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Antitumor agents 281. Design, synthesis, and biological activity of substituted 4-amino-7,8,9,10-tetrahydro-2*H*-benzo[*h*]chromen-2-one analogs (ATBO) as potent in vitro anticancer agents

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ABSTRACT

In our exploration of new biologically active chemical entities, we designed and synthesized a novel class of antitumor agents, substituted 4-amino-7,8,9,10-tetrahydro-2*H*-benzo[*h*]chromen-2-one (ATBO) analogs. We evaluated their cytotoxic activity against seven human tumor cell lines from different tissues, and established preliminary structure–activity relationships (SAR). All analogs, except **8**, **9**, and **25–27**, displayed potent tumor cell growth inhibitory activity. Especially, compounds **15** and **33** with a 4-methoxyphenyl group at position C-4 were extremely potent with ED_{50} values of 0.008–0.064 and 0.035–0.32 μ M, respectively. Compound **15** was the most potent analog compared with structurally related neo-tanshinlactone (e.g., **1**) and 4-amino-2*H*-benzo[*h*]chromen-2-one (ABO, e.g., **4**) analogs, and thus merits further exploration as an anti-cancer drug candidate.

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One of the most challenging areas of research in both industry and academia is the discovery and development of new medicines.^{1,2} In 2009, 103 new drugs were approved by the FDA's Center for Drug Evaluation and Research (CDER).³ Nineteen new molecular entities (NMEs) and six biologics license applications (BLAs) were filed among these approvals.³ The pharmaceutical industry's demand for new leads with new scaffolds has never been greater.⁴ These facts prompted us to design and discover new biologically active chemical entities.

Previously, our group successfully developed new cytotoxic chemical entities, including neo-tanshinlactone (**1**, Fig. 1) and its 4-ethyl analog **2**,^{5,6} for treating breast cancer, and these compounds are now in extended preclinical study. Structural simplification and optimization is a powerful tool for analog design and lead exploration. For example, this strategy was applied to the natural product halichondrin B, a potent mitotic inhibitor, which led to a new therapeutic medicine, eribulin mesylate.⁷ In our continuous exploration of new chemical entities, we also designed and developed several additional series of novel anticancer agents according to this strategy. These agents include 2-(furan-2-yl) naphthalen-1-ol (FNO),⁸ 6-phenyl-4*H*-furo[3,2-*c*]pyran-4-one (AFPO),⁹

tetrahydronaphthalene-1-ol (TNO),¹⁰ and 4-amino-2*H*-benzo[*h*]chromen-2-one (ABO, **3**, Fig. 1)¹¹ analogs. Lead compounds showed potent antitumor activity and different tumor tissue type selectivity. Braccio et al. first reported four compounds with the ABO scaffold and their cytotoxic activity against Ehrlich ascites tumor cells.¹² We also designed and explored this scaffold based on our studies of neo-tanshinlactone analogs (1 and 2). Compound 4 showed potent and broad antitumor activity compared with 1 and 2.11 Structure-activity relationship (SAR) studies on 3 indicated that (1) secondary amine (R^2 or $R^3 = H$) is preferred over tertiary amine (R^2 and $R^3 \neq H$), (2) bulky groups are favored at R^2/R^3 position, (3) 3'-bromophenyl group can cause dramatic loss of potency, and (4) hydrogen is better than an ethyl group at R¹ position. Our prior studies also suggested that a non-aromatic ring can greatly affect the antitumor activity and cancer cell line selectivity.¹⁰ Consequently, we have now designed scaffold 5 with a non-aromatic five- or six-membered A-ring (Fig. 1). Different amino (R^2/R^3) and A-ring (R^1) substituents were incorporated to establish SAR and identify potent analogs. This Letter reports the design, synthesis, and biological activity of 4-amino-7,8,9,10-tetrahydro-2H-benzo[h]chromen-2-one, (ATBO) analogs.

