

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 antibacterial e antifungal *in vitro*. A atividade antimicrobial foi avaliada confrontando nove cepas microbiais. Todos os compostos apresentaram boas atividades Gram positivas antibacterial e antifúngicas. Um dos compostos apresentou a melhor atividade antibacterial 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-allergic,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 possess 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

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on combining two or more different bioactive moieties in a single molecule to get the corresponding conjugate/hybrid molecules.¹⁹ 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.^{20,21} 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.²² As a part of our continued research for new antimicrobial agents by multicomponent reactions,²³ 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]xanthene-11-one, propargyl bromide and 4-azido-7-chloroquinoline/phenyl azide.

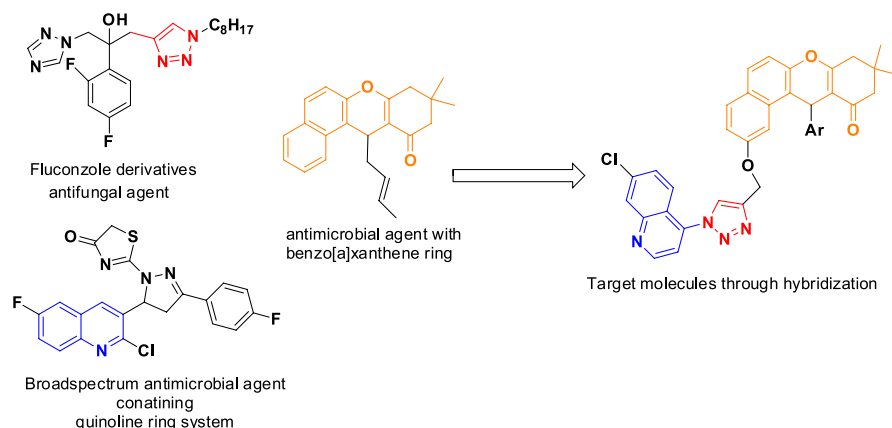
We describe herein an efficient one pot three-component synthesis of novel xanthene-triazole-quinoline conjugates namely, 2-((1-(7-chloroquinolin-4-yl)-1*H*-1,2,3-triazol-4-yl) methoxy)-12-aryl-8,9,10,12-tetrahydrobenzo[a]xanthene-11-one (**1a-1o**) and xanthene-triazole-phenyl conjugate namely, 9,9-dimethyl-12-phenyl-2-((1-phenyl-1*H*-1,2,3-triazol-4-yl) methoxy)-8,9,10,12-tetrahydrobenzo[a]xanthene-11-one (**1p**) using various 12-aryl-2-hydroxy-8,9,10,12-tetrahydrobenzo[a]xanthene-11-one derivatives, propargyl bromide and 4-azido-7-chloroquinoline/phenyl azide in the presence of 10 mol% CuSO₄·5H₂O and 20 mol% sodium ascorbate in polyethyleneglycol 400 (PEG-400) at 80 °C. All the novel compounds were evaluated for

in vitro antibacterial and antifungal activity. Compound **1a** showed the highest antibacterial and antifungal activity and therefore its mode of binding at the active site of topoisomerase II DNA gyrase B has been investigated.

Result and discussion

Chemistry

Initially, the starting 12-aryl-2-hydroxy-8,9,10,12-tetrahydrobenzo[a]xanthene-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 *p*TSA in refluxing ethanol.²⁴ 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.²⁵ We designed the synthesis of xanthene-triazole-quinoline conjugates via multicomponent reaction of 12-aryl-2-hydroxy-8,9,10,12-tetrahydrobenzo[a]xanthene-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]xanthene-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)-1*H*-1,2,3-triazol-4-yl)methyleneoxy)-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 was also attempted using KOH as a base in place of K₂CO₃, but the reaction required longer reaction time and gave inferior yield of the product (entry 10, 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]xanthene-11-ones

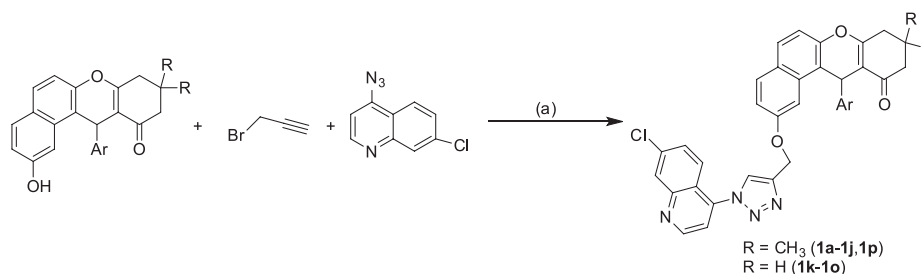
were carried out with propargyl bromide and 4-azido-7-chloroquinoline under above optimized conditions. All the reactions proceeded smoothly and were complete in 40 min, yielding corresponding xanthene-triazole-quinoline conjugates (**1b-1o**) in high yields (entries 2- 15, Table 2) (Scheme 2). The scope 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 dimerone 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^a

S. No.	Solvent	Base	Temperature / °C	time / min	Yield / %
1	Water	K ₂ CO ₃	80	80	68 ^b
2	Ethanol	K ₂ CO ₃	80	80	45 ^b
3	DMF	K ₂ CO ₃	80	65	85
4	<i>t</i> -BuOH-water (1:1)	K ₂ CO ₃	80	55	87
5	PEG-600	K ₂ CO ₃	80	40	84
6	CH ₂ Cl ₂ /H ₂ O (1:1)	K ₂ CO ₃	80	45	82
7	PEG-400	K ₂ CO ₃	80	35	92
8	PEG-400	K ₂ CO ₃	100	35	91
9	PEG-400	K ₂ CO ₃	60	80	70 ^b
10	PEG-400	KOH	80	70	84
11	PEG-400	K ₂ CO ₃	80	80	75 ^{b,c}

