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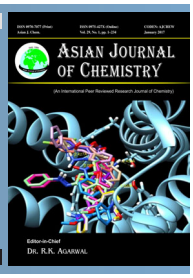

Synthesis of *N*-Alkylated and *N*-Acylated Derivatives of Girinimbine

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Girinimbine, a carbazole alkaloid has drawn an attention due to its wide range of pharmacological effects such as antiplatelet, antibacterial and anticancer activities. Five new derivatives of girinimbine were successfully semi-synthesized *via* modification on *N*-terminal of naturally isolated girinimbine. *N*-Benzylgirinimbine, *N*-butylgirinimbine and *N*-isopentylgirinimbine were obtained from alkylation reaction, whereas, *N*-butyrylgirinimbine and *N*-methylbutyrylgirinimbine were obtained from acylation reaction. The structures of synthesized girinimbine derivatives were confirmed by spectroscopic techniques.

Keywords: Girinimbine, Carbazole, *N*-Derivative, Alkylation, Acylation.

INTRODUCTION

Girinimbine (**1**) also called as 3,3,5-trimethyl-11*H*-pyrano-(3,2-a)carbazole was first isolated by Chakraborty *et al.* [1] from the stem bark of *Murraya koenigii* and then followed by Joshi *et al.* [2] from the leaves of *M. koenigii.* Furthermore, girinimbine (**1**) had been found in the roots of *Clausena heptaphylla* [3] and in the root bark of *Murraya euchrestifolia* [4].

This pyranocarbazole has drawn researcher's attention due to its wide range of pharmacological activities like antitumor, anti-inflammatory, anticancer, antiplatelet and antioxidant [5-9]. It was also reported that girinimbine (**1**) together with other carbazoles, mahanimbine (**2**) and murrayacine (**3**) were exhibited strong activity against *Aedes aegypti* [10]. According to Rao *et al*. [11], mahanimbine (**2**) and koenigine (**4**) from *M. koenigii* had also showed significantly antioxidant and radical-scavenging. The synthetic precursors, 3,8-dihydroxy-5-formylcarbazoles (**7**) and (**8**) exhibited strong antioxidant activities than its naturally isolated compounds, 3,8-dihydroxy-carbazoles carbazomadurin A (**5**) and B (**6**) respectively due to the reduced of formyl group at the C5 position on the benzene ring of carbazole [12] (Fig. 1).

Driven by the less number of *N*-protected of carbazole compound either from nature or synthetically synthesized and the potential of carbazole for bioactivity, therefore the aim of this study is to produce new derivatives of girinimbine *via* alkylation and acylation of *N*-terminal of naturally isolated girinimbine from *M. koenigii*.

EXPERIMENTAL

All chemicals are commercially available as analytical grade and used without purification unless otherwise stated. Thin layer chromatography was carried out on silica gel 60 F245 (Merck KGaA) precoated aluminium backed plates and visualized by UV and H_2SO_4 solution were used to monitored all reactions. All organic extracts were dried over Na_2SO_4 and evaporated using rotary evaporator. All crude products were purified by column chromatography in performed on silica gel 60. The melting point was recorded using the digital melting point equipment (Electrothermal IA9000 Series). Perkin-Elmer FT-IR model spectrum 100 series spectrophotometer was used for the IR spectra using UATR techniques. The adsorption bands were measured in range of 4000-280 cm^{-1}. The Shimadzu model QP5050A series was used to analyze the MS spectra. The 1D NMR and 2D NMR spectra were obtained on a JEOL machine (500 MHz). All chemical shifts, δ were recorded in ppm relative to TMS signal, whereas the coupling constants, *J* are given in Hz.

N-Alkylation

***N*-Benzylgirinimbine (12):** Potassium carbonate (1.90 mmol) was added to a stirred solution of girinimbine (**1**) (0.19 mmol) in acetonitrile (2 mL). The mixture was cooled to 0 °C and benzyl bromide (3.80 mmol) was added and stirred at room temperature for 5 days. The reaction mixture was quenched with water (10 mL) and extracted with ethyl acetate (3 × 10 mL). The combined organic layers were dried and solvent was removed.

