Med Chem Res
DOI 10.1007/s00044-015-1497-3

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and antiproliferative properties of isoxazole analogs containing dibenzosuberane moiety

Manjunath Moger[1] · Ashok Pradhan[1] · Apoorva Singh[1] · Darshan Raj Chenna Govindaraju[2] · Rama Mohan Hindupur[1] · Hari N. Pati[1]

Received: 14 October 2015 / Accepted: 18 December 2015

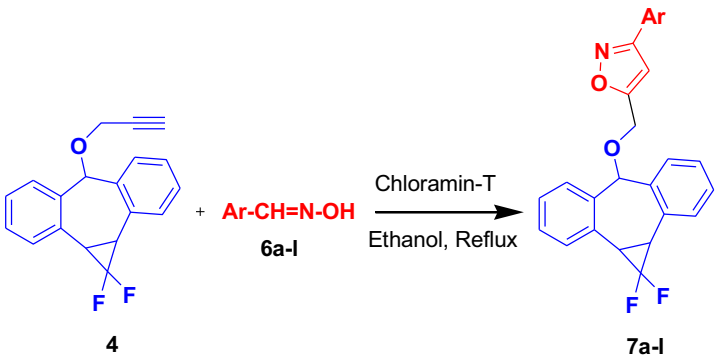
Abstract A series of twelve novel isoxazole analogs containing dibenzosuberane moiety were synthesized using convergent synthesis approach. Newly synthesized compounds were well characterized by mass spectroscopy, IR and NMR spectroscopy. All the compounds were screened for their antiproliferative property against HepG2 and HeLa cell lines. Among them, compounds **7a**, **7b**, **7c**, **7g** and **7h** were found active against both HepG2 and HeLa cell lines.

Graphical Abstract Twelve analogs of isoxazole containing dibenzosuberane moiety (**7a–l**) were synthesized, characterized and evaluated for their antiproliferative activity.

Keywords Dibenzosuberane · Isoxazole · Antidepressants · Antiproliferative activity

Introduction

Dibenzosuberones are class of compounds comprising of a tricyclic framework incorporating a cycloheptyl ring fused between two phenyl rings (Fig. 1a). Dibenzosuberones have been receiving large attention recently due to their multiple biological properties. For example, amitriptyline (Mao *et al*., 2011) (Fig. 1b) and nortriptyline (Wilens *et al*., 1993) (Fig. 1c) continue to be used as first-line agents for treating the depressive disorders and are also known for treating painful conditions like migraine headache, functional dyspepsia and irritable bowel syndrome (Verdu *et al*., 2008). Besides, dibenzosuberenone shares a structural resemblance with a key dibenzazepine intermediate, which has been exploited in synthesis of several compounds with antidepressant properties (Koeberle *et al*., 2012). In addition, a dibenzosuberane analog, skepinone-L



4 7a-l

Electronic supplementary material The online version of this article (doi:10.1007/s00044-015-1497-3) contains supplementary material, which is available to authorized users.

✉ Hari N. Pati
hari.pati@advinus.com

[1] Advinus Therapeutics Ltd., 21 & 22, Phase II, Peenya Industrial Area, Bangalore 560058, Karnataka, India

[2] Department of Chemistry, P A College of Engineering, Nadupadavu, Mangalore 574153, Karnataka, India

