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Synthesis and characterization of β-carboline fatty alcohol hybrids as potential biological active and antioxidant molecules

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Abstract

Nine new β-carboline fatty alcohol hybrids were synthesized from natural amino acid L-tryptophan and hybridized with 10-undecenol 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, antibio film and anticancer. Compounds **7d** and **7f** showed most potent antimicrobial activity against *B.subtilis* MTCC 121, *M.luteus* MTCC 2470, *S.aureus* MTCC 96 and *C.albicans* MTCC 3017 bacterial 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 good antioxidant molecule than all the tested compounds.

Keywords: β-carboline; L-tryptophan; antimicrobial; anticancer; pharmacophore

Introduction

Organic molecules synthesized from naturally available renewable materials for various applications especially, biological and pharmaceutical applications are an emerging trend in modern synthetic chemistry [1, 2]. During the last two decades, numerous kinds of alkaloids were isolated from the natural sources and studied their importance in biological and pharmaceutical applications [3, 4]. Clinically natural alkaloids are famous to treat central nervous system (CNS) disorders for instance atropine, physostigmine morphine, anatabine and papaverine etc [5]. Studies have revealed that wide range of natural alkaloids are biologically active molecules and have been using to treat many pathogenic diseases [6]. β-carboline alkaloids (βCAs; 9 H-pyrido-(3,4-β)indole) are naturally available plant-derived indole alkaloids from *Peganum harmala* (*Zygophillaceae*), structurally βCAs are constituted with five and six member ring structures along with two nitrogen atoms as a part of aromatic ring [7,8]. βCAs have been found to be associated with diverse biological and pharmaceutical activities including antialzheimer, antimicrobial, anti-inflammatory, antidepressant, neuroprotective and antioxidant [9-12].

In an endeavor to develop new βCA derivatives Lopes-Ortiz *et al*., synthesized imide βCA and carbomethoxy βCA derivatives and their pharmacological properties were studied [13]. In another study, Sireesha *et al*., synthesized new benzimidazole/benzoxazole linked β-carbolines by joining two different anti-cancer fragments, these hybrid β-carbolines showed high fold cytotoxic activity against MCF-7 cell lines [14]. The data available on βCAs suggest there is still lot of potential to develop new molecules combining with other bioactive moieties. In this

account, combining of βCA with other bio active molecules such as fatty acid (FA) derivatives is the aim of our present work. Structurally fatty acids are long chain hydrocarbons containing polar carboxylic acid moiety, formed from the hydrolysis of oils and fats. Variety of modified fatty acids and fatty acid derivatives are significant molecules act against many pathogenic microbes. Combination of FA with various bio active molecules is 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 [15]. 1-β-D-Arabinofuranosylcytosine (Ara-C, Cytarabine) well known drug used in the treatment of cancer, due to its lower lipophilicity Ara-C exhibited low bioavailability. Liu *et al*., further synthesized a series of Ara-C derivatives by combining fatty acid and amino acid in order to enhance lipophilicity and bioavailability [16]. Moreover, FA analogues containing hetrocyclic moieties such as oxadiazole, triazole and thiadiazole were exhibited promising antidepressant and antimicrobial activity [17]. 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). There have been many reports on 10-undecenoic acid derived compounds synthesis and utilization for diverse applications mainly pharmaceutical and polymer applications [18, 19].

Pharmacophore conjugation is an efficient technique for covalently adjoining two biological potent moieties in to one conjugate molecule [20]. The conjugated molecules demonstrate divergent action mechanism, might lead to synergistic effect with high affinity and selectivity [21]. 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-