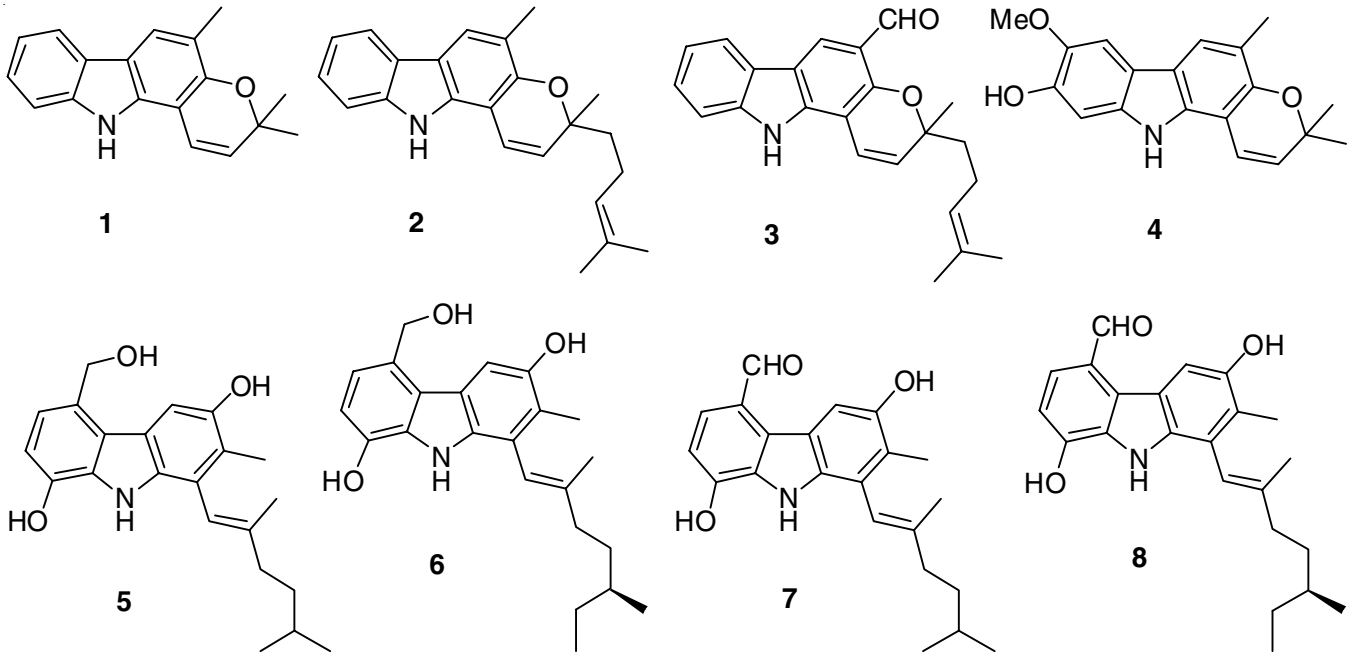


Fig. 1. Girinimbine (**1**) and some bioactive carbazoles

The crude product was purified by column chromatography (hexane:ethyl acetate, 9:1).

