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# Synthesis and evaluation of a class of new coumarin triazole derivatives as potential antimicrobial agents

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ABSTRACT

A series of new coumarin-based 1,2,4-triazole derivatives were designed, synthesized and evaluated for their antimicrobial activities in vitro against four Gram-positive bacteria (*Staphylococcus aureus*, MRSA, *Bacillus subtilis* and *Micrococcus luteus*), four Gram-negative bacteria (*Escherichia coli*, *Proteus vulgaris*, *Salmonella typhi* and *Shigella dysenteriae*) as well as three fungi (*Candida albicans*, *Saccharomyces cerevisiae* and *Aspergillus fumigatus*) by two-fold serial dilution technique. The bioactive assay showed that some synthesized coumarin triazoles displayed comparable or even better antibacterial and antifungal efficacy in comparison with reference drugs Enoxacin, Chloromycin and Fluconazole. Coumarin bis-triazole compounds exhibited stronger antibacterial and antifungal efficiency than their corresponding mono-triazole derivatives.



Coumarins and their derivatives have attracted considerable attention due to their extensively biological activities such as antibacterial, antifungal, antiviral, anti-tubercular, anti-malarial, anticoagulant, anti-inflammatory, anticancer, antioxidant properties and so on. Numerous efforts, including the separation and purification of naturally occurring coumarins from a variety of plants as well as artificial synthesis of coumarin compounds with novel structures and properties, have been focusing on the research and development of coumarins as potential drugs. So far some coumarins, for example, Warfarin, Acenocoumarol, Armillarisin A, Hymecromone and Carbochromen have been approved for therapeutic purposes in clinic. More importantly, an increasing number of coumarin compounds have displayed great potency in the treatment of various types of diseases.[1–3]

Coumarin compounds, containing 1,2-benzopyrone skeleton structurally similar to clinical anti-infective quinolone drugs with benzopyridone backbone, as a new type of antibiotics have received specific interest along with the dramatically rising prevalence of multi-drug resistant microbial infections. It is well known that the quinolone anti-infectives are of wholly synthetic origin and not modeled knowingly after any natural antibiotics. Of all the totally synthetic antimicrobial agents, the quinolones have proved to be most successful economically and clinically. As predominant antibacterial drugs, quinolones have been widely used in clinic with orally and parenterally active properties, broad antimicrobial spectrum including many frequently encountered pathogens, and bactericidal behavior in clinically achievable doses. Clearly, quinolone anti-infectives have been playing important roles in the past fifty years in the unending struggles against morbidity and mortality caused by microbial pathogens.[4] However, the prevalently clinical use of this class of anti-infectives has led to increasingly worrisome resistance at a disturbing rate, which has posed serious problems in the treatment of infectious diseases.[5] This trend has highlighted the urgent need for designing and developing powerful antimicrobial compounds, especially for the exploration of new quinolone-like compounds. Reasonably, a great deal of work has recently directed towards coumarin compounds which have similar structural framework to quinolones. Some naturally occurring coumarins such as Novobiocin, Coumermycin $A_1$ and Chlorobiocin have been found to be an unprecedented class of antibiotics, but they are not used in clinic owing to their relatively weak activity towards Gram-negative bacteria, poor water solubility and side effects.[6] However, wholly synthetic coumarin compounds have drawn renewed attention because of their prominent antibacterial properties, specifically against methicillin-resistant *Staphylococcus aureus* (MRSA).[1–3] In recent years, some works have manifested that coumarin backbone in combination with some nitrogen-containing heterocyclic moieties such as azetidine, thiazolidine, thiazole and so on could significantly increase the antimicrobial efficiency and broaden their antimicrobial spectrum.[7] In this work, we would like to introduce 1,2,4-triazolyl ring into coumarin.

As important aromatic nitrogen-containing heterocycle, 1,2,4-triazole compounds have aroused special interest due to their excellent pharmacokinetic characteristics, favorable safety profile, as well as the latent ability for the formation of hydrogen bonds

