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Click reaction based synthesis, antimicrobial, and cytotoxic activities of new 1,2,3-triazoles

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ABSTRACT

Three–motif pharmacophoric models **20a–e** and **21–25** were prepared in good yields by CuAAC of two azido substrates **2** and **11** with seven terminal acetylenic derivatives including chalcones **17a–e**, theophylline **18** and cholesterol **19**. The structure of these compounds was elucidated by NMR, MS, IR spectroscopy and micro analyses. This series was screened as antimicrobial and cytotoxic agents *in vitro*. Most derivatives showed appreciable antibacterial activity, but they displayed weak cytotoxic, and antifungal activities. Notably, conjugate **25** (cream of the crop) was found to be more active than Ampicillin against *E.coli* and *S. aureus* and showed appreciable antifungal and cytotoxic activities as well.

Keywords: Click reaction; Quinoline; Chalcone; Cholesterol; Theophylline; Biological activity

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Abbreviations: (CuAAC), copper catalyzed azide–alkyne cycloaddition; (RuAAC), Ruthenium II catalyzed azide–alkyne cycloaddition; HMBC, Heteronuclear multiple bond correlation; (HDACIs), histone deacetylase; *p*–TsOH, *p*–toluenesulphonic acid; (SRB), sulforhodamine B; *E.coli*, *Escherichia coli*; *S. aureus*, *Staphylococcus aureus*; *A. flavus*, *Aspergillus flavus*; *Candida albicans*, *C. albicans*; PZQ, Praziquantel; Dox, Doxorubicin; ELISA, enzyme–linked immunosorbent assay; ANOVA, analysis of variance.

1,3-Dipolar azide-alkyne [3+2] cycloaddition Scheme 1 is a major synthetic approach for 1,2,3-triazoles. Originally, the thermal Husigen cycloadditions are conflicted with simple reaction conditions and stereospecificity due to formation of both 1,4- and 1,5-disubstituted-1,2,3-triazoles, therefore, it is confined to symmetrical alkynes.[1] Mock *et al.* demonstrated the regioselective synthesis of 1,4-disubstituted-1,2,3-triazoles by cycloadditions catalyzed by amines encapsulated with cucurbituril[2] In a breakthrough, Sharpless[3] and Meldal[4] independently disclosed the CuAAC for exclusive formation of the 1,4-disubstituted-1,2,3-triazoles giving rise to a new era for Click-Chemistry. The orientation in disubstituted triazoles was recently studied and distinguished by $^{1}H-^{15}N$ HMBC.[5]

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Scheme 1. Conditions–based regioselectivity of Azide–Alkyne cyclocondensation.

Cu (I) salts were generally used as catalyst in the CuAAC in the presence of a base as stabilizer and to assist in the ionization of the terminal acetylene. However, *in situ* generation of Cu (I) salts from Cu(II)SO_4 reduced by sodium ascorbate without the need for a base was more convenient and more commonly used procedure. The reaction was well performed at low temperatures or even at ambient conditions in aqueous or polar solvents like THF and DMF. However, acetonitrile was avoided for its tendency to coordinate with Cu(I) salts, furthermore, halogenated solvents were not recommended.[6] Epoxides in the presence of NaN_3 and acetylenes could be used as three component system to perform the reaction.[7] Fused tetrazoles were also used as azide surrogates in Click reactions.[8] Certain azide substrates such as 2-azidomethylquinolines were able to be clicked with terminal alkynes in alcohols and in absence of a reducing agent due to intramolecular chelation–assisted cycloadditions.[9] Fokin *et al.* found that ruthenium II catalysts gave solely the 1,5-disubstituted triazoles (RuAAC).[10]

The easiness of the CuAAC, high yield, regioselectivity and specificity without environmental constraints made it indispensible and evolutionary synthetic tool in chemical research fields. These fields included polymer chemistry, electrode surfaces, gold surfaces, nanoparticles, nanotubes, silica particles, porous beads, magnetic metal oxides, microcontact printing, corrosion

retardants, optical brighteners, light stabilizers, fluorescent whiteners,[11–19] and development of sensors for detection of metal ions.[20]

In biochemistry, the reaction could be performed on cellular scale to modify biomolecules and cell surfaces for imaging purposes and functioning for physiological investigations.[21–24]

In pharmacology, formidable designs endowed with plethora of biological significances are constantly appearing by clicking assorted azide and terminal acetylenic structure motifs to discover new leads. Theses designs included activities as antibacterial,[25–27] for example Tozabactam is a commercial triazole based β–lactamase inhibitor,[28,29] antifungal,[30] and antiviral[31] including multidrug–resistant HIV–1 protease variants.[32] Clicked triazoles were reported as anti–leishmanial,[33] anti–*Trypanosoma crusi*,[34] and antimalarial[35] too. Many reports presented their activities as anti human carbonic anhydrase,[36] Pim kinases[37] and histone deacetylase (HDACIs)[38,39] with concurrent potential antiproliferative, anti–inflammatory and cognition–enhancing activities. Furthermore, several 1,2,3-triazoles were active as antiallergic, antiepileptic[7] and epigenetic modulators of gene transcription.[39] The pharmacological significances of 1,2,3-triazoles were reviewed[40,41] and a computer algorithm called, AutoClickChem, was capable of performing many Click–reactions *in Silico* to produce large combinatorial libraries of compound models for use in virtual screens.[42]

