Bioorganic & Medicinal Chemistry Letters xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Anticancer phytochemical analogs 37: Synthesis, characterization, molecular docking and cytotoxicity of novel plumbagin hydrazones against breast cancer cells

Prasad Dandawate [a], Aamir Ahmad [b,*], Jyoti Deshpande [c], K. Venkateswara Swamy [c], Ejazuddin M. Khan [a], Madhukar Khetmalas [c], Subhash Padhye [a,b,*], Fazlul Sarkar [b]

[a] ISTRA, Department of Chemistry, MCE Society's Abeda Inamdar Senior College of Arts, Science and Commerce, University of Pune, Pune 411001, India
[b] Department of Pathology, Barbara Ann Karmanos Cancer Institute and Wayne State University School of Medicine, Karmanos Cancer Institute, 707 HWCRC, 4100 John R St, Detroit, MI 48201, USA
[c] Department of Bioinformatics and Computer Science, Dr. D. Y. Patil Biotechnology and Bioinformatics Institute, Dr. D. Y. Patil Vidyapeeth, Pune 411044, India

ARTICLE INFO

Article history:
Received 18 November 2013
Revised 19 March 2014
Accepted 23 April 2014
Available online xxxx

Keywords:
Plumbagin
Breast cancer
NF-κB
Molecular docking

ABSTRACT

We have synthesized, structurally characterized and examined cytotoxicity of novel plumbagin hydrazones against estrogen and progesterone receptor positive (ER+/PR+) MCF-7 and triple negative MDA-MB-231 breast cancer cell lines in order to evaluate the potential of these novel phytochemical analogs. Compounds were docked into the protein cavity of p50-subunit of NF-κB protein revealing better fit and better binding energies than the parent plumbagin compound. This was also reflected in their superior cytotoxicities which were found to be mediated by inhibition of NF-κB expression. These compounds can provide a starting point for the development of novel drug molecules against triple negative breast cancers.

Even after extensive research on cancer cell pathways, target identification and anticancer drug discovery, cancer still remains a major deadly disease in the world.[1] It is now well established that multiple pathways are involved in cancer growth and metastasis,[2] while drug resistance is the major problem in the cancer treatment.[3,4] It is now clear that inhibition of single pathway is not a useful approach for controlling cancer growth and hence, use of 'Multi-targeted Drugs' has been thought to be the right approach for cancer treatment.

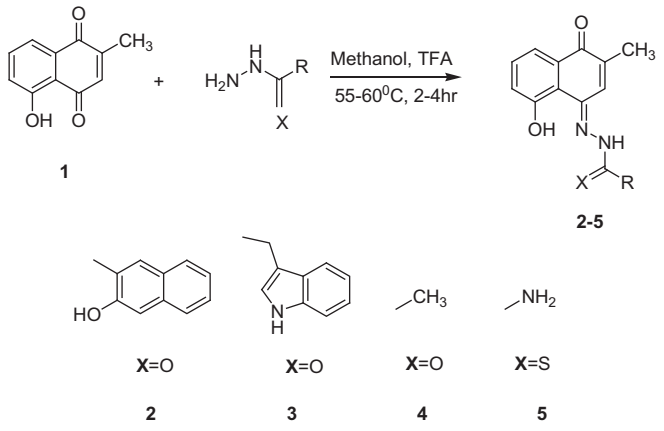
The use of naturally occurring phytochemicals for treatment of cancer has gained more attention in recent years due perhaps to their multi-targeting ability and largely non-toxic nature. Although phytochemicals have great potential in combating cancer their limited water solubility and metabolic instability are the major problems that need to be addressed while tailoring them for the clinical use.[5,6] In our group, we have been reviewing[7–12] and working on anticancer properties of phytochemical analogs of several bioactive compounds like genistein,[13] curcumin,[14,15] thymoquinone[16] and resveratrol[17] and have found that such analog approach involving modification of the parent phytochemical can yield more potent compounds capable of entering clinical trials. For example, the difluorinated benzylidene analog of curcumin known as CDF has shown superior anticancer activity with enhanced water solubility and metabolic stability than curcumin.[18,19]

Hence in the present work, we describe the use of such approach in case of plumbagin (5-hydroxy-2-methyl-1,4-naphthoquinone), which is one such multi-targeting phytochemical isolated from *Plumbago zeylanica*,[20] that is, found to induce apoptosis in TNBC and BRCA1 defective ovarian cancer cell lines. We have recently summarized the chemistry and biological activities of plumbagin.[10] Despite its promising activities, the compound has not been explored for generating analogs with optimized anticancer activity. There have been some reports describing the C-3 substituted plumbagin derivatives containing cyano, chloro, bromo and the *N*-acetyl amino acids moieties[21–25] as well as their metal complexes.[26–29]

In the present study, we have extended our structural studies on plumbagin by synthesizing its analogs containing bicyclic, heterocyclic, aliphatic hydrazones and thiosemicarbazone pharmacophore and evaluating their antiproliferative activities against hormone responsive and non-responsive triple negative breast cancer cell lines. We have rationalized these studies through molecular docking of all synthesized compounds into p50 subunit

* Corresponding authors. Tel.: +1 313 576 8315; fax: +1 313 576 8389 (A.A.); tel.: +91 8390025533; fax: +91 20 26446970 (S.P.).
E-mail addresses: ahmada@karmanos.org (A. Ahmad), sbpadhye@hotmail.com (S. Padhye).

http://dx.doi.org/10.1016/j.bmcl.2014.04.100
0960-894X/



Scheme 1. Schematic representation for synthesis of plumbagin hydrazones (**2**–**5**).
TFA = Trifluoroacetic acid.

of the NF-κB transcription protein and examining their inhibitory activities directly on p65-subunit of NF-κB in MDA-MB-231 breast cancer cells commonly recognized as one of the triple negative breast cancer lines.

