Bioorganic & Medicinal Chemistry Letters 20 (2010) 6504–6507

Synthesis and antihyperlipidemic activity of novel coumarin bisindole derivatives

Koneni V. Sashidhara [a,*], Abdhesh Kumar [a], Manoj Kumar [a], Anuj Srivastava [b], and Anju Puri [b]

Medicinal and Process Chemistry Division [a], Central Drug Research Institute, (CDRI-CSIR), Lucknow, 226 001, India

Biochemistry Division [b], Central Drug Research Institute, (CDRI- CSIR), Lucknow, 226 001, India

ABSTRACT

A series of novel coumarin bisindole heterocycles were synthesized following an uncommon method and evaluated for their antihyperlipidemic activity in hyperlipidemic hamster model. Among 12 compounds tested, the compound **5e** showed potent antihyperlipidemic activity and was found to decrease the plasma triglyceride levels (TG) by 55%, total cholesterol (TC) by 20%, accompanied by an increase in HDL-C/TC ratio by 42% in hyperlipidemic rats to a greater degree than some of the reference statins.

Keywords: Synthesis, Coumarin, Indole, Statins, Cholesterol lowering, Hypolipidemic Agents.

*Corresponding author. Tel.: +91 9919317940; Fax: +91-522-2623405.

Email: sashidhar123@gmail.com, kv_sashidhara@cdri.res.in (K. V. Sashidhara)

Cholesterol plays a major role in the assembly of membranes and performs other important biological functions in human heart health. However, when plasma cholesterol exceeds the level required for these functions, it results in the development of atherosclerotic cardiovascular disease such as coronary heart disease and stroke.[1] Hyperlipidemia may also induce other abnormalities like oxidation of free fatty acids, leading to the formation of ketone bodies as well as masking liver and muscles resistance to insulin which initiates the progress of diabetes in patients.[2]

Recent treatment guidelines for hyperlipidemia emphasize the importance of modifying lipid metabolism beyond lowering low-density lipoprotein- cholesterol (LDL-C), mainly by lowering TG and raising high-density lipoprotein- cholesterol (HDL-C).[3] HMG-CoA reductase is responsible for converting HMG-CoA to mevalonate, these results in a decrease in mevalonate, a precursor of cholesterol, and a subsequent decrease in hepatic cholesterol levels and increase in uptake of LDL cholesterol. Statins represent the major class of hypolipidemic drugs on the market. They (such as atorvastatin, lovastatin, fluvastatin, simvastatin and pravastatin)[4] act through the inhibition of HMG-CoA reductase, a pivotal enzyme in the cholesterol biosynthetic pathway, thus leading to a reduction of cholesterol concentration and a subsequent increase in expression of the low density lipoprotein receptor (LDLR), the main receptor involved in the hepatic clearance of LDL cholesterol.[5] The beneficial effect of statins on plasma lipoproteins is reflected by a significant reduction in lesions progression and major cardiovascular events (by 25-40%) as demonstrated by many prospective primary and secondary prevention trials.[6] However, most patients still experience adverse coronary events despite statin therapy. In addition, recent reports of undesirable side effects (myopathy) of some 'super statins' indicate that the scope of improving the potency of this class of drugs may be modest.[7] Therefore, there is a constant need for a improving their potency to treat hyperlipidemia without severe side effects.

Many drugs contain indole moiety, either as a basic template or as an attached group to invoke particular properties. Indole and their derivatives are known to exert antihypertension[8], antitubercular,[9] anticancer activity,[10] antiviral,[11] Alzheimer disease & antioxidant properties,[12] and free radical induced lipid peroxidation.[13] Furthermore, fluvastatin, which is a synthetic member of the statins class of compounds contains indole moiety in its molecular makeup.

On the other hand, natural as well as synthetic coumarins have recently drawn much attention due to its diverse pharmacological activities. Many coumarins and their derivatives underwent extensive investigations aimed to assess their potential beneficial effects on human health,[14,15] such as anti-HIV,[16] anticancer,[17,18] anticoagulant,[19] and antimicrobial.[20] In addition many coumarin derivatives have the special ability to scavenge reactive oxygen species (ROS) and to influence processes involving free radical injury.[21] Furthermore, coumarins (umbelliferone) and its derivatives are shown to have lipid lowering potential.[22,23] The recognition of key structural features within coumarin family is crucial for the design and development of new analogues with improved activity and for the characterization of their mechanism of action and potential side effects. The

different substituent's in the coumarin nucleus strongly influence the biological activity of the resulting derivatives.

