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ORIGINAL ARTICLE

Synthesis of β-carboline fatty alcohol hybrid molecules and characterization of their biological and antioxidant activities

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Abstract Nine new β-carboline fatty alcohol hybrids were synthesized from natural amino acid L-tryptophan by the conjugation of 10-undecen-1-ol via ester linkage. All the synthesized products were characterized in each step by spectral techniques (^{1}H, ^{13}C NMR mass and HRMS). Synthesized β-carboline derivatives (**7a-i**) were screened for biological activities such as antimicrobial, antifungal, anti-biofilm and anticancer. Compounds **7d** and **7f** showed most potent antimicrobial activity against *Bacillus subtilis* MTCC 121, *Micrococcus luteus* MTCC 2470, *Staphylococcus aureus* MTCC 96 and *Candida albicans* MTCC 3017 microbial strains ranged from 2.8 to 28.3 μg/mL. Biofilm inhibition assay showed that the compound **7f** exhibited better activity against three bacterial stains with IC_{50} values from 1.8 to 2.9 μM. All the β-carboline derivatives showed cytotoxic activity, among them compounds **7f** and **7h** (IC_{50} values 9.1 and 11.4 μM) were exhibited potential activity. Free radical scavenging activity via DPPH assay revealed that compound **7g** acted as a good antioxidant molecule than all the tested compounds.

1. Introduction

Organic molecules synthesized from naturally available renewable materials for various applications especially, biological and pharmaceutical applications is an emerging trend in modern synthetic chemistry (Wang et al., 2019; George et al., 2019). During the last two decades, numerous kinds of alkaloids were isolated from the natural sources and their importance in biological and pharmaceutical applications were studied (Ticona et al., 2020; Masi et al., 2019). Clinically natural alkaloids are famous to treat central nervous system

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(CNS) disorders for instance atropine, physostigmine morphine, anatabine and papaverine (Lin et al., 2020). Studies have revealed that wide ranges of natural alkaloids are biologically active molecules and they have been used to treat many pathogenic diseases (Jain and Parihar, 2018). β-carboline alkaloids (βCAs; 9H-pyrido-(3,4-β)indole) are naturally available indole alkaloids which are widespread in nature such as in plants, marine organisms, insects and mammals, structurally βCAs are constituted with five and six member ring structures along with two nitrogen atoms as part of aromatic ring (Fyhrquist et al., 2017; Devi et al., 2018; Piechowska et al., 2019; Szabo et al., 2021). βCAs have been found to be associated with diverse biological and pharmaceutical activities including anti-Alzheimer, antimicrobial, anti-inflammatory, antidepressant, neuroprotective and antioxidant (Dai et al., 2018; Wu et al., 2010; Nenaah, 2010; Manasa et al., 2020).

In an endeavor to develop new βCA derivatives Lopes-Ortiz et al., (2020) synthesized imide βCA and carbomethoxy βCA derivatives and their pharmacological properties were studied. In another study, Sireesha et al. (2021) synthesized new benzimidazole/benzoxazole linked β-carbolines by joining two different anticancer fragments, these hybrid β-carbolines showed excellent cytotoxic activity against MCF-7 cell lines. The data available on βCAs suggest there is still a lot of potential to develop new molecules combining with other bioactive moieties. Considering the above facts, combination of βCA with other bioactive molecules such as fatty acid (FA) derivatives is the aim of our present work. Variety of modified fatty acids and fatty acid derivatives are potential molecules to act against many pathogenic microbes such as *Vibrio harveyi* MTCC 3438, *V. parahaemolyticus* ATCC 17802, *V. vulnificus* MTCC 1145 and *V. alginolyticus* ATCC 17749 (Santhakumari et al., 2017). Combinations of FA with various bioactive molecules are called as fatty acid hybrid molecules (FHM). FHMs provide diverse advantages including improved oral bioavailability, improved targeting to the lymphatic system, enhanced tumor targeting and reduced toxicity (Irby et al., 2017). 1-β-D-arabinofuranosyl cytosine (Ara-C, cytarabine) is well known drug used in the treatment of cancer, due to its lower lipophilicity Ara-C exhibited low bioavailability. Liu et al. (2009) further synthesized a series of Ara-C derivatives by combining fatty acid and amino acid in order to enhance lipophilicity and bioavailability. Moreover, FA analogues containing heterocyclic moieties such as oxadiazole, triazole and thiadiazole were exhibited promising antidepressant and antimicrobial activities (Jubie et al., 2012). 10-undecenoic acid (UDA) is a versatile fatty acid containing terminal double bond derived from renewable feed stock castor oil (pyrolysis of ricinoleic acid ((9Z, 12R)-12-hydroxy-9-octadecenoic acid). Many reports available on 10-undecenoic acid derived compounds synthesis and utilization for pharmaceutical and polymer applications (Laskar et al., 2017; Valverde et al., 2018). 10-undecenoic fatty alkyl chain is a monounsaturated fatty alkyl chain with an uneven carbon chain length (C_{11}). 10-undecenoic alkyl chain exhibits many biological activities such as antifungal, antibacterial and antiviral activity etc. (Van der Steen and Stevens, 2009). In a report, different fatty alkyl chain substituted esters of apocynin oxime were synthesized and their antimicrobial and antifungal activities were studied. Results found that the 10-undecenyl substituted compound exhibited higher activity than other alky chain substituted compounds. Comparing to the unsaturated alkyl chains oleic fatty alkyl chain is less reactive than 10-undecenyl alkyl chain (Sammaiah et al., 2015). This suggests that the position of double has impact on biological activity.

Pharmacophore conjugation is an efficient technique for covalently adjoining two biological potent moieties in to one conjugate molecule (Nagarsenkar et al., 2016). The conjugated molecules demonstrate divergent action mechanism, might lead to synergistic effect with high affinity and selectivity (Viegas et al., 2007). Combined with the benefits of βCA, and fatty acid derivatives we report the synthesis, characterization, biological evaluation and antioxidant performance of fatty alcohol hybrid β-carboline derivatives. Herein, we synthesized β-carboline by taking natural amino acid L-tryptophan and various aldehydes and hybridized with 10-undecenol (a derivative of 10-undecenoic acid) by ester linkage.

2. Experimental

2.1. Materials

L-tryptophan, BOC anhydride, benzaldehyde, 4-fluorobenzaldehyde, 4-nitrobenzaldehyde, 4-hydroxybenzaldehyde, 4-formylbenzonitrile, 4-methoxybenzaldehyde, 3,4-dihydroxybenzaldehyde, 4-hydroxy-3-methoxybenzaldehyde and 3,4-dimethoxybenzaldehyde, DDQ, DCC and DMAP were purchased from M/s Sigma Aldrich (St. Louis, USA). Silica gel (60–120 mesh) for column chromatography was procured from M/s Acme synthetic chemicals (Mumbai, India). Highest grade purity solvents were purchased from M/s SD Fine Chemicals (Mumbai, India).

2.2. Characterization

The synthesized compounds were characterized by ^{1}H and ^{13}C NMR spectra using TMS as internal standard on Varian 500 and 125 MHz, respectively. Waters e2695 separators module mass spectrometer was used to identify the mass. HRMS spectra obtained from an Exactive Orbitrap mass spectrometer (Thermo Scientific, Waltham, MA, USA).

2.3. Synthesis of BOC tryptophan (2)

For the synthesis of product 2, a reported procedure was followed with slight modifications (Ellames et al., 2001). Briefly, L-tryptophan (5 g, 24 mmol) was dissolved with stirring in mixture of 1,4-dioxane, water (1:1 v/v, 20 mL) and 1 N NaOH. The mixture was cooled using ice bath then BOC anhydride (5.34 g, 24 mmol) was added to the solution. The cooled reaction mixture was stirred for 30 min and then reaction was brought to room temperature and stirred for further one hour. The reaction mixture was acidified with 1 N HCl to pH 2–3, extracted with ethyl acetate. Organic phase was washed with water dried over Na_2SO_4 and concentrated. The resulted crude product was passed through silica gel column running with hexane and ethyl acetate (92:8, v/v) to get colorless solid, yield obtained 84% (6.2 g); ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.1 (s, 1H), 9.84 (s, 1H), 8.15 (s, 1H), 7.60 (d, J = 7.8 Hz, 1H), 7.32 (d, J = 8.6 Hz, 1H), 7.18 (d, J = 8.6 Hz, 1H), 7.11(d, J = 8.0 Hz, 2H), 4.72 (t, J = 6.8 Hz, 1H), 3.34 (d, J = 7.6 Hz, 2H), 1.36 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$)

δ 174.6 (-*C*(O)-OH), 164. 7 (-NH-*C*(O)-), 136.5 (-NH-*C*-H = CH-), 127.4, 122.8, 118.6, 110.1, 79.5 (-C(O)-O(*C*-CH_3), 59.2 (-NH-*C*H-C(O)-OH), 29.6 (-CH-*C*H_2-), 26.4–25.2 (-*C*H_3); ESIMS (*m*/*z*): 305 $[M + H]^+$, 327 $[M + Na]^+$.

2.4. Esterification of Boc-Try (3)

To a solution of 2 (1 g, 3.2 mmol), DCC (0.67 g, 3.2 mmol) and DMAP (0.2 g, 1.6 mmol) in acetonitrile (10 mL) were added and the resulted solution was stirred for 8 h at room temperature. The progress of the reaction was monitored by TLC, after completion of the reaction the reaction mixture was extracted in to ethyl acetate and washed four times with water (50 mL). The resulted crude product was passed through silica gel column running with hexane and ethyl acetate (92:8, v/v) to get colorless solid, yield obtained 90% (1.32 g); ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.76 (s, 1H), 8.12 (s, 1H), 7.62 (d, J = 7.8 Hz, 1H), 7.31 (d, J = 8.6 Hz, 1H), 7.16 (d, J = 8.6 Hz, 1H), 7.12 (d, J = 8.0 Hz, 2H), 5.80–5.75 (m, 1H), 4.95–4.91 (m, 2H), 4.69 (t, J = 6.8 Hz, 1H), 4.08 (t, J = 8.0 Hz, 2H), 3.32 (d, J = 7.6 Hz, 2H), 2.14 (q, J = 8.6, 2H), 1.62–1.58 (m, 2H), 1.38 (s, 9H), 1.24–1.20 (m, 14H, CH_2); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.4 (-*C*(O)-OCH_2), 163. 1 (-NH-*C*(O)-), 139.7 (-*C*H = CH_2), 136.0 (-NH- *C*H = CH-), 126.8, 122.5, 117.9, 114.5 (-CH = *C*H_2), 110.3, 79.3 (-C(O)-O(*C*-CH_3), 65.2 (-*C*H_2-OC(O)), 58.4 (-NH-*C*H-C(O)-OCH_2), 34.6, 29.6 (-CH-*C*H_2-), 26.4–25.2 (-*C*H_3); ESIMS (*m*/*z*): 456 $[M + H]^+$, 478 $[M + Na]^+$.

