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Synthesis of benzo[3,4]azepino[1,2-*b*]isoquinolin-9-ones from 3-arylisoquinolines via ring closing metathesis and evaluation of topoisomerase I inhibitory activity, cytotoxicity and docking study

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ABSTRACT

Benzo[3,4]azepino[1,2-*b*]isoquinolinones were designed and developed as constraint forms of 3-arylisoquinolines with an aim to inhibit topoisomerase I (topo I). Ring closing metathesis (RCM) of 3-arylisoquinolines with suitable diene moiety provided seven membered azepine rings of benzoazepinoisoquinolinones. Spectral analyses of these heterocyclic compounds demonstrated that the methylene protons of the azepine rings are nonequivalent. The shielding environment experienced by these geminal hydrogens differs unusually by 2.21 ppm. As expected, benzoazepinoisoquinolinones displayed potent cytotoxicity. However, cytotoxic effects of the compounds were not related to topo I inhibition which is explained by non-planar conformation of the rigid compounds incapable of intercalating between DNA base pairs. In contrast, flexible 3-arylisoquinoline **8d** attains active conformation at drug target site to exhibit topo I inhibition identical to cytotoxic alkaloid, camptothecin (CPT).

1. Introduction

Chemotherapeutic treatment has been considered as an effective method for healing cancer over the last several decades.[1,2] Recently, synthesis and conformational analysis of medium sized heterocycles exhibiting promising pharmacological activities have received more attentions.[3–5] N-Containing tetracyclic chemical entities such as indeno[1,2-*c*]isoquinolines 1,[6,7] isoindolo[2,1-*b*]isoquinolines 2,[8] benz[*b*]oxepines 3,[9] benzo[*c*]phenanthridinones 4,[10] and protoberberines 5[10] have been studied extensively as plausible antitumor agents (Fig. 1). Interestingly, these compounds share a common 3-arylisoquinoline scaffold and have been successfully synthesized from 3-arylisoquinolones as key precursors. In addition to structural similarity, these diversely modified 3-arylisoquinoline analogs display prominent level of pharmacological activities such as cytotoxicity and topo I inhibitory activity.[11–14]

Topo I is an enzyme which solves superhelical tension and other topological consequences that occur during separation of DNA strands. It relieves torsional stress of DNA supercoil generated during various DNA metabolic processes as replication, transcription, recombination, chromatin condensation and chromosome partitioning in cell division.[15–17] Because of the pivotal role of topo I in these vital processes of cell cycle and its elevated level in solid tumors, it has been a promising target for treatment of cancers.

Development of 3-arylisoquinoline based potent antitumor agents targeting topo I strategically involves the process of anchoring the 3-aryl group to the isoquinoline moiety with rings of various sizes. Constrained forms of 3-arylisoquinolines have advantages over flexible ones in term of target receptor specificity and efficacy as rigid structures have little conformational entropy and fit well into active site of the receptor.[18] In fact, significant increases in the topo I inhibitory activity were observed through conversion of flexible three aromatic rings to rigid forms, and molecular docking studies were used to explain the rise of potency of non-flexible derivatives.[11] Specifically, the 3-arylisoquinoline analogs with restricted rotation of 3-aryl rings are generally flat which in turn have maximum π–π stacking interaction between the molecule and DNA base pairs planks.

In spite of sharing similarity in terms of chemical structure and topo I selectivity, 3-arylisoquinoline derivatives bridged by new

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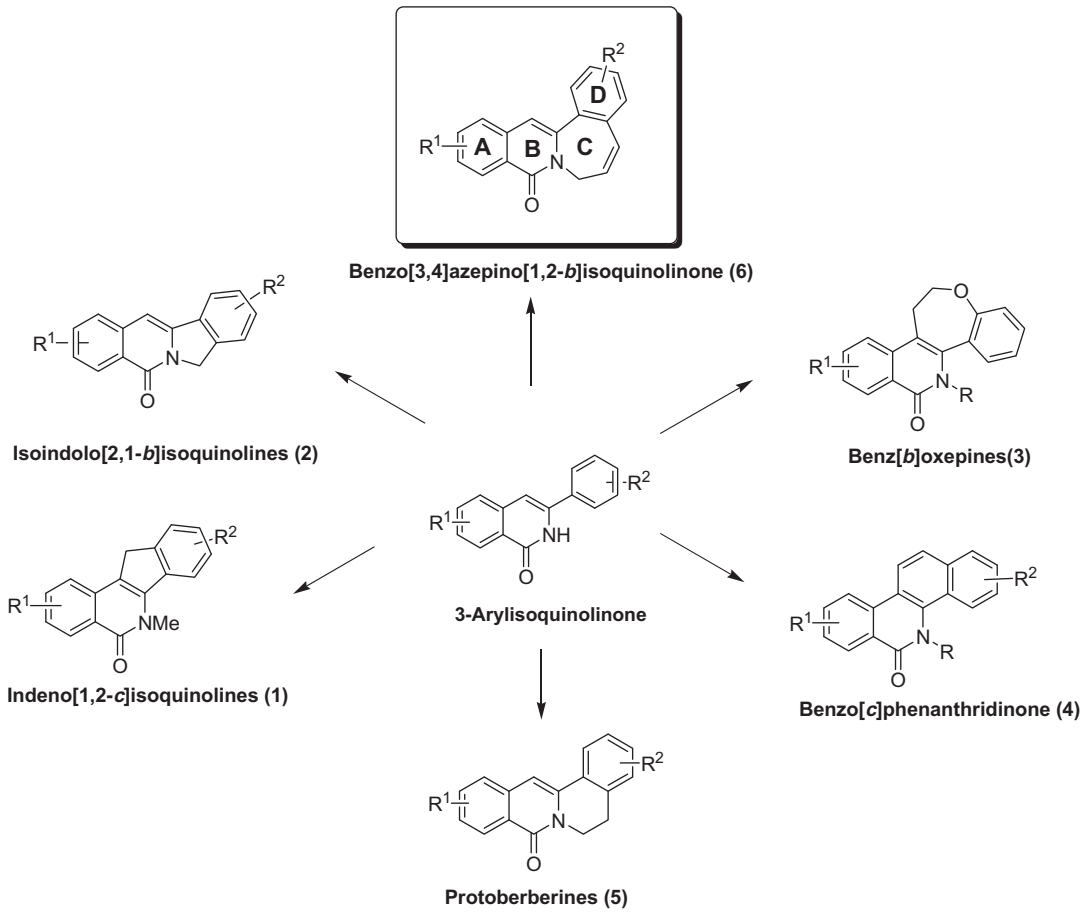


Figure 1. Structural modification of 3-arylisoquinolinone to heterocyclic compounds.

rings of various sizes differ in the level of potency. The difference is possibly due to their special molecular 3D geometric shapes and sizes which alter the orientation of the main polycyclic core and radiating functional groups at the drug binding pocket of the target. Thus, structural alterations can provide enough room for discovery of new chemical entities manifesting high degree of target selectivity and efficacy. Charmed with this aspect, we have been performing activity and molecular modeling score guided diverse modifications of 3-arylisoquinoline frame. Based on our reported procedure for the synthesis of protoberberine alkaloids via 3-arylisoquinolines as key intermediates,[19] we applied RCM for synthesis of benzo[3,4]azepino[1,2-*b*]isoquinolinones **6** with seven-membered C ring.

2. Results and discussion

2.1. Chemistry

2.1.1. Synthesis plan

The formation of cyclic rings from acyclic dienes is accomplished by RCM reaction catalyzed by transition metal.[20–33] In the similar manner, benzo[3,4]azepino[1,2-*b*]isoquinolinone **6** would form from olefin compound **7** by RCM method (Scheme 1). The RCM precursor **7** could be obtained through chemical modification of 3-arylisoquinolone **8**, which could be prepared by cycloaddition of lithiated toluamide **9** and benzonitrile **10**.

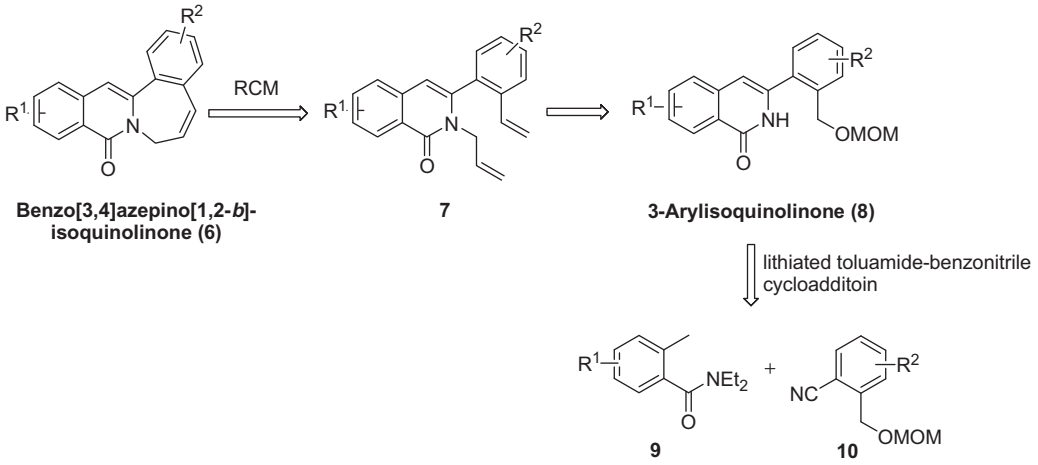
2.1.2. Synthesis of benzoazepinoisoquinolinones

Synthesis of benzoazepinoisoquinolinones was initiated by coupling *N*,*N*-diethyltoluamides **9** and benzonitriles **10** into 3-arylisoquinolines **8** (Scheme 2). The advantages of 3-arylisoquinoline synthesis methodology are the easy accessibility to starting materials with diverse aromatic ring substitutions and a one-pot procedure for construction of all essential carbon atoms of the target molecules. The versatile scaffold generated by coupling reaction of *o*-toluamides with benzonitriles has been well exploited for synthesis of natural isoquinoline alkaloids like benzophenanthridinones and protoberberines[10,34–40] as well as large array of heterocyclic compounds including 3-arylisoquinolinamines,[41] indeno[1,2-*c*]isoquinolines, isoindolo[2,1-*b*]isoquinolinones, 12-oxobenzo[*c*]phenanthridinones[9] and benz[*b*]oxepines with topo I inhibition and cytotoxicity property.

