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Synthesis and evaluation of small libraries of triazolylmethoxy chalcones, flavanones and 2-aminopyrimidines as inhibitors of mycobacterial FAS-II and PknG

Namrata Anand [a], Priyanka Singh [a,b], Anindra Sharma [a], Sameer Tiwari [c], Vandana Singh [c], Diwakar K. Singh [c], Kishore K. Srivastava [c], B. N. Singh [c], Rama Pati Tripathi [a,*]

[a] Medicinal & Process Chemistry Division, Central Drug Research Institute, CSIR, PO Box 173, Chattar Manzil, Mahatma Gandhi Marg, Lucknow 226001, India
[b] National Institute of Pharmaceutical Education and Research, Raebareli 229010, India
[c] Microbiology Division, Central Drug Research Institute, CSIR, PO Box 173, Chattar Manzil, Mahatma Gandhi Marg, Lucknow 226001, India

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ABSTRACT

A synthetic strategy to access small libraries of triazolylmethoxy chalcones **4**{1–20}, triazolylmethoxy flavanones **5**{1–10} and triazolylmethoxy aminopyrimidines **6**{1–17} from a common substrate 4-propargyloxy-2-hydroxy acetophenone using a set of different reactions has been developed. The chalcones and flavanones were screened against mycobacterial FAS-II pathway using a recombinant mycobacterial strain, against which the most potent compound showed ~88% inhibition in bacterial growth and substantially induction of reporter gene activity at 100 μM concentration. The triazolylmethoxy aminopyrimdines were screened against PknG of *Mycobaceterium tuberculosis* displaying moderate to good activity (23–53% inhibition at 100 μM), comparable to the action of a standard inhibitor.

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1. Introduction

The concept of 'molecular hybridization' in drug design aims primarily to combat drug resistance and to enrich existing arsenals of anti-infective agents.[1,2] It usually involves the combination of two or more pharmacophores or chemical entities either linked with one another or fused together to create a new molecule.[3] The selection of the pharmacophores is based upon their known bioprofiles, with the hope that the resulting hybrid molecules may exhibit synergistic or additive pharmacological activities.[4,5] The development of efficient synthetic strategies to access such molecules is therefore warranted.

Chalcones are versatile molecular scaffolds in nature and the laboratory, exhibiting numerous beneficial biological activities such as cytotoxicity to pathogenic organisms[6–10] and cancer cells,[11] anti-inflammatory properties,[12] inhibition of key enzymes,[13] and antioxidant behavior.[14,15] Of particular interest to us is the antitubercular activity of natural and synthetic chalcones, via inhibition of FAS-II pathway enzymes such as enoyl-ACP-reductase, β-ketoacyl-ACP reductase and β-hydroxyacyl-ACP-dehydratase (ACP = acyl carrier protein).[4] We sought to augment the activity of chalcones against FAS-II enzymes by pairing them with other pharmacophores, including 1,2,3-triazoles (which have been used in potent anti-TB compounds[16,17] via blocking the biosynthesis of certain bacterial lipids), flavanones (which exhibit a wide range of relevant biological activities[18–23]), and aminopyrimidines (important as inhibitors of different types of kinases[24–26] among many other effects[27–29]). Very recently we have shown a 2-aminopyrimidine derivative to possess potent antitubercular activity.[30] Furthermore, a comparison of the antitubercular activity of triazolylmethoxy chalcones with their cyclic counterparts, the flavanones, was of interest. Figure 1 shows the approach to a small library of the above motifs starting from a common 4-propargyloxy acetophenone precursor. No sophisticated instrumentation, conditions, or reagents are required for the transformations, all of which take place under mild conditions.

The resulting triazolylmethoxy chalcones and flavanones were screened for their antitubercular activity via FasII pathway inhibition while triazolylmethoxy aminopyrimidines were evaluated for mycobacterial serine-threonine protein kinase (STPK) inhibitory activity followed by in vitro evaluation against *M. tuberculosis* $H_{37}Rv$.

2. Results and discussion

2.1. Chemistry

The intermediate 2-hydroxy 4-propynyloxy acetophenone (**1**) was prepared by the reaction of 2,4-dihydroxy acetophenone (**A**)

* Corresponding author.
E-mail address: rpt.cdri@gmail.com (R.P. Tripathi).

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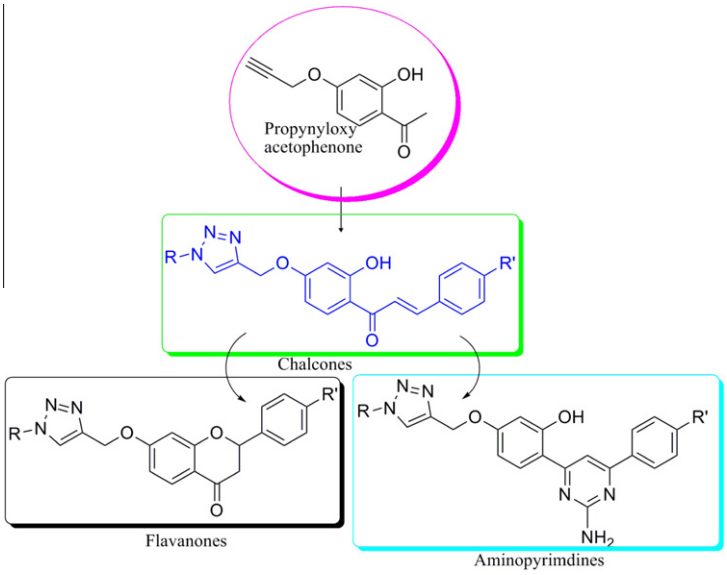
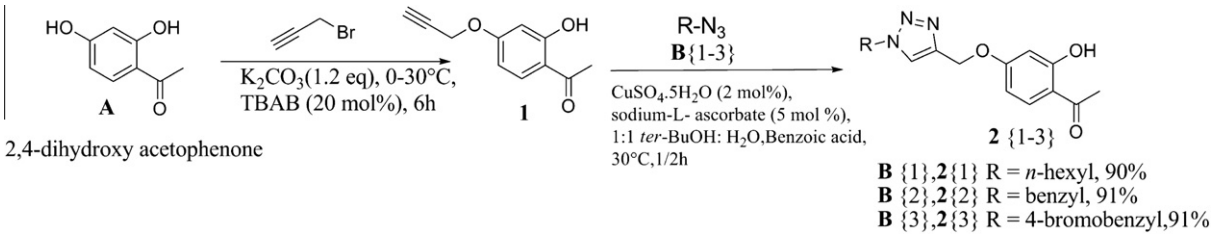


Figure 1. Preparation of three different prototypes from a common scaffold 2-hydroxy-4-propynyloxy acetophenone.

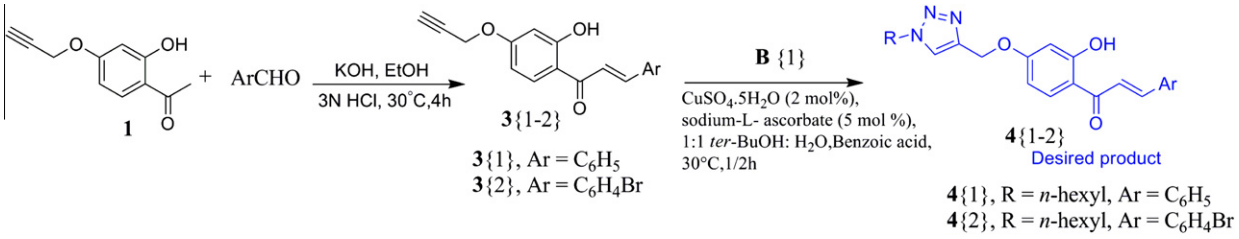


Scheme 1. Preparation of 2-hydroxy-4-(1-araalkyl/alkyl-1*H*-1,2,3-triazol-4-yl)-methoxy acetophenone.

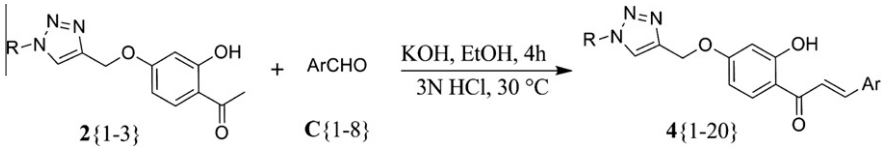
with propargyl bromide, in the presence of anhydrous K_2CO_3 and tetrabutylammonium bromide (20 mol %) in acetone at ambient temperature as recently reported by us[31] in quantitative yield. 2-Hydroxy-4-propynyloxy acotophenone (**1**) was elaborated with azides (**B**){1–3} and aromatic aldehydes (**C**){1–8}in standard transformations to give triazoles **2**{1–3} and triazolylmethoxy chalcones **4**{1–20}. The 'click reaction' of 2-hydroxy 4-propynyloxy acetophenone (**1**) with appropriate azides viz *n*-hexyl azide, benzyl azide and 4-bromobenzyl azide **A**{1–3} at ambient temperature in presence of $CuSO_4.5H_2O$ (2 mol %) and sodium-L-ascorbate (5 mol %) in a mixture of 1:1 *tert*-BuOH:H_2O, led to the formation of respective triazolylmethoxy acetophenones **2**{1–3} regioselectively in very good yields (Scheme 1). The presence of the group 1,2,3-triazole was indicated by a singlet in the ^{1}H NMR spectra at approximately 7.6 ppm, and signals for triazolyl C-4 and C-5 at approximately 143 and 132 ppm, respectively, in ^{13}C NMR.[16,31]

2.1.1. Synthesis of triazolylmethoxy chalcones

There are two possible routes to prepare triazolylmethoxy chalcones, In our experiments with route (a) reaction of propynyloxy



Scheme 2. Preparation of triazolylmethoxy chalcones via route (a).



Scheme 3. Synthesis of 1-[2-hydroxy-4-(1-araalkyl/alkyl-1*H*-1,2,3-triazol-4-yl)-methoxyl phenyl]-3-phenylprop-2-en-1-one.

Table 1
Library of 1-[2-hydroxy-4-(1-araalkyl/alkyl-1*H*-1,2,3-triazol-4-yl)-methoxyl phenyl]-3-phenylprop-2-en-1-ones

Compound	R	Ar	Mp (°C)	Yield (%)
4{1}	*n*-Hexyl	Phenyl	144–146	91
4{2}	*n*-Hexyl	4-Br-phenyl	154–156	92
4{3}	*n*-Hexyl	4-Cl-phenyl	150–153	93
4{4}	*n*-Hexyl	2-Cl,6-F-phenyl	156–158	93
4{5}	*n*-Hexyl	4-OCH_3-phenyl	150–152	93
4{6}	*n*-Hexyl	4-Isopropylphenyl	146–148	92
4{7}	*n*-Hexyl	4-Propargyloxyphenyl	149–152	82
4{8}	*n*-Hexyl	Naphthyl	142–145	92
4{9}	benzyl	Phenyl	144–146	90
4{10}	Benzyl	4-Cl-phenyl	152–154	91
4{11}	Benzyl	2-Cl,6-F-phenyl	156–158	92
4{12}	Benzyl	4-OCH_3-phenyl	148–150	88
4{13}	Benzyl	4-Isopropylphenyl	142–145	91
4{14}	Benzyl	4-Propargyloxyphenyl	150–152	94
4{15}	Benzyl	Naphthyl	151–153	94
4{16}	4-Br-benzyl	Phenyl	146–148	94
4{17}	4-Br-benzyl	4-Br-Phenyl	156–158	93
4{18}	4-Br-benzyl	2-Cl,6-F-phenyl	157–159	92
4{19}	4-Br-benzyl	4-Isopropylphenyl	144–146	93
4{20}	4-Br-benzyl	Naphthyl	150–152	92

acetophenone (**1**) with aromatic aldehydes followed by click reaction of the preformed 1-(2-hydroxy-4-(propynyloxy) aryl)-3-phenylpropen-1-one **3**{1–2} with *n*-hexyl azide **B**{1} gave triazolylmethoxy chalcone **4**{1} in 73% and **4**{2} in 71% yields along with several minor products which could not be isolated and characterized (Scheme 2). With route (b) Claisen-Schmidt condensation of 1-(4-((1-hexyl-1*H*-1,2,3-triazole-4-yl) methoxy)-2-hydroxyphenyl) ethanone **2**{1} with the benzaldehyde **C**{1} in presence of 10% aqueous KOH in ethanol at 30 °C followed by acidification with cold 3 N HCl led to the formation of respective triazolylmethoxy chalcone **4**{1} in 91% isolated yield. No side products were observed during reaction.

Similarly, triazolylmethoxy acetophenones **2**{2–3} were condensed with electron rich and electron deficient aromatic aldehydes **C**{1–8} under the above conditions for different intervals of time to give the respective triazolylmethoxy chalcones **4**{2–20} in good yields (Scheme 3,Table 1). These synthons have been utilized to prepare the corresponding flavanones and 2-aminopyrimidines.

The *trans*-(*E*) geometry of the chalcone double bond was evident by the large olefinic coupling constant between the relevant signals in the ^{1}H NMR spectrum (J = 15 Hz). Details of these synthetic methods and spectroscopic data are given in Supplementary data.

2.1.2. Synthesis of triazolylmethoxy flavanone

The reaction of the above selected triazolylmethoxy chalcones **4**{1}, **4**{2}, **4**{5}, **4**{6}, **4**{9}, **4**{10}, **4**{13}, **4**{16}, **4**{17} and **4**{19} separately with sodium acetate in ethanol and water (1:1) at 70–80 °C for different time intervals gave the respective triazolylmethoxy phenyl chromanone **5**{1–10} in good yields (Scheme 4). The effect of substituents on yields of the products **5**{1–10} is dependent on the nature of substitution in aryl ring at C-3 in the chalcone. In general, presence of electron releasing group **4**{5, 6, 13 and 19} in aryl ring enhanced the yield of products **5**{3, 4, 7 and 10} whereas

Table 2
Synthesized triazolylmethoxy flavanone

Compound	R	R′	Mp (°C)	Yield (%)
5{1}	*n*-Hexyl	Phenyl	120–123	79
5{2}	*n*-Hexyl	4-Br-phenyl	133–135	75
5{3}	*n*-Hexyl	4-OCH_3-phenyl	128–130	85
5{4}	*n*-Hexyl	4-Isopropylphenyl	115–117	82
5{5}	Benzyl	Phenyl	118–120	85
5{6}	Benzyl	4-Cl-phenyl	135–138	50
5{7}	Benzyl	4-Isopropylphenyl	123–125	87
5{8}	4-Br-benzyl	Phenyl	125–127	80
5{9}	4-Br-benzyl	4-Br-phenyl	132–135	60
5{10}	4-Br-benzyl	4-Isopropylphenyl	120–124	88

presence of electron deficient groups **4**{2, 10 and 17} decreased the yield of corresponding flavanones **5**{2, 6 and 9} as compared to unsubstituted **4**{1, 9, and 16} aryl ring (Table 2). Structures of these flavanones were established on the basis of their spectroscopic data and microanalyses. The characteristic benzylic protons (OC*H*) of the dihydrochromanone moiety were visible as *dd* at around δ 5.4 with with J_1 = 3.0 Hz and J_2 = 13.0 Hz; while the two methylene protons of the same were observed as *dd* at two different field strengths at round δ 3.0 (J_1 = 13.0 Hz and J_2 = 17.0 Hz), and at δ 2.87 (J_1 = 3.0 Hz and J_2 = 17.0 Hz) respectively. The other proton and carbon signals were observed as usual.

2.1.3. Synthesis of triazolylmethoxy amino-pyrimidines

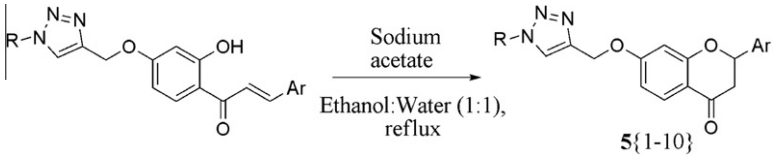
The triazolylmethoxy aminopyrimidines **6**{1–17} were prepared in a simple and straight forward manner by the reaction of their corresponding triazolylmethoxy chalcones **4**{1}, **4**{3–13}, **4**{15–17} and **4**{19–20} with guanidine hydrochloride in presence of NaH in anhydrous DMF as a solvent in moderate to good yields (Scheme 5). It was observed that aromatic substitutents in the chalcones do not have any substantial effect on the yields of the resulting 2-aminopyrimidines (Table 3).

