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Design and synthesis of novel 2*H*-chromen-2-one derivatives bearing 1,2,3-triazole moiety as lead antimicrobials

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ABSTRACT

A series of novel 2*H*-chromen-2-one derivatives decorated with 1,2,3-triazole moiety were designed and synthesized using the click reaction of azidoalkyloxy-2*H*-chromen-2-ones with different propargylamines. Propargylamines were obtained by alkylation of various heterocyclic amines with propargyl bromide. Newly synthesized compounds and intermediates were evaluated for their antifungal activity against four fungi (*Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus flavus* and *Candida albicans*). Antibacterial studies were also carried out against three Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis* and *Staphylococcus epidermis*) and four Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Klebsiella pneumoniae*). In vitro, bioassay results showed that all the synthesized compounds exhibited excellent activity against fungal strains *Aspergillus fumigatus*, *Aspergillus flavus* and *Candida albicans*. Interestingly, all the compounds have shown even superior activity than the reference drug miconazole against *Aspergillus fumigatus*. Morpholine and *N*-acetyl piperazine containing compounds **10c** and **10e** have shown promising activity against various bacterial strains. Compound **10e** was found to be most active against *Pseudomonas aeruginosa*. Based on, in silico pharmacokinetic studies, compounds **10a**–**e** were identified as lead compounds for future investigation due to their lower toxicity, high drug score values and good oral bioavailability as per OECD guidelines.

Life threatening infections, caused by pathogenic microorganisms, are reported to cause high morbidity and mortality despite the recent advances in antimicrobial chemotherapeutic regimen.[1] Emergence of new resistant species of fungi and bacteria, in addition to the poor safety profile and pharmacokinetics, challenges the clinicians in their own way to handle the microbial infections. This situation highlights the need to identify lead molecules, from newer classes of compounds, in order to develop novel and effective antifungal and antibacterial agents in future.

Among various classes of chemical moieties, 2*H*-chromen-2-ones (coumarins) have been reported to show diverse and interesting biological and pharmacological activities. Some naturally occurring coumarins such as Novobiocin **1** and Clorobiocin **2** were known as an unprecedented class of antibiotics, but they have several limitations, that is, weak activity towards Gram-negative bacteria, poor water solubility besides some side effects.[2]

In recent years, some studies have shown that coumarin incorporated with some nitrogen-containing heterocyclic moieties such as azetidine, thiazolidine, thiazole and oxadiazole not only significantly increases the antimicrobial efficiency but also broadens their antimicrobial spectrum.[3–6] Recently a report has been cited in literature which has suggested that incorporation of a 1,2,4-triazole moiety in coumarin is very essential for antimicrobial activity.[7] In view of this, we chose to incorporate the 1,2,3-triazole moiety into chromenone to obtain a new category of triazolylchromenones. 1,2,3-Triazoles are reported to possess a wide range of biological activities such as anti-HIV,[8,9] antimicrobial,[10] anticoccidiostats,[11] anticonvulsant,[12] antimalarial,[13] antiviral[14] and antimycobacterial.[15] Recent studies on 1,2,3-triazoles have revealed that the hydrogen bonding and dipole interactions of the triazole core can favour their binding to biomolecular targets and hence results in improving their solubility.[16,17] Triazoles have been used to improve the pharmacokinetic properties of the desired drug. For example, cephalosporins endowed with good oral availability were obtained by linking the triazole moiety to the cephalosporin core **3**.[18] Besides 1,2,3-triazoles, heterocyclic amines like morpholine, thiomorpholine, piperidine, thiazolidine-2, 4-dione, pyrrolidine piperazine, isoindoline-1,3-dione and their derivatives are also known to be present in many biologically[19–24] and commercially important compounds and play a key role in terms of activities of the resulting compounds. Many examples of

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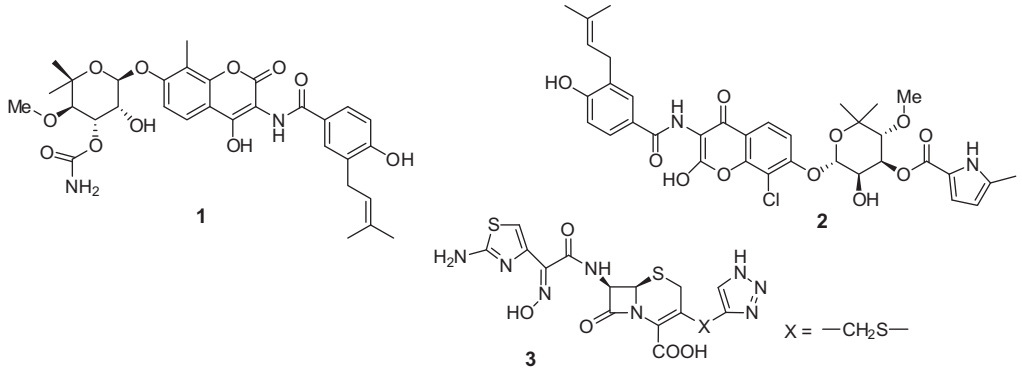
biologically active coumarins containing these heterocyclic amines have been cited in the literature.[25–33] Prompted by the above observations and in continuation to our research for finding new and effective antimicrobial agents, we envisioned, that incorporation of 1,2,3-triazole, heterocyclic amines and coumarin along with a lipophilic spacer in a single molecular framework, might result in compounds having potential antimicrobial activities. Alkyl chains when used as linkers, can introduce lipophilicity in the molecule, which is very essential to cross the cell membrane and to reach the target. Therefore we designed and synthesized a small library of new triazolylchromenones (**7a**–**d**) and (**10a**–**h**) containing alkyl chain as linkers using click reaction (Scheme 1). 7-Hydroxy-4-methyl-2*H*-chromen-2-one was synthesized readily via Pechmann condensation using resorcinol and ethyl acetoacetate.[34]