In the design of new drugs, the development of hybrid molecules through the combination of different pharmacophores in one frame may lead to compounds with interesting biological profiles. Adopting this approach, several research groups have recently reported hybrid molecules by coupling coumarins with different bioactive molecules like: resveratrol, maleimide and alpha-lipoic acid; these studies resulted in new compounds showing antiplatelet, antioxidant and anti-inflammatory activities.[24-26] Following this paradigm, we have designed and synthesized a series of novel compounds that have both coumarin and indole entities in one molecule and have evaluated them for their antihyperlipidemic activity. Figure 1 shows the chemical structures of some potent hyperlipidemia molecules that either contains a coumarin or indole in their molecular makeup and form the basis of our designed prototype.

Thus, in continuation of our on going drug discovery programme, for developing new antidyslipidemic drugs, we embarked on the synthesis of novel coumarin bisindole hybrids as potential antidyslipidemic agents. [27]

The route followed for the preparation of coumarin bisindole hybrids are illustrated in scheme 1. The Duff reaction on naphthalen-1-ol **1** gave compound **2,** which was engaged in a Knoevenagel type reaction with appropriate active methylene compounds, resulting in the formation of coumarinic compounds (**3**–**4**). Furthermore, an efficient electrophilic substitution of suitable indoles with these coumarin aldehydes derivatives using iodine in acetonitrile furnished coumarin bisindole hybrids (**5a**–**5h**)[28] (Scheme 1). Similarly, another series of coumarin bisindole hybrids were prepared starting from 2-*sec*-butylphenol **6** which was subjected to same series of above mentioned transformations resulting in another set of coumarin bisindole hybrids (**9a**–**9d**) (Scheme 1). The structures of the compounds were substantiated by ^{1}H NMR, ^{13}C NMR, Mass spectrometry and IR spectroscopy. The purity of these compounds was ascertained by TLC and spectral analysis (see supporting information).

In the present study, we carried out experiments to investigate the antidyslipidemic activity of coumarin bisindole hybrids (**5a**–**5h** & **9a**–**9d**) in the high fat diet (HFD) fed dyslipidemic hamster model, which has been reported as an ideal in vivo model for evaluating antidyslipidemic drugs.[29-30] The synthesized coumarin bisindole hybrids were administered orally at the dose of 10 mg/kg body weight for seven consecutive days. Normal hamsters fed with HFD and given drug vehicle (water) only served as control animals. No significant differences were observed in the food intake and weight gain between the groups. The results of the biological screening have been summarized in Table 1. Among 12 compounds tested, three compounds (**5a**, **5e** and **9d)** showed potent antihyperlipidemic activity either by lowering of TG and TC levels while increasing HDL-C/TC ratio. The higher TG levels and lower HDL–C increase the risk of coronary heart disease (CHD).[31] The HDL–C mediate the reverse transport of cholesterol from peripheral tissues to the liver for disposal by excretion into bile. This process will disallow the slow accumulation of lipids in artery walls. The compounds **5a** and **5e** exhibited both the above-mentioned properties. Thus, the compounds **5a**, **5e** and **9d** significantly lowered the plasma triglycerides by 50%, 55% & 57%, total cholesterol by 12%, 20% & 43% and increased the HDL-C/TC ratio by 30%, 42% & 39% respectively, which is considered a beneficiary effect in the treatment of dyslipidemia condition. These

data are comparable with standard drug atorvastatin at the same dose of 10 mg/kg body weight decreased the levels of TG by 63%, total cholesterol by 14% and increase in HDL-C/TC ratio by 35% (Figure 2). While, lovastatin in the same model at a higher dose of 25 mg/kg body weight decreased the level of TG by 29%, total cholesterol by 9% and increase in HDL-C/TC ratio by 12%. The lipid lowering effect of other coumarin bisindole hybrids was modest when compared to **5a**, **5e** and **9d** derivatives.