Published online: 07 January 2016

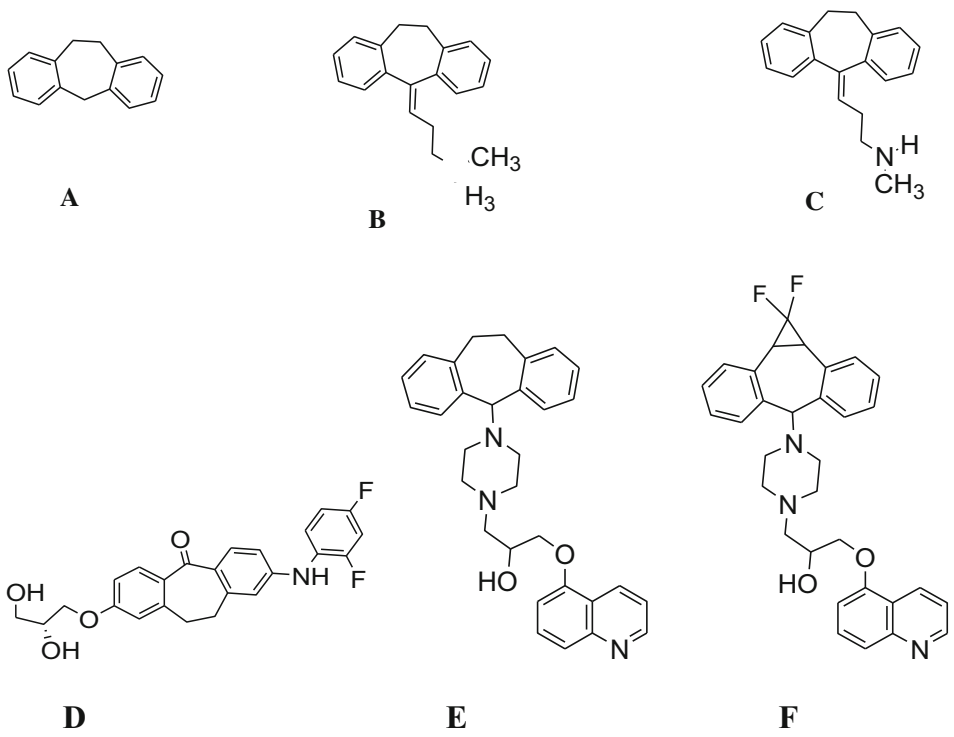


Fig. 1 Structures of dibenzosuberane (**a**), amitriptyline (**b**), nortriptyline (**c**), skepinone-L (**d**), MS-073 (**e**), LY335979 (**f**)

(Fig. 1d), is known as potent inhibitor of p38 mitogen-activated protein kinase (MAPK).

On the other hand, dibenzosuberones are identified for their ability to reverse the multi-drug resistance (MDR) by competitive inhibition of the antitumor agent binding to the P-glycoprotein (Klopman *et al*., 1997). In this regard, compound MS-073 (Fig. 1e) and an analog designed by annelation of difluorocyclopropyl moiety to the cycloheptyl ring in dibenzosuberone, i.e., LY335979 (Fig. 1f), are of potential interest in cancer chemotherapy (Pfister *et al*., 1995).

However, as per our knowledge there are no reports in the literature dealing with the exploration of dibenzosuberenone moiety or its derivatives for anticancer properties. In this study, we aimed to investigate dibenzosuberenone-based analogs for anticancer properties.

Structures of compounds in these new hybrid series molecules incorporated the skeleton derived from dibenzosuberone moiety and isoxazole, a key moiety present in antitumor drug acivicin (Poster *et al*., 1981).

In continuation of our work on dibenzosuberenone analogs (Moger *et al*., 2014), in this report we aimed to investigate a new series of hybrid molecules of dibenzosuberenone-based isoxazole analogs for anticancer properties. Results of the synthesis and antiproliferative properties of the synthesized compounds are discussed.

