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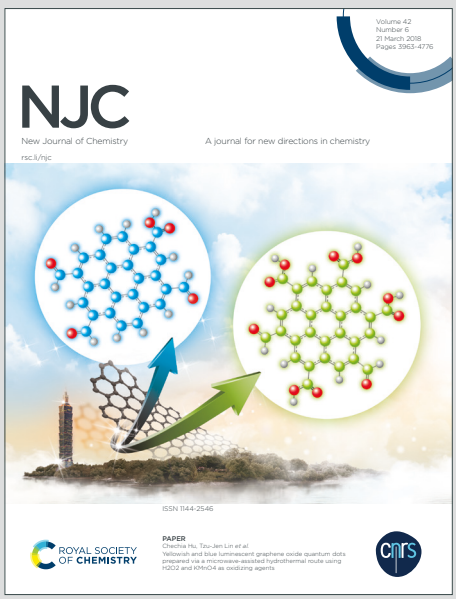

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ARTICLE

Synthesis, cytotoxic activity, ADMET and molecular docking study of quinoline-based hybrid compounds of 1,5-benzothiazepines

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Some α,β-unsaturated ketones **4a-g** of 3-acetyl-4-hydroxyquinolin-2(1*H*)-one were prepared by its reaction with (hetero)aromatic aldehydes with yields of 61−87% using piperidine as catalyst. These ketones reacted with *o*-aminothiophenol in the presence of acetic acid to afford a series of new hybrid compounds, quinoline-benzothiazepine, **6a-g**. The yields of benzothiazepines **6a-g** were 62−85%. All the synthesized compounds **6a-g** were screened for their *in vitro* anticancer activity against human hepatocellular carcinoma HepG2 and squamous cell *carcinoma* KB cancer lines. Compounds **6d** and **6g** had the best activity in the series, with IC_{50} values of 0.25 and 0.27 μg/mL, respectively against HepG2, and of 0.26 and 0.28 μM, respectively, against KB cell lines. ADMET properties showed that compounds **6c** and **6g** possessed the drug-likeness behavior. Cross-docking results indicated that residues GLN778(A), DA12(F), and DG13(F) in the binding pocket as potential ligand binding hot-spot residues for compounds **6c** and **6g**.

Introduction

Heterocycles containing nitrogen and sulphur as heteroatoms undoubtedly constitute an important class of highly applicable bioactive molecules because of their interesting biological activities and uses as key structural motif for the synthesis of various products of pharmaceutical interest. Benzothiazepine is a heterocyclic compound that contains a benzene ring fused with a seven membered ring having nitrogen and sulphur atoms. Benzothiazepine derivatives are of three types: 1,4-, 4,1- and 1,5-benzothiazepines[1, 2]. 1,5-Benzothiazepine is one of these three possible benzo-condensed derivatives. It is known that 1,5-benzothiazepine itself has not hitherto been described in the literature for its pharmacological properties[1, 3]. However, substituted 1,5-benzothiazepines are of particular interest for lead discovery because they have been found active against different families of targets[1, 2]. 1,5-Benzothiazepines play a unique role in drug discovery programs, as they display a wide spectrum of biological activities such as antibacterial[4], antifungal[5], antiviral[6], anticancer[7], antihypertensive[8] activities, and so on. 1,5-Benzothiazepine derivatives are also used as calcium channel modulators[9], either inhibitors for adenosine kinase [10], cholinesterase[11], antagonists[12], vasodilators[13], etc. Fig. 1 represented some bioactive benzothiazepine scaffold. Diltiazem, **A**,[14] was used clinically in USA from 1982[15], followed by clentiazem, **B**,[16] for their cardiovascular actions. Other 1,5-benzothiazepine derivatives were also used clinically for CNS disorders including thiazesim, **C**,[17] and quetiapine fumarate, **D**[18]. Therefore, their beneficial properties have prompted several groups to study these compounds. Owing to their importance from a pharmacological and synthetic point of view, several approaches have been reported for the synthesis of 1,5-benzothiazepines. Some 1,5-benzothiazepine derivatives were synthesized efficiently and environmentally from 2-aminobenzenethiols with α,β-unsaturated ketones[19] in good to excellent yield under solvent-free microwave irradiations using sulfuric acid on silica or basic alumina respectively as solid supports[20], under microwave irradiation *via* Mannich condensation[21], from chalcones and *o*-aminothiophenol in the presence of 10 mol% catalyst of ammonium cerium(IV) nitrate under ultrasonic irradiation[13], via a one-pot thia-Michael-cyclization sequence by the reaction of various *o*-aminothiophenols with chalcones in ionic liquid media, such as 1-octyl-3-methyl imidazolium thiocyanate ([omim]SCN) at 60 °C and 1-octyl-3-methyl imidazolium chloride ([omim]Cl) medium in room temperature[22], in PEG-400 catalyzed by acetic acid[23]. Another pathway to 1,5-benzothiazepine derivatives was the reaction of ω-bromoacetophenones and aromatic aldehydes[21], chalcone analogues of dehydroacetic acid[24], 2-aminophenyldisulfide with itaconic anhydride and dimethylitacone[25], α,β-unsaturated ketones with bis(2-nitrophenyl)disulphide in the presence of $TiClO_4$/Sm[26], nitrodisulphides and α,β-unsaturated ketones by SmI_2[27], photochemical reaction of 2-phenylbenzothiazole with ethoxy acetylene/ethoxy propyne[28], microwave activated reaction between 3-(4′-fluoro-2′-methylbenzoyl)-2-propenoic acid with

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2-aminothiolphenol[29], etc. The most convenient method for the synthesis of these compounds involves the treatment of 2-aminophenols (as dinucleophiles) with α,β-unsaturated ketones.

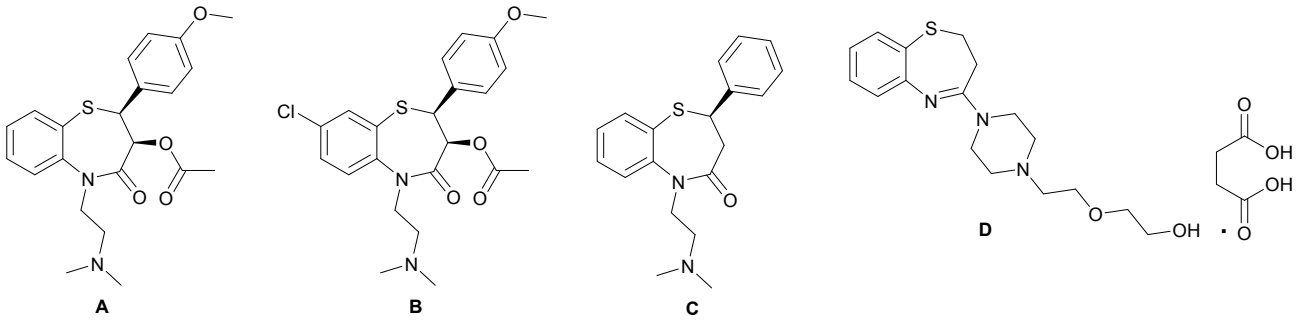

Figure 1. Some bioactive benzothiazepine derivatives.

We have been interested in 1,5-benzodiazepines and 1,5-benzothiazepine derivatives for a few years, and recently, have prepared a series of 1,5-benzothiazepines[30]. Inspired by the manifold applications of 1,5-benzothiazepine nuclei and in continuation of our interest in the synthesis and biological evaluation studies of heterocyclic compounds containing nitrogen and sulphur as heteroatoms, we report herein the synthesis, characterization, cytotoxic activities of some novel benzothiazepines having quinoline ring.

Results and discussion

Based on above-mentioned literature methods, we have chosen the synthetic pathway of target 1,5-benzothiazepines from α,β-unsaturated ketones of 3-acetyl-4-hydroxy-1-methylquinolin-2(1*H*)-one (**3**). This initial material was prepared from *N*-methylaniline. First, pyronoquinoline **2**, hydroxy-6-methyl-2*H*-pyrano[3,2-*c*]quinoline-2,5(6*H*)-dione, was prepared by using Kappe's and Stadlbauer's procedures[31, 32] by reaction *N*-methylaniline with diethyl malonate. In Stadlbauer's procedure diphenyl ether was used as solvent, but Kappe's procedure used excess diethyl malonate and this diester played the reagent and solvent roles. The former gave higher yield of pyronoquinoline **2**. The ring opening of **2** by sodium hydroxide in glycerol and subsequent spontaneous decarboxylation gave 3-acetyl-4-hydroxy-1-methylquinolin-2(1*H*)-one (**3**). α,β-Unsaturated ketones **5a-g**, substituted (*E*)-4-hydroxy-3-(3-(aryl)acryloyl)-1-methylquinolin-2(1*H*)-ones, were prepared by Claisen-Schmidt reaction of 3-acetylquinolone **3** with some appropriate aromatic and heteroaromatic aldehydes **4a-g** (Scheme 1). The reaction was carried out by heating under reflux in absolute ethanol as solvent for 25–50 h. Piperidine was used as catalyst for this process. The molar ratios of **3** and aldehydes **4a-g** were 1:1. Initially, the starting materials were dissolved completely in reaction solvent, then the product was separated as yellow-colour precipitates during the reaction. Products were obtained with yields of 61–87%.

