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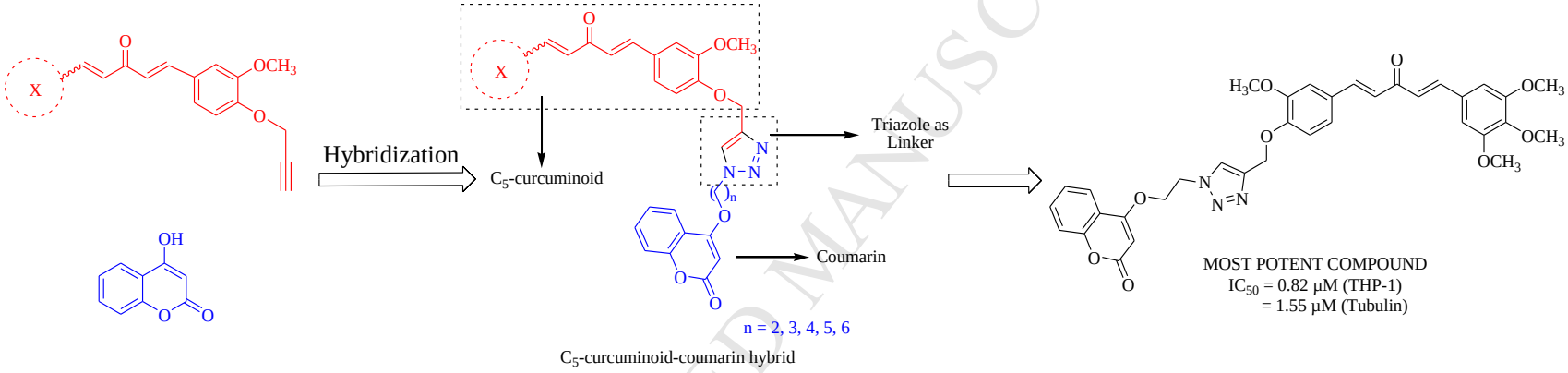

# Triazole tethered $C_5$-curcuminoid-coumarin based molecular hybrids as novel antitubulin agents: Design, synthesis, biological investigation and docking studies

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## 1. Introduction

Many of the currently available antitumor drugs are unable to differentiate between normal and neoplastic cells or to overcome primary or secondary resistance mechanisms evolved in the tumor cells [1-4]. Thus there is a pressing need for new antitumor agents with high potency, less toxicity in non-cancerous cells, and unique targets of action. Currently tumor therapy interfering with a single biological molecule or pathway has been successfully utilized. However, there is general belief that agents modulating more than one target or multiple sites on solitary target could have superior efficacy compared to solo target drugs.

Therefore, modulating various targets or more than one site on distinct target simultaneously can be achieved by the combination of several drugs with different targets or by single chemical entity that could modulate several sites of a particular target. As a result, there is increasing interest in the discovery of hybrids that concomitantly address more than one biological target for tumor treatment [5-7]. Keeping in view the limitations of the molecules modulating one target, these hybrids can give new dimensions to the molecules modulating more than one target site and can thus be extremely beneficial for the society which at the moment is striving hard to fight with this dreadful disease.

Tubulin is one of the most useful and strategic molecular targets for antitumor drugs. The dynamic process of microtubule assembly and disassembly can be blocked by various agents that bind to distinct sites in the β-tubulin subunit. By interfering with microtubule function, these agents arrest cells in mitosis, eventually leading to cell death, by both apoptosis and necrosis [8-10].

Triazole tethered bi-functional hybrids have been an area of focus for developing new cytotoxic agents in the last several years. Recently published reports on the cytotoxic potential of these hybrids attracted researchers worldwide towards their selection as a linker for the two functionalities [11-14].

Curcumin, a naturally occurring polyphenolic compound has shown tremendous success in various forms of therapeutic activities, among which, the chemotherapeutic effect in diverse forms of tumor is well documented and the most successful one. But, its poor solubility, absorption, bioavailability and rapid metabolism in human limit its clinical efficacy. To

overcome such limitations, $C_5$-curcuminoids have been synthesized [15-18] and some of the recently developed $C_5$-curcuminoid based molecular hybrids (**1** and **2**) (Fig. 1), exhibit potent antitumor activity [13, 14], which further supports its insertion as a vital pharmacophore in bi-functional molecular hybrids.

Coumarins, an elite class of naturally occurring compounds with promising therapeutic perspectives [19-21]. Sufficient numbers of recent reports on the cell damage potential of coumarin based molecules rationalize their inclusion in bi-functional molecular hybrids [22-24].

Therefore, the present study is targeted at the design (Fig. 2) and synthesis of triazole tethered $C_5$-curcuminoid-coumarin bifunctional hybrids via simple synthetic procedures and evaluation of these synthesized hybrids against a panel of human tumor cell lines using sulforhodamine B assay. Current reports on $C_5$-curcuminoid and coumarin based antitumor molecules (**2** and **3**) (Fig. 1) suggest tubulin as one of their primary targets [14, 24], therefore, hybrids exhibiting significant cytotoxicity were further tested for *in vitro* tubulin polymerization inhibition. *In vitro* microtubule polymerization inhibition by the most potent hybrid was also streamlined by exploring its key binding interactions with amino acid residues of tubulin using docking studies.

## 2. **Results and discussion**

### 2.1. Chemistry

Target hybrids were synthesized via a sequence of reactions (Scheme 1) starting from vanillin, where it was treated with acetone in the presence of 40% KOH at room temperature. The desired product vinyldenacetone (VDA) was thus formed. VDA was then treated with propargyl bromide in DMF as a solvent, in the presence of $K_2CO_3$ at room temperature to get the desired product i.e. propargylated vinyldenacetone (PVDA). PVDA was further treated with various substituted aldehydes in methanol as a solvent and the presence of 5% NaOH at room temperature in order to obtain the desired product wiz propargylated $C_5$-curcuminoid analogues.

Concurrently, 4-hydroxy coumarin was treated with diverse 1,2-dibromoalkanes in the presence of $K_2CO_3$, in DMF as a solvent at room temperature to get the desired product (4-(2-bromoalkoxy)-2H-chromen-2-one), which was further reacted with $NaN_3$ in DMF as a solvent, at room temperature to yield 4-(2-azidoalkoxy)-2H-chromen-2-one.

These 4-(2-azidoalkoxy)-2H-chromen-2-ones were treated with propargylated $C_5$-curcuminoid analogues in the presence of copper sulfate and sodium ascorbate, in DMF as a solvent, at room temperature to gain the desired triazole linked $C_5$-curcuminoid-coumarin hybrids.

In view of our recent report on triazole linked mono carbonyl curcumin-isatin bi-functional hybrids having antitubulin potential [14], only those $C_5$-curcuminoids have been synthesized and tethered to coumarin through triazole which earlier showed significant cytotoxicity when linked to isatin via triazole. Moreover, only those four cell lines i.e. THP-1, COLO-205, HCT-116 and PC-3 out of eight cell lines, in the previous study, have been employed for biological investigation which were sensitive towards triazole linked mono carbonyl curcumin-isatin bi-functional hybrids.

**2.2. Bioassay**

All the synthetics were investigated for *in-vitro* cytotoxicity against four human cancer cell lines (THP-1, COLO-205, HCT-116 and PC-3) using sulforhodamine B19 [25, 26]. The cells were allowed to proliferate in the presence of test material for 48 h. All the synthesized hybrids were screened against the cell lines at 50 µM. The hybrids showing percentage inhibition (Table 1) of greater than 70% at least against one cell line were only evaluated at different concentrations and $IC_{50}$ values (Table 2) were calculated. In contrary to our previous report [14], among the four cancer cell lines, THP-1, COLO-205 and HCT-116 were sensitive towards the designed hybrids whereas PC-3 was found to be resistant. More surprisingly, among the three sensitive cell lines, THP-1 cancer cell line instead of HCT-116 (most sensitive towards triazole linked mono carbonyl curcumin-isatin bi-functional hybrids) was the most sensitive one towards these hybrids. Compounds **A-2, A-3** and **A-7** displayed significant cytotoxicity with $IC_{50}$ value ranging from 0.82-4.68 µM, 2.34-6.78 µM and 4.48-9.95 µM respectively, against THP-1, HCT-116 and COLO-205 cell lines. The most active hybrid **A-2** with trimethoxy phenyl ring as Ring X ($IC_{50}$ = 0.82 µM) was almost three folds more active than **A-3** (dimethoxy phenyl ring as Ring X, $IC_{50}$= 2.34 µM), the second most potent hybrid against THP-1 cell line. Cytotoxicity results in Table 1 revealed an interesting structure activity relationship (Fig. 3) for these designed hybrids: (i) methoxy substituted phenyl ring as Ring X remarkably enhances the cytotoxic potential (compare **A-1** with **A-2** to **A-6**); (ii) placement of a hateroaryl ring such as furan and thiophene in place of the unsubstituted phenyl ring as Ring X improved the activity profile (compare **A-1**

with **A-8** and **A-9**); (iii) an enhanced effect was observed with the increased number of methoxy substituents on phenyl ring as Ring X such as trimethoxy phenyl > dimethoxy phenyl > monomethoxy phenyl (compare **A-2** and **A-3** with **A-4**, **A-5** and **A-6**); (iv) placement of naphthyl ring as Ring X behaved as a surrogate for dimethoxy substituted phenyl ring (compare **A-3** with **A-7**); (v) cytotxicity of hybrids with monomethoxy substituted phenyl ring as Ring X was found similar to the heteroaryl ring substituted hybrids (compare **A-4**, **A-5** and **A-6** with **A-8** and **A-9**). Thus, the overall preference order of Ring X is established as follows: trimethoxy phenyl > dimethoxy phenyl = naphthyl > monomethoxy phenyl = furan = thiophene > phenyl.

Additionally, the length of carbon-bridge connecting triazole ring with coumarin moiety considerably influences the activity. With the increase in chain length of carbon-bridge, cytotoxicity decreases significantly (compare **A** with **B**, **C** and **D**).

The most active compounds (**A-2**, **A-3** and **A-7**) among the series were evaluated for their inhibitory effects on tubulin polymerization as per the reported assay using a cytoskeleton tubulin polymerization assay kit [27, 28]. Compound **A-2** (most potent hybrid) with a trimethoxy phenyl ring (Ring X) also displayed the most potent antitubulin activity with $IC_{50}$ value of 1.55 µM. **A-3** also exhibited significant inhibition of tubulin polymerization with an $IC_{50}$ value of 2.88 µM. **A-7** with naphthyl ring (Ring X) was found to be endowed with weak inhibitory potential for tubulin polymerization. The results of the *in-vitro* tubulin polymerization assay clearly indicate that both **A-2** and **A-3** exert their cytotoxic effects through tubulin inhibition (Table 3).

### 2.3. Molecular docking

Docking study revealed that **A-2** fits well at the interface of β1/α2 subunits of tubulin and stabilized by various electrostatic interactions. Residues from the both subunits (β1and α2) were involved in D-R interactions (Fig. 4). The trimethoxy-phenyl ring (ring A) gets positioned in a cavity formed byArg2α2 (polar residue), Pro72β1, Ala99β1 and leu242α2 (a nonpolar residues). Here, -NH function of Arg2α2 (H-bond donor) is involved in a hydrogen bond interaction with –$OCH_3$ (-O: H=bond acceptor; d= 2.995Å). The presence of three methoxy groups (-$OCH_3$) in the aromatic phenyl ring (ring A) provides a unique blend polarity and hydrophobicity to the inhibitor **A-2**. Such functional group significantly increases the protein binding of inhibitors via van der Waals and dispersion interactions [29]. The trimethoxy-phenyl group is also present in

potent tubulin inhibitors such as colchicine, podophyllotoxin and combretastatin A-4. Therefore, this can be suggested that the presence of trimethoxy-phenyl group in **A-2** may be attributed for its superior activity. The ring B was found to be sandwiched between two polar residues namely Gln11β1 and Glu254α2. The $–OCH_3$ group of ring B was oriented towards the side chain carboxylate ionic head of Glu254α2 and involved H-bond interactions (d=2.404Å). Here, $–OCH_3$ is H-bond donor while carboxylate oxygen is H-bond acceptor. The $Mg^{2+}$ has shown two polar interactions with two oxygen linked at 3 and 4 position of ring B (ring B: $3–OCH_3$, d=2.377Å and $4–OCH_2–$ function, d=2.510Å).The triazole ring (ring C) was located near the carboxylate ionic head of Asp197β1.Here, this can be suggested that it is involved in polar interaction with Asp197β1. The ring D/E (2H-chromen-2-one moiety) was located in a cavity formed by Pro222β1, Thr223β1 and Thr224β1. Here, ring E has shown face-to-face π-π stacking interaction with Tyr224β1 (d=3.479Å). The face-to-face π-π stacking interaction play important role in binding of aromatic compounds with their target receptors and also contribute in their target specificity [30].

The docking study was helpful to explain the plausible mechanism of **A-2** mediated tubulin inhibition. Microtubules are dynamic polymers of αβ-tubulin that form various cellular structures including mitotic spindle for cell division. The microtubule-associated proteins (MAPs) and motor proteins regulate the growth and shrinkage of microtubule by selective binding to distinct conformations of polymerized or un-polymerized αβ-tubulin [31, 32]. The αβ-tubulin can adopt distinct conformation which contributes to the polymerization dynamics of microtubules. The diverse MAPs identify the distinct αβ-Tubulin conformations to build proper microtubule networks. This can be suggested that binding of **A-2** at the interface of β1-α2 heterodimer restrict the tubulin to adopt MAP-recognizable conformations and thus inhibit the tubulin polymerization into microtubule assembly. This results in G2/M arrest within the cell cycle and eventually cell death [31-34].

## 3. Conclusion

In continuation of our search for novel antitubulin bi-functional hybrids, $C_5$-curcuminoid-coumarin hybrids were designed, synthesized and evaluated for cytotoxicity against a panel of human cancer cell lines in the present study. Tubulin inhibitory potential of the most potent compound **A-2** (with trimethoxy phenyl ring as ring X and n = 2) was confirmed by *in vitro*

tubulin assay which clearly indicates that **A-2** exert its cytotoxic effect through tubulin inhibition. The worth mentioning inhibition of tubulin by compound **A-2** was also streamlined by docking studies.