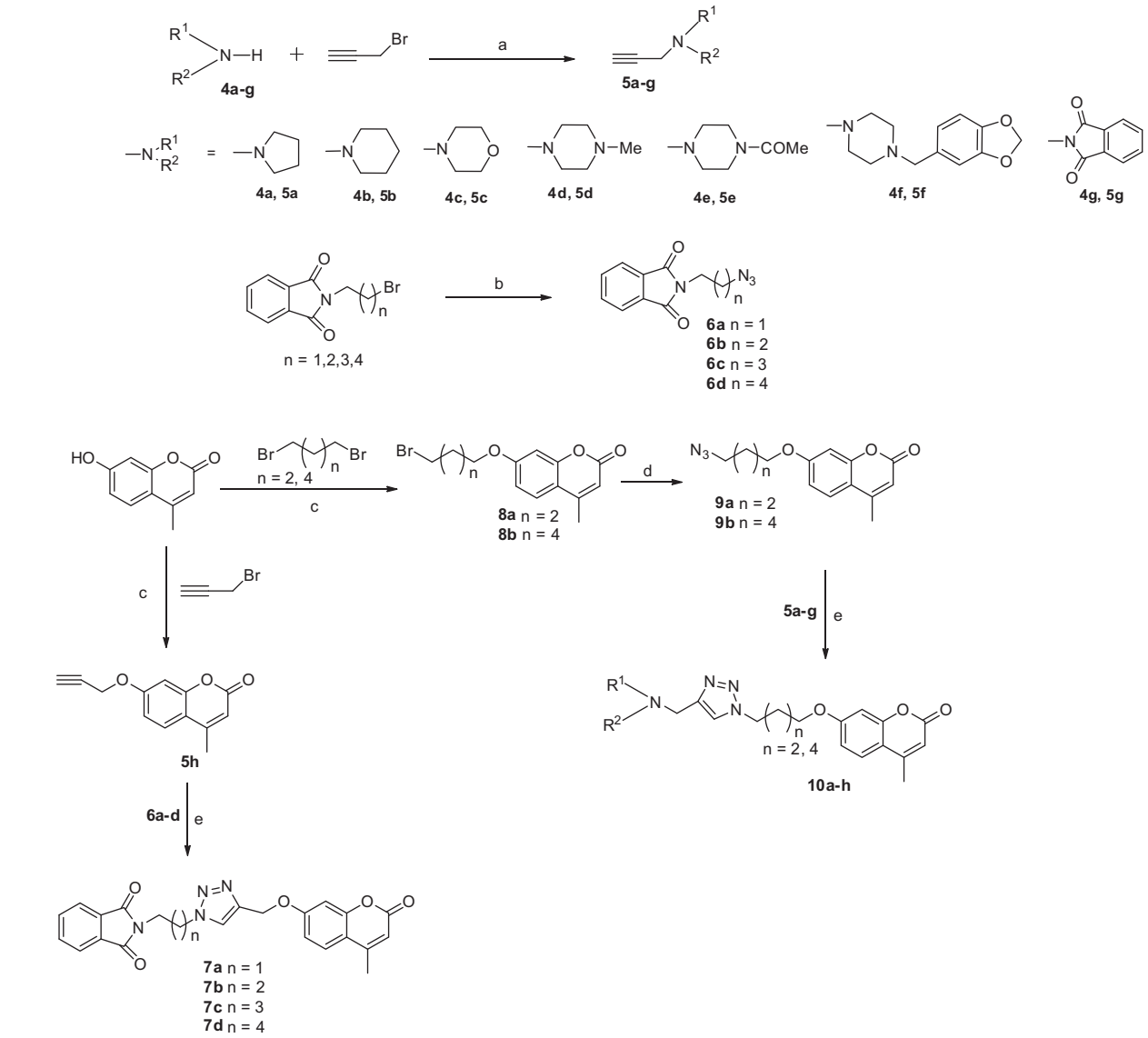


7-Hydroxy-4-methyl-2*H*-chromen-2-one was reacted with 1,4-dibromobutane or 1,6-dibromohexane under controlled conditions to give 7-(4-bromobutyloxy)-4-methyl-2*H*-chromen-2-one[35] (**8a**) and 7-(6-bromohexyloxy)-4-methyl-2*H*-chromen-2-one (**8b**), respectively. Compounds **8a** and **8b** were then converted to their corresponding azides[36] **9a** and **9b**. Required alkynes were then synthesized using propargyl bromide and heterocyclic amines **4a**–**g** to obtain corresponding propargyl amines **5a**–**g** (see SI). Finally compound **10a** was synthesized from 7-(6-azidohexyloxy)-4-methyl-2*H*-chromen-2-one (**9b**) and 1-(prop-2-yn-1-yl)pyrrolidine (**5a**) using the 'click reaction' in 87% yield (Scheme 1, Table 1).[37] The main evidence for the formation of compound **10a** came from the absence of absorption band at 2096 cm^{-1} in IR spectrum, for the azido functionality indicating that azide had been consumed completely in the reaction. This observation was also supported by its ^{1}H NMR spectrum which showed a singlet for triazole proton at δ 7.54 ppm, thus confirming its formation. Also characteristic singlets at δ 6.11 ppm and δ 2.38 ppm were observed for the C-3 olefinic proton and C-4 methyl group of the pyrone ring. The C-4 methyl carbon appeared at δ 18.6 ppm in the ^{13}C NMR spectrum. A multiplet at δ 2.63–2.60 ppm for four protons was due to pyrrolidine protons and singlet at δ 3.80 ppm for methylene protons (>N—CH_2—C=) linking pyrrolidine with triazole. Further the ^{1}H NMR spectrum displayed two triplets in the downfield region at δ 4.34 ppm and δ 3.98 ppm, each integrating for two protons, assigned to methylenes attached to oxygen of coumarin unit and nitrogen of triazole unit respectively. Methylene protons of the alkyl chain and pyrrolidine nucleus were observed as multiplets in the upfield region between δ 1.97 and 1.39 ppm. Its mass spectrum displayed (M^++1) peak at 411 which is in accordance with its molecular formula $C_{23}H_{30}N_4O_3$.

Azides (**6a**–**d**) were synthesized from the reaction of corresponding *N*-(bromoalkyl)isoindoline-1,3-dione with sodium azide in DMF and spectras were comparable to those reported in literature.