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Novel benzosuberone conjugates as potential anti-proliferative agents: Design, synthesis and molecular docking studies

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ABSTRACT

A series of novel benzosuberone derivatives bearing hexahydrospiro[indoline-pyrrolizin]-ones have been synthesized efficiently by the reaction of corresponding (*E*)-3-(9-chloro-2,3-dimethyl-6,7-dihydro-5*H*-benzo[7]annulen-8-yl)-1-phenylprop-2-en-1-ones with *N*-substituted isatins and L-proline in methanol. All the synthesized derivatives were evaluated for their anti-proliferative activity against A549, SKNSH, HeLa, HepG2 and MCF7 human cancer cell lines. The compounds **7h**, **7l**, **7n**, **7p**, **7q** and **7r** exhibited promising activity against all the cell lines and notably, compounds **7l** and **7n** showed the most potent activity against SKNSH with IC_{50} values of **4.61** and **5.04** μM. Further, *in silico* molecular docking [DNA (PDB ID: 1N37)] results stipulated a sign of good correlation between experimental activity and calculated binding affinity, indicating that all the synthesized compounds in particularly compounds **7h**, **7l**, **7n**, **7p**, **7q** and **7r** could be considered as good DNA intercalators. This is the first report on synthesis, *in vitro* anti-proliferative evaluation of hexahydrospiro[indoline-pyrrolizin]-one hybrids.

1. Introduction

Natural products are unique source for the development of novel effective cytotoxic agents. The derivatives of natural products, such as doxorubicin, paclitaxel and vinblastine are rich source of cancer treatment as well as some have been increasingly become major players in anticancer drug discovery [1–9]. A large volume of research has been carried out on benzosuberone from a natural product chemistry. In recent years, plant-derived benzosuberones (Colchicine) (**I**) has attracted public attention because of its beneficial[10a–c] effects in benzosuberone revealed relevant antitumor effects and cytotoxic activity towards various types of cancer cell lines [11a-c]. Theaflavin (**II**) is a promising anti cancer active compound in black tea. In *in-vitro* laboratory investigations, Theaflavins has been found to act on numerous points by regulating cancer cell growth, survival and metastasis [12]. Cycloheptadibenzofuran (**III**) is a new and unreported natural product skeleton from Malagasy plant *Busseasakalava*was found to be active against ovarian cancer cell line (the A2780) with an IC_{50} value of 45 μM [13]. Brussonol (**IV**), a natural product isolated from *Saliviabroussonetii* which showed potent anti cancer activity [14] against murine leukaemia cells line (P388) with $IC_{50} = 1.9$ μM.

Spiro compounds are attractive derivatives due to their structural and biological properties, particularly anticonvulsant [15], anti-tubercular [16], anti-cancer [17], antibacterial [18] and pain-relieving activities [19]. Isatin and its derivatives have proved to be versatile starting materials for the synthesis of heterocyclic products in particular spiro-oxindoles, which are attractive targets in organic synthesis due to their prominent biological activities [20a-h]. Apart from this, some spiro compounds have proved to be promising agents in the fields of agriculture and industry. In addition, they are used as antifungal agents [21], pesticides [22], laser dyes [23] and in electroluminescent devices [24]. Among them, heterocyclic spiro-oxindoles are attractive targets in organic and pharmaceutical chemistry owing to their potency and wide

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spectrum of biological activities [25a-e]. The spiro-oxindole system is the core structure in many pharmacological agents and alkaloids. Naturally occurring spiro-oxindole alkaloids (Fig. 1), such as horsfiline (**V**) and elacomine (**VI**) are used as indigenous medicines [26] and cytostatic alkaloids such as spirotrypsostanins[27a-b] and pteropodine has been shown to moderate the function of muscarinic and serotonin receptors [28]. Spiro-oxindole heterocycles in which the indole ring is linked to another heterocyclic system through a spiro carbon atom at C-3 shows an increased spectrum of biological activities [29] (Fig. 1).

Since combination of two or more active structural moieties possibly augment the bioactivity, so it was of interest to hybridize benzosuberone nucleus with isatin and L-proline fragments resulting in formation of hexahydrospiro[indoline-pyrrolizin]-one, hoping to develop potent anti-cancer agents. Our previous studies showed that the incorporation of 1, 3, 4-thiadiazole, oxadiazole and 1, 2, 4-triazole moieties at the β-chloroaldehyde position of benzosuberone which exhibited better inhibitory effect on growth of cancer cells [30a-c]. In an effort to further develop this class of promising compounds for anticancer treatment, the present study introduce a series of hexahydrospiro[indoline-pyrrolizin]-one hybrids (Fig. 2) (see Fig. 3).

In order to better understand their structure-activity relationships, molecular docking studies of synthesized compounds **7a-r** with therapeutic target of DNA of cancer was observed. Numerous anti-cancer drugs in clinical use (anthracyclines, dactinomycin) interact with DNA through intercalation. Intercalation of a molecule into DNA is the first step in a series of events which may eventually lead to its biological effects [31]. Once the molecule has been sandwiched between the DNA base pairs, the stability of complex is optimized by a number of non-covalent interactions, including van der Waals, $\pi-\pi$ interactions and hydrogen bond interactions [32]. Intercalation of DNA with spiropyrans [33] and spiro oxazines[34a-b] has been demonstrated previously.

In this context, molecular docking studies were performed to evaluate the possible intercalating potency of the synthesized compounds with DNA structure obtained from Protein data bank. Doxorubicin belongs to the anthracycline family of antitumor antibiotics has been taken as standard, the protein structure bound to respinomycin D was retrieved, as it belongs to same family of antitumor antibiotics.

The clinical agent doxorubicin is well-studied of this class but has relatively simple molecular architecture in which the pendant daunosamine sugar residue in the DNA minor groove. The compounds **7a-7r** were evaluated *insilico* (docking) to identify their hypothetical binding mode using the NMR structure of respinomycin D intercalation complex with a double stranded DNA molecule (AGACGTCT) 2 complex in solution [35] derived from NOE restraints, molecular dynamics simulations and molecular docking studies on DNA intercalates which supported the biological data.

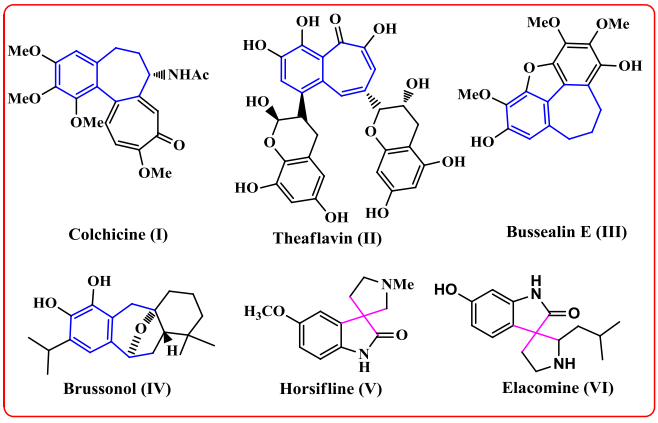


Fig. 1. Representative natural products bearing seven-membered ring fused to aromatic ring showed anti-cancer activity (I-IV) and drugs containing a spiro-oxindole moiety (V&VI).

