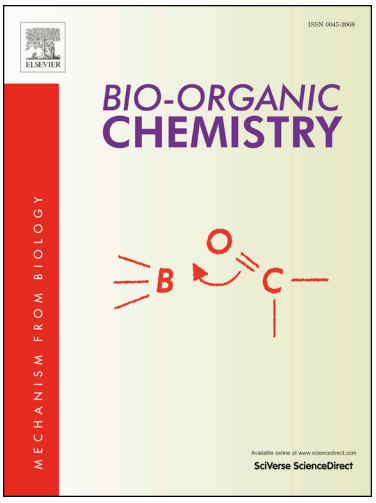

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Click chemistry based multicomponent approach in the synthesis of spirochromenocarbazole tethered 1,2,3-triazoles as potential anticancer agents

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Abstract: A series of spirochromenocarbazole tethered 1,2,3–triazoles were synthesized *via* click chemistry based one-pot, five component reaction between N-propargyl isatins, malononitrile, 4-hydroxycarbazole, aralkyl halides and sodium azide using cellulose supported CuI nanoparticles (Cell-CuI NPs) as the heterogeneous catalyst. Antiproliferative activity of all the synthesized compounds was investigated against panel of cancer cell lines such as MCF-7, MDA-MB-231, HeLa, PANC-1, A-549, and THP-1. Many of the synthesized compounds exhibited good anti-proliferative activity against breast (MCF-7 and MDA-MB-231) and cervical (HeLa) cancer cells with IC_{50} values less than 10 μM. In case of MCF-7 cells, among the nine compounds that showed good anti-proliferative activity, compounds **6f** and **6j** were found to be highly potent (IC_{50} = 2.13 μM and 4.80 μM, respectively). In case of MDA-MB-231, three compounds (**6k, 6j** and **6s**) showed antiproliferative activity amongst which **6k** was the most potent one (IC_{50} = 3.78 μM)**.** On the other hand, in cervical cancer HeLa cells, compounds **6b**, **6g**, **6s** and **6u** showed excellent antiproliferative activity (IC_{50} = 4.05, 3.54, 3.83, 3.35 μM, respectively). All the compounds were found to be nontoxic to the human umbilical vein endothelial cells (HUVECs). AO and EtBr staining and fluorescence microscopy studies of the active compounds (IC_{50} < 5 μM) suggested that these compounds induce cell death by apoptosis.

Keywords: Click chemistry, 1,2,3-triazolylspirochromenocarbazole, anticancer, cytotoxicity, apoptotic assay, heterogeneous catalysis, multicomponent synthesis

Introduction

Cancer is a multifaceted disease caused due to uncontrolled growth of abnormal cells and after cardiovascular disease it is the most common cause of death worldwide [1,2]. While great strides have been made in the treatment of cancer, it still continues to be a major health concern

[3]. In the treatment of almost all types of cancers, after surgery the use of chemotherapeutics that inhibit hyper proliferation of cancer cells is routinely practisized [4,5]. However, this therapy is plagued with the side effects that include off-target toxicity and drug resistance [6]. Consequently, the discovery of new drugs that can inhibit proliferation or induce death of cancer cells without disturbing normal cells is highly desirable.

Many heterocyclic compounds containing isatin, thiazolidinone, benzimidazole, oxindole, β-carboline, etc. as the structural motif serve as potential anticancer agents [7-10]. In the recent years based upon the hypothesis that, the molecules synthesized by the combination of heterocyclic pharmacophores can have enhanced biological potential [11,12] the most attractive strategy in the design of new anticancer agents involves combination of pharmacophoric templates through molecular hybridization [13-16]. With all these concepts in mind and our continued interest in multicomponent synthesis as well as in the synthesis of hybrid molecules [17-20], we planned to undertake the synthesis of novel hybrid molecules as potential anti-cancer agents.

Carbazoles are probably the most widely spread nitrogen heterocycles in nature [21, 22]. Especially aryl and hetero annulated carbazoles like pyridocarbazoles, indolocarbazoles, pyrrolocarbazoles, pyranocarbazoles, etc. are distributed in numerous natural products with diverse biological activities [23-26]. The hetero annulated carbazoles of synthetic origin have attracted growing attention these days due to their antidiabetic, antimicrobial, antioxidant, anticancer, antitubercular as well as anticonvulsant activities [27-32].

Alike carbazole, 1,2,3-triazole is another medicinally privileged scaffold serving as a key structural feature in many bioactive molecules as well as pharmaceuticals [33]. Many molecules containing 1,2,3-triazole as the structural motif are known to possess anti-tubercular, anti-fungal, anti-allergic, anti-viral, anti-tumor, anti-malarial as well as anti-HIV properties [34-42] while many 1,2,3-triazole conjugates have already been reported to exhibit potent anticancer activity [43-49]. For instance, carboxyamidotriazole is a commercially available anticancer drug that contains 1,2,3-triazole as the structural unit [50]. On the other hand, isatin is a medicinally privileged scaffold that has led to the generation of large number of structurally related compounds that exhibit anticancer agents [51]. Representative biologically potent compounds containing carbazole, triazole and isatin are shown in **Fig.1.** Considering the biological potential of compounds containing carbazole, isatin as well as 1,2,3-triazole as the structural units, we surmised that hybrid molecules containing all these structural units may possess enhanced anticancer activity.

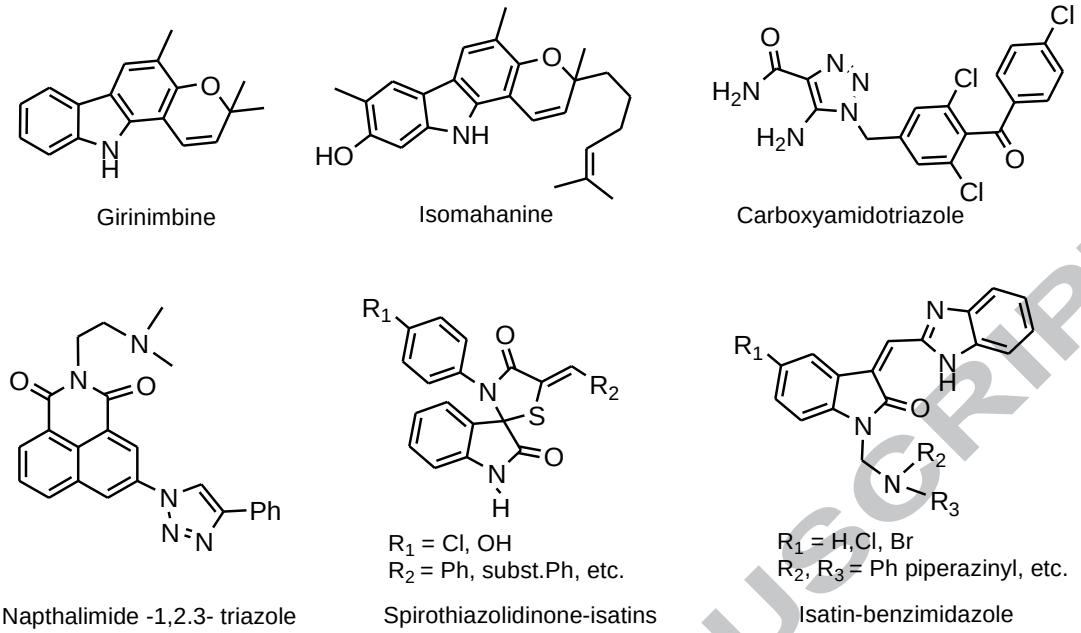
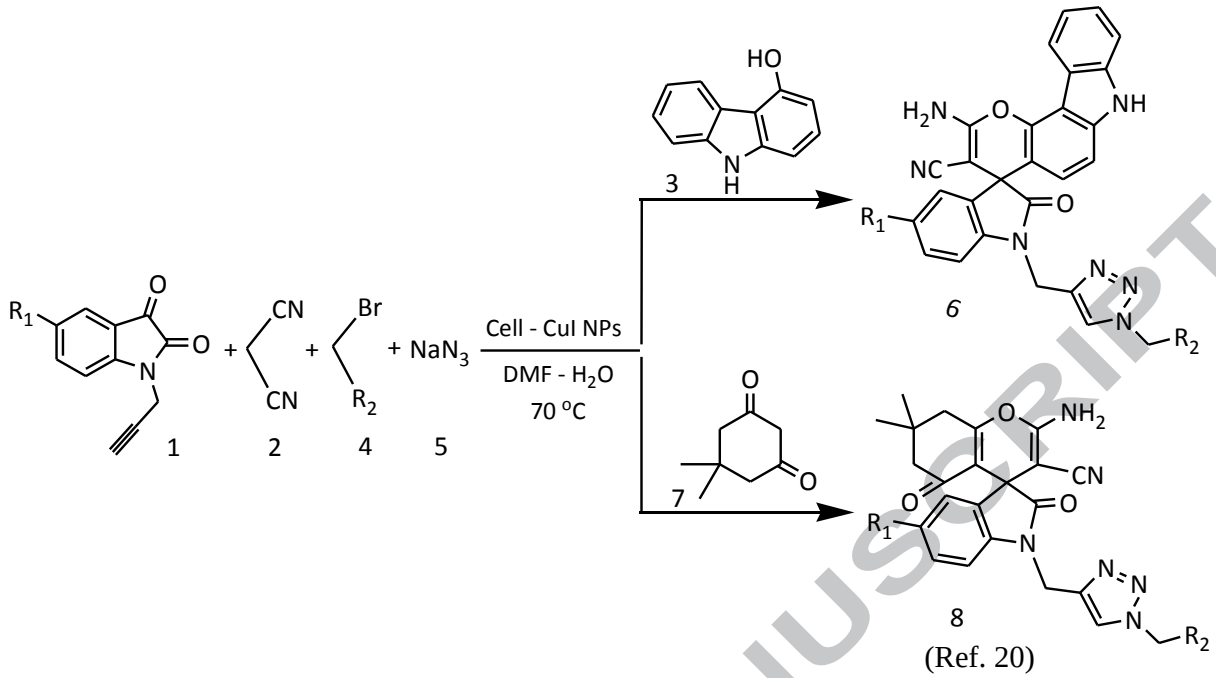


Fig. 1 Biologically active compounds containing 1,2,3 triazole, isatin and carbazole moieties

We have earlier reported cellulose supported CuI nanoparticles (Cell-CuI NPs) as a heterogeneous catalyst in the synthesis of 1,2 3- triazoles [52] and as an extension to that work, we have also reported the synthesis of spirochromene tethered 1,2,3–triazoles, **8 (Scheme 1A)** by the reaction between N-propargyl isatin, malononitrile, dimedone, aralkyl halide and sodium azide. The synthesized compounds were found to exhibit potential anti-tubercular activity [20]. Based on these results and the focused literature survey on biological potential of compounds containing pyranocarbazole, 1, 2, 3 - triazole as well as isatin as the structural units **(Fig. 1)** we speculated that, replacement of dimedone component from the aforementioned multicomponent reaction by 4-hydroxycarbazole would furnish spirochromenocarbazole tethered 1,2,3- triazoles with anti-cancer activity. As an outcome of this philosophy, herein we report the synthesis of spirochromenocarbazole tethered 1,2,3-triazoles by one-pot, five component reaction between N-propargyl isatins, malononitrile, 4-hydroxycarbazole, aralkyl halides and sodium azide using cellulose supported CuI nanoparticles (Cell-CuI NPs) as the catalyst. The studies on their anticancer activity have also been described **(Scheme 1B).**



(Ref. 20)