All these compounds were characterized on the basis of their spectroscopic (IR, ESI-MS, ^{1}H NMR and ^{13}C NMR) data and microanalyses. In general, the IR spectra of the compounds exhibited characteristic strong absorbance in the range of 3490–3200 cm^{-1} for the -NH_2 group. In the ^{1}H NMR spectra of these compounds a singlet at around δ 7.80 accounted the triazolyl proton while the two exchangeable NH_2 protons were observed at around δ 6.20.

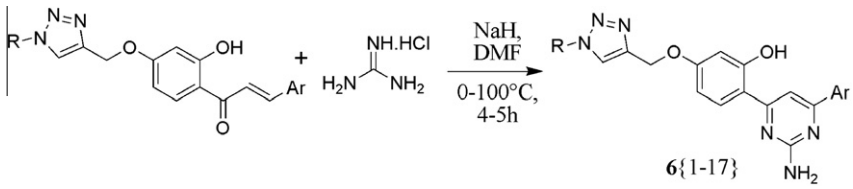
2.2. Biology

2.2.1. FAS-II inhibitory activity of triazolyl methoxy chalcones and flavanones

FAS-II inhibitory activity was analyzed using a recombinant non-pathogenic mycobacterial strain, *Mycobacterium aurum*, which contains the *M. tuberculosis* H_{37}Rv *kas* operon promoter in fusion with an *E. coli lacZ* reporter gene.[32,33] The recombinant strain shows continued expression of the reporter gene under the influence of the *kas* operon promoter during basal conditions, but the promoter responds to the inhibition of FAS-II pathway by inducing the additional quantifiable expression of the reporter gene. The inducibility rendered by the promoter is pathway specific and is not singularly dependent on the selective inhibition of a candidate



Scheme 4. Synthesis of 7-(1-araalkyl-1*H*-1,2,3-triazol-4-yl)methoxy-2-phenylchroman-4-one.



Scheme 5. Synthesis of 2-(2-amino-6-phenylpyrimidine-4-yl)-5-(1-araalkyl/alkyl-1*H*-1,2,3-triazol-4-yl)-methoxy phenol.

Table 3
Synthesized triazolylmethoxy aminopyrimidines

Compound	R	Ar	mp (°C)	Yield (%)
6{1}	*n*-Hexyl	Phenyl	155–156	55
6{2}	*n*-Hexyl	4-Cl-phenyl	192–194	54
6{3}	*n*-Hexyl	2-Cl,6-F-phenyl	185–187	51
6{4}	*n*-Hexyl	4-OCH_3-phenyl	180–182	52
6{5}	*n*-Hexyl	4-Isopropyl phenyl	164–166	59
6{6}	*n*-Hexyl	4-Propargyloxyphenyl	172–175	49
6{7}	*n*-Hexyl	Naphthyl	178–180	56
6{8}	Benzyl	Phenyl	185–187	54
6{9}	Benzyl	4-Cl-phenyl	200–202	51
6{10}	Benzyl	2-Cl,6-F-phenyl	>200	48
6{11}	Benzyl	4-OCH_3-phenyl	180–182	52
6{12}	Benzyl	4-Isopropyl phenyl	178–180	54
6{13}	Benzyl	Naphthyl	196–198	49
6{14}	4-Br-Benzyl	Phenyl	190–193	60
6{15}	4-Br-Benzyl	4-Br-phenyl	210–213	59
6{16}	4-Br-Benzyl	4-Isopropyl phenyl	183–185	56
6{17}	4-Br-Benzyl	Naphthyl	188–190	51

gene product. Notably, the FAS-II pathway in mycobacteria is inhibited by isoniazid (INH), ethionamide (ETH) and thiolactomycin (TLM). INH and ETH target inhA while TLM targets KasA, but all three drugs were shown to induce the reporter gene expression by virtue of their inhibition of the FAS-II pathway.[32]

Therefore, this screening system allows for the identification of compounds that inhibit the mycobacterial FAS-II pathway at any of several points. The cytotoxicity of the potent compounds was determined according to method reported by O'Brien et al with a slight modification.

In the present study, preliminary screening with triazolylmethoxy chalcones and flavanones was performed to score the inhibition of bacterial growth by measuring the decline in colony forming units (cfu) at different concentrations of drugs (Table 4, Fig. 2B).

As shown in Table 4, eleven compounds showed ⩾50% inhibition of recombinant *M. aurum* growth at 50 μM. These compounds were assessed by the β-gal enzyme assay to monitor the inducibility under treated conditions. Enhanced level of β-gal enzyme activity was observed after treatment with seven of these compounds, [**4**{4}, **4**{9}, **4**{16}, **4**{18}, **5**{2}, **5**{3} and **5**{7}] with respect to the untreated control, with the response somewhat greater at higher concentration (100 μM) **(**Fig. 2**A).** This enhanced reporter gene activity is indicative of FAS-II pathway inhibition, which is sensed by the *kas* promoter. Conversely, when the same experiment was performed using another *M. aurum* recombinant strain carrying the *hsp60* promoter, no reporter gene inducibility was observed under similar conditions **(**Fig. 2**A).** The *hsp60* promoter is nonresponsive to inhibition of the FAS-II pathway[33] and the decline in reporter gene activity in the treated samples is in line with the diminishing viability of bacterial cells as the treatment dose increased. Isoniazid (INH), a known FAS-II pathway inhibitor was used as a positive control in both conditions. Four compounds [**2**{1}, **2**{3}, **4**{2}, **5**{9}] which inhibited the bacterial growth did not show enhanced levels of β-gal enzyme in comparison to the untreated control (Fig. 2**A**), suggesting activity by a pathway other

Table 4
Percent inhibition of bacterial growth by synthesized triazolyl -methoxy chalcones and flavanones with CC_{50} values of the potent compounds

Compound	% Inhibition (50 μM)	% Inhibition (100 μM)	CC_{50} (μM)
1	2.0 ± 1.0	35.6 ± 3.0	ND
2{1}	93.1 ± 2.7	91.8 ± 1.7	ND
2{2}	23.7 ± 7.9	75.9 ± 0.3	ND
2{3}	70.4 ± 2.4	73.7 ± 1.2	ND
4{1}	16.8 ± 4.6	57.7 ± 2.0	ND
4{2}	**69.6 ± 3.6**	**76.5±4.0**	>100.0
4{3}	6.1 ± 4.2	59.0 ± 1.9	ND
4{4}	**71.6 ± 0.8**	**79.1 ± 4.2**	>100.0
4{5}	6.2 ± 0.2	44.7 ± 5.0	ND
4{6}	0	31.4 ± 5.9	ND
4{7}	39.6 ± 10.2	56.0 ± 7.2	ND
4{8}	5.5 ± 0.2	18.8 ± 6.5	ND
4{9}	**49.9 ± 4.7**	**88.0 ± 0.8**	80.0 ± 13.27
4{10}	11.6 ± 1.9	28.0 ± 0.6	ND
4{11}	17.6 ± 3.2	74.5 ± 1.5	ND
4{12}	25.5 ± 2.0	68.7 ± 7.0	ND
4{13}	0	12.7 ± 3.0	ND
4{14}	0	22.0 ± 4.3	ND
4{15}	2.6 ± 0.1	51.6 ± 5.7	ND
4{16}	**53.8 ± 2.5**	**85.3 ± 5.5**	71.7 ± 5.77
4{17}	11.6 ± 1.9	28.0 ± 0.6	ND
4{18}	**53.1 ± 0.4**	**83.0 ± 6.5**	65.8 ± 2.58
4{19}	0	17.8 ± 3.2	ND
4{20}	0	63.2 ± 3.2	ND
5{1}	19.0 ± 7.2	70.2 ± 6.8	ND
5{2}	**68.9 ± 1.9**	**75.1 ± 1.7**	20.3 ± 2.57
5{3}	**55.9 ± 0.8**	**65.7 ± 1.0**	57.5 ± 25.98
5{4}	34.1 ± 2.5	56.4 ± 3.0	ND
5{5}	27.0 ± 2.3	74.8 ± 2.5	ND
5{6}	46.9 ± 3.6	79.8 ± 0.8	ND
5{7}	**54.7 ± 3.8**	**79.3 ± 5.1**	23.8 ± 3.33
5{8}	15.8 ± 2.3	66.1 ± 2.5	ND
5{9}	**50.0 ± 6.4**	**70.6 ± 4.6**	87.5 ± 17.68
5{10}	31.0 ± 3.3	72.5 ± 7.2	ND

CC_{50} = cytotoxic concentration of compounds, ND = not done.

than FAS-II inhibition. Although the inhibitory concentrations used here are high, these tests provide lead structures with a potential unique mechanism of action that can be considered for further development into more potent antitubercular compounds.

Analysis of structure–activity correlations of these molecules shows that triazolylmethoxy acetophenones having an *n*-hexyl group as the triazol-1-yl substitutent exhibited greater growth inhibition of recombinant *M. aurum* than those with benzyl or 4-bromobenzyl groups. The presence of more than one halogen (Cl and F) in the flavanone C-2 aryl ring also appeared to impart better activity (compounds **4**{4}, **4**{11} and **4**{18}) than the other substituents. No other general trends could be discerned.With respect to other substituents. In general, the growth inhibitory activity and β-gal inducing power of the flavanones **5** were better than their precursor chalcones. The triazolylmethoxy acetophenones **2**{2–3} were found to inhibit bacterial growth without inhibiting the FAS-II pathway, whereas their corresponding chalcones and flavanones showed more potent FAS-II pathway inhibition. This suggests that the pharmacophore hybridization concept may be quite fruitful in antitubercular development**.**

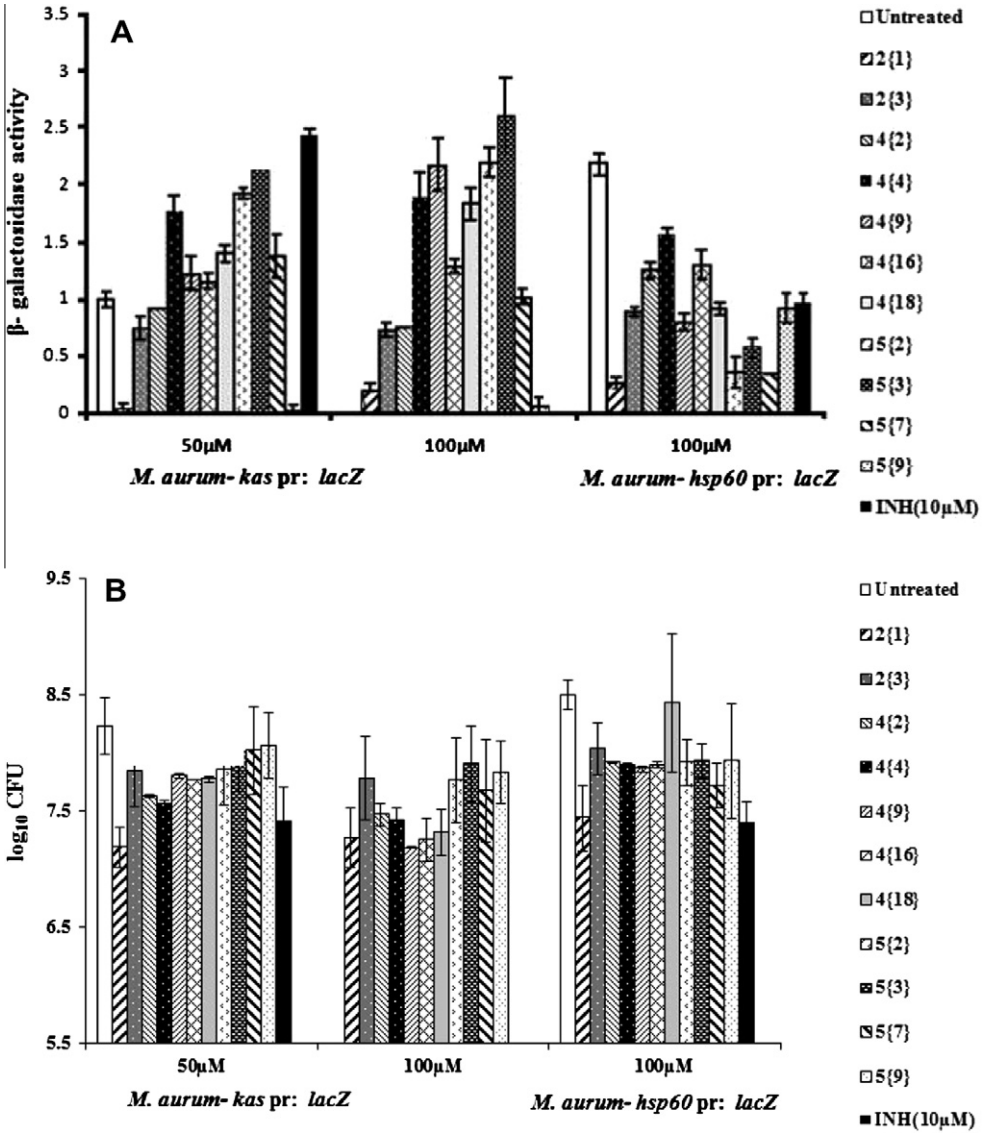


Figure 2. Reporter gene expression and viability assay after treatment with the indicated compounds. **(A)** Induced levels of β-gal activity in treated samples of recombinant strain *M. aurum- kaspr:lacZ* with respect to untreated control. INH as a positive control shows maximum inducibility. **(B)** Induced levels of β-gal activity in treated samples of recombinant *M. aurum* strain with *hsp60* promoter, *M. aurum-hsp60pr:lacZ*, showing a decline in β-gal activity in all cases, including INH, with respect to untreated control. Both recombinant strains exhibit similar declines in overall viability; greater inhibition is observed with increasing concentration of compounds.

2.2.2. Screening of compounds against kinase activity

Serine/threonine protein kinases (STPKs) have been shown to be important virulence factors in various pathogenic bacteria. Reversible protein phosphorylation by these STPKs plays a key role in regulating many cellular processes including stress response, regulation of cell cycle, and development. This regulatory phenomenon is unambiguously preserved during the course of evolution in all forms of life. The genome sequence of *Mycobacterium tuberculosis* H_{37}Rv revealed the presence of 11 STPKs. Some of these kinases have been implicated in the pathogenesis and survival of the tubercle bacillus within host, particularly PknA, PknB and PknG (Pkn = mycobacterial serine-threonine protein kinase). The last is of particular interest, since its structure contains inhibitor-binding pocket that is not present in any human kinase.[34]The compound AX20017 specifically binds to this region of PknG, and is therefore a useful tool for comparison to new candidates in screens for active compounds.[34,35] The compounds prepared above were therefore also screened as inhibitors of the enzymatic activity of purified PknG with myelin basic protein (MBP) as a substrate[36] using **AX20017** as positive control (Fig. 3).

PknG enzymatic activity was determined by quantification of the ADP generated by the kinase using the ADP–Glo luciferase reporter kit (Promega, USA). The triazolylmethoxy acetophenones and chalcones proved to be inactive, but aminopyrimidine derivatives **6**{2}, **6**{8}, **6**{9}, **6**{11}, **6**{13}, **6**{14} and **6**{17} showed respectively 43%, 29%, 53%, 43%, 35%, 41% and 34% inhibition against (STPK), while the standard inhibitor **AX20017** (41%) at 100 μm. (Table 5) However, only the aminopyrimidine **6**{1} was able to inhibit the growth of *M. tuberculosis* H37Rv (MIC = 50 μg/mL), suggesting that the level of PknG inhibition exhibited by the other compounds was insufficient, or that they could not reach the enzyme in the organism.

