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Original article

Synthesis and cytotoxicity evaluation of (tetrahydro-β-carboline)-1,3,5-triazine hybrids as anticancer agents

Ravi Kumar [a], Leena Gupta [a], Pooja Pal [b], Shahnawaz Khan [a], Neetu Singh [b], Sanjay Babu Katiyar [a], Sanjeev Meena [b], Jayanta Sarkar [b], Sudhir Sinha [b], Jitendra Kumar Kanaujiya [b], Savita Lochab [b], Arun Kumar Trivedi [b], Prem M.S. Chauhan [a,*]

[a] Division of Medicinal & Process Chemistry, Central Drug Research Institute, CSIR, Lucknow 226001, India
[b] Drug Target Discovery & Development Division, Central Drug Research Institute, CSIR, Lucknow 226001, India

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ABSTRACT

A series of tetrahydro-β-carbolines and 1,3,5-triazine hybrids have been synthesized and evaluated for their cytotoxicity against a panel of eight human cancer cell lines and normal human fibroblasts (NIH3T3). It led us to discovery of racemic compounds **69**, **71** and **75**, which are selectively cytotoxic towards KB (oral cancer) cell line with IC_{50} values of 105.8, 664.7 and 122.2 nM, respectively; while their enantiopure forms are less active and not selective. Enantiopure compound **42** showed 2.5 times more selectivity towards MCF7 cells over normal fibroblast NIH3T3 cells with an IC_{50} value of 740 nM, also arrests cell cycle in G_1 phase and induces apoptosis in MCF7 and MDA MB231cell lines.

1. Introduction

Cancer is a major health problem worldwide and is the leading cause of human mortality exceeded only by cardiovascular diseases [1]. Therefore, development of new anticancer drugs and more effective treatment strategies for cancer are of utmost importance as traditionally prescribed chemotherapeutic agents have problems with toxicity and drug resistance [2]. Although, numerous kinase inhibitors have been discovered recently and several have been successfully developed for treatment of cancer including Gleevec, [3] Iressa, [4] Tarceva, [5] Tykerb, [6] and Sutent, [7] still there is strong demand for discovery of improved cytotoxic agents. As most of the solid human cancer tumor are multi causal in nature and their treatment with "mechanism-based" agents alone is unlikely to be successful, so a combination of these inhibitors with a better cytotoxic drug is likely to be a good strategy [8].

The β-carboline nucleus that possess a common tricyclic pyrido[3,4-*b*]indole ring structure, is a recurring motif in both natural and synthetic cytotoxic compounds which can act through multiple mechanisms [9]. In addition to simple, substituted harman and norharman derivatives, [10] more complex structures such as manzamine(**1**) [11], eudistomine K (**2**) [12], azatoxin (**3**) [13], fascaplysine (**4**) [14], and picrasidine L (**5**) [15], display potent cytotoxic activities against various cancer cell lines (Fig. 1).

Cytotoxicity of 1,3,5-triazine derivatives is well known as hexamethylmelamine (HMM) (**6**) was discovered as an effective agent against breast, lung and ovarian cancer but it causes many adverse effects such as nausea, vomiting, anorexia and abdominal cramps [16]. Irsogladine (**7**) has been shown to have anti-tumor activity in murine xenograft models of epidermoid cancer and glioma [17]. More recently, the effect of compound (**7**) was also investigated in a human breast cancer athymic nude mouse system and the results suggested that irsogladine can be useful in the breast cancer adjuvant setting [18]. Moon et al. reported compound (**8**) as a microtubule destabilizing agent with potent growth inhibition against U936 cells ($GI_{50} = 1$ μM) [19]. p38 MAP kinase inhibitory activity [20] of (**9**), inhibitory potency of (**10**) against various cyclin dependent kinases, [21] and VEGF-R2 (KDR) tyrosine kinase inhibitory activity [22] of (**11**) has also been reported recently (Fig. 2).

* Corresponding author. Tel.: +91 522 2262411; fax: +91 522 2623405. CDRI comm., No. 7880.
E-mail addresses: premsc58@hotmail.com, prem_chauhan_2000@yahoo.com (P.M.S. Chauhan).

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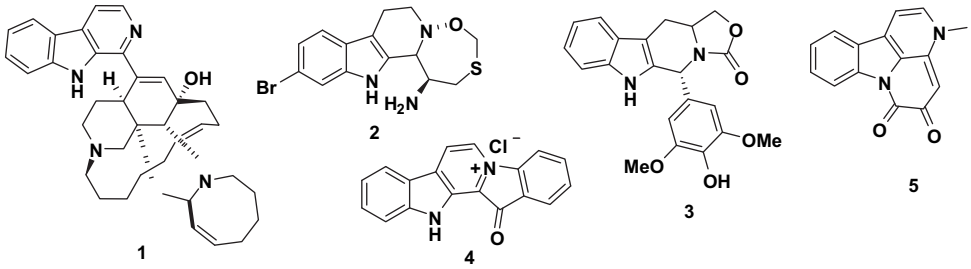


Fig. 1. Cytotoxic natural products containing β-carboline nucleus.

Now a days, there is an increased interest in use of hybrid molecules for drug discovery against multitude of disease indications [23]. Lesser degree of conformational flexibility and less likely interaction of tetrahydro-β-carbolines with DNA may also lead to reduced toxicity and increased anticancer activity [24]. These observations and our previous experience [25,26] led us to hypothesize that (tetrahydro-β-carboline)-1,3,5-triazine hybrid molecules will be more efficacious and selective cytotoxic agents. Herein, the synthesis and cytotoxic activities of a series of (tetrahydro-β-carboline)-1,3,5-triazine hybrid derivatives against a panel of human cancer cell lines are described.

2. Chemistry

The synthetic strategy followed for synthesis of (tetrahydro-β-carboline)-1,3,5-triazine hybrids is depicted in Scheme 1. The tetrahydro-β-carbolines (**12**–**32**) were obtained via Pictet–Spengler cyclization [26] of methyl ester of tryptophan with various substituted benzaldehydes under acidic conditions. Two diastereomers so obtained were separated by flash column chromatography. Spectroscopic data of the obtained tetrahydro-β-carbolines were identical to that of reported earlier [24,27]. The (tetrahydro-β-carboline)-1,3,5-triazine hybrids (**33**–**75**) enlisted in Table 1 were synthesized in a two step sequence [26] – (i) a nucleophilic substitution of one chloro group of cyanuric chloride with N-2 of different tetrahydro-β-carbolines, (ii) displacement of remaining two chloro groups with various amines (Scheme 1). Compound **76** was also synthesized according the same procedure except that tryptamine was used instead of methyl ester of tryptophan in step (i) of above mentioned method (Scheme 2). Treatment of cyanuric chloride with excess of *N*-methyl piperazine resulted in formation of compound **77** (Scheme 2).

3. Pharmacology

1–50 μM concentrations of (tetrahydro-β-carboline)-1,3,5-triazine hybrids were tested for in vitro cytotoxic activity on human cancer cell lines representing breast cancer (MCF7), colon (SW620), prostate (DU145), oral (KB), ovary (PA1), leukemia (K562), pancreas (MiaPaCa-2), lung (A549) and normal fibroblasts (NIH3T3) by MTT assay [28].

4. Results and discussion

Initially, anticancer activity of all the previously reported (tetrahydro-β-carboline)-1,3,5-triazine [24] hybrids by our group and synthesized analogues (**33**–**40**) was assessed in vitro against a panel of 8 human cancer cell lines and the results are summarized in Table 2. Previously reported (tetrahydro-β-carboline)-1,3,5-triazine hybrids (general structure **11a**) [24] were found to be inactive. But we were pleased to find that compound **34** obtained from DL-tryptophan, having trans stereochemistry, 4-methoxy group as R and *N*-methylpiperazino group as R_1 showed IC_{50} value of 1.87 and 4.78 μM against KB and NIH3T3 cell lines, respectively. It was 2.5 times more selectively toxic towards KB cells than NIH3T3 cell line, while its cis isomer **33** was moderately cytotoxic against all the tested cell lines. Butylamino group as R_1 as in compounds **35** and **36**, was found to be detrimental to cytotoxic activity. Also the combination of 4-methyl group as R and aminoethanol as R_1 has resulted in complete loss of activity in compounds **37** and **38**. Cytotoxicity profiles of **39** and **40** also indicated that trans isomer was preferred over cis for cytotoxicity. Excited by these findings, and to rapidly develop SAR around 1,3,5-triazine moiety, enantiopure β-carboline unit having 4-methoxy group as R, obtained from methyl ester of L-tryptophan was kept intact while R_1 was varied

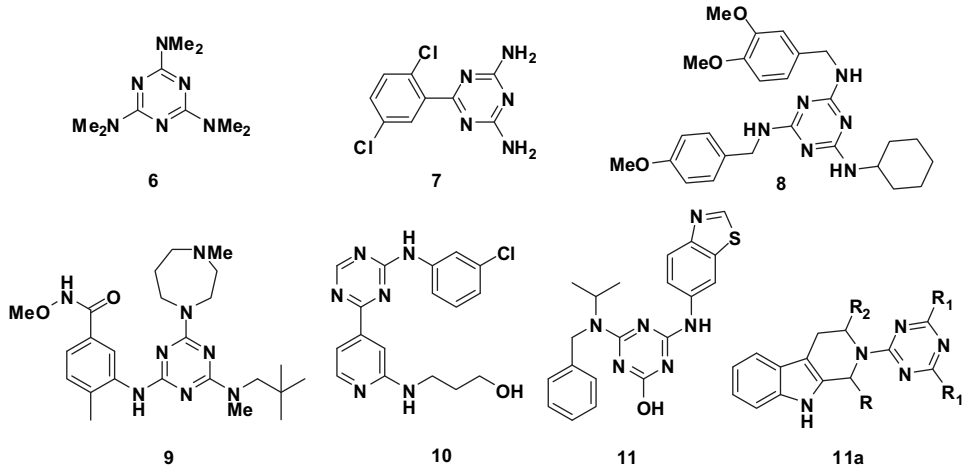
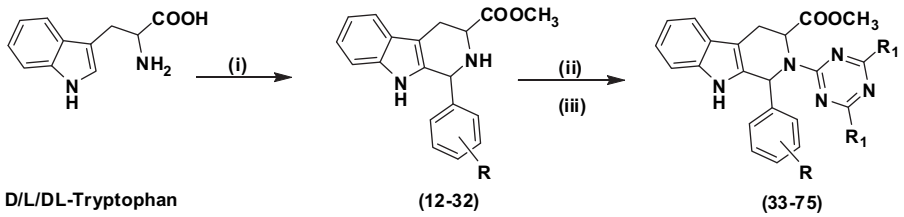


Fig. 2. Previously reported anticancer 1,3,5-triazine derivatives.



Scheme 1. Preparation of (tetrahydro-β-carboline)-1,3,5-triazine hybrids (**33**–**75**). For (**12**) R = 4-methyl, Isomer = DL, Trans; (**13**) R = 4-methyl, Isomer = DL, Cis; (**14**) R = 4-methyl, Isomer = L, Trans; (**15**) R = 4-methyl, Isomer = L, Cis; (**16**) R = 4-methyl, Isomer = D, Trans; (**17**) R = 4-methyl, Isomer = D, Cis; (**18**) R = 4-methoxy, Isomer = DL, Trans; (**19**) R = 4-methoxy, Isomer = DL, Cis; (**20**) R = 4-methoxy, Isomer = L, Trans; (**21**) R = 4-methoxy, Isomer = L, Cis; (**22**) R = 4-methoxy, Isomer = D, Trans; (**23**) R = 4-methoxy, Isomer = D, Cis; (**24**) R = 3,4,5-trimethoxy, Isomer = DL, Trans; (**25**) R = 3,4,5-trimethoxy, Isomer = DL, Cis; (**26**) R = 3,4,5-trimethoxy, Isomer = L, Trans; (**27**) R = 3,4,5-trimethoxy, Isomer = L, Cis; (**28**) R = 4-chloro, Isomer = DL, Trans; (**29**) R = 4-isopropyl, Isomer = L, Trans; (**30**) R = 4-isopropyl, Isomer = L, Cis; (**31**) R = 4-isopropyl, Isomer = D, Trans; (**32**) R = 4-isopropyl, Isomer = D, Cis. *Reagents and Conditions*: (i) Thionyl chloride, MeOH, various benzaldehydes, (ii) Cyanuric chloride, K_2CO_3, THF, 0 °C – r.t. (iii) Amines, K_2CO_3, THF, Reflux.

using various amine nucleophiles. Cytotoxic evaluation of (**41**–**49**) demonstrated that every substituent except *N*-methylpiperazino group as R_1 had detrimental effect on cytotoxicity against human cancer cell lines. To our surprise compound **42**, which is enantiopure form of **34**, was not selective towards KB cells but was 2.6 times selectively toxic towards MCF7 cell line in comparison to NIH3T3 cells. On the other hand **41**, the cis isomer of **42**, was generally cytotoxic to all cell lines and has similar cytotoxicity profile as **33**. Subsequently, we tried few other combinations, hybrids (**50**–**61**) having R = 4-isopropyl, 3,4,5-trimethoxy, 4-methyl, 4-methoxy, 3-hydroxy and R_1 = various cyclic amines, were synthesized starting from L-tryptophan and evaluated for cytotoxicity. But again, only compounds having *N*-methylpiperazino group as R_1 were found to be cytotoxic. Interestingly, cytotoxic activity was also found to be decreasing with increase in carbon chain length at N-4 of piperazine unit as evidenced by the cytotoxicity profile of **60** and **61**. Compound **60** with *N*-ethylpiperazino group as R_1 exhibited better cytotoxicity than *N*-propylpiperazine derivative **61**, whereas both were less active than **34**. In the meantime, compound **42** was studied for its effect on cell cycle in MCF7 and MDA MB231 cell lines and found to arrest mitotic cycle in G_1 phase. DNA fragmentation analysis and Hoechst staining was also carried out in MCF7 and MDA MB231 cell lines to confirm apoptosis by **42**.

