Int J Pharm Bio Sci 2013 Apr; 4(2): (P) 263 - 270

Research Article Medicinal Chemistry

International Journal of Pharma and Bio Sciences ISSN 0975-6299

MOLECULAR DOCKING STUDIES, SYNTHESIS AND ANTI-BACTERIAL PROPERTIES OF NEW MANNICH BASES

ONKARA.P[1], A.SUNIL KUMAR [2], S.KANAKARAJU [2],B.PRASANNA,[2] Y.PYDISETTY,[3] AND G.V.P.CHANDRAMOULI[1*]

[1]Department of Biotechnology,
[2]Department of Chemistry,
[3]Department of Chemical Engineering,National Institute of Technology, Warangal- 506 004, (A.P.), India

ABSTRACT

A series of 3-substituted aminophenyl-4-hydroxycoumarins 4a-h were synthesized *via* Mannich reaction by using [BMIM]BF_4 ionic liquid under microwave irradiation. Compounds were characterized by IR, ^{1}H NMR and mass spectroscopy. All the compounds were tested for their antibacterial activity against B.*subtilis,* Bacillus.*sp,* E. *coli and* P.*putida*. Most of the compounds showed moderate to good antibacterial activity. Docking studies of the synthesized compounds was done with the help of VLife MDS 3.5 software using GA docking method to study their observed activity.

KEYWORDS: 4-Hydroxy coumarin, Mannich base, Ionic liquid, Molecular docking, Antibacterial activity

G. V. P. CHANDRAMOULI
Department of Biotechnology, National Institute of Technology, Warangal- 506 004, (A.P.), India

INTRODUCTION

Docking is a method of molecular modeling, which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. Molecular docking can be defined as an optimization problem, which would describe the "best-fit" orientation of a ligand that binds to a particular protein of interest and is used to predict the structure the structure of the intermolecular complex formed between two or more molecules. The most interesting case is the protein ligand interaction, because of its applications in medicines. Ligand is a small molecule, which interacts with protein's binding sites. There are several possible mutual conformations in which binding may occur. These are commonly called binding modes. In modern drug designing, molecular docking is routinely used for understanding drug information about drug receptor interactions and is frequently used to predict the binding orientation of small molecule drug candidates to their protein targets in order to predict the affinity and activity of the small molecule. 4-Hydroxy coumarin derivatives exhibit a wide range of biological activities such as anti-HIV, antimalarial, insecticides, antioxidants, rodenticides, anticoagulants, pesticides. bactericides, fungicides, anti-inflammatory, cytotoxic and anti-tumor activities.[1-11] Among various derivatives of 4-hydroxy coumarin, 3-(benzyl)-substituted compounds have been used in important clinical applications.[12-15] These are also used as an important synthons in synthetic organic chemistry[16,17]. Therefore, we tried to synthesize 3-(benzyl)-substituted coumarins *via* Mannich type reaction. In continuation of our work on biologically active heterocycles, we here in reported synthesis and anti bacterial activity of novel 3-substituted aminophenyl-4-hydroxycoumarins along with the molecular docking studies.

MATERIALS AND METHODS

Melting points were measured in open capillary on Buchi melting point B-540 apparatus and were uncorrected. IR spectra were recorded on Simadzu FTIR-8400 spectrometer using KBr pellets. ^{1}H NMR (300 MHz) spectra recorded in $CDCl_3$ on a Bruker AVANCE 300 instrument with the TMS as an internal standard. All the chemical shifts values were recorded as *δ* ppm. Mass spectra (EI-MS) were taken on Perkin Elmer (SCIEX API-2000, ESI) at 12.5 eV. CHN analysis was carried out on Carlo Erba E A 1108 automatic analyzer. The ionic liquid used is 1-Butyl-3-methylimidazolium tetrafluoroborate [BMIM]BF_4. The progress of each reaction was monitored and purity of the compounds was checked by thin layer chromatography.

