Drug Designing: Open Access

ISSN: 2169-0138

Chandrashekhar et al., Drug Des 2017, 6:3
DOI: 10.4172/2169-0138.1000155

Research Article Open Access

Molecular Docking Studies of Hydrazide-Hydrazone Derivatives of Gossypol against Bcl-2 Family Anti-Apoptotic Targets

Chandrashekhar R[1], Bhavani Ram[2*], Lakshmi Bhavani N[1] and Vasudha Bakshi[3]

[1]*Pharmacognosy and Plant Molecular genetics Lab, Department of Botany, University College of Science, Osmania University, Saifabad, Hyderabad, Telangana State, India*

[2]*Green Evolution Laboratories, Wangapally Village, Nalgonda, Telangana State, India*

[3]*Department of Pharmacology, Anurag Group of Institutions, School of Pharmacy, Venkatapur, Ghatkesar, Telangana State-500088, India*

[*]**Corresponding author:** Dr. Ram Bhavani, Director, Green Evolution Laboratories, Medicinal Chemistry Lab, Wangapally Village, Nalgonda District, Telangana State-500085, India, Tel: +91 8179802942; E-mail: ram_19_73@yahoo.co.in

Received date: November 07, 2017; **Accepted date:** November 27, 2017; **Published date:** December 04, 2017

Abstract

Background: In tumor progression, BH3 domain containing Bcl-2 family members, anti-apoptotic proteins, are potential targets for cancer therapy. BH3 domain inhibitors or BH3 mimetics, a novel class of anti-cancer drugs, promote the apoptosis by inhibiting Bcl-2 family proteins that are highly conserved mitochondrial intrinsic apoptotic pathway members.

Methods: In the present study, we have designed 54 different gossypol derivatives and evaluated their potency by molecular docking studies. Molecular interaction between popular BH3 domain containing targets Bcl-2, Bcl-w, Bcl-xL, Mcl-1 and gossypol derivatives were investigated by dock score function.

Results: 54 chemo sensitive hydrazide-hydrazone gossypol derivatives (3a-3r) were designed to evaluate their binding interaction stability with the antiapoptotic targets Bcl-2, Bcl-w, Bcl-xL, and Mcl-1. Among interactions, Bcl-2 and gossypol derivative 3k has shown better interaction. Finally, pharmacokinetic property of each lead molecule against specific target was further probed to assess the drug likeliness.

Conclusion: Bcl-2 and gossypol derivative 3k complex has shown better interaction among Bcl-2 family members. Top ranked hydrazide-hydrazone gossypol derivatives against each anti-apoptotic target were further probed for ADME properties.

Keywords: Gossypol; Hydrazide-hydrazone; Molecular docking; Cancer targets; Anti-cancer agents; Anti-apoptotic inhibitors; BH3-mimetics

Introduction

The comprehensive process of cell division and cell death is tightly regulated in multicellular organisms. Dysregulation of either of these biological processes affect the normal development and homeostasis that often progresses to cancer. During cancer progression cell division pathways are elevated and programmed cell death or apoptotic pathways are dropped, therefore, repertoire of these failed mechanisms are hallmarks of cancer [1,2]. Since the inception of apoptosis; it is studied in various processes including normal cell turnover, embryonic development, morphogenesis, aging, cell population regulation and tumor progression [3,4].

Although apoptosis is very common mechanism to eliminate the unwanted, damaged or dangerous cells; but there are other flavours of such mechanisms anoikis, necroptosis, entosis, netosis, pyroptosis or ferroptosis are also equally responsible for programmed cell death [5]. Apoptosis can be triggered either by exposure to extrinsic factors like viral infections, xenobiotic and pollutants, or intrinsic factors like DNA damage, reactive oxygen species (ROS) and reactive nitrogen species (RNS) [6]. In addition, apoptosis also take part in elimination of immune cells that are damaged by disease or noxious agents in immune reactions [7].

Excessive cell division and evasion of cell death is characteristic features of the cancer. Plethora of literature explains the importance of targeting apoptotic pathway in cancer therapy by small molecular compounds [8]. The desirable feature of apoptosis or cell death is inhibited by a class of anti-apoptotic proteins. The anti-apoptotic family of Bcl-2 genes and proteins are over expressed in the tumor progression [9].

