Bioorganic & Medicinal Chemistry Letters 26 (2016) 3652–3657

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Synthesis and cytotoxic evaluation of novel indenoisoquinoline-substituted triazole hybrids

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ARTICLE INFO

Article history:
Received 8 March 2016
Revised 26 May 2016
Accepted 31 May 2016
Available online 1 June 2016

Keywords:
Indenoisoquinolines
Triazole
AZT
Hybrids
Cytotoxic agents

ABSTRACT

The synthesis of various substituted triazole–indenoisoquinoline hybrids was performed based on a CuI-catalyzed 1,3-cycloaddition between propargyl-substituted derivatives and the azide-containing indenoisoquinoline. Besides, a variety of *N*-(alkyl)propargylindenoisoquinolines was used as substrates for the construction of triazole–indenoisoquinoline–AZT conjugated via a click chemistry-mediated coupling with 3′-azido-3′-deoxythymidine (AZT). Thus, twenty three new indenoisoquinoline-substituted triazole hybrids were successfully prepared and evaluated as cytotoxic agents, revealing an interesting anticancer activity of four triazole linker–indenoisoquinoline–AZT hybrids in KB and HepG2 cancer cell lines.

Pharmacophore hybridization, in which two or more bioactive entities are linked into a single hybrid molecule, has been known as an efficient and comfortable method to give new compounds with anticancer properties. Molecular hybridization can deliver a synthetic advantage through selective chemical modification of the more reactive entity within hybrid systems. Moreover, when bioactive agents are combined, new hybrid structures might display both a biological and a synthetic benefit. In the pursuit of potential new compounds, the selection of molecules with high biological activities for hybridization is occupying an important role.

Thus, indenoisoquinolines are one of the key structural units, which have been attracting considerable interest because of their various biological activities, particularly cytotoxicity against human cancer cell lines.[1] The lead compound of this class is NCS 314622 (**1**) (Fig. 1), which was at first prepared in 1978 and was found to be a mammalian topoisomerase I (Top1) inhibitor.[1b,2] Its cytotoxicity profile revealed a strong resemblance with camptothecin derivatives by stabilizing its covalent complex with DNA, the Top1-DNA cleavage complex (Top1cc), preventing further DNA religation and thus leading to the accumulation of DNA breaks.[3] During the past decade considerable effort has been made to improve the potencies and pharmacokinetics properties of these indenoisoquinolinediones through the contributions of the indenone ring, isoquinoline ring and lactam side chain.[1f,1g,4–6] It was demonstrated that the introduction of heterocycles, which possess a heteroatom capable of serving as a hydrogen-bond acceptor at physiological pH (e.g., hydroxyl, imidazole), in the *N*-functionalized lactam chains significantly enhances the biological activity.[5a,5c,5d,7–14] Well-known examples are anticancer topoisomerase I (Top1) inhibitors indotecan (LMP400, **2**) and indimitecan (LMP776, **5**), which were ultimately promoted to clinical study at the National Cancer Institute.[1i,15] Recently, hydroxylated analogus of the indotecan and indimitecan **4**–**11**, which contain various functionalized at A and D rings and the imidazole or morpholine group in *N*-lactam side chain, have been prepared and found to be very potent Top1 inhibitor and antiproliferative agents.[9] All of them, unlike camptothecin, appear to be stable and are powerful, cytotoxic Top1 poisons that induce long-lasting DNA breaks and overcome the drug resistance issues associated with the camptothecins.[1i,15,16] In addition, in our previous work, a novel of indenoisoquinoline-propan-2-ols **12**–**15** with high cytotoxicity against KB and HepG2 was designed by incorporating an *N*-functionalized three-carbon side chain,

