-O-C}$); 1060 ($\nu_{sym\ C-O-C}$).

4. Conclusions

We discussed herein the importance of click chemistry in the process of drug discovery through several examples of the literature. We also presented the synthesis of a new functionalized triarylmethane using this chemical method in order to develop bioactive scaffolds. We are currently optimizing the synthesis and studying their antibacterial activity.

5. Acknowledgments

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Author Contributions

All authors contributed to the drafting and revision of the article and approved the final version.

Conflicts of Interest

The authors declare no conflict of interest.

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