2.5. Deprotection of BOC (4)

3 (1 g, 2.2 mmol) was dissolved with stirring in dry DCM, the mixture was cooled using ice bath and trifluoro acetic acid was added to the solution then stirred for 30 min. The reaction mixture brought to room temperature and stirred for another 30 min. The reaction mixture was washed with $NaHCO_3$ and dried over Na_2SO_4. Crude product was passed through silica gel column running with hexane and ethyl acetate (92:8, v/v), colorless solid obtained with 93% (0.73 g) yield; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.84 (s, 1H), 7.64 (d, J = 8.0 Hz, 1H), 7.40 (d, J = 8.6 Hz, 1H), 7.18 (d, J = 8.6 Hz, 1H), 7.16 (d, J = 7.8 Hz, 2H), 5.75–5.72 (m, 1H), 5.08 (s, 1H), 4.90–4.86 (m, 2H), 4.36 (t, J = 7.4 Hz, 1H), 4.04 (t, J = 7.6 Hz, 2H), 3.32 (d, J = 6.8 Hz, 2H), 2.16 (q, J = 8.5, 2H), 1.62–1.57 (m, 2H), 1.26–1.20 (m, 14H, CH_2); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.5 (-*C*(O)-OCH_2), 139.6 (-*C*H = CH_2), 136.4 (-NH-*C*H = CH-), 126.4, 122.2, 118.0, 115.3 (-CH = *C*H_2), 110.2, 65.8 (-*C*H_2-OC(O)), 56.4 (-*C*H-NH_2), 33.7, 29.6 (-CH-*C*H_2-); ESIMS (*m*/*z*): 356 $[M + H]^+$, 378 $[M + Na]^+$.

2.6. General procedure for the synthesis of Schiff bases (5a-i)

4 (1 g, 2.8 mmol), aldehyde (4.2 mmol) were dissolved in 25 mL of ethanol and refluxed for 8 h at 80-85 °C. The reaction mixture was cooled to room temperature and ethanol was evaporated, then diluted with dichloromethane (25 mL) and washed thrice with water (30 mL). The resulted pale yellow color reaction mixture was purified by column chromatography eluting with hexane and ethyl acetate (92:8, v/v) to get pure Schiff bases (pale yellow color solid) with good yield 81–90%.

5a: Quantities of substrates taken 4 (1 g, 2.8 mmol), benzaldehyde (0.45 g, 4.2 mmol) yield obtained 90% (1.12 g); ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.84 (s, 1H), 8.41 (s, 1H), 7.64 (d, J = 8.6 Hz, 2H), 7.57 (d, J = 8.0 Hz, 1H), 7.53 (d, J = 8.6 Hz, 3H), 7.31 (d, J = 7.8 Hz, 1H), 7.18 (d, J = 8.6 Hz, 1H), 7.14 (d, J = 7.4 Hz, 2H), 5.80–5.70 (m, 1H), 4.94–4.89 (m, 2H), 4.28 (t, J = 7.0, Hz 1H), 4.06 (t, J = 6.6 Hz, 2H), 3.34 (d, J = 7.5 Hz, 2H), 2.18 (q, J = 8.6, 2H), 1.64–1.60 (m, 2H), 1.28–1.21 (m, 14H, CH_2); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.7 (-*C*(O)-OCH_2), 163.6 (-N = *C*H-), 139.6 (-*C*H = CH_2), 134.5 (-NH-*C*H = CH-), 129.2, 126.4, 122.4, 118.4, 115.7 (-CH = *C*H_2), 75.6 (-*C*H-N =), 64.6 (-*C*H_2-OC(O)), 33.7, 29.6 (-CH-*C*H_2-); ESIMS (*m*/*z*): 445 $[M + H]^+$, 467 $[M + Na]^+$.

5b: Quantities of substrates taken 4 (1 g, 2.8 mmol), 4-fluorobenzaldehyde (0.52 g, 4.2 mmol) yield obtained 88% (1.15 g); ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.86 (s, 1H), 8.43 (s, 1H), 7.66 (d, J = 8.6 Hz, 2H), 7.58 (d, J = 8.4 Hz, 1H), 7.53 (d, J = 8.0 Hz, 1H), 7.33 (d, J = 8.6 Hz, 2H), 7.18 (d, J = 7.4 Hz, 1H), 7.16 (d, J = 7.5 Hz, 2H), 5.80–5.72 (m, 1H), 4.96–4.90 (m, 2H), 4.25 (t, J = 7.5 Hz, 1H), 4.06 (t, J = 6.6 Hz, 2H), 3.36 (d, J = 7.0 Hz, 2H), 2.21 (q, J = 8.4, 2H), 1.64–1.61 (m, 2H), 1.28–1.21 (m, 14H, CH_2); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.6 (-*C*(O)-OCH_2), 165.2, 164.6 (-N = *C*H-), 139.6 (-*C*H = CH_2), 133.8 (-NH-*C*-H = CH-), 128.8, 127.6, 122.4, 118.4, 116.3 (-CH = *C*H_2), 74.3 (-*C*H-N =), 65.2 (-*C*H_2-OC(O)), 33.7, 29.6 (-CH-*C*H_2-); ESIMS (*m*/*z*): 463 $[M + H]^+$, 485 $[M + Na]^+$.

5c: Quantities of substrates taken 4 (1 g, 2.8 mmol), 4-nitrobenzaldehyde (0.63 g, 4.2 mmol) yield obtained 87% (1.20 g); ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.88 (s, 1H), 8.46 (s, 1H), 8.23 (d, J = 8.6 Hz, 2H), 8.02 (d, J = 8.0 Hz, 2H), 7.56 (d, J = 7.6 Hz, 1H), 7.31 (d, J = 8.6 Hz, 1H), 7.18 (d, J = 8.0 Hz, 1H), 7.15 (d, J = 7.5 Hz, 2H), 5.78–5.71 (m, 1H), 4.86–4.78 (m, 2H), 4.26 (t, J = 7.4 Hz, 1H), 4.08 (t, J = 6.8 Hz, 2H), 3.34 (d, J = 8.0 Hz, 2H), 2.23 (q, J = 8.5, 2H), 1.66–1.61 (m, 2H), 1.28–1.21 (m, 14H, CH_2); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.6 (-*C*(O)-OCH_2), 166.6 (-N = *C*H-), 150.3, 138.9 (-*C*H = CH_2), 135.8 (-NH-*C*-H = CH-), 127.8, 126.6, 122.8, 119.4, 115.3 (-CH = *C*H_2), 73.9 (-*C*H-N =), 67.2 (-*C*H_2-OC(O)), 35.7, 29.6 (-CH-*C*H_2-); ESIMS (*m*/*z*): 490 $[M + H]^+$, 512 $[M + Na]^+$.

5d: Quantities of substrates taken 4 (1 g, 2.8 mmol), 4-hydroxybenzaldehyde (0.51 g, 4.2 mmol) yield obtained 85% (1.10 g); ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.96 (s, 1H), 8.65 (s, 1H), 7.78 (d, J = 8.6 Hz, 2H), 7.60 (d, J = 8.0 Hz, 1H), 7.32 (d, J = 8.5 Hz, 1H), 7.18 (d, J = 7.5 Hz, 1H), 7.12 (d, J = 8.0 Hz, 2H), 6.85 (d, J = 7.4 Hz, 2H), 5.88–5.84 (m, 1H), 4.95–4.91 (m, 2H), 4.32 (t, J = 6.6 Hz, 1H), 4.13 (t, J = 8.2 Hz, 2H), 3.56 (d, J = 7.4 Hz, 2H), 2.44 (q, J = 8.6, 2H), 1.68–1.63 (m, 2H), 1.28–1.21 (m, 14H, CH_2); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.3 (-*C*(O)-OCH_2), 160.8 (-N = *C*H-), 139.1 (-*C*H = CH_2), 136.5 (-NH-*C*H = CH-), 130.6, 127.7, 123.4, 118.8, 111.8 (-CH = *C*H_2), 75.7 (-*C*H-NH =), 66.4 (-*C*H_2-OC(O)), 36.7, 29.6 (-CH-*C*H_2-); ESIMS (*m*/*z*): 461 $[M + H]^+$, 483 $[M + Na]^+$.

5e: Quantities of substrates taken 4 (1 g, 2.8 mmol), 4-formylbenzonitrile (0.55 g, 4.2 mmol) yield obtained 86% (1.13 g); ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.98 (s, 1H), 8.64 (s, 1H), 8.03 (d, J = 8.5 Hz, 2H), 7.63 (d, J = 8.1 Hz, 1H), 7.56 (d, J = 7.5 Hz, 2H), 7.36 (d, J = 8.0 Hz, 1H), 7.18 (d, J = 8.0 Hz, 1H), 7.12 (d, J = 8.4 Hz, 2H), 5.92–5.86 (m,

1H), 4.96–4.83 (m, 2H), 4.35 (t, J = 7.4 Hz, 1H), 4.06 (t, J = 7.6 Hz, 2H), 3.48 (d, J = 6.8 Hz, 2H), 2.36 (q, J = 8.4, 2H), 1.58–1.53 (m, 2H), 1.28–1.21 (m, 14H, CH_2); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.3 (-C(O)-OCH_2), 161.4 (-N = CH-), 140.7 (-CH = CH_2), 139.1 (-NH-CH = CH-), 132.3, 127.7, 121.7, 115.9, 111.8 (-CH = CH$_2$), 76.4 (-CH-N =), 65.3 (-CH$_2$-OC(O)), 36.7, 29.6 (-CH-CH$_2$-); ESIMS (m/z): 470 $[M + H]^+$, 492 $[M + Na]^+$.

