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Original article:

SYNTHESIS OF NOVEL ANTIMICROBIAL ARYL HIMACHALENE DERIVATIVES FROM NATURALLY OCCURRING HIMACHALENES

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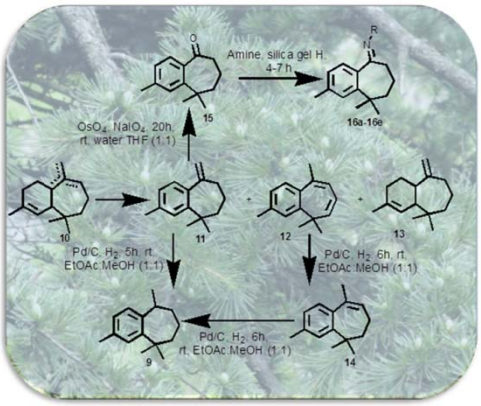
graphical ABSTRACT

Five new 2,9,9-trimethyl-6,7,8,9-tetrahydro-benzocyclohepten-5-ylidene-amine derivatives (**16a-16e**) were synthesized from *α*-dehydro-*ar*-himachalene (**11**) that was originally prepared from an isomeric mixture of *α*, *β* and *γ* himachalenes (**10**), the abundant sesquiterpenes of *Cedrus deodara* essential oil. In addition, different aryl himachalenes derivatives (**9**, **12, 14** and **15**) were also formed from **11**. The structures of the synthesized compounds were confirmed on the basis of their NMR, IR and mass spectral data. The prepared compounds were tested against a group of sixteen organisms including gram positive and gram negative bacterial and fungal strains. The introduction of a series of substituted imine groups in to aryl himachalenes at 5th position (**16a-16e**) enhanced antimicrobial activity as compared to the aromatized derivatives (**9**, **12, 14** and **15**) against gram-positive bacteria *Bacillus subtilis*, *Micrococcus luteus* and *Staphylococcus aureus*, and mycotoxigenic fungi *Aspergillus parasiticus*, *A. ochraceous* and *A. sydowii*.



Keywords: himachalenes, *Cedrus deodara*, essential oil, antimicrobial activity

INTRODUCTION

The essential oil of the Himalayan Cedar (*Cedrus deodara*) plays an important role of starting material in the fragrance and pharmaceutical industries (Hossini et al., 2011; Bhushan et al., 2006). This oil contains three major sesquiterpenes named *α-cis*-, *β*-, and *γ-cis*-himachalenes having hexahydrobenzocycloheptene as basic skeleton (Chaudhary et al., 2009). Benzocycloheptene and their derivatives are the biologically potent class of bicyclic framework and are attractive syn-

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thetic targets for organic and medicinal chemists (Chaudhary et al., 2012). These derivatives have been prepared by different routes, such as the enlargement of six-membered rings, cyclization (Lynch and Macdonald, 2009; Hattori and Tanaka, 2002) and coupling reactions or from benzocycloheptanone (Bohlmann et al., 2006) involving either oxime formation, reductive amination, through azide formation, α-bromination or cyanoboration (Nedelec et al., 1979, Nakano et al., 2010; Chow et al., 2009; Tandon et al., 2004). Nitrogen containing substituted benzocycloheptenes have been found to act as ORL-1 receptor agonists **1** (Figure 1) and β_3 adrenergic receptor agonists **2**, α-sympathomimetic, anorexigenic **3**, antidepressant **4**, analgesic and antiarrhythmic agents **5**. These were also reported for modulation of small-conductance calcium-activated potassium channels **6**, for treatment of psoriasis **7**, cardiovascular **8**, and neurodegenerative diseases (Chaudhary et al., 2012; Sorensen et al., 2008; Zaratin et al., 2004; Tandon et al., 2004).

The reactivity of himachalenes has been studied extensively since their isolation by Dev and co-workers and subjected to various chemical modifications such as cyclopropanation, oxidation, hydroxylation and epoxidation and total syntheses to improve their biological activities (Chaudhary et al., 2012; Daoubi et al., 2005; Hossini et al., 2011; Joseph and Dev, 1961). On reaction with various aromatizing agents, mixture of products was yielded (Shankaranarayan et al., 1977). Chemical analysis of the pheromone gland extract of sandfly and flea beetles revealed *ar*-himachalene **9**, himachalene and their methyl derivatives as sex pheromones (Figure 1) (Zilkowski et al., 2006). The insecticidal principles, himachalol and β-himachalene showed potency against pulse beetle and housefly (Singh and Agarwal, 1988). Hydroxylated derivatives of β-himachalene possess promising antifungal potential against phytopathogen *Botrytis cinerea* (Daoubi et al., 2005).

In continuation of our previous work (Chaudhary et al., 2012) to synthesize nitrogen containing benzocycloheptene analogues, herein, we have developed a simple and practical pathway for the synthesis of important similar benzocycloheptene analogues from α-dehydro-*ar*-himachalene (**11**) (Figure 2) and evaluated them for antimicrobial activities against opportunistic human bacterial and fungal pathogens.

