Supporting Information

***Rec. Nat. Prod*. 16:5 (2022) 493-498**

Secondary Metabolites from *Thraustochytrium* and their Biological Activity

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Compound **1** (ergosterol) :

^{1}H NMR (600 MHz, $CDCl_3$) δ ppm: 0.63 (3H, s, H_3-19), 0.82 (3H, d, *J* = 6.6 Hz, H_3-27), 0.84 (3H, d, *J* = 6.6 Hz, H_3-26), 0.91 (3H, d, *J* = 6.6 Hz, H_3-28), 0.94 (3H, s, H_3-18), 1.04 (3H, d, *J* = 6.6 Hz, H_3-21), 3.63 (1H, m, H-3), 5.17 (1H, dd, *J* = 7.8, 15.3 Hz, H-22), 5.23 (1H, dd, *J* = 7.2, 15.3 Hz, H-23), 5.38 (1H, dd, *J* = 5.4, 3.0 Hz, H-7), 5.57 (1H, dd, *J* = 6.0, 2.4 Hz, H-6).

^{13}C NMR (150 MHz, $CDCl_3$) δ ppm: 38.4 (C-1), 32.0 (C-2), 70.4 (C-3), 40.8 (C-4), 139.8 (C-5), 119.6 (C-6), 116.3 (C-7), 141.3 (C-8), 46.2 (C-9), 37.0 (C-10), 21.1 (C-11), 39.1 (C-12), 42.9 (C-13), 54.5 (C-14), 23.0 (C-15), 28.3 (C-16), 55.7 (C-17), 12.1 (C-18), 16.0 (C-19), 40.4 (C-20), 21.1 (C-21), 135.5 (C-22), 132.0 (C-23), 42.8 (C-24), 33.1 (C-25), 19.9 (C-26), 19.6 (C-27), 17.6 (C-28). [F. Matteo, T. Pasquale, L. Rafael (2010). Phytosterol from *Dunaliella tertiolecta* and *Dunaliella salina*: A potentially novel industrial application, *Bioresour. Technol.* **101**, 4144-4150.]

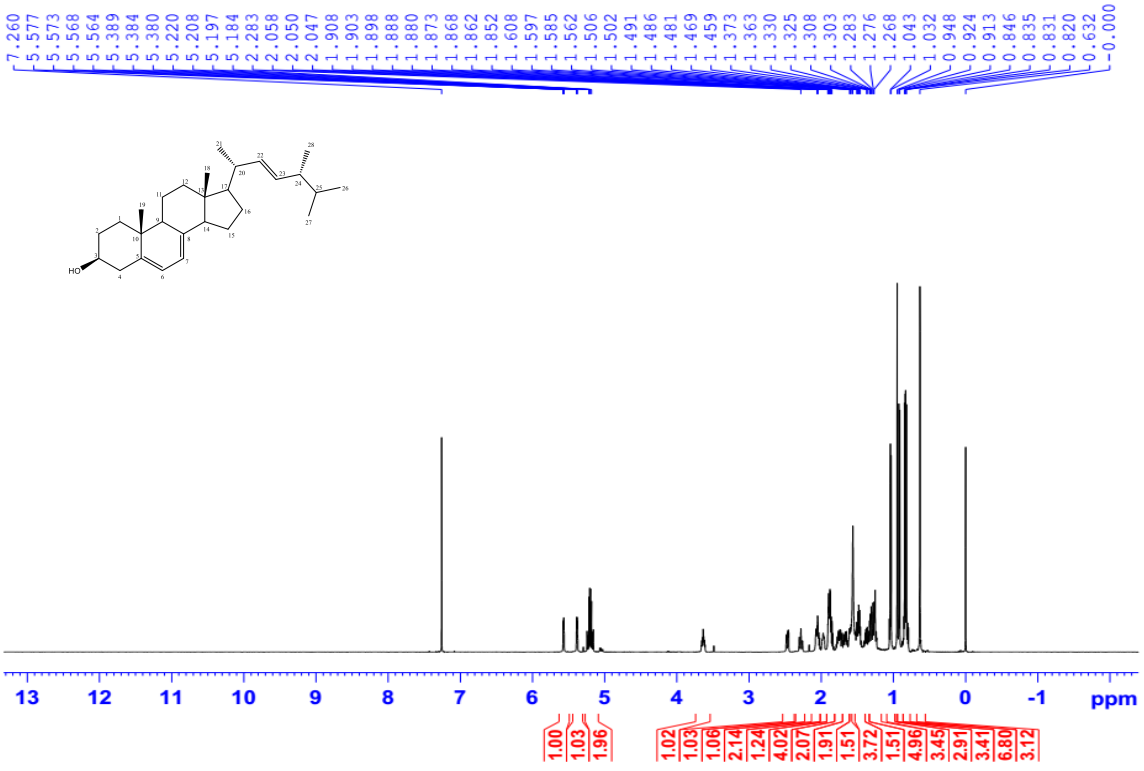

Figure S1: ^{1}H-NMR (500 MHz, $CDCl_3$) spectrum of **1** (ergosterol)

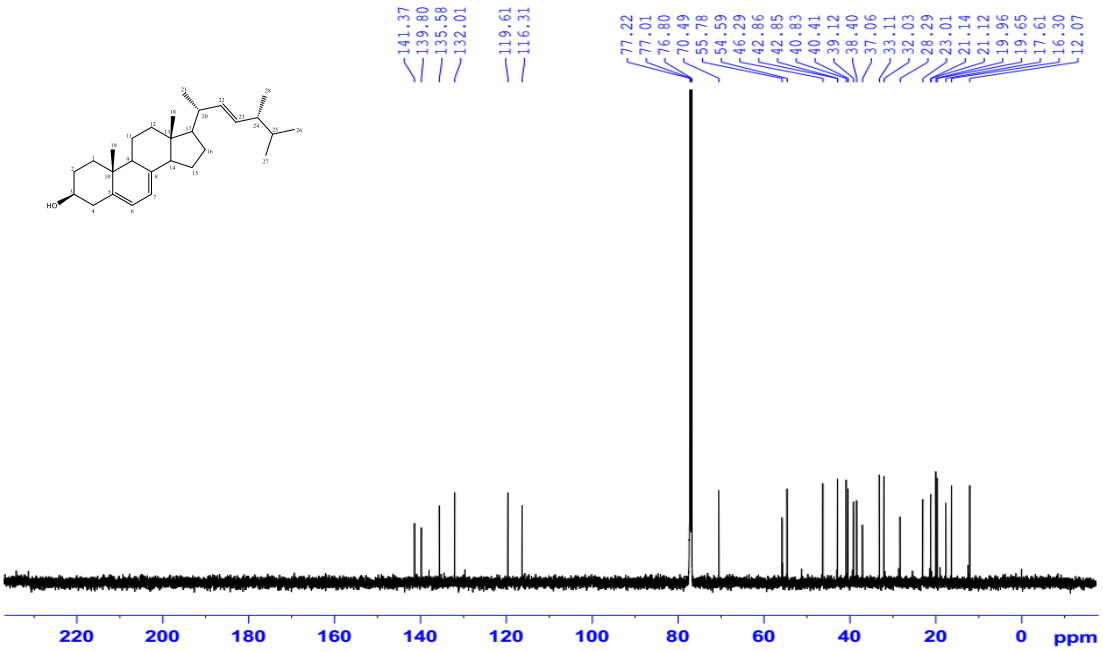

Figure S2: ^{13}C-NMR (125 MHz, $CDCl_3$) spectrum of **1** (ergosterol)

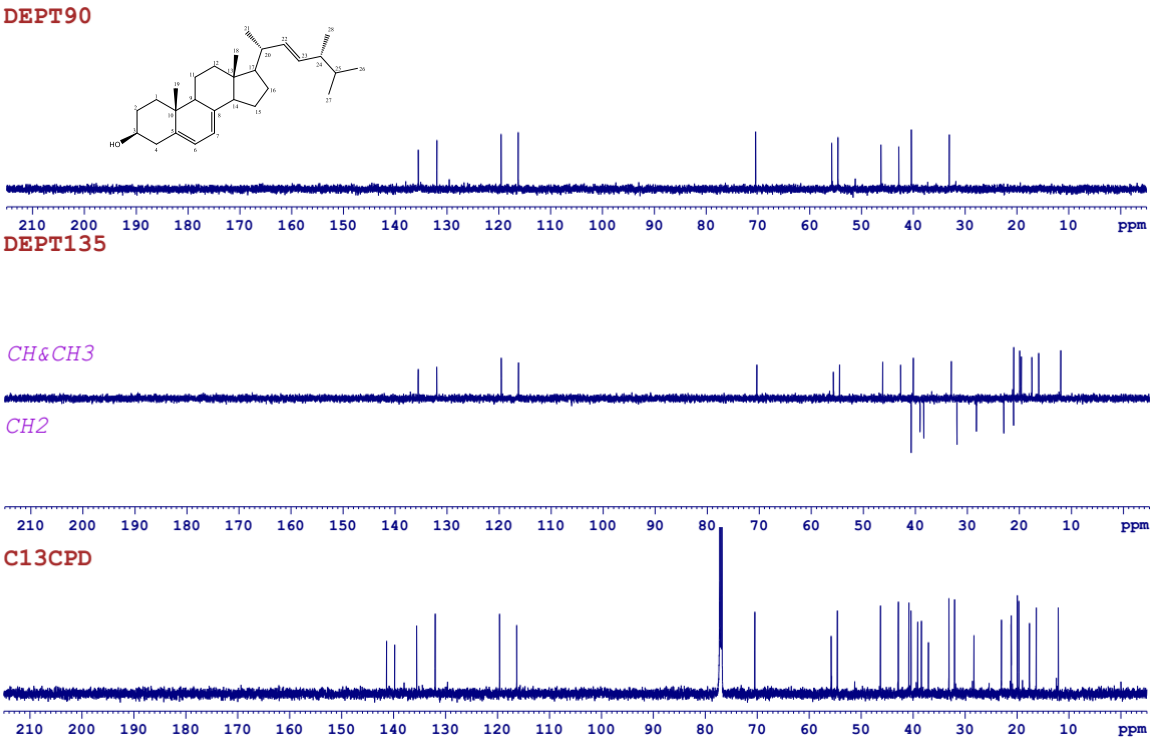

Figure S3: DEPT 90&135 spectrum of **1** (ergosterol)