## 4. Experimental section

### 4.1. Materials and measurements

The reagents were purchased from Sigma-Aldrich, Loba and CDH, India and used without further purification. All yields refer to isolated products after purification. Products were characterized by comparison with authentic samples and by spectroscopic data ($^{1}$H and $^{13}$C NMR, Elemental Analysis). $^{1}$H NMR and $^{13}$C NMR Spectra were recorded on Avance III HD 500 MHz BrukerBiospin Nuclear Magnetic Resonance Spectrometer. The spectra were measured in $CDCl_3$ and DMSO-$d_6$ relative to TMS (0.00 ppm). In $^{1}$H NMR chemical shifts were reported in δ values using tetramethylsilane as internal standard with number of protons, multiplicities (s-singlet, d-doublet, t-triplet, q-quartet, m-multiplet, dd-double doublet) and coupling constants (*J*) in Hz (Hertz) in the solvent indicated.

### 4.2. General procedure for the synthesis of vinyldenacetone (VDA)

Vanillin (20 g) was dissolved in acetone (50 ml) in a round bottom flask and 40% KOH (5 ml) was added to this reaction mixture. It was kept on stirring at room temperature. After the completion of reaction (monitored by TLC), reaction mixture was acidified with dilute hydrochloric acid until pH 7 was achieved. It was then poured in ice-cold water, filtered and dried to get the desired product i.e. vinyldenacetone (VDA). The physical data of vinyldenacetone is given below:

**4.2.1. (E)-4-(4-hydroxy-3-methoxyphenyl)but-en-2-one (VDA)**: Yield 72%; mp 71-73$^{o}$C. $^{1}$H NMR ($CDCl_3$, 500 MHz, δ, TMS = 0): 2.36 (s, 3H), 3.91 (s, 3H), 6.58 (d, *J* = 16.5 Hz, 1H), 6.92 (d, *J* = 7.5 Hz, 1H), 7.04–7.08 (m, 2H), 7.45 (d, *J* = 16.5 Hz, 1H). $^{13}$C NMR ($CDCl_3$, 125 MHz, δ, TMS = 0): 27.22, 55.84, 109.56, 114.99, 123.52, 124.82, 126.79, 144.03, 147.08, 148.51, 198.72.

### 4.3. General procedure for the synthesis of propargylated vinyldenacetone (PVDA)

VDA (1 eq) was dissolved in DMF, propargyl bromide (1 eq) and $K_2CO_3$ (1.5 eq) were added. Reaction mixture was kept on stirring at room temperature. After the completion of reaction (monitored by TLC), reaction mixture was poured on crushed ice and kept aside for some time. Then it was filtered and dried to obtain the desired product i.e. propargylated vinyldenacetone (PVDA).The physical data of propargylated vinyldenacetone is given below:

**4.3.1. (E)-4-(3-methoxy-4-(prop-2-ynyloxy)phenyl)but-3-en-2-one (PVDA)**: Yield 74%; mp 74-77$^{o}$C. $^{1}$H NMR ($CDCl_3$, 500 MHz, δ, TMS = 0): 2.38 (s, 3H). 2.55 (s, 1H), 3.92 (s, 3H), 4.81 (s, 2H), 6.63 (d, *J* = 16.5 Hz, 1H), 7.05 (d, *J* = 7.5 Hz, 1H), 7.08–7.14 (m, 2H), 7.47 (d, *J* = 16.5 Hz, 1H). $^{13}$C NMR ($CDCl_3$, 125 MHz, δ, TMS = 0): 27.38, 55.93, 56.62, 76.28, 77.94, 110.30, 113.75, 122.45, 125.71, 128.50, 143.28, 149.00, 149.83, 198.33.

**4.4. General procedure for the synthesis of propargylated $C_5$-curcuminoidanalogs**

PVDA (1 eq) was dissolved in methanol, 3,4-dimethoxy benzaldehyde (1 eq) and 5% NaOH (0.5 ml) were added. Reaction mixture was kept on stirring at room temperature. After the completion of reaction (monitored by TLC), reaction mixture was poured on crushed ice and kept aside for some time. Then it was filtered and dried to obtain the desired product i.e. propargylated $C_5$-curcuminoid analog (M-3).

**4.4.1 (1E, 4E)-1-(3-methoxy-4-(prop-2-ynyloxy)phenyl)-5-(3,4-dimethoxyphenyl)penta-1,4-dien-3-one (M-3):** Yield 71%; mp 76-78 $^{o}$C. $^{1}$H NMR ($CDCl_3$, 500 MHz, δ, TMS = 0): 2.88 (s, 1H), 3.91 (s, 3H), 3.93 (s, 6H), 4.81 (s, 2H), 6.94 (d, *J* = 8.5 Hz, 1H), 7.03–7.08 (m, 3H), 7.23–7.25 (m, 4H), 7.66-7.70 (m, 2H). $^{13}$C NMR ($CDCl_3$, 125 MHz, δ, TMS = 0): 60.75, 61.38, 64.92, 81.90, 83.25, 114.94, 115.01, 115.55, 116.18, 118.70, 127.90, 128.14, 128.53, 129.01, 132.96, 133.72, 147.40, 147.81, 153.72, 154.00, 154.50, 156.12, 175.48, 193.29.

All the other propargylated $C_5$-curcuminoid analogs were synthesized by following the above mentioned procedure, using various substituted aromatic aldehydes.

**4.5. General procedure for the synthesis of 4-(2-bromoalkoxy)-2H-chromen-2-one**

4-Hydroxy coumarin (1 eq) was dissolved in DMF, dibromoethane (1 eq) and $K_2CO_3$ (1.5 eq) were added. Reaction mixture was kept on stirring at room temperature. After the completion of reaction (evident by TLC), reaction mixture was poured on crushed ice and kept aside for some

time. Then it was filtered and dried to obtain the desired product i.e. 4-(2-bromoethoxy)-2H-chromen-2-one. All the other 4-(2-bromoalkoxy)-2H-chromen-2-ones were synthesized by following the above mentioned procedure, using various dibromoalkanes.

**4.6. General procedure for the synthesis of 4-(2-azidoalkoxy)-2H-chromen-2-one**

4-(2-bromoethoxy)-2H-chromen-2-one (1 eq) was dissolved in DMF and sodium azide (1 eq) was added. Reaction mixture was kept on stirring at room temperature. After the completion of reaction (evident by TLC), reaction mixture was poured on crushed ice and kept aside for some time. Then it was filtered and dried. Crude product thus obtained was impure, therefore, subjected to column chromatography, using chloroform as an eluent, to get the desired product i.e. 4-(2-azidoethoxy)-2H-chromen-2-one (HCE).

**4.6.1 4-(2-azidoethoxy)-2H-chromen-2-one (HCE):** Yield 91%; mp 92-94$^{o}$C. $^{1}$H NMR ($CDCl_3$, 500 MHz, δ, TMS = 0): 3.77 (t, 2H, *J* = 5 Hz), 4.47 (t, 2H, *J* = 5 Hz), 5.68 (s, 1H), 7.28-7.35 (m, 2H), 7.57-7.60 (m, 1H), 7.89 (d, 1H, *J* = 7.5 Hz). $^{13}$C NMR ($CDCl_3$, 125 MHz, δ, TMS = 0): 27.57, 30.90, 68.48, 90.89, 115.32, 116.80, 123.10, 124.06, 132.67, 153.35, 162.56, 164.90, 206.99.

All the other 4-(2-azidoalkoxy)-2H-chromen-2-ones were synthesized by following the above mentioned procedure, using various 4-(2-bromoalkoxy)-2H-chromen-2-ones.

**4.7. General procedure for the synthesis of triazole linked $C_5$-curcuminoid-coumarin hybrids**

$C_5$-curcuminoid (1 eq) and 4-(2-azidoalkoxy)-2H-chromen-2-one (1 eq) were dissolved in DMF, catalytic amount of copper sulphate and sodium ascorbate were added in it. Reaction mixture was kept aside at room temperature. After the completion of reaction (evident by TLC), reaction mixture was filtered on crushed ice to remove the excess of copper sulphate and sodium ascorbate and then kept aside for some time. Then it was filtered and dried to obtain the desired product i.e. triazole tethered $C_5$-curcuminoid-coumarin hybrids (A, B, C, D). The physical data of all the synthesized bi-functional hybrids is given below:

**4.7.1 (1E,4E)-1-phenyl-5-(3-methoxy-(4(1-(2-(2-oxo-2H-chromen-4-yloxy)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)penta-1,4-dien-3-one (A-1):** Yield 81%; mp 174-176$^{o}$C; MW

549.57. $^{1}$H NMR ($d_6$-DMSO, 500 MHz, δ, TMS = 0): 3.81 (s, 3H), 4.65 (s, 2H), 4.91 (s, 2H), 5.21 (s, 2H), 5.93 (s, 1H), 7.13-7.18 (m, 3H), 7.21-7.31 (m, 6H), 7.46-7.58 (m, 5H), 7.79-7.84 (m, 2H), 8.31 (s, 1H). $^{13}$C NMR ($d_6$-DMSO, 125 MHz, δ, TMS = 0): 48.90, 49.03, 56.01, 61.90, 68.36, 91.42, 110.88, 111.21, 112.17, 113.73, 115.31, 116.80, 123.18, 123.30, 123.71, 126.41, 128.01, 128.76, 135.20, 142.90, 143.23, 149.40, 149.57, 150.01, 151.51, 153.06, 162.06, 164.71, 188.69. Anal. Calcd for $C_{32}H_{27}N_3O_6$: C, 69.93; H, 4.95; N, 7.65; Found: C, 70.05; H, 4.75; N, 7.71.

**4.7.2 (1E,4E)-1-(3,4,5-trimethoxyphenyl)-5-(3-methoxy-(4((1-(2-(2-oxo-2H-chromen-4-yloxy)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)penta-1,4-dien-3-one (A-2):** Yield 89%; mp 130-132$^{o}$C; MW 639.65. $^{1}$H NMR ($d_6$-DMSO, 500 MHz, δ, TMS = 0): 3.71 (s, 3H), 3.79 (s, 3H), 3.85 (s, 6H), 4.64 (s, 2H), 4.95 (s, 2H), 5.21 (s, 2H), 5.97 (s, 1H), 7.12 (s, 2H), 7.21-7.39 (m, 7H), 7.64-7.73 (m, 4H), 8.30 (s, 1H). $^{13}$C NMR ($d_6$-DMSO, 125 MHz, δ, TMS = 0): 48.93, 56.11, 56.55, 60.61, 62.09, 68.31, 91.58, 106.58, 111.39, 113.78, 115.39, 116.87, 123.25, 123.43, 124.30, 124.66, 125.84, 130.79, 133.27, 140.10, 143.05, 143.20, 149.70, 150.19, 153.19, 153.59, 161.89, 164.71, 188.63. Anal. Calcd for $C_{35}H_{33}N_3O_9$: C, 65.72; H, 5.20; N, 6.57; Found: C, 65.58; H, 5.40; N, 6.46.

**4.7.3 (1E,4E)-1-(3,4-dimethoxyphenyl)-5-(3-methoxy-(4((1-(2-(2-oxo-2H-chromen-4-yloxy)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)penta-1,4-dien-3-one (A-3):** Yield 81%; mp 150-152$^{o}$C; MW 609.63. $^{1}$H NMR ($d_6$-DMSO, 500 MHz, δ, TMS = 0): 3.78 (s, 3H), 3.80 (s, 3H), 3.83 (s, 3H), 4.62 (s, 2H), 4.94 (s, 2H), 5.20 (s, 2H), 5.93 (s, 1H), 7.02 (d, 1H, *J* = 7.5 Hz), 7.19-7.38 (m, 9H), 7.64-7.70 (m, 4H), 8.41 (s, 1H). $^{13}$C NMR ($d_6$-DMSO, 125 MHz, δ, TMS = 0): 48.93, 49.05, 56.07, 56.08, 61.95, 68.30, 91.48, 110.92, 111.25, 112.10, 113.68, 115.32, 116.85, 123.23, 123.36, 123.73, 124.21, 124.71, 127.96, 133.33, 142.95, 143.19, 149.44, 149.63, 150.05, 151.55, 153.12, 162.00, 164.75, 188.69. Anal. Calcd for $C_{34}H_{31}N_3O_8$: C, 66.99; H, 5.13; N, 6.89; Found: C, 66.72; H, 5.28; N, 6.78.

**4.7.4 (1E,4E)-1-(4-methoxyphenyl)-5-(3-methoxy-(4((1-(2-(2-oxo-2H-chromen-4-yloxy)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)penta-1,4-dien-3-one (A-4):** Yield 84%; mp 165-167$^{o}$C; MW 579.60. $^{1}$H NMR ($d_6$-DMSO, 500 MHz, δ, TMS = 0): 3.73 (s, 6H), 4.66 (s, 2H), 4.94 (s, 2H), 5.23 (s, 2H), 5.95 (s, 1H), 7.04-7.15 (m, 5H), 7.19-7.26 (m, 5H), 7.34-7.45 (m, 3H), 7.77-7.80 (m, 2H), 8.33 (s, 1H). $^{13}$C NMR ($d_6$-DMSO, 125 MHz, δ, TMS = 0): 48.92,

56.15, 61.97, 68.84, 91.54, 89.38, 110.67, 111.68, 113.55, 115.28, 117.58, 118.79, 119.81, 120.94, 121.58, 123.55, 125.60, 126.86, 128.47, 128.59, 128.79, 136.28, 142.47, 149.05, 150.55, 152.77, 162.04, 164.78, 188.67. Anal. Calcd for $C_{33}H_{29}N_3O_7$: C, 68.38; H, 5.04; N, 7.25; Found: C, 68.48; H, 4.95; N, 7.37.

