Efficient One Pot Synthesis of Xanthene-Triazole-Quinoline/Phenyl Conjugates and Evaluation of their Antimicrobial Activity

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Novos conjugados xanteno-triazol-quinolina/fenila foram sintetizados via condensação ecologicamente amigável one pot de três componentes, 12-aril-2-hidroxi-tetrahidrobenzo[a]xanteno-11-ona, brometo propargílico e 4-azido-7-cloroquinolina/fenil azida usando polietilenoglicol 400 (PEG-400) como meio de reação, com o intuito de explorar seu efeito no crescimento in vitro de microorganismos que causam infecção microbial. Todos os conjugados xanteno-triazol-quinolina/fenila sintetizados foram caracterizados e avaliados quanto sua atividade antibacteriana e antifúngica in vitro. A atividade antimicrobiana foi avaliada confrontando nove cepas microbicas. Todos os compostos apresentaram boas atividades Gram positivas antibacteriana e antifúngica. Um dos compostos apresentou a melhor atividade antibacteriana e antifúngica. Posteriormente, os modos de ligação deste composto no sítio ativo da enzima topoisomerase II DNA gyrase B foram investigados.

Novel xanthene-triazole-quinoline/phenyl conjugates were synthesized by eco-friendly one pot three-component condensation of 12-aryl-2-hydroxy-tetrahydrobenzo[a]xanthene-11-one, propargyl bromide and 4-azido-7-chloroquinoline/phenyl azide using polyethylene glycol (PEG-400) as a reaction medium with an aim to explore their effect on the in vitro growth of microorganisms causing microbial infection. All newly synthesized xanthene-triazole-quinoline/phenyl conjugates were fully characterized and were evaluated for in vitro antibacterial and antifungal activity. Antimicrobial activity was evaluated against nine microbial strains. All compounds showed good Gram positive antibacterial and antifungal activity. One of the compounds showed best antibacterial and antifungal activity. Further, binding mode of this compound at the active site of enzyme topoisomerase II DNA gyrase B has also been investigated.

Keywords: 1,2,3-triazoles, quinolines, benzo[a]xanthenes, antimicrobial activity, multicomponent reactions, PEG-400

Introduction

During the past few years, the incidence of bacterial and fungal infection has increased to alarming levels because of the resistance to existing drugs and they are collectively a major cause of morbidity and mortality, especially in immunocompromised patients.1 Therefore, discovery of new classes of antimicrobial agents is crucial to combat multi-drug resistant infections. Benzo[a]xanthene and their derivatives are important heterocyclics with interesting biological activities, such as antibacterial,2 anti-inflammatory,3 antiviral,4 antimalarial,5 and antitumor.6 Some classes of xanthenes have also been used as antagonists for paralyzing the action of zoxazolamine and in photodynamic therapy. Nitrogen containing heterocycles such as triazoles and quinolines are common structural motifs in pharmacologically important molecules and alkaloids with activities spanning a diverse range of targets. 1,2,3-triazoles have occupied special place in medicinal chemistry due to their numerous biological activities such as anti-fungal,7 anti-bacterial,8 anti-alergic,9 anti-HIV,10,11 anti-tubercular.12,13 The 1,2,3-triazole derivatives can be easily synthesized using click chemistry through copper catalyzed azide alkyne cycloaddition.14 Quinolines have been of interest as they possesses useful pharmacological activities such as anti-malarial,15 anti-HIV,16 anti-tumor,17 and anti-bacterial.18 Literature survey reveals that hybridization is a classic strategy in drug design based
on combining two or more different bioactive moieties in a single molecule to get the corresponding conjugate/hybrid molecules. These conjugates generally show much better activity compared to their precursors and can work by the same or different mechanisms of action compared to the precursors. Thus, more effective antimicrobial compounds can be designed by joining two or more biologically active heterocyclic systems together in a single molecular framework. Multicomponent reactions combined with the use of environmental friendly reaction medium are valuable tools for the preparation of structurally diverse conjugates/hybrids of drug-like heterocyclic compounds without additional impact on environment.

As a part of our continued research for new antimicrobial agents by multicomponent reactions, and inspired by the biological activity of compounds containing benzo[a]xanthene, 1,2,3-triazoles and quinolines as pharmacophores, we designed the potentially bioactive target molecules by combining these pharmacophores in a single molecule as shown in Scheme 1. We attempted the synthesis of conjugates consisting of benzo[a]xanthene, 1,2,3-triazoles and quinolines moieties by condensation of 12-aryl-2-hydroxy-tetrahydrobenzo[a]xanthen-11-one, propargyl bromide and 4-azido-7-chloroquinoxine/phenyl azide.

We describe herein an efficient one pot three-component synthesis of novel xanthene-triazole-quinoline conjugates namely, 2-((1-(7-chloroquinolin-4-yl)-1H-1,2,3-triazol-4-yl)methoxy)-12-aryl-8,9,10,12-tetrahydrobenzo[a]xanthen-11-one derivatives, propargyl bromide and 4-azido-7-chloroquinine/phenyl azide.

Initially, the starting 12-aryl-2-hydroxy-8,9,10,12-tetrahydrobenzo[a]xanthen-11-one derivatives were synthesized by one pot three-component condensation reaction of various aldehydes, 2,7-dihydroxynaphthol and dimedone/cyclohexane-1,3-dione in the presence of catalytic amount of pTSA in refluxing ethanol. 4-azido-7-chloroquinoline was synthesized by reaction of 4,7-dichloroquinoline with sodium azide in dimethylformamide (DMF) at 60 °C as reported in literature. We designed the synthesis of xanthene-triazole-quinoline conjugates via multicomponent reaction of 12-aryl-2-hydroxy-8,9,10,12-tetrahydrobenzo[a]xanthen-11-one, propargyl bromide and 4-azido-7-chloroquinoline. The optimum reaction conditions for this three-component condensation were established using 12-(4-bromophenyl)-2-hydroxy-9,9-dimethyl-8,9,10,12-tetrahydrobenzo[a]xanthen-11-one (1.0 mmol), propargyl bromide (1.2 mmol), and 4-azido-7-chloroquinoline (1.0 mmol) as standard components for model reaction. The model reaction was attempted in different solvents like water, DMF, ethanol, tert-butyl alcohol-H2O (t-BuOH-H2O), PEG-600, CH2Cl2-H2O and PEG-400 in presence of catalytic amount of Cu(1), generated in situ from copper sulphate and sodium ascorbate, and K2CO3 (1.0 eq.) as a base as shown in Table 1. However, the best results were obtained when the reaction was carried out in PEG-400 as a medium at 80 °C. The reaction was complete in 35 min, yielding 92% of 2-((1-(7-chloroquinolin-4-yl)-1H-1,2,3-triazol-4-yl)methylenoxy)-12-(4-bromophenyl)-8,9,10,12-

**Result and discussion**

**Chemistry**

Initially, the starting 12-aryl-2-hydroxy-8,9,10,12-tetrahydrobenzo[a]xanthen-11-one derivatives were synthesized by one pot three-component condensation reaction of various aldehydes, 2,7-dihydroxynaphthol and dimedone/cyclohexane-1,3-dione in the presence of catalytic amount of pTSA in refluxing ethanol. 4-azido-7-chloroquinoline was synthesized by reaction of 4,7-dichloroquinoline with sodium azide in dimethylformamide (DMF) at 60 °C as reported in literature. We designed the synthesis of xanthene-triazole-quinoline conjugates via multicomponent reaction of 12-aryl-2-hydroxy-8,9,10,12-tetrahydrobenzo[a]xanthen-11-one, propargyl bromide and 4-azido-7-chloroquinoline. The optimum reaction conditions for this three-component condensation were established using 12-(4-bromophenyl)-2-hydroxy-9,9-dimethyl-8,9,10,12-tetrahydrobenzo[a]xanthen-11-one (1.0 mmol), propargyl bromide (1.2 mmol), and 4-azido-7-chloroquinoline (1.0 mmol) as standard components for model reaction. The model reaction was attempted in different solvents like water, DMF, ethanol, tert-butyl alcohol-H2O (t-BuOH-H2O), PEG-600, CH2Cl2-H2O and PEG-400 in presence of catalytic amount of Cu(1), generated in situ from copper sulphate and sodium ascorbate, and K2CO3 (1.0 eq.) as a base as shown in Table 1. However, the best results were obtained when the reaction was carried out in PEG-400 as a medium at 80 °C. The reaction was complete in 35 min, yielding 92% of 2-((1-(7-chloroquinolin-4-yl)-1H-1,2,3-triazol-4-yl)methylenoxy)-12-(4-bromophenyl)-8,9,10,12-