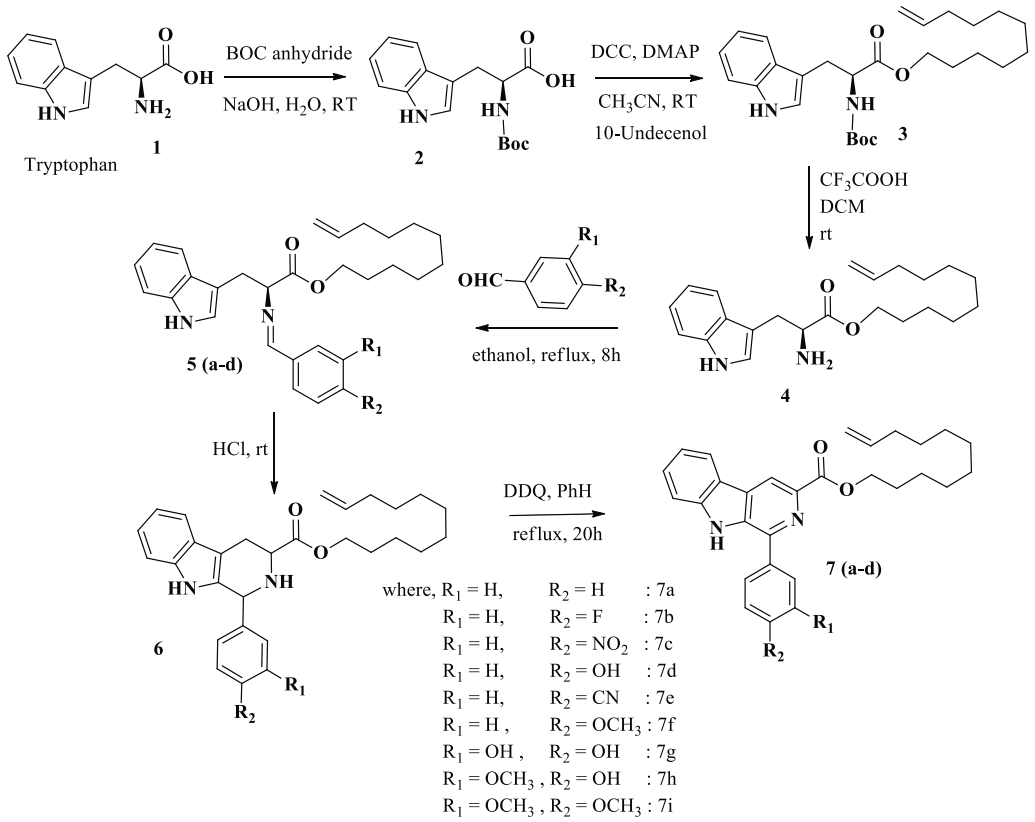
tryptophan and various aldehydes and hybridized with 10-undecenol (a derivative of 10-undecenoic acid) by ester linkage.

Results and Discussion

Synthesis of β-carboline derivatives

Synthesis of novel β-carboline derivatives was carried out in a six step reaction procedure as outlined in 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 ^{1}H and ^{13}C NMR spectrum indicate the presence of methyl groups. 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 ^{1}H 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 and171.4 ppm indicates the appearance of unsaturated and ester carbons, respectively. The esterified product on acid treatment undergone 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 ^{1}H and ^{13}C NMR spectra. In the subsequent step amine group was condensed with various types of aldehydes to attain Schiff bases (**5a-i**). In ^{1}H NMR spectra, the characteristic singlet peak appeared at 8.41-8.66 ppm for the Schiff bases (**5a-i**) were indicates the presence of -N=CH proton and it confirmed 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 indicate cyclisation. The cyclic products finally aromatized to obtain titled products (**7a-i**). The signals disappeared at 5.32-5.62 ppm and the appearance of new signals at 7.64-7.84 ppm in the ^{1}H NMR spectra and the peaks observed at 144.5-148.0 ppm and110.5-113.4 ppm in ^{13}C NMR spectra confirmed the formation of products **7a-i**. Further, the expected molecular weights observed in mass spectra positively confirm the synthesis of β-carboline derivatives.



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

Antimicrobial activity

Antimicrobial activity of the β-carboline derivatives were evaluated using well diffusion method [22] against different pathogenic reference strains. The synthesized products undergone screening for antimicrobial activity against eight pathogenic bacterial strains namely *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. The MIC (minimum inhibitory concentration) was determined by comparing the standard Ciprofloxacin and Miconazole 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. The results obtained from the antimicrobial activity analysis were presented in 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 antibacterial 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 antibacterial 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. On the basis of antimicrobial data, further evaluation of minimum bactericidal concentration (MBC) and biofilm inhibition assay was carried out on four bacterial strains [23]. The synthesized six β-carboline derivatives which are 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 Gram-positive bacterial strains, namely *B.subtilis* MTCC 121, *M.luteus* MTCC 2470, *S.aureus* MTCC 96 and *C.albicans* MTCC 3017. The results tabulated in table 2 shows that all the tested

compounds were showed activity against *B.subtilis* MTCC 121, compounds **7d** and **7f** being most potent showed MBC values 2.8, 4.7 µg/mL whereas, compound **7g** and **7h** showed good activity 13.8, 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.

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 (Standard)	0.9	0.9	0.9	0.9	0.9	0.9	0.9	>125
11	Miconazole (Standard)	>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.

Table 2: Minimum bactericidal concentration (MBC) data.

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

[a] B. subtilis MTCC 121. -[a] no activity.

[b] M. luteus MTCC 2470.

[d] S. aureus MTCC 96.

[h] C. albicans MTCC 3017.