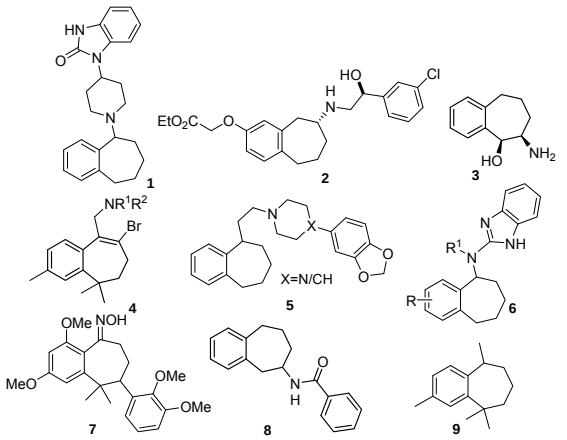

Figure 1: Structures of biologically potent benzocycloheptene analogues

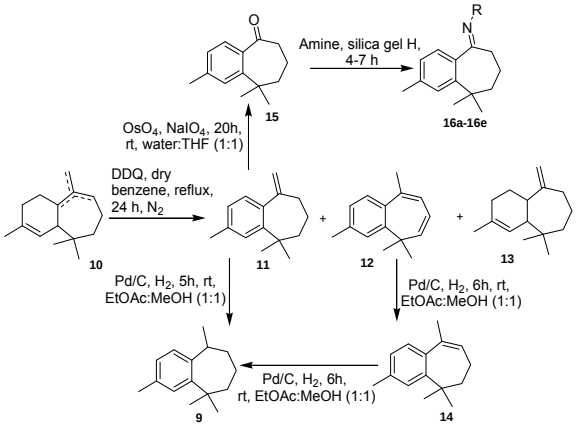

Figure 2: Synthesis of ar-himachalene derivatives from α-dehydro-ar-himachalene

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MATERIALS AND METHODS

General

All NMR spectra were recorded on a Bruker Avance-300 instrument (300 MHz ^{1}H, 75.46 MHz ^{13}C) using $CDCl_3$ and TMS as solvent and reference, respectively. Chemical shifts (δ) were given in parts per million. Silica gel (60–120 mesh) for column chromatography, TLC silica gel 60 F_{254} plates, silica gel (H) and all other chemicals and solvents used were purchased from Merck India Ltd. DMSO and ampicillin and nystatin was purchased from Sigma Aldrich Co., MO, USA and HiMedia Laboratories Pvt. Ltd., Mumbai, India respectively. GC-MS analysis was conducted on a GC-MS (QP2010) Shimadzu gas chromatograph mass spectrometer. A carbowax phase, BP-20 capillary column (30 m × 0.25 mm i.d. with film thickness 0.25 μm) was used with helium as a carrier gas at a flow rate of 1.1 ml/min on split mode (1:50). The injector temperature was programmed from 40-220 °C at 4 °C/min rise with 4 min hold at 40 °C and 15 min hold at 220 °C and interface temperatures were 250 °C. Ion source temperature was 200 °C. Sample (20 μl) was dissolved in 2 ml GC grade dichloromethane and sample injection volume was 2 μl. IR spectra were obtained on a Nicolet 5700 FTIR (Thermo, USA) spectrophotometer in the region 4000-400 cm^{-1} using KBr discs. Mass spectra were recorded on a Waters QTOF-MS with ESI using Waters Mass lynx 4.1 software.

*General procedure for synthesis of 5H-benzocycloheptene **11-13***

To a solution of himachalenes (**10**) (1 g, 4.902 mmol) in dry benzene (30 ml) was added DDQ (1.1 g, 9.804 mmol) and the mixture was stirred at reflux for 24 h under N_2. The solvent was removed under reduced pressure. The reaction was then quenched by adding 5 % sodium bicarbonate solution and extracted with ethyl acetate. Organic layer was finally concentrated and chromatographed on silica gel (heptane 100 %) to afford **11**, **12** and **13** as colorless oil.

*2,9,9-Trimethyl-5-methylene-6,7,8,9-tetrahydro-5H-benzocycloheptene **11***

The spectroscopic data of compound **11** was reported earlier (Chaudhary et al., 2012).

*3,5,5,9-Tetramethyl-5H-benzocycloheptene **12***

Colorless oil (yield 21 %). IR (KBr): λ_{max} = 2955, 2924, 2873, 1721, 1283, 774 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.59 (1H, d, J = 7.6 Hz), 7.26 (1H, s), 7.16 (1H, d, J = 7.5 Hz), 6.40-6.42 (1H, m), 6.00-6.05 (1H, m), 5.61 (1H, d, J = 10.2 Hz), 2.47 (6H, s), 1.41 (6H, s). ^{13}C NMR (75.4 MHz, $CDCl_3$) δ = 144.9, 139.5, 138.4, 138.1, 134.0, 126.8, 125.6, 125.1, 124.2, 123.8, 37.9, 29.3, 25.5, 21.0. GC–MS (70 eV): t_R = 35.704 min, *m/z* 200 [M^+, $C_{15}H_{18}$], 198, 183, 168, 153, 141, 128, 115, 83.