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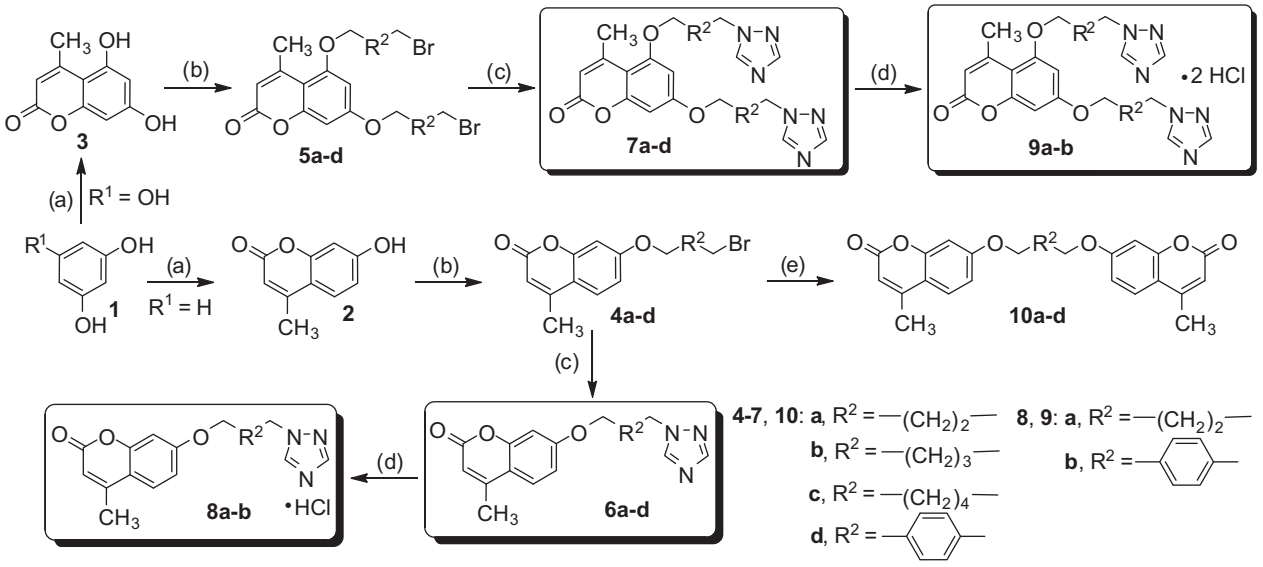
with other active molecules. A number of triazole drugs including Fluconazole, Itraconazole and Voriconazole have been prevalently used in the anti-infective therapy.[8,9] Recently, some triazole derivatives have been reported to exhibit good anti-MRSA potency.[10] Although the extensively clinical use of triazole anti-infective agents has ever revolutionized the treatment of many infectious diseases, some of them are still limited by poor activities towards intractable fungi, high frequency of renal toxicity and several adverse effects. Therefore, these situations have been served as impetus for developing new triazole antimicrobial agents.

Prompted by above observations and in continuation of our ongoing interest in the development of new antimicrobial agents,[10–12] herein we designed and synthesized a class of new coumarin triazole compounds and evaluated their antibacterial and antifungal activities in vitro. In order to investigate the effect of heterocyclic triazole moiety on antimicrobial activity, coumarin bis-triazoles **7a**–**d**, mono-triazoles **6a**–**d** as well as non-triazole bis-coumarins **10a**–**d** and coumarin bromides **4**–**5** were prepared. A lot of researches have provided evidences that the linkers could modulate the physicochemical properties of the whole molecule and thereby affect biological activities.[13,14] Based on this, and with the aim of better understanding of structure–activity relationship, various types of linkers including alkyl and aralkyl ones, as well as the spacers with different lengths of aliphatic chains were introduced into the target compounds. Recent studies also found that the transformation of azoles into their corresponding salts could enhance antimicrobial efficacy remarkably due to the improvement of water solubility and membrane permeability.[13–15] Thus, some representative coumarin mono-triazoles **6a** and **6d** as well as bis-triazoles **7a** and **7d** were converted into the corresponding hydrochlorides **8a**–**b** and **9a**–**b**.

The desired coumarin compounds were prepared from commercially available phenols, and the synthetic route was outlined in Scheme 1. The cyclization of resorcinol and phloroglucinol, respectively, with ethyl acetoacetate in the presence of oxalic acid formed the corresponding 7-hydroxy-coumarin **2** with excellent yield of 92% and 5,7-dihydroxy-coumarin **3** in 90% yield. Hydroxyl-coumarin **2** or **3** reacted with alkyl or aralkyl dibromides separately in acetone using potassium carbonate as base to produce intermediate coumarin bromides **4**–**5** in good yields (73–85%). The N-alkylation of compounds **4**–**5** with 1,2,4-triazole, respectively, in the presence of potassium carbonate gave the target coumarin triazoles **6**–**7** in satisfactory yields ranging from 71% to 83%. The treatment of coumarin mono-triazoles **6a** and **6d** as well as bis-triazoles **7a** and **7d** with hydrochloric acid were almost quantitatively transformed into corresponding hydrochlorides **8a**–**b** and **9a**–**b**. The coupling of coumarin halides **4a**–**d** with hydroxy-coumarin **2** generated bis-coumarins **10a**–**d** with yields of 81–83%. All new compounds were characterized by $^{1}$H NMR, $^{13}$C NMR, IR, MS, HRMS spectra and elemental analyses.[16]