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http://dx.doi.org/10.1016/j.bmcl.2016.05.092
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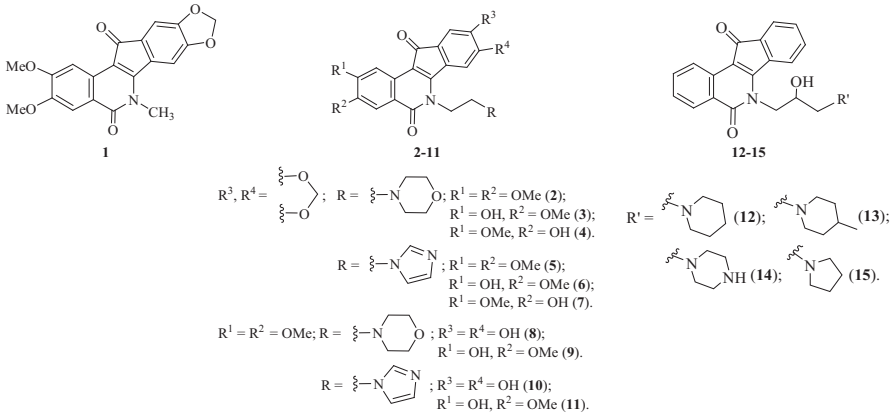


Figure 1. Chemical structure of several bioactive indenoisoquinoline derivatives.

possessing a 2′-hydroxyl group, combined with pyrrolidinyl, piperazine and piperidine units.[7]

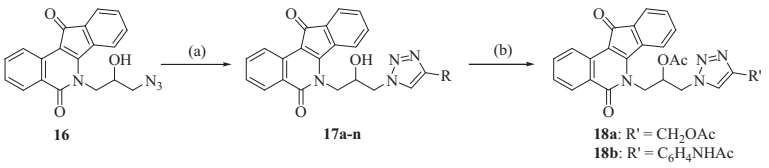
Another nitrogen-containing heterocycle 1,2,3-triazole has been known as a part of biologically active agent. Triazole has a high aromatic stabilization and high dipole moment, which might participate actively in hydrogen bond formation and in dipole–dipole and π stacking interactions.[17] Triazole is relatively resistant to metabolic degradation,[18] stable to acid and basic hydrolysis as well as reductive and oxidative conditions. Planar heteroaromatic triazole might lead to a more facile interaction with DNA, proteins, or cells.[19] Besides, triazole is easy synthesized by 'click' chemistry and exhibits a broad-spectrum of anticancer activities[20] and antiproliferative properties.[21] Therefore the combining 1,2,3-triazole with other pharmacophores becomes one of the most importance medicinal chemistry strategies. By this way a number of compounds with potent antitumor activity have been synthesized and are now available in the market such as tazobactam,[22] cefatrizine[23] and carboxyamidotriazole.[24]

From the multitude of contributions in the scientific literature, it is clear that indenoisoquinolines and triazoles can be considered as valuable compounds, both from a medicinal and a synthetic point of view. Moreover, consideration for the chemical structure of indimitecan, the addition of one more nitrogen atom to the imidazole ring could give more interesting bioactivities. All together, these findings have led to the hypothesis that the introduction of triazole group into *N*-functionalized three-carbon side chain of indenoisoquinoline, especially indenoisoquinoline-propan-2-ols, could give the promising potent biological compounds. However, the combination of these two bioactive moieties into hybrid molecules has not been reported in the literature so far. Having been inspired by the biological importance of 1,2,3-triazoles and indenoisoquinoline-propan-2-ols as anticancer agents and in continuation of our interest in pharmacophore hybridization we herein reported the synthesis of novel triazole–indenoisoquinolines hybrids. The anticancer activity evaluation results revealed that the 1,2,3-triazole–indenoisoquinoline hybrids exhibited potent anticancer activity.

The strategies for the formation of five-membered heterocyclic ring systems contain 1,3-dipolar cycloaddition reactions, which have gained major interest for several decades in view of the large numbers of potential dipoles and dipolarophiles.[25] As one such possibility, Cu(I)-catalyzed azide–alkyne cycloaddition is a widely utilized, reliable and powerful way to form 1,4-disubstituted 1,2,3-triazole.[26] In that respect, a synthetic approach toward novel triazole–indenoisoquinoline hybrids was devised based on a CuI-catalyzed 1,3-cycloaddition between propargyl-substituted derivatives and the azide-containing indenoisoquinoline.

