Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

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

Document heading

Synthesis, Molecular docking and Biological evaluation of 4-Cycloalkylidineamino 1, 2-Naphthoquinone Semicarbazones as Anticancer agents

Shubhanjali Shukla[1], Radhey Shyam Srivastava[*1], Sushant Kumar Shrivastava[1], Ajit Sodhi[2], Pankaj Kumar[3]

[1] *Department of Pharmaceutics, I.T., Banaras Hindu University, Varanasi, 221005*
[2] *School of Biotechnology, Faculty of Science, Banaras Hindu University, Varanasi, 221005*
[3] *School of Pharmaceutical science, Siksha 'O' Anusandhan University, Bhubaneswar*

ARTICLE INFO

Article history:
Received 2 December 2011
Received in revised form 6 December 2011
Accepted 9 January 2012
Available online 28 September 2012

Keywords:
Hep-G2
MG-63
MCF-7
anticancer activity
docking.

ABSTRACT

Objective: In an effort to etablish new candidates with improved antineoplastic activity, 4-cycloalkylidineamino 1,2-naphthoquinone semicarbazones were synthesized, characterized and evaluated for anticancer activity. **Method:** The desired compounds were synthesized by condensation of 4- amino1, 2-naphthoquinone with cyclic ketones and further subsequent reaction of this product with semicarbazide hydrochloride. Compounds were characterized by FT-IR, 1H NMR, 13C NMR, elemental analysis and screened for antiproliferative activity against three human cancer cell lines (HepG2, MG-63 and MCF-7) by MTT assay using doxorubicin as standard. Docking was performed by using the Glide 5.7 integrated with Maestro 9.2 (SchrÖdinger, LLC, 2011) to understand the binding preference of synthesized compounds with target enzyme topoisomerase-II. **Results:** 4-(cyclohexylideneamino) [1, 2] naphthoquinone 2-semicarbazone was found to be most active cytotoxic agent against all cancer cell lines with IC_{50} values in the range of 5.58 - 6.31 μM. Results of molecular docking were also well correlated in vitro cytotoxicity assay. Insilico ADME studies revealed the drug likeliness of compounds. **Conclusions:** The synthesized compounds were found to have significant anticancer activity and able to enter in higher phases of the drug development process due to favorable pharmacokinetic properties.

1. Introduction

Cancer treatment has been a major endeavor of research and development in academia and pharmaceutical industry from the last many years as it is one of the leading causes of death [1-3]. Although major advances have been made by medicinal chemists in the chemotherapeutic management but the medical need is still largely unmet due to many factors among which the lack of selectivity of conventional drugs leading to toxicity, the metastatic spreading, and multi-drug resistance; MDR [4-6]. Therefore, the search for novel and selective anticancer agents is urgently required due to problems associated with currently available anticancer drugs. The National Cancer Institute (NCI), Bethesda, USA, is still playing an articular role in this field and identified the quinone as an important pharmacophore for anticancer activity [7-8].

Literature survey revealed that large number of naturally occurring1, 2-naphthoquinones like rhinacanthone [9-11], beta lapachone [12-13], mansonones [14-15] etc.(Fig.1.a, b, c) and synthetic 1, 2-naphthoquinones including prenyl 1,2-naphthohydroquinone [16], 1,2-naphthoquinone semicarbazone, 1,2-furanonaphthoquinones and 1,2-pyranonaphthoquinones are found to have anticancer activity [17-19] (Fig.1.d, e, f). Several 1, 2-napthoquinone derivatives act as topoisomerase II inhibitor and induce the DNA-TOPO II-mediated cleavages of DNA, this effect is crucial for the cytotoxicity of these compounds [20-22].

Semicarbazones also received considerable attention due to anticancer potential associated with them [23-24] and in combination with 1, 2-naphthoquinone they are found to have synergestic action [25]. In our earlier study, we also reported the synthesis and evaluation of antiproliferative

*Corresponding author: Dr. Radhey Shyam Srivastava, Prof. of Pharmaceutical Chemistry Department of Pharmaceutics, Institute of Technology Banaras Hindu University, Varanasi, U.P. INDIA
Phone no. +91542-26702749,
Fax No. +915422368428
Email-ID: rssriitbhu@yahoo.com, shubpharma@rediffmail.com

activity of 1, 2-naphthouinone and its derivatives [26].

In continuation of our previous efforts we aimed to synthesize 4-cycloalkylidineamino 1, 2-naphthoquinone semicarbazones and their dose dependant antiproliferative efficiency is determined in MCF-7, Hep-G2 and MG-63 cell lines. Further to simulate their binding preference with target enzyme (ATPase domain of Topoisomerase-II), molecular docking was conducted and in silico pharmacokinetic properties were also predicted to check the drug likeliness of synthesized compounds.