All target compounds **8–27**, **32**, and **33** were synthesized from the related 4-hydroxy compounds, **6**, **28**, and **29**, respectively, according to the methods reported before (Scheme 1). Chlorides **7**, **30**, and **31** were synthesized by treatment of **6**, **28**, and **29**,

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Figure 1. Structures of neo-tanshinlactone (1), 4-ethyl neo-tanshinlactone (2), previously reported scaffold 3 and its analog 4, and newly designed scaffold 5.

respectively, with POCl₃.^{11,12} Various amino derivatives, **8–27**, **32**, and **33**, were newly prepared by substitution reactions of **7**, **30**, and **31** with different amines according to reported procedures.^{12,13}

All synthesized analogs **8–27**, **32**, and **33** were tested for in vitro cytotoxic activity against a panel of human tumor cell lines according to previously published methods (Table 1).^{8,14,15} Cell lines included A549 (non small cell lung cancer), DU145 (prostate cancer cell line), KB (nasopharyngeal carcinoma), KB-VIN (vincristine-resistant MDR KB subline), MDA-MB-231 (estrogen receptor negative breast cancer), SK-BR-3 (estrogen receptor negative, HER2 over-expressing breast cancer), and ZR-75-1 (estrogen receptor positive breast cancer).

Regarding ATBO analogs with aliphatic R_2/R_3 amino substituents, **11** with a cyclohexyl group showed greater activity than the methyl, propyl, and cyclopropyl derivatives **8–10**. Analog **10** with a cyclopropyl group displayed tumor cell line selectivity against KB, KB-VIN, and ZR-75-1, while **8** and **9** showed greatly

reduced or no activity against all tested cell lines. These findings suggested that a cyclic group is better than a linear group for cytotoxic activity, and a bulky amino group is favored. The tertiary amines **25–27** were not active, regardless of cyclic or linear amino groups; thus, a secondary amine is crucial for antitumor activity. The above results are consistent with our previous studies for ABO analogs.¹⁰ Interestingly, **8** and **9** showed unique selectivity against ZR-75-1 compared with other cell lines tested, although the inhibitory potency was only moderate. In addition, **11** with a cyclohexyl group showed similar potency to **12** with a phenyl group, which indicated that aromaticity of the amino substituent is not an essential factor.

Regarding aromatic R_2/R_3 substituents, we explored different groups at the 2', 3', and 4' positions. Generally, all aromatic derivatives **12–24** showed significant cytotoxicity against the human tumor cell lines tested. Among them, 4'-MeO **15** was the most potent analog against all tested tumor cell lines (ED₅₀ values of 0.008–0.064 µM). It was 2- to 3-fold more potent than the related



Scheme 1. Reagents and conditions: (a) POCl₃, Et₃N, reflux, 1 h; (b) aliphatic amines, EtOH, reflux, 2 h; (c) aromatic amines, ethylene glycol, 160 °C, 1 h.

Table 1			
Cytotoxicity of 8-2	27, 32–33 against humai	n tumor cell line	panel ^a