Compound **2** (7-dehydroporiferasterol)

^{1}H NMR (500 MHz, $CDCl_3$) δ ppm: 0.63 (3H, s, H_3-19), 0.79 (3H, d, *J* = 6.5 Hz, H_3-27), 0.82 (3H, t, *J* = 7.5 Hz, H_3-29), 0.84 (3H, d, *J* = 6.5 Hz, H_3-26), 0.94 (3H, s, H_3-18), 1.04 (3H, d, *J* = 6.6 Hz, H-21), 3.63 (1H, m, H-3), 5.17 (1H, dd, *J* = 7.5, 15.0 Hz, H-22), 5.23 (1H, dd, *J* = 7.5, 15.0 Hz, H-23), 5.38 (1H, dd, *J* = 5.5, 2.5 Hz, H-7), 5.57 (1H, dd, *J* = 5.0, 2.0 Hz, H-6). ^{13}C NMR (125 MHz, $CDCl_3$) δ ppm: 38.4 (C-1), 32.0 (C-2), 70.4 (C-3), 40.8 (C-4), 139.8 (C-5), 119.6 (C-6), 116.3 (C-7), 141.3 (C-8), 46.2 (C-9), 37.0 (C-10), 21.1 (C-11), 39.1 (C-12), 42.8 (C-13), 54.6 (C-14), 23.0 (C-15), 28.3 (C-16), 55.7 (C-17), 12.1 (C-18), 16.3 (C-19), 40.7 (C-20), 21.3 (C-21), 138.0 (C-22), 129.6 (C-23), 51.2 (C-24), 31.8 (C-25), 18.9 (C-26), 20.9 (C-27), 25.3 (C-28), 12.4 (C-29). [F. Matteo, T. Pasquale, L. Rafael (2010). Phytosterol from *Dunaliella tertiolecta* and *Dunaliella salina*: A potentially novel industrial application, *Bioresour. Technol.* **101**, 4144-4150.]

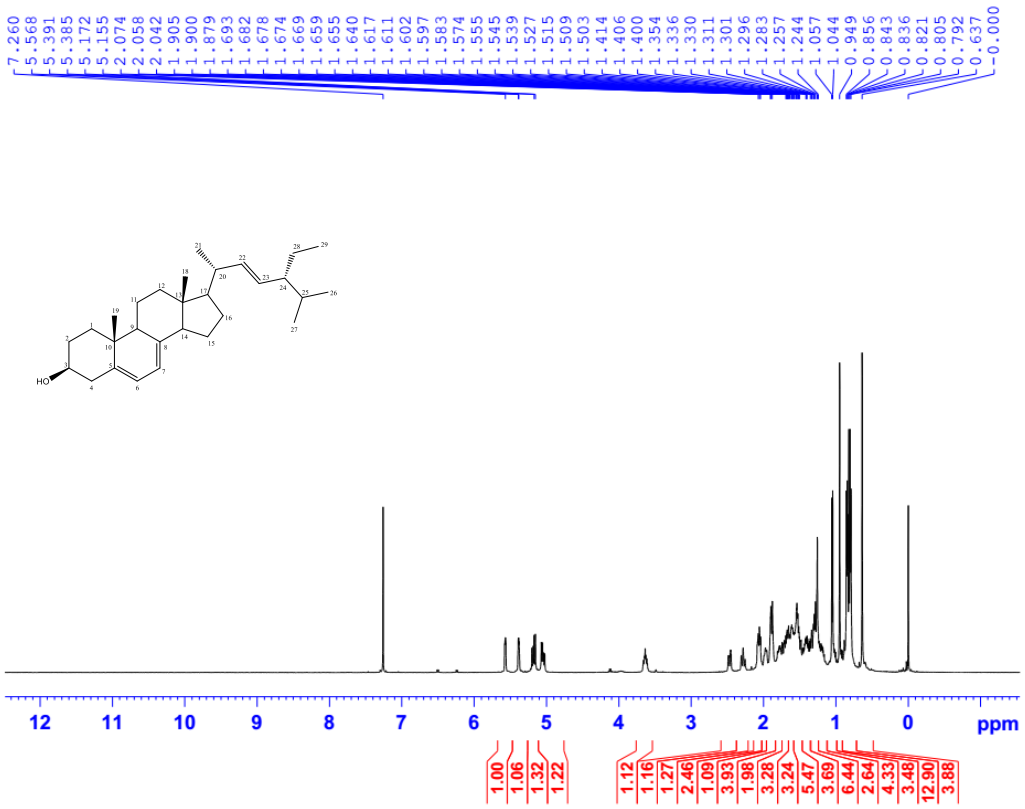

Figure S4: ^{1}H-NMR (500 MHz, $CDCl_3$) spectrum of **2** (7-dehydroporiferasterol)

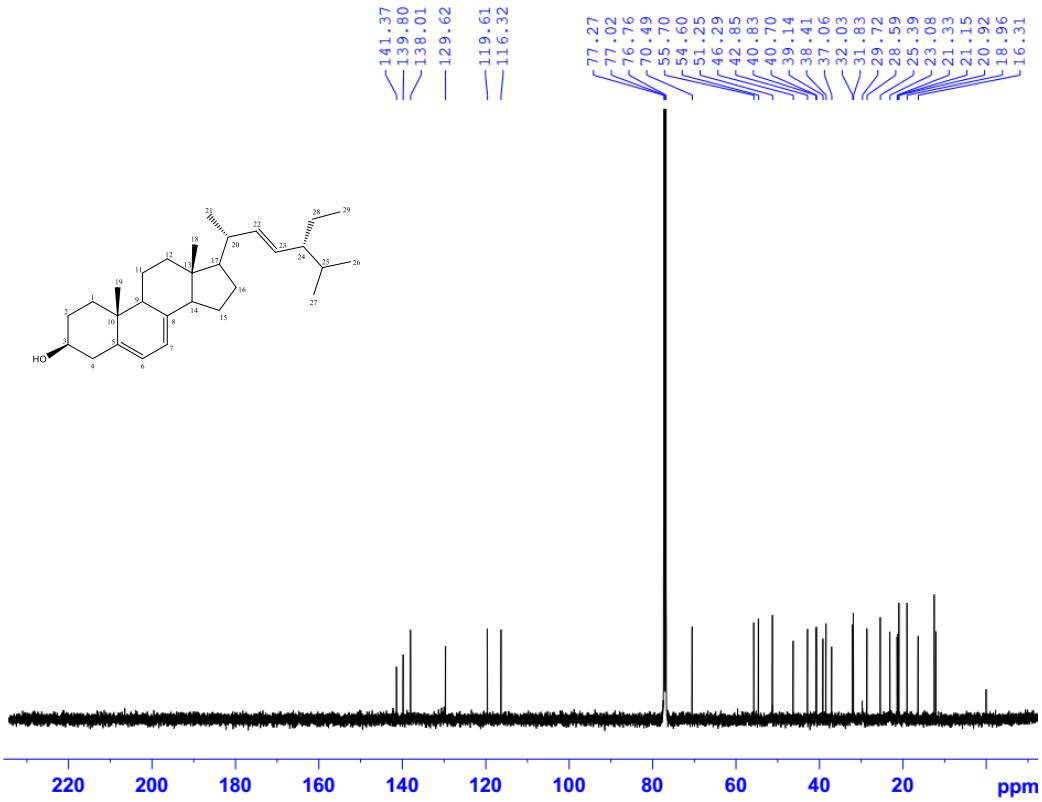

Figure S5: ^{13}C-NMR (125 MHz, $CDCl_3$) spectrum of **2** (7-dehydroporiferasterol)