These synthesized compounds were screened for their antimicrobial activities in vitro against four Gram-positive bacteria (*S. aureus* ATCC 25923, *S. aureus* N 315, *B. subtilis* ATCC 6633 and *M. luteus* ATCC 4698), four Gram-negative bacteria (*E. coli* ATCC 25922, *P. vulgaris* ATCC 6896, *S. typhi* ATCC 9484 and *S. dysenteriae* ATCC 49550) as well as three fungi (*C. albican* ATCC 76615, *S. cerevisiae* ATCC 9763 and *A. fumigatus* ATCC 96918) using two-fold broth dilution method in 96-well micro-test plates recommended by National Committee for Clinical Laboratory Standards (NCCLS).[17] Clinically antimicrobial drugs Enoxacin, Chloromycin and Fluconazole were used as the positive control. The obtained results, depicted in Table 1, revealed that these prepared coumarin compounds **2**–**10** could effectively, to some extent, inhibit the growth of all tested strains in vitro, except for bis-coumarin **10d** with an aralkyl spacer, which had poor water solubility. The prepared coumarin triazole compounds **6**–**9** demonstrated moderate to excellent antimicrobial activities towards the tested microorganisms, including methicillin-resistant *S. aureus* and Fluconazole-insensitive *A. fumigatus*, which showed broad antimicrobial spectrum. Noticeably, some triazole derivatives gave comparable or superior MIC values to standard drugs Chloromycin, Enoxacin and Fluconazole. Especially, bis-triazole **7a** and its corresponding hydrochloride **9a** gave quite low inhibitory concentrations (1–4 μg/mL), which suggested that they were potential as antimicrobial agents in comparison with clinical drugs Chloromycin (MIC = 4–16 μg/mL), Enoxacin (MIC = 1–4 μg/mL) and Fluconazole (MIC = 1–128 μg/mL). It was also observed that Gram-positive microorganisms were more sensitive than Gram-negative bacteria to hydroxyl-coumarins **2**–**3**, bromides **4**–**5**, bis-coumarins **10a**–**c** as well as mono-triazoles **6a**–**d** and **8a**–**b**. Furthermore, MRSA was known to be the most distraught and virulent organism that caused a broad array of problems to hospitalized and community-acquired patients, and showed multi-drug resistance to numerous currently available agents including the standard drugs




**Scheme 1.** Reagents and conditions: (a) ethyl acetoacetate, oxalic acid, 90–100 °C, 10–16 h; (b) alkyl or aralkyl dibromide, $K_2CO_3$, acetone, reflux, 10–14 h; (c) 1,2,4-triazole, $K_2CO_3$, $CH_3CN$, rt, 14–22 h; (d) 4 mol/L HCl, ethyl ether/chloroform, rt, 10–14 h; (e) compound **2**, NaOH, $CH_3CN$, 50 °C, 8–12 h.