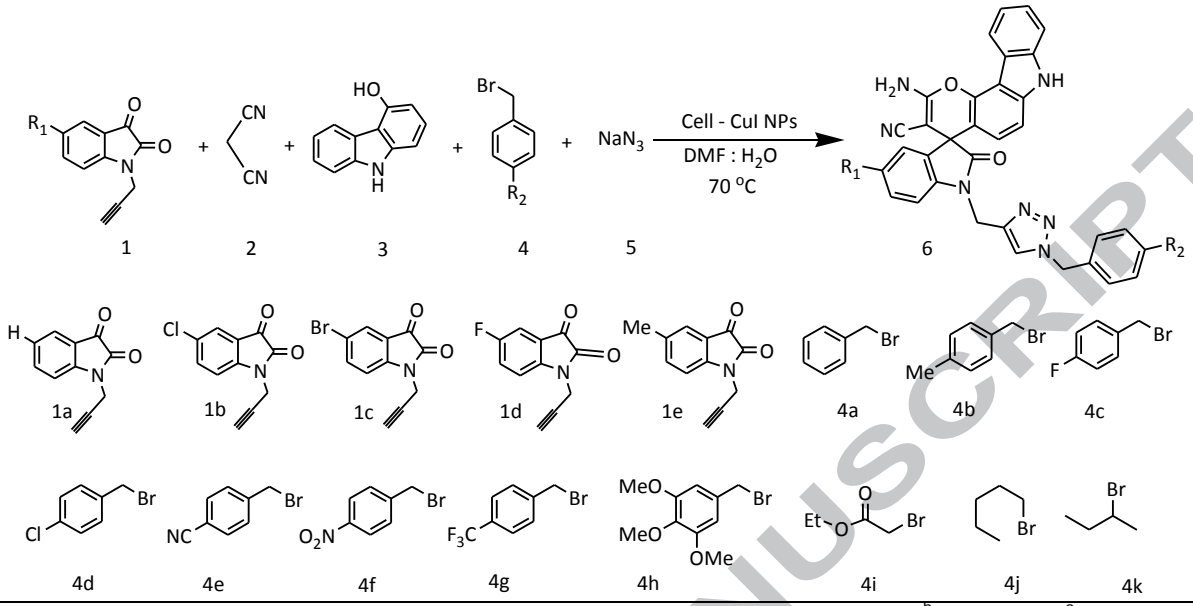
Scheme1. One-pot, five component synthesis of spirochromenocarbazole tethered 1,2,3- triazoles

Result and discussion:

Chemistry

The synthetic route was initiated with the preparation of N-propargyl isatins. Various substituted N-propargylated isatins were prepared by potassium carbonate catalyzed propargylation of isatins with propargyl bromide using DMF as solvent and TBAB as the phase transfer catalyst. We then turned our attention to examine the first model reaction for the synthesis of **6a.** In this regard, we decided to employ the reaction conditions described earlier by us for the synthesis of spirochromene tethered 1, 2, 3- triazoles [20]. Accordingly, to a well stirred solution of N-propargyl isatin, malononitrile, 4-hydroxycarbazole, benzyl bromide (1 mmol, each) and sodium azide (1.1 mmol) in DMF: H_2O (1: 2, v / v) was added Cell- CuI NPs (200 mg, 7.4 mol % CuI) as the catalyst. The reaction mixture was heated with stirring at 70 ^oC and the progress of the reaction was monitored by TLC. Upon completion of the reaction followed by work-up, it was truly gratifying to notice the formation of expected spirochromenocarbazole tethered 1,2,3 -triazole, **6a**, in excellent yield (92 %). So as to examine the scope of the developed protocol, few reactions were then performed by replacement of benzyl bromide component from the model reaction with various aralkyl as well as alkyl bromides. In each case, respective spirochromenocarbazole tethered 1,2,3-triazole was obtained in acceptable yield **(Table1, entry 6b to 6k)**. So as to explore generality of the reaction conditions, keeping malononitrile, 4-hydroxycarbazole and sodium azide components from the model reaction same, various N-propargylated isatins **(1b – 1e)** were allowed to react with various aralkyl bromides **(4a-4d).** Once again, the reactions furnished expected spirochromenocarbazole tethered 1,2,3-triazoles in decent yields **(Table1, entry 6l to 6aa).**

Table 1 : Cell-CuI NPs catalyzed synthesis of spiro [indoline-3,4'-pyrano[3,2-*c*] carbazole tethered 1,2,3-triazoles[a]



Entry	N–Propargyl isatin(1)	Aralkyl or alkyl bromide(4)	Product (6)	Time (h)	Yield[b] (%)	M.P. (^{o}C)
1	1a	4a	6a	3	92	160 - 162
2	1a	4b	6b	3	90	185 - 187
3	1a	4c	6c	3	87	190 - 192
4	1a	4d	6d	4	89	195 - 197
5	1a	4e	6e	4	84	168 - 170
6	1a	4f	6f	4	87	178 - 180
7	1a	4g	6g	4	85	160 - 162
8	1a	4h	6h	5	83	176 - 178
9	1a	4i	6i	3	91	165 - 167
10	1a	4j	6j	5	86	158 - 160
11	1a	4k	6k	5	84	156 - 157
12	1b	4a	6l	3	92	197 - 199
13	1b	4b	6m	4	87	160 - 165
14	1b	4c	6n	3	89	190 - 192
15	1b	4d	6o	3	90	187 - 189
16	1c	4a	6p	4	85	182 - 184
17	1c	4b	6q	3	87	202 - 204
18	1c	4c	6r	4	88	190 - 192
19	1c	4d	6s	5	86	185 - 187
20	1d	4a	6t	4	87	168 - 170
21	1d	4b	6u	4	85	175 - 177
22	1d	4c	6v	5	83	180 - 182
23	1d	4d	6w	4	81	170 - 172
24	1e	4a	6x	3	88	201 - 203
25	1e	4b	6y	4	86	218 - 220
26	1e	4c	6z	4	89	205 - 208
27	1e	4d	6aa	4	84	190-192

a: Reaction conditions: N-propargyl isatin, malononitrile, 4 - hydroxyl carbazole, aralkyl bromide(1 mmol, each) and NaN_3 (1.1 mmol), Cell-CuI (7 mol %), DMF - water– (1: 2:, v/v, 6 mL), 70 ^{o}C.

After the library synthesis of spirochromenocarbazole tethered 1,2,3- triazoles, **6a – 6aa**, it was planned to undertake screening of the synthesized compounds for their anti-cancer activity. In this regard it was essential that the synthesized compounds are free from copper content and the same in the synthesized compounds is possible if the catalyst undergoes leaching under the employed reaction conditions. Hence, it was decided to examine the leaching behavior of the catalyst. Thus, upon completion of the model reaction, the reaction mixture was diluted with water and resultant solid was filtered. Resultant filtrate was analyzed for it's copper content by AAS. Results being negative it was inferred that the synthesized compounds are suitable for the biological assay.

Biological evaluation

All the synthesized compounds were evaluated for their antiproliferative activity against a panel of six human cancer cells namely MCF-7 and MDA-MB-231 (Breast Carcinoma), HeLa (Cervical Carcinoma), PANC-1 (Pancreas Carcinoma), A-549 (Lung Carcinoma) and THP-1 (acute monocytic leukemia). In addition, non-cancerous HUVEC cell line was chosen to compare the effect of synthesized compounds between cancerous and non-cancerous cells. Paclitaxel and Doxorubicin were used as a positive control.

Cells were treated with different concentration (0-100 µg/ml) of all the synthesized compounds and incubated for 48 h. Screening studies revealed that, most of the compounds were active against breast cancer cells MCF-7 and MDA-MB-231 (ER, PR positive) and cervical cancer cells, HeLa (HPV-positive) with IC_{50} values in low micro-molar range (> 10 µM). However, they did not exhibit any cytotoxicity against PANC-1, A-549 and THP-1 cancer cell lines **(SI, Table 1).** IC_{50} values of all the synthesized compounds with respect to MCF-7, MDA-MB-231 and HeLa cancer cell lines and one, normal HUVEC cell line, are summarized in **Table 2**.

Among the synthesized compounds, **6a, 6f, 6g, 6h, 6j, 6p, 6q,** and **6r** were notably active against MCF-7 cells with IC_{50} values in the range 2.1 – 7.06 µM. Especially compounds **6f** and **6j** were highly active (IC_{50} = 2.13 and 4.80 µM, respectively). Similarly, compounds **6j, 6k** and **6s** showed very good activity against MDA-MB-231 cells with IC_{50} values in the range 3.78 - 9.35 µM and among these **6k** showed excellent activity (IC_{50} = 3.78 µM). On the other hand, compounds **6b, 6g, 6j, 6s, 6u and 6z** showed very good activity against HeLa cells with IC_{50} values in the range 3.35 - 9.2 µM and among these compound **6u** was noticed to be the most active one **(**IC_{50} = 3.35 µM). During the screening studies it was noticed that, compound **6j,** shows pronounced activity against all the aforementioned cell lines with IC_{50} in the range 4.8 - 9.2 µM. In order to check the biocompatibility of the synthesized compounds towards normal cell, MTT assay was also performed with HUVEC cell and it was truly delighting that all the synthesized compounds exhibited biocompatibility above 100 µM concentrations (**Table 2**). These results reveal that, synthesized compounds are nontoxic to noncancerous cell line.

Table 2: Anticancer activity data of spirochromenocarbazole tethered 1,2,3-triazoles on various human cancer cell lines.

Sr. No.	Compound Codes	MCF-7 Cells	MDA-MB-231	HeLa Cell	HUVEC
		IC_{50} (μM) ± SD	IC_{50} (μM) ± SD	IC_{50} (μM) ± SD	IC_{50} (μM) ± SD
1	**6a**	**7.02 ± 0.93**	17.25 ± 0.08	12.35 ± 0.17	>100
2	6b	25.56 ± 2.12	12.74 ± 2.32	**4.05** ± 0.69	>100
3	6c	14.37 ± 0.95	13.33 ± 0.48	>100	>100
4	6d	>100	>100	>100	>100
5	6e	25.51 ± 0.32	37.66 ± 0.55	>100	>100
6	**6f**	**2.13** ± 0.16	24.89 ± 0.74	>100 ± 1.6	>100
7	**6g**	**6.31** ± 0.63	14.86 ± 0.12	**3.54** ± 1.24	>100
8	**6h**	**6.97 ± 0.83**	23.66 ± 1.02	15.88 ± 1.12	>100
9	6i	18.00 ± 0.32	41.64 ± 0.04	10.83 ± 0.6	>100
10	**6j**	**4.80** ± 0.25	**8.39** ± 1.84	**9.21** ± 0.34	>100
11	**6k**	51.16 ± 0.53	**3.78** ± 0.74	61.89 ± 2.53	>100
12	6l	>100	>100	>100	>100
13	6m	>100	>100	>100	>100
14	6n	62.74 ± 0.17	71.26 ± 1.18	75.81 ± 0.61	>100
15	6o	42.50 ± 0.67	60.82 ± 0.38	13.25 ± 0.37	>100
16	**6p**	**9.40** ± 0.36	42.37 ± 0.55	32.49 ± 0.15	>100
17	**6q**	**7.06** ± 0.35	30.33 ± 3.8	61.29 ± 1.67	>100
18	**6r**	**6.57 ± 0.26**	25.38 ± 0.69	42.77 ± 0.22	>100
19	**6s**	14.81 ± 0.61	**9.35** ± 1.10	**3.83** ± 0.51	>100
20	**6t**	**9.72** ± 0.89	99.59 ± 0.55	>100	>100
21	6u	>100	75.01 ± 0.28	**3.35** ± 0.17	>100
22	6v	>100	63.08 ± 2.14	37.87 ± 4.11	>100
23	6w	>100	>100	>100	>100
24	6x	88.85 ± 0.06	>100	59.12 ± 0.32	>100
25	6y	>100	>100	>100	>100
26	6z	16.54 ± 0.51	27.88 ± 1.67	**7.80** ± 0.58	>100
27	6aa	23.69 ± 1.45	50.32 ± 0.59	94.77 ± 0.20	>100
28	**Paclitaxel**	0.0026±0.00	0.1 ± 0.07	0.0061±0.00	**>10**
29	**Doxorubicin**	4.63±0.41	0. 55 ± 0. 01	2.66±0.32	**>10**

Structure activity relationship:

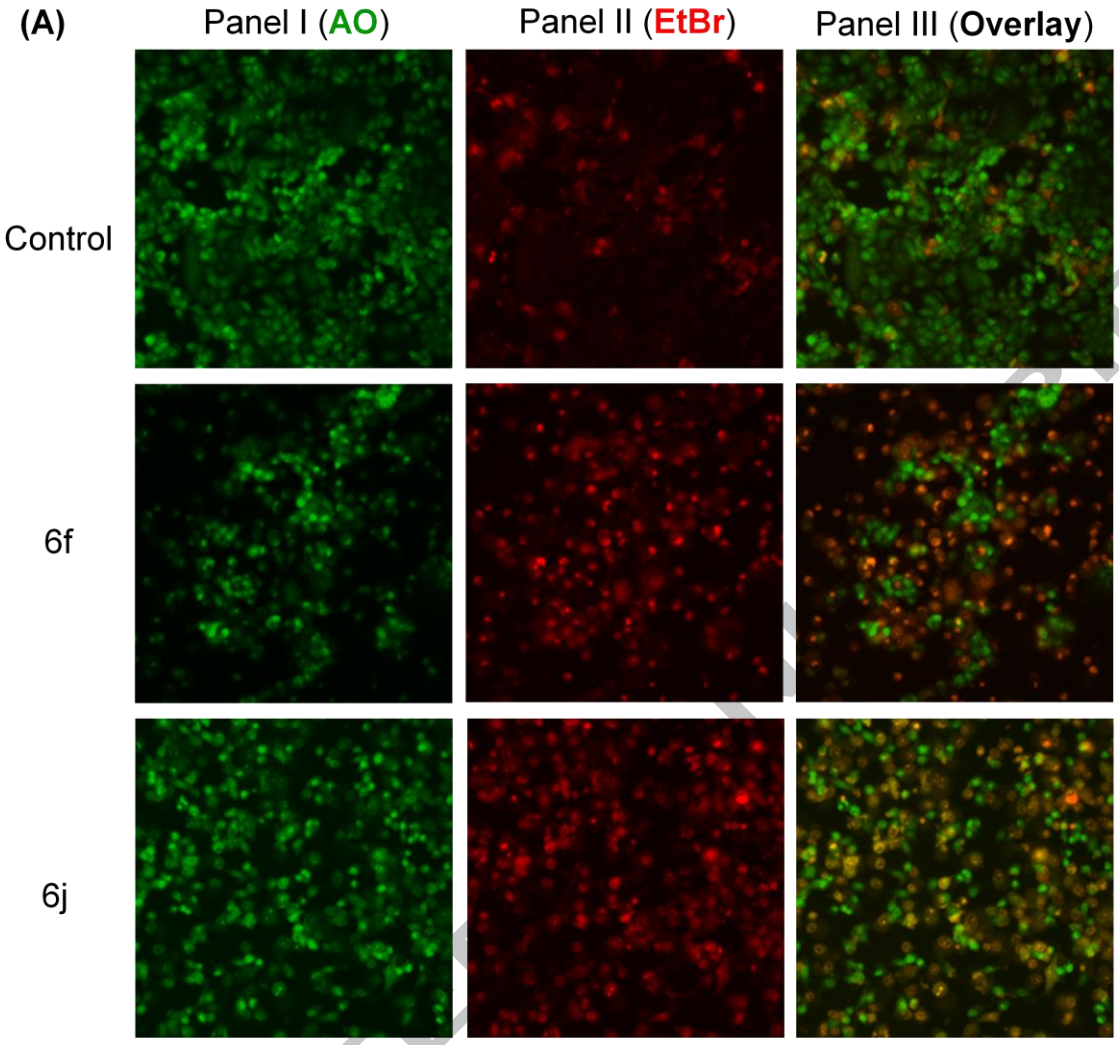
So as to arrive at the relationship between the structures of the synthesized compounds and their anticancer activity it would be rational to categorize these compounds in to two groups: i) Those prepared using N-propargyl isatin (**6a-6k**) and, ii) prepared using substituted N-propargyl isatins (**6l-6aa**). Among the twenty seven compounds screened for their anti-cancer activity, nine compounds **(6a, 6f, 6g, 6h, 6j, 6p, 6q, 6r and 6t)** were prominently active against MCF- 7 cells, six compounds **(6b, 6g, 6j, 6u, 6s and 6z)** showed excellent activity against HeLa cells and only three compounds **(6j, 6k and 6s)** showed good activity against MDA-MB-231 cells. It is worthy to note that among these eighteen compounds, ten active compounds **[6a, 6b, 6f, 6h, 6k, 6g (two cell lines) and 6j (three cell lines)]** were prepared using N-propargyl isatin as the starting compound. On the other hand, remaining eight compounds **(6p, 6q, 6r, 6s, 6t, 6u, and 6z)** were prepared using 5-chloro, 5-bromo, 5-fluoro and 5-methyl-N-propargyl isatin as the starting materials. Furthermore out of these eight compounds, five compounds **[6p, 6q, 6r, 6s (two cell lines)**] were prepared using 5-bromo-N-propargyl isatin as the starting material, the remaining three compounds **6t and 6u** were prepared using 5-fluoro-N-propargyl isatin while **6z** was prepared using 5-methyl-N-propargyl isatin. On analysis of this data it could broadly be concluded that, most active compounds are derived from N-propargyl isatin and 5-bromo-N-propargyl isatin whereas substitution of chloro, fluro as well as methyl group on N-propargyl isatin does not assist in improvement of anticancer activity. As regards the effect of aralkyl or alkyl bromide component on anticancer activity is concerned it was noticed that, compounds prepared using benzyl bromides bearing electron-withdrawing groups (**6f and 6g**) were more potent than those prepared using benzyl bromide **(6a)** or the one bearing electron-donating group **(6h)** and the one prepared using aliphatic long chain bromide, **6k**, was active against Breast as well as cervical cancer cells.

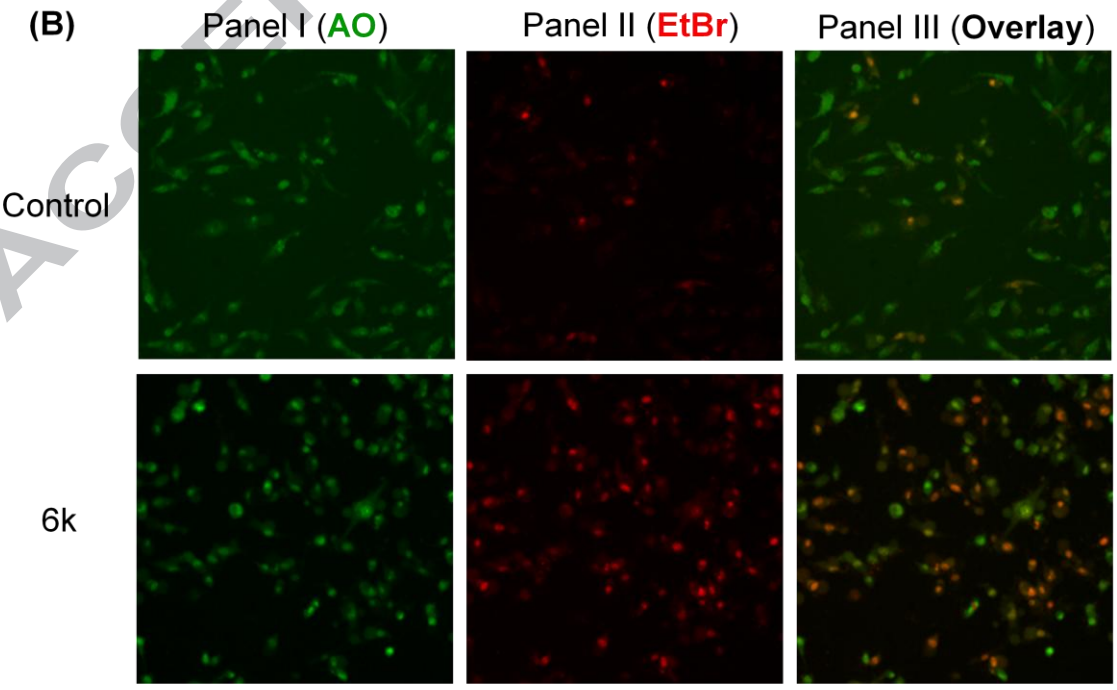
In summary, in the multicomponent synthesis of spirochromenocarbazole tethered 1,2,3-triazoles as anticancer agents, the use of unsubstituted n-propargyl isatin, **1a**, in combination with benzyl bromides bearing electron-withdrawing group, **4f and 4g,** or aliphatic bromides, **4j** and **4k** has furnished the compounds with excellent anticancer activity against MCF-7 cells. In case HeLa cells, excellent anticancer activity was noticed by four compounds **(6b, 6g, 6s and 6u)** and it was interesting to note that, out of these four compounds three compounds **(6g, 6s and 6u)** that showed excellent and comparable activity were containing fluorine or bromine substituent in them. Thus, presence of halogen substituent may be responsible for the improvement of anticancer activity against HeLa cells.

Based upon these studies we propose that, there certainly exists scope for the synthesis novel spirochromenocarbazole tethered 1,2,3-triazoles with improved anticancer activity by using difluoro or dibromo isatin in combination with 4-triflorobenzyl bromide, 4-nitrobenzyl bromide and aliphatic long chain bromides.

Apoptosis assays:

To gain insight of the mechanisms by which the tested compounds reduced proliferation of breast cancer cells, the compounds showing significant anti-proliferative activity were assessed for cell death. Thus, MCF-7, MDA-MB-231 and HeLa cells were treated at the concentrations in the range of IC_{50} for 48 h and after staining with acridine orange and ethidium bromide (AO/EB) were assessed by ArrayScan™ 4.0 High content screening system. Depending upon the overall morphological characteristics and the cell membrane integrity, the cells were distinguished from one another as necrotic, apoptotic or live cells. Apoptotic cells containing apoptotic bodies (condensed nucleus) were observed as yellow / orange colored cells while the necrotic cells were observed as red colored cells. Interestingly, compared to compounds **6a,6g, 6h,6p, 6q, 6r** and **6t (SI Fig 1)** the number of apoptotic and necrotic events were significantly high in case of **6f** and **6j** treated MCF-7 cells **(Fig. 2A).** Similarly, compared to compounds **6j** and **6s (SI, Fig. 2), 6k** treated MDA MB-231 cells showed higher apoptotic and necrotic population **(Fig. 2B).** On the other hand, in case of HeLa cells, compared to **6b, 6g, 6j, 6u and 6z (SI Fig.3), 6s** treated cells **(Fig. 2C)** showed significant increase in apoptotic events. However, the morphological assessment of **6b, 6g, 6j, 6u, 6z** treated Hela Cells revealed a decrease in cell number by cell cycle arrest. The results of the most active compounds in the respective cell line are summarized in **Table 3** and the same of all the compounds are summarized in **SI, Table 3**. The results obtained are in agreement with the results of MTT assay**.** In summary, we could assume that under the applied conditions apoptotic cell death was the major cause of reduced proliferation of the breast cancer cells but not in the cervical cancer cells.





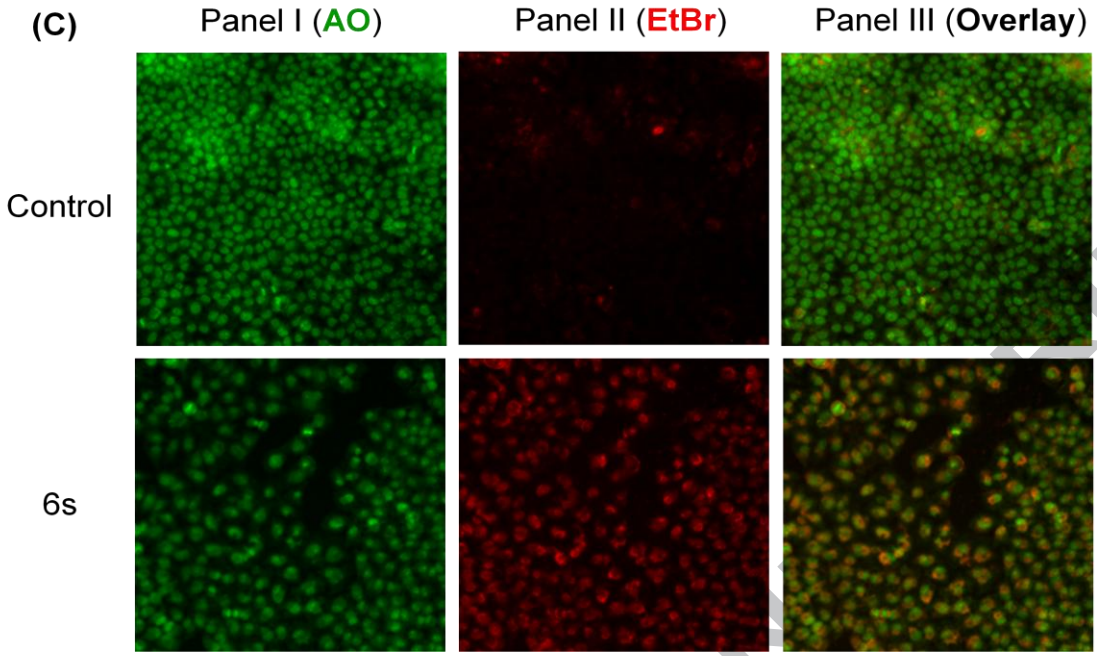


Figure 2. Fluorescent images showing apoptosis induced by selected chromenocarbazole tethered 1,2,3-triazoles derivatives in (**A**) MCF-7, (**B**) MDA MB 231 and **(C)** HeLa cells. Control and treated cells were stained with Acridine orange (AO) / Ethidium bromide (EtBr); and the staining pattern was monitored by The ArrayScan™ 4.0 system. For detection of AO (green) (Panel I) and EtBr (red) (Panel II), mercury-xenon lamp was excited at 488 nm and 590 nm, respectively. Panel III represents the merge images

Table 3. Percent of viable, apoptotic and necrotic cell population as measured by AO/EB fluorescent staining studies