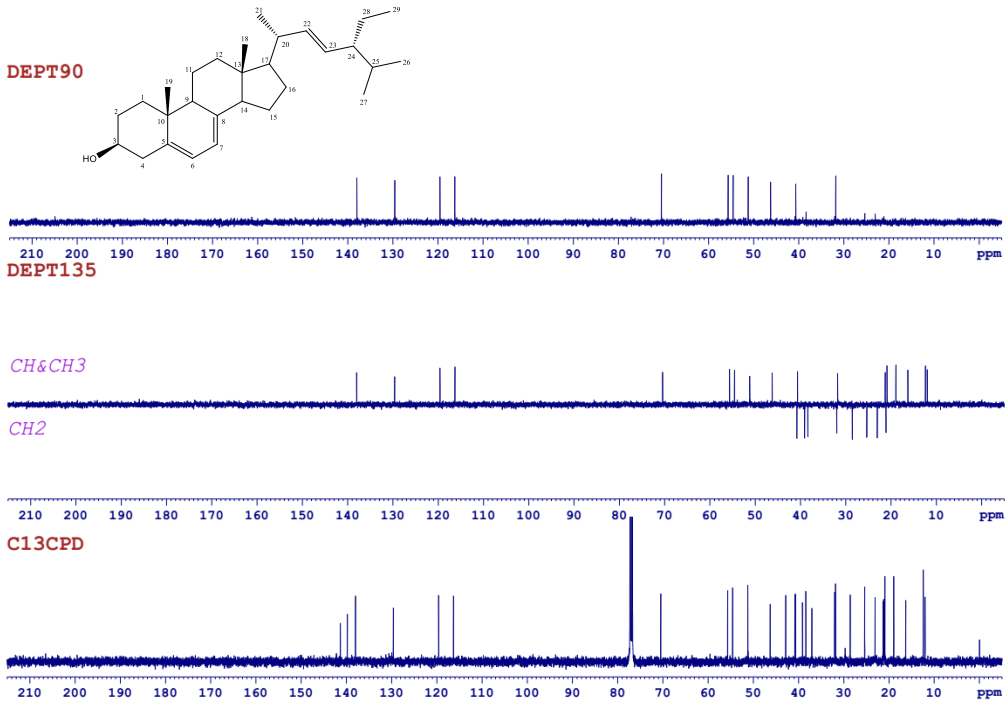

Figure S6: DEPT 90&135 spectrum of **2** (7-dehydroporiferasterol)

Compound **3** ((22*E*,24*R*)-ethylcholesta-7,22-dien-3*β*,5*α*,6*β*-triol)

^{1}H NMR (500 MHz, $CDCl_3$+CD_3OD) δ ppm: 0.60 (3H, s, H_3-18), 0.79 (3H, d, J = 6.5 Hz, H_3-27), 0.82 (3H, t, J = 7.5 Hz, H_3-29), 0.85 (3H, d, J = 6.5 Hz, H_3-26), 1.04 (3H, d, J = 6.5 Hz, H_3-21), 1.08 (3H, s, H_3-19), 3.62 (1H, br.s, H-6), 4.08 (1H, m, H-3), 5.04 (1H, dd, J = 8.5, 15.0 Hz, H-23), 5.16 (1H, dd, J = 8.5, 15.0 Hz, H-22).5.34 (1H, dd, J = 2.0, 5.5 Hz, H-7).

^{13}C NMR (125 MHz, $CDCl_3$+CD_3OD) δ ppm: 32.9 (C-1), 30.8 (C-2), 67.7 (C-3), 39.2 (C-4), 75.9 (C-5), 73.6 (C-6), 117.5 (C-7), 144.0 (C-8), 43.4 (C-9), 37.1 (C-10), 22.0 (C-11), 39.5 (C-12), 43.7 (C-13), 54.7 (C-14), 22.9 (C-15), 28.1 (C-16), 55.9 (C-17), 12.3 (C-18), 18.8 (C-19), 40.6 (C-20), 21.3 (C-21), 137.7 (C-22), 129.8 (C-23), 51.2 (C-24), 31.8 (C-25), 18.9 (C-26), 20.9 (C-27), 25.3 (C-28), 12.4 (C-29). [M. Anna, P. Vincenzo, S. Donato (1989). New polyhydroxysterols from the dictyoceratid sponges *Hippospongia communis*, *Spongia officinalis*, *Ircinia variabilis*, *and Spongionella gracilis*, *J. Nat. Prod.* **52**(5), 952-961].

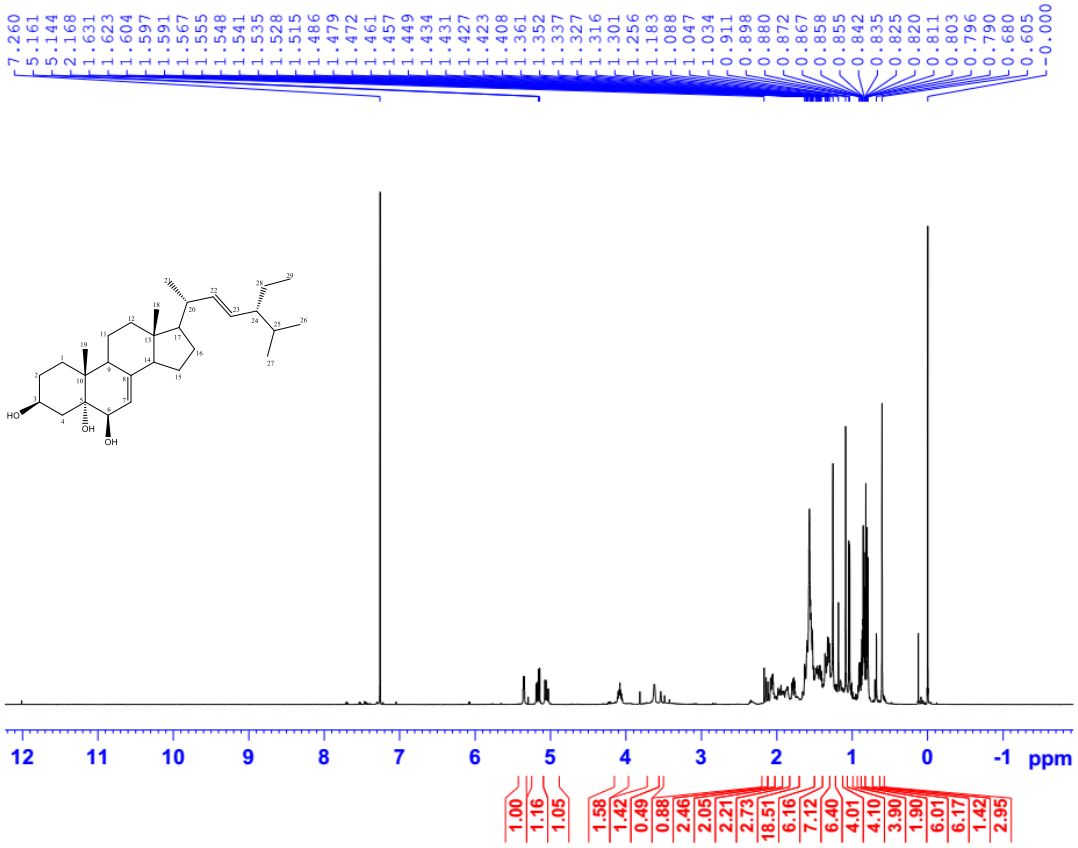

Figure S7: ^{1}H-NMR (500 MHz, $CDCl_3$) spectrum of **3** ((22*E*,24*R*)-ethylcholesta-7,22-dien-3*β*,5*α*,6*β*-triol)

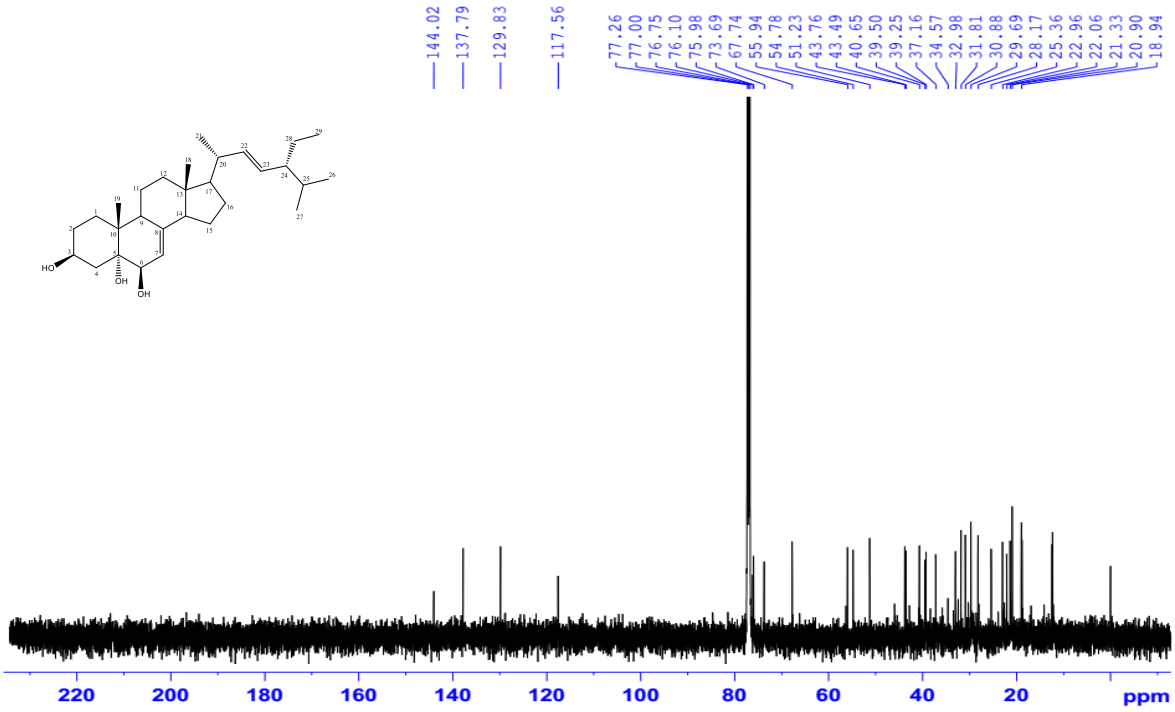

Figure S8: ^{13}C-NMR (125 MHz, $CDCl_3$) spectrum of **3** ((22*E*,24*R*)-ethylcholesta-7,22-dien-3*β*,5*α*,6*β*-triol)