Antibacterial studies

The anti-bacterial activity of newly synthesized compounds 4a-h was determined by well plate method[18] in Muller-Hinton Agar. The *in vitro* antibacterial activity was carried out against 24 h old cultures of bacterial strains. In this work, B. *subtilis* (MTCC 121), Bacillus *sp*. (MTCC 10616), P. *putida* (MTCC 10617), and E. *coli* (MTCC 1652) were used to investigate the antibacterial activity. The test compounds were dissolved in dimethyl sulphoxide (DMSO) at concentration of 1mg/mL. Twenty milliliters of sterilized agar media was poured into each pre-sterilized Petri dish. Excess of suspension was decanted and plates were dried by placing in an incubator at 37 ^{0}C for an hour. About 60 µL of 24 h old culture suspension was poured and neatly swabbed with the pre-sterilized cotton swabs. Six millimeters diameter well was then punched carefully using a sterile cork borer and 30 µL of test solutions of different concentrations were added into each labeled well. The plates were incubated for 24 h at 37 ^{0}C. The inhibition zone that appeared after 24 h around the well in each plate was measured as zone of inhibition in mm. The antibacterial

results were compared with Chloramphenicol and summarized in Table 3.

General procedure for the synthesis of 3-substituted aminophenyl-4-hydroxy-coumarin (Mannich bases)

a) Procedure for using only [BMIM]BF_4

A mixture of aldehyde (4.7 mmole), aniline (4.7 mmole), and 4-hydroxycoumarin (or its analogue) (5.0 mmole) in ionic liquid [BMIM]BF_4 (1mL) was stirred at room temperature in a round-bottomed flask fitted with a condenser. After certain interval of time the reaction mixture became viscous and solidified. At this stage the time was noted, the reaction mixture was diluted with 10 mL of water and extracted with Et_2O (4X5 mL) and the combined ether fractions were evaporated. The ionic liquid being soluble in water comes in the water layer.

b) Procedure for using [BMIM]BF_4 and Microwave irradiation

The procedure is similar to above except that the reaction mixture was microwave irradiated at 300W in different time intervals 15 seconds each for 6-12 minutes. The working up procedure is also similar.

Scheme 1

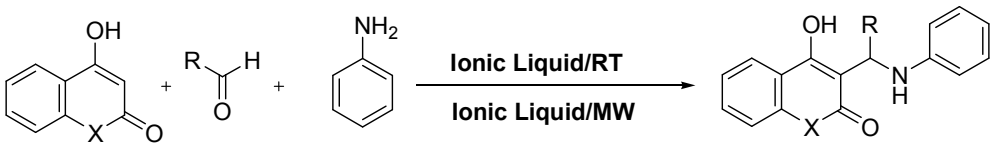
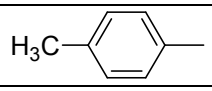
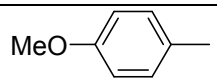
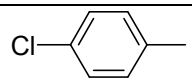
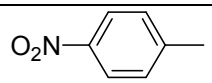
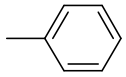
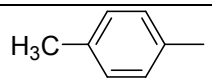
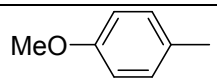


Table 1

Synthesis of 3-substituted aminophenyl-4-hydroxycoumarins and its analogues (Mannich bases) by using [BMIM]BF_4 and [BMIM]BF_4 and Microwave.

			using [BMIM]BF_4		using[BMIM]BF_4 and Microwave	
Entry	**R**	**X**	**Time (h)**	**Yield (%)**	**Time (min)**	**Yield (%)**
4a		O	9	72	10	82
4b		O	11	70	12	75
4c		O	8	78	10	90
4d		O	7	81	8	92
4e		O	8	74	9	84
4f	H	O	6	75	6	86
4g		S	9	72	10	83
4h		S	11	68	12	79

Molecular docking studies

a) Ligand Preparation

The structure of 3-substituted-aminophenyl-4-hydroxy-coumarin derivatives was used as the template to build the molecules in the dataset in V Life MDS 3.5. The ligand geometries were optimized by energy minimization using MMFF94 force field and Gasteiger-Marsili charges for the atoms, till a gradient of 0.001 kcal/mol/A° was reached, maintaining the template structure rigid during the minimization.

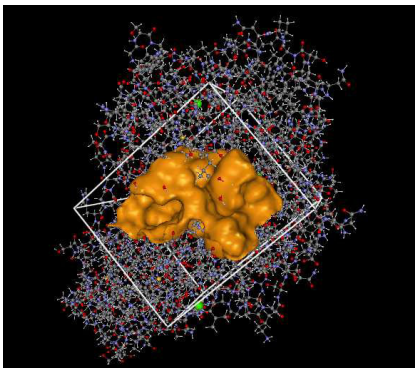


Figure. 1

Grid map generated for alpha amylase (1BAG) by VLife MDS 3.5

b) Preparation of the grid file

The grid map was centered at the active pocket of the protein by VLife MDS 3.5 (Fig. 1). The grid map, which was centered at the following residues of the protein Asp 212 (A), Lys 179 (A), His 180(A), Arg 174(A), Asp 269(A), His 268 (A),Tyr59(A),Gln 63 (A), Asp 273(A) which were predicted from the ligplot (Fig.2).