*3,5,5-Trimethyl-9-methylene-2,4a,5,6,7,8,9,9a-octahydro-1H-benzocycloheptene **13***

Colorless oil (yield 15 %). IR (KBr): λ_{max} = 3056, 1606, 1643, 1362, 720, 945 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ = 5.50 (1H, s), 4.79 (1H, s), 4.74 (1H, s), 2.83 (1H, m), 1.77-2.16 (7H, m), 1.69 (3H, s), 1.18-1.40 (4H, m), 1.02 (6H, s); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ = 157.9, 133.9, 123.8, 111.3, 47.9, 40.0, 36.7, 38.4, 36.6, 32.2, 28.3, 26.7, 25.2, 24.2. GC–MS (70 eV): t_R = 31.627 min, *m/z* 204 [M^+, $C_{15}H_{24}$], 189, 175, 161, 147, 134, 119, 105, 93, 79, 69, 55.

*2,5,9,9-Tetramethyl-6,7,8,9-tetrahydro-5H-benzocycloheptene **9***

To a solution of **11** (45 mg, 0.225 mmol) in 2 ml of ethyl acetate and methanol (1:1, v/v) was added 40 mg of 10 % palladium on activated carbon. The mixture was stirred for 5 h under hydrogen, the reaction mixture was filtered, and the filtrate was evaporated. The crude product was purified by silica gel column chromatography (heptane 100 %) to

give compound **9** as colorless oil (yield 80 %). IR (KBr): λ_{max} = 3043, 2876, 2853, 1566, 1292, 905 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ = 7.29 (1H, s), 7.22 (1H, d, J = 7.8 Hz), 7.08 (1H, d, J = 7.7 Hz), 3.34-3.39 (1H, m), 2.41 (3H, s), 1.85-1.92 (2H, m), 1.57-1.63 (2H, m), 1.52 (3H, s), 1.45 (2H, m) 1.43 (6H, s). ^{13}C NMR (75.4 MHz, $CDCl_3$) δ = 147.9, 141.4, 135.1, 128.4, 126.7, 125.6, 41.3, 39.7, 36.7, 34.7, 34.2, 24.3, 21.4, 21.2. GC–MS (70 eV): t_R = 34.401 min, *m/z* 202 [M^+, $C_{15}H_{22}$], 187, 159, 145, 131, 119, 105, 91, 77, 57.

3,5,5,9-Tetramethyl-6,7-dihydro-5H-benzocycloheptene ***14***

To a solution of **12** (46 mg, 0.232 mmol) in 2 ml of ethyl acetate and methanol (1:1, v/v) was added 40 mg, of 10 % palladium on activated carbon. The mixture was stirred for 6 h under hydrogen, the reaction mixture was filtered, and the filtrate was evaporated. The crude product was purified by silica gel column chromatography (heptane 100 %) to give compound **14** as colorless oil (yield 75 %). The compound **14** also yielded compound **9** by method as described above (yield 80 %). IR (KBr): λ_{max} = 3103, 2955, 2876, 1450, 1281, 872 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ = 7.19-7.29 (2H, m), 7.05-7.14 (1H, m), 5.94 (1H, m), 2.44 (3H, s), 2.40 (2H, m), 2.03 (3H, s), 1.84 (2H, m), 1.40 (6H, s). ^{13}C NMR (75.4 MHz, $CDCl_3$) δ = 146.6, 138.1, 137.3, 135.8, 127.7, 126.7, 126.5, 48.0, 38.2, 31.4, 26.3, 24.2, 21.6. GC–MS (70 eV): t_R = 34.085 min, *m/z* 200 [M^+, $C_{15}H_{20}$], 185, 171, 157, 143, 128, 115, 105, 91, 77.

2,9,9-Trimethyl-6,7,8,9-tetrahydro-benzocyclohepten-5-one ***15***

The compound **11** (95 mg, 0.475 mmol) and osmium tetroxide (1 mol %) in THF (0.5 ml) were added over a period of 30 min to a solution of sodium periodate (550.9 mg, 2.575 mmol) in water (0.5 ml). The mixture was stirred for further 20 h at room temperature. Extraction with ethyl acetate and diethyl ether followed by filtration through basic alumina and evaporation gave a yellow semisolid which on purification by silica gel column chromatography (hexane:EtOAc 97:3) gave **15** as light yellow semisolid (yield 73 %). IR (KBr): λ_{max} = 2967, 2925, 2850, 1730, 800 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ = 7.60-7.67 (1H, m), 7.52-7.56 (1H, m), 7.38-7.41 (1H, m), 3.06 (2H, m), 2.69 (3H, s), 2.45-2.52 (2H, m), 2.18-2.24 (2H, m), 1.66 (6H, s). ^{13}C NMR (75.4 MHz, $CDCl_3$) δ = 208.5, 147.0, 140.7, 138.0, 128.5, 126.7, 126.3, 42.5, 40.1, 38.5, 31.6, 23.3, 21.4. HR-ESI-MS: 203.3000 ([M+H]$^+$, $C_{14}H_{18}O^+$; calc. m/z 203.3001).