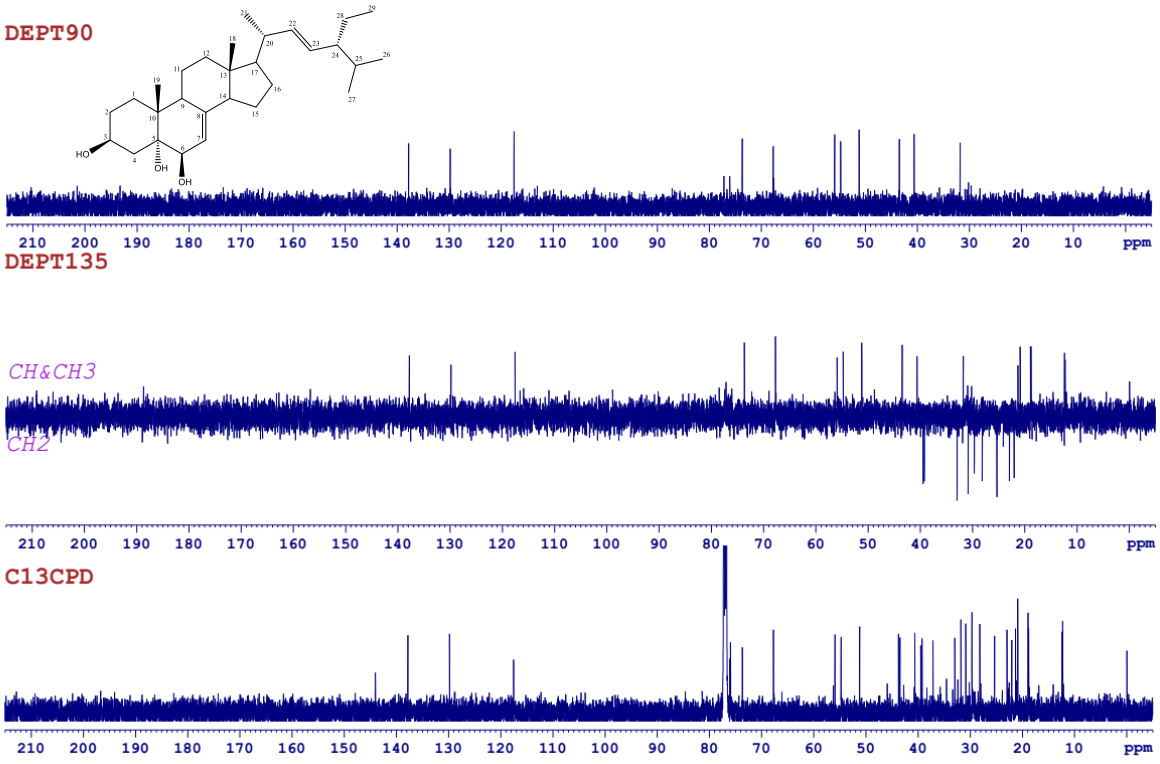

Figure S9: DEPT 90&135 spectrum of **3** ((22*E*,24*R*)-ethylcholesta-7,22-dien-3*β*,5*α*,6*β*-triol)

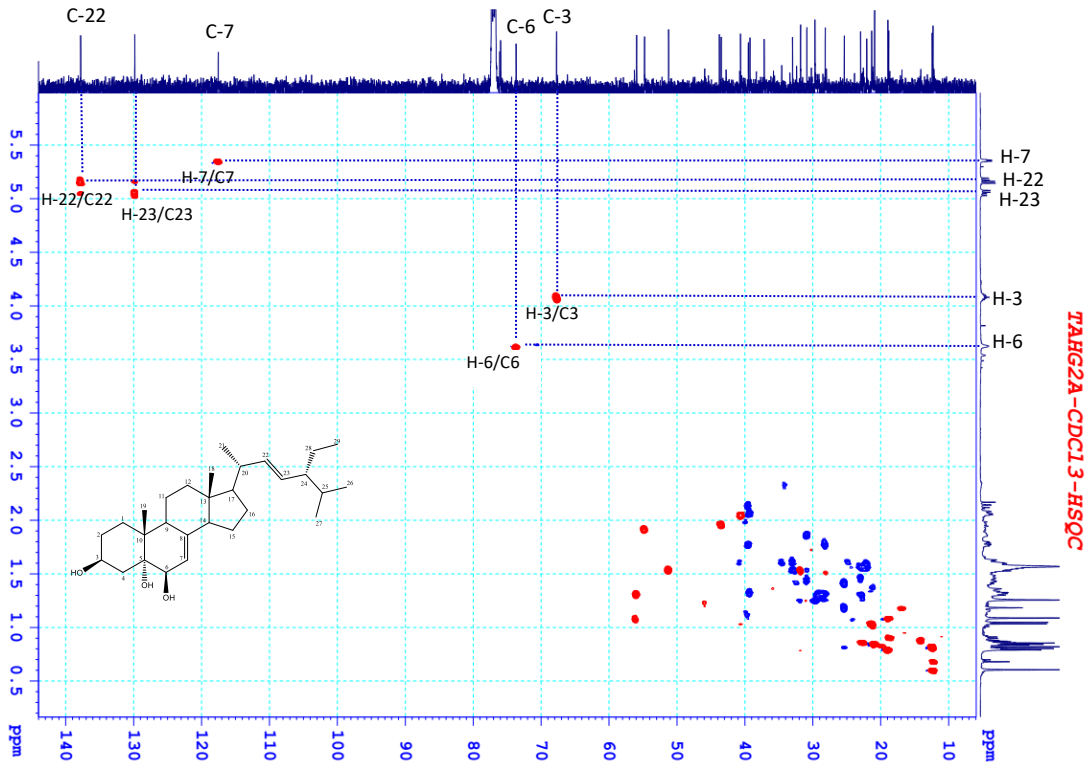

Figure S10: HSQC spectrum of **3** ((22*E*,24*R*)-ethylcholesta-7,22-dien-3β,5α,6β-triol)

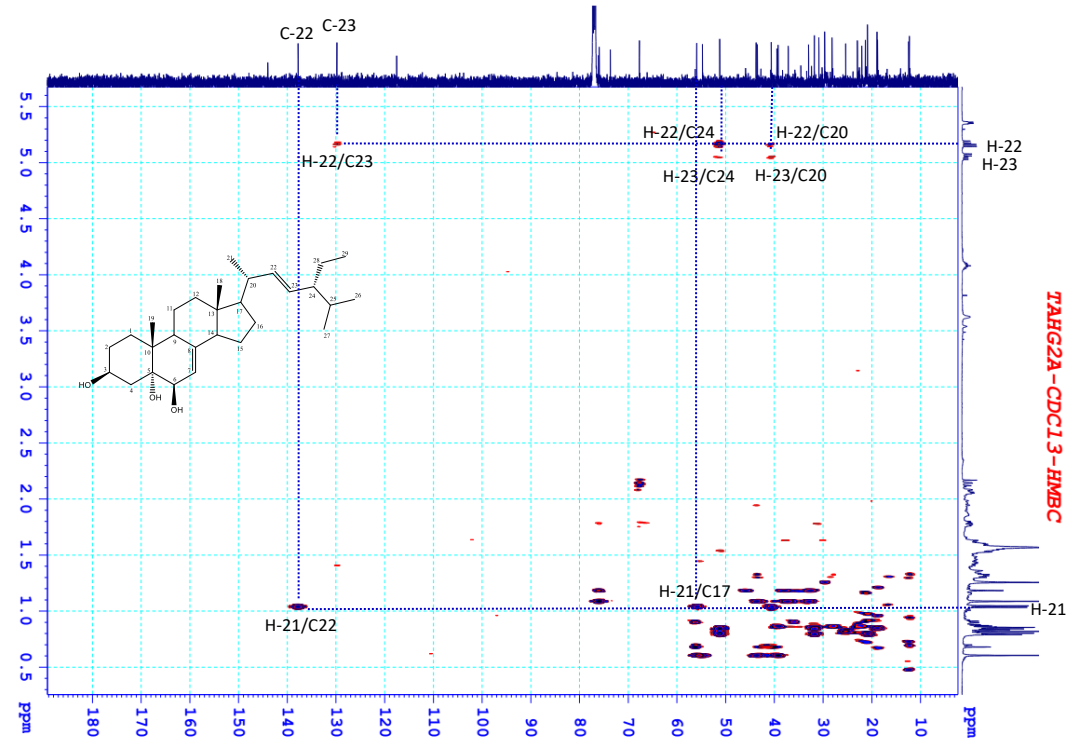

Figure S11: HMBC spectrum of **3** ((22*E*,24*R*)-ethylcholesta-7,22-dien-3β,5α,6β-triol)