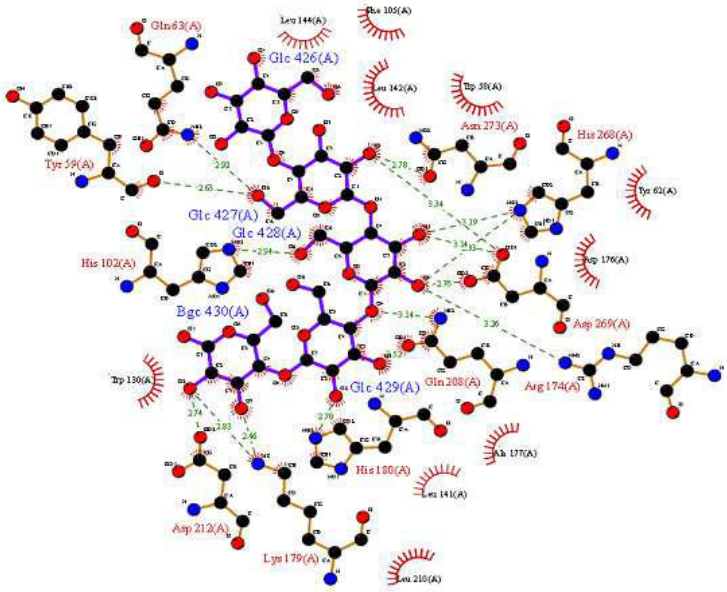


Figure 2

Ligplot results for alpha amylase showing interaction with ligand Glc 426(A) to BGC 430(A).

c) Preparation of the docking file

Over activation of receptor Alpha Amylase from Bacillus subtilis complexed with maltopentose (1BAG) signaling pathways is strongly associated with antibacterial activity. On this basis, we selected 1BAG as a biological target for docking study of synthesized compounds. The crystal structure of 1BAG was obtained from the Protein Data Bank (www.rcsb.org/pdb). The optimized receptor was then saved as .mol file and used for docking simulation. The 2D structure of the compounds were built and then converted into the 3D with the help of VLife MDS 3.5 software. The 3D structures were then energetically minimized upto the rms gradient of 0.01 using Merck Molecular Force Field (MMFF). Conformers of all the synthesized ligands were selected and were then energetically minimized upto the rms gradient of 0.01 and then saved in a separate folder. The active site selection was done by choosing the cavity having maximum hydrophobic surface area. Docking simulation was done by GA docking method. All the conformers were virtually docked at the defined cavity of the receptor. The parameters fixed for docking simulation was like this number of placements: 30, rotation angle: 30°, exhaustive method, scoring function: dock score. By rotation angle, the ligand gets rotated for different poses. By placements, the method will check all the 30 possible placements into the active site pocket and results out few best placements out of 30. For each ligand, all the conformers with their best placements and their dock score will be saved in output folder. The method also highlights the best placement of best conformer of one particular ligand which is having best (minimum) dock score. In the results of docking, we have listed only best conformers and its dock score for each ligand in Table 2**.** After docking simulation, the best docked conformer of each ligand and receptor were merged and aggregated by defining the radius of 4 Å. The receptor complexes were then energetically minimized along with the docked ligand. Stepwise aggregation was done first with hydrogen, second side chains and finally the backbone of receptor.[19] The optimized complexes were then checked for various interaction of ligand with receptor like hydrogen bonding, hydrophobic bonding and vander Waal's interaction.

RESULTS AND DISCUSSION

The *in vitro* antibacterial activity of newly synthesized compounds 4a-h was determined by well plate method. The antibacterial screening revealed that some of the tested compounds showed good inhibition against various tested microbial strains. The results indicated that among the tested compounds, 4d and 4g showed excellent activity against all tested organisms. Compound 4e showed good antibacterial activity against E. *coli* and P. *putida*. For compounds 4b and 4h Bacillus.*sp* showed resistance, whereas P. *putida* showed resistance to compound 4c and 4h. Comparative docking of alpha amylase with the ligand molecules was done in correlation to *in vitro* antibacterial activity. The molecular docking of ligand molecules with alpha amylase revealed that (Table 2), the compound 4g has exhibited the interaction with the other amino acids in the active pockets which is showed in Fig. 3. For docking calculations, Gasteigere-Marsili partial charges were assigned to the ligands and nonpolar hydrogen atoms were merged. All torsions were allowed to rotate during docking. The systematic and Monte-Carlo methods were applied for minimization, using default parameters. Theoretically all the ligand molecules showed encouraging docking score. Among the eight molecules, docking of alpha amylase with 4d and 4g revealed that their docking scores were -5.185860 and -4.727389 k.cal mol^{-1}, respectively, and it can be predicted as good inhibitor of alpha amylase.