IR spectra of these α,β-unsaturated ketones **5a-g** had characteristic absorption bands of *trans*-vinyl group in range of 998–967 cm^{-1}. Absorption band appeared at 1680–1634 cm^{-1} belonged carbonyl group of lactam function. Their ^1H NMR spectra indicated the presence of this *trans*-vinyl group through two signals at δ = 8.59–8.41 ppm and δ = 7.95–7.87 ppm for protons H-2' and H-3', respectively. These signals had the roof effects that showed the coupling constants *J* = 15.5–16 Hz. These values indicated that the resulting alkene had *trans* configuration. In compounds **5a-g** the phenolic hydroxyl group on position 4 of quinolone ring had no chemical shift downfield in DMSO-d_6 solvent, possibly due to hydrogen-bonding formation of this group to oxygen atom of carbonyl ketone (Fig. 2)[33]. In their IR spectra, weak and narrow absorption bands presented in region at 3462–3150 cm^{-1} that showed this intramolecular hydrogen bonding between this hydroxyl group and carbonyl oxygen atom. ^1H NMR spectrum of compound **5f** had signal at δ = 9.81 ppm that belonged 4''-hydroxyl group on benzene ring.

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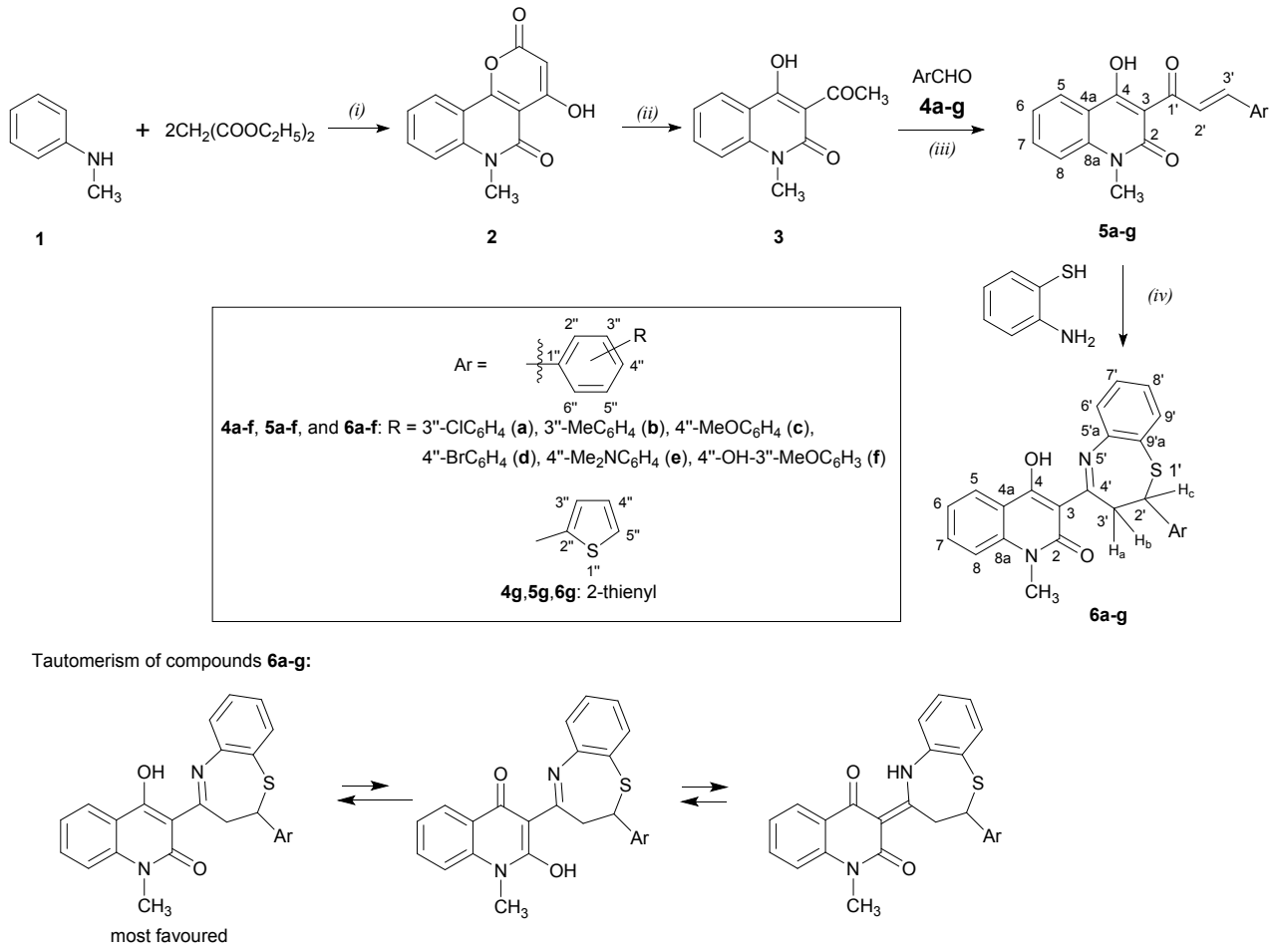

Scheme 1. Synthetic pathway for 1,5-benzothiazepines. Reaction conditions: (*i*) Diphenyl ether, 5 h, under reflux; (*ii*) 40% NaOH in water, glycerol, under reflux for 1h; (*iii*) Piperidine, abs. EtOH, under reflux for 25–50 h; (*iv*) Glacial acetic acid, abs. EtOH, under reflux for 5–7 h.

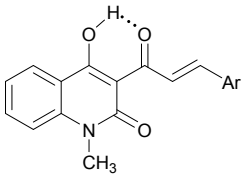

Figure 2. The hydro-bonding formation in compounds **5a-g**.

Next, substituted benzothiazepines **6a-g** were synthesized by ring-closure condensation reaction of obtained substituted α,β-unsaturated ketones **5a-g** with *o*-aminothiophenol. Reaction was carried out under reflux in absolute ethanol (Scheme 1) via Michael addition. At the beginning of the reaction, the initial materials were dissolved in solvent, and after about 3–4 h, the product precipitate began to appear. The ring-closure condensation process occurred over 5–7 h with obtained product yields of 65–82%. Structurally, the formation of benzothiazepines **6a-g** from α,β-unsaturated ketones **5a-g** of 3-acetyl-4-hydroxy-1-methylquinolin-2(1*H*)-one **3** could be confirmed by spectral data (IR, NMR, and MS). In IR spectra of these compounds, characteristic absorption band for out-of-plane bending vibration of C–H of *trans*-alkene group in α,β-unsaturated ketones **5a-g** in the region at 998–967 cm^{-1} disappeared. Hydroxyl group on position 4 of quinoline-2-one ring had chemical shift at δ = 14.78–14.76 ppm. This is the difference when compared to the case of α,β-unsaturated ketones above, in which the phenolic hydroxyl group on position 4 of quinolone ring had no chemical shift downfield in DMSO-d_6 solvent, and could be used as evidence to show that benzothiazepine ring was formed in the reaction

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of α,β-unsaturated ketones **5a-g** with 2-aminophenol. In ^{1}H NMR spectra of these benzothiazepines, the disappearance of signals at δ = 8.59–8.41 ppm and δ = 7.95–7.87 ppm for protons H-2′ and H-3′, respectively, also suggested that the benzothiazepine ring was formed.

Cytotoxic activity

All the synthesized of 3-(2-amino-6-arylpyrimidin-4-yl)-4-hydroxy-1-methylquinolin-2(1*H*)-ones **6a-g** were screened for their *in vitro* anticancer activity against two representatives: human squamous cell carcinoma (KB) and hepatocellular carcinoma (HepG2) cell lines. Ellipticine was used as reference. Evaluated results for **6a-g** were given in Table 1 and Figs. 3 and 4. In general, it has been observed that almost all tested compounds showed remarkable activity against the tested cancer cell lines, KB and HepG2, in comparison with the IC_{50} values of the reference compound (ellipticine). The results in Table 1 showed that almost all novel molecule exhibited anticancer activity against the tested KB cell line at wide range of concentrations. With the exception of compounds **6d** and **6e** that displayed negligible inhibitory effect on the KB carcinoma cell line (IC_{50} >128 μM), the remaining compounds had a good inhibitory effect. The order of the inhibitory effect of these compounds was **6c** ~ **6g** > **6f** > **6b** > **6a** > **6d**,**6e** of which compounds **6c** and **6g** had the best activity in the series, with IC_{50} values equal to 0.25 and 0.27 μg/mL, respectively, when compared to IC_{50} value with 0.28 μM of a positive reference drug ellipticine. Compound **6f** had significant inhibitory activity with IC_{50} of 0.8 μM.

For cancer cell line HepG2, compounds **6a-g** exhibited weak or insignificant activity, and two compounds **6c** and **6g** showed the highest activity in this sequence, with IC_{50} values equal to 0.26 and 0.28 μM (Table 1), respectively, when compared to the IC_{50} value of ellipticine (with IC_{50} = 0.36 μM). Compound **6f** exhibited medium activity with IC_{50} value of 2.10 μM. The remaining compounds in the series had a negligible activity for liver cancer cell lines HepG2. The order of inhibitory activity was as follows: **6c ~ 6g > 6f > 6b > 6e > 6a >6d**.

