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Synthesis, structure and antimicrobial evaluation of a new gossypol triazole conjugates functionalized with aliphatic chains and benzyloxy groups

Krystian Pyta [a,*], Marietta Blecha [a], Anna Janas [a], Katarzyna Klich [a], Paulina Pecyna [b], Marzena Gajecka [b,c], Piotr Przybylski [a]

[a] Faculty of Chemistry, A. Mickiewicz University, Umultowska 89b, 61-614 Poznan, Poland
[b] Department of Genetics and Pharmaceutical Microbiology, Poznan University of Medical Sciences, Swiecickiego 4, 60-781 Poznan, Poland
[c] Institute of Human Genetics, Polish Academy of Science, Strzeszynska 32, 60-479 Poznan, Poland

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ABSTRACT

Synthetic limitations in the copper-catalyzed azide alkyne cycloaddition (CuAAC) on gossypol's skeleton functionalized with alkyne (**2**) or azide (**3**) groups have been indicated. Modified approach to the synthesis of new gossypol–triazole conjugates yielded new compounds (**24**–**31**) being potential fungicides. Spectroscopic studies of triazole conjugates **24**–**31** have revealed their structures in solution, i.e., the presence of enamine–enamine tautomeric forms and π–π stacking intramolecular interactions between triazole arms. Biological evaluation of the new gossypol–triazole conjugates revealed the potency of **30** and **31** derivatives, having triazole–benzyloxy moieties, comparable with that of miconazole against *Fusarium oxysporum*. The results of HPLC evaluation of ergosterol content in different fungi strains upon treatment of gossypol and its derivatives enabled to propose a mechanism of antifungal activity of these compounds.

Gossypol (Scheme 1), is a yellow pigment, present in various parts of cotton plants acting as plant's defense system against pathogenic fungi and insects.[1] This natural bisesquiterpene has drawn the attention of many scientists because of its wide range of biological activities including contraceptive,[2] anticancer,[3] antiviral[4] or antimicrobial.[5] Unfortunately, the use of gossypol in medical therapy is limited because of its side effects.[6] A convenient way to obtain less toxic compounds, with no compromise to antimicrobial activity is to convert gossypol into its Schiff bases,[7] hydrazones[7a,c,f,h] or oximes.[7a]

In the 1960s a new cycloaddition reaction between alkyne and azide in the presence of Cu^+ cation leading to 1,2,3-triazoles was discovered.[8] The Meldal variant of cycloaddition with the use of Cu^+-catalyst with a classical antioxidant system as, e.g., sodium ascorbate, called the copper(I)-catalyzed azide–alkyne cycloaddition (CuAAC),[9] is one of the most convenient and efficient examples of '*click chemistry*'. This synthetic strategy of organic chemistry was fully described by Kolb, Finn and Sharpless in a landmark review published in 2001.[10] Applications of '*click chemistry*' are wide-ranging as they allow attachment of many structurally diverse triazole blocks to various biomolecules. The use of 1,3-Dipolar Huisgen cycloaddition has enabled to synthesize a number of new bioactive compounds and to modify agents of medical interest.[11]

In this work we used the copper-catalyzed dipolar cycloaddition to modify gossypol molecule with triazole moieties because triazole derivatives are well-known antifungal agents on their own.[12] Firstly, in our synthetic approach gossypol was subjected to reactions with primary amines containing azide or alkyne functions. These reactions in ethanol gave with very good yields (~85%) symmetrically substituted Schiff base products **2** and **3** (Scheme 2). Multiple attempts at direct conversion of compounds **2** and **3** with respective alkyne or azide reagents into triazole derivatives have failed. No expected products in the reaction mixture, even after long reaction time (48 h) or increased amount of the Cu^+-catalyst (2:1 mixture of Cu^+ with **2** or **3**), could be explained by fact that both amines and acidic phenolic groups [especially at C(1) and C (1′)] within the structures of **2** and **3** took part in coordination of Cu^+-catalyst in the transition state of dipolar cycloaddition reaction. Therefore, we changed our strategy and prepared the triazole blocks separately (Scheme 2). First, we converted phthalimide *N*-alkyl bromides (compounds **4** and **5**) into respective phthalimide *N*-alkyl azides (derivatives **6** and **7**). These derivatives were further

* Corresponding author. Tel.: +48 61 8291693; fax: +48 61 829 1505.
E-mail address: k.pyta@amu.edu.pl (K. Pyta).