^aReactions 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%); ^bincomplete reaction; ^creaction 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-one 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 ^a	time / min	Yield / %
1	4-BrC ₆ H ₄	CH ₃	1a	7.80	35	92
2	4-FC ₆ H ₄	CH ₃	1b	7.13	30	90
3	4-NO ₂ C ₆ H ₄	CH ₃	1c	7.27	30	91
4	4-CH ₃ O C ₆ H ₄	CH ₃	1d	6.84	35	88
5	3,4-(CH ₃ O) ₂ C ₆ H ₃	CH ₃	1e	6.72	30	90
6	4-CH ₃ C ₆ H ₄	CH ₃	1f	7.46	30	85
7	3-NO ₂ C ₆ H ₄	CH ₃	1g	7.16	25	93
8	C ₆ H ₅	CH ₃	1h	6.97	35	89
9	4-ClC ₆ H ₄	CH ₃	1i	7.53	30	84
10	1-naphthyl	CH ₃	1j	7.97	40	87
11	4-CH ₃ C ₆ H ₄	H	1k	6.96	30	91
12	1-naphthyl	H	1l	7.17	35	85
13	4-BrC ₆ H ₄	H	1m	7.01	25	92
14	4-(CH ₃) ₂ CHC ₆ H ₄	H	1n	7.40	35	90
15	C ₆ H ₅	H	1o	6.81	35	84
16	4-ClC ₆ H ₄ ^b	CH ₃	1p	6.99	50	88

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

ring appeared at δ 5.54 as singlet. The two aromatic protons of the chloroquinoline ring containing nitrogen were observed as two distinct doublets, each integrating one proton at δ 9.03 and at δ 8.22. The one proton of triazole ring appeared at δ 7.88 as singlet. The rest of the 12 aromatic protons were seen in the range of δ 7.86-7.09. In the ¹³C NMR spectrum of **1a** signal at 196.85 accounted for the carbonyl group, while the two methylene carbons, i.e., CH₂CMe₂ and CH₂C=O were observed at δ 50.77 and δ 41.34, respectively. The signal due to methylene group of linker (OCH₂) was observed at δ 61.69. Signal for methine carbon of benzo[a]xanthene ring was observed at δ 34.57, while the two methyl carbons and the quaternary carbon bearing two methyl groups were observed at δ 27.13, 29.26 and 32.24, respectively. The rest of the carbons were seen in the range of δ 164.02-103.97. Electron spray ionization-high resolution mass spectrometry (ESI-HRMS) of compound **1a**, displayed m/z = 693.0106 [M+H]⁺ corresponding to its molecular formula C₃₈H₃₁ClN₄O₄. The structure of compound **1d** and **1p** was further confirmed by single crystal X-ray diffraction. Ortep diagrams of **1d** and **1p** are shown in Figure 1. The crystallographic data collection and structure refinement details for compound **1d** and **1p** are summarized in Table 3 and Table 4, respectively.

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]xanthene-11-one with propargyl bromide in presence of K₂CO₃ resulted in formation of propargylated-benzo[a]xanthene 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(I) by reaction of CuSO₄·5H₂O with sodium ascorbate,²⁶ 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]xanthene-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]xanthene-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

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

Identification code	shelxl
Empirical formula	C ₃₈ H ₃₁ ClN ₄ O ₄
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) Å ³
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 mm ³
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 F ²
Data/restraints/parameters	5500/0/424
Goodness-of-fit on F ²	1.001
Final R indices [I > 2sigma(I)]	R ₁ = 0.0689, wR ₂ = 0.0870
R indices (all data)	R ₁ = 0.1632, wR ₂ = 0.1184
Largest diff. peak and hole	0.210 and -0.228 e.Å ⁻³

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.