A closure look into the structure activity relationship indicates that both the series of coumarin bisindole hybrids synthesized (**5a**–**5h**) and (**9a**–**9d**), show significant activity. Furthermore, in both the series of compounds, as far as coumarin pharmacophore is considered, it revealed that the substitution at position 3 play a pivotal role, the presence of ethyl ester over methyl is preferred for pronounced activity. On the other hand, cursory look at the lower indole pharmacophore highlight that the unsubstituted indoles (**5a**, **5e**) have good activity profile compared to substituted indoles (**5b**, **5c**, **5d**, **5f**, **5g**, **5h**). However, the opposite is true for second series (**9a**–**9d**) of coumarin indole derivatives derived from 2-*sec*-butylphenol **6.**

In conclusion, a series of novel substituted coumarin bisindole hybrids (**5a–5h & 9a–9d**) have been synthesized following an uncommon method. Among the synthesized compounds, the compound **5e** was found to be the most potent in the hamster model and was several times better than that of the reference standard lovastatin and atorvastatin. Initial studies indicate compound **5e** to be devoid of cytotoxicity in normal cells. Furthermore, dose dependent studies on **5e** done at different doses 2.5, 5, 10, and 25 mg/kg b wt, revealed that the optimum dose for compound **5e** was at 10 mg/kg b wt. Compound **5e** merits further detailed investigation in our continuing program to generate and develop lipid lowering agents.

Acknowledgments

Instrumentation facilities from SAIF, CDRI are gratefully acknowledged. A.K. and M.K. are thankful to CSIR, New Delhi, India for financial support. This is CDRI publication number 7953.

References and notes:

1. Inoue, T.; Hayashi, M.; Takayanagi, K.; Morooka, S. *Artherosclerosis.* **2002**, *160*, 369.
2. Stehouwer, C. D. A.; Lambert, J.; Donker, A. J. M.; van Hindbergh, V. W. M. *Cardiovasc. Res.* **1997**, *43*, 55.
3. Hubert, S.; Winfried, M. *Current Topics in Medicinal Chemistry*, **2005**, *5*, 233.
4. Ahmad, S.; Madsen, C. S.; Stein, P. D.; Janovitz, E.; Huang, C.; Ngu, K.; Bisaha, S.; Kennedy, L. J.; Chen, B. C.; Zhao, R.; Sitkoff, D.; Monshizadegan, H.; Yin, X.;. Ryan, C. S.; Zhang, R.; Giancarli, M.; Bird, E.; Chang, M.; Chen, X.; Setters, R.; Search, D.; Zhuang, S.; Tran, V. N.; Cuff, C. A.; Harrity, T.; Darienzo, C. J.; Li, T.; Reeves, R. A.; Blanar, M. A.; Barrish, J. C.; Zahler, R.; Robl J. A. *J. Med. Chem.* **2008,** *51,* 2722.
5. Brusq, J. M.; Ancellin, N.; Grondin, P.; Guillard, R.; Martin, S.; Saintillan, Y.; Issandou, M. *Journal of Lipid Research*, **2006**, *47*, 1281.
6. Malyszko, J.; Malyszko, J. S.; Mysliwiec, M. *Transpl Int.* **2003**, *16*, 53.
7. Graham, D. J.; Staffa, J. A.; Shatin, D.; Andrade, S. E.; Schech, S. D.; La Grenade, L.; Gurwitz, J. H.; Chan, K. A.; Goodman, M. J.; Platt, R. *JAMA.* **2004**, *292,* 2585.