***N*-Benzylgirinimbine (12):** White solid (56 mg, 83 %); m.p.: 162-163 °C; IR (UATR) 3054, 2972, 2924, 1637, 1595, 1456, 1351, 1128, 739 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 7.96 (1H, d, *J* = 8.0 Hz, H-aromatic), 7.76 (1H, s, H-aromatic), 7.29 (3H, *t*, *J* = 6.9 Hz, H-aromatic), 7.25 (1H, d, *J* = 6.9 Hz, H-aromatic), 7.19 (2H, *t*, *J* = 8.0 Hz, H-aromatic), 7.14 (2H, d, *J* = 6.9 Hz, H-aromatic), 6.67 (1H, d, *J* = 10.4 Hz, HC=CH), 5.60 (2H, s, CH_2), 5.49 (1H, d, *J* = 10.3 Hz, HC=CH), 2.35 (3H, s, CH_3), 1.43 (6H, s, CH_3); δ_C (125 MHz, $CDCl_3$) 151.1, 141.8, 138.2, 136.1, 129.2, 128.9, 127.6, 126.0, 124.6, 123.6, 121.6, 119.7, 119.3, 119.0, 118.9, 117.4, 108.8, 106.0, 75.0, 49.2, 27.2, 16.5; *m/z* (EIMS) 353 (M^+, $C_{25}H_{23}NO$ requires 353).

***N*-Butylgirinimbine (13) and *N*-isopentylgirinimbine (14):** K_2CO_3 (1.90 mmol) was added to a stirred solution of girinimbine (**1**) (0.19 mmol) in DMF (2 mL). Butyl bromide/ isopentyl bromide (3.80 mmol) was added and the mixture was stirred at 60 °C for 3 days. The reaction mixture was quenched with water (10 mL) and extracted with ethyl acetate (3 × 10 mL). The combined organic layers were dried and solvent was removed. The crude product was purified by column chromatography (hexane:ethyl acetate, 9:1).

***N*-Butylgirinimbine (13):** Yellow oil (43 mg, 72 %); IR (UATR) 3049, 2926, 2865, 1637, 1587, 1460, 1350, 1130, 739 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 7.92 (1H, d, *J* = 6.9 Hz, H-aromatic), 7.71 (1H, s, H-aromatic), 7.34 (1H, *t*, *J* = 8.1 Hz, H-aromatic), 7.31 (1H, d, *J* = 8.1 Hz, H-aromatic), 7.16 (1H, *t*, *J* = 6.9 Hz, H-aromatic), 6.98 (1H, d, *J* = 10.3 Hz, HC=CH), 5.70 (1H, d, *J* = 10.3 Hz, HC=CH), 4.36 (2H, *t*, *J* = 6.9 Hz, N-CH_2), 2.34 (3H, s, CH_3), 1.86 (2H, q, *J* = 6.9 Hz, CH_2), 1.49 (6H, s, CH_3), 1.43 (2H, m, CH_2), 0.98 (3H, *t*, *J* = 6.9 Hz, CH_3); δ_C (125 MHz, $CDCl_3$) 150.9, 141.5, 135.3, 129.1, 124.3, 123.3, 121.5, 119.2, 119.1, 119.0, 118.7, 117.5, 108.6, 105.8, 74.9, 45.2, 32.3, 27.3, 20.5, 16.5, 14.1; *m/z* (EIMS) 319 (M^+, $C_{22}H_{25}NO$ requires 319).

***N*-Isopentylgirinimbine (14):** Brown oil (44 mg, 70 %); IR (UATR) 2954, 1635, 1587, 1461, 1353, 1126, 739 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 7.90 (1H, d, *J* = 8.0 Hz, H-aromatic), 7.69 (1H, s, H-aromatic), 7.33 (1H, *t*, *J* = 6.9 Hz, H-aromatic), 7.28 (1H, d, *J* = 8.1 Hz, H-aromatic), 7.14 (1H, *t*, *J* = 6.9 Hz, H-aromatic), 6.96 (1H, d, *J* = 9.2 Hz, HC=CH), 5.69 (1H, d, *J* = 10.3 Hz, HC=CH), 4.35 (2H, t, *J* = 6.9 Hz, N-CH_2), 2.31 (3H, s, CH_3), 1.74 (2H, q, *J* = 6.9 Hz, CH_2), 1.73 (1H, m, CH), 1.47 (6H, s, CH_3), 1.01 (6H, d, *J* = 6.9 Hz, CH_3); δ_C (125 MHz, $CDCl_3$) 150.9, 141.3, 135.3, 129.0, 124.3, 123.4, 121.5, 119.2, 119.1, 118.9, 118.6, 117.4, 108.4, 105.8, 74.9, 43.9, 38.6, 27.3, 26.6, 22.9, 16.5; *m/z* (EIMS) 333 (M^+, $C_{23}H_{27}NO$ requires 333).