In this way, the key starting material, 6-(3-azido-2-hydroxypropyl)indenoisoquinoline **16**, or shortly azidoindenoisoquinoline **16**, was prepared by four-step methodology in our previous report.[7] Azidoindenoisoquinoline **16** exhibits an excellent precursor for the efficient generation of triazole–indenoisoquinoline hybrids **17a**–**n** in high yields (60–80%) upon treatment with the appropriate 1-propargyl derivative, as illustrated in Scheme 1.[27] This reaction was carried out in refluxing tetrahydrofuran in the presence of *N*,*N*-diisopropylethylamine (DIPEA) and CuI. The chemical structures of the obtained indenoisoquinolines **17a**–**n** were confirmed by means of spectral data (^{1}H NMR, ^{13}C NMR, IR and MS). In the previous work, it was shown that the hydroxy group in the *N*-lactam side chain of indenoisoquinoline-propan-2-ols increases antiproliferative activity.[7] In order to persuade this hypothesis, the hydroxy group in side chain was acylated by treatment with acetic anhydride to obtain esters **18a**,**b**, respectively (Scheme 1).

As a part of our ongoing work, the introduction of bioactive moieties into *N*-lactam side chain of indenoisoquinolines is also studied in this Letter. 3′-Azido-3′-deoxythymidine (AZT, zidovudine) has known as a nucleoside reverse transcriptase inhibitor used for the treatment of HIV infections. AZT has also been exhibited pronounced anticancer activity, especially in combination with other antitumor agents, for example, such as 5-fluorouracil, cisplatin, paclitaxel[28] and triterpenoids.[29,30] Thus, considering the documented anticancer activity of indenoisoquinolines, AZT and functionalized triazole, it is reasonable to suggest that the



Scheme 1. Synthesis of triazole–indenoisoquinoline hybrids **17a**–**n** and **18a**,**b**. Reagents and conditions: (a) 1.1 equiv ethynyl derivatives, 0.2 equiv DIPEA, 0.1 equiv CuI, THF, reflux, 24 h; (b) 3 equiv Ac_2O, 2 equiv Et_3N, DMF, rt, 24 h.

construction of triazole–indenoisoquinoline–AZT hybrids might show good cytotoxicity activities. For this purpose, *N*-propargyl indenoisoquinoline **20**, prepared from indenobenzopyran **19**,[7] was treated with AZT in *tert*-BuOH in the presence of CuI at 70 °C to afford 1,2,3-triazole–indenoisoquinoline–AZT conjugate **21** in good yield (Scheme 2). On the other hand, in order to investigate the substituted group on *N*-lactam side chain and containing triazole bridge the synthesized indenoisoquinoline acids **22a–e** were esterified by propargyl bromide in DMF/THF (1:1) in the presence of Cs_2CO_3 to furnish the corresponding esters **23a–e**, which were conjugated with AZT by the above-described click chemistry methodology. This approach resulted in novel indenoisoquinoline–AZT hybrids **24a–e** in a good yield (Scheme 2).[31] The chemical structures of indenoisoquinolines **20**–**24** were confirmed using ^{1}H NMR, ^{13}C NMR, MS and IR techniques.

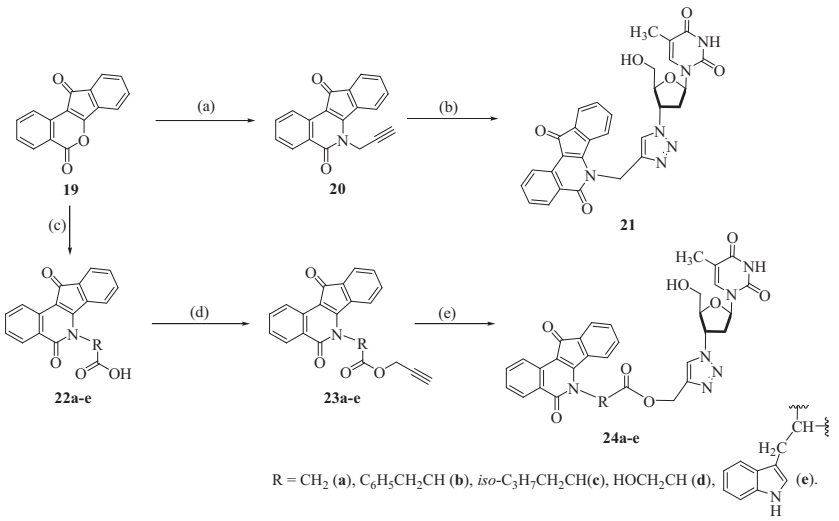
In continuation of our interest on hybridization of indenoisoquinolines via triazole linker, another bioactive coupling partner concerns imatinib and analogs, tyrosine-kinase inhibitors used in the treatment of multiple cancers, most notably chronic myelogenous leukemia,[32] have been taken into account. So the triazole hybrid between *N*-propargyl indenoisoquinoline **20** and imatinib derivative 4-(azidomethyl)-*N*-(4-methyl-3-(4-pyridin-3-yl)pyrimidin-2-ylamino)phenyl)benzamide **25** was synthesized as outlined in Scheme 3.

