Mass Spectrometry (LC-MS-MS) as a Tool in the Maillard Reaction Optimisation and Characterisation of New 6-methoxy-tetrahydro-β-carboline Derivatives

(Spektometri Jisim (LC-MS-MS) Sebagai Alat dalam Pengoptimuman Tindak Balas Maillard
dan Pencirian Terbitan Baru 6-metoksi-tetrahidro-β-karbolina)

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ABSTRACT

*Four new 6-methoxy-tetrahydro-β-carboline derivatives (**1**-6- methoxy-1-phenyl-2,3,4,9-tetrahydro-1H-pyrido[3,4-b] indole, **2**-6-methoxy-1-(4-methoxyphenyl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole, **3**-6-methoxy-1-methyl-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole and **4**-2-methoxy-4-(6-methoxy-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indol-1-yl) phenol) were prepared via the Maillard reaction using 5-methoxytryptamine and various aldehydes in water. The synthesis reaction conditions were optimised in catalyst loading, temperature and time using LC-MS for optimum yields. Surface response methodology and contour plot was selected as an approach for optimisation. The optimum yield could be achieved below 50ºC within 5 h at 7 mole % catalyst loading at yields > 70%. The β-carboline compounds produced were characterised using electrospray ionization mass spectrometry (ESI-MS) and electrospray tandem mass (ESI-MS/MS). The mass fragmentation patterns of this group of heterocyclic tetrahydro-β-carboline compounds are described herein.*

Keywords: Catalyst loading; heterocyclic; optimization; 5-methoxytryptamine; 6-methoxy-tetrahydro-β-carboline

ABSTRAK

*Empat terbitan baru 6-metoksi-tetrahidro-β-karbolina (**1**-6- methoksi-1-fenil-2,3,4,9-tetrahidro-1H-pirido[3,4-b]indol, **2**-6-metoksi-1-(4-metoksifenil)-2,3,4,9-tetrahidro-1H-pirido[3,4-b]indol, **3**-6-metoksi-1-metil-2,3,4,9-tetrahidro-1H-pirido[3,4-b]indol dan **4**-2-metoksi-4-(6-metoksi-2,3,4,9-tetrahidro-1H-pirido[3,4-b]indol-1-yl)fenol) telah disediakan melalui tindak balas Maillard dengan menggunakan 5-metoksitriptamin dan pelbagai aldehid dalam air. Keadaan tindak balas sintesis telah dioptimumkan daripada segi muatan mangkin, suhu dan masa dengan menggunakan LC-MS untuk hasil yang optimum. Kaedah gerak balas permukaan dengan plot kontur telah dipilih sebagai satu pendekatan untuk pengoptimuman. Hasil yang optimum telah dicapai pada suhu bawah 50ºC dalam masa 5 jam pada muatan mangkin 7% mol dengan hasil pemerolehan > 70%. Sebatian β-karbolina yang dihasilkan telah dicirikan dengan menggunakan spektrometri jisim pengionan semburan elektron (ESI-MS) dan jisim seiring semburan elektron (ESI-MS/MS). Corak fragmentasi kumpulan sebatian heterosiklik tetrahidro-β-karbolina telah diterangkan di sini.*

Kata kunci: Heterosiklik; muatan mangkin; pengoptimuman; 5-metoksitriptamin; 6-metoksi-tetrahidro-β-karbolina

INTRODUCTION

β-Carbolines are naturally occurring compounds produced during food preparation, processing and storage (Herraiz & Galisteo 2004; Herraiz et al. 2003; Teik Beng et al. 2009, 2003). It is well established that these alkaloids may be generated under mild conditions (pH > 5.0 and temperature below 100ºC) in foods from a Maillard condensation reaction of indoleamines, such as L-tryptophan, with short aliphatic aldehydes (Herraiz & Galisteo 2004; Herraiz et al. 2003). These β-carboline compounds are consumed diet and are stored and transported in biological fluids. These compounds may protect the body from radical damage (Herraiz et al. 2003) and may play a role in the protection of the human eye lens against oxidative stress (Pari et al. 2000). Aromatic aldehydes may produce novel tetrahydro-β-carbolines in foods during processing or cooking through a condensation reaction with L-tryptophan (Herraiz & Galisteo 2004; Herraiz et al. 2003).

β-Carboline was previously prepared using the Maillard reaction to condense tryptophan and formaldehyde in H_2O using mineral acid, HCl, as a catalyst, which provided a reasonable yield (Herraiz et al. 2003). However, the reaction is very slow, requiring longer time to complete even at an elevated temperature of 70ºC. Furthermore, using tryptophan as the starting material results in the formation of the cis and trans isomers in an equivalent ratio. Thus, an additional separation step utilising chiral chromatography is necessary to isolate the correct enantiomer. Benjamin et al. (2006) demonstrated that the reaction of tryptamine with formaldehyde does not favour the endo-cyclisation of aldimines but gives only the homo-aldol condensation product, which leads to imine formation. 5-methoxytryptamine was chosen as one of the starting materials for this reaction since it was hypothesised that the 5-methoxytryptamine might be more reactive than tryptamine and might favour cyclisation by

virtue of Thorpe-Ingold effect to produce β-carbolines. The reaction was performed under aqueous conditions due to the significant enhancement in the rate of the reaction in water as reported in the literature (Larghi et al. 2005; Saha et al. 2007) In addition, water is a safe, economical and environmentally friendly solvent to work with.

In this study, we use various aldehydes, including acetaldehyde, benzaldehyde, anisaldehyde and vanillin to give the targeted substituent group of methyl, phenyl, methoxyphenyl and hydroxymethoxyphenyl at position C-1 of the 6-methxoy scaffold of β-carboline nucleus (Figure 1), employing trifluoroacetic acid (TFA) as the catalyst. These compounds were prepared since these derivatives have not yet been synthesised and reported in the literature. Previous studies have demonstrated the aforementioned reaction scheme on other tetrahydro-ß-carboline analogues with excellent yield (Herraiz et al. 2003), but no full MS-MS spectral fragmentation characterisation and identification have been performed on 6-methoxy scaffold ß-carboline derivatives. An additional goal of this study was to optimise the existing Maillard reaction process with a more reactive 5-methoxytryptamine as a precursor to prepare a variety of 6-methoxy β-carbolines derivatives at a higher yield and conversion rate (> 90%) within shorter reaction times (<300 min) under relatively mild reaction conditions (< 50°C). All of the compounds were subjected to LC-MS/MS fragmentation analysis.
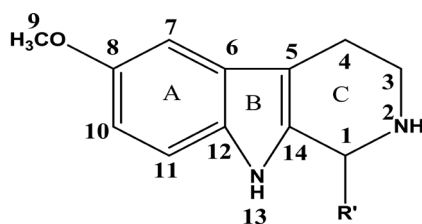
R' =Phenyl ring, methyl, methoxyphenyl and hydroxyphenyl

FIGURE 1. 6-methoxy-tetrahydro-ß-carbolines

MATERIALS AND METHODS

GENERAL

The DSC Pyris 6 (PerkinElmer Inc., Waltham, Massachusetts, USA) differential scanning calorimetry with STARe software (PerkinElmer Inc.) was used for melting point measurement. IR spectra were recorded as KBr pellets on a Thermal Scientific Nicolet 6700 spectrophotometer with Omnic software (Franklin, USA). All NMR was performed on a Bruker 500.13 MHz FT-NMR spectrometer (Bruker, Bremen, Germany) interfaced with a BBO 5 mm-1H probe operating at 25°C and the chemical shifts were referenced to tetramethylsilane (0 ppm) and the residual solvent signal (CDCl$_3$: δ H 7.26, δ C 77.0; MeOD: δ H 3.0, δ H 5.0 and δ C 50.0). The samples were dissolved in 600 μL of CD$_3$Cl or MeOD containing 1 mM tetramethylsilane (TMS) and transferred to 5 mm NMR tubes for analysis.