Compound **4** (poriferasterol glucoside) :

^{1}H NMR (600 MHz, DMSO-d_6) δ ppm: 0.67 (3H, s, H_3-18), 0.77 (3H, d, J = 7.0 Hz, H_3-27), 0.78 (3H, t, J = 7.0 Hz, H-29), 0.82 (3H, d, J = 6.6 Hz, H_3-26), 0.92 (3H, s, H_3-19), 1.00 (3H, d, J = 7.0 Hz, H_3-21), 3.45 (1H, m, H-3), 5.32 (1H, m, H-6), 5.03 (1H, dd, J = 8.7, 15.3 Hz, H-23), 5.17 (1H, dd, J = 8.7, 15.3 Hz, H_3-22), 4.21 (1H, d, J = 7.5 Hz, H-1′), 2.91 (1H, m, H-2′), 3.11 (1H, m, H-3′), 3.02 (1H, m, H-4′), 3.08 (1H, m, H-5′), 3.64 (1H, ddd, J = 2.0, 5.5, 11.5 Hz, H-6′a), 3.40 (1H, m, H-6′b.).

^{13}C NMR (150 MHz, DMSO-d_6) δ ppm: 36.2 (C-1), 26.2 (C-2), 76.9 (C-3), 28.3 (C-4), 140.4 (C-5), 121.1 (C-6), 38.3 (C-7), 31.4 (C-8), 49.6 (C-9), 36.8 (C-10), 20.5 (C-11), 39.0 (C-12), 41.7 (C-13), 56.2 (C-14), 23.8 (C-15), 29.2 (C-16), 55.3 (C-17), 11.8 (C-18), 19.0 (C-19), 40.0 (C-20), 20.7 (C-21), 137.9 (C-22), 128.8 (C-23), 50.5 (C-24), 31.2 (C-25), 18.7 (C-26), 21.0 (C-27), 24.7 (C-28), 12.2 (C-29), 100.7 (C-1′), 73.4 (C-2′), 76.7 (C-3′), 70.1 (C-4′), 76.7 (C-5′), 61.0 (C-6′). [K. Mahbuba, B. Mirajum and M. A. Quader (2012). Sterols and sterol glucoside from *Phyllanthus* species, *Dhaka Univ. J. Sci.* **60**(1), 5-10].

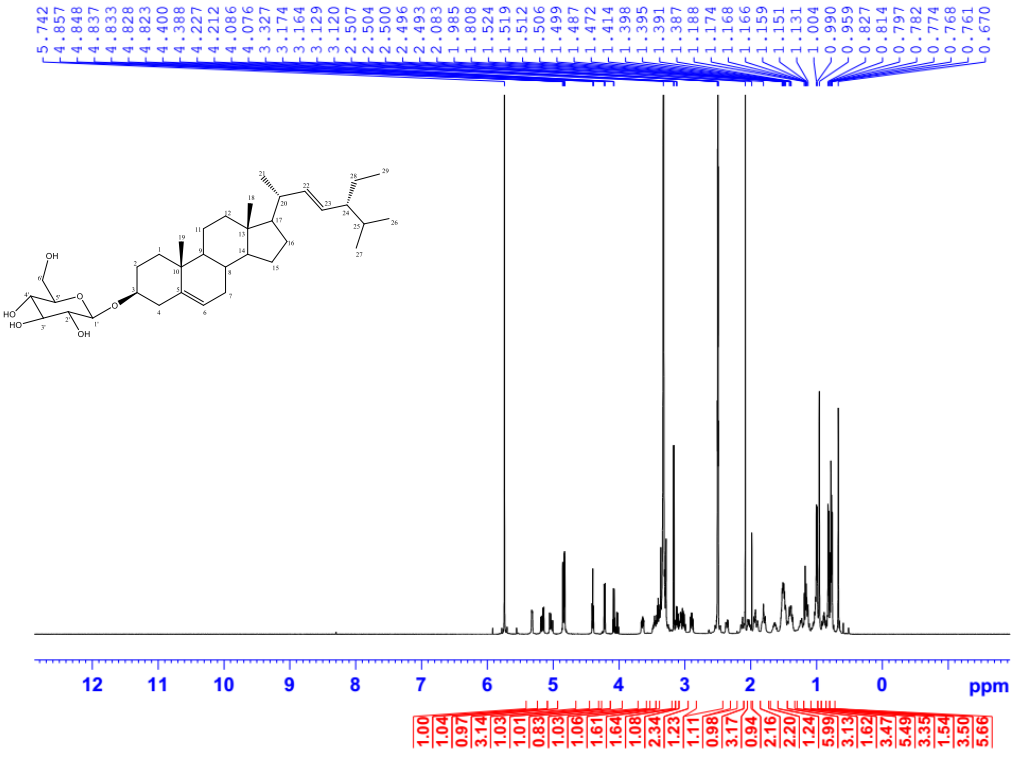

Figure S12: ^{1}H-NMR (500 MHz, DMSO-d_6) spectrum of **4** (poriferasterol glucoside)

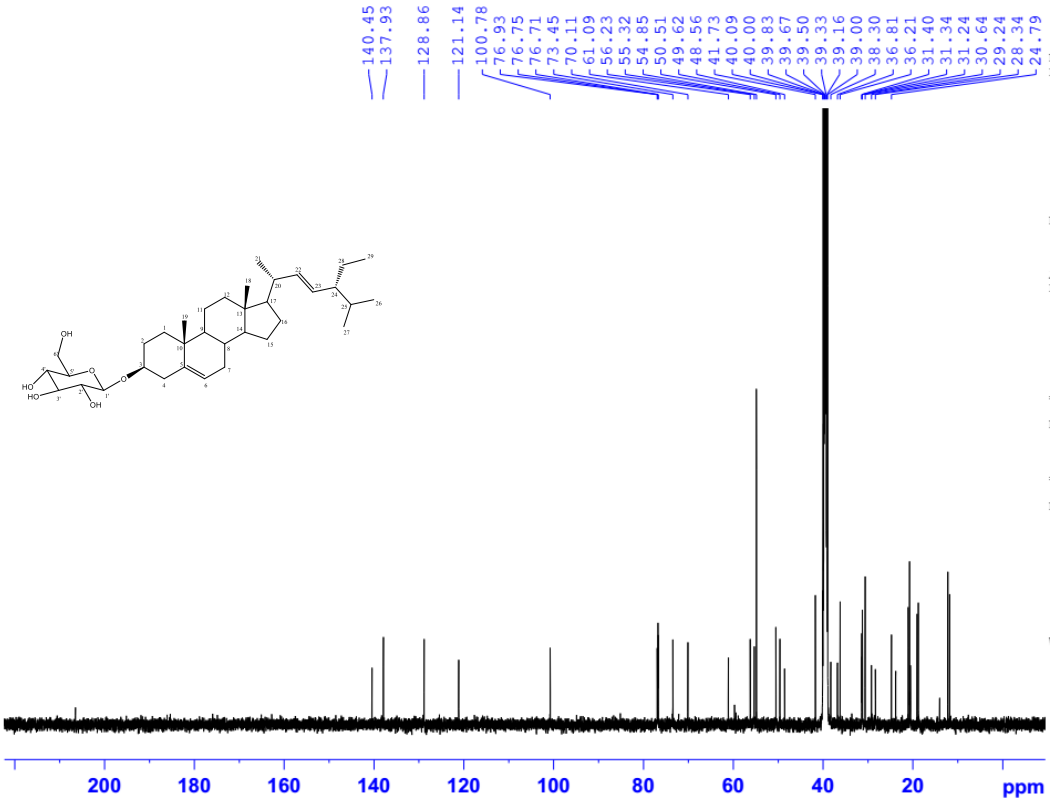

Figure S13: ^{13}C-NMR (125 MHz, DMSO-d_6) spectrum of **4** (poriferasterol glucoside)

