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Design, synthesis and bioactivity of catechin/epicatechin and 2-azetidinone derived chimeric molecules

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ABSTRACT

A new class of chimeric molecules have been developed. These are based on polyphenols like catechin and epicatechin and monocyclic β-lactams. The two units are joined via a triazole linker using the 'Click Chemistry' conditions. The compounds showed good to weak antibacterial activity against *Escherichia coli* as well as moderate inhibition of RNase A.

Diseases caused by pathogenic bacteria still attract significant attention of medicinal chemists and biologists because of growing antibacterial resistance. For example, *Staphylococcus aureus* which is one of the major causes of hospital- and community-acquired infections worldwide like wound infections, bacteraemia and sepsis is associated with a high mortality rate.[1] This is because Staphylococcal resistance to wide spectrum β-lactam antibiotics, such as methicillin, oxacillin and flucloxacillin, emerged soon after the introduction of the first drug in this class and there has been a steady rise in the incidence of methicillin resistant S. aureus (MRSA) clinical isolates.[2] Staphylococci show a strong tendency to accumulate antibiotic resistant genes and the majority of MRSA isolates are now resistant to a range of antibiotics.[3] Literature survey revealed that there has been an alternative approach to fight this nightmare of bacterial resistance. It is based on the identification of agents that have no intrinsic antibacterial activity but are able to sensitize the pathogen to a previously ineffective antibiotic. Such modification of the bacterial phenotype has been exploited with Augmentin, an antibacterial combination that relies on the co-administration of a β-lactamase inhibitor (clavulanate) and a β-lactam antibiotic (amoxicillin). The polyphenolic compounds (−)-epicatechin gallate (ECG) and (−)-epigallocatechin gallate (EGCG), major constituents of Japanese green tea and to a smaller extent other catechins have been shown to sensitize MRSA to methicillin and other β-lactam antibiotics.[4] However naturally occurring catechin gallates, such as the potent modifier ECG, are rapidly degraded in vivo to inactive products due to the presence of esterase-susceptible linkage groups.[5] Recently a couple of amides were synthesized as ECG analogues[6] in which the hydrolytically susceptible ester bond was replaced with an inherently more stable amide linkage. These were found to reduce the oxacillin resistance of the MRSA strain. These results prompted us to design a chimera comprising catechin/epicatechin and the β-lactam moiety (**1**–**2**) (Fig. 1).

Our inspiration has also come from two recent publications: one from our own laboratory reporting[7] the RNase A inhibition activity of nucleobase-polyphenol chimera and the other by Chen et al. reporting[8] weak antibacterial activity of 6-trizolyl penicillin. These results encouraged us to adopt the essentially chemically inert triazole linker to join the two pharmacophores, namely the polyphenol and the monocyclic β-lactam. The resulting chimeric molecules were tested against *E. coli* and also for inhibition of RNase A (Fig. 2).

Our first challenge was to suitably protect (+)-catechin and (−)-epicatechin. Acid or base sensitive protecting groups needed to be avoided because of possible racemization at C-2 of catechin and epicatechin involving a quinine methide intermediate.[9] Our previous experience of synthesis of chimeric molecules containing nucleobase and polyphenol,[7] guided us to protect the polyphenols as their tetrabenzyl ethers **9** and **10** respectively.[10] This is because of possible deprotection of the benzyl ether under neutral condition by simple hydrogenolysis.