OPTIMISATION PROCEDURE

Approximately 1.0-1.5 equivalent mol of aldehyde (Acetaldehyde, Benzaldehyde, Anisaldehyde and Vanilin) were used by this modified Maillard reaction to prepare the new 6-methoxy-tetrahydro-ß-carboline derivatives. The experiments were performed under various reaction conditions: At temperature (25, 40, 60, 70°C), time (60, 180, 300, 700 min) and TFA (Trifluoroacetic acid) as catalyst (1, 5, 10 mole %) in aqueous to prepare for (1), (2), (3) and (4). The progress of reaction was monitored by LCMS. Yields of the four compounds were obtained by LCMS analysis. The three independent variables were reaction temperature, catalyst loading and time and the dependant variable was yields. A three–dimensional (3D) surface plot was generated according to a quadratic general linear model best fit for the data of this three factors' full factorial design. Minitab 16 statistical software was utilized for the optimization of synthesis reaction conditions in order to investigate the effects of temperature, time and catalyst loading factors on the product yields (Teik Beng et al. 2013).

The LCMS system consisted of a Daltonic Bruker Microtof-Q II 10277 Separation Module and a Hystar software system (BrukerDaltonik GmbH, Bremen, Germany). A reversed-phase Acclaim Polar Advantage II C18 column (2.1×150 mm, 3 μmi.d. particle size) connected with a Sentry guard column (20×2.0 mm i.d., 2.5 μm particle size) from Waters (Milford, MA, USA) was used. The column was thermostatically set at 35°C. The binary mobile phase used consisted of (A) water-formic acid (99.9:0.1% v/v) and (B) CH$_3$CN. The gradient elution program was set to 10–90% B (0–5 min), 90–10% B (5–15 min), 90–10% A (15–25 min) and 10–90% A (25–30 min). The mobile phase was prepared fresh, filtered through a 0.22 μm polytetraflouroethylene membrane filter and degassed by sonication for 20 min before used. The flow rate was maintained at 0.20 mL/min. The standard and samples were prepared at a concentration of 1 mg/mL in the B phase mobile phase. The injection volume was 20 μL.

The high resolution mass (HR-MS) was obtained using Daltonic Bruker Microtof-Q II 10277 LC-MS (Bruker, Germany) using electrospray ionization (ESI) in positive mode. Nominal mass was obtained by API 2000 quadrupole mass detector (Bruker, Germany) with a cone voltage of 50 V, collision energy of 10 eV, quadrupole ion energy of 5.0 eV, nebulizer of 0.20 bar and a calibrated mass range of 49 to 1501 m/z.

RESULTS AND DISCUSSION

When the L-tryptophan was replaced with a more reactive 5-methoxytryptamin in this modified Maillard reaction, the reaction time was shortened to 5 h and we managed to obtain the 6-methoxy-tetrahydro-β-carboline products in good yields (> 70%) and with negligible impurities (< 5.00%) as confirmed by LC-ESI-MS-MS accurate mass and fragmentation data (Tables 2-3), under

a moderate reaction temperature (50ºC) within 300 min. This indicates 5-methoxytryptamine favors cyclization to form 6-methoxy-tetrahydro-ß-carboline derivatives. The reaction temperature was 50°C, which is 20ºC lower than that reported by Herraiz and Galisteo (2004). The reaction can be completed at 25ºC within 60 min if an aliphatic acetaldehyde is used. This was not unexpected since Benjamin et al. (2006) has demonstrated in a pioneering study that 5-methoxytryptamine was a more reactive substrate than tryptamine in promoting the endo-cyclization of aldimines to form ß-carboline. This outcome was especially striking when we obtained yields of up to 70% in reactions with the electron poor aromatic benzaldehyde, anisaldehyde and vanillin. However, the 6-methoxy-tetrahydro-β-carbolines end products ((1), (2) and (4)) produced from the electron poor aromatic benzaldehyde, anaisaldehyde and vanillin, respectively; required higher temperature (50ºC) compared to (3), which derived from electron rich aliphatic acetaldehyde at room temperature (25ºC). The yield, enantiomer purity and rate of this Maillard reaction were significantly improved even though milder reaction conditions were employed (Table 1) when compared to those reported by Herraiz et al. (2003). Replacing 0.10 N HCl with 10 % TFA may have contributed to the more favourable results (Table 1) (Herraiz & Galisteo 2004).

Herein, we report the condensation of an amine and an aldehyde to give to 6-methoxy-tetrahydro-β-carbolines in aqueous condition in the presence of trifluoroacetic acid catalyst to be a mild, convenient and simple procedure. This approach is different from the traditional Maillard reaction protocol involving aprotic solvents (dichloromethane and chloroform) wherein aldehydes bearing electron-donating substituents failed to undergo cyclisation to form ß-carboline (Larghi et al. 2005; Saha et al. 2007). This outcome might be attributed to the decrease in the reactivity of the iminium ion intermediate derived from aldehydes bearing the electron-donating group, which in turn preventing the formation of tetrahydro-β-carbolines (Larghi et al. 2005; Saha et al. 2007). Thus, the aqueous reaction condition appears to be a more efficient medium than toluene and dichloromethane in promoting the formation of 6-methoxy-tetrahydro-β-carbolines (Larghi et al. 2005; Saha et al. 2007). Although this study focused on finding a mild and facile laboratory scale route to new 6-methoxy scaffold β-carbolines synthesis, this work might has potential value in controlling the β-carboline formation during food processing.

DOE optimisation of organic synthesis condition (Arisseto et al. 2008; Erica et al. 2013; Farah et al. 2012; Jintao et al. 2000; Teik Beng et al. 2013) showed that temperature and catalyst was significant factors that determine the yield of this Maillard reaction (Figures 2-3). It was evident that both the temperature and time exhibit positive effects on the product yield of 6-methoxy-tetrahydro-β-carboline. Generally, a temperature of more than 50ºC is required to obtain satisfactory yield (> 70%) within 5 h. Therefore, temperature is a determinant factor for optimum yields. Longer time is significantly important to obtain satisfactory yield (> 90%) involving aliphatic acetaldehyde at ambient temperature (25ºC). However, with aromatic aldehydes (vanillin, anisaldehyde and benzaldehyde), elevated temperature are required. In conclusion, the yields of 6-methoxy-tetrahydro-β-carboline derivatives ((1), (2), (3) and (4)) are dependant on the synergistic effects of both the time and temperature.