**Scheme 1.** Structure of bioactive molecules containing heterocycles of interest and target molecules.
tetrahydrobenzo[a]xanthen-11-one (1a) after work up (entry 7, Table 1). Reaction was not complete even after 80 min when attempted in water and ethanol (entries 1 and 2, Table 1). The reactions in DMF, t-BuOH-water (1:1), PEG-600 and CH₂Cl₂-H₂O (1:1) required longer reaction times under identical conditions and gave inferior yields (entries 3-6, Table 1). The reaction in PEG-400 did not have any advantage when attempted at 100 °C. However, the reaction carried out at 60 °C was incomplete even after 80 min (entries 8 and 9, Table 1). The reaction employing CuI as catalyst in place of CuSO₄·5H₂O and sodium ascorbate was incomplete even after 80 min (entry 11, Table 1). Therefore, it can be inferred from Table 1 that condensation of three components using K₂CO₃ (1 eq.), CuSO₄·5H₂O (10 mol%) and sodium ascorbate (20 mol%) as catalysts in PEG-400 (5 mL) at 80 °C proved to be the optimum condition for the targeted molecules.

Subsequently, reactions of different 12-aryl-2-hydroxy-8,9,10,12-tetrahydrobenzo[a]xanthen-11-ones were carried out with propargyl bromide and 4-azido-7-chloroquinoline under above optimized conditions. All the reactions proceeded smoothly and were complete in 40 min, yielding corresponding xanthene-triazole-quinoline conjugates (1b-1o) in high yields (entries 2-15, Table 2) (Scheme 2). The scope of reaction was further examined by replacing 4-azido-7-chloroquinoline with phenyl azide. Reaction of 12-(4-chlorophenyl)-2-hydroxy-9,9-dimethyl-8,9,10,12-tetrahydrobenzo[a]xanthen-11-one and propargyl bromide with phenyl azide required 50 min for completion and gave the product (1p) in 88% yield (entry 16, Table 2).

The structures of all novel compounds (1a-1o) were confirmed by infrared (IR), ¹H nuclear magnetic resonance (NMR), ¹³C NMR, mass spectra and elemental analysis. The ¹H NMR spectra of compound 1a showed two singlets for three protons at δ 0.94 and δ 1.10, corresponding to two methyl groups. Two methylene protons adjacent to carbonyl group act as AB system and appeared at δ 2.24 and δ 2.27, while another methylene group of dimeredone ring appeared as singlet at δ 2.54. The methylene group adjacent to oxygen atom also acts as AB system and appeared at δ 5.41 and δ 5.30. The one methine proton of benzo[a]xanthene

Table 1. Optimization of reaction conditions for the synthesis of xanthene-triazole-quinoline conjugates

| S. No. | Solvent                          | Base    | Temperature / °C | time / min | Yield / % |
|--------|----------------------------------|---------|------------------|------------|-----------|
| 1      | Water                            | K₂CO₃   | 80               | 80         | 68b       |
| 2      | Ethanol                          | K₂CO₃   | 80               | 80         | 45b       |
| 3      | DMF                              | K₂CO₃   | 80               | 65         | 85        |
| 4      | t-BuOH-water (1:1)               | K₂CO₃   | 80               | 55         | 87        |
| 5      | PEG-600                          | K₂CO₃   | 80               | 40         | 84        |
| 6      | CH₂Cl₂/H₂O (1:1)                 | K₂CO₃   | 80               | 45         | 82        |
| 7      | PEG-400                          | K₂CO₃   | 80               | 35         | 92        |
| 8      | PEG-400                          | K₂CO₃   | 100              | 35         | 91        |
| 9      | PEG-400                          | K₂CO₃   | 60               | 80         | 70b       |
| 10     | PEG-400                          | KOH     | 80               | 70         | 84        |
| 11     | PEG-400                          | K₂CO₃   | 80               | 80         | 75c       |

[a] Reactions carried out using 12-(4-bromophenyl)-2-hydroxy-9,9-dimethyl-8,9,10,12-tetrahydrobenzo[a]xanthen-11-one (1.0 eq.), propargyl bromide (1.2 eq.), 4-azido-7-chloroquinoline (1.0 eq.) in the presence of base (1.0 eq.), CuSO₄·5H₂O (10 mol%) and Na ascorbate (20 mol%); [b] incomplete reaction; [c] reaction carried out in the presence of CuI rather than CuSO₄·5H₂O and Na ascorbate.

Scheme 2. Multicomponent synthesis of 2-((1-(7-chloroquinolin-4-yl)-1H-1,2,3-triazol-4-yl)methoxy)-12-aryl-8,9,10,12-tetrahydrobenzo[a]xanthen-11-ones derivatives. Reagents and conditions: (a) K₂CO₃ (1 eq.), CuSO₄·5H₂O (10 mol%), sodium ascorbate (10 mol%) PEG-400, 80 °C.
Table 2. Synthesis of xanthene-triazole-quinoline conjugates (1a-1p)

| S. No. | Ar            | R          | Product | log P*          | time / min | Yield / % |
|-------|---------------|------------|---------|----------------|------------|-----------|
| 1     | 4-BrC₆H₄      | CH₃        | 1a      | 7.80           | 35         | 92        |
| 2     | 4-FC₆H₄       | CH₃        | 1b      | 7.13           | 30         | 90        |
| 3     | 4-NO₂C₆H₄     | CH₃        | 1c      | 7.27           | 30         | 91        |
| 4     | 4-CH₂O C₆H₄  | CH₃        | 1d      | 6.84           | 35         | 88        |
| 5     | 3,4-(CH₂O)₂C₆H₄ | CH₃   | 1e      | 6.72           | 30         | 90        |
| 6     | 4-CH₃C₆H₄     | CH₃        | 1f      | 7.46           | 30         | 85        |
| 7     | 3-NO₂C₆H₄     | CH₃        | 1g      | 7.16           | 25         | 93        |
| 8     | C₆H₄          | CH₃        | 1h      | 6.97           | 35         | 89        |
| 9     | 4-ClC₆H₄      | CH₃        | 1i      | 7.53           | 30         | 84        |
| 10    | 1-naphthyl    | CH₂        | 1j      | 7.97           | 40         | 87        |
| 11    | 4-CH₂C₆H₄     | H          | 1k      | 6.96           | 30         | 91        |
| 12    | 1-naphthyl    | H          | 1l      | 7.17           | 35         | 85        |
| 13    | 4-BrC₆H₄      | H          | 1m      | 7.01           | 25         | 92        |
| 14    | 4-(CH₃)₂CH₂C₆H₄ | H    | 1n      | 7.40           | 35         | 90        |
| 15    | C₆H₄          | H          | 1o      | 6.81           | 35         | 84        |
| 16    | 4-ClC₆H₄⁺     | CH₂        | 1p      | 6.99           | 30         | 88        |

*logP values were calculated using OSIRIS property explorer software; reaction was performed using phenyl azide (1 eq.) instead of 4-azido-7-chloroquinoline.

The plausible reaction mechanism for one pot synthesis of xanthene-triazole-quinoline conjugates is provided in Scheme 3. Initially, the reaction between 12-aryl-2-hydroxy-8,9,10,12-tetrahydrobenzo[a]xanthen-11-one with propargyl bromide in presence of K₂CO₃ resulted in formation of propargylated-benzo[a]xanthen derivative.

Figure 1. Ortep diagram of compound 1d and 1p drawn with 30% ellipsoid probability.

(II). This is followed by Huisgen 1,3-dipolar cycloaddition reaction between II and 4-azido-7-chloroquinoline in presence of in situ generated Cu(II) by reaction of CuSO₄·5H₂O with sodium ascorbate, leading to formation of xanthene-triazole-quinoline conjugates.