Compd	KB	KB-VIN	A549	DU145	SKBR-3	ZR-75-1	MDA-MB-231
1	>30	>30	>30	>30	0.95	0.95	>30
4	0.11 ± 0.01	0.13 ± 0.02	0.17 ± 0.03	0.11 ± 0.02	0.13 ± 0.01	0.008 ± 0.003	0.025 ± 0.003
8	>30	>30	>30	>30	>30	7.86 ± 0.22	>30
9	>30	>30	>30	>30	>30	7.04 ± 0.19	>30
10	2.24 ± 0.26	3.28 ± 1.24	19.59 ± 10.48	8.37 ± 2.22	8.76 ± 2.57	2.16 ± 0.12	>30
11	1.23 ± 0.12	1.29 ± 0.42	2.00 ± 0.14	1.21 ± 0.01	1.49 ± 0.15	1.11 ± 0.17	>30
12	1.15 ± 0.43	2.01 ± 0.57	1.53 ± 0.50	1.35 ± 0.69	1.33 ± 0.61	2.51 ± 0.38	>30
13	0.91 ± 0.29	1.36 ± 0.46	1.64 ± 0.71	1.52 ± 0.69	1.29 ± 0.73	1.28 ± 0.39	27.54 ± 1.97
14	0.16 ± 0.03	0.12 ± 0.02	0.15 ± 0.03	0.16 ± 0.02	0.44 ± 0.28	10.34 ± 1.25	14.11 ± 0.63
15	0.037 ± 0.005	0.046 ± 0.002	0.049 ± 0.005	0.038 ± 0.005	0.064 ± 0.027	0.024 ± 0.002	0.008 ± 0.002
16	1.04 ± 0.26	1.02 ± 0.68	1.02 ± 0.36	0.96 ± 0.38	1.23 ± 0.41	14.24 ± 0.32	>30
17	0.63 ± 0.16	$0.52 \pm \pm 0.01$	0.53 ± 0.20	0.51 ± 0.20	0.76 ± 0.22	10.00 ± 1.08	6.76 ± 0.54
18	0.80 ± 0.20	2.41 ± 1.30	1.14 ± 0.32	2.43 ± 1.32	5.61 ± 4.63	2.43 ± 0.13	7.54 ± 1.64
19	0.87 ± 0.21	0.96 ± 0.27	1.08 ± 0.36	0.81 ± 0.23	0.96 ± 0.12	0.69 ± 0.03	7.48 ± 0.31
20	0.20 ± 0.06	0.20 ± 0.06	0.16 ± 0.05	0.11 ± 0.03	0.41 ± 0.15	0.18 ± 0.02	0.13 ± 0.01
21	0.24 ± 0.02	0.18 ± 0.02	0.24 ± 0.02	0.15 ± 0.04	0.51 ± 0.28	1.84 ± 0.10	3.87 ± 0.13
22	0.22 ± 0.05	0.16 ± 0.01	0.25 ± 0.02	0.22 ± 0.06	0.31 ± 0.15	1.53 ± 0.09	3.52 ± 0.16
23	0.83 ± 0.05	1.08 ± 0.17	1.52 ± 0.10	1.29 ± 0.33	1.02 ± 0.17	0.49 ± 0.05	4.59 ± 0.54
24	0.48 ± 0.16	0.33 ± 0.004	0.79 ± 0.21	0.50 ± 0.11	0.86 ± 0.26	1.26 ± 0.06	3.08 ± 0.44
25	20.44 ± 3.80	19.58 ± 4.60	26.41 ± 1.96	15.86 ± 1.29	>30	17.06 ± 1.34	>30
26	>30	>30	>30	>30	>30	18.37 ± 1.06	>30
27	>30	23.52 ± 9.78	>30	>30	27.90 ± 3.39	19.30 ± 1.05	>30
32	0.11 ± 0.02	0.10 ± 0.03	0.13 ± 0.01	0.090 ± 0.01	0.15 ± 0.03	0.39 ± 0.07	0.27 ± 0.04
33	0.035 ± 0.02	0.044 ± 0.01	0.049 ± 0.001	0.043 ± 0.01	0.037 ± 0.02	0.32 ± 0.06	0.23 ± 0.02

^a Mean $ED_{50} \pm SD$ (μM), from 2 or more independent tests.

ABO analog **4** against all cell lines, except ZR-75-1. Importantly, **15** also exhibited the highest cytotoxicity against MDA-MB-231 (ER negative breast cancer) compared with other cell lines. Analogs **10–13**, **19**, **23**, and **24** were less potent against MDA-MB-231 and **14**, **16**, **21**, **22** were less potent against both MDA-MB-231 and ZR-75-1 compared with other tested cell lines, which indicated different cancer cell line selectivity among ATBO analogs.

We further investigated the effect of ring-A. Analogs **15** and **32**, with an unsubstituted six and five-membered ring, respectively, as well as analog **33**, with a six-membered ring-A with *gem*-dimethyl substitution, displayed significant inhibition against all tested human tumor cell lines, but different selectivity against tumor cell lines from different tissues. Against KB, KB-VIN, A549, DU145, and SKBR-3, **15** and **33** were at least 2-fold more potent than **32**. Against ZR-75-1 and MDA-MB-231, **15** (ED₅₀ 0.024 and 0.008 μ M, respectively) showed much higher activity as compared with **32** and **33**. These results together with our previous findings indicated that a non-aromatic ring-A may lead to changes in both potency and cancer cell line selectivity of certain analogs. The size and identity of ring-A substituents are also important factors.