These six compounds were further considered for evaluation of biofilm inhibition assay according to a reported protocol [24]. According to the report the bacterial strain which caused biofilm formation were appeared to tolerate antibiotics which posed a major threat in the treatment of bacterial infections [25]. The compounds synthesized in the current study were

composed with β-carboline and fatty alcohol is expected to have potential to inhibit the biofilm formation. In this regard the compounds which are potent in antimicrobial assay were tested for biofilm inhibition assay and the obtained results were placed in 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**.

Table 3: Anti-biofilm activity of the selected compounds.

Test compounds	IC_{50} values in (μM)			
	B.s[a]	*M.l*[b]	*S.a*[d]	*C.a*[h]
7a	8.6 ± 0.31	12.5 ± 0.44	_a	_a
7d	2.2 ± 0.38	3.6 ± 0.65	4.2 ± 0.46	7.8 ± 0.57
7f	1.8 ± 0.42	2.6 ± 0.56	2.9 ± 0.28	_a
7g	6.8 ± 0.73	14.4 ± 0.48	18.4 ± 0.34	16.5 ± 0.74
7h	8.8 ± 0.46	18 ± 0.35	28 ± 0.86	_a
7i	_a	21.2 ± 0.93	_a	22 ± 0.52
Ciprofloxacin (Standard)	0.5 ± 0.10	0.5 ± 0.08	0.3 ± 0.11	0.4 ± 0.09

[a] B. subtilis MTCC 121.

[b] M. luteus MTCC 2470.

[d] S. aureus MTCC 96.

[h] C. albicans MTCC 3017.

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 3017. From the above result the tested compounds were considered to evaluate antifungal activity against various fungal strains and the results were presented in 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 183, *C. albicans* MTCC 3958 and *C. parapsilosis* MTCC 1744. Moreover, 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 showed promising activity was 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.

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 3018	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

Structure–activity relationship

β-carboline is a naturally occurring versatile 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. Amino acids especially, a tryptophan derivative with high antimicrobial activity and low toxicity makes the molecules highly effective as novel candidates for both topical and systemic treatment of bacterial infections [26]. 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**) were 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 conclude 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.

Cytotoxic activity

Fatty alcohol conjugated β-carboline moiety was expected to show cytotoxic activity, β-carboline derivatives were further screened for their anticancer activity [27]. The compounds **7a** to **7i** including doxorubicin as positive control were screened against five cell lines and the results obtained were presented in 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. Compounds with lower or near IC_{50} values were considered as good anticancer activity. 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, compounds **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.

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	23.6 ± 0.62	29.6 ± 0.46	86.2 ± 0.45	12.6 ± 0.24	16.5 ± 0.18
2	7b	48.4 ± 0.54	44.8 ± 0.52	22.7 ± 0.28	22.5 ± 0.53	22.4 ± 0.87

3	7c	56.8 ± 0.67	44.8 ± 0.98	25.4 ± 0.67	24.3 ± 0.71	34.7 ± 0.28
4	7d	54.3 ± 0.46	58.4 ± 0.64	65.2 ± 0.79	23.6 ± 0.84	20.6 ± 0.49
5	7e	36.4 ± 0.27	86.2 ± 0.25	34.6 ± 0.56	26.4 ± 0.25	18.4 ± 0.28
6	7f	11.6 ± 0.34	38.6 ± 0.43	22.4 ± 0.73	9.1 ± 0.45	12.5 ± 0.18
7	7g	28.7 ± 0.76	64.7 ± 0.43	36.7 ± 0.42	20.4 ± 0.32	16.8 ± 0.47
8	7h	13.8 ± 0.63	9.4 ± 0.45	26.3 ± 0.64	11.4 ± 0.64	11.8 ± 0.56
9	7i	48.4 ± 0.49	96.5 ± 0.67	46.6 ± 0.47	27.8 ± 0.82	28.6 ± 0.51
10	Doxorubicin (Control)	0.8 ± 0.15	0.7 ± 0.14	0.7 ± 0.16	0.8 ± 0.14	0.8 ± 0.12

Antioxidant activity

The anti-oxidant activity of the synthesized β-carboline derivatives were screened by the well-established DPPH radical scavenging assay. The DPPH is widely using stable free radical to estimate the free radical scavenging capability of the compounds. The results obtained from the test were presented in table 6 and the obtained results were compared with the reference anti-oxidants α-tocopherol (α-TP) and tert-butylhydroquinone (TBHQ). 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 moiety [28].