A careful SAR of these aminopyrimidine reveals that the compounds **6**{1} and **6**{2} having *n*-hexyl as triazolyl ring subtituent and phenyl/4-Cl-phenyl as 6-aryl substituent were found to be moderate activity against mycobacterial PknG. Other compound

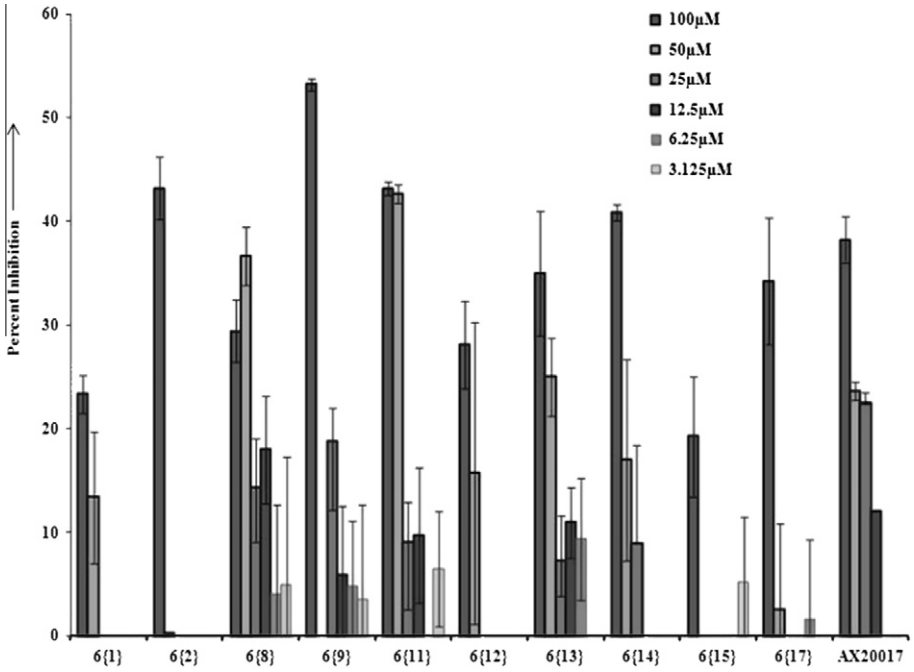
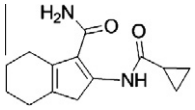


Figure 3. ADP-Glo kinase assay on PknG with MBP as substrate, in the presence of aminopyrimidines at different concentration (Dose Response). AX20017 was used as a positive control.

Table 5
Bio-evaluation of compounds against mycobacterial PknG

Compound	% Inhibition at 100 μm (Mean ± SE)	% Inhibition at 12.5 μm (Mean ± SE)	CC_{50} (μM)
6{1}	23 ± 1.86	0	ND
6{2}	43 ± 2.94	ND	30.3 ± 2.57
6{3}	11 ± 0.32	ND	ND
6{4}	4 ± 0.35	ND	ND
6{5}	5 ± 0.78	ND	ND
6{6}	17 ± 0.27	ND	ND
6{7}	11 ± 0.76	ND	ND
6{8}	29±3.03	18 ± 5.22	76.5 ± 16.26
6{9}	53 ± 0.61	5 ± 6.32	66.0 ± 29.46
6{10}	12 ± 0.34	ND	ND
6{11}	43 ± 0.61	10 ± 6.49	42.7 ± 8.52
6{12}	28 ± 4.20	0	>100.0
6{13}	35 ± 5.97	11 ± 3.38	71.3 ± 1.77
6{14}	41 ± 0.78	0	>100.0
6{15}	19 ± 5.84	0	ND
6{16}	5 ± 0.41	ND	ND
6{17}	34 ± 6.10	0	>100.0
 AX20017	41 ± 0.02	12 ± 0.07	

CC_{50} = cytotoxic concentration of compounds. ND = not done.

6{8}, **6**{9}, **6**{11}, **6**{13}, **6**{14} and **6**{17} with benzyl group as triazolyl substituent coupled with phenyl, naphthyl and 4-Cl-phenyl as 6-aryl substituent also displayed only moderate inhibition of the enzyme.

3. Conclusion

The FAS-II pathway in mycobacteria is responsible for the elongation of carbon chain length of mycolic acid, and is absent in humans. It is therefore an excellent target for the development of small molecules as new chemotherapeutic agents against tuberculosis. We describe the synthesis of new triazolylmethoxy hybrid molecules in good yields from a common scaffold using different sets of reactions. Several chalcone and flavanone derivatives showed moderate to good inhibition of the mycobacterial FAS-II pathway. These compounds were ineffective against protein kinase G, another attractive mycobacterial target, but several triazolylmethoxy aminopyrimidines showed moderate anti-PknG activity. Further studies on the application of these methods in the synthesis of biologically relevant compounds are in progress.

4. Experimental

4.1. Chemistry

Commercially available reagent grade chemicals were purchased from Sigma–Aldrich or Spectrochem Pvt Ltd and were used as received. All reactions were followed by TLC on E. Merck Kieselgel 60 F254, with detection by UV light. Column chromatography was performed on silica gel (60–120 mesh, E. Merck). IR spectra were recorded as thin films or in chloroform with a Perkin–Elmer Spectrum RX-1 (4000–450 cm^{-1}) spectrophotometer. ^1H and ^{13}C NMR spectra were recorded on a Brucker DRX-300 in $CDCl_3$. Chemical shift values are reported in ppm relative to $SiMe_4$ as internal reference and J in hertz. MS were performed using a mass Spectrometer Jeol SX-102 and ESIMS were performed using Quattro II (Micromass). Elemental analyses were performed on a Perkin–Elmer 2400 II elemental analyzer

4.1.1. 4-Propargyloxy-2-hydroxyacetophenone (1)

To a stirring mixture of 2,4-dihydroxy acetophenone (1 g, 6.5 mmol) and K_2CO_3 (1.07 g, 1.2 equiv) in acetone (15 mL), propargyl bromide (0.59 mL, 6.3 mmol) was slowly added at ambient temperature. Catalytic amount of TBAB (0.41 g, 20 mol %) was added to the reaction mixture and stirred for 6 h. The reaction mixture was extracted with ethylacetate, dried over anhydrous Na_2SO_4 and solvent was evaporated under reduced pressure. Crude product was purified by column of silica gel using 9:1 Hexane/EtOAc as eluent to give compound **1**. White solid, mp 197 °C, yield 1.11 g (89.1%); IR ν_{max} cm^{-1} 3411, 2357, 1640.6; ^1H NMR (300 MHz, $CDCl_3$) δ 12.6 (s, 1H, OH), 7.63 (dd, J = 7.05 and 2.3 Hz,

1H, ArH), 6.46–6.43 (m, 2H, -ArH), 4.71 (d, J = 2.3 Hz, 2H, OCH_2), 2.55 (s, 3H, CH_3), 2.53 (d, J = 2.28 Hz, 1H, C≡CH); MS (ESI+) m/z (M+H): 191.1; Anal. Calcd for $C_{11}H_{10}O_3$: C, 69.46; H, 5.30. Found C, 69.41; H, 5.38.

4.1.2. Triazolylmethoxy acetophenones 2{1–3}

4.1.2.1. 1-[4-{(1-Hexyl-1*H*-1,2,3-triazol-4-yl)methoxy}-2-hydroxyphenyl]ethanone 2{1}. A mixture of 2-hydroxy-4-propargyloxy acetophenone (1 g, 5.2 mmol) and *n*-hexyl azide (0.66 g, 5.2 mmol) in 1:1 *tert*-BuOH:H_2O (10 mL) was stirred at ambient temperature. A freshly prepared solution (500 μL) of sodium ascorbate (0.05 g, 0.2 mmol) in water was added followed by addition of freshly prepared aqueous solution (200 μL) of $CuSO_4{\cdot}5H_2O$ (0.02 g, 0.09 mmol). This heterogeneous mixture was stirred vigorously for 4 h at room temperature after which the reaction mixture was extracted with ethyl acetate and water. Ethyl acetate layer was dried (anhyd Na_2SO_4) and evaporated under reduced pressure to give a crude product. This was purified by silica gel (60–120) column chromatography using hexane/EtOAc (1:1) as eluent to give compound **2**{1} as white solid, mp 50–52 °C, yield 1.5 g (90%); IR ν_{max} cm^{-1} 3376 , 1640.6, 1365 ; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 12.6 (s, 1H, OH), 7.62 (dd, J = 6.09 and 3.81 Hz, 1H, ArH), 7.59 (s, 1H, triazolyl-CH), 6.50 (dd, J = 5.97, J = 2.34 Hz, 2H, ArH), 5.21 (s, 2H, OCH_2), 4.35 (t, J = 7.23 Hz, 2H, NCH_2), 2.54 (s, 1H, CH_3), 1.94–1.89 (t, J = 6.7 Hz, 2H, CH_2), 1.32 (br s, 6H, 3 × CH_2), 0.90- 0.89 (m, 3H, CH_3); ^{13}C NMR (300 MHz, $CDCl_3$) δ: 202.1, 165.1, 164.5, 142.9, 132.2, 122.4, 114.28, 107.5, 102.0, 62.1, 50.3, 31.1, 30.2, 26.1, 26.0, 22.4, 13.9; MS (ESI+) m/z (M+H): 318.0; Anal. Calcd for $C_{17}H_{23}N_3O_3$: C, 64.33; H, 7.30; N, 13.24. Found C, 64.9; H, 6.39; N, 13.19.

4.1.2.2. 1-[4-{(1-Benzyl-1*H*-1,2,3-triazol-4-yl)methoxy}-2-hydroxyphenyl]ethanone 2{2}. The reaction of **1** (1 g, 5.2 mmol) and 4-bromobenzyl azide (0.70 g, 5.2 mmol) as described above gave white solid, 89–92 °C, yield 1.5 g (91%); IR ν_{max} cm^{-1} 3382, 1638. NMR (300 MHz, $CDCl_3$) δ: 12.63 (s, 1H, OH), 7.60 (m, 1H, ArH), 7.54 (s, 1H, triazolyl-CH,), 7.36 (m, 2H, ArH), 7.26 (m, 2H, ArH), 6.46 (d, J = 6.87, Hz, 2H, ArH), 5.51 (s, 2H, OCH_2), 5.15(s, 2H, NCH_2), 2.52 (s, 1H, CH_3), MS (ESI+) m/z (M+H): 324.1; Anal. Calcd for $C_{18}H_{17}N_3O_3$: C, 66.86; H, 5.30; N, 13.00. Found C, 66.79; H, 5.37; N, 12.91.

4.1.2.3. 1-[4-{(1-Bromobenzyl-1*H*-1,2,3-triazol-4-yl)methoxy}-2-hydroxyphenyl] ethanone 2{3}. The reaction of **1** (1 g, 5.2 mmol) and 4-bromobenzyl azide (0.96 g, 5.2 mmol) as described above gave white solid, 120–123 °C, yield 1.9 g (91%); IR ν_{max} cm^{-1} 3459, 1629.4, 1256 ; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 12.6 (s, 1H, OH), 7.62 (m, 1H, ArH), 7.54 (m, 3H, triazolyl-CH, 2ArH), 6.50 (d, J = 7.4 Hz, 2H, ArH), 5.49 (s, 2H, OCH_2), 5.20 (s, 2H, NCH_2), 2.55 (s, 1H, CH_3); MS (ESI+) m/z (M+H): 402.4 and 404.3; Anal. Calcd for $C_{18}H_{16}BrN_3O_3$: C, 53.73; H, 4.01; N, 10.45. Found C, 53.69; H, 4.10; N, 10.39.

4.1.3. Propargyloxy chalcones 3{1–2}

4.1.3.1. (*E*)-1-{2-Hydroxy-4-(pro-2-ynyloxy)phenyl}-3-phenyl prop-en-1-one 3{1}. To a mixture of compound **2**{1} (1 g, 5.3 mmol) and benzaldehyde (0.67 mL, 6.3 mmol) in ethanol (10 mL), pellets of KOH (1.2 equiv) was added. Reaction was stirred at ambient temperature for 4 h then reaction was carefully neutralized with 3 N HCl and precipitate so obtained was filtered and recrystallized from ethanol as colorless solid; mp 210 °C, yield, 1.01 g (67%); IR (KBr) cm^{-1}: 3264, 2365, 1633, 1365, 976; ^{1}H NMR (300 MHz, $CDCl_3$) δ 13.28 (s, 1H, OH), 7.90–7.82 (m, 2H, ArH), 7.65–7.64 (m, 2H, ArH), 7.55 (d, J = 15.4 Hz, 1H, -COCH=CH-), 7.43–7.41 (m, 3H, ArH), 6.53–6.50 (m, 2H, ArH), 4.47 (d, 2H, J = 2.2 Hz, OCH_2), 2.50 (d, J = 2.2 Hz,1H, C≡CH); MS (ESI+) m/z (M+H) 279.3: Anal. Calcd for $C_{18}H_{14}O_3$: C, 77.68; H, 5.07. Found C, 77.57; H, 5.18.

4.1.3.2. (*E*)-3-(4-Bromophenyl)-1-{2-hydroxy-4-(prop-2-ynyloxy)phenyl}prop-2-en-1-one 3{2}. Colorless solid; mp >200 °C, yield, 1.27 g (65%); IR (KBr) cm^{-1}: 3278, 2368, 1642, 1356, 791; ^{1}H NMR (300 MHz, $CDCl_3$) δ 13.21 (s, 1H, OH), 7.82–7.78 (m, 2H, ArH), 7.65–7.64 (m, 2H, ArH), 7.55–7.52 (m, 5H, ArH), 6.53 (br s, 2H, ArH), 4.74 (s, 2H, OCH_2), 2.55 (s,1H, C≡CH); MS (ESI+) m/z (M+H) 357.3 and 359.3: Anal. Calcd for $C_{18}H_{13}BrO_3$: C, 60.52; H, 3.67. Found C, 60.57; H, 3.71.

4.1.4. Triazolylmethoxy chalcones 4{1–20}

4.1.4.1. (*E*)-1-[4-{(1-Hexyl-1*H*-1,2,3-triazol-4-yl)methoxy}-2-hydroxyphenyl]-3-phenyl prop-2-en-1-one 4{1}. To a mixture of compound **2**{1} (1 g, 3.0 mmol) and benzaldehyde (0.38 mL, 3.6 mmol) in ethanol (10 mL), pellets of KOH (1.2 equiv) was added. Reaction was stirred at ambient temperature for 4 hours then reaction was carefully neutralized with 3 N HCl and precipitate so obtained was filtered and recrystallized from ethanol as yellow crystal; mp 144–146 °C, 1.15 g, yield (90%); IR (KBr) cm^{-1}: 3401.5, 2367.6, 1647.3, 1580.0; ^{1}H NMR (300 MHz, $CDCl_3$) δ 13.38 (s, 1H, OH), 7.92 (d, J = 15.13 Hz, 1H, -COCH=CH-), 7.86 (s, 1H, triazolyl-CH), 7.66–7.63 (m, 3H, ArH), 7.61 (d, J = 15.5 Hz, 1H, –COCH=CH–), 7.45–7.43 (m, 3H, ArH), 6.60 (d, J = 6.48 Hz, 2H, ArH), 5.27 (s, 2H, OCH_2), 4.40 (t, J_1 = 7.20, J_2 = 7.26 Hz, 2H, NCH_2), 1.95–1.90 (m, 2H, CH_2CH_3), 1.32 (br s, 6H, 3xCH_2), 0.91 (d, J = 6.4 Hz, 3H, CH_3); MS (ESI+) m/z (M+H): 406.2; Anal. Calcd for $C_{24}H_{27}N_3O_3$: C, 71.09; H, 6.71; N, 10.36. Found C, 71.07; H, 6.80; N, 10.39.