General procedure for the synthesis of compounds ***16a-16e***

A mixture of **15** (56 mg, 0.277 mmol) and benzylamine (32.6 mg, 0.305 mmol) was uniformly adsorbed on the surface of activated silica gel (0.5 g) by dropwise addition under stirring, and the mixture was then stirred at 60 °C for 6 h to allow complete formation of the corresponding imine. The reaction was monitored by TLC and the reaction mixture was extracted with ethyl acetate. The organic layer finally concentrated and then chromatographed on silica gel (hexane:EtOAc 90:10) to afford **16a**.

Benzyl-(2,9,9-trimethyl-6,7,8,9-tetrahydro-benzocyclohepten-5-ylidene)-amine ***16a***

Light brown semisolid (yield 74 %). IR (KBr): λ_{max} = 3009, 2838, 2828, 1669, 1629, 1369, 843 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ = 6.86-7.50 (8H, m), 4.37-4.63 (1H, m), 2.38 (3H, s), 1.77-1.84 (2H, m), 1.45 (2H, m), 1.30 (6H, s), 1.24 (2H, m). ^{13}C NMR (75.4 MHz, $CDCl_3$) δ = 176.8, 146.5, 141.0, 138.3, 135.8, 128.8, 128.2, 127.4, 127.2, 126.9, 126.7, 57.9, 41.8, 38.9, 32.2, 30.2, 25.4, 22.0. HR-ESI-MS: 292.4380 ([M+H]$^+$, $C_{21}H_{25}N^+$; calc. 292.4378).

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*Cyclohexyl-(2,9,9-trimethyl-6,7,8,9-tetrahydro-benzocyclohepten-5-ylidene)-amine **16b***

Prepared as described for **16a**; starting from **15** (50 mg, 0.248 mmol) and cyclohexyl amine (27 mg, 0.272 mmol) and after purification with silica gel column chromatography (hexane:EtOAc, 90:10) to afford **16b** as light brown semisolid (yield 78 %). IR (KBr): λ_{max} = 3177, 2992, 2813, 1708, 1633, 1197, 920 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ = 7.15 (1H, m), 7.01 (1H, m), 6.83 (1H, m), 2.57 (1H, m), 2.38 (3H, s), 2.05-2.07 (2H, m), 1.70-1.76 (4H, m), 1.58-1.65 (7H, m), 1.40-1.42 (3H, m), 1.28 (6H, s). ^{13}C NMR (75.4 MHz, $CDCl_3$) δ = 174.0, 140.0, 138.1, 136.0, 129.2, 127.4, 126.6, 51.0, 41.4, 38.9, 32.3, 32.1, 31.4, 30.1, 25.3, 23.0, 21.9. HR-ESI-MS: 284.4588 ($[M+H]^+$, $C_{20}H_{29}N$; calc. 284.4589).

*Isobutyl-(2,9,9-trimethyl-6,7,8,9-tetrahydro-benzocyclohepten-5-ylidene)-amine **16c***

Prepared as described for **16a**; starting from **15** (99 mg, 0.491 mmol) and isobutyl amine (39.5 mg, 0.539 mmol) and after purification with silica gel column chromatography (hexane:EtOAc 90:10) to afford **16c** as light brown semisolid (yield 71 %). IR (KBr): λ_{max} = 3044, 2872, 2990, 1523, 1657, 1284, 791 cm^{-1}. ^1H NMR(300 MHz, $CDCl_3$) δ = 7.03 (2H, m), 6.85 (1H, m), 2.38 (3H, s), 2.12 (1H, m), 1.92 (1H, m), 1.70(4H, m), 1.41 (2H, m), 1.27 (12H, s). ^{13}C NMR (75.4 MHz, $CDCl_3$) δ = 174.0, 145.8, 138.0, 136.1, 129.1, 127.3, 126.6, 61.8, 41.0, 38.9, 32.0, 31.7, 31.2, 30.3, 25.0, 21.8. IR. HR-ESI-MS: 258.4210 ($[M+H]^+$, $C_{21}H_{25}N^+$; calc. 258.4216).

*Methyl-(2,9,9-trimethyl-6,7,8,9-tetrahydro-benzocyclohepten-5-ylidene)-amine **16d***

Prepared as described for **16a**; starting from **15** (100 mg, 0.495 mmol), methyl amine (17 mg, 0.545 mmol) and after purification with silica gel column chromatography (hexane:EtOAc 95:05) to afford **16d** as light brown semisolid (yield 79 %). IR (KBr): λ_{max} = 3020, 2845, 2880, 1683, 1697, 1254, 870 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ = 7.06-7.20 (3H, m), 2.36 (3H, s), 2.12 (3H, s), 1.96 (2H, m), 1.72 (4H, m), 1.34 (6H, s). ^{13}C NMR (75.4 MHz, $CDCl_3$) δ = 174.3, 147.8, 136.7, 130.4, 128.8, 126.9, 126.6, 41.0, 38.4, 37.8, 31.6, 30.1, 26.5, 21.9. HR-ESI-MS: 216.3414 ($[M+H]^+$, $C_{15}H_{22}N^+$; calc. 216.3419).