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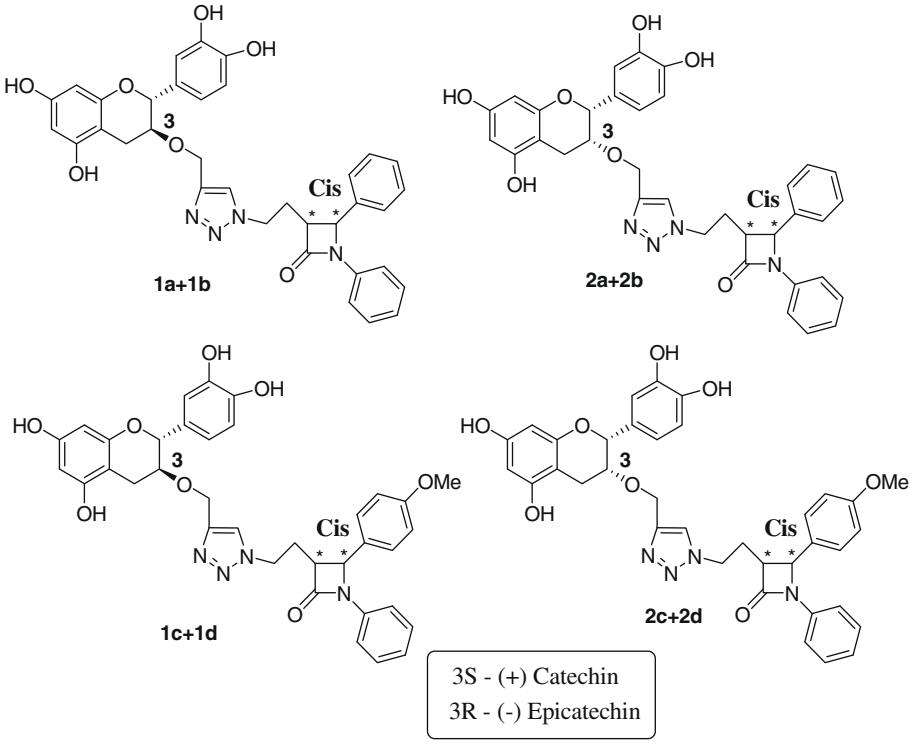


Figure 1. Target chimeric molecules.

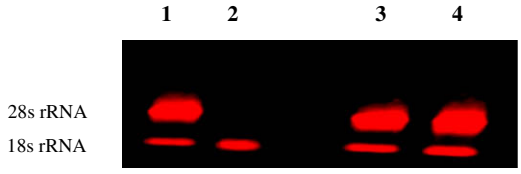
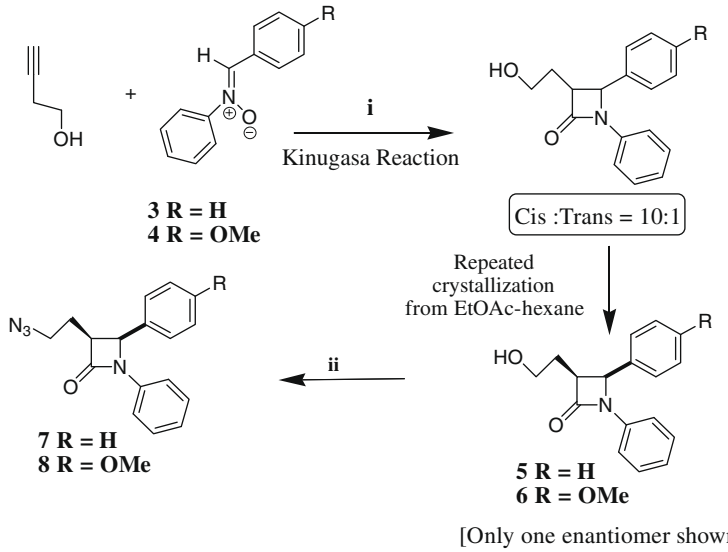


Figure 2. Gel electrophoresis experiment. Lane 1: RNA + water + Methanol, Lane 2: RNA + RNase A + Methanol, Lane 3: RNA + RNase A + [**1a+1b**] (0.460 mM, MeOH), Lane 4: RNA + RNase A + [**2c+2d**] (0.456 mM, MeOH).

Our next task was to introduce the alkyne and the azide functionalities separately in protected polyphenol and β-lactam components. For the alkyne component, tetrabenzyl catechin (TBC) or epicatechin (TBEC)[10] was converted to 3-*O*-propargyl catechin or epicatechin by reacting with propargyl bromide in presence of sodium hydride. The azido β-lactams (**7** and **8**) were prepared from the corresponding alcohols via mesylation followed by nucleophilic displacement with azide. The alcohols were prepared by the Kinugasa reaction[11] between 3-butyn-1-ol and nitrones using a published protocol[12] from our laboratory (Scheme 1).