[38–40] Compound **7a** was synthesized from 4-methyl-7-(prop-2-ynyloxy)-2*H*-chromen-2-one (**5h**) and 2-(2-azidoethyl)isoindoline-1,3-dione (**6a**) in 86% yield, exploiting again the click reaction[41] (Scheme 1, Table 2). The absorption band at 2096 cm^{-1} for the azido functionality was absent in the IR spectrum of compound **7a**. ^{1}H NMR spectrum displayed a singlet for triazole proton at δ 7.90 ppm, thus confirming its presence. The presence of absorption bands at 1772 and 1708 cm^{-1} in IR spectrum and double doublets at δ 7.86–7.85 ppm and δ 7.76–7.74 ppm each integrating for two protons indicated the presence of isoindoline-1,3-dione moiety. ^{1}H NMR spectra also displayed a singlet in the downfield region at δ 5.15 ppm which was due to methylene linking chromen-2-one with triazole nucleus while two triplets at δ 4.64 ppm and at δ 4.02 ppm each integrating for two protons were assigned to methylenes of the alkyl chain attached to nitrogen atom of triazole (>NCH_2) and isoindoline-1,3-dione moieties, respectively. In its mass spectrum (M^++1) peak was observed at *m*/*z* 431 corresponded to its molecular formula, $C_{23}H_{18}N_4O_5$. Similarly compounds **10b**–**h** and **7b**–**d** were synthesized and characterized on the basis of their detailed spectral studies.

The newly synthesized triazolylchromenones **10a**–**h**, **7a**–**d** and azido precursors **9a**,**b** were screened for their in vitro antibacterial activity against three Gram-positive bacteria *Staphylococcus aureus* MTCC 096, *Bacillus subtilis* MTCC 441 and *Staphylococcus epidermis* MTCC 435 as well as four Gram-negative bacteria *Escherichia coli* MTCC 443, *Pseudomonas aeruginosa* MTCC 424, *Salmonella typhi* MTCC 733, and *Klebsiella pneumoniae* MTCC 432. They were also evaluated for their in vitro antifungal studies against *Aspergillus niger* MTCC 282, *Aspergillus fumigatus* MTCC 343, *Aspergillus flavus* MTCC 277, and *Candida albicans* MTCC 227.

Cup plate method was employed for the determination of antibacterial and antifungal activity[42,43] of the synthesized compounds at 100 μg/mL concentration. Inocculated müller Hinton agar for bacteria and sabouraud dextrose agar for fungi was poured onto the sterilized petri dishes (25–30 mL: each petri dish). The poured material was allowed to set (30 min) and thereafter the 'CUPS' (08 mm diameter) was made by punching into the agar surface with a sterile cork borer and scooping out the punched part of the agar. The test solution (0.1 mL) was added into the cups with the help of a micro pipette. The plates were incubated at 37 °C and the results were recorded for antibacterial activity after 14 h and for antifungal activity after 30 h. The test solution was



a = K_2CO_3 /DMF/rt, b = NaN_3 /K_2CO_3 /DMF/80 °C, c = K_2CO_3 /acetone/reflux, d =NaN_3 /DMF/120 °C,
e = $CuSO_4.5H_2O$/NaAsc/THF:H_2O(3:1)/rt.