2. Materials and methods

2.1. Biological evaluation

2.1.1. Chemicals

Dulbecco's modified eagle medium (DMEM), MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide], trypsin-EDTA, antibiotic antimycotic solution, phosphate buffered saline (Ca^{2+}, Mg^{2+} free; PBS), dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich Chemicals Co. (St. Louis, MO, USA), Foetal bovine serum (FBS) was purchased from Gibco, USA. Cell culture 96 well plates and plastic ware were obtained from Techno Plastic Products (TPP) (CH-8219, Trasadingen, Switzerland). All other chemicals were obtained locally and were of analytical grade.

2.1.2. Methods: cell culture

A549 derived from human lung adenocarcinoma cells (ATCC No. CCL-185), HeLa derived from human cervical cancer cells (ATCC No. CCL-2), SK-N-SH derived from human neuroblastoma cells (ATCC No. HTB-11), HepG2 derived from hepatocellular carcinoma cells (ATCC No. HB-8065) and MCF7 derived from mammary gland adenocarcinoma cells (ATCC No. HTB-22) were obtained from American Type Culture Collection (ATCC) (Manassas, VA, USA). The cell lines were grown in DMEM culture medium supplemented with 10% FBS, 0.3% sodium bicarbonate, 10 m$props_value{liter-Pattern}/L antibiotic antimycotic solution (10,000 U/mL penicillin, 10 mg/L streptomycin and 25 μg/mL amphotericin B), culture was maintained in CO_2 incubator at 37 °C with a 90% humidified atmosphere and 5% CO_2.

2.1.3. Preparation of samples for MTT assay

Test compounds of 1–18 were taken in 10 mg/mL of DMSO and various dilutions were made with sterile PBS (1 ×) to get desired concentrations. All formulations were filtered with 0.22 μm sterile filter and 20 min of UV eradication before adding to the 96 well plates containing cells.

2.1.4. Cytotoxic evaluation (MTT assay)

All synthesized compounds were screened for *in vitro* cytotoxic activity against a panel of human tumour cell lines. Cytotoxicity of formulations was assessed using MTT assay to determine the cell viability according to a method described by Hansen [36]. The assay is based on the reduction of MTT by the mitochondrial dehydrogenase of viable cells into purple formazan crystals which gets dissolved in DMSO and read at 570 nm. Briefly, 5×10^3 exponentially growing cells were seeded into each 96 well plate (counted by Trypan blue exclusion dye method) allowed to grow till 60–70% confluence then compounds (name of the compounds if applicable) were added to the culture medium with the final concentrations ranging from of 0.1, 1, 5 and 10 μM and along with controls (negative (without compound) and positive (Doxorubicin)) incubated in CO_2 incubator at 37 °C and 90% humidified atmosphere containing 5% CO_2 for 24 h. Then the media of the wells were replaced with 90 μL of fresh serum free media and 10 μL of MTT (5 mg/mL of PBS), plates were incubated at 37 °C for 2 h, there after the above media was discarded allow to dry for 30 min 100 μL of DMSO was added in each well at 37 °C for 5min. The purple formazan crystals were dissolved and immediately read absorbance at 570 nm was measured using Spectra Max plus 384 UV–Visible plate readers

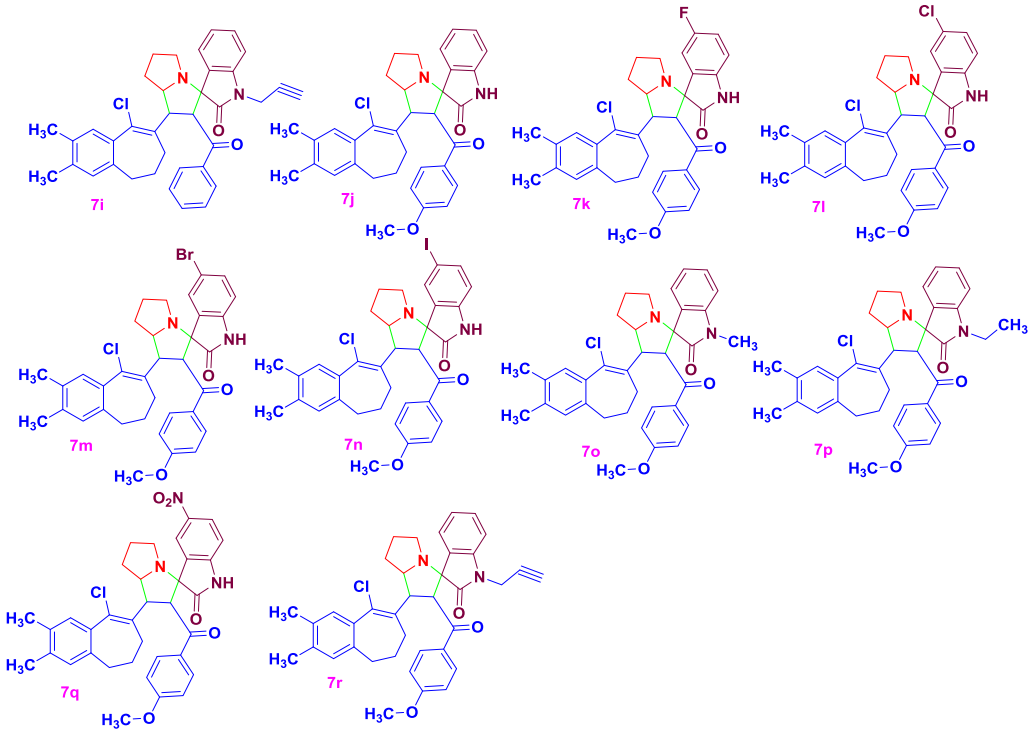


Fig. 2. Hexahydrospiro[indoline-pyrrolizin]-one hybrids 7a-r.

