European Journal of Medicinal Chemistry 46 (2011) 2152–2156

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Chemoenzymatic synthesis with lipase catalyzed resolution and evaluation of antitumor activity of (*R*/*S*)-2-[2-hydroxy-3-(4-phenylpiperazin-1-*yl*)propyl]-1*H*-pyrrolo[3,4-*b*]quinolin-3(2*H*)-one

Lingaiah Nagarapu*, Hanmant K. Gaikwad, Rajashaker Bantu, Sheeba Rani Manikonda

Organic Chemistry Division II, Indian Institute of Chemical Technology (CSIR), Tarnaka, Hyderabad 500607, India

ARTICLE INFO

Article history:
Received 16 December 2010
Received in revised form
21 February 2011
Accepted 26 February 2011
Available online 5 March 2011

Keywords:
Lipase
Cytotoxicity
Neuroblastoma cell line (SK–N–SH)
Lung carcinoma A549 cell line

ABSTRACT

Abstract: Synthesis, characterization, resolution and evaluation of novel (*R*/*S*)-2-[2-hydroxy-3-(4-phenylpiperazin-1-*yl*)propyl]-1*H*-pyrrolo[3,4-*b*]quinolin-3(2*H*)-one derivatives are described. Enantiomerically pure compounds were isolated in good to excellent yield with high enantiomeric excess under mild reaction conditions by using *Candida antarctica* B (CAL-B) and *Candida rugosa* (CRL) Lipases. Newly synthesized and resolved compounds were screened for their antitumor activity against cancer cells such as human neuroblastoma SK–N–SH and human lung carcinoma A549 cell line *in vitro*. The results have shown that the compound **1** S-(−) alcohol was more effective in inhibiting the tumor cell growth.

1. Introduction

Cancer is a one of the deathliest disease in the medical field worldwide, the majority of cancer patients treated today will receive either preoperative or postoperative chemotherapy over a past few decades, and drug discovery has played a greater role in development of therapeutic agents [1–6]. Many antitumor compounds available in the market, some of in clinical trials which are natural as well as synthetic. The successes of the new agents and treatments have limitations related to their side effects and drug resistance [7]. Active new chiral therapeutic agents with fewer side effects are still needed [8]. The natural alkaloids such as Camptothecin, Luotonin A and their analogues contain pyrroloquinolinone ring and they possess cytotoxic activity [9,10]. According to Malawska et al. [11,12] and Williams [13] compound 1-[2-hydroxy-3-(4-phenyl-1-piperazinyl)-propyl]-pyrrolidin-2-one prevented adrenaline and barium chloride induced arrhythmia, so it could be considered as a class **Ia** antiarrhythmic drug [14]. In past few decades, progress in understanding the biochemical pharmacology of β-blockers has lead to a more rational approach in designing new drug combinations involving this hydroxyl propyl pyrroloquinolinone [15,16]. In our on-going research programme [17–19] on the to discover and develop tumor growth inhibitors and apoptosis inducers as potential new anticancer agents, we obtained a novel compound (*R*/*S*)-2-[2-hydroxy-3-(4-phenylpiperazin-1-*yl*)propyl]-1*H*-pyrrolo[3,4-*b*]quinolin-3(2*H*)-one (**1**) which has antitumor activity (Fig. 1).

Chiral drugs have been used as therapeutic agents in their racemic form in most of the cases. In industries, it is used to synthesize new chiral drugs eco-friendly synthetic routes and usages of biocatalysts are being implemented [20–23]. Immobilized enzymes are highly stable in organic solvents and we can reuse the enzymes which is always interesting in economical terms [9,24]. Herein we would like to report synthesis of antitumor therapeutic agents by utilizing chemoenzymatic approach for the enzymatic resolution of compound **1** in terms of enzyme, acyl donor and other parameters which have influence on biocatalytic process and screened their antitumor activity against human cancer cell lines *in vitro*.