8. Velezheva, V. S.; Brennan, P. J.; Marshakov, V. Y.; Lisichkina, D. V. G. I. N.; Peregudov, A. S.; Tchernousova, L. N.; Smirnova, T. G.; Andreevskaya, S. N.; Medvedev, A. E. *J. Med. Chem.* **2004,** *47,* 3455.
9. Andreani, A.; Burnelli, S.; Granaiola, M.; Leoni, A.; Locatelli, A.; Morigi, R.; Rambaldi, M.; Varoli, L.; Landi, L.; Prata, C.; Berridge, M. V.; Grasso, C.; Fiebig, H. H.; Kelter, G.; Burger, A. M.; Kunkel, M. W. *J. Med. Chem.* **2008,** *51,* 4563.
10. Routier, S.; Peixoto, P.; Mérour, J. Y.; Coudert, G.; Dias, N.; Pierré, C. B. A.; Léonce, S.; Caignard, D. H. *J. Med. Chem.* **2005,** *48,* 1401.
11. Regina, G. L.; Coluccia, A.; Piscitelli, F.; Bergamini, A.; Sinistro, A.; Cavazza, A.; Maga, G.; Samuele, A.; Zanoli, S.; Novellino, E.; Artico, M.; Silvestri, R. *J. Med. Chem.* **2007,** *50,* 5034.
12. Franco, M. I. R.; Bachiller, M. I. F.; Pérez, C.; Ledesma, B. H.; Bartolomé, B. *J. Med. Chem.* **2006,** *49,* 459.
13. Knott, K. E.; Auschill, S.; Jäger, A.; Knölker, H. J. *Chem. Commun*. **2009**, 1467.
14. Kennedy, R. O.; Thornes, R. D. *Coumarins. Biology, Applications and Mode of Action, Wiley, New York*. **1997.**
15. Hoult, J. R. S.; Paya, M. *Gen. Pharmacol.* **1996**, *27*, 713.
16. Ma, T.; Liu, L.; Xue, H.; Li, L.; Han, C.; Wang, L.; Chen, Z.; Liu, G. *J. Med. Chem.* **2008,** *51,* 1432.
17. Kostova, I. *Curr. Med. Chem.* **2005**, *5*, 29.
18. Musa, M. A.; Cooperwood, J. S. *Curr. Med. Chem.* **2008**, *15*, 2664.
19. Kidane, A. G.; Salacinski, H.; Tiwari, A.; Bruckdorfer, K. R.; Seifalian, A. M. *Biomacromolecules.* **2004**, *5*, 798.
20. Appendino, G.; Mercalli, E.; Fuzzati, N.; Arnoldi, L.; Stavri, M.; Gibbons, S.; Ballero, M.; Maxia, A. *J. Nat. Prod.* **2004,** *67*, 2108.
21. Lin, H .C.; Tsai, S. H.; Chen, C .S.; Chang, Y. C.; Lee, C. M.; Lai, Z. Y.; Lin, C. M. *Biochem Pharmacol.* **2008**, *75*, 1416.
22. Yuce, B.; Danis, O.; Ogan, A.; Sener, G.; Bulut, M.; Yarat, A. *Arzneimittel-Forschung-Drug Research.* **2009**, *59*, 129.
23. Madhavan, G. R.; Balraju, V.; Mallesham, B.; Chakrabarti, R.; Lohray, V. B. *Bioorg. Med. Chem. Lett*. **2003**, *13*, 2547.
24. Vilar, S.; Quezada, E.; Santana, L.; Uriarte, E.; Yanez, M.; Fraiz, N.; Alcaide, C.; Cano, E.; Orallo, F. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 257.
25. Song, H. Y.; Ngai, M. H.; Song, Z. Y.; MacAry, P. A.; Hobley, J.; Lear, M. J. *Org. Biomol. Chem.* **2009**, *7*, 3400.
26. Melagraki, G.; Afantitis, A.; Igglessi, M. O.; Detsi, A.; Koufaki, M.; Kontogiorgis, C.; Hadjipavlou L. D. *Eur. J. Med. Chem.* **2009**, *44*, 3020.
27. (a) Sashidhara, K.V.; Kumar, A.; Kumar, M.; Sonkar, R; Bhatia, G.; Khanna, A. K. *Bioorg. Med. Chem. Lett*. **2010,** *20*, 4248. (b) Sashidhara, K.V.; Rosaiah, J. N.; Kumar, A.; Bhatia, G.; Khanna, A. K. *Bioorg. Med. Chem. Lett*. **2010,** *20*, 3065. (c) Sashidhara, K. V.; Kumar, A.; Bhatia, G.; Khan, M. M.; Khanna, A. K.; Saxena, J. K. *Eur. J. Med. Chem*. **2009**, *44*, 1813. (d) Sashidhara, K. V.; Rosaiah, J. N.; Bhatia, G.; Saxena, J. K. *Eur. J. Med. Chem*. **2008**, *43*, 2592.
28. Representative procedure for the synthesis of compound **5a** (6-[Bis-(1H-indol-3-yl)-methyl]-2-oxo-2H-benzo[h]chromene-3-carboxylic acid methyl ester) : A mixture of Methyl 6-formyl-2-oxo-2H-benzo[h]chromene-3-carboxylate **3** (300 mg, 1.06 mmol), indole (248.93 mg, 2.12 mmol) and I_2 (53.83 mg, 0.21 mmol) in acetonitrile (20 ml) was stirred at room temperature for 30 min. After completion of the reaction, the mixture treated with aq. $Na_2S_2O_3$ solution (5%, 10 mL) and the product was extracted with chloroform (3×25 mL). The combined organic layers were dried with anhydrous sodium sulphate, concentrated in vacuo and purified by column chromatography to afford the pure compound **5a** (6-[Bis-(1H-indol-3-yl)-methyl]-2-oxo-2H-benzo[h]chromene-3-carboxylic acid methyl ester) (Bandgar, B. P.; Shaikh, K. A. *Tetrahedron Lett.* **2003**, *44*, 1959.). White solid, yield: 92%; mp >300 ^{o}C; IR (KBr): 3387, 2981,1761, 1596, 1564, 1040 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ: 10.85 (s, 2H, -NH), 8.75 (s, 1H), 8.47-8.39 (m, 2H), 7.74-7.71 (m,