	Viable	Apoptotic	Necrotic
MCF-7			
Control	89.5 ± 2.3	5.4± 1.4	5.1 ± 1.8
6f	42.0 ± 2.3	14.3± 1.4	38.6 ± 1.8
6j	49.6 ± 10.2	43.2 ±2.4	7.2 ± 4.1
MDA-MB-231			
Control	92.8 ± 1.8	5.6 ±1.3	1.6 ± 0.01
6k	42.8 ± 2.2	36.1 ±1.8	21.1 ± 2.1
HeLa			
Control	97.8 ± 1.2	1.4 ±0.01	0.8 ± 0.2
6s	41.4 ± 2.8	46.2 ±2.3	12.4 ± 7.2

Conclusions:

Taking clues from the structures of the compounds with proven biological activities and our experiences in click chemistry as well as multicomponent reactions, studies were undertaken on the synthesis of novel anticancer agents. During the studies, we have designed and synthesized for the first time a series of spirochromenocarbazole tethered 1,2,3- triazoles by one-pot, five component condensation reaction between N-propargyl isatins, malononitrile, 4-hydroxycarbazole, alkyl / arylalkyl halides and sodium azide using cellulose supported CuI nanoparticles (Cell-CuI NPs) as the heterogeneous catalyst. Simple experimental procedure, wide scope and reusability of the catalyst are the main merits of the developed protocol. The synthesized compounds were found to be selectively cytotoxic against breast (MCF-7, MDA-MB-231) and cervical (HeLa) cancer cells. Among all screened compounds, **6f, 6k, 6g, 6s** and **6u** showed excellent activity towards MCF-7, MDA-MB-231 and HeLa cancer cell lines. Especially compound **6j** showed good activity for all three cancer cell lines. The synthesized compounds being nontoxic towards normal cells, they may be useful towards specific cancer cell lines. Furthermore, AO/EB staining suggested that under the applied conditions apoptotic cell death was the major cause of reduced proliferation of the breast cancer cells but not in the cervical cancer cells.

Acknowledgments

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Experimental

General

Isatins (Aldrich), benzyl halides (Aldrich), sodium azide (S. D. Fine-Chem. Limited, Mumbai), 4-hydroxycarbazole, malononitrile, copper (I) iodide (Spectrochem, Mumbai) and microcrystalline cellulose (SRL, Mumbai) were used, as received. The catalyst Cell-CuI NPs was prepared according to the procedure developed and reported by us earlier [20]. Melting points were recorded using Kumar melting point apparatus. IR spectra were recorded as neat using Thermo Scientific Nicolet S10 FT-IR Spectrometer. ^{1}H-NMR (300 MHz) and ^{13}C-NMR (75 MHz) spectra were recorded using Bruker Avance – II spectrometer. High resolution mass spectra (HRMS) were recorded using Thermo Scientific Q-Exactive, Accela 1250 pump, instrument.

Representative procedure for the preparation of N - propargylation of isatin:

Isatin (1mmol), potassium carbonate (1 mmol), propargyl bromide (1.3 mmol) and TBAB (0.05 mmol) were placed in a round bottom flask. DMF (3 mL) was added and the reaction

mixture was stirred at room temperature for 24 h. After completion of reaction (TLC), crushed ice was added and stirring was continued for 15 min more. Resultant solid was filtered and washed with water (4 x 5 mL). It was dried in air and recrystallized from ethanol.

Representative procedure for one-pot synthesis of spirochromenocarbazole tethered 1,2,3-triazoles [1,4-disubstituted-1,2,3–triazolyl spiroindoline pyrano [3,2-c] carbazoles, 6a-6aa]:

N-Propargyl isatin (1 mmol), malononitrile (1 mmol), 4-hydroxycarbazole (1 mmol), benzyl bromide (1 mmol), and sodium azide (1.1 mmol) were placed in a round bottom flask. DMF-Water (1: 2, 6 mL, v / v) and cellulose-CuI NPs catalyst (0.2 g) were added and the reaction mixture was heated at 70 °C. After completion of the reaction (TLC), the reaction mixture was filtered and the filter was washed with ethyl acetate (4 x 10 mL). The organic extract was washed with water, brine and dried over Na_2SO_4. Removal of solvent under vacuum furnished corresponding 1,4-disubstituted-1,2,3-triazolyl spiroindoline pyrano [3, 2-c] carbazole as a solid product. The resultant solid was successively washed using a mixture of hexane - chloroform (80: 20 v/v), and dried. The resultant product did not require any further purification. The recovered catalyst was washed with acetone, dried in air. Reusability studies showed that the catalyst can be reused for five consecutive runs without significant loss in its activity.

Biological methods

Cytotoxicity assay

Six human cancer cell lines, MCF-7 and MDA-MB-231, HeLa, PANC-1, A-549, THP-1 were used to check the cytotoxicity of the synthesized compounds. The cell lines were obtained from National Center for Cell Science (NCCS), Pune and maintained in T25 flasks with 10 % (v/v) fetal bovine serum (FBS) containing Dulbecco's Modified Eagle Medium (DMEM). HUVECs cells were purchased from Life Technologies and maintained in basal medium M200 (Life Technologies) containing 10 % FBS and growth factors (LSGS, Life Technologies). Cell line containing T25 flasks were maintained at 37°C under 5 % CO_2 and 95 % air in a humidified atmosphere. Medium were replaced twice a week.

The cytotoxicity of the synthesized compounds was tested against the mentioned cell lines by using modified MTT assay [53]. Briefly, cells were seeded at the density of 1×10^4 cells/mL in a 96 well plate. The plates were incubated overnight into CO_2 incubator (37 ºC under 5 % CO_2 and 95% air in a humidified atmosphere) to adhere the cells. Next day, cells were treated with different concentration of test compounds (100-0 µg/ml) and incubated for additional 48 h. Post incubation, cell medium was replaced with MTT (0.5 mg/mL) - PBS medium and incubated for 2-4 h to form Formazan crystals. The crystals were solubilized by addition of acidified *iso*-propanol. The optical density was read on a micro plate reader at 570 nm filter against a blank prepared from cell-free wells. Absorbance given by cells treated with the vehicle alone was taken as 100% cell growth. IC_{50} and MIC values were calculated by using Origin Pro

software. The viability and growth in the presence of test material is calculated by using the following formula: Percent cytotoxicity = [(average absorbance of control − absorbance of compound) / (absorbance of control – absorbance of blank)] × 100, where control is the culture medium with cells and DMSO while blank is the culture medium without cells. Paclitaxel and Doxorubicin were used as a positive control.

Morphological assessment by acridine orange and ethidium bromide double staining

The dual-fluorescence staining using acridine orange (AO) and ethidium bromide (EtBr) is a nuclear staining method used to assess apoptotic cell morphology. Stain AO is able to permeate into both live and dead cells to stain all the nucleated cells and excite green fluorescence, while stain EtBr enters only into dead cells with damaged membranes and generates red fluorescence. Thus, late apoptotic and necrotic cells take up both stains. Briefly, MCF-7, MDA-MB-231 and HeLa cells were seeded at the density of 1×10^4 cells/mL in a 96 well plate. The plates were incubated overnight into CO_2 incubator. Next day, the cells were treated with IC_{50} concentration of test compounds for additional 48 h. Post incubation, cell medium was replaced with AO and EtBr solution mixture (1 part each of 10 µg mL^{-1} of AO and EtBr in PBS) and examined under Array Scan VTI HCS 600-type high content live cell imaging system. The Fluorescence microphotographs in the green, and red channels of at least 20 fields were acquired in each well with a 20X objective, corresponding to at least 2000 cells counted. An analysis of the fluorescence intensity was performed for AO and EtBr stained samples by the HCS Assay Scan software.

Spectral data

All the synthesized compounds are new and were fully characterized by spectral methods.

2-Amino-4,7-dihydro-1'((1-(benzyl)-1H-1,2,3-triazol-4-yl)methyl)-2'oxo-spiro[indoline-3',4-chromene] pyrano [3,2-c]carbazole-3-carbonitrile, ***6a****:* Off-white solid; M.P.: 160 -162 °C; **IR (KBr):**3310, 3187, 2927, 2190, 1708, 1652, 1607, 1398, 1338, 1098, 748 cm^{-1}; **^{1}H NMR (300 MHz, DMSO - d_6):** δ 4.97 (m, 2H,indolinyl-CH_2), 5.58 (s, 2H, benzylic-CH_2), 6.35 (br s, 1H, Ar-H), 7.04 (s, 1H, Ar-H), 7.14 (d, 2H, *J* = 8.4 Hz, Ar-Hs), 7.21 (d, 1H, *J* = 7.8 Hz, Ar-Hs), 7.29 (m, 3H, Ar-Hs), 7.34 - 7.42 (m, 4H, Ar- Hs), 7.45 (d, 2H, *J* = 7.2 Hz, Ar-Hs), 7.51 (d, 1H, *J* = 8.1 Hz, Ar-H), 7.59 (s, 2H, NH_2), 8.08 (s, 1H, triazolyl-H), 8.58 (d, 1H, *J* = 7.8 Hz, Ar-H), 11.55 (s, 1H, NH); **^{13}C NMR (75.4MHz, DMSO-d_6):** δ 35.70, 50.37, 53.31, 54.55, 108.50, 109.51, 110.04, 110.59, 111.42, 119.25, 119.74, 120.65, 123.68, 128.31, 128.59, 129.21, 129.39, 130.20, 136.41, 140.08, 140.77, 142.47, 142.76, 144.44, 161.86, 177.87 ppm; **HRMS:** mass calculated for [$C_{33}H_{23}N_7O_2$]: 550.1991 $[M + H]^+$ and 572.1811 $[M + Na]^+$; obs. mass: 550.1986 $[M + H]^+$ and 572.1805 $[M + Na]^+$.

2-Amino-4,7-dihydro-1'((1-(4-methylbenzyl)-1H-1,2,3-triazol-4-yl)methyl)-2'oxo-spiro[indoline-3',4- chromene] pyrano[3,2-c] carbazole-3-carbonitrile, ***6b****:* cream colored solid; M.P.: 185 -187

°C; **IR (KBr):** 3308, 3190, 2928, 2195, 1710, 16525, 1609, 1390, 1335, 1094, 745 cm^{-1}; **^{1}H NMR (300 MHz, DMSO-d_6):** δ 2.26 (s, 3H, Ar-CH_3), 5.10 (m, 2H, indolinyl-CH_2), 5.18 - 5.56 (m, 2H, benzylic-CH_2), 6.37 (d, 1H, *J* = 8.4 Hz, Ar-H), 7.06 (d, 2H, *J* = 8.4 Hz, Ar-Hs), 7.13 (m, 4H, Ar-Hs), 7.19 (d, 2H, *J* = 8.1 Hz, Ar-Hs), 7.24 (d, 1H, *J* = 7.5 Hz, Ar-H), 7.34 (d, 1H, *J* = 7.8 Hz, Ar-H), 7.42 (d, 1H, *J* = 7.8 Hz, Ar-H), 7.47 – 7.51 (m, 1H, Ar-H), 7.54 (br s, 2H, NH_2), 8.02 (s, 1H, triazolyl-H), 8.57 (d, 1H, *J* = 7.8 Hz, Ar-H), 11.55 (s, 1H, NH); **^{13}C NMR (75.4 MHz, DMSO-d_6):** δ 21.14, 35.70, 50.37, 53.13, 54.50, 108.53, 109.51, 110.03, 110.60, 111.43, 119.25, 119.77, 120.64, 123.64, 123.93, 125.18, 126.30, 128.34, 129.41, 129.74, 133.34, 135.17, 137.96, 140.08, 140.78, 142.46, 142.71, 144.44, 161.87, 177.88 ppm; **HRMS:** mass calculated for [$C_{34}H_{25}N_7O_2$]: 564. 2148 [M + H] $^+$ and 586.1968 [M + Na] $^+$; obs. mass: 564.2142 [M + H] $^+$ and 586.1962 [M + Na] $^+$.