We have also carried out the sequential synthesis of 1a in two steps as depicted in Scheme 4 to demonstrate the advantages of multicomponent reactions in the synthesis of target molecules over sequential process. The reaction of 12-(4-bromophenyl)-2-hydroxy-9,9-dimethyl-8,9,10,12-tetrahydrobenzo[a]xanthen-11-one (1.0 mmol) with propargyl bromide (1.2 mmol) in presence of K₂CO₃ in PEG-400 at 80 °C gave 12-(4-bromophenyl)-9,9-dimethyl-2-(prop-2-ynyloxy)-8,9,10,12-tetrahydrobenzo[a]xanthen-11-one (3) in 82% yield. Structure of the intermediate 3 was confirmed by ¹H NMR, ¹³C NMR and mass spectra. The reaction of 3 was then carried out with 4-azido-7-chloroquinoline in PEG-400 in the presence of CuSO₄·5H₂O (10 mol%) and sodium ascorbate (20 mol%) and gave the desired product 4 in 89% yield. The overall
sequential reaction required longer reaction time and gave poorer overall sequential reaction yield of 74% compared to the multicomponent process (92%), thereby demonstrating its advantage.

**Biological studies**

For the evaluation of antibacterial and antifungal activity of all compounds, total nine microbial strains, three Gram positive bacteria (*Staphylococcus aureus* (MTCC 96), *Bacillus subtilis* (MTCC 121) and *Bacillus cereus* (MTCC 430)); three Gram negative bacteria (*Escherichia coli* (MTCC 1652), *Pseudomonas aeruginosa* (MTCC 741) and *Enterobacter aerogenes* (MTCC 111)) and three fungi, (*Aspergillus niger* (MTCC 282), *Aspergillus flavus* (MTCC 277) and *Penicillium sp.* (MTCC 9062)) were selected. Standard antibacterial drug, ciprofloxacin and antifungal drug, fluconazole were used as positive controls.

All xanthene-triazole-quinoline/phenyl conjugates (1a-1p) showed good antibacterial activity, against Gram positive (*Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus*) bacteria. However, none of the compounds showed activity against Gram negative bacteria as shown in Figure 2. Compounds 1a-1p showed growth of inhibition zone diameter in the range of 24.6 mm-14.6 mm against *Staphylococcus aureus* as compared to standard ciprofloxacin (26.6 mm), 22.6 mm-13.3 mm against *Bacillus subtilis* as compared to standard ciprofloxacin (24.0 mm) and 21.3 mm-14.3 mm against *Bacillus cereus* as compared to standard ciprofloxacin (23.0 mm). Compound 1a was found to be most effective antibacterial agent with growth of inhibition zone diameter of 24.6 mm against *Staphylococcus aureus* was found to be most effective antibacterial agent with growth of inhibition zone diameter of 24.6 mm against *Staphylococcus aureus*, 22.6 mm against *Bacillus subtilis* and 21.3 mm against *Bacillus cereus* bacteria.

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of all compounds (1a-1p) were measured against Gram positive bacteria. MIC is the lowest concentration of an antimicrobial compound that inhibits the visible growth of a microorganism after overnight incubation, while MBC is the lowest concentration of an antibacterial agent that reduces the viability of the initial bacterial inoculum by ≥ 99.9%. The MIC and MBC values of all compounds are shown in Table 5. All compounds showed MIC values in the range of

### Table 3. Crystal data and structure refinement for 1d

| Identification code | shelxl |
|---------------------|--------|
| Empirical formula   | C₇₆H₇₈Cl₇N₇O₇ |
| Formula weight      | 643.20 |
| Temperature         | 293 K  |
| Wavelength          | 0.71073 Å |
| Crystal system      | Triclinic |
| Space group         | P-1    |
| Unit cell dimensions | a = 9.8780(10) Å, α = 105.339(7)° |
|                      | b = 11.6884(10) Å, β = 90.715(7)° |
|                      | c = 14.3841(11) Å, γ = 101.896(8)° |
| Volume              | 1563.3(3) Å³ |
| Z                   | 2      |
| Density (calculated) | 1.366 mg m⁻³ |
| Absorption coefficient | 0.172 mm⁻¹ |
| F(000)              | 672    |
| Crystal size        | 0.38 × 0.19 × 0.09 mm³ |
| Theta range for data collection | 2.94 to 25.00° |
| Index ranges        | –11 ≤ h ≤ 11, –13 ≤ k ≤ 13, –17 ≤ l ≤ 17 |
| Reflections collected | 20163 |
| Independent reflections | 5500 [R(int) = 0.0914] |
| Completeness to theta = 25.00° | 99.8% |
| Max. and min. transmission | 1.00000 and 0.99083 |
| Refinement method   | Full-matrix least-squares on F² |
| Data/restraints/parameters | 55000/0/424 |
| Goodness-of-fit on F² | 1.001 |
| Final R indices [I > 2σ(I)] | R₁ = 0.0689, wR₁ = 0.0870 |
| R indices (all data) | R₁ = 0.1632, wR₁ = 0.1184 |
| Largest diff. peak and hole | 0.210 and –0.228 e.Å⁻³ |

### Table 4. Crystal data and structure refinement for 1p

| Identification code | shelxl |
|---------------------|--------|
| Empirical formula   | C₇₆H₇₈Cl₇N₇O₇ |
| Formula weight      | 652.90 |
| Temperature         | 293(2) K |
| Wavelength          | 0.71073 Å |
| Crystal system      | Triclinic |
| Space group         | P-1    |
| Unit cell dimensions | a = 10.3161(6) Å, α = 74.275(5)° |
|                      | b = 11.3199(7) Å, β = 85.032(5)° |
|                      | c = 12.9621(6) Å, γ = 76.875(5)° |
| Volume              | 1418.46(14) Å³ |
| Z                   | 2      |
| Density (calculated) | 1.316 mg m⁻³ |
| Absorption coefficient | 0.175 mm⁻¹ |
| F(000)              | 588    |
| Crystal size        | 0.38 × 0.18 × 0.09 mm³ |
| Theta range for data collection | 3.25 to 26.00° |
| Index ranges        | –12 ≤ h ≤ 12, –13 ≤ k ≤ 13, –15 ≤ l ≤ 15 |
| Reflections collected | 20177 |
| Independent reflections | 5558 [R(int) = 0.0211] |
| Completeness to theta = 26.00° | 99.8% |
| Max. and min. transmission | 0.9844 and 0.9364 |
| Refinement method   | Full-matrix least-squares on F² |
| Data/restraints/parameters | 55580/0/372 |
| Goodness-of-fit on F² | 1.046 |
| Final R indices [I > 2σ(I)] | R₁ = 0.0393, wR₁ = 0.0913 |
| R indices (all data) | R₁ = 0.0448, wR₁ = 0.0943 |
| Largest diff. peak and hole | 0.232 and –0.355 e.Å⁻³ |
16-128 µg mL\(^{-1}\) and MBC in the range of 32-256 µg mL\(^{-1}\) against *Staphylococcus aureus*, *Bacillus subtilis* and *Bacillus cereus* bacteria as compared to standard drug ciprofloxacin.

The results of antibacterial activity revealed that among all compounds, compound 1a was found to be most potent antibacterial agent with MIC value of 16 µg mL\(^{-1}\) against *Staphylococcus aureus*, *Bacillus subtilis* and 32 µg mL\(^{-1}\) against *Bacillus cereus*. Compound 1a showed MBC of 32 µg mL\(^{-1}\) against *Staphylococcus aureus*, *Bacillus subtilis* and *Bacillus cereus* bacteria. The structure activity relationship (SAR) for compounds (1a-1p) revealed that compounds (1a, 1b, 1i, and 1m) with halogen substituent over phenyl ring of xanthene moiety showed higher activity as compared to other compounds. The replacement of halogen atom with nitro or alkyl group results in a decrease
in the antibacterial activity. The position of substituent over phenyl ring also affects the antibacterial activity of compound. Compound 1c having nitro group at para position over phenyl ring of xanthene moiety showed MIC 64 µg mL⁻¹ against Staphylococcus aureus, while compound 1g with nitro group at meta position showed MIC 128 µg mL⁻¹ against Staphylococcus aureus. The presence of chloroquinoline moiety also enhances the antibacterial activity of these compounds as replacing chloroquinoline moiety with phenyl ring (compound 1p) resulted in decrease in the antibacterial activity (Figure 2 and Table 5). All compounds were found to be inactive against Gram negative bacteria (Escherichia coli, Pseudomonas aeruginosa and Enterobacter aerogenes). The possible reason for inactivity of these compounds against Gram negative bacteria can be explained by the fact that the susceptibility of microorganisms to a drug depends on the physicochemical characteristics of that drug (hydrophobicity and hydrosolubility) and on the composition of microbial membranes. The outer layer of the outer membrane of Gram negative bacteria is composed of lipopolysaccharide molecules that form a hydrophilic environment providing protection against hydrophobic molecules. The synthesized compounds (1a-1p) are highly hydrophobic as indicated by their high log P values as shown in Table 2 which makes them inactive against Gram negative bacteria.