**4.7.5 (1E,4E)-1-(3-methoxyphenyl)-5-(3-methoxy-(4((1-(2-(2-oxo-2H-chromen-4-yloxy)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)penta-1,4-dien-3-one (A-5):** Yield 84%; mp 163-165$^{o}$C; MW 579.60. $^{1}$H NMR ($d_6$-DMSO, 500 MHz, δ, TMS = 0): 3.72 (s, 3H), 3.79 (s, 3H), 4.64 (s, 2H), 4.90 (s, 2H), 5.20 (s, 2H), 5.93 (s, 1H), 7.04-7.14 (m, 6H), 7.22-7.34 (m, 7H), 7.64 (m, 2H), 8.39 (s, 1H). $^{13}$C NMR ($d_6$-DMSO, 125 MHz, δ, TMS = 0): 48.89, 56.09, 61.91, 68.78, 91.49, 89.30, 110.65, 111.62, 113.50, 115.20, 117.52, 118.73, 119.74, 120.90, 121.54, 123.49, 125.56, 126.82, 128.43, 128.54, 128.77, 136.20, 142.40, 149.00, 150.52, 152.74, 162.05, 164.74, 188.63. Anal. Calcd for $C_{33}H_{29}N_3O_7$: C, 68.38; H, 5.04; N, 7.25; Found: C, 66.21; H, 5.14; N, 6.68.

**4.7.6 (1E,4E)-1-(2-methoxyphenyl)-5-(3-methoxy-(4((1-(2-(2-oxo-2H-chromen-4-yloxy)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)penta-1,4-dien-3-one (A-6):** Yield 84%; mp 161-163$^{o}$C; MW 579.60. $^{1}$H NMR ($d_6$-DMSO, 500 MHz, δ, TMS = 0): 3.70 (s, 6H), 4.62 (s, 2H), 4.87 (s, 2H), 5.18 (s, 2H), 5.85 (s, 1H), 7.04-7.14 (m, 6H), 7.18-7.25 (m, 7H), 8.12 (s, 1H), 8.39 (m, 2H). $^{13}$C NMR ($d_6$-DMSO, 125 MHz, δ, TMS = 0): 48.96, 56.15, 61.96, 68.79, 91.39, 111.60, 114.24, 115.00, 117.54, 119.76, 120.98, 121.06, 121.56, 123.40, 125.56, 126.87, 127.49, 128.48, 128.56, 129.00, 142.44, 149.08, 149.56, 152.74, 157.76, 162.10, 164.79, 188.74. Anal. Calcd for $C_{33}H_{29}N_3O_7$: C, 68.38; H, 5.04; N, 7.25; Found: C, 66.42; H, 5.18; N, 6.86.

**4.7.7 (1E,4E)-1-naphthyl-5-(3-methoxy-(4((1-(2-(2-oxo-2H-chromen-4-yloxy)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)penta-1,4-dien-3-one (A-7):** Yield 85%; mp 140-142$^{o}$C; MW 599.63. $^{1}$H NMR ($d_6$-DMSO, 500 MHz, δ, TMS = 0): 3.83 (s, 3H), 4.67 (s, 2H), 4.94 (s, 2H), 5.23 (s, 2H), 5.91 (s, 1H), 7.14-7.19 (m, 3H), 7.24-7.34 (m, 5H), 7.43-7.53 (m, 5H), 7.77-7.84 (m, 5H), 8.31 (s, 1H). $^{13}$C NMR ($d_6$-DMSO, 125 MHz, δ, TMS = 0): 48.94, 49.00, 56.06, 61.97, 68.40, 91.46, 110.93, 111.26, 112.24, 113.79, 115.37, 116.87, 123.26, 123.37, 123.77, 124.00, 126.02, 126.34, 126.41, 128.01, 128.76, 128.80, 135.20, 142.96, 143.26, 149.47, 149.63, 150.11, 151.58, 153.16, 162.08, 164.75, 188.60. Anal. Calcd for $C_{36}H_{29}N_3O_6$: C, 72.11; H, 4.87; N, 7.01; Found: C, 72.21; H, 4.77; N, 7.21.

**4.7.8 (1E,4E)-1-(furan-2-yl)-5-(3-methoxy-(4((1-(2-(2-oxo-2H-chromen-4-yloxy)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)penta-1,4-dien-3-one (A-8):** Yield 80%; mp 155-157$^{o}$C; MW 539.54. $^{1}$H NMR ($d_6$-DMSO, 500 MHz, δ, TMS = 0): 3.80 (s, 3H), 4.60 (s, 2H), 4.95 (s, 2H), 5.22 (s, 2H), 5.95 (s, 1H), 7.13-7.16 (m, 3H), 7.32-7.43 (m, 6H), 7.69-7.77 (m, 5H), 8.42 (s, 1H). $^{13}$C NMR ($d_6$-DMSO, 125 MHz, δ, TMS = 0): 48.95, 56.14, 61.99, 68.80, 91.58, 111.40, 111.60, 112.72, 115.20, 117.50, 119.73, 120.90, 121.5, 123.45, 125.55, 126.72, 128.58, 129.45, 139.00, 142.42, 145.90, 149.02, 149.70, 151.62, 152.76, 162.05, 164.68, 188.66. Anal. Calcd for $C_{30}H_{25}N_3O_7$: C, 66.78; H, 4.67; N, 7.79; Found: C, 66.82; H, 4.59; N, 7.86.

**4.7.9 (1E,4E)-1-(thiophen-2-yl)-5-(3-methoxy-(4((1-(2-(2-oxo-2H-chromen-4-yloxy)ethyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)penta-1,4-dien-3-one (A-9):** Yield 85%; mp 147-149$^{o}$C; MW 555.60. $^{1}$H NMR ($d_6$-DMSO, 500 MHz, δ, TMS = 0): 3.83 (s, 3H), 4.61 (s, 2H), 4.96 (s, 2H), 5.25 (s, 2H), 5.97 (s, 1H), 7.09-7.14 (m, 3H), 7.36-7.45 (m, 6H), 7.67-7.77 (m, 5H), 8.41 (s, 1H). $^{13}$C NMR ($d_6$-DMSO, 125 MHz, δ, TMS = 0): 48.92, 56.12, 61.97, 68.82, 91.56, 111.41, 111.64, 112.76, 115.27, 117.55, 119.77, 120.95, 121.4, 123.46, 125.54, 126.77, 128.54, 129.44, 137.87, 142.43, 145.96, 149.06, 149.74, 151.67, 152.77, 162.03, 164.62, 188.65. Anal. Calcd for $C_{30}H_{25}N_3O_6S$: C, 64.85; H, 4.54; N, 7.56; S, 5.77; Found: C, 64.92; H, 4.46; N, 7.67; S, 5.62.

**4.7.10 (1E,4E)-1-phenyl-5-(3-methoxy-(4((1-(2-(2-oxo-2H-chromen-4-yloxy)propyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)penta-1,4-dien-3-one (B-1):** Yield 80%; mp 158-160$^{o}$C; MW 563.60. $^{1}$H NMR ($d_6$-DMSO, 500 MHz, δ, TMS = 0): 2.11 (s, 2H), 3.75 (s, 3H), 4.61 (s, 2H), 4.92 (s, 2H), 5.25 (s, 2H), 5.94 (s, 1H), 7.15-7.21 (m, 3H), 7.27-7.36 (m, 6H), 7.43-7.53 (m, 5H), 7.78-7.84 (m, 2H), 8.34 (s, 1H). $^{13}$C NMR ($d_6$-DMSO, 125 MHz, δ, TMS = 0): 28.16, 48.98, 56.25, 63.49, 72.73, 86.14, 110.73, 111.70, 113.56, 115.27, 117.64, 118.84, 119.83, 120.96, 121.57, 123.49, 125.55, 126.93, 128.49, 128.82, 129.84, 135.29, 142.49, 149.07, 149.78, 150.25, 152.84, 162.08, 164.88, 188.74. Anal. Calcd for $C_{33}H_{29}N_3O_6$: C, 70.33; H, 5.19; N, 7.46; Found: C, 70.43; H, 5.29; N, 7.32.

**4.7.11 (1E,4E)-1-(3,4,5-trimethoxyphenyl)-5-(3-methoxy-(4((1-(2-(2-oxo-2H-chromen-4-yloxy)propyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)penta-1,4-dien-3-one (B-2):** Yield 89%; mp 117-119$^{o}$C; MW 653.68. $^{1}$H NMR ($d_6$-DMSO, 500 MHz, δ, TMS = 0): 2.15 (s, 2H), 3.73 (s, 3H), 3.81 (s, 3H), 3.82 (s, 6H), 4.65 (s, 2H), 4.95 (s, 2H), 5.21 (s, 2H), 5.88 (s, 1H), 7.03 (s, 2H),

7.14-7.21 (m, 7H), 7.65-7.81 (m, 4H), 8.31 (s, 1H). $^{13}$C NMR ($d_6$-DMSO, 125 MHz, δ, TMS = 0): 28.60, 48.90, 56.20, 63.40, 72.60, 86.04, 103.97, 111.60, 115.20, 117.55, 119.72, 120.97, 121.53, 123.46, 125.56, 126.87, 128.45, 128.54, 129.50, 138.49, 142.44, 149.00, 149.70, 150.26, 150.70,152.75, 162.13, 165.23, 188.65. Anal. Calcd for $C_{36}H_{35}N_3O_9$: C, 66.15; H, 5.40; N, 6.43; Found: C, 66.18; H, 5.30; N, 6.56.

**4.7.12 (1E,4E)-1-(3,4-dimethoxyphenyl)-5-(3-methoxy-(4((1-(2-(2-oxo-2H-chromen-4-yloxy)propyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)penta-1,4-dien-3-one (B-3):** Yield 78%; mp 133-135$^o$C; MW 623.65. $^1$H NMR ($d_6$-DMSO, 500 MHz, δ, TMS = 0): 2.14 (s, 2H), 3.75 (s, 3H), 3.83 (s, 3H), 3.85 (s, 3H), 4.64 (s, 2H), 4.96 (s, 2H), 5.23 (s, 2H), 5.96 (s, 1H), 7.03-7.13 (m, 6H), 7.22-7.34 (m, 6H), 7.62-7.67 (m, 2H), 8.41 (s, 1H). $^{13}$C NMR ($d_6$-DMSO, 125 MHz, δ, TMS = 0): 28.06, 48.94, 56.20, 63.40, 72.66, 111.62, 115.26, 117.54, 119.70, 120.98, 121.56, 123.46, 125.56, 126.88, 128.44, 128.56, 142.45, 149.70, 150.22, 152.77, 162.00, 164.75, 188.69. Anal. Calcd for $C_{35}H_{33}N_3O_8$: C, 67.41; H, 5.33; N, 6.74; Found: C, 66.59; H, 5.29; N, 6.79.

**4.7.13 (1E,4E)-1-(4-methoxyphenyl)-5-(3-methoxy-(4((1-(2-(2-oxo-2H-chromen-4-yloxy)propyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)penta-1,4-dien-3-one (B-4):** Yield 84%; mp 150-152$^o$C; MW 593.63. $^1$H NMR ($d_6$-DMSO, 500 MHz, δ, TMS = 0): 2.15 (s, 2H), 3.79 (s, 6H), 4.63 (s, 2H), 4.92 (s, 2H), 5.21 (s, 2H), 5.92 (s, 1H), 7.14-7.23 (m, 5H), 7.26-7.33 (m, 5H), 7.43-7.48 (m, 3H), 7.67-7.72 (m, 2H), 8.35 (s, 1H). $^{13}$C NMR ($d_6$-DMSO, 125 MHz, δ, TMS = 0): 28.15, 48.95, 55.97, 56.27, 63.45, 72.67, 86.19, 110.74, 111.73, 113.56, 115.27, 117.64, 118.83, 119.84, 120.96, 121.57, 123.49, 125.59, 126.93, 128.49, 128.63, 129.84, 136.28, 142.50, 149.06, 149.78, 150.25, 152.84, 162.13, 164.87, 188.76. Anal. Calcd for $C_{34}H_{31}N_3O_7$: C, 68.79; H, 5.26; N, 7.08; Found: C, 68.89; H, 5.16; N, 7.24.

**4.7.14 (1E,4E)-1-(3-methoxyphenyl)-5-(3-methoxy-(4((1-(2-(2-oxo-2H-chromen-4-yloxy)propyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)penta-1,4-dien-3-one (B-5):** Yield 80%; mp 146-148$^o$C; MW 593.63. $^1$H NMR ($d_6$-DMSO, 500 MHz, δ, TMS = 0): 2.13 (s, 2H), 3.77 (s, 6H), 4.65 (s, 2H), 4.95 (s, 2H), 5.22 (s, 2H), 5.97 (s, 1H), 7.13-7.25 (m, 6H), 7.29-7.40 (m, 7H), 7.71-7.80 (m, 2H), 8.42 (s, 1H). $^{13}$C NMR ($d_6$-DMSO, 125 MHz, δ, TMS = 0): 28.12, 48.91, 55.90, 56.20, 63.44, 72.67, 86.09, 110.68, 111.64, 113.51, 115.21, 117.57, 118.78, 119.77, 120.90, 121.51, 123.44, 125.55, 126.88, 128.45, 128.56, 129.77, 136.20, 142.44, 149.00, 149.70,

150.20, 152.77, 162.05, 164.81, 188.70. Anal. Calcd for $C_{34}H_{31}N_3O_7$: C, 68.79; H, 5.26; N, 7.08; Found: C, 66.82; H, 5.31; N, 6.97.

**4.7.15 (1E,4E)-1-(2-methoxyphenyl)-5-(3-methoxy-(4((1-(2-(2-oxo-2H-chromen-4-yloxy)propyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)penta-1,4-dien-3-one (B-6):** Yield 83%; mp 145-147°C; MW 593.63. $^1$H NMR ($d_6$-DMSO, 500 MHz, δ, TMS = 0): 2.16 (s, 2H), 3.73 (s, 6H), 4.64 (s, 2H), 4.97 (s, 2H), 5.25 (s, 2H), 5.94 (s, 1H), 7.23-7.30 (m, 6H), 7.40-7.48 (m, 7H), 7.79-7.84 (m, 2H), 8.40 (s, 1H). $^{13}$C NMR ($d_6$-DMSO, 125 MHz, δ, TMS = 0): 28.06, 48.97, 56.20, 56.35, 63.45, 72.67, 86.06, 111.60, 114.23, 115.00, 115.23, 117.53, 119.70, 120.90, 121.06, 121.56, 123.40, 125.52, 126.80, 127.40, 128.04, 128.50, 129.02, 142.48, 149.08, 150.24, 152.70, 157.78, 162.08, 164.82, 188.78. Anal. Calcd for $C_{34}H_{31}N_3O_7$: C, 68.79; H, 5.26; N, 7.08; Found: 68.60; H, 5.36; N, 7.00.