Table 1. Cytotoxic activity against KB and HepG2 cancer cell line in IC_{50} of compounds **6a-g**

Compds.	Ar	IC_{50} (μM)	
6a	3′′-ClC_6H_4	80.0	45.71
6b	3′′-MeC_6H_4	8.0	32.0
6c	4′′-$MeOC_6H_4$	0.25	0.26
6d	4′′-BrC_6H_4	>128	>128
6e	4′′-$Me_2NC_6H_4$	>128	32.0
6f	4′′-OH-3′′-$MeOC_6H_3$	0.8	2.10
6g	2′′-Thienyl	0.27	0.28
Ellipticine		0.28	0.36

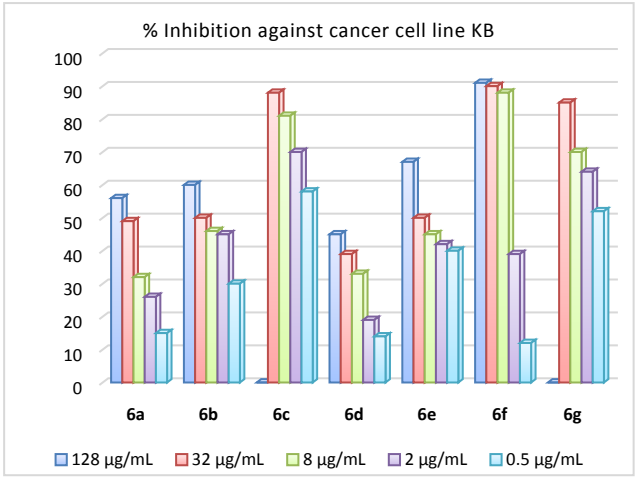

Figure 3. Dose-dependent cell growth inhibition percentages against KB cell line by the synthesized compounds **6a-g**.

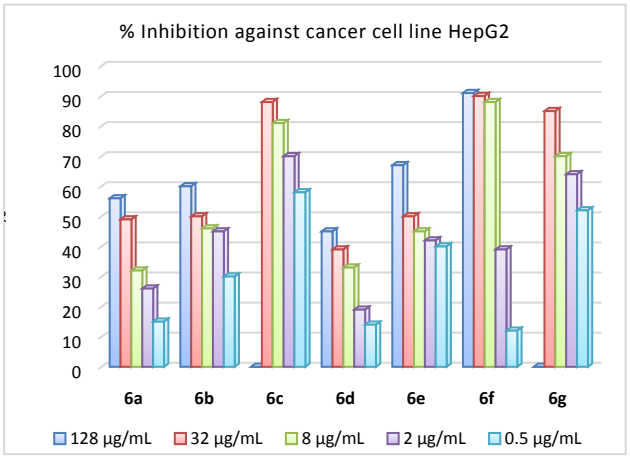

Figure 4. Dose-dependent cell growth inhibition percentages against HepG2 cell line by the synthesized compounds **6a-g**.

ADMET studies

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A variety of key ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) properties had been calculated *in silico* to estimate the drug-likeness of the compounds (Table 2)[34]. The predictions were carried out for synthesized compounds **6a-g** were screened using PreADMET online software[35] (for HIA, Caco2) and SwissADME online softwares[36] (for TPSA, n-ROTB, n-ON, n-OHNH, LogP, Lipinski's violations and Veber's violation). From Table 2, it could be observed that all the synthesized compounds have shown promising human intestinal percentage absorption (HIA = 95.71–98.59%). These values showed that these compounds had good human intestinal absorption. The most active compounds **6c** and **6g** showed 96.90% and 96.92% absorption, respectively. The designed structures were tested for compliance with rules evaluating bioavailability of a compound after oral administration Lipinski's rule of five and Veber's filter. The first one assumed that compounds having $LogP_{o/w}$ (octanol/water partition coefficient) lower than 5 (with values of 3.95–4.98), molecular weight (MW) below 500, less than 10 H-bond acceptors (n-ON), and less than 5 H-bond donors (n-OHNH) were more likely to show favourable bioavailability[37]. The Veber rule extended the range of parameters by rotatable bonds (preferably n-ROTB <10) and topological polar surface area with values of 79.89–109.35 (preferably TPSA ≤ 140 Å^2)[38]. The Egan rule considered good bioavailability for compounds with TPSA ≤ 132 Å^2 and −1 < LogP < 6[39]. These compounds had middle cell permeability with Caco2 values in ranges of 24.51–49.87 mm/s.

Table 2. Physicochemical properties, lipophilicity and drug-likeness of compounds **6a-g**

Entry	Ar	MW[a]	TPSA[b]	n-ROTB[c]	n-ON[d]	n-OHNH[e]	LogP[f]	L.V.[g]	V.V.[h]	Caco2[i]	%HIA[j]
6a	3′′-ClC_6H_4	446.95	79.89	2	3	1	4.94	1	0	49.87	97.37
6b	3′′-MeC_6H_4	426.53	79.89	2	3	1	4.66	1	0	31.03	97.17
6c	4′′-$MeOC_6H_4$	442.53	89.12	3	4	1	4.41	0	0	29.33	96.90
6d	4′′-BrC_6H_4	491.40	79.89	2	3	1	4.98	1	0	37.73	97.59
6e	4′′-$Me_2NC_6H_4$	455.57	83.13	3	3	1	4.35	0	0	30.31	97.17
6f	4′′-OH-3′′-$MeOC_6H_3$	458.53	109.35	3	5	2	3.95	0	0	24.51	95.71
6g	2′′-Thienyl	418.53	108.13	2	3	1	4.41	0	0	34.01	96.92

Note. [a] MW: molecular weight (<500, expressed as Dalton); [b] TPSA: topological polar surface area (Å^2); [c] n-ROTB: number of rotatable bonds; [d] n-ON: number of hydrogen bond acceptors (≤10); [e] n-OHNH: number of hydrogen bond donors (≤5); [f] LogP: logarithm of partition coefficient (<5) of compound between n-octanol and water [37]; [g] L.V.: Lipinski's violations [37]; [h] V.V.: Veber's violation [38]; [i] Caco-2: Caco-2 cell permeability (P_{Caco-2} (nm/s), <4: low, 4–70: middle, >70: high); [j] HIA: human intestinal absorption (0–20=poor, 20–70=moderate, 70–100=good).

Molecular Modelling

Molecular docking simulations were performed in order to better understand the molecular basis for the inhibition of topoisomerases by ellipticine (reference drug) and compounds (ligands, herein) **6a-g** against above- mentioned cancer cell lines. Based on the results obtained from the enzymatic assays, molecular modelling studies were performed as a step toward understanding the interaction mode of these compounds as inhibitors. It is known that ellipticine (5,11-dimethyl-6*H*-pyrido[4,3-*b*]carbazole, an alkaloid from extracted from trees of the species *Ochrosia elliptica* and *Rauvolfia sandwicensis* [40]) is a potent antineoplastic agent exhibiting multimodal mechanism of action. This compound inhibited the enzyme topoisomerase II via intercalative binding to DNA. The prevalent mechanisms of ellipticine antitumor, mutagenic and cytotoxic activities were suggested to be intercalation into DNA and inhibition of DNA topoisomerase II activity[41, 42]. Human topoisomerase IIβ in complex with DNA and mitoxantrone was a targeting anticancer drug, and so we have chosen it for docking study[43, 44]. This enzyme was retrieved with PDB code 4G0V. The structure has a resolution of 2.6 Å with intercalated drug mitoxantrone, and consisted of 7 chains: A, B, C, D, E, and F, two molecules of mitoxantrone had complexed with these chains. Two of the most active benzothiazepine compounds (**6c** and **6g**), one moderately active compound (**6f**), and one of the least active compound (**6d**) were chosen for docking examination. The favourably docked molecules were ranked according to the XP Glide Score. Obtained docking results (glide score, in kcal/mol) were represented in Table 3. The order of glide scores was **6c** > **6g** > **6f** > **6d**, which indicated that ligands **6c** and **6g** were docked nicely fitted into the active site. The active pocked was formed from chains A, D, F.

Table 3. Molecular docking analysis of protein target 6QXG with ligands **6c,6f and 6g**

Ligands	Ar	Glide score[a]
6c	4-$MeOC_6H_4$	−7.964
6d	4-BrC_6H_4	−7.555
6f	4-OH-3-$MeOC_6H_3$	−7.876
6g	2-Thienyl	−7.948
Ellipticine		−8.102

[a] In kcal/mol.

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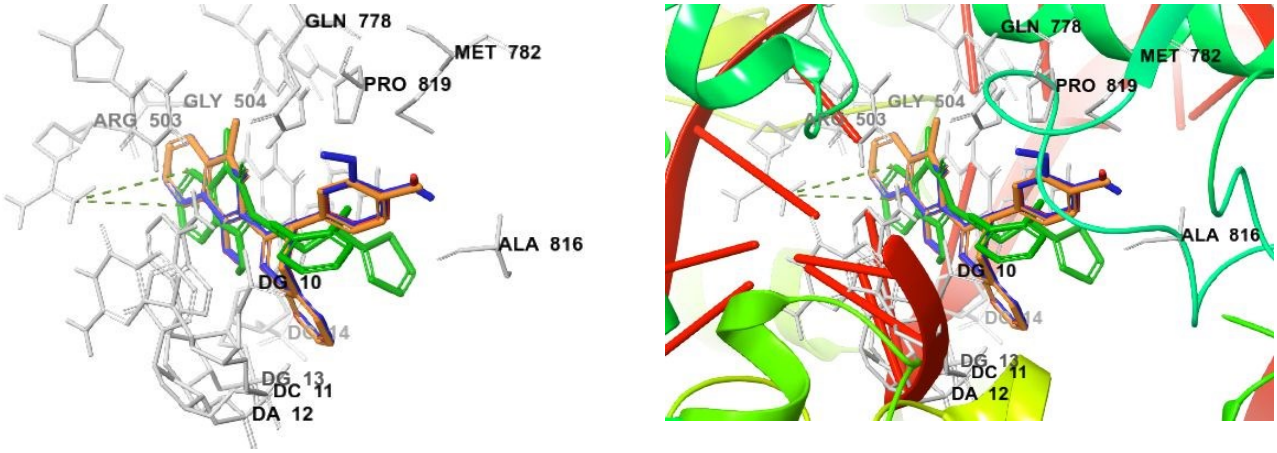

Figure 5. Docked (superimposed) poses showed ellipticine (magenta), **6c** (red), (**6d**, orange), **6f** (blue), and **6g** (green), all binding at the same position on molecular pocket of human topoisomerase IIβ in complex with DNA (4G0V) and showing common interactions with residues GNL A:778, ARG A:503, DA F:12, DG F:13.