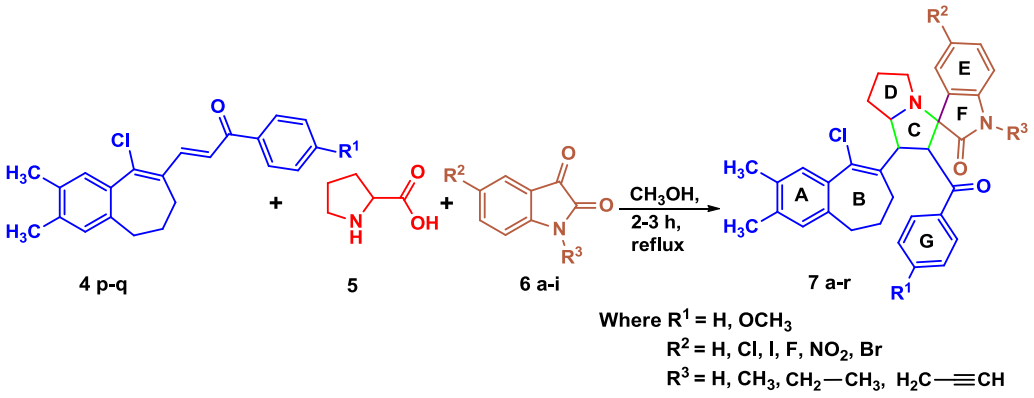


Fig. 3. Design strategy for novel hexahydrospiro[indoline-pyrrolizin]-one hybrids.

(Molecular Devices, Sunnyvale, CA, USA). IC−50 values were determined by probit analysis software package of MS-excel.% Cell viability (from control) versus concentration.

2.2. Molecular docking studies

To gain more insight into the interactions of novel synthesized compounds **7a-7r**, molecular docking studies were performed. NMR structure of double stranded DNA molecule (AGACGTCT) 2 complex in solution (PDB id: 1N37) was retrieved from protein data bank (www.rcsb.org). To identify their hypothetical binding mode, interactions of the docked DNA with ligands were analysed.

All the molecular docking studies were performed using Schrödinger Suite 2010 [37] on Linux operating system. Protein preparation wizard was applied for the preparation of the DNA by applying default parameters. Grid was generated around the active site by selecting the co-crystallized ligand. Receptor van der Waals scaling for the nonpolar atoms was kept at 0.9.Molecules were built using Maestro build panel and prepared by Lig Prep OPLS_2005 force field. GLIDE 5.6[38] was used for molecular docking. Low energy conformation of the ligands was selected and docked into the grid using standard precision (SP) and extra precision (XP) docking modes.

2.3. General information

All the solvents and reagents were purchased from commercial suppliers and were used without further purification. Melting points were measured with a Fischer-Johns melting point apparatus and were uncorrected. Nuclear Magnetic Resonance spectra were recorded on 300 (Bruker), 400 (Bruker) and 500 MHz (Varian) spectrometers in appropriate solvents using TMS as internal standard or the solvent signals as secondary standards and the chemical shifts are represented in δ scales. Multiplicities of NMR signals are

designated as s (singlet), d (doublet), t (triplet), br (broad), m (multiplet, for unresolved lines), etc. ^{13}C NMR spectra were recorded on 75, 100 and 125 MHz spectrometer. IR spectra were recorded on Perkin-Elmer model 683 or 1310 spectrometers with sodium chloride optics or KBr pellets with neat. ESI-MS were recorded on ThermoFinnigan LCQ ion trap mass spectrometer equipped with electron spray ionization. High-resolution mass spectra were obtained by using ESI-QTOF mass spectrometer. All the experiments were monitored by analytical thin layer chromatography (TLC) performed on silica gel G F_{254} pre-coated plates. After elution, the plate was visualized for the spots on TLC plates which was achieved either by exposure to UV (254 nm) light, iodine vapour and/or by dipping the plates in phosphomolybdic acid-ceric (IV) sulfate-sulfuric acid solution (PMA solution) and heating the plates at 110 °C. Appropriate names (if possible) for all the new compounds were given with the help of ChemBioOffice v12.0; 2012.

3. Results and discussion

3.1. Chemistry

One of the key intermediates (*E*)-3-(9-chloro-2,3-dimethyl-6,7-dihydro-5*H*-benzo[7]annulen-8-yl)-1-phenylprop-2-en-1-one (**4p-q**) achieved in excellent yields by the reaction of 9-chloro-2,3-dimethyl-6,7-dihydro-5*H*-benzo [7] annulene-8-carbaldehyde with acetophenones (**3p-q**) in the presence of 40% NaOH in ethanol at room temperature for 30 min. Subsequently, a convenient multicomponent reaction strategy was developed for the synthesis of hexahydrospiro[indoline-pyrrolizin]-one hybrids **7a-r** with various substituted isatins and L-proline at reflux temperature in methanol, with good to excellent yields (Scheme 1).

The structure of the final compounds **7a-r** was confirmed by ^{1}H NMR, ^{13}C NMR, ESI/LCMS, HRMS and IR. The Spectral data of all these synthesized compounds were in good agreement with proposed structures. Based on the ^{1}H NMR spectra, the characteristic multiplet, triplet and doublet signals for three C–H protons (new C–C bonds) appeared in the range of δ 3.75–4.85 ppm. The structures for all these compounds were further confirmed by HRMS analysis. For instance, **7a** displayed a molecular ion peak at *m/z*537.23,033 [M+H]+suggesting the molecular formula of $C_{34}H_{34}O_2N_2Cl$. Additionally, the ^{13}C NMR spectra for the target compounds **7a-r** exhibited characteristic peak at δ 72.21–73.93 ppm for spiro carbon.