5f: Quantities of substrates taken 4 (1 g, 2.8 mmol), 4-methoxybenzaldehyde (0.57 g, 4.2 mmol) yield obtained 90% (1.20 g); ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.98 (s, 1H), 8.65 (s, 1H), 7.84 (d, J = 8.6 Hz, 2H), 7.61 (d, J = 7.5 Hz, 1H), 7.34 (d, J = 8.1 Hz, 1H), 7.18 (d, J = 8.6 Hz, 1H), 7.14 (d, J = 7.4 Hz, 2H), 7.10 (d, J = 8.3 Hz, 2H), 5.86–5.78 (m, 1H), 4.98–4.91 (m, 2H), 4.32 (t, J = 7.0 Hz, 1H), 4.08 (t, J = 7.4 Hz, 2H), 3.83 (s, 3H), 3.36 (d, J = 6.6 Hz, 2H), 2.28 (q, J = 8.5, 2H), 1.60–1.53 (m, 2H), 1.28–1.21 (m, 14H, CH_2); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.3 (-C(O)-OCH_2), 162.9, 161.8 (-N = CH-), 138.4 (-CH = CH_2), 136.2 (-NH-CH = CH-), 131.3, 128.4, 120.9, 116.3, 112.7 (-CH = CH$_2$), 77.5 (-CH-N =), 66.8 (-CH$_2$-OC(O)), 56.1 (-OCH$_3$), 36.7, 29.6 (-CH-CH$_2$-); ESIMS (m/z): 475 $[M + H]^+$, 497 $[M + Na]^+$.

5 g: Quantities of substrates taken 4 (1 g, 2.8 mmol), 3,4-dihydroxybenzaldehyde (0.57 g, 4.2 mmol) yield obtained 87% (1.16 g); ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.96 (s, 1H), 8.65 (s, 1H), 7.60 (d, J = 8.6 Hz, 1H), 7.34 (d, J = 7.5 Hz, 1H), 7.30 (d, J = 8.0 Hz, 1H), 7.26 (d, J = 7.4 Hz, 1H), 7.18 (d, J = 7.3 Hz, 1H), 7.12 (d, J = 8.0 Hz, 2H), 6.85 (d, J = 8.4 Hz, 1H), 5.95–5.86 (m, 1H), 4.96–4.85 (m, 2H), 4.36 (t, J = 6.8 Hz, 1H), 4.09 (t, J = 7.2 Hz, 2H), 3.48 (d, J = 7.0 Hz, 2H), 2.35 (q, J = 8.4, 2H), 1.66–1.58 (m, 2H), 1.28–1.21 (m, 14H, CH_2); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.1 (-C(O)-OCH_2), 160.4 (-N = CH-), 149.5, 139.4 (-CH = CH_2), 136.2 (-NH-CH = CH-), 133.1, 128.4, 120.9, 116.3, 112.7 (-CH = CH$_2$), 76.5 (-CH-NH =), 65.4 (-CH$_2$-OC(O)), 36.7, 29.6 (-CH-CH$_2$-); ESIMS (m/z): 477 $[M + H]^+$, 499 $[M + Na]^+$.

5 h: Quantities of substrates taken 4 (1 g, 2.8 mmol), 4-hydroxy-3-methoxybenzaldehyde (0.63 g, 4.2 mmol) yield obtained 82% (1.13 g); ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.98 (s, 1H), 8.66 (s, 1H), 7.60 (d, J = 8.6 Hz, 1H), 7.52 (d, J = 8.0 Hz, 1H), 7.34 (d, J = 8.5 Hz, 1H), 7.30 (d, J = 7.5 Hz, 1H), 7.18 (d, J = 7.6 Hz, 1H), 7.08 (d, J = 8.1 Hz, 2H), 6.91 (d, J = 7.6 Hz, 1H), 5.94–5.84 (m, 1H), 4.98–4.86 (m, 2H), 4.34 (t, J = 7.6 Hz, 1H), 4.12 (t, J = 6.8 Hz, 2H), 3.84 (s, 3H), 3.56 (d, J = 8.0 Hz, 2H), 2.38 (q, J = 8.1, 2H), 1.66–1.55 (m, 2H), 1.28–1.21 (m, 14H, CH_2); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.2 (-C(O)-OCH_2), 160.8 (-N = CH-), 151.2, 149.5, 139.2 (-CH = CH_2), 135.2 (-NH-CH = CH-), 133.1, 127.4, 121.9, 116.8, 112.5 (-CH = CH$_2$), 76.9 (-CH-NH =), 65.6 (-CH$_2$-OC(O)), 56.1 (-OCH$_3$), 36.7, 29.6 (-CH-CH$_2$-); ESIMS (m/z): 491 $[M + H]^+$, 513 $[M + Na]^+$.

5i: Quantities of substrates taken 4 (1 g, 2.8 mmol), 3,4-dimethoxybenzaldehyde (0.69 g, 4.2 mmol) yield obtained 81% (1.15 g); ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.98 (s, 1H), 8.64 (s, 1H), 7.60 (d, J = 8.6 Hz, 1H), 7.58 (d, J = 8.1 Hz, 1H), 7.40 (d, J = 7.6 Hz, 1H), 7.32 (d, J = 8.1 Hz, 1H), 7.18 (d, J = 7.4 Hz, 1H), 7.12 (d, J = 8.4 Hz, 2H), 6.85 (d, J = 8.0 Hz, 1H), 5.95–5.84 (m, 1H), 4.96–4.85 (m, 2H), 4.36 (t, J = 7.0 Hz, 1H), 4.10 (t, J = 6.4 Hz, 2H), 3.82 (s, 6H), 3.56 (d, J = 7.2 Hz, 2H), 2.38 (q, J = 8.3, 2H), 1.66–1.57 (m, 2H), 1.28–1.21 (m, 14H, CH_2); ^{13}C NMR (125 MHz, $CDCl_3$) δ 174.3 (-C(O)-OCH_2), 160.6 (-N = CH-), 152.3, 149.8, 138.6 (-CH = CH_2), 136.2 (-NH- CH = CH-), 132.8, 127.9, 121.7, 115.8, 112.5 (-CH = CH$_2$), 76.7 (-CH-NH =), 65.5 (-CH$_2$-OC(O)), 56.3 (-OCH$_3$), 36.7, 29.6 (-CH-CH$_2$-); ESIMS (m/z): 505 $[M + H]^+$, 527 $[M + Na]^+$.

2.7. General procedure for the cyclisation of Schiff bases (6a-i)

Schiff base 5a-i (1 g) was dissolved in methanol (15 mL) and acidified with 2 M aqueous HCl (5 mL) and stirred for 4 h at room temperature after completion of reaction, the reaction mixture was extracted in to ethyl acetate and washed with water (50 mL) until mixture get neutralized and dried over Na_2SO_4. Crude products were purified by column chromatography eluting with hexane and ethyl acetate (92:8, v/v) to get colorless solid.

6a: Quantities of substrates taken 5a (1 g, 2.2 mmol), 2 M HCl (5 mL) yield obtained 86% (0.85 g); ^{1}H NMR (500 MHz, $CDCl_3$) δ 12.38 (s, 1H), 7.54 (d, J = 8.5 Hz, 1H), 7.43 (d, J = 7.4 Hz, 1H), 7.37 (d, J = 8.0 Hz, 2H), 7.33 (d, J = 7.4 Hz, 3H), 7.06 (d, J = 8.6 Hz, 2H), 5.80–5.71 (m, 1H), 5.56 (s, 1H), 4.94–4.86 (m, 2H), 4.04 (t, J = 7.4 Hz, 2H), 3.82 (t, J = 6.8 Hz, 1H), 3.27 (d, J = 7.0 Hz, 2H), 2.18 (q, J = 8.4, 2H), 1.62–1.56 (m, 2H), 1.28–1.21 (m, 14H, CH_2); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.4 (-C(O)-OCH_2), 141.4, 139.6 (-CH = CH_2), 136.2, 128.2, 127.4, 121.4, 119.2, 118.4, 115.7 (-CH = CH$_2$), 106.5, 65.2 (-CH$_2$-OC(O)), 61.4, 57.5 (-CH-NH-), 33.7, 29.6 (-CH-CH$_2$-); ESIMS (m/z): 445 $[M + H]^+$, 467 $[M + Na]^+$.

6b: Quantities of substrates taken 5b (1 g, 2.1 mmol) 2 M HCl (5 mL) yield obtained 85% (0.83 g); ^{1}H NMR (500 MHz, $CDCl_3$) δ 12.23 (s, 1H), 7.58 (d, J = 8.6 Hz, 1H), 7.45 (d, J = 7.5 Hz, 1H), 7.21 (d, J = 8.1 Hz, 2H), 7.14 (d, J = 8.0 Hz, 2H), 7.06 (d, J = 7.4 Hz, 2H), 5.80–5.70 (m, 1H), 5.54 (s, 1H), 4.88–4.81 (m, 2H), 4.07 (t, J = 7.4 Hz, 2H), 3.82 (t, J = 6.6 Hz, 1H), 3.28 (d, J = 7.2 Hz, 2H), 2.24 (q, J = 8.2, 2H), 1.64–1.56 (m, 2H), 1.28–1.21 (m, 14H, CH_2); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.8 (-C(O)-OCH_2), 161.8, 139.6 (-CH = CH_2), 136.7, 133.8, 127.4, 121.4, 118.4, 115.3 (-CH = CH$_2$), 105.6, 65.2 (-CH$_2$-OC(O)), 62.1, 33.9, 29.6 (-CH-CH$_2$-); ESIMS (m/z): 463 $[M + H]^+$, 485 $[M + Na]^+$.

6c: Quantities of substrates taken 5c (1 g, 2.0 mmol) 2 M HCl (5 mL) yield obtained 82% (0.81 g); ^{1}H NMR (500 MHz, $CDCl_3$) δ 12.54 (s, 1H), 8.16 (d, J = 8.6 Hz, 2H), 7.57 (d, J = 8.1 Hz, 1H), 7.48 (d, J = 8.6 Hz, 2H), 7.42 (d, J = 7.5 Hz, 1H), 7.12 (d, J = 8.1 Hz, 2H), 5.78–5.69 (m, 1H), 5.62 (s, 1H), 4.86–4.80 (m, 2H), 4.08 (t, J = 7.6 Hz, 2H), 3.76 (t, J = 7.4 Hz, 1H), 3.28 (d, J = 8.0 Hz, 2H), 2.23 (q, J = 8.0, 2H), 1.66–1.58 (m, 2H), 1.28–1.21 (m, 14H, CH_2); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.6 (-C(O)-OCH_2), 147.8, 146.3, 138.7 (-CH = CH_2), 136.5, 128.8, 127.6, 122.8, 119.4, 115.3 (-CH = CH$_2$), 106.5, 67.2 (-CH$_2$-OC(O)), 61.6, 35.7, 29.6 (-CH-CH$_2$-); ESIMS (m/z): 490 $[M + H]^+$, 512 $[M + Na]^+$.