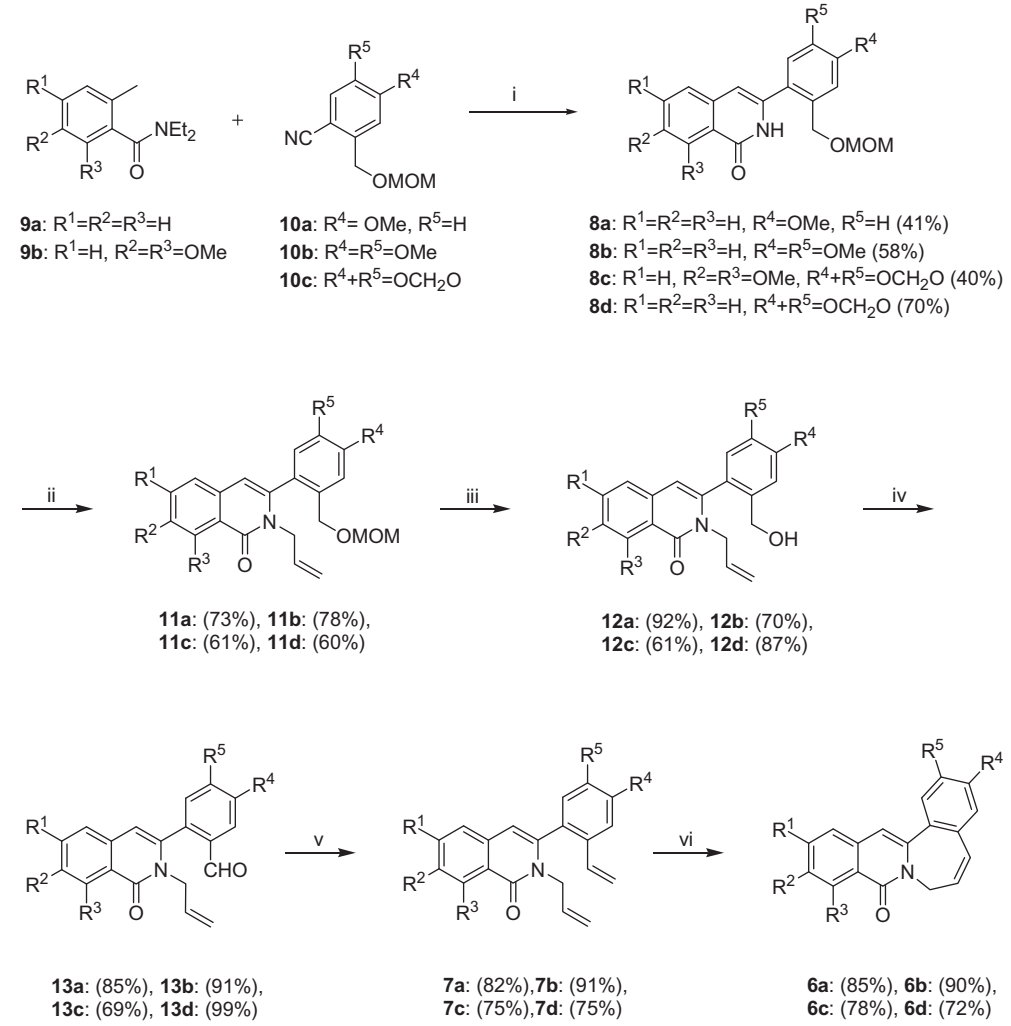
In the next step, selective N-allylation of amides **8** was achieved with allyl bromide in presence of K_2CO_3 in DMF. When we tried to introduce an alkyl group such as methyl or PMB, only N-alkylated compounds were obtained. MOM of **11** was readily removed with 10% HCl to give deprotected alcohols **12**, which were then oxidized by Cornforth reagent (pyridinium dichromate, PDC) to give the corresponding benzaldehydes **13**. Wittig reaction of the aldehydes **13** with Ph_3PCH_3Br and *n*-BuLi in THF provided the desired olefins **7**. Finally, RCM reaction of **7** was performed with 1st generation Grubbs catalyst in CH_2Cl_2 to give the desired cyclized compounds **6**.

2.1.3. Spectral data analysis

The structures of benzoazepinoisoquinolinones were confirmed by IR, mass, 1D ^1H, ^{13}C NMR and 2D ^1H–^{13}C HSQC spectra. Examination of ^1H NMR spectra of compound **6a** showed that the methylene protons of azepine ring exhibited geminal coupling. Interestingly, the geminal protons labeled as H7α and H7β signaled at δ 5.74 (dd, J = 8, 13.5 Hz, 1H) and 3.53 (ddd, J = 1.5, 6.5, 13.5 Hz, 1H), respectively (Fig. 2). The pronounced difference in the



Scheme 1. Retrosynthetic pathway of benzo[3,4]azepino[1,2-*b*]isoquinolinone **6**.



Scheme 2. Synthesis of benzo[3,4]azepino[1,2-*b*]isoquinolinones **6**. Reagents and conditions: (i) *n*-BuLi, THF, −78 °C; (ii) allyl bromide, K_2CO_3, DMF; (iii) 10% HCl; (iv) PDC, CH_2Cl_2; (v) Ph_3PCH_3Br, *n*-BuLi, THF; (vi) 1st generation Grubbs catalyst, CH_2Cl_2, reflux.

chemical shifts ($\Delta\delta$ = 2.21) of the methylene protons was verified by Heteronuclear Single Quantum Coherence (HSQC) experiments. HSQC cross peaks of H7α/C7 and H7β/C7 supported that the protons are attached to the same carbon C7 (Fig. 3).

1D and 2D spectral data revealed that the methylene protons H7α and H7β as an AB system, approached as an AX system with large difference in chemical shifts. This unusual behavior of the geminal protons is possibly due to deshielding effects of anisotropy

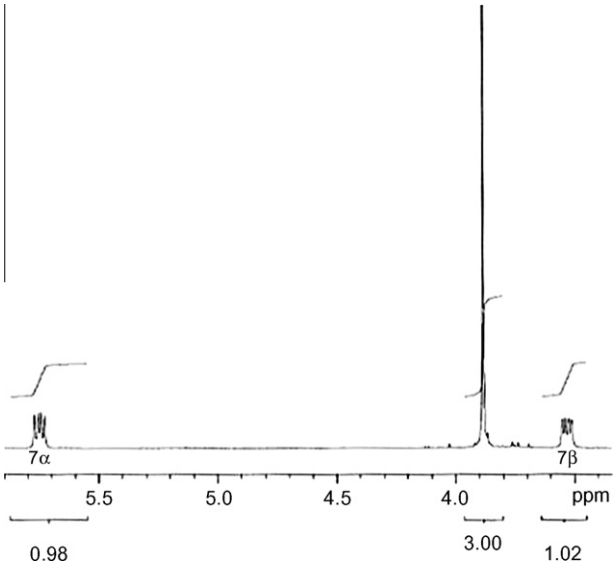


Figure 2. A portion of ^{1}H NMR spectrum of **6a** showing nonequivalence of geminal protons H7α and H7β.

of azepine ring current as well as of anisotropic magnetic field and electric field of neighboring carbonyl group on H7α.

The unusual behavior of geminal protons can be readily explained on the basis of reasonable assumptions about the lowest energy conformation of the compound **6a** (Fig. 4). Energetically minimized molecular model of **6a** shows that the azepine ring of the benzoazepinoisoquinolinone **6a** exists in boat conformation (Fig. 5a). At this stable conformation, flagpole proton H7β being held over and towards center of the seven membered azepine ring experiences shielding effect due to ring current while bowsprit proton H7α projecting outwards of the ring is deshielded by the same anisotropic effect.

Newman projection about the C7 and isoquinolone ring plane (Fig. 5b) shows that the proton H7α nearly tends to eclipse

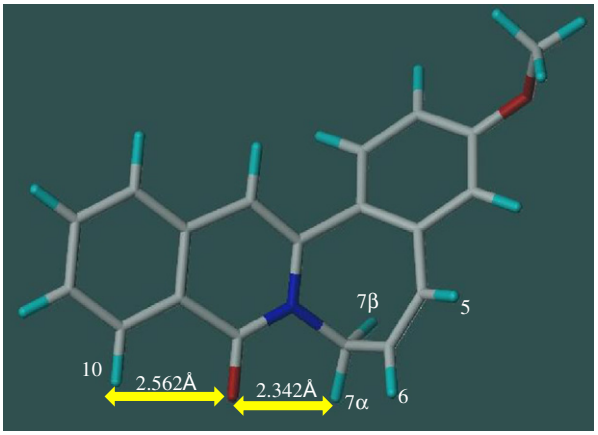


Figure 4. Minimized structure of **6a**. Sybyl software package was used to construct the energy minimized model.