2. Results and discussion

2.1. Chemistry

Some of the synthetic procedures available for the preparation of enantiopure compounds are Sharpless asymmetric dihydroxylation (AD), hydrolytic kinetic resolution (HKR) methods, enzyme (biocatalysts) catalyzed reactions but as far we know there are only

* Corresponding author. Tel.: +91 40 27191509/10/11; fax: +91 40 27193382.
E-mail address: lnagarapuiict@yahoo.com (L. Nagarapu).

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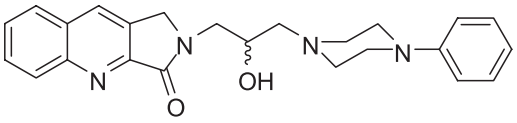
doi:10.1016/j.ejmech.2011.02.069



1 (±)

Fig. 1. Structure of 2-[2-hydroxy-3-(4-phenylpiperazin-1-*yl*)propyl]-1*H*-pyrrolo[3,4-*b*] quinolin-3(2*H*)-one.

few reports on the use of biocatalysts [25–27]. The lipase having high enantioselectivities towards a wide range of secondary alcohols and primary amines are used in the preparation of pharmaceutical formulations [28–30]. The enzyme catalysed reactions work at mild reaction conditions such as room temperature, atmospheric pressure etc.

The synthesis of racemic alcohol **(±)-1** was obtained in good yields from allyl amine. We have synthesized a starting material 2-allyl-1*H*-pyrrolo[3,4-*b*]quinolin-3(2*H*)-one (**7**) from the Michael addition reaction of allyl amine with ethyl acrylate in ethanol, followed by pyrrolidine ring formation with diethyl oxalate, sodium methoxide in diethyl ether at room temperature (87%, yield). Then hydrolysis followed by decarboxylation of compound (**5**) with aq. 10% HCl to gave *N*-allyl pyrrolidin-2,3-dione (**6**), which on Friedlander condensation with *o*-aminobenzaldehyde to gave compound (**7**) (63% yield, Scheme 1). The structure of this compound was confirmed from its spectral and analytical data. Based on $[M + H]^+$ 225 its molecular formula was established as $C_{12}H_{14}N_2O$. ^1H NMR spectrum (300 MHz) of compound (**7**) recorded in $CDCl_3$ exhibited signals arising due to typical *N*-allyl pyrroloquinolinone. The spectrum contained one doublet appeared at δ 4.3 for two proton, one singlet appeared at δ 4.44 for two proton, one doublet of doublet at δ 5.24–5.34 for two olefin proton, one multiplet appeared at δ 5.80–5.96 for one olefin proton and remaining splitting at δ 7.52–8.31 which were assigned to the five aromatic protons, respectively.

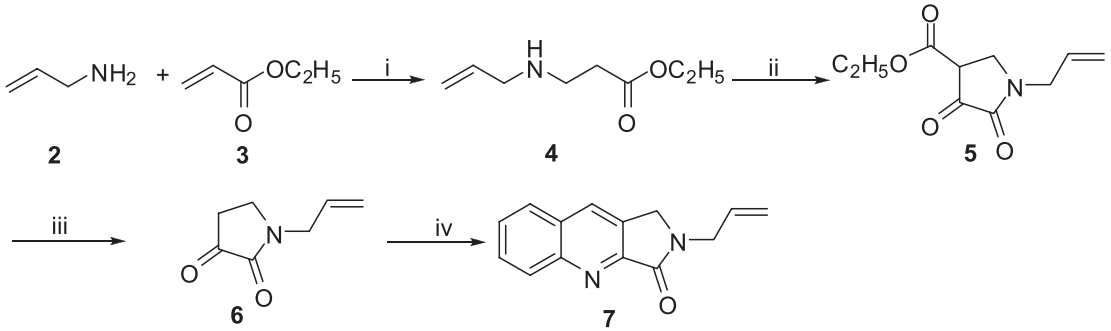
We have attempted to introduce stereo center by using Sharpless asymmetric dihydroxylation [26,27] (AD mix-β) on olefin compound (**7**). The oxidation of (**7**) with commercially available AD mix-β which is a mixture of potassium osmate $K_3[Fe(CN)_6]$ K_2CO_3 and $(DHQD)_2$-PHAL gave dihydroxy compound (**8**). The diol (**8**) obtained was easily converted to epoxide (**9**) using the process based on acetoxonium ion-mediated formation of acetate ester of halohydrin, subsequent base mediated ester saponification and cyclization yields epoxide. The aminolysis of compound (**9**) with 1-phenylpiperazine in methanol gave the racemic compound **(±)-1** in about 71% yield (Scheme 2). The structure of compound **1** was confirmed from their spectral and analytical data. Based on HRMS $[M + H]^+$ 403.2134 its molecular formula was established as $C_{24}H_{26}N_4O_2$. ^1H NMR spectrum (300 MHz, DMSO-d_6) revealed, signals arising due to typical secondary alcohol. The spectrum contained two doublet of doublet, one appeared at δ 3.51–3.64 for two proton, second appeared at δ 3.73–3.86 for two proton and one multiplet at δ 5.24–5.34 for one proton. The two multiplets appeared at δ 2.55–2.71 and δ 3.01–3.16 for piperazine protons and remaining splitting at δ 6.76–8.60 which were assigned to the 10 aromatic protons, respectively.