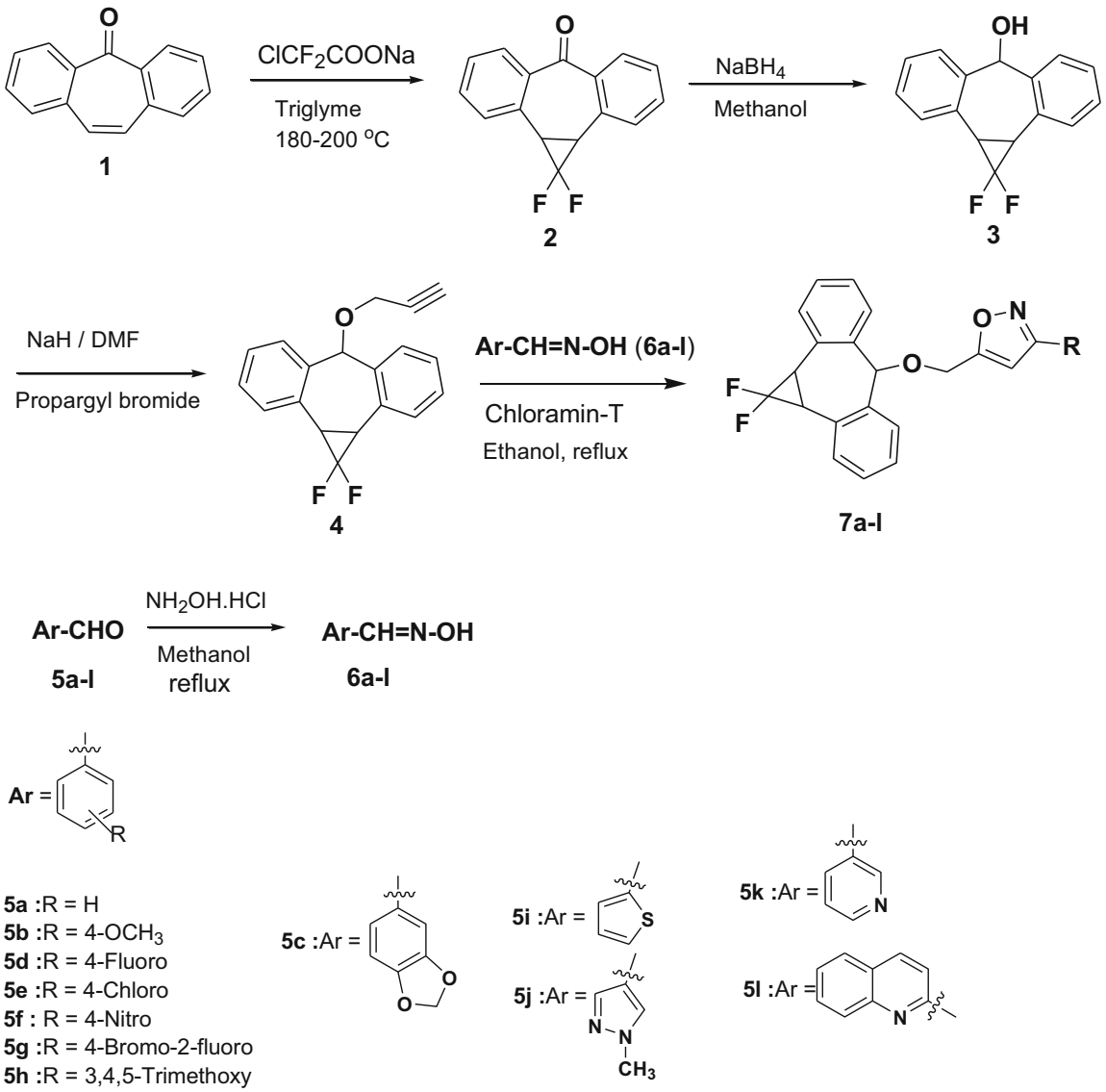
Results and discussion

Chemistry

Compounds **7a–l** were synthesized using convergent approach as depicted in Scheme 1. Commercially available dibenzosuberenone **1** was heated with chlorodifluoroacetate in triglyme at 180–200 °C to afford compound **2** in 80 % yield using a literature procedure (Astleford *et al.*, 2003). Compound **2** was reduced by using $NaBH_4$ in methanol to give compound **3** in 90 % yield. The intermediate **3** was alkylated with propargyl bromide in the presence of sodium hydride to give the key intermediate **4**. Then, the intermediate **4** was treated with oximes **6a–l** and chloramine-T in ethanol to yield the title compounds **7a–l** in 60–75 % yield.

The required oximes **6a–l** were prepared by heating the substituted aldehydes **5a–l** and $NH_2OH.HCl$ by using literature-reported procedure (Corniere *et al*., 2002).

All the synthesized compounds were well characterized by their ^1H NMR, ^{13}C NMR, IR and mass spectral analysis and screened for their antiproliferative activity against HepG2 and HeLa-ccl-13 cell lines, and the results are summarized in Table 1.



Scheme 1 Synthesis of isoxazole derivatives **7a–l**

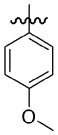
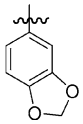
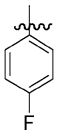
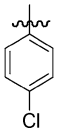
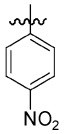
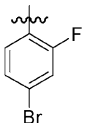
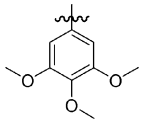
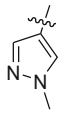
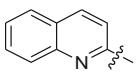
Antiproliferative activity

Compounds **7a–7l** were investigated for their antiproliferative activity against two cell lines, HepG2 and HeLa. In particular, antiproliferative activity of the compounds was determined by two different studies, namely percentage inhibition and IC_{50} using two cell lines HepG2 and HeLa, and the results are presented in Table 1. For determining percentage inhibition against HepG2 and HeLa cell lines, compounds **7a–7l** were used at 25 μg/mL concentration.

Results of percentage inhibition against HepG2 cell lines indicated that compound **7d** showed highest percentage inhibition value (89.13 %), followed by other compounds **7k** (88.17 %) >**7a** (87.87 %) >**7i** (85.43 %) >**7h** (85.28 %) in the decreasing order of their potency. On the other hand, the percentage inhibition values against HeLa cell line ranged between 60 and 89 %. Compound **7c** showed highest percentage of inhibition with 89.12 % followed by **7h** (79.12 %), **7g** (78.26 %), **7f** (76.87 %) and **7b** (76.26 %).