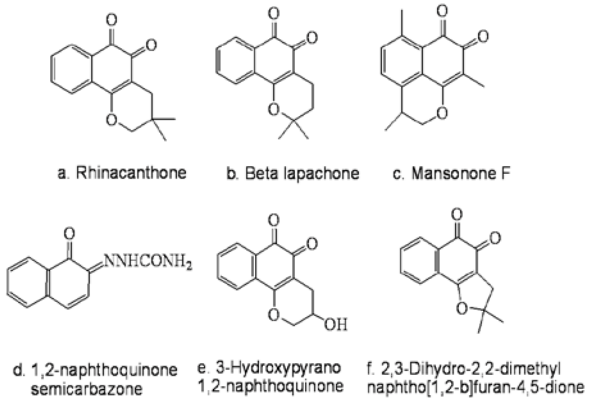

Figure 1. Structures of some naturally occurring and synthetic 1, 2-naphthoquinones.

2. Materials and Methods

2.1 Chemicals

All the chemicals and reagents used to carry out the research work were procured from Merck Genei (Bangalore), S.D. Fine and Sigma Aldrich.

2.2 Synthesis of 4-Cycloalkylidineamino 1, 2-naphthoquinone semicarbazone

4-amino1, 2-naphthoquinone(ANQ), the key intermediate for the synthesis of target compounds was synthesized by the method previously described in our recent published paper [26]. Condensation of ANQ with cyclic ketones (cyclopentanone and cyclohexanone) by refluxing with ethanol & conc. H_2SO_4 as catalyst afforded the 4-cycloalkylidineamino 1, 2-naphthoquinone (CYANQ). Further reaction of 4-cycloalkylidineamino 1, 2-naphthoquinone with semicarbazide hydrochloride leads to synthesis of desired compounds.

2.3 Characterization

Melting points were determined by BARNSTEAD/Electrothermal Stuart-SMP10, open capillary melting point apparatus and were uncorrected. IR spectra's were recorded on KBr disks using SHIMADZU Infrared-spectrophotometer, FTIR-8400S. The 1H-NMR and 13C NMR spectra's were measured by JEOL, AL300, FT-NMR spectrophotometer with Operating frequency, 300 MHz at temperature 25 ℃ using DMSO-d6 as solvent containing TMS as internal standard and reported chemical shift in δ values (ppm). Elemental analysis has been performed with Exeter Analytical Inc., USA, CE-440 elemental analyzer.

General procedure for synthesis of 4-Cycloalkylidineamino 1, 2-naphthoquinone (CYANQ):

A mixture of equimolar quantities of cyclic ketones (0.01 mol) and 4-amino 1, 2-naphthoquinone (0.01 mol) in ethanol (20 ml) was refluxed on a water bath for 8-10 h in the presence of few drops of conc. H_2SO_4 as catalyst. The progress of reaction was monitored by TLC at appropriate time interval. After completion of reaction, the solution was cooled, separated solid was filtered and washed with ice-cold water and dried. Finally, the product thus obtained was recrystallized from ethyl acetate and ethanol in different proportions.

General procedure for synthesis of 4-Cycloalkylidineamino 1, 2-naphthoquinone 2-semicarbazones:

Semicarbazone derivatives of CYANQ were synthesized by refluxing the boiling solution of 4-cycloalkylidineamino 1, 2-naphthoquinone (0.01mol) with semicarbazide hydrochloride (previously dissolved in 10 ml of water, 0.01 mol) in 50 ml of ethanol for 7-9 h. After cooling the solid product is filtered off and recrystallized with methanol.

4-(cyclohexylideneamino) [1, 2] naphthoquinone 2-semicarbazone (Scz A)

Yield: 59.1 %.M.P. 209-211 ℃; IR (KBr, υ max cm-1): 3371.82(-NH2 str.), 3259.72(-NH str.), 3093.71 (Aromatic C-H str.), 2963.56-2882.22(C-H str. of cylohexyl group), 1702.30,1673.22(C=O str. of semicarbazone and quinone),1622.39, 1581.89 (C=N str.); 1H NMR (DMSO-d6, 300 MHz) δ (ppm): 13.74 (s,1H,NH), 8.75,8.42 (2s,2H,NH2), 1.25-1.50 (m,10H, cyclohexylidene-H), 7.08-7.62 (m, 4H, Ar - H); 13C NMR (DMSO-d6) δ (ppm): 15.48-21.35(Cyclohexyl -C), 117.45-123.94(Aromatic-C),153.41, 148.18 (-C=N), 165.11(C=O of semicarbazone), 179.13 (C=O of quinone); anal. calcd. For $C_{17}H_{18}N_4O_2$ (%): C, 65.79; H, 5.85; N, 18.05. Found (%): C, 65.75; H, 5.89; N, 18.01.