This piece of work describes the synthesis of ten 1,4-disubstituted-1,2,3-triazoles as three-motif pharmacophoric derivatives based on CuAAC of chloroquinoline and glucose azide substrates with a set of terminal acetylenes including chalcones, theophylline and cholesterol. While, chloroquinolines are wide spectrum pharmacophores,[43] glucose was selected as surface recognition tag.[38] Time since, chalcones[44] and theophylline[45] are well documented for their diverse pharmacological profiles. Recently, cholesterol derivatives are progressively emerging as pharmacologically giant targets. Cholesterol can be functionalized at the double bond and the 3-β-OH group as well, to afford quite effective antimicrobial and cytotoxic probes. An explanation for this potency is attributed to the propensity of bacteria[46,47] and cancerous cells to elevate serum cholesterol level.[48] While, bacteria do so to incorporate it in its own membrane to acquire resistance against the human immune defense and antibiotics,[46] cancerous cells use it to build membranes of new cells.[48] This feature was exploited to develop cholesterol based antimicrobial agents,[49] besides drug carriers[50] and antiproliferative agents[51] in cancer research. Also,

cholesterol-peptide conjugates were reported as effective antiviral agents,[52] while cholesterol metal complexes were reviewed for their rule in managing Alzheimer`s disease[53] and cholesterol glycosides displayed immunostimulant activities.[54] Thus, the confluence of these activities prompted us to merge cholesterol in part of the target architectures through propargylation of the 3-OH group as it is compatible with our strategy that depended on CuAAC. The antimicrobial and cytotoxic activities of these architectures *in vitro* were screened to discover the optimum combination as a new lead structure for prospective investigations.

Azidolysis of chloroquinoline derivative **1**[55] Scheme 2 with NaN_3 in hot DMF afforded **2** in very good yield as the first substrate for CuAAC. To prepare the second substrate **11**, commercially available spacer–linker azidohexanol **8** was prepared from diol **3** or aminohexanol **4**. Thus, selective monobromination of **3** ensued by 0.5 equivalents of CBr_4/PPh_3 or tosylation in the presence of 0.75 equivalents of *p*–TsCl in Et_3N, added in portions, afforded **5** and **6** in moderate yields, respectively. Azidolysis of both **5** and **6** under the same conditions for **1** afforded **8** in excellent yields. Azide **8** was also prepared from **4** according to the diazo–transfer procedure in high yield.[56] IR and ^1H NMR spectra of **8** were in agreement with the reported data.[57] On the other hand, azidolysis of 6–chloro–1–hexanol was incomplete, as shown by ^1H NMR even after prolonged reaction times. The conversion in this case also was conflicted by difficult purification due to polarity resemblance between the substrate and **8**. This problem was′t encountered in the case of azidolysis of both **5** and **6**.

Coupling of glycosyl acceptor **8** with donor **9**[58] that is anomerically activated by the trichloroacetimidate as leaving group in the presence of TMSOTf as promotor afforded the expected pure β–glycoside **10** in 70% yield. A high $J_{1,2}$ 7.8 Hz value at δ 4.49 ppm precluded compound **10** to be an α–anomer since such glycosides have $J_{1,2}$ values less than 5.0 Hz. Saponification of **10** under the mild transesterification conditions of Zémplen[59] yielded the target second azide substrate **11** in low yield. All of the previous azides showed the diagnostic medium N_3 stretching vibration band at 2100 cm^{-1}.

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Scheme 2. *Reagents and conditions: (a)* NaN_3, DMF, 90–100 °C [(**2**, 73%); (**5**→**8**, 90%); (**6**→**8**, 85%)]; *(b)* CBr_4/PPh_3, DCM–THF (55%); *(c) p*–TsCl (0.75 eq), Et_3N, THF (**6**, 47%, **7**, 53%); *(d)* Tf_2O, NaN_3, $CuSO_4.5H_2O$, $NaHCO_3$ (90%); *(e)* TMSOTf (0.02 eq), DCM, rt (98%); *(f)* NaOMe/MeOH (31%).