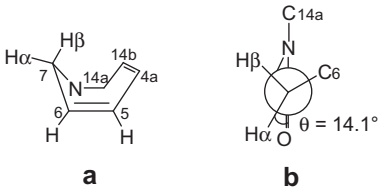


Figure 5. (a) Boat conformation of azepine ring; (b) Newman projection about C7 and isoquinolone ring.

carbonyl functional group with a dihedral angle of $\theta = 14.1°$. Whereas, proton H7β lies at an angle of 133.6° from C9═O group. In other words, H7α lies in the plane of carbonyl group while H7β erects above the plane. Due to this unique orientation, H7α resonates at lower magnetic field than H7β as commonly accepted, conventional model of anisotropic magnetic field of carbonyl group states that a nucleus in the plane of C═O is deshielded, and in the

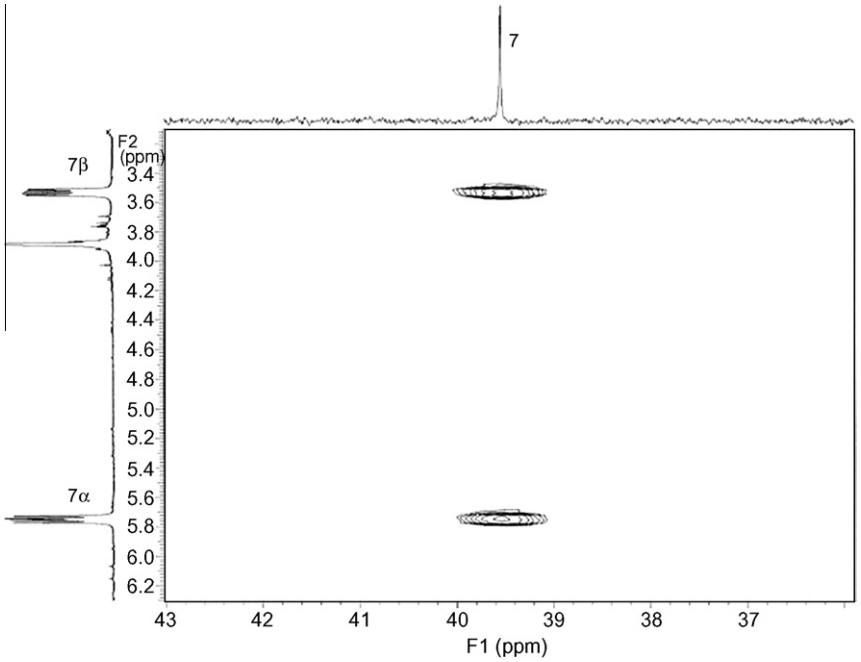


Figure 3. HSQC spectrum of **6a** showing correlations between geminal protons H7α and H7β with C7.

Table 1
IC_{50} cytotoxicity (μM) and topo I inhibitory activity of the compounds

No.	Compound	A549	HCT15	SKOV-3	SK-MEL-2	Topo I[a]
1	**6a**	6.48	12.57	24.34	7.74	++
2	**6b**	14.76	21.34	10.98	6.23	++
3	**6c**	7.45	6.34	2.80	2.54	++
4	**6d**	17.10	20.13	29.11	28.55	++
5	**7a**	17.23	22.76	43.82	11.63	++
6	**7b**	43.22	75.16	35.53	47.66	++
7	**7c**	>100	>100	>100	>100	–
8	**7d**	19.32	11.17	13.62	9.36	++
9	**8a**	45.26	27.46	75.50	97.63	++
10	**8b**	85.13	73.71	87.73	55.21	++
11	**8d**	7.93	13.11	22.90	64.47	++++
12	**11a**	>100	27.76	>100	42.63	++
13	**11b**	88.13	78.71	37.73	75.01	++
14	**11c**	29.92	28.17	>100	27.37	–
15	**11d**	19.79	36.13	29.50	15.44	++
16	**13a**	88.13	56.47	65.53	88.92	++
17	**13b**	76.26	77.46	85.50	67.63	++
18	**13c**	85.13	63.91	61.73	71.21	++
19	**13d**	87.53	54.11	56.90	>100	++
20	CPT	0.091	0.166	2.544	7.86	++++

[a] Activity is expressed semi-quantitatively as follows: –, no inhibitory activity; ++, weak activity; ++++, similar activity as CPT.

conical regions above and below the trigonal plane of carbonyl is shielded.[42] Moreover, the proton H7α which lies at a distance of 2.342 Å from carbonyl group is further deshielded by electric field of carbonyl oxygen. These results are in consistency with those observed for peri proton H10 which is deshielded by anisotropic magnetic, electric fields and steric effects of carbonyl[43] to appear downfield (δ 8.42) compared to other aromatic protons occurring at a range of δ 7.68–6.80.

3. Biological evaluation and docking study

Cytotoxicity test was assessed by the MTT assay on four different cell lines originating from human tumors: A549 (lung), HCT15 (colon), SKOV-3 (ovarian), and SK-MEL-2 (melanoma).[44] Cytotoxicity results are reported as IC_{50} values in Table 1. Topo I inhibition was evaluated by measurement of topo I-dependent DNA cleavage at two concentrations, and the inhibition data are expressed semi-quantitatively as following: –, no inhibitory activity; ++, weak activity; ++++, similar activity as CPT (Fig. 6, Table 1).[41,45] Docking study of selected compounds was performed by molecular modeling software, Surflex-Dock, on crystallographic structure of topo I, DNA duplex and indenoisoquinoline MJ-II-38 ternary complex (PDB code 1SC7). Representative 3-arylisoquinoline derivatives were docked into topo I–DNA complex to support the biological test results by determining their binding mode at the drug target site.

Benzoazepinoisoquinolinones **6a–d** exhibited the strongest antiproliferative activity among the series of 3-arylisoquinolines (both flexible and rigid) derivatives subjected for cytotoxicity assay. The cytotoxicity of the seven membered heterocyclic compounds **6** ranged between 2.54 and 29.11 μM against four different tumor cell lines. Most notably, compound **6c** had comparable and superior toxic effect on ovarian and melanoma cancer cells than CPT. However, these benzoazepinoisoquinolinones were weak topo I inhibitors. Hypothetical binding model of **6c** in a ternary complex with DNA and topo I did not show any stabilizing hydrogen bonding/ionic interaction with either amino acids or nucleotides (Fig. 7). More disappointingly, the isoquinolone moiety which is proved to be responsible for intercalation between +1 and −1 DNA base pairs,[8] was expelled out from the layers of DNA base pairs planks. This may be related to molecular geometry of the compound. Comparison of molecular shape of energetically stable conformer of compound **6a** and its five membered ring analog 9-methoxy-7*H*-isoindolo[2,1-*b*]isoquinolin-5-one (with topo I inhibition comparable to CPT),[8] reveals that planarity of tetracyclic chromophore is important for inhibiting function of topo I (Fig. 8a and b). Similar observation has also been reported for synthetic lamellarin 501 (LMD-501) with non-planar dihydro isoquinoline system.[46]

3-Arylisoquinolines **8a**, **8b** showed moderate cytotoxicity and low topo I inhibition activity. Interestingly, compound **8d** exhibited topo I inhibition comparable to CPT with strong cytotoxicities ranging between 7.93 and 64.47 μM. This is the first incident which demonstrates flexible 3-arylisoquinolone as topo I inhibitor during our decade long effort to develop selective and effective anticancer agents targeting topo I. Docking model of **8d** illustrates that 3-aryl rings of **8d** are well positioned in the binding sites of DNA–topo I ternary complex (Figs. 9 and 10). The isoquinoline ring intercalates between the −1 and +1 bases, parallel to the plate of the bases. Furthermore, the lactam carbonyl of compound **8d** associates with Arg 364 by hydrogen bond. The cumulative effect of intercalation and hydrogen bond interaction of the ligand **8d** with DNA–topo I complex ultimately freeze the topo I–DNA–drug ternary complex and prevent the religation of cleaved DNA strands. This remarkable effect of **8d** verifies that flexible ligands, in spite of high conformational entropy, can attain active conformation within the drug binding site.

N-Allylated isoquinolines **11**, in general, showed low cytotoxicity as well as topo I activity compared to unsubstituted compounds **8**. Similar results have been found to be reported for various N-alkylated isoquinolones when their cytotoxicity profiles are examined closely.[9,47,48] Unfortunately, aldehydes **13** and dienes **7** did not show any significant biological efficacy.