Scheme 1. Synthesis of triazolylchromenones **10a–h** and **7a–d**.

prepared using DMSO as solvent. Clinically antimicrobial drugs Ciprofloxacin and Miconazole were used as the reference drugs and DMSO was used for blank. Each experiment was carried out in triplicate and the results were recorded as the average diameter of inhibition zones[44,45] of bacterial or fungal growth around the disks in mm. The results for antimicrobial studies depicted in Tables 3 and 4 revealed that the tested compounds displayed variable inhibitory effects on the growth of the bacterial and fungal pathogens. In general, compounds were found to exhibit an excellent activity against most of the fungal pathogens while moderate to good activity was displayed against the various bacterial pathogens. Most of the compounds, exhibited activity comparable to standard against *A. fumigatus*, *A. flavus* and *C. albicans*. Amongst all, **7d** containing phthalimide moiety at the end of the lipophilic chain (C5) was strikingly the most active against *A. fumigatus*. Another compound of the phthalimide series **7a** having (C2) alkyl chain was found to be the most active against *C. albicans*. Compounds **7a** and **7d** could be taken up further for suitable modifications and for the development of more promising analogues. Notably compounds **10g** and **10h** which also contained phthalimide but at a slightly different position have also exhibited significant actvity against *A. fumigatus*. The results indicated the importance of the phthalimide nucleus and its effect on biological properties. Furthermore, compounds containing secondary amines **10a–f** have also shown reasonably good activity against *A. fumigatus* and *C. albicans*. One of the interesting result from the Table 3 is the activity displayed by the azidoalkoxy derivatives. Both **9a** and **9b** have shown promising activity against fungal strains *A. fumigatus*, *C. albicans* and *A. flavus*. Against, the bacterial strains compounds **10a–f** have shown better activity as compared to phthalimide containing **7a–d** and **10g–h**. The intermediate **9b** (C6 alkyl chain) was also found to exhibit good activity against *S. aureus*. Compound **10c** containing morpholine moiety has shown promising activity against *B. subtilis*, *P. aeruginosa* and *K. pneumonia* while **10e** containing *N*-acetylpiperazine moiety was found to possess significant activity against various bacterial strains and especially it showed comparable activity to that of standard against *P. aeruginosa*. The above results therefore suggest that

Table 1
Synthesis of **10a–h** from the reaction of **5a–g** with **9a,b**

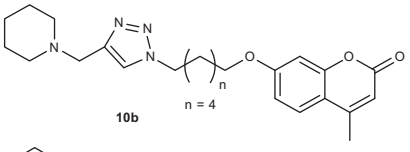
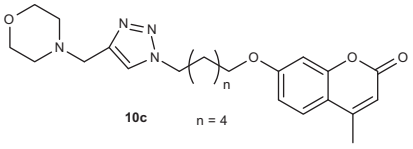
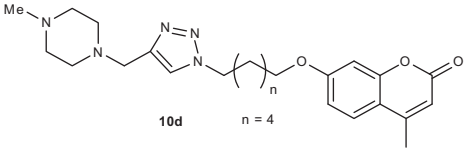
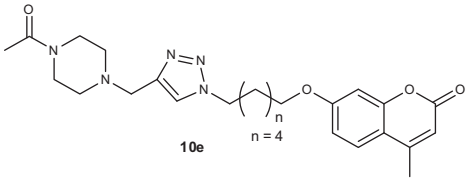
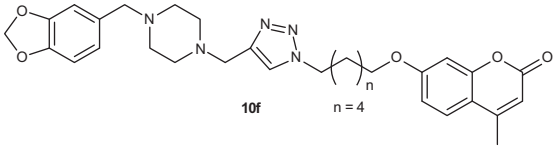
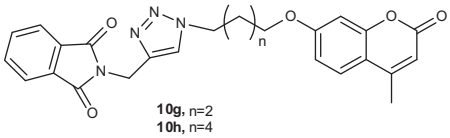
Product	Yield (%)	Mp (°C)
10a n = 4	87	Oil
 **10b** n = 4	86	52–54
 **10c** n = 4	85	64–66
 **10d** n = 4	84	Oil
 **10e** n = 4	85	Oil
 **10f** n = 4	86	Oil
 **10g**, n=2 **10h**, n=4	90 89	75–76 79–80

Table 2
Synthesis of compounds **7a–d** from **5h** and **6a–d**

S. no	Reactant (alkyne)	Reactant (azides)	Product	Yield (%)	Mp (°C)
1	**5h**	**6a**	**7a**	85	122–123
2	**5h**	**6b**	**7b**	88	117–118
3	**5h**	**6c**	**7c**	86	121–122
4	**5h**	**6d**	**7d**	84	124–125

different substituents, type of linkers and the length and position of aliphatic chains markedly influence the antimicrobial efficacy of the compounds.

A molecule to be a probable drug, besides having a good biological activity, must also have optimal pharmacokinetic properties. To assess the pharmacokinetic profile of synthesized molecules, we used, well validated in silico tools: Osiris, Advanced Chemistry Development (ACD) and Catalyst.[46–50] These tools have been

Table 3
Antifungal evaluation of **10a–h**, **7a–d** and **9a–b** (zone of inhibition in mm) all values are mean of three independent experiments. Zone of inhibition is measured by vernier caliper.