In the next part of this work, the newly prepared compounds were subjected to in vitro biological assessment against two human cancer cell lines (KB-CCL-17, HepG2-HB-8065)[33] in order to evaluate their potential as cytotoxic agents, and the results are summarized in Table 1. These results indicated that most of the derivatives possess at least moderate cytotoxic activity, and some triazole–indenoisoquinoline conjugates even display a promising activity profile. It is important to note that these separate pharmacophores display considerably less potent cytotoxic activities (IC_{50}-values ranging from 42 to >400 μM) as compared to the most promising conjugates **17l**, **21** and **24b** (IC_{50}-values between 0.4 and 1 μM) showing a reasonable activity against both cancer cells.

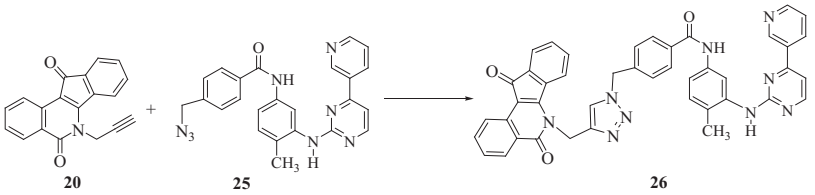
Hybrid triazole–indenoisoquinolines **17a**,**b**,**l**,**m**,**n**, **21**, **24b**,**c**,**d** and **26** exhibit potent cytotoxicity against both cell lines with IC_{50} < 10 μM, pointing that the introduction of functionalized 1,2,3-triazole into *N*-lactam side chain of indenoisoquinoline actually enhances biological activities. On the other hand, as compared to compounds **17a**,**e** containing 2-hydroxypropane side chain, the synthesized compounds **18a**,**b** by acylation display low cytotoxicity against both cell lines with IC_{50}-values ranging from 18 to >234 μM. It is demonstrated that the acylation of hydroxyl and amine groups decreases cytotoxicity activity of these hybrids.

The triazole–indenoisoquinoline hybrids with AZT and imatinib derivative also possess a high cytotoxic against both cell lines with IC_{50} < 1 μM. Moreover, hybrid **24b** can be identified as the most promising compound with IC_{50}-values below 0.5 μM against both cell lines (0.49 and 0.48 μM, respectively), thus representing a suitable template for further elaboration and optimization toward potent cytotoxic agents. These biological results clearly indicate the added value of merging AZT and indenoisoquinoline into single hybrid compounds in terms of their anticancer properties.

In conclusion, 6-(3-azido-2-hydroxypropyl)indenoisoquinoline was converted to various triazole–indenoisoquinoline hybrids by CuI-catalyzed 1,3-cycloaddition. In addition, a series of new triazole–indenoisoquinoline–AZT hybrids was prepared starting from diverse propargyl indenoisoquinolines. Subsequently, the 23 triazole–indenoisoquinoline hybrids thus prepared were subjected to in vitro cytotoxic evaluation against two human cancer cell lines,

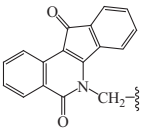
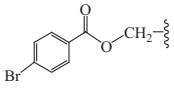
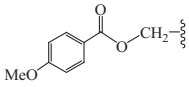
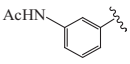
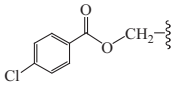
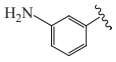
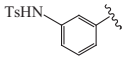
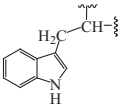
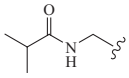
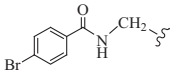
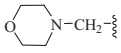