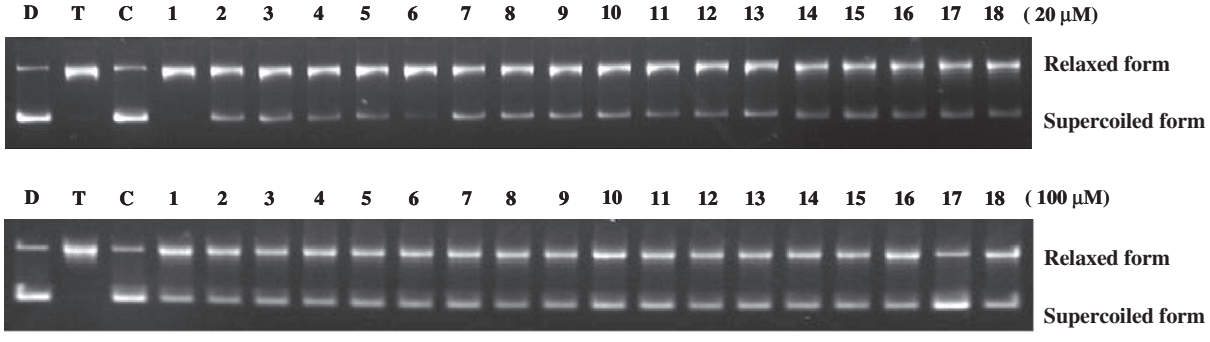


Figure 6. Topo I inhibitory activity of compounds. Compounds were examined at the final concentrations of 20 and 100 μM, respectively. Lane D: pBR322 only; lane T: pBR322 + topo I; lane C: pBR322 + topo I + CPT; lanes 1–18: pBR322 + topo I + compounds at the designated concentration (1: **11a**, 2: **6a**, 3: **7d**, 4: **11d**, 5: **6d**, 6: **7a**, 7: **13a**, 8: **13b**, 9: **7b**, 10: **13c**, 11: **6c**, 12: **13d**, 13: **11c**, 14: **6b**, 15: **11b**, 16: **8b**, 17: **8d**, 18: **8a**).

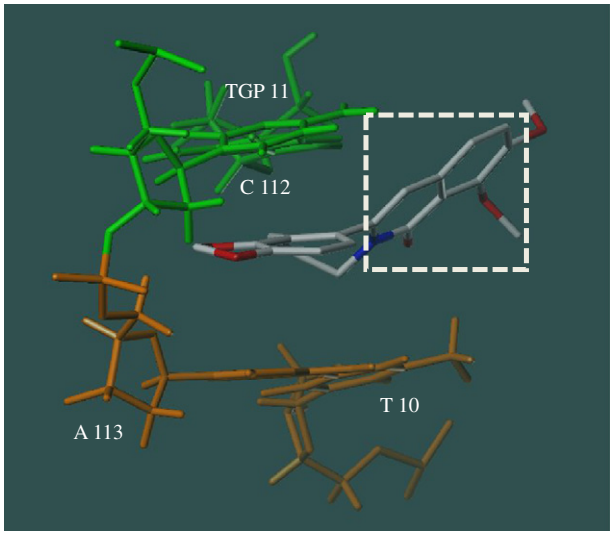


Figure 7. The docking model of compound **6c** in active site. Isoquinoline ring is enclosed within box with broken lines.

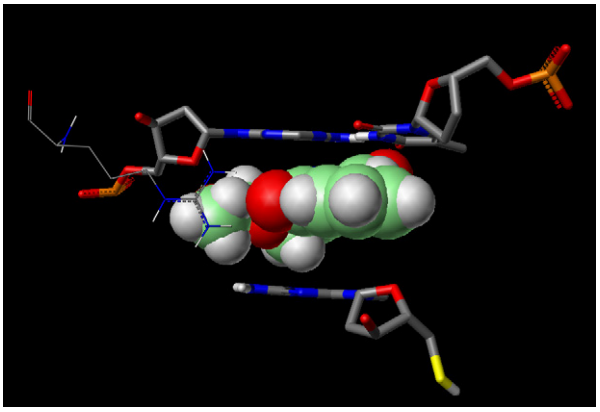


Figure 10. Space-filling model of **8d**.

4. Conclusion

In summary, we successfully synthesized benzo[3,4]azepino[1,2-*b*]isoquinolinones as rigid forms of 3-arylisoquinolines. The synthesis of the azepine derivatives involved (a) intermolecular cyclization of toluamides and benzonitriles to prepare the 3-arylisoquinolones, (b) series of chemical alterations to construct the basic diene precursors **7** and (c) finally, transition metal catalyzed RCM of the olefins to the desired seven-membered heterocyclic azepine derivatives **6**. The profound difference in chemical shifts of geminal protons H7α and H7β of azepine ring could be the unique conformation of the ring due to which H7β is shielded by ring current of azepine ring, whereas H7α is deshielded by magnetic field of carbonyl group and azepine ring. Benzoazepinoisoquinolinones exhibited potent cytotoxicity but showed only moderate topo I inhibition. The lack of correlation between anti-topo I activity and cytotoxicity is due to non-planar

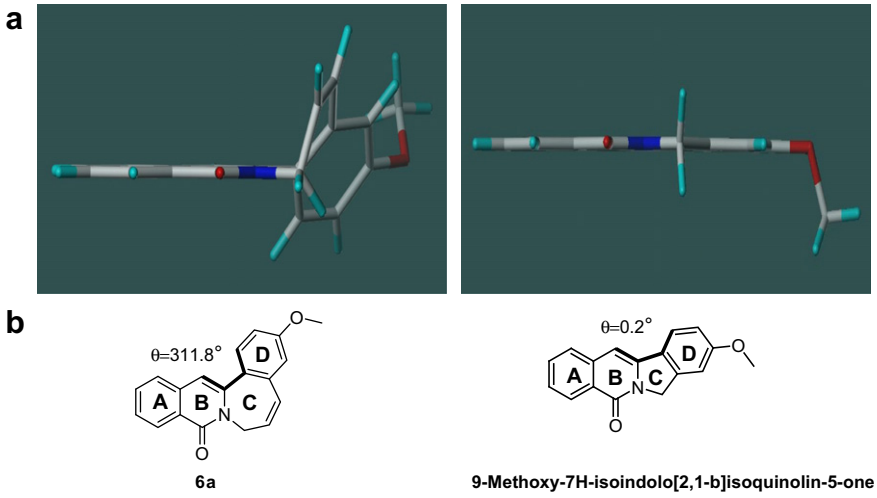


Figure 8. (a) Conformations (side views) displaying non-planarity and planarity of benzoazepinoisoquinolinone **6a** and isoindenoisoquinoline, respectively. (b) Structures showing dihedral angles between isoquinolone and D rings.

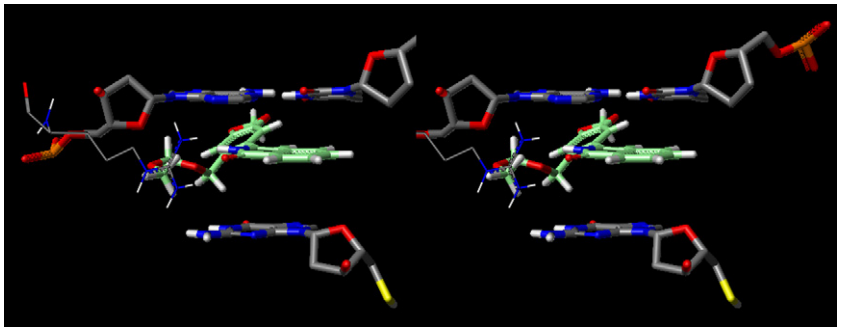


Figure 9. Wall-eyed viewing model of compound **8d**.

conformation of the constraint tetracyclic species. Despite of high degree of flexibility, 3-arylisoquinoline **8d** showed potent topo I inhibitory activity similar to CPT. The unexpected result is plausibly due to its ability to adjust to desired active conformation at the ligand binding site of receptor.

We believe that the synthetic pathway, structure–activity relationships and molecular models of the benzoazepinoisoquinolinones and related 3-aryoisoquinolines will provide a framework for the further design and development of potent and selective heterocyclic topo I inhibitors.

5. Experimental section

5.1. General considerations

Melting points were determined by the capillary method with an Electrothermal IA9200 digital melting point apparatus and were uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded with Varian 300 or Kjui 500-Inova 500 FT spectrometers at the Korea Basic Science Institute. Chemical shifts for ^{1}H NMR were reported in ppm, downfield from the peak of the internal standard, tetramethylsilane. The data are reported as follows: chemical shift, multiplicity, number of protons (s: singlet, d: doublet, t: triplet, q: quartet, m: multiplet, bs: broad singlet). HSQC spectra were obtained using Kjui 500-Inova 500 FT spectrometer. IR spectra were recorded on a JASCO-FT IR spectrometer using $CHCl_3$ or KBr pellets. Mass spectra were obtained on JEOL JNS-DX 303 using the electron-impact (EI) method. Column chromatography was performed on Merck silica gel 60 (70–230 mesh). TLC was performed using plates coated with silica gel 60 F254 (Merck). Chemical reagents were purchased from Aldrich Chemical Co. and used without further purification. Solvents were distilled prior to use; THF and ether were distilled from sodium/benzophenone.

5.2. Chemistry

5.2.1. 4-Methoxy-2-methoxymethoxymethylbenzonitrile (10a)

To a solution of 2-hydroxymethyl-4-methoxybenzonitrile (4.08 g, 25 mmol) in CH_2Cl_2 (20 mL) was added diisopropylethylamine (DIPEA) (6.53 g, 50 mmol) and chloromethylmethyl ether (4.02 g, 50 mmol) at 0 °C. After the reaction was over, CH_2Cl_2 was removed in vacuo and the residue was purified by column chromatography with *n*-hexane–ethyl acetate (3:1) to give benzonitrile **10a** as yellow oil (4.70 g, 91%). IR (cm^{-1}): 2222 (CN). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.59 (d, J = 8.4 Hz, 1H), 7.10 (d, J = 2.6 Hz, 1H), 6.90 (dd, J = 2.6, 8.6 Hz, 1H), 4.77 (s, 2H), 4.74 (s, 2H), 3.87 (s, 3H), 3.44 (s, 3H). EIMS: *m/z* 207 (M^+, 86).