In order to investigate a plausible mechanism of action of the most active compound 1a against bacteria S. aureus, docking studies of 1a into active site of enzyme topoisomerase II DNA gyrase B were performed. Bacterial DNA gyrase is an established and validated target for the development of novel antibacterials. The protein ligand complex was constructed based on the X-ray structure of topoisomerase II DNA gyrase with its bound inhibitor ciprofloxacin that is available through the RCSB Protein Data Bank (PDB entry 2XCT). Docking was performed using auto dock 4 program. The binding mode of inhibitor ciprofloxacin at the active site of topoisomerase II DNA gyrase B is shown in Figure 3. Ciprofloxacin formed two hydrogen bonds with Arg-458 and Ser-1084 residues at the active site of enzyme. Binding mode of 1a at the active site of topoisomerase II DNA gyrase B is shown in Figures 4 and 5. Compound 1a binds in a similar fashion as ciprofloxacin at the active site with binding energy of −5.86 kcal mol⁻¹. Compound 1a formed hydrogen bonds with Ser1084 residue and

Table 5. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of all compounds against Gram positive bacteria

| Product | MIC / (µg mL⁻¹) | MBC / (µg mL⁻¹) |
|---------|----------------|----------------|
|         | Staphylococcus aureus | Bacillus subtilis | Bacillus cereus | Staphylococcus aureus | Bacillus subtilis | Bacillus cereus |
| 1a      | 16             | 16             | 32             | 32             | 32             |
| 1b      | 64             | 64             | 64             | 64             | 64             | 64             | 64             | 64             | 64             | 64             | 64             | 64             | 64             |
| 1c      | 64             | 128            | 128            | 128            | 128            | 128            | 128            | 128            | 128            | 128            | 128            | 128            | 128            |
| 1d      | 128            | 128            | 128            | 128            | 128            | 128            | 128            | 128            | 128            | 128            | 128            | 128            | 128            |
| 1e      | 128            | 64             | 128            | 128            | 128            | 128            | 128            | 128            | 128            | 128            | 128            | 128            | 128            |
| 1f      | 128            | 64             | 128            | 128            | 128            | 128            | 128            | 128            | 128            | 128            | 128            | 128            | 128            |
| 1g      | 128            | 128            | 128            | 128            | 128            | 128            | 128            | 128            | 128            | 128            | 128            | 128            | 128            |
| 1h      | 128            | 128            | 128            | 128            | 128            | 128            | 128            | 128            | 128            | 128            | 128            | 128            | 128            |
| 1i      | 32             | 32             | 64             | 64             | 64             | 64             | 64             | 64             | 64             | 64             | 64             | 64             | 64             |
| 1j      | 128            | 128            | 128            | 128            | 128            | 128            | 128            | 128            | 128            | 128            | 128            | 128            | 128            |
| 1k      | 128            | 128            | 128            | 128            | 128            | 128            | 128            | 128            | 128            | 128            | 128            | 128            | 128            |
| 1l      | 64             | 128            | 64             | 64             | 64             | 64             | 64             | 64             | 64             | 64             | 64             | 64             | 64             |
| 1m      | 32             | 64             | 64             | 64             | 64             | 64             | 64             | 64             | 64             | 64             | 64             | 64             | 64             |
| 1n      | 128            | 128            | 128            | 128            | 128            | 128            | 128            | 128            | 128            | 128            | 128            | 128            | 128            |
| 1o      | 128            | 64             | 128            | 128            | 128            | 128            | 128            | 128            | 128            | 128            | 128            | 128            | 128            |
| 1p      | 128            | 128            | 128            | 128            | 128            | 128            | 128            | 128            | 128            | 128            | 128            | 128            | 128            |
| Ciprofloxacin | 6.25      | 6.25          | 6.25       | 6.25         | 6.25        | 6.25         | 6.25        | 6.25        | 6.25        | 6.25        | 6.25        | 6.25        | 6.25        |
DC13 chain at the active site of enzyme topoisomerase II DNA gyrase B. Compound 1a is surrounded by GLY-1174, ALA-1180, GLY-1178 and VAL-1177 amino acid residues at active site of enzyme topoisomerase II DNA gyrase B. Compound 1a ‘bridges’ the DNA and a transient non-catalytic pocket at the GyrA dimer interface. Because of formation of this bridge, the active site seems poised to cleave the DNA and results in inhibition of bacterial growth.28

All compounds (1a-1p) were also tested for their in vitro antifungal activity against three fungal strains, namely, *A. niger*, *A. flavus* and *Penicillum sp*. The standard drug fluconazole was used for comparison of the antifungal activity shown by the compounds and results were recorded as a percentage of mycelial growth inhibition and MIC. The results of antifungal activity of all compounds are shown in Table 6. It can be inferred from Table 6 that all the compounds showed good antifungal activity against the three pathogens. From a careful comparison of the results, it is observed that all compounds (1a-1p) showed more than 50% inhibition of mycelial growth against *A. niger*, *A. flavus* and *Penicillum sp.* species. Compounds (1a, 1b, 1i, and 1m), having a halogen substituent over phenyl ring of xanthene moiety, showed higher antifungal activity as inferred from Table 4. Compound 1a showed excellent antifungal activity against all three strains with mycelial growth inhibition of 72.2% against *Aspergillus niger*, 65.5% against *Aspergillus flavus* and 57.7% against *Penicillum sp*. Compound 1a showed MIC of 16 µg mL\(^{-1}\) against *Aspergillus niger* as compared to fluconazole with MIC of 12.5 µg mL\(^{-1}\). Compound 1a also showed a lowest MIC value (32 µg mL\(^{-1}\)) against *Aspergillus flavus* and *Penicillum sp.*

**Conclusion**

In conclusion, we have reported an efficient synthesis of novel xanthene-triazole-quinoline/phenyl conjugates (1a-1p) by one pot three-component condensation of 12-aryl-2-hydroxy-tetrahydrobenzo[a]xanthene-11-ones, propargyl bromide and 4-azido-7-chloroquinoline/phenyl azide in the presence of K\(_2\)CO\(_3\), as a base and 10 mol% CuSO\(_4\)-5H\(_2\)O and 20 mol% sodium ascorbate in PEG-400 at 80 °C. All newly synthesized compounds were evaluated for antimicrobial activity against nine microbial strains including six bacterial strains and three fungal strains. All compounds exhibited good antibacterial activity and antifungal activity. All compounds showed good Gram positive antibacterial activity and antifungal activity. Compound 1a was found to be most potent antibacterial and antifungal agent with highest activity among all compounds. Binding mode of compound 1a at the active site of enzyme topoisomerase II DNA gyrase B has also been investigated.
Experimental

All chemicals were purchased from Sigma-Aldrich, Spectrochem and were used as received. F$_{254}$ precoated aluminium plates with silica gel 60 from Merck were used to monitor reaction progress. IR (KBr) spectra were recorded on Perkin Elmer FTIR spectrophotometer and the values are expressed as $\nu_{\text{max}}$/cm$^{-1}$. The NMR ($^1$H and $^{13}$C) spectra were recorded on Jeol JNM ECX-400P at 400 MHz and 100 MHz, respectively. The chemical shift values are recorded on $\delta$ scale and the coupling constants ($J$) are in Hertz. The mass spectra were recorded on an Agilent 6520-QTOF LCMS having ESI source in positive mode. Elemental analyses were recorded on VarioEL III elemental analyzer in CHNS mode. Single crystal X-Ray intensity data was collected on Oxford Diffraction Xcalibur CCD diffractometer with graphite monochromatic Mo K$\alpha$ radiation ($\lambda = 0.71073$ Å) at temperature 298 K.