In addition, IC_{50} values of **7a–l** against HepG2 and HeLa cell lines were determined at 200 μg/mL concentration, wherein seven compounds showed IC_{50} values at below 100 μM concentration. Compound **7f** showed least IC_{50} value of 36 μM followed by **7b** (65 μM), **7c** (74 μM), **7j** (76 μM), **7d** (81 μM), **7k** (87 μM) and **7h**

Table 1 Antiproliferative activity of compounds **7a–l** (by MTT and trypan blue assay)

Entry	Substitution (R)	HepG2 (% inhibition)	IC_{50} (μM)	HeLa (% inhibition)	IC_{50} (μM)
7a		87.87 ± 1.65	132	74.87 ± 2.23	106
7b		83.43 ± 2.52	65	76.26 ± 6.10	98
7c		81.26 ± 3.11	74	89.12 ± 3.22	78
7d		89.13 ± 2.23	81	63.12 ± 2.46	132
7e		79.41 ± 3.18	109	60.45 ± 2.22	102
7f		76.57 ± 3.15	36	76.87 ± 7.23	85
7g		82.23 ± 2.46	125	78.26 ± 2.10	76
7h		85.28 ± 2.21	92	79.12 ± 5.22	87
7i		85.43 ± 3.58	120	63.12 ± 2.56	92
7j		76.83 ± 1.22	76	74.44 ± 4.67	115
7k		88.17 ± 2.41	87	72.45 ± 2.18	132
7l		84.22 ± 1.82	103	71.45 ± 1.62	113

(92 μM) in the order of decreasing activity. Likewise, six compounds showed IC_{50} values <100 μM against HeLa cell lines. Compound **7g** showed least IC_{50} with 76 μM concentration followed by **7c** (78 μM), **7f** (85 μM), **7h** (87 μM), **7i** (92 μM) and **7b** (98 μM) in the order of decreasing activity.

SAR study based on IC_{50} values against HepG2 cell lines revealed that substitution on phenyl ring connected to the isoxazole ring ('R' group) resulted in improvement of IC_{50} values of **7b**, **7d**, **7e** and **7f** as compared to compound **7a** (R=Ph). Compound **7f** (R = *p*-nitrophenyl) was found to be the best among all. Additionally, compounds **7i–7l** (R=heterocyclic substituents) were found to be more potent than the compound **7a** (R=Phenyl).

On the other hand, based on IC_{50} values against HeLa cell lines, substitution of phenyl ring connected to the isoxazole ring ('R' group) resulted in decreased activities of compounds **7b**, **7d**, **7e** and **7f** as compared to compound **7a** (R=Phenyl). However, replacing substituent in **7a** (R=Phenyl) with heterocyclic substituents as in **7j**, **7k** and **7l** resulted in decreased activities, in comparison with compound **7a**.

Conclusion

A series of twelve novel 1, 2-isoxazole analogs containing dibenzosuberane moiety (**7a–7l**) having various substituents on the phenyl ring attached to 1, 2-isoxazole ring and heterocyclic rings were first time synthesized and well characterized. All the analogs were investigated for antiproliferative activities. In general, all the compounds showed moderate to good activity against both the cell lines. Compounds **7a**, **7b**, **7c**, **7d**, **7g**, **7h**, **7i**, **7k** and **7l** showed >80 % inhibition against HepG2 cell line, while compounds **7b, 7c**, **7f** and **7g** showed >75 % inhibition against HeLa cell line. Compounds **7a**, **7b**, **7c**, **7g** and **7h** were exhibited good activity against both HepG2 and HeLa cell lines. Electron donating groups like methoxy (**7b**), trimethoxy (**7h**) and methylenedioxy (**7c**) on phenyl ring attached to the isoxazole ring contribute significantly for enhanced inhibition against both the cell lines.

Experimental

Materials and methods

All chemicals used for the synthesis were of reagent grade and procured from Sigma-Aldrich, Bangalore, India. ^{1}H and ^{13}C NMR spectra were recorded on AS 400 MHz Varian NMR spectrometer using TMS as an internal standard. IR spectra were recorded by using PerkinElmer Spectrum 100 Series FT-IR spectrometer. Mass spectra were recorded on Agilent 1200 Series LC/MSD VL system. Melting points were determined by using Buchi melting point B-545 instrument and are uncorrected. All the reactions were monitored by thin-layer chromatography (TLC) using pre-coated silica 60 F254, 0.25-mm aluminum plates (Merck). The crude compounds were purified by column chromatography using silica gel (100–200 mesh) and gradient (0–50 %) ethyl acetate in hexane as the eluent system.