**4.7.16 (1E,4E)-1-naphthyl-5-(3-methoxy-(4((1-(2-(2-oxo-2H-chromen-4-yloxy)propyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)penta-1,4-dien-3-one (B-7):** Yield 80%; mp 124-126°C; MW 613.66. $^1$H NMR ($d_6$-DMSO, 500 MHz, δ, TMS = 0): 2.14 (s, 2H), 3.77 (s, 3H), 4.63 (s, 2H), 4.91 (s, 2H), 5.22 (s, 2H), 5.93 (s, 1H), 7.15-7.21 (m, 3H), 7.25-7.34 (m, 5H), 7.42-7.52 (m, 5H), 7.75-7.82 (m, 5H), 8.34 (s, 1H). $^{13}$C NMR ($d_6$-DMSO, 125 MHz, δ, TMS = 0): 28.20, 48.96, 49.05, 56.04, 61.94, 68.46, 91.52, 110.91, 111.27, 112.27, 113.83, 115.42, 116.94, 123.32, 123.43, 123.83, 124.06, 126.07, 126.39, 126.48, 128.04, 128.78, 128.86, 135.28, 142.99, 143.30, 149.53, 149.67, 150.19, 151.59, 153.21, 162.13, 164.79, 188.63. Anal. Calcd for $C_{37}H_{31}N_3O_6$: C, 72.42; H, 5.09; N, 6.85; Found: C, 72.49; H, 6.96; N, 6.95.

**4.7.17 (1E,4E)-1-(furan-2-yl)-5-(3-methoxy-(4((1-(2-(2-oxo-2H-chromen-4-yloxy)propyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)penta-1,4-dien-3-one (B-8):** Yield 83%; mp 139-141°C; MW 553.56. $^1$H NMR ($d_6$-DMSO, 500 MHz, δ, TMS = 0): 2.14 (s, 2H), 3.73 (s, 3H), 4.66 (s, 2H), 4.95 (s, 2H), 5.23 (s, 2H), 5.91 (s, 1H), 7.19-7.25 (m, 4H), 7.43-7.49 (m, 7H), 7.80-7.84 (m, 3H), 8.42 (s, 1H). $^{13}$C NMR ($d_6$-DMSO, 125 MHz, δ, TMS = 0): 28.60, 48.95, 56.25, 63.45, 72.65, 86.05, 111.45, 111.63, 112.79, 115.20, 117.50, 119.75, 120.91, 121.56, 123.45, 125.55, 126.87, 128.49, 128.56, 129.43, 139.00, 142.40, 145.94, 149.06, 149.70, 150.24, 151.67, 152.77, 162.08, 164.82, 188.78. Anal. Calcd for $C_{31}H_{27}N_3O_7$: C, 67.26; H, 4.92; N, 7.59; Found: C, 67.37; H, 4.81; N, 7.61.

**4.7.18 (1E,4E)-1-(thiophen-2-yl)-5-(3-methoxy-(4((1-(2-(2-oxo-2H-chromen-4-yloxy)propyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)penta-1,4-dien-3-one (B-9):** Yield 83%; mp 130-132$^{o}$C; MW 569.63. $^{1}$H NMR ($d_6$-DMSO, 500 MHz, δ, TMS = 0): 2.16 (s, 2H), 3.76 (s, 3H), 4.62 (s, 2H), 4.97 (s, 2H), 5.27 (s, 2H), 5.96 (s, 1H), 7.12-7.22 (m, 3H), 7.41-7.49 (m, 7H), 7.82-7.88 (m, 4H), 8.40 (s, 1H). $^{13}$C NMR ($d_6$-DMSO, 125 MHz, δ, TMS = 0): 28.64, 48.97, 56.29, 63.42, 72.61, 86.06, 111.42, 111.68, 112.80, 115.24, 117.55, 119.70, 120.93, 121.58, 123.42, 125.60, 126.90, 127.19, 128.20, 128.56, 137.83, 138.50, 142.45, 145.99, 149.10, 149.77, 150.30, 151.66, 152.80, 162.10, 164.94, 188.79. Anal. Calcd for $C_{31}H_{27}N_3O_6S$: C, 65.36; H, 4.78; N, 7.38; S, 5.63; Found: C, 65.43; H, 4.68; N, 7.52; S, 5.53.

**4.7.19 (1E,4E)-1-phenyl-5-(3-methoxy-(4((1-(2-(2-oxo-2H-chromen-4-yloxy)butyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)penta-1,4-dien-3-one (C-1):** Yield 85%; mp 136-138$^{o}$C; MW 577.63. $^{1}$H NMR ($d_6$-DMSO, 500 MHz, δ, TMS = 0): 1.83 (s, 2H), 2.07 (s, 2H), 3.86 (s, 3H), 4.27 (s, 2H), 4.54 (s, 2H), 5.21 (s, 2H), 5.88 (s, 1H), 7.03-7.08 (m, 3H), 7.23-7.38 (m, 6H), 7.45-7.57 (m, 5H), 7.77-7.84 (m, 2H), 8.35 (s, 1H).$^{13}$C NMR ($d_6$-DMSO, 125 MHz, δ, TMS = 0): 24.85, 28.00, 52.55, 56.27, 65.64, 72.67, 86.04, 111.65, 115.2, 117.53, 119.76, 120.93, 121.56, 123.45, 125.56, 126.40, 126.80, 128.09, 128.56, 128.74, 135.20, 142.40, 149.00, 149.75, 150.24, 152.78, 128.43, 162.12, 165.38, 188.55. Anal. Calcd for $C_{34}H_{31}N_3O_6$: C, 70.70; H, 5.41; N, 7.27; Found: C, 70.80; H, 5.30; N, 7.38.

**4.7.20 (1E,4E)-1-(3,4,5-trimethoxyphenyl)-5-(3-methoxy-(4((1-(2-(2-oxo-2H-chromen-4-yloxy)butyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)penta-1,4-dien-3-one (C-2):** Yield 89%; mp 96-98$^{o}$C; MW 667.70. $^{1}$H NMR ($d_6$-DMSO, 500 MHz, δ, TMS = 0): 1.81 (s, 2H), 2.05 (s, 2H), 3.71 (s, 3H), 3.82 (s, 3H), 3.85 (s, 6H), 4.25 (s, 2H), 4.50 (s, 2H), 5.20 (s, 2H), 5.89 (s, 1H), 7.13 (s, 2H), 7.24-7.41 (m, 7H), 7.66-7.83 (m, 4H), 8.31 (s, 1H). $^{13}$C NMR ($d_6$-DMSO, 125 MHz, δ, TMS = 0): 25.49, 26.83, 49.46, 56.01, 56.51, 60.60, 62.09, 69.22, 91.03, 106.49, 111.18, 113.67, 115.67, 116.91, 123.33, 123.56, 124.25, 124.68, 125.83, 130.79, 133.82, 139.97, 143.09, 143.27, 149.65, 150.20, 153.20, 153.57, 162.14, 165.36, 188.64. Anal. Calcd for $C_{37}H_{37}N_3O_9$: C, 66.56; H, 5.59; N, 6.29; Found: C, 66.46; H, 5.73; N, 6.15.

**4.7.21 (1E,4E)-1-(3,4-dimethoxyphenyl)-5-(3-methoxy-(4((1-(2-(2-oxo-2H-chromen-4-yloxy)butyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)penta-1,4-dien-3-one (C-3):** Yield 85%; mp 112-114$^{o}$C; MW 637.68. $^{1}$H NMR ($d_6$-DMSO, 500 MHz, δ, TMS = 0): 1.81 (s, 2H), 2.05 (s,

2H), 3.82 (s, 6H), 3.84 (s, 3H), 4.25 (s, 2H), 4.50 (s, 2H), 5.19 (s, 2H), 5.89 (s, 1H), 7.03 (d, 1H, *J* = 7.5 Hz), 7.25-7.40 (m, 8H), 7.65-7.83 (m, 5H), 8.31 (s, 1H). $^{13}$C NMR ($d_6$-DMSO, 125 MHz, δ, TMS = 0): 25.49, 26.83, 49.46, 56.04, 56.08, 69.21, 79.61, 91.48, 110.89, 111.17, 112.08, 113.68, 115.67, 116.91, 123.33, 123.46, 123.73, 124.26, 124.40, 124.67, 133.21, 142.92, 143.13, 149.44, 149.64, 150.12, 151.52, 153.20, 162.15, 165.36, 188.58. Anal. Calcd for $C_{36}H_{35}N_3O_8$: C, 67.81; H, 5.53; N, 6.59; Found: C, 67.92; H, 5.43; N, 6.68.

**4.7.22 (1E,4E)-1-(4-methoxyphenyl)-5-(3-methoxy-(4((1-(2-(2-oxo-2H-chromen-4-yloxy)butyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)penta-1,4-dien-3-one (C-4):** Yield 82%; mp 126-128$^o$C; MW 607.65. $^1$H NMR ($d_6$-DMSO, 500 MHz, δ, TMS = 0): 1.86 (s, 2H), 2.04 (s, 2H), 3.80 (s, 3H), 3.82 (s,3H), 4.24 (s, 2H), 4.45 (s, 2H), 5.23 (s, 2H), 5.86 (s, 1H), 7.07-7.16 (m, 5H), 7.20-7.27 (m, 5H), 7.38-7.42 (m, 3H), 7.75-7.80 (m, 2H), 8.32 (s, 1H). $^{13}$C NMR ($d_6$-DMSO, 125 MHz, δ, TMS = 0): 25.45, 26.80, 49.43, 56.10, 56.08, 69.23, 79.64, 91.52, 110.67, 111.69, 113.59, 115.29, 117.62, 118.82, 119.79, 120.98, 121.58, 123.53, 125.59, 126.90, 128.49, 128.56, 129.58, 136.30, 142.49, 149.14, 149.77, 152.77, 162.19, 165.35, 188.57. Anal. Calcd for $C_{35}H_{33}N_3O_7$: C, 69.18; H, 5.47; N, 6.92; Found: C, 69.31; H, 5.37; N, 7.06.

**4.7.23 (1E,4E)-1-(3-methoxyphenyl)-5-(3-methoxy-(4((1-(2-(2-oxo-2H-chromen-4-yloxy)butyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)penta-1,4-dien-3-one (C-5):** Yield 79%; mp 124-126$^o$C; MW 607.65. $^1$H NMR ($d_6$-DMSO, 500 MHz, δ, TMS = 0): 1.84 (s, 2H), 2.08 (s, 2H), 3.80 (s, 3H), 3.82 (s, 3H), 4.22 (s, 2H), 4.48 (s, 2H), 5.23 (s, 2H), 5.86 (s, 1H), 7.12-7.19 (m, 6H), 7.35-7.43 (m, 7H), 7.69-7.82 (m, 2H), 8.36 (s, 1H). $^{13}$C NMR ($d_6$-DMSO, 125 MHz, δ, TMS = 0): 25.43, 26.79, 49.41, 56.05, 56.06, 69.18, 79.59, 91.46, 110.60, 111.62, 113.55, 115.23, 117.56, 118.78, 119.72, 120.90, 121.54, 123.47, 125.50, 126.85, 128.41, 128.51, 129.53, 136.26, 142.42, 149.06, 149.70, 152.73, 162.11, 165.32, 188.54. Anal. Calcd for $C_{35}H_{33}N_3O_7$: C, 69.18; H, 5.47; N, 6.92; Found: C, 67.98; H, 5.52; N, 6.83.

**4.7.24 (1E,4E)-1-(2-methoxyphenyl)-5-(3-methoxy-(4((1-(2-(2-oxo-2H-chromen-4-yloxy)butyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)penta-1,4-dien-3-one (C-6):** Yield 82%; mp 122-124$^o$C; MW 607.65. $^1$H NMR ($d_6$-DMSO, 500 MHz, δ, TMS = 0): 1.82 (s, 2H), 2.06 (s, 2H), 3.81 (s, 6H), 4.21 (s, 2H), 4.44 (s, 2H), 5.22 (s, 2H), 5.85 (s, 1H), 7.15-7.23 (m, 6H), 7.39-7.50 (m, 7H), 7.65-7.76 (m, 2H), 8.34 (s, 1H). $^{13}$C NMR ($d_6$-DMSO, 125 MHz, δ, TMS = 0): 25.41, 26.78, 49.40, 56.06, 56.07, 69.18, 79.59, 91.46, 111.66, 114.23, 115.20, 117.56, 119.72,

120.91, 121.50, 123.43, 125.55, 126.88, 127.40, 128.47, 129.00, 142.42, 149.06, 149.72, 150.24, 152.70, 157.78, 162.11, 165.32, 188.54. Anal. Calcd for $C_{35}H_{33}N_3O_7$: C, 69.18; H, 5.47; N, 6.92; Found: C, 69.28; H, 5.32; N, 6.98.

**4.7.25 (1E,4E)-1-naphthyl-5-(3-methoxy-(4((1-(2-(2-oxo-2H-chromen-4-yloxy)butyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)penta-1,4-dien-3-one (C-7):** Yield 82%; mp 102-104$^o$C; MW 627.69. $^1$H NMR ($d_6$-DMSO, 500 MHz, δ, TMS = 0): 1.81 (s, 2H), 2.08 (s, 2H), 3.87 (s, 3H), 4.23 (s, 2H), 4.55 (s, 2H), 5.24 (s, 2H), 5.83 (s, 1H), 7.17-7.25 (m, 3H), 7.29-7.34 (m, 5H), 7.41-7.50 (m, 5H), 7.76-7.81 (m, 5H), 8.32 (s, 1H). $^{13}$C NMR ($d_6$-DMSO, 125 MHz, δ, TMS = 0): 24.85, 28.00, 52.55, 56.07, 65.64, 72.67, 86.04, 111.65, 115.2, 117.53, 119.76, 120.93, 121.56, 123.45, 124.03, 125.56, 126.05, 126.34, 126.40, 126.80, 128.09, 128.43, 128.87, 128.56, 128.74, 135.20, 142.40, 149.00, 149.75, 150.24, 152.78,162.12, 165.38, 188.55. Anal. Calcd for $C_{38}H_{33}N_3O_6$: C, 72.71; H, 5.30; N, 6.69; Found: C, 72.81; H, 5.25; N, 6.74.