**Table 1**
In vitro antimicrobial activities for some synthetic coumarin compounds **2–10** expressed as MIC (μg/mL)

| Compds | Gram-positive bacteria | | | | Gram-negative bacteria | | | | Fungi | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | *S. aureus* | MRSA | *B. subtilis* | *M. luteus* | *E. coli* | *P. vulgaris* | *S. typhi* | *S. dysenteriae* | *C. albicans* | *S. cerevisiae* | *A. fumigatus* |
| **2** | 32 | 64 | 32 | 32 | 128 | 256 | 128 | 512 | 64 | 128 | 128 |
| **3** | 32 | 64 | 64 | 64 | 128 | 128 | 256 | 256 | 64 | 256 | 256 |
| **4a** | 128 | 256 | 128 | 128 | 256 | 256 | 256 | 512 | 128 | 256 | 512 |
| **4b** | 128 | 256 | 128 | 256 | 512 | 512 | 512 | 256 | 128 | 512 | 256 |
| **4c** | 64 | 256 | 128 | 64 | 512 | 256 | 256 | 512 | 256 | 256 | 512 |
| **4d** | 256 | 512 | 256 | 256 | 512 | 512 | 512 | 512 | 256 | 256 | 256 |
| **5a** | 256 | 512 | 128 | 256 | 512 | 256 | 256 | 256 | 128 | 256 | 256 |
| **5b** | 128 | 256 | 128 | 128 | 512 | 256 | 256 | 256 | 128 | 256 | 128 |
| **5c** | 256 | 512 | 128 | 128 | 512 | 512 | 512 | 512 | 128 | 128 | 128 |
| **5d** | 256 | 512 | 128 | 256 | 512 | 512 | 256 | 512 | 256 | 256 | 256 |
| **6a** | 2 | 8 | 2 | 4 | 8 | 16 | 16 | 16 | 2 | 4 | 16 |
| **6b** | 4 | 16 | 4 | 8 | 32 | 32 | 16 | 32 | 8 | 16 | 16 |
| **6c** | 8 | 16 | 8 | 8 | 32 | 32 | 32 | 32 | 16 | 32 | 32 |
| **6d** | 32 | 64 | 32 | 32 | 64 | 64 | 64 | 64 | 32 | 64 | 64 |
| **7a** | 1 | 2 | 2 | 1 | 1 | 2 | 1 | 1 | 1 | 2 | 4 |
| **7b** | 2 | 8 | 4 | 4 | 8 | 4 | 4 | 4 | 2 | 4 | 32 |
| **7c** | 8 | 16 | 4 | 8 | 8 | 8 | 16 | 8 | 4 | 4 | 32 |
| **7d** | 32 | 64 | 64 | 64 | 64 | 32 | 32 | 64 | 32 | 32 | 64 |
| **8a** | 2 | 8 | 2 | 2 | 4 | 8 | 16 | 16 | 2 | 4 | 8 |
| **8b** | 8 | 16 | 8 | 8 | 16 | 16 | 16 | 16 | 8 | 16 | 16 |
| **9a** | 1 | 2 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 2 | 2 |
| **9b** | 8 | 16 | 16 | 16 | 8 | 16 | 16 | 16 | 8 | 16 | 16 |
| **10a** | 128 | 512 | 128 | 128 | 256 | 256 | 256 | 512 | 512 | 256 | 512 |
| **10b** | 128 | 256 | 256 | 128 | 256 | 512 | 512 | 512 | 512 | 256 | 512 |
| **10c** | 256 | 512 | 128 | 128 | 256 | 256 | 512 | 512 | 512 | 256 | 512 |
| **10d** | >512 | >512 | >512 | >512 | >512 | >512 | >512 | >512 | >512 | 512 | >512 |
| Enoxacin | 1 | 4 | 1 | 1 | 1 | 1 | 2 | 1 | — | — | — |
| Chloromycin | 4 | 16 | 8 | 4 | 8 | 8 | 4 | 4 | — | — | — |
| Fluconazole | — | — | — | — | — | — | — | — | 1 | 2 | 128 |

Chloromycin and Enoxacin. Unexpectedly, in our work, coumarin triazole compounds **6a–c**, **7a–c**, **8a–b** and **9a–b** demonstrated good anti-MRSA activity with MIC values in the range of 2–16 μg/mL. Among all tested compounds including the positive control Fluconazole, only coumarin trizoles could significantly inhibit the growth of *A. fumigatus* (MIC = 2–64 μg/mL). These implied that coumarin triazoles were potential as new antimicrobial agents towards all these tested bacterial and fungal strains.

Mono-hydroxyl-coumarin **2** and dihydroxy-coumarin **3** showed moderate activities against *C. albicans* and Gram-positive bacteria with MIC values of 32–64 μg/mL. However, the incorporation of bromoalkyl or bromoaralkyl groups into coumarins resulted in dramatic decrease in inhibiting the growth of the tested strains in coumarin bromides **4a–b**, **4d** and **5a–d** (MIC = 128–512 μg/mL), this suggested that these bromoalkyl and bromoaralkyl moieties were not beneficial for antimicrobial efficacy.

Surprisingly, the introduction of triazole ring instead of bromo moiety in coumarin bromides **4a–d**, which yielded corresponding coumarin mono-triazole compounds **6a–d**, contributed to an unexpected enhancement for antimicrobial efficiency. Mono-triazoles **6a–d** exhibited better activities than their precursor coumarin bromides **4a–d** as well as hydroxyl-coumarin **2**. Particularly, compound **6a** could efficiently inhibit the growth of all tested Gram-positive bacteria at the concentrations of 2–8 μg/mL, which was more effective than Chloromycin (4–16 μg/mL) and almost equipotent to Enoxacin (MIC = 1–4 μg/mL), and its antifungal potency against *C. albicans* and *S. cerevisiae* (MIC = 2–4 μg/mL) was also comparable to positive control Fluconazole (MIC = 1 μg/mL). Nevertheless, the substitution of triazolyl group in mono-triazoles **6a–d** by coumarin ring caused remarkable decrease or total loss of antimicrobial properties for bis-coumarins **10a–d**. These showed that nitrogen-containing heterocyclic 1,2,4-triazole moiety exerted important influence on antimicrobial activities. Furthermore, bis-triazoles **7a–d** displayed much stronger antimicrobial efficacy in contrast to mono-triazoles **6a–d**. It was particularly pointed out that, among these tested bis-triazoles, compound **7a** with a $(CH_2)_4$ linker possessed the strongest antimicrobial activities, and it gave the best anti-MRSA efficacy with MIC value of 2 μg/mL, which was two-fold and eight-fold more active than Enoxacin (MIC = 4 μg/mL) and Chloromycin (MIC = 16 μg/mL), respectively. These results further indicated that triazole moiety was specifically favorable for antimicrobial activities, which could not only broaden the antimicrobial spectrum, but also increase the bioactivity significantly. It was also suggested that coumarin bis-triazole **7a** was worthy for further investigations as potential antimicrobial agent.