Synthesis of 1,1-Difluoro-1,1 a, 6, 10b-tetrahydro- (1 a, 6, 10b)- dibenzo [a,e] cyclopropa[c] cyclohepten-6-yloxypropyne (**4**) To the stirred solution of **3** (2.0 g, 7.75 mmol) in DMF (20 mL) was added sodium hydride (279 mg, 11.62 mmol) at 0 °C and stirred for 15 min. Propargyl bromide solution (80 % in toluene, 1.7 mL, 11.62 mmol) was added dropwise and stirred for 30 min. Completion of the reaction was monitored by TLC. The reaction mass was poured on crushed ice (100 g); precipitated solid was filtered, washed with water (10 mL) and dried at vacuum to yield the title compound **4** as off-white solid.

Yield: 2.0 g; ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.53–3.56 (m, 3H), 4.41 (s, 2H), 6.37 (s, 1H), 7.21–7.27 (m, 6H), 7.36 (d, $J = 6.4$ Hz, 2H).

General procedure for the synthesis of isoxazole derivatives 7a–l

To a stirred solution of compound **4** (200 mg, 1.02 mmol) in ethanol (10 mL) was added oxime derivative **6a-j** (1.02 mmol, 1.0 eq) followed by chloramine–T (575 mg, 2.04 mmol), and the resultant mixture was heated to reflux for 16 h. Completion of the reaction was monitored by TLC. The reaction mass was cooled to room temperature and evaporated the solvent under reduced pressure; the residue was stirred with ice-cold water (10 mL); and the precipitated solid was filtered and dried at vacuum. The crude product was purified by column chromatography [silica gel (100–200 mesh), 0–50 % ethyl acetate in hexane as eluent].

1,1-Difluoro-1,1 a, 6, 10b-tetrahydro- (1 a, 6, 10b)-dibenzo [a,e] cyclopropa[c] cyclohepten-6-yloxymethyl-3-phenyl-isoxazole (**7a**) Yield 62 %; purity by HPLC: 97.4 %; white solid; m.p. 203–205 °C; IR (KBr) ν_{max}/cm^{-1}: 3009, 1617, 1459, 1168, 751; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.53 (d, $J = 13.2$ Hz, 2H), 4.93 (s, 2H), 6.47 (s, 1H), 7.20–7.29 (m, 7H), 7.46–7.55 (m, 5H), 7.90–7.93 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 27.3, 62.6, 77.3, 102.4, 122.6, 126.8, 127.1, 127.8, 128.3, 128.8, 129.6, 130.7, 131.8, 142.8, 162.4, 169.9; MS (ESI) *m/z*: 416 $[M + H]^+$.

1,1-Difluoro-1, 1a,6, 10b-tetrahydro-(1a,6, 10b)-dibenzo [a,e]cyclopropa[c] cyclohepten-6-yloxymethyl-3-(4-methoxylphenyl)-isoxazole (**7b**) Yield 60 %; purity by HPLC: 97.3 %; white solid; m.p. 206–209 °C; IR (KBr) ν_{max}/cm^{-1}: 3010, 1611, 1460, 1169, 750; ^{1}H NMR (400 MHz,

$CDCl_3$): δ 3.53 (d, $J = 13.6$ Hz, 2H), 3.81 (s, 3H), 4.91 (s, 2H), 6.46 (s, 1H), 7.07 (d, $J = 8.8$ Hz, 2H), 7.20–7.28 (m, 7H), 7.46 (d, $J = 6.8$ Hz, 2H), 7.84 (d, $J = 8.8$ Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 27.4, 55.7, 62.6, 77.3, 102.2, 114.9, 121.2, 122.6, 126.8, 127.8, 128.3, 128.6, 131.8, 142.8, 161.2, 161.9, 169.5; MS (ESI) *m/z*: 446.1 $[M + H]^+$.

1,1-Difluoro-1,1 a, 6, 10b-tetrahydro- (1 a, 6, 10b)-dibenzo [a,e] cyclopropa[c] cyclohepten-6-yloxymethyl-3-(benzo–[1,3]dioxol-5yl)-isoxazole (**7c**) Yield 50 %; purity by HPLC: 99.2 %; off-white solid; m.p. 219–221 °C; IR (KBr) v_{max}/cm^{-1}: 3008, 1616, 1474, 1168, 749; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.22 (d, $J = 13.2$ Hz, 2H), 4.90 (s, 2H), 6.04 (s, 2H), 6.35 (s, 1H), 6.61(s, 1H), 6.88 (d, $J = 8.0$ Hz, 1H), 7.19–7.35 (m, 8H), 7.53 (d, $J = 6.8$ Hz, 2H); MS (ESI) *m/z*: 460.1 $[M + H]^+$.