5.2.2. 4,5-Dimethoxy-2-methoxymethoxymethylbenzonitrile (10b)

The procedure described for compound **10a** was used with 4,5-dimethoxy-2-hydroxymethylbenzonitrile (5.5 g, 28.5 mmol), DIPEA (7.35 g, 57 mmol), and chloromethylmethyl ether (4.59 g, 57 mmol) to afford benzonitrile **10b** as white solid (6.7 g, 99%). mp: 54.5–56.4 °C. IR (cm^{-1}): 2222 (CN). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.07 (s, 1H), 7.03 (s, 1H), 4.76 (s, 2H), 4.71 (s, 2H), 3.95 (s, 3H), 3.90 (s, 3H), 3.44 (s, 3H). EIMS: *m/z* 237 (M^+, 100).

5.2.3. 6-Methoxymethoxymethyl-benzo[1,3]dioxole-5-carbonitrile (10c)

Synthesis of **10c** was previously reported.[36]

5.2.4. 3-(4-Methoxy-2-methoxymethoxymethylphenyl)-2*H*-isoquinolin-1-one (8a)

A solution of *N,N*-diethylbenzamide **9a** (1.68 g, 8.8 mmol) and benzonitrile **10a** (1.52 g, 7.3 mmol) in dry THF (20 mL) was added drop wise to a solution of *n*-butyllithium (6 mL of 2.5 M in hexane, 15 mmol) in THF (20 mL) at −78 °C, and then the reaction mixture was stirred at the same temperature for 6 h. The reaction was quenched with water, extracted with ethyl acetate and dried over sodium sulfate. After removal of the solvent, the residue was purified by column chromatography with *n*-hexane–ethyl acetate (1:1) to afford compound **8a** as yellow oil (985 mg, 41%). IR (cm^{-1}): 3447 (NH), 1655 (C=O). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.79 (s, 1H), 8.40 (d, 1H), 7.67 (m, 1H), 7.56 (m, 1H), 7.48 (m, 2H), 7.03 (m, 1H), 6.97 (m, 1H), 6.52 (s, 1H), 4.80 (s, 2H), 4.56 (s, 2H), 3.87 (s, 3H), 3.43 (s, 3H). EIMS: *m/z* 325 (M^+, 65). HRMS-EI (calcd for $C_{19}H_{19}NO_4$): 325.1314, found 325.1321.

5.2.5. 3-(4,5-Dimethoxy-2-methoxymethoxymethylphenyl)-2*H*-isoquinolin-1-one (8b)

The procedure described for compound **8a** was used with toluamide **9a** (1.85 g, 9.7 mmol) and benzonitrile **10b** (1.8 g, 7.6 mmol) in the presence of 1.6 M *n*-BuLi in hexane (14 mL, 22.3 mmol) to give compound **8b** as yellow solid (2.0 g, 58%). mp: 122.5–124.5 °C. IR (cm^{-1}): 3447 (NH), 1655 (C=O). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 10.32 (bs, 1H), 8.38 (d, J = 8.1 Hz, 1H), 7.67 (t, J = 7.5 Hz, 1H), 7.57 (d, J = 7.5 Hz, 1H), 7.48 (t, J = 8.1 Hz, 1H), 7.06 (s, 1H), 7.00 (s, 1H), 6.59 (s, 1H), 4.79 (s, 2H), 4.56 (s, 2H), 3.97 (s, 3H), 3.96 (s, 3H), 3.43 (s, 3H). EIMS: *m/z* 355 (M^+, 100). HRMS-EI (calcd for $C_{20}H_{21}NO_5$): 355.1420, found 355.1431.

5.2.6. 7,8-Dimethoxy-3-(6-methoxymethoxymethylbenzo[1,3]-dioxol-5-yl)-2*H*-isoquinolin-1-one (8c)

The procedure described for compound **8a** was used with *N, N*-diethyl-2,3-dimethoxy-6-methylbenzamide **9b** (1.96 g, 9.4 mmol) and benzonitrile **10c** (1.4 g, 6.3 mmol) in the presence of *n*-BuLi (9 mL of 2.5 M in hexane, 22.5 mmol) to give compound **8c** as yellow solid (1.01 g, 40%). mp: 151.0–154.2 °C. IR (cm^{-1}): 3400 (NH), 1650 (C=O). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.34 (d, J = 9.0 Hz, 1H), 7.27 (d, J = 9.0 Hz, 1H), 6.96 (s, 1H), 6.93 (s,1H), 6.37 (s, 1H), 6.04 (s, 2H), 4.83 (s, 2H), 4.47 (s, 2H), 3.98 (s, 3H), 3.97 (s, 3H), 3.43 (s, 3H). EIMS, *m/z* (%): 399 (M^+, 18), 354 (42), 336 (70), 222 (100), 162 (38). HRMS-EI (calcd for $C_{21}H_{21}NO_7$): 399.1318, found 399.1321.

5.2.7. 3-(5-((Methoxymethoxy)methyl)benzo[*d*][1,3]dioxol-6-yl)-isoquinolin-1(2*H*)-one (8d)

The procedure described for compound **8a** was used with toluamide **9a** (1.34 g, 7 mmol) and benzonitrile **10c** (1.1 g, 5 mmol) in the presence of *n*-BuLi (6 mL of 2.5 M in hexane, 15 mmol) to give compound **8d** as bright yellow solid (1.19 g, 70%). mp: 132–135 °C. IR (cm^{-1}): 3400 (NH), 1657 (C=O). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 9.7 (s, 1H), 8.40 (m, 1H), 7.65 (m, 1H), 7.49 (m, 2H), 6.98 (s, 1H), 6.95 (s, 1H), 6.51 (s, 1H), 6.0 (s, 2H), 4.77 (s, 2H), 4.46 (s, 2H), 3.42 (s, 3H). EIMS: *m/z* 339 (M^+, 100). HRMS-EI (calcd for $C_{19}H_{17}NO_5$): 339.1107, found 339.1110.

5.2.8. 2-Allyl-3-(4-methoxy-2-methoxymethoxymethylphenyl)-2*H*-isoquinolin-1-one (11a)

To a solution of 3-arylisoquinoline **8a** (985 mg, 3 mmol) and K_2CO_3 (1.38 g, 10 mmol) in DMF (20 mL) was added allyl bromide (720 mg, 6 mmol). The mixture was stirred at room temperature overnight and then quenched with water and extracted with ethyl acetate. The combined ethyl acetate extracts were washed with water and brine and dried over anhydrous sodium sulfate. After removing the solvent in vacuo, the residue was purified by column chromatography on silica gel with *n*-hexane–ethyl acetate (2:1) to give compound **11a** as yellow oil (800 mg, 73%). IR (cm^{-1}): 1650 (C=O). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.46 (d, J = 7.9 Hz, 1H), 7.64 (t, J = 7.5 Hz, 1H), 7.52–7.45 (m, 2H), 7.21 (d, J = 8.4 Hz, 1H), 7.12 (d, J = 2.6 Hz, 1H), 6.89 (dd, J = 2.7, 8.4 Hz, 1H), 6.40 (s, 1H),

5.84–5.73 (m, 1H), 5.04 (dd, *J* = 1.3, 10.3 Hz, 1H), 4.83–4.69 (m, 2H), 4.59 (d, *J* = 2.1 Hz, 2H), 4.39 (s, 2H), 4.15 (dd, *J* = 5.4, 15.3 Hz, 1H), 3.88 (s, 3H), 3.26 (s, 3H). EIMS: *m/z* 365 (M^+, 78). HRMS-EI (calcd for $C_{22}H_{23}NO_4$): 365.1627, found 365.1629.

5.2.9. 2-Allyl-3-(4,5-dimethoxy-2-methoxymethoxymethylphenyl)-2*H*-isoquinolin-1-one (11b)

The procedure described for compound **11a** was used with 3-arylisoquinoline **8b** (1.2 g, 3.4 mmol), K_2CO_3 (970 mg, 7 mmol) in DMF (20 mL) and allyl bromide (847 mg, 7 mmol) to give compound **11b** as yellow oil (1.05 g, 78%). IR (cm^{-1}): 1650 (C=O). ^1H NMR (300 MHz, $CDCl_3$) δ: 8.47 (d, *J* = 7.9 Hz, 1H), 7.68–7.63 (m, 1H), 7.53–7.46 (m, 2H), 7.05 (s, 1H), 6.79 (s, 1H), 6.43 (s, 1H), 5.89–5.80 (m, 1H), 5.06 (dd, *J* = 1.3, 10.3 Hz, 1H), 4.83 (dd, *J* = 1.4, 17.1 Hz, 1H), 4.74 (dd, *J* = 5.0, 15.4 Hz, 1H), 4.58 (d, *J* = 2.1 Hz, 2H), 4.37 (s, 2H), 4.21–4.13 (m, 1H), 3.97 (s, 3H), 3.85 (s, 3H), 3.25 (s, 3H). EIMS: *m/z* 395 (M^+, 100). HRMS-EI (calcd for $C_{23}H_{25}NO_5$): 395.1733, found 395.1743.