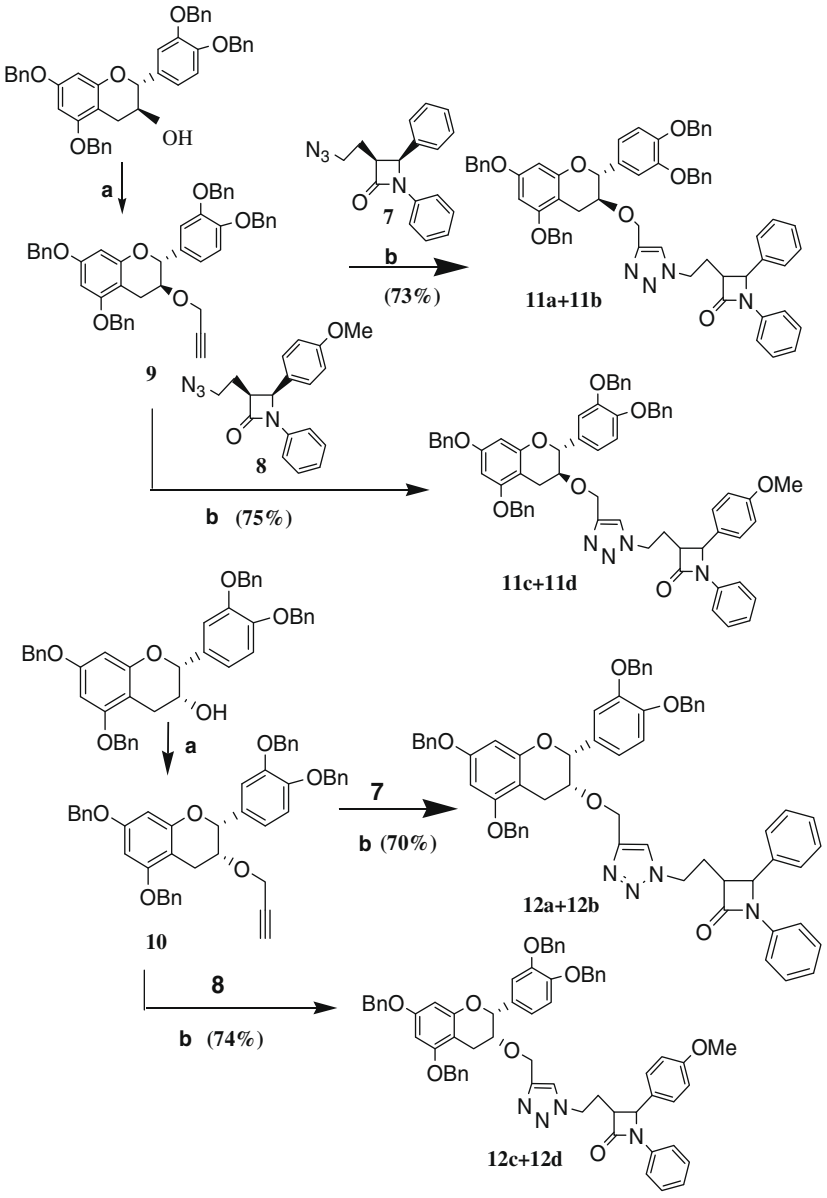


Scheme 1. Synthesis of azido β-lactams. Reagents and conditions: (i) CuI, Et_3N, degassed acetonitrile, rt, (70%); (ii) (a) MsCl, Et_3N, dry DCM, 0 °C, (90%); (b) NaN_3, dry DMF, 50 °C, (80%).

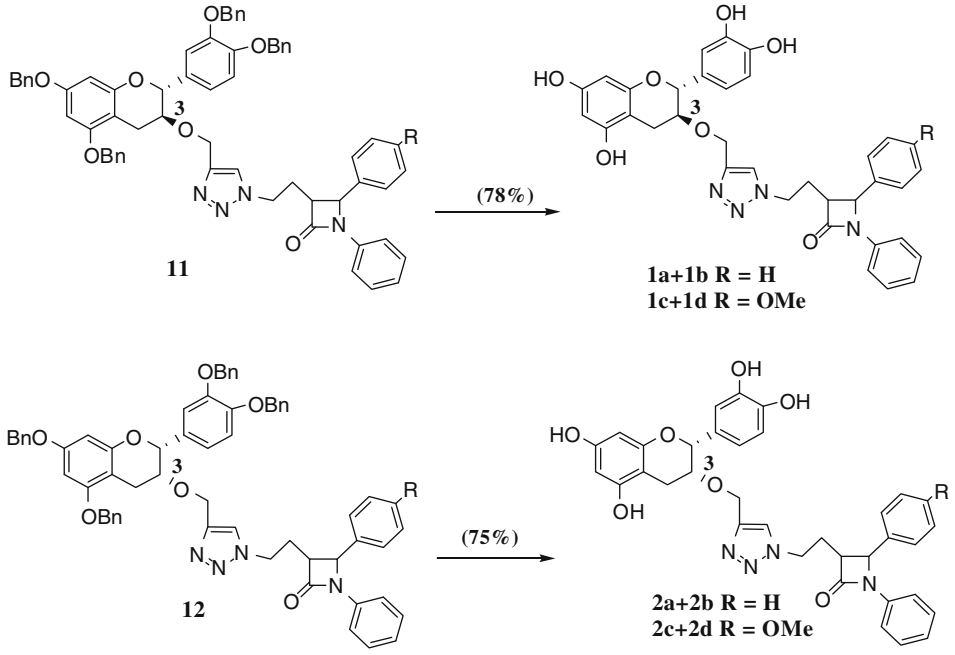
With the alkyne and the azide components in hand, the 'click' methodology[13] was utilized to join the two arms together to generate the desired chimera. The click reaction was performed in presence of Cu (I) made in situ by reduction of $CuSO_4$ with L-ascorbate in acetonitrile–water (1:1) solvent systems (Scheme 2). The protected chimeric molecules were finally deprotected by hydrogenolysis in presence of Pearlman catalyst.[14] The free phenols were isolated in about 80% yield (Scheme 3).