1,1-Difluoro-1, 1a,6, 10b-tetrahydro-(1a,6, 10b)-dibenzo [a,e]cyclopropa[c] cyclohepten-6-yloxymethyl-3-(4-fluorophenyl)-isoxazole (**7d**) Yield 65 %; purity by HPLC: 99.8 %; white solid; m.p. 202–205 °C; IR (KBr) v_{max}/cm^{-1}: 3007, 1610, 1459, 1166, 748; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.22 (d, $J = 13.2$ Hz, 2H), 4.92 (s, 2H), 6.35 (s, 1H), 6.67 (s, 1H), 7.14–7.26 (m, 8H), 7.53 (d, $J = 7.6$ Hz, 2H), 7.81 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 27.3, 62.9, 77.3, 100.8, 115.9, 116.1, 122.4, 126.1, 127.5, 128.1, 128.7, 131.6, 141.9, 161.5, 162.6, 165.1, 169.7; MS (ESI) *m/z*: 434.1 $[M + H]^+$.

1,1-Difluoro-1, 1a,6, 10b-tetrahydro-(1a,6, 10b)-dibenzo [a,e]cyclopropa[c] cyclohepten-6-yloxymethyl-3-(4-chlorophenyl)-isoxazole (**7e**) Yield 60 %; purity by HPLC: 97.7 %; white solid; m.p. 235–238 °C; IR (KBr) v_{max}/cm^{-1}: 3007, 1610, 1459, 1166, 748; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.22 (d, $J = 13.2$ Hz, 2H), 4.92 (s, 2H), 6.35 (s, 1H), 6.67 (s, 1H), 7.19–7.28 (m, 6H), 7.44–7.46 (s, 2H), 7.53 (d, $J = 6.8$ Hz, 2H), 7.76–7.78 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 27.33, 63.0, 76.7, 100.9, 122.4, 126.1, 127.3, 127.8, 128.1, 128.2, 129.2, 131.7, 136.2, 141.9, 161.5, 169.9; MS (ESI) *m/z*: 450 $[M + H]^+$.

1,1-Difluoro-1, 1a,6, 10b-tetrahydro-(1a,6, 10b)-dibenzo [a,e]cyclopropa[c] cyclohepten-6-yloxymethyl-3-(4-nitrophenyl)-isoxazole (**7f**) Yield 55 %; purity by HPLC: 98.1 %; off-white solid; m.p. 210–213 °C; IR (KBr) v_{max}/cm^{-1}: 3009, 1600, 1524, 1165, 751; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.53 (d, $J = 13.2$ Hz, 2H), 4.97 (s, 2H), 6.48 (s, 1H), 7.23–7.30 (m, 6H), 7.45 (m, 3H), 8.20 (m, 2H), 8.37 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 27.2, 62.9, 77.3, 101.1, 122.3, 124.2, 126.1, 127.6, 127.7, 128.2, 131.7, 134.8, 141.7, 148.7, 160.7, 170.7; MS (ESI) *m/z*: 461 $[M + H]^+$.

1,1-Difluoro-1, 1a,6, 10b-tetrahydro-(1a,6, 10b)-dibenzo [a,e]cyclopropa[c] cyclohepten-6-yloxymethyl-3-(4-bromo-2-fluorophenyl)-isoxazole (**7g**) Yield 63 %; purity by HPLC: 99.0 %; off-white solid; m.p. 217–219 °C; IR (KBr) v_{max}/cm^{-1}: 3011, 1611, 1437, 1168, 750; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.22 (d, $J = 13.2$ Hz, 2H), 4.93 (s, 2H), 6.36 (s, 1H), 6.81 (d, $J = 4.0$ Hz, 1H), 7.19–7.28 (m, 6H), 7.38–7.43 (m, 2H), 7.53 (d, $J = 7.2$ Hz, 2H), 7.87 (t, $J = 8.8$ Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 27.3, 62.8, 79.4, 103.4, 103.5, 119.9, 120.2, 122.4, 126.1, 127.3, 128.1, 130.0, 131.5, 141.9, 157.1, 158.6, 161.1, 169.5; MS (ESI) *m/z*: 514 $[M + 2H]^+$.