6d: Quantities of substrates taken 5d (1 g, 2.1 mmol) 2 M HCl (5 mL) yield obtained 86% (0.84 g); ^{1}H NMR (500 MHz, $CDCl_3$) δ 12.36 (s, 1H), 7.56 (d, J = 8.6 Hz, 1H), 7.46 (d, J = 7.8 Hz, 1H), 7.13 (d, J = 8.0 Hz, 2H),

7.08 (d, J = 8.1 Hz, 2H), 6.65 (d, J = 7.4 Hz, 2H), 5.88–5.76 (m, 1H), 5.54 (s, 1H), 4.95–4.88 (m, 2H), 4.10 (t, J = 7.4 Hz, 2H), 3.75 (t, J = 6.6 Hz, 1H), 3.26 (d, J = 7.0 Hz, 2H), 2.36 (q, J = 8.2, 2H), 1.68–1.58 (m, 2H), 1.28–1.21 (m, 14H, CH_2); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.4 (-C(O)-OCH_2), 156.4, 139.1 (-CH = CH_2), 134.5, 133.6, 128.7, 124.2, 119.7, 118.8, 111.8 (-CH = CH$_2$), 104.8, 66.8 (-CH$_2$-OC(O)), 61.4, 34.8, 29.6 (-CH-CH$_2$-); ESIMS (m/z): 461 $[M + H]^+$, 483 $[M + Na]^+$.

6e: Quantities of substrates taken 5e (1 g, 2.1 mmol) 2 M HCl (5 mL) yield obtained 83% (0.82 g); ^{1}H NMR (500 MHz, $CDCl_3$) δ 12.28 (s, 1H), 7.58 (d, J = 8.6 Hz, 1H), 7.48 (d, J = 8.5 Hz, 2H), 7.45 (d, J = 7.3 Hz, 1H), 7.32 (d, J = 8.4 Hz, 2H), 7.12 (d, J = 7.5 Hz, 2H), 5.92–5.85 (m, 1H), 5.54 (s, 1H), 4.96–4.89 (m, 2H), 4.06 (t, J = 7.0 Hz, 2H), 3.84 (t, J = 7.4 Hz, 1H), 3.48 (d, J = 8.2 Hz, 2H), 2.36 (q, J = 8.3, 2H), 1.58–1.53 (m, 2H), 1.28–1.21 (m, 14H, CH_2); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.6 (-C(O)-OCH_2), 146.2, 140.7 (-CH = CH_2), 137.2, 134.5, 132.3, 128.7, 121.7, 118.7, 115.9, 113.8 (-CH = CH$_2$), 106.2, 65.3 (-CH$_2$-OC(O)), 56.8, 37.4, 29.6 (-CH-CH$_2$-); ESIMS (m/z): 470 $[M + H]^+$, 492 $[M + Na]^+$.

6f: Quantities of substrates taken 5f (1 g, 2.1 mmol) 2 M HCl (5 mL) yield obtained 80% (0.80 g); ^{1}H NMR (500 MHz, $CDCl_3$) δ 12.21 (s, 1H), 7.58 (d, J = 8.6 Hz, 1H), 7.49 (d, J = 7.5 Hz, 1H), 7.18 (d, J = 8.0 Hz, 2H), 7.12 (d, J = 7.4 Hz, 2H), 6.88 (d, J = 8.1 Hz, 2H), 5.86–5.78 (m, 1H), 5.54 (s, 1H), 4.98–4.93 (m, 2H), 4.08 (t, J = 6.8 Hz, 2H), 3.86–3.48 (t, J = 8.0 Hz, 4H), 3.36 (d, J = 7.4 Hz, 2H), 2.26 (q, J = 8.0, 2H), 1.63–1.56 (m, 2H), 1.28–1.21 (m, 14H, CH_2); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.6 (-C(O)-OCH_2), 158.4, 139.4 (-CH = CH_2), 135.2, 133.1, 128.4, 121.7, 115.3, 113.7 (-CH = CH$_2$), 105.8, 66.8 (-CH$_2$-OC(O)), 56.9, 56.1 (-OCH$_3$), 36.7, 29.6 (-CH-CH$_2$-); ESIMS (m/z): 475 $[M + H]^+$, 497 $[M + Na]^+$.

6 g: Quantities of substrates taken 5f (1 g, 2.1 mmol) 2 M HCl (5 mL) yield obtained 81% (0.81 g); ^{1}H NMR (500 MHz, $CDCl_3$) δ 12.36 (s, 1H), 7.58 (d, J = 8.6 Hz, 1H), 7.46 (d, J = 7.5 Hz, 1H), 7.11 (d, J = 8.1 Hz, 2H), 6.86 (d, J = 7.3 Hz, 1H), 6.67 (d, J = 8.2 Hz, 2H), 5.95–5.87 (m, 1H), 5.46 (s, 1H), 4.96–4.85 (m, 2H), 4.09 (t, J = 7.4 Hz, 2H), 3.78 (t, J = 7.8 Hz, 1H), 3.36 (d, J = 8.2 Hz, 2H), 2.44 (q, J = 8.3, 2H), 1.66–1.57 (m, 2H), 1.28–1.21 (m, 14H, CH_2); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.8 (-C(O)-OCH_2), 145.8, 139.4 (-CH = CH_2), 134.2 , 133.1, 130.4, 121.6, 119.9, 116.3, 112.7 (-CH = CH$_2$), 106.3, 65.4 (-CH$_2$-OC(O)), 60.7, 36.7, 29.6 (-CH-CH$_2$-); ESIMS (m/z): 477 $[M + H]^+$, 499 $[M + Na]^+$.

6 h: Quantities of substrates taken 5f (1 g, 2.0 mmol) 2 M HCl (5 mL) yield obtained 80% (0.78 g); ^{1}H NMR (500 MHz, $CDCl_3$) δ 12.24 (s, 1H), 7.57 (d, J = 8.5 Hz, 1H), 7.46 (d, J = 7.6 Hz, 1H), 7.16 (d, J = 8.0 Hz, 2H), 6.89 (d, J = 7.4 Hz, 1H), 6.71 (d, J = 8.1 Hz, 1H), 6.62 (d, J = 7.5 Hz, 1H), 5.94–5.82 (m, 1H), 5.32 (s, 1H), 4.98–4.84 (m, 2H), 4.06 (t, J = 7.2 Hz, 2H), 3.82 (t, J = 7.4 Hz, 4H), 3.34 (d, J = 8.0 Hz, 2H), 2.40 (q, J = 8.0, 2H), 1.68–1.55 (m, 2H), 1.28–1.21 (m, 14H, CH_2); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.3 (-C(O)-OCH_2), 147.2, 139.2 (-CH = CH_2), 135.2, 133.1, 130.4, 127.4, 121.9, 116.8, 112.5 (-CH = CH$_2$), 104.2, 66.4 (-CH$_2$-OC(O)), 58.3, 56.1 (-OCH$_3$), 36.7, 29.6 (-CH-CH$_2$-); ESIMS (m/z): 491 $[M + H]^+$, 513 $[M + Na]^+$.

6i: Quantities of substrates taken 5f (1 g, 1.9 mmol) 2 M HCl (5 mL) yield obtained 83% (0.84 g); ^{1}H NMR (500 MHz, $CDCl_3$) δ 12.18 (s, 1H), 7.60 (d, J = 8.4 Hz, 1H), 7.48 (d, J = 7.6 Hz, 1H), 7.16 (d, J = 8.0 Hz, 2H), 6.91 (d, J = 7.5 Hz, 1H), 6.78 (d, J = 8.0 Hz, 1H), 6.67 (d, J = 7.5 Hz, 1H), 5.95–5.87 (m, 1H), 5.34 (s, 1H), 4.96–4.84 (m, 2H), 4.10 (t, J = 7.4 Hz, 2H), 3.83–3.56 (m, 7H), 3.46 (d, J = 7.0 Hz, 2H), 2.28 (q, J = 8.0, 2H), 1.64–1.53 (m, 2H), 1.28–1.21 (m, 14H, CH_2); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.2 (-C(O)-OCH_2), 149.6, 147.8, 138.6 (-CH = CH_2), 136.4, 132.8, 130.6, 127.3, 121.7, 118.6, 115.8, 113.5, 112.2 (-CH = CH$_2$), 106.8, 65.5 (-CH$_2$-OC(O)), 57.8, 56.3 (-OCH$_3$), 36.7, 29.6 (-CH-CH$_2$-); ESIMS (m/z): 505 $[M + H]^+$, 527 $[M + Na]^+$.

2.8. General procedure for aromatization of cyclic products (7a-i)

6a-i (1 g), DDQ were dissolved in benzene and refluxed for 20 h the progress of the reaction was monitored by TLC, after completion of reaction the reaction mixture was extracted in to ethyl acetate and washed with water and dried over Na_2SO_4. Crude products were purified by column chromatography eluting with hexane and ethyl acetate (92:8, v/v) to get colorless solid.

7a: Quantities of substrates taken 6a (1 g, 2.2 mmol), DDQ (0.99 g, 4.4 mmol) yield obtained 80% (0.78 g); ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.86 (s, 1H), 8.19 (d, J = 8.4 Hz, 1H), 7.96 (d, J = 8.0 Hz, 2H), 7.84 (s, J = 7.5 Hz, 1H), 7.62 (d, J = 7.4 Hz, 1H), 7.54 (d, J = 8.0 Hz, 4H), 7.26 (d, J = 8.5 Hz, 1H), 5.80–5.73 (m, 1H), 4.94–4.86 (m, 2H), 4.26 (t, J = 7.4 Hz, 2H), 2.18 (q, J = 8.2, 2H), 1.78–1.64 (m, 2H), 1.29–1.24 (m, 14H, CH_2); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.3 (-C(O)-OCH_2), 158.2, 146.9, 141.4, 139.6 (-CH = CH_2), 132.4, 129.2, 127.4, 123.5, 121.4, 119.2, 118.4, 115.7 (-CH = CH$_2$), 111.3, 103.5, 65.2 (-CH$_2$-OC(O)), 33.7, 29.6 (-CH-CH$_2$-); ESIMS (m/z): 441 $[M + H]^+$, 463 $[M + Na]^+$; HRMS (m/z): $[M + H]^+$ calcd for $C_{29}H_{32}N_2O_2$, 440.24647; found, 440.24596.