2.2. Enzymatic resolution

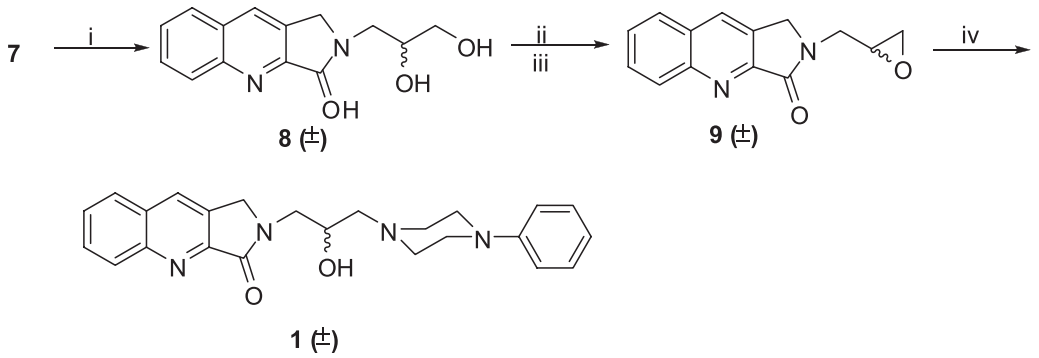
The enzymatic resolution of **(±)-1** was attempted using *Candida antarctica* lipase type B (CAL-B) and lipase from *Candida rugosa* (CRL) with use of vinyl acetate as a acyl donor at mild reaction condition (Scheme 3). The enzymatic acylation was performed in neat vinyl acetate because the poor solubility of **(±)-1** in MTBE (Methyl *tert*-Butyl Ether), Toluene and there is no conversion observed in Chloroform, 1,4-Dioxane, Acetonitrile, DMF (Dimethyl formamide) solvents. Controlling the reaction conditions is very important in enzymatic process, since it is necessary to adjust time and conversion. We have analyzed varying the reaction times to reach 50% conversion values at two different ratios of enzyme with substrate. Thus the chemoenzymatic resolution of (±)-**1** by CAL-B and CRL in 1:1 ratio led to **1** ***S***-(−) alcohol and **1** ***R***-(+) acetate with high enantiomeric excess (>98%), in good isolated yield (Table 1). The enzyme CAL-B shows high enantioselectivity towards resolution of (±)-**1** (Table 1, entry 1). The influence of the enzyme loading is analyzed by recovering the product as a single enantiomer. When the amount of enzyme with respect to substrate is decreased the purity of enantiomer also decreased. The enantiomeric excess of the compound was determined by HPLC using Chiralcel OD column [31]. The mobile phases were used for Chiralcel OD column is n-hexane/EtOH (85/10, v/v), n-hexane/EtOH/H_2O (85/10/0.2, v/v/v) and flow rate was 1.2 mL/min. The absolute configurations of the products obtained by the lipase-mediated resolution can be assigned [32] with the help of empirical Kazlauskas rule [33,34] to predict which enantiomer reacts faster during the resolution of secondary chiral alcohols.