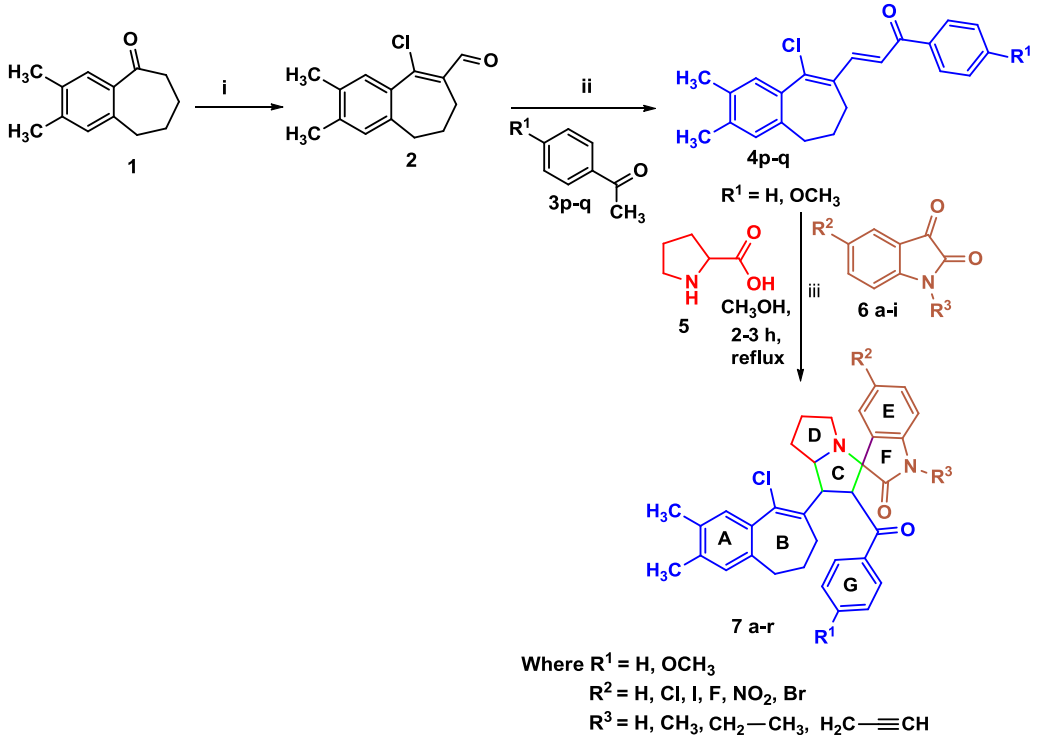
3.2. Chemical synthesis

3.2.1. Synthesis of (E)-3-(9-chloro-2,3-dimethyl-6,7-dihydro-5H-benzo[7]annulen-8-yl)-1-phenylprop-2-en-1-one *(4p-q)*

Acetophenones (**3p-q**) (1 equiv) and 9-chloro-2, 3-dimethyl-6, 7-dihydro-5*H*-benzo[7]annulene-8-carbaldehyde (1 equiv) are stirred in ethanol (20 mL) and to it 10 mL of 40% NaOH solution was added. The mixture was stirred for 30 min at room temperature. The contents were poured on crushed ice and acidified with 1 M HCl. The solid was filtered, dried and recrystallised from ethanol.

3.2.2. Synthesis of 2′-benzoyl-1'-(9-chloro-2,3-dimethyl-6,7-dihydro-5H-benzo[7]annulen-8-yl)-1′,2′,5′,6′,7′,7a'-hexahydrospiro [indoline-3,3′-pyrrolizin]-2-one (7a-r)

To a 25 mL round bottom flask, isatin**6a-i** (1 equiv), L-proline **5** (1.2 equiv) and (*E*)-3-(9-chloro-2,3-dimethyl-6,7-dihydro-5*H*-benzo[7]annulen-8-yl)-1-phenylprop-2-en-1-one **4p-q** (1 equiv) in methanol (10 mL) were added successively. The reaction mixture was stirred at reflux and monitored periodically by TLC.Upon completion (normally 2–3 h) of reaction, cooled to room temperature.The solid formed in the reaction mixture was filtered to obtain the pure product and recrystallized from ethanol to afford hexahydrospiro[indoline-pyrrolizin]-one hybrids(**7a-r**).



Scheme 1. Synthesis of (*E*)-3-(9-chloro-2,3-dimethyl-6,7-dihydro-5*H*-benzo[7]annulen-8-yl)-1-phenylprop-2-en-1-one derivatives 4p-q and novel series of hexahydrospiro [indoline-pyrrolizin]-one hybrids 7a-r.

*3.2.3. (E)-3-(9-chloro-2,3-dimethyl-6,7-dihydro-5H-benzo[7] annulen-8-yl)-1-phenylprop-2-en-1-one **(4p)***

Yield 90%; m.p 158–160 °C; ^{1}H NMR(400 MHz, $CDCl_3$): 2.15–2.22 (m, 2H, CH_2), 2.28 (s, 6H, $2CH_3$), 2.33 (d, J = 6.9, 7.0 Hz, 2H, CH_2), 2.59 (t, J = 7.0, 6.9 Hz, 2H, CH_2), 6.98 (s, 1H, Ar-H), 7.12 (d, J = 15.5 Hz, 1H, Ar-H), 7.37 (s, 1H, Ar-H), 7.47–7.53 (m, 2H, Ar-H), 7.54–7.60 (m, 1H, Ar-H), 7.95–8.00 (m, 2H, Ar-H), 8.23 (d, J = 15.5 Hz, 1H, Ar-H). ^{13}C NMR (100 MHz, $CDCl_3$):δ19.38, 19.67, 26.43, 31.33, 33.45, 123.10, 128.47, 128.56, 129.78, 130.01, 132.62, 133.61, 134.60, 136.36, 137.75, 138.37, 138.48, 141.76, 191.18.

*3.2.4. (E)-3-(9-chloro-2,3-dimethyl-6,7-dihydro-5H-benzo[7] annulen-8-yl)-1-(4-methoxyphenyl)prop-2-en-1-one **(4q)***

Yield 92%; m.p 172–174 °C; ^{1}H NMR(400 MHz, $CDCl_3$): δ2.16–2.21 (m, 2H, CH_2), 2.28 (s, 6H, $2CH_3$), 2.32 (t, J = 6.9, 7.0 Hz, 2H, CH_2), 2.58 (t, J = 7.09 Hz, 2H, CH_2), 3.88 (s, 3H, CH_3), 6.96–7.0 (m, 3H, Ar-H), 7.13 (d, J = 15.5 Hz, 1H, Ar-H), 7.37 (s, 1H, Ar-H), 7.97–8.02 (m, 2H, Ar-H), 8.22 (d, J = 15.4 Hz, 1H, Ar-H); ^{13}C NMR (100 MHz, $CDCl_3$):δ19.38, 19.65, 31.31, 31.86, 33.52, 55.47, 126.52, 128.73, 129.35, 129.75, 129.48, 130.78, 133.64, 134.12, 136.84, 137.72, 139.00, 140.21, 140.59, 140.88, 189.24.

*3.2.5. 2′-Benzoyl-1'-(9-chloro-2,3-dimethyl-6,7-dihydro-5Hbenzo [7]annulen-8-yl)-1′,2′,5′,6′,7′,7a'-hexahydrospiro[indoline- 3,3′-pyrrolizin]-2-one **(7a)***

Yield 89%; m.p 228–230 °C; IR (KBr) υ in cm^{-1}: 1015, 1715, 2815, 3448;^{1}H NMR(500 MHz, $CDCl_3$): δ 1.85–1.95 (m, 4H, $2CH_2$), 2.02–2.10 (m, 4H, $2CH_2$), 2.22 (s, 3H, CH_3), 2.25 (s, 3H, CH_3), 2.32–2.35 (m, 2H, CH_2), 2.60–2.65 (m, 2H, CH_2), 3.95–3.99 (m, 1H, CH), 4.49 (t, J = 10.0, 11.5 Hz, 1H, CH), 4.78 (d, J = 11.59 Hz, 1H, CH), 6.52 (d, J = 7.6 Hz, 1H, Ar-H, 6.87 (s, 1H, Ar-H), 7.02–7.06 (m, 1H, Ar-H), 7.12 (t, J = 7.6 Hz, 1H, Ar-H), 7.21 (t, J = 7.6, 7.9 Hz, 2H, Ar-H), 7.31–7.34 (m, 2H, Ar-H), 7.35–7.39 (m, 2H, Ar-H), 7.42 (d, J = 7.1 Hz, 1H, Ar-H), 7.7 (bs, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$):δ19.36, 19.47, 26.41, 27.13, 30.32, 30.94, 38.69, 48.30, 61.09, 68.13, 73.55, 110.04, 122.50, 123.07, 127.37, 127.78, 128.13, 128.44, 128.54, 128.76, 129.48, 129.75, 130.00, 130.84, 132.61, 132.72, 133.58, 134.06, 134.25, 134.77, 141.76, 167.72, 180.90, 196.99; ESI-MS: m/z = 537 [M+H] $^+$, HRMS cacld for $C_{34}H_{34}O_2N_2Cl$: 537.23,033, Found 537.23,003.