Seven terminal alkynes **17a–e**, **18** and **19** Scheme 3 were prepared as complement for azides **2** and **11** to ensue variations of CuAAC reactions. Phenolic chalcones **13a,b,d,e**[60–62] and theophylline **14** were propargylated by stirring with propargyl bromide in DMF at ambient temperature in the presence of K_2CO_3 to afford **17a,b,d,e** and **18** in acceptable yields. To prepare **17c**, compound **12** was propargylated as previously described to afford intermediate **16** followed by condensation with *p*–dimethylaminobenzaldehyde under Claisen–Schmidt conditions. This procedure was used to avoid quenching of propargyl bromide as quaternary ammonium salt by the dimethylamino group if the relevant phenolic chalcone was propargylated as described for **17a**,**b**,**d**,**e**. Cholesterol **15** was propargylated by propargyl bromide in DMF–Et_2O at its secondary OH group in the presence of NaH to afford **19** in excellent yield.

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Scheme 3. *Reagents and conditions: (a)* Propargyl bromide, K_2CO_3, DMF, rt [**16** (47%); **17a** (R = Ph, 95%); **17b** (R = *p*-MeOPh, qu); **17d** (R = 2-Furanyl, 53%); **17e** (R = 2-Thiofuranyl, 58%); **18** (78%)]; *(b)* NaH, DMF–Et_2O (**19,** 93%); *(c) p*-dimethylaminobenzaldehyde, NaOH, EtOH, rt (**17c**, R = *p*-dimethylaminophenyl, 97%).

The IR spectra of these acetylenes displayed strong ≡C–H and weak C≡C stretching vibration bands at ~ 3220 and 2110 cm^{-1}, respectively. The shielded ≡C–H signal, ^1H NMR, was observed at ~ 2.56 ppm as doublet due to extended weak coupling, *J* 2.4 Hz, with the methylene protons which appeared at ~ 4.77 ppm.

The first target 1,2,3-triazole architectures **20a–e, 21 and 22** Scheme 4 in this study were prepared by clicking azidoquinoline **2** with propargylated chalcones **17a–e**, theophylline **18** and cholesterol **19**. Structurally, these 1,2,3-triazoles have chloroquinoline as permanent arm at N1 and the basic skeleton of **17a–e**, **18** and **19** as variable motifs at C–4. The reactions proceeded properly in short time in the presence of a catalytic amount of $CuSO_4.5H_2O$ reduced *in situ* by

L–ascorbic acid. THF–H_2O combination was a good solvent mixture to bring all components into solution. No reaction was observed if H_2O was not added since the copper salt was out of the solution phase. Yields were generally high upon smooth work up and chromatographic purification by virtue of the high polarity of the resulting triazoles compared with **2** and the propargyl substrates **17a–e**, **18** and **19**.

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Scheme 4. *Reagents and conditions: (a)* **17**–**19**, $CuSO_4$. $5H_2O$, L-Ascorbic acid, THF–H_2O 4:1, rfx. [**20a**, R = Ph (92%); **20b**, R = *p*-C_6H_4-OMe (73%); **20c**, R = *p*-C_6H_4-NMe_2 (61%); **20d**, R = Furan-2-yl (87%); **20e**, R = Thiophen-2-yl, (63%); **21** (75%); **22** (76%)].

The strong ≡C–H and weak C≡C stretching vibration bands disappeared in these triazoles and the ≡C–H ^{1}H NMR signal as well. The OCH_2 protons of the parent propargyl group that positioned at C–4 of the clicked triazole rings was shifted downfield to δ ~ 5.5 ppm in most cases due to the magnetic anisotropic effect of the triazole ring and appeared as singlet. The enone moieties in **20a–e** retained their red shift for the C=O stretching vibration bands at ~ 1640 cm^{-1} and high *vicinal* $J_{H,H}$ values of ~ 15.6 Hz corresponding to their s–*trans* configuration. However, chalcones **17a**, **20b** and **20d** are existing as *s–cis* / *s–trans* mixtures since they displayed a second $C{=}O_{str}$ band at ~1710 cm^{-1}. The disappearance of the acetylenic IR and ^{1}H NMR spectroscopic features precluded the possibility of azide–alkene cyclocondensation at the enone moiety which was reported under tetrabutyammonium hydrogensulfate catalysis in absence of terminal acetylenic groups.[63] Theophylline modified triazole **21** showed two C=O stretching vibrations at 1653 and 1696 cm^{-1} for the conjugated C=O at position 6 and that at position 2, respectively. Cholesterolyl triazole **22** showed the fingerprint ^{1}H NMR signals of cholesterol at δ5.4 ppm for C–6, 3.45 ppm for C–3, while, all CH_3 residues appeared with their expected multiplicities and they were well resolved within the chemical shift range δ 1.03–0.68 ppm. Exceptionally, the triazole C–4 methylene group retained the chemical shift value of the parent alkyne **19**.

The second set of triazoles Scheme 5 embraced three triazoles having a fixed β–hexylglucpyranoside arm at N1 and a variety of motifs at C–4 including a chalcone **23**, theophylline **24** and cholesterol **25**. Structurally, this set included the following essential pharmacophoric motifs, glucose as surface recognition cap, a six–carbon arm as recommended spacer for efficient recognition and a bifunctional pharmacophore Figure 1. Glucose, as ligand for lectins, act as homing cap for the terminal pharmacophore at cellular surfaces to augment the potential of their activities.[38]

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Figure 1. Schematic representation of the three–motif pharmacophoric design of compounds **23-25**.