2.3. Effects of the compounds on the viability of human cancer cells

The *in vitro* antitumor activity of these compounds was evaluated against SK–N–SH (human neuroblastoma cell line) and A549 (human lung carcinoma cell line) cells by the standard MTT assay method [35–37]. The potential of various human tumor cell growth inhibitors was determined as described [38]. The inhibitory action was expressed in micro molar concentrations of the compound which causes 50% inhibition per unit of enzyme (IC_{50}) under the assay conditions and Doxorubicin (DOX) is used as a reference drug, it is widely used to treat tumors. The data obtained from MTT assay



Scheme 1. Reagents and Conditions: i) Ethanol, rt, 48 h, 96%; ii) NaOMe, diethyl oxalate, Et_2O, rt, 12 h, 70%; ii) 10% HCl, reflux, 2 h, 61%; iv) *o*-aminobenzaldehyde, *p*-TSA, toluene, reflux, 12 h, 63%.



Scheme 2. Reagents and Conditions: i) AD Mix-β, *t*-BuOH/H_2O, 0 °C, 20 h, 75%; ii) $MeC(OMe)_3$, Me_3SiCl/CH_2Cl_2, 1 h; iii) K_2CO_3, CH_3OH, rt, 2 h, 72%; iv) 1-phenylpiperazine, MeOH, 50 °C, 12 h, 71%.

shows that most of the prepared compounds are exhibiting significant cytotoxicity against SK–N–SH and A549 cell lines. Antitumor potency of the compounds was indicated by % cell viability assay and IC_{50} values that were calculated by linear regression analysis. The potency of chiral compound **1*S*** was shown to be more effective inhibiting the cancer cell growth ($IC_{50} = 16.2$). The chiral compound showed significantly inhibited the SK–N–SH cell growth than the compound **1** and **1*R***. (Table 2)**.**

3. Conclusion

In summary, an efficient novel chemoenzymatic approach has been developed for the preparation of enantiopure compound **1**. The enantiopure ***R*** and ***S*** enantiomers have been achieved using CAL-B and CRL in vinyl acetate at mild reaction conditions. The chiral compounds screened in viability assay against human neuroblastoma (SK–N–SH) and lung carcinoma (A549) cells *in vitro*. The prepared compounds had shown significantly cytotoxicity activity against human cancer cell lines. Compound **1*S*** has shown potent antitumor against the SK–N–SH tumor cell line.

4. Experimental section

4.1. Chemistry

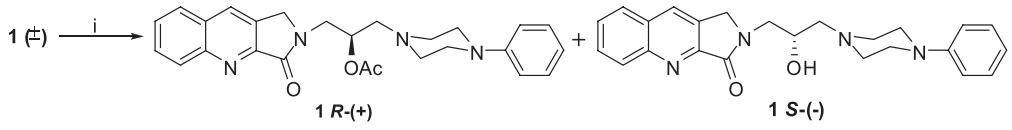
All commercial reagents and solvents were used as received without further purification unless specified and reaction solvents were used distilled. The reactions were monitored and Rf value were determined using analytical thin layer chromatography (TLC) with Merck Silica gel 60 and F_{254} precoated plates (0.25 mm thickness). Spot on the TLC plates were visualized using ultraviolet light (254 nm). Flash column chromatography was performed with Merck silica gel 60 (100–200 mesh). Melting points were determined in capillaries and are uncorrected. ^{1}H NMR spectra were recorded on Bruker DRX-300, Varian 400 and Varian-500 NMR spectrometers. ^{13}C NMR spectra were recorded on Bruker DRX-300. Proton chemical shifts are reported in ppm (δ) relative to internal tetramethylsilane (TMS, δ 0.00 or with the solvent reference relative to TMS employed as the internal standard (CDCl3, δ 7.26 ppm; DMSO-d_6 δ 2.54 ppm) and Multiplicities of NMR signals are designated as s (singlet), d (doublet), t (triplet), q (quartet), br (broad), m (multiplet, for unresolved lines), etc.Infrared (IR) spectra were recorded on a Perkin Elmer FT-IR 400 spectrometer; data are reported in wave numbers (cm^{-1}). Mass spectra were recorded on Agilent Technologies 1100 Series (Agilent Chemistation Software). High-resolution mass spectra (HRMS) were obtained by using ESI-QTOF mass spectrometry.