3.3. Pharmacology

The *in vitro* cytotoxic activity of the novel hexahydrospiro [indoline-pyrrolizin]-one hybrids was examined in five human cancer cell lines: A549 (ATCC No. CCL-185), SKNSH (ATCC No. HTB-11), HeLa (ATCC No. CCL-2), HepG2 (ATCC No. HB-8065) and MCF7 (ATCC No. HTB-22) using the MTT assay. Doxorubicin was included in the experiments as a positive control. Based on results, the synthesized compounds **7a-r** showed prominent to moderate inhibition on cancer cell growth with IC_{50} values ranging from **3.46** to

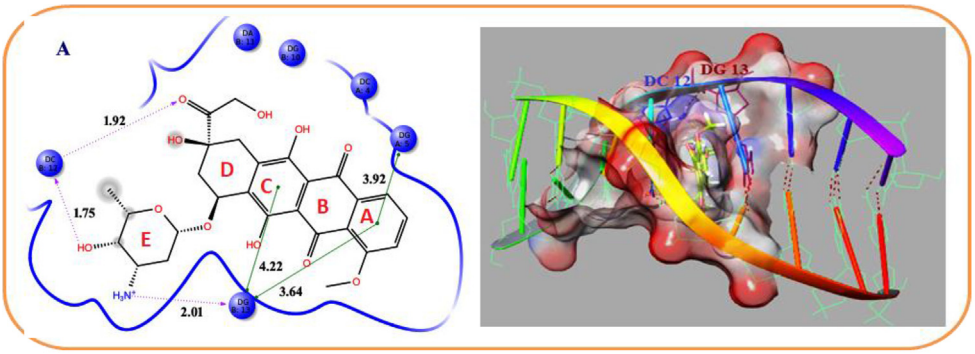
Table 2
SP dock scores of compounds.

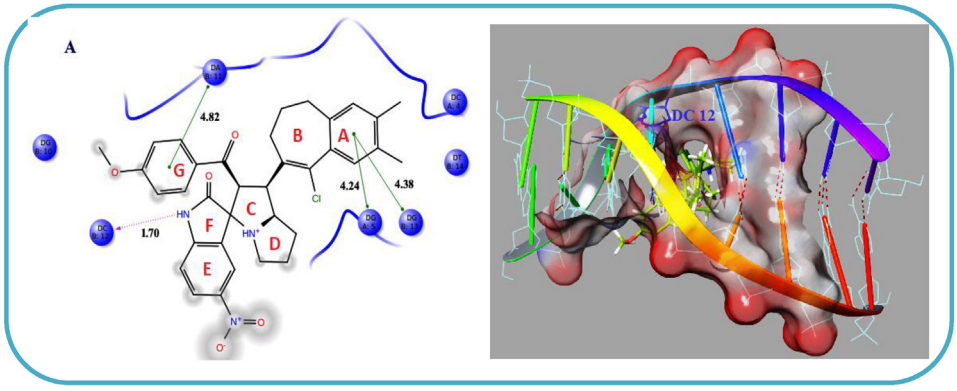
S No	Compound	SP dock score (kcal/mol)
1	7a	−3.517
2	7b	−5.208
3	7c	−5.566
4	7d	−2.471
5	7e	−1.663
6	7f	−5.784
7	7g	−5.960
8	7h	−5.925
9	7i	−4.313
10	7j	−3.276
11	7k	−4.489
12	7l	−5.812
13	7 m	−3.395
14	7n	−4.136
15	7°	−1.138
16	7p	−6.663
17	7q	6.160
18	7r	−5.925
19	Doxorubicin	−10.412

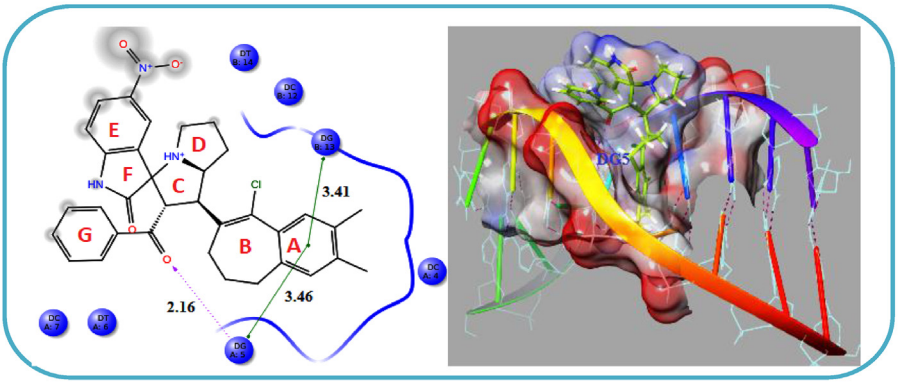
Table 1
IC_{50} (μM)[a] values of the tested compounds against five human cancer cell lines and dock scores.