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	_a
2	7b	_a
3	7c	_a
4	7d	79.5 ± 0.44
5	7e	_a
6	7f	_a
7	7g	85.7 ± 0.72
8	7h	82.4 ± 0.56
9	7i	26.6 ± 0.62
10	α-TP	90.2 ± 0.54
11	TBHQ	92.3 ± 0.71

Conclusion

In the present study, β-carboline hybrids were synthesized by joining 10-undecenyl alcohol chain and evaluated their biological activities. The synthesized fatty alcohol linked β-carboline derivatives (**7a-i**) containing two pharmacophoric features. Among the synthesized derivatives compounds **7d** and **7f** were found to be most active in all the activities conducted. 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%. Therefore, all the studies revealed that β-carboline fatty alcohol hybrids have the potential to develop new biological active and antioxidant molecules.

Experimental

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 of solvents was purchased from M/s SD Fine Chemicals (Mumbai, India).

Synthesis of BOC tryptophan (2)

L-tryptophan (5 g, 0.024 mol) was dissolved with stirring in mixture of 1,4-dioxane, water (20 ml) and 1N NaOH. The mixture was cooled using ice bath then BOC anhydride (5.34 g, 0.024 mol) was added to the solution. The cooled reaction mixture was stirred for 30 min, then reaction was brought to room temperature and stirred for further one hour. The reaction mixture was acidified with 1N HCl to pH 2-3, aqueous layer was extracted with ethyl acetate and 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), 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, 1H), 3.34 (d, 2H), 1.36 (s, 9H); ^{13}C NMR (75 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*): 304 $[M + H]^+$, 326 $[M + Na]^+$

Esterification of Boc-Try (3)

To a solution of 2 (1g, 0.0032 mol), DCC (0.67g, 0.0032 mol) and DMAP (0.2 g, 0.0016 mol) in acetonitrile were added and the resulted solution was stirred for 8h 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 with water. The resulted crude product was passed through silica gel column running with hexane and ethyl acetate (92:8, v/v), 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 (m, 1H), 4.95 (m, 2H), 4.69 (t, 1H), 4.08 (t, 2H), 3.32 (d, 2H), 2.14 (q, 2H), 1.62 (m, 2H), 1.38 (s, 9H), 1.24–1.20 (m, 14H, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.4 (-*C*(O)-OCH_3), 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_3), 34.6, 29.6 (-CH-*C*H_2-), 26.4-25.2 (-*C*H_3); ESIMS (*m/z*): 456 $[M + H]^+$, 478 $[M + Na]^+$

Deprotection of Boc (4)

3 (1g, 0.0022 mol) was dissolved with stirring in dry DCM, the mixture was cooled using ice bath and trifluro 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) yield obtained 93% (0.73 g); ^{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 = 7.8Hz, 2H), 5.75 (m, 1H), 5.08 (s, 1H), 4.90 (m, 2H), 4.36 (t, 1H), 4.04 (t, 2H), 3.32 (d, 2H), 2.16 (q, 2H), 1.62 (m, 2H), 1.26–1.20 (m, 14H, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.5 (-*C*(O)-OCH_3), 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 $[M + Na]^+$

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

4 (1 g, 0.0028 mol), aldehyde (0.003 mol) were dissolved in 25 mL of ethanol and refluxed for 8 h at 80-85 °C. The reaction mixture was cooled to room temperature, then extracted in to dichloromethane and washed with water. The resulted mixture was purified by column chromatography eluting with hexane and ethyl acetate (92:8, v/v) to get pure Schiff bases with good yield 81-90%.

5a: Quantities of substrates taken 4 (1 g, 0.0028 mol), benzaldehyde (0.45 g, 0.042 mol) 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 (m, 1H), 4.94 (m, 2H), 4.28 (t, 1H), 4.06 (t, 2H), 3.34 (d, 2H), 2.18 (q, 2H), 1.64 (m, 2H), 1.28–1.21 (m, 14H, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.7 (-*C*(O)-OCH_3), 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*): 440 $[M + H]^+$, 462 $[M + Na]^+$

5b: Quantities of substrates taken 4 (1 g, 0.0028 mol), 4-fluorobenzaldehyde (0.52 g, 0.042 mol) 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 (m, 1H), 4.96 (m, 2H), 4.25 (t, 1H), 4.06 (t, 2H), 3.36 (d, 2H), 2.21 (q, 2H), 1.64 (m, 2H), 1.28–1.21 (m, 14H, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.6 (-*C*(O)-OCH_3), 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*): 462 $[M + H]^+$, 484 $[M + Na]^+$

5c: Quantities of substrates taken 4 (1 g, 0.0028 mol), 4-nitrobenzaldehyde (0.63 g, 0.042 mol) 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 (m, 1H), 4.86 (m, 2H), 4.26 (t, 1H), 4.08 (t, 2H), 3.34 (d, 2H), 2.23 (q, 2H), 1.66 (m, 2H), 1.28–1.21 (m, 14H, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.6 (-*C*(O)-OCH_3), 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*): 489 $[M + H]^+$, 511 $[M + Na]^+$