Table 1
(Tetrahydro-β-carboline)-1,3,5-triazine hybrid analogues (**33**–**75**).

Entry	Compound no.	Isomer	*R*	R_1
1	**33**	DL, Cis	4-Methoxy	*N*-methylpiperazine
2	**34**	DL, Trans	4-Methoxy	*N*-methylpiperazine
3	**35**	DL, Cis	4-Methoxy	butylamine
4	**36**	DL, Trans	4-Methoxy	butylamine
5	**37**	DL, Cis	4-Methyl	2-aminoethanol
6	**38**	DL, Trans	4-Methyl	2-aminoethanol
7	**39**	DL, Trans	3,4,5-Trimethoxy	*N*-methylpiperazine
8	**40**	DL, Cis	3,4,5-Trimethoxy	*N*-methylpiperazine
9	**41**	L, Cis	4-Methoxy	*N*-methylpiperazine
10	**42**	L, Trans	4-Methoxy	*N*-methylpiperazine
11	**43**	L, Cis	4-Methoxy	*N*-phenylpiperazine
12	**44**	L, Trans	4-Methoxy	*N*-phenylpiperazine
13	**45**	L, Cis	4-Methoxy	morpholine
14	**46**	L, Trans	4-Methoxy	morpholine
15	**47**	L, Trans	4-Methoxy	cyclohexylamine
16	**48**	L, Cis	4-Methoxy	o-toluidine
17	**49**	L, Trans	4-methoxy	o-toluidine
18	**50**	L, Cis	4-Isopropyl	*N*-methylpiperazine
19	**51**	L, Trans	4-Isopropyl	*N*-methylpiperazine
20	**52**	L, Cis	4-Isopropyl	*N*-phenylpiperazine
21	**53**	L, Trans	4-Isopropyl	*N*-phenylpiperazine
22	**54**	L, Cis	4-Isopropyl	Morpholine
23	**55**	L, Trans	4-Isopropyl	Morpholine
24	**56**	L, Trans	4-Isopropyl	*N*,*N*-dimethylamine
25	**57**	L, Cis	4-Isopropyl	cyclohexylamine
26	**58**	L, Trans	3,4,5-Trimethoxy	*N*-methylpiperazine
27	**59**	L, Cis	3,4,5-Trimethoxy	*N*-methylpiperazine
28	**60**	L, Trans	4-Methoxy	*N*-ethylpiperazine
30	**61**	L, Trans	4-Methoxy	*N*-propylpiperazine
31	**62**	D, Cis	4-Methoxy	*N*-methylpiperazine
32	**63**	D, Trans	4-methoxy	*N*-methylpiperazine
33	**64**	D, Cis	-H	*N*-methylpiperazine
34	**65**	D, Trans	-H	*N*-methylpiperazine
35	**66**	D, Cis	4-Methyl	*N*-methylpiperazine
36	**67**	D, Trans	4-Methyl	*N*-methylpiperazine
37	**68**	DL, Trans	4-Chloro	*N*-methylpiperazine
38	**69**	DL, Trans	4-Methyl	*N*-methylpiperazine
39	**70**	DL, Cis	4-Methyl	propylamine
40	**71**	DL, Trans	4-Methyl	Propylamine
41	**72**	DL, Trans	4-Methyl	Ethylamine
42	**73**	DL, Trans	4-Methoxy	Ethylamine
43	**74**	DL, Cis	4-Methoxy	Propylamine
44	**75**	DL, Trans	4-Methoxy	Propylamine

4.1. Arrest of MCF7 and MDA MB231 cells in G_1 and inhibition of mitosis

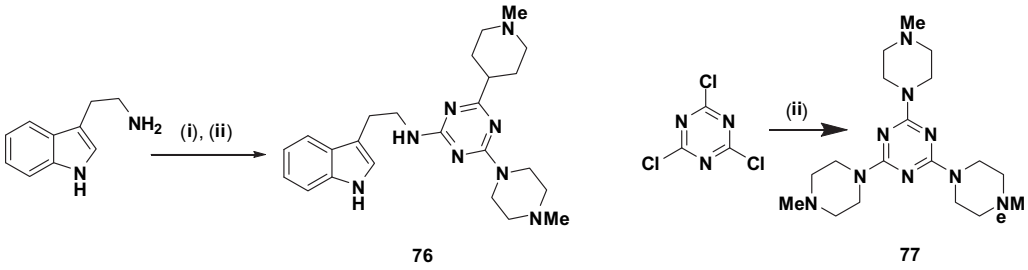
To understand the mechanism of cell death, compound **42** was evaluated for its effect on cell cycle. Breast cancer cells MCF7 and MDA MB231 were exposed to two concentrations of 0.50 and 0.74 μM for 24 h, which were then stained with propidium iodide and analyzed by flow cytometry. DNA contents of the live population of MCF7 cells were 54.35, 0.01, and 45.64 for untreated cells to 76.68, 0.12, 23.20% for the cells treated with 0.74 μM of compound **42** after 24 h in G_1, S and G_2 phase, respectively (Fig. 3). While, at a concentration of 0.50 μM, 64.4% accumulation of MCF7 cells was observed in comparison to that of 54.4% of untreated control cells. Similar results were obtained with MDA MB231 cells but increase in percentage accumulation in G_1 phase was less in comparison to that of MCF7 cells. Hence, compound **42** at 1 μM concentration enhances mitotic arrests in G_1 phase.

4.2. DNA fragmentation analysis

A nuclear associated event in apoptosis is the degradation of DNA into nucleosomal sized fragments of approximately 180–200 bp which forms a ladder pattern when subjected to gel electrophoresis and is indeed one of the important diagnostic biomarker of apoptosis. Both MCF7 and MDA MB231 cells were treated with varying concentrations of compound **42** ranging from 0.25 to 0.74 μM for 24 h. DNA fragmentation analysis was carried out to evaluate apoptosis in both cell lines. DNA fragmentation was observed in both cell lines at all the concentrations tested (Fig. 4).

4.3. Hoechst staining for visualization of apoptosis

For visualization of apoptosis both MCF7 and MDA MB231 cells were treated with 0.50 and 0.74 μM concentration of compound **42**



Scheme 2. Preparation of 2,4,6-triamino-1,3,5-triazines. *Reagents and Conditions*: (i) Cyanuric chloride, K_2CO_3, THF, 0 °C – r.t. (ii) *N*-methylpiperazine, K_2CO_3, THF, Reflux.

for 24 h and Hoechst staining was performed. Compound **42** causes apoptosis, was demonstrated by unevenly displayed and fragmented micronuclei in significant number of cells (Fig. 5).

At that time it was thought that cytotoxicity profile of other enantiomers of **41** and **42** i.e. **62** and **63** will be interesting. So, a few analogues (**62**–**67**) were also synthesized starting with methyl ester of D-tryptophan. They also showed same SAR as that of compounds obtained from methyl ester of L-tryptophan, i.e. only compounds having *N*-methylpiperazino group as R_1 were found to be cytotoxic but they differed from **34** in their selectivity towards KB cells. A little decrease in cytotoxicity was observed when R = 4-methoxy in **63** was replaced with H (**65**). Similar was the case with 4-methyl derivative **67**, as it also showed decreased toxicity and selectivity. From the above data it was evident that racemates are more selective towards KB cell lines than their enantiopure forms. Keeping in view this fact, we synthesized a few analogues (**68**–**75**) having 4-methyl, 4-methoxy, 4-chloro group as R at C-1 phenyl ring of tetrahydro-β-carboline ring system and alkyl amino groups such as *N*-methylpiperazino, propylamino and ethylamino groups as R_1, starting from methyl ester of DL-tryptophan. Cytotoxicity evaluation of (**68**–**75**) led us to discovery of compounds **69**, **71** and **75**, which are selectively cytotoxic towards KB cells with IC_{50} values of 105.8, 664.7, 122.2 nM, respectively, and in accordance to previous SAR their cis isomers were inactive. Compound **69** having 4-methyl group as R and *N*-methylpiperazino group as R_1 combination is selectively cytotoxic towards KB cells, while compound **34** having the combination of 4-methoxy group as R and *N*-methylpiperazino group as R_1 was not selectively cytotoxic. Both compound **71** and **75** have propylamino group as R_1, but differs in R group. In this case 4-methoxy and propylamino group combination as R and R_1 was proven to be more active than 4-methyl and propylamino group combination. Replacing β-carboline with tryptamine in **69** resulted in formation of 2,4,6-triamino-1,3,5-triazine **76**, which was found to be inactive, indicating that β-carboline part is essential for cytotoxicity. Substituting all three chloro groups of cyanuric chloride with *N*-methyl piperazine, another inactive compound **78** was obtained, proving our hypothesis correct. More selectivity of racemic mixture in comparison to pure enantiomers indicates that both the enantiomers may be acting on different targets, and their cumulative effect leads to selectivity, while one enantiomer alone is not selective at all. Further studies are required to delineate the mode of action and subsequent improvement in selectivity towards cancer cells.

5. Conclusion

In summary, we have described a series of (tetrahydro-β-carboline)-1,3,5-triazine hybrid molecules, some of them have shown good cytotoxicity against a panel of human cancer cell lines. Compounds **69**, **71** and **75** are selectively toxic towards KB (oral cancer) cell line with IC_{50} values of 105.8, 664.7 and 122.2 nM, while their enantiopure forms are less active and not selective.

Table 2
In Vitro cytotoxicity data of (tetrahydro-β-carboline)-1,3,5-triazine hybrids and 2,4,6-triamino-1,3,5-triazine derivatives.[a]

Entry	Compound no.	IC_{50} (μM)								
		MCF7	SW620	DU145	KB	PA1	K562	Miapaca2	A549	NIH3T3
1	**33**	6.06	3.32	5.35	1.46	2.72	7.13	7.37	6.30	2.33
2	**34**	12.44	4.26	10.47	1.87	2.03	NA	6.35	5.83	4.78
3	**39**	NA	3.15	2.01	2.43	2.75	3.00	7.54	5.67	2.17
4	**40**	5.96	ND	5.22	17.88	5.32	ND	ND	9.84	6.71
5	**41**	9.00	1.19	10.31	7.54	2.77	4.83	3.62	3.13	1.62
6	**42**	0.74	1.35	6.75	8.06	1.82	4.6	2.3	2.1	1.96
7	**50**	5.93	1.93	2.87	6.88	0.27	6.43	1.81	1.75	1.17
8	**51**	6.73	5.92	NA	3.73	2.89	2.24	NA	1.76	2.63
10	**60**	1.34	NA	1.67	1.22	NA	ND	ND	1.61	1.86
11	**61**	2.07	NA	7.11	3.54	NA	ND	ND	1.67	2.24
12	**62**	12.27	3.27	2.45	1.82	3.34	4.48	5.26	11.46	5.28
13	**63**	10.97	2.15	2.58	0.99	2.05	6.00	6.23	3.00	3.16
14	**64**	8.28	3.24	7.76	1.83	1.79	6.45	5.88	8.62	11.38
15	**65**	6.21	2.84	2.81	1.98	1.93	5.34	6.11	3.21	6.00
16	**67**	5.89	2.65	2.14	1.86	6.91	3.22	7.00	2.34	2.16
17	**68**	NA	2.73	NA	1.98	6.39	5.01	7.12	6.55	2.22
18	**69**	NA	NA	NA	0.1058	NA	NA	NA	NA	NA
19	**71**	NA	NA	NA	0.6647	NA	NA	NA	NA	NA
20	**75**	NA	NA	NA	0.1222	NA	NA	NA	NA	NA

IC_{50} = compound concentration required to inhibit tumor cell proliferation by 50%. Data are expressed as mean from the dose response curves of at least two independent experiments with three determinations in each.

[a] NA = not active, that is, the IC_{50} is greater than 25 μM. ND = not done.