In conclusion, our structural modification and optimization resulted in a novel class of in vitro anti-cancer agents, ATBO. The results indicated that the non-aromatic ring-A of **5** is critical to both potency and cancer cell line selectivity. Based on this study, the preliminary SAR findings for the ATBO class were similar to those with ABO analogs. (1) Secondary amine (R_2 or $R_3 = H$) is preferred over tertiary amine (R_2 and $R_3 \neq H$), (2) bulky groups are favored at R_2/R_3 position, (3) aromaticity is not required at R_2/R_3 position, and (4) non-aromatic ring-A can increase potency and cancer cell line selectivity for certain analogs, such as **15**. Compound **15** was the most potent analog (ED₅₀ values of 0.008–0.064 μ M) among all ABO and ATBO derivatives, and thus, is a promising new lead compound for further development toward a potential clinical trials candidate.

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References and notes

- Bleicher Konrad, H.; Bohm, H.-J.; Muller, K.; Alanine Alexander, I. Nat. Rev. Drug Disc. 2003, 2, 369.
- 2. Vallance, P.; Williams, P.; Dollery, C. Clin. Pharmacol. Ther. 2010, 87, 525.
- 3. Hughes, B. Nat. Rev. Drug Disc. 2010, 9, 89.
- Crommelin, D.; Stolk, P.; Besancon, L.; Shah, V.; Midha, K.; Leufkens, H. Nat. Rev. Drug Disc. 2010, 9, 99.
- Wang, X.; Bastow, K. F.; Sun, C. M.; Lin, Y. L.; Yu, H. J.; Don, M. J.; Wu, T. S.; Nakamura, S.; Lee, K. H. J. Med. Chem. 2004, 47, 5816.
- Wang, X.; Nakagawa-Goto, K.; Bastow, K. F.; Don, M. J.; Lin, Y. L.; Wu, T. S.; Lee, K. H. J. Med. Chem. 2006, 49, 5631.
- Kim, D. S.; Dong, C. G.; Kim, J. T.; Guo, H.; Huang, J.; Tiseni, P. S.; Kishi, Y. J. Am. Chem. Soc. 2009, 131, 15636.
- Dong, Y.; Shi, Q.; Liu, Y.-N.; Wang, X.; Bastow, K. F.; Lee, K.-H. J. Med. Chem. 2009, 52, 3586.
- Dong, Y.; Shi, Q.; Nakagawa-Goto, K.; Wu, P.-C.; Morris-Natschke, S. L.; Brossi, A.; Bastow, K. F.; Lang, J.-Y.; Hung, M.-C.; Lee, K.-H. *Bioorg. Med. Chem.* 2010, *18*, 803.
- Dong, Y.; Shi, Q.; Nakagawa-Goto, K.; Wu, P. C.; Bastow, K. F.; Morris-Natschke, S. L.; Lee, K. H. Bioorg. Med. Chem. Lett. 2009, 19, 6289.