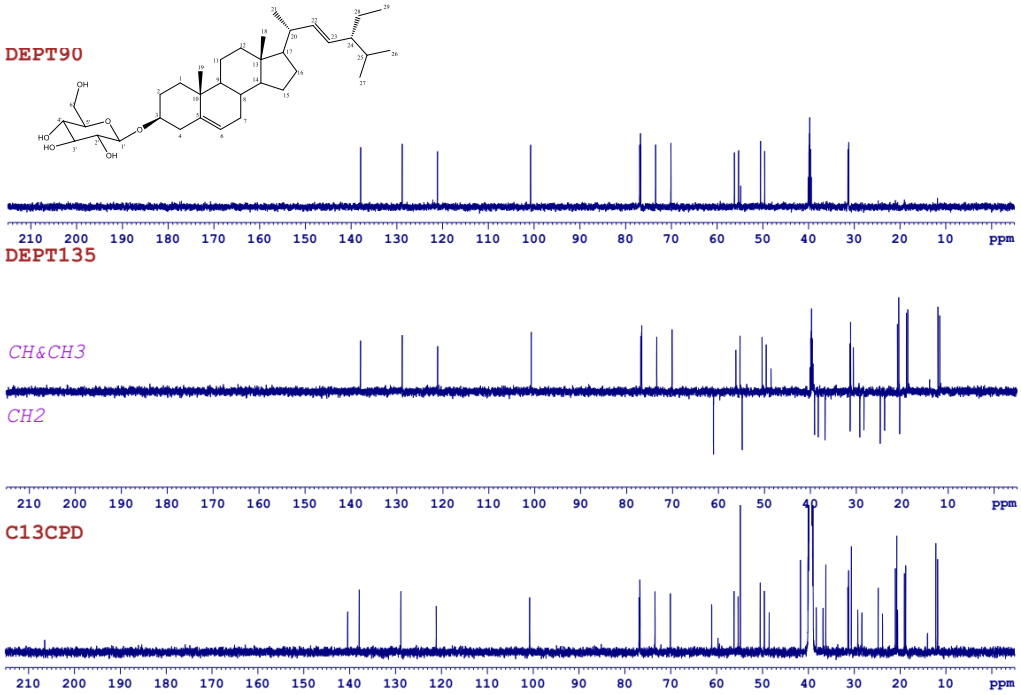

Figure S14: DEPT 90&135 spectrum of **4** (poriferasterol glucoside)

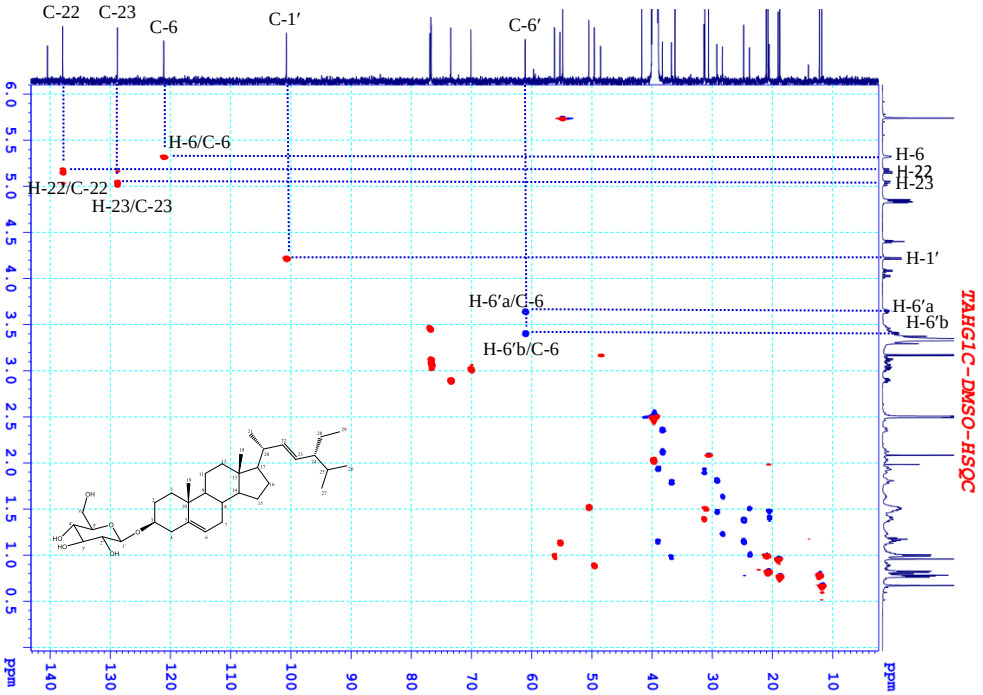

Figure S15: HSQC spectrum of **4** (poriferasterol glucoside)

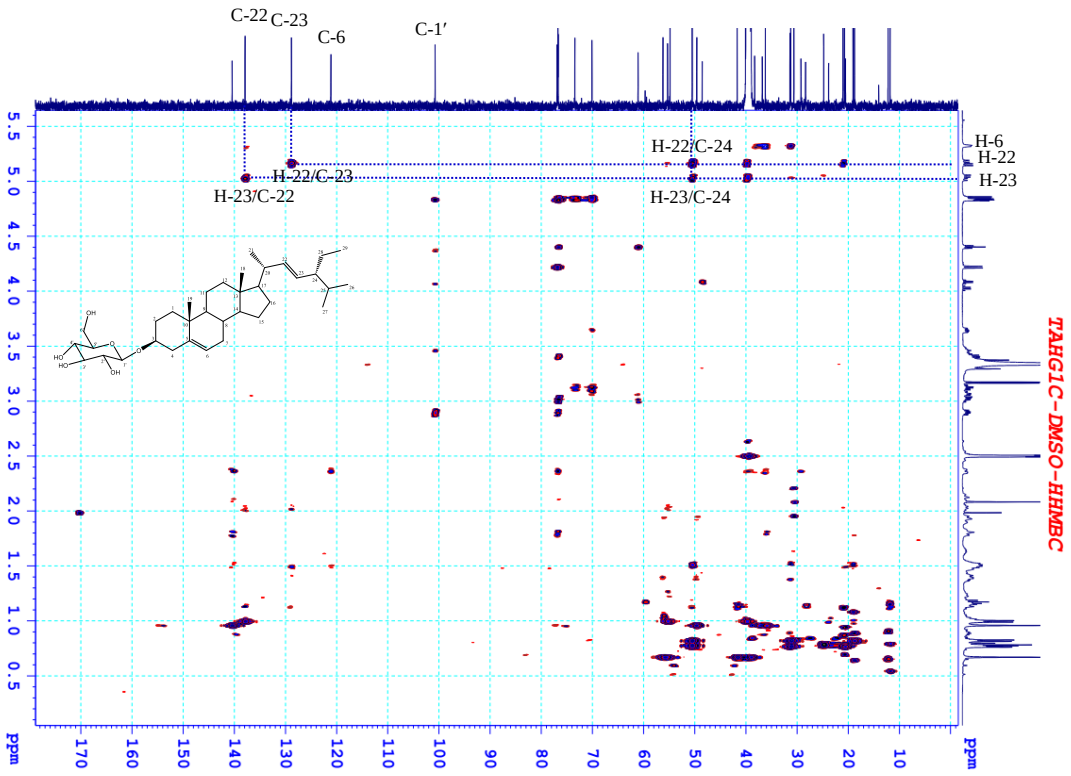

Figure S16: HMBC spectrum of **4** (poriferasterol glucoside)

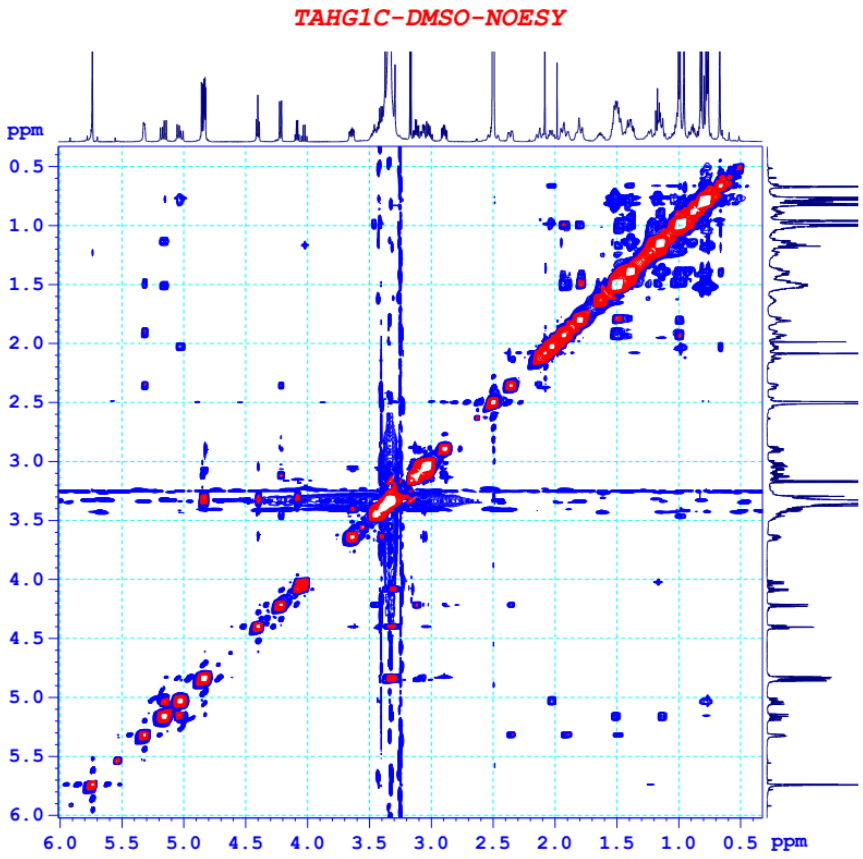

Figure S17: NOESY spectrum of **4** (poriferasterol glucoside)

Compound **5** (perlolyrine) :

^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 8.36 (1H, d, J = 5.0 Hz, H-3), 8.05 (1H, d, J = 5.0 Hz, H-4), 8.25 (1H, d, J = 8.0 Hz, H-5), 7.28 (1H, dt, J = 1.0, 8.0 Hz, H-6), 7.59 (1H, dt, J = 1.0, 8.0 Hz, H-7), 7.75 (1H, d, J = 8.0 Hz, H-8), 7.19 (1H, d, J = 3.0 Hz, H-3′), 6.57 (1H, d, J = 3.0 Hz, H-4′), 4.65 (2H, d, J = 4.0 Hz, H-6′).