1,1-Difluoro-1, 1a,6, 10b-tetrahydro-(1a,6, 10b)-dibenzo [a,e]cyclopropa[c] cyclohepten-6-yloxymethyl-3-(3,4,3-trimethoxyphenyl)-isoxazole (**7h**) Yield 72 %; purity by HPLC: 97.6 %; white solid; m.p. 190–193 °C; IR (KBr) v_{max}/cm^{-1}: 3009, 1585, 1463, 1131, 750; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.52 (d, $J = 13.2$ Hz, 2H), 3.72 (s, 3H), 3.87 (s, 6H), 4.92 (s, 2H), 6.46 (s, 1H), 7.19 (s, 2H), 7.24–7.28 (m, 6H), 7.32 (s, 1H), 7.46 (d, $J = 7.2$ Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 27.3, 56.5, 60.5, 62.6, 77.3, 102.7, 104.6, 122.6, 124.3, 126.8, 127.8,

1,1-Difluoro-1,1a,6,10b-tetrahydro-(1a,6,10b)-dibenzo[a,e] cyclopropa[c]cyclohepten-6-yloxymethyl-3-thiophene- isoxazole (**7i**) Yield 65 %; purity by HPLC: 97.0 %; white solid; m.p. 189–192 °C; IR (KBr) v_{max}/cm^{-1}: 3082, 1610, 1462, 1168, 751; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.23 (d, $J = 13.2$ Hz, 2H), 4.91 (s, 2H), 6.35 (s, 1H), 6.63 (s, 1H), 7.12 (m, 1H), 7.19–7.28 (m, 6H), 7.43 (m, 1H), 7.49 (m, 1H), 7.53 (d, $J = 8.0$ Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 29.6, 62.9, 77.1, 101.0, 122.4, 126.1, 127.5, 127.6, 127.7, 128.1, 130.5, 131.6, 141.9, 157.7, 169.5; MS (ESI) *m/z*: 422.1 $[M + H]^+$.

1,1-Difluoro-1, 1a,6, 10b-tetrahydro-(1a,6, 10b)-dibenzo [a,e]cyclopropa[c] cyclohepten-6-yloxymethyl-3-(1-methyl-1H-pyrazole)-isoxazole (**7j**) Yield 60 %; purity by HPLC: 97.2 %; off-white solid; m.p. 246–248 °C; IR (KBr) v_{max}/cm^{-1}: 3008, 1615, 1458, 1167, 749; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.22 (d, $J = 13.6$ Hz, 2H), 3.97 (s, 3H), 4.89 (s, 2H), 6.34 (s, 1H), 6.50 (s, 1H), 7.19–7.28 (m, 6H), 7.52 (d, $J = 7.2$ Hz, 2H), 7.82 (d, $J = 8.8$ Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 27.3, 39.2, 62.3, 77.3, 101.1, 121.3, 126.1, 127.5, 128.1, 128.8, 131.6, 138.6, 141.9, 154.9, 169.1; MS (ESI) *m/z*: 420.1 $[M + H]^+$.

1,1-Difluoro-1, 1a,6, 10b-tetrahydro-(1a,6, 10b)-dibenzo [a,e]cyclopropa[c] cyclohepten-6-yloxymethyl-3-pyridyl-isoxazole (**7k**) Yield 62 %; purity by HPLC: 99.3 %; off-white solid; m.p. 221–223 °C; IR (KBr) v_{max}/cm^{-1}: 3014, 1685, 1458, 1167, 750; ^{1}H NMR (400 MHz, $CDCl_3$): δ

3.23 (d, $J = 13.2$ Hz, 2H), 4.95 (s, 2H), 6.37 (s, 1H), 6.99 (s, 1H), 7.20–7.34 (m, 6H), 7.52 (m, 2H), 7.82 (m, 1H), 8.16 (m, 1H), 8.72 (m, 1H), 9.04 (m, 1H); MS (ESI) *m/z*: 417.1 $[M + H]^+$.

1,1-Difluoro-1, 1a,6, 10b-tetrahydro-(1a,6, 10b)-dibenzo [a,e]cyclopropa[c] cyclohepten-6-yloxymethyl-3-(quinoline-2yl)-isoxazole (**7l**) Yield 70 %; purity by HPLC: 95.3 %; off-white solid; m.p. 232–234 °C; IR (KBr) ν_{max}/cm^{-1}: 3011, 1602, 1458, 1167, 747; ^1H NMR (400 MHz, $CDCl_3$): δ 3.24 (d, $J = 13.2$ Hz, 2H), 4.98 (s, 2H), 6.39 (s, 1H), 7.19–7.28 (m, 6H), 7.57–7.62 (m, 3H), 7.75 (m, 1H), 7.87 (d, $J = 8.4$ Hz, 1H), 8.16–8.22 (m, 2H), 8.27 (d, $J = 8.8$ Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 27.3, 62.8, 77.3, 102.3, 119.1, 122.5, 126.1, 127.4, 127.5, 127.7, 128.2, 128.4, 129.7, 129.9, 131.6, 137.0, 142.0, 148.0, 148.4, 163.7, 169.6; MS (ESI) *m/z*: 467.1 $[M + H]^+$.