- 11. Dong, Y.; Nakagawa-Goto, K.; Lai, C. Y.; Morris-Natschke, S. L.; Bastow, K. F.; Lee, K. H. Bioorg. Med. Chem. Lett. **2010**, 20, 4085.
- Di Braccio, M.; Grossi, G.; Roma, G.; Marzano, C.; Baccichetti, F.; Simonato, M.; Bordin, F. Farmaco 2003, 58, 1083.
- 13. Alberola, A.; Alvaro, R.; Ortega, A. G.; Sadaba, M. L.; Sanudo, M. C. *Tetrahedron* 1999, 55, 13211.
- 14. Solubility of **12–14** and **16–18** in DMSO is not very great. Work is ongoing to solve the solubility issue and results will be reported in due course.
- Spectroscopic data. 4-(methylamino)-7,8,9,10-tetrahydro-2H-benzo/h]chromen-2-one (8): ¹H NMR (400 MHz, CDCl₃): δ 7.16 (d, 1H, J = 8.4 Hz, Ar-H), 6.96 (d, 1H, J = 8.4 Hz, Ar-H), 5.28 (s, 2H, NH & 3-H), 3.00 (d, 3H, J = 4.8 Hz, NCH₃), 2.90 (t, 2H, J = 6.0 Hz, 10-H), 2.83 (t, 2H, J = 6.0 Hz, 7-H), 1.82 (m, 4H, 8 & 9-H). MS m/ z 228 (M⁺-1). 4-(propylamino)-7,8,9,10-tetrahydro-2H-benzo[h]chromen-2-one (9): ¹H NMR (400 MHz, CDCl₃): δ 7.18 (d, 1H, J = 8.0 Hz, Ar-H), 6.96 (d, 1H, J = 8.0 Hz, Ar-H), 5.28 (s, 1H, 3-H), 5.19 (br s, 1H, NH), 3.22 (q, 2H, J = 5.2 Hz, NCH₂), 2.90 (t, 2H, J = 6.0 Hz, 10-H), 2.82 (t, 2H, J = 6.0 Hz, 7-H), 1.78 (m, 6H, 8, 9 & 2'-H), 1.04 (t, 3H, J = 7.2 Hz, 3'-H). MS m/z 258 (M*+1). 4-(cyclopropylamino)-7,8,9,10-tetrahydro-2H-benzo[h]chromen-2-one (**10**): ¹H NMR (400 MHz, CDCl₃): δ 7.10 (d, 1H, *J* = 8.4 Hz, Ar-H), 6.94 (d, 1H, *J* = 8.0 Hz, Ar-H), 5.72 (s, 1H, 3-H), 5.41 (br s, 1H, NH), 2.90 (t, 2H, J = 5.6 Hz, 10-H), 2.83 (t, 2H, J = 5.6 Hz, 7-H), 2.58 (m, 1H, NCH), 1.83 (m, 4H, 8 & 9-H), 0.91 (dd, 2H, J = 5.6, 5.2 Hz, NCHCH₂), 0.68 (dd, 2H, J = 5.2, 4.4 Hz, NCHCH₂). MS m/z 254 (M⁺-1). 4-(cyclohexylamino)-7,8,9,10-tetrahydro-2H-benzo[h]chromen-2-one (**11**): ¹H NMR (400 MHz, CDCl₃): δ 7.15 (d, 1H, J = 8.0 Hz, Ar-H), 6.95 (d, 1H, J = 8.4 Hz, Ar-H), J = 6.0 Hz, 10-H), 2.81 (t, 2H, J = 6.0 Hz, 7-H), 2.12 (m, 2H, 2' & 6'-H), 1.78 (m, 6H, 8, 9 & 2' & 6'-H), 1.28 (m, 6H, 3' & 4'& 5'-H). MS m/z 298 (M⁺+1). 4phenylamino-7,8,9,10-tetrahydro-2H-benzo[h]chromen-2-one (12): ¹H NMR (400 MHz, DMSO- d_6): δ 9.20 (br s, 1H, NH), 7.96 (d, 1H, J = 8.2 Hz, Ar-H), 7.48 (dd, 1H, J = 8.2 and 7.4 Hz, Ar-H), 7.36 (d, 1H, J = 8.2 Hz, Ar-H), 7.36 (d, 1H,