4.2. Synthesis of ethyl 3-(allylamino)propanoate (*4*)

To a stirred solution of freshly distilled ethyl acrylate (35 g, 0.35 mol) in absolute ethanol (200 mL), allyl amine (20 g, 0.350 mol) was added and allowed to stand at room temperature for 48 h. The solvent was concentrated under reduced pressure to obtain compound **4** (53 g, 96.45%) as yellow oil.. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.26 t, $J = 7.55$ Hz, 3H, CH_3), 2.18 (br, 1H, NH), 2.49 (t, $J = 6.79$ Hz, 2H, CH_2), 2.86 (t, $J = 6.79$ Hz, 2H, CH_2), 3.25 zHH(d, $J = 6.03$ Hz, 2H, CH_2), 4.13 (q, $J = 7.55$ Hz, 2H, CH_2), 5.06–5.23 (dd, $J = 15.86, 12.08$ Hz, 2H, $=CH_2$), 5.79–5.94 (m, 1H, =CH); EI-MS: $m/z = 157$ [M^+].

4.3. Synthesis of ethyl 1-allyl-4,5-dioxopyrrolidine-3-carboxylate (*5*)

To a stirred solution of freshly prepared sodium methoxide (17.2 g, 0.32 mol) in diethyl ether (300 mL), diethyl oxalate (43.2 mL, 0.32 mol) was added at room temperature, followed by compound **4** (50 g, 0.32 mol) was added and the resulting reaction mixture refluxed for 2.0 h. The solvent was removed under reduced pressure. The residual slurry obtained was diluted with warm water (300 mL) and acidified with 6N HCl. The reaction mixture was then cooled and allowed to stand till to complete the precipitation. The precipitate of compound **5** was filtered out and recrystallized form ethanol to obtained white needles. Yield 70%, mp 82 °C. IR (KBr, ν cm^{-1}): 2986, 2854, 1737, 1689, 1602, 1557, 1451, 1257, 1160, 1052, 932, 798, 763. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.35 (t, $J = 7.17$ Hz, 3H), 3.85 (s, 1H, CH), 3.94 (s, 2H, CH_2), 4.10 (d, $J = 6.04$ Hz, 2H, CH_2), 4.31 (q, $J = 7.17$ Hz, 2H, OCH_2), 5.18–5.28 (m, 2H, $=CH_2$), 5.71–5.86 (m, 1H, =CH); ESI-MS: $m/z = 212$ [$M + H$]$^+$



Scheme 3. Reagents and Conditions: i) Lipase (CAL-B/CRL), vinyl acetate, rt, 48 h.

Table 1
Enzymatic Resolution of **(±)-1** using Vinyl Acetate at 30 °C at 210 rpm.

Entry	Enzyme	Ratio[a]	Time (h)	ee_p (%)[b]	ee_S (%)[b]	c (%)[c]	E[d]
1	CAL-B	1:1	48	96(36)	>98(41)	50	>200
2	CRL	1:1	48	89	95	52	74
3	CAL-B	1:0.5	64	92	96	50	175
4	CRL	1:0.5	64	84	89	51	70

[a] Ratio substrate vs. enzyme in weight.
[b] Determined by HPLC. Isolated yield in parentheses.
[c] $c = ee_s/(ee_s+ee_p)$.
[d] $E = \ln[(1-c)(1-ee_s)]/\ln[(1-c)(1+ee_p)]$.