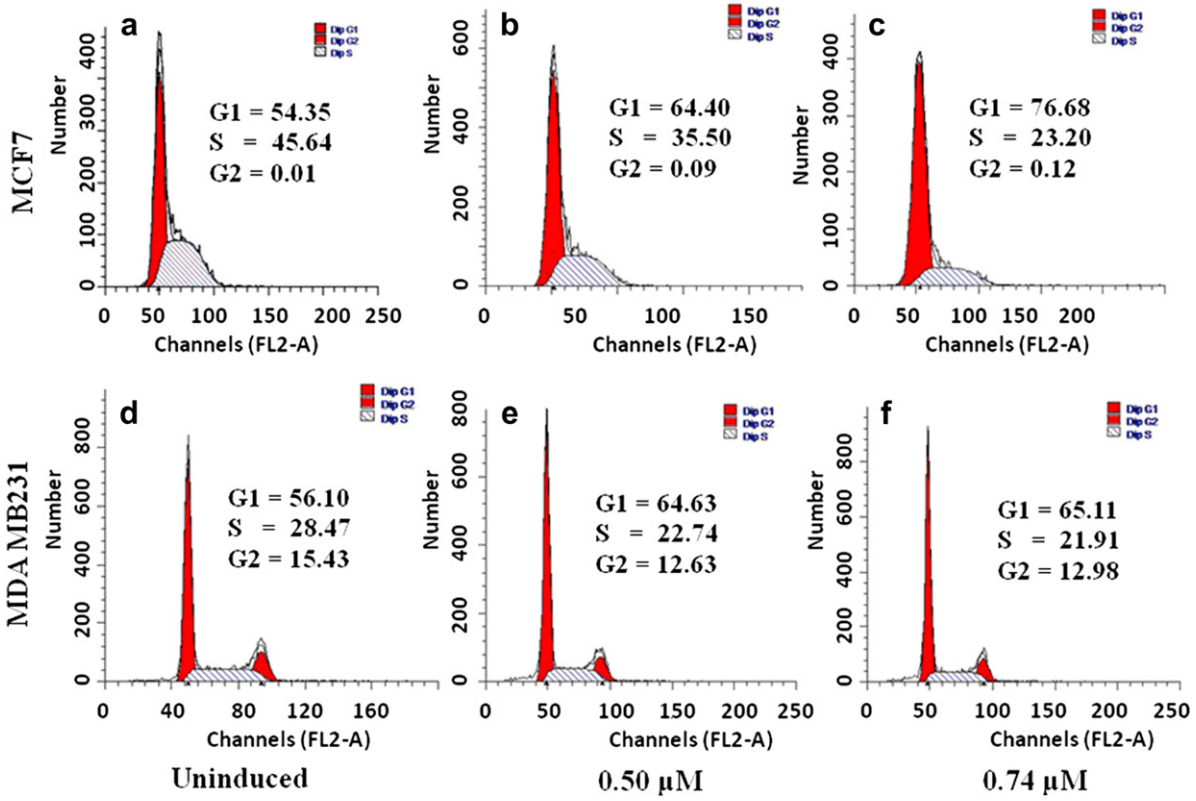


Fig. 3. Effect on the cell cycle as determined by flow cytometry. Uninduced MCF7 and MDA MB231 cells were taken as controls. Cells were treated with 0.50 and 0.74 μM concentrations of compound **42** (b, c for MCF7 & e, f for MDA MB231).

Compound **42**, however, is not as good as **69**, **71** or **75** but is 2.5 times more cytotoxic towards MCF7 cells than normal fibroblasts NIH3T3 with an IC_{50} value of 740 nM. Compound **42** arrests the cell cycle in G_1 phase in MCF7 as well as MDA MB231 cell lines, ultimately leading to cell death which was confirmed by DNA fragmentation analysis and Hoechst staining. In vivo studies, additional SAR, further lead optimization, and studies regarding mode of action are progress in our laboratories and will be reported in due course. These (tetrahydro-β-carboline)-1,3,5-triazine hybrids should be considered as important lead compounds for potential application in anticancer chemotherapy.

6. Experimental

6.1. Chemistry

All non-indigenous chemicals were purchased from Sigma–Aldrich. All reactions were monitored by thin layer chromatography conducted on E. Merck TLC plates (Silica gel 60 F-254, Aluminium Back) and visualized with UV light or iodine. The crude products were purified by column chromatography using silica gel as adsorbent. Nuclear magnetic spectra were recorded on 200 MHz and 300 MHz spectrometers. In the case of multiplets, the signals

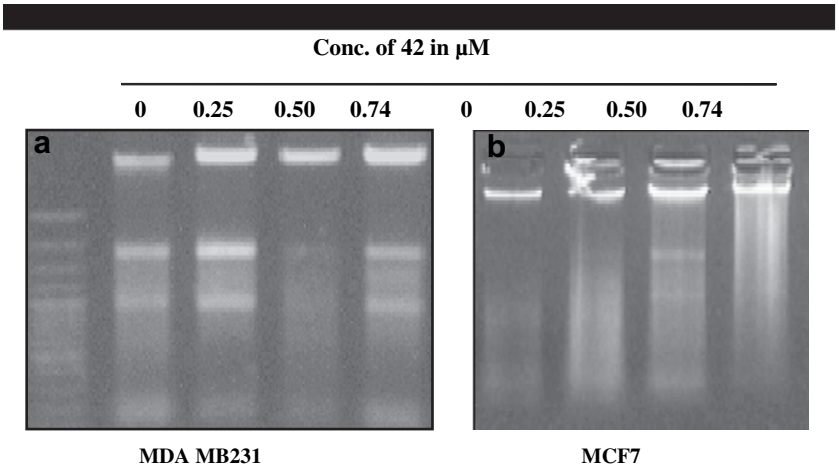


Fig. 4. DNA fragmentation analysis. Depiction of DNA fragmentation pattern observed with treatment of MDA MB231 and MCF7 cells with **42** at conc. of 0.5, 0.74 μM concentrations, respectively.

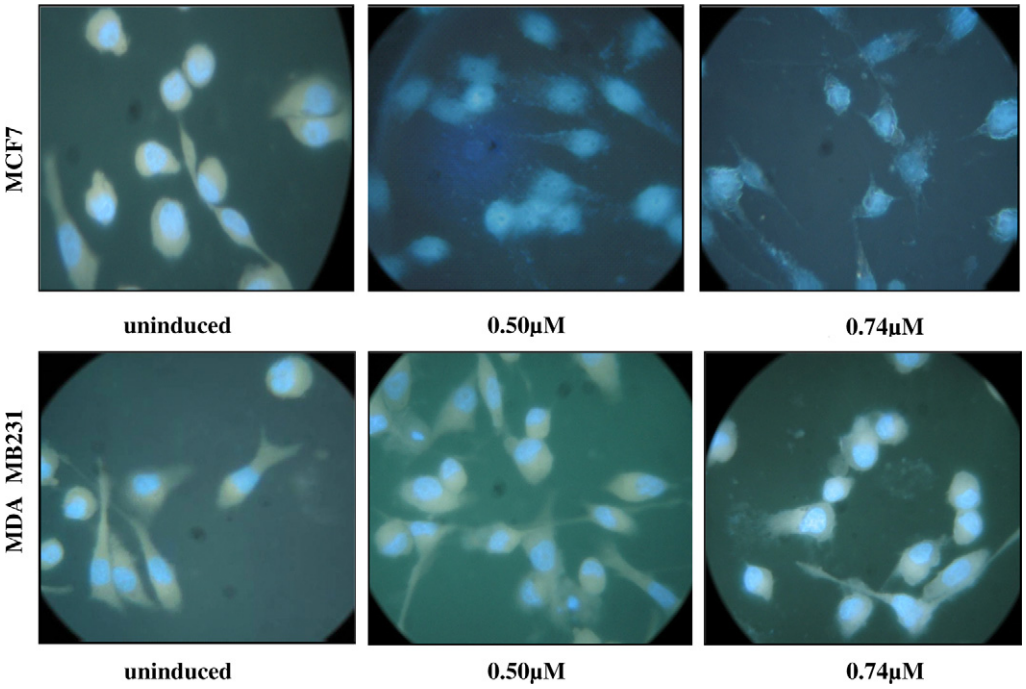


Fig. 5. Hoechst staining for visualization of apoptosis. Untreated MCF7 and MDA MB231 cells were taken as control.

are reported as intervals. Signals were abbreviated as s, singlet; d, doublet; t, triplet; m, multiplet. The electron spray mass spectra were recorded on a triple quadrupole mass spectrometer. The samples (dissolved in suitable solvents such as methanol/acetonitrile/water) were introduced into the ESI source through a syringe pump at the rate of 5 μL/min. The ESI capillary was set at 3.5 kV, and the cone voltage was 40 V. The spectra were collected in 6 s scans, and the printouts are averaged spectra of 6–8 scans. Infrared spectra were recorded using a Perkin–Elmer RX-1 spectrometer; the values were reported in cm-1. The purity of all tested compounds was ascertained on the basis of their elemental analysis and was ≥95%.

*6.1.1. General procedure for synthesis of compounds (**12**–**32**)*

To the stirred solution of 2.04 g (10 mmol) DL/L/D-tryptophan in methanol at 0 °C, 1.34 ml (1.1 equivalent, 11 mmol) of thionyl chloride was added drop-wise. The reaction mixture was then refluxed for 2 h. An appropriate aromatic aldehyde (1.1 equivalent) was added to the reaction mixture and was then refluxed for 8 h. The solvent was removed under vacuum and the solid residue so resulted was dissolved in water, neutralized with saturated sodium bicarbonate solution and extracted with ethyl acetate. The organic layer was washed with brine solution (three times), water (three times), and dried over sodium sulfate. The solvent was removed in vacuo and the cis and trans isomers were separated by flash column chromatography using silica gel as adsorbent. Spectroscopic data of the obtained tetrahydro-β-carbolines were identical to that of reported earlier [24,31].

*6.1.2. General procedure for synthesis of compounds (**33**–**75**)*

To the stirred mixture of cyanuric chloride (0.92 g, 5 mmol) and K_2CO_3 (0.56 g, 5 mmol) in dry THF (50 ml) at 0 °C was added the solution of tetrahydro-β-carboline (5 mmol) in dry THF (30 ml) dropwise for 1 h. After completion of the reaction, solvent was evaporated under vacuum and solid mass was obtained. The solid residue was dissolved in $CHCl_3$ (100 mL), washed with water (three times), dried over anhydrous Na_2SO_4, concentrated in vacuo and used further without purification. Then 2 mmol of resulting solid, 4 mmol of required amine and K_2CO_3 (0.55 g, 4 mmol) in dry THF (40 ml) were refluxed for 3–6 h. The reaction mixture was filtered and evaporated the solvent under vacuum. The solid residue was dissolved in $CHCl_3$ (75 mL), washed with water (three times), dried over anhydrous Na_2SO_4, purified with column chromatography using silica gel as adsorbent.

*6.1.3. (±)cis-2-[4,6-Bis-(4-methylpiperazin-1-yl)-[1,3,5]-triazin-2-yl]-1-(4-methoxyphenyl)-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylic acid methyl ester (**33**)*

Yield: 67%; mp. >210 °C; ESMS: 612 (M + 1); IR (KBr): 3402, 3182, 2936, 2848, 2802, 1737, 1542, 1436, 1362 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz): δ (ppm) 8.63 (bs, 1H), 7.59–7.13 (m, 7H), 6.57 (d, 2H, J = 8.7 Hz), 6.22 (dd, 1H, J = 6.3, 2.4 Hz), 3.78 (t, 8H, J = 4.9 Hz), 3.75 (s, 3H), 3.61–3.54 (m, 2H), 3.06 (s, 3H), 2.43 (t, 8H, J = 5.4 Hz), 2.31 (s, 6H). ^{13}C NMR ($CDCl_3$, 50 MHz): 21.62, 43.44, 46.63, 50.03, 51.55, 51.97, 55.32, 55.72, 108.92, 111.08, 113.75, 118.98, 119.57, 122.12, 127.36, 130.43, 131.83, 134.03, 136.74, 159.29, 165.33, 165.95, 173.19. Anal. calcd. for $C_{33}H_{41}N_9O_3$: C 64.79, H 6.76, N 20.61; Found: C 64.68, H 6.80, N 20.56%.

