Tetrahedron Letters 57 (2016) 466–471

Contents lists available at ScienceDirect

Tetrahedron Letters

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

Synthesis and cytotoxic evaluation of novel indenoisoquinoline-propan-2-ol hybrids

Tham Pham Thi [a,b], Lena Decuyper [c], Tan Luc Quang [a], Chinh Pham The [a,b], Tuyet Anh Dang Thi [a], Ha Thanh Nguyen [a], Thuy Giang Le Nhat [a], Tra Nguyen Thanh [a], Phuong Hoang Thi [a], Matthias D'hooghe [c], Tuyen Van Nguyen [a,*]

[a] Institute of Chemistry, Vietnam Academy of Science and Technology, 18-Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam
[b] Thai Nguyen University of Science, Tan Thinh, Thai Nguyen, Vietnam
[c] SynBioC Research Group, Department of Sustainable Organic Chemistry and Technology, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, B-9000 Ghent, Belgium

ARTICLE INFO

Article history:
Received 30 October 2015
Revised 2 December 2015
Accepted 8 December 2015
Available online 9 December 2015

Keywords:
Indenoisoquinolines
β-Amino alcohols
2-Carboxybenzaldehyde
Phthalide
Cytotoxicity

ABSTRACT

The synthesis of *N*-substituted indenoisoquinolines was performed by applying a two-step condensation between 2-carboxybenzaldehyde and phthalide, followed by treatment with various primary amines. *N*-allylindenoisoquinoline was subsequently selected as a substrate for hydroxybromination, providing 6-(3-bromo-2-hydroxy)indenoisoquinoline as a key intermediate for derivatization in the lactam side chain. In this way, a series of 6-(2-hydroxypropyl)indenoisoquinolines bearing various functional groups at the 3′-position were prepared, which can be considered as novel indenoisoquinoline-propan-2-ol hybrid molecules. Subsequent cytotoxic evaluation of 28 indenoisoquinolines against two human cancer cell lines (Hep-G2 and KB) demonstrated a moderate to high antiproliferative activity displayed by 11 indenoisoquinolines thus synthesized. In particular, the introduction of the 2-hydroxypropyl side chain was shown to be beneficial for the overall cytotoxic activity, pointing to the potential relevance of these novel indenoisoquinoline-propan-2-ol hybrids.

Indenoisoquinolines represent a versatile class of compounds, known for their anticancer activity resulting from the inhibition of DNA topoisomerase I (Top1), a universal and essential enzyme for the relaxation of supercoiled DNA during important cellular processes.[1] Top1 had previously emerged as a valuable molecular target for the development of anticancer agents,[1e,1f,2] such as the pentacyclic alkaloid camptothecin **1** (Fig. 1) and its semisynthetic analogues.[1f,k,3] However, in spite of the established anticancer activity, the application and efficiency of the camptothecins was hampered by several pharmacological and clinical limitations, moving forward the class of the indenoisoquinolines as valuable alternatives.[1f,4] NSC 314622 **2** was developed as a lead compound[5] and in the years thereafter, many derivatives followed, two of which have been promoted into the stage of clinical trials, indimitecan **3** and indotecan **4**.[6] In view of these promising results, medicinal chemistry research on indenoisoquinolines is of present interest in order to obtain new inhibitors that (i) overcome the limitation of instability and the resulting poor bioavailability of the camptothecins, (ii) are more selective toward tumor cells versus normal cells and therefore have improved toxicity profiles, and (iii) are less subject to the emergence of resistance and have improved pharmacokinetics.[7]

Functionalized propanes are often part of biologically active agents. Particularly, the class of the β-amino propanols consists of multiple representatives with *inter alia* antimalarial, anticancer, Src kinase inhibiting, antimicrobial, and antifungal properties.[8] Famous examples include propranolol **5** and timolol **6**, depicted in Figure 1. Both compounds are commercially available β-blockers, prescribed for the treatment of hypertension and the eye disease glaucoma, respectively.[9] Besides, functionalized 3-amino-2-propanol derivatives have been evaluated as potent sodium channel blockers for the treatment of stroke,[10] while others represent green volatile corrosion inhibitors for brass.[11] In addition, amino propanols comprise important intermediates or precursors in the chemical synthesis of therapeutically or biologically relevant compounds,[12] concluding that this constitutes a versatile class of functionalized propanes with promising applications in divergent areas.

The nature of the indenoisoquinoline lactam side chains is known to have a significant impact on the biological effects of these molecules.[13] In that respect, this Letter presents the

* Corresponding author.