For tested coumarin mono-triazoles **6a–d** and bis-triazoles **7a–d**, the structural parameters of spacers including the types of linkers and the lengths of aliphatic chains markedly influenced their antimicrobial efficacy. Alkyl compounds **6a–c** and **7a–c** were more active (MIC = 1–32 μg/mL) than their corresponding aralkyl triazoles **6d** and **7d** (MIC = 32–64 μg/mL). Moreover, the antimicrobial potency for triazoles **6a–c** and **7a–c** depended on the lengths of aliphatic chains, and the activities seemed to decrease with the increase of aliphatic chain length. Obviously, the bridged linkers have large effect on their antimicrobial efficiency, and further works are essential in order to deduce the structure–activity relationship.

It was also observed that coumarin triazole hydrochlorides **8b** and **9b** displayed stronger antibacterial and antifungal efficacy in comparison with their poor water-soluble aralkyl triazole precursors **6d** and **7d**. The result was consistent with the literatures. This transformation of triazole compounds into their hydrochlorides might modulate the lipid/water partition coefficient, affect their diffusion in bacterial cells as well as interaction with bacterial cells and tissues, and thereby improve the pharmacological properties. Further studies were reasonable to understand their mechanism.

In summary, a series of coumarin triazoles were successfully synthesized through an easy, convenient and economic synthetic procedure starting from commercially available resorcinol and phloroglucinol, and were confirmed by $^1$H NMR, $^{13}$C NMR, IR, MS,

HRMS spectra and elemental analyses. The in vitro antibacterial and antifungal evaluation showed that most synthesized coumarin compounds could effectively inhibit the growth of all tested bacteria and fungi including methicillin-resistant *S. aureus* and Fluconazole-insensitive *A. fumigatus*, and some coumarin triazoles displayed excellent antimicrobial activities in contrast to their positive control. Particularly, bis-triazole **7a** and its hydrochloride **9a** containing a $(CH_2)_4$ linker gave the most potent antimicrobial efficacy (MIC = 1–4 μg/mL) among these tested substances including the standard drugs. Moreover, the anti-MRSA activity for coumarin triazole compounds **6a**–**c**, **7a**–**c**, **8a**–**b** and **9a**–**b** were comparable or superior to currently clinical antibacterial drugs Enoxacin and Chloromycin, which suggested that further investigations are necessary to optimize these potentially leading compounds as more efficacious antibacterial agents. Compared to antifungal drug Fluconazole, all title compounds displayed much stronger inhibition towards *A. fumigatus*, which should be a good starting point for further extension of triazole derivatives as anti-*A. fumigatus* drugs. These results confirmed that the incorporation of 1,2,4-triazole moiety was greatly helpful for the antimicrobial activities, which could not only increase the inhibition remarkably, but also broaden their antimicrobial spectrum. Other related works, including the in vivo bioactive evaluation along with toxicity investigation, the effect factors on antimicrobial activities such as other heterocyclic azole rings (benzotriazole, imidazole, benzimidazole and their derivatives, etc.) as well as spacers with different types of linkers (alkyl, aralkyl, aryl and heterocyclic types and their lengths of chains) are active in progress. All these will be discussed in the future paper.