BH domain containing Bcl-2 family members Bcl-2, Bcl-w, Bcl-xL and Mcl-1are essential for survival of the cells, and any loss or defective function of these proteins cause premature cell death. Bcl-2 family, BH3 domain, inhibitors were investigated in diverse cancers: leukemia, hepatocellular carcinoma, prostate cancer, gastric cancer, lung cancer, and head and neck squamous cell carcinoma [10-13]. Therefore, a category of therapeutics, 'BH3-mimetics', have been developed to counter resistant the mechanisms of Bcl2 family members [14-16]. Despite Gossypol popularized for reproductive damage and male infertility [17,18], some evidence says it is a BH3 mimetic [19].

Gossypol (1,1', 6,6', 7,7'-hexahydroxy-3,3'-dimethyl-5,5'-diisopropyl-2,2'-binaphthyl-8,8'-dialdehyde) is yellow colored polyphenolic compound found in pigment glands of cotton plant (*Gossypium* sp.) and the tropical tree *Thespesia populnea* L.

The phytochemical gossypol is majorly found in leaves, flowers, seed hulls, bark, and roots of the cotton plant. Gossypol comprises toxic dimeric sesquiterpenoid often called hemi gossypol, contains three isoprene units from each terpene monomer and generally protects plant from pathogens and insects as part of the plant chemical defense strategy. The molecule has been in the spotlight after the study of antifertility properties in China [20].

Later studies also revealed the anticancer role of natural gossypol, racemic mixture of (+) and (-) enantiomers, on colon cancer [21]. The (-) enantiomer has more cytotoxicity than (+) form of enantiomer [22]. Furthermore, gossypol derivatives underwent in phase I/III clinical trials in breast cancer treatment [23].

Therefore the researchers have started investigating the molecular interaction mechanisms in the cancer cells for the gossypol targets. Anti-apoptotic mechanism of gossypol interactions with BH3 domain of Bcl-2 family proteins was explained elsewhere [24]. In addition, a small variant of gossypol called apogossypol and its derivatives have also been found to possess anti-tumor genic properties on antiapoptotic Bcl-2 family proteins [25].

Previously reported gossypol derivatives have shown improved anti-tumor activity over natural gossypol on breast cancer and prostate cancer [26,27], water solubility in colon cancer [28], and reduced toxicity in lung cancer cell lines [25].

In the present study, we have designed a new set of hydrazide-hydrazone derivatives (Hydrazide Schiff's base) of gossypol in order to obtain improved chemotherapeutically sensitive and less toxic gossypol derivatives. These derivatives were investigated on BH3 domains of Bcl-2 family proteins. To the best of our knowledge and based on the previous literature precedence, the above aforementioned investigation has not been reported in the literature so far. So this study enumerates the molecular interaction affinities between anti apoptotic targets Bcl-2, Bcl-w, Bcl-xL, Mcl-1 and hydrazide-hydrazone derivatives of gossypol based on molecular docking. Furthermore, top lead of each target was analysed for ADME properties.

Materials and Methods

In the present study (molecules that inhibit BH3 domains of Bcl-2 family proteins are potential anti-cancer therapeutics, in fact gossypol, a BH3-mimetic, bind to Bcl-2, Bcl-xL, Bcl-w and Mcl-1), protein data bank (PDB), X-ray crystal structures - 4IEH, 2YXJ, 2Y6W, and 5FDO – of aforementioned targets are used for docking.

Molecular docking reveals the preferred orientation and conformation of gossypol derivative in BH3 domain pocket after forming stable complex. Scoring function of the Molegro virtual docker 6.0 reports the binding affinity score or docking score between protein and ligand [29]. Except Bcl-w, remaining targets' active site or binding site co-ordinates were obtained from PDB database, while Bcl-w active site residues have been analyzed in Pocket Query, CASTp and Phyre online tools as shown in Table 1 along with a protein sequence database Uniport, and structure database PDB identifiers.

The designed hydrazide-hydrazone gossypol derivatives were sketched in Marvin sketch 5 that supports the stereo chemical bonding between atoms. Before evaluation, the feasibility of the synthesis of these derivatives were sketched in sdf chemical file format were considered based on Schiff base reaction. All sketched 2D molecules were converted to 3D structures with explicit hydrogens.

Finally, these newly designed hydrazide-hydrazone compounds (3a-3r) were screened for top ranked hit in Molegro virtual docker. Scoring function – MolDock score and searching algorithm MolDock SE is selected with grid resolution of 0.2 Å. Energy of the docked complex was minimized along with H-Bonds.