Reactions were easily performed by clicking **11** with the relevant alkynes **17c**, **18** and **19** exactly as described for the synthesis of **20a-e, 21** and **22**. They were purified by simple flash chromatography using nonhalogenated solvent mixtures. Yields were a little low in the case of **23** and **24**, while, **25** was obtained in high yield.

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Scheme 5. *Reagents and conditions: (a)* **17d**, **18** or **19**, $CuSO_4$. $5H_2O$, L-Ascorbic acid, THF-H_2O 4:1, rfx. [**23** (44% from **17d**); **24** (36% from **18**); **25** (78% from **19**)].

The ^{1}H NMR spectra of these triazoles showed clearly the signals of the sugar moiety in all triazoles and the hexyl arm in the case of **23** and **24**, while, it was overlapped with the cholesterol protons in **25**. Compound **25** afforded confirmation of the trazole´s H–5 chemical shift at δ 7.94 ppm. All intermediates and target triazoles synthesized in this series showed molecular ion peaks at different intensities by EI–MS. Fortunately, the three glucose containing triazoles **23**–**25** afforded their molecular ion peaks under this harsh ionization conditions. Finally, **25** could be obtained by CuAAC of **10** and **19** followed by deacetylation in better yield compared with the coupling of **11** with **19.**

The antimicrobial activity of the ten target triazoles **20a–e** and **21**–**25** was tested *in vitro* against four microorganisms namely *Escherichia. coli* ATCC 11775, *Staphylococcus. aureus*

ATTC 12600, *Aspergillus flavus* Link and *Candida. albicans* ATCC 7102. Screening was done according to the Kirby–Bauer disc diffusion method.[64] Structurally, these triazoles were divided into two groups according to their triazole`s N1 domain. Group A comprised a common quinoline domain **20a–e, 21** and **22**, while group B had a common glucopyranosylhexyl domain **23–25**. In both groups, there were variations, such as, chalcones, theophylline and cholesterol as triazole`s C4 substituents. Inhibition zone diameters were calculated and compared with the control; Ampicillin in case of bacteria and Amphoterecin B in case of fungi Figure 2.

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Figure 2. *In vitro* antimicrobial activity of compounds **20a–e** and **21–25** against *E.coli*, *S. aureus. A. flavus* and *C. albicans*. Ampicillin was used as positive control in case of *E. coil* and *S. aureus*, while, Amphotericin B was used in case of *A. flavus* and *C. albicans*. Different letters on the column for each parameter varied significantly at $p \leq 0.05$.

As shown in Figure 2, all triazoles were active against *E. coli* and varied significantly with the control. Compound **25** from group B was the unique to cross the activity of the control by 18%. In the same group, compounds **23** and **24** were equally 59% less active than the control reflecting the preference of the cholesterolyl ring over the chalcone and theophylline moieties in developing the activity of this pharmacophoric model. In group A compounds, chalcone **20b** showed an activity of 9% less than the control but putting it in front of the seven triazoles in this group and giving a preference for the methoxy substituent on Ring B of the chalcone entity. Chalcones **20a**,**c**,**e** were varied significantly with the control but varied insignificantly with each other and they were 18, 23 and 32% less active than Ampicillin, respectively. Triazole **21** from group A varied significantly with the control and it was 41% less active than the control. Derivatives **22**, **23** and **24** varied significantly with the control without significant variations with each other. Theophylline containing triazole **22** was 45 % less active than Ampicillin, Thus, the impact of the theophylline moiety in **21** and **24** was weak against this bacterial strain.

In case of *S. aureus*, compound **25** also showed the best activity among the ten triazoles. It varied significantly compared with the control and it was 55% more active than it. Triazoles **20b,c,e, 21, 22** and **23** varied insignificantly with the control. Derivatives **20a** and **24** varied

significantly with the control and they were the least active triazoles (55%). They varied insignificantly with each other. Compound **20d** was the unique inactive triazole and comparing the structure of **20d** with **20e** denotes to the impact of the sulfur atom of the thiofuranyl moiety in case of **20e** over the oxygen atom of the furanyl entity in **20d** on its activity toward this bacterial strain. This structural variation was the unique difference between them.

Concerning the tested fungi, only **20b** and **25** showed antifungal activity against *A. flavus*. They varied significantly with the control and with each other. Compound **25** was 12% less active than the control, while **20b** was less active by 41%.

On the other hand, triazole derivatives **20a**,**b**, **22**, **23** and **25** showed antifungal activity against *C. albicans* and varied significantly with the control. Compound **25** from group B was the most active derivative, 12% less active than the control. Other triazoles varied insignificantly with each other and they were 30–47% less active than the control.