***N*-Acylation**

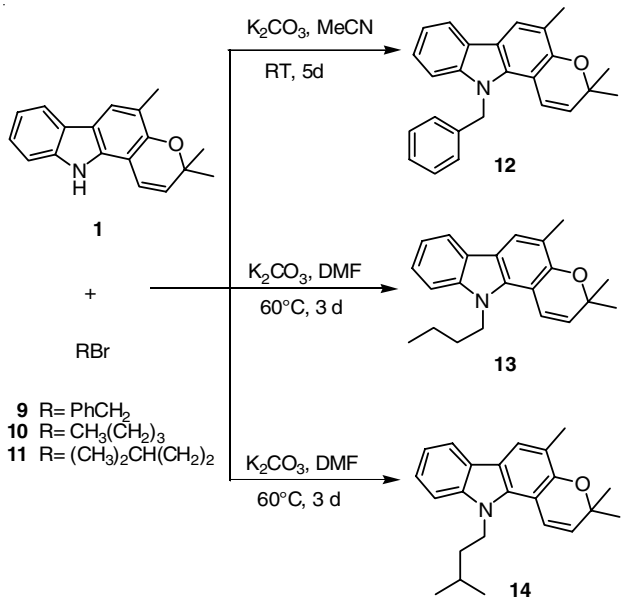
***N*-Butyrylgirinimbine (17) and *N*-methylbutyrylgirinimbine (18):** Butyryl chloride/2-methylbutyryl chloride (1.90 mmol) was added to a solution of girinimbine (**1**) (0.19 mmol) in 1 mL anhydrous toluene and refluxed under nitrogen atmosphere for 5-24 h. The reaction mixture was allowed to cool to room temperature and the solvent was evaporated. Ether (30 mL) was added and washed with saturated $NaHCO_3$ (10 mL) and brine (10 mL). The organic layer was dried and solvent was removed. The crude was purified by column chromatography (hexane:ethyl acetate, 9:1).

***N*-Butyrylgirinimbine (17):** Yellow oil (37 mg, 59 %); IR (UATR) 3049, 2966, 1701, 1637, 1595, 1424, 1367, 1141, 747 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 8.04 (1H, d, *J* = 8.0 Hz, H-aromatic), 7.80 (1H, d, *J* = 6.9 Hz, H-aromatic), 7.61 (1H, s, H-aromatic), 7.33 (1H, *t*, *J* = 8.0 Hz, H-aromatic), 7.29 (1H, *t*, *J* = 6.9 Hz, H-aromatic), 6.42 (1H, d, *J* = 9.2 Hz, HC=CH), 5.69 (1H, d, *J* = 10.4 Hz, HC=CH), 2.82 (2H, *t*, *J* = 6.9 Hz, O=C-CH_2), 2.32 (3H, s, CH_3), 1.72 (2H, m, CH_2), 1.51 (6H, s, CH_3), 0.87 (3H, *t*, *J* = 6.9 Hz, CH_3); δ_C (125 MHz, $CDCl_3$) 175.3, 151.4, 140.0, 134.7, 128.1, 126.5, 126.0, 123.6, 122.7, 121.5, 121.2, 120.4, 118.9, 115.0, 110.5, 75.6, 40.7, 27.2, 19.5, 16.4, 13.9; *m/z* (EIMS) 333 (M^+, $C_{22}H_{23}NO_2$ requires 333).