*6.1.4. (±)trans-2-[4,6-Bis-(4-methylpiperazin-1-yl)-[1,3,5]-triazin-2-yl]-1-(4-methoxyphenyl)-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylic acid methyl ester (**34**)*

Yield: 71%; mp. >210 °C; ESMS: 612 (M + 1); IR (KBr): 3368, 3181, 2936, 2849, 2798, 1739, 1544, 1428, 1354 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz): δ (ppm) 8.75 (bs, 1H), 7.56–6.85 (m, 7H), 6.84 (d, 2H, J = 8.1 Hz), 4.41 (dd, 1H, J = 8.7, 4.1 Hz), 3.78 (s, 3H), 3.76 (t, 8H, J = 4.5 Hz), 3.57 (s, 3H), 3.49–3.07 (m, 2H), 2.37 (t, 8H, J = 5.6 Hz), 2.29 (s, 6H). ^{13}C NMR ($CDCl_3$, 50 MHz): δ (ppm) 22.44, 43.53, 46.55, 52.18, 53.63, 55.32, 55.70, 56.15, 109.95, 111.23, 114.25, 118.78, 119.82, 122.22, 127.39, 129.67, 133.74, 133.97, 136.77, 159.51, 165.29, 166.67, 172.39. Anal. calcd. for $C_{33}H_{41}N_9O_3$: C 64.79, H 6.76, N 20.61; Found: C 64.62, H 6.74, N 20.58%.

*6.1.5. (±)cis-2-[4,6-Bis-(butylamino)-[1,3,5]-triazin-2-yl]-1-(4-methoxyphenyl)-2,3,4,9-tetra-hydro-1H-β-carboline-3-carboxylic acid methyl ester (**35**)*

Yield: 65%; mp. 132–134 °C (dec.); FAB-MS: 558 (M + 1); IR (KBr): 3415, 3339, 3025, 2953, 2837, 1733, 1595, 1489, 1375 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ (ppm) 7.71 (bs, 1H), 7.60–7.13 (m, 7H), 6.75 (d, 2H, $J = 8.6$ Hz), 6.27 (dd, 1H, $J = 6.2$, 2.1 Hz), 3.75 (s, 3H), 3.49 (s, 3H), 3.39–3.12 (m, 6H), 1.75–1.64 (m, 4H), 1.44–1.34 (m, 4H), 0.93 (t, 6H, $J = 6.1$ Hz); ^{13}C NMR (50 MHz, $CDCl_3$): δ (ppm) 14.31, 20.42, 22.85, 32.55, 40.83, 53.44, 55.47, 56.65, 57.23, 109.11, 111.34 114.21, 118.72, 119.86, 122.40, 127.29, 129.47, 134.42, 136.36, 159.82, 166.12, 166.43, 172.56. Anal. calcd. for $C_{31}H_{39}N_7O_3$: C 66.76, H 7.05, N 17.58; Found: C 66.67, H 6.98, N 17.55%.

*6.1.6. (±)trans-2-[4,6-Bis-(butylamino)-[1,3,5]-triazin-2-yl]-1-(4-methoxyphenyl)-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylic acid methyl ester (**36**)*

Yield: 65%; mp. 238–240 °C (dec.); FAB-MS: 558 (M + 1); IR (KBr); 3418, 3315, 3060, 2965, 2853, 1734, 1591, 1489, 1378 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ (ppm) 7.84 (bs, 1H), 7.56–7.15 (m, 6H), 6.87–6.79 (m, 3H), 4.57 (dd, 1H, $J = 6.2$, 1.9 Hz), 3.76 (s, 3H), 3.59 (s, 3H), 3.52–3.44 (m, 2H), 3.39–3.21 (m, 4H), 1.50–1.27 (m, 8H), 0.94 (t, 6H, $J = 6.4$ Hz); ^{13}C NMR (50 MHz, $CDCl_3$): δ (ppm) 14.29, 20.48, 22.91, 32.36, 40.85, 52.29, 54.27, 55.65, 56.30, 109.13, 111.43, 114.25, 118.70, 119.97, 122.32, 127.35, 129.30, 134.46, 136.90, 159.32, 166.29, 166.90, 172.68. Anal. calcd. for $C_{31}H_{39}N_7O_3$: C 66.76, H 7.05, N 17.58; Found: C 66.65, H 6.94, N 17.52%.

*6.1.7. (±)cis-2-[4,6-Bis-(2-hydroxyethylamino)-[1,3,5]-triazin-2-yl]-1-p-tolyl-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylic acid methyl ester (**37**)*

Yield: 72%; mp. 202–205 °C (dec.); FAB-MS: 518 (M + 1); IR (KBr): 3445, 3333, 3028, 2965, 2855, 1734, 1599, 1479, 1395 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ (ppm) 7.95 (bs, 1H), 7.58–7.03 (m, 9H), 6.10 (dd, 1H, $J = 6.2$, 2.1 Hz), 3.71–3.51 (m, 8H), 3.18–3.03 (m, 2H), 3.01 (s, 3H), 2.25 (s, 3H); ^{13}C NMR (50 MHz, $CDCl_3$): δ (ppm) 21.44, 21.77, 43.74, 51.05, 52.00, 52.63, 62.61, 108.49, 111.37, 118.83, 119.85, 122.38, 127.06, 129.11, 129.46, 131.64, 136.73, 138.07, 165.69, 166.18, 172.92. Anal. calcd. for $C_{27}H_{31}N_7O_4$: C 62.65, H 6.04, N 18.94; Found: C 62.57, H 6.13, N 18.85%.

*6.1.8. (±)trans-2-[4,6-Bis-(2-hydroxyethylamino)-[1,3,5]-triazin-2-yl]-1-p-tolyl-2,3,4,9-tetr-ahydro-1H-β-carboline-3-carboxylic acid methyl ester (**38**)*

Yield: 69%; mp. 218–220 °C; FAB-MS: 518 (M + 1); IR (KBr): 3451, 3315, 3055, 2965, 2853, 1735, 1559, 1472, 1378 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ (ppm) 7.91 (bs, 1H), 7.51–7.10 (m, 8H), 6.88 (s, 1H), 4.60 (dd, 1H, $J = 6.5$, 2.1 Hz), 3.59 (s, 3H), 3.52–3.16 (m, 10H), 2.28 (s, 3H); ^{13}C NMR (50 MHz, $CDCl_3$): δ (ppm) 21.25, 22.75, 43.37, 52.23, 54.33, 56.44, 62.16, 109.47, 111.30, 118.48, 119.73, 122.16, 126.84, 127.25, 129.43, 134.12, 136.65, 137.34, 138.12, 166.24, 172.63. Anal. calcd. for $C_{27}H_{31}N_7O_4$: C 62.65, H 6.04, N 18.94; Found: C 62.62, H 6.12, N 18.86%.

*6.1.9. (±)trans-2-[4,6-Bis-(4-methylpiperazin-1-yl)-[1,3,5]-triazin-2-yl]-1-(3,4,5-trimethoxyphenyl)-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylic acid methyl ester (**39**)*

Yield: 71%; mp. 169–171 °C; ESMS: 672 (M + 1); IR (KBr): 3415, 3058, 2937, 2849, 2797, 1741, 1658, 1542, 1432, 1353 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz): δ (ppm) 8.78 (bs, 1H), 7.56 (d, 1H, $J = 8.8$ Hz), 7.48 (d, 1H, $J = 6.6$ Hz), 7.34–7.07 (m, 3H), 6.89 (s, 1H), 6.58 (d, 2H, $J = 8.8$ Hz), 5.10 (dd, 1H, $J = 6.2$, 2.2 Hz), 4.11 (s, 3H), 3.85–3.67 (m, 17H), 3.42–3.31 (m, 1H), 3.21–3.11 (m, 1H), 2.38–2.29 (m, 14H). ^{13}C NMR ($CDCl_3$, 50 MHz): δ (ppm) 23.36, 43.35, 46.57, 51.44, 52.21, 55.28, 55.81, 56.37, 61.25, 105.74, 107.82, 111.50, 118.35, 119.72, 121.95, 122.30, 126.59, 129.33, 134.90, 136.19, 142.04, 150.33, 152.98, 165.16, 166.44, 174.72. Anal. calcd. for $C_{33}H_{45}N_9O_5$: C 62.58, H 6.75, N 18.76; Found: C 62.47, H 6.81, N 18.73%.

*6.1.10. (±)cis-2-[4,6-Bis-(4-methylpiperazin-1-yl)-[1,3,5]-triazin-2-yl]-1-(3,4,5-trimethoxyphenyl)-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylic acid methyl ester (**40**)*

Yield: 68%; mp. 142–144 °C; ESMS: 672 (M + 1); IR (KBr): 3372, 3062, 2936, 2847, 2799, 1741, 1593, 1435, 1357, 1279 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz): δ (ppm) 8.38 (s, 1H), 7.64 (d, 1H, $J = 8.8$ Hz), 7.29–7.12 (m, 4H), 6.71 (d, 2H, $J = 8.8$ Hz), 6.23 (dd, 1H, $J = 6.2$, 2.1 Hz), 3.95–3.77 (m, 20H), 3.62–3.53 (m, 2H), 2.42–2.25 (m, 14H). ^{13}C NMR ($CDCl_3$, 50 MHz): δ (ppm) 21.67, 43.44, 46.62, 50.27, 51.97, 52.65, 55.30, 56.46, 109.16, 111.14, 119.06, 119.68, 119.96, 122.26, 127.32, 129.61, 131.42, 133.91, 136.79, 137.44, 153.28, 165.50, 165.97, 173.36. Anal. calcd. for $C_{33}H_{45}N_9O_5$: C 62.58, H 6.75, N 18.76; Found: C 62.45; H 6.83, N 18.70%.

*6.1.11. (1S, 3S)-2-[4,6-Bis-(4-methylpiperazin-1-yl)-[1,3,5]-triazin-2-yl]-1-(4-methoxyphenyl)-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylic acid methyl ester (**41**)*

Yield: 60%; mp. 150–152 °C; ESMS: 612 (M + 1); IR (KBr): 3390, 3180, 2929, 2850, 2798, 1739, 1537, 1433, 1359 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz): δ (ppm) 8.38 (bs, 1H), 7.62–7.12 (m, 7H), 6.76 (d, 2H, $J = 7.9$ Hz), 6.28 (dd, 1H, $J = 6.1$, 2.6 Hz), 3.78 (s, 3H), 3.75 (t, 8H, $J = 4.8$ Hz), 3.61–3.54 (m, 1H), 3.18–3.09 (m, 1H), 3.01 (s, 3H), 2.41 (t, 8H, $J = 5.9$ Hz), 2.29 (s, 6H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ (ppm) 21.62, 43.28, 46.60, 50.05, 51.56, 51.97, 55.31, 55.72, 108.08, 111.06, 113.74, 118.96, 119.52, 122.09, 129.53, 130.42, 131.82, 134.08, 136.75, 159.27, 165.29, 165.96, 173.19. Anal. calcd. for $C_{33}H_{41}N_9O_3$; C 64.79, H 6.76, N 20.61; Found: C 64.66, H 6.72, N 20.57%.

*6.1.12. (1R, 3S)-2-[4,6-Bis-(4-methylpiperazin-1-yl)-[1,3,5]-triazin-2-yl]-1-(4-methoxyphenyl)-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylic acid methyl ester (**42**)*

Yield: 68%; mp. 110–112 °C; ESMS: 612 (M + 1); IR (KBr): 3389, 3180, 2937, 2854, 2798, 1739, 1542, 1442, 1357 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz): δ (ppm) 8.16 (bs, 1H), 7.56–7.06 (m, 8H), 6.84 (d, 1H, $J = 8.2$ Hz), 4.48 (dd, 1H, $J = 7.4$, 3.3 Hz), 3.78 (s, 3H), 3.75 (t, 8H, $J = 4.2$ Hz), 3.58 (s, 3H), 3.17–3.10 (m, 2H), 2.40 (t, 8H, $J = 5.1$ Hz), 2.31 (s, 6H). ^{13}C NMR ($CDCl_3$, 50 MHz): δ (ppm) 22.45, 43.44, 46.52, 52.18, 53.66, 55.26, 55.69, 56.17, 109.83, 111.23, 114.24, 118.75, 119.79, 122.20, 127.36, 129.63, 133.77, 133.97, 136.77, 159.49, 165.24, 166.67, 172.38. Anal. calcd. for $C_{33}H_{41}N_9O_3$: C 64.79, H 6.76, N 20.61; Found: C 64.67, H 6.69, N 20.54%.

*6.1.13. (1S, 3S)-2-[4,6-Bis-(4-phenylpiperazin-1-yl)-[1,3,5]-triazin-2-yl]-1-(4-methoxyphenyl)-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylic acid methyl ester (**43**)*

Yield: 72%; mp. 218–220 °C; FAB-MS: 736 (M + 1); IR (KBr): 3340, 3056, 2944, 2829, 1739, 1597, 1544, 1438, 1370, 1174 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz): δ (ppm) 7.73 (bs, 1H), 7.36–6.76 (m, 19H), 6.24 (dd, 1H, $J = 6.7$, 2.3 Hz), 3.99 (t, 8H, $J = 4.8$ Hz), 3.75 (s, 3H), 3.61–3.39 (m, 2H), 3.22 (t, 8H, $J = 5.1$ Hz), 3.04 (s, 3H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ (ppm) 21.82, 43.67, 49.92, 50.48, 51.93, 52.08, 55.74, 109.33, 111.21, 113.86, 117.01, 119.01, 119.97, 120.62, 122.47, 127.35, 129.65, 130.35, 131.78, 133.77, 136.68, 151.86, 159.36, 165.29, 165.63, 166.09, 173.20. Anal. calcd. for $C_{43}H_{45}N_9O_3$: C 70.18, H 6.16, N 17.13; Found C 70.07, H 6.19, N: 16.99%.