Superimposed poses in Fig. 5 showed that four ligands **6c** (red), (**6d**, orange), **6f** (blue), and **6g** (green), all bound at the same position that the reference ellipticine (magenta) did on molecular pocket of human topoisomerase IIβ in complex with DNA. Ligands **6c** (with 4-methoxyphenyl group), **6f** (4-hydroxy-3-methoxyphenyl group), and **6g** (with 2-thienyl group) gave better glide scores when compared with other five compounds for the target protein, with binding score of −7.964, −7.876, and −7.948 kcal/mol, respectively. These values could be compared with the one of ellipticine (−8.102 kcal/mol). The worst active ligand **6d** with 5-bromophenyl group had glide score of −7.555 kcal/mol.

The important intermolecular protein-ligand interactions of compounds **6c** and **6g** are depicted in Fig. 6 (top). The lowest energies pose the highest active compounds **6c** and **6g** obtained by Glide 8.1 software are reported in Fig. 6 (bottom), similar to the docking position of ellipticine (Fig. 6, middle). Ellipticine itself had some active interactions with the residues in active site, such as π-cation (ARG503 on chain A), π-π stacking (DA12 and DG13 on chain F; DC8 on chain C; DT9, on chain D). There was not any hydrogen bonding interaction of this drug with the residues in active site. The ligands **6c**, **6d**, **6f**, and **6g** had common interactions with the residues ARG503 (on chain A), GLN778(on chain A), DA12 (on chain F), and DG13 (on chain F). There were some intermolecular residue interactions with compounds **6c** and **6g**, some interactions were the same ones in case of ellipticine: π-cation (ARG503 on chain A), π-π stacking (DA12 and DG13 on chain F). Another ligand-amino acid interactions of residue GLN778 on chain A of enzyme 4G0V took place in these ligands. This interaction was hydrogen bonding of GLN778 with C=O (lactam) group of 4-hydroxyquinolin-2(1*H*)-one moiety (Fig. 6). Additionally, ligand **6g** had more π-π stacking interactions than ligand **6c**. This one could explain the obtained higher glide score value of this ligand (Table 3).

Experimental

Melting points were determined by open capillary method on STUART SMP3 instrument (BIBBY STERILIN, UK) and are uncorrected. IR spectra (KBr disc) were recorded on an Impact 410 FT-IR Spectrometer (Nicolet, USA). ^{1}H and ^{13}C NMR spectra were recorded on Bruker Avance Spectrometer AV500 (Bruker, Germany) at 500 MHz and 125 MHz, respectively, using DMSO-d_6 as solvent and TMS as an internal standard. ESI-mass spectra were recorded on LTQ Orbitrap XL^{TM} (Thermo Fisher Scientific Co., USA) and ESI-MSD-Trap-SL (Agilent Technologies, Inc., USA) mass spectrometers) mass spectrometers. All reactions were monitored by thin layer chromatography, carried out on silica gel 60 $WF_{254}S$ aluminium sheets (Merck, Germany) and was visualized with UV light. Chemical reagents in high purity were purchased from the Merck Chemical Company (in Viet Nam). All materials were of reagent grade for organic synthesis. 4-Hydroxy-6-methyl-2*H*-pyrano[3,2-*c*]quinoline-2,5(6*H*)-dione (**2**, pyronoquinoline) was prepared from *N*-methylaniline according to known method[32]. 3-Acetyl-4-hydroxy-1-methylquinolin-2(1*H*)-one was prepared by modified procedure according to literatures[45], in which glycerol was used instead ethylene glycol, as follows.

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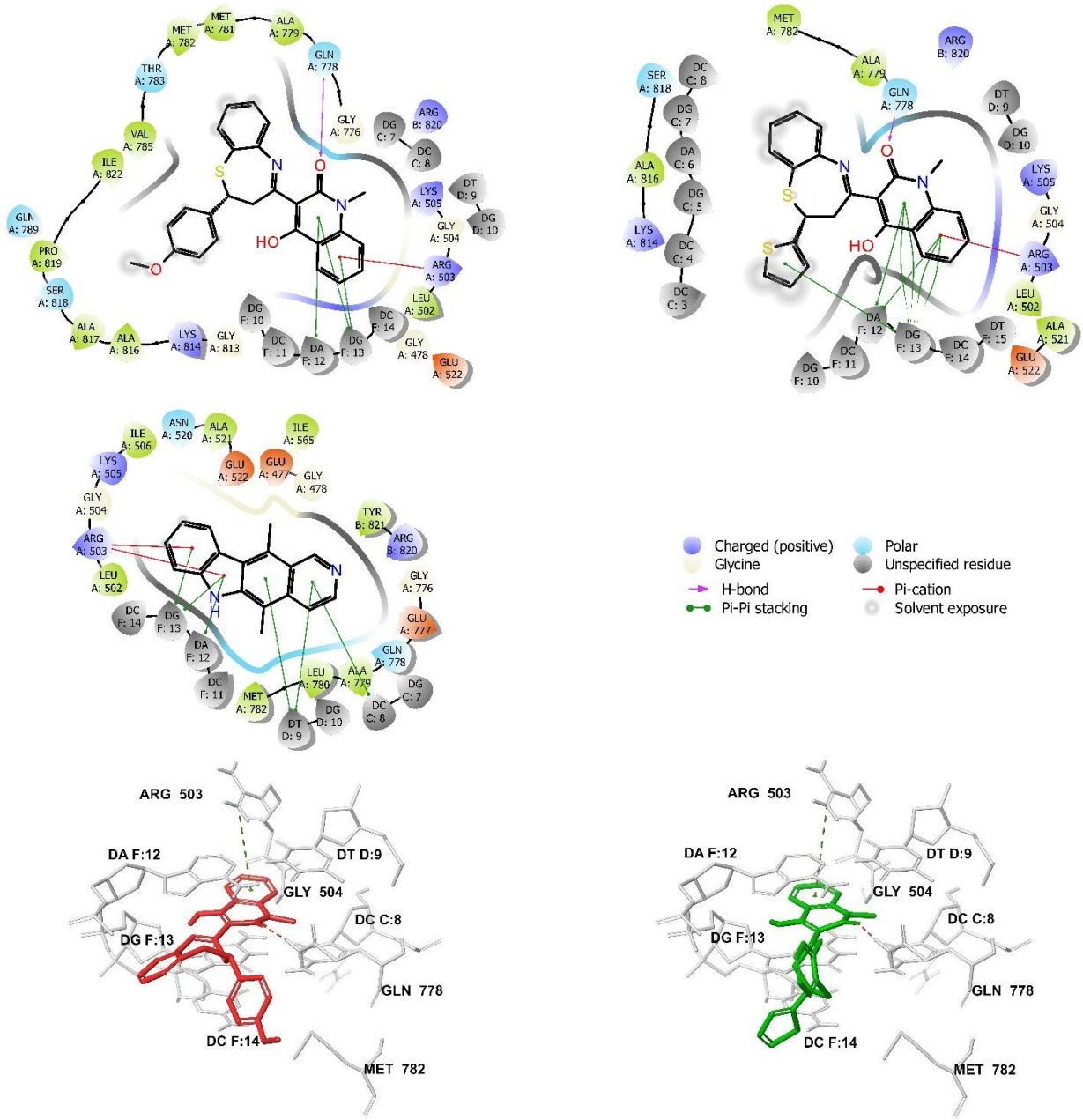

Figure 6. Residues involved in intermolecular interactions of ligands **6c** (top left), **6g** (top right), and ellipticine (middle left) in active pocked of human topoisomerase IIβ in complex with DNA (4G0V). The molecular 4G0V interactions of **6c** and **6g** (bottom) were depicted as dashed lines (orange – hydrogen bonds, green – π/ π stacking).

Synthesis of 3-acetyl-4-hydroxy-1-methylquinolin-2(1*H*)-one (**3**)

This compound was prepared by modified procedure[45]. To suspension of pyronoquinoline **2** (0.103 mol, 25 g) in glycerol (321 mL) a 40% aqueous solution of sodium hydroxide (0.515 mol, 32.1 mL) was added. Reaction mixture was boiled within 1 hour. The obtained solution was cooled in ice bath and poured into cold water (642 mL), and neutralized the solution by concentrated HCl until the precipitate completely separated (to acidic medium, pH 3). The separated precipitates were filtered, washed with water to pH 7, dried at temperature of 80°C, and crystallized from 96% ethanol. Yield: 12.51 g (56%).

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M.p. 141–142°C, ref.[32]: 141–142.5°C. IR (KBr, ν, cm^{-1}): 3468 (ν_{OH}), 1650 ($\nu_{C=O\ lactam}$), 1620 ($\nu_{C=O\ conj.\ acetyl}$). ^1H NMR (500 MHz, DMSO-d_6), δ (ppm): 2.71 (3H, s, 3-$COCH_3$), 3.54 (s, 3H, *N*-CH_3), 7.31 (t, *J* = 8.0 Hz, 1H, H-6), 7.50 (d, *J* = 8.0 Hz, 1H, H-5), 7.79 (td, *J* = 8.0, 1.5 Hz, 1H, H-7), 8.06 (dd, *J* = 8.0, 1.5 Hz, 1H, H-8).