4.1.4.2. (*E*)-3-(4-Bromophenyl)-1-(4-{(1-hexyl-1*H*-1,2,3-triazol-4-yl)methoxy}-2-hydroxy phenyl] prop-2-en-1-one 4{2}. The reaction of **2**{1} (1 g, 3.0 mmol) and 4-bromobenzaldehyde (0.70 g, 3.6 mmol) as described above gave yellow solid; mp 154–156 °C, yield, 1.42 g (93%); IR (KBr) cm^{-1}: 3455.0, 2366.1, 1638.3, 1575.0; ^{1}H NMR (300 MHz, $CDCl_3$) δ 13.25 (s, 1H, OH), 7.78 (d, J = 13.95 Hz, 1H, –COCH=CH–), 7.75 (s, 1H, triazolyl-CH), 7.53–7.35 (m, 6H, ArH, –COCH=CH–), 6.54 (s, 2H, ArH), 5.24 (s, 2H, OCH_2), 4.36 (s, 2H, NCH_2), 1.92 (br s, 2H, CH_2CH_3), 1.32 (m, 6H, 3xCH_2), 0.89 (br s, 3H, CH_3); ^{13}C NMR (300 MHz, $CDCl_3$) δ 191.2, 166.6, 164.7, 142.9, 133.7, 132.2(2C), 131.3, 129.8(2C), 125.0, 120.9, 114.4, 107.8, 102.3, 93.2, 62.4, 50.5, 31.1, 30.3, 26.1(2C), 22.4, 13.9; MS (ESI+) m/z (M+H): 483.9 and 485.9; Anal. Calcd for $C_{24}H_{26}BrN_3O_3$: C, 59.51; H, 5.41; N, 8.67. Found C, 59.43; H, 5.45; N, 8.72.

4.1.4.3. (*E*)-3-(4-Chlorophenyl)-1-[4-{(1-hexyl-1*H*-1,2,3-triazol-4-yl)methoxy}-2-hydroxy phenyl]prop-2-en-1-one 4{3}. The reaction of **2**{1} (1 g, 3.0 mmol) and 4-chlorobenzaldehyde (0.51 g, 3.6 mmol) as described above gave yellow solid; mp 150–153 °C, yield, 1.26 g (91%); IR (KBr) cm^{-1}: 3428.9, 2363.6, 1640.8, 1568.4; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 13.25 (s, 1H, OH), 7.84–7.79 (m, 2H, –COCH=CH–, triazolyl-CH), 7.60–7.57 (m, 3H, ArH), 7.55 (d, J = 15.7 Hz, 1H, –COCH=CH–), 7.40 (d, J = 8.07 Hz, 2H, ArH), 6.56–6.54 (d, J = 6.45 Hz, 2H, ArH), 5.26 (s, 2H, OCH_2), 4.37 (t, J_1 = 7.17 Hz, J_2 = 7.14 Hz, 2H, NCH_2), 1.93–1.91 (m, 2H, CH_2CH_3), 1.34 (br s, 6H, 3 × CH_2), 0.92–0.90 (d, J = 6.4 Hz, 3H, CH_3); ^{13}C NMR (300 MHz, $CDCl_3$) δ: 191.2, 166.6, 164.7, 142.9(2C), 136.6, 133.2, 131.1, 129.6(2C), 129.2(2C), 120.7, 114.4, 107.7, 102.3, 62.1, 50.3, 31.1, 30.2, 26.1(2C), 22.4, 13.9; MS (ESI+) m/z (M+H): 440.0 and 442.0; Anal. Calcd for $C_{24}H_{26}ClN_3O_3$: C, 65.52; H, 5.96; N, 9.55. Found C, 65.48; H, 6.11; N, 9.42.

4.1.4.4. (*E*)-3-(2-Chloro-6-fluorophenyl)-1-[4-{(1-Hexyl-1*H*-1,2,3-triazol-4-yl)methoxy}-2-hydroxy phenyl] prop-2-en-1-one 4{4}. The reaction of **2**{1} (1 g, 3.0 mmol) and 2-Chloro, 6- flourobenzaldehyde (0.42 g, 3.6 mmol) as described above gave yellow solid; mp 156–158 °C, yield, 1.32 g (92%); IR (KBr) cm^{-1}: 3448.5, 2367.0, 1641.3, 1573.5; ^{1}H NMR (300 MHz, $CDCl_3$) δ:

13.20 (s, 1H, OH), 8.07 (d, J = 15.81 Hz, 1H, –COCH=C*H*–), 7.81 (s, 1H, triazolyl-C*H*=), 7.79–7.75 (m, 1H, ArH), 7.61 (s, 1H, ArH), 7.28 (d, J = 1.89 Hz, 2H, ArH), 7.11–7.04 (m, 1H, ArH), 6.54 (d, J = 1.98 Hz, 2H, ArH), 5.24 (s, 2H, OCH_2), 4.38–4.33 (t, J_1 = 7.23, J_2 = 7.20 Hz, 2H, NCH_2), 1.92–1.790 (m, 2H, CH_2CH_3), 1.32 (br s, 6H, 3xCH_2), 0.90 (d, J = 6.2 Hz, 3H, CH_3); MS (ESI+) m/z (M+H): 458.0 and 460.0; Anal. Calcd for $C_{24}H_{25}ClFN_3O_3$: C, 62.95; H, 5.50; N, 9.18. Found C, 62.91; H, 5.55; N, 9.09.

4.1.4.5. (*E*)-1-[4-{(1-Hexyl-1*H*-1,2,3-triazol-4-yl)methoxy}-2-hydroxyphenyl]-3-(4-methoxy phenyl)prop-2-en-1-one 4{5}. The reaction of **2**{1} (1 g, 3.0 mmol) and 4-methoxy benzaldehyde (0.46 mL, 3.6 mmol) as described above gave yellow solid; mp 150–152 °C, yield 1.20 g (93%); IR (KBr) cm^{-1}: 3621.8, 2364.8, 1639.8, 1564.0; ^{1}H NMR (300 MHz, $CDCl_3$) δ 13.47 (s, 1H, OH), 7.85 (m, 2H, –CO*CH*=C*H*–, triazolyl-C*H*=), 7.60 (m, 3H, ArH), 7.44 (d, J = 15.18, 1H, –CO*CH*=CH–), 6.93 (d, J = 8.13 Hz, 2H, ArH), 6.55 (br s, 2H, ArH), 5.24 (s, 2H, OCH_2), 4.36 (t, J = 6.69 Hz, 2H, NCH_2), 1.92 (br s, 2H, CH_2CH_3), 1.32 (m, 6H, 3 × CH_2), 0.89 (br s, 3H, CH_3); ^{13}C NMR (300 MHz, $CDCl_3$) δ: 191.7, 166.5, 164.4, 161.8, 144.4(2C), 131.3, 130.4(2C), 127.5, 117.7, 114.6, 114.4(2C), 107.6, 102.3, 62.4, 55.3, 50.6, 31.1, 30.3, 26.2(2C), 22.4, 14.0; MS (ESI+) m/z (M+H): 436.0; Anal. Calcd for $C_{25}H_{29}N_3O_3$: C, 68.95; H, 6.71; N, 9.65. Found C, 68.89; H, 6.75; N, 9.61.

4.1.4.6. (*E*)-1-[4-{(1-Hexyl-1*H*-1,2,3-triazol-4-yl)methoxy}-2-hydroxyphenyl]-3-(4-isopropyl phenyl) prop-2-en-1-one 4{6}. The reaction of **2**{1} (1 g, 3.0 mmol) and 4-isopropyl benzaldehyde (0.39 mL, 3.6 mmol) as described above gave yellow solid; mp 146–148 °C, yield 1.28 g, 92%; IR (KBr) cm^{-1}: 3419.6, 2371.9, 1644.0, 1569.2; ^{1}H NMR (300 MHz, $CDCl_3$) δ 13.40 (s, 1H, OH), 7.89–7.82 (m, 2H, –CO*CH*=C*H*, triazolyl-C*H*), 7.62–7.56 (m, 3H, ArH), 7.55 (d, J = 15.5 Hz, -CO*CH*=CH-), 7.28 (d, J = 6.57, 2H, ArH), 6.54 (br s, 2H, ArH), 5.25 (s, 2H, OCH_2), 4.36 (t, J = 7.2 Hz, 2H, NCH_2), 2.98–2.94 (m, 1H, –CH–), 1.91 (br s, 2H, CH_2CH_3), 1.31–1.28 (m, 12H, 3xCH_2, 2xCH_3), 0.90–0.89 (d, J = 3.7 Hz, 3H, CH_3); ^{13}C NMR (300 MHz, $CDCl_3$) δ: 191.8, 166.5, 164.6, 152.1, 144.7(2C), 132.4, 131.3, 128.7(2C), 127.1(2C), 122.6, 119.2, 114.6, 107.6, 102.2, 62.2, 50.5, 34.2, 31.1, 30.2, 26.1(2C), 23.8, 22.4, 13.9; MS (ESI+) m/z (M+H): 448.1; Anal. Calcd for $C_{27}H_{33}N_3O_3$: C, 72.46; H, 7.43; N, 9.39. Found C, 72.43; H, 7.48; N, 9.32.

4.1.4.7. (*E*)-1-[4-{(1-Hexyl-1*H*-1,2,3-triazol-4-yl)methoxy}-2-hydroxyphenyl]-3-(4-(prop-2-ynyloxy) phenyl)prop-2-en-1-one 4{7}. The reaction of **2**{1} (1 g, 3.0 mmol) and 4-propynyloxy-benzaldehyde (0.58 g, 3.6 mmol) as described above gave yellow solid; mp 149–152 °C, yield, 1.36 g (82%); IR (KBr) cm^{-1}: 3462.0, 2367.3, 1635.7, 1578.5; ^{1}H NMR (300 MHz, $CDCl_3$) δ 13.40 (s, 1H, OH), 7.88–7.83 (m, 2H, –CO*CH*=C*H*–, triazolyl-C*H*), 7.64–7.60 (m, 3H, ArH), 7.46 (d, J = 15.36 Hz, 1H, –CO*CH*=CH–), 7.03 (d, J = 8.67 Hz, 2H, ArH), 6.55 (d, J = 7.26 Hz, 2H, ArH), 5.26 (s, 2H, OCH_2), 4.76 (d, J = 2.28 Hz, 2H, OCH_2), 4.36 (t, J_1 = 7.20, J_2 = 7.21 Hz, 2H, NCH_2), 2.57–2.52 (m, 1H, C*H*), 1.96–1.92 (m, 2H, CH_2CH_3), 1.34 (br s, 6H, 3xCH_2), 0.93–0.90 (d, J = 6.5 Hz, 3H, CH_3); MS (ESI+) m/z (M+H): 460.0; Anal. Calcd for $C_{27}H_{29}N_3O_4$: C, 70.57; H, 6.36; N, 9.14. Found C, 70.53; H, 6.41; N, 9.10

4.1.4.8. (*E*)-1-[4-{(1-Hexyl-1*H*-1,2,3-triazol-4-yl)methoxy}-2-hydroxyphenyl]-3-(naphthalene-1-yl) prop-2-en-1-one 4{8}. The reaction of **2**{1} (1 g, 3.0 mmol) and 1-naphthaldehyde (0.51 mL, 3.6 mmol) as described above gave yellow solid; mp 142–145 °C, yield, 1.35 g (92%); IR (KBr) cm^{-1}: 3135.8, 2359.9, 1724.8, 1582.9; ^{1}H NMR (300 MHz, $CDCl_3$) δ 13.40 (s, 1H, OH), 8.73 (d, J = 15.18 Hz, 1H, –COCH=C*H*–), 8.26 (d, J = 8.22 Hz, 1H, ArH), 7.94–7.85 (m, 4H, triazolyl-C*H*, ArH), 7.67 (d, J = 15.3 Hz, 1H,–CO*CH*=CH–), 7.61–7.49 (m, 4H, ArH), 6.58 (d, J = 8.64 Hz, 2H, ArH), 5.26 (s, 2H, OCH_2), 4.36 (t, J = 7.20 Hz, 2H, NCH_2), 1.93–1.90 (m, 2H, CH_2CH_3), 1.33 (s, 6H, 3 × CH_2), 0.92–0.90 (m, 3H, CH_3); ^{13}C NMR (300 MHz, $CDCl_3$) δ 191.4, 166.6, 164.7, 141.3, 133.7, 132.2, 131.8, 131.4, 130.9, 128.7, 127.0(2C), 126.3, 125.3(2C), 125.1, 123.5, 122.8, 114.5, 107.7, 102.3, 62.2, 50.4, 31.1, 30.2, 26.1, 22.4, 14.0; MS (ESI+) m/z (M+H): 456.1; Anal. Calcd for $C_{28}H_{29}N_3O_3$: C, 73.82; H, 6.42; N, 9.22. Found C, 73.83; H, 6.48; N, 9.11.

4.1.4.9. (*E*)-1-[4-{(1-Benzyl-1*H*-1,2,3-triazol-4-yl)methoxy}-2-hydroxyphenyl]-3-phenyl prop-2-en-1-one 4{9}. The reaction of **2**{2} (1 g, 3.1 mmol) and benzaldehyde (0.38 mL, 3.7 mmol) as described above gave yellow solid; mp 144–146 °C, yield,1.20 g (90.5%); IR (KBr) cm^{-1}: 3408.2, 2366.7, 1645.8, 1581.1; ^{1}H NMR (300 MHz, $CDCl_3$) δ 13.21 (s, 1H, OH), 7.79 (m, 2H, -COCH=C*H*-, triazolyl-C*H*), 7.56–7.52 (m, 3H, ArH), 7.51 (d, J = 15.63 Hz, 1H, -CO*CH*=CH-), 7.33–7.26 (m, 6H, ArH), 7.19 (d, J = 7.23 Hz, 2H, ArH,), 6.44 (d, J = 2.13 Hz, 2H, ArH), 5.45 (s, 2H, OCH_2), 5.12 (s, 2H, NCH_2); MS (ESI+) m/z (M+H): 412.0; Anal. Calcd for $C_{25}H_{21}N_3O_3$: C, 72.98; H, 5.14; N, 10.21. Found C, 72.88; H, 5.21; N, 10.12.

4.1.4.10. (*E*)-1-[4-{(1-Benzyl-1*H*-1,2,3-triazol-4-yl)methoxy}-2-hydroxyphenyl]-3-(4-chlorophenyl) prop-2-en-1-one 4{10}. The reaction of **2**{2}(1 g, 3.1 mmol) and 4-chlorobenz-aldehyde (0.52 g, 3.7 mmol) as described above gave yellow solid; mp 152–154 °C, yield 1.32 g (91%); IR (KBr) cm^{-1}: 3444.7, 2367.2, 1618.0, 1577.8; ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO-d_6) δ 8.00 (s, 1H, triazolyl-C*H*=), 7.84 (d, J = 16.4 Hz 1H, CO*CH*=C*H*–), 7.61–7.39 (m, 4H, ArH, and –CO*CH*=CH–), 7.10–6.98 (m, 7H, ArH), 6.26 (br s, 1H, ArH), 5.26 (s, 2H, OCH_2), 4.87 (s, 2H, NCH_2); MS (ESI+) m/z (M+H): 445.9 and 448.0; Anal. Calcd for $C_{25}H_{20}ClN_3O_3$: C, 67.34; H, 4.52; N, 9.42. Found C, 67.28; H, 4.55; N, 9.38.

4.1.4.11. (*E*)-1-(4-{(1-Benzyl-1*H*-1,2,3-triazol-4-yl)methoxy}-2-hydroxyphenyl]-3-(2-chloro-6-fluoro phenyl) prop-2-en-1-one 4{11}. The reaction of **2**{2} (1 g, 3.1 mmol) and 2-chloro-6-fluorobenzaldehyde (0.58 g, 3.7 mmol) as described above gave yellow solid; mp 152–154 °C, yield, 1.32 g (91%); IR (KBr) cm^{-1}: 3423.3, 2366.4, 1638.9, 1576.6; ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO-d_6) δ: 8.27 (s, 1H, triazolyl-C*H*=), 7.92 (m, 3H, ArH, –CO*CH*=C*H*–), 7.49–7.39 (m, 2H, ArH), 7.37–7.29 (m, 6H, ArH, and –CO*CH*=CH–), 6.67 (d, J = 1.98 Hz, 1H, ArH), 6.61 (dd, J_1 = 2.1, J_2 = 8.9 Hz, 1H, ArH), 5.61 (s, 2H, OCH_2), 5.24 (s, 2H, NCH_2); ^{13}C NMR (300 MHz, $CDCl_3$+ DMSO-d_6) δ: 192.2, 170.5, 170.0, 147.4, 140.9, 140.5, 138.2, 137.4, 137.0, 133.9(2C), 133.3, 133.1(2C), 131.4, 129.9, 126.6, 126.4, 120.7, 120.3119.4, 113.3, 107.1, 66.8, 58.1; MS (ESI+) m/z (M+H): 463.9 and 466.0; Anal. Calcd for $C_{25}H_{19}ClFN_3O_3$: C, 64.73; H, 4.13; N, 9.06. Found C, 64.69; H, 4.21; N, 9.01.