J = 7.4 Hz, Ar-H), 7.28 (dd, 1H, J = 7.4 & 7.2 Hz, Ar-H), 7.12 (d, 1H, J = 8.2 Hz, Ar-H), 5.27 (s, 1H, 3-H), 2.86-2.74 (m, 4H, 7 & 10-H), 1.85-1.72 (m, 4H, 8 & 9-H). MS m/z 292 (M*+1). 4-[(4-methylphenyl)amino]-7,8,9,10-tetrahydro-2H-benzo[h]chromen-2-one (**13** $): ¹H NMR (400 MHz, DMSO-d₆): <math>\delta$ 9.14 (br s, 1H, NH), 7.95 (d, 1H, J = 8.3 Hz, Ar-H), 7.28 (d, 2H, J = 8.2 Hz, Ar-H), 7.23 (d, 2H, r = 8.2 Hz, Ar-H), 5.19 (s, 1H, 3-H), 2.86–2.74 (m, 4H, 7 &10-H), 2.34 (s, 3H, Ar-CH₃), 1.84-1.71 (m, 4H, 8 & 9-H). MS m/z 306 (M⁺+1). 4-[(4'ethylphenyl)amino]-7,8,9,10-tetrahydro-2H-benzo[h]chromen-2-one (14): NMR (400 MHz, DMSO-d₆): δ 9.14 (br s, 1H, NH), 7.95 (d, 1H, J = 8.4 Hz, Ar-H), 7.31 (d, 2H, J = 8.4 Hz, Ar-H), 7.25 (d, 2H, J = 8.4 Hz, Ar-H), 7.11 (d, 1H, J = 8.4 Hz, Ar-H), 5.20 (s, 1H, 3-H), 2.86-2.73 (m, 4H, 7 & 10-H), 2.64 (q, 2H, J = 7.5 Hz, Ar-CH₂CH₃), 1.84-1.72 (m, 4H, 8 & 9-H), 1.21 (t, 3H, J = 7.5 Hz, Ar-CH₂CH₃). MS m/z 320 (M⁺+1). 4-((4'-methoxyphenyl)amino)-7,8,9,10-tetrahydro-2H-benzo[h]chromen-2-one (**15**): ¹H NMR (400 MHz, DMSO-d₆): δ 9.09 (br s, 1H, NH), 7.94 (d, 1H, J = 8.8 Hz, Ar-H), 7.27 (d, 2H, J = 8.0 Hz, Ar-H), 7.10 (d, 1H, J = 8.0 Hz, Ar-H), 7.04 (d, 2H, J = 8.0 Hz, Ar-H), 5.05 (s, 1H, 3-H), 3.79 (s, 3H, OCH₃), 2.82 (t, 2H, J = 5.6 Hz, 10-H), 2.76 (t, 2H, J = 5.6 Hz, 7-H), 1.78 (m, 4H, 8 & 9-H). MS m/z 320 (M⁺-1). 4-[(4'-fluorophenyl)amino]-7,8,9,10-tetrahydro-2Hbenzo[h]chromen-2-one (16): ¹H NMR (400 MHz, DMSO-d₆): δ 9.18 (br s, 1H, NH), 7.93 (d, 1H, J = 8.2 Hz, Ar-H), 7.42-7.36 (m, 2H, Ar-H), 7.35-7.28 (m, 2H, Ar-H), 7.12 (d, 1H, J = 8.2 Hz, Ar-H), 5.16 (s, 1H, 3-H), 2.86-2.73 (m, 4H, 7 & 10-H), 1.85-1.71 (m, 4H, 8 & 9-H). MS m/z 310 (M⁺+1). 4-[(4'-bromophenyl)amino]-7,8,9,10-tetrahydro-2H-benzo[h]chromen-2-one (17): ¹H NMR (400 MHz, DMSOd₆): δ 9.21 (br s, 1H, NH), 7.92 (d, 1H, J = 8.3 Hz, Ar-H), 7.68–7.62 (m, 2H, Ar-H), 7.36-7.31 (m, 2H, Ar-H), 7.12 (d, 1H, J = 8.3 Hz, Ar-H), 5.34 (s, 1H, 3-H), 2.86-2.74 (m, 4H, 7 &10-H), 1.85-1.72 (m, 4H, 8 & 9-H). MS m/z 368 and 370 (1:1, M⁺-1). 