http://dx.doi.org/10.1016/j.tetlet.2015.12.045
0040-4039/

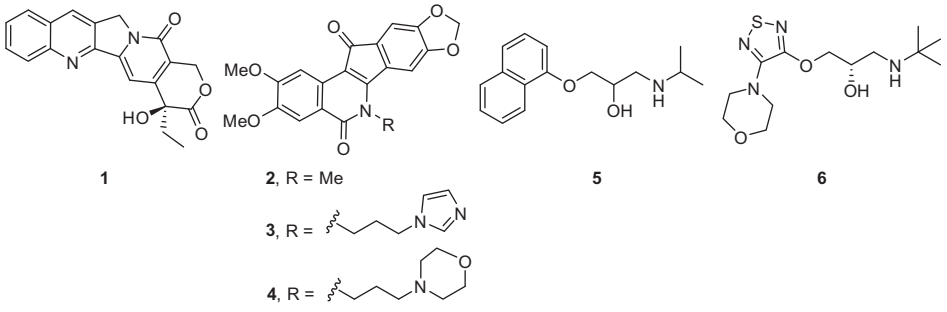


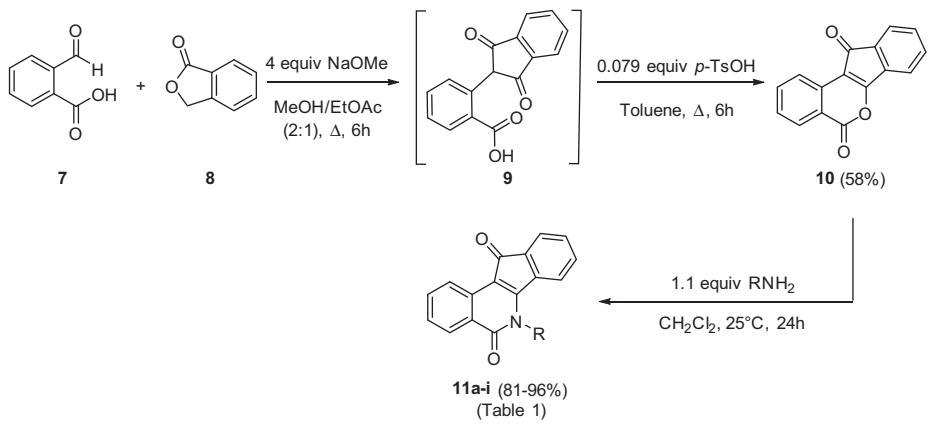
Figure 1.

synthesis of a variety of novel indenoisoquinolines combining the indenoisoquinoline scaffold with the promising functionalized 2-hydroxypropane unit, linked together via the indenoisoquinoline lactam nitrogen, followed by a preliminary evaluation of the cytotoxic potential of these derivatives against two human tumor cell lines. However, at first, a series of different indenoisoquinolines, *N*-functionalized with diverse heteroatom-containing side chains, was synthesized in order to be able to compare the influence of a functionalized 2-hydroxypropane unit with other side chains on the cytotoxicity in a later stage. The main objective of this work thus comprised the synthesis of indenoisoquinoline-propan-2-ol hybrids as new molecular entities and their preliminary cytotoxic evaluation to assess the potential of these new scaffolds as templates for more elaborate studies (e.g., as Top1 inhibitors) in the future.

The synthesis of the indenoisoquinoline skeleton has been performed by many methods in the literature. Some recently reported procedures include (i) the condensation of primary amines with the appropriately substituted indenoisochromenones,[14] (ii) the Suzuki–Miyaura cross-coupling followed by ring-closing metathesis,[15] (iii) the oxidative cyclization of the *cis* acid resulting from the condensation of homophthalic anhydrides and benzylidene Schiff bases,[2e,14a,16] (iv) cobalt-catalyzed dual annulation of *o*-halobenzaldimines with suitable alkynes,[17] (v) the combinational photochemical and carbocationic cyclization of adequately functionalized *N*-styryl benzamides,[18] and (vi) lithiated toluamide-benzonitrile cycloaddition followed by intramolecular radical cyclization.[19] The synthesis of the indenoisoquinolines was pursued in the present work by applying the first, widely used method of Morrell et al.[14d]

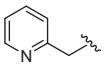
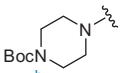
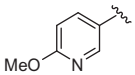
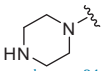
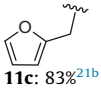
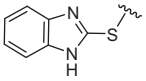
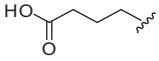
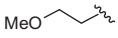
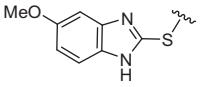
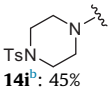
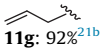
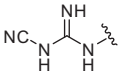
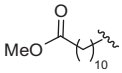
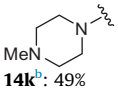
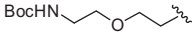
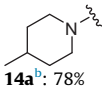
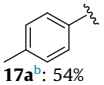
In this way, the key starting material, benz[*d*]indeno[1,2-*b*]pyran-5,11-dione **10**, or shortly indenobenzopyran **10**, was synthesized using a two-step methodology in a multi-gram scale. Condensation of 2-carboxybenzaldehyde **7** with phthalide **8** in the presence of sodium methoxide in methanol/ethyl acetate (2:1) under reflux furnished intermediate **9**, which could, after dehydrative acid-catalyzed lactonization in toluene, efficiently be converted to indenobenzopyran **10** in 58% yield after recrystallization from ethyl acetate. This compound **10** is an excellent precursor for the efficient generation of indenoisoquinolines **11** in high yields (81–96%) upon treatment with the appropriate primary amines, as illustrated in Scheme 1 (Table 1).[14d,20] In this way, the synthesis of four new derivatives was efficiently performed, giving rise to a series of nine variously substituted indenoisoquinolines at the lactam nitrogen atom. The molecular structures of the obtained indenoisoquinolines **11** were confirmed by means of spectral data (^{1}H NMR, ^{13}C NMR, IR and MS), some of which were identical to those reported in the literature.[13c,14c,21b,25]