2-Amino-4,7-dihydro-1'((1-(4-fluorobenzyl)-1H-1,2,3-triazol-4-yl)methyl)-2'oxo-spiro[indoline-3',4- chromene] pyrano[3,2-c]carbazole-3-carbonitrile, ***6c****:* yellow solid; M.P.: 190 - 192 °C; **IR (KBr):** 3317, 3190, 2915, 2198, 1714, 1625, 1610, 1398, 1331, 1092, 745cm^{-1}; **^{1}H NMR (300 MHz, DMSO -d_6):** δ 4.98 (m, 2H, indolinyl-CH_2), 5.57 (br s, 2H, benzylic-CH_2), 6.38 (d, 1H, *J* = 8.4 Hz, Ar-H), 7.07 (d, 2H, *J* = 8.4 Hz, Ar-Hs), 7.13 (d, 1H, *J* = 6 Hz, Ar-H), 7.18 (d, 2H, *J* = 8.7 Hz, Ar-Hs), 7.23 - 7.26 (m, 2H, Ar-Hs), 7.31 - 7.37 (m, 3H, Ar-Hs), 7.42 - 7.47 (m, 1H, Ar-H), 7.51 (d, 1H, *J* = 8.1 Hz, Ar-H), 7.57 (br s, 2H, NH_2), 8.17 (s, 1H, triazolyl-H), 8.57 (d, 1H, *J* = 7.8 Hz, Ar-H), 11.54 (s, 1H, NH); **^{13}C NMR (75.4 MHz, DMSO-d_6):** δ 35.70, 50.36, 52.50, 54.57, 108.51, 109.50, 110.03, 110.59, 111.42, 115.88, 116.16, 119.62, 119.73, 120.64, 123.67, 123.76, 123.84, 125.19, 126.27, 129. 40, 130.59, 130.70, 132.63, 135.17, 140. 08, 140.78, 142.48, 142.79, 144.45, 160.70, 161.85, 177.87 ppm; **HRMS:** mass calculated for [$C_{33}H_{22}FN_7O_2$]: 568.1897 [M + H] $^+$ and 590.1717 [M + Na] $^+$; obs. mass: 568.1892 [M + H] $^+$ and 590.1711 [M + Na] $^+$.

2-Amino-4,7-dihydro-1'((1-(4-chlorobenzyl)-1H-1,2,3-triazol-4-yl)methyl)-2'oxo-spiro[indoline-3',4- chromene] pyrano[3,2-c]carbazole-3 carbonitrile, ***6d****:* brown solid; M.P.: 195 - 197 °C; **IR KBr):** 3317, 3190, 2915, 2198, 1714, 1625, 1610, 1398, 1331, 1092, 745 cm^{-1}; **^{1}H NMR (300 MHz, DMSO-d_6):** δ 5.03 - 5.05 (m, 2H, indolinyl-CH_2), 5.59 (s, 2H, benzylic-CH_2), 6.38 (d, 1H, *J* = 8.4 Hz, Ar-H), 7.07 (d, 2H, *J* = 8.1 Hz, Ar-Hs), 7.14 (d, 1H, *J* = 6.9 Hz, Ar-Hs), 7.21 (d, 2H, *J* = 7.5 Hz, Ar-Hs),7.31- 7.37 (m, 3H, Ar-Hs), 7.40 - 7.47 (m, 3H, Ar-Hs), 7.51 (d, 2H, *J* = 7.8 Hz, Ar-Hs), 7. 58 (s, 2H, NH_2), 8.10 (s, 1H, triazolyl-H), 8.57 (br s, 1H, Ar-H), 11.55 (s, 1H, NH); **^{13}C NMR (75.4 MHz, DMSO - d_6):** δ 35.69, 50.36, 52.47, 54.58, 108.51, 109.50, 110.02, 110.59, 111.41, 119.20, 119.71, 120.65, 123.67, 123.87, 125.20, 126.25, 129. 17, 129.38, 130.23, 133.28, 135.18, 135.42, 138.25, 140. 08, 140.78, 142.49, 142.80, 144.45, 147.88, 161.84, 177.85 ppm; **HRMS:** mass calculated for [$C_{33}H_{22}ClN_7O_2$]: 584.1602 [M+ H] $^+$ and 606.1422 [M + Na]$^+$; obs. mass: 584.1596 [M+H] $^+$ and 606.1416 [M + Na] $^+$.

2-Amino-4,7-dihydro-1'((1-(4-cyanobenzyl)-1H-1,2,3-triazol-4-yl)methyl)-2'oxo-spiro[indoline-

3',4 chromene] pyrano [3,2-c]carbazole-3-carbonitrile, ***6e****:* brown solid; M. P.: 168 - 170 °C; **IR (KBr):** 3313, 3183, 2968, 2189, 1987, 1714, 1653, 1608, 1365, 1340, 1214, 1051, 749 cm^{-1}; **^{1}H NMR (300 MHz, DMSO- d_6):** δ 5.05 **-** 5.06 (br s, 2H, indolinyl-CH_2), 5.72 (s, 2H, benzylic-CH_2,), 6.39 (d, 1H, *J* = 8.4 Hz, Ar-H), 7.07 (d, 1H, *J* = 7.8 Hz, Ar-H), 7.14 (d, 1H, *J* = 7.9 Hz, Ar-H), 7.2 (m, 2H, Ar-Hs), 7.32 (d, 1H, *J* = 7.2 Hz, Ar-H), 7.39 - 7.47 (m, 4H, Ar-Hs), 7.51 (d, 1H, *J* = 8.1 Hz, Ar-H), 7.59 (s, 2H, NH_2), 7.81 (d, 2H, *J* = 7.8 Hz, Ar-Hs), 8.17 (s, 1H, triazolyl-H), 8.57 (d, 1H, *J* = 7.8 Hz, Ar-H), 11.55 (s, 1H, NH); **^{13}C NMR (75.4MHz, DMSO-d_6):** δ 35.66, 50.36, 52.65, 54.54, 108.53, 109.47, 110.05, 110.58, 111.34, 111.43, 118.99, 119.21, 119.76, 120.63, 123.66, 123.89, 123.92, 124.36, 125.21, 126. 28, 129.01, 129.42, 133.15, 135.17, 140. 07, 140.78, 141.91, 142.47, 142.89, 144.45, 161.84, 177.89 ppm; **HRMS:** mass calculated for [$C_{34}H_{22}N_8O_2$]: 575.1944 $[M + H]^+$ and 597.1764 $[M + Na]^+$; obs. mass: 575.1938 $[M + H]^+$ and 597.1758 $[M + Na]^+$.

2-Amino-4,7-dihydro-1'((1-(4-nitrobenzyl)-1H-1,2,3-triazol-4-yl)methyl)-2'oxo-spiro[indoline-3',4-chromene] pyrano [3,2-c]carbazole-3-carbonitrile, ***6f****:* brown solid; M.P.: 178 - 180 °C; **IR (KBr):** 3315, 3173, 2978, 2179,1985, 1714, 1650, 1610, 1360, 1338, 1212, 1055, 750 cm^{-1}; **^{1}H NMR (300 MHz, DMSO-d_6):** δ 5.06 (s, 2H, indolinyl-CH_2), 5.78 (s, 2H, benzylic-CH_2), 6.41 (d, 1H, *J* = 8.4 Hz, Ar-H), 7.08 - 7.14 (m, 3H, Ar-Hs), 7.24 (d, 2H, *J* = 7.0 Hz, Ar-Hs), 7.32 (d, 1H, *J* = 7.5 Hz, Ar-H), 7.44 - 7.53 (m, 4H, Ar-Hs), 7.58 (s, 2H, NH_2), 8.19 (s, 1H, triazolyl-H), 8.24 (br s, 2H, Ar-Hs), 8.57 (d, 1H, *J* = 7.8 Hz, Ar-H), 11.54 (s, 1H, NH); **^{13}C NMR (75.4MHz, DMSO-d_6):** δ 35.65, 50.36, 52.65, 54.54, 108.54, 109.47, 110.05, 110.58, 111.34, 111.43, 118.99, 119.74, 120.63, 123.66, 123.92, 124.32, 124.42, 125.22, 126.29, 129. 33, 129.42, 133.15, 135.17, 140.07, 140.78, 141.91, 142.47, 142.89, 144.45, 161.84, 177.89 ppm; **HRMS:** mass calculated for [$C_{33}H_{22}N_8O_4$]: 595.1842 $[M+H]^+$ and 617.1662 $[M+Na]^+$; obs. mass: 595.1837 $[M + H]^+$ and 617.1662 $[M + Na]^+$.

2-Amino-4,7-dihydro-1'((1-(4-trifluoromethylbenzyl)-1H-1,2,3-triazol-4-yl)methyl)-2'oxo-spiro[indoline-3',4 chromene] pyrano[3,2-c]carbazole-3-carbonitrile, ***6g****:* brown solid; M. P.: 160 - 162 °C; **IR (KBr):** 3315, 3173, 2978, 2179,1985, 1714, 1650, 1610, 1360, 1338, 1212, 1055, 750 cm^{-1}; **^{1}H NMR (300 MHz, DMSO-d_6):** δ 5.06 (s, 2H, indolinyl-CH_2), 5.72 (s, 2H, benzylic-CH_2), 6.41 (d, 1H, *J* = 8.1 Hz, Ar-H) , 7.08 (d, 2H, *J* = 7.8 Hz, Ar-Hs), 7.14 (br s, 1H, Ar-H), 7.24 (br s, 2H, Ar-Hs), 7.32 (d, 1H, *J* = 7.2 Hz, Ar-H), 7.46 - 7.50 (m, 3H, Ar-Hs), 7.51 (d, 1H, *J* = 7.8 Hz, Ar-H), 7.58 (s, 2H, NH_2), 7.70 (d, 2H, *J* = 7.2 Hz, Ar-Hs), 8.17 (s, 1H, triazolyl-H), 8.57 (d, 1H, *J* = 7.5 Hz, Ar-H), 11.55 (s, 1H, NH); **^{13}C NMR (75.4 MHz, DMSO-d_6):** δ 35.68, 50.38, 52.62, 54.59, 108.54, 109.50, 110.05, 110.60, 111.43, 119.23, 119.74, 120.64, 122.73, 123.67, 123.91, 124.29, 125.21, 126. 07, 126.11, 126.28, 128.95, 129.31, 129.41, 135. 19, 140.10, 140.79, 141.10, 142.49, 142.87, 144.46, 161.86, 177.90 ppm; **HRMS:** mass calculated for [$C_{34}H_{22}F_3N_7O_2$]: 618.1865 $[M+H]^+$ and 640.1685 $[M+Na]^+$; obs. mass: 618.1860 $[M+H]^+$ and 640.1679 $[M + Na]^+$.

2-Amino-4,7-dihydro-1'((1-(3,4,5-trimethoxybenzyl)-1H-1,2,3-triazol-4-yl)methyl)-2'oxo-spiro[indoline-3',4- chromene] pyrano[3,2-c]carbazole-3-carbonitrile, ***6h****:* brown solid; **M.P**.: 176 - 178 °C; **IR (KBr):** 3315, 3173, 2978, 2179, 1985, 1714, 1650, 1610, 1360, 1338, 1055, 750 cm^{-1}; **^{1}H NMR (300 MHz, DMSO-d_6):** δ 3.57 (s, 3H, Ar-OCH_3), 3.66 (s, 6H, 2xAr-OCH_3), 5.03 - 5.06 (br s, 2H, indolinyl-CH_2), 5.49 (s, 2H, benzylic-CH_2), 6.39 (d, 1H, *J* = 8.4 Hz, Ar-H), 6.55 - 6.68 (m, 3H, Ar-Hs), 7.04 (d, 1H, *J* = 8.4 Hz, Ar-H), 7.13 (d, 1H, *J* = 6.0 Hz, Ar-H), 7.17 (d, 1H, *J* = 7.8 Hz, Ar-H), 7.24 (d, 1H, *J* = 7.5 Hz, Ar-H), 7.28 (d, 1H, *J* = 7.5 Hz, Ar-H), 7.42 (d, 1H, *J* = 7.8 Hz, Ar-H), 7.51 (d, 1H, *J* = 8.4 Hz, Ar-H), 7.57 (s, 2H, NH_2), 8.10 (s, 1H, triazolyl-H), 8.53 (d, 1H, *J* = 7.2 Hz, Ar-H), 11.70 (s, 1H, NH); **^{13}C NMR (75.4 MHz, DMSO-d_6):** δ 35.65, 50.38, 53.51, 56.26, 60.44, 105.70, 105.98, 108.48, 109.48, 110.00, 111.45, 119.27, 120.62, 123. 67, 123.83, 123.87, 123.96, 125.79, 126. 28, 129.33, 131.76, 131.84, 135.22, 137.62, 140.08, 140.76, 142.48, 142.69, 144.46, 153.03, 153.44, 161.81, 178.11 ppm; **HRMS:** mass calculated for [$C_{36}H_{29}N_7O_5$]: 640.2308 [M + H] $^+$ and 662.2128 [M + Na]$^+$; obs. mass: 640.2303 [M + H] $^+$ and 662.2122 [M + Na] $^+$.