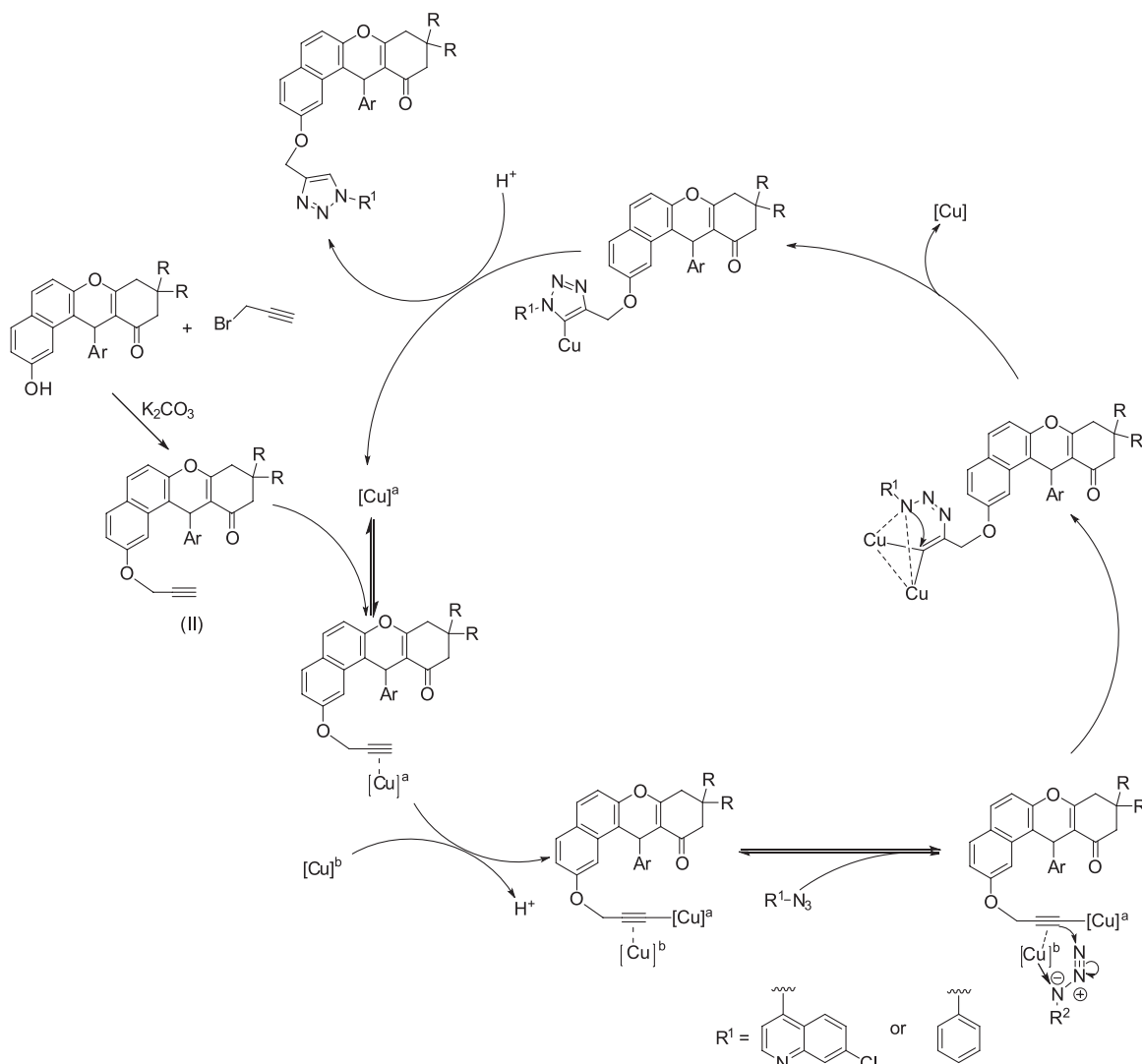
All xanthene-triazole-quinoline/phenyl conjugates (**1a-1p**) showed good antibacterial activity, against Gram positive (*Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus cereus*) bacteria. However, none of the compounds

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

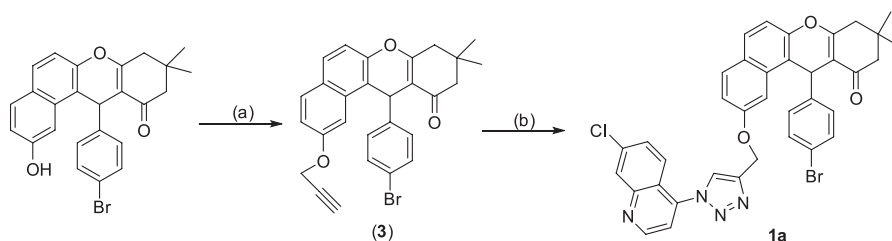
Identification code	shelxl
Empirical formula	C ₃₄ H ₂₈ ClN ₃ O ₃
Formula weight	562.90
Temperature	293(2) K
Wavelength	0.71073 Å
Crystal system	Triclinic
Space group	P-1
Unit cell dimensions	a = 10.3161(6) Å α = 74.275(5)° b = 11.3199(7) Å β = 85.032(5)° c = 12.9621(6) Å γ = 76.875(5)°
Volume	1418.46(14) Å ³
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 mm ³
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 F ²
Data/restraints/parameters	5558/0/372
Goodness-of-fit on F ²	1.046
Final R indices [I > 2sigma(I)]	R ₁ = 0.0393, wR ₂ = 0.0913
R indices (all data)	R ₁ = 0.0448, wR ₂ = 0.0943
Largest diff. peak and hole	0.232 and -0.355 e.Å ⁻³

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*, 22.6 mm against *Bacillus subtilis* bacteria 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 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



Scheme 3. Plausible reaction mechanism for synthesis of xanthene-triazole-quinoline conjugates.



Scheme 4. Sequential synthesis of **1a**. Reagents and conditions: (a) PEG-400, K_2CO_3 (1 eq.), $80^\circ C$; (b) PEG-400, 4-azido-7-chloroquinoline (1.0 eq.), $CuSO_4 \cdot 5H_2O$ (10 mol%), sodium ascorbate (15 mol%), $80^\circ C$.

16-128 $\mu g\ mL^{-1}$ and MBC in the range of 32-256 $\mu g\ mL^{-1}$ 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 $\mu g\ mL^{-1}$ against *Staphylococcus aureus*, *Bacillus subtilis* and 32 $\mu g\ mL^{-1}$

against *Bacillus cereus*. Compound **1a** showed MBC of 32 $\mu g\ mL^{-1}$ 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

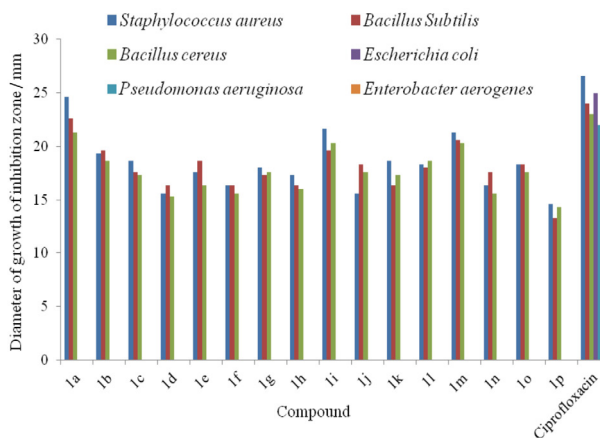


Figure 2. Graphical representation of diameter of growth of inhibition zone (mm) of all compounds (**1a-1p**).