Compounds	A549[b]		SKNSH[c]		HELA[d]		HEPG2[e]		MCF7[f]		DOCK SCORE (XP)
	IC_{50} (μM)	pIC_{50}	IC_{50} (μM)	pIC_{50}	IC_{50} (μM)	pIC_{50}	IC_{50} (μM)	pIC_{50}	IC_{50} (μM)	pIC_{50}	
7a	13.8 ± 2.4	4.860	13.4 ± 1.6	4.870	11.4 ± 2.6	4.940	9.1 ± 0.6	5.030	12.8 ± 0.8	4.890	−3.121
7b	42.8 ± 4.2	4.360	10.5 ± 0.2	4.970	4.7 ± 1.0	5.320	20.±1.2	4.690	14.2 ± 1.0	4.840	−4.985
7c	452.2 ± 23.6	3.340	9.4 ± 0.6	5.020	3.4 ± 0.8	5.460	45.4 ± 32	4.340	10.8 ± 0.4	4.960	−5.154
7d	47.8 ± 3.8	4.320	10.1 ± 1.2	4.990	80.1 ± 5.0	4.090	47.4 ± .4	4.320	37.7 ± 2.4	4.420	−2.561
7e	724.1 ± 32.4	3.140	20.1 ± 2.3	4.690	10.6 ± 1.2	4.970	16.7 ± .0	4.770	14.7 ± 1.6	4.830	−1.442
7f	658.6 ± 32.9	3.180	8.7 ± 0.7	5.050	5.7 ± 1.0	5.230	14.9 ± .2	4.820	15.8 ± 1.0	4.790	−5.443
7g	238.2 ± 16.4	3.620	7.7 ± 0.6	5.100	14.0 ± 1.9	4.850	18.7 ± 2.1	4.720	9.6 ± 0.7	5.010	−5.236
7h	5.6 ± 0.6	5.240	11.0 ± 0.9	4.950	3.8 ± 0.7	5.410	20.0 ± 2.8	4.690	7.3 ± 0.4	5.130	−6.324
7i	8.7 ± 1.2	5.050	19.5 ± 1.4	4.700	20.0 ± 3.6	4.690	7.5 ± 0.8	5.120	33.9 ± 3.1	4.460	−3.391
7j	28.9 ± 1.7	4.530	7.3 ± 0.3	5.130	10.8 ± 1.4	4.960	26.3 ± 3.1	4.570	29.0 ± 2.1	4.530	−3.839
7k	13.1 ± 1.4	4.880	33.0 ± 2.6	4.480	4.3 ± 0.8	5.360	25.1 ± 1.8	4.600	17.3 ± 1.2	4.760	−4.937
7l	16.8 ± 1.2	4.770	4.6 ± 0.6	5.330	3.6 ± 0.4	5.430	13.3 ± 0.7	4.870	7.8 ± 0.6	5.100	−5.786
7m	65.0 ± 3.6	4.180	9.7 ± 0.8	5.010	30.6 ± 2.6	4.510	22.2 ± 2.8	4.650	50.7 ± 2.4	4.290	−4.047
7n	15.9 ± 2.6	4.790	5.0 ± 0.4	5.290	3.8 ± 1.0	5.410	8.7 ± 0.9	5.050	8.3 ± 0.9	5.070	−5.746
7o	16.4 ± 2.8	4.780	11.8 ± 0.9	4.920	22.3 ± 2.4	4.650	9.2 ± 0.9	5.030	11.9 ± 1.4	4.920	−1.873
7p	9.4 ± 0.9	5.020	6.8 ± 0.9	5.160	6.0 ± 1.4	5.220	8.3 ± 0.6	5.080	8.2 ± 1.1	5.080	−6.387
7q	9.4 ± 1.0	5.020	9.9 ± 1.0	5.000	6.1 ± 1.4	5.210	20.5 ± 3.1	4.680	16.3 ± 1.0	4.780	−6.888
7r	6.9 ± 0.6	5.150	13.2 ± 1.2	4.870	6.2 ± 1.2	5.200	10.6 ± 0.9	4.970	13.3 ± 2.0	4.870	−5.825
Dox[g]	0.22 ± 0.03	6.650	6.3 ± 1.0	5.200	1.8 ± 0.0	5.740	5.4 ± 0.6	5.260	6.3 ± 0.3	5.190	−10.925

[a] Each data represents mean ± SD of at least three independent experiments.
[b] A549 derived from human lung adenocarcinoma cells (ATCC No. CCL-185).
[c] SK-N-SH derived from human neuroblastoma cells (ATCC No. HTB-11).
[d] HeLa derived from human cervical cancer cells (ATCC No. CCL-2).
[e] HepG2 derived from hepatocellular carcinoma cells (ATCC No. HB-8065).
[f] MCF7 derived from mammary gland adenocarcinoma cells (ATCC No. HTB-22).
[g] Doxorubicin was used as a positive control.







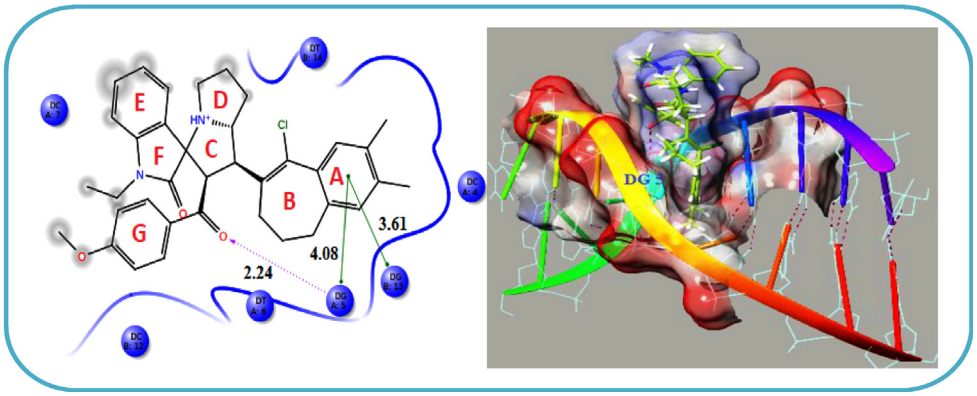


Fig. 4. a. Super imposition in DNA-intercalation site of Doxorubicin (Bond length in Å). b. Super imposition in DNA-intercalation site of 7q (Bond length in Å). c. Super imposition in DNA-intercalation site of 7h (Bond length in Å). d. Super imposition in DNA-intercalation site of 7p (Bond length in Å).

724.16 μM. Notably, the compounds **7h**, **7l**, **7n**, **7p**, **7q** and **7r**exhibited promising activity against all the cell lines. Particularly, compounds **7l** and **7n** showed the most potent activity against SKNSH with IC_{50} values of **4.61** and **5.04** μM. It was noteworthy that, modification on benzosuberone pharmacophore on ring E [NO_2, Cl and I], ring G [4-Methoxy] and *N*-alkyl on ring F was associated with a relevant increase in the inhibitory effect against all human cancer cell lines. These results and structural-activity relationship perspective, proved that the introduction of bulky group(s) on the ring E of benzosuberone and electron-donating group (methoxy) on the *para*-position of the phenyl group (ring G) may be favorable for the inhibitory activity against cells.

3.4. Docking study

To gain more insight into the binding modes and corresponding interaction energies, the newly synthesized molecules (**7a-r**) were docked into the active site of DNA. The list of molecules (**7a-r** & **doxorubicin**) along with their pIC_{50} values (pIC_{50} = - log IC_{50}) SP docking scores (Table. ST1) and XP docking scores have been tabulated in Table 1. Binding energies of the molecules ranged between −1.442 and −6.888 kcal mol^{-1} and doxorubicin was found to be −10.925 kcal mol^{-1}.(see Table 2)

Analysis of dock pose revealed that among all the tested compounds **7q, 7h, 7p** and **Doxorubicin** molecules have been sandwiched between the DNA base pairs. In this context, the stability of complexes was optimized by the hydrogen bond interactions and π−π interactions. Fig. 4a shows the interaction of doxorubicin with DNA. The molecule showed three hydrogen bond interactions; briefly, one with C═O group bonded at D ring, OH group at E ring of doxorubicin with DC12 nucleotide having a bond distances of 1.92 Å and 1.75 Å respectively, third H-bond between NH group of E ring with DG13 nucleotide, with a bond distance of 2.01 Å. Two π − π interactions were also seen between the π electron cloud of aromatic A & C rings with DG13 nucleotide with bond distances of 3.64 & 4.22 Å respectively.