**4.7.26 (1E,4E)-1-(furan-2-yl)-5-(3-methoxy-(4((1-(2-(2-oxo-2H-chromen-4-yloxy)butyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)penta-1,4-dien-3-one (C-8):** Yield 82%; mp 119-121$^o$C; MW 567.59. $^1$H NMR ($d_6$-DMSO, 500 MHz, δ, TMS = 0): 1.84 (s, 2H), 2.04 (s, 2H), 3.82 (s, 3H), 4.62 (s, 2H), 4.94 (s, 2H), 5.20 (s, 2H), 5.97 (s, 1H), 7.15-7.28 (m, 4H), 7.39-7.47 (m, 7H), 7.72-7.80 (m, 3H), 8.33 (s, 1H). $^{13}$C NMR ($d_6$-DMSO, 125 MHz, δ, TMS = 0): 25.44, 26.77, 49.41, 56.07, 56.08, 69.19, 79.57, 91.46, 111.43, 111.68, 112.77, 115.25, 117.50, 119.77, 120.90, 121.57, 123.44, 125.52, 126.88, 128.40, 128.56, 129.45, 139.00, 142.40, 145.99, 149.03, 149.72, 150.20, 151.67, 152.75, 162.12, 165.31, 188.52. Anal. Calcd for $C_{32}H_{29}N_3O_7$: C, 67.71; H, 5.15; N, 7.40; Found: C, 67.80; H, 4.98; N, 7.55.

**4.7.27 (1E,4E)-1-(thiophen-2-yl)-5-(3-methoxy-(4((1-(2-(2-oxo-2H-chromen-4-yloxy)butyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)penta-1,4-dien-3-one (C-9):** Yield 84%; mp 111-113$^o$C; MW 583.65. $^1$H NMR ($d_6$-DMSO, 500 MHz, δ, TMS = 0): 1.87 (s, 2H), 2.06 (s, 2H), 3.85 (s, 3H), 4.68 (s, 2H), 4.96 (s, 2H), 5.24 (s, 2H), 5.93 (s, 1H), 7.12-7.17 (m, 3H), 7.35-7.44 (m, 7H), 7.75-7.83 (m, 4H), 8.37 (s, 1H). $^{13}$C NMR ($d_6$-DMSO, 125 MHz, δ, TMS = 0): 25.48, 26.82, 49.48, 56.13, 56.18, 69.24, 79.64, 91.49, 111.48, 111.72, 112.79, 115.31, 117.56, 119.83, 120.97, 121.63, 123.47, 125.55, 127.11, 128.20, 128.56, 137.85, 138.50, 141.75, 146.04, 149.10, 149.78, 150.27, 151.73, 152.79, 162.15, 165.37, 188.57. Anal. Calcd for $C_{32}H_{29}N_3O_6S$: C, 65.85; H, 5.01; N, 7.20; S, 5.49; Found: C, 65.92; H, 4.93; N, 7.29; S, 5.38.

**4.7.28 (1E,4E)-1-phenyl-5-(3-methoxy-(4((1-(2-(2-oxo-2H-chromen-4-loxy)pentyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)penta-1,4-dien-3-one (D-1):** Yield 81%; mp 122-124$^{o}$C; MW 591.65. $^{1}$H NMR ($d_6$-DMSO, 500 MHz, δ, TMS = 0): 1.46 (s, 2H), 1.83-1.91 (m, 4H), 3.81 (s, 3H), 4.18 (s, 2H), 4.45 (s, 2H), 5.12 (s, 2H), 5.89 (s, 1H), 7.06-7.10 (m, 3H), 7.20-7.31 (m, 6H), 7.42-7.51 (m, 5H), 7.76-7.84 (m, 2H), 8.33 (s, 1H). $^{13}$C NMR ($d_6$-DMSO, 125 MHz, δ, TMS = 0): 25.42, 26.73, 49.32, 56.03, 69.64, 79.67, 90.93, 111.69, 114.25, 115.14, 115.29, 117.59, 119.76, 120.95, 121.15, 121.57, 123.46, 125.58, 126.41, 128.04, 128.75, 135.20, 142.49, 149.10, 149.79, 150.29, 152.79, 157.78, 162.25, 165.48, 188.64. Anal. Calcd for $C_{35}H_{33}N_3O_6$: C, 71.05; H, 5.62; N, 7.10; Found: C, 71.10; H, 5.52; N, 7.22.

**4.7.29 (1E,4E)-1-(3,4,5-trimethoxyphenyl)-5-(3-methoxy-(4((1-(2-(2-oxo-2H-chromen-4-yloxy)pentyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)penta-1,4-dien-3-one (D-2):** Yield 87%; mp 80-82$^{o}$C; MW 681.73. $^{1}$H NMR ($d_6$-DMSO, 500 MHz, δ, TMS = 0): 1.45 (s, 2H), 1.85-1.93 (s, 4H), 3.71 (s, 3H), 3.82 (s, 3H), 3.85 (s, 6H), 4.19 (s, 2H), 4.43 (s, 2H), 5.18 (s, 2H), 5.87 (s, 1H), 7.12 (s, 2H), 7.23-7.41 (m, 7H), 7.65-7.77 (m, 4H), 8.28-8.31 (m, 1H). $^{13}$C NMR ($d_6$-DMSO, 125 MHz, δ, TMS = 0): 22.81, 27.74, 29.73, 49.70, 56.05, 56.50, 60.60, 69.62, 79.62, 90.95, 116.48, 111.18, 113.64, 115.69, 116.89, 123.26, 123.54, 124.24, 124.62, 125.82, 130.79, 133.17, 139.97, 143.08, 143.26, 149.44, 149.64, 150.21, 153.20, 153.57, 162.15, 165.38, 188.62. Anal. Calcd for $C_{38}H_{39}N_3O_9$: C, C, 66.95; H, 5.77; N, 6.16; Found: C, 66.86; H, 5.88; N, 6.10.

**4.7.30 (1E,4E)-1-(3,4-dimethoxyphenyl)-5-(3-methoxy-(4((1-(2-(2-oxo-2H-chromen-4-loxy)pentyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)penta-1,4-dien-3-one (D-3):** Yield 77%; mp 99-101$^{o}$C; MW 651.70. $^{1}$H NMR ($d_6$-DMSO, 500 MHz, δ, TMS = 0): 1.44 (s, 2H), 1.85-1.93 (m, 4H), 3.81 (s, 9H), 4.19 (s, 2H), 4.43 (s, 2H), 5.17 (s, 2H), 5.87 (s, 1H), 7.03 (s, 1H), 7.23-7.40 (m, 9H), 7.68-7.77 (m, 4H), 8.27-8.31 (m, 1H). $^{13}$C NMR ($d_6$-DMSO, 125 MHz, δ, TMS = 0): 25.42, 26.72, 49.40, 56.04, 56.06, 69.62, 79.62, 90.95, 110.89, 111.17, 112.07, 113.65, 115.69, 116.90, 123.26, 123.45, 123.72, 124.40, 124.63, 128.47, 133.17, 142.91, 143.12, 149.44, 149.64, 150.13, 151.52, 153.20, 162.15, 165.38, 188.57. Anal. Calcd for $C_{37}H_{37}N_3O_8$: C, 68.19; H, 5.72; N, 6.45; Found: C, 68.30; H, 5.80; N, 6.55.

**4.7.31 (1E,4E)-1-(4-methoxyphenyl)-5-(3-methoxy-(4((1-(2-(2-oxo-2H-chromen-4-loxy)pentyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)penta-1,4-dien-3-one (D-4):** Yield 86%; mp 108-110$^{o}$C; MW 621.68. $^{1}$H NMR ($d_6$-DMSO, 500 MHz, δ, TMS = 0): 1.44 (s, 2H), 1.82-

1.94 (m, 4H), 3.81 (s, 6H), 4.13 (s, 2H), 4.44 (s, 2H), 5.13 (s, 2H), 5.87 (s, 1H), 7.06-7.16 (m, 5H), 7.21-7.28 (m, 5H), 7.36-7.42 (m, 3H), 7.73-7.80 (m, 2H), 8.31 (s, 1H). $^{13}$C NMR ($d_6$-DMSO, 125 MHz, δ, TMS = 0): 25.45, 26.77, 49.42, 56.03, 56.07, 69.69, 79.67, 90.90, 110.67, 111.66, 113.59, 115.27, 117.68, 118.75, 119.79, 120.97, 121.58, 123.48, 125.59, 126.94, 128.49, 128.64, 129.86, 136.32, 142.46, 149.09, 150.26, 172.84, 162.18, 165.47, 188.59. Anal. Calcd for $C_{36}H_{35}N_3O_7$: C, 69.55; H, 5.67; N, 6.76; Found: C, 69.35; H, 5.78; N, 6.67.

**4.7.32 (1E,4E)-1-(3-methoxyphenyl)-5-(3-methoxy-(4((1-(2-(2-oxo-2H-chromen-4-loxy)pentyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)penta-1,4-dien-3-one (D-5):** Yield 84%; mp 106-108$^o$C; MW 621.68. $^1$H NMR ($d_6$-DMSO, 500 MHz, δ, TMS = 0): 1.45 (s, 2H), 1.84-1.94 (m, 4H), 3.83 (s, 6H), 4.16 (s, 2H), 4.41 (s, 2H), 5.15 (s, 2H), 5.88 (s, 1H), 7.13 (s, 6H), 7.33-7.40 (m, 7H), 7.68-7.77 (m, 2H), 8.25-8.30 (m, 1H). $^{13}$C NMR ($d_6$-DMSO, 125 MHz, δ, TMS = 0): 25.44, 26.73, 49.45, 56.03, 56.05, 69.63, 79.65, 90.97, 110.67, 111.66, 113.56, 115.21, 117.59, 118.70, 119.74, 120.90, 121.51, 123.40, 125.52, 126.88, 128.45, 128.56, 129.77, 136.26, 142.42, 149.07, 150.20, 172.76, 162.17, 165.40, 188.58. Anal. Calcd for $C_{36}H_{35}N_3O_7$: C, 69.55; H, 5.67; N, 6.76; Found: C, 69.65; H, 5.58; N, 6.86.

**4.7.33 (1E,4E)-1-(2-methoxyphenyl)-5-(3-methoxy-(4((1-(2-(2-oxo-2H-chromen-4-loxy)pentyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)penta-1,4-dien-3-one (D-6):** Yield 81%; mp 105-107$^o$C; MW 621.68. $^1$H NMR ($d_6$-DMSO, 500 MHz, δ, TMS = 0): 1.46 (s, 2H), 1.82-1.92 (m, 4H), 3.86 (s, 6H), 4.14 (s, 2H), 4.43 (s, 2H), 5.13 (s, 2H), 5.87 (s, 1H), 7.17 (s, 6H), 7.28-7.37 (m, 7H), 7.61-7.73 (m, 2H), 8.26-8.31 (m, 1H). $^{13}$C NMR ($d_6$-DMSO, 125 MHz, δ, TMS = 0): 25.41, 26.71, 49.39, 56.03, 56.05, 69.63, 79.65, 90.97, 111.67, 114.22, 115.08, 115.25, 117.55, 119.73, 120.91, 121.08, 121.50, 123.40, 125.52, 126.81, 127.40, 128.45, 128.56, 129.01, 142.46, 149.08, 149.77, 150.25, 152.77, 157.74, 162.18, 165.42, 188.59. Anal. Calcd for $C_{36}H_{35}N_3O_7$: C, 69.55; H, 5.67; N, 6.76; Found: C, 69.66; H, 5.54; N, 6.89.

**4.7.34 (1E,4E)-1-naphthyl-5-(3-methoxy-(4((1-(2-(2-oxo-2H-chromen-4-loxy)pentyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)penta-1,4-dien-3-one (D-7):** Yield 81%; mp 84-86$^o$C; MW 641.71. $^1$H NMR ($d_6$-DMSO, 500 MHz, δ, TMS = 0): 1.47 (s, 2H), 1.82-1.93 (m, 4H), 3.84 (s, 3H), 4.15 (s, 2H), 4.47 (s, 2H), 5.15 (s, 2H), 5.86 (s, 1H), 7.15-7.20 (m, 3H), 7.29-7.34 (m, 5H), 7.42-7.49 (m, 5H), 7.77-7.83 (m, 5H), 8.33 (s, 1H). $^{13}$C NMR ($d_6$-DMSO, 125 MHz, δ, TMS = 0): 25.42, 26.73, 49.32, 56.03, 69.64, 79.67, 90.93, 111.69, 114.25, 115.14, 115.29, 117.59,

119.76, 120.95, 121.15, 121.57, 123.46, 124.06, 125.58, 126.06, 126.34, 126.41, 128.04, 128.75, 128.84, 135.20, 142.49, 149.10, 149.79, 150.29, 152.79, 157.78, 162.25, 165.48, 188.64. Anal. Calcd for $C_{39}H_{35}N_3O_6$: C, 72.99; H, 5.50; N, 6.55; Found: C, 72.89; H, 5.66; N, 6.39.

**4.7.35 (1E,4E)-1-(furan-2-yl)-5-(3-methoxy-(4((1-(2-(2-oxo-2H-chromen-4-loxy)pentyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)penta-1,4-dien-3-one (D-8):** Yield 78%; mp 104-106$^{o}$C; MW 581.62. $^{1}$H NMR ($d_6$-DMSO, 500 MHz, δ, TMS = 0): 1.43 (s, 2H), 1.81-1.91 (m, 4H), 3.81 (s, 3H), 4.13 (s, 2H), 4.41 (s, 2H), 5.13 (s, 2H), 5.84 (s, 1H), 7.17-7.25 (m, 4H), 7.34-7.46 (m, 7H), 7.66-7.78 (m, 3H), 8.26-8.31 (m, 1H). $^{13}$C NMR ($d_6$-DMSO, 125 MHz, δ, TMS = 0): 25.46, 26.73, 49.37, 56.04, 69.67, 79.69, 90.93, 111.40, 111.65, 112.78, 115.26, 117.54, 119.36, 119.70, 120.93, 123.44, 125.54, 126.84, 128.56, 129.45, 139.00, 142.48, 145.94, 149.06, 149.77, 150.20, 151.60, 152.70, 162.20, 165.46, 188.60. Anal. Calcd for $C_{33}H_{31}N_3O_7$: C, 68.15; H, 5.37; N, 7.22; Found: C, 68.26; H, 5.19; N, 7.33.