General procedure for synthesis of (*E*)-4-hydroxy-3-(3-(aryl/hetaryl)acryloyl)-1-methylquinolin-2(1*H*)-ones (**5a-g**)

To a mixture of 3-acetyl-4-hydroxy-*N*-methyl-2(*1H*)-quinolone (**3**, 5 mmol) and appropriate (un)substituted benzaldehydes **4a-f** or thiophene-2-carboxaldehyde **4g** (5 mmol) in absolute ethanol (25 mL) was added piperidine (1 mol%, 0.1 mL). The reaction mixture was heated under reflux for 25–50 h. After reaction, solvent was led to evaporate to half a volume. Separated solid product was filtered, washed with a little of 96% ethanol (2×2 mL), recrystallized from appropriate solvents to afford the titled compounds **5a-g**.

(*E*)-4-Hydroxy-3-(3-(3-chlorophenyl)acryloyl)-1-methylquinolin-2(1*H*)-one (5a)

From **3** (5 mmol, 1085 mg) and **3a** (Ar=3''-ClC_6H_4, 5 mmol, 702 mg), under reflux for 35 h. Yield: 1324 mg (78%) of **5a** as pale-yellow crystals. M.p.: 168–169°C (96% ethanol/DMF 5:1 by volume); R_f = 0.70 (*n*-hexane/ethyl acetate = 5:3 by volume). IR (KBr), ν (cm^{-1}): 3102, 1650, 1623, 1596, 1542, 983. ^1H NMR (500 MHz, DMSO-d_6), δ (ppm): 8.57 (d, *J* = 16.0 Hz, 1H, H-3'), 8.17 (dd, *J* = 8.0 and 1.5 Hz, 1H, H-5), 7.87 (d, *J* = 16.0 Hz, 1H, H-2'), 7.81 (td, *J* = 8.0 and 1.5 Hz, 1H, H-7), 7.76 (s, 1H, H-2''), 7.71–7.69 (m, 1H, H-6''), 7.56 (d, *J* = 8.0 Hz, 1H, H-8), 7.52–7.51 (m, 2H, H-4'', H-5''), 7.34 (t, *J* = 8.0 Hz, 1H, H-6), 3.62 (s, 3H, *N*-CH_3). ESI-MS (+): calcd. for $C_{19}H_{14}{}^{35}ClNO_3$, M=339.1 Da, found: *m/z* 399.9 $[M]^+$.

(*E*)-4-Hydroxy-3-(3-(3-methylphenyl)acryloyl)-1-methylquinolin-2(1*H*)-one (5b)

From **3** (5 mmol, 1085 mg) and **4b** (Ar=3''-MeC_6H_4, 5 mmol, 600 mg), under reflux for 32 h. Yield: 1308 mg (87%) of **5b** as yellow crystals. M.p.: 172–173°C (96% ethanol); R_f = 0.82 (*n*-hexane/ethyl acetate = 5:3 by volume). IR (KBr), ν (cm^{-1}): 3110, 3034, 2917, 2849, 1633, 1625, 980, 870, 797, 750. ^1H NMR (500 MHz, DMSO-d_6), δ (ppm): 8.57 (d, *J* = 16.0 Hz, 1H, H-3'), 8.13 (d, *J* = 8.0 Hz, 1H, H-5), 7.88 (d, *J* = 16.0 Hz, 1H, H-2'), 7.80 (t, *J* = 8.0 Hz, 1H, H-7), 7.53–7.51 (m, 3H, H-8, H-2'', H-6''), 7.38-7.29 (m, 3H, H-6, H-4'', H-5''), 3.58 (s, 3H, *N*-CH_3), 2.36 (s, 3H, 3''-CH_3).

(*E*)-4-Hydroxy-3-(3-(4-methoxyphenyl)acryloyl)-1-methylquinolin-2(1*H*)-one (5c)

From **3** (5 mmol, 1085 mg) and **4c** (Ar=4''-$MeOC_6H_4$, 5 mmol, 680 mg), under reflux for 30 h. Yield: 1423 mg (85%) of **5c** as yellow crystals. M.p.: 178–179°C (96% ethanol/DMF 5:1 by volume); R_f = 0.81 (*n*-hexane/ethyl acetate = 5:3 by volume). IR (KBr), ν (cm^{-1}): 3103, 2971, 2933, 2838, 1643, 1599, 1532, 1504, 980. ^1H NMR (500 MHz, DMSO-d_6), δ (ppm): 8.51 (d, *J* = 15.5 Hz, 1H, H-2'), 8.13 (d, *J* = 8.0 Hz, 1H, H-5), 7.93 (d, *J* = 15.5 Hz, 1H, H-3'), 7.81 (t, *J* = 8.0 Hz, 1H, H-7), 7.72 (d, 2H, *J* = 8.5 Hz, H-3'', H-5''), 7.55 (d, *J* = 8.0 Hz, 1H, H-8), 7.33 (t, *J* = 8.0 Hz, 1H, H-6), 7.06 (d, 2H, *J* = 8.5 Hz, H-2'', H-6''), 3.83 (s, 3H, 4''-OCH_3), 3.59 (s, 3H, *N*-CH_3). ESI-MS (+): calcd. for $C_{20}H_{17}NO_4$, M=335.1 Da, found: *m/z* 335.9 $[M]^+$.

(*E*)-4-Hydroxy-3-(3-(4-bromophenyl)acryloyl)-1-methylquinolin-2(1*H*)-one (5d)

From **3** (5 mmol, 1085 mg) and **4d** (R =4'-Br, 5 mmol, 925 mg), under reflux for 25 h. Yield: 1632 mg (80%) of **5d** as pale-yellow crystals. M.p.: 200–201°C (96% ethanol/DMF 5:1 by volume); R_f = 0.68 (*n*-hexane/ethyl acetate = 5:3 by volume). IR (KBr), ν (cm^{-1}): 3088, 3045, 2925, 1680, 1616, 1535, 979, 756. ^1H NMR (500 MHz, DMSO-d_6), δ (ppm): 8.58 (d, *J* = 16.0 Hz, 1H, H-3'), 8.12 (dd, *J* = 8.0, 1.0 Hz, 1H, H-5), 7.86 (d, *J* = 16.0 Hz, 1H, H-2'), 7.81 (td, *J* = 8.0, 1.5 Hz, 1H, H-7), 7.72-7.67 (m, 4H, H-2'', H-3'', H-5'', H-6''), 7.55 (d, *J* = 8.5 Hz, 1H, H-8), 7.33 (t, *J* = 8.5 Hz, 1H, H-6), 3.58 (s, 3H, *N*-CH_3). ESI-MS (-): calcd. for $C_{19}H_{14}{}^{79}BrNO_3$, M-H=382.0 Da, found: *m/z* 381.9 $[M-H]^+$.

(*E*)-4-Hydroxy-3-(3-(4-(*N*,*N*-dimethylamino)phenyl)acryloyl)-1-methylquinolin-2(1*H*)-one (5e)

From **3** (5 mmol, 1085 mg) and **4e** (Ar=4''-$Me_2NC_6H_4$, 5 mmol, 820 mg), under reflux for 42 h. Yield: 1061 mg (61%) of **5e** as red brown crystals. M.p.: 220–221°C (96% ethanol/DMF 5:2 by volume); R_f = 0.78 (*n*-hexane/ethyl acetate = 5:3 by volume). IR (KBr), ν (cm^{-1}): 3110, 2910, 2807, 2538, 1636, 1595, 1504, 979, 762. ^1H NMR (500 MHz, DMSO-d_6), δ (ppm): 8.43 (d, *J* = 15.5 Hz, 1H, H-3'), 8.10 (d, *J* = 8.0 Hz, 1H, H-5), 7.94 (d, *J* = 15.5 Hz, 1H, H-2'), 7.75 (t, *J* = 8.0 Hz, 1H, H-7), 7.58 (s, 2H, *J* = 8.5 Hz, H-3'', H-5''), 7.50 (d, *J* = 8.0 Hz, 1H, H-8), 7.28 (t, *J* = 8.0 Hz, 1H, H-6), 6.77 (d, 2H, *J* = 8.5 Hz, H-2'', H-6''), 3.56 (s, 3H, *N*-CH_3), 3.02 [s, 6H, 4'-$N(CH_3)_2$].

(*E*)-4-Hydroxy-3-(3-(4-hydroxy-3-methoxyphenyl)acryloyl)-1-methylquinolin-2(1*H*)-one (5f)

From **3** (5 mmol, 1085 mg) and **4f** (Ar=4''-OH-3''-$MeOC_6H_3$, 5 mmol, 706 mg), under reflux for 38 h. Yield: 1440 mg (82%) of **5f** as yellow crystals. M.p.: 232–233°C (96% ethanol/DMF 5:1 by volume); R_f = 0.81 (*n*-hexane/ethyl acetate = 5:3 by volume). IR (KBr), ν (cm^{-1}): 3365, 3116, 3010, 2962, 2838, 1634, 1616, 1597, 1505, 998, 747. ^1H NMR (500 MHz, DMSO-d_6), δ (ppm): 9.81 (s, 1H, 4''-OH), 8.43 (d, *J* = 16.0 Hz, 1H, H-3'), 8.07 (d, *J* = 8.0 Hz, 1H, H-5), 7.88 (d, *J* = 16.0 Hz, 1H, H-2'), 7.74 (td, *J* = 8.0, 1.5 Hz, 1H, H-7), 7.47 (d, *J* = 8.0 Hz, 1H, H-8), 7.28-7.21 (m, 3H, H-6, H-2'', H-5''), 6.88 (d, 1H, *J* = 8.0 Hz, H-6''), 3.85 (s, 3H, 3''-OCH_3), 3.53 (s, 3H, *N*-CH_3).