Absorption, distribution, metabolism and excretion (ADME) prediction

ADME properties were calculated using QikProp v 3.0 tool of Schrodinger software [30]. QikProp provides ranges for comparing an exact molecule's properties with those of 95% of known drugs. QikProp also flags 30 types of reactive functional groups that may cause false positives in high throughput screening assays. It also evaluates the suitability of analogs based on Lipinski's rule of five [31], which is essential to ensure drug-like pharmacokinetic profile while using rational drug design. All the analogs were neutralized before being used by QikProp v3.0.

Results and Discussion

Objectives of the study: (1) To design some new hydrazide-hydrazone derivatives of gossypol from naturally occurring phenolic carboxylic acids (2) To evaluate the molecular interaction affinities between anti apoptotic targets Bcl-2, Bcl-w, Bcl-xL, Mcl-1 and hydrazide-hydrazone derivatives of gossypol based on molecular docking (3) To predict the binding modes of ligands in active sites with reference to anti apoptotic targets (4) To determine the ADME properties of lead molecule against each anti apoptotic target.

The hydrazide-hydrazone derivatives of gossypol (3a-3r, Figure 1) were designed by coupling the corresponding hydrazide derivatives (2a-2r, R-group with –CO-NH-NH2 functional group) with the parent gossypol ring structure (possessing –CHO functional group). The hydrazide derivatives 2a-2r (R-group with –CO-NH-NH_2) can be easily prepared from the corresponding phenolic carboxylic acids (-COOH functional group) as starting materials.

It is interesting to note that most of these phenolic carboxylic acids are abundantly available from plant resources [32], as an example, hydrazide compounds such as 2a, 2b, 2h and 2k are derived from the phenolic carboxylic acids viz., p-hydroxy benzoic acid, α-Resorcyclic acid, Pinosylvic acid and Digallic acid respectively. For the purpose of molecular docking, (-) gossypol was used to design the hydrazide-hydrazone derivatives 3a-3r (Figure 1). The synthesis, characterization and *in vitro*/*in vivo* studies of these derivatives are the future scope of this research work.

In order to show the molecular interaction affinities between anti-apoptotic targets, Bcl2 family proteins, Bcl-2 (PDB id: 4IEH), Bcl-xL (PDB id: 2YXJ), Bcl-w (PDB id: 2Y6W), Mcl-1 (PDB id: 5FDO) and hydrazide-hydrazone derivatives of gossypol, a series of molecular dockings were carried out on Molegro Virtual docker. This integrated tool prepares the protein molecule and small molecules for docking that are essential for prior to docking. Binding modes of ligands are predicted in active site (co-ordinates are shown in Table 1).

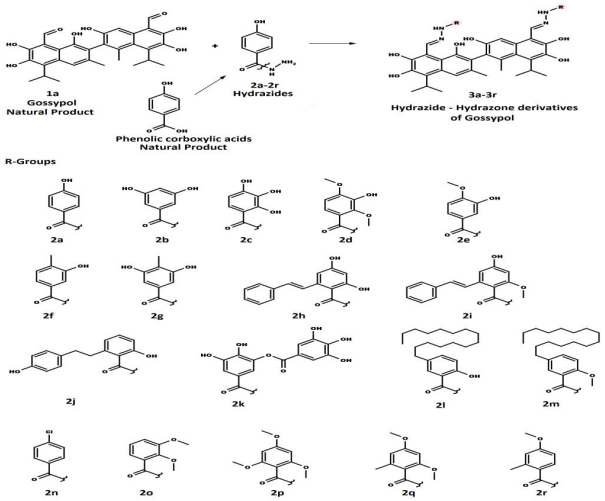

Figure 1: Structure of various hydrazide-hydrazone derivatives (3a-3r) of Gossypol.

Target Name	Uniprot ID	PDB ID	Active Site Co-ordinates
Bcl-2	P10415	4IEH	13.00, 27.45, 10.48
Bcl-xL	Q07817	2YXJ	-11.27, -12.62, 7.93
Bcl-w	Q92843	2Y6W	-19.78, 12.85, 5.21
Mcl-1	Q07820	5FDO	6.12, 22.76, -13.57

Table 1: Active site co-ordinates of Bcl-2 family proteins that are used in molecular docking

Active site residues of each target as follows – Bcl2: Phe63, Arg66, Tyr67, Met74, Leu96, Gly104, Ala108, Tyr161; Bcl-xL: Phe97, Tyr101, Ala104, Gly138, Ala142, Tyr195; Bcl-W: Leu86 which is predicted based on active site predicting tools; Mcl-1: Met231, Phe228, Met250, Leu267, Arg263, Phe270. BH3 domain is highly conserved in Bcl-2 family proteins, and it contains 5 alpha helices (Figure 2a).