5d: Quantities of substrates taken 4 (1 g, 0.0028 mol), 4-hydroxybenzaldehyde (0.51 g, 0.042 mol) 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 (m, 1H), 4.95 (m, 2H), 4.32 (t, 1H), 4.13

(t, 2H), 3.56 (d, 2H), 2.44 (q, 2H), 1.68(m, 2H), 1.28–1.21 (m, 14H, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.3 (-*C*(O)-OCH_3), 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*): 460 $[M + H]^+$, 482 $[M + Na]^+$

5e: Quantities of substrates taken 4 (1 g, 0.0028 mol), 4-formylbenzonitrile (0.55 g, 0.042 mol) yield obtained 86% (1.13 g); ^1H 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 (m, 1H), 4.96 (m, 2H), 4.35 (t, 1H), 4.06 (t, 2H), 3.48 (d, 2H), 2.36 (q, 2H), 1.58 (m, 2H), 1.28–1.21 (m, 14H, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.3 (-*C*(O)-OCH_3), 161.4 (-N=*C*H-), 140.7 (-*C*H=CH_2), 139.1 (-NH- *C*H=CH-), 132.3, 127.7, 121.7, 115.9, 111.8 (-CH=*C*H_2), 76.4 (-*C*H-N=), 65.3 (-*C*H_2-OC(O)), 36.7, 29.6 (-CH-*C*H_2-); ESIMS (*m/z*): 469 $[M + H]^+$, 491 $[M + Na]^+$

5f: Quantities of substrates taken 4 (1 g, 0.0028 mol), 4-methoxybenzaldehyde (0.57 g, 0.042 mol) yield obtained 90% (1.20 g); ^1H 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 (m, 1H), 4.98 (m, 2H), 4.32 (t, 1H), 4.08 (t, 2H), 3.83 (s, 3H), 3.36 (d, 2H), 2.28 (q, 2H), 1.60 (m, 2H), 1.28–1.21 (m, 14H, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.3 (-*C*(O)-OCH_3), 162.9, 161.8 (-N=*C*H-), 138.4 (-*C*H=CH_2), 136.2 (-NH- *C*H=CH-), 131.3, 128.4, 120.9, 116.3, 112.7 (-CH=*C*H_2), 77.5 (-*C*H-N=), 66.8 (-*C*H_2-OC(O)), 56.1 (-O*C*H_3), 36.7, 29.6 (-CH-*C*H_2-); ESIMS (*m/z*): 474 $[M + H]^+$, 496 $[M + Na]^+$

5g: Quantities of substrates taken 4 (1 g, 0.0028 mol), 3,4-dihydroxybenzaldehyde (0.57 g, 0.042 mol) yield obtained 87% (1.16 g); ^1H 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 (m, 1H), 4.96 (m, 2H), 4.36 (t, 1H), 4.09 (t, 2H), 3.48 (d, 2H), 2.35 (q, 2H), 1.66 (m, 2H), 1.28–1.21 (m, 14H, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.1 (-*C*(O)-OCH_3), 160.4 (-N=*C*H-), 149.5, 139.4 (-*C*H=CH_2), 136.2 (-NH- *C*H=CH-), 133.1, 128.4, 120.9, 116.3, 112.7 (-CH=*C*H_2), 76.5 (-*C*H-NH=), 65.4 (-*C*H_2-OC(O)), 36.7, 29.6 (-CH-*C*H_2-); ESIMS (*m/z*): 476 $[M + H]^+$, 498 $[M + Na]^+$

5h: Quantities of substrates taken 4 (1 g, 0.0028 mol), 4-hydroxy-3-methoxybenzaldehyde (0.63g, 0.042 mol) yield obtained 82% (1.13 g); ^1H 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 (m, 1H), 4.98 (m, 2H), 4.34 (t, 1H), 4.12 (t, 2H), 3.84 (s, 3H), 3.56 (d, 2H), 2.38 (q, 2H), 1.66 (m, 2H), 1.28–1.21 (m, 14H, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.2 (-*C*(O)-OCH_3), 160.8 (-N=*C*H-), 151.2, 149.5, 139.2 (-*C*H=CH_2), 135.2 (-NH- *C*H=CH-), 133.1, 127.4, 121.9, 116.8, 112.5 (-CH=*C*H_2), 76.9 (-*C*H-NH=), 65.6 (-*C*H_2-OC(O)), 56.1 (-O*C*H_3), 36.7, 29.6 (-CH-*C*H_2-); ESIMS (*m/z*): 490 $[M + H]^+$, 512 $[M + Na]^+$