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(*E*)-4-Hydroxy-3-(3-(2-thienyl)acryloyl)-1-methylquinolin-2(1*H*)-one (5g)

From **3** (5 mmol, 1085 mg) and **4g** (Ar =2''-thienyl, 5 mmol, 701 mg), under reflux for 50 h. Yield: 1078 mg (70%) of **5g** as bright yellow crystals. M.p.: 211−212°C (96% ethanol); R_f = 0.80 (*n*-hexane/ethyl acetate = 5:3 by volume). IR (KBr), ν (cm^{-1}): 3106, 3086, 1655, 1607, 1597, 1532, 967, 755. ^{1}H NMR (500 MHz, DMSO-d_6), δ (ppm): 8.41 (d, *J* = 15.5 Hz, 1H, H-3'), 8.12−8.10 (m, 2H, H-5, H-2'), 7.79−7.78 (m, 2H, H-7, H-5''), 7.61 (d, 1H, *J* = 3.5 Hz, H-3''), 7.53 (d, *J* = 8.0 Hz, 1H, H-8), 7.31 (t, *J* = 8.0 Hz, 1H, H-6), 7.20 (td, 1H, *J* = 3.5, 1.0 Hz, H-4''), 3.58 (s, 3H, *N*-CH_3).

General procedure for synthesis of 3-(2-aryl/hetaryl-1,5-benzothiazepin-4-yl)-4-hydroxy-1-methyl-quinolin-2(1*H*)-ones (**6a-g**)

A reaction mixture of appropriate α,β-(un)saturated ketone **5a-g** (1 mmol) and *o*-aminothiophenol (1 mmol, 125 mg, 0.1 mL) in absolute ethanol (30 mL). Glacial acetic acid (0.05 mL, 5 mol%) was added to this mixture. Obtained reaction mixture was heated under reflux for 5−7 h. Separated products were filtered and recrystallized from a mixture of DMF and 96% ethanol (in appropriate volume rations) to afford the compounds **6a-g**.

3-(2-(3-Chorophenyl)-1,5-benzothiazepin-4-yl)-4-hydroxy-1-methyl-quinolin-2(1*H*)-one (6a)

From **5a** (Ar=3''-ClC_6H_4, 1 mmol, 339 mg) under reflux for 6 h. Yield: 299 mg (67%) of **6a** as white crystals. M.p.: 222−223°C (from 96% ethanol/DMF=5:1 in volume), R_f = 0.80 (TLC solvent system: *n*-hexane/acetone 5:3). IR (KBr), ν (cm^{-1}): 3428 (ν_{OH}), 3061 (ν_{C-H} aryl), 2940, 2887 (ν_{C-H} alkyl), 1628 ($\nu_{C=O}$ lactam), 1590, 1550, 1478 ($\nu_{C=C}$ arene). ^{1}H NMR (500 MHz, DMSO-d_6), δ (ppm): 14.76 (s, 1H, 4-OH), 8.16−8.12 (m, 1H, H-5), 7.69−7.61 (m, 3H, H-7, H-6', H-8'), 7.53 (d, *J* = 7.5 Hz, 1H, H-9'), 7.43−7.31 (m, 6H, H-8, H-7', H-2'', H-4'', H-5'', H-6''), 7.23 (t, *J* = 7.5 Hz, 1H, H-6), 5.33−5.30 (m, 1H, H_c), 4.34−4.32 (m, 1H, H_b), 3.52 (s, 3H, *N*-CH_3), 2.75−2.70 (m, 1H, H_a); ^{13}C NMR (125 MHz, DMSO-d_6), δ (ppm): 145.7 (C-4), 134.8 (C-6'), 134.8 (C-3''), 134.0 (C-7), 132.8 (C-2''), 130.3 (C-8'), 130.1 (C-6''), 127.7 (C-7'), 127.0 (C-5''), 126.7 (C-5), 126.3 (C-1''), 125.7 (C-4''), 124.7 (C-9'), 124.4 (C-6), 116.9 (C-4a), 114.3 (C-8), 101.0 (C-3), 54.7 (C-2'), 54.9 (OCH_3), 38.2 (C-3'), 28.4 (*N*-CH_3); ESI-MS: calcd. for $C_{25}H_{19}{}^{35}ClN_2O_2S$/$C_{25}H_{19}{}^{37}ClN_2O_2S$, M=446.94/448.94 Da, found: *m/z* 447 (100%) $[M-H]^+$ and 449 (32%) $[M+2-H]^+$.

3-(2-(3-Methylphenyl)-1,5-benzothiazepin-4-yl)-4-hydroxy-1-methyl-quinolin-2(1*H*)-one (6b)

From **5b** (Ar=3''-MeC_6H_4, 1 mmol, 319 mg) under reflux for 7 h. Yield: 349 mg (82%) of **6b** as white crystals. M.p.: 208−209°C (from 96% ethanol/DMF=5:1 in volume), R_f=0.76 (TLC solvent system: *n*-hexane/acetone 5:2). IR (KBr), ν (cm^{-1}): 3444 (ν_{OH}), 2933 (ν_{C-H} alkyl), 1627 ($\nu_{C=O}$ lactam), 1600, 1548, 1479 ($\nu_{C=C}$ arene). ^{1}H NMR (500 MHz, DMSO-d_6), δ (ppm): 14.78 (s, 1H, 4-OH), 8.16−8.10 (m, 1H, H-5), 7.69−7.60 (m, 3H, H-7, H-6', H-8'), 7.53 (d, *J* = 7.5 Hz, 1H, H-9'), 7.44−7.40 (m, 2H, H-8, H-7'), 7.24−7.08 (m, 5H, H-6, H-2'', H-4'', H-5'', H-6''), 5.26 (dd, *J* = 13.0, 4.5 Hz, 1H, H_c), 4.44 (dd, *J* = 11.0, 3.0 Hz, 1H, H_b), 3.51 (s, 3H, *N*-CH_3), 2.73-2.67 (m, 1H, H_a), 2.28 (s, 3H, 3''-CH_3); ^{13}C NMR (125 MHz, DMSO-d_6), δ (ppm): 143.4 (C-4), 134.9 (C-6'), 133.6 (C-7), 130.1 (C-8'), 128.0 (C-4''), 127.7 (C-5''), 127.6 (C-7'), 127.5 (C-3''), 127.2 (C-5), 126.3 (C-1''), 124.6 (C-9'), 122.7 (C2''), 121.0 (C-6), 119.8 (C-4a), 114.2 (C-8), 55.7 (C-2'), 38.4 (C-3'), 28.4 (*N*-CH_3), 20.56 (3''-CH_3); ESI-MS: calcd. for $C_{26}H_{22}N_2O_2S$, M=426.53 Da, found: *m/z* 427.0 $[M+H]^+$.

3-(2-(4-Methoxyphenyl)-1,5-benzothiazepin-4-yl)-4-hydroxy-1-methyl-quinolin-2(1*H*)-one (6c)

From **5c** (Ar=4''-$MeOC_6H_4$, 1 mmol, 335 mg) under reflux for 5 h. Yield: 318 mg (72%) of **6c** as white crystals. M.p.: 213−214°C (from 96% ethanol/DMF=5:2 in volume), R_f = 0.68 (TLC solvent system: *n*-hexane/acetone 5:3). IR (KBr), ν (cm^{-1}): 3415 (ν_{OH}), 3061 (ν_{C-H} aryl), 2948, 2836 (ν_{C-H} alkyl), 1631 ($\nu_{C=O}$ lactam), 1590, 1550, 1478 ($\nu_{C=C}$ arene). ^{1}H NMR (500 MHz, DMSO-d_6), δ (ppm): 14.77 (s, 1H, 4-OH), 8.17 (d, 1H, H-5), 7.68-7.66 (m, 2H, H-7, H-6'), 7.61 (t, *J* = 7.5 Hz, 1H, H-8'), 7.51 (d, *J* = 7.5 Hz, 1H, H-9'), 7.42 (t, *J* = 7.5 Hz, 2H, H-8, H-7'), 7.27 (d, *J* = 7.5 Hz, 2H, H-2'', H-6''), 7.23 (t, *J* = 8.0 Hz, 1H, H-6), 6.89 (d, *J* = 7.5 Hz, 2H, H-3'', H-5''), 5.28 (d, 1H, H_c), 4.44-4.36 (m, 1H, H_b), 3.75 (s, 3H, 4''-OCH_3), 3.54 (s, 3H, *N*-CH_3), 2.73-2.71 (m, 1H, H_a); ^{13}C NMR (125 MHz, DMSO-d_6), δ (ppm): 162.5 (C-2 & C-4'), 158.4 (C-4''), 139.9 (C-4), 139.5 (C-9a'), 135.9 (C-1''), 135.0 (C-6'), 133.7 (C-7), 130.2 (C-8'), 127.8 (C-7'), 127.2 (C-5a'), 126.9 (C-2'', C-6''), 125.9 (C-5), 124.7 (C-9'), 121.2 (C-6), 120.1 (C-4a), 114.4 (C-8), 113.7 (C-3'', C-5''), 91.5 (C-3), 55.5 (C-2'), 54.9 (4''-OCH_3), 39.4 (C-3'), 28.5 (*N*-CH_3); ESI-MS: calcd. for $C_{26}H_{22}N_2O_3S$, M=442.52 Da, found: *m/z* 441 (100%) $[M-H]^+$.