2H), 7.57 (s, 1H), 7.38-7.30 (m, 4H), 7.05 (t, *J* = 7.2 Hz, 2H), 6.87 (t, *J* = 7.4 Hz, 2H); 6.79 (brs, 2H), 6.64 (s, 1H), 3.79 (s, 3H); ESI-MS: (*m*/z): 499 $(M+H)^+$.

29. Rizvi, R.; Puri, A.; Bhatia, G.; Khanna, A. K.; Wulf, E. M.; Rastogi, A. K.; Chandra, R. *Biochem. Biophys. Res. Commun.* **2003**, *305*, 215.
30. Bhatia, G.; Rizvi, F.; Saxena, R.; Puri, A.; Khanna, A. K.; Chander, R.; Wulf, E. M.; Chandra, R.; Rastogi, A. K. *Indian J. Exp. Biol.* **2003**, *41*, 1456.
31. Gordon, D. J.; Probstfield, J. L.; Garrison, R. J.; Neatom, J. D.; Jacob, D. R.; Bangdiwala, S.; Tyroler, H. A. *Circulation*, **1989**, *79*, 8.
32. McCarthy, P. A., DeNinno, M. P.; Morehouse, L.A.; Chandler, C. E.; Bangerter, F. W.; Wilson, T. C.; Urban, F. G.; Walinsky, S. W.; Cosgrove, P. G.; Duplantier, K.; Etienne, J. B.; Fowler, M. A.; Lambert, J. F.; O'Donnell, J. P.; Pezzullo, S. L.; Watson, H. A, Jr.; Wilkins, R. W.; Zaccaro, L. M.; Zawistoski, M. P. *J. Med. Chem.* **1996**, *39*, 1935.
33. Knott, K. E.; Auschill, S.; Jäger, A.; Knölker, H. J. *Chem. Commun.*, **2009**, 1467.
34. Hoult, J. R. S.; Payá, M. *General Pharmacology: The Vascular System* **1996**, *27*, 713.