*6.1.14. (1R, 3S)-2-[4,6-Bis-(4-phenylpiperazin-1-yl)-[1,3,5]-triazin-2-yl]-1-(4-methoxyphenyl)-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylic acid methyl ester (**44**)*

Yield: 75%; mp. 168–170 °C; FAB-MS: 736 (M + 1); IR (KBr): 3338, 3146, 2945, 2848, 1730, 1593, 1519, 1433, 1352, 1168 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz): δ (ppm) 7.84 (bs, 1H), 7.35–6.83 (m, 19H),

4.53 (dd, 1H, $J = 7.6, 4.7$ Hz), 3.88 (t, 8H, $J = 4.5$ Hz), 3.78 (s, 3H), 3.59 (s, 3H), 3.53–3.46 (m, 2H), 3.15 (t, 8H, $J = 5.1$ Hz); ^{13}C NMR ($CDCl_3$, 50 MHz): δ (ppm) 22.67, 43.59, 49.85, 52.30, 54.01, 55.72, 56.34, 109.72, 119.35, 113.74, 114.36, 116.95, 118.81, 120.12, 120.56, 122.48, 127.37, 129.27, 129.61, 134.10, 136.81, 151.85, 159.48, 165.55, 166.86, 172.62. Anal. calcd. for $C_{43}H_{45}N_9O_3$: C 70.18, H 6.16, N 17.13; Found: C 70.03, H 6.15, N: 17.19%.

*6.1.15. (1S, 3S)-2-[4,6-Bis-(morpholin-4yl)-[1,3,5]-triazin-2-yl]-1-(4-methoxyphenyl)-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylic acid methyl ester (**45**)*

Yield: 73%; mp. 203–205 °C; FAB-MS: 586 (M + 1); IR (KBr): 3317, 2924, 2850, 1737, 1539, 1436, 1257, cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.69 (bs, 1H), 7.60 (d, 1H, $J = 5.6$ Hz), 7.32–7.13 (m, 6H), 6.77 (d, 2H, $J = 8.5$ Hz), 6.19 (dd, 1H, $J = 6.2, 2.1$ Hz), 3.95–3.63 (m, 22H), 3.07–3.03 (m, 4H); ^{13}C NMR (50 MHz, $CDCl_3$): δ (ppm) 21.77, 43.92, 44.18, 50.47, 52.05, 55.71, 67.26, 107.91, 109.19, 111.22, 113.82, 114.56, 118.96, 119.95, 122.46, 127.29, 128.68, 129.20, 130.28, 131.74, 133.67, 136.69, 159.35, 165.70, 172.33. Anal. calcd. for $C_{31}H_{35}N_7O_5$: C 63.58, H 6.02, N 16.74; Found: C 63.46, H 6.09, N 16.71%.

*6.1.16. (1R, 3S)-2-[4,6-Bis-(morpholin-4yl)-[1,3,5]-triazin-2-yl]-1-(4-methoxyphenyl)-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylic acid methyl ester (**46**)*

Yield: 76%; mp. 203–205 °C; FAB-MS: 586 (M + 1); IR (KBr): 3346, 2923, 2852, 1726, 1550, 1442, 1357, 1259, 1172 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.85 (bs, 1H), 7.55 (d, 1H, $J = 8.1$ Hz), 7.31–7.10 (m, 5H), 6.88 (t, 3H, $J = 6.7$ Hz), 4.66 (dd, 1H, $J = 6.8, 2.1$ Hz), 3.80 (s, 3H), 3.69 (bs, 8H), 3.60 (s, 3H), 3.55 (bs, 8H), 3.52–3.46 (m, 1H), 3.22–3.15 (m, 1H); ^{13}C NMR (50 MHz, $CDCl_3$): δ (ppm) 22.61, 44.11, 52.25, 53.93, 55.69, 56.34, 67.25, 109.66, 111.33, 114.31, 118.77, 120.09, 122.48, 127.32, 129.24, 134.01, 136.85, 137.55, 159.46, 165.30, 166.72, 172.54; Anal. calcd. for $C_{31}H_{35}N_7O_5$: C 63.58, H 6.02, N 16.74; Found: C 63.44, H 5.98, N 16.83%.

*6.1.17. (1R, 3S)-2-[4,6-Bis-(cyclohexylamino)-1,3,5-triazin-2-yl]-1-(4-methoxyphenyl)-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylic acid methyl ester (**47**)*

Yield: 65%; mp. 151–153 °C; FAB-MS: 610 (M + 1); IR (KBr): 3203, 3082, 2931, 2858, 1735, 1558, 1454, 1352, 1245, 1170 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.83 (bs, 1H), 7.57–6.75 (m, 9H), 4.74 (dd, 1H, $J = 7.5, 2.2$ Hz), 3.79 (s, 3H), 3.65 (s, 3H), 3.12–2.92 (m, 2H), 1.94–1.18 (m, 22H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ (ppm) 24.78, 30.11, 32.37, 33.63, 49.41, 50.23, 52.21, 54.44, 55.67, 111.41, 113.40, 114.10, 114.27, 118.72, 119.70, 121.70, 127.30, 127.53, 131.24, 136.71, 137.16, 158.26, 159.05, 166.45, 179.74. Anal. calcd. for $C_{35}H_{43}N_7O_3$: C 68.94, H 7.11, N 16.08; Found: C 68.87, H 6.92, N 16.14%.

*6.1.18. (1S, 3S)-2-[4,6-Bis-(o-tolylamino)-1,3,5-triazin-2-yl]-1-(4-methoxyphenyl)-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylic acid methyl ester (**48**)*

Yield: 64%; mp. 188–190 °C; ESMS: 626 (M + 1); IR (KBr): 3267, 2925, 2839, 1739, 1591, 1452, 1245 cm^{-1}. ^{1}H NMR ($CDCl_3$, 200 MHz): δ (ppm) 7.85 (bs, 1H), 7.57–7.17 (m, 17H), 6.66 (dd, 1H, $J = 6.4, 2.5$ Hz), 3.74 (s, 3H), 3.72 (s, 3H), 3.56–3.34 (m, 2H), 2.17 (s, 6H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ (ppm) 18.57, 21.66, 50.41, 52.15, 52.41, 55.67, 109.15, 111.21, 113.63, 118.99, 119.99, 122.55, 123.98, 125.03, 126.73, 127.18, 130.11, 131.48, 132.76, 136. 72, 137.17, 159.40, 165.22, 166.45, 172.75. Anal. calcd. for $C_{37}H_{35}N_7O_3$: C 71.02, H 5.64, N 15.67; Found: C 70.93, H 5.67, N 15.69%.

*6.1.19. (1R, 3S)-2-[4,6-Bis-(o-tolylamino)-1,3,5-triazin-2-yl]-1-(4-methoxyphenyl)-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylic acid methyl ester (**49**)*

Yield: 70%; mp. 188–190 °C; FAB-MS: 640 (M + 1); IR (KBr): 3244, 2949, 2842, 1741, 1569, 1515, 1448, 1244 cm^{-1}. ^{1}H NMR ($CDCl_3$, 200 MHz): δ (ppm) 7.91 (bs, 1H), 7.53–7.06 (m, 17H), 4.88 (dd, 1H, $J = 6.4, 2.5$ Hz), 3.75 (s, 3H), 3.72 (s, 3H), 3.56–3.35 (m, 2H), 2.18 (s, 6H); ^{13}C NMR ($CDCl_3$, 50 MHz): δ (ppm) 19.82, 21.49, 40.20, 42.71, 50.54, 56.51, 57.18, 107.83, 110.00, 111.46, 111.62, 114.25, 118.75, 120.22, 122.56, 124.98, 126.18, 126.90, 127.04, 128.73, 129.73, 131.07, 136. 27, 142.78, 159.39, 165.63, 169.42, 173.56. Anal. calcd. for $C_{37}H_{35}N_7O_3$: C 71.02, H 5.64, N 15.67; Found: C 70.89, H 5.56, N 15.70%.

*6.1.20. (1S, 3S)-2-[4,6-Bis-(4-methylpiperazin-1-yl)-[1,3,5]-triazin-2-yl]-1-(4-isopropylphenyl)-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylic acid methyl ester (**50**)*

Yield: 68%; mp 166–168 °C; MS: 624 (M + 1); IR (KBr): 3417, 3066, 2937, 2852, 1741, 1666, 1541, 1436, 1365 cm^{-1}. ^{1}H NMR ($CDCl_3$, 200 MHz): δ (ppm) 7.99 (bs, 1H), 7.62–7.07 (m, 9H), 6.24 (dd, 1H, $J = 6.3, 2.5$ Hz), 3.77 (s, 3H), 3.35 (t, 8H, $J = 4.9$ Hz), 3.08–2.97 (m, 2H), 2.84–2.78 (m, 1H), 2.34 (t, 8H, $J = 8.3$ Hz), 2.04 (s, 6H), 1.17 (d, 6H, $J = 6.0$ Hz). ^{13}C ($CDCl_3$, 50 MHz): 21.65, 24.52, 34.35, 43.41, 46.05, 46.67, 49.91, 51.80, 54.62, 11.34, 108.87, 118.94, 119.52, 122.04, 126.35, 129.12, 131.75, 136.86, 139.35, 148.64, 161.23, 165.34, 165.65, 165.93, 173.20. Anal. calcd. for $C_{35}H_{45}N_9O_2$: C 67.39, H 7.27, N 20.21; Found: C 67.32, H 7.30, N 20.19%.

*6.1.21. (1R, 3S)-2-[4,6-Bis-(4-methylpiperazin-1-yl)-[1,3,5]-triazin-2-yl]-1-(4-isopropylphenyl)-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylic acid methyl ester (**51**)*

Yield: 68%; mp 186–188 °C; ESMS: 624 (M + 1); IR (KBr): 3373, 3055, 2939, 2854, 1739, 1666, 1539, 1438, 1357 cm^{-1}. ^{1}H NMR ($CDCl_3$, 200 MHz): δ (ppm) 8.38 (bs, 1H), 7.55–7.30 (m, 8H), 6.90 (s, 1H), 4.64 (dd, 1H, $J = 5.1, 3.8$ Hz), 3.53 (s, 3H), 3.47 (t, 8H, $J = 7.7$ Hz), 3.21–3.09 (m, 2H), 2.91–2.84 (m, 1H), 2.28 (t, 8H, $J = 8.8$ Hz), 2.12 (s, 6H), 1.22 (d, 6H, $J = 6.8$ Hz). ^{13}C ($CDCl_3$, 50 MHz): 22.82, 24.54, 34.25, 43.61, 49.92, 52.45, 54.34, 55.23, 56.58, 109.23, 111.34, 116.87, 118.81, 120.12, 120.26, 122.44, 127.12, 127.34, 127.67, 129.56, 136.58, 139.87, 148.54, 151.91, 165.33, 167.02, 172.83. Anal. calcd. for $C_{35}H_{45}N_9O_2$: C 67.39, H 7.27, N 20.21; Found: C 67.35, H 7.29, N 20.24%.

*6.1.22. (1S, 3S)-2-[4,6-Bis-(4-phenylpiperazin-1-yl)-[1,3,5]-triazin-2-yl]-1-(4-isopropylphenyl)-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylic acid methyl ester (**52**)*

Yield: 66%; mp 178–180 °C; ESMS: 748 (M + 1); IR (KBr): 3408, 2924, 2817, 1737, 1602, 1541, 1431, 1366 cm^{-1}. ^{1}H NMR ($CDCl_3$, 200 MHz): δ (ppm) 7.95 (bs, 1H), 7.92–6.86 (m, 19H), 6.26 (dd, 1H, $J = 6.4, 2.4$ Hz), 3.71 (s, 3H), 3.75–3.03 (m, 16H), 2.93–2.63 (m, 3H), 1.23 (d, 6H, $J = 10.0$ Hz). ^{13}C ($CDCl_3$, 50 MHz): 21.28, 24.46, 34.23, 43.56, 49.87, 50.27, 51.93, 52.20, 109.45, 11.23, 117.02, 119.04, 119.92, 120.61, 122.53, 126.62, 129.03, 129.21, 129.56, 131.16, 136.27, 139.03, 148.57, 151.88, 165.67, 166.32, 173.11. Anal. calcd. for $C_{45}H_{49}N_9O_2$: C 72.26, H 6.60, N 16.85; Found: C 72.12, H 6.66, N 16.89%.