4.1.4.12. (*E*)-1-[4-{(1-Benzyl-1*H*-1,2,3-triazol-4-yl)methoxy}-2-hydroxyphenyl]-3-(4-methoxy phenyl)prop-2-en-1-one 4{12}. The reaction of **2**{2} (1 g, 3.1 mmol) and 4-methoxy-benzaldehyde (0.45 mL, 3.7 mmol) as described above gave yellow solid; mp 148–150 °C, yield, 1.26 g (88%); IR (KBr) cm^{-1}: 3408.2, 2366.7, 1645.8, 1581.1; ^{1}H NMR (300 MHz, $CDCl_3$) δ 13.41 (s, 1H, OH), 7.87 (d, J = 15.21 Hz, 1H, -COCH=C*H*–), 7.79 (s, 1H, triazolyl-C*H*), 7.61–7.58 (d, J = 8.5 Hz, 2H, ArH), 7.52 (s, 1H, ArH), 7.48 (d, J = 15.3 Hz, 1H, –CO*CH*=CH–), 7.33–7.26 (m, 6H, ArH) 6.97 - 6.88 (m, 2H, ArH,), 6.51 (d, J = 6.42 Hz, 2H, ArH), 5.55 (s, 2H, OCH_2), 5.23 (s, 2H, NCH_2) 3.91- 3.85 (m, 1H, OCH_3); ^{13}C NMR (300 MHz, $CDCl_3$) δ: 192.2, 166.5, 164.3, 144.3, 131.0, 130.3(3C), 129.2(3C), 128.8(2C), 128.0(3C), 127.5, 117.7, 114.6, 114.4(2C), 107.4, 102.3, 62.1, 55.2, 54.2; MS (ESI+) m/z (M+H): 442.0; Anal. Calcd for $C_{26}H_{23}N_3O_4$: C, 70.73; H, 5.25; N, 9.52. Found C, 70.69; H, 5.29; N, 9.51.

4.1.4.13. (*E*)-1-[4-{(1-Benzyl-1*H*-1,2,3-triazol-4-yl)methoxy}-2-hydroxyphenyl]-3-(4-isopropyl phenyl)prop-2-en-1-one 4{13}. The reaction of **2**{2} (1 g, 3.1 mmol) and 4-isopropyl benzaldehyde (0.56 mL, 3.7 mmol) as described above gave yellow solid; mp 142–145 °C, yield 1.30 g, 91%; IR (KBr) cm^{-1}: 3393.3, 2362.5, 1628.9, 1569.6; ^1H NMR (300 MHz, $CDCl_3$) δ: 13.5 (s, 1 H, OH), 7.91 (d, J = 15.7 Hz, 1H, –COCH=C*H*–), 7.83 (s, 1H, triazolyl-C*H*), 7.61–7.58 (m, 2H, ArH), 7.57 (d, 1H, J = 15.4 Hz, –CO*CH*=CH–), 7.44–7.29 (m, 7H, ArH), 6.57–6,54 (m, 2H, ArH), 5.56 (s, 2H, OCH_2), 5.22 (s, 2H, NCH_2) 2.98–2.94 (m, 1H, –CH–), 1.28 (d, J = 6.9 Hz, 6H, 2 × CH_3); ^{13}C NMR (300 MHz, $CDCl_3$) δ: 191.9, 166.5, 164.5, 152.1, 144.7, 134.4, 132.4, 131.3, 129.2(2C), 128.9, 128.8(2C), 128.7(2C), 128.1(2C), 127.1(2C), 122.8, 119.2, 114.5, 107.7, 102.2, 62.0, 54.2, 34.1, 23.7 (2C); MS (ESI+) m/z (M +H): 454.0; Anal. Calcd for $C_{28}H_{27}N_3O_4$: C, 74.15; H, 6.00; N, 9.27. Found C, 74.11; H, 6.07; N, 9.21.

4.1.4.14. (*E*)-1-[4-{(1-Benzyl-1*H*-1,2,3-triazol-4-yl)methoxy}-2-hydroxyphenyl]-3-{4-(prop-2-yny loxyl) phenyl} prop-2-en-1-one 4{14}. The reaction of **2**{2} (1 g, 3.1 mmol) and 4-propynyloxy benzaldehyde (0.59 g, 3.7 mmol) as described above gave solid; mp 150–152 °C, yield, 1.29 g (84%); IR (KBr) cm $^{-1}$: 3408.2, 2366.7, 1645.8, 1581.1; ^1H NMR (300 MHz, $CDCl_3$) δ: 13.42 (s, 1H, OH), 7.89 (d, J = 15.63 Hz, 1H, –COCH=C*H*–), 7.81 (s, 1H, triazolyl-C*H*), 7.64–7.48 (m, 3H, ArH), 7.40–7.37 (m, 3H, ArH,–CO*CH*=CH–), 7.30–7.27 (m, 3H, ArH) 7.03 (d, J = 13.05 Hz, 2H, ArH), 6.53–6.47 (m, 2H, ArH), 5.55 (s, 2H, OCH_2), 5.22 (s, 1H, OCH_2), 4.75 (d, J = 3.1 Hz, 2H, NCH_2), 2.55 (m, 1H, C*H*); MS (ESI+) m/z (M+H): 466.0; Anal. Calcd for $C_{28}H_{23}N_3O_4$: C, 72.24; H, 4.98; N, 9.03. Found C, 72.20; H, 5.01; N, 9.01.

4.1.4.15. (*E*)-1-[4-{(1-Benzyl-1*H*-1,2,3-triazol-4-yl)methoxy}-2-hydroxyphenyl]-3-(naph thalen-1-yl)prop-2-en-1-one 4{15}. The reaction of **2**{2} (1 g, 3.1 mmol) and 1-naphthaldehyde (0.50 mL, 3.7 mmol) as described above gave yellow solid; mp 151–153 °C, yield, 1.32 g (94.4%); IR (KBr) cm^{-1}: 3455.2, 2367.1, 1636.6, 1575.9; ^1H NMR (300 MHz, $CDCl_3$ + DMSO-d_6) δ: 8.64 (d, J = 15.2 Hz, 1H, –COCH=C*H*–), 8.25 (d, J = 8.28 Hz, 1H, ArH), 8.17 (m, 3H, triazolyl-C*H*, ArH), 7.99–7.91 (m, 3H, ArH), 7.61–7.52 (m, 3H, ArH, CO*CH*=CH–), 7.31 (br s, 5H, ArH), 6.61 - 6.57 (m, 2H, ArH), 5.58 (s, 2H, OCH_2), 5.21 (s, 2H, NCH_2); MS (ESI+) m/z (M+H): 462.2; Anal. Calcd for $C_{29}H_{23}N_3O_3$: C, 75.47; H, 5.07; N, 9.10. Found C, 75.49; H, 5.19; N, 9.07.

4.1.4.16. (*E*)-1-[4-{(1-(4-Bromobenzyl)-1*H*-1,2,3-triazol-4-yl)methoxy}-2-hydroxyphenyl]-3-phenyl)prop-2-en-1-one 4{16}. The reaction of **2**{3} (1 g, 2.5 mmol) and benzaldehyde (0.30 mL, 3.0 mmol) as described above gave yellow solid; mp 146–148 °C, yield, 1.10 g (91%); IR (KBr) cm^{-1}: 3408.2, 2366.7, 1645.8, 1581.1; ^1H NMR (300 MHz, $CDCl_3$+ DMSO-d_6) δ: 7.90 (d, J = 9.63 Hz, 1 H, ArH), 7.80 (s, 1H, triazolyl-C*H*), 7.83 (d, J = 15.6 Hz, 1H, –COCH=C*H*–), 7.65–7.60 (m, 3H, ArH), 7.47–7.38 (m, 5H, ArH, –CO*CH*=CH–), 7.18 (d, J = 8.19 Hz, 2H, ArH), 6.52 (d, J = 2.07 Hz, 2H, ArH), 5.50 (s, 2H, OCH_2), 5.18 (s, 2H, NCH_2); MS (ESI+) m/z (M+H): 490.3, 492.3; Anal. Calcd for $C_{25}H_{20}BrN_3O_3$: C, 61.24; H, 4.11; N, 8.57. Found C, 61.18; H, 4.15; N, 8.49.

4.1.4.17. (*E*)-1-[4-{(1-(4-Bromobenzyl)-1*H*-1,2,3-triazol-4-yl)methoxy}-2-hydroxy phenyl]-3-(4-bromo phenyl)prop-2-en-1-one 4{17}. The reaction of **2**{3} (1 g, 2.5 mmol) and 4-bromobenzaldehyde (0.55 g, 3.0 mmol) as described above gave yellow solid; mp 156–158 °C, yield,1.30 g (93%); IR (KBr) cm^{-1}: 3408.2, 2366.7, 1645.8, 1581.1; ^1H NMR (300 MHz, $CDCl_3$+ DMSO-d_6) δ 8.23–8.19 (m, 2H, ArH, triazolyl-C*H*), 7.96 (d, J = 15.42 Hz, 1H, COCH=C*H*–), 7.81–7.73 (m, 2H, ArH, –CO*CH*=CH–), 7.60 (d, J = 8.28 Hz, 2H, ArH), 7.52 (d, J = 8.19 Hz, 2H, ArH), 7.28 (d, J = 8.07 Hz, 2H, ArH), 6.62–6.56 (m, 2H, ArH), 5.59 (s, 2H, OCH_2), 5.23 (s, 2H, NCH_2); MS (ESI+) m/z (M+H): 568.05, 570.05 and 572.05; Anal. Calcd for $C_{25}H_{19}Br_2N_3O_3$: C, 52.75; H, 3.36; N, 7.38. Found C, 52.69; H, 3.42; N, 9.31.

4.1.4.18. (*E*)-1-[4-{(1-(4-Bromobenzyl)-1*H*-1,2,3-triazol-4-yl)methoxy}-2-hydroxy phenyl]-3-(2-chloro-6-fluorophenyl) prop-2-en-1-one 4{18}. The reaction of **2**{3} (1 g, 2.5 mmol) and 2-chloro-6-fluorobenzaldehyde (0.47 g, 3.0 mmol) as described above gave yellow solid; mp 157–159 °C, yield, 1.25 g (92%); IR (KBr) cm^{-1}: 3441.2, 2374.8, 1638.7, 1573.7; ^1H NMR (300 MHz, $CDCl_3$ + DMSO-d_6) δ 8.26 (s, 1H, triazolyl-C*H*), 7.92 (m, , 3H, CO*CH*=CH–, ArH), 7.53 (d, J = 8.37 Hz, 4H, –COCH=C*H*–, ArH), 7.32–7.27 (m, 3H, ArH,), 6.66 (d, J = 2.37 Hz, 1H, ArH), 6.63 (dd, J_1 = 8.88, J_2 = 2.4 Hz, 1H, ArH), 5.59 (s, 2H, OCH_2), 5.23 (s, 2H, NCH_2); ^{13}C NMR (300 MHz, $CDCl_3$+ DMSO-d_6) δ: 196.2, 170.6, 170.0, 147.4, 140.5, 140.2, 138.3, 137.3(2C), 136.9, 136.8(2C), 135.3(2C), 132.8, 131.3, 130.0, 126.8, 126.6, 120.7, 119.4, 113.3, 107.1, 66.7, 57.4; MS(ESI+) m/z (M+H): 541.8 and 543.8; Anal. Calcd for $C_{25}H_{18}BrClFN_3O_3$: C, 55.32; H, 3.34; N, 7.74. Found C, 55.28; H, 3.39; N, 7.69.

4.1.4.19. (*E*)-1-[4-{(1-(4-Bromobenzyl)-1*H*-1,2,3-triazol-4-yl)methoxy}-2-hydroxy phenyl]-3-(4-isopropyl phenyl) prop-2-en-1-one 4{19}. The reaction of **2**{3} (1 g, 2.5 mmol) and 4-isopropylbenzaldehyde (0.45 mL, 3.0 mmol) as described above yellow solid; mp 144–146 °C, yield, 1.22 g (93%); IR (KBr) cm^{-1}: 3457.9, 2367.8, 1633.7, 1570.2; ^1H NMR (300 MHz, $CDCl_3$) δ: 13.38 (s, 1H, OH), 7.88 (d, J = 15.3 Hz, 2H, –COCH=C*H*–, triazolyl-C*H*), 7.59–7.51 (m, 5H, ArH, –CO*CH*=CH), 7.29–7.02 (m, 5H, ArH,), 6.54 (br s, 2H, ArH), 5.51 (s, 2H, OCH_2), 5.24 (s, 2H, NCH_2) 2.95 (m, 1H, -*CH*-), 1.30 (d, J = 6.63 Hz, 6H, 2 x CH_3); MS (ESI+) m/z (M+H): 532.2 and 534.2; Anal. Calcd for $C_{28}H_{26}BrN_3O_3$: C, 63.16; H, 4.92; N, 7.89. Found C, 63.11; H, 4.98; N, 7.86.

4.1.4.20. (*E*)-1-[4-{(1-(4-Bromobenzyl)-1*H*-1,2,3-triazol-4-yl) methoxy}-2-hydroxy phenyl]-3-(naphthalene-1-yl)prop-2-en-1-one 4{20}. The reaction of **2**{3} (1 g, 2.5 mmol) and 4-bromobenzaldehyde (0.40 g, 3.0 mmol) as described above gave yellow solid; mp 150–152 °C, yield, 1.25 g (92%); IR (KBr) cm^{-1}: 3449.5, 2372.4, 1632.1, 1576.5; ^1H NMR (300 MHz, $CDCl_3$ + DMSO-d_6) δ: 8.67 (d, J = 15.24 Hz, 1H, –COCH=C*H*–), 8.25 (d, J = 7.02 Hz, 1H, ArH), 8.11 (br s, 3H, triazolyl-C*H*, ArH), 7.98–7.87 (m, 3H, ArH), 7.55–7.48 (m, 5H, ArH, -CO*CH*=CH–), 7.27 (m, 2H, ArH), 6.60 (m, 2H, ArH), 5.56 (s, 2H, OCH_2), 5.21 (s, 2H, NCH_2); MS (ESI+) m/z (M+H): 539.9 and 541.9; Anal. Calcd for $C_{29}H_{22}BrN_3O_3$: C, 64.45; H, 4.10; N, 7.78. Found C, 64.41; H, 4.13; N, 7.69.

4.1.5. Triazolylmethoxy flavanones 5{1–10}

4.1.5.1. 7-{(1-Hexyl-1*H*-1,2,3-triazol-4-yl)methoxy}-2-phenyl chroman-4-one 5{1}. To a stirred solution of triazolymethoxychalcone **4**{1} (0.5 g, 1.23 mmol) in minimum amount of a mixture of EtOH/H_2O (1:1) and NaOAc (0.40 g, 4.92 mmol) was added. The reaction mixture was refluxed till the disappearance of the starting materials (TLC). After completion of reaction, the reaction mixture was allowed to cool to room temperature. The reaction mixture was extracted with ethyl acetate and water. The combined organic phases were washed with brine, dried over anhydrous sodium sulphate, and concentrated under reduced pressure. Crude product was purified by recrystallization in ethanol. light yellow solid, mp 120–123 °C, yield, 0.38 g (79%); IR (KBr) cm^{-1} 3754, 2834, 1708, 1451, 1252, 697; ^1H NMR (300 MHz, $CDCl_3$ + DMSO-d_6) δ: 7.88 (d, J = 8.7 Hz, 1H, ArH), 7.62 (s, 1H, triazolyl C*H*=), 7.47–7.42 (m, 2H, ArH), 6.70 (dd, J_1 = 2.1 Hz, J_2 = 8.8 Hz, 1H, ArH), 6.64–6.59 (m, 1H, ArH), 5.48 (dd, J_1 = 2.7 Hz, J_2 = 13.1 Hz, 1H, O*CH*), 5.24 (s, 2H, OCH_2), 4.37 (t, J = 7.1 Hz, 2H, NCH_2), 3.05 (dd,

J = 13.2 Hz, J = 16.8 Hz, 1H, H_a, CH_2), 2.85 (dd, J_1 = 2.8 Hz, J_2 = 16.8 Hz, 1H, H_b, CH_2), 1.92 (br s, 2H, CH_2), 1.32 (m, 6H, 3x CH_2), 0.89 (br s, 3H. CH_3); ^{13}C NMR (50 MHz, $CDCl_3$ +DMSO-d_6) δ: 190.2, 164.6, 163.4, 143.06, 138.1, 128.8(3C), 126.1(2C), 122.4(2C), 110.5, 107.6, 101.9, 79.9, 62.3, 50.4, 44.4, 31.1, 30.2, 26.1(2C), 22.4, 13.9; MS (ESI+) *m/z* (M+H): 406.3; Anal. Calcd for $C_{24}H_{27}N_3O_3$: C, 71.09; H, 6.71; N, 10.36. Found C, 71.11; H, 6.79; N, 10.28.