The interesting properties of 3-aminopropan-2-ols and indenoisoquinolines as such, together with the fact that an improvement of the biological activity resulting from specific lactam side chain features has been observed previously, motivated the synthesis of indenoisoquinoline-propan-2-ols as a new class of hybrid molecules. More specifically, the advantages associated with the incorporation of an alkyl side chain containing two to four methylene units into the indenoisoquinoline core structure has been described earlier.[13c,14a,21a,22] Besides, multiple indenoisoquinoline structure–activity relationships have demonstrated that the presence of hydrogen bonding groups (e.g., hydroxyl) on the lactam side chains correlates well with an increase in biological activity.[14c,22b,c] In particular, the (*S*)-2,3-dihydroxypropyl lactam side chain has recently been proven to exert significant influence on the Top1 poisoning and cytotoxic activity, as the 2′-hydroxyl group may form a crucial hydrogen bond in the active site.[2d,13a] Moreover, a compound in which the terminal hydroxyl group is



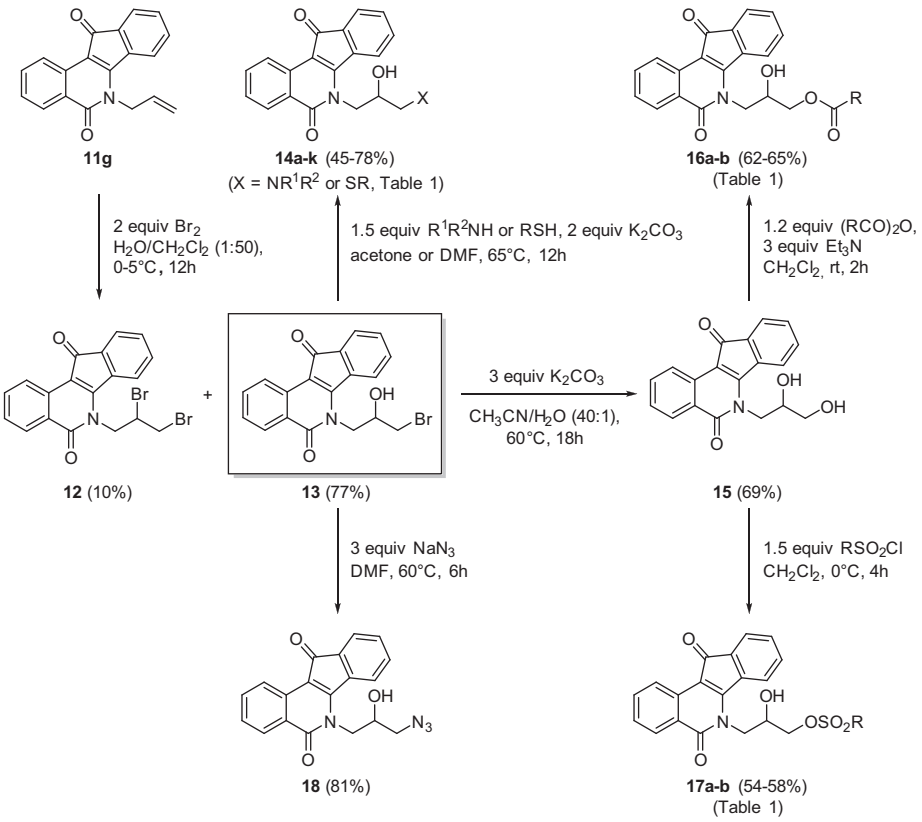
Scheme 1. Synthesis of indenoisoquinolines **11a–i**.

Table 1
Synthesis and cytotoxicity evaluation (IC_{50}) of indenoisoquinoline derivatives **11–18**

Entry	R or X **Compound**: Yield[a]	Cytotoxicity (μM)		Entry	R or X **Compound**: Yield[a]	Cytotoxicity (μM)	
		KB	Hep-G2			KB	Hep-G2
1	Ellipticine	1.42	1.62				
2	 **11a**: 86%[13c]	> 379	> 379	16	 **14d**[b]: 66%	31.4	32.9
3	 **11b**: 83%[20]	> 362	> 362	17	 **14e**[b]: 60%[24]	0.82	0.69
4	 **11c**: 83%[21b]	> 391	309	18	 **14f**[b]: 64%	73.2	68.3
5	 **11d**: 81%[25]	> 384	> 384	19	 **14g**[b]: 68%	2.35	3.44
6	 **11e**: 96%	> 420	> 420	20	 **14h**[b]: 65%	> 128	> 128
7	 **11f**: 81%[21b]	> 449	> 449	21	 **14i**[b]: 45%	15.3	14.8
8	 **11g**: 92%[21b]	> 446	> 446	22	 **14j**[b]: 65%	2.50	2.99
9	 **11h**: 82%	> 294	> 294	23	 **14k**[b]: 49%	1.51	1.19
10	 **11i**: 86%	1.82	1.41	24	 **15**[b]: 69%[14c]	26.6	27.1
11	**12**[b]: 10%	3.40	2.05	25	 **16a**[b]: 62%	10.6	8.33
12	**13**[b]: 77%	5.20	3.30	26	 **16b**[b]: 65%	54.9	52.1
13	 **14a**[b]: 78%	0.82	0.47	27	 **17a**[b]: 54%	4.33	7.19
14	 **14b**[b]: 73%	1.79	2.23	28	 **17b**[b]: 58%	57.1	52.1
15	 **14c**[b]: 76%	1.67	1.15	29	 **18**[b]: 81%	42.0	45.0