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16. *Experimental*: Melting points are uncorrected and were determined on X-6 melting point apparatus. IR spectra were determined on a Bio-Rad FTS-185 spectrophotometer in the range of 400–4000 $cm^{-1}$. $^1H$ NMR and $^{13}C$ NMR spectra were recorded on a Bruker AV 300 or Varian 400 spectrometer using TMS as an internal standard. The mass spectra were confirmed on FINIGAN TRACE GC/MS and HRMS. Elemental analyses were carried out on Carlo Erba model EA 1106 elemental analyzer. Some characteristics were given for some representative compounds.
*Synthesis of 7-(4-((1H-1,2,4-triazol-1-yl)methyl) benzyloxy)-4-methyl-2H-chromen-2-one* (**6d**). A mixture of triazole (0.42 g, 6 mmol) and potassium carbonate (0.83 g, 6 mmol) in $CH_3CN$ (20 mL) was stirred for 1 h at room temperature, and then compound **4d** (1.79 g, 5 mmol) was added. The resulting mixture was stirred at room temperature for 16 h (monitored by TLC, eluent, chloroform/acetone, 3/1, V/V). The solvents were evaporated under reduced pressure, and the residue was treated with water (50 mL) and extracted with chloroform (3 × 50 mL). The organic layers were combined, dried with anhydrous $Na_2SO_4$, and concentrated under reduced pressure. The crude product was purified via silica gel column chromatography (eluent, chloroform/acetone, 3/1, V/V) and recrystallized from the mixture solvent of chloroform/petroleum ether (1/2, V/V) to afford compound **6d** (1.40 g) as white solid. Yield 81%; mp 156–157 °C; IR (KBr) *v*: 3115, 3067 (Ar-H), 2976, 2957 ($CH_2$), 1704 (C=O), 1619, 1573, 1511, 1506 (aromatic frame), 1389, 1370, 1296, 1150, 1006, 990, 940, 870, 827, 786, 712 $cm^{-1}$; $^1H$ NMR (400 MHz, $CDCl_3$) *δ*: 8.07 (s, 1H, triazole 3-*H*), 7.83 (s, 1H, triazole 5-*H*), 7.53–7.50 (d, 1H, coumarin 5-*H*), 7.18–7.00 (m, 4H, Ar-*H*), 6.92–6.91 (d, 1H, coumarin 6-*H*), 6.66 (s, 1H, coumarin 8-*H*), 6.13 (s, 1H, coumarin 3-*H*), 5.15 (s, 2H, coumarin-O$CH_2$), 4.99 (s, 2H, triazole-$CH_2$), 2.40 (s, 3H, coumarin-$CH_3$) ppm; $^{13}C$ NMR (100 MHz, DMSO-$d_6$) *δ*: 161.4 (coumarin 2-*C*), 161.1 (coumarin 7-*C*), 155.2 (coumarin 9-*C*), 152.5 (coumarin 4-*C*), 151.8 (triazole 3-*C*), 145.8 (triazole 5-*C*), 136.0 ($OCH_2$Ph 1-*C*), 135.1 ($OCH_2$Ph 4-*C*), 131.7 ($OCH_2$Ph 3,5-*C*), 131.0 ($OCH_2$Ph 2,6-*C*), 128.8 (coumarin 5-*C*), 115.5 (coumarin 3-*C*), 114.9 (coumarin 10-*C*), 114.0 (coumarin 6-*C*), 104.6 (coumarin 8-*C*), 73.8 (coumarin-O$CH_2$), 54.6 (triazole-$CH_2$), 20.4 (coumarin-$CH_3$) ppm; MS (*m*/*z*): 347 $[M]^+$; HRMS (TOF) calcd for $C_{20}H_{18}N_3O_3$ $[M+H]^+$, 348.1348; found, 348.1352; Anal. Calcd for $C_{20}H_{17}N_3O_3$: C, 69.15; H, 4.93; N, 12.10. Found: C, 69.19; H, 4.89; N, 12.07.
*Synthesis of 5,7-bis(4-(1H-1,2,4-triazol-1-yl)butoxy)-4-methyl-2H-chromen-2-one* (**7a**). Prepared according to the procedure described for the preparation of compound **6d**, starting from compound **5a** (2.38 g, 5 mmol), triazole (0.83 g, 12 mmol), potassium carbonate (1.67 g, 12 mmol) and $CH_3CN$ (30 mL), the crude product was obtained and further purification by chromatography (chloroform/methanol, 6/1, V/V) produced bis-triazole **7a** (1.67 g) as white solid. Yield 77%; mp 121–123 °C; IR (KBr) *v*: 3118, 3076 (Ar-H), 2987, 2944 ($CH_2$), 1707 (C=O), 1633, 1600, 1543, 1477 (aromatic frame), 1306, 1220, 1190, 1084, 1002, 848, 804, 731, 715 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) *δ*: 8.10 (s, 2H, triazole 3-*H*), 7.94 (s, 2H, triazole 5-*H*), 6.36 (s, 1H, coumarin 6-*H*), 6.20 (s, 1H, coumarin 8-*H*), 5.91 (s, 1H, coumarin 3-*H*), 4.28–4.23 (t, 4H,coumarin-O$CH_2$), 4.00–3.96 (t, 4H, triazole-$CH_2$), 2.47 (s, 3H, coumarin-$CH_3$), 2.14–2.07 (m, 4H, coumarin-$OCH_2CH_2$), 1.89–1.75 (m, 4H, triazole-$OCH_2CH_2$) ppm; $^{13}C$ NMR (75 MHz, DMSO-$d_6$) *δ*: 160.8 (coumarin 2-*C*), 159.0 (coumarin 7-*C*), 157.0 (coumarin 5-*C*), 155.3 (coumarin 9-*C*), 153.0 (coumarin 4-*C*), 150.0 (2C, triazole 5-*C*), 142.0 (2C, triazole 3-*C*), 109.4 (coumarin 3-*C*), 102.9 (coumarin 10-*C*), 94.6 (coumarin 6-*C*), 92.3 (coumarin 8-*C*), 67.4 (2C, coumarin-O$CH_2$), 47.7 (2C, triazole-$CH_2$), 28.1 (2C, coumarin-$OCH_2CH_2$), 27.2 (2C, triazole-$CH_2CH_2$), 22.9 (coumarin-$CH_3$) ppm; MS (*m*/*z*): 461 $[M+Na]^+$, 439 $[M+H]^+$; HRMS (TOF) calcd for $C_{22}H_{27}N_6O_4$ $[M+H]^+$, 439.2088; found, 439.2083; Anal. Calcd for $C_{22}H_{26}N_6O_4$: C, 63.26; H, 5.98; N, 19.17. Found: C, 63.18; H, 5.99; N, 19.16.
*Synthesis of 5,7-bis(6-(1H-1,2,4-triazol-1-yl)hexyloxy)-4-methyl-2H-chromen-2-one* (**7c**). Compound **7c** was prepared according to the experimental procedure for compound **6d**. Starting from compound **5c** (2.58 g, 5 mmol), triazole (0.83 g, 12 mmol), potassium carbonate (1.67 g, 12 mmol) and $CH_3CN$ (30 mL), the crude product was obtained and purified by chromatography (chloroform/methanol, 6/1, V/V) to give compound **7c** (1.95 g) as white solid. Yield 79%; mp 79–80 °C; IR (KBr) *v*: 3121, 3090 (Ar-H), 2983, 2957 ($CH_2$), 1709 (C=O), 1620, 1602, 1512, 1490 (aromatic frame), 1249, 1225, 1208, 1145, 1116, 879, 860, 848, 842, 804, 745, 646 $cm^{-1}$; $^1H$ NMR (300 MHz, $CDCl_3$) *δ*: 8.07 (s, 2H, triazole 3-*H*), 7.94 (s, 2H, triazole 5-*H*), 6.36 (s, 1H, coumarin 6-*H*), 6.20 (s, 1H, coumarin 8-*H*), 5.93 (s, 1H, coumarin 3-*H*), 4.23–4.19 (t, 4H,coumarin-O$CH_2$), 3.96–3.94 (t, 4H, triazole-$CH_2$), 2.47 (s, 3H, coumarin-$CH_3$), 1.97–1.92 (m, 4H, coumarin-$OCH_2CH_2$), 1.88–1.82 (m, 4H, triazole-$OCH_2CH_2$), 1.51–1.47 (m, 8H, coumarin-$OCH_2CH_2CH_2CH_2$) ppm; $^{13}C$ NMR (75 MHz, $CDCl_3$) *δ*: 162.2 (coumarin 2-*C*), 161.0 (coumarin 7-*C*), 158.2 (coumarin 5-*C*), 156.8 (coumarin 9-*C*), 154.2 (coumarin 4-*C*), 151.9 (2C, triazole 5-*C*), 142.9 (2C, triazole 3-*C*), 111.3 (coumarin 3-*C*), 104.6 (coumarin 10-*C*), 96.1 (coumarin 6-*C*), 93.8 (coumarin 8-*C*), 68.5 (2C, coumarin-O$CH_2$), 49.2 (2C, triazole-$CH_2$), 29.1 (2C, coumarin-$OCH_2CH_2$), 28.6 (2C, triazole-$CH_2CH_2$), 24.3 (2C, coumarin-$OCH_2CH_2CH_2$), 23.2 (2C, triazole-$CH_2CH_2CH_2$), 22.9 (coumarin-$CH_3$) ppm; MS (*m*/*z*): 517 $[M+Na]^+$, 495 $[M+H]^+$; HRMS (TOF) calcd for $C_{26}H_{35}N_6O_4$ $[M+H]^+$, 495.2720; found, 495.2724; Anal. Calcd for $C_{26}H_{34}N_6O_4$: C, 63.14; H, 6.93; N, 16.99. Found: C, 63.22; H, 4.89; N, 16.86.
*Synthesis of 7-(4-(1H-1,2,4-triazol-1-yl)butoxy)-4-methyl-2H-chromen-2-one hydrochloride* (**8a**). To a solution containing compound **6a** (0.60 g, 2 mmol) in ethyl ether/chloroform (4/1, V/V, 10 mL), diluted hydrochloric acid (4 mol/L) was added dropwise until no more precipitate formed. The precipitate was filtered and washed with chloroform to afford hydrochloride **8a** (0.67 g) as white solid. Yield 99%; mp 152–154 °C; IR (KBr) *v*: 3088, 3026 (Ar-H), 2919, 2794 ($CH_2$), 1711 (C=O), 1607, 1566, 1543 (aromatic frame), 1349, 1269, 1204, 1158, 1045, 907, 849, 812, 804, 750, 646, 623 $cm^{-1}$; $^1H$ NMR (400 MHz, DMSO-$d_6$) *δ*: 8.77 (s, 1H, triazole 3-*H*), 8.17 (s, 1H, triazole 5-*H*), 7.69–7.67 (d, 1H, coumarin 5-*H*), 6.95–6.93 (d, 1H, coumarin 6-*H*), 6.91 (s, 1H, coumarin 8-*H*), 6.21 (s, 1H, coumarin 3-*H*), 4.41–4.38 (t, 2H, coumarin-O$CH_2$), 4.09–4.06 (t, 2H, triazole-$CH_2$), 2.38 (s, 3H, coumarin-$CH_3$), 2.26–2.22 (d, 2H, coumarin-$OCH_2CH_2$), 2.19–2.14 (d, 2H, triazole-$CH_2CH_2$) ppm; $^{13}C$ NMR (100 MHz, DMSO-$d_6$) *δ*: 162.4 (coumarin 2-*C*), 159.3 (coumarin 7-*C*), 155.7 (coumarin 9-*C*), 154.7 (coumarin 4-*C*), 152.0 (triazole 3-*C*), 143.6 (triazole 5-*C*), 127.9 (coumarin 5-*C*), 113.4 (coumarin 3-*C*), 112.8 (coumarin 10-*C*), 112.1 (coumarin 6-*C*), 108.2 (coumarin 8-*C*), 68.7 (coumarin-O$CH_2$), 45.9 (triazole-$CH_2$), 33.1 (coumarin-$OCH_2CH_2$), 30.8 (triazole-$CH_2CH_2$), 20.2 (coumarin-$CH_3$) ppm; MS