*6.1.23. (1R, 3S)-2-[4,6-Bis-(4-phenylpiperazin-1-yl)-[1,3,5]-triazin-2-yl]-1-(4-isopropylphenyl)-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylic acid methyl ester (**53**)*

Yield: 69%; mp. 170–172 °C; ESMS: 748 (M + 1); IR (KBr): 3406, 3361, 2922, 2854, 1735, 1542, 1436, 1369 cm^{-1}. ^{1}H NMR ($CDCl_3$, 200 MHz): δ (ppm) 7.83 (bs, 1H), 7.37–6.92 (m, 18H), 6.84 (s, 1H), 4.25 (dd, 1H, $J = 6.4, 2.1$ Hz), 3.86 (s, 3H), 3.60–3.31 (m, 16H), 3.25–3.14 (m, 3H), 1.44 (d, 6H, $J = 6.0$ Hz); ^{13}C ($CDCl_3$, 50 MHz): δ (ppm) 22.78, 24.34, 34.12, 43.25, 49.68, 52.34, 54.32, 56.58, 109.01, 111.23, 116.78, 118.82, 120.11, 120.50, 122.43, 127.04, 127.32, 127.41, 127.67, 129.56, 134.34, 136.82, 139.48, 148.56, 151.82, 165.51, 166.69, 172.82; Anal. calcd. for $C_{45}H_{49}N_9O_2$: C 72.26, H 6.60, N 16.85; Found: C 72.19, H 6.65, N 16.80%.

*6.1.24. (1S, 3S)-2-[4,6-Bis-(morpholin-4-yl)-[1,3,5]-triazin-2-yl]-1-(4-isopropylphenyl)-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylic acid methyl ester (**54**)*

Yield: 67%; mp 180–182 °C; ESMS: 598 (M + 1); IR (KBr): 3379, 2927, 2854, 1720, 1539, 1434, 1361 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz): δ (ppm) 7.77 (bs, 1H), 7.28–7.08 (m, 9H), 6.19 (dd, 1H, $J = 6.8$, 2.4 Hz), 3.72 (s, 3H), 3.02–2.85 (m, 19H), 1.18 (d, 6H, $J = 6.0$ Hz). ^{13}C ($CDCl_3$, 50 MHz): 21.13, 24.25, 34.31, 44.20, 50.44, 51.92, 52.25, 67.34, 109.32, 11.20, 118.87, 119.92, 122.45, 126.61, 129.03, 129.34, 131.53, 136.74, 138.91, 148.62, 165.84, 166.16, 172.97. Anal. calcd. for $C_{33}H_{39}N_7O_4$: C 66.31, H 6.58, N 16.40; Found: C 66.29, H 6.60, N 16.37%.

*6.1.25. (1R, 3S)-2-[4,6-Bis-(morpholin-4-yl)-[1,3,5]-triazin-2-yl]-1-(4-isopropylphenyl)-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylic acid methyl ester (**55**)*

70%; mp 210–212 °C; ESMS: 598 (M + 1); IR (KBr): 3379, 2958, 2852, 1732, 1542, 1431, 1361 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz): δ (ppm) 7.81 (bs, 1H), 7.35–7.10 (m, 8H), 6.90 (s, 1H), 4.80 (dd, 1H, $J = 7.8$, 4.0 Hz), 3.66 (s, 3H), 3.58–3.47 (m, 16H), 3.44–3.21 (m, 2H), 2.90–2.83 (m, 1H), 1.21 (d, 6H, $J = 8.0$ Hz). ^{13}C ($CDCl_3$, 50 MHz): 22.86, 24.44, 34.25, 44.12, 52.35, 54.42, 56.93, 67.22, 108.97, 111.34, 118.82, 120.15, 122.43, 127.02, 127.35, 127.52, 134.24, 136.81, 139.86, 148.53, 165.45, 165.91, 172.72. Anal. calcd. for $C_{33}H_{39}N_7O_4$: C 66.31, H 6.58, N 16.40; Found: C 66.34, H 6.60, N 16.35%.

*6.1.26. (1S, 3S)-2-[4,6-Bis-(dimethylamino)-1,3,5-triazin-2-yl]-1-(4-isopropylphenyl)-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylic acid methyl ester (**56**)*

Yield: 60%; mp 176–178 °C; ESMS: 514 (M + 1); IR (KBr): 3408, 2945, 2817, 1737, 1541, 1431, 1362 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz): δ (ppm) 7.80 (bs, 1H), 7.35 (d, 1H, $J = 8.0$ Hz), 7.28–7.11 (m, 7H), 6.94 (s, 1H), 4.52 (dd, 1H, $J = 8.0$, 4.2 Hz), 3.59 (s, 3H), 3.25–2.93 (m, 15H), 1.22 (d, 6H, $J = 6.0$ Hz). ^{13}C ($CDCl_3$, 50 MHz): 24.10, 24.46, 34.22, 36.53, 53.22, 55.91, 108.93, 111.21, 118.76, 119.98, 123.31, 126.72, 126.91, 127.84, 133.32, 136.45, 138.83, 148.75, 165.83, 166.92, 173.71. Anal. calcd. for $C_{29}H_{35}N_7O_2$: C 67.81, H 6.87, N 19.09; Found: C 67.78, H 6.86, N 19.12%.

*6.1.27. (1S, 3S)-2-[4,6-Bis-(cyclohexylamino)-1,3,5-triazin-2-yl]-1-(4-isopropylphenyl)-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylic acid methyl ester (**57**)*

Yield: 60%; mp 170–173 °C; ESMS: 622 (M + 1); IR (KBr): 3386, 2940, 2853, 1740, 1545, 1457, 1355 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz): δ (ppm) 7.73 (bs, 1H), 7.61 (d, 1H, $J = 4.0$ Hz), 7.58–7.09 (m, 8H), 6.25 (dd, 1H, $J = 6.2$, 2.2 Hz), 4.79 (bs, 2H), 3.63 (s, 3H), 2.94–2.82 (m, 5H), 1.53–1.12 (m, 26H). ^{13}C ($CDCl_3$, 50 MHz): 21.62, 24.45, 25.31, 26.23, 33.71, 34.32, 47.78, 48.52, 49.55, 52.24, 109.41, 111.22, 118.94, 119.42, 122.40, 126.53, 127.44, 129.32, 131.67, 136.73, 138.91, 148.65, 165.87, 166.12, 173.21; Anal. calcd. for $C_{37}H_{47}N_7O_2$: C 71.47, H 7.62, N 15.77; Found: C 71.37, H 7.66, N 15.69%.

*6.1.28. (1R, 3S)-2-[4,6-Bis-(4-methylpiperazin-1-yl)-[1,3,5]-triazin-2-yl]-1-(3,4,5-trimethoxyphenyl)-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylic acid methyl ester (**58**)*

Yield: 67%; mp. 148–150 °C; ESMS: 672 (M + 1); IR (KBr): 3364, 3060, 2937, 2851, 2799, 1739, 1658, 1549, 1439, 1352 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz): δ (ppm) 8.72 (s, 1H), 7.56–7.06 (m, 6H), 6.92 (s, 1H), 4.65 (dd, 1H, $J = 6.1$, 2.2 Hz), 3.72 (bs, 17H), 3.57 (s, 3H), 3.51–3.44 (m, 1H), 3.23–3.13 (m, 1H), 2.34 (t, 8H, $J = 5.9$ Hz), 2.21 (s, 6H). ^{13}C NMR ($CDCl_3$, 50 MHz): 21.64, 43.41, 46.60, 50.25, 51.97, 52.68, 55.32, 56.45, 109.15, 111.13, 119.05, 119.67, 119.97, 122.27, 127.30, 129.67, 133.82, 131.41, 136.79, 137.45, 153.29, 165.49, 165.96, 173.38. Anal. calcd. for $C_{33}H_{45}N_9O_5$: C 62.58, H 6.75, N 18.75; Found: C 62.52, H 6.78, N 18.64%.

*6.1.29. (1S, 3S)-2-[4,6-Bis-(4-methylpiperazin-1-yl)-[1,3,5]-triazin-2-yl]-1-(3,4,5-trimethoxyphenyl)-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylic acid methyl ester (**59**)*

Yield: 65%; mp. 140–142 °C; ESMS: 672 (M + 1); IR (KBr): 3373, 3061, 2936, 2848, 2798, 1739, 1593, 1435, 1357, 1278 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz): δ (ppm) 8.36 (s, 1H), 7.62 (d, 1H, $J = 8.7$ Hz), 7.29–7.13 (m, 4H), 6.70 (d, 2H, $J = 8.8$ Hz), 6.22 (dd, 1H, $J = 6.2$, 2.3 Hz), 3.94–3.78 (m, 20H), 3.61–3.51 (m, 2H), 2.42–2.25 (m, 14H). ^{13}C NMR ($CDCl_3$, 50 MHz): δ (ppm) 21.64, 43.41, 46.60, 50.25, 51.97, 52.67, 55.32, 56.45, 109.15, 111.12, 119.05, 119.66, 119.97, 122.27, 127.30, 129.62, 131.41, 133.91, 136.79, 137.45, 153.29, 165.49, 165.96, 173.38. Anal. calcd. for $C_{33}H_{45}N_9O_5$: C 62.58, H 6.75, N 18.75; Found: C 62.51; H 6.82, N 18.73%.

*6.1.30. (1R, 3S)-2-[4,6-Bis-(4-ethylpiperazin-1-yl)-[1,3,5]-triazin-2-yl]-1-(4-methoxyphenyl)-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylic acid methyl ester (**60**)*

Yield: 72%; mp. 162–164 °C; ESMS: 640 (M + 1); IR (KBr): 3401, 3183, 2935, 2813, 1739, 1550, 1439, 1350 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz): δ (ppm) 8.57 (bs, 1H), 7.56 (d, 1H, $J = 8.8$ Hz), 7.35–7.27 (m, 3H), 7.20–7.09 (m, 2H), 7.01 (s, 1H), 6.86 (d, 2H, $J = 8.8$ Hz), 4.48 (dd, 1H, $J = 8.9$, 3.0 Hz), 3.80 (s, 3H), 3.76 (t, 8H, $J = 4.9$ Hz), 3.60 (s, 3H), 3.56–3.46 (m, 1H), 3.18–3.12 (m, 1H), 2.43 (bs, 12H), 1.11 (t, 6H, $J = 6.0$ Hz). ^{13}C NMR ($CDCl_3$, 50 MHz): δ (ppm) 12.13, 22.48, 43.40, 52.15, 52.77, 53.11, 53.71, 55.69, 56.16, 109.91, 111.18, 114.25, 118.76, 119.85, 122.25, 127.39, 129.56, 133.91, 136.73, 159.51, 165.13, 166.68, 172.40. Anal. calcd. for $C_{35}H_{45}N_9O_3$: C 65.71, H 7.09, N 19.70; Found: C 65.62, H 7.14, N 19.65%.

*6.1.31. (1R, 3S)-2-[4,6-Bis-(4-propylpiperazin-1-yl)-[1,3,5]-triazin-2-yl]-1-(4-methoxyphenyl)-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylic acid methyl ester (**61**)*

Yield: 72%; mp. 158–160 °C; ESMS: 668 (M + 1); IR (KBr): 3400, 3184, 2935, 2811, 1740, 1547, 1439, 1352 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz): δ (ppm) 8.50 (bs, 1H), 7.57 (d, 1H, $J = 6.4$ Hz), 7.35–7.27 (m, 3H), 7.20–7.12 (m, 2H), 7.01 (s, 1H), 6.86 (d, 2H, $J = 8.8$ Hz), 4.48 (dd, 1H, $J = 6.4$, 2.1 Hz), 3.80 (s, 3H), 3.76 (t, 8H, $J = 4.9$ Hz), 3.60 (s, 3H), 3.54–3.46 (m, 1H), 3.18–3.12 (m, 1H), 2.43 (t, 8H, $J = 5.7$ Hz), 2.29 (t, 4H, $J = 5.9$ Hz), 1.54 (m, 4H), 1.11 (t, 6H, $J = 6.0$ Hz). ^{13}C NMR ($CDCl_3$, 50 MHz): 12.32, 20.17, 22.48, 43.37, 52.15, 53.53, 55.68, 56.16, 61.15, 71.83, 109.94, 111.18, 114.27, 118.77, 119.84, 122.24, 127.40, 129.58, 133.91, 136.73, 159.49, 165.14, 166.67, 172.40. Anal. calcd. for $C_{37}H_{49}N_9O_3$: C 66.54, H 7.40, N 18.88; Found: C 66.47, H 7.44, N 18.91%.