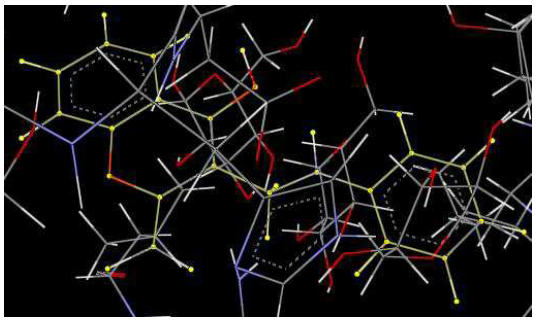


Figure 3
Docking profile of compound 4g

Table 2
Molecular docking results with alpha amylase

S.No	Molecule	Dock score	No.of Hydrogen Bonds	Bonding Residues
1	4a	-4.077394	1	GLC 427A, GLC 428A, GLC 429A, BGC 430A
2	4b	-3.932703	0	GLC 427A, GLC 428A, GLC 429A, BGC 430A
3	4c	-3.175717	0	GLC 427A, GLC 428A, GLC 429A, BGC 430A
4	4d	-5.185860	1	GLC 426A, GLC 427A, GLC 428A, GLC 429A, BGC 430A
5	4e	-4.378602	0	GLC 427A, GLC 428A, GLC 429A, BGC 430A
6	4f	-4.343019	0	GLC 427A, GLC 428A, GLC 429A, BGC 430A
7	4g	-4.727389	1	GLC 426A, GLC 427A, GLC 428A, GLC 429A, BGC 430A
8	4h	-3.778533	2	GLC 427A, GLC 428A, GLC 429A, BGC 430A
9	Chloramphenicol	-4.792215	2	GLC 426A, GLC 427A, GLC 428A, GLC 429A, BGC 430A

Table 3
Zone of inhibition (in mm)

Compd	B.*subtilis* (MTCC 121)	Bacillus.*sp* (MTCC 10616)	E. *coli* (MTCC 1652)	P.*putida* (MTCC 10617)
4a	23	20	22	35
4b	20	R	19	21
4c	14	17	19	R
4d	35	38	32	46
4e	26	21	30	42
4f	24	19	22	27
4g	34	37	34	48
4h	17	R	20	R
Chloramphenicol	33	34	30	42

"R = resistance"

Antibacterial activity of tested compounds 4a-h

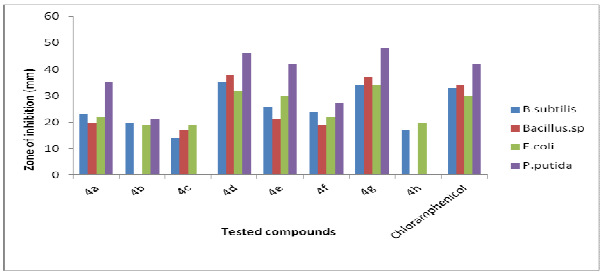


Figure 4
Antibacterial activity of 4a-h

CONCLUSION

3-substituted aminophenyl-4-hydroxy-coumarin derivatives (Mannich bases) were synthesized and characterized by analytical IR, ^{1}H NMR, and mass spectral studies. All the compounds were screened for their antibacterial property. All the compounds show significant antibacterial activity when compared to chloramphenicol. Few of the bacteria showed resistance to some compounds. The synthesized molecules seem to be useful as specific antibacterial agents against specific bacteria. Further evaluation is necessary for their clinical use.