5i: Quantities of substrates taken 4 (1 g, 0.0028 mol), 3,4-dimethoxybenzaldehyde (0.69 g, 0.042 mol) yield obtained yield obtained 81% (1.15 g); ^1H 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 (m, 1H), 4.96 (m, 2H),4.36 (t, 1H), 4.10 (t, 2H), 3.82 (s, 6H), 3.56 (d, 2H), 2.38 (q, 2H), 1.66 (m, 2H), 1.28–1.21 (m, 14H, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.3 (-*C*(O)-OCH_3), 160.6 (-N=*C*H-), 152.3, 149.8, 138.6 (-*C*H=CH_2), 136.2 (-NH- *C*H=CH-), 132.8, 127.9, 121.7, 115.8,

112.5 (-CH=*C*H_2), 76.7 (-*C*H-NH=), 65.5 (-*C*H_2-OC(O)), 56.3 (-O*C*H_3), 36.7, 29.6 (-CH-*C*H_2-); ESIMS (*m/z*): 504 $[M + H]^+$, 526 $[M + Na]^+$

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

Schiff base 5a-i (1g) was acidified with dilute HCl 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 and dried over Na_2SO_4. Crude products were purified by column chromatography eluting with hexane and ethyl acetate (92:8, v/v).

6a: ^{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 (m, 1H), 5.56 (s, 1H), 4.94 (m, 2H), 4.04 (t, 2H), 3.82 (t, 1H), 3.27 (d, 2H), 2.18 (q, 2H), 1.62 (m, 2H), 1.28–1.21 (m, 14H, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.4 (-*C*(O)-OCH_3), 141.4, 139.6 (-*C*H=CH_2), 136.2, 128.2, 127.4, 121.4, 119.2, 118.4, 115.7 (-CH=*C*H_2), 106.5, 65.2 (-*C*H_2-OC(O)), 61.4, 57.5 (-*C*H-NH-), 33.7, 29.6 (-CH-*C*H_2-); ESIMS (*m/z*): 444 $[M + H]^+$, 466 $[M + Na]^+$

6b: ^{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 = 8.0Hz, 2H), 7.06 (d, J = 7.4 Hz, 2H), 5.80 (m, 1H), 5.54 (s, 1H), 4.88 (m, 2H), 4.07 (t, 2H), 3.82 (t, 1H), 3.28 (d, 2H), 2.24 (q, 2H), 1.64 (m, 2H), 1.28–1.21 (m, 14H, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.8 (-*C*(O)-OCH_3), 161.8, 139.6 (-*C*H=CH_2), 136.7, 133.8, 127.4, 121.4, 118.4, 115.3 (-CH=*C*H_2), 105.6, 65.2 (-*C*H_2-OC(O)), 62.1, 33.9, 29.6 (-CH-*C*H_2-); ESIMS (*m/z*): 462 $[M + H]^+$, 484 $[M + Na]^+$

6c: ^{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 (m, 1H), 5.62 (s, 1H), 4.86 (m, 2H), 4.08 (t, 2H), 3.76 (t, 1H), 3.28 (d, 2H), 2.23 (q, 2H), 1.66 (m, 2H), 1.28–1.21 (m, 14H, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.6 (-*C*(O)-OCH_3), 147.8, 146.3, 138.7 (-*C*H=CH_2), 136.5, 128.8, 127.6, 122.8, 119.4, 115.3 (-CH=*C*H_2), 106.5, 67.2 (-*C*H_2-OC(O)), 61.6, 35.7, 29.6 (-CH-*C*H_2-); ESIMS (*m/z*): 489 $[M + H]^+$, 511 $[M + Na]^+$

6d: ^{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 (m, 1H), 5.54 (s, 1H), 4.95 (m, 2H), 4.10 (t, 2H), 3.75 (t, 1H), 3.26 (d, 2H), 2.36 (q, 2H), 1.68 (m, 2H), 1.28–1.21 (m, 14H, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.4 (-*C*(O)-OCH_3), 156.4, 139.1 (-*C*H=CH_2), 134.5, 133.6, 128.7, 124.2, 119.7, 118.8, 111.8 (-CH=*C*H_2), 104.8, 66.8 (-*C*H_2-OC(O)), 61.4, 34.8, 29.6 (-CH-*C*H_2-); ESIMS (*m/z*): 460 $[M + H]^+$, 482 $[M + Na]^+$