Figures 2-3 show that in order to obtain satisfactory yields (> 70%) with a temperature less than 50ºC, more than 7.0 mole % trifluoroacetic acid (catalyst) is required. Only (3) can achieve relative good yield (> 70%) at room temperature (25°C) with 7.0 mole % catalyst. The yield dependency on catalyst and temperature was on the order of (4) > (2) > (1) > (3). Maillard reaction with aromatic aldehydes (vanillin, anisaldehyde, benzaldehyde) required higher catalyst loading and temperature to achieve the required yield compared to aliphatic acetaldehyde. The contour plot (Figure 3) shows that the synergistic effects of both catalyst loading and temperature are crucial and significant factors to achieve good yields.

Identifications of the compounds (1), (2), (3) and (4) were done by comparing the HRMS-ESI protonated precursor molecular mass values with the references in the ChemSpider library and their retention indexes in the base peak chromatogram (BPC) (Table 2). (1) was observed as a protonated parent molecular mass of 279.1533 at a retention time of 8.90 min, (3) with a protonated parent molecular mass of 217.1349 at 7.60 min, (2) with an protonated parent molecular mass of 309.1630 was at 9.10 min retention time and a found experimental protonated parent molecular mass of 325.1556 was represented by compound 4 at 6.40 min retention time as shown in Table 2. The observed and calculated molecular mass agreed within 0.1 % for all of the four compounds (Table 2). Among the pure compounds, (3) was obtained with the highest yield of 92.30 %, followed by (1) with 84.40 %, (2) with 78.36 % and (4) at 74.26 % (Table 2).

TABLE 1. Summary of optimum reaction condition and yields

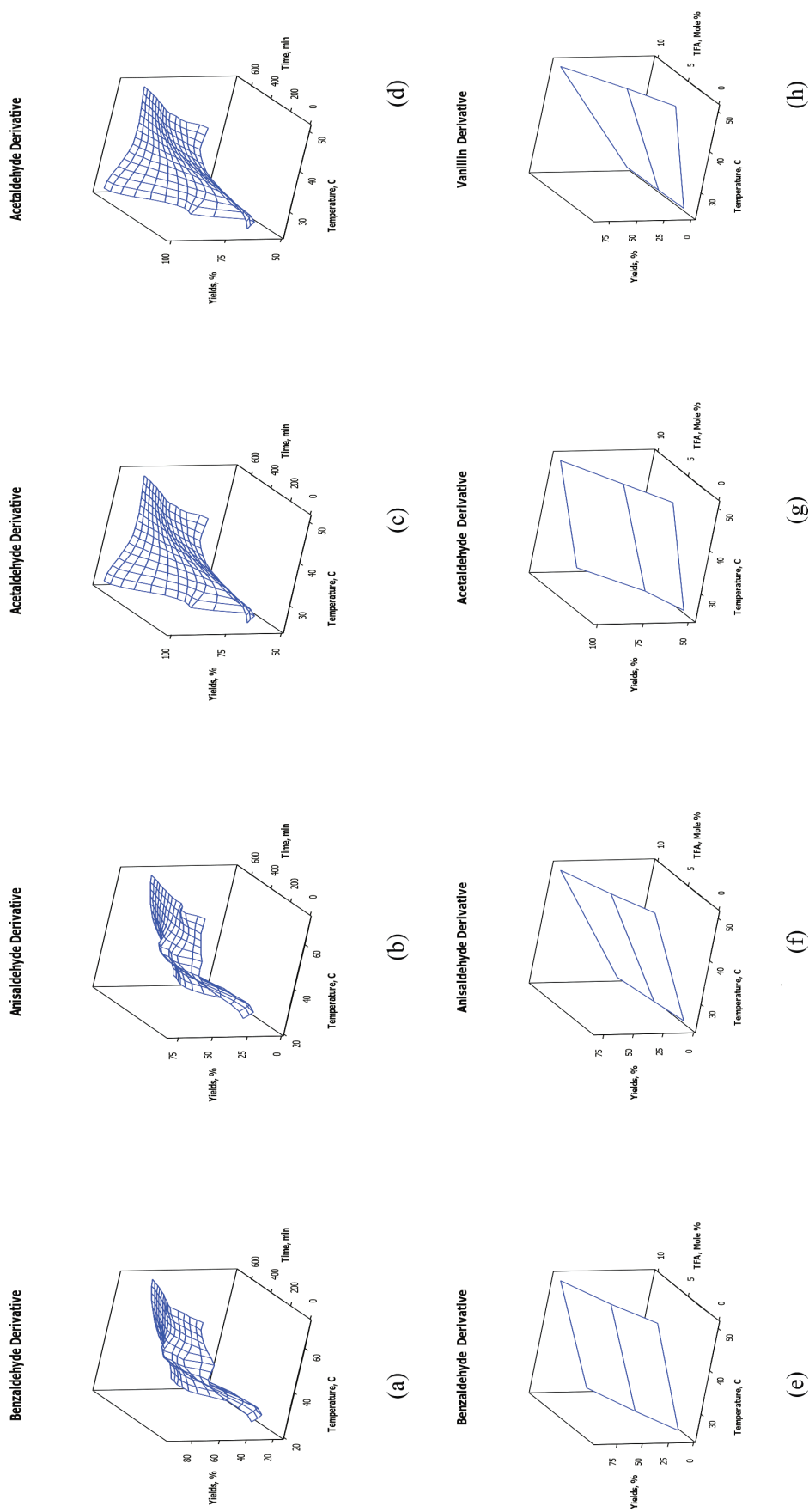| No	Compound	Reaction conditions	Purity, %	Yield, %	Conversion, %
1	(1)	50 ºC, 5 h, 10 mol % TFA	98.02	84.40	100.0
2	(2)	50 ºC, 5 h, 10 mol % TFA	98.85	78.36	100.0
3	(3)	25 ºC, 5 h, 10 mol % TFA	98.22	92.30	100.0
4	(4)	50 ºC, 5 h, 10 mol % TFA	97.67	74.26	100.0