Interacting site is partly hydrophilic (red and blue) and aromatic, and "phe and Leu" are conserved residues (Figure 2b). Steric favorable (green), hydrogen acceptor favorable (cyan), hydrogen donor favorable (yellow), and electrostatic favorable (red and blue) regions of the BH3 domain are show in Figure 2c. It is inferred from the Figure 2c that BH3 domain is mostly steric favorable (green); thus, gossypol (1a) and its hydrazide-hydrazone derivatives (3a-3r) are most suitable ligands. We have designed 54 different gossypol derivatives to see their interaction ability against four antiapoptotic targets, interactions are shown in Figure 3.

Protein Bcl-2 residues; Asp62, Arg65, Leu96, Asp99, Asn102, and Arg105; are interacting with gossypol derivative 3k through H-bonds. Likewise, target Bcl-w residues – Arg56, Thr60, Glu85 and Pro91 – and gossypol derivative 3b; Bcl-xL residues – Glu129 and Gly138 – and gossypol derivative 3h; and Mcl-1 residues – Lys234 and Arg263 – and gossypol derivative 3a; are showing H-bond interactions as shown in the Figure 3.

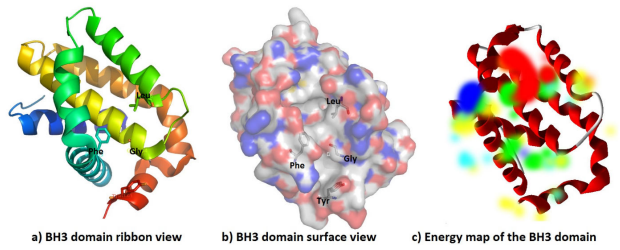

Figure 2: Molecular structure and active site of BH3 domain: 2a) Ribbon view of the BH3 domain, N-ter is shown in blue color and C-ter is shown in red color and conserved residues are shown in stick view (Phe, Tyr, Leu and Gly); 2b) Surface view of BH3 domain, electrostatic charges are shown in blue (positive) and red (negative) along with active site residues; 2c) Energy map of the BH3 domain – steric favorable (green), hydrogen acceptor favorable (cyan), hydrogen donor favorable (yellow) and electrostatic favorable (red and blue) are shown.

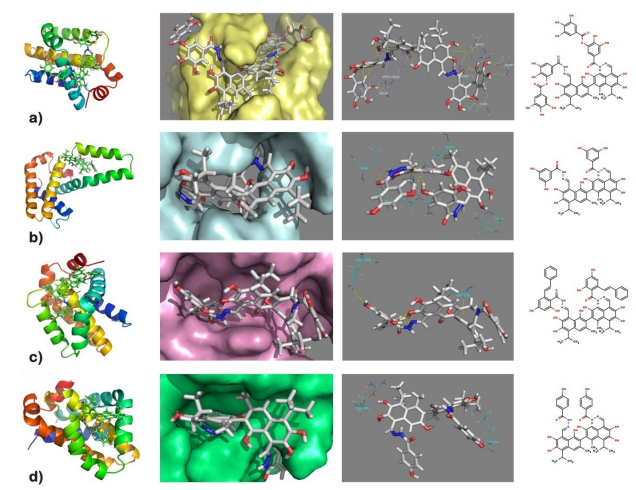

Figure 3: Docked pose of gossypol derivatives in BH3 domains of Bcl2 family proteins, H-bond interacting residues are shown in protein ligand complex: 3a) Molecular interaction between gossypol derivative 3k and Bcl-2 protein; 3b) Molecular interaction between gossypol derivative 3b and Bcl-w protein; 3c) Molecular interaction between gossypol derivative 3h and Bcl-xL protein; 3d) Molecular interaction between gossypol derivative 3a and Mcl-1 protein.

MolDock scores of Bcl-2, Bcl-w, Bcl-xL and Mcl-1 are reported -250.364, -196.753, -234.244 and -188.749 Kilo Joules respectively. Among all the apoptotic targets Bcl-2 and gossypol derivative 3k complex has shown better interaction when compared to others; followed by Bcl-xL and gossypol derivative 3h, Bcl-w and gossypol derivative 3b and Mcl-1 and gossypol derivative 3a.