General procedure for synthesis of 2-((1-(7-chloroquinolin-4-yl/phenyl)-1H-1,2,3-triazol-4-yl)methyleneoxy)-12-aryl-8,9,10,12-tetrahydrobenzo[a]xanthen-11-one (1a-1p)

A mixture of 12-aryl-2-hydroxy-tetrahydrobenzo[a]xanthen-11-one (1 mmol), propargyl bromide (1.0 mmol), and 4-azido-7-chloroquinoline/phenyl azide (1.0 mmol), K$_2$CO$_3$ (1.0 mmol), CuSO$_4$.5H$_2$O (10 mol%), sodium ascorbate (20 mol%) and PEG 400 (5 mL) was placed in a 50 mL round-bottomed flask. The reaction mixture was stirred at 80 °C for appropriate time as mentioned in Table 2. The progress of the reaction was monitored by thin layer chromatography (TLC) using ethyl acetate: petroleum ether (30:70, v/v) as eluent. After completion of the reaction as indicated by TLC, water (5 mL) was added to the reaction mixture. The precipitate formed was collected by filtration at pump and washed with water. The product obtained, was recrystallized from ethanol to yield the pure product (1a-1p) in high yield (Table 2).

Procedure for synthesis of 12-(4-bromophenyl)-9,9-dimethyl-2-(prop-2-ynyloxy)-9,10,11,12-tetrahydrobenzo[a]xanthen-11-one (3)

A mixture of 12-(4-bromophenyl)-2-hydroxy-9,9-dimethyl-9,10,11,12-tetrahydrobenzo[a]xanthen-11-one (1 mmol), propargyl bromide (1.0 mmol), K$_2$CO$_3$ (1.0 mmol), and PEG 400 (5 mL) was placed in a 50 mL round-bottomed flask. The reaction mixture was stirred at 60 °C for 30 min. The progress of the reaction was monitored by TLC using ethyl acetate: petroleum ether (20:80, v/v) as eluent. After completion of the reaction as indicated by TLC, water (5 mL) was added to the reaction mixture. The precipitate formed was collected by filtration at pump and washed with water. The product so obtained, was recrystallized from ethanol to yield 3 in 82% yield.

Table 6. Antifungal activity of all compounds (1a-1p)

| Product | Mycelial growth inhibition / % | MIC / (µg mL$^{-1}$) |
|---------|--------------------------------|-----------------------|
|         | Aspergillus niger | Aspergillus flavus | Penicillium sp. | Aspergillus niger | Aspergillus flavus | Penicillium sp. |
| 1a      | 72.2              | 65.5               | 57.7            | 16               | 32               | 32              |
| 1b      | 63.3              | 58.8               | 55.5            | 64               | 64               | 64              |
| 1c      | 58.8              | 51.1               | 53.3            | 64               | 128              | 128             |
| 1d      | 41.1              | 55.5               | 50.0            | 256              | 64               | 128             |
| 1e      | 57.7              | 51.1               | 48.8            | 64               | 128              | 128             |
| 1f      | 52.2              | 47.7               | 44.4            | 128              | 128              | 128             |
| 1g      | 51.1              | 44.4               | 45.5            | 128              | 64               | 128             |
| 1h      | 65.5              | 48.8               | 43.3            | 32               | 128              | 128             |
| 1i      | 59.1              | 55.5               | 51.1            | 64               | 64               | 128             |
| 1j      | 53.3              | 55.5               | 48.8            | 128              | 128              | 128             |
| 1k      | 53.3              | 58.8               | 55.5            | 128              | 64               | 128             |
| 1l      | 54.4              | 49.7               | 51.1            | 128              | 128              | 128             |
| 1m      | 60.7              | 59.2               | 55.5            | 64               | 64               | 64              |
| 1n      | 54.4              | 58.8               | 52.2            | 128              | 64               | 128             |
| 1o      | 51.1              | 53.3               | 47.7            | 128              | 128              | 128             |
| 1p      | 54.4              | 57.7               | 55.5            | 64               | 64               | 64              |
| Fluconazole | 81.1            | 77.7               | 78.8            | 12.5             | 6.25             | 6.25            |

Colorless solid; mp 185-187 °C; IR (KBr) $\nu_{\text{max}}$/cm$^{-1}$ 3227, 2962, 2173, 1646, 1221; $^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ 7.68 (d, 2H, J 9.52, Ar-H), 7.22-7.29 (m, 6H, Ar-H), 7.17 (d, 1H, J 8.8, Ar-H), 7.04-7.07 (dd, 1H, J 6.6 and 2.2, Ar-H).
5.53 (s, 1H, CH), 4.66-4.68 (dd, 2H, J 2.2, OCH₂), 2.58 (s, 2H, CH₃), 2.45-2.46 (t, 1H, J 2.2, CH₂), 2.20 and 2.31 (AB system, 2H, J 16.1 and 12.48, CH₂=CHCO), 1.11 (s, 3H, CH₃), 0.95 (s, 3H, CH₂); ¹³C NMR (CDCl₃, 100 MHz): δ 196.87, 163.97, 156.42, 148.30, 143.63, 132.43, 131.32, 130.30, 129.08, 128.75, 127.09, 120.06, 117.56, 116.11, 114.88, 113.60, 104.02, 78.01, 75.97, 55.76, 50.83, 41.36, 34.36, 32.22, 29.23, 27.16; HRMS (ESI) m/z, calcd. for C₁₉H₁₈BrO₂: 346.0904, found: 347.0912; Anal. calcd. for C₁₉H₁₈BrO₂⋅C₂H₅OH: C 69.00, H 4.76, found: C 69.07, H 4.80.

Procedure for synthesis of 2-((1-(7-chloroquinolin-4-yl)-1H-1,2,3-triazol-4-yl) methoxy)-12-(4-bromophenyl)-9,9-dimethyl-8,9,10,12-tetrahydrobenzo[a]xanthen-11-one (1a) from 3

A mixture of 12-(4-bromophenyl)-9,9-dimethyl-2-(prop-2-ynyloxy)-9,10,11,12-tetrahydrobenzo[a]xanthene (1 mmol), 4-azido-7-chloroquinoline (1.0 mmol), CuSO₄·5H₂O (10 mol%), sodium ascorbate (20 mol%) and PEG 400 (5 mL) was placed in a 50 mL round-bottomed flask. The reaction mixture was stirred at 80 °C for 45 min. The progress of the reaction was monitored by TLC using ethyl acetate: petroleum ether (30:70, v/v) as eluent. After completion of the reaction as indicated by TLC, water (5 mL) was added to the reaction mixture. The precipitate formed was collected by filtration at pump and washed with water. The product so obtained, was recrystallized from ethanol to yield pure product 1a in 89% yield.

Spectral data for compounds 1a-1p

2-((1-(7-chloroquinolin-4-yl)-1H-1,2,3-triazol-4-yl) methoxy)-12-(4-bromophenyl)-9,9-dimethyl-8,9,10,12-tetrahydrobenzo[a]xanthen-11-one (1a)

Colorless solid; mp 172-176 °C; IR (KBr) v/cm⁻¹ 2957, 1641, 1598, 1375, 1220, 1208; ¹H NMR (CDCl₃, 400 MHz): δ 9.03 (d, 1H, J 4.4, Ar-H), 8.22 (d, 1H, J 2.2, Ar-H), 7.88 (s, 1H, triazole-H), 7.86 (d, 1H, J 9.52, Ar-H), 7.72 (d, 1H, J 1.48, Ar-H), 7.70 (d, 1H, J 1.48 Hz, Ar-H), 7.45 (d, 1H, J 5.12, Ar-H), 7.32 (s, 1H, Ar-H), 7.21 (m, 6H, Ar-H), 7.12-7.09 (m, 1H, Ar-H), 5.54 (s, 1H, CH), 5.41 and 5.30 (AB system, 2H, J 12.44, OCH₂), 2.54 (s, 2H, CH₂), 2.27 and 2.24 (AB system, 2H, J 16.12, CH₂=CHCO), 1.10 (s, 3H, CH₃), 0.94 (s, 3H, CH₂); ¹³C NMR (CDCl₃, 100 MHz): δ 196.85, 164.02, 156.86, 151.40, 150.14, 149.56, 148.43, 144.57, 143.60, 140.76, 136.89, 132.49, 131.37, 130.34, 130.18, 129.48, 128.95, 128.80, 127.09, 124.52, 124.46, 120.55, 120.17, 117.45, 116.10, 115.03, 113.60, 103.97, 61.69, 50.77, 41.34, 34.57, 32.24, 29.26, 27.13; HRMS (ESI) m/z, calcd. for C₁₉H₁₈BrCIN₃O₃⋅[M+H]⁺: 693.0106, found: 694.0723, 665.0033 [M+H- N₂]⁺; Anal. calcd. for C₁₉H₁₈BrCIN₃O₃: C 64.22, H 4.08, N 8.10, found: C 64.25, H 4.11, N 8.16.