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 $64 \mu\text{g mL}^{-1}$ against *Staphylococcus aureus*, while compound **1g** with nitro group at *meta* position showed MIC $128 \mu\text{g mL}^{-1}$ 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 hydrossolubility) 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.²⁷ 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.²⁸ 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.²⁹ 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.²⁸

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 $-5.86 \text{ kcal mol}^{-1}$. 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 / ($\mu\text{g mL}^{-1}$)			MBC / ($\mu\text{g mL}^{-1}$)		
	<i>Staphylococcus aureus</i>	<i>Bacillus subtilis</i>	<i>Bacillus cereus</i>	<i>Staphylococcus aureus</i>	<i>Bacillus subtilis</i>	<i>Bacillus cereus</i>
1a	16	16	32	32	32	32
1b	64	64	64	64	128	128
1c	64	128	128	128	128	256
1d	128	128	128	128	256	256
1e	128	64	128	256	128	256
1f	128	64	128	256	128	128
1g	128	128	128	128	256	128
1h	128	128	128	256	128	256
1i	32	32	64	64	64	64
1j	128	128	128	128	256	256
1k	128	128	128	256	128	256
1l	64	128	64	64	256	128
1m	32	64	64	64	64	128
1n	128	64	128	128	128	128
1o	128	64	128	256	64	256
1p	128	128	128	256	128	256
Ciprofloxacin	6.25	6.25	6.25	6.25	6.25	6.25

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

Product	Mycelial growth inhibition / %			MIC / ($\mu\text{g mL}^{-1}$)		
	<i>Aspergillus niger</i>	<i>Aspergillus flavus</i>	<i>Penicillium sp.</i>	<i>Aspergillus niger</i>	<i>Aspergillus flavus</i>	<i>Penicillium sp.</i>
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	128	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

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 $\nu_{\text{max}}/\text{cm}^{-1}$. The NMR (^1H 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 δ 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 ($\lambda = 0.71073 \text{ \AA}$) at temperature 298 K.

General procedure for synthesis of 2-((1-(7-chloroquinolin-4-yl)/phenyl)-1*H*-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]xanthene-11-one (1 mmol), propargyl bromide (1.0 mmol), and 4-azido-7-chloroquinoline/phenyl azide (1.0 mmol), K_2CO_3 (1.0 mmol), $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (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_2CO_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.

Colorless solid; mp 185-187 °C; IR (KBr) $\nu_{\text{max}}/\text{cm}^{-1}$ 3227, 2962, 2173, 1646, 1221; ^1H NMR (CDCl_3 , 400 MHz): δ 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, CH₂), 2.45-2.46 (t, 1H, J 2.2, CH), 2.20 and 2.31 (AB system, 2H, J 16.1 and 12.48, CH_aH_bCO), 1.11 (s, 3H, CH₃), 0.95 (s, 3H, CH₃); ¹³C NMR (CDCl₃, 100 MHz): δ 196.87, 163.97, 156.42, 148.30, 143.63, 132.43, 131.32, 130.30, 130.08, 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 C₂₈H₂₃BrO₃ [M+H]⁺: 486.0904, found: 487.0912; Anal. calcd. for C₂₈H₂₃BrO₃: C 69.00, H 4.76, found: C 69.07, H 4.80.

Procedure for synthesis of 2-((1-(7-chloroquinolin-4-yl)-1*H*-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]xanthen-1-one (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)-1*H*-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) ν /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, CH_aH_bCO), 1.10 (s, 3H, CH₃), 0.94 (s, 3H, CH₃); 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 C₃₇H₂₈BrClN₄O₃ [M+H]⁺: 693.0106, found: 694.0723, 665.0033 [M+H- N₂]⁺; Anal. calcd. for C₃₇H₂₈BrClN₄O₃: 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)-1*H*-1,2,3-triazol-4-yl) methoxy)-12-(4-fluorophenyl)-9,9-dimethyl-8,9,10,12-tetrahydrobenzo[a]xanthen-11-one (**1b**)

Colorless solid; mp 212-216 °C; IR (KBr) ν /cm⁻¹ 2961, 1639, 1619, 1560, 1372; ¹H NMR (CDCl₃, 400 MHz): δ 9.03 (d, 1H, J 5.12, Ar-H), 8.22 (d, 1H, J 1.96, Ar-H), 7.89 (s, 1H, triazole-H), 7.86 (d, 1H, J 8.8, Ar-H), 7.71 (d, 1H, J 3.64, Ar-H), 7.69 (d, 1H, J 3.68, Ar-H), 7.54-7.51 (m, 1H, Ar-H), 7.43 (d, 1H, J 4.4, Ar-H), 7.33-7.27 (m, 3H, Ar-H), 7.20 (d, 1H, J 8.8, Ar-H), 7.11-7.08 (m, 1H, Ar-H), 6.80-6.78 (t, 2H, J 8.8, Ar-H), 5.57 (s, 1H, CH), 5.42 and 5.27 (AB system, dd, 2H, J 12.44, OCH₂), 2.54 (s, 2H, CH₂), 2.27 and 2.19 (AB system, dd, 2H, J 16.12, CH_aH_bCO), 1.10 (s, 3H, CH₃), 0.93 (s, 3H, CH₃); ¹³C NMR (CDCl₃, 100 MHz): δ 196.93, 163.89, 156.84, 151.36, 150.13, 148.46, 144.60, 140.79, 136.94, 132.53, 130.32, 129.95, 129.87, 129.51, 128.97, 128.71, 128.08, 127.12, 124.50, 124.13, 120.57, 117.42, 116.45, 115.20, 115.04, 114.99, 113.97, 103.97, 61.63, 50.81, 41.24, 34.10, 32.24, 29.13, 26.93; HRMS (ESI) m/z , calcd. for C₃₇H₂₈FCIN₄O₃ [M+H]⁺: 631.1906, found: 631.1916, 603.1823 [M+H- N₂]⁺; Anal. calcd. for C₃₇H₂₈FCIN₄O₃: C 70.42, H 4.47, N 8.88, found: C 71.48, H 4.52, N 8.92.