*6.1.32. (1R, 3R)-2-[4,6-Bis-(4-methylpiperazin-1-yl)-[1,3,5]-triazin-2-yl]-1-(4-methoxyphenyl)-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylic acid methyl ester (**62**)*

Yield: 63%; mp. 125–127 °C; ESMS: 612 (M + 1); IR (KBr): 3392, 3180, 2928, 2850, 2796, 1739, 1537, 1432, 1359 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz): δ (ppm) 8.70 (bs, 1H), 7.62–7.12 (m, 7H), 6.76 (d, 2H, $J = 8.5$ Hz), 6.20 (dd, 1H, $J = 6.1$, 2.6 Hz), 3.80 (s, 3H), 3.75 (t, 8H, $J = 4.8$ Hz), 3.53–3.10 (m, 2H), 3.07 (s, 3H), 2.61 (t, 8H, $J = 5.4$ Hz), 2.30 (s, 6H). ^{13}C NMR ($CDCl_3$, 50 MHz): δ (ppm) 21.64, 43.38, 46.59, 50.13, 51.63, 51.96, 55.31, 55.71, 108.95, 111.07, 113.75, 118.96, 119.60, 122.14, 129.43, 130.40, 131.83, 134.03, 136.73, 159.29, 165.55, 165.55, 173.19. Anal. calcd. for $C_{33}H_{41}N_9O_3$: C 64.79, H 6.76, N 20.61; Found: C 64.67, H 6.84, N 20.59%.

*6.1.33. (1S, 3R)-2-[4,6-Bis-(4-methylpiperazin-1-yl)-[1,3,5]-triazin-2-yl]-1-(4-methoxyphenyl)-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylic acid methyl ester (**63**)*

Yield: 71%; mp. 125–128 °C; ESMS: 612 (M + 1); IR (KBr): 3372, 3180, 2937, 2849, 2797, 1739, 1548, 1441, 1356 cm^{-1}. ^1H NMR ($CDCl_3$, 200 MHz): δ (ppm) 8.40 (bs, 1H), 7.56–7.09 (m, 6H), 6.97 (s,

1H), 6.84 (d, 2H, J = 8.4 Hz), 4.47 (dd, 1H, J = 7.7, 4.1 Hz), 3.78 (s, 3H), 3.75 (t, 8H, J = 4.2 Hz), 3.54 (s, 3H), 3.49–3.46 (m, 1H), 2.64–2.58 (m, 1H), 2.35 (t, 8H, J = 5.8 Hz), 2.27 (s, 6H). ^{13}C NMR ($CDCl_3$, 50 MHz): δ (ppm) 22.50, 43.43, 46.53, 53.77, 54.63, 55.31, 55.70, 56.18, 109.82, 111.23, 114.25, 118.76, 119.89, 122.27, 127.37, 129.47, 133.94, 136.77, 159.46, 161.14, 165.26, 166.65, 172.43. Anal. calcd. for $C_{33}H_{41}N_9O_3$: C 64.79, H 6.76, N 20.61; Found: C 64.73, H 6.81, N: 20.56%.

*6.1.34. (1R, 3R)-2-[4,6-Bis-(4-methylpiperazin-1-yl)-[1,3,5]-triazin-2-yl]-1-phenyl-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylic acid methyl ester (**64**)*

Yield: 73%; mp. 120–122 °C; ESMS: 582 (M + 1); IR (KBr): 3405, 3062, 2937, 2850, 2798, 1738, 1539, 1489, 1436, 1363 cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm) 8.43 (s, 1H), 7.62 (d, 1H, J = 7.8 Hz), 7.45–7.14 (m, 9H), 6.22 (dd, 1H, J = 7.8, 2.5 Hz), 3.84 (t, 8H, J = 6.1 Hz), 3.63–3.56 (m, 1H), 3.22–3.02 (m, 1H), 2.98 (s, 3H), 2.43–2.26 (m, 14H). ^{13}C NMR ($CDCl_3$, 50 MHz): δ (ppm) 21.64, 43.26, 46.63, 50.18, 51.82, 52.14, 55.29, 108.99, 111.05, 119.01, 119.58, 122.15, 127.33, 127.88, 128.54, 129.17, 131.47, 136.75, 141.83, 165.30, 166.04, 173.09. Anal. calcd. for $C_{32}H_{39}N_9O_2$: C 66.07, H 6.76, N 21.67; Found: C 66.02, H: 6.88, N 21.62%.

*6.1.35. (1S, 3R)-2-[4,6-Bis-(4-methylpiperazin-1-yl)-[1,3,5]-triazin-2-yl]-1-phenyl-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylic acid methyl ester (**65**)*

Yield: 75%; mp. 144–146 °C; ESMS: 582 (M + 1); IR (KBr): 3394, 2936, 2851, 2799, 1739, 1548, 1437, 1356 cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm) 8.18 (s, 1H), 7.56 (d, 1H, J = 8.1 Hz), 7.42–7.12 (m, 8H), 6.91 (s, 1H), 4.76 (dd, 1H, J = 6.3, 2.6 Hz), 3.73 (t, 8H, J = 5.9 Hz), 3.60 (s, 3H), 3.56–3.48 (m, 1H), 3.24–3.18 (m, 1H), 2.44–2.29 (m, 14H). ^{13}C NMR ($CDCl_3$, 50 MHz): δ (ppm) 22.60, 43.46, 46.45, 52.24, 54.00, 55.33, 56.84, 109.53, 111.24, 118.80, 119.85, 122.26, 127.34, 127.95, 128.17, 128.54, 128.93, 133.80, 136.82, 141.75, 142.14, 165.17, 166.79, 172.51. Anal. calcd. for $C_{32}H_{39}N_9O_2$: C 66.07, H 6.76, N 21.67; Found: C 65.96, H 6.83, N 21.61%.

*6.1.36. (1R, 3R)-2-[4,6-Bis-(4-methylpiperazin-1-yl)-[1,3,5]-triazin-2-yl]-1-p-tolyl-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylic acid methyl ester (**66**)*

Yield: 67%; mp. 150–152 °C; ESMS: 596 (M + 1); IR (KBr): 3425, 2937, 2849, 2800, 1738, 1589, 1541, 1436, 1355, 1277 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$): δ 9.05 (bs, 1H), 7.60–6.94 (m, 9H), 6.21 (dd, 1H, J = 6.8, 1.9 Hz), 3.78 (t, 8H, J = 4.6 Hz), 3.61–3.53 (m, 1H), 3.10–3.03 (m, 1H), 2.82 (s, 3H), 2.81–2.01 (m, 14H); ^{13}C NMR (50 MHz, $CDCl_3$): δ (ppm) 21.46, 21.67, 43.34, 46.61, 50.25, 51.77, 51.94, 55.31, 108.85, 111.06, 118.95, 119.55, 122.08, 127.36, 128.05, 129.10, 129.58, 131.82, 133.82, 136.75, 137.44, 138.80, 165.34, 165.03, 173.16; Anal. calcd. for $C_{33}H_{41}N_9O_2$: C 66.53, H 6.94, N 21.16; Found: C 66.42, H 6.95, N 20.98%.

*6.1.37. (1S, 3R)-2-[4,6-Bis-(4-methylpiperazin-1-yl)-[1,3,5]-triazin-2-yl]-1-p-tolyl-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylic acid methyl ester (**67**)*

Yield: 72%; mp. 138–140 °C; ESMS: 596 (M + 1); IR (KBr): 3368, 2937, 2852, 2798, 1739, 1549, 1439, 1353 cm^{-1}. ^{1}H NMR ($CDCl_3$, 200 MHz): δ (ppm) 8.68 (bs, 1H), 7.54 (d, 1H, J = 6.6 Hz), 7.30–7.09 (m, 7H), 6.95 (s, 1H), 4.52 (dd, 1H, J = 6.3, 2.2 Hz), 3.70 (t, 8H, J = 4.9 Hz), 3.70 (s, 3H), 3.57–3.43 (m, 1H), 3.18–3.11 (m, 1H), 2.34 (m, 17H). ^{13}C NMR ($CDCl_3$, 50 MHz): δ (ppm) 21.55, 22.44, 43.55, 46.57, 52.19, 53.84, 55.39, 56.53, 109.70, 111.20, 118.77, 119.78, 122.18, 127.39, 128.29, 129.59, 133.95, 136.80, 137.69, 138.81, 165.21, 166.75, 172.44. Anal. calcd. for $C_{33}H_{41}N_9O_2$: C 66.53, H 6.94, N 21.16; Found: C 66.38, H 6.98, N 21.18%.

*6.1.38. (±)trans-2-[4,6-Bis-(4-methylpiperazin-1-yl)-[1,3,5]-triazin-2-yl]-1-(4-chlorophenyl)-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylic acid methyl ester (**68**)*

Yield: 68%; mp. 224–227 °C (dec.); ESMS: 616 (M + 1); IR (KBr): 3367, 2937, 2852, 2799, 1738, 1549, 1488, 1440, 1354, 1277 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.35 (bs, 1H), 7.54 (d, 1H, J = 6.0 Hz), 7.37–7.10 (m, 7H), 6.84 (s, 1H), 4.86 (dd, J = 7.0, 2.3 Hz), 3.70 (t, 8H, J = 4.7 Hz), 3.58 (s, 3H), 3.51–3.43 (m, 1H), 3.22–3.12 (m, 1H), 2.35–2.27 (m, 14H); ^{13}C NMR (50 MHz, $CDCl_3$): δ (ppm) 22.67, 43.40, 46.54, 52.29, 54.11, 55.28, 56.29, 109.48, 111.34, 118.85, 120.02, 122.48, 127.26, 129.07, 129.35, 133.34, 133.61, 136.90, 141.06, 165.17, 166.76, 172.46. Anal. calcd. for $C_{32}H_{38}ClN_9O_2$: C 62.38, H 6.22, N 20.46; Found: C 62.29, H 6.19, N 20.42%.

*6.1.39. (±)trans-2-[4,6-Bis-(4-methylpiperazin-1-yl)-[1,3,5]-triazin-2-yl]-1-p-tolyl-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylic acid methyl ester (**69**)*

Yield: 63%; mp. 160–162 °C; FAB-MS: 596 (M + 1); IR (KBr): 3387, 3078, 2936, 2855, 1739, 1543, 1443, 1359, 1259 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.45 (bs, 1H), 7.54–7.08 (m, 8H), 6.92 (s, 1H), 4.61 (dd, 1H, J = 7.7, 2.6 Hz), 3.90 (t, 8H, J = 5.1 Hz), 3.59 (s, 3H), 3.19–3.10 (m, 2H), 2.36 (t, 8H, J = 4.9 Hz), 2.32 (s, 3H), 2.25 (s, 6H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 21.49, 22.63, 43.34, 46.55, 53.99, 55.31, 56.57, 57.78, 109.53, 111.24, 118.76, 119.91, 122.28, 127.39, 128.00, 129.58, 134.09, 136.82, 137.54, 139.11, 165.23, 166.81, 172.54. Anal. calcd. for $C_{33}H_{41}N_9O_2$: C 66.53, H 6.94, N 21.16; Found: C 66.47, H 6.98, N 20.97%.

*6.1.40. (±)cis-2-[4,6-Bis-(propylamino)-[1,3,5]-triazin-2-yl]-1-p-tolyl-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylic acid methyl ester (**70**)*

Yield: 70%; mp. 138–140 °C; FAB-MS: 514 (M + 1); IR (KBr): 3408, 3311, 3060, 2968, 2855, 1736, 1570, 1449, 1378 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 7.72 (bs, 1H), 7.62–7.03 (m, 9H), 6.28 (dd, 1H, J = 6.2, 1.9 Hz), 3.39–3.30 (m, 4H), 3.15–3.05 (m, 2H), 2.97 (s, 3H), 2.28 (s, 3H), 1.69–1.58 (m, 4H), 0.96 (t, 6H, J = 7.3 Hz); ^{13}C NMR (50 MHz, $CDCl_3$): δ (ppm) 11.88, 21.50, 22.95, 23.45, 42.88, 50.16, 51.83, 52.68, 109.06, 111.33, 118.90, 119.85, 122.35, 127.03, 129.08, 129.46, 131.65, 136.73, 137.52, 138.22, 165.78, 173.16; Anal. calcd. for $C_{29}H_{35}N_7O_2$: C 67.81, H 6.87, N 19.09; Found: C 67.73, H 6.96, N 19.12%.

*6.1.41. (±)trans-2-[4,6-Bis-(propylamino)-[1,3,5]triazin-2-yl]-1-p-tolyl-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylic acid methyl ester (**71**)*

Yield: 63%; mp. 180–183 °C; FAB-MS: 514 (M + 1); IR (KBr): 3430, 3315, 3033, 2951, 2860, 1733, 1575, 1480, 1378 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 7.89 (bs, 1H), 7.48–7.02 (m, 8H), 6.87 (s, 1H), 4.60 (dd, 1H, J = 6.5, 2.1 Hz), 3.56 (s, 3H), 3.41–3.32 (m, 4H), 3.29–3.15 (m, 2H), 2.31 (s, 3H), 1.67–1.55 (m, 4H), 0.97 (t, 6H, J = 7.2 Hz); ^{13}C NMR (50 MHz, $CDCl_3$): δ (ppm) 11.82, 21.49, 22.95, 23.47, 42.87, 52.27, 54.39, 56.60, 109.68, 111.33, 118.73, 119.99, 122.31, 126.74, 127.82, 129.57, 134.41, 136.85, 137.44, 138.55, 162.85, 166.35, 172.73; Anal. calcd. for $C_{29}H_{35}N_7O_2$: C 67.81, H 6.87, N 19.09; Found: C 67.78, H 6.75, N 19.23%.