[a] After purification by column chromatography (SiO_2).
[b] Obtained as a racemic mixture.

replaced with an amino group, exerts even higher cytotoxic activity.[14c,22c] Furthermore, it was demonstrated that the incorporation of heterocycles into the indenoisoquinoline lactam side chain, possessing a heteroatom capable of serving as a hydrogen-bond acceptor at physiological pH, generally provides good biological results.[14d,22b,c,23] All together, these findings led



Scheme 2. Transformation of *N*-allylindenoisoquinoline **11g** toward 6-(2-hydroxypropyl)indenoisoquinolines **12**–**18**.

to the hypothesis that an efficacious indenoisoquinoline could be designed by incorporating an *N*-functionalized three-carbon side chain, possessing a 2′-hydroxyl group, combined with a terminal hydroxyl, ester, sulfonyl or amino group, substituted or integrated in various heterocycles. In that respect, the current work is the first in combining those extensively reported and promising features, concretized by the formation of indenoisoquinoline–propan-2-ol hybrids as novel structures.

For this purpose, we opted to derivatize *N*-allyl-substituted indenoisoquinoline **11g** via hydroxybromination using 2 equiv of bromine in water/dichloromethane (1:50). A mixture of 6-(2,3-dibromopropyl)indenoisoquinoline **12** (10%) and 6-(3-bromo-2-hydroxypropyl)indenoisoquinoline **13** (77%) was formed, after which the latter was considered as the central building block for conversion into a variety of *O*-, *N*- or *S*-based functional groups linked to the 3′-carbon of the propyl side chain. Firstly, 6-(3-bromo-2-hydroxypropyl)indenoisoquinoline **13** was converted into 6-(3-amino/thio-2-hydroxypropyl)indenoisoquinolines **14** in moderate to good yields (45–78%) after base-catalyzed nucleophilic substitution of the primary bromide by a series of primary or secondary amines or primary thiols in acetone or DMF at 65 °C (Table 1, Scheme 2).[24] Furthermore, intermediate **13** could be converted to the corresponding 2,3-propanediol **15** via K_2CO_3-catalyzed nucleophilic substitution by water. It should be mentioned, however, that 6-(2,3-dihydroxypropyl)indenoisoquinolines **15** can also be synthesized via condensation of indenobenzopyran **10** with the corresponding commercially available amino alcohols.[14c] Compound **15** was then further acylated using acetic and isobutyric anhydride in the presence of 3 equiv of triethylamine to provide esters **16** in 62–65% yield. On the other hand, treatment of 2,3-propanediol **15** with tosyl or mesyl chloride resulted in the formation of the monosulfonylated diols **17** (54–58%). A final option involved the reaction of 6-(3-bromo-2-hydroxypropyl)indenoisoquinoline **13** with 3 equiv of sodium azide in order to furnish the corresponding azide **18** in good yield (81%). The synthesized indenoisoquinolines **12**–**18** were analyzed using ^{1}H NMR, ^{13}C NMR, MS, and IR techniques in order to confirm the proposed molecular frameworks. Additionally, single crystal X-ray analysis of compound **13** secured the formation of this new scaffold (Fig. 2).

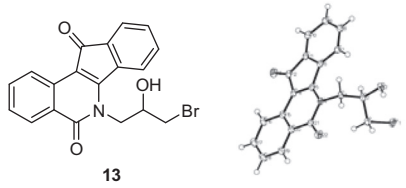


Figure 2. Single crystal X-ray analysis of compound **13**.

Subsequently, as compared to indenoisoquinolines **11a**–**i** lacking a 2-hydroxypropane side chain, the synthesized indenoisoquinolines **12**–**18** were evaluated in terms of their cytotoxicity profile against two human cancer cell lines, KB and Hep-G2.[26,27] Ellipticine, a compound with known cytotoxic activity, was added as a reference compound. The results of this biological assessment, summarized in Table 1, clearly indicate that the majority of the compounds exhibit at least moderate cytotoxicity against both cancer cell lines. Eleven of the prepared indenoisoquinolines display IC_{50} values of around or less than 5 μM (**11i**, **12**, **13**, **14a**,**b**,**c**, **e**,**g**,**j**,**k**, **17a**). Furthermore, it should be noticed that derivatives **11i** and **14a**,**c**,**e**,**k** exhibit equal cytotoxic activity as compared to the reference compound ellipticine, with 4-methylpiperidin-1-yl- and piperazin-1-ylindenoisoquinolines **14a** and **14e** being the most promising (IC_{50} values of 0.82 and 0.47 μM and 0.82 and 0.69 μM, respectively, against KB and Hep-G2).