4-(cyclopentylideneamino) [1, 2] naphthoquinone 2-semicarbazone (Scz B)

Yield: 44.5 %.M.P. 217-218 ℃; IR (KBr, υ max cm^{-1}): 3388.18(-NH2 str.), 3221.23(-NH str.), 3054.59 (Aromatic C-H str.), 2961.23-2878.13(-C-H str. of cylopentyl group), 1703.76, 1674.12(C=O str. of quinone),1628.84,1585.54 (C=N str.);1H NMR (DMSO-d6, 300 MHz) δ (ppm):13.71 (s,1H,NH), 8.91,8.62(2s,2H,NH2),1.33-1.75(m, 8H, cyclopentylidene-H), 7.10-7.51 (m, 4H, Ar - H); ^{12}C NMR (DMSO-d6) δ (ppm): 22.10-27.65 (Cyclopentyl-C), 118.49-134.26 (Aromatic-C), 153.13,147.10 (C=Ngroup), 167.49 (C=O of semicarbazone), 178.67 (C=O of quinone); anal. calcd. For $C_{16}H_{16}N_4O_2$ (%): C, 64.85; H, 5.44; N, 18.91. Found (%): C, 64.88; H, 5.39; N, 18.88.

2.4. Biological activity

2.4.1. Cell lines and culture medium

The cell lines MCF-7 (human breast cancer), Hep-G2 (liver sarcoma), MG-63 (Osteosarcoma) were procured from National Centre for Cell Sciences Repository, Pune, India. The cells were maintained in Dulbecco's minimum essential medium supplemented with 10% heat-inactivated fetal calf serum and 0.1% antibiotic solution (complete medium-CM) at 37 ℃ in humidified air containing 5% CO_2 in the CO_2 incubator.

2.4.2. In vitro anticancer activity

The synthesized compounds were screened for their cytotoxic activity against a panel of three cancer cell lines (MCF-7 for human breast cancer, Hep-G2 for liver sarcoma, and MG-63 for Osteosarcoma) by MTT assay. Doxorubicin, a quinonoidal drug was used as positive control. These cells were seeded in 96 well plates at the density of approximately 20,000 cells per well and incubated for 24 h to get a uniform monolayer of cells. Compounds were dissolved in DMSO and then diluted with complete medium-CM to obtain various concentrations of the compounds (1, 2.5, 5, 10, 15, 20, 25 μM). Each concentration of compound was added to triplicate in all cancer cell lines and incubated at 37 ℃ in a CO_2 incubator for 48h. After completion of treatment, 30 μl of 5 mg/ml MTT solution was added to the monolayers and incubated for 4hr at 37 ℃.

The MTT reaction was terminated by the addition of 0.04N HCl in isopropanol. The absorbance of MTT formazan formed was measured on ELISA plate reader at 540 nm.

2.4.3. Statistical analysis

Statistical analysis was performed using Graph Pad prism software, version 5 (Graph Pad software) by using nonlinear regression analysis. IC_{50} values were calculated from dose-response curves for triplicate dose of each compound. Level of significance checked by using one way ANOVA followed by Dunnett's t test.

2.5 Molecular docking

The computation was carried out using Schrödinger 2011 molecular modeling software package. Docking was performed by using the Glide 5.7 integrated with Maestro 9.2 (Schrödinger, LLC, 2011) interface on the linux operating system. The structure of the human topoisomerase-II (TP-II) co-crystallized with AMP-PNP complex [PDB: 1ZXM] was retrieved from the Protein Data Bank (www.rcsb.org) and the protein (TP-II complex) was modified with the "protein preparation wizard" by deleting the substrate cofactor and crystallographically observed water molecules. The protein with least energy (0.6 kcal/mol) was selected for docking calculations and receptor grid was generated at the active site of protein. Then single low energy 3D structure of ligands with correct chiralities was docked with the binding site using the 'extra precision' Glide algorithm in Schrodinger.

2.6 In silico ADME predictions

Pharmacokinetic properties of synthesized compounds were predicted by QikProp 3.4 (V34111) program to get an idea whether the compounds are able to enter higher phases of the drug development process or not.

3. Results

3.1 Synthesis of compounds

The synthetic route of the target compounds is illustrated in Figure 2 and revealed the reaction of 4-cycloalkylidineamino 1, 2-naphthoquinones (2a and 2b) with semicarbazide hydrochloride afforded the synthesis of desired compounds.