Fig. 4b represents the interaction of molecule **7q** with DNA havingone hydrogen bond interaction between NH group of F ring with DC12 nucleotide with a bond distance of 1.70 Å. The molecule also has three π − π interactions between the π electron cloud of aromatic A ring with DG5 & DG13 nucleotide and π electron cloud of aromatic G ring with DA11 nucleotide with bond distances of4.24, 4.38& 4.82 Å respectively.

Fig. 4c demonstrates the interaction of molecule **7h** with DNA having one hydrogen bond interaction between C═O group of C ring with DG5 nucleotide with a bond distance of 2.16 Å. The molecule also hastwo π − π interactions between the π electron clouds of aromatic A ring with DG5 & DG13 nucleotide having bond distances of 3.46 & 3.41 Å respectively.

Analysis of these dock poses revealed that high potential of activity of a molecule can be explained in terms of H-bond interactions and π − π interactions with nucleotides present in DNA. We found that compound **Doxorubicin** (SP docking score = −10.412 kcal mol^{-1}, XP docking score = −10.925 kcal mol^{-1}) showed more hydrogen bond interactions than compounds**7q** (SP docking score = −6.160 kcal mol^{-1}, XP docking score = −6.888 kcal mol^{-1}) and **7p** (SP docking score = −6.663 kcal mol^{-1}, XP docking score = −6.387 kcal mol^{-1}) as well as **7h** (SP docking score = −5.925 kcal mol^{-1}, XP docking score = −6.324 kcal mol^{-1}). Compound **7q** showed more H-bond and π − π interactions than compounds **7p** and **7h.** Molecules **7p** and **7h** showed similar interactions (Fig. 4d illustrated the interaction of molecule **7p** with DNA). It obviously indicates that increasing H-bond acceptors &donor groups and increasing π electron cloud in the title compounds lead to enhance the activity and it is also corroborated by docking results.

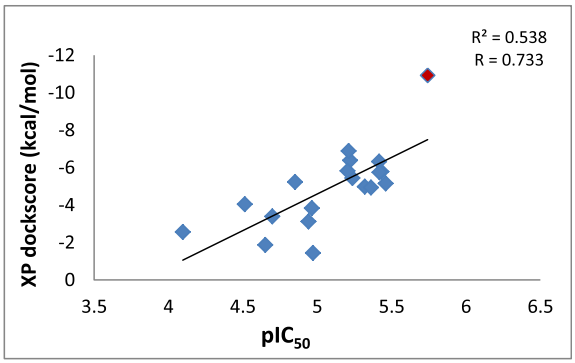


Fig. 5. Scatter plot of pIC_{50} versus XP dockscore. (Dark red and blue diamonds represent for doxorubicin and 7a-7r molecules respectively).

A regression analysis between XP dock score (binding affinity) and experimental pIC_{50} (HeLa) of the molecules were carried out. It gave a correlation coefficient values in acceptable range of 73% (r = 0.76 and r^2 = 0.53), which shows a significant correlation between DNA binding (dock score) and anti-proliferative activity. The scatter plot of pIC_{50} versus XP docks score shown in Fig. 5. Fig. 4c and d and 5 are depicted in supporting information.

4. Conclusion

In summary, we have synthesized and evaluated for their anti-proliferation of novel analogues of hexahydrospiro[indoline-pyrrolizin]-one hybrids in good to excellent yields. Further, docking studies were also performed on these derivatives **7a-r** to validate our wet-lab anti-proliferative studies. In general, the majority of target compounds displayed moderate to promising activity against the tested cancer cell lines. Particularly, compounds **7h**, **7l**, **7n**, **7p**, **7q** and **7r** exhibited relevant activity against all the cell lines with IC_{50} values ranging from **3.69** to **20.59** μM. In addition, the compounds **7l** and **7n** showed the most potent activity against SKNSH with IC_{50} values of **4.61** and **5.04** μM. Docking studies showed that the compound **7q** shows strong H-bond interactions with high binding energies, while compounds **7h**, **7l**, **7n**, **7p** and **7r** showed good H-bond interactions with moderate binding energies. Taken together, analogues bearing nitro, chloro, iodo substituents on ring E, ethyl and propargyl substituents on ring F and 4-methoxy substituent on the phenyl ring (G) of benzosuberon moiety showed potent activity. Insight of the above mentioned findings, compounds **7h**, **7l**, **7n**, **7p**, **7q** and **7r** could serve as a promising lead compounds for further optimization as anti-proliferative agents.

Conflicts of interest

The authors have no conflict of interest to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.molstruc.2018.11.072.