*6.1.42. (±)trans-2-[4,6-Bis-(ethylamino)-[1,3,5]-triazin-2-yl]-1-p-tolyl-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylic acid methyl ester (**72**)*

Yield: 65%; mp. 232–234 °C (dec.); FAB-MS: 486 (M + 1); IR (KBr): 3439, 3315, 3035, 2975, 2865, 1735, 1580, 1483, 1385 cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 7.86 (bs, 1H), 7.46–7.02 (m, 8H), 6.92 (s, 1H), 4.56 (dd, 1H, J = 6.6, 2.1 Hz), 3.58 (s, 3H), 3.44–3.31 (m, 4H), 3.25–3.12 (m, 2H), 2.29 (s, 3H), 1.12 (t, 6H, J = 7.2 Hz); ^{13}C NMR (50 MHz, $CDCl_3$): δ (ppm) 15.58, 21.69, 22.60, 36.07, 52.31, 54.49,

56.78, 109.78, 111.63, 118.89, 119.73, 122.39, 126.84, 127.62, 129.88, 134.61, 136.87, 137.64, 138.45, 162.90, 166.75, 172.68; Anal. calcd. for $C_{27}H_{31}N_7O_2$: C 66.78, H 6.43, N 20.19; Found: C 66.65, H 6.66, N 20.05%.

*6.1.43. (±)trans-2-[4,6-Bis-(ethylamino)-[1,3,5]-triazin-2-yl]-1-(4-methoxyphenyl)-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylic acid methyl ester (**73**)*

Yield: 61%; mp. >200 °C (dec.); FAB-MS: 502 (M + 1); IR (KBr); 3429, 3318, 3029, 2971, 2859, 1732, 1585, 1483, 1380 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ (ppm) 7.88 (bs, 1H), 7.56–7.15 (m, 6H), 6.86–6.78 (m, 3H), 4.54 (dd, 1H, $J = 6.2$, 2.3 Hz), 3.77 (s, 3H), 3.62 (s, 3H), 3.52–3.23 (m, 6H), 1.17 (t, 6H, $J = 6.8$ Hz); ^{13}C NMR (50 MHz, $CDCl_3$): δ (ppm) 15.64, 21.97, 36.11, 52.30, 54.47, 55.43, 56.79, 109.26, 111.25, 118.92, 119.87, 122.39, 127.32, 129.12, 129.37, 131.69, 136.72, 137.57, 138.37, 163.14, 165.98, 173.42; Anal. calcd. for $C_{27}H_{31}N_7O_3$: C 64.65, H 6.23, N 19.55; Found: C 64.57, H 6.32, N 19.45%.

*6.1.44. (±)cis-2-[4,6-Bis-(propylamino)-[1,3,5]-triazin-2-yl]-1-(4-methoxyphenyl)-2,3,4,9-tetra-hydro-1H-β-carboline-3-carboxylic acid methyl ester (**74**)*

Yield: 72%; mp. 140–143 °C (dec.); FAB-MS: 530 (M + 1); IR (KBr): 3429, 3315, 2953, 2829, 1732, 1599, 1479, 1380 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ (ppm) 7.89 (bs, 1H), 7.59–7.15 (m, 7H), 6.83 (d, 2H, $J = 8.6$ Hz), 6.25 (dd, 1H, $J = 6.1$, 2.1 Hz), 3.73 (s, 3H), 3.52 (s, 3H), 3.42–3.35 (m, 4H), 3.30–3.10 (m, 2H), 1.57–1.49 (m, 4H), 0.98 (t, 6H, $J = 6.8$ Hz); ^{13}C NMR (50 MHz, $CDCl_3$): δ (ppm) 15.57, 21.49, 21.62, 36.08, 50.16, 51.19, 52.01, 52.06, 109.25, 111.24, 118.95, 119.86, 122.38, 127.34, 129.09, 129.36, 131.64, 136.75, 137.58, 138.36, 165.97, 166.74, 173.11; Anal. calcd. for $C_{29}H_{35}N_7O_3$: C 65.76, H 6.66, N 18.51; Found: C 65.72, H 6.78, N 18.44%.

*6.1.45. (±)trans-2-[4,6-Bis-(propylamino)-[1,3,5]-triazin-2-yl]-1-(4-methoxyphenyl)-2,3,4,9-tetrahydro-1H-β-carboline-3-carboxylic acid methyl ester (**75**)*

Yield: 68%; mp. 220–223 °C; FAB-MS: 530 (M + 1); IR (KBr): 3419, 3340, 3060, 2959, 2870, 1734, 1562, 1459, 1353 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ (ppm) 7.89 (bs, 1H), 7.57–7.13 (m, 7H), 6.86–6.75 (m, 3H), 4.69 (dd, 1H, $J = 6.3$, 2.1 Hz), 3.76 (s, 3H), 3.59 (s, 3H), 3.53–3.45 (m, 2H), 3.40–3.22 (m, 4H), 1.52–143 (m, 4H), 0.91 (t, 6H, $J = 7.2$ Hz); ^{13}C NMR (50 MHz, $CDCl_3$ + DMSO-d_6): δ (ppm) 11.64, 22.76, 23.22, 42.52, 52.04, 54.20, 54.46, 56.07, 111.48, 113.92, 118.18, 119.28, 121.66, 126.92, 128.90, 134.69, 136.76, 136.92, 158.97, 165.85, 166.51, 173.16. Anal. calcd. for $C_{29}H_{35}N_7O_3$: C 65.76, H 6.66, N 18.51; Found: C 65.63, H 6.70, N 18.40%.

*6.1.46. Typical procedure for synthesis of compound **76***

To 1.5 equivalent of cyanuric chloride in dry THF was added dropwise a solution of tryptamine (1 equivalent) at 0 °C in dry THF over a period of half an hour. The reaction mixture was stirred for 1 h and solvent was removed in vacuo, washed with water and extracted with chloroform. Organic layers were combined, dried with Na_2SO_4 and solvent was evaporated under reduced pressure. Resulting solid was refluxed with 2 equivalent of *N*-methylpiperazine in THF. When TLC analysis showed completion of reaction, solvent was evaporated and resulted solid was column chromatographed to get pure compounds.

*6.1.47. N-[2-(1H-indol-3-yl)ethyl]-4,6-bis-(morpholin-4-yl)-1,3,5-triazin-2-amine (**76**)*

Yield: 76%; mp. 160–162 °C; ESMS: 436 (M + 1); IR (KBr): 2934, 2852, 2800, 1534, 1497, 1444, 1278 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 8.01 (bs, 1H), 7.62 (d, 1H, $J = 7.8$ Hz), 7.33 (d, 1H, $J = 7.8$ Hz), 7.19–7.08 (m, 3H), 5.08 (s, 1H), 3.54 (t, 8H, $J = 4.5$ Hz), 2.44 (t, 8H, $J = 4.5$ Hz), 2.31 (s, 6H); ^{13}C NMR (50 MHz, $CDCl_3$): δ (ppm) 26.18, 41.43, 43.36, 46.66, 55.39, 111.59, 113.85, 119.26, 119.61, 122.41, 123.02, 127.89, 136.82, 165.63, 166.76; Anal. calcd. for $C_{23}H_{33}N_9$: C 63.42, H 7.64, N 28.94; Found: C 63.25, H 7.66, N 28.97%.

*6.1.48. Typical procedure for synthesis of compound **77***

To a solution of cyanuric chloride in THF was added 3.3 equivalents of *N*-methylpiperazine and refluxed for 4 h. Solvent was removed in vacuo and resulting solid was crystalized from ethanol to get pure compound **77**.

*6.1.49. 2,4,6-Tris(4-methylpiperazin-1-yl)-1,3,5-triazine (**77**)*

Yield: 86%; mp. 160–162 °C; ESMS: 376 (M + 1); IR (KBr): 2934, 2852, 2800, 1534, 1497, 1444, 1278 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): 3.21 (t, 8H, $J = 4.5$ Hz), 2.46 (t, 8H, $J = 4.5$ Hz), 2.28 (s, 6H); ^{13}C NMR (50 MHz, DMSO-d_6): δ (ppm) 46.66, 53.56, 58.24, 179.62; Anal. calcd. for $C_{18}H_{33}N_9$: C 57.57, H 8.86, N 33.57; Found: C 57.42, H 8.89, N 33.45%.

6.2. Biological assays

MTT, PI, DAPI, guanosine 5′-triphosphate (GTP), and PIPES were obtained from Sigma, U.S.A. DMEM (Dulbecco's modified eagles medium) and fetal bovine serum (FBS) were procured from Gibco BRL, U.S.A. DMSO was from Merck, India, and antibiotic solution (containing penicillin and streptomycin) was obtained from Hyclone, U.S.A. Human cancer cell lines representing breast cancer (MCF7), colon (SW620), prostate (DU145), oral (KB), ovary (PA1), Leukemia (K562), pancreas (MiaPaCa-2), Lung (A549) and normal fibroblasts (NIH3T3) were procured from NCCS, Pune, India.

6.2.1. Cytotoxicity assay

Various concentrations of (tetrahydro-β-carboline)-1,3,5-triazine hybrids were tested for in vitro cytotoxic activity on human cancer cell lines representing breast cancer (MCF7), colon (SW620), prostate (DU145), oral (KB), ovary (PA1), leukemia (K562), pancreas (MiaPaCa-2), lung (A549) and normal fibroblasts (NIH3T3). Cytotoxicity was measured by MTT assay, which is based on the principle of uptake of MTT by the metabolically active cells, where it is metabolized by active mitochondria into a blue-colored formazan product that is read spectrophotometrically [29]. Briefly, tumor cells were seeded (5000–10000 cells/well) in 96-well culture plates and incubated at 37 °C in a CO_2 incubator with various concentrations of (tetrahydro-β-carboline)-1,3,5-triazine hybrids analogues ranging from 1 to 100 μg/mL, with relevant controls in triplicate wells. After 72 h, the assay was terminated by the addition of 25 μL of MTT solution (5 mg/mL) in each well. Percentage cytotoxicity was calculated as given below. The IC_{50} values were determined by nonlinear regression using Prism software v 4.01.

$$\text{percentage cytotoxicity} = 100\times[1-(X/R_1)]$$

where X = absorbance of treated sample at 540 nm and R_1 = absorbance of control sample at 540 nm.

6.2.2. Analysis of cell cycle by flow cytometry

For cell cycle analysis of MCF7 and MDA MB231 cells, 1×10^5 cells/ml were plated in 6-well plate. After treatment of cells with the compound **42** and incubation, cells were trysinized and washed with 1 × PBS, 2 ml of cold 70% ethanol was added, and cell suspension was fixed on ice for at least 30 min. Cells were centrifuged for 5 min at 1200 rpm and washed with 1 × PBS. Cell pellets were resuspended in 500 μl of freshly made propidium iodide (PI) staining solution (0.1% Triton X-100, 0.1% sodium citrate, 50 μg/mL PI and 10 μg/mL of RNase A in PBS (with Ca^{2+} and Mg^{2+}) [30]. Cells were analyzed in Flow Cytometer (FACS Calibur, Beckon Dickinson, USA).

6.2.3. Analysis of apoptosis by DNA fragmentation

The DNA fragmentation assay was performed according to the protocol [31] described previously by Nianyu et al. MCF7 and MDA MB231 cells were treated with different concentration of compound **42**. After 24 h, 1×10^5 cells were trypsinized and washed with PBS (4 °C, pH 7.4) and collected by centrifugation at 1200 rpm for 5 min. The pellet was then treated with 0.5 ml of lysis buffer (10 mM Tris-HCL, pH 7.4, 10 mM EDTA, 0.5% sodium dodecyl sulfate) for 10 min on ice. After treatment with RNase A (final concentration, 100 μg/mL) for 1 h at 37 °C, the cells were incubated at 50 °C for 4 h in the presence of 100 μg/mL proteinase K. DNA was precipitated by addition of 50 μl of 3 M sodium acetate (pH 5.2) and 1 ml of cold (4 °C) 100% ethanol to the solution. DNA was then collected and dissolved in TE buffer (10 mM Tris pH 8.0, EDTA 1 mM). For analysis, 10–20 μl of DNA was loaded on a 1.5% agarose gel containing 10 μg/mL ethidium bromide. DNA was visualized under ultraviolet light and photographed.

6.2.4. Visualization of apoptotic bodies by Hoechst staining

For Hoechst staining in MCF7 and MDA MB231, 1×10^4 cells were plated in 6-well plate on coverslip. After treatment of cells with compound **42**, they were washed with 1 × PBS and fixed with 4% paraformaldehyde for 15 min. After fixing cells were stained with 1.0ug/ml of Hoechst stain for 15 min at 4 °C. Excess stain was washed with 1 × PBS, coverslips were mounted with fluorescent mounting solution. Cells were visualized under fluorescent microscope [32].

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