*(2-Morpholin-4-yl-ethyl)-(2,9,9-trimethyl-6,7,8,9-tetrahydro-benzocyclohepten-5-ylidene)-amine **16e***

Prepared as described for **16a**; starting from **15** (106 mg, 0.525 mmol), methyl amine (75.2 mg, 0.578 mmol) and after purification with silica gel column chromatography (hexane:EtOAc 50:50) to afford **16e** as light brown semisolid (yield 65 %). IR (KBr): λ_{max} = 2817, 2791, 1650, 1629, 1486, 1397, 1107 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ = 6.90-7.27 (3H, m), 3.57 (6H, m), 2.65 (2H, m), 2.31 (4H, m), 2.23 (3H, s), 1.50-1.98 (6H, m), 1.20 (6H, s). ^{13}C NMR (75.4 MHz, $CDCl_3$) δ = 170.6, 147.1, 140.5, 136.8, 129.7, 127.3, 126.0, 66.7, 61.2, 53.5, 48.7, 40.8, 38.5, 36.2, 31.0, 25.9, 21.1. HR-ESI-MS: 315.4709 ($[M+H]^+$, $C_{20}H_{31}N_2O^+$; calc. 315.4729).

Antimicrobial assays

Antimicrobial activity was tested against a panel of sixteen organisms: gram-positive bacteria- *Bacillus subtilis* (MTCC 121), *Micrococcus luteus* (MTCC 2470), *Staphylococcus aureus* (MTCC 96), Gram-negative bacteria- *Burkholderia cepacea* (MTCC 438), *Escherichia coli* (MTCC 43), *Klebsiella pneumoniae* (MTCC 109), *Pseudomonas aeruginosa* (MTCC 424), *Enterobacter cloacae* (MTCC 509) and fungal strains *Candida albicans* (MTCC 3017), *Issatchenkia orientalis* (MTCC 231), *Aspergillus flavus* (MTCC 277), *A. niger* (MTCC 404), *A. parasiticus* (MTCC 2797), *A. sydowii* (MTCC 4335) and *A. ochraceous* (MTCC 4893), *Trichophyton rubrum* (MTCC 296) procured from the Microbial Type Culture Collection at the Institute of

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Microbial Technology, India. Broth microdilution method was used for the determination of minimum inhibitory concentration (MIC) and minimum microcidal concentration (MMC) in triplicates (Cos et al., 2006; Sharma et al., 2009). Test compounds were dissolved in DMSO to prepare the stock solutions. Two-fold dilution series of test compounds were prepared for the dose range 3000-23.4 µg/ml in sterilized Mueller-Hinton broth (MHB) for bacteria and Sabouraud dextrose broth (SDB) for fungi in 96-well microtiter plates. Freshly grown bacterial and fungal cultures adjusted to 1.0×10^5 cfu/ml with sterile normal saline were used to inoculate the plates. The uninoculated sterilized medium with and without DMSO served as control. Ampicillin and nystatin served as positive controls for bacteria and fungi, respectively. The plates were incubated at 37 °C for 24 h for bacteria, 28 °C for 24 h for *C. albicans*, and 28 °C for 5 days for rest of the fungi. The plates were incubated for 12 h after addition of 5 µl resazurin indicator solution (5mg/ml) to each well. The experiment included three replicates for each plate. The lowest concentration which prevented colour change from purple to pink was recorded as MIC, while the lowest concentration completely killing the inoculated microorganism was recorded as MMC.

RESULTS AND DISCUSSION

To the best of our knowledge, the himachalenes have not yet been thoroughly investigated, and there are few methods reported involving synthesis of benzocycloheptene derivatives from himachalenes by simple pathway (Chaudhary et al., 2012; Pandey and Dev, 1968). The synthesis was carried out by the conversion of himachalenes **10** into benzocycloheptene derivatives namely *α*-dehydro-*ar*-himachalene **11**, bisdehydro-*ar*-himachalene **12** and *α*-himachalene **13** by oxidative aromatization. In this study, *α*-dehydro-*ar*-himachalene **11** is used as precursor for the synthesis of substituted benzocycloheptenone. The benzocycloheptenone acts as an intermediate for synthesis of benzocycloheptene moiety and have been investigated for a wide spectrum of biological activities like cytotoxic, anticancer, antimicrobial and antagonistic activity (Bohlmann et al., 2006). It was mostly synthesized by cyclization of phenyl pentanoic acid (Liu et al., 2008). However, here, for the first time we conducted the synthesis of benzocycloheptenone starting from *α*-dehydro-*ar*-himachalene **11**. Oxidation of terminal alkene of **11** was attempted with different oxidizing agents such as $KMnO_4$, $Hg(OAc)_2$, $RuCl_3$/$NaIO_4$, $Pd(OAc)_2/O_2$ that led to the formation of mixture of products. Finally, the oxidation of exocyclic double bond of **11** was optimized with $NaIO_4$ and OsO_4 in water:THF (1:1, v/v) for 20 h at room temperature to produce corresponding benzocycloheptenone (**15**) in 73 % yield (Figure 2).

As nitrogen containing amino and imino benzocycloheptenes have been investigated to show diverse range of biological activities, the conversion of carbonyl moiety of benzocycloheptenone **15** into imino substituted benzocycloheptenes **16** was tried with reported conditions in several solvents such as toluene, benzene, DMF, THF but no successful results were obtained. Using dry silica gel (H) as a lewis acid and an appropriate amine gave good conversion of imine derivatives of benzocycloheptene (Figure 2) and no work up was required before purification through column chromatography. The mixture was directly transferred to column and purification was performed with hexane:EtOAc (90:10). With the optimized reaction conditions in hand, the scope of this reaction was then probed with a range of different amines (Table 1). Under similar conditions, the reactions of benzocyclohepten-5-one **15** with corresponding amines proceeded smoothly to form novel imine compounds **16a-16e** in 65-79 % yields (Table 1, entries 1-5).