4-[(2'-methylphenyl)amino]-7,8,9,10-tetrahydro-2H-benzo[h]chromen-2one (18): ¹H NMR (400 MHz, DMSO-d₆): δ 9.10 (br s, 1H, NH), 7.96 (d, 1H, J = 8.2 Hz, Ar-H), 7.44–7.39 (m, 1H, Ar-H), 7.36–7.31 (m, 2H, Ar-H), 7.29–7.24 (m, 1H, Ar-H), 7.12 (d, 1H, J = 8.2 Hz, Ar-H), 4.54 (s, 1H, 3-H), 2.86-2.74 (m, 4H, 7 &10-H), 2.18 (s, 3H, Ar-CH₃), 1.85–1.72 (m, 4H, 8 & 9-H). MS m/z 306 (M⁺+1). 4-[(2'-methoxyphenyl)amino]-7,8,9,10-tetrahydro-2H-benzo[h]chromen-2-one (19): ¹H NMR (400 MHz, DMSO- d_6): δ 8.90 (br s, 1H, NH), 7.96 (d, 1H, J = 8.2 Hz, Àr-H), 7.42–7.34 (m, 1H, Ar-H), 7.32–7.26 (m, 1H, Ar-H), 7.20 (d, 1H, J = 8.2 Hz, Ar-H), 7.10 (d, 1H, J = 8.2 Hz, Ar-H), 7.09-7.05 (m, 1H, Ar-H), 4.68 (s, 1H, 3-H), 3.79 (s, 3H, OCH₃), 2.96-2.72 (m, 4H, 7 &10-H), 1.86-1.70 (m, 4H, 8 & 9-H). MS m/z 322 (M⁺+1). 4-((2'-bronophenyl)amino]-7,8,9,10-tetrahydro-2H-benzo[h]chromen-2-one (**20**): ¹H NMR (400 MHz, DMSO-d₆): δ 9.21 (br s, 1H, NH), 7.98-7.90 (m, 1H, Ar-H), 7.70-7.62 (m, 1H, Ar-H), 7.50-7.24 (m, 3H, Ar-H), 7.12 (d, 1H, J = 8.3 Hz, Ar-H), 5.35 (s, 1H, 3-H), 2.86-2.74 (m, 4H, 7 & 10-H), 1.85-1.72 (m, 4H, 8 & 9-H). MS m/z 368 and 370 (1:1, M⁺-1). 4-[(3' methylphenyl)amino]-7,8,9,10-tetrahydro-2H-benzo[h]chromen-2-one (21): NMR (400 MHz, DMSO-d₆): δ 9.15 (br s, 1H, NH), 7.95 (d, 1H, J = 8.3 Hz, ArH), 7.36 (t, 1H, J = 7.7 Hz, Ar-H), 7.19-7.08 (m, 3H, Ar-H), 5.26 (s, 1H, 3-H), 2.86-2.74 (m, 4H, 7 &10-H), 2.36 (s, 3H, Ar-CH₃), 1.85-1.72 (m, 4H, 8 & 9-H). MS m/z (M⁺+1). 306 4-[(3'-methoxyphenyl)amino]-7,8,9,10-tetrahydro-2Hbenzo[h]chromen-2-one (22): ¹H NMR (400 MHz, DMSO-d₆): δ 9.16 (br s, 1H, NH), 7.94 (d, 1H, J = 8.4 Hz, Ar-H), 7.38 (t, 1H, J = 8.1 Hz, Ar-H), 7.11 (d, 1H, J = 8.4 Hz, Ar-H), 6.98-6.92 (m, 1H, Ar-H), 6.92-6.89 (m, 1H, Ar-H), 6.85 (dd, 1H, J = 8.1 and 2.5 Hz, Ar-H), 5.35 (s, 1H, 3-H), 3.79 (s, 3H, OCH₃), 2.86-2.74 (m, 4H, 7 &10-H), 1.85-1.72 (m, 4H, 8 & 9-H). MS m/z 322 (M⁺+1). 4-[(3'bromophenyl)amino]-7,8,9,10-tetrahydro-2H-benzo[h]chromen-2-one (23): 1H NMR (400 MHz, DMSO-d₆): δ 9.24 (br s, 1H, NH), 7.91 (d, 1H, J = 8.2 Hz, Ar-H), 7.48-7.59-7.56 (m, 1H, Ar-H), 7.40 (m, 3H, Ar-H), 7.13 (d, 1H, J = 8.2 Hz, Ar-H), 5.39 (s, 1H, 3-H), 2.86-2.74 (m, 4H, 7 & 10-H), 1.85-1.72 (m, 4H, 8 & 9-H). MS m/z 368 and 370 (1:1, M^{*}-1). 