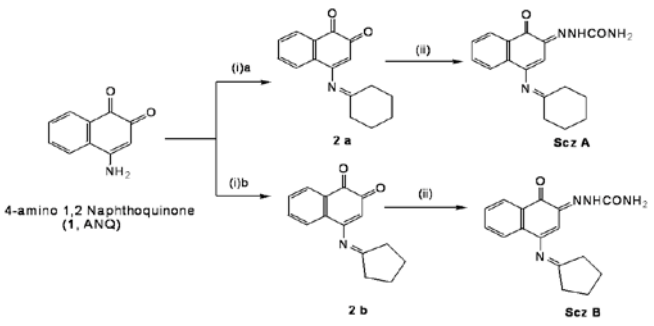

Figure 2. Scheme for synthesis of 4-Cycloalkylidineamino 1, 2-naphthoquinone semicarbazones, Reagents and conditions (i) a. Cyclohexanone, conc.H_2SO_4/ Ethanol, reflux, 8.5 h (i) b. Cyclopentanone, conc.H_2SO_4/Ethanol, reflux, 9.5 h (ii) $NH_2NHCONH_2$. HCl,Ethanol,7-9hr.

3.2 Characterization of compounds

Substitution of one of the quinone carbonyl oxygens in 4-cycloalkylidineamino 1, 2-naphthoquinone with semicarbazide side chain is confirmed by the presence of additional peaks in the range of 3221-3259 and 3371-3388 cm^{-1} in the IR spectrum of synthesized compounds which reveals the asymmetric and symmetric stretches of the amino group. Strong imine absorption at 1581-1585 cm^{-1} due to C=N linkage also confirms the successful derivatization. Compounds are showing bands in the range of 2878-2963 cm-1 which confirms C-H stretching of cycloalkyl group.

In 1H NMR spectrum, multiplet observed in the range of 1.25-1.75 ppm confirms the presence of cycloalkyl group in the compounds which are further confirmed by peaks at 15-27 ppm in 13C NMR spectra.

3.3 In vitro anticancer activity

All the compounds having cycloalkylidine group are showing significant cytotoxicity against all the cancer cell lines. The results are represented graphically in Figure 3, 4, 5 and 6 and IC_{50} values are shown in Table 1.

Table 1

Docking score and IC_{50} of compounds in MCF-7, Hep-G2 and MG-63 cell lines.

Compounds	Docking score	IC_{50} (μM)		
		MCF-7	Hep-G2	MG-63
1(ANQ)	-5.23	14.8	15.68	24.6
2 a	-5.35	9.3**	7.37**	11.52*
2 b	-5.45	9.64**	7.77**	11.88*
Scz A	-7.63	5.58**	5.74**	6.31**
Scz B	-7.45	6.21**	7.29**	6.07**
Dox	-8.06	2.67***	1.69***	3.17***

IC_{50} (μM): dose of the compound which caused 50% reduction of cell survival. Values were calculated from dose-response curves for triplicate dose of each compound. Level of significance checked by using one way ANOVA followed by Dunnett's t test .

* $P<0.05$, ** $P<0.01$, *** $P<0.001$, ns: non significant

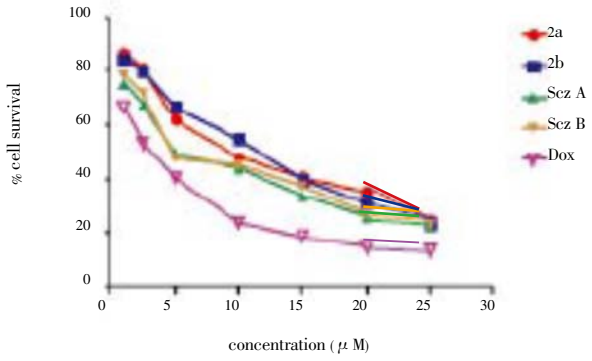

Fig. 3. Effect of concentrations (1 μM to 25 μM) of compounds 2a, 2 b, Scz A, Scz B & doxorubicin on viability of human breast cancer (MCF-7) cell line.

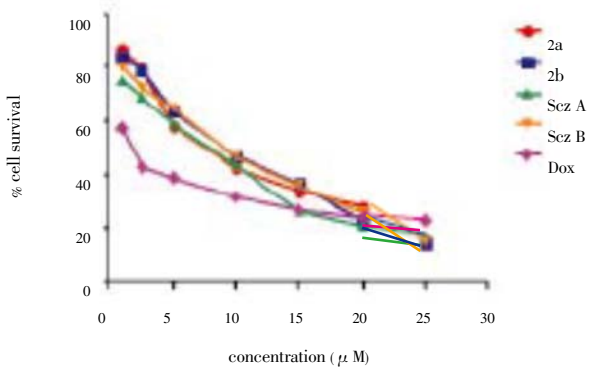

Fig. 4. Effect of concentrations (1 μM to 25 μM) of compounds 2a, 2 b, Scz A, Scz B & doxorubicin on viability of liver sarcoma (Hep-G2) cell line.