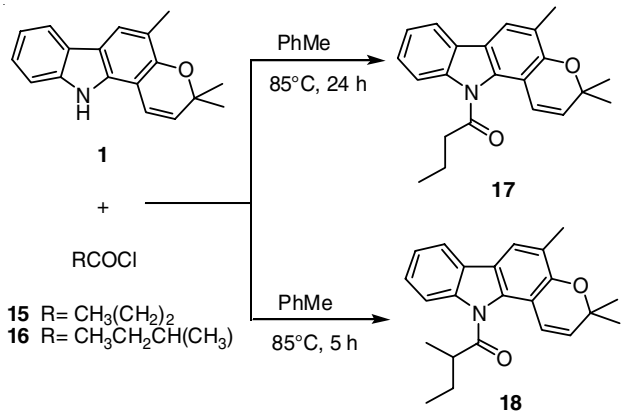
***N*-Methylbutyrylgirinimbine (18):** Brown oil (32 mg, 48 %); IR (UATR) 2969, 2923, 1701, 1637, 1608, 1454, 1377, 1128, 746 cm^{-1}; δ_H (500 MHz, $CDCl_3$) 8.02 (1H, d, *J* = 8.0 Hz, H-aromatic), 7.81 (1H, d, *J* = 6.9 Hz, H-aromatic), 7.63 (1H, s, H-aromatic), 7.33 (1H, *t*, *J* = 8.0 Hz, H-aromatic), 7.28 (1H, *t*, *J* = 6.9 Hz, H-aromatic), 6.42 (1H, d, *J* = 10.3 Hz, HC=CH), 5.70 (1H, d, *J* = 10.3 Hz, HC=CH), 3.28 (1H, m, O=C-CH), 2.33 (3H, s, CH_3), 1.53 (6H, s, CH_3), 1.39 (2H, q, *J* = 6.9 Hz, CH_2), 1.13 (3H, d, *J* = 6.9 Hz, CH_3), 0.70 (3H, *t*, *J* = 6.9 Hz, CH_3); δ_C (125 MHz, $CDCl_3$) 179.8, 151.2, 140.4, 134.5, 128.5, 126.1, 126.0, 123.4, 122.4, 121.7, 120.8, 120.3, 118.9, 114.4, 110.1, 75.6, 43.1, 27.6, 26.8, 16.4, 16.2, 11.6; *m/z* (EIMS) 347 (M^+, $C_{23}H_{25}NO_2$ requires 347).

RESULTS AND DISCUSSION

The synthesis of girinimbine derivatives was achieved through alkylation or acylation reaction of *N*-carbazole unit with the corresponding primary alkyl bromides or acid chlorides. *N*-Alkylation reactions were produced *N*-benzylgirinimbine (**12**), *N*-butylgirinimbine (**13**) and *N*-isopentylgirinimbine (**14**), whereas, *N*-butyrylgirinimbine (**17**) and *N*-methylbutyryl-girinimbine (**18**) were obtained from *N*-acylation reaction (**Schemes I** and **II**).



Scheme-I: *N*-Alkylation of girinimbine (**1**)



Scheme-II: *N*-Acylation of girinimbine (**1**)

***N*-Alkylation:** Three new alkylated girinimbines were prepared according to Gawande *et al.* [13] protocol (**Scheme-I**). Treatment of 1:20 ratio of girinimbine (**1**) and alkyl bromides (**9**-**11**) in CH_3CN or DMF [14,15] gave 70-83 % of *N*-alkylated girinimbines (**12**-**14**) (Table-1, entry I-III). The results showed that the longer the alkyl chain of bromides **10** and **11**, the higher the temperature needed for the reaction. It was observed that the presence of aromatic ring gave a higher yield compared to the aliphatic alkyl bromide.