2-((1-(7-chloroquinolin-4-yl)-1H-1,2,3-triazol-4-yl) methoxy)-12-(4-fluorophenyl)-9,9-dimethyl-8,9,10,12-tetrahydrobenzo[a]xanthen-11-one (1b)
2-((1-(7-chloroquinolin-4-yl)-1H-1,2,3-triazol-4-yl)methoxy)-12-(3,4-dimethoxyphenyl)-9,9-dimethyl-8,9,10,12-tetrahydrobenzo[a]xanthen-11-one (1d)

Colorless solid; mp 211-214 °C; IR (KBr) ν/cm⁻¹ 2958, 1653, 1641, 1534, 1375; ¹H NMR (CDCl₃, 400 MHz): δ 9.01 (d, 1H, J 4.4, Ar-H), 8.21 (d, 1H, J 1.48, Ar-H), 7.81 (d, 1H, J 8.76, Ar-H), 7.78 (s, 1H, triazole-H), 7.70-7.67 (m, 2H, Ar-H), 7.50-7.47 (m, 2H, Ar-H), 7.40-7.39 (m, 2H, Ar-H), 7.21-7.18 (m, 2H, Ar-H), 7.09-7.06 (m, 1H, Ar-H), 6.59 (d, 2H, J 8.8, Ar-H), 5.52 (s, 1H, CH), 5.43 and 5.33 (AB system, 2H, J 12.44, OCH₃), 2.53 (s, 2H, CH₂), 2.28 and 2.23 (AB system, 2H, J 16.12, CH₂H₂CO), 1.09 (s, 3H, CH₃), 0.93 (s, 3H, CH₃), 1.09 (s, 3H, CH₃), 0.94 (s, 3H, CH₃); ¹³C NMR (CDCl₃, 100 MHz) δ 197.40, 163.60, 157.73, 156.63, 151.36, 148.46, 144.58, 140.72, 136.95, 135.88, 132.65, 130.08, 129.45, 129.37, 128.84, 128.24, 127.09, 124.53, 124.45, 120.55, 117.44, 116.74, 116.15, 115.05, 114.23, 113.52, 104.15, 61.22, 54.82, 50.86, 41.11, 33.10, 32.04, 29.05, 27.08; HRMS (ESI) m/z, calcd. for C₇₁H₇₈ClO₃N₅O₂ [M+H⁺]: 627.2158, found 599.2058 [M+H⁺ - N₂]⁺; Anal. calcd. for C₇₁H₇₆ClO₃N₅O₂: C 72.78, H 4.98, N 8.93, found: C 72.85, H 4.92, N 8.89.

2-((1-(7-chloroquinolin-4-yl)-1H-1,2,3-triazol-4-yl)methoxy)-12-(3-nitrophenyl)-9,9-dimethyl-8,9,10,12-tetrahydrobenzo[a]xanthen-11-one (1g)

Colorless solid; mp 191-195 °C; IR (KBr) ν/cm⁻¹ 2957, 1645, 1602, 1561, 1374; ¹H NMR (CDCl₃, 400 MHz) δ 9.06 (d, 1H, J 4.4, Ar-H), 8.24 (d, 1H, J 2.2, Ar-H), 8.17 (d, J = 2.2 Hz, 1H, Ar-H), 8.05 (s, 1H, triazole-H), 7.94 (d, 1H, J 9.52, Ar-H), 7.89-7.82 (m, 2H, Ar-H), 7.76-7.73 (m, 2H, Ar-H), 7.59-7.56 (dd, 1H, J 2.2 and 7.32, Ar-H), 7.48 (d, 1H, J 4.4, Ar-H), 7.38-7.34 (m, 2H, Ar-H), 7.26-7.25 (m, 1H, Ar-H), 7.16-7.13 (m, 1H, Ar-H), 5.72 (s, 1H, CH), 5.42 and 5.28 (AB system, 2H, J 12.44, OCH₃), 2.61 (s, 2H, CH₂), 2.31 and 2.26 (AB system, 2H, J 16.84, CH₂H₂CO), 1.13 (s, 3H, CH₃), 0.96 (s, 3H, CH₃); ¹³C NMR (CDCl₃, 100 MHz) δ 196.83, 164.58, 157.13, 151.36, 150.16, 148.44, 148.35, 146.64, 144.39, 140.78, 136.97, 134.78, 132.29, 130.51, 129.54, 129.31, 129.17, 129.88, 127.15, 124.63, 124.51, 123.24, 121.61, 120.53, 117.40, 116.06, 115.19, 115.15, 113.02, 103.70, 61.62, 50.70, 41.33, 35.08, 32.31, 29.22, 27.15; HRMS (ESI) m/z, calcd. for C₇₁H₇₈ClO₃N₅O₂ [M+H⁺]: 658.1852, found 630.1801 [M+H⁺ - N₂]⁺; Anal. calcd. for C₇₁H₇₆ClO₃N₅O₂: C 67.53, H 4.29, N 10.64, found: C 67.60, H 4.30, N 10.69.

2-((1-(7-chloroquinolin-4-yl)-1H-1,2,3-triazol-4-yl)methoxy)-12-(3-phenyl)-9,9-dimethyl-8,9,10,12-tetrahydrobenzo[a]xanthen-11-one (1h)

Colorless solid; mp 210-213 °C; IR (KBr) ν/cm⁻¹ 2956, 1648, 1618, 1513, 1374; ¹H NMR (CDCl₃, 400 MHz) δ 9.01 (d, 1H, J 4.4, Ar-H), 8.22 (d, 1H, J 2.2, Ar-H), 7.81 (d, 1H, J 8.8, Ar-H), 7.76 (s, 1H, triazole-H), 7.69 (d, 2H, J 8.8, Ar-H), 7.52-7.49 (m, 1H, Ar-H), 7.41-7.40 (m, 2H, Ar-H), 7.31 (d, 2H, J 7.32, Ar-H), 7.20 (d, 1H, J 8.8, Ar-H), 7.09-7.02 (m, 3H, Ar-H), 6.85-6.82 (m, 1H, Ar-H),
5.58 (s, 1H, CH), 5.42 and 5.28 (AB system, 2H, J 12.44, OCH$_3$), 2.54 (s, 2H, CH$_2$), 2.30 and 2.19 (AB system, 2H, J 16.84, CH$_3$H$_2$CO), 1.09 (s, 3H, CH$_3$), 0.92 (s, 3H, CH$_3$); $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$ 196.10, 163.86, 160.23, 156.75, 151.11, 150.20, 148.96, 144.56, 132.56, 131.25, 130.26, 129.49, 128.92, 128.55, 128.42, 128.26, 127.13, 126.23, 124.53, 120.65, 119.36, 117.55, 116.72, 116.11, 114.10, 112.90, 111.20, 103.40, 61.50, 50.84, 41.39, 35.03, 32.25, 29.42, 27.13; HRMS (ESI) m/z, calcld. for C$_{37}$H$_{40}$ClN$_{2}$O$_{2}$ [M+H$^+$]: 663.2158, found: 635.2056 [M+H- N$_2$]$^+$; Anal. calcld. for C$_{37}$H$_{40}$ClN$_{2}$O$_{2}$: C 74.26, H 4.71, N 8.45, found: C 74.35, H 4.75, N 8.50.