4.4. Synthesis of 1-allylpyrrolidine-2,3-dione (**6**)

A solution of compound **5** (25 g, 0.118 mol) in 10% HCl (530 mL) was refluxed for 2 h. The reaction mixture was cooled to room temperature and extracted with CH_2Cl_2 (3 × 300 mL). The combined organic phases was dried over sodium sulphate and concentrated under reduced pressure to afford **6** (10 g, 61%) as yellow oil. IR (KBr, ν cm^{-1}): 3012, 2896, 1692, 1586, 1500, 1445, 1249, 1163, 1018, 927, 798, 673. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.69 (t, J = 5.86 Hz, 2H, CH_2), 3.63 (t, J = 5.86 Hz, 2H, CH_2), 4.10 (d, J = 6.59 Hz, 2H, CH_2), 5.25–5.35 (m, 2H, = CH_2), 5.72–5.85 (m, 1H, = CH); EI-MS: m/z = 139 [M^+]

4.5. Synthesis of 2-allyl-1H-pyrrolo [3,4-b]quinolin-3(2H)-one (**7**)

To a stirred solution of compound **6** (9.5 g, 0.068 mol) in toluene (25 mL), o-aminobenzaldehyde (9.5 g, 0.068 mol) and *p*-toluenesulfonic acid (0.58 mg, 0.0034 mol) was added at room temperature. The reaction mixture was refluxed under Dean–Stark apparatus until no more water was collected and then reaction mixture was concentrated under reduced pressure. The residue obtained was triturated with diethyl ether and the precipitate of compound **7** was filtered out and purified by column chromatography over silica gel to obtained brownish solid. Yield 63%, mp 153–155 °C. IR (KBr, ν cm^{-1}): 3028, 2919, 1698, 1572, 1500, 1414, 1239, 1133, 1001, 925, 785, 628. ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.30 (d, J = 6.23 Hz, 2H, CH_2), 4.44 (s, 2H, CH_2), 5.24–34 (m, 2H, = CH_2), 5.80–5.96 (m, 1H, = CH), 7.52–7.60 (m, 1H, Ar–H), 7.69–7.70 (m, 2H, Ar–H), 8.07 (s, 1H, Ar–H), 8.31 (d, J = 8.30 Hz, 1H, Ar–H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 45.24, 46.52, 118.39, 127.36, 127.44, 127.91, 129.45(2), 129.94, 130.14, 131.78(2), 148.22, 150.64; ESI-MS: m/z = 225 [M + H]$^+$

Table 2
In vitro cytotoxicity activity of compounds on SK–N–SH and A549 cell line.[a]

Compounds	Concentration (μM)	% Cell viability assay		% IC_{50} (μM)	
		SK–N–SH	A549	SK–N–SH	A549
Control		100	100		
1	10	78.31 ± 1.89	96.52 ± 2.45	–	–
	25	73.53 ± 1.48	85.48 ± 2.27	–	–
	50	70.54 ± 2.68	78.71 ± 3.38	65.1	63.2
1R	10	95.51 ± 0.85	98.43 ± 1.83	–	–
	25	84.19 ± 2.68	85.61 ± 2.24	–	–
	50	79.35 ± 3.55	83.29 ± 4.13	67.8	72.7
1S	10	76.79 ± 3.43	90.66 ± 1.40	–	–
	25	69.10 ± 0.64	81.75 ± 2.34	62.5	–
	50	49.39 ± 1.88	74.66 ± 2.41	16.2	54.8
Doxorubicin[b]	0.1	72.50 ± 0.30	76.60 ± 1.05	–	–
	1	60.79 ± 0.72	65.33 ± 1.74	43	–
	5	50.97 ± 3.01	33.88 ± 6.37	19.9	–

[a] Exponentially growing cells were treated with different concentrations of test compounds for 48 h and cell growth inhibition was analyzed through MTT assay. A viability assay was carried out. Experiments were performed in triplicate; Data are expressed as means means ± SEM from three independent determinations of a representative experiment, the % cell viability of untreated cells [100%].
[b] Doxorubicin is a reference drug.