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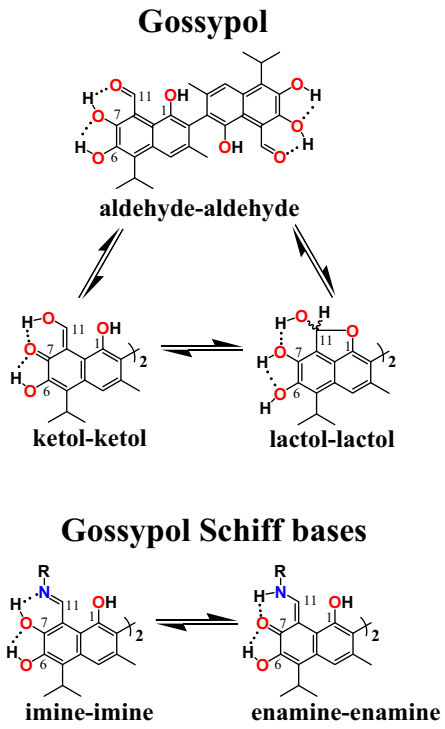
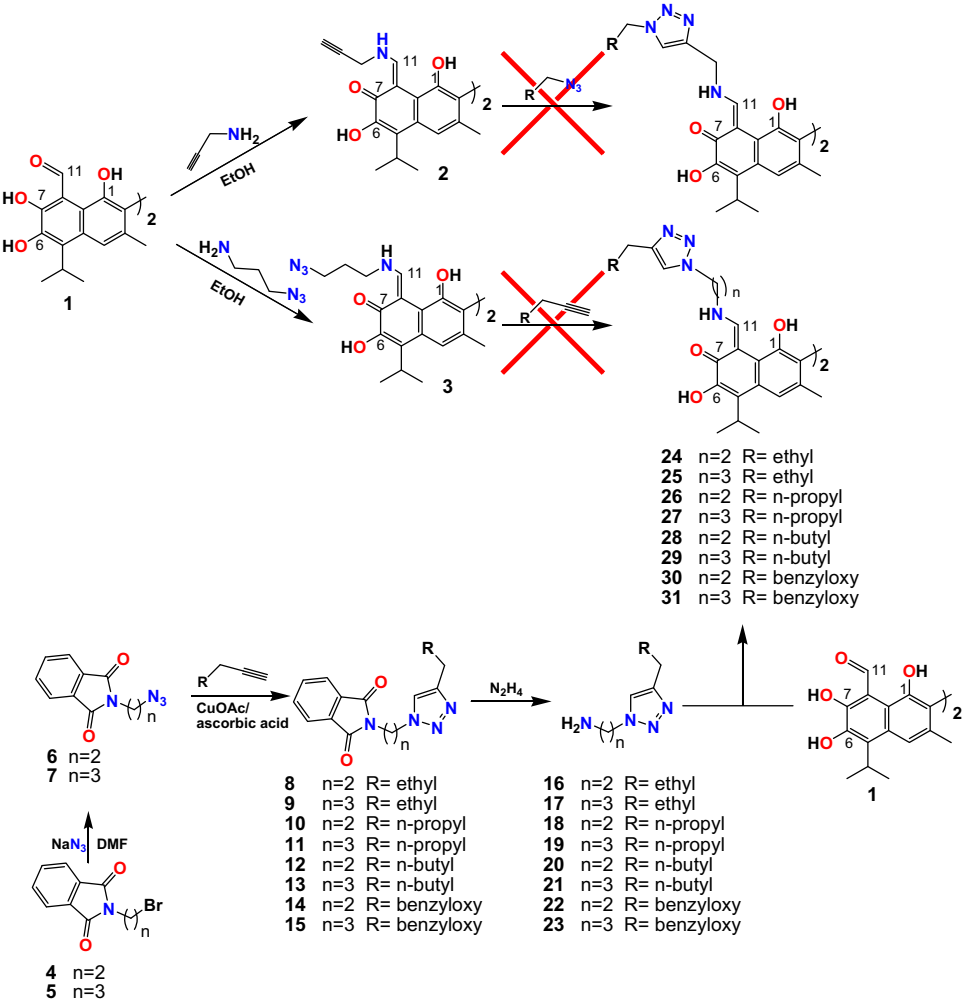


Scheme 1. Structure and tautomeric forms of gossypol and its Schiff bases.

modified into new triazole products **8**–**15** via dipolar cycloaddition using various alkynes in the presence of CuOAc as a catalyst (Scheme 2). Removal of phthalimide protection group in the time-consuming reaction with hydrazine (72 h) yielded various new amine–triazole intermediates **16**–**23** with a moderate or good yields (60–75%). Further condensation of the obtained intermediates, containing triazoles and different length alkyl chains or benzyloxy groups, with gossypol molecule has given new Schiff base products **24**–**31** at good yields (~80%). All synthesized products (**2**–**31**) were characterized in detail by ESI MS, HR-MALDI-TOF and 1D and 2D NMR methods (Supplementary data). In view of earlier reports describing the presence of different tautomeric forms within gossypol as well as within gossypol derivatives it was necessary to determine the structures of newly obtained chemical entities **2**, **3** and **24**–**31**. In all ^{1}H NMR spectra of **24**–**31** derivatives, the characteristic doublets at about 9.5 ppm appeared (Fig. 1S). ^{1}H–^{13}C HMBC correlation (Fig. 2S) revealed that these signals should be assigned to H(11) protons. Thus, taking into regard the fact that H(11) signal is a doublet, it was clear that all synthesized derivatives **2**, **3** and **24**–**31** were present as symmetric enamine–enamine tautomers. Furthermore, C(7) carbon atom signals recorded in the ^{13}C NMR spectra were in the range 173.0–173.6 ppm (Supplementary data), characteristic of quinone like ketone carbonyl groups as previously reported.[13] Structures of compounds **2**, **3** and **24**–**31** in solution are stabilized by the presence of collective intramolecular H-bond systems, as indicated by the chemical shifts observed for O(1)H, O(6)H and NH protons in



Scheme 2. Synthetic approach to obtain novel Schiff bases of gossypol containing triazole ring.