**4.7.36 (1E,4E)-1-(thiophen-2-yl)-5-(3-methoxy-(4((1-(2-(2-oxo-2H-chromen-4-loxy)pentyl)-1H-1,2,3-triazol-4-yl)methoxy)phenyl)penta-1,4-dien-3-one (D-9):** Yield 78%; mp 95-97$^{o}$C; MW 597.68. $^{1}$H NMR ($d_6$-DMSO, 500 MHz, δ, TMS = 0): 1.46 (s, 2H), 1.84-1.96 (m, 4H), 3.86 (s, 3H), 4.17 (s, 2H), 4.46 (s, 2H), 5.16 (s, 2H), 5.87 (s, 1H), 7.12-7.23 (m, 3H), 7.39-7.48 (m, 7H), 7.76-7.84 (m, 4H), 8.28-8.32 (m, 1H). $^{13}$C NMR ($d_6$-DMSO, 125 MHz, δ, TMS = 0): 25.42, 26.70, 49.31, 56.01, 69.62, 79.63, 90.87, 111.37, 111.61, 112.73, 115.19, 117.47, 119.29, 119.65, 120.91, 123.39, 127.10, 128.14, 137.79, 138.46, 141.71, 145.89, 149.00, 149.67, 150.13, 151.55, 152.64, 162.11, 165.42, 188.58. Anal. Calcd for $C_{33}H_{31}N_3O_6S$: C, 66.32; H, 5.23; N, 7.03; S, 5.36; Found: C, 66.21; H, 5.34; N, 6.92; S, 5.51.

### 4.8. *In-vitro* cytotoxic assay

*In vitro* cytotoxicity against four human cancer cell lines was determined using 96-well tissue culture plate. The cells were allowed to grow in carbon dioxide incubator (37ºC) for 24 h. Test materials in complete growth medium (100 ml) were added after 24 h of incubation to the wells containing cell suspension. The plates were stained with sulforhodamine B dye (0.4% in 1% acetic acid, 100 ml) for 30 min. The plates were washed five times with 1% trichloroacetic acid and then air-dried. The adsorbed dye was dissolved in Tris-HCl buffer (100 mL, 0.01 M, pH 10.4), and the plates were gently stirred for 10 min. The plates were further incubated for 48 h in

a carbon dioxide incubator. The cell growth was stopped by gentle layering trichloroacetic acid (50%, 50 ml) on top of the medium in all the wells. The plates were incubated at 4ºC for 1 h to fix the cells attached to the bottom of the wells. The liquid of all the wells was gently pipette out and discarded. The plates were washed five times with distilled water to remove trichloroacetic acid, growth medium low molecular weight metabolites, serum on a mechanical stirrer. The optical density (OD) was recorded on ELISA reader at 540 nm. The cell growth was determined by subtracting the mean OD value of respective blank from the mean OD value of the experimental set. Percent growth in presence of test material was calculated considering the growth in the absence of any test material as 100%, and in turn, percent growth inhibition in presence of test material was calculated [25, 26].

### 4.9. *In-vitro* tubulin polymerization assay

A cytoskeleton tubulin polymerization assay kit was purchased from Labex India. For assessing the tubulin polymerization inhibition potential, 100 mM stock solution of each compound was made in molecular biology grade DMSO. Further dilutions (50 mM, 25 mM, 10 mM) of each compound were made by diluting the stock solution. General tubulin buffer was prepared by reconstituting the constituents (available in kit) in 10 mL distilled water. General Tubulin Buffer (1 mL) was supplemented with 10 mL of 100 mM GTP solution and 10 mg Tubulin was suspended in it and mixture was stored at 4ºC until used. Before starting the experiment 96 well plate and Elisa plate reader were warmed at 37ºC as this temperature is required to achieve the proper polymerization of tubulin. Tubulin polymerization buffer was prepared by mixing General tubulin buffer (750 mL), Tubulin glycerol buffer (250 mL) and 100 mM GTP (10 mL). 10 mL General tubulin buffer was added in the wells, where first two wells were kept as control (without any test compound), and different concentrations of compounds were subsequently added in previously marked wells and plate was kept in plate reader for 2 min at 37ºC. Meanwhile tubulin suspension was diluted with tubulin polymerization buffer and 100 mL of diluted tubulin was added in each well. Plate was again kept in plate reader at 37ºC for 1 h and absorbance was measured at 340 nm (Lee and Timasheff, 1977). Percentage inhibition and $IC_{50}$ values were calculated according to the absorbance values obtained after 1 h [27, 28].

### 4.10. Docking simulations

The X-ray structure coordinates of tubulin bound with colchicine was obtained from protein data bank (PDB entry: 1SA0; Resolution 3.5Å) [35]. The X-ray structure of tubulin consists of two tubulin heterodimers in a curved complex capped by the stathmin-likedomain (SLD). In each tubulin heterodimer, a colchicine molecule was found in complexed form at the interface of $\alpha$ and $\beta$ subunits. Chakraborti et al. investigated the tubulin-binding mode of few curcumin analogs and reported that curcumin binds at the interface of *β1-α2* heterodimer which is approximately 32 Å away from the colchicine-binding site [36].

To explore the tubulin binding mode of the synthesized compounds, we have docked **A-2** at the curcumin binding site of tubulin as reported by Chakraborti et al. The docking study was carried out using the GOLD 5.3.0 software [37]. Chem score was applied as fitness function to rank various docked conformations. Chem score estimates total free energy change that occurs on ligand binding by considering parameters like hydrogen bond energies, atom radii and polarisabilities, torsion potentials, hydrogen bond directionalities, lipophilicity, etc [38]. The 3D conformation of **A-2** was generated in ChemDraw software 2010. The 2D structure of **A-2** was drawn in ChemDraw Ultra (2010) and energy minimized using the MM2 force field in Chem 3D Ultra software. The compound was docked ten times and conformation associated with highest scoring value was considered to analyze various drug-receptor (D-R) interactions between **A-2** and tubulin.

## Conflict of interest

The authors confirm that this article content has no conflicts of interest.

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**Captions**

**Fig. 1.** $C_5$-curcuminoid based molecular hybrids (**1** & **2**) and coumarin derivative with cell damage potential (**3**)

**Fig. 2.** Design strategy for $C_5$-curcuminoid-coumarin hybrids

**Scheme 1.** Synthesis of triazole linked $C_5$-curcuminoid-coumarin hybrids. Reagents and conditions: (a) Acetone, 40% KOH, 2 hrs, stir, rt; (b) Propargyl bromide, $K_2CO_3$, DMF, 2 hrs, stir, rt; (c) Aldehydes, 5% NaOH, MeOH, 2 hrs, stir, rt; (d) Dibromoalkane, $K_2CO_3$, DMF, 2 hrs, stir, rt; (e) $NaN_3$, DMF, 1 hr, stir, rt; (f) Sodium ascorbate, $CuSO_4$, DMF, 15 mins, rt.

**Table 1** Various substituted bi-functional hybrids with their %age inhibition against eight human cancer cell lines at 50 µM

**Table 2** $IC_{50}$ values of active hybrids against sensitive human cancer cell lines

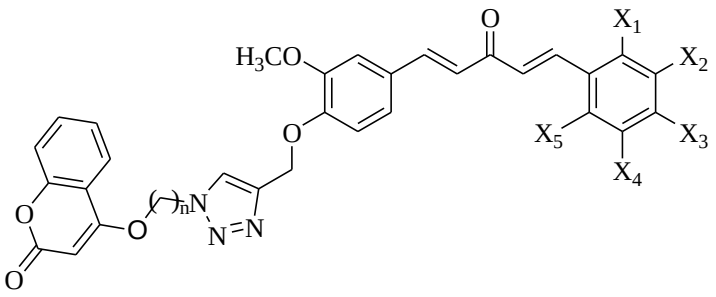
**Fig. 3.** Structure activity relationship

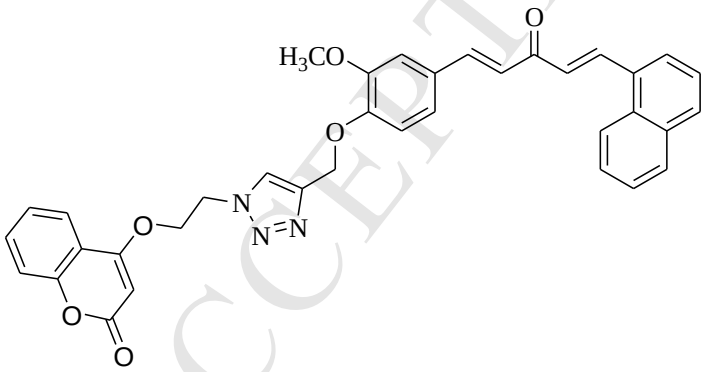
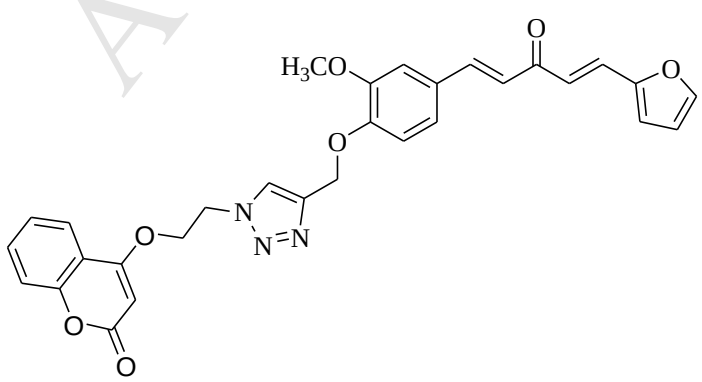
**Table 3** Tubulin polymerization inhibition potential of most active hybrids

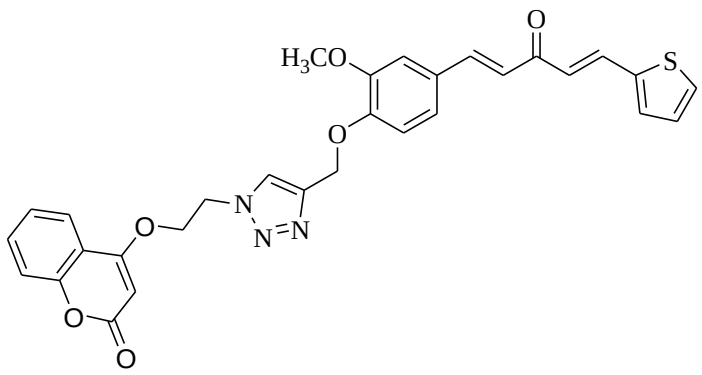
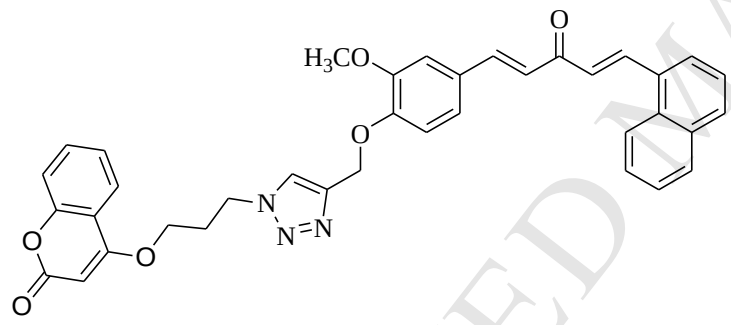
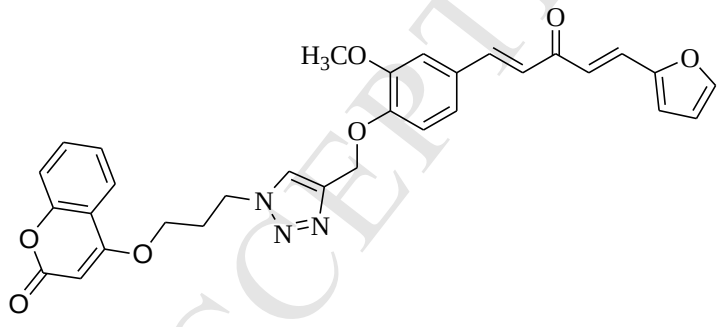
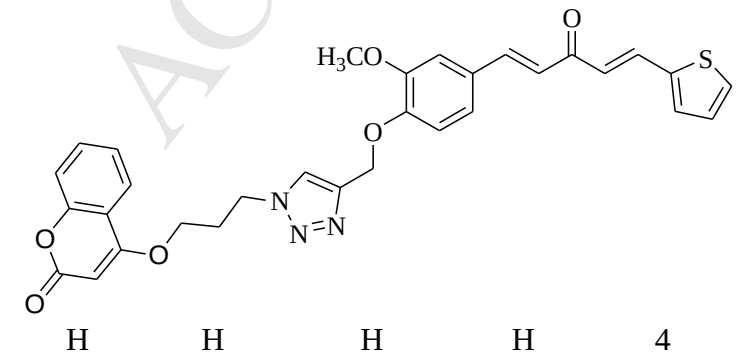
**Fig. 4.** (**a**) Docked conformation of compound **A-2** (cyan) at the interface of β1-α2 subunits of tubulin complexed with colchicine (red) (PDB id 1SA0); (**b**) Residues involved in drug-receptor interactions between **A-2** and tubulin (**A-2**: carbon atoms are shown in green; only hydrogen which is involved in H-bond interactions are shown)