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FIGURE 2. 3D Surface response plot showing synergistic effects of temperature (°C) and time (min.) on yields (%) [a-d; a-(1), b-(2),c-(3), d-(4)] and synergistic effects of catalyst loading (mole %) and temperature on yields (%) [a-d; a-(1), b-(2),c- (3), d- 4)] of Maillard reaction in water using 5-methoxytryptamine
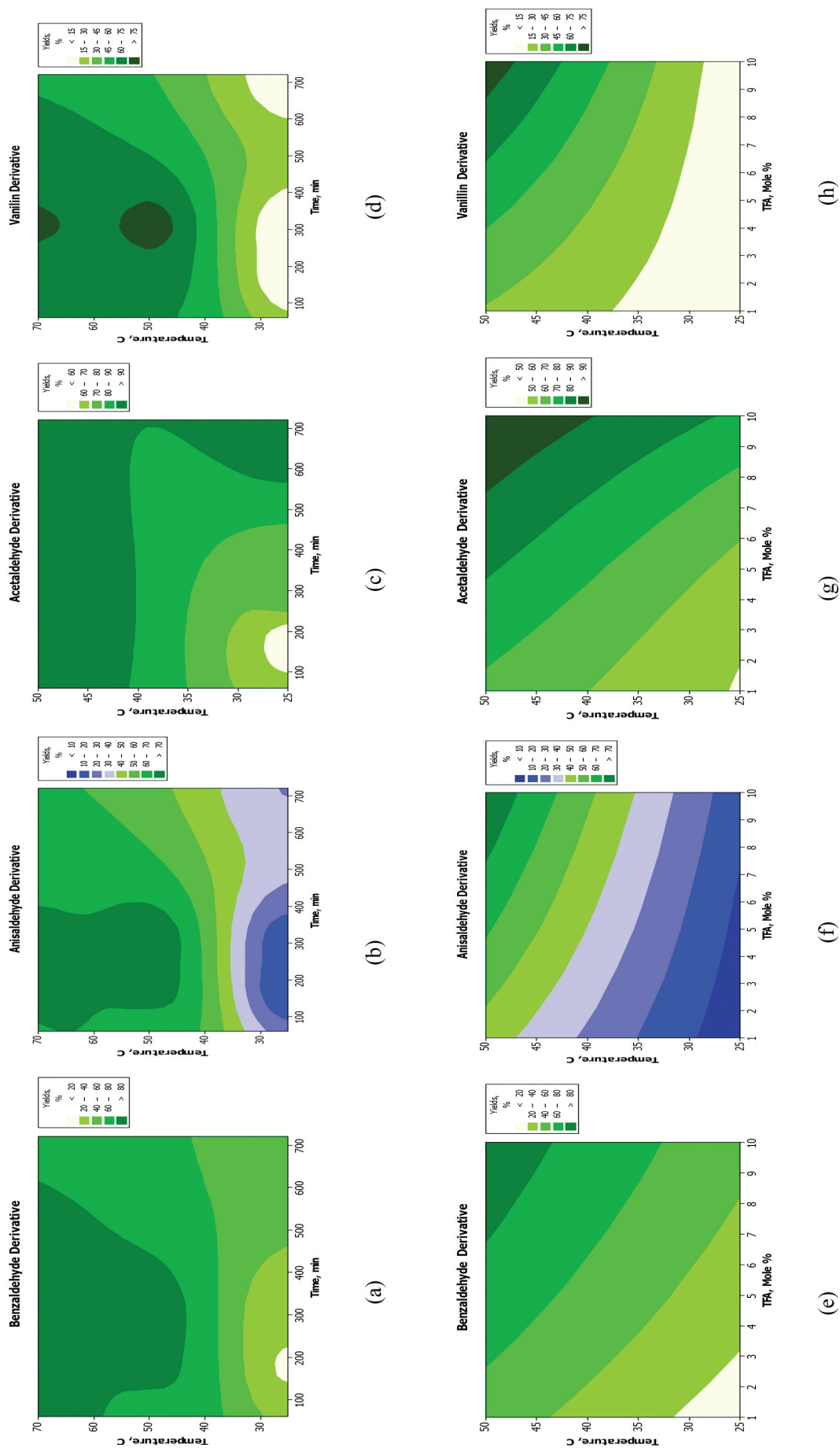
FIGURE 3. Contour plot for synergistic effects of temperature (°C) and time (min.) on yields (%) [(a-d; a-(1), b-(2),c-(3), d-(4)] and synergistic effects of catalyst loading and temperature on yields (%) [a-d; a-(1), b-(2),c- (3), d-(4)]] of Maillard reaction in water using 5-methoxytryptamine

TABLE 2. Comparison of the protonated HR-LCMS found value, calculated protonated MS value and BPC retention time indexes of the pure 6-methoxy-tetrahydro-ß-carboline derivatives

No.	Bioactive compounds		Parameters
1	Compound (1)		
	a)	Protonated HR-MS value	279.1553
	b)	Calculated protonated MS value	279.3555
	c)	% difference of (a) and (b)	< 0.10
	d)	BPC retention time, min	8.90
	e)	Yield, %	84.40
2	Compound (2)		
	a)	Protonated HR-MS value	309.1630
	b)	Calculated protonated MS value	309.3814
	c)	% difference of (a) and (b)	< 0.10
	d)	BPC retention time, min	9.10
	e)	Yield, %	78.36
3	Compound (3)		
	a)	Protonated HR-MS value	217.1349
	b)	Calculated protonated MS value	217.2862
	c)	% difference of (a) and (b)	< 0.10
	d)	BPC retention time, min	7.60
	e)	Yield, %	92.30
4	Compound (4)		325.1606
	a)	Protonated HR-MS value	325.3808
	b)	Calculated protonated MS value	< 0.10
	c)	% difference of (a) and (b)	6.40
	d)	BPC retention time, min	74.26
	e)	Yield, %	

Remarks: Data obtained from S1,S4, S7 and S10

(1) has a molecule formula of $C_{18}H_{18}N_2O$ with high resolution (ESI-TOF-HR-MS-MS) accurate protonated parent molecular weight $[M+1]^+$ m/z (%) 279.1533 (100) (Table 2) and its mass spectrum showed fragment ion peaks at m/z 262.1239 $[M + H – 17]^+$ (5), 250.1254 $[M + H - 28]^+$ (80), 236.1082 $[M + H -43]^+$ (10) (Table 3). Loss of $[CNH_3]^+$ from the protonated parent molecular ion gave a higher abundance peak at m/z 250.1254 $[M + H - 29]^+$(80) (Figure 4 & Table 3). Their relative intensity depends on the products ion stability, m/z 250.1254 is the most stable products ion compared to m/z 236.1082 and m/z 262.1239. The molecular formula of $C_{18}H_{16}N_2O$ supports eleven degree of unsaturation. The mass fragmentation theoretical pathway was as proposed in Figure 4.

(2) has a molecular formula of $C_{19}H_{20}N_2O_2$ with high resolution (ESI-TOF-HR-MS-MS) accurate protonated molecular weight $[M+1]^+$ m/z (%) 309.1630 (100) (Table 2). Its mass spectrum showed fragment ion peaks at m/z 292.1339 $[M + H – 17]^+$ (10), 280.1340 $[M + H – 29]^+$ (30), 266.1180 $[M + H – 43]^+$ (20) (Table 3). The molecular formula of $C_{18}H_{16}N_2O$ supports eleven degree of unsaturation. The postulated fragmentation pathway is as per listed in Figure 5.