The aryl himachalene was earlier prepared from mixture of himachalenes or 3-methylacetophenone (Abouhamza et al., 2001). While in the present study aryl himachalene **9** was obtained by catalytic hydro-

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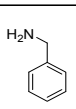
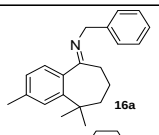
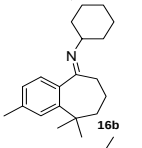
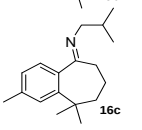
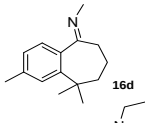
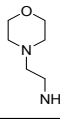
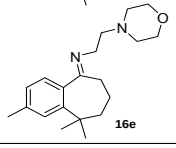
genation of *α*-dehydro-*ar*-himachalene **11** with Pd/C in ethyl acetate and methanol (1:1, v/v) in 80 % yield. Aryl himachalene **9** was also obtained by dual reduction of bisdehydro-*ar*-himachalene **12** with Pd/C in ethyl acetate and methanol via γ-dehydro-*ar*-himachalene **14** (Figure 2). The structures of synthesized compounds were established on the basis of IR, NMR and mass spectrometry.

The antimicrobial activity of all the himachalene derivatives (**9-15**, **16a-16e**) were tested against a panel of sixteen organisms: gram-positive bacteria- *B. subtilis*, *M. luteus*, *S. aureus*, gram-negative bacteria- *B. cepacea*, *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *E. cloacae* and fungal strains *C. albicans*, *I. orientalis*, *A. flavus*, *A. niger*, *A. parasiticus*, *A. sydowii*, *A. ochraceous* and *T. rubrum*. It was observed that the aromatized himachalenes (*ar*-himachalene **9**, *α*-dehydro-*ar*-himachalene **11,** bisdehydro-*ar*-himachalene **12** and *γ*-dehydro-*ar*-himachalene **14**) did not show antimicrobial activity against the gram-negative bacteria *B. cepacea*, *E. coli*, *K. pneumoniae* and *P. aeruginosa* even at a high concentration of 3000 µg/ml (Table 2). However, bis dehydro-*ar*-himachalene **12** exhibited antibacterial activity against the gram-positive bacteria *M. luteus* at MIC 625 µg/ml, and *ar*-himachalene **9** and *γ*-dehydro-*ar*-himachalene **14** against *B. subtilis* at MIC 375 µg/ml and 1500 µg/ml, respectively. Non aromatic *α*-himachalene **13** was more active than aromatic himachalenes against both bacterial and fungal cultures. Insertion of carbonyl group at 5th position of *α*-dehydro-*ar*-himachalene **11** slightly enhanced the activity against *B. subtilis*, *M. luteus*, and *S. aureus*. Conversely, the substitution of carbonyl group of substituted benzocycoheptenone **15** by imine group (**16a-16e**) displayed a noticeable improvement in their antibacterial activity against *B. subtilis*, *M. luteus* and *S. aureus* with MIC ranging from 46.8-750 µg/ml. Antifungal activity of these compounds against *A. sydowii*, *A. parasiticus* and *A. ochraceous* was also high with MIC 23.4-187.5 µg/ml as reported against *A. fumigatus* for himachalol and other derivatives from *Cedrus* (Parveen et al., 2010; Chowdhry et al., 1997).

The aromatized compounds, however, showed low activity against *Aspergillus* spp. High antimicrobial activity observed for compounds **16a-16e** indicated that the nature of imine group at C-5 position markedly affects the activity profile of those compounds. The imine substituent **16c** exhibited antimicrobial activity higher than **16a** possibly due to its electron releasing effect. No activity was observed against *C. albicans* and *I. orientalis* except for the compound **16c** which exhibited a broad spectrum antifungal activity. Antimicrobial activity of *Cedrus* extracts and essential oils has been previously reported particularly against *S. aureus*, *B. subtilis*, *E. coli*, *P. aeruginosa* and fungi of genus *Aspergillus* (Chowdhry et al., 1997; Pawar et al., 2007; Zeng et al., 2011). However, there are no reports on the antimicrobial properties of mentioned aryl himachalene derivatives.