6e: ^{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 (m, 1H), 5.54 (s, 1H), 4.96 (m, 2H), 4.06 (t, 2H), 3.84 (t, 1H), 3.48 (d, 2H), 2.36 (q, 2H), 1.58 (m, 2H), 1.28–1.21 (m, 14H, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.6 (-*C*(O)-OCH_3), 146.2, 140.7 (-*C*H=CH_2), 137.2, 134.5, 132.3, 128.7, 121.7, 118.7, 115.9, 113.8 (-CH=*C*H_2), 106.2, 65.3 (-*C*H_2-OC(O)), 56.8, 37.4, 29.6 (-CH-*C*H_2-); ESIMS (*m/z*): 469 $[M + H]^+$, 491 $[M + Na]^+$

6f: ^{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 (m, 1H), 5.54 (s, 1H), 4.98 (m, 2H), 4.08 (t, 2H), 3.86-3.48 (t, 4H), 3.36 (d, 2H), 2.26 (q, 2H), 1.63 (m, 2H), 1.28–1.21 (m, 14H, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.6 (-*C*(O)-OCH_3), 158.4, 139.4

(-*C*H=CH_2), 135.2, 133.1, 128.4, 121.7, 115.3, 113.7 (-CH=*C*H_2), 105.8, 66.8 (-*C*H_2-OC(O)), 56.9, 56.1 (-O*C*H_3), 36.7, 29.6 (-CH-*C*H_2-); ESIMS (*m/z*): 474 $[M + H]^+$, 496 $[M + Na]^+$

6g: ^{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 (m, 1H), 5.46 (s, 1H), 4.96 (m, 2H), 4.09 (t, 2H), 3.78 (t, 1H), 3.36 (d, 2H), 2.44 (q, 2H), 1.66 (m, 2H), 1.28–1.21 (m, 14H, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.8 (-*C*(O)-OCH_3), 145.8, 139.4 (-*C*H=CH_2), 134.2 , 133.1, 130.4, 121.6, 119.9, 116.3, 112.7 (-CH=*C*H_2), 106.3, 65.4 (-*C*H_2-OC(O)), 60.7, 36.7, 29.6 (-CH-*C*H_2-); ESIMS (*m/z*): 476 $[M + H]^+$, 498 $[M + Na]^+$

6h: ^{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 = 8.0Hz, 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 (m, 1H), 5.32 (s, 1H), 4.98 (m, 2H), 4.06 (t, 2H), 3.82-3.52 (t, 4H), 3.34 (d, 2H), 2.40 (q, 2H), 1.68 (m, 2H), 1.28–1.21 (m, 14H, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.3 (-*C*(O)-OCH_3), 147.2, 139.2 (-*C*H=CH_2), 135.2 , 133.1, 130.4, 127.4, 121.9, 116.8, 112.5 (-CH=*C*H_2), 104.2, 66.4 (-*C*H_2-OC(O)), 58.3, 56.1 (-O*C*H_3), 36.7, 29.6 (-CH-*C*H_2-); ESIMS (*m/z*): 490 $[M + H]^+$, 512 $[M + Na]^+$

6i: ^{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 = 8.0Hz, 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 (m, 1H), 5.34 (s, 1H), 4.96 (m, 2H), 4.10 (t, 2H), 3.83-3.56 (m, 7H), 3.46 (d, 2H), 2.28 (q, 2H), 1.64 (m, 2H), 1.28–1.21 (m, 14H, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.2 (-*C*(O)-OCH_3), 149.6, 147.8, 138.6 (-*C*H=CH_2), 136.4, 132.8, 130.6, 127.3, 121.7, 118.6, 115.8, 113.5, 112.2 (-CH=*C*H_2), 106.8, 65.5 (-*C*H_2-OC(O)), 57.8, 56.3 (-O*C*H_3), 36.7, 29.6 (-CH-*C*H_2-); ESIMS (*m/z*): 504 $[M + H]^+$, 526 $[M + Na]^+$

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

6a-i (1g), DDQ (0.6g, 0.0026 mol) 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).