4.6. Synthesis of 2-(2,3-dihydroxypropyl)-1H-pyrrolo [3,4-b] quinolin-3(2H)-one (**8**)

To a solution of *tert*-butanol (100 mL) and water (100 mL), 20 g of AD mix β was added and stirred at room temperature to produce two clear phases. The reaction mixture cooled at 0 °C then compound **7** (5 g, 0.022 mol) was added at once and the heterogeneous slurry was stirred at 0–3 °C for 20 h (Progress of reaction was monitored by TLC). Solid Na_2SO_3 (20 g) was added to the reaction mixture at room temperature and stirred for 30 min. The reaction mixture was diluted with CH_2Cl_2, the organic phases was separated, dried over sodium sulphate and concentrated under reduced pressure. The residue obtained was purified by column chromatography over silica gel. Yield 75%, mp 197–199 °C. IR (KBr, ν cm^{-1}): 3413, 3345, 2919, 1690, 1579, 1459, 1405, 1214, 1041, 776, 628. ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.44–3.53 (m, 2H, CH_2), 3.72–3.85 (m, 2H, CH), 3.89–4.00 (m, 1H, CH), 4.22–4.36 (m, 1H. CH), 4.79 (s, 2H, CH_2), 7.64 (t, J = 7.55 Hz, 1H, Ar–H), 7.78 (t, J = 7.55 Hz, 1H, Ar–H), 7.95 (d, J = 7.93 Hz, 1H, Ar–H), 8.27 (d, J = 8.68 Hz, 1H, Ar–H), 8.33 (s, 1H, Ar–H); ESI-MS: m/z = 281 [M + Na]$^+$

4.7. Synthesis of 2-(oxiran-2-ylmethyl)-1H-pyrrolo [3,4-b]quinolin-3(2H)-one (**9**)

To a stirred solution of compound **8** (4.5 g, 0.017 mol) in CH_2Cl_2, trimethylsilyl chloride (2.2 mL, 0.017 mol) and trimethylorthoacetate (2.17 mL, 0.017 mol) was added at 0 °C. The reaction mixture was stirred for 1 h and then concentrated under reduced pressure. The crude product obtained was dissolved in dry methanol and K_2CO_3 (4.7 g, 0.034 mol) was added to the reaction mixture. The suspension was stirred for 2 h, then precipitate filtered out. The filtrate obtained was diluted with water, extracted with ethyl acetate and then aqueous phase was further extracted with ethyl acetate. The combined organic phases dried over sodium sulphate and concentrated under reduced pressure. The residue obtained was purified by column chromatography over silica gel. Yield 72%, mp 136–138 °C. IR (KBr, ν cm^{-1}): 2936, 1699, 1576, 1462, 1415, 1304, 1246, 1024, 913, 775, 633. ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.65–2.69 (dd, J = 3.02 and 2.64 Hz, 1H, CH), 2.79–2.84 (dd, J = 4.34 and 4.54 Hz, 1H, CH), 3.24–3.32 (m, 1H, CH), 3.57–3.66 (dd, J = 6.23 and 6.04 Hz, 1H, CH), 3.97–4.06 (dd, J = 3.39 and 3.40 Hz, 1H, CH), 4.74 (s, 2H, CH_2), 7.74 (t, J = 8.09 Hz, 1H, Ar–H), 7.87 (t, J = 8.21 Hz, 1H, Ar–H), 8.13 (d, J = 8.12 Hz, 1H, Ar–H), 8.21 (d, J = 8.49 Hz, 1H, Ar–H), 8.62 (s, 1H, Ar–H); ESI-MS: m/z = 241 [M + H]$^+$