In conclusion, the present Letter describes an efficient synthetic approach toward nine variously substituted indenoisoquinolines with regard to the lactam nitrogen, starting from the two-step condensation of 2-carboxybenzaldehyde and phthalide, followed by the reaction with the appropriate primary amines. In addition, a series of new functionalized 6-(2-hydroxypropyl)indenoisoquinolines was prepared via diverse methods of functionalization starting from 6-(3-bromo-2-hydroxypropyl)indenoisoquinoline. Subsequently, the 28 indenoisoquinolines thus prepared were subjected to in vitro cytotoxic evaluation against two human cancer cell lines, resulting in moderate to high bioactivity values of 11 representatives, especially those comprising the 3-amino-2-hydroxypropyl unit. The potential relevance of this novel class of indenoisoquinolines, incorporating the promising β-amino alcohol side chain, was thus proven, pointing to the importance of further, more elaborate biological assays and optimization of the most promising compounds.

Acknowledgements

The authors are indebted to the Vietnamese National Foundation for Science and Technology Development (NAFOSTED, code: 104.01-2014.38), Vietnam Academy of Science and Technology (Vast-04.07/14-15) and to Ghent University – Belgium (BOF) for financial support.

References and notes

1. (a) Wang, J. C. *Annu. Rev. Biochem.* **1996**, *65*, 635–692; (b) Wang, J. C. *Nat. Rev. Mol. Cell Biol.* **2002**, *3*, 430–440; (c) Fortune, J. M.; Osheroff, N. *Prog. Nucleic Acid Res. Mol. Biol.* **2000**, *64*, 221–253; (d) Wilstermann, A. M.; Osheroff, N. *Curr. Top. Med. Chem.* **2003**, *3*, 321–338; (e) Champoux, J. J. *Annu. Rev. Biochem.* **2001**, *70*, 369–413; (f) Pommier, Y. *Nat. Rev. Cancer* **2006**, *6*, 789–802; (g) Pommier, Y.; Barcelo, J. M.; Rao, V. A.; Sordet, O.; Jobson, A. G.; Thibaut, L.; Miao, Z.-H.; Seiler, J. A.; Zhang, H.; Marchand, C.; Agama, K.; Nitiss, J. L.; Redon, C. *Prog. Nucleic Acid Res. Mol. Biol.* **2006**, *81*, 179–229; (h) Stewart, L.; Redinbo, M. R.; Qiu, X.; Hol, W. G. J.; Champoux, J. J. *Science* **1998**, *279*, 1534–1540; (i) Seol, Y.; Zhang, H.; Pommier, Y.; Neuman, K. C. *Proc. Natl. Acad. Sci. U.S.A.* **2012**, *109*, 16125–16130; (j) Koster, D. A.; Palle, K.; Bot, E. S. M.; Bjornsti, M.-A.; Dekker, N. H. *Nature* **2007**, *448*, 213–217; (k) Pommier, Y. *Chem. Rev.* **2009**, *109*, 2894–2902.
2. (a) Singh, S.; Das, S.; Pandey, A.; Paliwal, S.; Singh, R. *J. Chem.* **2013** (849793/1-849793/10); (b) Zhi, Y.; Yang, J.; Tian, S.; Yuan, F.; Liu, Y.; Zhang, Y.; Sun, P.; Song, B.; Chen, Z. *Int. J. Mol. Sci.* **2012**, *13*, 6009–6025; (c) Reinhold, W. C.; Mergny, J.-L.; Liu, H.; Ryan, M.; Pfister, T. D.; Kinders, R.; Parchment, R.; Doroshow, J.; Weinstein, J. N.; Pommier, Y. *Cancer Res.* **2010**, *70*, 2191–2203; (d) Beck, D. E.; Agama, K.; Marchand, C.; Chergui, A.; Pommier, Y.; Cushman, M. *J. Med. Chem.* **2014**, *57*, 1495–1512; (e) Cinelli, M. A.; Reddy, P. V. N.; Lv, P.-C.; Liang, J.-H.; Chen, L.; Agama, K.; Pommier, Y.; van Breemen, R. B.; Cushman, M. *J. Med. Chem.* **2012**, *55*, 10844–10862; (f) Pommier, Y.; Cushman, M. *Mol. Cancer Ther.* **2009**, *8*, 1008–1014; (g) Pommier, Y.; Marchand, C. *Nat. Rev. Drug Disc.* **2012**, *11*, 25–36; (h) Marchand, C.; Antony, S.; Kohn, K. W.; Cushman, M.; Ioanoviciu, A.; Staker, B. L.; Burgin, A. B.; Stewart, L.; Pommier, Y. *Mol. Cancer Ther.* **2006**, *5*, 287–295; (i) Bailly, C. *Curr. Med. Chem.* **2000**, *7*, 39–58.
3. (a) Hsiang, Y. H.; Liu, L. F. *Cancer Res.* **1988**, *48*, 1722–1726; (b) Takimoto, C. H.; Wright, J.; Arbuck, S. G. *Biochim. Biophys. Acta* **1998**, *1400*, 107–119.
4. (a) Teicher, B. A. *Biochem. Pharmacol.* **2008**, *75*, 1262–1271; (b) Schaeppi, U.; Fleischman, R. W.; Cooney, D. A. *Cancer Chemother. Rep., Part 3* **1974**, *5*, 25–36; (c) Mi, Z.; Burke, T. G. *Biochemistry* **1994**, *33*, 10325–10336; (d) Staker, B. L.; Hjerrild, K.; Feese, M. D.; Behnke, C. A.; Burgin, A. B., Jr.; Stewart, L. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 15387–15392; (e) Brangi, M.; Litman, T.; Ciotti, M.; Nishiyama, K.; Kohlhagen, G.; Takimoto, C.; Robey, R.; Pommier, Y.; Fojo, T.; Bates, S. E. *Cancer Res.* **1999**, *59*, 5938–5946.
5. (a) Cushman, M.; Cheng, L. *J. Org. Chem.* **1978**, *43*, 3781–3783; (b) Kohlhagen, G.; Paull, K. D.; Cushman, M.; Nagafuji, P.; Pommier, Y. *Mol. Pharmacol.* **1998**, *54*, 50–58.
6. Antony, S.; Agama, K. K.; Miao, Z.-H.; Takagi, K.; Wright, M. H.; Robles, A. I.; Varticovski, L.; Nagarajan, M.; Morrell, A.; Cushman, M.; Pommier, Y. *Cancer Res.* **2007**, *67*, 10397–10405.
7. Xu, X.; Liu, F.; Zhang, S.; Jia, J.; Li, Z.; Guo, X.; Yang, Y.; Sun, H.; You, Q. *Biomed. Pharmacother.* **2013**, *67*, 715–722.
8. (a) Chennakesava Rao, K.; Arun, Y.; Easwaramoorthi, K.; Balachandran, C.; Prakasam, T.; Eswara Yuvaraj, T.; Perumal, P. T. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 3057–3063; (b) Robin, A.; Brown, F.; Bahamontes-Rosa, N.; Wu, B.; Beitz, E.; Kun, J. F. J.; Flitsch, S. L. *J. Med. Chem.* **2007**, *50*, 4243–4249; (c) Sharma, D.; Sharma, R. K.; Bhatia, S.; Tiwari, R.; Mandal, D.; Lehmann, J.; Parang, K.; Olsen, C. E.; Parmar, V. S.; Prasad, A. K. *Biochimie* **2010**, *92*, 1164–1172; (d) de Almeida, C. G.; Reis, S. G.; de Almeida, A. M.; Diniz, C. G.; da Silva, V. L.; Le Hyaric, M. *Chem. Biol. Drug Des.* **2011**, *78*, 876–880.
9. (a) Bago Basaganas, S. ES 371488 A1, Oct 16 1971.; (b) Arrigoni-Martelli, E. GB 1509778 A, Oct 29 975.
10. Seki, M.; Tsuruta, O.; Aoyama, Y.; Soejima, A.; Shimada, H.; Nonaka, H. *Chem. Pharm. Bull.* **2012**, *60*, 488–498.
11. (a) Gao, G.; Liang, C. *J. Electrochem. Soc.* **2007**, *154*, C144–C151; (b) Gao, G.; Liang, C. *Electrochim. Acta* **2007**, *52*, 4554–4559.