As the promising and most active triazole in this consideration, the antimicrobial activity of **25** was further quantified as MIC_{90} values[65] and compared with the controls.

The compound recorded an MIC_{90} value of 122 mM against *E.coli* compared with a value of 162 mM for Ampicillin, *i.e.* it was 25% more active than the control. Also, its MIC_{90} value was 94 mM against *S. aureus*, thus, it was 52% more active than the control that recorded a value of 194 mM.

In case of fungi, this triazole was less active than the control; it showed against *A. flavus* an MIC_{90} value of 268 mM compared with 228 mM for Amphoterecin B. Thus, it was about 18% less active than the control, relatively as in case of *C. albicans*, where it gave a value of 239 mM compared with a value of 198 mM for Amphoterecin B.

In conclusion, these results were compatible with the inhibition zone diameter results and demonstrated clearly the value of 1,2,3-triazoles decoration with the glucopyranosylhexyl and cholesteroyl moieties to develop the antimicrobial activities of **25** which emerged from this study as the most promising triazole of reproducible antimicrobial activity.

Target triazoles **20a–e** and **21–25** were screened *in vitro* as cytotoxic agents against human prostate cancer cell line PC3 using sulforhodamine B colorimetric (SRB) assay and doxorubicin as positive control.[66]

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Figure 3. Cytotoxicity effect of compounds **20a–e** and **21–25** on PC3 cell line. Doxorubicin (Dox) was used as positive control. Different letters on the column varied significantly at $p \leq 0.05$.

As shown in Figure 3, all derivatives showed different levels of cytotoxicity against prostate cancer PC3 cell line and they were varied significantly with their control (Doxorubicin). The best activity was observed for cholesterol derivative **25** from group B which was 2.3–fold less active than the control. The activity of **23** that retain glucose as surface recognition tag and a chalcone terminal instead of cholesterol was 3.6–fold less active than the control and nearly twice less active than **25**.

Theophylline containing triazole **24** was the least active probe in this consideration, 8–fold less active than the control. On the other side, compounds **20a**,**d** from group A were 3–fold and 3.1–fold less active than the control, respectively. Despite, they varied insignificantly with **25**. Chalcones **20b,c**,**e** varied insignificantly with each other but they varied significantly with chalcones **20a**,**d** and were less active than them.

Both compounds **21** and **22** were 5.4 and 5.3–folds less active than the control, respectively. In conclusion, theophylline had low impact on developing cytotoxic effects, while, merging of cholesterol with glucose as in compound **25** gave better cytotoxicity over merging with quinoline as in compound **22**. It might be deduced from this structure activity relationship that, clicking of more propargylated chalcone derivatives either with azidoquinoline or azidoglucose might lead to more potential cyctotoxic probes. Also, substitution of D-glucopyranose in **25** by other glycans might lead to more promising antimicrobial and cyctotoxic effects.

In conclusion, a set of three–motif pharmacophoric probs **20a–e** and **21–25** was prepared by CuAAC of relevant propargyl chalcones, theophylline or cholesterol with azidoquinoline or glucopyranosylhexyl azide. This set was screened as antimicrobial, and cytotoxic agents *in vitro*.

Triazole **25** showed moderate antifungal activity against *A. flavus* and *C. albicans*, while it was more active than Ampicillin against *E. coli* and *S. aureus*. Also, compound **25** showed the best cytotoxic activity against prostate cancer PC3 cell line *in vitro*. These results gave evidences for the value of tagging triazolylcholesterol with glucose, as surface recognition tag, in developing new probes of potential pharmacologic activities. Assuming that **25** is a new antibacterial lead structure encouraged us to substitute glucose with chitobiose, in a progressing work, for potential targeting of *E. coli* K1. This strain is the causative agent of bacterial meningitis. Further modifications of cholesterol are going in due course as well.