The ESI-HR-MS-MS spectrum of (3) exhibited a protonated parent molecular ion peak at m/z 217.1349 (Table 2) that corresponds to the molecular formula $C_{13}H_{16}N_2O$. Its mass spectrum showed fragment ion peaks at m/z 200.1076 $[M + H – 17]^+$(90), 188.1070 $[M + H – 29]^+$ (55), 174.0907 $[M + H – 43]^+$ (5) (Figure 6 and Table 3). The molecular formula of $C_{18}H_{16}N_2O$ supports seven degree of unsaturation. The LC-ESI-TOF-HR-MS-MS mass spectrum contains a signal corresponding to the product ion at m/z = 200.1076 and indicates a loss of 12 Da to form the ion with m/z = 188.1070 (Figure 6). Cyclic ring (Figure 6 and Table 3). The postulated fragmentation pathway is as per listed in Figure 6.

The observed protonated parent molecule $[M+1]^+$ of (4) has an m/z of 325.1556 (Table 2) corresponding to empirical formula of $C_{19}H_{20}N_2O_3$. Other characteristic VAN principal mass peak are $[M+1-17]^+$ at 308.1286, $[M+1-29]^+$ at 296.1281, $[M+1-43]^+$ at 282.1134 and $[M+1-OH-136]^+$ at 189.1034. Intensity of m/z 308.1286, 296.1281, 282.1134 and 189.1034 are 10, 30, 25 and 5%, respectively, (Figure 7 and Table 3). The molecular formula of $C_{18}H_{16}N_2O$ supports eleven degree of unsaturation. In the mass spectrum, the basic peak at m/z = 308.1286 is formed after the apparent loss of the $[NH3]^+$ group by an inductive cleavage (Figure 7 & Table 3). The postulated fragmentation pathway is as per listed in Figure 7. The protonated molecular ion peak is often the most abundant signal for heterocyclic compounds such as ß-carbolines and the spectrum often does not show many characteristic fragments in ESI-MS-MS. The pattern and sequence of the fragmentation pathway was relatively similar among the four compounds, except that the signal
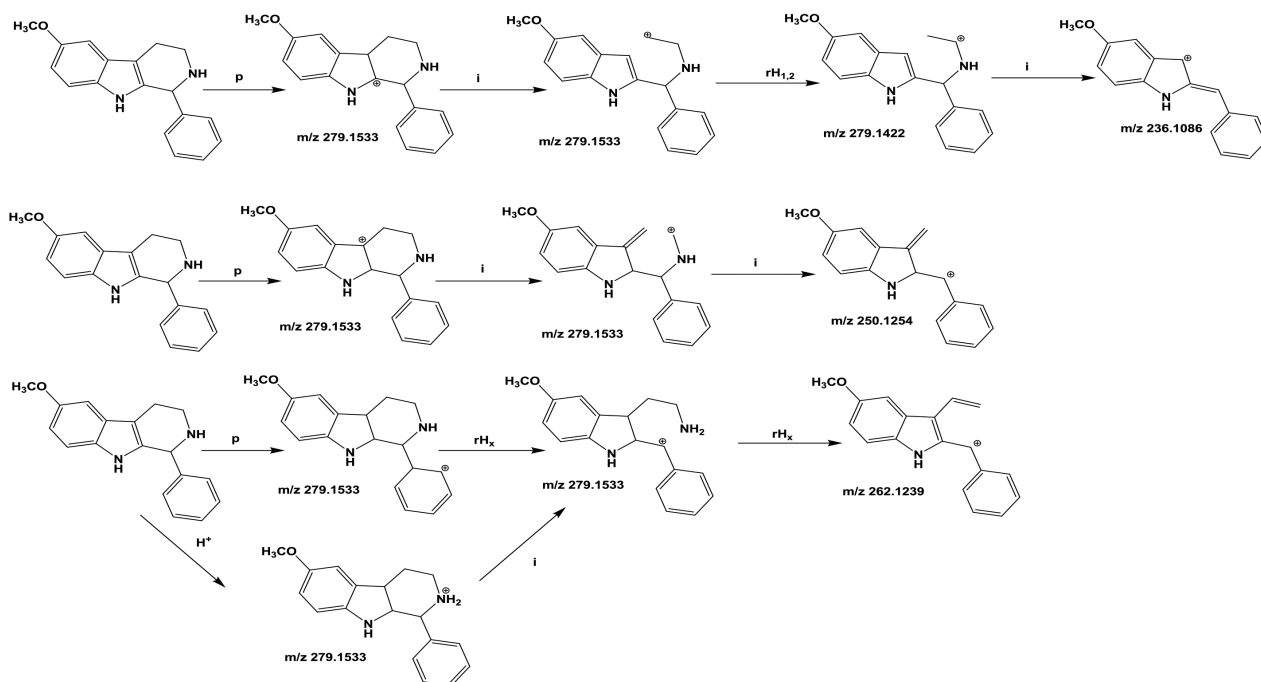
FIGURE 4. Proposed mass fragmentation pathway for (1) (Symbols : π referred to ionization on π bond, i referred to inductive cleavage, rHx referred to hydrogen rearrangement or hydrogen transfer in atom and rH1,2 referred to hydrogen 1,2-rearrangement or Whitmore 1,2-shift)

TABLE 3. HR-ESI-MS-MS mass fragments of compound 1-4

Type of compounds	Mass fragments (Intensity) m/z (%)
Compound 1	$[M+1]^+$ 279.1533 (100); $[M + H - 17]^+$ 262.1239 (5); $[M + H - 29]^+$ 250.1254 (80); $[M + H -43]^+$ 236.1082 (10)
Compound 2	$[M+1]^+$ 309.1630 (100); $[M + H - 17]^+$ 292.1339 (10); $[M + H - 29]^+$ 280.1340 (30); $[M + H - 43]^+$ 266.1180 (20)
Compound 3	$[M+1]^+$ 217.1349 (100); $[M + H - 17]^+$ 200.1076 (90); $[M + H - 29]^+$ 188.1070 (55); $[M + H - 43]^+$ 174.0907 (5)
Compound 4	$[M+1]^+$ 325.1556; $[M+1-17]^+$ 308.1286 (10); $[M+1-29]^+$ 296.1281 (30); $[M+1-43]^+$ 282.1134 (25)

intensity of MSMS spectrum of (3) and (1) for product ion m/z 200.1076 and 250.1254, respectively, is higher with approximately 80% relative abundance, probably due to the stability of these products ion.