(*m*/*z*): 299 [M−HCl]$^+$; HRMS (TOF) calcd for $C_{16}H_{18}ClN_3O_3$ [M−HCl]$^+$, 299.1270; found, 299.1269; Anal. Calcd for $C_{16}H_{18}ClN_3O_3$: C, 57.23; H, 5.40; N, 10.56. Found: C, 57.28; H, 5.36; N, 10.59.

*Synthesis of 5,7-bis(4-(1H-1,2,4-triazol-1-yl)butoxy)-4-methyl-2H-chromen-2-one dihydrochloride* (**9a**). According to the synthetic procedure of compound **8a**, hydrochloride **9a** was prepared starting from bis-triazole **7a** (0.88 g, 2 mmol). The hydrochloride **9a** (1.00 g) was obtained as white solid. Yield 98%; mp 220–222 °C; IR (KBr) *v*: 3099, 3021 (Ar-H), 2926, 2875 ($CH_2$), 1706 (C=O), 1643, 1610, 1509, 1487 (aromatic frame), 1356, 1208, 1100, 1001, 911, 833, 796, 731, 698 cm$^{-1}$; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$: 8.91 (s, 2H, triazole 3-*H*), 8.24 (s, 2H, triazole 5-*H*), 6.89 (s, 1H, coumarin 6-*H*), 6.52 (s, 1H, coumarin 8-*H*), 6.11 (s, 1H, coumarin 3-*H*), 4.68–4.63 (t, 4H,coumarin-OC$H_2$), 4.22–4.16 (t, 4H, triazole-C$H_2$), 2.50 (s, 3H, coumarin-C$H_3$), 2.20–2.16 (m, 4H, coumarin-OCH$_2$C$H_2$), 2.09–2.03 (m, 4H, triazole-OCH$_2$C$H_2$) ppm; $^{13}$C NMR (75 MHz, DMSO-$d_6$) $\delta$: 161.2 (coumarin 2-*C*), 158.8 (coumarin 7-*C*), 156.1 (coumarin 5-*C*), 154.9 (coumarin 9-*C*), 152.7 (coumarin 4-*C*), 150.8 (2C, triazole 5-*C*), 141.7 (2C, triazole 3-*C*), 109.2 (coumarin 3-*C*), 103.0 (coumarin 10-*C*), 94.5 (coumarin 6-*C*), 92.0 (coumarin 8-*C*), 68.1 (2C, coumarin-OC$H_2$), 47.5 (2C, triazole-C$H_2$), 27.9 (2C, coumarin-OCH$_2$C$H_2$), 27.4 (2C, triazole-CH$_2$C$H_2$), 22.9 (coumarin-C$H_3$) ppm; MS (*m*/*z*): 438 [M−2HCl]$^+$; HRMS (TOF) calcd for $C_{22}H_{28}Cl_2N_6O_4$ [M−2HCl]$^+$, 438.2016; found, 438.2018; Anal. Calcd for $C_{22}H_{28}Cl_2N_6O_4$: C, 51.67; H, 5.52; N, 16.43. Found: C, 51.54; H, 5.48; N, 16.49.

17. National Committee for Clinical Laboratory Standards Approved standard Document. M27-A2, Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts, National Committee for Clinical Laboratory Standards, Wayne, PA, 2002.