References

[1] T. Beghyn, R. Deprez-Poulain, N. Willand, B. Folleas, B. Deprez, Chem. Biol. Drug Des. 72 (2008) 3.
[2] J.W. Chen, Q.H. Wu, D.C. Rowley, A.M. Al-Kareef, H. Wang, J. Asian Nat. Prod. Res. 17 (2015) 199.
[3] J.E. John, Chem. Biol. Drug Des. 73 (2009) 367.
[4] L. Pan, H. Chai, A.D. Kinghorn, Phytochem. Lett. 3 (2010) 1.
[5] G.M. Cragg, D.J. Newman, K.M. Snader, J. Nat. Prod. 60 (1997) 52.
[6] B.B. Mishra, V.K. Tiwari, Eur. J. Med. Chem. 46 (2011) 4769.
[7] B.M.F. Goncalves, J.A.R. Salvador, S. Marin, M. Cascante, Eur. J. Med. Chem. 114 (2016) 101.
[8] V.I.S. Mendes, G.A. Bartholomeusz, M. Ayres, V. Gandhi, J.A.R. Salvador, Eur. J. Med. Chem. 123 (2016) 317.
[9] D. Rodriguez-Hernandeza, A.J. Demunera, L.C.A. Barbosaa, L. Hellerc, R. Csuk, Eur. J. Med. Chem. 115 (2016) 257.
[10] (a) D.J. Chaplin, S.A. Hills, Int. Radiat. Oncol. Biol. Phys. 54 (2002) 1491;
(b) G.F. Falasca, Clin. Dermatol. 24 (2006) 498;
(c) J.E. Koenig, T. Senge, E.P. Allhoff, W. Koenig, Prostate 58 (2004) 121.
[11] a) P. Vicini, M. Incerti, I.A. Doytchinova, P. La Colla, B. Busonera, R. Loddo, Eur. J. Med. Chem. 41 (2006) 624;
b) N. Terzioglu, A. Gursoy, Eur. J. Med. Chem. 38 (2003) 781;
c) A. Abadi, A.A.H. Eiss, G.S. Hassan, Chem. Pharm. Bull. 51 (2003) 838.
[12] C.S. Yang, J.D. Lambert, J. Ju, G. Lu, S. Sang, Toxicol. Appl. Pharmacol. 224 (2007) 265.
[13] E. Pan, L. Harinantenaina, P.J. Brodie, J.S. Miller, M.W. Callmander, S. Rakotonandrasana, E. Rakotobe, V.E. Rasamison, D.G.I. Kingston, J. Nat. Prod. 73 (2010) 1792.
[14] A. Yutaka, T. Yoshinao, F. Haruhiko, T. Koichi, A. Ritsuo, M. Takeshi, H. Shusuke, K. Teruo, Chem. Pharm. Bull. 54 (2006) 1602.
[15] K. Krzysztof, O. Jolanta, D. Malgorzata, Eur. J. Med. Chem. 43 (2008) 53.
[16] S.M. Chande, S.R. Verma, A.P. Barve, R.R. Khanwelkar, B.R. Vaidya, B.K. Ajaikumar, Eur. J. Med. Chem. 40 (2005) 1143.
[17] C. Young-Won, S. Angela, S. Bao-Ning, M. Qiuwen, C. Hee- Byung, R. Soedarsono, K. Leonardus, R. Agus, F. Norman, S. Steven, K. Douglas, J. Nat. Prod. 3 (2008) 390.
[18] S. Van der Sar, J. Blunt, M. Munro, Org. Lett. 8 (2006) 2059.
[19] R. Frank, M. Reich, R. Jostock, G. Bahrenberg, H. Schick, B. Henkel, H. Sonnenschein, US Pat. 20080269271 (App. USPTO: 514278), 2008.
[20] (a) J.W. Daly, T.W.F. Spande, N. Whittaker, R.J. Highet, D. Feigl, T. Nishimori, T. Tokuyama, C.W. Myers, J. Nat. Prod. 49 (1986) 265;
(b) S.T. Hilton, T.C.T. Ho, G. Pljevaljcic, K. Jones, Org. Lett. 2 (2000) 2639;
(c) V. Nair, S. Mathai, A. Augustine, S. Viji, K.V. Radhakrishnan, Synthesis 16 (2004) 2617;
(d) C. Marti, E.M. Carreira, Eur. J. Org Chem. (2003) 2209;
(e) M. Toyota, M. Ihara, Nat. Prod. Rep. 15 (1998) 327;
(f) A.B. Dounary, K. Hatanaka, J.J. Kodanko, M. Oestreich, L.A. Pfeifer, M.M. Weiss, J. Am. Chem. Soc. 12 (2003) 56261;
(g) J.F.M. Da Silva, S.J. Garden, A.C. Pinto, J. Braz. Chem. Soc. 12 (2001) 273;
(h) J.R. Liddel, Nat. Prod. Rep. 13 (1996) 187.
[21] H. Hejiao, G. Huijuan, L. Erwei, L. Xingzhong, Z. Yuguang, C. Yongsheng, F.I. Decaspirones, J. Nat. Prod. 69 (2006) 1672.
[22] S. Lindell, U. Sanft, M. T. Thonessen, Int. Pat. WO/*2001/ 011968* (App. PCT/EP2000/007851), 2001.
[23] W. Kreuder, N. Yu, J. Salbeck, Int. Pat. WO/*1999/040655* (App. PCT/EP1999/000441), 1999.d
[24] D. Lupo, J. Salbeck, H. Schenk, T. Stehlin, R. Stern, A. Wolf, *US Pat. 5840217* (App. USPTO: 08/417390), 2008.
[25] (a) B.M. Trost, C. Jiang, Synthesis (2006) 369;
(b) R.M. Williams, R. Cox, Acc. Chem. Res. 36 (2003) 127;
(c) V.C. Galliford, A.K. Scheidt, Angew. Chem. Int. Ed. 46 (2007) 8748;
(d) C. Marti, M.E. Carreira, Eur. J. Org Chem. (2003) 2209;
(e) H. Lin, J.S. Danishefsky, Angew. Chem. 42 (2003) 36.
[26] S.B. Singh, K. Tiwari, P.V. Kumar, M. Srivastava, K.P. Tiwari, J. Singh, Supramol. Chem. 25 (2013) 255.
[27] (a) M.M. Khafagy, A.H. El-Wahas, F.A. Eid-El, A.M. Agrody, Farmaco 57 (2002) 715;
(b) P.R. Sebahar, R.M. Williams, J. Am. Chem. Soc. 122 (2000) 5666.
[28] T.H. Kang, K. Matsumoto, Y. Murakami, H. Takayama, M. Kitajima, N. Aimi, H. Watanabe, Eur. J. Pharmacol. 444 (2002) 39.
[29] S. Edmondson, J.S. Danishefsky, L. Sepp Lorenzino, N. Rosen, J. Am. Chem. Soc. 121 (1999) 2147.
[30] a) B. Yadagiri, H. Uma Devi, B. Rajashaker, N. Lingaiah, G. Vijayacharan, P. Sowjanya, J. Nishanth, Bioorg. Med. Chem. 24 (2014) 5041;
b) B. Yadagiri, G. Srikanth, B. Rajashaker, N. Lingaiah, P. Sowjanya, G. Srujana, J. Nishant, Bioorg. Med. Chem. 25 (2015) 2220;
c) B. Yadagiri, H. Uma Devi, B. Rajashaker, N. Lingaiah, C. Ganesh Kumar, P. Sujitha, B. Sridhar, Eur. J. Med. Chem. 79 (2014) 260.
[31] M.F. Brana, M. Cacho, A. Gradillas, B. de Pascual-Teresa, A. Ramos, Curr. Pharmaceut. Des. 7 (2001) 1745.
[32] F. Gago, Methods 14 (1998) 277.
[33] J. Andersson, S. Li, P. Lincoln, J. Andreasson, J. Am. Chem. Soc. 130 (2008) 11836.
[34] (a) V. Lokshin, A. Samat, A.V. Metelitsa, Russ. Chem. Rev. 71 (2002) 89;
(b) H. Durr, H. Bouas-Laurent Elsevier, New York, U.S.A., 1990.
[35] M.S. Searle, A.J. Maynard, H.E. Williams, Org. Biomol. Chem. 7 (2003) 60.
[36] M.B. Hansen, S.E. Nielsen, K. Berg, J. Immunol. Methods 119 (1989) 203.
[37] Schrodinger Suite, Induced Fit Docking Protocol, Glide, version 5.6, LLC, New York, NY, 2011, 2011.
[38] R.A. Friesner, J.L. Banks, R.B. Murphy, T.A. Halgren, J.J. Klicic, D.T. Mainz, M.P. Repasky, E.H. Knoll, M. Shelley, J.K. Perry, D.E. Shaw, P. Francis, P.S. Shenkin, J. Med. Chem. 47 (2004) 1739–1749.