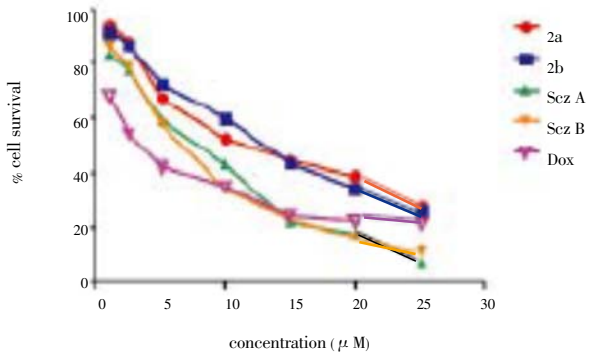

Fig 5. Effect of concentrations (1 μM to 25 μM) of compounds 2a, 2 b, Scz A, Scz B & doxorubicin on viability of Osteosarcoma (MG-63) cell line.

Activity data revealed that conversion of 4-amino1, 2-napthoquinone into 4-cycloalkylidineamino 1, 2-naphthoquinone drastically reduces the IC_{50} (almost 50%) values of compounds. Incorporation of semicarbazide side chain in one of the quinone carbonyl group further improves the activity of synthesized compounds and enhances its selectivity towards MG-63 cell lines.

3.4 Molecular docking

Molecular basis of interactions between target enzyme and synthesized ligands can be understand with the help of docking analysis and docking scores were summarized in Table 1. It is pertinent to note that the more active compounds Scz A and Scz B are showing nice docking (glide) scores, -7.63 & -7.45 respectively. Glide predictions revealed that semicarbazone derivatives are having greater binding affinity within the ATPase domain of topoisomerse II in comparison to 4-cycloalkylidineamino 1, 2-naphthoquinone. These dry lab findings are well supported by results of in vitro anticancer activity. The binding pattern of Scz A with topoisomerase II was depicted in Fig.7 and clearly revealed that the semicarbazide side chain showed major binding interactions with Gly 161, Lys 378 and Asn 163. The oxygen of free carbonyl group of 1, 2-naphthoquinone also showed hydrogen bond interaction with tyrosine 165.

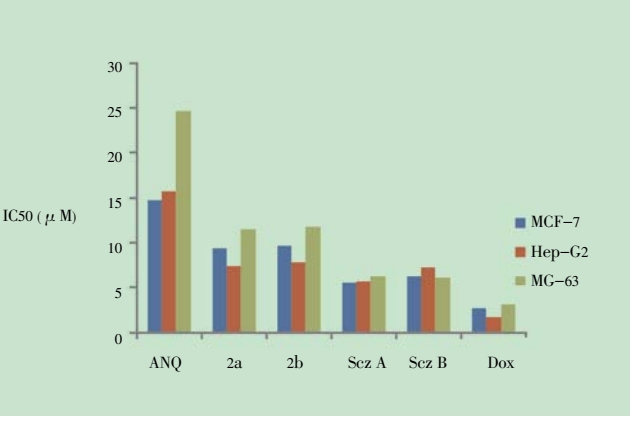

Fig.6. Comparative IC_{50} values of compound ANQ, 2a, 2 b, Scz A, Scz B & doxorubicin against MCF-7, HepG2 and MG-63 cell lines.

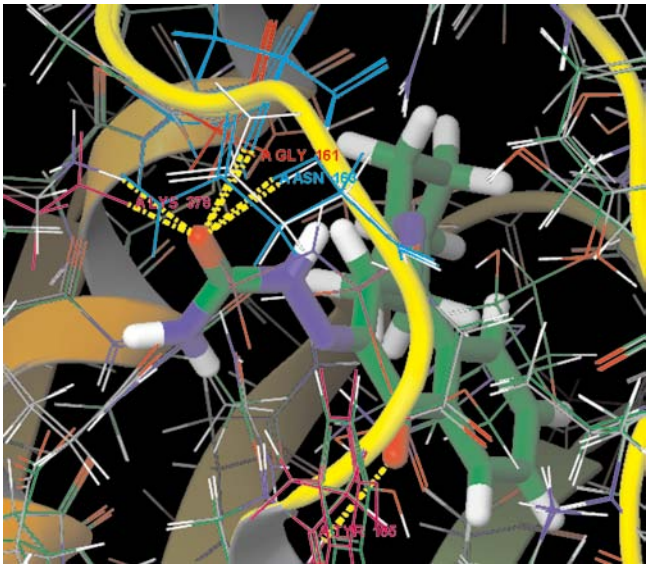

Fig 7. Glide predicted binding pose for compound Scz A within the ATPase domain of Topoisomerase-II, i.e. Oxygen of semicarbazide side chain bind with the Glycine(GLY) 161, Lysine(LYS) 378 and Asparagine (ASN) 163 as well as oxygen of free carbonyl group bind with the Tyrosine (TYR) 165.