12. (a) Cossy, J.; Pardo, D. G.; Dumas, C.; Mirguet, O.; Dechamps, I.; Metro, T.-X.; Burger, B.; Roudeau, R.; Appenzeller, J.; Cochi, A. *Chirality* **2009**, *21*, 850–856; (b) Lalithamba, H. S.; Manohara, S. R.; Siddlingeshwar, B.; Shivakumaraiah *J. Mol. Liq.* **2014**, *198*, 94–100.
13. (a) Beck, D. E.; Abdelmalak, M.; Lv, W.; Reddy, P. V. N.; Tender, G. S.; O'Neill, E.; Agama, K.; Marchand, C.; Pommier, Y.; Cushman, M. *J. Med. Chem.* **2015**, *58*, 3997–4015; (b) Kiselev, E.; Sooryakumar, D.; Agama, K.; Cushman, M.; Pommier, Y. *J. Med. Chem.* **2014**, *57*, 1289–1298; (c) Morrell, A.; Placzek, M. S.; Steffen, J. D.; Antony, S.; Agama, K.; Pommier, Y.; Cushman, M. *J. Med. Chem.* **2007**, *50*, 2040–2048; (d) Staker, B. L.; Feese, M. D.; Cushman, M.; Pommier, Y.; Zembower, D.; Stewart, L.; Burgin, A. B. *J. Med. Chem.* **2005**, *48*, 2336–2345.
14. (a) Conda-Sheridan, M.; Reddy, P. V. N.; Morrell, A.; Cobb, B. T.; Marchand, C.; Agama, K.; Chergui, A.; Renaud, A.; Stephen, A. G.; Bindu, L. K.; Pommier, Y.; Cushman, M. *J. Med. Chem.* **2013**, *56*, 182–200; (b) Nguyen, T. X.; Morrell, A.; Conda-Sheridan, M.; Marchand, C.; Agama, K.; Bermingham, A.; Bermingam, A.; Stephen, A. G.; Chergui, A.; Naumova, A.; Fisher, R.; O'Keefe, B. R.; Pommier, Y.; Cushman, M. *J. Med. Chem.* **2012**, *55*, 4457–4478; (c) Peterson, K. E.; Cinelli, M. A.; Morrell, A. E.; Mehta, A.; Dexheimer, T. S.; Agama, K.; Antony, S.; Pommier, Y.; Cushman, M. *J. Med. Chem.* **2011**, *54*, 4937–4953; (d) Morrell, A.; Antony, S.; Kohlhagen, G.; Pommier, Y.; Cushman, M. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1846–1849.
15. Lebrun, S.; Couture, A.; Deniau, E.; Grandclaudon, P. *Tetrahedron Lett.* **2011**, *52*, 1481–1484.
16. (a) Cushman, M.; Gentry, J.; Dekow, F. W. *J. Org. Chem.* **1977**, *42*, 1111–1116; (b) Ryckebusch, A.; Garcin, D.; Lansiaux, A.; Goossens, J.-F.; Baldeyrou, B.; Houssin, R.; Bailly, C.; Henichart, J.-P. *J. Med. Chem.* **2008**, *51*, 3617–3629; (c) Zhang, X.; Wang, R.; Zhao, L.; Lu, N.; Wang, J.; You, Q.; Li, Z.; Guo, Q. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 1276–1281; (d) Ahn, G.; Schifano-Faux, N.; Goossens, J.-F.; Baldeyrou, B.; Couture, A.; Grandclaudon, P.; Lansiaux, A.; Ryckebusch, A. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 2259–2263.
17. Liu, C.-C.; Hsieh, J.-C.; Korivi, R. P.; Cheng, C.-H. *Chem. Eur. J.* **2015**, *21*, 9544–9549.
18. Dubois, M.; Deniau, E.; Couture, A.; Grandclaudon, P. *Synlett* **2012**, 1047–1051.
19. (a) Van, H. T. M.; Le, Q. M.; Lee, K. Y.; Lee, E.-S.; Kwon, Y.; Kim, T. S.; Le, T. N.; Lee, S.-H.; Cho, W.-J. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 5763–5767; (b) Cho, W.-J.; Le, Q. M.; Van, H. T. M.; Lee, K. Y.; Kang, B. Y.; Lee, E.-S.; Lee, S. K.; Kwon, Y. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3531–3534; (c) Cho, W.-J.; Park, M.-J.; Imanishi, T.; Chung, B.-H. *Chem. Pharm. Bull.* **1999**, *47*, 900–902.
20. *6-(6-Methoxypyridin-3-yl)-5H-indeno[1,2-c]isoquinoline-5,11(6H)-dione* (**11b**): 6-Methoxypyridin-3-amine (275 mg, 1.1 equiv) was added to a solution of indeno[1,2-*c*]*iso*chromene-5,11-dione (**10**; 500 mg, 2.02 mmol) in anhydrous CH_2Cl_2 (20 mL). The solution was allowed to stir at room temperature for 24 h. Afterward, the reaction mixture was washed with brine (20 mL). Drying of the organic phase ($MgSO_4$), filtration of the drying agent, and evaporation of the solvent in vacuo afforded indenoisoquinoline **11b** as a racemic mixture, which was then purified by column chromatography on silica gel (Hexane–EtOAc, 8:2) to obtain an analytically pure sample; Red crystals; Mp 253–254 °C; yield: 83%. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.72 (d, *J* = 8.0 Hz, 1H), 8.34 (d, *J* = 8.0 Hz, 1H), 8.21 (d, *J* = 2.5 Hz, 1H), 7.77 (t × d, *J* = 1.0, 8.0 Hz, 1H), 7.65 (d × d, *J* = 2.5, 8.5 Hz, 1H), 7.57 (d, *J* = 8.0 Hz, 1H), 7.49 (t × d, *J* = 2.5, 8.0 Hz, 1H), 7.26 (t, *J* = 2.5, 7.5 Hz, 1H), 7.10 (t, *J* = 1.0, 8.0 Hz, 1H), 7.01 (d, *J* = 8.5 Hz, 1H), 5.80 (d, *J* = 2.5 Hz, 1H), 4.08 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 190.5, 164.6, 163.8, 155.3, 146.4, 138.8, 137.0, 134.6, 134.3, 132.8, 132.7, 130.8, 128.7, 127.7, 127.4, 123.8, 123.7, 123.0, 122.2, 112.0, 108.5, 54.1. IR (KBr): 3076, 2943, 1789, 1693, 1662, 1606, 1573, 1548, 1485, 1411, 1375, 1182, 1012, 817, 759 cm^{-1}. MS: *m*/*z* (%) = 355 (100) [M^++1]. HRMS (ESI): *m*/*z* [M+H]$^+$ calcd for $C_{22}H_{15}N_2O_3$: 355.1077; found: 355.1081.
21. (a) Cushman, M.; Jayaraman, M.; Vroman, J. A.; Fukunaga, A. K.; Fox, B. M.; Kohlhagen, G.; Strumberg, D.; Pommier, Y. *J. Med. Chem.* **2000**, *43*, 3688–3698; (b) Wawzonek, S. *Org. Prep. Proced. Int.* **1982**, *14*, 163–168.
22. (a) Antony, S.; Jayaraman, M.; Laco, G.; Kohlhagen, G.; Kohn, K. W.; Cushman, M.; Pommier, Y. *Cancer Res.* **2003**, *63*, 7428–7435; (b) Nagarajan, M.; Morrell, A.; Ioanoviciu, A.; Antony, S.; Kohlhagen, G.; Agama, K.; Hollingshead, M.; Pommier, Y.; Cushman, M. *J. Med. Chem.* **2006**, *49*, 6283–6289; (c) Nagarajan, M.; Xiao, X.; Antony, S.; Kohlhagen, G.; Pommier, Y.; Cushman, M. *J. Med. Chem.* **2003**, *46*, 5712–5724.
23. Nagarajan, M.; Morrell, A.; Fort, B. C.; Meckley, M. R.; Antony, S.; Kohlhagen, G.; Pommier, Y.; Cushman, M. *J. Med. Chem.* **2004**, *47*, 5651–5661.
24. *6-[2-Hydroxy-3-(piperazin-1-yl)propyl]-5H-indeno[1,2-c]isoquinoline-5,11(6H)-dione* (**14e**): A mixture of piperazine (100 mg, 1.5 equiv) and K_2CO_3 (215 mg, 2 equiv) was stirred for 30 min at room temperature in 5 mL of DMF. Then, 6-(3-bromo-2-hydroxypropyl)-5*H*-indeno[1,2-*c*]isoquinoline-5,11(6*H*)-dione (**13**; 300 mg, 0.78 mmol) was added, after which the reaction was carried out at 65 °C for 12 h. Afterward, the reaction mixture was poured in water and extracted with EtOAc (3 × 5 mL). The combined organic phases were washed with water (10 mL) and brine (10 mL), dried ($MgSO_4$), filtrated, and evaporated in vacuo to afford the crude product as a racemic mixture, which was then