2-((1-(7-chloroquinolin-4-yl)-1H-1,2,3-triazole-4-yl)methoxy)-12-(4-methylphenyl)-8,9,10,12-tetrahydrobenzo[a]xanthene-11-one (1f)

Colorless solid; mp 210-214 °C; IR (KBr) v/cm$^{-1}$: 2945, 1639, 1619, 1560, 1376; $^{1}H$ NMR (CDCl$_3$, 400 MHz): $\delta$ 9.03 (s, 1H, J 5.12, Ar-H), 8.2 (s, 1H, J 1.08, Ar-H), 7.74 (d, 2H, J 10.28, Ar-H), 7.70 (s, 1H, triazole-H), 7.67 (s, 1H, Ar-H), 7.47-7.44 (m, 1H, Ar-H), 7.42-7.39 (m, 2H, Ar-H), 7.24-7.19 (m, 3H, Ar-H), 7.05-7.08 (m, 1H, Ar-H), 6.86 (d, 2H, J 8.04, Ar-H), 5.56 (s, 1H, CH), 5.40 and 5.30 (AB system, 2H, J 12.44, OCH$_3$), 2.74-2.59 (m, 2H, CH$_2$), 2.44-2.30 (m, 2H, CH$_2$), 2.15 (s, 3H, CH$_3$), 2.06-1.91 (m, 2H, CH$_2$); $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$ 197.11, 165.43, 156.53, 151.33, 150.11, 148.40, 144.49, 142.02, 140.79, 136.91, 135.81, 132.62, 130.21, 129.47, 129.02, 128.90, 128.43, 128.35, 127.09, 124.64, 124.48, 120.55, 117.53, 116.94, 116.07, 115.50, 114.99, 104.24, 61.53, 36.98, 34.59, 27.68, 20.80, 20.13; HRMS (ESI) m/z, calcld. for C$_{38}$H$_{35}$ClN$_{2}$O$_{2}$ [M+H$^+$]: 599.1845, found: 571.1796 [M+H- N$_2$]$^+$; Anal. calcld. for C$_{38}$H$_{35}$ClN$_{2}$O$_{2}$: C 72.18, H 4.54, N 9.35, found: C 72.28, H 4.60, N 9.38.

2-((1-(7-chloroquinolin-4-yl)-1H-1,2,3-triazole-4-yl)methoxy)-12-(naphthalen-1-yl)-8,9,10,12-tetrahydrobenzo[a]xanthene-11-one (1f)

Colorless solid; mp 181-185 °C; IR (KBr) v/cm$^{-1}$: 2943, 1647, 1617, 1565, 1375; $^{1}H$ NMR (CDCl$_3$, 400 MHz): $\delta$ 8.90 (d, 1H, J 4.4, Ar-H), 8.19 (d, 1H, J 2.2, Ar-H), 7.71 (s, 1H, triazole-H), 7.68 (d, 1H, J 8.8, Ar-H), 7.59 (d, 1H, J 8.04, Ar-H), 7.55 (d, 1H, J 8.04, Ar-H), 7.51-7.50 (m, 3H, Ar-H), 7.45-7.37 (m, 4H, Ar-H), 7.25 (d, 1H, J 8.01, Ar-H), 7.17-7.10 (m, 3H, Ar-H), 7.05-7.02 (m, 1H, Ar-H), 7.57 (s, 1H, CH), 5.43 and 5.30 (AB system, 2H, J 12.44, OCH$_3$), 2.77-2.62 (m, 2H, CH$_2$), 2.38-2.36 (m, 2H, CH$_2$), 2.05-1.87 (m, 2H, CH$_2$); $^{13}$C NMR (CDCl$_3$, 100 MHz) $\delta$ 197.04, 165.54, 156.54, 151.25, 150.01, 148.53, 144.34, 142.45, 140.51, 136.84, 133.14, 132.63, 131.91, 130.28, 129.30, 128.82, 128.67, 128.01, 127.61, 127.18, 127.12, 127.05, 126.88, 125.83, 125.43, 124.39, 124.32, 120.43, 117.74, 116.61, 115.87, 115.19, 115.04, 104.26, 61.39, 36.95, 35.18, 27.72, 20.12; HRMS (ESI) m/z, calcld. for C$_{39}$H$_{38}$ClN$_{2}$O$_{2}$ [M+H$^+$]: 635.1846, found: 607.1754 [M+H-
2-((1-(7-chloroquinolin-4-yl)-1H-1,2,3-triazol-4-yl)methoxy)-12-(4-bromophenyl)-8,9,10,12-tetrahydrobenzo[a]xanthen-11-one (1m)

Colorless solid; mp 211-215 °C; IR (KBr) ν/cm⁻¹: 2923, 1647, 1617, 1548, 1388; ¹H NMR (CDCl₃, 400 MHz): δ 9.02 (d, 1H, J 5.12, Ar-H), 8.21 (d, 1H, J 1.48, Ar-H), 7.88 (s, 1H, triazole-CH₃), 7.83 (d, J = 8.8 Hz, 2H, Ar-H), 7.70 (d, J = 8.8 Hz, 1H, Ar-H), 7.50-7.47 (m, 1H, Ar-H), 7.43 (d, 1H, J 4.4, Ar-H), 7.29 (d, 1H, J 2.2, Ar-H), 7.24-7.18 (m, 5H, Ar-H), 7.11-7.08 (m, 1H, Ar-H), 5.56 (s, 1H, CH), 5.42 and 5.29 (AB system, 2H, J 12.44, OCH), 2.73-2.59 (m, 2H CH₂), 2.45-2.31 (m, 2H, CH₂), 2.06-1.89 (m, 2H, CH₂);
¹³C NMR (CDCl₃, 100 MHz) δ 197.04, 165.69, 156.83, 151.38, 150.12, 148.41, 147.52, 144.82, 143.87, 140.73, 136.85, 132.44, 131.37, 130.31, 123.44, 128.91, 128.79, 127.07, 124.53, 124.45, 120.51, 120.17, 117.41, 116.07, 114.94, 114.48, 103.99, 61.64, 36.91, 34.49, 27.68, 20.21; HRMS (ESI) m/z, calcd. for C₃₆H₂₇BrCINO₃ [M+H⁺]: 663.0793, found: 653.0785 [M+H⁻Ni⁺]; Anal. calcd. for C₃₆H₂₇BrCINO₃: C 63.31, H 3.64, N 8.44, found: C 63.37, H 3.65, N 8.47.

12-(4-chlorophenyl)-9,9-dimethyl-2-((1-phenyl-1H-1,2,3-triazol-4-yl)methoxy)-8,9,10,12-dihydro-8-H-benzo[a]xanthen-11-one (1p)

Colorless solid; mp 185-187 °C; IR (KBr) ν/cm⁻¹: 2922, 1646, 1618, 1560, 1198; ¹H NMR (CDCl₃, 400 MHz): δ 7.93 (s, 1H, triazole-H), 7.67-7.70 (m, 3H, Ar-H), 7.50 (t, 2H, J 7.32, Ar-H), 7.42 (d, 1H, J 7.36Hz, Ar-H), 7.20-7.31 (m, 4H, Ar-H), 7.18 (d, 1H, J 8.8, Ar-H), 7.08 - 7.11 (m, 3H, Ar-H), 5.55 (s, 1H, CH), 5.22 and 5.33 (AB system, 2H, J 12.44, OCH₂), 2.27 and 2.23 (AB system, 2H, J 16.12, CH₂CH₂CO), 1.10 (s, 3H, CH₃), 0.94 (s, 3H, CH₃); ¹³C NMR (CDCl₃, 100 MHz) δ 196.87, 163.93, 157.10, 148.35, 144.42, 143.09, 137.01, 132.55, 131.98, 130.20, 129.90, 129.76, 128.85, 128.74, 128.42, 127.04, 120.89, 120.62, 117.37, 116.20, 114.84, 113.75, 103.83, 61.89, 50.80, 41.35, 34.48, 32.32, 29.66, 27.11; HRMS (ESI) m/z, calcd. for C₃₆H₃₅BrCINO₃ [M+H⁺]: 585.1688, found: 557.1685 [M+H⁻Ni⁺]; Anal. calcd. for C₃₆H₃₅BrCINO₃: C 71.85, H 4.31, N 9.58, found: C 71.92, H 4.34, N 9.60.

Experimental procedure for antibacterial activity

The antibacterial activity of all compounds was evaluated by the agar well diffusion method. All the microbial cultures were adjusted to 0.5 McFarland standard, which is visually comparable to a microbial suspension of approximately 1.5 × 10⁶ cfu mL⁻¹. 20 mL of Mueller Hinton agar medium was poured into each Petri plate and plates were swabbed with 100 µL inocula of the test microbial cultures were adjusted to 0.5 McFarland standard, and plates were loaded with a 100 µL volume with concentration of 2.0 mg mL⁻¹ of each compound reconstituted in dimethyl sulphoxide (DMSO).
All the plates were incubated at 37 °C for 24 h. Antibacterial activity of each compound was evaluated by measuring the zone of growth inhibition against the test organisms with zone reader (HiAntibiotic zone scale). DMSO was used as a negative control whereas ciprofloxacin was used as positive control. This procedure was performed in three replicate plates for each organism and the mean values of the diameter of inhibition zones ± standard deviations were calculated.