4.8. Synthesis of 2-[2-hydroxy-3-(4-phenyl-piperazin-1-yl)-propyl]-1,2-dihydro- pyrrolo [3,4-b]quinolin-3-one (**1**)

To a solution of compound **9** (500 mg, 2.08 mmol) and 1-phenylpiperazine (338 mg, 2.08 mmol) in methanol was stirred at 50 °C for 12 h. Then the solvent was concentrated under reduced pressure and the crude product was cooled down. The residue obtained was crystallized from a mixture of *n*-hexane and ethyl acetate to afford compound (**±**)-**1.** Yield 71%, mp 188–190 °C. IR (KBr, ν cm^{-1}): 3395, 2937, 1689, 1597, 1501, 1383, 1150, 1116, 750, 687. ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.55–2.71 (m, 4H, 2 × CH_2), 3.01–3.16 (m, 4H, 2 × CH_2), 3.51–3.64 (dd, J = 8.12 and 6.98 Hz, 2H, CH_2), 3.73–3.86 (dd, J = 3.21 and 4.91 Hz 2H, CH_2), 4.01–4.17 (m, 1H, CH),

4.76 (s, 2H, CH_2), 4.96–5.12 (br, 1H, O–H), 6.76 (t, $J = 6.98$ Hz, 1H, Ar–H), 6.90 (d, $J = 8.12$ Hz, 2H, Ar–H), 7.11–7.25 (m, 2H, Ar–H), 7.72 (t, $J = 7.36$ Hz, 1H, Ar–H), 7.86 (t, $J = 7.26$ Hz, 1H, Ar–H), 7.11 (d, $J = 8.12$ Hz, 1H, Ar–H), 8.20 (d, $J = 8.49$ Hz, 1H, Ar–H), 8.60 (s, 1H, Ar–H); HRMS (ESI^+) calcd. for $C_{24}H_{26}N_4O_2$ $(M + H)^+$: 403.2168; found: 403.2134.

4.9. Typical procedure for the enzymatic resolution of compound (*1*)

To a suspension of racemic alcohol **16** (50 mg, 0.12 mmol) and CAL-B (50 mg) in vinyl acetate (2 mL) was added under nitrogen atmosphere. The reaction mixture was stirred at 30 °C and 210 rpm, taking regularly aliquots that were analyzed by HPLC until around 50% conversion was reached. Then the reaction was stopped, and the enzyme filtered with $CHCl_3$. The solvents was concentrated under reduced pressure and the residue obtained was purified by flash chromatography on silica gel to afford (*R*)-(+) **1** {36% isolated yield and >98% *ee*, $[\alpha]_D^{25} = +2.3$ (c 0.35, $CHCl_3$)} and (*S*)-(−) **1**{41% isolated yield and 94% *ee*, $[\alpha]_D^{25} = -2.5$ (c 0.31, $CHCl_3$)}.

4.10. In vitro antitumor activity evaluation by MTT Proliferation assay

The cancer cell lines (SK–N–SH, A549) were obtained from ATCC. The cytotoxic activity *in vitro* was measured using the MTT assay. The cells were plated in 24-well plates at a density 2.0×10^4 in 500 μL of medium per well of 24-well plate and treated with drugs in triplicates. The cells were incubated for 48 h at 37 °C under a 5% CO_2 atmosphere. The MTT [3-(4,5-dimethylthiazol-2-*yl*)-2,5-diphenyltetrazolium bromide] solution 100 μg/mL was added in RPMI-1640 media, after cells were treated with the drug for 48 h and cells were incubated for further 2–3 h at 37 °C. The purple formazan crystals were dissolved in 100 μL DMSO. After 10 min the optical density was measured at 540 nm on ELISA plate reader. The % cell viability calculated by comparing the absorbance of treated cell versus untreated cells. The standard drug doxorubicin was used for reference.

Acknowledgements

Authors thank the Director and Head, Organic Chemistry Division-II, IICT for their support. Hanmant K. Gaikwad thanks University Grant Commission (UGC), New Delhi for research fellowship. Authors also thank Dr. K. ShasiVardhan, Chemical Biology Division, IICT for their biological support.

Appendix. Supplementary data

Supplementary data related to this article can be found online at doi:10.1016/j.ejmech.2011.02.069.

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