Table 1: Synthesis of novel imine derivatives (**16a**-**16e**) of aryl himachalene

Entry	Amine	Product (**16**)	Yield[a] (%)
1		 **16a**	74
2		 **16b**	78
3		 **16c**	71
4	CH_3NH_2	 **16d**	79
5		 **16e**	65

[a]Isolated yield

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Table 2: Antimicrobial activity of derivatives of *α*-dehydro-*ar*-himachalene by broth microdilution method against standard microbial cultures (µg/ml)

Test organism		Test compound												
		10	11	12	13	14	9	15	16a	16b	16c	16d	16e	Ampicillin / Nystatin[b]
E. coli	MIC	-	-	-	-	-	-	-	3000	-	3000	-	-	31.3
	MMC	-	-	-	-	-	-	-	-	-	-	-	-	62.5
E. cloacae	MIC	-	-	-	312.5	-	-	-	3000	-	1500	-	-	2000
	MMC	-	-	-	625	-	-	-	-	3000	3000	-	-	-
B. subtilis	MIC	-	-	-	312.5	375	1500	187.5	187.5	187.5	46.8	187.5	-	7.8
	MMC	-	-	-	625	750	3000	375	375	375	93.75	375	-	15.6
B. cepacea	MIC	-	-	-	312.5	-	-	-	-	-	-	-	-	2000
	MMC	-	-	-	625	-	-	-	-	-	-	-	-	-
M. luteus	MIC	-	-	625	156.2	3000	-	375	750	750	93.75	187.5	-	3.9
	MMC	-	-	2500	1250	-	-	750	1500	1500	187.5	375	-	7.8
S. aureus	MIC	-	-	-	312.5	-	-	750	750	750	375	3000	-	3.9
	MMC	-	-	-	625	-	-	3000	3000	3000	750	-	-	3.9
P. aeruginosa	MIC	-	-	-	2500	-	-	-	-	-	3000	-	-	1000
	MMC	-	-	-	-	-	-	-	-	-	-	-	-	2000
K. pneumoniae	MIC	-	-	-	-	-	-	-	-	-	-	-	-	2000
	MMC	-	-	-	-	-	-	-	-	3000	-	-	-	-
A. niger	MIC	-	-	-	-	3000	3000	3000	750	750	187.5	1500	750	15.6
	MMC	-	-	-	-	-	-	-	1500	1500	1500	-	-	62.5
A. sydowii	MIC	312.5	1250	1250	625	750	750	187.5	23.4	187.5	46.8	375	375	3.9
	MMC	-	-	-	-	-	-	1500	750	750	187.5	3000	3000	3.9
A. parasiticus	MIC	1250	-	1250	1250	1500	375	750	187.5	93.75	46.8	750	375	100
	MMC	-	-	-	-	-	3000	-	3000	3000	750	-	-	200
A. ochraceous	MIC	156.2	1250	625	625	750	375	750	187.5	93.75	46.8	375	375	62.5
	MMC	-	-	-	-	-	-	-	3000	3000	375	-	-	125
A. flavus	MIC	1250	-	2500	2500	1500	750	1500	750	375	375	1500	1500	62.5
	MMC	-	-	-	-	-	-	-	-	-	3000	-	-	62.5
T. rubrum	MIC	-	1250	1250	1250	1250	1250	1250	1250	1250	625	-	-	31.3
	MMC	-	-	2500	2500	-	2500	-	-	-	1250	-	-	62.5
C. albicans	MIC	-	-	-	-	-	-	-	-	-	187.5	-	-	7.8
	MMC	-	-	-	-	-	-	-	-	-	750	-	-	7.8
I. orientalis	MIC	-	-	-	1250	-	-	-	-	-	187.5	-	-	31.3
	MMC	-	-	-	-	-	-	-	-	-	375	-	-	31.3

[a]Antibiotic against gram positive and gram negative bacteria; [b]Antibiotic against fungi
-: No activity; MIC= Minimum inhibitory concentration; MMC = Minimum microcidal concentration

CONCLUSION

In conclusion, analogues of aryl himachalenes were synthesized from readily available naturally occurring isomeric mixture of himachalenes via *α*-dehydro-*ar*-himachalene. Aromatization of himachalenes slightly increased the antibacterial activity which was further enhanced after insertion of imine moiety in novel imine compounds (**16a-16e**). The imine substituent containing isobutyl group **16c** showed the highest activity amongst tested compounds. These de-

rivatives could find potential in biological applications for drug design and development.

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REFERENCES

Abouhamza B, Allaoud, S, Karim A. *ar*-Himachalene. Molecules. 2001;6:M236.

Bhushan S, Singh J, Rao JM, Saxena AK, Qazi GN. A novel lignan composition from *Cedrus deodara* induces apoptosis and early nitric oxide generation in human leukemia Molt-4 and HL-60 cells. Nitric Oxide. 2006;14:72-88.

Bohlmann R, Kroll J, Kuenzer H, Hegele-Hartung C, Lessl M, Lichtner R et al. Benzocycloheptenes, process for their production, pharmaceutical preparations that contain the latter as well as their use for the production of pharmaceutical agents. US Patent 7145015 B2, 2006.

Chaudhary A, Kaur P, Singh B, Pathania V. Chemical composition of hydrodistilled and solvent volatiles extracted from woodchips of Himalayan Cedrus: *Cedrus deodara* (Roxb.) Loud. Nat Prod Commun. 2009;4:1257-60.

Chaudhary A, Das P, Mishra A, Kaur P, Singh B, Goel RK. Naturally occurring himachalenes to benzocycloheptene amino vinyl bromide derivatives: as antidepressant molecules. Mol Divers. 2012;16: 357-66.