The four compounds share some common mass losses often found for heterocyclic ß-carboline indole derivatives and these can be explained by identical elimination of NH_3, C and subsequently CH_2 by inductive cleavage, suggesting a common fragmentation pathway. In conclusion, the LC-ESI-TOF-HR-MS/MS mass spectra looks remarkably similar with the molecular ion form the base peak and mass fragmentation loss of 17 Da, 29 Da and 43 Da which corresponds to product ions of $[NH_3]^+$, $[CNH_3]^{+\cdot}$ and $[C_2H_2NH_3]^+$. The mechanism of fragmentation for all the four compounds was initiated by ionization of π bond at indole double bond and phenyl ring as well as protonation

of NH (2) (Figures 4 to 7), followed by inductive cleavage and hydrogen rearrangement. The suggested mass fragmentation pathway of these β-carbolines are well in agreement with those reported in literature (Antônio et al. 2010; Herraiz et al. 2003; Maurício et al. 2005).

Previous studies have demonstrated the aforementioned reaction scheme on other tetrahydro-ß-carboline analogues with excellent yield (Herraiz et al. 2003), but no full MS/MS spectral fragmentation pattern characterisation has been performed on heterocyclic 6-methoxy-tetrahydro-ß-carboline derivatives, knowledge about which is lacking and need to be bridged up. The mass fragmentation patterns of this group of heterocyclic tetrahydro-ß-carboline compounds described herein provide a facile approach for the identification of 6-methoxy-tetrahydro-β-carboline derivatives.
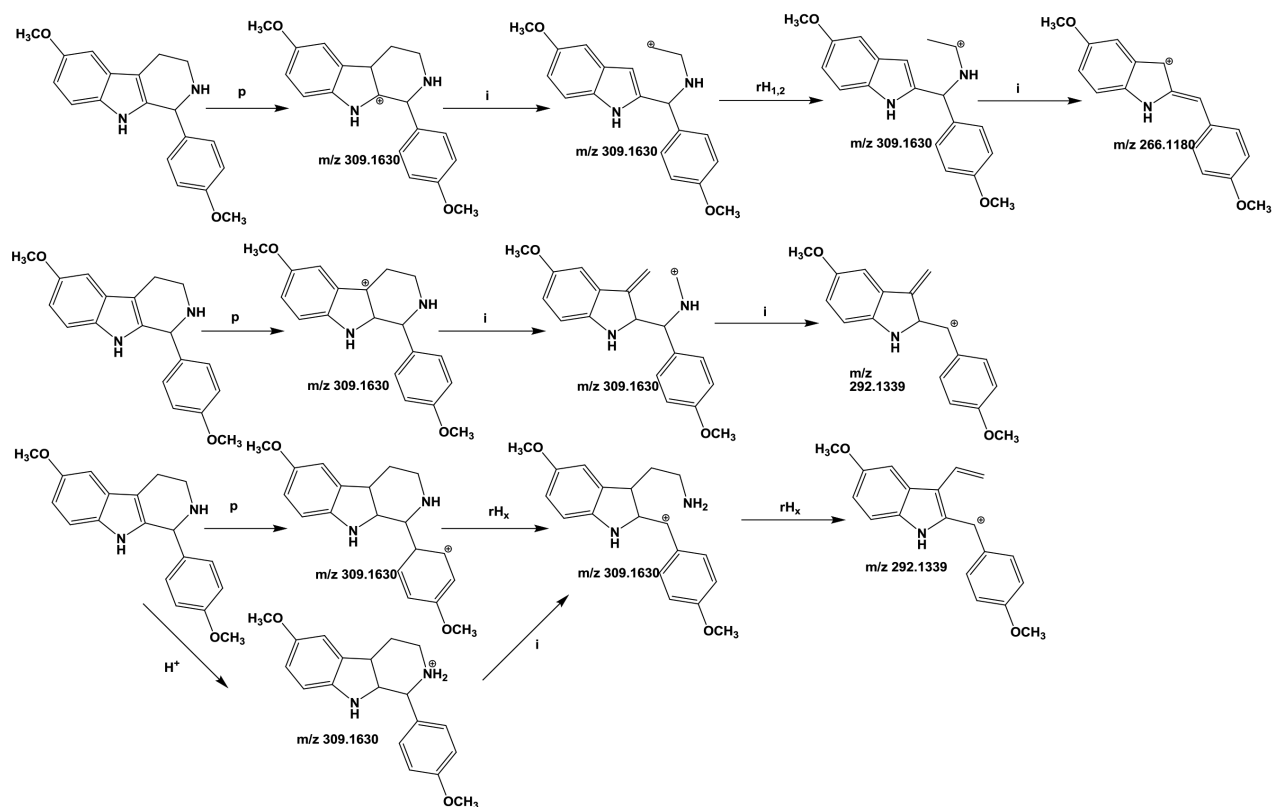
FIGURE 5. Proposed mass fragmentation pathway for (2) (Symbols : π referred to ionization on π bond, i referred to inductive cleavage, rHx referred to hydrogen rearrangement or hydrogen transfer in atom and rH1,2 referred to hydrogen 1,2-rearrangement or Whitmore 1,2-shift)
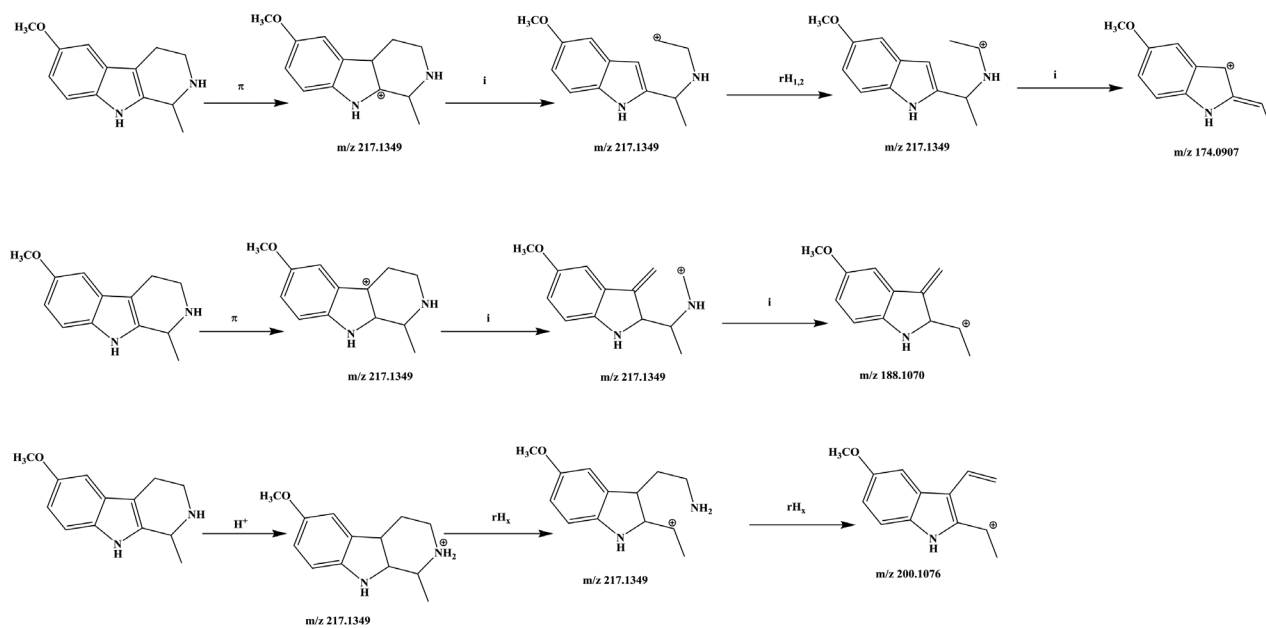