7b: Quantities of substrates taken 6b (1 g, 2.1 mmol), DDQ (0.95 g, 4.2 mmol) yield obtained 79% (0.76 g); ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.91 (s, 1H), 8.64 (d, J = 8.6 Hz, 2H), 8.12 (d, J = 7.6 Hz, 1H), 7.84 (s, J = 8.0 Hz, 1H), 7.62 (d, J = 8.1 Hz, 1H), 7.48 (d, J = 7.5 Hz, 1H), 7.28 (d, J = 7.3 Hz, 3H), 5.80–5.71 (m, 1H), 4.88–4.79 (m, 2H), 4.24 (t, J = 7.0 Hz, 2H), 2.24 (q, J = 8.0, 2H), 1.64–1.55 (m, 2H), 1.28–1.21 (m, 14H, CH_2); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.4 (-C(O)-OCH_2), 160.5, 158.2, 148.0, 141.3, 139.6 (-CH = CH_2), 137.0, 136.7, 133.8, 130.4, 127.4, 123.6, 121.4, 118.4, 116.3 (-CH = CH$_2$), 111.2, 105.6, 65.2 (-CH$_2$-OC(O)), 33.9, 29.6 (-CH-CH$_2$-); ESIMS (m/z): 459 $[M + H]^+$, 481 $[M + Na]^+$; HRMS (m/z): $[M + H]^+$ calcd for $C_{29}H_{31}FN_2O_2$, 458.23685; found, 458.23459.

7c: Quantities of substrates taken 6c (1 g, 2.0 mmol), DDQ (0.90 g, 4.0 mmol) yield obtained 78% (0.76 g); ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.84 (s, 1H), 8.38 (d, J = 8.4 Hz, 2H), 8.22 (d, J = 7.5 Hz, 2H), 8.06 (d, J = 8.6 Hz, 1H), 7.84 (s, J = 8.3 Hz, 1H), 7.62 (d, J = 7.5 Hz, 1H), 7.52 (d, J = 8.0 Hz, 1H), 7.26 (d, J = 7.6 Hz, 1H), 5.78–5.72 (m, 1H), 4.86–4.80 (m, 2H), 4.26 (t, J = 7.4 Hz, 1H), 2.23 (q, J = 8.4, 2H), 1.66–1.60 (m, 2H), 1.28–1.21 (m, 14H, CH_2);

^{13}C NMR (125 MHz, $CDCl_3$) δ 166.4 (-*C*(O)-OCH_2), 158.1, 147.8, 146.3, 141.2, 138.7 (-*C*H = CH_2), 132.6, 128.8, 126.6, 124.8, 121.3, 119.4, 115.3 (-CH = *C*H_2), 113.4, 106.5, 103.4, 67.2 (-*C*H_2-OC(O)), 35.7, 29.6 (-CH-*C*H_2-); ESIMS (*m*/*z*): 486 $[M + H]^+$, 508 $[M + Na]^+$; HRMS (*m*/*z*): $[M + H]^+$ calcd for $C_{29}H_{31}N_3O_4$, 485.23155; found, 485.23064.

7d: Quantities of substrates taken 6d (1 g, 2.1 mmol), DDQ (0.95 g, 4.2 mmol) yield obtained 81% (0.78 g); ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.86 (s, 1H), 8.10 (d, *J* = 8.6 Hz, 1H), 7.96 (d, *J* = 7.6 Hz, 2H), 7.74 (s, *J* = 8.0 Hz, 1H), 7.62 (d, *J* = 7.5 Hz, 1H), 7.50 (d, *J* = 8.0 Hz, 1H), 7.26 (d, *J* = 7.4 Hz, 1H), 6.74 (d, *J* = 8.1 Hz, 2H), 5.88–5.81 (m, 1H), 4.95–4.84 (m, 2H), 4.24 (t, *J* = 7.0 Hz, 2H), 2.36 (q, *J* = 8.3, 2H), 1.68–1.60 (m, 2H), 1.28–1.21 (m, 14H, CH_2); ^{13}C NMR (125 MHz, $CDCl_3$) δ 164.8 (-*C*(O)-OCH_2), 158.4, 156.2, 146.7, 141.3, 139.1 (-*C*H = CH_2), 134.5, 132.6, 129.6, 123.0, 119.1, 116.8, 114.8 (-CH = *C*H_2), 111.4, 105.8, 66.8 (-*C*H_2-OC(O)), 34.8, 29.6 (-CH-*C*H_2-); ESIMS (*m*/*z*): 457 $[M + H]^+$, 479 $[M + Na]^+$; HRMS (*m*/*z*): $[M + H]^+$ calcd for $C_{29}H_{32}N_2O_3$, 456.24139; found, 456.24084.

7e: Quantities of substrates taken 6e (1 g, 2.1 mmol), DDQ (0.95 g, 4.2 mmol) yield obtained 78% (0.76 g); ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.72 (s, 1H), 8.36 (d, *J* = 8.4 Hz, 2H), 8.04 (d, *J* = 7.6 Hz, 1H), 7.72 (s, *J* = 8.0 Hz, 3H), 7.58 (d, *J* = 7.4 Hz, 1H), 7.30 (d, *J* = 8.1 Hz, 1H), 7.24 (d, *J* = 7.4 Hz, 1H), 5.925.86 (m, 1H), 4.96–4.88 (m, 2H), 4.20 (t, *J* = 7.2 Hz, 2H), 2.28 (q, *J* = 8.0, 2H), 1.58–1.50 (m, 2H), 1.28–1.21 (m, 14H, CH_2); ^{13}C NMR (125 MHz, $CDCl_3$) δ 162.8 (-*C*(O)-OCH_2), 156.4, 148.2, 144.5, 139.7 (-*C*H = CH_2), 132.7, 123.7, 121.7, 118.7, 115.9, 113.8 (-CH = *C*H_2), 109.8, 104.8, 65.3 (-*C*H_2-OC(O)), 37.4, 29.6 (-CH-*C*H_2-); ESIMS (*m*/*z*): 466 $[M + H]^+$, 488 $[M + Na]^+$; HRMS (*m*/*z*): $[M + H]^+$ calcd for $C_{30}H_{31}N_3O_2$, 465.24164; found, 465.24107.

7f: Quantities of substrates taken 6f (1 g, 2.1 mmol), DDQ (0.95 g, 4.2 mmol) yield obtained 79% (0.78 g); ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.64 (s, 1H), 8.10 (d, *J* = 8.5 Hz, 1H), 8.04 (d, *J* = 7.6 Hz, 2H), 7.68 (s, *J* = 8.1 Hz, 1H), 7.54 (d, *J* = 7.4 Hz, 1H), 7.43 (d, *J* = 8.3 Hz, 1H), 7.18 (d, *J* = 7.4 Hz, 1H), 7.02 (d, *J* = 8.0 Hz, 2H), 5.86–5.78 (m, 1H), 4.98–4.90 (m, 2H), 4.18 (t, *J* = 7.4 Hz, 2H), 3.83 (s, 3H), 2.26 (q, *J* = 8.2, 2H), 1.63–1.54 (m, 2H), 1.28–1.21 (m, 14H, CH_2); ^{13}C NMR (125 MHz, $CDCl_3$) δ 164.2 (-*C*(O)-OCH_2), 159.6, 148.2, 141.0, 139.4 (-*C*H = CH_2), 133.2, 132.7, 128.4, 123.6, 121.7, 115.3, 113.7 (-CH = *C*H_2), 110.5, 105.8, 66.4 (-*C*H_2-OC(O)), 56.1 (-O*C*H_3), 36.7, 29.6 (-CH-*C*H_2-); ESIMS (*m*/*z*): 471 $[M + H]^+$, 493 $[M + Na]^+$; HRMS (*m*/*z*): $[M + H]^+$ calcd for $C_{30}H_{34}N_2O_3$, 470.25684; found, 470.25670.

7 g: Quantities of substrates taken 6 g (1 g, 2.1 mmol), DDQ (0.95 g, 4.2 mmol) yield obtained 75% (0.74 g); ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.85 (s, 1H), 8.08 (d, *J* = 8.6 Hz, 1H), 7.78 (s, *J* = 7.8 Hz, 1H), 7.64 (s, *J* = 8.0 Hz, 1H), 7.58 (d, *J* = 7.4 Hz, 2H), 7. 46 (d, *J* = 8.1 Hz, 1H), 7.18 (d, *J* = 7.6 Hz, 1H), 6. 75 (d, *J* = 7.6 Hz, 1H), 5.95–5.86 (m, 1H), 4.96–4.88 (m, 2H), 4.25 (t, *J* = 7.6 Hz, 2H), 2.44 (q, *J* = 8.4, 2H), 1.66–1.58 (m, 2H), 1.28–1.21 (m, 14H, CH_2); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.4 (-*C*(O)-OCH_2), 158.6, 148.2, 145.4, 141.0, 139.4 (-*C*H = CH_2), 132.1, 130.4, 121.6, 119.7, 116.2, 112.9 (-CH = *C*H_2), 111.0, 106.6, 65.4 (-*C*H_2-OC(O)), 36.7, 29.6 (-CH-*C*H_2-); ESIMS (*m*/*z*): 473 $[M + H]^+$, 495 $[M + Na]^+$; HRMS (*m*/*z*): $[M + H]^+$ calcd for $C_{29}H_{32}N_2O_4$, 472.23516; found, 472.23385.