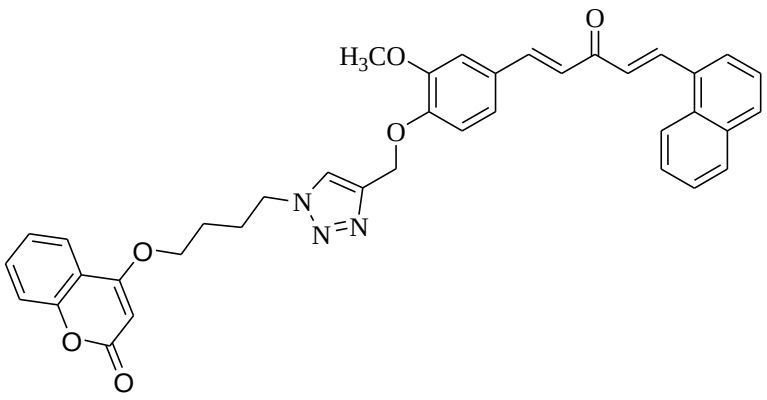
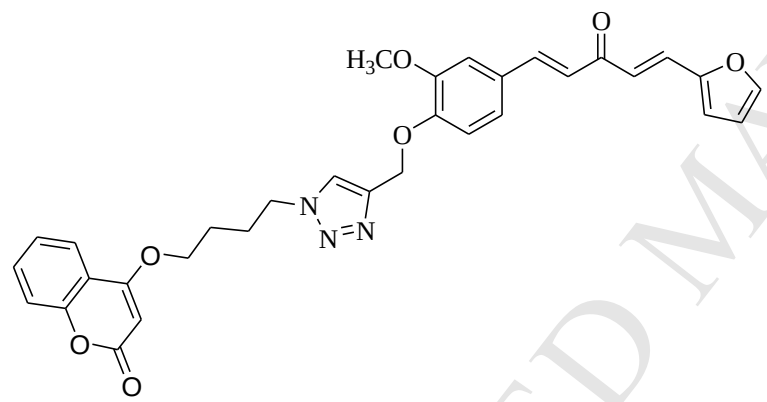
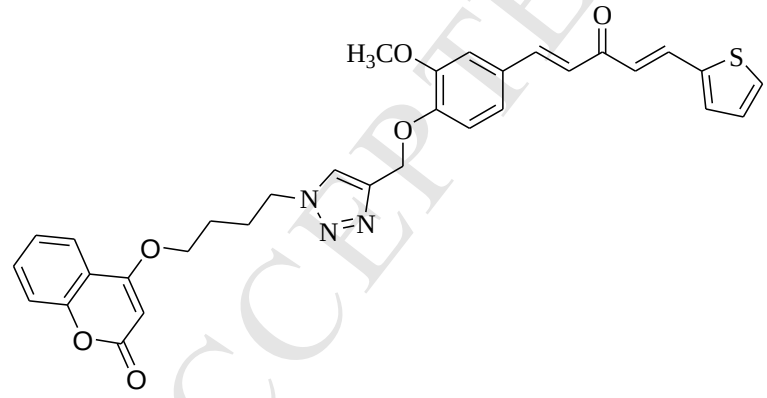
**Table 1**

Various substituted bi-functional hybrids with their %age inhibition against eight human cancer cell lines at 50 µM




| CODE | $X_1$ | $X_2$ | $X_3$ | $X_4$ | $X_5$ | n | Percentage growth inhibition (50 µM) | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | HCT-116 (Colon) | COLO-205 (Colon) | THP-1 (Leukemia) | PC-3 (Prostate) |
| A-1 | H | H | H | H | H | 2 | 56 | 54 | 61 | 10 |
| A-2 | H | $OCH_3$ | $OCH_3$ | $OCH_3$ | H | 2 | 89 | 86 | 99 | 22 |
| A-3 | H | $OCH_3$ | $OCH_3$ | H | H | 2 | 82 | 80 | 90 | 19 |
| A-4 | H | H | $OCH_3$ | H | H | 2 | 71 | 68 | 78 | 15 |
| A-5 | H | $OCH_3$ | H | H | H | 2 | 70 | 69 | 77 | 13 |
| A-6 | $OCH_3$ | H | H | H | H | 2 | 70 | 67 | 75 | 11 |
| A-7 |  | | | | | | 83 | 85 | 89 | 20 |
| A-8 |  | | | | | | 72 | 68 | 78 | 14 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| A-9 |  | | | | | | 71 | 66 | 76 | 12 |
| B-1 | H | H | H | H | H | 3 | 45 | 43 | 48 | NA |
| B-2 | H | $OCH_3$ | $OCH_3$ | $OCH_3$ | H | 3 | 76 | 74 | 79 | 12 |
| B-3 | H | $OCH_3$ | $OCH_3$ | H | H | 3 | 67 | 65 | 73 | 9 |
| B-4 | H | H | $OCH_3$ | H | H | 3 | 54 | 53 | 56 | NA |
| B-5 | H | $OCH_3$ | H | H | H | 3 | 55 | 52 | 58 | NA |
| B-6 | $OCH_3$ | H | H | H | H | 3 | 53 | 57 | 50 | NA |
| B-7 |  | | | | | | 66 | 64 | 71 | 8 |
| B-8 |  | | | | | | 51 | 59 | 57 | NA |
| B-9 |  | | | | | | 58 | 55 | 54 | NA |
| C-1 | H | H | H | H | H | 4 | 34 | 31 | 36 | NA |
| C-2 | H | $OCH_3$ | $OCH_3$ | $OCH_3$ | H | 4 | 63 | 60 | 65 | 5 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| C-3 | H | $OCH_3$ | $OCH_3$ | H | H | 4 | 55 | 53 | 59 | NA |
| C-4 | H | H | $OCH_3$ | H | H | 4 | 40 | 49 | 45 | NA |
| C-5 | H | $OCH_3$ | H | H | H | 4 | 44 | 46 | 47 | NA |
| C-6 | $OCH_3$ | H | H | H | H | 4 | 43 | 47 | 40 | NA |
| C-7 |  | | | | | | 56 | 50 | 54 | NA |
| C-8 |  | | | | | | 47 | 43 | 49 | NA |
| C-9 |  | | | | | | 41 | 40 | 44 | NA |
| D-1 | H | H | H | H | H | 5 | 24 | 22 | 26 | NA |
| D-2 | H | $OCH_3$ | $OCH_3$ | $OCH_3$ | H | 5 | 53 | 51 | 55 | NA |
| D-3 | H | $OCH_3$ | $OCH_3$ | H | H | 5 | 45 | 43 | 47 | NA |
| D-4 | H | H | $OCH_3$ | H | H | 5 | 35 | 30 | 39 | NA |
| D-5 | H | $OCH_3$ | H | H | H | 5 | 36 | 34 | 37 | NA |
| D-6 | $OCH_3$ | H | H | H | H | 5 | 33 | 30 | 37 | NA |

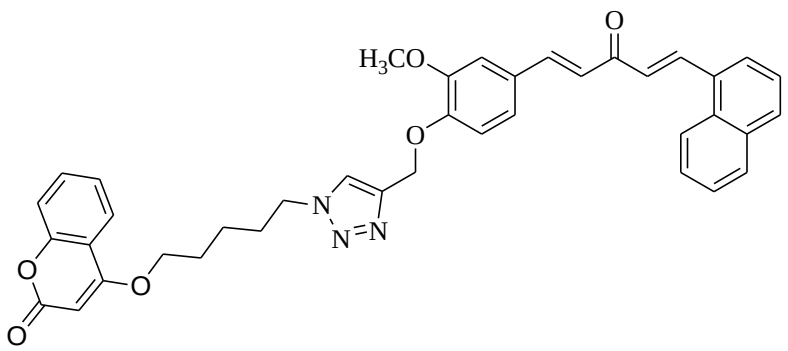
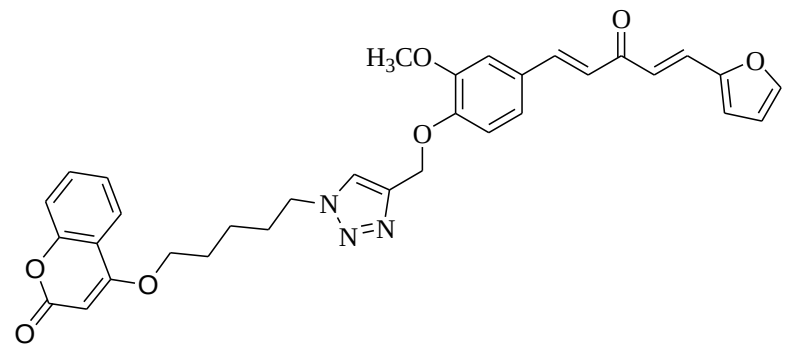
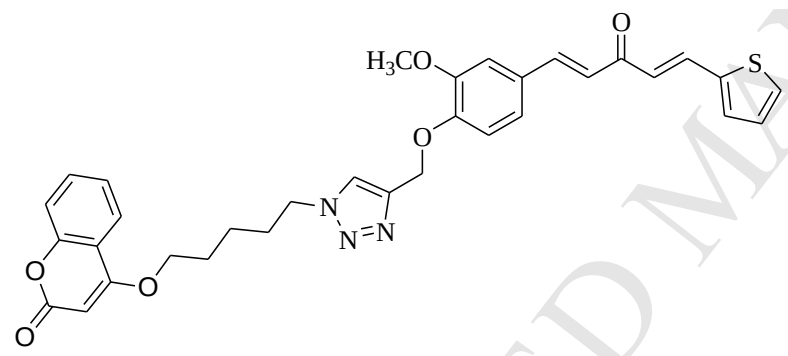
| | | | | | |
|---|---|---|---|---|---|
| D-7 |  | 42 | 40 | 44 | NA |
| D-8 |  | 37 | 33 | 39 | NA |
| D-9 |  | 31 | 30 | 34 | NA |

**Table 2**

$IC_{50}$ values of active hybrids against sensitive human cancer cell lines

| Code | $IC_{50}$ (μM) | | |
|---|---|---|---|
| | THP-1 (Leukemia) | COLO-205 (Colon) | HCT-116 (Colon) |
| A-2 | 0.82 | 4.68 | 2.21 |
| A-3 | 2.34 | 6.78 | 4.71 |
| A-4 | 7.41 | 11.29 | 9.29 |
| A-5 | 7.13 | 11.87 | 9.76 |
| A-6 | 7.33 | 11.45 | 9.49 |
| A-7 | 4.48 | 9.95 | 6.54 |
| A-8 | 7.87 | 11.79 | 9.18 |
| A-9 | 7.14 | 11.43 | 9.29 |
| B-2 | 5.43 | 10.61 | 8.65 |
| B-3 | 8.51 | 12.99 | 10.97 |
| B-7 | 8.41 | 12.65 | 10.43 |

**Table 3**

Tubulin polymerization inhibition potential of most active hybrids

| Compound | Inhibition of tubulin polymerization $IC_{50}$ (μM) |
|---|---|
| A-2 | 1.55 |
| A-3 | 2.88 |
| A-7 | 7.65 |
| CA-4 (Combrtastatin A-4) | 1.2[a] |

[a]Literature value[27]

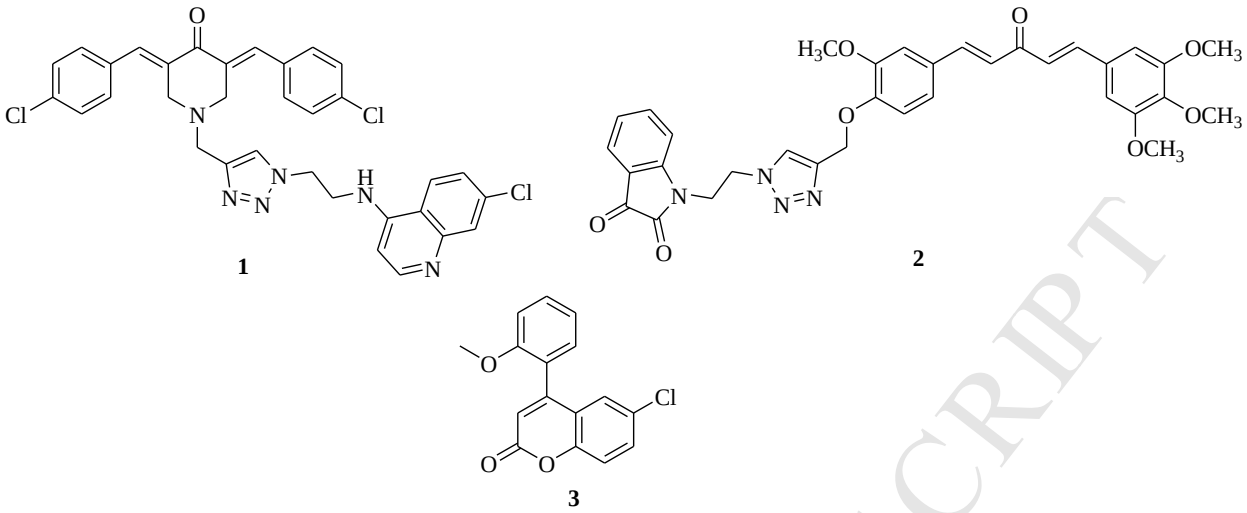



**Fig. 1.** $C_5$-curcuminoid based molecular hybrids (**1** & **2**) and coumarin derivative with cell damage potential (**3**)