4-(naphthalen-1-ylamino)-7,8,9,10-tetrahydro-2H-benzo[h]chromen-2-one (**24**): ¹H NMR (400 MHz, DMSO- d_6): δ 9.54 (br s, 1H, NH), 8.13 (d, 1H, J = 8.2 Hz, Ar-H), 8.05 (d, 1H, J = 7.4 Hz, Ar-H), 8.01 (d, 1H, J = 8.2 Hz, Ar-H), 7.81 (d, 1H, J = 8.2 Hz, Ar-H), 7.65 (dd, 1H, J = 8.2 and 7.4 Hz, Ar-H), 7.64-7.53 (m, 3H, Ar-H), 7.18 (d, 1H, J = 8.2 Hz, Ar-H), 4.50 (s, 1H, 3-H), 2.90-2.75 (m, 4H, 7 &10-H), 1.86-1.74 (m, 4H, 8 & 9-H). MS m/z 342 (M⁺+1). 4-(dipropylamino)-7,8,9,10-tetrahydro-2H-benzo[h]chromen-2-one (25): ¹H NMR (400 MHz, CDCl₃): δ 7.37 (d, 1H, J = 8.4 Hz, Ar-H), 6.94 (d, 1H, J = 8.4 Hz, Ar-H), 5.59 (s, 1H, 3-H), 3.27 (t, 4H, J = 6.0 Hz, N(CH₂)₂), 2.91 (t, 2H, J = 5.6 Hz, 10-H), 2.82 (t, 2H, J = 5.6 Hz, 7-H), 1.83 (m, 4H, 8 & 9-H), 1.68 (m, 4H, N(CH₂ CH₂ CH₃)₂), 0.90 (t, 6H, J = 7.6 Hz, N(CH₂ CH₂ CH₃)₂). MS m/z 300 (M⁺+1). 4-(piperidin-1-yl)-7,8,9,10-tetrahydro-2H-benzo[h]chromen-2-one (26): ¹H NMR (400 MHz, CDCl₃): δ 7.32 (d, 1H, J = 8.0 Hz, Ar-H), 6.94 (d, 1H, J = 8.4 Hz, Ar-H), 5.64 (s, 1H, 3-H), 3.20 (t, 4H, J = 5.6 Hz, N(CH₂)₂), 2.90 (t, 2H, J = 5.6 Hz, 10-H), 2.82 (t, 2H, J = 5.6 Hz, 7-H), 1.81 (m, 8H, 8 & 9 & 3' & 5'-H), 1.72 (m, 2H, NCH₂ CH₂ CH₂). MS m/z 284 (M⁺+1). 4-morpholino-7,8,9,10-tetrahydro-2H-benzo[h]chromen-2-one (**27**): ¹H NMR (400 MHz, CDCl₃): δ 7.32 (d, 1H, J = 8.4 Hz, Ar-H), 6.96 (d, 1H, J = 8.4 Hz, Ar-H), 5.69 (s, 1H, 3-H), 3.92 (t, 4H, J = 4.8 Hz, O(CH₂)₂), 3.23 (t, 4H, J = 4.8 Hz, N(CH₂)₂), 2.90 (t, 2H, J = 5.6 Hz, 10-H), 2.83 (t, 2H, J = 5.6 Hz, 7-H), 1.83 (m, 4H, 8 & 9-H). MS m/z 286 (M⁺+1). 4-((4'methoxyphenyl)amino)-8,9-dihydrocyclopenta[h]chromen-2(7H)-one (**32**): ^{1}H NMR (400 MHz, DMSO- d_6): δ 9.13 (br s, 1H, NH), 8.00 (d, 1H, J = 8.0 Hz, Ar-H), 7.27 (m, 3H, Ar-H), 7.04 (d, 2H, J = 8.4 Hz, Ar-H), 5.04 (s, 1H, 3-H), 3.79 (s, 3H, OCH₃), 3.00 (m, 4H, 7 & 9-H), 2.13 (m, 2H, 8-H). MS m/z 306 (M⁺-1). 4-((4'methoxyphenyl)amino)-7,7-dimethyl-7,8,9,10-tetrahydro-2H-benzo[h]chromen-2-one (33): ¹H NMR (400 MHz, DMSO-d₆): δ 9.10 (br s, 1H, NH), 8.00 (d, 1H, J = 8.4 Hz, Ar-H), 7.41 (d, 1H, J = 8.4 Hz, Ar-H), 7.25 (d, 2H, J = 8.4 Hz, Ar-H), 7.04 (d, 2H, J = 8.8 Hz, Ar-H), 5.04 (s, 1H, 3-H), 3.79 (s, 3H, OCH₃), 2.76 (t, 2H, J = 6.0 Hz, 10-H), 1.79 (m, 2H, 9-H), 1.65 (m, 2H, 8-H), 1.30 (s, 6H, (CH₃)₂). MS m/z 348 (M⁺-1).