2-Amino-4,7-dihydro-1'((1-(carboethoxy)-1H-1,2,3-triazol-4-yl)methyl)-2'oxo-spiro[indoline-3',4-chromene] pyrano[3,2-c]carbazole-3-carbonitrile, ***6i****:* brown solid; M.P.: 165 - 167 °C; **IR (KBr):**3313, 3186, 2926, 2190,1985, 1739, 1650, 1608, 1518, 1459, 1367, 1214, 1098, 745 cm^{-1}; **^{1}H NMR (300 MHz, DMSO-d_6):** δ 1.19 (t, 3H, *J* = 7.2 Hz, CH_3)**,** 4.17 (q, 2H,*J* = 7.2 Hz, CH_3CH_2), 5.07 (q, 2H, *J* = 39.3 Hz, indolinyl-CH_2), 5.39 (s, 2H, $COCH_2$), 6.37 (d, 1H, *J* = 8.4 Hz, Ar-H), 7.05 – 7. 53 (m, 8H, Ar-Hs), 7.60 (s, 2H, NH_2), 8.07 (s, 1H, triazolyl-H), 8.57 (d, 1H, *J* = 7.8 Hz, Ar-H), 11.56 (s, 1H, NH); **^{13}C NMR (75.4 MHz, DMSO-d_6):** δ 14.39, 35.61, 50.37, 50.89, 54.52, 61.95, 108.59, 109.57, 110.06, 110.56, 111.41, 119.21, 119.73, 120.64, 123.67, 123.90, 125.17, 126.26, 129.37, 135. 20, 140.06, 140.77, 142.47, 144.40, 161.87, 167.58, 177.89 ppm; **HRMS:** mass calculated for [$C_{30}H_{23}N_7O_4$]: 546.1890 [M + H] $^+$ and 568.1710 [M + Na]$^+$; obs. mass: 546.1884 [M + H] $^+$ and 568.1704 [M + Na].$^+$

2-Amino-4,7-dihydro-1'((1-(pentyl)-1H-1,2,3-triazol-4-yl)methyl)-2'oxo-spiro[indoline-3',4-chromene] pyrano[3,2-c]carbazole-3-carbonitrile, ***6j****:* brown solid; M.P.: 158 - 160 °C; **IR (KBr):** 3313, 3186, 2926, 2190, 1985, 1739, 1650, 1608, 1518, 1459, 1367, 1214, 1098, 745 cm^{-1}; **^{1}H NMR (300 MHz, DMSO-d_6):** δ 0.80 (t, 3H,*J* = 7.2 Hz, CH_3)**,** 1.09 - 1.26 (m, 4H, CH_2), 1.74 - 1.78 (m, 2H, CH_2), 4.31 (t, 2H, *J* = 6.9 Hz CH_2), 5.04 (m, 2H, indolinyl-CH_2), 6.39 (d, 1H, *J* = 8.4 Hz, Ar-H), 7.08 (m, 2H, Ar-Hs), 7.12 – 7.20 (m, 2H, Ar-Hs), 7.24 (d, 1H, *J* = 7.2 Hz, Ar-H), 7.30 (d, 1H, *J* = 7.8 Hz, Ar-H), 7.42 (d, 1H, *J* = 7.2 Hz, Ar-H), 7.50 (d, 1H, *J* = 8.1 Hz, Ar-H), 7.56 (s, 2H, NH_2), 7.98 (s, 1H, triazolyl-H), 8.56 (d, 1H, *J* = 7.8 Hz, Ar-H), 11.53 (s, 1H, NH); **^{13}C NMR (75.4 MHz, DMSO-d_6):** δ 14.19, 21.90, 28.34, 29.70, 35.86, 49.84, 50.38, 108.51, 109.46, 110.05, 110.57, 111.42, 119.34, 119.76, 120.62, 123.41, 123.65, 123.88, 123.90, 125.16, 126.29, 129.37, 135.21, 140.06, 140.77, 142.39, 144.43, 161.82, 177.88 ppm; **HRMS:** mass calculated for [$C_{31}H_{27}N_7O_2$]: 530.2304 [M + H] $^+$ and 552.2124 [M + Na] $^+$; obs. mass:

530.2299 $[M + H]^+$ and 552.2118$[M + Na].^+$

2-Amino-4,7-dihydro-1'((1-(secondarybutyl)-1H-1,2,3-triazol-4-yl)methyl)-2'oxo-spiro[indoline-3',4-chromene] pyrano[3,2-c] carbazole-3-carbonitrile, ***6k****:* brown solid; M.P.: 156-158 °C; **IR (KBr):** 3307, 3184, 2967, 2189, 1709, 1652, 1608, 1458, 1397, 1305, 1051, 746 cm^{-1}; **^{1}H NMR (300 MHz, DMSO-d_6):** δ 0.63 - 0.70 (m, 3H, CH_3)**,** 1.43 (dd, 3H, *J* = 6.6 and 6.1 Hz, CH_3), 1.74 - 1.80 (m, 2H, CH_2), 4.56 (sextet, 1H, *J* = 6.6 Hz, CH), 5.04 (br s, 2H, indolinyl-CH_2), 6.38 (d, 1H, *J* = 8.4 Hz, Ar-H), 7.07 (m, 2H, Ar-H), 7.15 (d, 1H, *J* = 8.4 Hz, Ar-H), 7.19 (d, 2H, *J* = 7.2 Hz, Ar-H), 7.31 - 7.36 (m, 1H, Ar-H), 7.44 (d, 1H, *J* = 7.2 Hz, Ar-H), 7.50 (d, 1H, *J* = 7.8 Hz, Ar-H), 7.59 (s, 2H, NH_2), 7.99 (s, 1H, triazolyl-H), 8.57 (d, 1H, *J* = 7.8 Hz, Ar-H), 11.54 (s, 1H, NH); **^{13}C NMR (75.4 MHz, DMSO-d_6):** δ 10.56, 20.99, 29.92, 35.85, 50.39, 54.59, 58.50, 108.51, 109.43, 110.05, 110.58, 111.42, 119.37, 119.75, 120.64, 121.56, 123.66, 123.89, 123.91, 125.16, 126.28, 129.38, 135.27, 140.06, 140.78, 142.37, 142.37, 142.42, 144.45, 161.78, 177. 89 ppm; **HRMS:** mass calculated for $[C_{30}H_{25}N_7O_2]$: 516.2148 $[M + H]^+$ and 538.1968 $[M + Na]^+$; obs. mass: 516.2142 $[M + H]^+$ and 538.1962$[M + Na]^+$.

2-Amino-4,7-dihydro-1'((1-(benzyl)-1H-1,2,3-triazol-4-yl)methyl)-2'oxo-spiro[5'-chloroindoline-3',4- chromene] pyrano[3,2-c] carbazole-3-carbonitrile, ***6l****:* brown solid; M.P.: 197-199 °C; **IR (KBr):** 3307, 3184, 2967, 2189, 1709, 1652, 1608, 1458, 1397, 1305, 1051, 746 cm^{-1}; **^{1}H NMR (300 MHz, DMSO-d_6):** δ 5.00 (s, 2H, indolinyl-CH_2), 5.49 (br s, 2H, benzylic-CH_2), 6.34 (d, 1H, *J* = 8.4 Hz, Ar-H), 7.00 (d, 2H, *J* = 8.4 Hz, Ar-Hs), 7.11 (d, 2H, *J* = 8.4 Hz, Ar-Hs), 7.17 (d, 2H, *J* = 8.4 Hz, Ar-Hs), 7.23 - 7.33 (m, 4H, Ar-Hs), 7.36 - 7.38 (br s, 2H, NH_2), 7.43 (d, 1H, *J* = 8.1 Hz, Ar-H), 7.90 (br s, 1H, Ar-H), 8.00 (s, 1H, triazolyl-H), 8.50 (d, 1H, *J* = 7.8 Hz, Ar-H), 11.30 (s, 1H, NH); **^{13}C NMR (75.4MHz, DMSO-d_6):** δ 35.85, 50.57, 53.62, 54.00, 108.33, 108.49, 110.80, 111.15, 111.25, 119.17, 119.57, 120.70, 123.43,123.51, 123.63, 125.18, 125.96, 128.12, 128.34, 128.50, 129.03, 129.11, 135.71, 136.97, 140.03, 140.92, 140.99, 142.40, 144.44, 161. 93, 177.59 ppm; **HRMS:** mass calculated for $[C_{33}H_{22}ClN_7O_2]$: 584.1602 $[M + H]^+$; obs. mass: 584.1596 $[M + H]^+$.

2-Amino-4,7-dihydro-1'((1-(4-methylbenzyl)-1H-1,2,3-triazol-4-yl)methyl)-2'oxo-spiro[5'-chloroindoline-3',4-chromene] pyrano[3,2-c]carbazole-3-carbonitrile, ***6m****:* brown solid; M.P.: 160 - 165 °C; **IR (KBr):** 3307, 3184, 2923, 2190, 1777, 1705, 1650, 1608, 1487, 1454, 1335, 1216, 1107, 749 cm^{-1}; **^{1}H NMR (300 MHz, DMSO-d_6):** δ 2.26 (s, 3H, Ar-CH_3), 4.99 (s, 2H, indolinyl-CH_2), 5.44 (s, 2H, benzylic-CH_2), 6.36 (d, 1H, *J* = 8.4 Hz, Ar- H), 7.03 (d, 2H, *J* = 8.7 Hz, Ar-Hs), 7.11 - 7.15 (m, 4H, Ar-Hs), 7.18 (d, 1H, *J* = 7.5 Hz, Ar- H), 7.26 (d, 1H, *J* = 8.4 Hz, Ar- H), 7.35 (d, 2H, *J* = 7.2 Hz, Ar- Hs), 7.44 (br s, 2H, NH_2), 7.88 (d, 1H, *J* = 9.0 Hz), 8.07 (s, 1H, triazolyl-H), 8.52 (d, 1H, *J* = 7.8 Hz, Ar-H), 11.36 (s, 1H, NH); **^{13}C NMR (75.4 MHz, DMSO-d_6):** δ 21.18, 35.91, 50.56, 53.44, 54.12, 108.33, 108.45, 110.80, 111.13, 111.20, 119.16, 119.53, 120.71, 123.29, 123.45, 123.63, 125.17, 125.93, 128.14, 128.26, 128.32, 129.07, 129.60, 132. 64, 136.95, 137.99, 140.02, 140.92, 140.99, 144.45, 161.91, 177.55 ppm; **HRMS:** mass

calculated for $[C_{34}H_{24}ClN_7O_2]$: 598.1758[M+ H]$^+$; obs. mass: 598.1753 [M + H] $^+$.

2-Amino-4,7-dihydro-1'((1-(4-fluorobenzyl)-1H-1,2,3-triazol-4-yl)methyl)-2'oxo-spiro[5'-chloroindoline-3',4 -chromene] pyrano[3,2-c]carbazole-3-carbonitrile, ***6n****:* brown solid; M.P.: 190 - 192 °C; **IR (KBr):** 3307, 3184, 2923, 2190, 1777,1705, 1650, 1608, 1487, 1454, 1335, 1216, 1107, 749 cm^{-1}; **^{1}H NMR (300 MHz, DMSO-d_6):** δ 5.04 (br s, 2H, indolinyl-CH_2), 5.58 (s, 2H, benzylic-CH_2), 6.41 (d, 1H, *J* = 8.4 Hz, Ar-H), 7.09 - 7.12 (d, 1H, *J* = 8.7 Hz, Ar-H), 7.15 - 7.28 (m, 2H, Ar-Hs), 7.21 - 7.25 (m, 3H, Ar-Hs), 7.33 (d, 2H, *J* = 6.3 Hz, Ar-Hs), 7.35 (d, 1H, *J* = 6.0 Hz, Ar-H), 7.43 (d,1H, *J* = 9.0 Hz, Ar-H), 7.52 (d, 1H, *J* = 7.8 Hz, Ar-H), 7.64 (s, 2H, NH_2), 8.10 (s, 1H, triazolyl-H), 8.57 (d, 1H, *J* = 7.8 Hz, Ar-H), 11.57 (s, 1H, NH); **^{13}C NMR (75.4 MHz, DMSO-d_6):** δ 35.80, 50.62, 52.51, 53.91, 108.68, 110.64, 110.45, 111.74, 115.89, 116.17, 119.13, 119.79, 120.63, 123.71, 123.82, 125.32, 126.34, 128.01, 129.42, 130.59, 130.70, 132.61, 132.65, 137.15, 140.08, 140.90, 141.45, 142.50, 144.43, 160.71, 161.86, 163.95, 177.66 ppm; **HRMS:** mass calculated for $[C_{33}H_{21}ClFN_7O_2]$:602. 1507 [M + H] $^+$; obs. mass: 602.1502 [M + H].$^+$