5.2.10. 2-Allyl-7,8-dimethoxy-3-(6-methoxymethoxymethylbenzo[1,3]dioxol-5-yl)-2*H*-isoquinolin-1-one (11c)

The procedure described for compound **11a** was used with 3-arylisoquinoline **8c** (330 mg, 0.83 mmol) and K_2CO_3 (350 mg, 2.5 mmol) in DMF (20 mL) and allyl bromide (200 mg, 1.7 mmol) to give compound **11c** as yellow oil (221 mg, 61%). IR (cm^{-1}): 1650 (C=O). ^1H NMR (300 MHz, $CDCl_3$) δ: 7.33 (d, *J* = 8.7 Hz, 1H), 7.18 (d, *J* = 8.6 Hz, 1H), 7.02 (s, 1H), 6.73 (s, 1H), 6.26 (s, 1H), 6.03 (dd, *J* = 1.3, 6.4 Hz, 2H), 5.88–5.79 (m, 1H), 5.04 (dd, *J* = 1.3, 10.2 Hz, 1H), 4.81 (dd, *J* = 1.4, 17.2 Hz, 1H), 4.65 (dd, *J* = 5.4, 15.3 Hz, 1H), 4.57 (s, 2H), 4.30 (s, 2H), 4.18 (dd, *J* = 5.3, 15.4 Hz, 1H), 4.01 (s, 3H), 3.95 (s, 3H), 3.27 (s, 3H). EIMS: *m/z* 439 (M^+, 45). HRMS-EI (calcd for $C_{24}H_{25}NO_7$): 439.1631, found 439.1635.

5.2.11. 2-Allyl-3-(6-((methoxymethoxy)methyl)benzo[*d*][1,3]-dioxol-5-yl)isoquinolin-1(2*H*)-one (11d)

The procedure described for compound **11a** was used with 3-arylisoquinoline **8d** (800 mg, 2.36 mmol), K_2CO_3 (1.24 g, 9 mmol) in DMF (20 mL) and allyl bromide (570 mg, 4.7 mmol) to afford compound **11d** as oil (537 mg, 60%). IR (cm^{-1}): 1650 (C=O). ^1H NMR (300 MHz, $CDCl_3$) δ: 8.46 (d, *J* = 7.9 Hz, 1H), 7.67–7.62 (m, 1H), 7.52–7.46 (m, 2H), 7.03 (s, 1H), 6.75 (s, 1H), 6.41 (s, 1H), 6.04 (dd, *J* = 1.3, 6.0 Hz, 2H), 5.86–5.77 (m, 1H), 5.07 (dd, *J* = 1.3, 10.2 Hz, 1H), 4.84 (dd, *J* = 1.4, 17.1 Hz, 1H), 4.70 (dd, *J* = 5.4, 16.9 Hz, 1H), 4.56 (s, 2H), 4.30 (s, 2H), 4.23 (dd, *J* = 5.2, 15.4 Hz, 1H), 3.25 (s, 3H). EIMS: *m/z* 379 (M^+, 81). HRMS-EI (calcd for $C_{22}H_{21}NO_5$): 379.1420, found 379.1427.

5.2.12. 2-Allyl-3-(2-hydroxymethyl-4-methoxyphenyl)-2*H*-isoquinolin-1-one (12a)

To a solution of compound **11a** (800 mg, 2.2 mmol) in THF (15 mL) was added 10% HCl (10 mL) and the reaction was refluxed for 2 h. After cooling to room temperature, the reaction mixture was poured into water and extracted with ethyl acetate. The ethyl acetate extracts were washed with water and brine and dried over anhydrous sodium sulfate. After removal of the solvent in vacuo, the residue was purified by column chromatography on silica gel with *n*-hexane–ethyl acetate (1:2) to produce the alcohol **12a** as white solid (650 mg, 92%). mp: 109–110 °C. IR (cm^{-1}): 3300 (OH), 1641 (C=O). ^1H NMR (300 MHz, $CDCl_3$) δ: 8.39 (d, *J* = 6.6 Hz, 1H), 7.65–7.59 (m, 1H), 7.47–7.42 (m, 2H), 7.19–7.15 (m, 2H), 6.86 (dd, *J* = 2.7, 8.4 Hz, 1H), 6.38 (s, 1H), 5.79–5.68 (m, 1H), 5.00 (d, *J* = 10.2 Hz, 1H), 4.73 (d, *J* = 17.8 Hz, 1H), 4.61 (dd, *J* = 5.5, 15.3 Hz, 1H), 4.48 (d, *J* = 5.5 Hz, 2H), 4.15 (dd, *J* = 5.2, 15.3 Hz, 1H), 3.87 (s, 3H), 2.74 (bs, 1H). EIMS: *m/z* 321 (M^+, 66). HRMS-EI (calcd for $C_{20}H_{19}NO_3$): 321.1365, found 321.1368.

5.2.13. 2-Allyl-3-(2-hydroxymethyl-4,5-dimethoxyphenyl)-2*H*-isoquinolin-1-one (12b)

The procedure described for compound **12a** was used with compound **11b** (1 g, 2.5 mmol) in THF (15 mL) and 10% HCl (10 mL) to afford the alcohol **12b** as white solid (615 mg, 70%). mp: 151–153 °C. IR (cm^{-1}): 3300 (OH), 1641 (C=O). ^1H NMR (300 MHz, $CDCl_3$) δ: 8.45 (d, *J* = 7.9 Hz, 1H), 7.69–7.63 (m, 1H), 7.53–7.47 (m, 2H), 7.12 (s, 1H), 6.77 (s, 1H), 6.43 (s, 1H), 5.89–5.79 (m, 1H), 5.06 (dd, *J* = 1.3, 11.6 Hz, 1H), 4.80 (dd, *J* = 1.4, 17.2 Hz, 1H), 4.63 (dd, *J* = 5.3, 15.4 Hz, 1H), 4.49 (s, 2H), 4.25 (dd, *J* = 5.2, 15.3 Hz, 1H), 3.98 (s, 3H), 3.86 (s, 3H), 1.83 (bs, 1H). EIMS: *m/z* 351 (M^+, 98). HRMS-EI (calcd for $C_{21}H_{21}NO_4$): 351.1470, found 351.1481.

5.2.14. 2-Allyl-3-(6-hydroxymethylbenzo[1,3]dioxol-5-yl)-7,8-dimethoxyisoquinolin-1(2*H*)-one (12c)

The procedure described for compound **12a** was used with compound **11c** (200 mg, 0.455 mmol) in THF (15 mL) and 10% HCl (10 mL) to give the alcohol **12c** as yellow oil (110 mg, 61%). IR (cm^{-1}): 3300 (OH), 1641 (C=O). ^1H NMR (300 MHz, $CDCl_3$) δ: 7.32 (d, *J* = 8.6 Hz, 1H), 7.18 (d, *J* = 8.7 Hz, 1H), 7.06 (s, 1H), 6.71 (s, 1H), 6.25 (s, 1H), 6.03 (dd, *J* = 1.3, 6.2 Hz, 2H), 5.89–5.80 (m, 1H), 5.04 (dd, *J* = 1.4, 10.2 Hz, 1H), 4.79 (dd, *J* = 1.4, 17.1 Hz, 1H), 4.54 (dd, *J* = 5.7, 15.3 Hz, 1H), 4.41 (s, 2H), 4.27 (dd, *J* = 4.9, 16.9 Hz, 1H), 4.00 (s, 3H), 3.94 (s, 3H) 1.88 (bs, 1H). EIMS: *m/z* 395 (M^+, 87). HRMS-EI (calcd for $C_{22}H_{21}NO_6$): 395.1369, found 395.1361.

5.2.15. 2-Allyl-3-(6-hydroxymethylbenzo[1,3]dioxol-5-yl)-2*H*-isoquinolin-1-one (12d)

The procedure described for compound **12a** was used with compound **11d** (480 mg, 1.26 mmol) in THF (15 mL) and 10% HCl (10 mL) to give compound **12d** as pale yellow oil (370 mg, 87%). IR (cm^{-1}): 3300 (OH), 1641 (C=O). ^1H NMR (300 MHz, $CDCl_3$) δ: 8.37 (d, *J* = 8.3 Hz, 1H), 7.64–7.59 (m, 1H), 7.47–7.42 (m, 2H), 7.10 (s, 1H), 6.69 (s, 1H), 6.40 (s, 1H), 6.02 (dd, *J* = 1.3, 6.6 Hz, 2H), 5.80–5.69 (m, 1H), 5.02 (dd, *J* = 1.3, 10.3 Hz, 1H), 4.77 (dd, *J* = 1.3, 17.1 Hz, 1H), 4.60 (dd, *J* = 5.5, 15.4 Hz, 1H), 4.38 (d, *J* = 1.7 Hz, 2H), 4.22 (dd, *J* = 5.0, 15.4 Hz, 1H). EIMS: *m/z* 335 (M^+, 78). HRMS-EI (calcd for $C_{20}H_{17}NO_4$): 335.1157, found 335.1152.