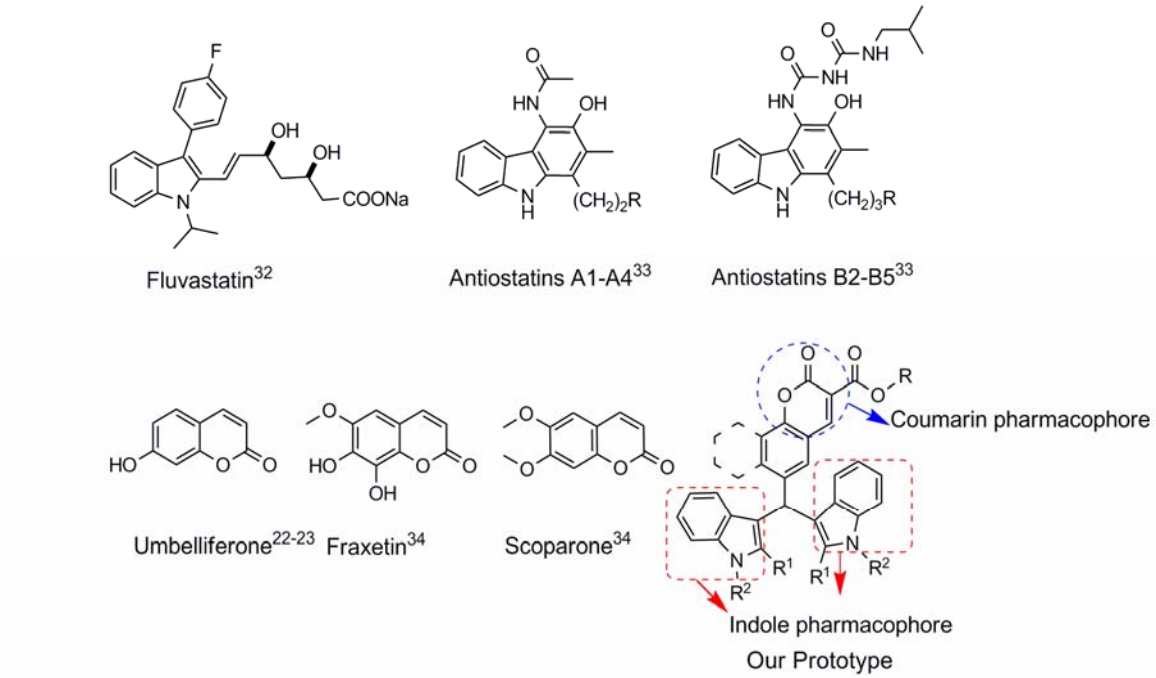
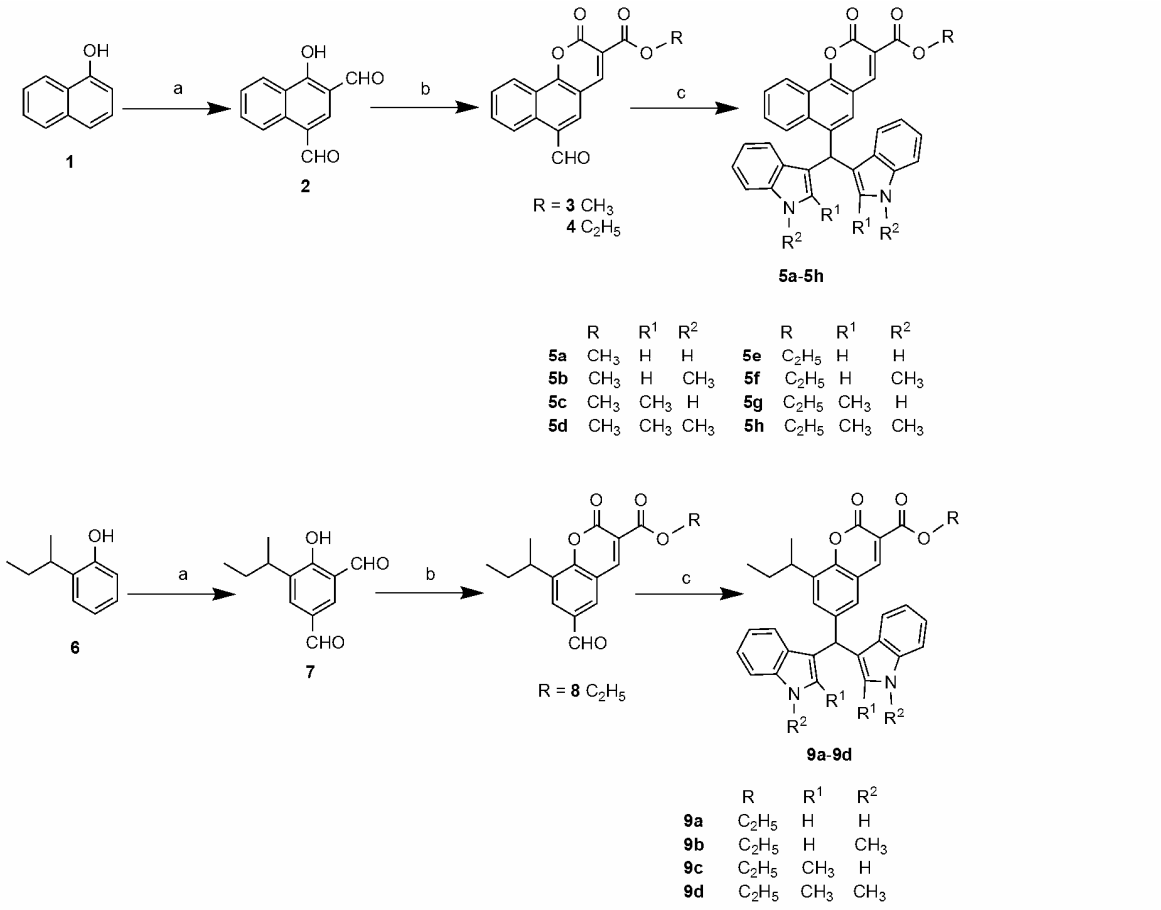


Figure 1. Chemical structure of some potent lipid modulating indoles and coumarins and general structure of our synthesized hybrids.