4.1.5.2. 7-{(1-Hexyl-1*H*-1,2,3-triazol-4-yl)methoxy}-2-(4-bromophenyl)chroman-4-one 5{2}. Light yellow solid, mp 133–135 °C, 0.37 g, yield 75%; IR (KBr) cm^{-1} 3754, 2834, 1698, 1451, 1252, 697; ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO-d_6) δ: 7.76–7.71 (m, 1H, ArH), 7.51 (s, 1H, triazolyl C*H*═), 7.29–7.26 (m, 2H, ArH), 7.18–7.16 (m, 2H, ArH), 6.55 (dd, J_1 = 2.3 Hz, J_2 = 8.8 Hz, 1H, ArH), 5.34–5.29 (m, 1H, ArH), 5.30 (m, 1H, OC*H*), 5.11 (s, 2H, OCH_2), 4.25 (t, J = 7.2 Hz, 2H, NCH_2), 2.94 (dd, J = 13.3 Hz, J = 16.8 Hz, 1H, H_a, CH_2), 2.72 (dd, J_1 = 2.7 Hz, J_2 = 16.8 Hz, 1H, H_b, CH_2), 1.82–1.79 (m, 2H, CH_2), 1.22 (m, 6H, 3 × CH_2), 0.81–0.79 (m, 3H. CH_3); ^{13}C NMR (50 MHz, $CDCl_3$+DMSO-d_6) δ: 190.1, 164.6, 163.3, 143.0, 138.7, 128.8(2C), 126.1(2C), 122.4(2C), 115.3, 110.5, 107.6, 101.9, 79.9, 62.3, 50.4, 44.4, 31.1, 29.6, 26.1(2C), 22.4, 13.9; MS (ESI+) *m/z* (M+H): 484.3 and 486.3; Anal. Calcd for $C_{25}H_{27}BrN_2O_3$: C, 59.51; H, 5.41; N, 8.67. Found C, 59.47; H, 5.52; N, 8.58.

4.1.5.3. 7-{(1-Hexyl-1*H*-1,2,3-triazol-4-yl)methoxy}-2-(4-methoxyphenyl)chroman-4-one 5{3}. Light yellow solid, mp 128–130 °C, yield, 0.42 g 85%; IR (KBr) cm^{-1} 3682, 2821, 1724, 1410, 1232, 692; ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO-d_6) δ: 7.87 (d, J = 8.8 Hz, 1H, ArH), 7.58 (m, 2H, triazolyl C*H*═, ArH), 7.39 (d, J = 8.6 Hz, 1H, ArH), 6.95–6.92 (m, 2H, ArH), 6.66 (dd, J_1 = 2.1 Hz, J_2 = 8.7 Hz, 1H, ArH), 6.58–6.55 (m, 1H, ArH), 5.40 (dd, J_1 = 2.7 Hz, J_2 = 13.0 Hz, 1H, OC*H*), 5.22 (s, 2H, OCH_2), 4.36 (t, J = 7.1 Hz, 2H, NCH_2), 3.83 (s, 3H, OCH_3), 3.03 (dd, J = 13.2 Hz, J = 16.8 Hz, 1H, H_a, CH_2), 2.78 (dd, J_1 = 2.8 Hz, J_2 = 16.8 Hz, 1H, H_b, CH_2), 1.92–1.90 (m, 2H, CH_2), 1.33 (m, 12H, 3x CH_2), 0.89 (br s, 3H, CH_3); ^{13}C NMR (50 MHz, $CDCl_3$ + DMSO-d_6) δ: 190.3, 164.4, 163.4, 159.9, 144.4, 143.0, 130.7, 130.3, 128.8, 127.5, 122.4, 114.5, 110.4, 107.5, 101.9, 79.7, 62.3, 55.2, 50.4, 44.1, 31.1, 30.2, 26.2, 22.4, 13.9; MS (ESI+) *m/z* (M+H): 436.; Anal. Calcd for $C_{25}H_{29}N_3O_4$: C, 68.95; H, 6.71; N, 9.65. Found C, 68.89; H, 6.69; N, 5.51.

4.1.5.4. 7-{(1-Hexyl-1*H*-1,2,3-triazol-4-yl)methoxy}-2-(4-isopropylphenyl)chroman-4-one 5{4}. Light yellow solid, mp 115–117 °C, 0.41 g, yield 82%; IR (KBr) cm^{-1} 3694, 2832, 1740, 1411, 1232, 697; ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO-d_6) δ: 7.86 (d, J = 8.8 Hz, 1H, ArH), 7.59 (s, 1H, triazolyl C*H*═), 7.55 (m, 1H, ArH), 7.39–7.36 (m, 2H, ArH),7.25 (s, 1H, ArH), 6.65 (dd, J_1 = 2.5 Hz, J_2 = 8.8 Hz, 1H, ArH), 6.58–6.54 (m, 1H, ArH), 5.42 (m, 1H, OC*H*), 5.21 (s, 2H, OCH_2), 4.35 (t, J = 7.1 Hz, 2H, NCH_2), 3.02 (dd, J = 13.2 Hz, J = 16.8 Hz, 1H, H_a, CH_2), 2.96–2.92 (m, 1H, CH), 2.80 (dd, J_1 = 2.6 Hz, J_2 = 16.8 Hz, 1H, H_b, CH_2), 1.92–1.89 (m, 2H, CH_2), 1.32 -1,27(m, 12H, 3x CH_2+ 2x CH_3), 0.89 (br s, 3H. CH_3); ^{13}C NMR (50 MHz, $CDCl_3$ + DMSO-d_6) δ: 190.1, 164.6, 163.4, 149.4, 144.6, 143.01, 136.1, 131.2, 128.8, 127.0, 126, 122.4, 119, 115.2, 110.4, 107.5, 101.9, 79.9, 62.3, 50.4, 44.2, 33.9, 31.1, 30.2, 26.1, 23.9, 22.4, 13.9; MS (ESI+) *m/z* (M+H): 448; Anal. Calcd for $C_{27}H_{33}N_3O_3$: C, 72.46; H, 7.43; N, 9.39. Found C, 72.39; H, 7.33; N, 7.38.

4.1.5.5. 7-{(1-Benzyl-1*H*-1,2,3-triazol-4-yl)methoxy}-2-phenyl chroman-4-one 5{5}. Light yellow solid, mp 118–120 °C, 0.68 g, yield 85%; IR (KBr) cm^{-1}: 3754, 3449, 2923, 1708, 1443, 1252, 696; ^{1}HNMR (300 MHz, $CDCl_3$ + DMSO-d_6) δ: 7.88 (m, 1H, ArH), 7.70 (s, 1H, triazolyl C*H*═), 7.63–7.53 (m, 3H, ArH), 7.45–7.36(m, 5H, ArH), 7.30–7.26 (m, 2H, ArH), 6.68 (dd, J_1 = 2.3 Hz, J_2 = 8.8 Hz, 1H, ArH), 6.60–6.53 (m, 1H, ArH), 5.54 (s, 2H, OCH_2), 5.41 (dd, J_1 = 3.3 Hz, J_2 = 13.0 Hz, 1H, OC*H*), 5.22 (s, 2H, NCH_2), 3.11 (dd, J = 13 Hz, J = 16.9 Hz, 1H, H_a, CH_2), 2.87 (dd, J_1 = 3.2 Hz, J_2 = 16.9 Hz, 1H, H_b, CH_2); MS (ESI+) *m/z* (M+H): 412; Anal. Calcd for $C_{25}H_{21}N_3O_3$: C, 72.98; H, 5.14; N, 10.21. Found C, 72.92; H, 5.17; N, 10.24.

4.1.5.6. 7-{(1-Benzyl-1*H*-1,2,3-triazol-4-yl)methoxy}-2-(4-chlorophenyl)chroman-4-one 5{6}. Light yellow solid, mp 135–138 °C, yield, 0.30 g (50%); IR (KBr) cm^{-1} 3454, 2934, 1731, 1398, 1282, 691; ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO-d_6) δ: 7.87 (m, 1H, ArH), 7.52 (s, 1H, triazolyl C*H*═, ArH), 7.45–7.28 (m, 9H, ArH), 6.66–6.54 (m, 2H, ArH), 5.50 (s, 2H, OCH_2), 5.46 (dd, J_1 = 2.5 Hz, J_2 = 13.2 Hz, 1H, OC*H*), 5.20 (s, 2H, NCH_2), 3.02 (dd, J = 13.3 Hz, J = 16.7 Hz, 1H, H_a, CH_2), 2.83 (dd, J_1 = 2.6 Hz, J_2 = 16.7 Hz, 1H, H_b, CH_2); MS (ESI+) *m/z* (M+H): 446.0 and 448; Anal. Calcd for $C_{25}H_{20}ClN_3O_3$: C, 67.34; H, 4.52; N, 9.42. Found C, 67.27; H, 4.59; N, 9.36.

4.1.5.7. 7-{(1-Benzyl-1*H*-1,2,3-triazol-4-yl)methoxy}-2-(4-isopropylphenyl)chroman-4-one 5{7}. Light yellow solid, mp 123–125 °C, yield, 0.44 g (87%); IR (KBr) cm^{-1}: 3454, 2834, 1758, 1398, 1282, 691; ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO-d_6) δ: 7.88 (m, 1H, ArH), 7.54–7.51 (m, 4H, triazolyl C*H*═, ArH), 7.40–7.38 (m, 2H, ArH), 7.30 (m, 1H, ArH), 7.18–7.15 (m, 2H, ArH), 6.67 (m, 1H, ArH), 5.34–5.29 (m, 1H, ArH), 5.50 (s, 2H, OCH_2), 5.45 (dd, J_1 = 2.3 Hz, J_2 = 13.0 Hz, 1H, OC*H*), 5.21 (s, 2H, NCH_2), 3.03 (dd, J = 13.1 Hz, J = 16.8 Hz, 1H, H_a, CH_2), 2.98–2.93 (m, 1H. CH), 2.70 (dd, J_1 = 2.6 Hz, J_2 = 16.8 Hz, 1H, H_b, CH_2), 1.31–1.28(m, 6H, 2xCH_3); MS (ESI+) *m/z* (M+H): 454.0; Anal. Calcd for $C_{28}H_{27}N_3O_3$: C, 74.15; H, 6.00; N, 9.27. Found C, 74.47; H, 6.17; N, 9.16.

4.1.5.8. 7-{(1-(4-Bromobenzyl)-1*H*-1,2,3-triazol-4-yl) methoxy-2-phenylchroman-4-one 5{8}. Light yellow solid, mp 125–127 °C, yield, 0.42 g (83%); IR (KBr) cm^{-1}: 3750, 3453, 2960, 1732, 1562, 1458, 1119, 799; ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO-d_6) δ: 7.88 (m, 1H, ArH), 7.66–7.38 (m, 8H, triazolylC*H*═, ArH), 7.18 (d, J = 8.2 Hz, 2H, ArH), 6.68 (dd, J_1 = 2.0 Hz, J_2 = 10.8 Hz, 1H, ArH), 6.59–6.52 (m, 1H, ArH), 5.50 (s, 2H, OCH_2), 5.45–5.44 (m, 1H, OC*H*), 5.21 (s, 2H, NCH_2), 3.07 (dd, J = 13.2 Hz, J = 16.8 Hz, 1H, H_a, CH_2), 2.86 (dd, J_1 = 2.9 Hz, J_2 = 16.9 Hz, 1H, H_b, CH_2); MS (ESI+) *m/z* (M+H): 490.0 and 492; Anal. Calcd for $C_{25}H_{20}BrN_3O_3$: C, 61.24; H, 4.11; N, 8.57. Found C, 61.22; H, 4.07; N, 8.54.

4.1.5.9. 7-{(1-(4-Bromobenzyl)-1H-1,2,3-triazol-4-yl) methoxy}-2-(4-bromophenyl) chroman-4-one 5{9}. Light yellow solid, mp 132–135 °C, yield, 0.30 g, (60%); IR (KBr) cm^{-1} 3453, 2963, 1725, 1562, 1458, 1215, 799; ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO-d_6) δ: 7.89–7.82 (m, 1H, ArH), 7.52 (s, 1H, triazolylC*H*═), 7.45–7.36 (m, 8H, ArH), 6.66–6.58 (m, 2H, ArH), 5.54 (s, 2H, OCH_2), 5.47–5.42 (m, 1H, OC*H*), 5.20 (s, 2H, NCH_2), 3.06–2.96 (m, 1H, H_a, CH_2), 2.84–2.78 (m, 1H, H_b, CH_2); MS (ESI+) *m/z* (M+H): 567.0, 569.0 and 571; Anal. Calcd for $C_{25}H_{19}Br_2N_3O_3$: C, 52.75; H, 3.36; N, 7.38. Found C, 52.72; H, 3.41; N, 7.34.

4.1.5.10. 7-{(1-(4-Bromobenzyl)-1*H*-1,2,3-triazol-4-yl) methoxy}-2-(4-isopropylphenyl) chroman-4-one 5{10}. Light yellow solid, mp 120–124 °C, yield, 0.44 g (88%); IR (KBr) cm^{-1}:3750, 3453, 2960, 1625, 1562, 1458, 1119, 799; ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO-d_6) δ: 7.87–7.85 (m, 1H, ArH), 7.61–7.50 (m, 4H, triazolylC*H*═, ArH), 7.42–7.38 (m, 1H, ArH), 7.30 (d, J = 7.5 Hz, 2H, ArH), 7.15 (d, J = 8.1 Hz, 2H, ArH), 6.67 (dd, J_1 = 1.8 Hz, J_2 = 8.7 Hz, 1H, ArH), 6.58 (m, 1H, ArH), 5.50 (s, 2H, OCH_2), 5.46 (dd, J_1 = 2.7 Hz, J_2 = 13.3 Hz, 1H, OC*H*), 5.21 (s, 2H, NCH_2), 3.04 (d, J = 13.1 Hz, 1H, H_a, CH_2), 2.97(m, 1H, CH), 2.85

(dd, J_1 = 2.6 Hz, J_2 = 16.6 Hz, 1H, H_b, CH_2), 1.30 (s, 3H, CH_3), 1.28(s, 3H, CH_3); MS (ESI+) *m/z* (M+H): 532.0 and 534.0 ; Anal. Calcd for $C_{28}H_{26}BrN_3O_3$: C, 63.16; H, 4.92; N, 7.89. Found C, 63.12; H, 5.01; N, 17.84.