The antibacterial activity was measured against *E. coli* by disc-diffusion assay.[15] In this assay, a colony of the organism was inoculated in 2–5 mL nutrient broth and grown for 2.5 h. The agar plates were dried and inoculated by spreading the bacterial suspension evenly over it. The sterile paper discs (6 mm) impregnated with fixed dose viz., 1 mmol of compound were placed on the pre-inoculated surface. The disc-bearing plates were incubated at 37 °C and examined at 48 h for zone of inhibition, if any, around the disc. Ampicillin was used in assay as a standard control drug. An additional negative control disc without any sample, but impregnated with equivalent amount of solvent (DMSO), was also used in the assay. The diameter of inhibition zone is directly proportional to the degree of sensitivity of bacterial strain and the concentration of compound under test. The results of antibacterial activity testing of the chimeric molecules are given in Table 1. It is interesting to note that the isolated monocyclic azido β-lactams **7** and **8** as well as the alcohols **5** and **6**, were found to be ineffective as antibacterial agents. Moreover free catechin and epicatechin do not possess any antibacterial activity. Thus the earlier ineffective monobactams have been successfully sensitized towards *E. coli* after conjugating with catechin/epicatechin.

The RNase A inhibition study[16] was done qualitatively by an agarose gel based assay, wherein the degradation of the 28s and 18s rRNA was studied. The ribonuclease A of concentration 0.011 μM was mixed with the synthesized compounds and incubated for 1 h at 37 °C. To these preincubated enzyme solutions, the RNA (0.22 μg/μl) was added as substrate and the degradation was allowed for 15 minutes. The resulting solution was mixed with 40% sucrose solution (made from RNase free water) and 0.25% bromophenol blue (dissolved in 1× TAE buffer) and loaded onto a 1.1%



Scheme 2. The click reaction. Reagents and conditions: (a) NaH, propargyl bromide, dry THF, 0 °C–rt, 10 h, (75%); (b) $CuSO_4{\cdot}5H_2O$, Na-ascorbate, MeCN–H_2O (1:1), rt, 80 h.



Scheme 3. Deprotection by hydrogenolysis. Reagents and conditions: H_2 [2 bar], 20% $Pd(OH)_2$-C, THF/MeOH/H_2O = 20:1:1, rt.

Table 1
Results of antibacterial activity testing by disc diffusion assay

Ampicillin	Compd **1a**+**1b**	Compd **2a**+**2b**	Compd **1c**+**1d**	Compd **2c**+**2d**
+++	++	++	+	+

+++ Highly active (inhibition zone diameter >12 mm).
++ Moderately active (inhibition zone diameter 9–12 mm).
+ Slightly active (inhibition zone diameter 6–9 mm); Control (inhibition zone diameter <6 mm).

agarose gel. The bands were visualized under a UV–transilluminator by virtue of the ethidium bromide (final concentration 0.5 μg/ml) present in the gel. Comparison of the gel pattern in various lanes indicated that these catechin and epicatechin and 2-azetidinone chimeric molecules are also good inhibitors of RNase A. It may be mentioned here that catechin and epicatechin themselves are very weak RNase A inhibitors thus pointing to the importance of the chimeric molecules as a whole in RNase A inhibition. The other important observation is that the inhibition activity appears to be valid for the 28s rRNA. The specific reason for this differential behaviour is not known at present.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.04.084.

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