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Supplementary data

Supplementary data related to this article can be found at

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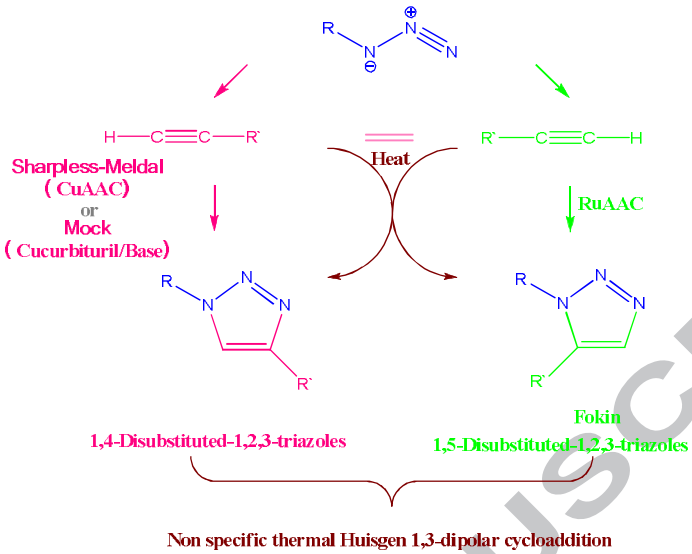
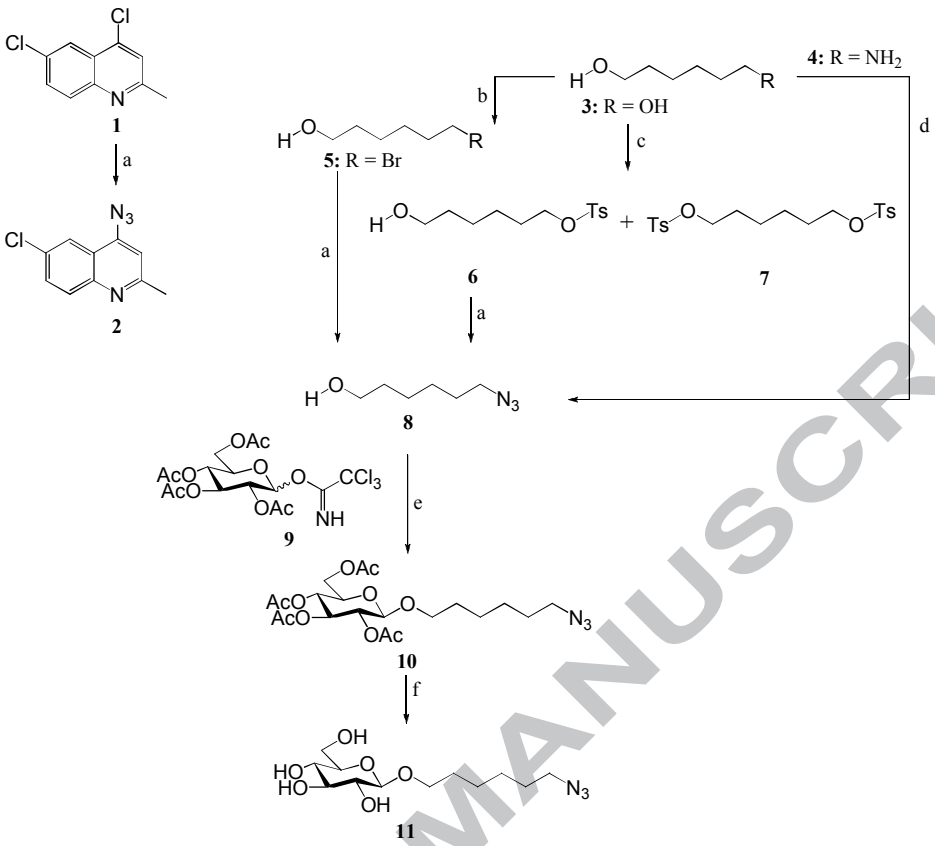
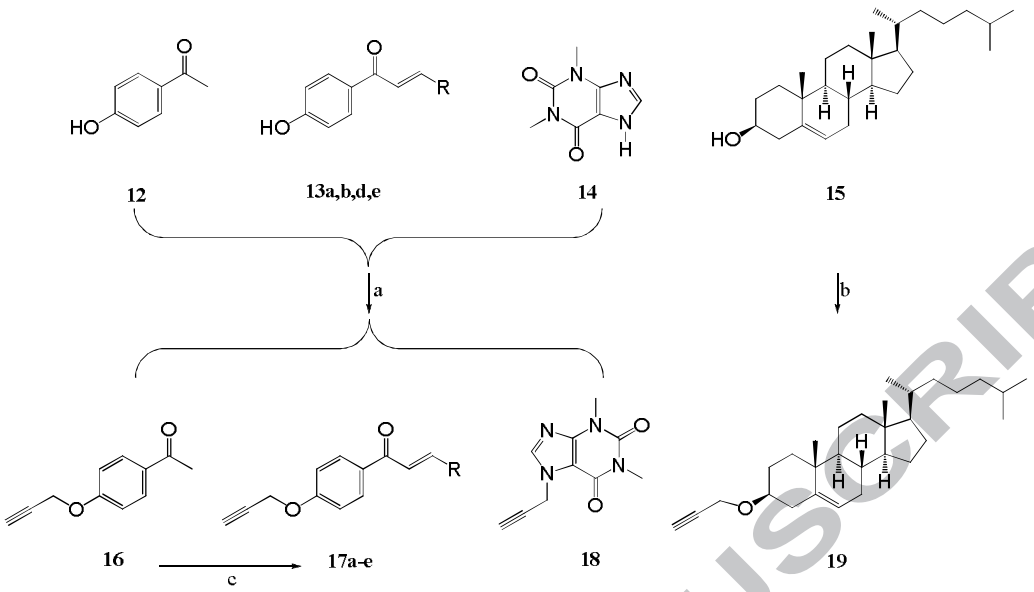