Compound	*A. niger*	*A. fumigatus*	*A. flavus*	*C. albicans*
10a	12.5 (±0.70)	18.5 (±0.70)	14.7 (±0.98)	14.4 (±0.63)
10b	11.9 (±1.27)	18.8 (±1.13)	12.5 (±0.70)	14.0 (±1.41)
10c	12.9 (±1.34)	16.9 (±1.27)	16.8 (±1.13)	13.5 (±2.12)
10d	14.0 (±1.41)	18.4 (±0.63)	14.4 (±0.56)	13.6 (±0.91)
10e	12.7 (±1.06)	18.2 (±1.76)	12.9 (±1.34)	14.7 (±0.98)
10f	11.9 (±1.27)	20.6 (±0.91)	15.0 (±1.41)	16.8 (±1.20)
10g	12.8 (±1.20)	19.0 (±1.41)	13.8 (±1.13)	16.5 (±0.70)
10h	13.9 (±1.27)	18.5 (±0.70)	15.2 (±1.69)	14.9 (±1.34)
7a	14.4 (±1.97)	19.5 (±2.12)	12.5 (±0.70)	20.2 (±1.69)
7b	13.2 (±1.76)	21.3 (±1.90)	16.4 (±0.56)	18.6 (±0.84)
7c	13.5 (±2.12)	18.9 (±1.34)	14.8 (±1.20)	16.6 (±0.91)
7d	12.3 (±1.90)	23.4 (±1.97)	14.5 (±0.77)	16.9 (±1.27)
9a	13.1 (±1.55)	18.6 (±0.84)	13.6 (±0.91)	19.3 (±1.83)
9b	13.8 (±1.31)	19.0 (±1.48)	16.4 (±0.63)	18.7 (±1.06)
Miconazole	18	15	17	19

Table 4
Antibacterial activities of the compounds **10a–h**, **7a–d** and **9a**,**b** (zone of inhibition in mm). All values are mean of three independent experiments. Zone of inhibition is measured by vernier caliper.

Compound	*S. aureus*	*B. subtilis*	*S. epidermis*	*E. coli*	*P. aeruginosa*	*S. typhi*	*K. pneumoniae*
10a	13.6 (±0.91)	14.0 (±1.41)	14.0 (±1.41)	11.7 (±1.06)	14.8 (±0.56)	15.2 (±0.28)	14.4 (±0.63)
10b	12.9 (±1.27)	12.6 (±0.91)	13.1 (±1.55)	12.8 (±1.20)	13.2 (±0.84)	14.4 (±0.56)	12.9 (±1.27)
10c	11.7 (±1.06)	15.4 (±0.56)	13.4 (±0.63)	14.4 (±0.56)	14.5 (±1.06)	13.5 (±0.77)	14.6 (±0.84)
10d	12.5 (±0.77)	12.4 (±0.56)	14.6 (±0.84)	14.7 (±1.06)	13.9 (±0.63)	13.1 (±0.21)	12.4 (±0.63)
10e	14.4 (±0.63)	12.4 (±0.63)	14.8 (±1.13)	13.3 (±0.49)	17.1 (±0.77)	12.1 (±0.14)	14.4 (±0.56)
10f	14.0 (±1.41)	12.5 (±0.70)	11.9 (±1.34)	12.3 (±0.42)	14.9 (±0.63)	14.1 (±0.14)	13.9 (±1.27)
10g	12.8 (±1.20)	12.9 (±1.34)	12.4 (±0.63)	12.7 (±0.98)	13.9 (±1.34)	12.2 (±0.28)	12.4 (±0.63)
10h	12.9 (±1.34)	12.8 (±1.13)	12.6 (±0.84)	13.4 (±0.63)	12.8 (±0.56)	12.4 (±0.56)	12.9 (±1.27)
7a	13.0 (±1.48)	10.5 (±0.70)	12.4 (±0.63)	14.5 (±2.12)	13.0 (±0.70)	11.9 (±0.07)	13.3 (±0.42)
7b	12.8 (±1.13)	12.5 (±0.77)	11.4 (±0.63)	12.6 (±0.84)	13.1 (±0.77)	12.3 (±0.42)	14.4 (±0.56)
7c	12.6 (±0.84)	11.6 (±0.91)	12.8 (±1.13)	12.4 (±0.56)	14.0 (±1.41)	12.5 (±0.77)	11.6 (±0.49)
7d	12.5 (±0.70)	13.6 (±0.84)	12.6 (±0.84)	12.5 (±0.70)	12.9 (±0.63)	12.7 (±1.06)	12.6 (±0.84)
9a	13.5 (±0.70)	11.3 (±0.42)	12.7 (±1.06)	13.0 (±1.41)	12.8 (±0.56)	12.9 (±1.27)	12.5 (±0.77)
9b	15.7 (±1.06)	12.5 (±0.70)	13.5 (±0.70)	14.3 (±1.90)	13.0 (±0.70)	12.4 (±0.63)	14.5 (±0.70)
Ciprofloxacin	19	18	16	20	18	19	17

Table 5
Pharmacokinetic parameters (Catalyst, Chemaxon and Osiris softwares)

Molecules	nHba	nHbd	nrotb	TPSA	MW	*c*Log*P*	Druglikeness	Drug score
10a	7	0	10	69.4	410	3.14	−22.5	0.23
10b	7	0	10	69.4	424	3.45	−22.0	0.21
10c	8	0	10	78.7	426	2.25	−23.7	0.24
10d	8	0	10	72.7	439	2.37	−18.6	0.24
10e	9	0	10	89.7	467	2.4	−19.1	0.22
10f	10	0	12	91.1	559	3.84	−19.0	0.15
10g	9	0	8	103.6	458	2.50	−16.8	0.17
10h	9	0	8	103.6	458	3.43	−33.2	0.14
7a	9	0	6	103.6	430	1.57	−25.3	0.19
7b	9	0	7	103.6	444	2.04	−25.2	0.18
7c	9	0	8	103.6	458	2.5	−26.3	0.17
7d	9	0	9	103.6	472	2.96	−28.2	0.16
9a	6	0	6	65.8	273	2.49	−21.7	0.27
9b	6	0	8	65.8	301	3.42	−25.9	0.24
Moxifloxacin	7	2	4	82.1	401	0.58	1.6	0.32
Ciprofloxacin	6	2	3	72.8	331	0.13	2.07	0.39
Miconazole	3	0	6	27.05	414	4.93	6.33	0.49
Fluconazole	7	1	5	81.65	306	−0.21	−1.13	0.46
Amp B	18	13	3	319.6	924	2.38	−0.14	0.26

Amp B is standard drug Amphotericin B.