The analogs **2**–**5** were synthesized[30] by condensing equimolar quantities of plumbagin and corresponding hydrazides (Scheme 1) in absolute methanol in presence of catalytic amount of trifluoroacetic acid with continuous stirring at 55–60 °C for 2–4 h. The precipitated products were washed three times with cold methanol, dried under vacuum and purified by column chromatography using chloroform/methanol (9.5:0.5) as eluting system and characterized by spectroscopic methods.[33]

All synthesized compounds were yellow–orange to brown in color and their compositional and spectral data is summarized in Table 1. The FTIR spectra of compounds **2**–**5** showed bands in the region 1580–1606 cm^{-1} corresponding to azomethine linkage confirming the formation of plumbagin hydrazinic Schiff bases.[31] The hydroxyl stretch of plumbagin occurring at 3292 cm^{-1} was found to be shifted in the range of 3306–3074 cm^{-1} in these analogs.[28–30] The free quinone carbonyl and hydrazinic carbonyl groups were found in the region 1680–1645 cm^{-1}, while the one observed at 1600 cm^{-1} could be ascribed to C=S stretch in case of compound **5**. The hydrazinic N—N stretch[31] was observed in the range 1058–1038 cm^{-1}, while the C—S vibration appeared at 823 cm^{-1} in the compound **5**. The phenolic C—O vibration could be observed at 1259 cm^{-1} for the free plumbagin ligand[28] which was shifted to higher frequency region 1280–1257 cm^{-1} in the hydrazone derivatives. The mass spectroscopic analysis showed molecular ion peaks corresponding to M^+ and M^++H ions

Table 1
Compositional and spectral data on plumbagin hydrazones (**2**–**5**)

Code	Mol formula	IR frequencies (cm^{-1})					Mass spectra	
		O—H	C=O	C=N	C—O	N—N	Calcd	Found
1	$C_{11}H_8O_3$	3445	1663, 1645	–	1229	–	–	–
2	$C_{22}H_{16}N_2O_4$	3306.10–3273.31	1680.05–1660.77	1600.97	1552.75	1057.03	372.37	372.3389
3	$C_{21}H_{17}N_3O_3$	3180.72–3111.28	1676.20–1645.33	1604.83	1257.63	1058.96	359.38	360.1341
4	$C_{13}H_{12}N_2O_3$	3217.3–3074.63	1676.20–1647.26	1606.76	1278.85	1045	244.08	245.0899
5	$C_{12}H_{11}N_3O_2S$	3282.95–3171.08	1600.97 (C=S)	1580	823.63 (C—S)	1049.31	261.30	261.0475

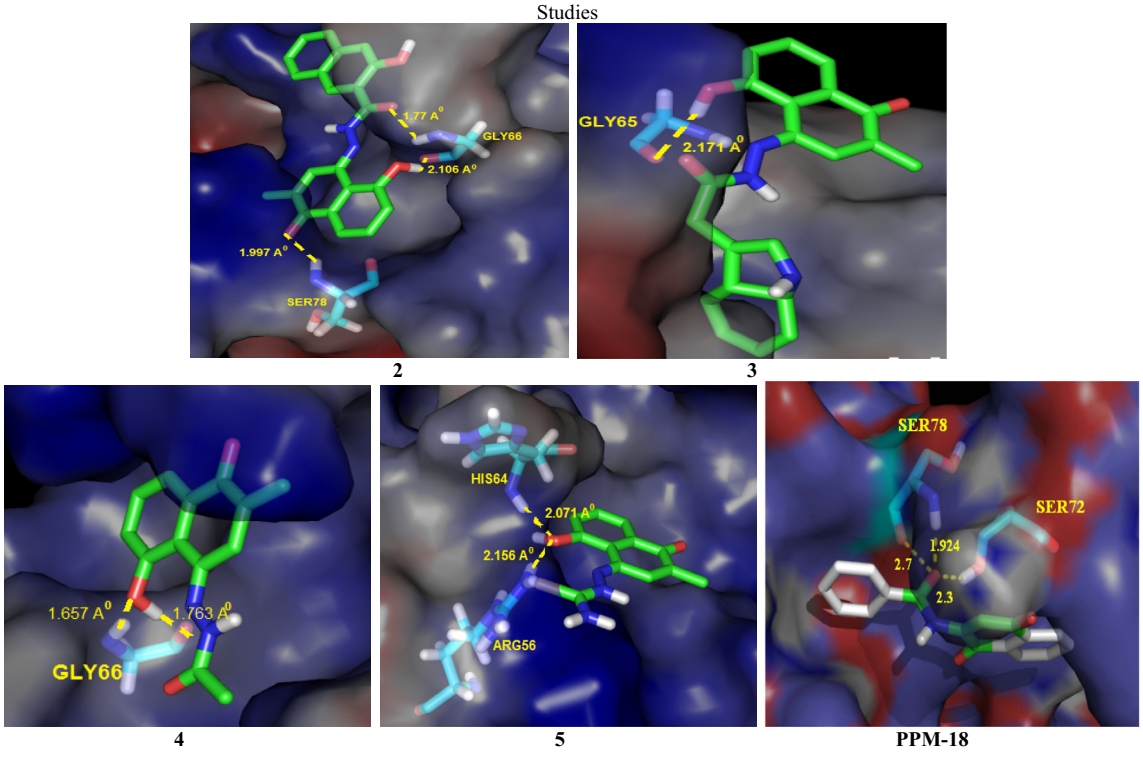


Figure 1. Binding of plumbagin Hydrazones (**2**–**5**) into the active site of p50 subunit of NF-κB as assessed by computer modeling studies.