TABLE-1
OPTIMIZED *N*-ALKYLATION AND *N*-ACYLATION REACTIONS OF GIRINIMBINE (**1**)

Entry	RBr/RCOCl (eq.)	Solvent	Temp. (°C)	Reaction time	Product (% yield)
I	**9** (20)	MeCN	RT	5 days	**12** (83)
II	**10** (20)	DMF	60	3 days	**13** (72)
III	**11** (20)	DMF	60	3 days	**14** (70)
IV	**15** (10)	PhMe	85	24 h	**17** (59)
V	**16** (10)	PhMe	85	5 h	**18** (48)

Based on the ^{1}H NMR spectra, a broad singlet peak of N-H at δ 7.84 ppm was disappeared for all alkylated girinimbine's derivatives. The substitution reaction was confirmed by the presence of a new singlet peak corresponding to the methylene protons, CH_2 of benzyl at δ 5.60 ppm for compound **12**. Meanwhile, a new triplet peak of methylene protons of butyl and isopentyl groups were observed at δ 4.36 ppm and δ 4.35 ppm respectively. The ^{1}H NMR of **12** displayed two triplet peaks at δ 7.29 ppm and δ 7.19 ppm were corresponded to the aromatic protons H13 and H14, while the doublet peak appeared at δ 7.14 ppm represent the aromatic proton of H12. The ^{13}C NMR spectrum showed the presence of 25 signals indicated 25 carbons in the structure. The DEPT spectrum of **12** displayed nine quaternary carbons at chemical shift 151.1, 141.8, 138.2, 136.1, 123.6, 119.0, 117.4, 106.0 and 75.0 ppm. MS spectrum of **12** exhibited a molecular ion peak at *m/z* 353 with molecular formulae $C_{25}H_{23}NO$, which matched with the calculated value for structure **12**.

The ^{13}C NMR of derivative **13** displayed four new peaks at δ 45.2 ppm (C10), δ 32.3 ppm (C11), δ 20.5 ppm (C12) and δ 14.1 ppm (C13) indicated that the alkylation reaction of butyl group at *N*-terminal had occurred. Meanwhile, compound **14** also showed four new peaks at higher chemical shift 43.9 ppm, 38.6 ppm, 26.6 ppm and 22.9 ppm which corresponding to C10, C11, C12, C13 and C14. The *N*-alkylation of girinimbine was confirmed by HMBC experiment that showed a ^3J correlation of C8a with H10 for all derivatives **12**, **13** and **14**. Compound **12** showed a ^2J and ^3J correlation of H13 with C12 and C11 respectively, while a ^3J correlation of H11 with C13 for both compounds **13** and **14** (Fig. 2).

***N*-Acylation:** This study was based on Arnold *et al.* [16] (**Scheme-II**). Reaction of girinimbine (**1**) with acid chlorides **15** and **16** (1:10 ratio), were managed to produce derivatives **17** and **18** in 59 and 48 % yields respectively (Table-1, entry IV and V). The *N*-acylation reaction seemed depending on the hindrance of acyl chloride where the straight chain of acyl chloride gave higher yield compared to the branched acyl chloride.