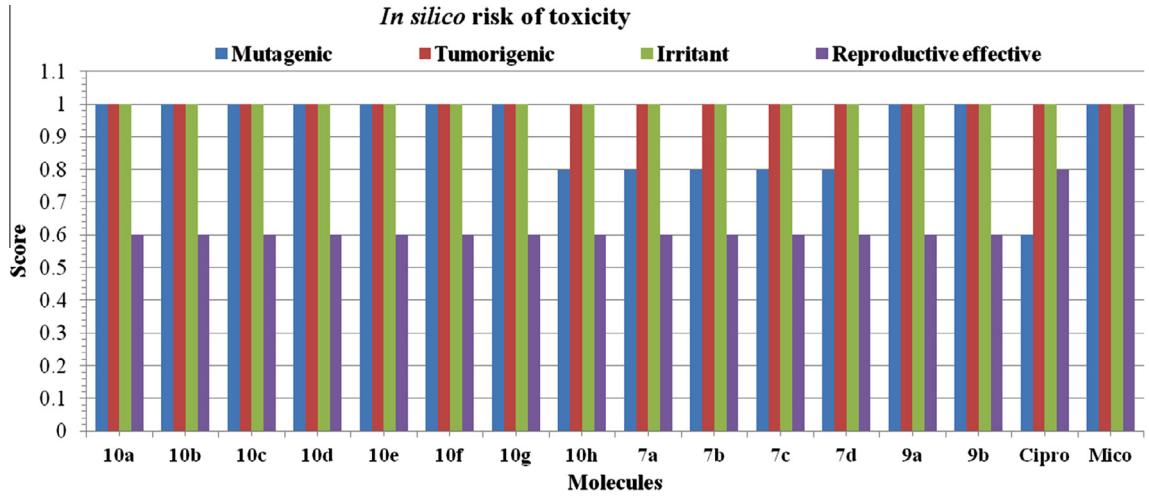


Figure 1. In silico drug safety analysis of molecules by using Osiris program. High score is indicator for safe drugs (score 1 is highly safe drug indicator, score 0.8 is low risk indicator and score 0.6 or less is moderate or high risk indicator). Cipro and mico are standard drug ciprofloxacin and miconazole, respectively.

validated with more than 7000 drug molecules available in market. As these compounds are considered for oral delivery, they were submitted to the analysis of Lipinski 'rule of five', druglikeness and drug score by using Catalyst, ACD and Osiris programme (Table 5).[46–48] The analysis of theoretical toxicity risks for the all derivatives using OSIRIS and ACD program shows that all

Table 6
In silico acute toxicity evaluation on mouse, rat and water flea (*Daphnia magna*)

Molecules	Acute toxicity: LD_{50} (mg/kg body weight)						OECD oral acute toxicity category	Aquatic toxicity LC_{50}
	Mouse IP	Mouse Oral	Mouse IV	Mouse SC	Rat IP	Rat oral	(probability)	(mg/L)
10a	340	800	49	250	350	990	4 or 5 (75%)	2
10b	300	720	41	240	300	1100	4 or 5 (75%)	2
10c	350	750	50	250	360	860	3 or 4 or 5 (86%)	2.1
10d	230	450	43	220	170	580	3 or 4 or 5 (89%)	3.8
10e	460	1100	53	300	390	980	4 or 5 (76%)	2.1
10f	270	340	35	160	75	320	3 or 4 (74%)	0.57
10g	1100	1200	57	160	490	1000	4 or 5 (74%)	0.54
10h	1000	1000	46	190	630	1200	4 or 5 (78%)	0.43
7a	1200	790	92	280	590	830	3 or 4 or 5 (87%)	0.27
7b	1400	830	70	190	530	870	3 or 4 or 5 (87%)	0.48
7c	1200	650	60	260	530	890	4 or 5 (76%)	0.27
7d	940	850	53	260	640	1100	4 or 5 (76%)	0.26
9a	110	260	44	69	390	690	3 or 4 or 5 (91%)	1.5
9b	99	230	32	60	380	770	4 or 5 (76%)	1.1
Ciprofloxacin	930	3500	120	1400	140	2600	4 or 5 (88%)	8.7
Miconazole	240	940	61	550	480	1400	4 or 5 (95%)	0.21
Moxifloxacin	660	1700	81	1100	100	1400	4 or 5 (86%)	1.4

Aquatic toxicity was calculated on water flea *Daphnia magna*. OECD is the Organisation for Economic Co-operation and Development. LC and LD are lethal concentration and lethal dose, respectively.