Although previous studies of gossypol derivatives have shown some improved anti-apoptotic activity, but none of them progressed to approval due to poor pharmacokinetic activity. The four important properties of chemical compound absorption, distribution, metabolism and excretion (ADME) were analyzed to assess drug likeliness. ADME

properties of lead molecule against each anti-apoptotic target are shown in Table 2.

The gossypol derivatives have shown poor ADME properties but these key molecules are indispensable for improving the future studies.

Compound	Target	Mol Formula	MW	Donor-HB	Acceptor-HB	PLog Po/W	Plog S	BBB Per meant
3k	Bcl-2	C58H50N4O22	1155.03	18	24	4.97	-11.3	No
3b	Bcl-w	C44H42N4O12	818.82	12	14	5.3	-9.18	No
3h	Bcl-xL	C60H54N4O12	1023.09	12	14	8.77	-13.77	No
3a	Mcl-1	C44H42N4O10	786.83	10	12	5.95	-9.45	No

Table 2: ADME properties of lead Gossypol derivatives (3a, 3b, 3h and 3k) in anti-apoptotic Bcl-2 family targets.

Conclusion

Natural gossypol is toxic, less soluble in water, and anti-fertility agent, therefore, 54 chemo sensitive hydrazide-hydrazone gossypol derivatives (3a-3r) were designed to evaluate their binding interaction stability with the antiapoptotic targets Bcl-2, Bcl-w, Bcl-xL and Mcl-1. Molecular interactions involving H-bonds between protein and ligands were deduced based on dock score functions. Bcl-2 and gossypol derivative 3k complex has shown better interaction among Bcl-2 family members. Top ranked hydrazide-hydrazone gossypol derivatives against each anti-apoptotic target were further probed for ADME properties. Although drug likeliness is not significant for the top ranked molecules, but they are good leads for the further improvement and synthesis of functionally valid BH3 mimetics.

Acknowledgement

Authors highly acknowledge to University Grants Commission (UGC), Government of India (Post-Doctoral Fellowship for SC/ST, Award Letter No#: PDFSS-2015-17-ST-TEL-10464) for funding this project. Authors acknowledge to Prof. S.K.Mahmood, Principal and Dr. P. Sushma (Head, Department of Botany), University College of Science, Osmania University, Saifabad, Hyderabad, Telangana for providing lab facility and instrumentation in the department of Botany to complete this work.

Authors Contribution

Dr. R Chandrashekhar carried out entire research process, gathers data, analyzed and interprets the results and also wrote research paper under the co-supervision of Dr. Ram Bhavani and supervision of Dr. N Lakshmi Bhavani. Dr. Vasudha Bakshi helped in the analysis of pharmacophore models and docking results. The manuscript final draft proofreads and approved by all of the authors