Determination of minimum inhibitory concentration (MIC) of chemical compounds

MIC of the all compounds against bacterial and yeast strains was determined using macrodilution tube method as recommended by the National Committee for Clinical Laboratory Standards (NCCLS).31 In this method, various test concentrations of newly synthesized compounds were prepared from 128 to 0.25 µg mL⁻¹ in sterile tubes No. 1-10. 100 µL sterile Mueller Hinton Broth (MHB) was poured in each sterile tube followed by the addition of 200 µL test compound in tube 1. Two fold serial dilutions were carried out from tube 1 to tube 12 and excess broth (100 µL) was discarded from the last tube No. 10. To each tube, 100 µL of standard inoculum (1.5 × 10⁸ cfu mL⁻¹) was added. Turbidity was observed after incubating the inoculated tubes at 37 °C for 24 h. Ciprofloxacin was used as positive control while DMSO was used as negative control.

Procedure for minimum bactericidal concentration (MBC)

MBC is the lowest concentration of antimicrobial compound that will prevent the growth of an organism after subculture on to antibiotic free media. MBCs were determined by spreading the 100 µL compound from one below MIC and MIC itself. All the tubes were incubated for 24 h at 37 °C. The growth was observed on each plate.32

Experimental procedure for antifungal activity

The antifungal activity all compounds was evaluated by poisoned food technique.33 The molds were grown on Sabouraud dextrose agar (SDA) at 25 °C for 7 days and used as inocula. The 15 mL of molten SDA (45 °C) was poisoned by the addition of 100 µL volume of each compound reconstituted in the DMSO, poured into a sterile Petri plate and allowed it to solidify at room temperature. The solidified poisoned agar plates were inoculated at the center with fungal plugs (8 mm diameter) obtained from the colony margins and incubated at 25 °C for 7 days. DMSO was used as the negative control whereas fluconazole was used as the positive control. The experiments were performed in triplicates. Diameter of fungal colonies was measured and expressed as percent mycelial inhibition.

Percent inhibition of mycelial growth = (dc – dt) / dc × 100

where dc = average diameter of fungal colony in negative control sets; dt = average diameter fungal colony in experimental sets.

MIC of all compounds was determined by the macrodilution broth method. A two fold serial dilution of compounds was prepared in Sabouraud dextrose broth to achieve a decreasing concentration range of 512 to 1 µg mL⁻¹ in sterile test tubes. Each dilution was seeded with 100 µL of the standardized fungal inoculums (2 × 10⁵ spores mL⁻¹). The inoculated culture tubes were incubated at 25 °C for 7 days. A set of tubes containing only broth was kept as control. After incubation, tubes were examined for changes in turbidity as an indicator of growth. The lowest concentration that did not permit any visible growth of a mold was considered as MIC of that compound.

Docking protocol

The automated docking studies were carried out using Auto Dock version 4.0.29 First, AutoGrid component of the program precalculates a three-dimensional grid of interaction energies based on the macromolecular target using the AMBER force field. Then automated docking studies were carried out to evaluate the binding free energy of the inhibitors within the macromolecules. The three-dimensional structures of the aforementioned compounds were constructed using Chem. 3D ultra 11.0 software [Chemical Structure Drawing Standard; Cambridge Soft corporation, USA (2009)], then they were energetically minimized by using MOPAC with 100 interations and minimum RMS gradient of 0.10. The Gasteiger-Hückel charges of ligands were assigned. The crystal structures of topoisomerase II DNA gyrase (PDB code: 2XCT) complex were retrieved from the RCSB protein data bank (http://www.rcsb.org/pdb/home/home.do). All bound waters and ligands were eliminated from the protein and the polar hydrogens and the Kollman-united charges were added to the proteins. AutoGrid component of the auto dock program pre-calculates a three-dimensional grid of interaction energies based on the macromolecular target using the AMBER force field. The cubic grid box of size 28 Å, 22 Å, 42 Å along x, y, z directions, respectively with a spacing of 0.375 Å and grid maps were created representing the catalytic active target site region where
the native ligand was embedded. Then automated docking studies were carried out to evaluate the binding free energy of the inhibitors within the macromolecules. The GALS search algorithm (genetic algorithm with local search) was chosen to search for the best conformers. The default parameters were set using the software ADT on PC, which is associated with Auto-Dock 4.0. Results differing by less than 0.5 Å in positional root-mean-square deviation (RMSD) were clustered together and the results of the most favorable free energy of binding were selected as the resultant complex structures.

**Supplementary material**

Copies of 1H, 13C NMR spectra of all compounds and crystallographic information file (CIF) of compound 1d and 1p can be found as supplementary information, free of charge at http://jpcs.sbq.org.br as PDF file. Crystallographic data (excluding structure factors) for the structure have been deposited with the Cambridge Crystallographic Data Center with CCDC No. 962611 (compound 1d), and 962663 (compound 1p). These data can be obtained free of charge from the CCDC via www.ccdc.cam.ac.uk/data_request/cif.

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Efficient One Pot Synthesis of Xanthene-Triazole-Quinoline/Phenyl Conjugates and Evaluation of their Antimicrobial Activity

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Figure S1. $^1$H NMR spectrum (CDCl$_3$, 400 MHz) of 1a.

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Figure S2. $^{13}$C NMR spectrum (CDCl$_3$, 100 MHz) of 1a.

Figure S3. $^1$H NMR spectrum (CDCl$_3$, 400 MHz) of 1b.
Figure S4. $^{13}$C NMR spectrum (CDCl$_3$, 100 MHz) of 1b.

Figure S5. $^1$H NMR spectrum (CDCl$_3$, 400 MHz) of 1c.
Figure S6. $^{13}$C NMR spectrum (CDCl$_3$, 100 MHz) of 1c.

Figure S7. $^1$H NMR spectrum (CDCl$_3$, 400 MHz) of 1d.
Figure S8. $^{13}$C NMR spectrum (CDCl$_3$, 100 MHz) of 1d.

Figure S9. $^1$H NMR spectrum (CDCl$_3$, 400 MHz) of 1e.
Figure S10. $^{13}$C NMR spectrum (CDCl$_3$, 100 MHz) of 1e.

Figure S11. $^1$H NMR spectrum (CDCl$_3$, 400 MHz) of 1f.
Figure S12. $^{13}$C NMR spectrum (CDCl$_3$, 100 MHz) of $\text{1f}$.

Figure S13. $^1$H NMR spectrum (CDCl$_3$, 400 MHz) of $\text{1g}$.
Figure S14. $^{13}$C NMR spectrum (CDCl$_3$, 100 MHz) of $1g$.

Figure S15. $^1$H NMR spectrum (CDCl$_3$, 400 MHz) of $1h$. 

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Figure S16. $^1$C NMR spectrum (CDCl$_3$, 100 MHz) of 1h.

Figure S17. $^1$H NMR spectrum (CDCl$_3$, 400 MHz) of 1h.
Figure S18. $^{13}$C NMR spectrum (CDCl$_3$, 100 MHz) of 1i.

Figure S19. $^1$H NMR spectrum (CDCl$_3$, 400 MHz) of 1j.
Figure S20. $^{13}$C NMR spectrum (CDCl$_3$, 100 MHz) of 1j.

Figure S21. $^1$H NMR spectrum (CDCl$_3$, 400 MHz) of 1k.
Figure S22. $^{13}$C NMR spectrum (CDCl$_3$, 100 MHz) of 1k.

Figure S23. $^1$H NMR spectrum (CDCl$_3$, 400 MHz) of II.
Figure S24. $^1$C NMR spectrum (CDCl$_3$, 100 MHz) of 1l.

Figure S25. $^1$H NMR spectrum (CDCl$_3$, 400 MHz) of 1m.
Figure S26. $^{13}$C NMR spectrum (CDCl$_3$, 100 MHz) of 1m.

Figure S27. $^1$H NMR spectrum (CDCl$_3$, 400 MHz) of 1n.
Figure S28. $^{13}$C NMR spectrum (CDCl$_3$, 100 MHz) of In.

Figure S29. $^1$H NMR spectrum (CDCl$_3$, 400 MHz) of 1o.
Figure S30. $^{13}$C NMR spectrum (CDCl$_3$, 100 MHz) of 1o.

Figure S31. $^1$H NMR spectrum (CDCl$_3$, 400 MHz) of 1p.
Figure S32. $^{13}$C NMR spectrum (CDCl$_3$, 100 MHz) of 1p.