7 h: Quantities of substrates taken 6 h (1 g, 2.0 mmol), DDQ (0.90 g, 4.0 mmol) yield obtained 78% (0.76 g); ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.76 (s, 1H), 8.08 (d, *J* = 8.6 Hz, 1H), 7.78 (s, *J* = 8.0 Hz, 1H), 7.68 (s, *J* = 7.5 Hz, 2H), 7.54 (d, *J* = 8.1 Hz, 1H), 7.46 (d, *J* = 7.6 Hz, 1H), 7.18 (d, *J* = 7.6 Hz, 1H), 6.86 (d, *J* = 8.1 Hz, 1H), 5.94–5.86 (m, 1H), 4.98–4.92 (m, 2H), 4.20 (t, *J* = 6.8 Hz, 2H), 3.82 (s, 3H), 2.40 (q, *J* = 8.3, 2H), 1.68–1.56 (m, 2H), 1.28–1.21 (m, 14H, CH_2); ^{13}C NMR (125 MHz, $CDCl_3$) δ 162.9 (-*C*(O)-OCH_2), 156.4,147.2, 141.6, 139.2 (-*C*H = CH_2),135.2, 132.1, 130.0, 128.4, 123.5, 121.9, 115.8, 113.5 (-CH = *C*H_2), 111.2, 106.5, 102.6, 66.4 (-*C*H_2-OC(O)), 55.8 (-O*C*H_3), 36.7, 29.6 (-CH-*C*H_2-); ESIMS (*m*/*z*): 487 $[M + H]^+$, 509 $[M + Na]^+$; HRMS (*m*/*z*): $[M + H]^+$ calcd for $C_{30}H_{34}N_2O_4$, 486.25172; found, 486.25024.

7i: Quantities of substrates taken 6i (1 g, 1.9 mmol), DDQ (0.86 g, 3.8 mmol) yield obtained 75% (0.71 g); ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.86 (s, 1H), 8.09 (d, *J* = 8.6 Hz, 1H), 7.78 (s, *J* = 8.0 Hz, 1H), 7.69 (s, *J* = 7.4 Hz, 2H), 7.58 (d, *J* = 7.5 Hz, 1H), 7.46 (d, *J* = 8.1 Hz, 1H), 7.18 (d, *J* = 8.6 Hz, 1H), 6.82 (d, *J* = 7.5 Hz, 1H), 5.95–5.84 (m, 1H), 4.96–4.89 (m, 2H), 4.28 (t, *J* = 7.6 Hz, 2H), 3.82 (s, 6H), 2.28 (q, *J* = 8.1, 2H), 1.64–1.55 (m, 2H), 1.28–1.21 (m, 14H, CH_2); ^{13}C NMR (125 MHz, $CDCl_3$) δ 162.8 (-*C*(O)-OCH_2), 156.4, 149.8, 147.8, 141.3, 138.6 (-*C*H = CH_2), 132.8, 130.6, 123.3, 121.7, 119.6, 115.8, 113.5 (-CH = *C*H_2), 111.0, 106.8,103.4, 65.5 (-*C*H_2-OC(O)), 56.3 (-O*C*H_3), 36.7, 29.6 (-CH-*C*H_2-); ESIMS (*m*/*z*): 501 $[M + H]^+$, 523 $[M + Na]^+$; HRMS (*m*/*z*): $[M + H]^+$ calcd for $C_{31}H_{36}N_2O_4$, 500.26642; found, 500.26488.

2.9. Biological activity

2.9.1. Antimicrobial activity

The antimicrobial activity of the β-carboline derivatives (**7a-7i**) were investigated by using well diffusion method as described in previous report (Madabhushi et al., 2014). Different pathogenic reference strains which were purchased from the Microbial Type Culture Collection (MTCC), CSIR-Institute of Microbial Technology, Chandigarh, India. The pathogenic reference strains were seeded on the surface of the media Petri plates, containing Muller-Hinton agar with 0.1 mL of previously prepared microbial suspensions individually containing 1.5×10^8 cfu ml^{-1} (equal to 0.5 McFarland). Wells of 6.0 mm diameter were prepared in the media plates using a cork borer and the synthesized derivatives at a dose range of 300–1.45 μM were added in each well under sterile conditions in a laminar air flow chamber. Standard antibiotic solution of ciprofloxacin at a dose range of 300 – 1.45 μM and the well containing methanol served as positive and negative controls, respectively. The plates were incubated for 24 h at 37 °C for bacterial strains and the well containing the least concentration showing the inhibition zone was considered as the minimum inhibitory concentration. All the experiments were carried out in triplicate and the average of the three values were reported.

2.9.2. Minimum bactericidal concentration (MBC) assay

Bactericidal assay (NCCLS, 2000) was conducted as described in previous report (Kumar et al., 2016). In sterile 2.0 mL

microfuge tubes against a panel of bacterial strains, including *Bacillus subtilis* MTCC 121, *Staphylococcus aureus* MTCC 96 and *Staphylococcus aureus* MLS-16 MTCC 2940 which were cultured overnight in Mueller Hinton broth. Serial dilutions of test compounds were prepared in Mueller Hinton broth with different concentrations ranging from 0 to 300 μM. To the test compounds, 100 μl of overnight cultured bacterial suspensions were added to reach a final concentration of 1.5×10^8 cfu ml^{-1} (equal to 0.5 McFarland) and incubated at 37 °C for 24 h. After 24 h of incubation, the minimum bactericidal concentration (MBC) was determined by sampling 10 μl of suspension from the tubes onto Mueller Hinton agar plates and were incubated for 24 h at 36 °C to observe the growth of test organisms. MBC are the lowest concentration of test compound required to kill a particular bacterium strain. All the experiments were carried in triplicate and the average of the three values were reported.

2.9.3. Biofilm inhibition assay

The test compounds were screened in sterile 96 well polystyrene microtiter plates using the modified biofilm inhibition assay as described in previous report (Venepally et al., 2018). A panel of pathogenic bacterial strains including *Staphylococcus aureus* MLS-16 MTCC 2940, *Staphylococcus aureus* MTCC 96, and *Bacillus subtilis* MTCC 121, which were cultured overnight in tryptone soy broth (supplemented with 0.5% glucose). The test compounds of predetermined concentrations ranging from 0 to 300 μM were mixed with the bacterial suspensions having an initial inoculum concentration of 5×10^5 CFU/ml. Aliquots of 100 μl were distributed in each well and then incubated at 37 °C for 24 h under static conditions. The medium was then discarded and washed with phosphate buffered saline to remove the non-adherent bacteria. Each well of the microtiter plate was stained with 100 μl of 0.1% crystal violet solution followed by 30 min incubation at room temperature. Later the crystal violet solution from the plates was discarded, thoroughly washed with distilled water for 3 to 4 times and air dried at room temperature. The crystal violet stained biofilm was solubilised in 95% ethanol (100 μl) and the absorbance was recorded at 540 nm using TRIAD multimode reader (Dynex Technologies, Inc, Chantilly, VA, USA). Blank wells were employed as background check. The inhibition data were interpreted from the dose–response curves, where IC_{50} value is defined as the concentration of inhibitor required to inhibit 50% of biofilm formation under the above assay conditions. All the experiments were carried out in triplicates and the values are indicated as mean ± S.D.

2.9.4. Cytotoxicity assay

Cytotoxicity of the β-carboline derivatives (**7a-7i**) was determined on the basis of measurement of in vitro growth inhibition of tumor cell lines in 96-well plates by cell-mediated reduction of tetrazolium salt to water insoluble formazan crystals using doxorubicin as a standard as mentioned in previous report (Reddy et al., 2016). The cytotoxicity as assessed against a panel of four different human tumor cell lines: HeLa derived from human cervical cancer cells (ATCC No. CCL-2), DU145 derived from prostate cancer cells (ATCC No. HTB-81) SKOV3 derived from human ovarian carcinomas cells (ATCC No. HTB-77), and MDA-MB-231 derived from human breast adenocarcinoma cells (ATCC No. HTB-26) using the MTT assay (Mosmann, 1983). The IC_{50} values (50% inhibitory concentration) were calculated from the plotted absorbance data for the dose–response curves. IC_{50} values (in μM) are expressed as the average of two independent experiments.