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

Colorless solid; mp 267-271 °C; IR (KBr) ν /cm⁻¹ 2929, 1648, 1618, 1542, 1376; ¹H NMR (CDCl₃, 400 MHz): δ 9.03 (d, 1H, J 4.4, Ar-H), 8.23 (d, 1H, J 2.2, Ar-H), 8.02 (d, 2H, Ar-H), 7.99 (s, 1H, triazole-H), 7.89 (d, 2H, J 8.8, Ar-H), 7.75-7.72 (m, 2H, Ar-H), 7.45 (d, 2H, J 4.4, Ar-H), 7.29 (d, 2H, J 2.2, Ar-H), 7.21 (s, 1H, Ar-H), 7.14-7.11 (m, 1H, Ar-H), 5.70 (s, 1H, CH), 5.39 and 5.27 (AB system, 2H, J 12.44, OCH₂), 2.57 (s, 2H, CH₂), 2.31 and 2.12 (AB system, 2H, J 16.12, CH_aH_bCO), 1.11 (s, 3H, CH₃), 0.93 (s, 3H, CH₃); ¹³C NMR (CDCl₃, 100 MHz): δ 196.72, 164.57, 157.16, 151.70, 151.36, 150.17, 148.49, 146.33, 144.49, 140.70, 136.96, 132.37, 130.48, 129.53, 129.39, 129.29, 129.02, 127.14, 124.60, 124.42, 123.65, 120.48, 117.36, 116.00, 115.22, 115.06, 112.90, 103.77, 61.75, 50.72, 41.37, 35.09, 32.26, 29.26, 27.06; HRMS (ESI) m/z , calcd. for C₃₇H₂₈ClN₅O₅ [M+H]⁺: 658.1832, found: 630.1796 [M+H- N₂]⁺; Anal. calcd. for C₃₇H₂₈ClN₅O₅: C 67.53, H 4.29, N 10.64, found: C 67.60, H 4.36, N 10.65.

2-((1-(7-chloroquinolin-4-yl)-1*H*-1,2,3-triazol-4-yl)methoxy)-12-(4-methoxyphenyl)-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 (CDCl_3 , 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, OCH_2), 2.53 (s, 2H, CH_2), 2.28 and 2.23 (AB system, 2H, *J* 16.12, $\text{CH}_a\text{H}_b\text{CO}$), 1.09 (s, 3H, CH_3), 0.93 (s, 3H, CH_3); ^{13}C NMR (CDCl_3 , 100 MHz): δ 197.40, 163.60, 157.73, 156.63, 151.36, 150.13, 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 $\text{C}_{38}\text{H}_{31}\text{ClN}_4\text{O}_4$ [$\text{M}+\text{H}$] $^+$: 643.2106, found: 615.2069 [$\text{M}+\text{H}-\text{N}_2$] $^+$; Anal. calcd. for $\text{C}_{38}\text{H}_{31}\text{ClN}_4\text{O}_4$: C 70.97, H 4.86, N 8.71, found: C 70.92, H 4.87, N 8.69.

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

Colorless solid; mp 223–225 °C; IR (KBr) ν/cm^{-1} 2956, 1685, 1648, 1560, 1374; ^1H NMR (CDCl_3 , 400 MHz): δ 9.04 (d, 1H, *J* 4.4, Ar-H), 8.21 (s, 1H, Ar-H), 7.85 (s, 1H, triazole-H), 7.83 (d, 1H, *J* 8.8, Ar-H), 7.71–7.68 (m, 2H, Ar-H), 7.51–7.48 (m, 1H, Ar-H), 7.43–7.41 (m, 2H, Ar-H), 7.19 (d, 1H, 8.8, Ar-H), 7.10–7.07 (m, 1H, Ar-H), 6.93 (s, 1H, Ar-H), 6.79 (d, 1H, *J* 8.8, Ar-H), 6.56 (d, 1H, *J* 8.8, Ar-H), 5.53 (s, 1H, CH), 5.43 and 5.32 (AB system, 2H, *J* 12.44, OCH_2), 3.74 (s, 3H, OCH_3), 3.62 (s, 3H, OCH_3), 2.54 (s, 2H, CH_2), 2.28 and 2.22 (AB system, 2H, *J* 16.12, $\text{CH}_a\text{H}_b\text{CO}$), 1.10 (s, 3H, CH_3), 0.95 (s, 3H, CH_3); ^{13}C NMR (CDCl_3 , 100 MHz) δ 197.05, 163.67, 156.60, 151.39, 150.17, 148.63, 148.38, 147.24, 144.60, 140.75, 137.30, 136.87, 132.72, 132.52, 130.23, 129.44, 128.92, 128.48, 127.07, 124.51, 124.46, 120.52, 117.40, 116.82, 116.05, 115.00, 114.21, 111.79, 110.75, 104.18, 61.64, 55.83, 55.55, 50.85, 41.36, 34.50, 32.24, 29.29, 27.07; HRMS (ESI) m/z , calcd. for $\text{C}_{39}\text{H}_{33}\text{ClN}_4\text{O}_5$ [$\text{M}+\text{H}$] $^+$: 673.2212, found: 645.2146 [$\text{M}+\text{H}-\text{N}_2$] $^+$; Anal. calcd. for $\text{C}_{39}\text{H}_{33}\text{ClN}_4\text{O}_5$: C 69.59, H 4.94, N 8.32, found: C 69.65, H 4.92, N 8.40.