^{13}C NMR (125 MHz, DMSO-d_6) δ ppm: 133.1 (C-1), 138.1 (C-3), 113.6 (C-4), 121.6 (C-5), 119.7 (C-6), 128.4 (C-7), 112.4 (C-8), 130.4 (C-10), 129.4 (C-11), 120.6 (C-12), 141.0 (C-13), 152.1 (C-2′), 109.6 (C-3′), 109.0 (C-4′), 156.7 (C-5′), 55.9 (C-6′). [B. Dassonneville, B. Witulski, H. Detert (2011). [2+2+2] Cycloadditions of Alkynylynamides - A Total Synthesis of Perlolyrine and the First Total Synthesis of “Isoperlolyrine”, *Eur. J. Org. Chem.* **2011**, 2836-2844.]

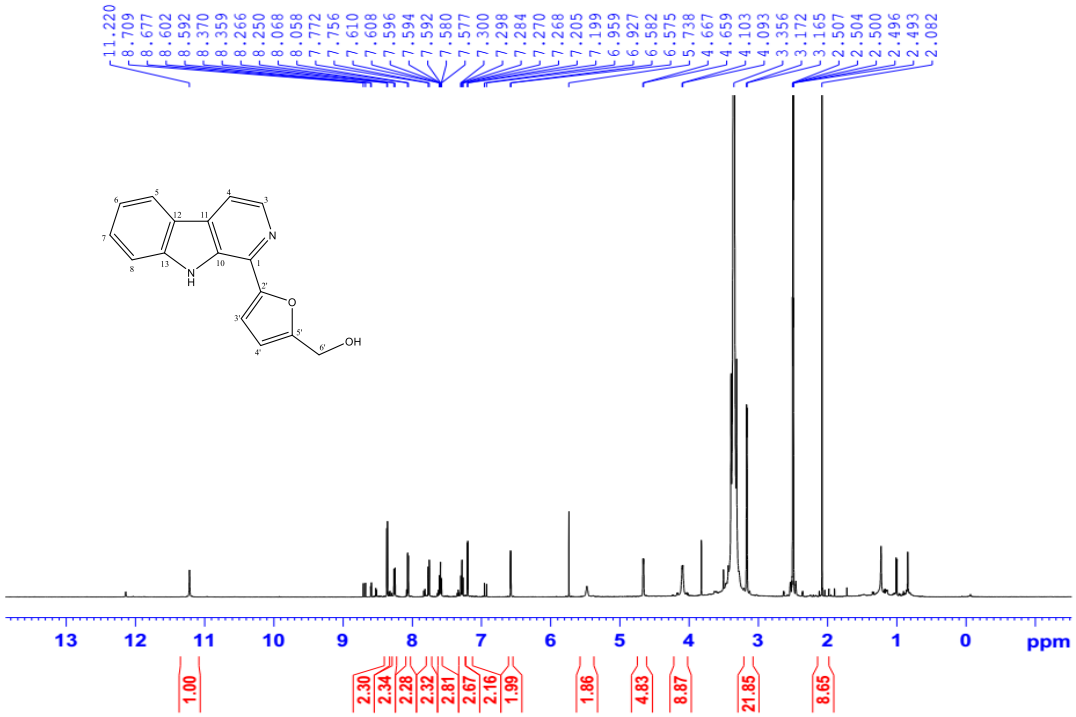

Figure S18: ^{1}H-NMR (500 MHz, DMSO-d_6) spectrum of **5** (perlolyrine)

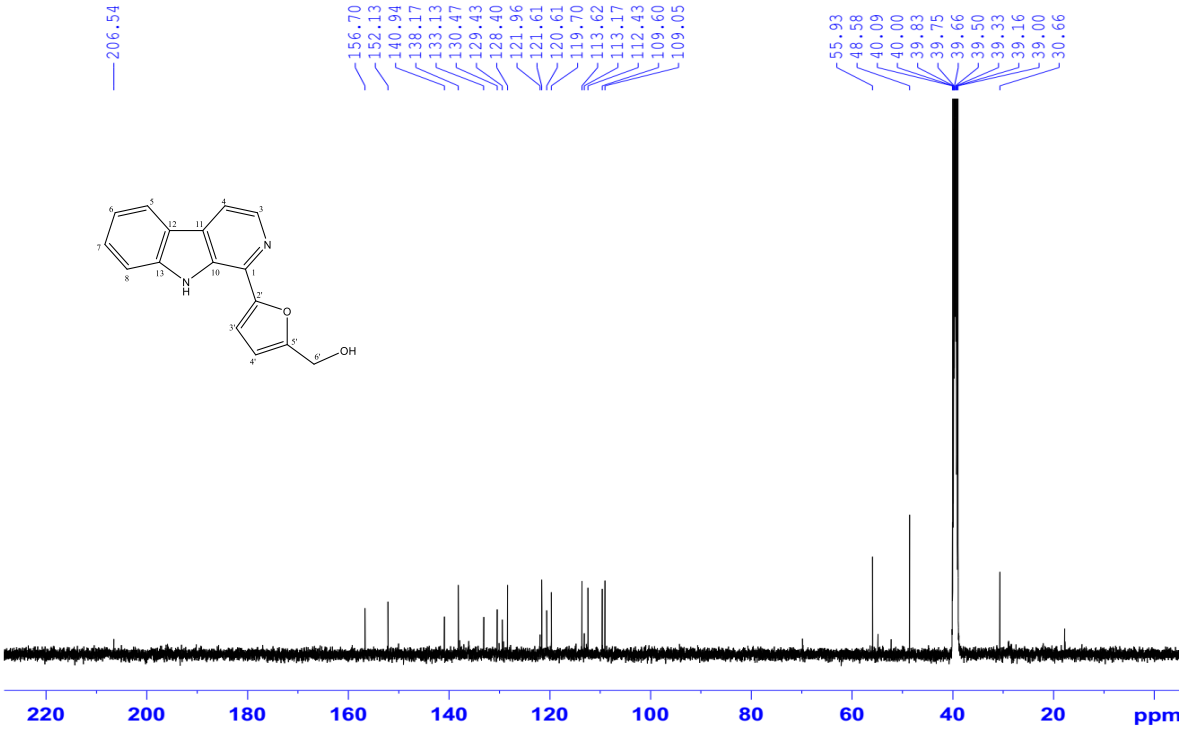

Figure S19: ^{13}C-NMR (125 MHz, DMSO-d_6) spectrum of **5** (perlolyrine)

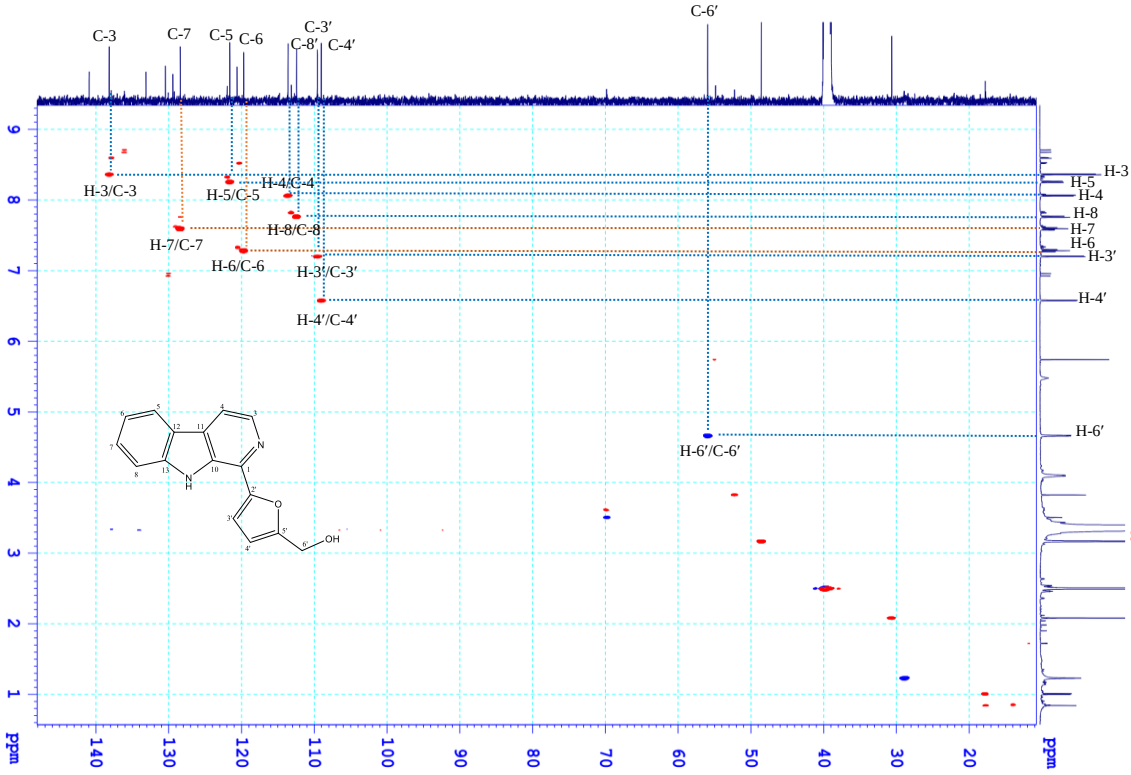

Figure S20: HSQC spectrum of **5** (perlolyrine)