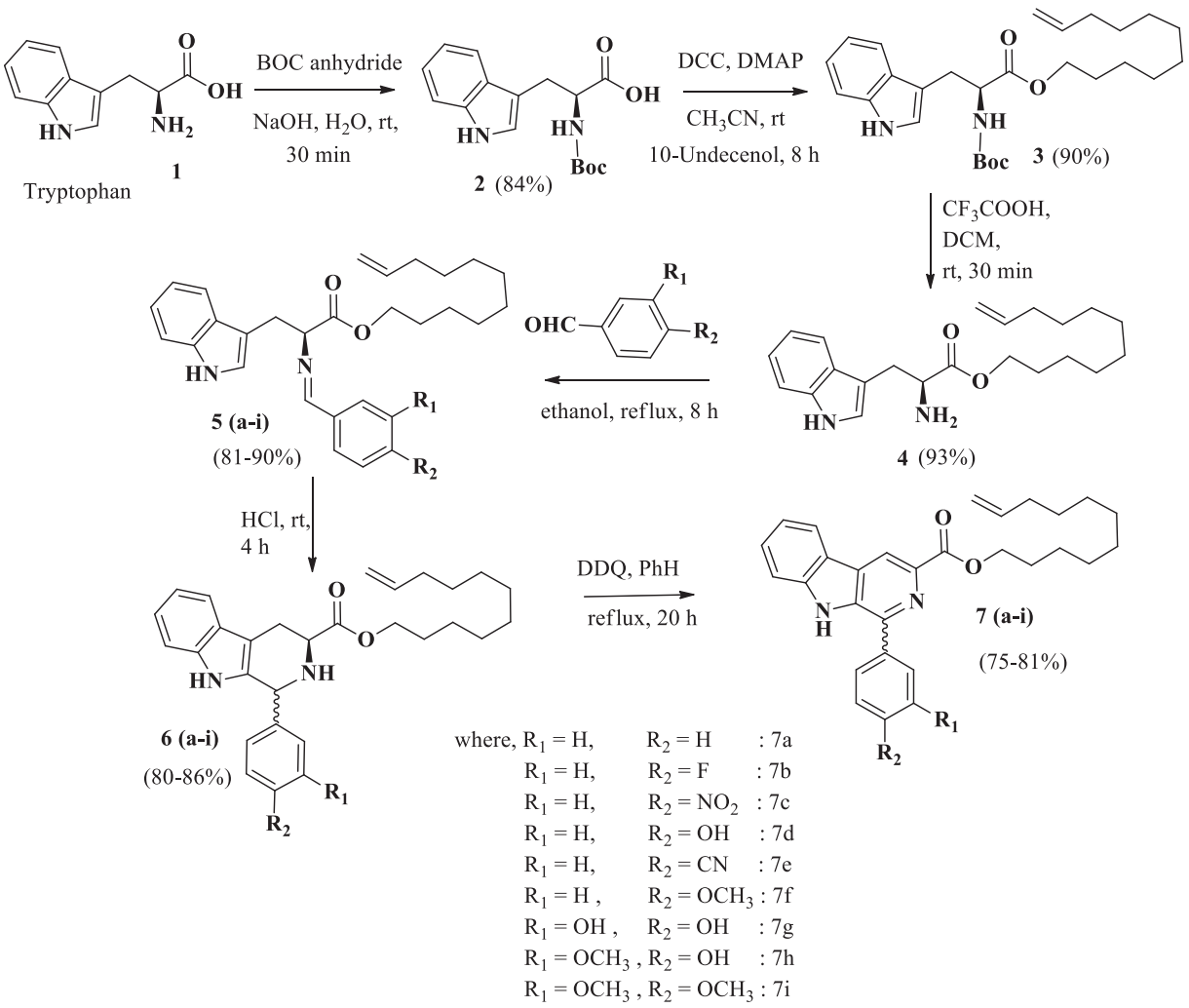
2.9.5. DPPH radical scavenging assay

Anti-oxidant activity of β-carboline derivatives (**7a-i**) was investigated by the radical scavenging potential by stable DPPH radical method as reported (Akowuah et al., 2006). Briefly, 200 μl of a methanolic solution of the synthesized β-carboline derivatives (1 mM concentrations) were added to 2 mL of a methanolic solution of the DPPH radical (1 mM concentration) and the total volume was made up to 3 mL with methanol. After 40 min of standing, the absorbance of the mixture was measured at 517 nm against methanol as blank sample. TBHQ and α-TP (1 mM concentration) were used as a positive control. The radical-scavenging activities (%) of the tested samples were analyzed by comparison with control (2 mL DPPH radical solution and 1 mL methanol). Each sample was evaluated in triplicate and the average of the three values was reported.

3. Results and discussion

3.1. Synthesis of β-carboline derivatives

Synthesis of novel β-carboline derivatives was carried out in a six step reaction procedure (Scheme 1). Synthesized products were analyzed in each step by NMR and mass spectroscopy. Initially, the amine group present on L-tryptophan (**1**) was protected with BOC anhydride (**2**) and the structure of the product **2** was confirmed by spectral studies. The signals appeared at 1.38 and 26.4 ppm in the ^1H and ^{13}C NMR spectrum indicate the presence of methyl groups of BOC anhydride. In the next step, the carboxylic group present on BOC tryptophan was esterified with 10-undecenol (**3**). The characteristic peaks at 4.08, 4.95 and 5.80 ppm in ^1H NMR spectrum indicate the appearance of protons α to the ester functional group and unsaturated protons of 10-undecenol. Carbon signals appeared at 115, 163.1 and 171.4 ppm indicate the appearance of unsaturated and ester carbons, respectively. The esterified product on acid treatment underwent for deprotection to obtain free amine (**4**). Product **4** was confirmed by the presence of free $–NH_2$ signal at 5.08 ppm and the carbon α to amine at 56.4 ppm in ^1H and ^{13}C NMR spectra. In the subsequent step amino group was condensed with various types of aldehydes to obtain Schiff bases (**5a-i**). In ^1H NMR spectra, the characteristic singlet peak appeared at 8.41–8.66 ppm for was due to the presence of –N = CH proton and it indicates the imine coupling. In addition, characteristic carbon peaks in the ^{13}C NMR appeared at 160.4–166.6 ppm due to N = CH group. In the subsequent step Schiff bases were cyclised to give cyclic products (**6a-i**). Cyclic products (**6a-i**) were confirmed by the singlet peak appeared at 5.32–5.62 ppm and the disappearance of the peaks in the range of 8.41–8.66 ppm. In ^{13}C spectrum disappearance of signal at 160.4–166.6 ppm and appearance of new signals at 56.8–62.2 ppm indicates cyclisation. The cyclic products were finally aromatized to give title products (**7a-i**). The disappeared signals at 5.32–5.62 ppm and the appearance of new signals at 7.64–7.84 ppm in the ^1H NMR spectra



Scheme 1 Synthetic procedure for the preparation of β-carboline fatty alcohol hybrids.

and the observed peaks at 144.5–148.0 ppm and 110.5–113.4 ppm in ^{13}C NMR spectra confirmed the formation of products **7a-i**. Further, the expected molecular masses observed in mass spectra positively confirmed the structures of β-carboline derivatives.

3.2. Antimicrobial activity

Synthesized β-carboline derivatives were screened for their antimicrobial activity using well diffusion method (Amsterdam, 1996) against eight microbial strains. *Bacillus subtilis* MTCC 121, *Micrococcus luteus* MTCC 2470, *Staphylococcus aureus* MLS-16 MTCC 2940, *S. aureus* MTCC 96, *Pseudomonas aeruginosa* MTCC 2453, *Escherichia coli* MTCC 739, *Klebsiella planticola* MTCC 530 and *Candida albicans* MTCC 3017 were taken to evaluate antimicrobial activity. The MIC (minimum inhibitory concentration) was studied by comparing the standard ciprofloxacin (Uzunbayir et al., 2020) and miconazole (Xu et al., 2020) as reference drugs for evaluating the antibacterial and antifungal activities, respectively. The tested compounds with MIC value less than 125 μg/mL were treated as antimicrobial active candidates and considered for further analysis (Table 1). From the table it was observed that among the all tested compounds six compounds showed activity. Among the six active compounds **7d** exhibited excellent antimicrobial activity with MIC values 2.8, 7.8, 18.2, 13.6, 2.8 μg/mL against *B. subtilis* MTCC 121, *M. luteus* MTCC 2470, *S. aureus* MLS-16 MTCC 2940, *S. aureus* MTCC 96 and *C. albicans* MTCC 3017, respectively. Compound **7f** showed good antimicrobial activity 4.7, 10.3, 28.3 and 8.6 μg/mL against *B. subtilis* MTCC 121, *M. luteus* MTCC 2470, *S. aureus* MTCC 96 and *C. albicans* MTCC 3017, respectively. Compounds **7g** and **7h** exhibited moderate antimicrobial activity 5.3, 11.8, 45.8 and 6.1, 12.6 μg/mL against *B. subtilis* MTCC 121, *M. luteus* MTCC 2470 and *B. subtilis* MTCC 121, *M. luteus* MTCC 2470, *C. albicans* MTCC 3017, respectively. Comparing to the MIC values of long-chain 2-amino-3-alkanols called as clavaminols, the compounds **7d** and **7f** exhibited similar activity (Reddy et al., 2016). On the basis of antimicrobial data, further evaluation of minimum bactericidal concentration (MBC) and biofilm inhibition assay was carried out on four bacterial strains. The synthesized six β-carboline derivatives which were potent as antimicrobial were evaluated for their MBC and it was found that the compound **7d** and **7f** showed good MBC values of 2.8, 7.8, 14.6, 7.8 and 4.7, 15.6, 28.3, 17.8 μg/mL respectively, against the four

Table 1 Antimicrobial activity of β-carboline derivatives **7 (a-i)**.

S. No	Test compounds	Minimum inhibitory concentration (μg/mL)							
		B.s[a]	*M.l*[b]	*S.a*[c]	*S.a*[d]	*P.a*[e]	*E.c*[f]	*K.p*[g]	*C.a*[h]
1	7a	7.8	13.8	> 125	> 125	> 125	> 125	> 125	> 125
2	7b	> 125	> 125	> 125	> 125	> 125	> 125	> 125	> 125
3	7c	> 125	> 125	> 125	> 125	> 125	> 125	> 125	> 125
4	7d	2.8	7.8	18.2	13.6	> 125	> 125	> 125	2.8
5	7e	> 125	> 125	> 125	> 125	> 125	> 125	> 125	> 125
6	7f	4.7	10.3	> 125	28.3	> 125	> 125	> 125	8.6
7	7g	5.3	11.8	> 125	> 125	> 125	> 125	> 125	45.8
8	7h	6.1	12.6	> 125	> 125	> 125	> 125	> 125	> 125
9	7i	7.7	> 125	> 125	> 125	> 125	> 125	> 125	> 125
10	Ciprofloxacin	0.9	0.9	0.9	0.9	0.9	0.9	0.9	0.9
11	Miconazole	> 125	> 125	> 125	> 125	> 125	> 125	> 125	7.8

[a] *B. subtilis* MTCC 121.
[b] *M. luteus* MTCC 2470.
[c] *S. aureus* MLS-16 MTCC 2940.
[d] *S. aureus* MTCC 96.
[e] *P. aeruginosa* MTCC 2453.
[f] *E. coli* MTCC 739.
[g] *K. planticola* MTCC 530.
[h] *C. albicans* MTCC 3017.

Gram-positive bacterial strains, namely *B. subtilis* MTCC 121, *M. luteus* MTCC 2470, *S. aureus* MTCC 96 and *S. aureus* MLS-16 MTCC 2940. From the Table 2 all the tested compounds showed activity against *B. subtilis* MTCC 121, compounds **7d** and **7f** being most potent showed MBC values of 2.8 and 4.7 μg/mL whereas, compound **7g** and **7h** showed good activity 13.8 and 16.4 μg/mL. The compounds **7a** and **7i** showed moderate activity 24.8, 32.3 μg/mL against *B. subtilis* MTCC 121. The obtained results were compared with Ciprofloxacin taking as standard drug which had MBC values ranged between 0.9 and 1.9 μg/mL.

Compounds **7a**, **7d**, **7f**, **7g**, **7h** and **7i** were taken for evaluation of biofilm inhibition activity as per the reported protocol (Venepally et al., 2018). According to the report the microbial strain which caused development of biofilm was appeared to tolerate antibiotics which possessed a major threat in the treatment of bacterial infections (Hall et al., 2004). The compounds synthesized in the current study were composed with β-carboline and fatty alcohol, the combination of these two moieties is expected to have potential to inhibit the development of biofilm. The compounds which are potent in antimicrobial assay were tested for biofilm inhibition assay (Table 3). From the results it was observed that the compound **7f** showed excellent anti-biofilm activity against *B. subtilis* MTCC 121, *M. luteus* MTCC 2470, *S. aureus* MTCC 96 of 1.8, 2.6 and 2.9 μM, respectively. Compounds **7d** and **7g** were showed activity against all the strains whereas, compound **7a** and **7h** showed moderate activity followed by **7i**.