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

Colorless solid; mp 189–193 °C; IR (KBr) ν/cm^{-1} 2958, 1653, 1618, 1534, 1375; ^1H NMR (CDCl_3 , 400 MHz): δ

9.02 (d, 1H, *J* 5.12, Ar-H), 8.21 (d, 1H, *J* 2.2, Ar-H), 7.77 (d, 1H, *J* 2.2, Ar-H), 7.75 (s, 1H, triazole-H), 7.68 (d, 2H, *J* 8.8, Ar-H), 7.47–7.41 (m, 3H, Ar-H), 7.21–7.19 (m, 4H, Ar-H), 7.08–7.05 (m, 1H, Ar-H), 6.85 (d, 1H, *J* 8.8, Ar-H), 5.53 (s, 1H, CH), 5.44 and 5.29 (AB system, 2H, *J* 12.44, OCH_2), 2.54 (s, 2H, CH_2), 2.26 and 2.22 (AB system, 2H, *J* 16.12, $\text{CH}_a\text{H}_b\text{CO}$), 2.0 (s, 3H, CH_3), 1.09 (s, 3H, CH_3), 0.94 (s, 3H, CH_3); ^{13}C NMR (CDCl_3 , 100 MHz) δ 196.93, 163.64, 156.34, 150.71, 148.18, 146.5, 145.37, 144.20, 141.73, 137.92, 136.23, 135.65, 133.24, 132.61, 129.94, 128.91, 128.41, 128.34, 127.07, 124.03, 123.52, 121.04, 120.37, 117.39, 117.05, 114.90, 114.30, 104.11, 55.82, 50.89, 41.38, 34.75, 32.26, 29.21, 27.28, 20.95; HRMS (ESI) m/z , calcd. for $\text{C}_{38}\text{H}_{31}\text{ClN}_4\text{O}_3$ [$\text{M}+\text{H}$] $^+$: 627.2158, found 599.2058 [$\text{M}+\text{H}-\text{N}_2$] $^+$; Anal. calcd. for $\text{C}_{38}\text{H}_{31}\text{ClN}_4\text{O}_3$: 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)-1*H*-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 (CDCl_3 , 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, OCH_2), 2.61 (s, 2H, CH_2), 2.31 and 2.26 (AB system, 2H, *J* 16.84, $\text{CH}_a\text{H}_b\text{CO}$), 1.13 (s, 3H, CH_3), 0.96 (s, 3H, CH_3); ^{13}C NMR (CDCl_3 , 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, 128.98, 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 $\text{C}_{37}\text{H}_{28}\text{ClN}_5\text{O}_5$ [$\text{M}+\text{H}$] $^+$: 658.1852, found: 630.1801 [$\text{M}+\text{H}-\text{N}_2$] $^+$; Anal. calcd. for $\text{C}_{37}\text{H}_{28}\text{ClN}_5\text{O}_5$: 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)-1*H*-1,2,3-triazol-4-yl)methoxy)-12-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 (CDCl_3 , 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, OCH₂), 2.54 (s, 2H, CH₂), 2.30 and 2.19 (AB system, 2H, J 16.84, CH_aH_bCO), 1.09 (s, 3H, CH₃), 0.92 (s, 3H, CH₃); ¹³C NMR (CDCl₃, 100 MHz) δ 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 , calcd. for C₃₇H₂₉ClN₄O₃ [M+H]⁺: 614.1115, found: 586.1025 [M+H- N₂]⁺; Anal. calcd. for C₃₇H₂₉ClN₄O₃: C 72.48, H 4.77, N 9.14, found: C 72.52, H 4.75, N 9.10.

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

Colorless solid; mp 219-224 °C; IR (KBr) ν /cm⁻¹ 2958, 1640, 1604, 1560, 1375; ¹H NMR (CDCl₃, 400 MHz): δ 9.04 (d, 1H, J 4.4, Ar-H), 8.22 (d, 1H, J 2.2, Ar-H), 7.89 (s, 1H, triazole-H), 7.85 (d, 1H, J 8.8, Ar-H), 7.72 (d, 1H, J 2.2, Ar-H), 7.69 (d, 1H, J 1.48, Ar-H), 7.52-7.50 (m, 1H, Ar-H), 7.43 (d, J = 4.4 Hz, 1H, Ar-H), 7.32 (s, 1H, Ar-H), 7.27 (d, 2H, J 8.08, ArH), 7.21 (d, 1H, J 8.75, Ar-H), 7.12 (m, 1H, Ar-H), 7.06 (m, 2H, Ar-H), 5.50 (s, 1H, CH), 5.44 and 5.31 (AB system, 2H, J 12.44, OCH₂), 2.54 (s, 2H, CH₂), 2.27 and 2.19 (AB system, 2H, J 16.12, CH_aH_bCO), 1.10 (s, 3H, CH₃), 0.93 (s, 3H, CH₃); ¹³C NMR (CDCl₃, 100 MHz) δ 196.87, 164.00, 156.87, 151.39, 150.16, 148.45, 144.59, 143.11, 140.78, 136.91, 132.51, 131.99, 130.34, 129.81, 129.49, 128.97, 128.80, 128.43, 127.11, 124.51, 124.48, 120.56, 117.56, 116.18, 116.09, 115.05, 113.68, 103.97, 61.69, 50.79, 41.35, 34.49, 32.25, 29.16, 27.12; HRMS (ESI) m/z , calcd. for C₃₇H₂₈Cl₂N₄O₃ [M+H]⁺: 647.1611, found: 619.1596 [M+H- N₂]⁺; Anal. calcd. for C₃₇H₂₈Cl₂N₄O₃: C 68.63, H 4.36, N 8.65, found: C 68.71, H 4.41, N 8.87.