2-Amino-4,7-dihydro-1'((1-(4-chlorobenzyl)-1H-1,2,3-triazol-4-yl)methyl)-2'oxo-spiro[5'-chloroindoline-3',4 chromene]pyrano[3,2-c]carbazole-3-carbonitrile, ***6o***: brown solid; M. P.: 187 - 189 °C; **IR (KBr):** 3307, 3184, 2923, 2190, 1777,1705, 1650, 1608, 1487, 1454, 1335, 1216, 1107, 749 cm^{-1}; **^{1}H NMR (300 MHz, DMSO-d_6):** δ 5.05 (br s, 2H, indolinyl-CH_2), 5.58 (s, 2H, benzylic-CH_2), 6.42 (d, 1H, *J* = 8.4 Hz, Ar- H), 7.10 (d, 1H, *J* = 8.4 Hz, Ar- H), 7.23 - 7.29 (m, 5H, Ar-Hs), 7.39 - 7.45 (m, 4H, Ar-Hs), 7.47 - 7.54 (m, 1H, Ar-H), 7.65 (s, 2H, NH_2), 8.10 (s, 1H, triazolyl-H), 8.56 (d, 1H, *J* = 7.8 Hz, Ar-H), 11.57 (s, 1H, NH); **^{13}C NMR (75.4 MHz, DMSO-d_6):** δ 35.78, 50.61, 52.52, 53.91, 108.65, 110.64, 111.47, 111.75, 119.13, 119.84 120.60, 123.67, 123.73, 124.00, 125.29, 126.38, 128.03, 129.18, 129.44, 130.21, 130.34, 133.32, 135.32, 137.13, 140.07, 140.90, 142.51, 142.60, 144.42, 161.87, 177.62 ppm; **HRMS:** mass calculated for $[C_{33}H_{21}Cl_2N_7O_2]$: 618. 1212 [M + H]; $^+$ obs. mass: 618.1207 [M + H].$^+$

2-Amino-4,7-dihydro-1'((1-(benzyl)-1H-1,2,3-triazol-4-yl)methyl)-2'oxo-spiro[5'-bromoindoline-3',4- chromene] pyrano[3,2-c] carbazole-3-carbonitrile, ***6p****:* brown solid; M.P.: 182 - 184 °C; **IR (KBr):** 3308, 3174, 2933, 2180, 1767, 1715, 1640, 1618, 1485, 1451, 1335, 1216, 1109, 749 cm^{-1}; **^{1}H NMR (300 MHz, DMSO-d_6):** δ 5.04 (s, 2H, indolinyl-CH_2), 5.59 (s, 2H, benzylic-CH_2), 6.40 (d, 1H, *J* = 8.0 Hz, Ar-H), 6.99 (br s, 1H, Ar-H), 7.25 - 7.35 (m, 9H, Ar-Hs), 7.54 (d, 2H, *J* = 4.0 Hz, Ar- Hs), 7.66 (s, 2H, NH_2), 8.10 (s, 1H, triazolyl-H), 8.59 (d, 1H, *J* = 8.0 Hz, Ar-H), 11.58 (s, 1H, NH); **^{13}C NMR (75.4 MHz, DMSO-d_6):** δ 35.25, 50.04, 52.79, 53.37, 108.14, 110.11, 110.92, 111.73, 115.19, 118.59 118.81, 119.26, 120.10, 123.14, 123.39, 125.82, 127.44, 127.76, 127. 82, 128.07, 128.68, 131.72, 135.83, 137.00, 139.55, 140.36, 141.30, 143.85, 143.88, 161.32, 176.94 ppm; **HRMS:** mass calculated for $[C_{33}H_{22}BrN_7O_2]$: 628.1096 [M + H]; $^+$ obs. mass: 628.1091 [M + H].$^+$

2-Amino-4,7-dihydro-1'((1-(4-methylbenzyl)-1H-1,2,3-triazol-4-yl)methyl)-2'oxo-spiro[5'-

bromoindoline-3',4-chromene] pyrano[3,2-c]carbazole-3-carbonitrile, ***6q***: brown solid; M. P.: 202 - 204 °C; **IR (KBr):** 3310, 3170, 2938, 2154, 1777,1710, 1645, 1608, 1495, 1471, 1365, 1226, 1106, 753 cm^{-1}; **^{1}H NMR (300 MHz, DMSO-d_6):** δ 2.26 (s, 3H, Ar-CH_3), 4.99 (s, 2H, indolinyl-CH_2), 5.36 (dd, 2H, *J* = 19.5 and 18.9 Hz benzylic-CH_2), 6.37 (d, 1H, *J* = 8.1 Hz, Ar-H), 7.02 (d, 2H, *J* = 8.4 Hz, Ar-H), 7.05 - 7.09 (m, 4H, Ar-Hs), 7.18 (s, 2H, NH_2), 7.33 - 7.38 (m, 3H, Ar-Hs), 7.42 (d, 2H, *J* = 8.1 Hz, Ar-H), 7.83 (s, 1H, triazolyl-H), 8.51 (d, 1H, *J* = 7.8 Hz, Ar-H), 11.26 (s, 1H, NH); **^{13}C NMR (75.4MHz, DMSO-d_6):** δ 21.19, 35.91, 50.52, 53.64, 54.17, 108.37, 108.45, 110.83, 111.10, 111.69, 115.85, 119.14, 119.53, 120.72, 123.27, 123.46, 123.64, 125.93, 127.90, 128.14, 129.61, 131.92, 132.60, 137.33, 138.00, 140.03, 140.93, 141.44, 144.46, 161.91, 177.43 ppm; **HRMS:** mass calculated for [$C_{34}H_{24}BrN_7O_2$]: 642.1253 [M + H]; $^+$ obs. mass: 642.1248 [M + H].$^+$

2-Amino-4,7-dihydro-1'((1-(4-flourobenzyl)-1H-1,2,3-triazol-4-yl)methyl)-2'oxo-spiro[5'-bromoindoline-3',4-chromene] pyrano[3,2-c] carbazole-3-carbonitrile, ***6r***: brown solid; M.P.: 190 - 192 °C; **IR (KBr):** 3320, 3174, 2959, 2191, 1710,1715, 1652, 1625, 1451, 1335, 1216, 1021, 750 cm^{-1}; **^{1}H NMR (300 MHz, DMSO -d_6):** δ 5.01 (s, 2H, indolinyl-CH_2), 5.50 (s, 2H, benzylic-CH_2), 6.40 (d, 1H, *J* = 8.4 Hz, Ar-H), 7.04 - 7.51 (m, 13H, 11-Ar-Hs, NH_2), 7.98 (s, 1H, triazolyl-H), 8.55 (d, 1H, *J* = 7.2 Hz, Ar-H), 11.38 (s, 1H, NH); **^{13}C NMR (75.4 MHz, DMSO-d_6):** δ 35.86, 50.52, 50.80, 54.10, 108.32, 108.47, 110.81, 111.14, 111.75, 115.70, 115.85, 115.99, 119.15, 119.56, 120.71, 123.48, 123.65, 125.97, 127.90, 130.29, 130.40, 131.85, 131.96, 137.35, 140.03, 140.93, 141.48, 142.43, 144.46, 160.77, 161.91, 164.03, 177.45 ppm; **HRMS:** mass calculated for [$C_{33}H_{21}BrFN_7O_2$]: 646.1002 [M + H];$^+$ obs. mass: 646.0997 [M + H].$^+$

2-Amino-4,7-dihydro-1'((1-(4-chlorobenzyl)-1H-1,2,3-triazol-4-yl)methyl)-2'oxo-spiro[5'-bromoindoline-3',4-chromene] pyrano[3,2-c] carbazole-3-carbonitrile, ***6s****:* brown solid; M. P.: 185 - 187 °C*;* **IR (KBr):** 3325, 3138, 2959, 2191, 1710, 1652, 1602, 1457, 1398, 1333, 1220, 1115, 1047, 749 cm^{-1}*;* **^{1}H NMR (300 MHz, DMSO-d_6)*:*** δ 5.03 (s, 2H*,* indolinyl-CH_2*),* 5.56 (s, 2H*,* benzylic-CH_2*),* 6.42 (d, 1H, *J* = 8.4 Hz*,* Ar-H*),* 7.07 (d, 1H, *J* = 8.4 Hz, Ar-H), 7.16 - 7.50 (m, 10H, Ar-Hs), 7.58 (s, 2H, NH_2), 8.06 (s, 1H*,* triazolyl-H*),* 8.55 (d, 1H, *J* = 7.8 Hz, Ar-H), 11.47 (s, 1H, NH); **^{13}C NMR (75.4 MHz, DMSO-d_6):** δ 35.80, 50.54, 52.61, 53.94, 108.49, 108.54, 110.73, 111.26, 112.02, 115.74, 119.12, 119.61, 120.69, 123.62, 123.71, 123.84, 126.08, 127.90, 129.07, 130.05, 132.08, 133.48, 135.05, 137.45, 140.06, 140.92, 141.71, 142.43, 144.45, 161.87, 177.43 ppm; **HRMS:** mass calculated for [$C_{33}H_{21}BrClN_7O_2$]: 662.0707 [M + H]; $^+$ obs. mass: 662.0701 [M + H].$^+$

2-Amino-4,7-dihydro-1'((1-(benzyl)-1H-1,2,3-triazol-4-yl)methyl)-2'oxo-spiro[5'-fluoroindoline-3',4- chromene] pyrano[3,2-c]carbazole-3-carbonitrile, ***6t****:* brown solid; M. P.: 168-170 °C; **IR (KBr):** 3328, 3148, 2951, 2198, 1717, 1657, 1612, 1458, 1398, 1338, 1227, 1105, 1049, 759 cm^{-1}; **^{1}H NMR (300 MHZ, DMSO-d_6):** δ 5.03 (dd, 2H, *J* = 24.6 Hz, indolinyl-CH_2), 5.57 (s, 2H, benzylic-CH_2), 6.37 (d, 1H, *J* = 8.7 Hz, Ar-H), 7.06 - 7.54 (m, 12H, Ar-Hs), 7.63 (s, 2H, NH_2),

8.07 (s, 1H, triazolyl-H), 8.56 (d, 1H, *J* = 7.8 Hz, Ar-H), 11.56 (s, 1H, NH); **^{13}C NMR (75.4 MHz, DMSO-d_6):** δ 35.78, 50.34, 53.34, 54.17, 108.63, 108.85, 110.63, 111.13, 111.24, 111.46, 112.81, 113.14, 115.74, 116.05, 119.15, 119.83, 120.60, 123.65, 123.87, 126.36, 128.29, 128.62, 129.22, 136.32, 136.92, 138.68, 140.07, 140.87, 142.58, 144.40, 158.04, 161.88, 177.84 ppm; **HRMS:** mass calculated for [$C_{33}H_{22}FN_7O_2$]: 568.1897 [M + H]$^+$ and 590.1717 [M + Na];$^+$ obs. mass: 568.1892 [M + H]$^+$ and 590.1711 [M + Na].$^+$

2-Amino-4,7-dihydro-1'((1-(4-methylbenzyl)-1H-1,2,3-triazol-4-yl)methyl)-2'oxo-spiro[5'-fluoroindoline-3',4 chromene] pyrano[3,2-c]carbazole-3-carbonitrile, ***6u****:* brown solid; M. P.: 175 - 178 °C; **IR (KBr):** 3321, 3138, 2942, 2182, 1727, 1656, 1617, 1448, 1395, 1339, 1228, 1145, 1039, 755 cm^{-1}; **^{1}H NMR (300 MHz, DMSO-d_6):** δ 2.29 (s, 3H, Ar-CH_3), 4.95 (dd, 2H, *J* = 23.4 Hz, indolinyl-CH_2), 5.50 (s, 2H, benzylic-CH_2), 6.38 (d, 1H, *J* = 8.4 Hz, Ar-H), 7.07 – 7.54 (m, 11H, Ar-Hs), 7.64 (s, 2H, NH_2), 8.02 (s, 1H, triazolyl-H), 8.55 (d, 1H, *J* = 7.5 Hz, Ar-H), 11.57 (s, 1H, NH); **^{13}C NMR (75.4 MHz, DMSO-d_6):** δ 21.14, 35.86, 50.79, 53.36, 54.17, 108.51, 110.75, 110.90, 111.22, 112.58, 112.91, 115.51, 115.83, 119.14, 119.65, 120.67, 123.49, 123.61, 126.05, 128.20, 129.64, 132.80, 136.72, 136.82, 137.98, 138. 38, 140.04, 140.91, 142.46, 144.44, 158.01, 161.20, 161.95, 177.84 ppm; **HRMS:** mass calculated for [$C_{34}H_{24}FN_7O_2$]: 582. 2054[M + H]$^+$ and 604.1874[M + Na];$^+$ obs. mass: 582.2048 [M + H]$^+$ and 604.1868[M + Na].$^+$