purified by means of column chromatography on silica gel (Hexane–EtOAc, 1:1) to obtain the analytically pure compound **14e**: red oil; Rf 0.61 (Hexane–EtOAc, 3:7); yield: 60%. ^{1}H NMR (CD_3OD, 500 MHz): δ = 8.55 (d, J = 8.5 Hz, 1H), 8.17 (d, J = 8.0 Hz, 1H), 7.87 (d, J = 7.5 Hz, 1H), 7.68 (d × d × d, J = 1.0, 8.0, 15 Hz, 1H), 7.48–7.35 (m, 4 H), 4.72–4.60 (m, 1H), 4.49–4.41 (m, 1H), 4.25–4.21 (m, 1H), 3.13–3.11 (m, 2H), 2.78–2.59 (m, 8H). ^{13}C NMR (CD_3OD, 125 MHz): δ = 192.0, 165.3, 138.7, 136.0, 135.0, 134.4, 133.5, 132.0, 129.1, 128.2, 125.6, 124.4, 124.2, 123.6, 109.4, 67.6, 63.1, 54.7, 52.1 (2 × C), 50.1, 45.0 (2 × C). IR (KBr): 3469, 3276, 3045, 2958, 2858, 1681, 1544, 1496, 1425, 1317, 1204, 1139, 799 cm^{-1}. MS: m/z (%) = 390 (100) [M$^+$+1]. HRMS (ESI): m/z [M+H]$^+$ calcd for $C_{23}H_{24}N_3O_3$: 390.1812; found: 390.1818.