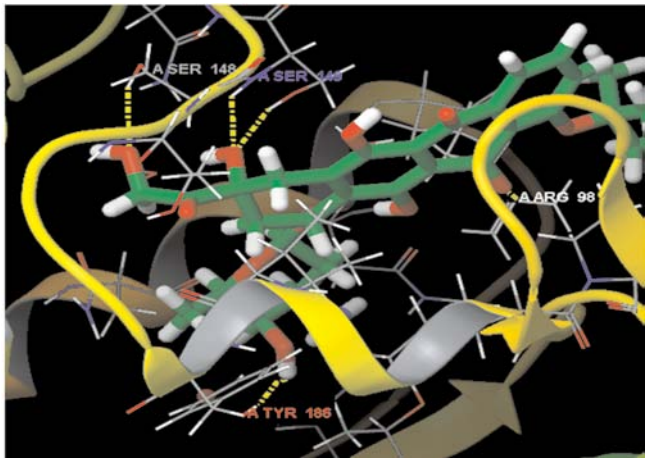

Fig 8. Glide predicted binding pose for standard drug doxorubicin which shows binding interaction with Arginine (ARG) 98, Tyrosine (TYR) 186, Serine (SER) 148 and Serine (SER) 149 residue at the active site of ATPase domain of Topoisomerase-II.

The standard drug doxorubicin showed binding interactions with Arg 98, Tyr 186, Ser 148 and Ser 149 with glide score -8.06 (Fig.8) which is close to docking scores of Scz A and Scz B.

3.5 In silico ADME predictions

The main objective of drug discovery is the development of new molecular scaffolds having high binding affinity towards the target with significant absorption, distribution, metabolism and excretion (ADME) profile and having less toxicity. The application of computational technology during drug discovery and development offers considerable potential for reducing the number of experimental studies required for compound selection and development. The QikProp program of Schrödinger, LLC, 2011 software was used to obtain the ADME properties of the ligands. It predicts both physically significant descriptors and Pharmaceutically relevant properties with a detailed analysis of the log P (Octanol/Water), % human oral absorption, CNS activity and permeability through MDCK (Madin-Darby Canine Kidney) cells in nm/sec etc. (MDCK cells are considered to be a good mimic for the blood-brain barrier) (cf. Table 2). All the compounds were found to be non toxic for CNS, value of permeability through MDCK Cells is < 500 in all cases so the compounds will not cross blood-brain barrier which is desirable. The predicted drug likeliness of the synthesized compounds follow the Lipinski "Rule of Five", all four parameter values for a compound i.e. Log P <5, H-bond donors <5 , H-bond acceptors <10 and molecular weight < 500 suggested that the compounds might have good absorption or permeability properties [27].

4. Discussion

Proposed compounds were synthesized and characterized successfully. All the synthesized compounds gave satisfactory analytical and spectroscopic data, which were

Table 2

Pharmacokinetic prediction of the synthesized compounds by QikProp 3.4

Comp code	% Human oral absorption[a]	QPlogPo/w[b]	QPPMDCK[c]	CNS[d]	M.wt[e]	donorHB[f]	acceptHB[g]	Rule of fiveviolation[h]
2 a	88.76	2.70	151.81	0	253	0	5	0
2 b	86.04	2.41	142.02	0	239	0	5	0
SczA	75.89	2.26	53.8	-2	310	2	3.5	0
Scz B	74.67	2.07	52.2	-2	296	2	3.5	0

a. % Human oral absorption: <25% is poor absorption. b. QPlogPo/w: Partition coefficient; recommended range -2.0 - 6.5. c. QPP MDCK: apparent MDCK permeability (nm/second) (<25 poor, >500 great). d. CNS: Predictive Central Nervous Activity -2 (inactive) to +2 (active). e. M.wt.: Molecular weight of the molecule < 500 is preferred. f. donor HB: Estimated number of hydrogen bonds that would be donated by the solute to water molecules in an aqueous solution, recommended range 0.0 - 6.0 g. accpt HB: Estimated number of hydrogen bonds that would be accepted by the solute from water molecules in an aqueous solution, recommended range 2 - 20. h. Rule of five: Number of violations of Lipinski's rule of five; recommended range 0 - 4

according to their depicted structures. In 1H NMR spectrum of semicarbazone derivatives a singlet is observed in the range of 13.71-13.74 ppm due to NH proton of semicarbazone and presence of 2 singlet at 8.75-8.91 and 8.42-8.62 ppm revealed that 2 protons of NH2 group are magnetically inequivalent. In 13C NMR of semicarbazone derivatives peak was observed in the range of 165-167 which is due to C=O group of semicarbazide side chain.