Characterization data

4-Hydroxy-3-(phenylamino-p-tolyl-methyl)-chromen-2-one (4a)

Yellow solid; m.p:174–176 ^{o}C; IR (KBr, cm^{-1}): 3429, 3325, 1735; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.34 (s, 3H), 4.95 (s, 1H), 6.59–7.10 (m, 6H), 7.25-7.35 (m, 4H), 7.40–7.50 (m, 3H); MS: 357 (M^+); Anal. Calcd. for $C_{23}H_{19}NO_3$: C, 77.29; H, 5.36; N, 3.92. Found : C, 77.31; H, 5.39; N, 3.88 %.

4-Hydroxy-3-[(4-methoxy-phenyl)-(phenylamino)-methyl]-chromen-2-one (4b)

Yellow solid; m.p:188–190 ^{o}C; IR (KBr, cm^{-1}): 3430, 3320, 1731; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.81 (s, 3H), 4.96 (s, 1H), 6.58–7.11 (m, 6H), 7.24–7.36 (m, 4H), 7.41–7.49 (m, 3H); MS: 373 (M^+); Anal. Calcd. for $C_{23}H_{19}NO_4$: C, 73.98; H, 5.13; N, 3.75. Found: C, 73.91; H, 5.19; N, 3.78 %.

4-Hydroxy-3-[(4-chloro-phenyl)-(phenylamino)-methyl]- chromen-2-one (4c)

White solid; m.p:179–181 ^{o}C; IR (KBr, cm^{-1}): 3432, 3321, 1730; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 4.96 (s, 1H), 6.59–7.12 (m, 6H), 7.26–7.38 (m, 4H), 7.46-7.51 (m, 3H); MS: 377 (M^+); Anal. Calcd for $C_{22}H_{16}NClO_3$: C, 69.94; H, 4.27; N, 3.71. Found: C, 69.91; H, 4.19; N, 3.78 %.

4-Hydroxy-3-[4-nitrophenyl-(phenylamino)-methyl]-chromen-2-one (4d)

Yellow solid; m.p:196–198 ^{o}C; IR (KBr, cm^{-1}): 3453, 3290, 1742; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 4.82 (s, 1H), 6.58–7.11 (m, 8H), 7.24–7.36 (m, 5H); MS: 388 (M^+); Anal. Calcd for $C_{22}H_{16}N_2O_5$: C, 68.04; H, 4.15; N, 7.21. Found: C, 68.11; H, 4.19; N, 7.28 %.

4-Hydroxy-3-[phenyl-(phenylamino)-methyl]-chromen-2-one (4e)

White solid; m.p:153–156 ^{o}C; IR (KBr, cm^{-1}): 3430, 3320, 1734; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 4.94 (s, 1H), 6.98–7.14 (m, 5H), 7.25–7.36 (m, 5H), 7.39–7.49 (m, 4H); MS: 343 (M^+); Anal. Calcd for $C_{22}H_{17}NO_3$: C, 76.95; H, 4.99; N, 4.08. Found: C, 76.91; H, 4.89; N, 4.18 %.

4-Hydroxy-3-phenylaminomethyl-chromen-2-one (4f)

White solid; m.p:164–166 ^{o}C; IR (KBr, cm^{-1}): 3435, 3322, 1732; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.51 (s, 2H), 6.58–7.11 (m, 5H), 7.24–7.36 (m, 4H); MS: 267 (M^+); Anal. Calcd for $C_{16}H_{13}NO_3$: C, 71.90; H, 4.90; N, 5.24. Found: C, 71.91; H, 4.89; N, 5.28 %.

4-Hydroxy-3-(phenylamino-p-tolyl-methyl)-thiochromen-2-one (4g)

Pale yellow solid; m.p:170–172 ^{o}C; IR (KBr, cm^{-1}): 3430, 3318, 1712; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.39 (s, 3H), 4.98 (s, 1H), 7.01–7.15 (m, 5H), 7.24–7.36 (m, 5H), 7.41-7.59 (m, 3H); MS: 373 (M^+); Anal.Calcd for $C_{23}H_{19}NO_2S$: C, 73.97; H, 5.13; N, 3.75. Found: C, 73.94; H, 5.11; N, 3.74 %.

4-Hydroxy-3-[(4-methoxy-phenyl)-(phenylamino)-methyl]-thiochromen-2-one (4h)

Pale yellow solid; m.p:191–194 ^{o}C; IR (KBr, cm^{-1}): 3430, 3320, 1732; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.82 (s, 3H), 4.95 (s, 1H), 7.09 -7.21 (m, 5H), 7.26-7.49 (m, 4H), 7.52–7.61 (m, 4H); MS: 389 (M^+); Anal.Calcd for $C_{23}H_{19}NO_3S$: C, 70.93; H, 4.92; N, 3.60. Found: C, 70.90; H, 4.91; N, 3.61 %.