4.1.6. Triazolylmethoxy aminopyrimidines 6{1–17}

4.1.6.1. 2-(2-Amino-6-phenylpyrimidin-4-yl)-5-{(1-hexyl-1*H*-1,2,3-triazol-4-yl) methoxy} phenol 6{1}. A solution of guanidine hydrochloride (0.13 g, 1.35 mmol) dissolved in DMF (1 mL) was added into a slurry of NaH (0.32 g, 1.35 mmol) in DMF (1 mL) at 0 °C, followed by the addition of chalcone **4**{1} (0.50 g, 1.23 mmol) in DMF (1 mL). The whole reaction mixture was stirred at 0 °C for about half an hour and after at 100 °C until reaction got completed (The reaction progress and completion was monitored by TLC). Reaction mixture was filtered over cellite pad using Ethylacetate as solvent. Organic layer was washed three times with water, dried over Na_2SO_4 and concentrated in vacuum. The viscous crude mass (sometimes solid) thus obtained was purified by column chromatography using ethylacetate/hexane as eluent in the 3:10 ratio, yellow solid, mp 155–156 °C, yield 0.30 g (55%); IR (KBr) cm^{-1}: 3416.4,. 3315.0, 2336.2, 1571.1; ^1H NMR (300 MHz, $CDCl_3$ + DMSO-d_6) δ: 8.03–8.01 (m, 2H, ArH), 7.80 (s, 1H, triazolyl C*H*═), 7.76 (s, 1H, ArH), 7.44–7.36 (m, 4H, ArH), 6.52–6.50 (m, 2H, ArH), 6.24 (s, 2H, NH_2), 5.16 (s, 2H, OCH_2), 4.34 (t, J_1 = 7.08, J_2 = 7.2 Hz, 2H, NCH_2), 1.87 (m, 2H, CH_2), 1.28 (br s, 6H, 3x-CH_2), 0.84 (br s, 3H, CH_3); ^{13}C NMR (300 MHz, $CDCl_3$ + DMSO-d_6) δ: 170.3, 167.6, 166.6, 165.9, 147.8, 142.4, 135.2.2, 133.3(2C), 131.9(2C), 128.0, 121.2, 116.1, 111.8, 107.6, 104.6, 66.4, 54.9, 36.6. 35.8, 34.9, 30.8, 27.1, 18.7; MS (ESI+) *m/z* (M+H): 445.0 Anal. Calcd for $C_{25}H_{28}N_6O_2$: C, 67.55; H, 6.35; N, 18.91. Found C, 67.51; H, 6.39; N, 18.94.

4.1.6.2. 2-(2-Amino-6-(4-chlorophenyl)pyrimidin-4-yl)-5-{(1-hexyl-1*H*-1,2,3-triazol-4-yl) methoxy} phenol 6{2}. The reaction of **4**{3} (0.5 g, 1.13 mmol) and Guanidine hydrochloride (0.19 g, 1.24 mmol) as described above gave yellow solid, mp 192–194 °C, yield 0.29 g (54%), IR (KBr) cm^{-1}: 3505.2, 3386.1, 2367.1, 1609.3; ^1H NMR (300 MHz, $CDCl_3$ + DMSO-d_6) δ: 8.06 (d, J = 8.4 Hz, 2H, ArH) 7.83–7.80 (m, 2H, triazolyl C*H*═, ArH), 7.42–7.38 (m, 3H, ArH), 6.52–6.49 (m, 4H, ArH, NH_2), 5.15 (s, 2H, OCH_2), 4.36 (t, J_1 = J_2 = 7.1 Hz, 2H, NCH_2), 1.89–1.85 (m, 2H, CH_2), 1.29 (br s, 6H, 3xCH_2), 0.87 (d, J = 6.2 Hz, 3H, CH_3); MS (ESI+) *m/z* (M+H): 479.1 and 481.1; Anal. Calcd for $C_{25}H_{27}ClN_6O_2$: C, 62.69; H, 5.68; N, 17.55. Found C, 62.64; H, 5.73; N, 17.51.

4.1.6.3. 2-(2-Amino-6-(2-chloro-6-fluorophenyl)pyrimidin-4-yl)-5-{(1-hexyl-1*H*-1,2,3-triazol-4-yl)methoxy} phenol 6{3}. The reaction of **4**{4} (0.5 g, 1.09 mmol) and Guanidine hydrochloride (0.11 g, 1.19 mmol) as described above gave yellow solid; mp 185–187 °C, yield, 0.24 g, 51%; IR (KBr) cm^{-1}: 3427.0, 3354.6, 2366.4, 1577.2; ^1H NMR (300 MHz, $CDCl_3$ + DMSO-d_6) δ: 7.82 (m, 1H, ArH), 7.67–7.59 (m, 2H, ArH), 7.41–7.30 (m, 2H, ArH), 6.98–6.48 (m, 3H, ArH, NH_2), 5.18 (s, 2H, OCH_2), 4.35 (t, J = 6.80 Hz , 2H, NCH_2), 1.88–1.89 (m, 2H, CH_2CH_3), 1.31 (br s, 6H, 3xCH_2), 0.87 (br s, 3H, CH_3); MS (ESI+) *m/z* (M+H): 497.0 and 499.1; Anal. Calcd for $C_{25}H_{26}ClFN_6O_2$: C, 62.42; H, 5.27; N, 16.91. Found C, 62.38; H, 5.33; N, 16.84.

4.1.6.4. 2-(2-Amino-6-(4-methoxyphenyl)pyrimidin-4-yl)-5-{(1-hexyl-1*H*-1,2,3-triazol-4-yl) methoxy} phenol 6{4}. The reaction of **4**{5} (0.5 g, 1.14 mmol) and Guanidine hydrochloride (0.12 g, 1.25 mmol) as described above gave yellow solid; mp 180–182 °C, yield, 0.28 g (52%); IR (KBr) cm^{-1}: 3427.0, 3354.6, 2366.4, 1577.2; ^1H NMR (300 MHz, $CDCl_3$ + DMSO-d_6) δ: 8.05 (d, J = 8.6 Hz, 2H, ArH), 7.83 (d, J = 8.07 Hz, 2H, ArH), 7.36 (s, 1H, ArH), 6.97 (d, J = 7.8 Hz, 2H, ArH), 6.54–6.52 (m, 2H, ArH), 6.38 (br s, 2H, NH_2), 5.17 (s, 2H, OCH_2), 4.34 (t, J_1 = 7.10, J_2 = 6.90 Hz , 2H, NCH_2), 3.85 (s, 3H, OCH_3), 1.88–1.86 (m, 2H, CH_2CH_3), 1.30 (br s, 6H, 3xCH_2), 0.86 (br s, 3H, CH_3); ^{13}C NMR (300 MHz, $CDCl_3$ + DMSO-d_6) δ: 169.8, 167.6, 166.7 166.6, 166.4, 165.9, 147.8, 134.7 , 133.8, 133.5(2C), 128.3, 118.7(2C), 116.1, 111.7, 107.7, 103.7, 66.5, 60.5, 54.7, 35.8, 34.9, 30.8, 27.1, 18.8; MS (ESI+) *m/z* (M+H): 475.2 Anal. Calcd for $C_{26}H_{30}N_6O_3$: C, 65.80; H, 6.37; N, 17.71. Found C, 65.77; H, 6.39; N, 17.74.

4.1.6.5. 2-{2-Amino-6-(4-isopropylphenyl)pyrimidin-4-yl}-5-{(1-hexyl-1*H*-1,2,3-triazol-4-yl) methoxy} phenol 6{5}. The reaction of **4**{6} (0.5 g, 1.11 mmol) and Guanidine hydrochloride (0.116 g, 1.21 mmol) as described above gave yellow solid; mp 164–166 °C, yield, 0.32 g (59%); IR (KBr) cm^{-1}: 3488.8, 3333.7, 2365.3, 1580.6; ^1H NMR (300 MHz, $CDCl_3$ + DMSO-d_6) δ: 8.05 (d, J = 8.64 Hz, 2H, ArH), 7.83 (d , J = 8.07 Hz, 2H, ArH, triazolyl C*H*═), 7.51 (s, 1H, ArH), 7.33 (d, J = 8.07 Hz, 2H, ArH), 6.85 (br s, 2H, NH_2), 6.56 (d, J = 6.03 Hz, 2H, ArH), 5.16 (s, 2H, OCH_2), 4.36 (t, J_1 = J_2 = 7.08 Hz, 2H, NCH_2), 2.98 -2.93 (m, 1H, -CH-), 1.86–1.82 (m, 2H, CH_2), 1.27–1.25 (m, 12H, 3xCH_2, 2xCH_3), 0.85 (m, 3H, CH_3); MS (ESI+) *m/z* (M+H): 487.1; Anal. Calcd for $C_{28}H_{34}N_6O_2$: C, 69.11; H, 7.04; N, 17.27. Found C, 69.08; H, 7.10; N, 17.31.

4.1.6.6. 2-{2-Amino-6-(4-propynyloxy)pyrimidin-4-yl}-5-{(1-hexyl-1*H*-1,2,3-triazol-4-yl) methoxy) phenol 6{6}. The reaction of **4**{7} (0.5 g, 1.0 mmol) and Guanidine hydrochloride (0.11 g, 1.20 mmol) as described above gave yellow solid; mp 172–175 °C, 0.27 g, yield 49%; IR (KBr) cm^{-1}: 3443.5, 3314.8, 2367.2, 1584.2; ^1H NMR (300 MHz, $CDCl_3$ + DMSO-d_6) δ: 8.12–8.10 (m, 4H, ArH, triazolyl C*H*═), 7.44–7.38 (m, 1H, ArH), 7.16–7.01 (m, 2H, ArH,), 6.56–6.54 (m, 4H, NH_2, ArH), 5.18 (s, 2H, OCH_2), 4.79 (s, 1H, OCH_2), 4.38 (t, J_1 = J_2 = 6.93 Hz, 2H, NCH_2), 3.02 (m, 1H, -C*H*), 1.88 (m, 2H, CH_2), 1.31 (m, 6H, 3xCH_2), 0.88 (m, 3H, CH_3); MS (ESI+) *m/z* (M+H): 499.1; Anal. Calcd for $C_{28}H_{30}N_6O_3$: C, 67.42; H, 6.06; N, 16.86. Found C, 67.38; H, 6.16; N, 16.84.

4.1.6.7. 2-{2-Amino-6-(4-napthalen-1-yl)pyrimidin-4-yl}-5-((1-hexyl-1*H*-1,2,3-triazol-4-yl) methoxy) phenol 6{7}. The reaction of **4**{8} (0.5 g, 1.09 mmol) and Guanidine hydrochloride (0.115 g, 1.20 mmol) as described above gave yellow solid; mp 178–180 °C, yield, 0.27 g, 56%; IR (KBr) cm^{-1}: 3488.8, 3333.7, 2362.3, 1575.6; ^1H NMR (300 MHz, $CDCl_3$ + DMSO-d_6) δ: 8.12(m, 1H, ArH), 7.97–7.76 (m, 2H, ArH, triazolyl C*H*═), 7.68–7.60 (d , 2H, ArH,), 7.56–7.45 (m, 3H, ArH), 7.20 (s, 1H, ArH), 6.85 (br s, 2H, NH_2), 6.48 (dd, J_1 = 2.34 Hz, J_2 = 8.82 Hz, 1H, ArH), 5.15 (s, 2H, OCH_2), 4.36 (t, J_1 = J_2 = 7.08 Hz, 2H, NCH_2), 1.86–1.78 (m, 2H, CH_2), 1.28 (m, 12H, 3xCH_2, 2xCH_3), 0.85–0.77 (m, 3H, CH_3); MS (ESI+) *m/z* (M+H): 495.1; Anal. Calcd for $C_{29}H_{30}N_6O_2$: C, 70.42; H, 6.11; N, 16.99. Found C, 70.38; H, 6.19; N, 16.94.

4.1.6.8. 2-(2-Amino-6-phenylpyrimidin-4-yl)-5-{(1-benzyl-1*H*-1,2,3-triazol-4-yl)methoxy} phenol 6{8}. The reaction of **4**{9} (0.5 g, 1.2 mmol) and Guanidine hydrochloride (0.127 g, 1.33 mmol) as described above gave yellow solid, mp 185–186 °C, yield, 0.30 g (54%); IR (KBr) cm^{-1}: 3491.3. 3356.5, 2366.2, 1572.1; ^1H NMR (300 MHz, $CDCl_3$ + DMSO-d_6) δ: 8.07–8.06 (m, 2H, ArH), 7.82 (s, 1H, triazolyl C*H*═), 7.66 (s, 1H, ArH), 7.48–7.28 (m, 9H, ArH), 6.55–6.53 (m, 2H, ArH), 6.27 (s, 2H, NH_2), 5.56 (s, 2H, OCH_2), 5.18 (s, 2H, NCH_2); MS (ESI+) *m/z* (M+H): 451.1; Anal. Calcd for $C_{26}H_{22}N_6O_2$: C, 69.32; H, 4.92; N, 18.66. Found C, 69.29; H, 4.96; N, 18.64.

4.1.6.9. 2-{2-Amino-6-(4-chlorophenyl)pyrimidin-4-yl}-5-{(1-benzyl-1*H*-1,2,3-triazol-4-yl)methoxy} phenol 6{9}. The reaction of **4**{10} (0.5 g, 1.10 mmol) and Guanidine hydrochloride (0.115 g, 1.21 mmol) as described above gave yellow solid, mp 200–203 °C, yield, 0.28 g, (52%); IR (KBr) cm^{-1}: 3496.9, 3370.8,

2365.8, 1580.0; ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO-d_6) δ: 8.14 (d, J = 6.9 Hz, 2H, ArH), 8.04 (s, 1H, triazolyl C*H*=), 7.94–7.92 (m, 1H, ArH), 7.50 (d, J = 2.2 Hz, 1H, ArH), 7.45 (d, J = 7.9 Hz, 2H, ArH), 7.33–7.31 (m, 5H, ArH), 6.79 (s, 2H, NH_2), 6.52 (d, J = 6.6 Hz, 2H, ArH), 5.57 (s, 2H, O*CH*$_2$), 5.14 (s, 2H, N*CH*$_2$); ^{13}C NMR (300 MHz, $CDCl_3$ + DMSO-d_6) δ: 170.3, 170.0, 167.6, 166.6, 166.1, 148.2, 142.5, 139.2, 136.7, 135.2(2C), 134.8(2C), 133.4(2C), 133.3(2C), 132.0 128.7, 127.0, 116.1, 111.7, 107.7, 104.5, 66.4, 57.7; MS (ESI+) m/z (M+H): 485.0 and 487.1; Anal. Calcd for $C_{26}H_{21}ClN_6O_2$: C, 64.40; H, 4.36; N, 17.33. Found C, 64.37; H, 4.39; N, 17.28.

4.1.6.10. 2-{2-Amino-6-(2-chloro-6-flourophenyl)pyrimidin-4-yl}-5-{(1-benzyl-1*H*-1,2,3-triazol-4-yl)methoxy} phenol 6{10}. The reaction of **4**{11} (0.5 g, 1.07 mmol) and Guanidine hydrochloride (0.113 g, 1.18 mmol) as described above gave yellow solid, mp >200 °C, yield, 0.26 g (48%); IR (KBr) cm^{-1}: 3487.2, 3365.3, 2384.1, 1568.0; ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO-d_6) δ: 13.52 (s, 1H, OH), 7.41 (s, 1H, triazolyl C*H*=), 7.41–7.12 (m, 1H, ArH), 6.78 (br s, 7H, ArH), 6.69–6.58 (m, 1H, ArH), 6.45–6.39 (m, 1H, ArH), 6.11–5.98 (s, 2H, NH_2), 5.03 (s, 2H, O*CH*$_2$), 4.62 (s, 2H, N*CH*$_2$); MS (ESI+) m/z (M+H): 503 and 505.2; Anal. Calcd for $C_{26}H_{21}ClFN_6O_2$: C, 62.09; H, 4.01; N, 16.71. Found C, 62.07; H, 4.09; N, 16.68.

4.1.6.11. 2-{2-Amino-6-(4-methoxyphenyl)pyrimidin-4-yl}-5-{(1-benzyl-1*H*-1,2,3-triazol-4-yl) methoxy} phenol 6{11}. The reaction of **4**{12} (0.5 g, 1.13 mmol) and Guanidine hydrochloride (0.12 g, 1.24 mmol) as described above gave yellow solid; mp 180–182 °C, 0.28 g, yield (52%); IR (KBr) cm^{-1}: 3496.9, 3370.8, 2365.8, 1580.0; ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO-d_6) δ: 8.12 (d, J = 6.78 Hz, 1H, ArH), 8.07 (s, 1H, triazolyl C*H*=), 7.98 (d, J = 9.51 Hz, 1H, ArH), 7.47(s, 1H, ArH), 7.27–7.24 (m, 5H, ArH), 6.94 (d, J = 8.85 Hz, 2H, ArH), 6.84 (br s, 2H, NH_2), 6.47(d, 2H, J = 7.2 Hz, ArH), 5.51 (s, 2H, O*CH*$_2$), 5.08 (s, 2H, N*CH*$_2$) 3.76 (s, 1H, O*CH*$_3$); ^{13}C NMR (300 MHz, $CDCl_3$ + DMSO-d_6) δ: 169.8, 169.6, 167.7, 166.7, 166.5, 166.1, 147.9, 140.9, 134.6, 134.2(2C), 133.9(2C), 133.2(2C), 133.1(2C), 129.7, 118.9(2C), 116.2, 111.9, 107.7, 103.5, 66.4, 60.4, 58.1; MS (ESI+) (M+H): 481.1; Anal. Calcd for $C_{27}H_{24}N_6O_2$: C, 67.49; H, 5.03; N, 17.49. Found C, 67.42; H, 5.07; N, 17.44.