Chow K, Fang, WK, Corpuz EG, Gil, DW, Garst ME. Substituted fluoroethyl ureas as alpha 2 adrenergic agents. US Patent 7598417 B2, 2009.

Chowdhry L, Khan ZK, Kulshrestha DK. Comparative *in vitro* and *in vivo* evaluation of himachalol in murine invasive aspergillosis. Indian J Expt Biol. 1997;35:727-34.

Cos P, Vlietinck AJ, Berghe BV, Maes L. Anti-infective potential of natural products: how to develop a stronger *in vitro* proof-of-concept. J Ethnopharmacol. 2006;106:290-302.

Daoubi M, Hernaandez-Galaan R, Benharref A, Collado IG. Screening study of lead compounds for natural product-based fungicides: Antifungal activity and biotransformation of 6*α*,7*α*-dihydroxy-*β*-himachalene by *Botrytis cinerea*. J Agri Food Chem. 2005; 53:6673-7.

Hattori K, Tanaka A. Benzocycloheptene derivatives. US Patent 6384072 B, 2002.

Hossini I, Harrad MA, Ali MA, Firdoussi LE, Karim A, Valerga P, et al. Friedel-Craft acylation of *ar*-himachalene: Synthesis of acyl-*ar*-himachalene and a new acyl-hydroperoxide. Molecules. 2011;16:5886-95.

Joseph TC, Dev S. Structure of himachalenes. Tetrahedron Lett. 1961;6:216-22.

Liu Y, Su J, Xiao JH, Jiang SB, Lu H, Zhong W, et al. Synthesis of benzocycloheptene derivatives as CCR5 antagonists with potent anti-HIV activity. Chinese Chem Lett. 2008;19:428-30.

Lynch KR, Macdonald TL. Benzocycloheptyl analogs having sphingosine 1-phosphate receptor activity. US Patent 0253761 A1, 2009.

Nakano M, Minoguchi M, Hanano T, Ono S-I, Horiuchi H, Teshima K. Benzimidazole compound and pharmaceutical use thereof. US Patent 0120841 A1, 2010.

Nedelec L, Pierdet A, Dumont C, Kannengiesser MH. 7-Amino-6,7-dihydro[5H]benzocycloheptene derivatives. US Patent 4148919, 1979.

Pandey RC, Dev S. Studies in sesquiterpenes-XXX. Synthesis of *ar*himachalene and himachalenes. Tetrahedron. 1968;24:3829-39.

Parveen R, Azmi MA, Tariq RM, Mahmood SM, Hijazi M, Mahmud S, et al. Determination of antifungal activity of *Cedrus deodara* root oil and its compounds against *Candida albicans* and *Aspergillus fumigatus*. Pak J Bot. 2010;42:3645-9.

Pawar VC, Thaker VS. Evaluation of the anti-*Fusarium oxysporum* f. sp cicer and anti-*Alternaria porri* effects of some essential oils. World J Microbiol Biotechnol. 2007;23:1099-106.

Shankaranarayan R, Bisarya SC, Dev S. Studies in sesquiterpenes-LIV: Oxidohimachalene, a novel sesquiterpenoid from the wood of *Cedrus deodara* Loud. Tetrahedron. 1977;33:1207-10.

Sharma N, Ghosh P, Sharma UK, Sood S, Sinha AK, Gulati A. Microwave-assisted efficient extraction and stability of juglone in different solvents from *Juglans regia*: Quantification of six phenolic constituents by validated RPHPLC and evaluation of antimicrobial activity. Anal Lett. 2009;42:2592-609.

Singh D, Agarwal SK. Himachalol and himachalene: insecticidal principles of Himalayan cedarwood oil. J Chem Ecol. 1988;14:1145-51.

Sorensen US, Teuber L, Peters D, Strobaek D, Johansen TH, Nielsen KS, et al. 2-Aminobenzimidazole derivatives and their use as modulators of small-conductance calcium-activated potassium channels. European Patent EP1776348 B1, 2008.

Tandon VK, Singh KA, Awasthi AK, Khanna JM, Lal B, Anand N. Chemo- and stereoselective synthesis of benzocycloheptene and 1-benzoxepin derivatives as α-sympathomimetic and anorexigenic agents. Bioorg Med Chem Lett. 2004;14:2867-70.

Zaratin PF, Petrone G, Sbacchi M, Garnier M, Fossati C, Petrillo P, et al. Modification of nociception and morphine tolerance by the selective opiate receptor-like orphan receptor antagonist (-)-*cis*-1-methyl-7-[[4-(2,6-dichlorophenyl)piperidin-1-yl]methyl]-6,7,8,9-tetrahydro-5H-benzocyclohepten-5-ol (SB-612111). J Pharmacol Exp Ther. 2004;308:454-61.

Zeng WC, Jia LR, Zhang Y, Cen JQ, Chen X, Gao H, et al. Antibrowning and antimicrobial activities of the water-soluble extract from pine needles of *Cedrus deodara*. J Food Sci. 2011;76:318-23.

Zilkowski BW, Bartelt RJ, Cosse AA, Petroski RJ. Male-produced aggregation pheromone compounds from the eggplant flea beetle (*Epitrix fuscula*): Identification, synthesis, and field biossays. J Chem Ecol. 2006;32:2543-58.