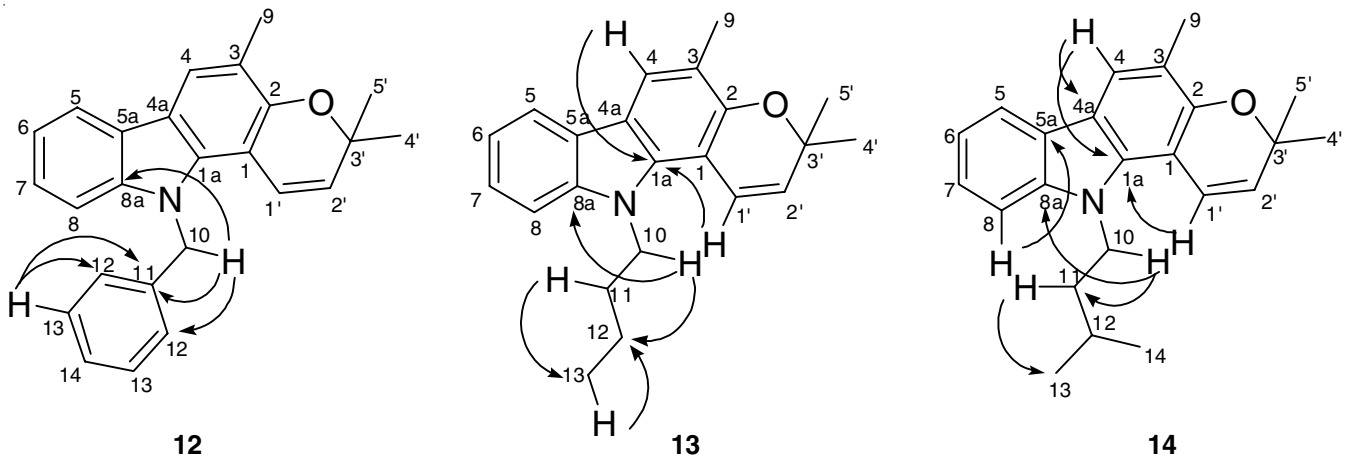


Fig. 2. Selected HMBC correlation of **12**, **13** and **14**

The ^{1}H NMR spectrum of compound **17** showed a new triplet peak at upfield region, δ 2.82 ppm (*J* 6.9 Hz) and δ 0.87 ppm (*J* 6.9 Hz) that corresponding to H11 and H13 respectively. Meanwhile, compound **18** displayed doublet peak at δ 1.13 ppm (*J* 6.9 Hz) and triplet peak at δ 0.70 ppm (*J* 6.9 Hz) corresponding to the protons H14 and H13. The ^{13}C NMR spectra of **17** and **18** showed a new peak at higher chemical shift indicated that C=O occurs at δ 175.3 ppm and δ 179.8 ppm, respectively. The presence of C11, C12 and C13 of compound **17** were observed at δ 40.7 ppm, δ 19.5 ppm and δ 13.9 ppm respectively. Four peaks at δ 179.8 ppm, δ 43.1 ppm, δ 26.8 ppm, δ 16.2 ppm and δ 11.6 ppm were assigned to methylbutyryl carbons of **18**.

The strong absorption was observed at 1701 cm^{-1} on the FTIR spectra indicated the existence of C=O of amide for the both compounds **17** and **18**. The *N*-acylation of girinimbine was confirmed by HMBC experiment that showed a ^{3}J correlation of carbonyl carbon, C10 with H12 for both acylated derivatives **17** and **18**. Compound **17** showed ^{2}J correlation of H12 with C11, while **18** showed a ^{2}J correlation of H12 with C11 and C13 (Fig. 3).

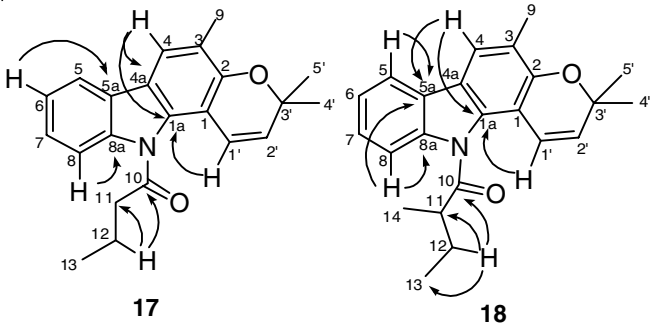


Fig. 3. Selected HMBC correlation of **17** and **18**

Conclusion

Five new derivatives of girinimbine were successfully semi-synthesized *via* alkylation and acylation reactions on the *N*-terminal of naturally isolated girinimbine. *N*-Benzylgirinimbine (83 %), *N*-butylgirinimbine (72 %) and *N*-isopentylgirinimbine (70 %) were obtained from alkylation reaction, whereas, *N*-butyrylgirinimbine (59 %) and *N*-methylbutyrylgirinimbine (48 %) were obtained from acylation reaction.

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