4.1.6.12. 2-{2-Amino-6-(4-isopropylphenyl)pyrimidin-4-yl}-5-{(1-benzyl-1*H*-1,2,3-triazol-4-yl) methoxy} phenol 6{12}. The reaction of **4**{13} (0.5 g, 1.10 mmol) and Guanidine hydrochloride (0.115 g, 1.21 mmol) as described above gave yellow solid; mp 178–180 °C, 0.29 g, yield (54%); IR (KBr) cm^{-1}: 3481.3, 3366.5, 2365.8, 1577.8; ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO-d_6) δ: 8.11–8.05 (m, 3H, triazolyl C*H*=), 7.98 (d, J = 7.65 Hz, 1H, ArH), 7.50 (s, 1H, ArH), 7.33–7.31 (m, 7H, ArH), 6.83 (br s, 2H, NH_2), 6.54 (d, J = 7.29 Hz, 2H, ArH), 5.58 (s, 2H, O*CH*$_2$), 5.15 (s, 2H, N*CH*$_2$), 2.98–2.91 (m, 1H, -CH-), 1.28–1.22 (m, 6H, 2xCH_3); MS (ESI+) m/z (M+H): 493.5; Anal. Calcd for $C_{29}H_{28}N_6O_2$: C, 70.71; H, 5.73; N, 17.06. Found C, 70.69; H, 5.76; N, 17.04.

4.1.6.13. 2-{2-Amino-6-(naphthalene-1-yl)pyrimidin-4-yl}-5-{(1-benzyl-1*H*-1,2,3-triazol-4-yl) methoxy} phenol 6{13}. The reaction of **4**{15} (0.5 g, 1.08 mmol) and Guanidine hydrochloride (0.113 g, 1.18 mmol) as described above gave yellow solid; mp 196–198 °C, yield, 0.30 g (54%); IR (KBr) cm^{-1}: 3473.2, 3305.3, 2371.4, 1572.1; ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO-d_6) δ: 8.22–8.20 (m, 1H, ArH), 7.97 (s, 1H, triazolyl C*H* =), 7.93–7.89 (m, 2H, ArH), 7.73 (d, J = 8.85 Hz, 1H, ArH), 7.63 (d, J = 6.15 Hz, 1H, ArH), 7.56–7.47 (m, 3H, ArH), 7.31–7.30 (m, 5 H, ArH), 7.19 (s, 1H, ArH), 6.80 (br s, 2H, NH_2), 6.54 -6.46 (m, 2H, ArH), 5.55 (s, 2H, O*CH*$_2$), 5.14 (s, 2 H, N*CH*$_2$); ^{13}C NMR (200 MHz, $CDCl_3$ + DMSO-d_6) δ: 173.0, 169.7, 167.7, 166.7, 165.9, 148.0, 142.1, 140.3, 138.5, 135.3, 134.3, 133.7(2C), 133.5, 133.2(2C), 132.9, 131.8, 131.4, 130.9, 130.5, 130.0, 128.9, 115.9, 111.9, 109.1, 107.8, 66.4, 58.4; MS (ESI+) m/z (M+H): 501.1; Anal. Calcd for $C_{30}H_{24}N_6O_2$: C, 71.98; H, 4.83; N, 16.79. Found C, 71.92; H, 4.87; N, 16.74.

4.1.6.14. 2-(2-Amino-6-phenylpyrimidin-4-yl)-5-{1-(4-bromobenzyl)-1*H*-1,2,3-triazol-4-yl} methoxy)phenol 6{14}. The reaction of **4**{16} (0.5 g, 1.02 mmol) and Guanidine hydrochloride (0.107 g, 1.12 mmol) as described above gave yellow solid, mp 190–193 °C, yield, 0.30 g (60%); IR (KBr) cm^{-1}: 3501, 3349, 1577, 691; ^{1}H NMR (300 MHz, $CDCl_3$+ DMSO-d_6) δ: 8.04 (br s, 2H, ArH), 7.91 (s, 1H, triazolyl C*H*=), 7.84–7.77 (m, 1H, ArH), 7.46–7.39 (m, 6H, ArH), 7.21 (d, J = 8.12 Hz, 2H, ArH), 6.47–6.39 (m, 4H, ArH, NH_2), 5.51 (s, 2H, O*CH*$_2$), 5.13 (s, 2H, N*CH*$_2$); ^{13}C NMR (300 MHz, $CDCl_3$+ DMSO-d_6) δ: 170.3, 170.0, 167.7, 166.6, 166.1, 148.2, 142.5, 139.4, 136.7(2C), 135.2, 134.8(2C), 133.5, 133.3(2C), 132.0(2C), 128.7, 127.0, 116.1, 111.8, 107.7, 104.5, 66.4, 57.7; MS (ESI+) m/z (M+H): 529, 531; Anal. Calcd for $C_{26}H_{21}BrN_6O_2$: C, 58.99; H, 4.00; N, 15.87. Found C, 58.92; H, 4.07; N, 15.84.

4.1.6.15. 2-{2-Amino-6-(4-bromophenyl) pyrimidin-4-yl}-5-{1-(4-bromobenzyl)-1*H*-1,2,3-triazol-4-yl)methoxy} phenol 6{15}. The reaction of **4**{17} (0.7 g, 1.33 mmol) and Guanidine hydrochloride (0.139 g, 1.46 mmol) as described above gave yellow solid, mp 210–212 °C, yield, 0.440 g (59%); IR (KBr) cm^{-1}: 3466, 3310, 1621,793; ^{1}H NMR (300 MHz, $CDCl_3$+ DMSO-d_6) δ: 14.2 (s, 1H, OH), 8.14 (d, J = 7.9 Hz, 2H, ArH), 8.07 (s, 1H, triazolyl C*H*=), 7.98 (m, 1H, ArH), 7.47 (m, 5H, ArH), 7.24 (d, J = 7.9 Hz, 2H, ArH), 6.77 (br s, 2H, NH_2), 6.53 (d, J = 6.6 Hz, 2H, ArH), 5.56 (s, 2H, O*CH*$_2$), 5.16 (s, 2H, N*CH*$_2$); ^{13}C NMR (300 MHz, $CDCl_3$+DMSO-d_6) δ: 170.3, 168.8, 167.4, 166.7, 166.1, 147.9, 140.9, 140.7, 140.1, 136.8(2C), 135.3(2C), 134.4, 133.9(2C), 133.8(2C), 129.8, 126.7, 116.0, 112.1, 107.7, 104.3, 66.2, 57.4; MS (ESI+) m/z (M+H): 607.3, 609.3 and 611.1; Anal. Calcd for $C_{26}H_{20}Br_2N_6O_2$: C, 51.34; H, 3.31; N, 13.82. Found C, 51.39; H, 3.43; N, 13.78.

4.1.6.16. 2-{2-Amino-6-(4-isopropyl)pyrimidin-4-yl}-5-{(1-(4-bromobenzyl)-1*H*-1,2,3-triazol-4-yl) methoxy} phenol 6{16}. The reaction of **4**{19} (0.5 g, 0.94 mmol) and Guanidine hydrochloride (0.98 g, 1.03 mmol) as described above gave yellow solid; mp 183–185 °C, 0.25 g, yield 56%; IR (KBr) cm^{-1}: 3438.6, 3333.7, 2368.2, 1576.5; ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO-d_6) δ: 8.05–7.91 (m, 3H, ArH), 7.51–7.20 (m, 8H, ArH, triazolyl C*H*=), 6.73–6.51 (m, 4H, NH_2, ArH), 5.57 (s, 2 H, O*CH*$_2$), 5.15 (s, 2 H, N*CH*$_2$); MS (ESI+) m/z (M+H): 571.0 and 573.1; Anal. Calcd for $C_{29}H_{27}BrN_6O_2$: C, 60.95; H, 4.76; N, 14.71. Found C, 60.91; H, 4.81; N, 14.64.

4.1.6.17. 2-{2-Amino-6-(naphthalen-1-yl)pyrimidin-4-yl}-5-{(1-(4-bromobenzyl)-1*H*-1,2,3-triazol-4-yl) methoxy} phenol 6{17}. The reaction of **4**{20} (0.5 g, 0.93 mmol) and Guanidine hydrochloride (0.98 g, 1.02 mmol) as described above gave yellow solid; mp 188–190 °C, yield, 0.26 g (51%); IR (KBr) cm^{-1}: 3428.6, 3339.7, 2369.9, 1576.9; ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO-d_6) δ: 8.22–8.14 (m, 1H, ArH), 8.11 (s, 1H, triazolyl C*H*=), 7.96–7.90 (m, 2H, ArH), 7.80 (d, J = 9.0 Hz, 1H, ArH), 7.64 (d, J = 6.24 Hz,1H, ArH), 7.57–7.46 (m, 6H, ArH), 7.26–7.23 (m, 3H, ArH), 7.04 (br s, 2H, N*H*$_2$), 6.54 (d, J = 0.21 Hz, 1H, ArH), 6.49 (dd, 1H, J_1 = 0.21, J_2 = 8.82 Hz, 1H, ArH), 5.55 (s, 2 H, O*CH*$_2$), 5.14 (s, 2 H, N*CH*$_2$); MS (ESI+) m/z (M+H): 579.0 and 581.0; Anal. Calcd for $C_{30}H_{23}BrN_6O_2$: C, 62.18; H, 4.00; N, 14.50. Found C, 62.21; H, 4.07; N, 14.54.

4.2. Biological assay

4.2.1. Material and method for Fas-II inhibitory screening

4.2.1.1. FAS-II inhibition assay. FAS-II inhibitory activity was assessed using a recombinant non-pathogenic mycobacterial

strain, *Mycobacterium aurum*, which contains *M. tuberculosis kas* operon promoter in fusion with *lacZ* reporter gene.[33] The strain shows continued expression of reporter gene under the influence of *kas* operon promoter during basal conditions, while an increased expression of the reporter gene is noticed only after treatment with FAS-II pathway inhibitors. The preliminary screening of the compounds shows FAS-II inhibitory activity at two different concentrations, 50 and 100 μM.

4.2.1.2. Bacterial strains and viability assay. The generation of recombinant *M. aurum* strains was described earlier.[33] *M. aurum* cultures were grown in Sauton's medium supplemented with 0.05% Tween-80 and kanamycin (25 μg/mL) and were plated on Nutrient-agar plates with 0.05% Tween-80 (NAT) supplemented with kanamycin. For post treatment viability assay, *M. aurum* was grown in Sauton's medium up to 0.6 OD_{600} and the culture was diluted to 0.05 OD with fresh medium. From these diluents, $\sim 1 \times 10^5$cells were inoculated into different tubes containing 5 ml fresh medium and added varying concentration of compounds. The cultures were allowed to grow for 12 h at 37 °C with continuous shaking at 180 rpm. The treated and untreated cultures were plated on NAT-Km plates using 10-fold serial dilution to count the number of viable cells. % inhibition was scored considering the number of bacterial colonies in untreated condition as 100%.

4.2.1.3. Reporter gene expression analysis. Recombinant *M. aurum* strains were grown in Sauton's medium with Kanamycin at 37 °C to 0.5 OD_{600} after which culture was diluted to 0.04–0.05 OD with fresh medium. Ten millilitre of diluted culture were distributed to separate tubes, equilibrated for 2 h at 37 °C and then varying concentrations (50 and 100 μM) of compounds were added to different tubes. Following 12 h incubation at 37 °C, 5 ml cultures from each tube were pelleted, washed and resuspended in PBS (Phosphate Buffer Saline, pH 7.2), sonicated at 4 °C and supernatant was collected by centrifugation at 13000 rpm for10 min at 4 °C. Protein contents were quantified using Bradford Assay reagent (Sigma B6916) as per manufacturer protocol. β-Gal assay was performed from total cellular protein as described earlier[1]. Briefly, same amount of protein were mixed with 200 μL of ONPG (4 mg/ml) and incubated for 30 min at 37 °C. Reaction was stopped by adding 500 μL of $1MNa_2CO_3$ and optical density was measured at 410 nm. Experiments were carried out in triplicates for each treatment and β-galactosidase units were calculated for each set individually. The culture at each point was also plated to confirm the decline in viability of cells after drug treatment. The whole experiment was repeated twice and similar trends in results were obtained. Mean value and standard deviation were calculated and plotted for each set of data.

4.2.2. Material and method for PknG inhibitory screening

4.2.2.1. Purification of Mycobacterium tuberculosis PKnG. The compounds were screened against the mycobacterial serine threonine protein kinase G (PknG). The recombinantly purified enzyme was used for the study.[36] Briefly the *Mycobacterium tuberculosis* (MTB) genomic DNA was used as a template for amplification of *pknG* gene by PCR. The gene was cloned in pTriEx4 vector using the primers containing the desired restriction enzyme sites. For expression in *E. coli*, *pknG* with *Hind*III flanking sites was subcloned in pTriEx4 vector. *E. coli* BL21 (DE3) cells were transformed with pTriEX4-*pknG* and transformants were grown in LB medium containing ampicillin (100 μg/ml) at 37 °C, till OD at 600 nm reached 0.6. IPTG was then added to a final concentration of 0.8 mM and cultures were further grown for an additional 4 h at 37 °C with shaking. Cells were harvested by centrifugation at 5000 × g for 15 min and resuspended in binding buffer [Sodium Phosphate 20 mM (pH 7.4), NaCl 50 mM, Imidazole 5 mM, PMSF 1 mM] and sonicated on ice for 2 min. After sonication TritonX-100 was added in cell lysate at a final concentration of 1% before centrifugation at 30000 × g for 30 min at 4 °C. Supernatant was loaded onto Ni2+-NTA column, washed with 60 mM Imidazole and 6-His-PknG was eluted with 200 mM Imidazole. Affinity purified 6-His-PknG was further purified by size exclusion chromatography using Sephacryl 200 column and AKTA Prime protein purification system (GE healthcare).

4.2.2.2. Screening of compounds against Kinase activity. The compounds were dissolved completely in DMSO. For the determination of primary efficacy, 100 μM concentration of each compound was screened using purified PknG as an enzyme and myelin basic protein as a substrate. The activity and the inhibition were determined by using luciferase activity mediated by ATP, by ADP–Glo (Promega, USA). Briefly, ADP-Glo™ Kinase Assay is a luminescent kinase assay that measures ADP formed from a kinase reaction. ADP is converted into ATP, which is converted into light by Ultra-Glo™ Luciferase.

4.2.3. Cytotoxicity Assay: CC_{50} Determination

The cytotoxicity assays was performed according to method reported by O'Brien et al.[37] with a slight modification. Macrophages were harvested from subconfluent monolayers. The suspended cells were seeded in 96-well microplates, at an approximate initial density of 1×10^6 cells per well, in RPMI-1640 medium. Compounds were added at the concentrations from 100 μM to 12.5 μM. Rifampicin was taken as positive control for showing viability at concentrations from 4 μg/ml to 0.5 μg/ml. 96-well plates were incubated for 48 h at 5% CO_2 at 37 °C. After 48 h, the resazurin was added and incubated for 4 h. The fluorescence and absorbance were measured in a spectrophotometer at 535/590 nm. Cytotoxicity was determined by comparing the resulting fluorescence with the mean fluorescence of the control wells (untreated cells), and was expressed as percentage of cell viability. The 50% cytotoxic concentration (CC_{50}) is defined as the quantity of compound generating 50% of cell viability, compared to the control. The values of the percentages of cell viability were plotted against the concentrations, and CC_{50} was determined. Experiments were carried out in triplicates.

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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.bmc.2012.07.009.

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