Scheme 2. Synthesis of triazole–indenoisoquinoline–AZT hybrids **21** and **24a**–**e**. Reagents and conditions: (a) 1.1 equiv propargyl amine, CH_2Cl_2, 40 °C, 24 h; (b) 0.9 equiv AZT, 0.2 equiv CuI, *tert*-BuOH, 70 °C, 24 h; (c) 1.1 equiv amino acid, CH_2Cl_2, 40 °C, 2–8 h; (d) 1.1 equiv propargyl bromide, 1.1 equiv Cs_2CO_3, DMF:THF (1:1), rt, 2–6 h; (e) 0.8 equiv AZT, 0.2 equiv DIPEA, 0.1 equiv CuI, THF, reflux, 24 h.



Scheme 3. Synthesis of triazole–indenoisoquinoline hybrid **26**. Reagents and condition: 0.9 equiv **25**, 0.2 equiv CuI, *tert*-BuOH, 70 °C, 24 h.

Table 1
Synthesis and cytotoxicity evaluation (IC_{50}) of triazole–indenoisoquinoline hybrids **17–26**

Entry	Compound R or R′: Yield, %	Cytotoxicity (μM)		Entry	Compound R or R′: Yield, %	Cytotoxicity (μM)	
		KB	HepG2			KB	HepG2
1	$HO\text{-}CH_2$- **17a**: 70	2.86	0.74	14	 **17n**: 79	2.89	2.29
2	 **17b**: 60	6.28	4.27	15	$AcOCH_2$- **18a**: 75	18.61	19.04
3	 **17c**: 55	11.19	11.86	16	 **18b**: 77	>234	>234
4	 **17d**: 46	10.46	9.75	17	**21**: 75	0.79	0.64
5	 **17e**: 75	15.33	31.96	18	CH_2 **24a**: 75	26.2	26.2
6	 **17f**: 77	89.89	69.76	19	$C_6H_5CH_2CH$ **24b**: 85	0.49	0.48
7	NH_2CH_2- **17g**: 50	50.03	195.43	20	*iso*-$C_3H_7CH_2CH$ **24c**: 82	0.75	2.25
8	$TsNHCH_2$- **17h**: 78	115.31	115.31	21	$HOCH_2CH$ **24d**: 78	0.35	1.18
9	$CH_3CONHCH_2$- **17i**: 80	14.07	9.04	22	 **24e**: 80	21.63	84.50
10	 **17j**: 70	123.23	22.1	23	**26**: 70	1.56	2.33
11	 **17k**: 50	56.74	43.83	24	**16**	42.0	45.0
12	$BrCH_2$- **17l**: 60	0.79	0.76	25	AZT	>479* 3.12** 9.17***	>479
13	 **17m**: 71	1.56	10.2	26	Ellipticine	1.26	1.46

* KB-CCL-17 (mouth epidermoid carcinoma).[29,30,33]
** KB (nasopharynx carcinoma).[34]
*** KB-3-1 (cervix epidermoid carcinoma).[35]

resulting in moderate to high bioactivity values of 10 representatives, especially those comprising the AZT unit. The potential relevance of this novel class of indenoisoquinolines, incorporating the promising triazole in lactam 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) for financial support.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.05.092.