	R	R^1	R^2		R	R^1	R^2
5a	CH_3	H	H	**5e**	C_2H_5	H	H
5b	CH_3	H	CH_3	**5f**	C_2H_5	H	CH_3
5c	CH_3	CH_3	H	**5g**	C_2H_5	CH_3	H
5d	CH_3	CH_3	CH_3	**5h**	C_2H_5	CH_3	CH_3

	R	R^1	R^2
9a	C_2H_5	H	H
9b	C_2H_5	H	CH_3
9c	C_2H_5	CH_3	H
9d	C_2H_5	CH_3	CH_3

Scheme 1. Reagents and conditions: (a) 1. HMTA / TFA, 120 ºC, 3h 2. 10% H_2SO_4, 90-100 ºC, 2h (b) $CH_2(COOR)_2$, ROH, piperidine, reflux, 30 min. (c) Different indole, I_2, CH_3CN, rt, 30 min.

Table 1. Percentage decrease/increase of plasma lipids with the treatment of novel coumarin bisindole hybrids **5a**–**5h** and **9a**–**9d** in dyslipidemic hamsters at the dose of 10 mg/kg body weight.

Compounds	TG (mM)	TC (mM)	HDL (mM)	HDL-C/TC
5a	**-50***	**-12**	**+13**	**+30**
5b	-35	+6	-17	+12
5c	-33	-17	+1	+24
5d	-29	-4	-3	+2
5e	**-55*****	**-20**	**+14**	**+42**
5f	-33	+3	+22	+18
5g	-35	-7	+14	+24
5h	-45	-38	-31	+11
9a	-10	+28	+11	-14
9b	-43	-36	-24	+18
9c	-39	-21	-13	+9
9d	**-57***	**-43**	**-20**	**+39**
Atorvastatin	-63**	-14	+15	+35
Lovastatin[a]	-29	-9	+3	+12

[a]Lovastatin at the dose of 25 mg/kg body weight.
Values represented are % change with respect to control HFD-fed hamster group (values are means ± SD of eight hamsters in each group).
*$p < 0.05$(*), $p < 0.01$(**), $p < 0.001$(***).

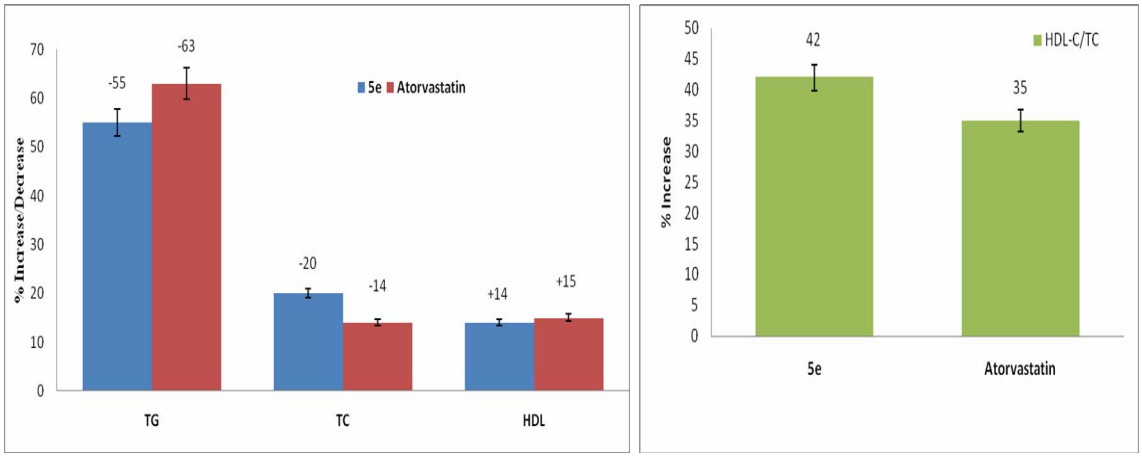


Figure 2. Effect of **5e** at 10 mg/kg body-wt dose as compared with atorvastatin on TG, TC, HDL and HDL-C/TC of hamster model.