25. Strumberg, D.; Pommier, Y.; Paull, K.; Jayaraman, M.; Nagafuji, P.; Cushman, M. *J. Med. Chem.* **1999**, *42*, 446–457.
26. *Cell culture and cell viability assay:* Two human cancer cell lines (epidermoid carcinoma cell line (KB) and hepatoma carcinoma cell line (Hep-G2)), obtained from the American Type Culture Collection (USA) ATCC, were used for cytotoxic evaluation. The cells were grown in RPMI 1640 medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin at 37 °C in a humidified atmosphere (95% air and 5% CO_2). The exponentially growing cells were used throughout the experiments. The inhibitory effects of the compounds on the growth of the human cancer cell lines were determined by measuring the metabolic activity using a *3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide* (MTT) assay.[27] Briefly, human cancer cell lines (1×10^5 cells/mL) were treated for 3 days with a series of concentrations of the compounds (in DMSO): 0.125, 0.5, 2.0, 8.0, 32.0, and 128.0 μg/mL. After incubation, 0.1 mg MTT solution (50 μL of a 2 mg/mL solution) was added to each well, and the cells were then incubated at 37 °C for 4 h. The plates were centrifuged at 1000 rpm for 10 min at room temperature, and the media were then carefully aspirated. Dimethylsulfoxide (150 μL) was added to each well to dissolve the formazan crystals. The plates were read immediately at 540 nm on a microplate reader (TECAN GENIOUS). All the experiments were performed three times, and the mean absorbance values were calculated. The results are expressed as the percentage of inhibition that produced a reduction in the absorbance by the treatment of the compounds compared to the untreated controls. A dose–response curve was generated, and the inhibitory concentration of 50% (IC_{50}) was determined for each compound as well as each cell line.
27. (a) Carmichael, J.; DeGraff, W. G.; Gazdar, A. F.; Minna, J. D.; Mitchell, J. B. *Cancer Res.* **1987**, *47*, 936–942; (b) Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55–63.