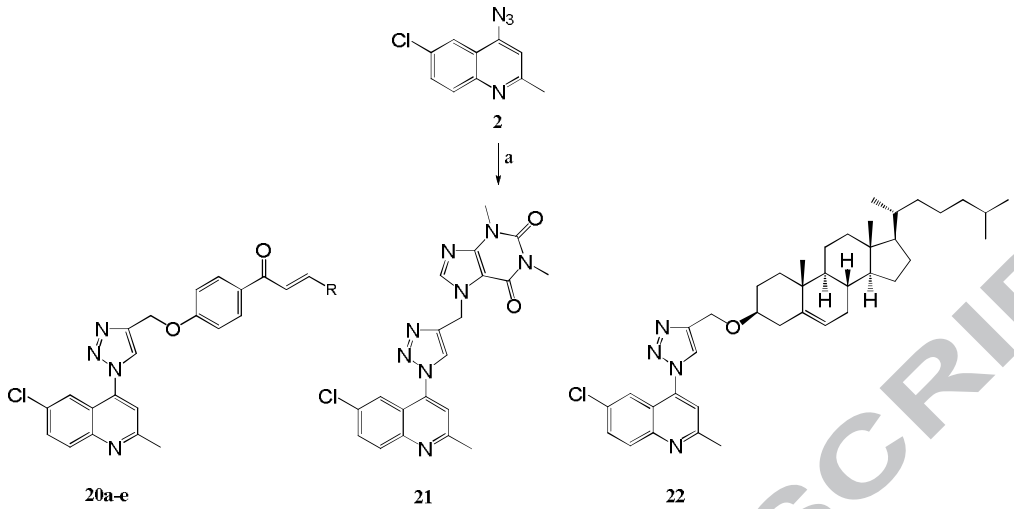
Scheme 1.



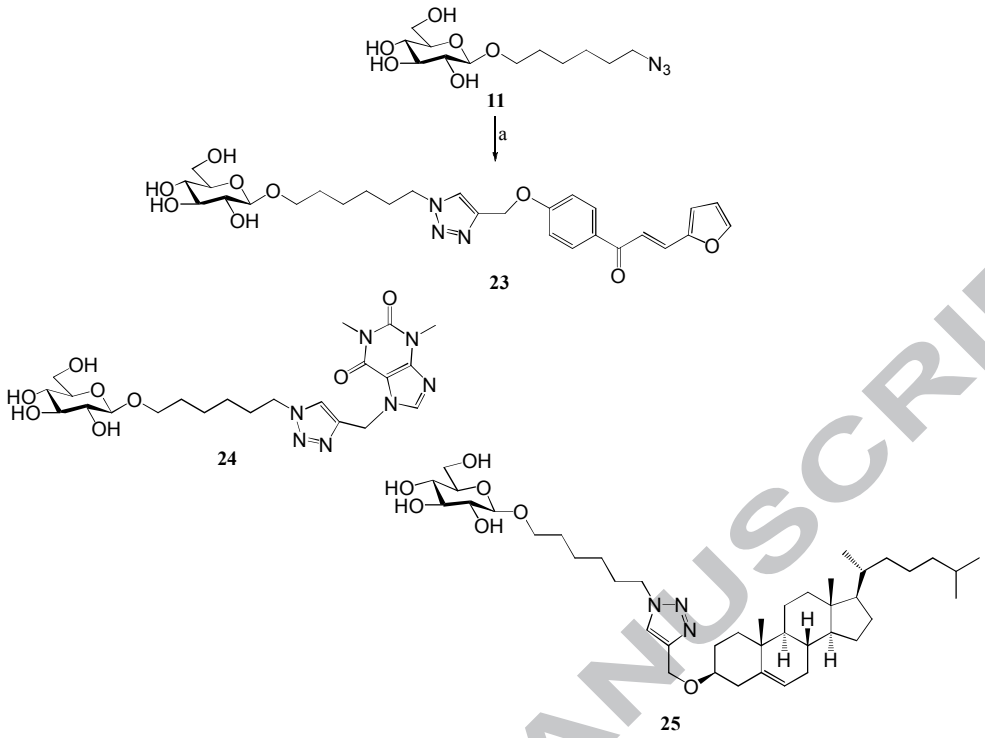
Scheme 2.



Scheme 3.



Scheme 4.



Scheme 5.

Captions List

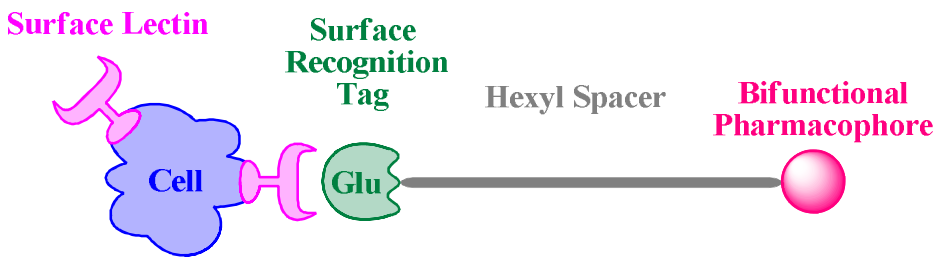


Figure 1.