2-Amino-4,7-dihydro-1'((1-(4-fluorobenzyl)-1H-1,2,3-triazol-4-yl)methyl)-2'oxo-spiro[5'-fluoroindoline-3',4 chromene] pyrano[3,2-c]carbazole-3-carbonitrile, ***6v***: brown solid; M. P.: 180 - 182 °C; **IR (KBr):** 3317, 2924, 2853, 2191, 1707, 1652, 1600, 1488, 1451, 1399, 1220, 1167, 1020, 786 cm^{-1}; **^{1}H NMR (300 MHz, DMSO-d_6):** δ 5.01 (dd, 2H, *J* = 20.1 and 21.0 Hz, indolinyl CH_2) , 5.55 (s, 2H, benzylic CH_2), 6.39 (d, 1H, *J* = 8.4 Hz, Ar-H), 7.07 - 7.54 (m, 11H, Ar-Hs), 7.63 (s, 2H, NH_2), 8.07 (s, 1H, triazolyl-H), 8.55 (d, 1H, *J* = 7.8 Hz, Ar-H), 11.56 (s, 1H, NH); **^{13}C NMR (75.4 MHz, DMSO-d_6):** δ 35.32, 50.33, 52.02, 53.59, 108.12, 108.40, 110.16, 110.95, 112.45, 115.29, 115.46, 115.63, 118.65, 119.26, 120.17, 123.21, 123.33, 125.82, 130.13, 130.20, 132.16, 136.43, 138.26, 139.60, 140.41, 142.15, 143.96, 158.20, 160.10, 160.88, 161.38, 162.82, 177.32 ppm; **HRMS:** mass calculated for [$C_{33}H_{21}F_2N_7O_2$]: 586.1803 [M + H]$^+$ and 608.1623 [M + Na];$^+$ obs. mass: 586.1798 [M + H]$^+$ and 608.1617 [M + Na].$^+$

2-Amino-4,7-dihydro-1'((1-(4-chlorobenzyl)-1H-1,2,3-triazol-4-yl)methyl)-2'oxo-spiro[5'-fluoroindoline-3',4-chromene] pyrano[3,2-c]carbazole-3-carbonitrile, ***6w****:* brown solid; M.P.: 170-172 °C; **IR (KBr):** 3310, 3164, 2930, 2185, 1757, 1705, 1645, 1608, 1485, 1459, 1385, 1216, 1129, 749 cm^{-1}; **^{1}H NMR (300 MHz, DMSO-d_6):** δ 5.03 (br s, 2H, indolinyl CH_2) , 5.59 (s, 2H, benzylic-CH_2), 6.43 (d, 1H, *J* = 7.8 Hz, Ar-H), 7.09 - 7.54 (m, 11H, Ar-Hs), 7.64 (s, 2H, NH_2), 8.11 (s, 1H, triazolyl-H), 8.56 (d, 1H, *J* = 7.8 Hz, Ar-H), 11.57 (s, 1H, NH); **^{13}C NMR (75.4MHz, DMSO-d_6):** δ 35.79, 50.84, 52.65, 54.16, 108.63, 108.85, 110.63, 111.45, 115.74, 119.12, 119.79, 120.63, 123.67, 123.72, 124.00, 126.34, 129.18, 129.40, 130.22, 133.31, 135. 36 136. 82, 136.90, 138.72, 140.08, 140.87, 142.64, 144.43, 158.16, 161.86, 177.81 ppm; **HRMS:**

mass calculated for $[C_{33}H_{21}ClFN_7O_2]$: 602.1507 $[M + H]^+$ and 624.1327 $[M + Na]^+$; obs. mass: 602.1502 $[M + H]^+$ and 624.1322 $[M + Na].^+$

2-Amino-4,7-dihydro-1'((1-(4-benzyl)-1H-1,2,3-triazol-4-yl)methyl)-2'oxo-spiro[5'-methylindoline-3',4 chromene] pyrano[3,2-c]carbazole-3-carbonitrile, ***6x****:* brown solid; M. P.: 201 - 203 °C; **IR (KBr):** 3318, 2933, 2180, 1767, 1715, 1640, 1618, 1485, 1451, 1335, 1216, 1109, 755 cm^{-1}; **^{1}H NMR (300 MHz, DMSO-d_6):** δ 2.21 (s, 3H, Ar-CH_3), 4.99 (dd, 2H, *J* = 27.0 and 27.9 Hz, indolinyl-CH_2) , 5.58 (s, 2H, benzylic-CH_2), 6.35 (d, 1H, *J* = 8.3 Hz, Ar-H), 6.96 (s, 1H, Ar-H), 7.03 – 7.51 (m, 11H, Ar-Hs), 7.56 (s, 2H, NH_2), 8.06 (s, 1H, triazolyl-H), 8.57 (d, 1H, *J* = 8.1 Hz, Ar-H), 11.55 (s, 1H, NH); **^{13}C NMR (75.4 MHz, DMSO-d_6):** δ 20.99, 35.73, 50.41, 53.29, 54.67, 108.54, 109.58, 109.82, 110.54, 111.41, 119.32, 119.73, 120.64, 123.66, 123.76, 123.89, 125.63, 126.25, 128.32, 128.59, 129.21, 129.64, 132.99, 135.42, 136.42, 139.99, 140.06, 140.72, 142.83, 144.36, 161.73, 177.79 ppm; **HRMS:** mass calculated for $[C_{34}H_{25}N_7O_2]$: 564.2148 $[M + H]^+$ and 586.1968 $[M + Na];^+$ obs. mass: 564.2142 $[M + H]^+$ and 586.1962$[M + Na].^+$

2-Amino-4,7-dihydro-1'((1-(4-methylbenzyl)-1H-1,2,3-triazol-4-yl)methyl)-2'oxo-spiro[5'-methylindoline-3',4-chromene] pyrano[3,2-c]carbazole- 3 carbonitrile, ***6y*** : brown solid; M. P.: 268 - 270 °C; **IR (KBr):** 3318, 3171, 2937, 2185, 1757,1705, 1630, 1628, 1488, 1441, 1338, 1220, 1169, 752 cm^{-1}; **^{1}H NMR (300 MHz, DMSO-d_6):** δ 2.12 (s, 3H, Ar-CH_3), 2.26 (s, 3H, Ar-CH_3), 4.98 (br s, 2H, indolinyl-CH_2), 5.51 (s, 2H, benzylic-CH_2), 6.36 (d, 1H, *J* = 8.4 Hz, Ar-Hs), 6.95 (br s, 1H, Ar-H), 7.06 – 7.44 (m, 10H, Ar-Hs), 7.55 (s, 2H, NH_2), 8.01 (s, 1H, triazolyl-H), 8.56 (d, 1H, *J* = 8.1 Hz, Ar-H), 11.54 (s, 1H, NH); **^{13}C NMR (75.4 MHz, DMSO-d_6):** δ 20.99, 21.15, 35.74, 50.41, 53.11, 54.67, 108.54, 109.58, 109.81, 110.55, 111.41, 119.33, 119.73, 120.64, 123.57, 123.66, 123.92, 125.63, 126.26, 128.37, 129. 65, 129.73, 132.99, 133.38, 135.41, 137.43, 140.00, 140.07, 140.73, 142.79, 144.37, 161.74, 177.86 ppm; **HRMS:** mass calculated for $[C_{35}H_{27}N_7O_2]$: 578.2304 $[M + H]^+$ and 600.2124 $[M + Na]^+;$ obs. mass: 578.2299 $[M + H]^+$ and 600.2118 $[M + Na].^+$

2-Amino-4,7-dihydro-1'((1-(4-fluorobenzyl)-1H-1,2,3-triazol-4-yl)methyl)-2'oxo-spiro[5'-methylindoline-3',4-chromene] pyrano[3,2-c]carbazole- 3 carbonitrile, ***6z***: brown solid; M. P.: 205 - 207 °C; **IR (KBr):** 3317, 2923, 2854, 2362, 2190, 1703, 1652, 1625, 1496, 1398, 1336, 1220, 1180, 1098, 809, 785, 750 cm^{-1}; **^{1}H NMR (300 MHz, DMSO-d_6):** δ 2.21 (s, 3H, Ar-CH_3), 4.98 (dd, 2H, *J* = 24.0 Hz, indolinyl-CH_2), 5.57 (s, 2H, benzylic-CH_2), 6.38 (d, 1H, *J* = 8.4 Hz, Ar-H), 7.06 – 7.51 (m, 11H, Ar-Hs), 7.55 (s, 2H, NH_2), 8.07 (s, 1H, triazolyl-H), 8.58 (d, 1H, *J* = 7.8 Hz, Ar-H), 11.54 (s, 1H, NH); **^{13}C NMR (75.4 MHz, DMSO - d_6):** δ 20.99, 35.74, 50.42, 52.49, 54.69, ,108.54, 109.58, 109.81, 110.56, 111.42, 115.88, 116.17, 119.32, 119.73, 120.65, 123.71, 123.94, 125.65, 126. 26, 129.65, 130. 61, 130.72, 132.64, 133.00, 135.41, 140.01, 140.07, 140.75, 142.87, 144.39, 160.70, 161.74, 163.94, 177.81 ppm; **HRMS:** mass calculated for $[C_{34}H_{24}FN_7O_2]$: 582.2054 $[M + H]^+$ and 604.1874$[M + Na];^+$ obs. mass: 582.2048

$[M + H]^+$ and 604.1868$[M + Na].^+$

2-Amino-4,7-dihydro-1'((1-(4-chlorobenzyl)-1H-1,2,3-triazol-4-yl)methyl)-2'oxo-spiro[5'-methylindoline-3',4-chromene] pyrano[3,2-c]carbazole-3-carbonitrile, ***6aa***: brown solid; M. P.: 190 - 192 °C; **IR (KBr):** 3308, 3174, 2933, 2180, 1767,1715, 1640, 1618, 1485, 1451, 1335, 1216, 1109, 749 cm^{-1}; **^{1}H NMR (300 MHz, DMSO-d_6):** δ 2.21 (s, 3H, Ar-CH_3), 5.00 (br s, 2H, indolinyl-CH_2), 5.59 (s, 2H, benzylic-CH_2), 6.39 (d, 1H, *J* = 8.4 Hz, Ar-H), 6.96 (s, 1H, Ar-H), 7.11 – 7.53 (m, 10H, Ar-Hs), 7.56 (s, 2H, NH_2), 8.08 (s, 1H, triazolyl-H), 8.58 (d, 1H, *J* = 7.8 Hz, Ar-H), 11.54 (s, 1H, NH); **^{13}C NMR (75.4 MHz, DMSO-d_6):** δ 21.00, 35.72, 50.41, 52.47, 54.70, ,108.54, 109.57, 109.81, 110.53, 111.41, 119.30, 119.72, 120.64, 123.66, 123.88, 123. 95, 125.64, 126.25, 129.17, 129.18, 129. 64, 130.24, 132.99, 133.29, 135.41, 140.00, 140.07, 140.74, 142.87, 144.38, 161.73, 177.80 ppm; **HRMS:** mass calculated for [$C_{34}H_{24}FN_7O_2$]: 598.1758 $[M + H]^+$ and 620.1578$[M + Na];^+$ obs. mass: 598.1753 $[M + H]^+$ and 620.1572$[M + Na].^+$

Supporting information: Original copies of ^{1}H, ^{13}C-NMR and HRMS spectra of all the synthesized compounds and morphological phase contrast microscopy results of selected compounds are provided in ESI.

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HIGHLIGHTS OF THE WORK

- A series of chromenocarbazole tethered 1, 2, 3 – triazoles were designed and synthesized by Click chemistry based one-pot five component reaction.
- Most of the synthesized compounds exhibited anti-proliferative activity against breast (MCF-7 and MDA-MB-231) and cervical (HeLa) cancer cells.
- The compounds **6f** and **6j** showed most potent activity against MCF-7 breast cancer cell with the IC_{50} values of 2.13 and 4.80 µM.
- The compounds **6k, 6j** and **6s** showed higher activity against MDA-MB-231 breast cancer cell while the compounds **6g**, **6s** and **6u** showed excellent activity against HeLa cervical cell.
- The subsequent apoptotic cell death was evidenced by AO / EB fluorescence microscopy analysis.
- The First report on the use of cellulose supported CuI as heterogeneous catalyst in the synthesis of 1, 2, 3- triazolylchromenocarbazole.

Graphical Abstract

Click chemistry based multicomponent approach in the synthesis of spirochromenocarbazole tethered 1, 2, 3 – triazoles as potential anticancer agents

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