5.2.16. 2-(2-Allyl-1-oxo-1,2-dihydroisoquinolin-3-yl)-5-methoxybenzaldehyde (13a)

To a solution of alcohol **12a** (600 mg, 1.87 mmol) in methylene chloride (30 mL) was added PDC (1.5 g, 4 mmol), and the mixture was stirred for 2 h at room temperature. The reaction mixture was filtered and the filtrate was washed with CH_2Cl_2. The solvent was evaporated and the residue was purified by column chromatography on silica gel with *n*-hexane–ethyl acetate (2:1) to afford the aldehyde **13a** as yellow oil (510 mg, 85%) IR (cm^{-1}): 1700, 1640 (C=O). ^1H NMR (300 MHz, $CDCl_3$) δ: 9.90 (s, 1H), 8.48 (d, *J* = 8.0 Hz, 1H), 7.70–7.66 (m, 1H), 7.55–7.47 (m, 3H), 7.38 (d, *J* = 8.3 Hz, 1H), 7.22 (dd, *J* = 2.7, 8.4 Hz, 1H), 6.43 (s, 1H), 5.85–5.72 (m, 1H), 5.04 (d, *J* = 10.2 Hz, 1H), 4.75 (d, *J* = 17.1 Hz, 1H), 4.50 (d, *J* = 5.4 Hz, 2H), 3.93 (s, 3H). EIMS: *m/z* 319 (M^+, 100). HRMS-EI (calcd for $C_{20}H_{17}NO_3$): 319.1208, found 319.1212.

5.2.17. 2-(2-Allyl-1-oxo-1,2-dihydroisoquinolin-3-yl)-4,5-dimethoxybenzaldehyde (13b)

The procedure described for compound **13a** was used with alcohol **12b** (660 mg, 1.9 mmol) and PDC (1.5 g, 4 mmol) in CH_2Cl_2 (30 mL) to afford the aldehyde **13b** as yellow solid (597 mg, 91%). mp: 135–137 °C. IR (cm^{-1}): 1700, 1640 (C=O). ^1H NMR (300 MHz, $CDCl_3$) δ: 9.81 (s, 1H), 8.48 (d, *J* = 8.0 Hz, 1H), 7.72–7.66 (m, 1H), 7.57–7.49 (m, 3H), 6.89 (s, 1H), 6.48 (s, 1H),

5.90–5.81 (m, 1H), 5.09 (dd, J = 1.2, 10.3 Hz, 1H), 4.81 (dd, J = 1.2, 17.2 Hz, 1H), 4.54–4.47 (m, 2H), 4.02 (s, 3H), 3.95 (s, 3H). EIMS: m/z 349 (M^+, 36). HRMS-EI (calcd for $C_{21}H_{19}NO_4$): 349.1314, found 349.1320.

5.2.18. 6-(2-Allyl-1,2-dihydro-7,8-dimethoxy-1-oxoisoquinolin-3-yl)benzo[*d*][1,3]dioxole-5-carbaldehyde (13c)

The procedure described for compound **13a** was used with alcohol **12c** (100 mg, 0.25 mmol) and PDC (190 mg, 0.5 mmol) in CH_2Cl_2 (20 mL) to give the aldehyde **13c** as white solid (68 mg, 69%). IR (cm^{-1}): 1700, 1640 (C=O). ^1H NMR (300 MHz, $CDCl_3$) δ: 9.75 (s, 1H), 7.44 (s, 1H), 7.35 (d, J = 8.7 Hz, 1H), 7.20 (d, J = 8.7 Hz, 1H), 6.84 (s, 1H), 6.29 (s, 1H), 6.15 (dd, J = 1.1, 6.3 Hz, 2H), 5.87–5.79 (m, 1H), 5.05 (dd, J = 1.2, 10.3 Hz, 1H), 4.79 (dd, J = 1.2, 17.2 Hz, 1H), 4.51–4.44 (m, 2H), 4.01 (s, 3H), 3.96 (s, 3H). EIMS: m/z 393 (M^+, 54). HRMS-EI (calcd for $C_{22}H_{19}NO_6$): 393.1212, found 393.1219.

5.2.19. 6-(2-Allyl-1,2-dihydro-1-oxoisoquinolin-3-yl)benzo[*d*]-[1,3]dioxole-5-carbaldehyde (13d)

The procedure described for compound **13a** was used with compound **12d** (340 mg, 1 mmol) and PDC (750 mg, 2 mmol) in CH_2Cl_2 (30 mL) to afford the aldehyde **13d** as pale yellow solid (330 mg, 99%). mp: 155–157 °C. IR (cm^{-1}): 1700, 1640 (C=O). ^1H NMR (300 MHz, $CDCl_3$) δ: 9.74 (s, 1H), 8.47 (d, J = 8.7 Hz, 1H), 7.68–7.65 (m, 1H), 7.56–7.46 (m, 3H), 6.86 (s, 1H), 6.44 (s, 1H), 6.16 (dd, J = 1.1, 5.7 Hz, 2H), 5.84–5.76 (m, 1H), 5.08 (dd, J = 1.2, 10.3 Hz, 1H), 4.81 (dd, J = 1.2, 17.1 Hz, 1H), 4.54–4.50 (m, 2H). EIMS: m/z 333 (M^+, 100). HRMS-EI (calcd for $C_{20}H_{15}NO_4$): 333.1001, found 333.1005.

5.2.20. 2-Allyl-3-(4-methoxy-2-vinyl-phenyl)-2*H*-isoquinolin-1-one (7a)

To a solution of methyltriphenylphosphonium bromide (1.42 g, 4 mmol) in dry THF (30 mL) was added n-butyllithium (1.6 mL of 2.5 M in hexane, 4 mmol) at 0 °C and the solution was stirred at 0 °C for 1 h. To this mixture was added the aldehyde **13a** (420 mg, 1.31 mmol) in THF (10 mL), and the resulting mixture was stirred at room temperature for 1 h and quenched with water followed by extraction with ethyl acetate. The combined organic layers were washed with water and brine and dried over sodium sulfate. After removing the solvent, the residue was purified by column chromatography with n-hexane–ethyl acetate (3:1) to afford the olefin **7a** as white solid (341 mg, 82%). mp: 119–120 °C. ^1H NMR (300 MHz, $CDCl_3$) δ: 8.46 (d, J = 8.0 Hz, 1H), 7.67–7.62 (m, 1H), 7.52–7.46 (m, 2H), 7.21 (d, J = 8.4 Hz, 1H), 7.17 (d, J = 2.6 Hz, 1H), 6.87 (dd, J = 2.6, 8.4 Hz, 1H), 6.50 (dd, J = 10.9, 17.4 Hz, 1H), 6.39 (s, 1H), 5.75–5.69 (m, 2H), 5.22 (d, J = 10.9 Hz, 1H), 5.00 (d, J = 10.2 Hz, 1H), 4.86–4.76 (m, 2H), 4.09–4.02 (m, 1H), 3.89 (s, 3H). EIMS: m/z 317 (M^+, 58). HRMS-EI (calcd for $C_{21}H_{19}NO_2$): 317.1415, found 317.1412.

5.2.21. 2-Allyl-3-(4,5-dimethoxy-2-vinylphenyl)-2*H*-isoquinolin-1-one (7b)

The procedure described for compound **7a** was used with the aldehyde **13b** (560 mg, 1.6 mmol) and methyltriphenylphosphonium bromide (2.85 g, 8 mmol) and n-butyllithium (5 mL of 1.6 M in hexane, 8 mmol) in dry THF (30 mL) to afford compound **7b** as brown oil (503 mg, 91%). ^1H NMR (300 MHz, $CDCl_3$) δ: 8.47 (d, J = 7.7 Hz, 1H), 7.68–7.63 (m, 1H), 7.52–7.48 (m, 2H), 7.15 (s, 1H), 6.77 (s, 1H), 6.53–6.42 (m, 2H), 5.86–5.73 (m, 1H), 5.63 (d, J = 17.4 Hz, 1H), 5.14 (d, J = 11.0 Hz, 1H), 5.03 (d, J = 10.8 Hz, 1H), 4.82 (d, J = 19.0 Hz, 2H), 4.11–4.04 (m,1H), 3.99 (s, 3H), 3.86 (s, 3H). EIMS: m/z 347 (M^+, 76). HRMS-EI (calcd for $C_{22}H_{21}NO_3$): 347.1521, found 347.1524.

5.2.22. 2-Allyl-7,8-dimethoxy-3-(6-vinylbenzo[1,3]dioxol-5-yl)-2*H*-isoquinolin-1-one (7c)

The procedure described for compound **7a** was used with the aldehyde **13c** (180 mg, 0.46 mmol) and methyltriphenylphosphonium bromide (890 g, 2.5 mmol) and n-butyllithium (1 mL of 2.5 M in hexane, 2.5 mmol) in dry THF (20 mL) to afford the olefin **7c** as yellow oil (137 mg, 75%). ^1H NMR (300 MHz, $CDCl_3$) δ: 7.32 (d, J = 8.6 Hz, 1H), 7.18 (d, J = 8.6 Hz, 1H), 7.11 (s, 1H), 6.71 (s, 1H), 6.43 (dd, J = 10.9, 17.4 Hz, 1H), 6.23 (s, 1H), 6.03 (dd, J = 1.2, 4.1 Hz, 2H), 5.85–5.72 (m, 1H), 5.57 (d, J = 17.3 Hz, 1H), 5.11 (d, J = 11.1 Hz, 1H), 5.01 (d, J = 10.2 Hz, 1H), 4.84–4.72 (m, 2H), 4.09–4.03 (m, 1H), 4.01 (s, 3H), 3.95 (s, 3H). EIMS: m/z 391 (M^+, 87). HRMS-EI (calcd for $C_{23}H_{21}NO_5$): 391.1420, found 391.1428.