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

Colorless solid; mp 161-163 °C; IR (KBr) ν /cm⁻¹ 2956, 1647, 1610, 1562, 1375; ¹H NMR (CDCl₃, 400 MHz): δ 8.91 (d, 1H, J 5.12, Ar-H), 8.18 (d, 1H, J 1.8, Ar-H), 7.74 (d, 1H, J 2.92, Ar-H), 7.70 (d, 1H, J 5.12, Ar-H), 7.68 (s, 1H, triazole-H), 7.57-7.56 (m, 2H, Ar-H), 7.51-7.28 (m, 4H, Ar-H), 7.27 (d, 1H, J 8.8, Ar-H), 7.20-7.10 (m, 5H, Ar-H), 7.05-7.02 (m, 1H, Ar-H), 5.75 (s, 1H, CH), 5.41 and 5.32 (AB system, 2H, J 12.44, OCH₂), 2.60 (s, 2H, CH₂), 2.26 and 2.20 (AB system, 2H, J 16.12, CH_aH_bCO), 1.09 (s, 3H, CH₃), 0.80 (s, 3H, CH₃); ¹³C NMR (CDCl₃, 100 MHz) δ 196.91, 163.87, 156.55, 151.24, 150.07, 148.46, 144.30,

142.08, 140.44, 136.69, 131.13, 132.66, 131.91, 130.28, 129.32, 128.82, 128.67, 128.01, 127.66, 127.17, 127.13, 127.01, 126.77, 125.81, 125.41, 124.43, 124.33, 120.43, 117.75, 116.66, 115.82, 115.10, 113.87, 104.24, 61.42, 50.81, 41.37, 35.19, 32.25, 29.22, 27.15; HRMS (ESI) m/z , calcd. for C₄₁H₃₁ClN₄O₃ [M+H]⁺: 663.2158, found: 635.2056 [M+H- N₂]⁺; Anal. calcd. for C₄₁H₃₁ClN₄O₃: 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)-1*H*-1,2,3-triazol-4-yl)methoxy)-12-(4-methylphenyl)-8,9,10,12-tetrahydrobenzo[a]xanthen-11-one (**1k**)

Colorless solid; mp 210-214 °C; IR (KBr) ν /cm⁻¹ 2945, 1639, 1619, 1560, 1376; ¹H NMR (CDCl₃, 400 MHz): δ 9.03 (d, 1H, J 5.12, Ar-H), 8.2 (d, 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, ArH), 5.56 (s, 1H, CH), 5.40 and 5.30 (AB system, 2H, J 12.44, OCH₂), 2.74-2.59 (m, 2H, CH₂), 2.44-2.30 (m, 2H, CH₂), 2.15 (s, 3H, CH₃), 2.06-1.91 (m, 2H, CH₂); ¹³C NMR (CDCl₃, 100 MHz) δ 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 , calcd. for C₃₆H₂₇ClN₄O₃ [M+H]⁺: 599.1845, found: 571.1796 [M+H- N₂]⁺; Anal. calcd. for C₃₆H₂₇ClN₄O₃: 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)-1*H*-1,2,3-triazol-4-yl)methoxy)-12-(naphthalen-1-yl)-8,9,10,12-tetrahydrobenzo[a]xanthen-11-one (**1l**)

Colorless solid; mp 181-185 °C; IR (KBr) ν /cm⁻¹ 2943, 1647, 1617, 1565, 1375; ¹H NMR (CDCl₃, 400 MHz): δ 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), 5.77 (s, 1H, CH), 5.43 and 5.30 (AB system, 2H, J 12.44, OCH₂), 2.77-2.62 (m, 2H, CH₂), 2.38-2.36 (m, 2H, CH₂), 2.05-1.87 (m, 2H, CH₂); ¹³C NMR (CDCl₃, 100 MHz) δ 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 , calcd. for C₃₉H₂₇ClN₄O₃ [M+H]⁺: 635.1846, found: 607.1754 [M+H-

$N_2]^+$; Anal. calcd. for $C_{39}H_{27}ClN_4O_3$: C 73.75, H 4.28, N 8.82, found: C 73.81, H 4.29, N 9.85.

2-((1-(7-chloroquinolin-4-yl)-1*H*-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 ($CDCl_3$, 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-H), 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), 2.73-2.59 (m, 2H CH_2), 2.45-2.31 (m, 2H, CH_2), 2.06-1.89 (m, 2H, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 197.04, 165.69, 156.83, 151.38, 150.12, 148.41, 147.52, 144.52, 143.87, 140.73, 136.85, 132.44, 131.37, 130.31, 130.25, 129.44, 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 $C_{35}H_{24}BrClN_4O_3$ $[M+H]^+$: 663.0793, found: 635.0785 $[M+H-N_2]^+$; Anal. calcd. for $C_{35}H_{24}BrClN_4O_3$: C 63.31, H 3.64, N 8.44, found: C 63.37, H 3.65, N 8.47.