References

1. Hanahan D, Weinberg RA (2011) Hallmarks of cancer: The next generation. Cell 144: 646-674.
2. Thang PM, Takano A, Yoshitake Y, Shinohara M, Murakami Y, Daigo Y (2016) Cell division cycle associated 1 as a novel prognostic biomarker and therapeutic target for oral cancer. Int J Oncol 49: 1385-1393.
3. Elmore S (2007) Apoptosis: A review of programmed cell death. Toxicol Pathol 35: 495-516.
4. Kerr JF (2002) History of the events leading to the formulation of the apoptosis concept. Toxicology 181: 471-474.
5. Cabon L, Martinez-Torres AC, Susin SA (2013) Programmed cell death comes in many flavors. Med Sci 29: 1117-1124.
6. Redza-Dutordoir M, Averill-Bates DA (2016) Activation of apoptosis signalling pathways by reactive oxygen species. Biochim Biophys Acta 1863: 2977-2992.
7. Norbury CJ, Hickson ID (2001) Cellular responses to DNA damage. Annu Rev Pharmacol Toxicol 41: 367-401.
8. Ke B, Tian M, Li J, Liu B, He G (2016) Targeting programmed cell death using small-molecule compounds to improve potential cancer therapy. Med Res Rev 36: 983-1035.
9. Yip KW, Reed JC (2008) Bcl-2 family proteins and cancer. Oncogene 27: 6398-6406.
10. Crawford M, Batte K, Yu L, Wu X, Nuovo GJ, et al. (2009) MicroRNA 133B targets pro-survival molecules MCL-1 and Bcl2L2 in lung cancer. Biochem Biophys Res Commun 388: 483-489.
11. Huerta-Yepez S, Baritaki S, Baay-Guzman G, Hernandez-Luna MA, Hernandez-Cueto A, et al. (2013) Contribution of either YY1 or BclXL-induced inhibition by the NO-donor detanonoate in the reversal of drug resistance, both *in vitro* and *in vivo*. YY1 and BclXL are overexpressed in prostate cancer. Nitric Oxide 29: 17-24.
12. Kontro M, Kumar A, Majumder MM, Eldfors S, Parsons A, et al. (2016) HOX gene expression predicts response to Bcl-2 inhibition in acute myeloid leukemia. Leukemia 31: 301-309.
13. Yin J, Li Y, Zhao H, Qin Q, Li X, et al. (2016) Copy-number variation of MCL1 predicts overall survival of non-small-cell lung cancer in a Southern Chinese population. Cancer Med 5: 2171-2179.
14. Delbridge AR, Grabow S, Strasser A, Vaux DL (2016) Thirty years of Bcl-2: Translating cell death discoveries into novel cancer therapies. Nat Rev Cancer 16: 99-109.
15. Delbridge AR, Strasser A (2015) The Bcl-2 protein family, BH3-mimetics and cancer therapy. Cell Death Differ 22: 1071-1080.
16. Labi V, Grespi F, Baumgartner F, Villunger A (2008) Targeting the Bcl-2-regulated apoptosis pathway by BH3 mimetics: A breakthrough in anticancer therapy? Cell Death Differ 15: 977-987.
17. El-Sharaky AS, Newairy AA, Elguindy NM, Elwafa AA (2010) Spermatotoxicity, biochemical changes and histological alteration induced by gossypol in testicular and hepatic tissues of male rats. Food Chem Toxicol 48: 3354-3361.
18. Santana AT, Guelfi M, Medeiros HC, Tavares MA, Bizerra PF, et al. (2015) Mechanisms involved in reproductive damage caused by gossypol in rats and protective effects of vitamin E. Biol Res 48: 43.
19. Kim NY, Han BI, Lee M (2016) Cytoprotective role of autophagy against BH3 mimetic gossypol in ATG5 knockout cells generated by CRISPR-Cas9 endonuclease. Cancer Lett 370: 19-26.
20. Coutinho EM (2002) Gossypol: A contraceptive for men. Contraception 65: 259-263.
21. Wang X, Wang J, Wong SC, Chow LS, Nicholls JM, et al. (2000) Cytotoxic effect of gossypol on colon carcinoma cells. Life Sci 67: 2663-2671.
22. Oliver CL, Miranda MB, Shangary S, Land S, Wang S, et al. (2005) (-)-Gossypol acts directly on the mitochondria to overcome Bcl-2- and Bcl-X(L)-mediated apoptosis resistance. Mol Cancer Ther 4: 23-31.

23. Van Poznak C, Seidman AD, Reidenberg MM, Moasser MM, Sklarin N, et al. (2001) Oral gossypol in the treatment of patients with refractory metastatic breast cancer: A phase I/II clinical trial. Breast Cancer Res Treat 66: 239-248.
24. Kang MH, Reynolds CP (2009) Bcl-2 inhibitors: Targeting mitochondrial apoptotic pathways in cancer therapy. Clin Cancer Res 15: 1126-1132.
25. Wei J, Kitada S, Rega MF, Emdadi A, Yuan H, et al. (2009) Apogossypol derivatives as antagonists of anti-apoptotic Bcl-2 family proteins. Mol Cancer Ther 8: 904-913.
26. Liang XS, Rogers AJ, Webber CL, Ormsby TJ, Tiritan ME, et al. (1995) Developing gossypol derivatives with enhanced antitumor activity. Invest New Drugs 13: 181-186.
27. Zhan Y, Jia G, Wu D, Xu Y, Xu L (2009) Design and synthesis of a gossypol derivative with improved antitumor activities. Arch Pharm 342: 223-229.
28. Yan F, Cao XX, Jiang HX, Zhao XL, Wang JY, et al. (2010) A novel water-soluble gossypol derivative increases chemotherapeutic sensitivity and promotes growth inhibition in colon cancer. J Med Chem 53: 5502-5510.
29. Thomsen R, Christensen MH (2006) MolDock: A new technique for high-accuracy molecular docking. J Med Chem 49: 3315-3321.
30. (2008) Schrodinger Inc., LLC, New York, USA.
31. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ (2012) Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev 64: 4-17.
32. Khadem S, Marles RJ (2010) Monocyclic phenolic acids; hydroxy and polyhydroxybenzoic acids: occurrence and recent bioactivity studies. Molecules 15: 7985-8005.