The potency of synthesized compounds as anticancer agents has been evaluated against three human cancer cell lines (MCF-7, Hep G2 and MG-63). Semicarbazone derivatives of 4-cycloalkylidineamino 1, 2-naphthoquinone were found to be more active than 4-cycloalkylidineamino 1, 2-naphthoquinones. The pharmacological data indicated that semicarbazone derivatives of 4-cycloalkylidineamino 1, 2-naphthoquinone Scz A and Scz B showed approximate 1.5-2 fold improvement in cytotoxicity against MCF-7 and MG-63 cell lines (IC50 5.58-6.31 μM) as compared with 4-cycloalkylidineamino 1, 2-naphthoquinones 2 a and 2 b (IC_{50} 9.3-11.88 μM).The results of antiproliferative activity are supported by docking analysis. The nice docking scores of semicarbazone derivatives reveals that these compounds are well accommodated in active site of enzyme and the binding pattern of compound Scz A shows that semicarbazide side chain is strongly interact within the active site of topoisomerse-II.

In silico pharmacokinetic prediction revealed that synthesized compounds are showing drug likeliness with marked lipophilicity, oral absorption and lack of CNS toxicity. None of compound is violated from Lipinski rule of 5, so they are able to enter in next phase of drug development.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

The authors are grateful to The Head, Department of Chemistry, Faculty of Science, Banaras Hindu University (BHU), Varanasi, India for providing the facilities of 1H NMR and 13C NMR spectroscopy. The financial assistance from Jawaharlal Nehru Memorial Fund, New Delhi (Ref: SU-A/165/2011-12/380), India to one of the author Shubhanjali Shukla is greatly acknowledged.

References

[1] Eckhardt S. Recent progress in the development of anticancer agents. *Curr Med Chem* 2002; **2**: 419-439.

[2] Mulcahy N. Cancer to become leading cause of death worldwide by 2010 by Medscape Medical News © 2008. http://www.medscape.com/ viewarticle/585098.

[3] Siegel R, Naishadham D, Jemal A. Cancer statistics, 2012. *CA Cancer J Clin* 2012; **62**:10-29.

[4] Ullah MF. Cancer multidrug resistance (MDR): A major impediment to effective chemotherapy. *Asian Pac J Cancer Prev* 2008; **9**:1-6.

[5] Gillet JP, Gottesman MM. Advances in the molecular detection of ABC transporters involved in multidrug resistance in cancer. *Curr Pharm Biotechnol* 2011;**12**: 686-692.

[6] Duan Z, Li X, Huang H, Yuan W, Zheng SL, Liu X, Zhang Z, Choy E, Harmon D, Mankin H, Hornicek F. Synthesis and evaluation of (2-(4-Methoxyphenyl)-4-quinolinyl)(2-piperidinyl)methanol (NSC23925) isomers to reverse multidrug resistance in cancer. *J Med Chem* 2012; **55**: 3113-3121.

[7] Driscoll JS, Hazard GF, Wood HB, Goldin A. Structure antitumor activity relationships among quinone derivatives. *Cancer Chemother Rep* 1974; **4**(2): 1-362.

[8] Liu KC, Li J, Sakya S. Synthetic approaches to the 2003 new drugs. *Mini-Rev Med Chem* 2004; **4**: 1105-1125.

[9] Thirumurugan RS, Kavimani S, Srivastava RS. Antitumour activity of rhinacanthone against Dalton's ascitic lymphoma. *Biol Pharm Bull* 2000; **23**(12):1438-1440.

[10]Siripong P, Hahnvajanawong C, Yahuafai J, Piyaviriyakul S, Kanokmedhakul K, Kongkathip N, Ruchirawat S, Oku N. Induction of apoptosis by Rhinacanthone isolated from Rhinacanthus nasutus roots in human cervical carcinoma cells. *Biol Pharm Bull* 2009; **32**(7):1251-1260.

[11]Pradidphol N, Kongkathip N, Sittikul P, Boonyalai N, Kongkathip B. First synthesis and anticancer activity of novel naphthoquinone amides. *Eur J Med Chem* 2012; **49**:253-270.

[12]Pardee AB, Li YZ, Li CJ. Cancer therapy with beta-lapachone. *Curr Cancer Drug Targets* 2002; **2**: 227- 242.