compounds were moderate to less toxic and can be used as therapeutic molecules (Fig. 1 and Table 6). We calculated *c*Log*P*, number of hydrogen bond acceptor (nHbd), number of hydrogen bond donor (nHba), (6–9), nHbd (0) and rotb (6–10) were almost similar to commercial drugs (Table 5). Finally, we evaluated all these molecules as potential drugs by calculating druglikeness and drug score. Druglikeness, which is related to the similarity with trade drugs were in the range of −16.8 to −28.2. Drug score of all the synthesized molecules were in the range of 0.14–0.27, whereas score of market available antibiotic amphotericin B, fluconazole, miconazole, ciprofloxacin and moxifloxacin were 0.26, 0.46, 0.49, 0.39 and 0.26, respectively (Table 5). We also calculated in silico toxicity of synthesized molecules by using Advanced Chemistry Development (ACD) software (Table 6). We measured LD_{50} on rodents and aquatic toxicity was measured on water flea. Most of the molecules were in 4 or 5 OECD oral acute toxicity category with high probability and having less aquatic toxicity (Table 6). All molecules except **10f** demonstrate moderate to less toxicity and showed the best values of drug-score, which suggest the need of further investigation on these molecules.

In summary, we have developed a convenient methodology by incorporating 2*H*-chromen-2-one, 1,2,3-triazole and heterocyclic amines in a single molecular frame and synthesized 12 novel triazolylchromenones in excellent yields. All the synthesized compounds were fully characterized on the basis of their detailed spectral studies. Phthalimide containing coumarin derivatives showed remarkable antifungal activity against various fungal strains. Compounds **7d** and **7a** possessed exceptional activity against *A. fumigatus* and *C. albicans*, respectively. Surprisingly phthalimide incorporated compounds showed only moderate activity against bacterial strains whereas morpholine and piperazine containing compounds **10c** and **10e** showed noticeable activity against various bacterial pathogens. Therefore, it can be concluded that substituent and, length and position of alkyl spacer has profound effect on the antimicrobial potency of these compounds. Compounds **10a**–**e** could be considered as the best candidates for further investigation. Since these compounds present the overall best parameters including: (a) promising activity against microbial strains, (b) low or moderate toxicity risks in in silico analysis, (d) good oral bioavailability according to the Lipinski 'rule of five' and (e) better druglikeness and drug-score values, nearly similar or better than some commercial antimicrobials such as amphotericin B. Compounds **7a** and **7d** could also be used for further studies as they also satisfy most of the conditions stated above except that they have slightly less drug score values.

In silico pharmacokinetic study showed that most of the compounds have the best drug score values with a lower or moderate toxicity risk, which points for further in vivo and ex vivo exploration. The methodology could be extended to synthesize more potent derivatives using different substituents and different linkers and their effect on different bacterial and fungal strains could be studied further to develop a suitable pharmacophore.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.02.027.