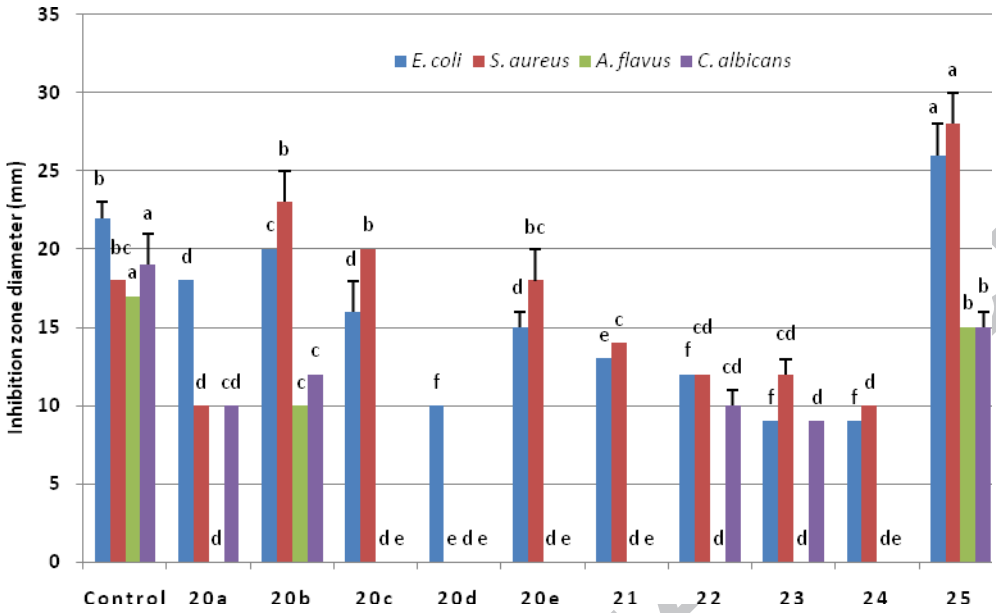


Figure 2.

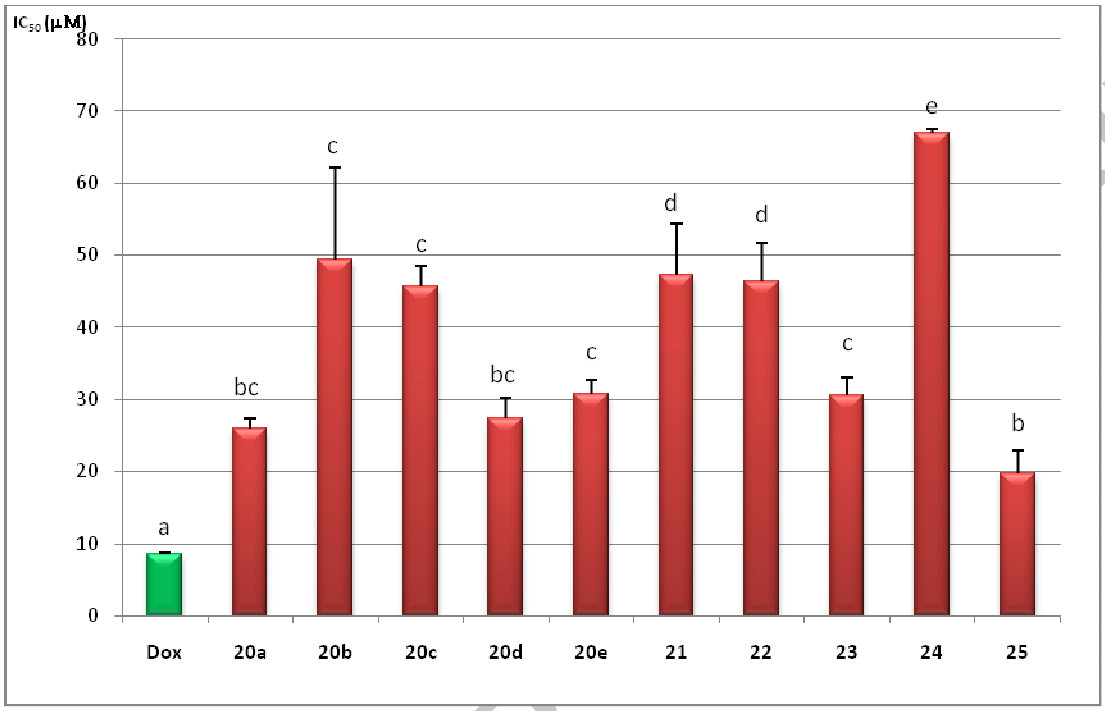


Figure 3.