3-(2-(4-Bromophenyl)-1,5-benzothiazepin-4-yl)-4-hydroxy-1-methyl-quinolin-2(1*H*)-one (6d)

From **5d** (Ar=4''-BrC_6H_4, 1 mmol, 384 mg) under reflux for 6 h. Yield: 382 mg (78%) of **6d** as white crystals. M.p.: 221−222°C (from 96% ethanol/DMF=5:1 in volume), R_f = 0.60 (TLC solvent system: *n*-hexane/acetone 5:2). IR (KBr), ν (cm^{-1}): 3525, 3320 (ν_{OH}), 3189, 3061 (ν_{C-H} aryl), 2977, 2858 (ν_{C-H} alkyl), 1628 ($\nu_{C=O}$ lactam), 1593, 1541, 1463 ($\nu_{C=C}$ arene). ^{1}H NMR (500 MHz, DMSO-d_6), δ (ppm): 14.76 (s, 1H, 4-OH), 8.18 (d, 1H, H-5), 7.70−7.67 (m, 2H, H-7, H-6'), 7.62 (t, *J* = 7.5 Hz, 1H, H-8'), 7.55-7.52 (m, 3H, H-3'', H-5'', H-9'), 7.44−7.41 (m, 2H, H-7', H-8), 7.36 (d, *J* = 8.0 Hz, 2H, H-2'', H-6''), 7.24 (t, *J* = 7.5 Hz, 1H, H-6), 5.34 (1H, d, H_c), 4.42 (1H, dd, H_b), 3.56 (s, 3H, *N*-CH_3), 2.75 (1H, t, H_a); ^{13}C NMR (125 MHz, DMSO-d_6), δ (ppm): 172.6 (C-4), 162.3 (C-4''), 143.2 (C-2), 141.0 (C-8a), 139.7 (C-9a'), 135.4 (C-1''), 134.1 (C-6'), 131.5 (C-3'', C-5''), 130.8 (C-8'), 128.3 (C-2'', C-

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6''), 127.0 (C-5a'), 126.5 (C-7'), 125.3 (C-5), 125.2 (C-9'), 121.6 (C-6), 120.5 (C-7), 120.1 (C-4a), 114.8 (C-8), 101.4 (C-3), 55,2 (C-2'), 39.5 (C-3'), 28.7 (*N*-CH_3); ESI-MS: calcd. for $C_{25}H_{19}{}^{79}BrN_2O_2S$/ $C_{25}H_{19}{}^{81}BrN_2O_2S$, M=490.52/492.52 Da, found: *m/z* 489 (9.7%) $[M-H]^+$ and 491 (9.5%) $[M+2-H]^+$.

3-(2-(4-(*N*,*N*-Dimethylamino)phenyl)-1,5-benzothiazepin-4-yl)-4-hydroxy-1-methyl-quinolin-2(1*H*)-one (6e)

From **5e** (Ar=4''-$Me_2NC_6H_4$, 1 mmol, 348 mg) under reflux for 7 h. Yield: 354 mg (78%) of **6e** as red brown crystals. M.p.: 228–229°C (from 96% ethanol/DMF=5:2 in volume), R_f = 0.81 (TLC solvent system: *n*-hexane/acetone 5:3). IR (KBr), ν (cm^{-1}): 3450 (ν_{OH}), 2895, 2794 (ν_{C-H} alkyl), 1630 ($\nu_{C=O}$ lactam), 1610, 1542, 1482 ($\nu_{C=C}$ arene); ^{1}H NMR (500 MHz, DMSO-d_6), δ (ppm): 14.77 (s, 1H, 4-OH), 8.17-8.11 (m, 1H, H-5), 7.69–7.52 (m, 4H, H-7, H-6', H-8', H-9'), 7.46–7.41 (m, 2H, H-8, H-7'), 7.23 (1H, t, *J* = 7.5 Hz, H-6), 7.13 (d, 2H, *J* = 8.5 Hz, H-3'', H-5''), 6.66 (d, 2H, *J* = 8.5 Hz, 2H, H-2'', H-6''), 5.25 (dd, 1H, *J* = 12.0, 4.0 Hz H_c), 4.42–4.28 (m, 1H, H_b), 3.52 (s, 3H, *N*-CH_3), 2.87 [s, 6H, $N(CH_3)_2$], 2.73–2.63 (m, 1H, H_a); ^{13}C NMR (125 MHz, DMSO-d_6), δ (ppm): 149.6 (C-4''), 139.9 (C-4), 134.9 (C-6'), 133.4 (C-7), 131.2 (C-1''), 129.9 (C-8'), 127.5 (C-7'), 126.2 (C-3'', C-5''), 125.9 (C-5), 124.5 (C-9'), 121.1 (C-6), 114.2 (C-8), 111.9 (C-2'', C-6''), 56.0 (CH_3), 55.9 (C-2'), 38.7 (C-3'), 28.4 (*N*-CH_3).

3-(2-(4-Hydroxy-3-methoxyphenyl)-1,5-benzothiazepin-4-yl)-4-hydroxy-1-methyl-quinolin-2(1*H*)-one (6f)

From **5f** (Ar=4''-OH-3''-$MeOC_6H_3$, 1 mmol, 351 mg) under reflux for 6 h. Yield: 284 mg (62%) of **6f** as white crystals. M.p.: 241–242°C (from 96% ethanol/DMF=5:1 in volume), R_f = 0.72 (TLC solvent system: *n*-hexane/acetone 5:2). IR (KBr), ν (cm^{-1}): 3475 (ν_{OH}), 2935, 2837 (ν_{C-H} alkyl), 1628 ($\nu_{C=O}$ lactam), 1595, 1552 ($\nu_{C=C}$ arene). ^{1}H NMR (500 MHz, DMSO-d_6), δ (ppm): 14.77 (s, 1H, C4-OH), 8.99 (s, 1H, C4'' -OH), 8.17-8.11 (m, 1H, H-5), 7.70-7.66 (m, 2H, H-7, H-6'), 7.61 (t, *J*=7.5 Hz, 1H, H-8'), 7.55 (d, *J*=7.5 Hz, 1H, H-9'), 7.47-7.41 (m, 2H, H-8, H-7'), 7.23 (1H, t, *J*=7.5 Hz, H-6), 6.90 (m, 1H, H-6''), 6.72 (m, 2H, 2H, H-2'', H-3''), 5.26-5.22 (m, 1H, Hc), 4.33-4.31 (m, 1H, Hb), 3.71 (s, 3H, 4''-OCH_3), 3.52 (s, 3H, *N*-CH_3), 2.75-2.60 (m, 1H, Ha); ^{13}C NMR (500 MHz, DMSO-d_6), δ (ppm): 180.5 (C-4'), 172.8 (C-4), 162.3 (C-4''), 147.4 (C-5''), 146.0 (C-2), 141.0 (C-8a), 139.7 (C-9a'), 135.4 (C-1''), 135.0 (C-6'), 134.0 (C-7), 130.5 (C-8'), 128.1 (C-7'), 127.6 (C-5a'), 126.1 (C-5), 125.0 (C-9'), 121.4 (C-6), 120.0 (C-4a), 118.2 (C-6''), 115.1 (C-3''), 114.7 (C-8), 110.2 (C-2''), 101.4 (C-3), 55.4 (C-2'), 55.9 (OCH_3), 39.3 (C-3'), 28.6 (*N*-CH_3).

3-(2-(2-Thienyl)-1,5-benzothiazepin-4-yl)-4-hydroxy-1-methyl-quinolin-2(1*H*)-one (6g)

From **5g** (Ar=2''-thienyl, 1 mmol, 312 mg) under reflux for 7 h. Yield: 356 mg (85%) of **6g** as white crystals. M.p.: 216–217°C (from 96% ethanol/DMF=5:1 in volume), R_f = 0.81 (TLC solvent system: *n*-hexane/acetone 5:2). IR (KBr), ν (cm^{-1}): 3450 (ν_{OH}), 3059 (ν_{C-H} aryl), 1630 ($\nu_{C=O}$ lactam), 1600, 1548, 1475 ($\nu_{C=C}$ arene). ^{1}H NMR (500 MHz, DMSO-d_6), δ (ppm): 8.17 (s, 1H, H-5), 7.70–7.61 (m, 3H, H-7, H-6', H-8'), 7.52 (dd, *J* = 8.0, 2.0 Hz, 1H, H-9'), 7.45–7.40 (m, 3H, H-8, H-7', H-5''), 7.24 (td, *J* = 8.0, 1.0 Hz, 1H, H-6), 7.05 (s, 1H, H-3''), 6.98 (td, *J* = 5.0, 3.5 Hz, 1H, H-4''), 5.60 (dd, *J* = 12.0, 4.5 Hz, 1H, H_c), 4.56 (s, 1H, H_b), 3.55 (s, 3H, *N*-CH_3), 2.69–2.63 (m, 1H, H_a); ^{13}C NMR (125 MHz, DMSO-d_6), δ (ppm): 148.5 (C-4), 147.1 (C-2''), 139.5 (C-9a'), 135.5 (C-6'), 133.8 (C-7), 130.5 (C-8'), 126.4 (C-5''), 126.3 (C-5), 126.0 (C-5a'), 125.9 (C-5), 124.7 (C-9'), 124.6 (C-3''), 123.6 (C-4''), 121.2 (C-6), 114.4 (C-8), 90.6 (C-3), 51.4 (C-2'), 39.0 (C-3'), 28.5 (*N*-CH_3); ESI-MS: calcd. for $C_{23}H_{18}N_2O_2S_2$, M=418.53 Da, found: *m/z* 419 $[M+H]^+$.