FIGURE 6. Proposed mass fragmentation pathway for (3) (Symbols : π referred to ionization on π bond, i referred to inductive cleavage, rHx referred to hydrogen rearrangement or hydrogen transfer in atom and rH1,2 referred to hydrogen 1,2-rearrangement or Whitmore 1,2-shift)
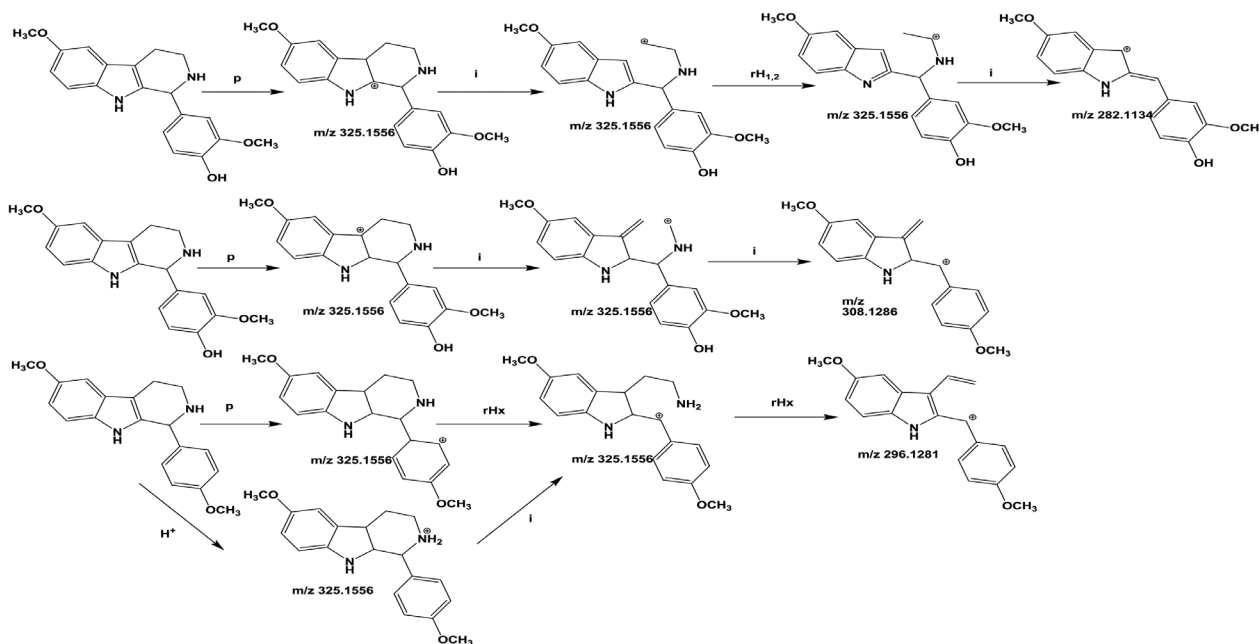
FIGURE 7. Proposed mass fragmentation pathway for (4) (Symbols : π referred to ionization on π bond,
i referred to inductive cleavage, rHx referred to hydrogen rearrangement or hydrogen transfer in atom
and rH1,2 referred to hydrogen 1,2- rearrangement or Whitmore 1,2-shift)

CONCLUSION

The optimum temperature for the Maillard reaction was 50°C , catalyst loading of 7 mole % and time within 5 h. Based on the LC-ESI-MS accurate mass and LC-ESI-MS/MS fragmentation pattern, the structural formula of compounds (1), (2), (3) and (4) was identified as 6-methoxy-1-phenyl-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole, 6-methoxy-1-(4-methoxyphenyl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b] indole, 6-methoxy-1-methyl-2,3,4,9- tetrahydro-1H-pyrido[3,4-b]indole and 2-methoxy-4-(6-methoxy-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indol-1-yl)phenol, respectively.

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Synthesis and analytical instrument characterisation data of (1), (2), (3) and (4)

6- methoxy-1-phenyl-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole (1)

5-methoxytryptamine (3.80g, 20 mmol) was dissolved in Trifluoroacetic acid (TFA) (1 mL) in 50 mL water before added and stirred with the corresponding benzaldehyde (2.12 g, 20 mmol) for 5 h at 50°C to give a crude precipitate that was subjected for medium pressure chromatography to obtain (1) (4.62 g, 92.00%) m.p. (DSC): 164-166°C. IR (KBr): 3311, 5, 3139.3, 2923.6, 1598.3, 1478.3, 1209.7 cm^{-1}. HRMS (ESI): m/z [M+H]$^+$ calcd for $C_{18}H_{18}N_2O$: 278.3483; found: 278.3455. ^{1}H NMR (500 MHz, CDCl$_3$) : δ 7.80 (br-s, 1H, N-H [8]), 7.29 (m, 3H, H-2', H-3'& H-4' overlapped), 7.23 (d, J = 7.09 Hz, 2H, H-1'& H-5'), 7.01 (d, J = 7.60 Hz, 1H, H-6), 6.98 (s, 1H, H-5), 6.76 (d, J = 8.75 Hz, 1H, H-7), 5.04 (s, 1H, H-1), 3.84 (s, 3H, 9-OCH$_3$), 3.29 (m, 1H, H-3ß), 3.06 (1H, m, H-3α), 2.86 (1H, m, H-4ß), 2.77 (1H, H-4α), 1.84(1H, br-s, N-H[2]). ^{13}C NMR (125 MHz, CDCl$_3$): 153.97 (C-8, C$_{Ar}$), 141.83 (C-1', C$_{Ar}$), 135.40 (C-1, C$_{ind}$), 130.99 (C-11, C$_{Ar}$), 128.75 (C-6, C$_{Ar}$), 128.50 (C-3' & C-5', CH$_{Ar}$), 128.12 (C-2' & C-6', CH$_{Ar}$), 127.71 (C-4', CH$_{Ar}$), 111.50 (C-9, CH$_{Ar}$), 111.43 (C-10, CH$_{Ar}$), 109.92 (C-5, C$_{ind}$), 100.48 (C-7, CH$_{Ar}$), 58.11 (C-2, CH$_{Al}$), 55.95 (C-12, OCH$_3$), 42.73 (C-3, CH$_2$), 22.55 (C-4, CH$_2$).