[13]Terai K, Dong GZ, Oh ET, Park MT, Gu Y, Song CW, Park HJ. Cisplatin enhances the anticancer effect of b-lapachone by upregulating NQO1. *Anticancer Drugs* 2009; **20** (10): 901-909.

[14]Wang D, Ming YX, Zheng C, Shinichi T, Satoshi O, Takashi I. Cytotoxic effects of Mansonone E and F isolated from Ulmus pumila. *Biol Pharm Bull* 2004; **27**(7):1025-1030.

[15]Wu WB, Ou JB, Huang ZH, Chen SB, Ou TM, Tan JH, Li D, Shen LL, Huang SL, Gu LQ, Huang ZS. Synthesis and evaluation of mansonone F derivatives as topoisomerase inhibitors. *Eur J Med Chem*. 2011; **46**(8):3339-3347.

[16]Molinari A, Oliva A, Ojeda C, Miguel del Corral JM, Castro MA, Cuevas C, Feliciano AS. Cytotoxic antineoplastic derivatives of

prenyl-1,2- naphthohydroquinone, Arch. Pharm. Chem. *Life Sci.* 2008; **341**: 301 - 306.

[17]Kongkathip N, Kongkathip B, Siripong P, Sangma C, Luangkamin S, Niyomdecha M, Pattanapa S, Piyaviriyakul S, Kongsaeree P. Potent antitumor activity of synthetic 1,2-Naphthoquinones and 1,4-Naphthoquinones. *Bioorg Med Chem* 2003; **11**: 3179-3191.

[18]Rocha DR, Souza ACG, Resende JALC, Santos WC, Santos EA, Pessoa C, Moraes MO, Costa-Lotufo LV, Montenegro RC, Ferreira VF. Synthesis of new 9-hydroxy- α - and 7-hydroxy- β -pyran naphthoquinones and cytotoxicity against cancer cell lines.*Org Biomol Chem*, 2011, **9,** 4315-4322.

[19]Su JC, Lin KL, Chien CM, Tseng CH, Chen YL, Chang LS, Lin SR. Furano-1,2-naphthoquinone inhibits EGFR signaling associated with G2/M cell cycle arrest and apoptosis in A549 cells. *Cell Biochem Funct* 2010; **28**(8):695-705.

[20]Frydman B, Marton LJ, Sun JS, Neder K, Witiak DT, Liu AA, Wang HM, Mao Y, Wu HY, Sanders MM, Liu LF. Induction of DNA topoisomerase II mediated DNA cleavage by beta lapachone and related naphthoquinones. *Cancer Res* 1997; **57**:620-627.

[21]Gurbani D, Kukshal V, Laubenthal J, Kumar A, Pandey A, Tripathi S, Arora A, Jain SK, Ramachandran R, Anderson D, Dhawank A. Mechanism of inhibition of the ATPase domain of human topoisomerase IIa by 1,4-Benzoquinone, 1,2-Naphthoquinone,1,4-Naphthoquinone, and 9,10-Phenanthroquinone. *Toxicol Sci* 2012; **126**(2): 372-390.

[22]Jiménez-Alonso S, Orellana HC, Estévez-Braun A, Ravelo AG, Pérez-Sacau E, Machín F. Design and synthesis of a novel series of pyranonaphthoquinones as topoisomerase II catalytic inhibitors. *J Med Chem* 2008; **51**: 6761-6772.

[23]Dutta S, Padhye S, Priyadarsini KI, Newton C. Antioxidant and antiproliferative activity of curcumin semicarbazone. *Bioorg Med Chem Lett* 2005; **15**(11): 2738-44.

[24]Ali SMM, Azad MAK, Jesmin M, Ahsan S, Rahman MM, Khanam JA, Islam MN, Shahriar SMS. In vivo anticancer activity of vanillin semicarbazone. *Asian Pac J Trop Biomed* 2012; **2**(6): 438-442.

[25]Afrasiabi Z, Sinn E, Lin W, Ma Y, Campana C, Padhye S. Nickel (II) complexes of naphthaquinone thiosemicarbazone and semicarbazone: Synthesis, structure, spectroscopy, and biological activity. *J Inorg Biochem* 2005; **99**: 1526-1531.

[26]Shukla S, Srivastava RS, Shrivastava SK, Sodhi A, Kumar P. Synthesis, characterization and antiproliferative activity of 1,2-naphthoquinone and its derivatives. *Appl Biochem Biotechnol.* 2012; doi: 10.1007/s12010-012-9551-9.

[27]Moorthy NSHN, Karthikeyan C, Trivedi P. Cytotoxic evaluation and in silico Pharmacokinetic Prediction of Some Benzo[a]Phenazine-5-sulfonic acid derivatives. *Med chem* 2009; **5**: 549-557.