Cytotoxicity assay

Dilution series (128, 32, 8, 2, and 0.5 µg/mL of each compound **6a-g**) were prepared and used for 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) assay [46]. Two cancer cell lines were seeded at a density of 3×10^4 cells/well and treated with a range of concentrations in triplicate in 96-well cell culture plates, whereupon cell proliferation was assessed using a standard MTT assay. Specifically, the growth inhibitory activity of benzothiazepines was determined using MTT, which correlates the cell number with the mitochondrial reduction of MTT to a blue formazan precipitate. In brief, the cells were plated in 96-well plates and allowed to attach overnight. The medium was then replaced with serum-free medium containing the test compounds and cells were incubated at 37°C for 72 h. The medium was then replaced with fresh medium containing 1 mg/mL MTT. Following incubation at 37°C for 2–4 h, the wells were aspirated, the dye was solubilized in DMSO and the absorbance was measured at 540 nm using a Tecan™ GENios® Microplate Reader (Conquer Scientific, USA). The viability of cells was compared with that of the control cells. The slope of the absorbance change was used for calculating the reaction rate. Negative controls were performed in the absence of enzyme and compound, and positive controls in the presence of enzyme and 100% DMSO. The percentage of residual activity was calculated as the difference in absorbance between the time 6 and 2 min, obtained by the average of two experiments carried out in triplicate. The obtained rate was related to the rate when the inhibitor was absent. IC_{50} values were calculated from linear extrapolations of reaction rate (as a function of the logarithm of the concentration). The IC_{50} values were determined with increasing concentrations of inhibitor (128, 32, 8, 2, and 0.5 µg/mL) versus % of inhibition, in triplicate in two independent experiments. The experimental data were analysed with TableCurve 2D Software (Systat Software, Inc.) and the IC_{50} values determined by linear regression. It is important to stress the fact that all compounds are soluble in the assay mixtures at the described experimental conditions.

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In silico physicochemical property calculation and druglikeness evaluation

PreADMET online software (https://preadmet.bmdrc.kr/)[35] and SwissADME online (http://www.swissadme.ch/)[36] prediction tools were applied for determination of physicochemical properties, lipophilicity, water solubility, pharmacokinetics, drug-likeness and medicinal chemistry parameters, and also Topological Polar Surface Area (TPSA), number of rotatable bonds, LogP, violations of Veber's rule[38], and violations of Lipinski's rule of five[37].

Molecular docking

The two-dimensional structures (.mae) of compounds **6a-g** (ligands), were drawn and the structure was analysed by using 2D sketcher and 3D builder of Maestro 11.8 (Schrödinger, LLC, New York, NY, USA) [47]. The three-dimensional structures of these compounds (ligands) were generated from three-dimensional structures prepared first using LigPrep 3.6 using OPLS-2005 force field. The tautomeric isomers for the ligands, as displayed in Scheme 1, were searched and energy minimizations were carries out by applying the OPLS 2005 force fields, at pH 7.0 ± 2.0. The Epik v.4.3 methodology was used when preparing the ligands. Then geometrically minimized with MacroModel 12.2 followed by conformational analysis using MMFFs force field. Monte Carlo Multiple Minimum (MCMM) conformational search was used with 2500 iterations and convergence threshold of 0.05 kJ/mol. Water was chosen as solvent. Truncated Newton Conjugate Gradient minimization was used with 2500 iterations and convergence threshold of 0.05 kJ/mol. Other parameters were used as default. Crystal structure of human topoisomerase IIβ in complex with DNA and mitoxantrone (code: 4G0V) was retrieved from the RCSB Protein Data Bank (https://www.rcsb.org/structure/4G0V). This structure was solved by X-ray crystallography at 2.6 Å resolution. Coordinates of the protein-ligand complex were fixed for errors in atomic representations and optimized using Protein Preparation Wizard Maestro v. 11.5 (Maestro, v. 11.5: Schrödinger, LLC, New York, NY, USA). The bond orders were assigned to residues, hydrogen atoms were added at pH 7.0 ± 2.0. The restrained minimizations were carried out using the OPLS 2005 force field with an RMSD cut-off value of 0.3 Å for heavy atom convergences. The molecular docking was accomplished and analysed via the Glide v. 8.1 docking tool[48]. The receptor grid was located in the centre based on the active site of the protein, using the receptor grid generation tool. The ligands were flexibly docked in grid box using Monte Carlo-based simulation algorithm and an XP (Extra Precision) method without any constraints was employed that generated binding poses based on energy. For preparation of protein, water molecules (559 molecules) and 6 magnesium ions were removed. Mitoxantrone molecule complexed with chains A, C, D, F was chosen for Protein Preparation Wizard used OPLS-2005 force field for structural optimization and minimization and for Receptor Grid Generation tool of Glide v.8.1. The Glide HTVS 8.1 algorithm (High-Throughput Virtual Screening Mode) was employed using a grid box volume of 10 × 10 × 10 Å. Briefly, Glide approximates a systematic search of positions, orientations and conformations of the ligand in the receptor binding site using a series of hierarchical filters. Docking progress used constraints of two H-bond donor on Arg503, GLU522 and GLU778, one H-bond acceptor on ASN520. Upon completion of each docking calculation, 32 poses per ligand were generated and the best docked structure was chosen using a GlideScore (Gscore) function. A flow chart for molecular docking calculation using Schrödinger suite was implemented Supplementary Information file in online version of this article.

Conclusions

A series of (*E*)-4-hydroxy-3-(3-(aryl)acryloyl)-1-methylquinolin-2(1*H*)-ones (**5a-g**) were prepared from 3-acetyl-4-hydroxy-1-methylquinolin-2(1*H*)-one with yields of 61–87%. These α,β-unsaturated ketones were converted into 3-(2-amino-6-arylpyrimidin-4-yl)-4-hydroxy-1-methylquinolin-2(1*H*)-ones (**6a-g**) by reaction with *o*-aminothiophenol in the presence of piperidine as catalyst. The yields of benzothiazepines **6a-g** were 62–85%. All synthesized compounds **6a-g** were evaluated for *in vitro* anticancer activity against two cancer cell lines, human squamous cell carcinoma (KB) and hepatocellular carcinoma (HepG2). Compounds **6c** and **6g** exhibited remarkable inhibitory activity against the tested cancer cell lines with MIC values of 0.25–0.27 and 0.26–0.28 μM, respectively. ADMET properties as well as drug-likeness behaviours showed that of compounds 6c, 6f, and 6g were evaluated. Docking results indicated that residues GLN778(A), DA12(F), and DG13(F) in the binding pocket as potential ligand binding hot-spot residues for compounds **6c** and **6g**.

Conflicts of interest

There are no conflicts to declare.

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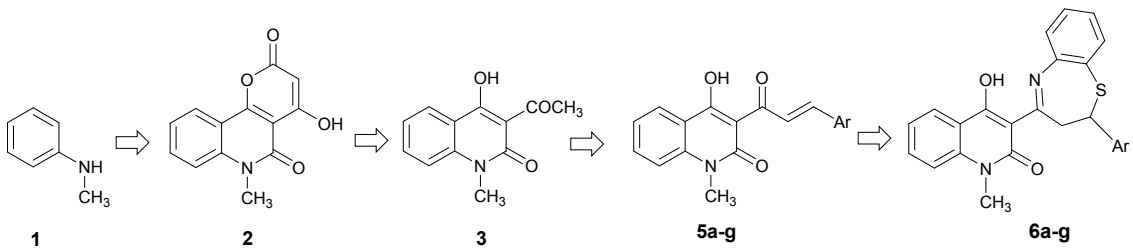
Table of Contents

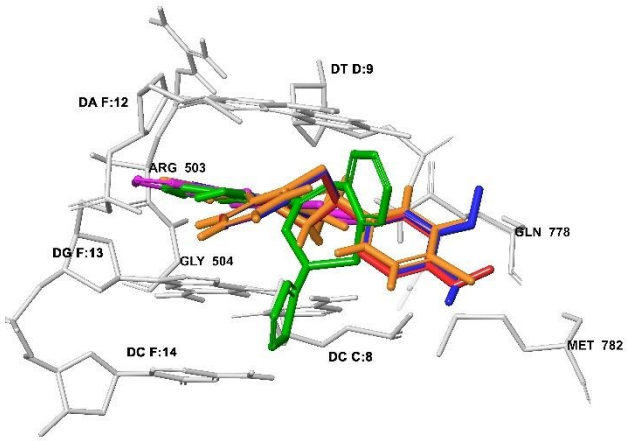
Synthesis, cytotoxic activity, ADMET and molecular docking study of quinoline-based hybrid compounds of 1,5-benzothiazepines

Duong Ngoc Toan,[a,b*] Nguyen Dinh Thanh,[b*] Mai Xuan Truong,[a] Duong Nghia Bang,[c] Mai Thanh Nga,[a] Nguyen Thi Thu Huong [b]

Some α,β-unsaturated ketones **4a-g** of 3-acetyl-4-hydroxyquinolin-2(1*H*)-one were prepared by its reaction with (hetero)aromatic aldehydes with yields of 61−87% using piperidine as catalyst. These ketones reacted with *o*-aminothiophenol in the presence of acetic acid to afford a series of new hybrid compounds, quinoline-benzothiazepine, **6a-g**. The yields of benzothiazepines **6a-g** were 62−85%. All the synthesized compounds **6a-g** were screened for their *in vitro* anticancer activity against human hepatocellular carcinoma HepG2 and squamous cell *carcinoma* KB cancer lines. Compounds **6d** and **6g** had the best activity in the series, with IC_{50} values of 0.25 and 0.27 μg/mL, respectively against HepG2, and of 0.26 and 0.28 μM, respectively, against KB cell lines. ADMET properties showed that compounds **6c** and **6g** possessed the drug-likeness behavior. Cross-docking results indicated that residues GLN778(A), DA12(F), and DG13(F) in the binding pocket as potential ligand binding hot-spot residues for compounds **6c** and **6g**.



1 **2** **3** **5a-g** **6a-g**



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