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35. *Representative procedure for the synthesis of 7-(4-bromobutyloxy)-4-methyl-2H-chromen-2-one (**8a**):* To a solution of 7-hydroxy-4-methyl-2*H*-chromen-2-one (5.67 mmol) in acetone (25 mL), oven dried potassium carbonate (17.02 mmol) and 1,4-dibromobutane (17.02 mmol) were added and the reaction mixture was refluxed for 24 h. The mixture was then filtered through celite and the solvent was evaporated under vacuum. The solid residue so obtained was purified by column chromatography to give **8a** as white crystalline solid; mp: 49–50 °C; IR ν_{max} (KBr): 2951, 1720, 1614, 1388, 1293, 1201, 1148, 1071, 1015, 847, 638 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ_H 7.48 (d, 1H, J = 8.7 Hz), 6.84–6.82 (dd, 1H, J = 8.6 and 2.7 Hz), 6.77 (d, 1H, J = 2.2 Hz), 6.11 (s, 1H), 4.04 (t, 2H, $-OCH_2$, J = 5.9 Hz), 3.48 (t, 2H, $-CH_2Br$, J = 6.4 Hz), 2.38 (s, 3H), 2.09–2.05 (m, 2H), 1.99–1.95 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ_C 161.8 (C-2, —C═O), 161.1, 155.1, 152.5, 125.5, 113.5, 112.4, 111.8, 101.2, 67.4 ($-OCH_2$), 33.3 ($-CH_2Br$), 29.3, 27.6, 18.6. Mass spectral data, TOF ES+ m/z (%): 312 (M^++2).
36. *Representative procedure for the synthesis of 7-(4-azidobutyloxy)-4-methyl-2H-chromen-2-one (**9a**):* To a stirred solution of sodium azide (9.64 mmol) in DMF (10 mL), a solution of **8a** (3.21 mmol) dissolved in 10 mL of DMF was added. The contents were heated at 120 °C for 10 h. The reaction mixture was then quenched with ice cold water and extracted with ethylacetate thrice. The organic extracts were combined, dried over anhydrous sodium suphate, filtered, and concentrated under reduced pressure to give **9a** as cream solid; IR ν_{max} (KBr): 3078, 2936, 2861, 2096, 1720, 1612, 1424, 1202, 1071, 1014, 847 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ_H 7.49 (d, 1H, J = 8.8 Hz), 6.86–6.83 (dd, 1H, J = 8.8 and 2.2 Hz), 6.79 (d, 1H, J = 2.2 Hz), 6.13 (s, 1H), 4.05 (t, 2H, J = 6.6 Hz, $-OCH_2$), 3.38 (t, 2H, J = 6.6 Hz, $-CH_2N_3$), 2.39 (s, 3H, $-CH_3$), 1.93–1.88 (m, 2H), 1.84–1.78 (m, 2H). ^{13}C NMR (δ, $CDCl_3$, 100 MHz): 161.8 (—C═O), 161.0, 155.1, 152.0, 125.2, 112.9, 112.2, 111.5, 101.0, 68.3 ($-OCH_2$), 51.2 ($-CH_2N_3$), 28.7, 26.5, 18.6. Mass spectral data, TOF ES+ m/z (%): 274 (M^++1). Anal. Calcd for $C_{14}H_{15}N_3O_3$: C, 61.53; H, 5.53; N, 15.38. Found: C, 61.81; H, 5.49; N, 15.36.
37. *Representative procedure for 4-methyl-7-(6-(4-(pyrrolidin-1-ylmethyl)-1H-1,2,3-triazol-1-yl)hexyloxy)-2H-chromen-2-one (**10a**):* To a vigorously stirred solution of **9b** (3.32 mmol) in THF/H_2O (3:1) (10 mL), **5a** (3.32 mmol) was added. The reaction was initiated by the addition of $CuSO_4{\cdot}5H_2O$ (0.33 mmol) and sodium ascorbate (1.32 mmol). The coloured suspension that formed was stirred at room temperature for 12–16 h. Progress of the reaction was monitored on TLC. After completion of the reaction, ice-cold water was added and the aqueous layer was extracted twice with CH_2Cl_2. The combined organic extracts were dried, evaporated under reduced pressure and purified by using a very short column of silica gel to afford pure **10a** as yellow oil; IR ν_{max} (film): 2924, 2853, 1719, 1612, 1459, 1388, 1369, 1293, 1201, 1145, 1071, 1016, 847 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ_H 7.54 (s, 1H, triazole), 7.47 (d, 1H, J = 9.1 Hz), 6.83–6.81 (dd, 1H, J = 8.8 and 3.6 Hz), 6.76 (d, 1H, J = 2.2 Hz), 6.11 (s, 1H), 4.34 (t, 2H, J = 7.3 Hz, $-NCH_2$), 3.98 (t, 2H, J = 6.6 Hz, $-OCH_2$), 3.80 (s, 2H, >N—CH_2—C═), 2.63–2.60 (m, 4H, 2× $-NCH_2$, pyrrolidine), 2.38 (s, 3H, $-CH_3$), 1.97–1.89 (m, 2H), 1.81–1.77 (m, 4H), 1.51–1.47 (m, 4H), 1.41–1.39 (m, 2H). ^{13}C NMR (δ, $CDCl_3$, 100 MHz): 162.0 (—C═O), 161.4, 155.2, 152.6, 143.5, 125.2, 122.3, 113.4, 112.6, 111.8, 101.3, 68.2 ($-OCH_2$), 53.9, 50.6, 50.1 30.2, 28.7, 26.2, 25.4, 23.4, 18.6. Mass spectral data, LCMS m/z (%): 411 (M^++1). Anal. Calcd for $C_{23}H_{30}N_4O_3$: C, 67.29; H, 7.37; N, 13.65. Found: C, 67.31; H, 7.35; N, 13.66.
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41. *Representative procedure for 7-(1-(2-isoindoline-1,3-dione-2-ylethyl)-1H-1,2,3-triazol-4-yl)methyloxy-4-methyl-2H-chromen-2-one (**7a**):* To a vigorously stirred solution of the 2-(2-azidoethyl)isoindoline-1,3-dione (1.0 equiv) in THF/H_2O (3:1) (10 mL) 4-methyl-7-(prop-2-ynyloxy)-2*H*-chromen-2-one (1.0 equiv) was added. The reaction was initiated by the addition of $CuSO_4{\cdot}5H_2O$ (0.1 equiv) and sodium ascorbate (0.4 equiv). The coloured suspension that formed was stirred at room temperature for 12–16 h. Progress of the reaction was monitored on TLC. After completion of the reaction, ice-cold water was added and the aqueous layer was extracted twice with CH_2Cl_2. The combined organic extracts were dried, evaporated under reduced pressure and purified by using a short column of silica gel to afford **7a** as white solid; mp: 122–123 °C. IR ν_{max} (KBr): 2926, 1772, 1717, 1708, 1614, 1437, 1396, 1258, 1135, 1038, 886, 794, 719 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ_H 7.90 (s, 1H, triazole), 7.86–7.85 (dd, 2H, J = 6.8 and 2.4 Hz), 7.76–7.74 (dd, 2H, J = 8.7 and 2.6 Hz), 7.50 (d, 1H, J = 8.6 Hz), 6.91–6.87 (m, 2H), 6.10 (s, 1H), 5.15 (s, 2H, $O-CH_2$), 4.64 (t, 2H, J = 5.4 Hz, >N–CH_2), 4.02 (t, 2H, J = 5.8 Hz, $(CO)_2N-CH_2$), 2.34 (s, 3H, $-CH_3$). ^{13}C NMR (δ, DMSO-d_6, 100 MHz): 165.6 (—C═O), 159.3 (—C═O), 158.6, 153.1, 151.1, 132.5, 132.0, 129.8, 124.3, 123.2, 121.4, 111.8, 110.9, 109.8, 99.9, 60.1 ($O-CH_2$), 45.9, 38.2, 18.7. Mass spectral data, TOF ES+ m/z (%): 431 (M^++1). Anal. Calcd for $C_{23}H_{18}N_4O_5$: C, 64.18; H, 4.22; N, 13.02. Found: C, 64.22; H, 4.19; N, 13.05.
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