7a: Quantities of substrates taken 6a (1 g, 0.0022 mol), DDQ (0.99 g, 0.0044 mol) yield obtained 87% (0.85 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 (m, 1H), 4.94 (m, 2H), 4.26 (t, 2H), 2.18 (q, 2H), 1.78 (m, 2H), 1.29–1.24 (m, 14H, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.3 (-*C*(O)-OCH_3), 158.2, 146.9, 141.4, 139.6 (-*C*H=CH_2), 132.4, 129.2, 127.4, 123.5, 121.4, 119.2, 118.4, 115.7 (-CH=*C*H_2), 111.3, 103.5, 65.2 (-*C*H_2-OC(O)), 33.7, 29.6 (-CH-*C*H_2-); ESIMS (*m/z*): 440 $[M + H]^+$, 462 $[M + Na]^+$; 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, 0.0021 mol), DDQ (0.95 g, 0.0042 mol) yield obtained 80% (0.81 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 (m, 1H), 4.88 (m, 2H), 4.24 (t, 2H), 2.24 (q, 2H), 1.64 (m, 2H), 1.28–1.21 (m, 14H, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.4 (-*C*(O)-OCH_3), 160.5, 158.2, 148.0, 141.3, 139.6 (-*C*H=CH_2), 137.0, 136.7, 133.8, 130.4, 127.4, 123.6, 121.4, 118.4, 116.3 (-CH=*C*H_2), 111.2, 105.6, 65.2 (-*C*H_2-OC(O)), 33.9, 29.6 (-CH-*C*H_2-); ESIMS (*m/z*): 458 $[M + H]^+$, 480 $[M + Na]^+$]$^+$; 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, 0.0020 mol), DDQ (0.90 g, 0.0040 mol) yield obtained 78% (0.84 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 (m, 1H), 4.86 (m, 2H), 4.26 (t, 1H), 2.23 (q, 2H), 1.66 (m, 2H), 1.28–1.21 (m, 14H, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.4 (-*C*(O)-OCH_3), 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*): 485 $[M + H]^+$, 507 $[M + Na]^+$; 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, 0.0021mol), DDQ (0.95 g, 0.0042 mol) yield obtained 81% (0.82 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 (m, 1H), 4.95 (m, 2H), 4.24 (t, 2H), 2.36 (q, 2H), 1.68 (m, 2H), 1.28–1.21 (m, 14H, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.8 (-*C*(O)-OCH_3), 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*): 456 $[M + H]^+$, 478 $[M + Na]^+$; 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, 0.0021mol), DDQ (0.95 g, 0.0042 mol) yield obtained 78% (0.80 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), 5.92 (m, 1H), 4.96 (m, 2H), 4.20 (t, 2H), 2.28 (q, 2H), 1.58 (m, 2H), 1.28–1.21

(m, 14H, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 162.8 (-*C*(O)-OCH_3), 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*): 465 $[M + H]^+$, 487 $[M + Na]^+$; 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, 0.0021mol), DDQ (0.95 g, 0.0042 mol) yield obtained 79% (0.82 g); ^1H 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 (m, 1H), 4.98 (m, 2H), 4.18 (t, 2H), 3.83 (s, 3H), 2.26 (q, 2H), 1.63 (m, 2H), 1.28–1.21 (m, 14H, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.2 (-*C*(O)-OCH_3), 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*): 470 $[M + H]^+$, 492 $[M + Na]^+$; HRMS (m/z): [M + H]+ calcd for $C_{30}H_{34}N_2O_3$, 470.25684; found, 470.25670.

7g: Quantities of substrates taken 6g (1 g, 0.0021mol), DDQ (0.95 g, 0.0042 mol) yield obtained 75% (0.78 g); ^1H 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 = 7.4Hz, 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 (m, 1H), 4.96 (m, 2H), 4.25 (t, 2H), 2.44 (q, 2H), 1.66 (m, 2H), 1.28–1.21 (m, 14H, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.4 (-*C*(O)-OCH_3), 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*): 472 $[M + H]^+$, 494 $[M + Na]^+$; HRMS (m/z): [M + H]+ calcd for $C_{29}H_{32}N_2O_4$, 472.23516; found, 472.23385.

7h: Quantities of substrates taken 6h (1 g, 0.0020 mol), DDQ (0.90 g, 0.0040 mol) yield obtained 71% (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 (m, 1H), 4.98 (m, 2H), 4.20 (t, 2H), 3.82 (t, 3H), 2.40 (q, 2H), 1.68 (m, 2H), 1.28–1.21 (m, 14H, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 162.9 (-*C*(O)-OCH_3), 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*): 486 $[M + H]^+$, 508 $[M + Na]^+$; 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, 0.0019 mol), DDQ (0.86 g, 0.0038 mol) yield obtained 65% (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 (m, 1H), 4.96 (m, 2H), 4.28 (t, 2H), 3.82 (s, 6H), 2.28 (q, 2H), 1.64 (m, 2H), 1.28–1.21 (m, 14H, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$) δ 162.8 (-*C*(O)-OCH_3), 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*): 500 $[M + H]^+$, 522 $[M + Na]^+$; HRMS (m/z): [M + H]+ calcd for $C_{31}H_{36}N_2O_4$, 500.26642; found, 500.26488. Biological activities and antioxidant assay methods were given in Supplementary data.

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