ACKNOWLEDGEMENTS

The authors thank the Director, National Institute of Technology, Warangal for providing research facilities.

REFERENCES

1. Stanchev S, Jensen F, Hinkov A, Atanasov V, Genova-Kalou P, Argirova R, Manolov I, Synthesis and Inhibiting Activity of Some 4-Hydroxycoumarin Derivatives on HIV-1 Protease. ISRN Pharm. 2011: 137637, (2011).
2. (a) Guilet D, Helesbeux J-J, Seraphin D, Sevenet T, Richomme P, Bruneton J, Novel Cytotoxic 4-Phenylfuranocoumarins from *Calophyllum dispar*, J Nat Prod, 64 (5): 563-568, (2001); (b) Rodriguez SA, Nazareno MA, Baumgartner MT, Effect of different C3-aryl substitutents on the antioxidant activity of 4-hydroxycoumarin derivatives, Bioorg Med Chem, 19 (21): 6233–6238, (2011).
3. Hermodson MA, Barker WM, Link KP, 4-Hydroxy coumarins. Synthesis of matabolites and some other derivatives of warfarin, J Med Chem, 14 (2): 167–169, (1971).
4. Jung, JC, Park OS, Molecules, Synthetic Approaches and Biological Activities of 4-Hydroxycoumarin derivatives, 14 (11): 4790–4803, (2009)
5. (a) Feuer G, The Metabolism and Biological Actions of Coumarins, Prog. Med. Chem. 10: 85–158, (1974); (b) Obaseki AO, Porter WR, Trager, WF, 4-Hydroxycoumarin/2-hydroxychromone tautomerism: Infrared spectra of 2-^{13}C and 3-D labeled 4-hydroxycoumarin and its anion, J Heterocycl Chem. 19 (2): 385–390, (1982);
6. El-Sayed AM, Abd-Allah OA, Synthetic and biologacal studies on coumarin hydrazone derivatives, Phosphorus, Sulfur and Silicon Relat Elem, 170 (1): 75–86, (2001).
7. El-Agrody AM, Abd El-Latif MS, El-Hady NA, Fakery AH, Bedair AH, Heteroaromatization with 4-hydroxycoumarin. Part II, Molecules, 6 (6): 519–527, (2001).
8. Luchini AC, Rodrigues-Orsi P, Cestari SH, Seito LN, Witaicenis A, Pellizzon CH, Di Stasi LC, Intestinal anti-inflammatory activity of coumarin and 4-hydroxycoumarin in the trinitrobenzenesulphonic acid model of rat colitis, Biol Pharm Bull, 31 (7): 1343–1350, (2008).
9. Nofal ZM, El-Zahar MI, Abd-El-Karim SS, Molecules, 5 (2): 99–113, (2000).
10. Stanchev S, Momekov G, Jensen F, Manolov I, Synthesis, computational study and cytotoxic activity of new 4-hydroxycoumarin derivatives, Eur J Med Chem, 43 (4): 694–706, (2008).
11. Raev L, Voinov E, Ivanov I, Popov D, Antitumour activity of some coumarin derivatives, Pharmazie, 45 (9): 696–698, (1990).
12. Hadler MR, Shadbolt RS, Novel 4-hydroxycoumarin anticoagulants active against resistant rats, Nature, 253 (5489): 275–277, (1975).
13. Mizuno T, Nishiguchi I, Hirashima T, Ogawa A, Kambe N, Sonoda N, Synthesis, (3): 257–259, (1988).
14. Clerici A, Porta O, Synthesis, (1): 99-102, (1993).
15. Fiedler VB, Schotholt J, Effects of carbocromene on myocardial oxygen consumption in isolated dog hearts, J Pharmacol Exp Ther 217 (2), 306–313, (1981).
16. Bezwada RS, Chemistry of Coumarins, Indofine Chemical Company, 2008.
17. Dike SY, Merchant JR, Novel Synthesis of oxazines from 4-hydroxycoumarin and Schiff bases, Tetrahedron Lett, 19 (38): 3607–3608, (1978).
18. Arthington-Skaggs BA, Motley M, Warnock DW, Morrison CJ, J Clin Microbiol, 38 (6), 2254–2260: (2000).
19. VLife MDS 3.5 documentation, Tutorial: Protein Complex Optimization using MDS, 1–12.