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

Colorless solid; mp 202-206 °C; IR (KBr) ν/cm^{-1} 2957, 1648, 1618, 1545, 1374; 1H NMR ($CDCl_3$, 400 MHz): δ 9.04 (d, 1H, J 4.4, Ar-H), 8.23 (d, 1H, J 1.48, Ar-H), 7.86 (s, 1H, triazole-H), 7.79 (d, 1H, J 8.8, Ar-H), 7.69 (d, 1H, J 8.8, Ar-H), 7.47-7.46 (m, 2H, Ar-H), 7.43 (d, 1H, J 2.2, Ar-H), 7.21-7.18 (m, 3H, Ar-H), 7.08-7.06 (m, 2H, Ar-H), 6.94 (d, 2H, J 8.04, Ar-H), 5.57 (s, 1H, CH), 5.43 and 5.28 (AB system, 2H, J 12.44, OCH_2), 2.76-2.56 (m, 3H, $CHCH_2$), 2.46-2.30 (m, 2H, CH_2), 2.06-1.94 (m, 2H, CH_2), 1.04 (dd, 6H, J 8.0, $CH(CH_3)_2$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 197.19, 165.50, 156.70, 151.36, 150.13, 148.42, 144.59, 142.17, 140.82, 136.92, 132.68, 130.16, 129.49, 128.39, 128.38, 128.25, 127.09, 126.37, 124.66, 124.42, 120.61, 119.29, 117.55, 117.15, 116.21, 115.67, 115.03, 104.48, 61.69, 36.99, 34.73, 30.91, 27.66, 23.76, 20.17; HRMS (ESI) m/z , calcd. for $C_{38}H_{31}ClN_4O_3$ $[M+H]^+$: 627.2158, found: 599.2054 $[M+H-N_2]^+$; Anal. calcd. for $C_{38}H_{31}ClN_4O_3$: C 72.78, H 4.98, N 8.93, found: C 72.85, H 5.01, N 8.97.

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

Colorless solid; mp 221-225 °C; IR (KBr) ν/cm^{-1} 2925, 1638, 1610, 1550, 1374; 1H NMR ($CDCl_3$, 400 MHz): δ 9.04

(d, 1H, J 4.4 Hz, Ar-H), 8.24 (d, 1H, J 2.2, Ar-H), 7.83 (d, 1H, J 8.8, Ar-H), 7.75 (s, 1H, triazole-H), 7.72 (d, 2H, J 8.8, Ar-H) 7.53-7.50 (m, 1H, Ar-H), 7.42 (d, 1H, J 5.12, Ar-H), 7.42 (d, 1H, J 2.2, Ar-H), 7.34 (d, 2H, J 6.6, Ar-H), 7.24 (d, 1H, J 8.8, Ar-H), 7.11-7.04 (m, 3H, Ar-H), 6.85 (t, 1H, J 7.32, Ar-H), 5.63 (s, 1H, CH), 5.45 and 5.30 (AB system, 2H, J 12.44, OCH_2), 2.76-2.63 (m, 2H, CH_2), 2.48-2.32 (m, 2H, CH_2), 2.08-1.92 (m, 2H, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 197.06, 165.54, 156.68, 151.32, 150.12, 148.49, 144.90, 144.47, 140.80, 136.91, 132.62, 130.24, 129.47, 128.92, 128.55, 128.48, 128.29, 127.08, 126.23, 124.55, 124.52, 120.61, 117.53, 116.71, 116.13, 115.21, 114.96, 104.08, 61.46, 36.97, 34.96, 27.70, 20.22; HRMS (ESI) m/z , calcd. for $C_{35}H_{25}ClN_4O_3$ $[M+H]^+$: 585.1688, found: 557.1685 $[M+H-N_2]^+$; Anal. calcd. for $C_{35}H_{25}ClN_4O_3$: C 71.85, H 4.31, N 9.58, found: C 71.92, H 4.34, N 9.60.

12-(4-chlorophenyl)-9,9-dimethyl-2-((1-phenyl-1*H*-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_{max}/cm^{-1} : 2922, 1646, 1618, 1560, 1198; 1H NMR ($CDCl_3$, 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 7.36 Hz, 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, OCH_2), 2.27 and 2.23 (AB system, 2H, J 16.12, CH_2H_2CO), 1.10 (s, 3H, CH_3), 0.94 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$, 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, 32.23, 29.66, 27.11; HRMS (ESI) m/z , calcd. for $C_{34}H_{29}N_3O_3$ $[M+H]^+$: 562.9242, found: 534.1856 $[M+H-N_2]^+$; Anal. calcd. for $C_{34}H_{29}N_3O_3$: C 77.40, H 5.54, N 9.10, found: C 77.45, H 5.58, N 9.09.

Experimental procedure for antibacterial activity

The antibacterial activity of all compounds was evaluated by the agar well diffusion method.³⁰ All the microbial cultures were adjusted to 0.5 McFarland standard, which is visually comparable to a microbial suspension of approximately 1.5×10^6 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 microorganisms and kept for 15 min for adsorption. Using sterile cork borer of 8 mm diameter, wells were bored into the seeded agar plates and these were loaded with a 100 μL volume with concentration of 2.0 $mg mL^{-1}$ of each compound reconstituted in dimethylsulphoxide (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 \pm 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 $\mu\text{g mL}^{-1}$ 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 (1.5×10^8 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.³² 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.

$$\text{Percent inhibition of mycelial growth} = (dc - dt) / dc \times 100$$

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 $\mu\text{g mL}^{-1}$ in sterile test tubes. Each dilution was seeded with 100 μL of the standardized fungal inoculums (2×10^5 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 iterations 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 , ^{13}C 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://jbcbs.sbg.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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