On the basis of preliminary antimicrobial data, it was observed that compounds **7d**, **7f** and **7g** exhibited antifungal activity with MIC values of 7.8, 10.3 and 12.6 μg/mL, respectively, against C. *albicans* MTCC 183. Above results were considered to evaluate antifungal activity of the tested compounds against various fungal strains (Table 4). Among all the tested β-carboline derivatives compound **7d** exhibited equal activity 7.8 μg/mL comparing to standard drug miconazole (MIC, 7.8 μg/mL) and also showed excellent activity against *C. albicans* MTCC 3958 and *C. parapsilosis* MTCC 1744. Moreover,

Table 2 Minimum bactericidal concentration (MBC) data.

Test compounds	MBC (μg/mL)			
	B.s[a]	*M.l*[b]	*S.a*[c]	*S.a*[d]
7a	24.8	na	na	na
7d	2.8	7.8	7.8	14.6
7f	4.7	15.6	17.8	28.3
7g	13.8	na	56.4	na
7h	16.4	26.9	na	na
7i	32.3	na	na	na
Ciprofloxacin	1.9	0.9	1.9	0.9

[a] *B. subtilis* MTCC 121. na = no activity.
[b] *M. luteus* MTCC 2470.
[c] *S. aureus* MLS-16 MTCC 2940.
[d] *S. aureus* MTCC 96.

Table 3 Anti-biofilm activity of the selected compounds.

Test compounds	IC_{50} values in (μM)			
	B.s[a]	*M.l*[b]	*S.a*[c]	*S.a*[d]
7a	8.62 ± 0.31	12.56 ± 0.44	na	na
7d	2.24 ± 0.38	3.60 ± 0.65	7.83 ± 0.57	4.23 ± 0.46
7f	1.80 ± 0.42	2.60 ± 0.56	na	2.90 ± 0.28
7 g	6.81 ± 0.73	14.42 ± 0.48	16.55 ± 0.74	18.44 ± 0.34
7 h	8.83 ± 0.46	18.05 ± 0.35	na	28.06 ± 0.86
7i	na	21.26 ± 0.93	22.07 ± 0.52	na
ciprofloxacin	0.50 ± 0.10	0.50 ± 0.08	0.40 ± 0.09	0.30 ± 0.11

[a] *B. subtilis* MTCC 121. na = no activity.
[b] *M. luteus* MTCC 2470.
[c] *S. aureus MLS-16* MTCC 2940.
[d] *S. aureus* MTCC 96.

Table 4 Antifungal activity of compounds **7d**, **7f** and **7g**.

S. No	Test organism	MIC (μg/mL)			
		7d	7f	7g	miconazole
1	*C. albicans* MTCC 183	7.8	10.3	12.6	7.8
2	*C. albicans* MTCC 227	13.6	15.9	18.4	7.8
3	*C. albicans* MTCC 854	27.4	31.2	46.9	7.8
4	C. albicans MTCC 1637	27.4	31.2	46.9	7.8
5	*C. albicans* MTCC 3017	13.6	15.9	18.4	7.8
6	*C. albicans* MTCC 3958	7.8	10.3	12.6	7.8
7	*C. albicans* MTCC 4748	13.6	15.9	18.4	7.8
8	*C. albicans* MTCC 7315	13.6	15.9	18.4	7.8
9	*C. parapsilosis* MTCC 1744	7.8	10.3	12.6	7.8
10	*C. aaseri* MTCC 1962	13.6	15.9	18.4	7.8
11	*C. glabrata* MTCC 3019	27.4	31.2	46.9	7.8
12	*C. krusei* MTCC 3020	13.6	15.9	18.4	7.8
13	Issatchenika hanoiensis MTCC 4755	13.6	15.9	18.4	7.8

it exhibited good antifungal activity against other strains with MIC values of 13.6 and 27.4 μg/mL. The synthesized β-carboline derivatives **7f** and **7g** showed good to moderate antifungal activity with MIC values ranging between 10.3 and 46.9 μg/mL. The compounds which were active in antifungal activity were further considered to test for their minimum fungicidal concentration (MFC). The results showed that the compound **7d** exhibited MFC value of 7.8 μg/mL against *C. parapsilosis* MTCC 1744.

3.3. Structure–activity relationship

β-carboline is a naturally occurring alkaloid having many diverse biological applications. The fatty alkyl chain (undecenyl (C-11)) in the β-carboline derivatives participate in increasing the lipophilicity. The enhancement in liphophilicity further increases the biological activity for instance antibacterial and antifungal activities. β-carboline derivative (**7d**) with a hydroxyl group on the phenyl ring exhibited promising antibacterial and antifungal activities. Compounds bearing two -OH groups (**7g**) and compound with -OH, $-OCH_3$ (**7h**) exhibited good antibacterial activity and also compound bearing $-OCH_3$ **7f** showed good antifungal activity. Remaining β-carboline derivatives **7a**, **7b**, **7c** and **7e** bearing H, -F, $-NO_2$ and -CN were did not showed considerable activity. The above discussion concludes that the β-carboline derivatives containing electron donating groups like -OH, $-OCH_3$ showed superior activity against antibacterial and antifungal screening compared than those with electron withdrawing groups such as -F, $-NO_2$ and -CN. Whereas, compound **7i** did not showed activity even though consisting two $-OCH_3$ groups.

3.4. Cytotoxic activity

Fatty alcohol conjugated β-carboline moiety was expected to show cytotoxic activity, β-carboline derivatives were further screened for their anticancer activity (Reddy et al., 2016). Compounds **7a** to **7i** including doxorubicin as positive control were screened against five cell lines (Table 5). The results showed that all the synthesized β-carboline derivatives were showed good to moderate activity, the activity was reported by comparing the IC_{50} value of Doxorubicin (Zheng et al., 2020; Zhang et al., 2019). Compounds with lower or near IC_{50} values comparing to Doxorubicin were considered as good anticancer active molecules. The compounds **7a**, **7f** and **7h** showed good anticancer activity against all the cell lines. Compounds **7e** and **7g** showed moderate activity against DU 145, SKOV3 and MDA-MB 231cell lines. Specifically, com-

Table 5 Anticancer activity of β-carboline derivatives **7 (a-i)**.

S. No	Test compounds	IC_{50} values in (μM)				
		DU145	HepG2	SKOV3	MDA-MB 231	MCF7
1	7a	23.60 ± 0.62	29.60 ± 0.46	86.26 ± 0.45	12.60 ± 0.24	16.51 ± 0.18
2	7b	48.43 ± 0.54	44.80 ± 0.52	22.71 ± 0.28	22.51 ± 0.53	22.44 ± 0.87
3	7c	56.80 ± 0.67	44.80 ± 0.98	25.40 ± 0.67	24.34 ± 0.71	34.70 ± 0.28
4	7d	54.33 ± 0.46	58.43 ± 0.64	65.23 ± 0.79	23.60 ± 0.84	20.60 ± 0.49
5	7e	36.45 ± 0.27	86.27 ± 0.25	34.60 ± 0.56	26.44 ± 0.25	18.43 ± 0.28
6	7f	11.61 ± 0.34	38.60 ± 0.43	22.45 ± 0.73	9.15 ± 0.45	12.55 ± 0.18
7	7g	28.70 ± 0.76	64.70 ± 0.43	36.70 ± 0.42	20.43 ± 0.32	16.80 ± 0.47
8	7h	13.80 ± 0.63	9.43 ± 0.45	26.33 ± 0.64	11.41 ± 0.64	11.80 ± 0.56
9	7i	48.46 ± 0.49	96.50 ± 0.67	46.60 ± 0.47	27.80 ± 0.82	28.60 ± 0.51
10	Doxorubicin	0.80 ± 0.15	0.70 ± 0.14	0.70 ± 0.16	0.80 ± 0.14	0.80 ± 0.12

pounds **7f** and **7h** exhibited superior activity than all the tested compounds with IC_{50} values 9.1 and 11.4 μM against cell line MDA-MB 231. β-carboline derivatives **7f** and **7h** exhibited similar activity comparing to fatty acid constituted Schiff bases human breast adenocarcinoma cell lines MDA-MB-231 and MCF7 (Mohini et al., 2013).

3.5. Antioxidant activity

The antioxidant activity of the synthesized β-carboline derivatives were screened by the well-established DPPH radical scavenging assay (Table 6). The DPPH is widely used stable free radical to estimate the free radical scavenging capability of the compounds. The results were compared with the reference antioxidants α-tocopherol (α-TP) (Zhang et al., 2015) and *tert*-butylhydroquinone (TBHQ) (Balasundram et al., 2006). Among all the tested β-carboline derivatives, the compounds **7d**, **7g** and **7h** exhibited antioxidant activity. Specifically, compound **7g** exhibited excellent free radical scavenging activity (FRSA) of 85%, which is nearly close to control α-TP. Compounds **7d** and **7h** exhibited good free radical scavenging activity (FRSA) of 79 and 82%, respectively. From the above results compounds with phenolic hydroxyl functional groups were active in DPPH assay. This could be due to the radical scavenging efficiency of phenolic hydroxyl moieties. The superior FRSA of compound **7g** is due to the presence of more than one phenolic hydroxyl moieties (Kaki et al., 2016).

Table 6 DPPH radical scavenging activity of β-carboline derivatives **7 (a-i)**.

S. No	Test compounds	FRSA (%) at 1.0 mM concentration
1	7a	na
2	7b	na
3	7c	na
4	7d	79.55 ± 0.44
5	7e	na
6	7f	na
7	7g	85.70 ± 0.72
8	7h	82.43 ± 0.56
9	7i	26.60 ± 0.62
10	α-TP	90.23 ± 0.54
11	TBHQ	92.30 ± 0.71

na = no activity.

4. Conclusion

In the present study, β-carboline hybrids were synthesized by joining 10-undecenyl alcohol chain and their biological activities were evaluated. Among the synthesized derivatives compounds **7d** and **7f** were found to be most active in all the conducted activities. Antimicrobial studies showed that compound **7d** exhibited promising activity with MIC values of 2.8, 7.8, 18.2, 13.6, 2.8 μg/mL, MBC value 2.8 μg/mL and also showed antifungal activity equal to the control 7.8 μg/mL. Whereas, compound **7f** showed activity in both biofilm inhibition (1.8 μM) and cytotoxic assay (IC_{50} value 9.1 μM). DPPH radical scavenging assay showed that some compounds were exhibited activity especially, compound **7g** showed most potent activity with FRSA 85%. All the studies revealed that β-carboline fatty alcohol hybrids have the potential as biological active and antioxidant molecules.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.arabjc.2021.103163.

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