5.2.23. 2-Allyl-3-(6-vinylbenzo[1,3]dioxol-5-yl)-2*H*-isoquinolin-1-one (7d)

The procedure described for compound **7a** was used with aldehyde **13d** (280 mg, 0.84 mmol) and methyltriphenylphosphonium bromide (940 g, 2.5 mmol) and n-butyllithium (1 mL of 2.5 M in hexane, 2.5 mmol) in dry THF (30 mL) to afford compound **7d** as pale yellow solid (171 mg, 75%). mp: 87–89 °C. ^1H NMR (300 MHz, $CDCl_3$) δ: 8.46 (d, J = 8.1 Hz, 1H), 7.67–7.61 (m, 1H), 7.52–7.46 (m, 2H), 7.13 (s, 1H), 6.73 (s, 1H), 6.47–6.37 (m, 2H), 6.03 (dd, J = 1.3, 3.8 Hz, 2H), 5.84–5.7 (m, 1H), 5.59 (dd, J = 0.6, 17.3 Hz, 1H), 5.11 (dd, J = 0.6, 10.9 Hz, 1H), 5.04 (dd, J = 1.3, 10.2 Hz, 1H), 4.87–4.78 (m, 2H), 4.16–4.08 (m, 1H). EIMS: m/z 331(M^+, 77). HRMS-EI (calcd for $C_{21}H_{17}NO_3$): 331.1208, found 331.1209.

5.2.24. 3-Methoxy-7*H*-benzo[3,4]azepino[1,2-*b*]isoquinolin-9-one (6a)

The reaction mixture of compound **7a** (150 mg, 0.5 mmol) and 1st generation Grubbs catalyst (40 mg) in CH_2Cl_2 (30 mL) was stirred for 2 h at room temperature and filtered. The filtrate was washed with CH_2Cl_2. The solvent was evaporated and the residue was purified by column chromatography on silica gel with n-hexane–ethyl acetate (2:1) to afford the azepine **6a** as white solid (123 mg, 85%). IR (cm^{-1}): 1640 (C=O). ^1H NMR (500 MHz, $CDCl_3$) δ: 8.42 (d, J = 8 Hz, 1H), 7.68 (d, J = 8.5 Hz, 1H), 7.61 (t, J = 7.5 Hz, 1H), 7.50 (d, J = 8 Hz, 1H), 7.44 (t, J = 7.5 Hz, 1H), 6.97 (dd, J = 2.5, 8.7 Hz, 1H), 6.84 (d, J = 10 Hz, 1H), 6.80 (d, J = 2.5 Hz, 1H), 6.54 (s, 1H), 6.49–6.45 (m, 1H), 5.74 (dd, J = 8, 13.5 Hz, 1H), 3.88 (s, 3H), 3.56 (ddd, J = 1.5, 6.5, 13.5 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 161.2, 160.0, 142.7, 137.4, 136.6, 134.4, 132.1, 131.1, 129.9, 128.6, 127.9, 126.2, 125.9, 124.0, 114.2, 113.3, 107.4, 55.4, 39.5. EIMS: m/z 289 (M^+, 100). HRMS-EI (calcd for $C_{19}H_{15}NO_2$): 289.1102, found 289.1103.

5.2.25. 2,3-Dimethoxy-7*H*-benzo[3,4]azepino[1,2-*b*]isoquinolin-9-one (6b)

The procedure described for compound **6a** was used with the olefin **7b** (100 mg, 0.29 mmol) and 1st generation Grubbs catalyst (25 mg) in CH_2Cl_2 (30 mL) to afford the azepine **6b** as white solid (82 mg, 90%). mp: 187–189 °C. IR (cm^{-1}): 1640 (C=O). ^1H NMR (300 MHz, $CDCl_3$) δ: 8.44 (d, J = 8.0 Hz, 1H), 7.63 (t, J = 7.4 Hz, 1H), 7.53 (d, J = 7.4 Hz, 1H), 7.46 (d, J = 7.5 Hz, 1H), 7.23 (s, 1H), 6.82 (d, J = 9.7 Hz, 1H), 6.78 (s, 1H), 6.57 (s, 1H), 6.45–6.37 (m, 1H), 5.77 (dd, J = 7.6, 13.3 Hz, 1H), 4.02 (s, 3H), 3.96 (s, 3H), 3.51 (ddd, J = 1.8, 6.5, 13.3 Hz, 1H). EIMS: m/z 319 (M^+, 97). HRMS-EI (calcd for $C_{20}H_{17}NO_3$): 319.1208, found 319.1207.

5.2.26. 10,11-Dimethoxy-2,3-[1,3-dioxol])-7*H*-benzo[3,4]azepino[1,2-*b*]isoquinolin-9-one (6c)

The procedure described for compound **6a** was used with the olefin **7c** (100 mg, 0.25 mmol) and 1st generation Grubbs catalyst (40 mg, 20%) in CH_2Cl_2 (30 mL) to afford the azepine **6c** as white

solid (71 mg, 78%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.32 (d, J = 8.7 Hz, 1H), 7.23 (d, J = 8.7 Hz, 1H), 7.17 (s, 1H), 6.76–6.73 (m, 2H), 6.45–6.37 (m, 2H), 6.06 (d, J = 2.1 Hz, 2H), 5.72 (dd, J = 7.5, 13.2 Hz, 1H), 4.01 (s, 3H), 3.94 (s, 3H), 3.44 (ddd, J = 1.6, 6.6, 13.3 Hz, 1H). EIMS: m/z 363 (M^+, 89). HRMS-EI (calcd for $C_{21}H_{17}NO_5$): 363.1107, found 363.1110.

5.2.27. 2,3-([1,3]Dioxol)-7*H*-benzo[3,4]azepino[1,2-*b*]isoquinolin-9-one (6d)

The procedure described for compound **6a** was used compound with **7d** (122 mg, 0.37 mmol) and 1st generation Grubbs catalyst (60 mg, 20%) in CH_2Cl_2 (30 mL) to produce the azepine **6d** as solid (81 mg, 72%). mp: 199–201 °C. IR (cm^{-1}): 1640 (C=O). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.43 (d, J = 8.0 Hz, 1H), 7.64–7.59 (m, 1H), 7.51–7.42 (m, 2H), 7.20 (s, 1H), 6.77–6.73 (m, 2H), 6.54 (s, 1H), 6.41–6.35 (m, 1H), 6.07 (dd, J = 1.2, 4.1 Hz, 2H), 5.74 (dd, J = 7.6, 13.3 Hz, 1H), 3.49 (ddd, J = 1.8, 6.5, 13.3 Hz, 1H). EIMS: m/z 303 (M^+, 90). HRMS-EI (calcd for $C_{19}H_{13}NO_3$): 303.0895, found 303.0787.

5.3. Biological evaluation

5.3.1. Cytotoxicity assay

Four different kinds of human tumor cells, A549, HCT15, SKOV-3, and SK-MEL-2, were seeded at 1×10^5 cells/mL in each well containing 100 μL of RPMI-1640 medium supplemented with 10% FBS in a 96-well plate. After 24 h, various concentrations of test samples were added. After 48 h, 50 μL of MTT (5 mg/mL stock solution, in PBS) were added per well and the plates were incubated for an additional 4 h. The medium was discarded and the formazan blue formed in the cells was dissolved with 100 μL of DMSO. The optical density was measured using a standard ELISA reader at 540 nm.

5.3.2. Topo I inhibition

Topo I inhibition was assayed by determining relaxation of supercoiled DNA pBR322. A mixture of 200 ng of plasmid pBR322 and 0.3 U calf thymus DNA topo I (Amersham) was incubated with the stock solutions of the compounds under test in final volume of 10 μL (in DMSO) at 37 °C for 30 min in relaxation buffer [35 mM Tris–HCl (pH 8.0), 72 mM KCl, 5 mM $MgCl_2$, 5 mM dithiothreitol, 2 mM spermidine, 0.01% bovine serum albumin]. The reaction was terminated by adding 2.5 μL of stop solution containing 10% SDS, 0.2% bromophenol blue, 0.2% xylene cyanol and 30% glycerol. DNA samples were then electrophoresed on 1% agarose gel for 10 h with Tris–borate–EDTA running buffer. Gels were stained for 30 min in an aqueous solution of ethidium bromide (0.5 μg/mL). DNA brands were visualized by transillumination with UV light and were quantitated using AlphaImager™ (Alpha Innotech Corporation).

5.4. Docking study

The docking study was performed using Surflex-Dock in Sybyl version 8.1.1 by Tripos Associates, operating under Red Hat Linux 4.0 with an IBM computer (Intel Pentium 4, 2.8 GHz CPU, and 1 GB memory). The structures of **6c** and **8d** were drawn into the Sybyl package and minimized with the Tripos force field and Gasteiger–Huckel charge. Crystallographic structure of topo I, DNA duplex and indenoisoquinoline MJ-II-38 complex, 1SC7 (PDB code), available at the Protein Data Bank was refined as follows: the phosphoester bond of G12 in 1SC7 was reconstructed, and the SH of G11 on the scissile strand was changed to OH. After running Surflex-Dock, 10 docked models were chosen. Among the conformers, the best score conformer was used to study the precise binding pattern in the active site.

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A. Supplementary data

Supplementary data (^{1}H NMR, ^{13}C NMR, ^{1}H–^{13}C HSQC of **6a**) associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.08.006.

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