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27. *6-(2-Hyroxy-3-(4-(hydroxymethyl)-1H-1,2,3-triazol-1-yl)propyl)-5H-indeno[1,2-c]isoquinoline-5,11(6H)-dione* (**17a**): Propargyl alcohol (1.1 equiv) was added to a solution of 6-(3-azido-2-hydroxypropyl)-5*H*-indeno[1,2-*c*]isoquinoline-5,11(6*H*)-dione (**16**) (1.0 equiv), DIPEA (0.2 equiv) and CuI (0.1 equiv) in THF (20 mL). The solution was stirred and heated to reflux for 24 h. Afterwards, the tetrahydrofuran was removed under reduced pressure and the residue was extracted with ethyl acetate. The combined organic phase was washed with brine, dried over $MgSO_4$ and concentrated in vacuum to give crude product **17a**. The target hybrid **17a** was obtained by column chromatography purification on silica gel using n-hexane-ethyl acetate (8:2, v/v) as an eluent. Yield: 70%. Mp 240 °C. IR (KBr) cm^{-1}: 3420, 3221, 2872, 1660, 1601, 1580, 1502, 1430, 1415.9, 1306, 1264, 1056, 971, 838, 756. ^1H NMR (DMSO-d_6, 500 MHz): 8.56 (1H, d, *J* = 9.0 Hz), 8.2 (1H, d, *J* = 8.0 Hz), 7.99 (1H, s, H-triazole), 7.79–7.82 (1H, m), 7.50–7.73 (2H, m), 7.41–7.44 (3H, m), 5.75 (1H, bs, OH), 5.25 (1H, bs, OH), 4.59–4.69 (3H, m), 4.55 (2H, s, CH_2OH), 4.40–4.44 (2H, m). ^{13}C NMR (DMSO-d_6, 125 MHz): 190.1, 162.8, 157.4, 147.7, 137.1, 134.3, 134.0, 133.4, 131.9, 130.9, 128.0, 127.0, 124.7, 124.1, 122.8, 122.5, 122.2, 107.1, 67.0, 55.0, 53.0, 47.9.
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31. *(1-((2S,3S,5R)-5-(Hydroxymethyl)-2-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)tetrahydrofuran-3-yl)-1H-1,2,3-triazol-4-yl)methyl 2-(5,11-dioxo-5,11-dihydro-6H-indeno[1,2-c]isoquinolin-6-yl)-3-phenylpropanoate* (**24b**): Prop-2-yn-1-yl 2-(5,11-dioxo-5,11-dihydro-6H-indeno[1,2-c]isoquinolin-6-yl)-3-phenylpropanoate (**23b**) (1.0 equiv) was added to a solution of AZT (0.8 equiv), DIPEA (0.2 equiv) and CuI (0.1 equiv) in THF (20 mL). The solution was stirred and heated to reflux for 24 h. Afterwards, the tetrahydrofuran was removed under reduced pressure and the residue was extracted with ethyl acetate. The combined organic phase was washed with brine, dried over $MgSO_4$ and concentrated in vacuum to give crude product **24b**. The target hybrid **24b** was obtained by column chromatography purification on silica gel using *n*-hexane-ethyl acetate (8:2, v/v) as an eluent. Yield: 85%. Mp 182 °C. IR (KBr) cm^{-1}: 3670, 3211, 2988, 2850, 1752, 1691, 1648, 1608, 1572, 1499, 1413, 1270, 1065, 759. ^1H NMR (DMSO-d_6, 500 MHz): 11.35 (1H, s, N*H*), 8.52 (1H, d, *J* = 6.5 Hz), 8.29 (1H, s, H-triazole), 8.2(1H, d, *J* = 6.5 Hz), 7.81 (3H, s), 7.57 (1H, s), 7.40 (1H, s), 7.36 (2H, s), 6.92 (4H, s), 6.40 (1H, s), 5.93 (1H, d, *J* = 5.5 Hz, O*H*), 5.29–5.36 (4H, m), 4.17 (1H, s), 3.51–3.67 (4H, m), 2.66 (2H, s), 1.81 (3H, s). ^{13}C NMR (DMSO-d_6, 125 MHz): 189.7, 168.0, 163.7, 162.4, 156.1, 150.4, 141.7, 136.2, 136.0, 135.8, 134.6, 133.5, 133.3, 131.5, 131.1, 129.4 (2C), 128.0 (2C), 127.9, 127.6, 126.7, 124.6, 123.7, 122.7, 122.4, 109.6, 107.4, 84.4, 83.9, 60.6, 60.4, 59.2, 58.2, 37.1, 33.6, 12.2.
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33. *Cell culture and cell viability assay*: Two human cancer cell lines epidermoid carcinoma cell line ((KB)-ATCC®CCL-17™) and hepatoma carcinoma cell line ((HepG2)-ATCC®HB-8065™), 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. 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.
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