Antiproliferative assay

The synthesized compounds **7a–l** were screened for their antiproliferative activity against HepG2 and HeLa cell lines. Cells were treated with compounds **7a–l** at a concentration of 25 μg/mL added to 96-well plates in antibiotic-free RPMI medium containing 10 % fetal calf serum. Compound treatment lasted for 48 h in 5 % CO_2 atmosphere at 37 °C with high humidity. After 48 h, 50 μL of 1 mg/mL solution of MTT in RPMI-1640 medium was added to each well. The culture plates were gently shaken and incubated for four more hours. MTT was removed carefully, and DMSO (100 μL) was added and shaken well. The absorbance was measured at 570 nm in an automated plate reader, and the percentage of cell growth inhibition was determined.

Cell viability was determined before and after the treatment with compounds was determined by trypan blue assay (Cetin and Bullerman, 2005). Cells were seeded in six-well plates prior to the addition of compounds **7a–l**. The cells were incubated with different doses of compounds **7a–l** along with 1 % DMSO as the solvent control. Cultures were harvested and monitored for cell number by counting cell suspensions using a hemocytometer. The results are given in Table 1.

The number of cells per ml and the total number of cells were calculated using the following formula:

$$\% \text{ viability} = (\text{live cell count/total cell count}) \times 100.$$

Acknowledgments This work was carried out as part of Mr. Manjunath Moger's Ph.D. work. The authors are grateful to Advinus Therapeutics Ltd, Bangalore, India, for the support and encouragement to higher education. The authors also thank the Department of Chemistry, Mangalore University, India.

References

Astleford BA, Barnett CJ, Kobierski ME, Wilson TM (2003) Process for preparing a 10, 11-methanodibenzosuberane derivative U.S. Patent No. 6,570,016 B1

Cetin Y, Bullerman L (2005) Evaluation of reduced toxicity of zearalenone by extrusion processing as measured by the MTT Cell Proliferation Assay. J Agric Food Chem 53:6558–6563

Corniere AR, Dijols S, Perollier C, Lefevre- Groboill CD, Boucher JL, Attias R, Sari M-A, Stuehs D, Mansuy D (2002) N-aryl N'-hydroxyguanidines, a new class of no-donors after selective oxidation by nitric oxide synthases: structure–activity relationship. J Med Chem 45:944–954

Klopman G, Shi LM, Ramu A (1997) Quantitative structure-activity relationship of multidrug resistance reversal agents. Mol Pharm 52:323–334

Koeberle SC, Romir J, Fisher S, Koeberle A, Schattel V, Albrecht W, Grutter C, Werz O, Rauh D, Stehle T, Laufer SA (2012) Skepinone-L is a selective p38 mitogen-activated protein kinase inhibitor. Nat Chem Bio 8:141–143

Mao X, Hou T, Cao B, Wang W, Li Z, Chen S, Fei M, Hurren R, Gronda M, Wu D, Trudel S, Schimmer AD (2011) The tricyclic antidepressant amitriptyline inhibits D-cyclintransactivation and induces myeloma cell apoptosis by inhibiting histone deacetylases: in vitro and in silicoevidence. Mol Pharma Fast Forward 79:672–680

Moger M, Satam VS, Govindraju DRC, Paniraj AS, Gopinath VS, Hindupur RM, Pati HN (2014) Synthesis and antimicrobial properties of 1,3,4-oxadiazole analogs containing dibenzosuberane moiety. J Braz Chem Soc 25:104–111

Pfister JR, Makra F, Muehldorf AV, Nelson JT, Slate DL (1995) Methanodibenzosuberylpiperazines as potent multidrug resistance reversal agents. Bioorg Med Chem Lett 3:2473–2476

Poster DS, Bruno S, Penta J, Neil GL, McGovren JP (1981) Acivicin: an antitumor antibiotic. Cancer Clin Trials 4:327–330

Verdu B, Decostered I, Buclin T, Stiefel F, Berney A (2008) Antidepressants for the treatment of chronic pain. Drugs 68:2611–2632

Wilens TE, Biederman J, Geist DE, Steingard R, Spencer T (1993) Nortriptyline in the treatment of ADHD: a chart review of 58 cases. J Am Acad Child Adolesc Psych 32:343–349