6-methoxy-1-(4-methoxyphenyl)-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole (2)

5-methoxytryptamine (3.80g, 20 mmol) was dissolved in TFA (1 mL) in 50 mL water before added and stirred with the corresponding Anisaldehyde (2.74 g, 20 mmol) for 5 h at 50°C to give a crude precipitate that was subjected for medium pressure chromatography hexane:ethyl acetate between 96: 4 v/v and 92: 8 v/v to obtain (2) (4.85 g, 78%). m.p. (DSC): 165-168°C. IR (KBr): 3182.3, 2886.9, 1608.4, 1512.1, 1244.6 cm^{-1}. HRMS (ESI): m/z [M+H]$^+$ calcd for $C_{19}H_{20}N_2O_2$: 308.3743; found 308.1562.^{1}H NMR (500 MHz, CDCl$_3$) : δ 7.59 (1H, br-s, N-H), 7.20 (2H, d, J = 8.57 Hz, H-1'& H-5'), 7.07 (1H, d, J = 8.74 Hz, H-7), 6.99 (1H, s, H-5), 6.85 (2H, d, J = 8.60 Hz H-2' & H-4'), 6.78 (1H, d, J = 8.70 Hz, H-6), 5.06 (1H, s, H-1), 3.86 (3H, s, 9-OCH$_3$), 3.78 (3H, s, 3'-OCH$_3$), 3.32 (1H, m, H-3ß), 3.09 (1H, m, H-3α), 2.87 (1H, m, H-4ß), 2.76 (1H, m, H-4α), 2.00 (1H, br-s, N-H). ^{13}C NMR (125 MHz, CDCl$_3$): 159.60 (C-4', C$_{Ar}$), 154.04 (C-8, C$_{Ar}$), 136.79 (C-1', C$_{Ar}$), 133.89 (C-1, C$_{ind}$), 130.96 (C-2'& C-7', CH$_{Ar}$), 129.64 (C-11, C$_{Ar}$), 127.82 (C-6, C$_{Ar}$), 114.13 (C-3'& C-6', CH$_{Ar}$), 111.50 (C-9, CH$_{Ar}$), 111.44 (C-10, CH$_{Ar}$), 109.92 (C-5, C$_{ind}$), 100.53 (C-7, CH$_{Ar}$), 57.55 (C-2, CH$_{Al}$), 56.01 (C-5', OCH$_3$), 55.34 (C-12, OCH$_3$), 42.89 (C-3, CH$_2$), 22.58 (C-4, CH$_2$).

6-methoxy-1-methyl-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indole (3)

5-methoxytryptamine (3.80g, 20 mmol) was dissolved in TFA (1 mL) in 50 mL water before added and stirred with the corresponding Acetaldehyde (0.88 g, 1.20 mL, 20 mmol) for 5 h at room temperature (25°C) to give a crude precipitate that was subjected to medium pressure chromatography at hexane: ethyl acetate between 92: 8 v/v and 90: 10 v/v to obtain (3) (3.98 g, 99.00%) m.p (DSC): 246-248°C. IR (KBr): 3320.5, 2969.2, 3439.3, 1493.1, 1440.9, 1123.7 cm^{-1}. HRMS (ESI): m/z [M+H]$^+$ calcd for $C_{13}H_{16}N_2O$: 216.2789; found 216.1293. ^{1}H NMR (500 MHz, MeOD) : δ 7.83 (1H, s, N-H), 7.32 (1H, d, J = 8.75 Hz, H-6), 6.99 (1H, s, H-5), 6.84 (H, d, J = 8.72 Hz, H-7), 4.71 (1H, q, H-1), 3.83 (3H, s, H-9), 3.10 (1H, m, H-3ß), 3.07 (1H, m, H-3α), 3.04 (1H, m, H-4ß), 2.98 (1H, m, H-4α), 1.96 (1H, s, NH[2]), 1.75 (3H, d, J = 6.85 Hz, H-1'). ^{13}C NMR (125 MHz, MeOD) : δ 155.06 (C-8, C$_{Ar}$), 133.26 (C-1, C$_{ind}$), 131.84 (C-11, C$_{Ar}$), 127.62 (C-6, C$_{Ar}$), 113.22 (C-9, CH$_{Ar}$), 113.16 (C-10, CH$_{Ar}$), 106.52 (C-5, C$_{ind}$), 101.42 (C-7, CH$_{Ar}$), 56.50 (C-12, OCH$_3$), 50.55 (C-2, CH$_{Al}$), 42.47 (C-3, CH$_2$), 19.37 (C-4, CH$_2$), 17.82 (C-1', CH$_3$).

2-methoxy-4-(6-methoxy-2,3,4,9-tetrahydro-1H-pyrido[3,4-b]indol-1-yl)phenol (4)

5-methoxytryptamine (3.80g, 20 mmol) was dissolved in TFA (1 mL) in 50 mL water before added and stirred with the corresponding Vanillin (3.08 g, 20 mmol) for 5 hat 50°C to give a crude precipitate that was subjected for medium pressure chromatography hexane:ethyl acetate between 90: 10 v/v and 88: 12 v/v to obtain (4) (4.62 g, 84%). m.p.(DSC): 242-245°C. IR (KBr): 3352.3, 2936.4, 1658.7, 1580.9, 1493.2, 1422.3, 1218.3 cm^{-1}. HRMS (ESI): m/z [M+H]$^+$ calcd for $C_{19}H_{20}N_2O_3$: 324.3737; found 324.1507.^{1}H NMR (500 MHz, MeOD): δ 7.78 (1H, br-s, N-H[8]), 5.25 (1H, s, H-3'), 7.02 (1H, d, J = 8.67 Hz, H-6), 6.86 (1H, s, H-5'), 6.76 (1H, s, H-5), 6.69 (1H, d, J = 8.08 Hz, H-2'), 6.64 (1H, d, J = 8.17, Hz, H-5), 6.61 (1H, d, J = 8.70, H-7), 4.98 (1H, s, H-1), 3.72 (3H, s, 4'-OCH$_3$), 3.68 (3H, s, 9-OCH$_3$), 3.31 (1H, m, H-3ß), 3.12 (1H, m, H-3α), 2.90 (1H, m, H-4ß), 2.79 (1H, m, H-4α), 1.84(1H, br-s, N-H [2]).^{13}C NMR (125 MHz, MeOD) : 155.42 (C-8, C$_{Ar}$), 149.39 (C-5', C$_{Ar}$), 147.10 (C-4', C$_{Ar}$), 133.42 (C-1, C$_{ind}$), 127.55 (C-11, C$_{Ar}$), 129.61 (C-1', C$_{Ar}$), 126.40(C-6, C$_{Ar}$), 123.94 (C-2', CH$_{Ar}$), 116.65 (C-3', CH$_{Ar}$), 113.83 (C-7', CH$_{Ar}$), 113.69 (C-10, CH$_{Ar}$), 113.19 (C-9, CH$_{Ar}$), 108.68 (C-5, C$_{ind}$), 101.27 (C-7, C$_{Ar}$), 58.33 (C-2, CH$_{Al}$), 56.31 (C-6', OCH$_3$), 52.21 (C-12, OCH$_3$), 42.10 (C-3, CH$_2$), 19.50 (C-4, CH$_2$).