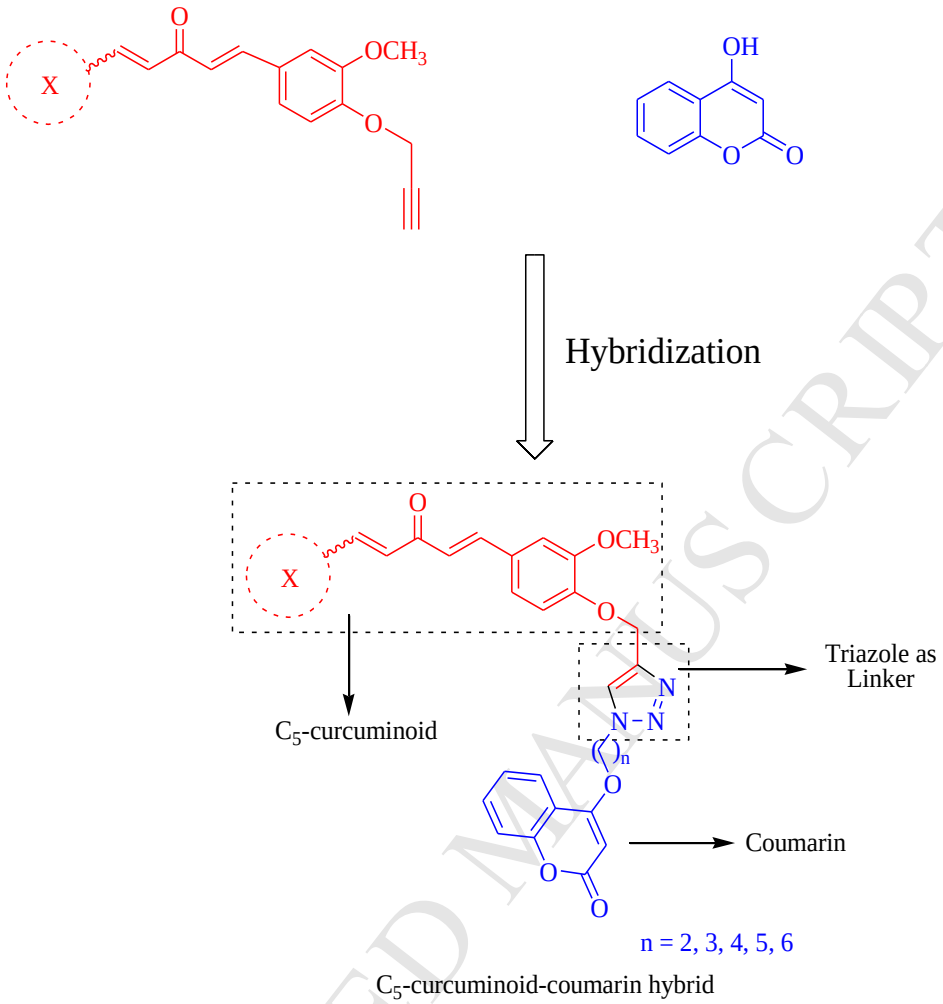
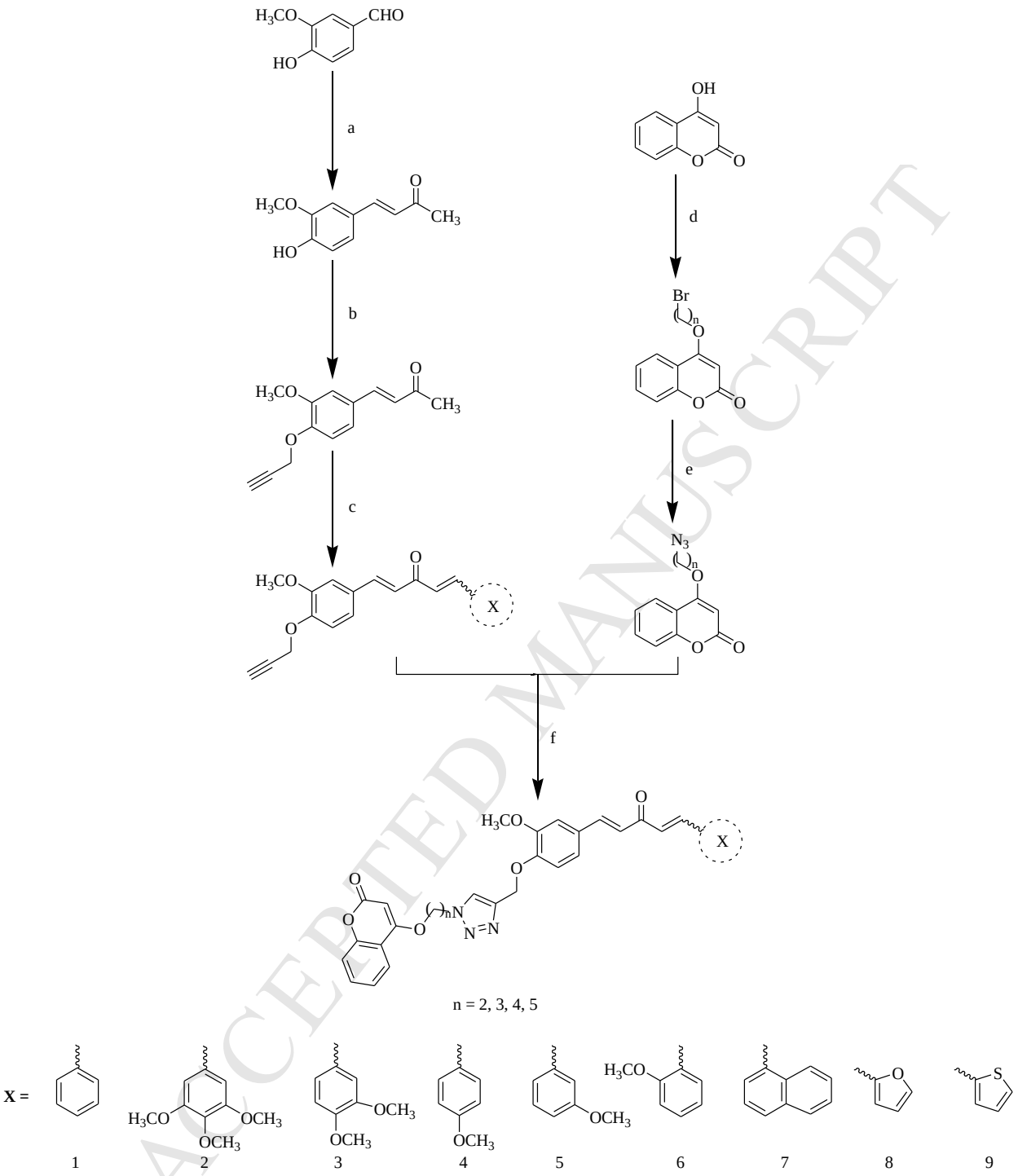



**Fig. 2.** Design strategy for $C_5$-curcuminoid-coumarin hybrids




**Scheme 1.** Synthesis of triazole linked $C_5$-curcuminoid-coumarin hybrids. Reagents and conditions: (a) Acetone, 40% KOH, 2 hrs, stir, rt; (b) Propargyl bromide, $K_2CO_3$, DMF, 2 hrs, stir, rt; (c) Aldehydes, 5% NaOH, MeOH, 2 hrs, stir, rt; (d) Dibromoalkane, $K_2CO_3$, DMF, 2 hrs, stir, rt; (e) $NaN_3$, DMF, 1 hr, stir, rt; (f) Sodium ascorbate, $CuSO_4$, DMF, 15 mins, rt.

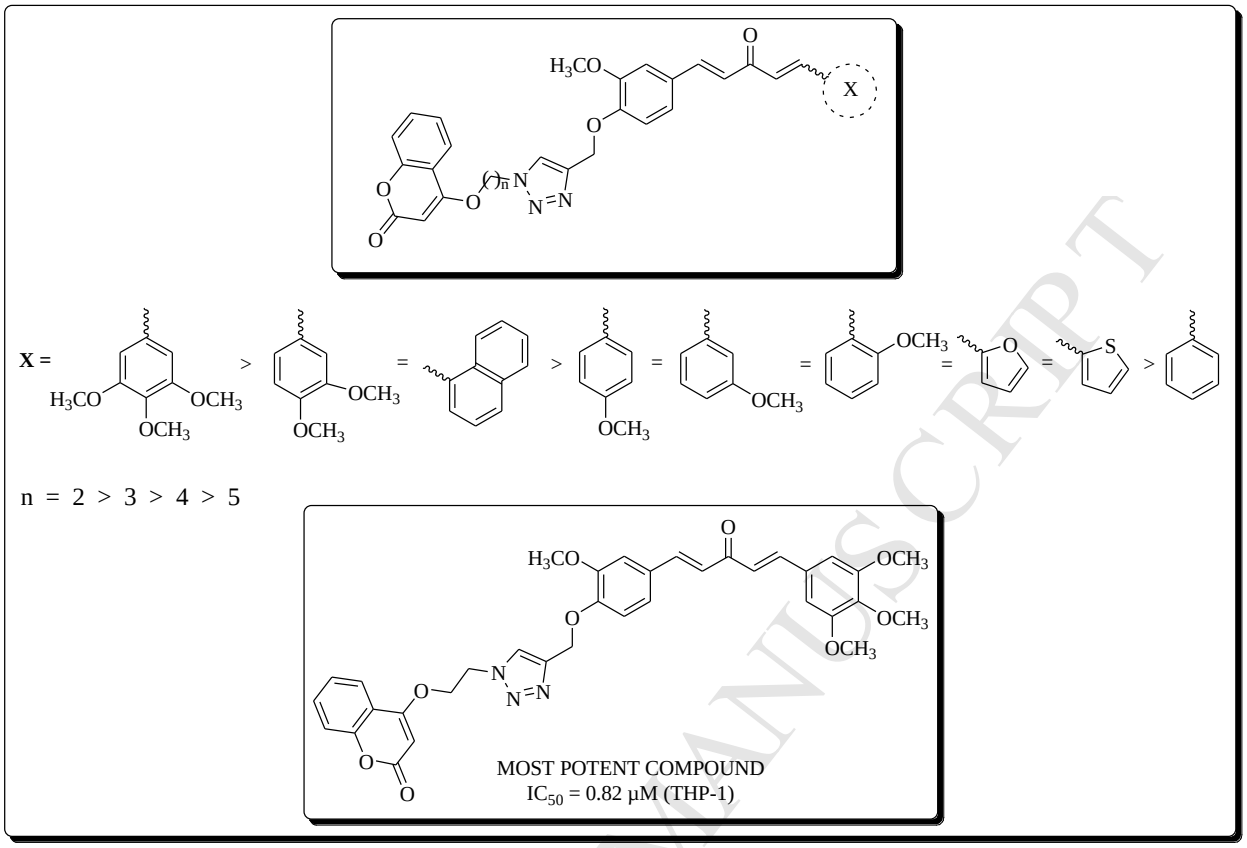



**Fig. 3.** Structure activity relationship

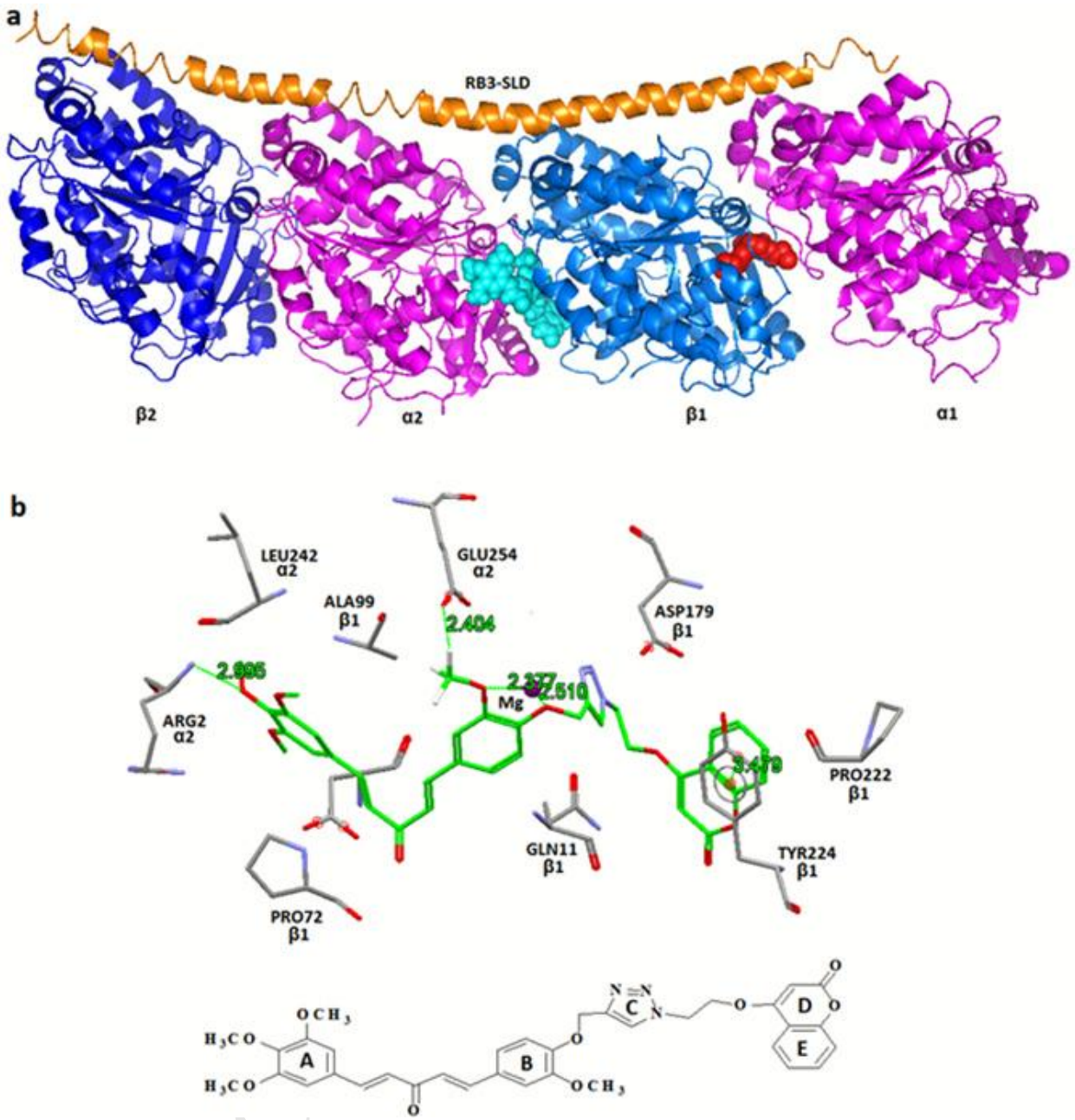



**Fig. 4.** (**a**) Docked conformation of compound **A-2** (cyan) at the interface of β1-α2 subunits of tubulin complexed with colchicine (red) (PDB id 1SA0); (**b**) Residues involved in drug-receptor interactions between **A-2** and tubulin (**A-2**: carbon atoms are shown in green; only hydrogen which is involved in H-bond interactions are shown)

- Design of $C_5$-curcuminoid-coumarin hybrids in view of cytotoxic effects of individual pharmacophore
- Synthesis of a series of 36 novel $C_5$-curcuminoid-coumarin hybrids using click chemistry approach
- Evaluation of $C_5$-curcuminoid-coumarin hybrids against a panel of human cancer cell lines
- Evaluation of the effect of most cytotoxic hybrids on tubulin polymerization
- Exploration of possible binding interactions between the most potent hybrid and the amino acid residues at curcumin binding site on tubulin