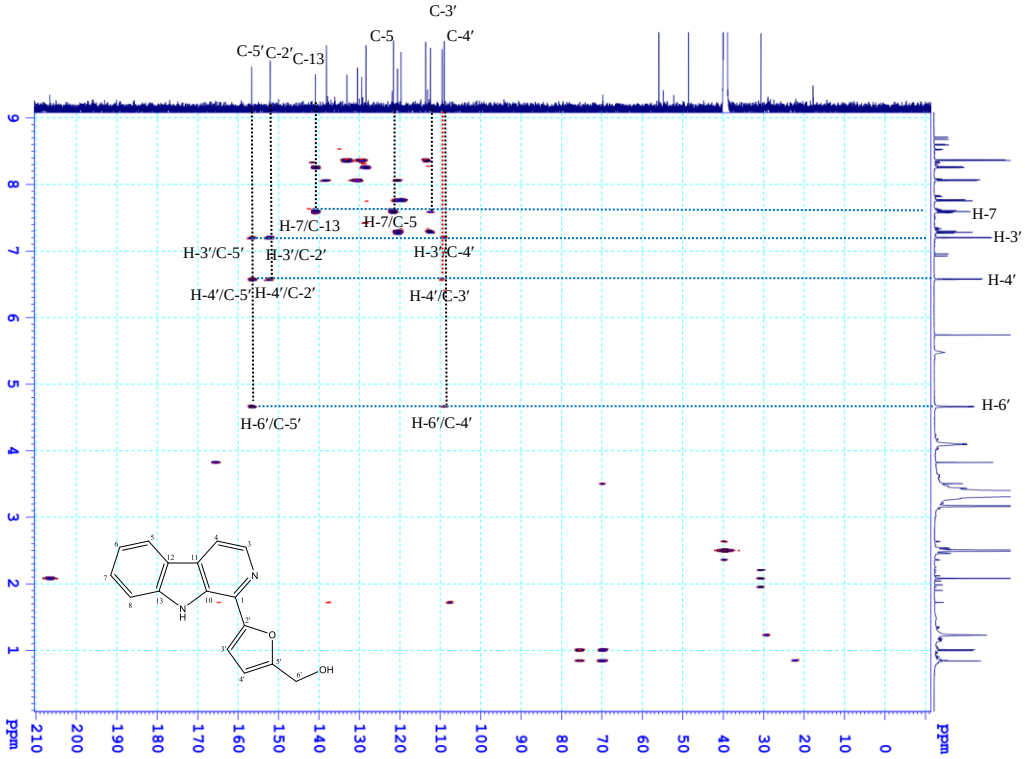

Figure S21: HMBC spectrum of **5** (perlolyrine)

Compound **6** (pyrrolezanthine-6-methyl ether)

^{1}H NMR (500 MHz, $CDCl_3$) δ ppm: 6.90 (1H, d,J = 4.0 Hz, H-3), 6.17 (1H, d, J = 4.0 Hz, H-4), 9.55 (1H, s, H-6), 7.01 (2H, d, J = 8.5 Hz, H-2′, H-6′), 6.74 (2H, d, J = 8.5 Hz, H-3′, H-5′), 2.94 (1H, t, J = 7.5 Hz, H-7′), 4.46 (1H, dd, J = 6.0, 7.5 Hz, H-8′), 4.14 (2H, s, H_2-1′′), 3.30 (3H, s, H_3-3′′).

^{13}C NMR (125 MHz, $CDCl_3$) δ ppm: 132.4 (C-2), 124.3 (C-3), 111.3 (C-4), 139.0 (C-5), 179.4 (C-6), 130.7 (C-1′), 130.2 (C-2′, C-6′), 115.4 (C-3′, C-5′), 154.4 (C-4′), 36.8 (C-7′), 47.9 (C-8′), 65.6 (C-1′′), 57.9 (C-3′′). [G-H. Xu, Y-H. Kim, S-J. Choo, I-J. Ryoo, J-K. Yoo1, J-S. Ahn, and I-D. Yoo (2009). Chemical Constituents from the Leaves of *Ilex paraguariensis* Inhibit Human Neutrophil Elastase, *Arch. Pharm. Res.* **32**, 1215-1220]

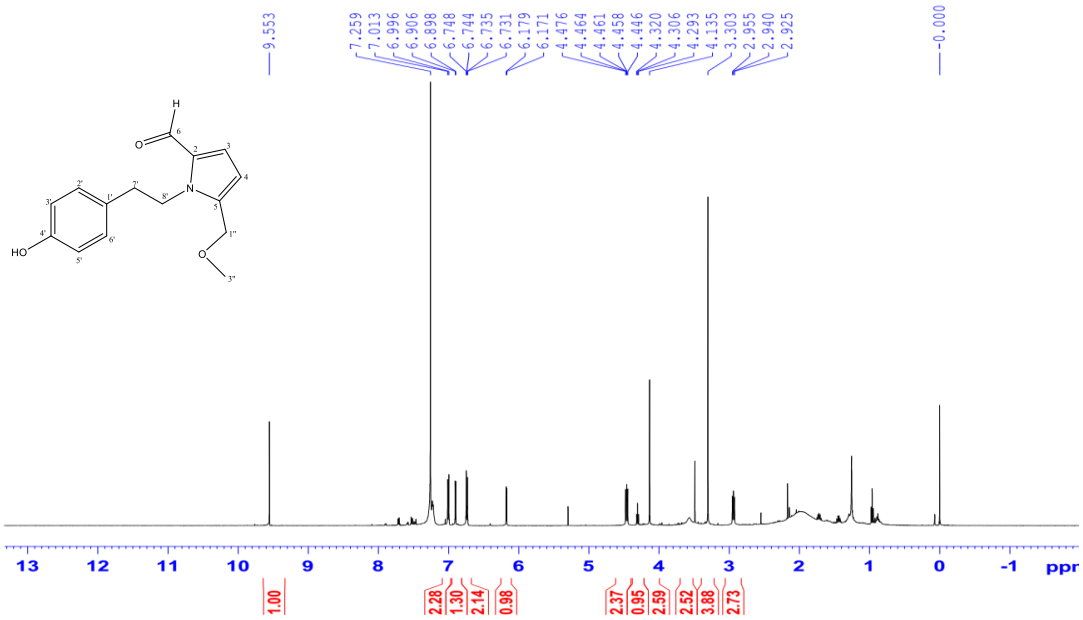

Figure S22: ^{1}H-NMR (500 MHz, $CDCl_3$) spectrum of **6** (pyrrolezanthine-6-methyl ether)

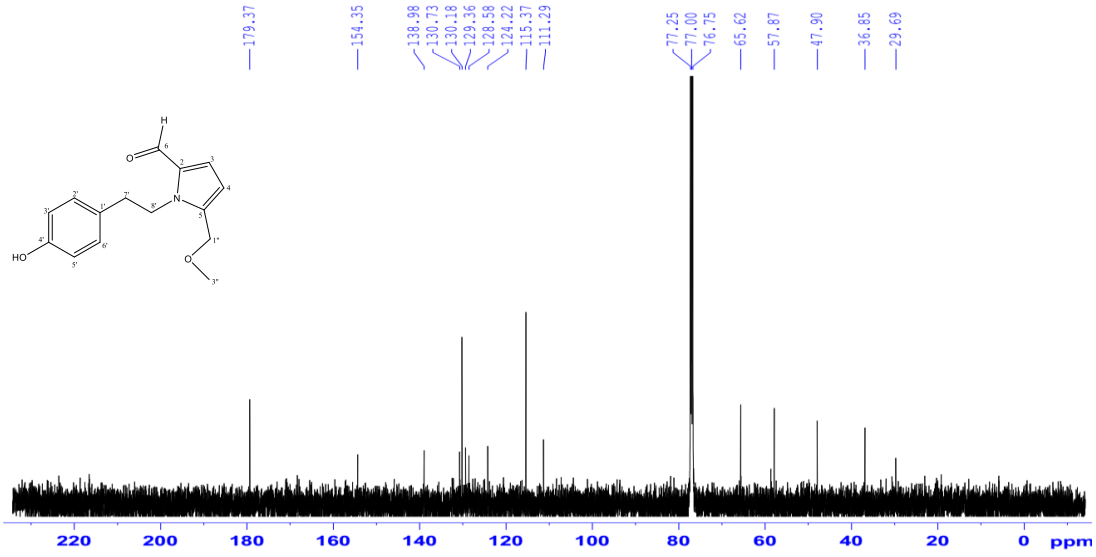

Figure S23: ^{13}C-NMR (125 MHz, $CDCl_3$) spectrum of **6** (pyrrolezanthine-6-methyl ether)

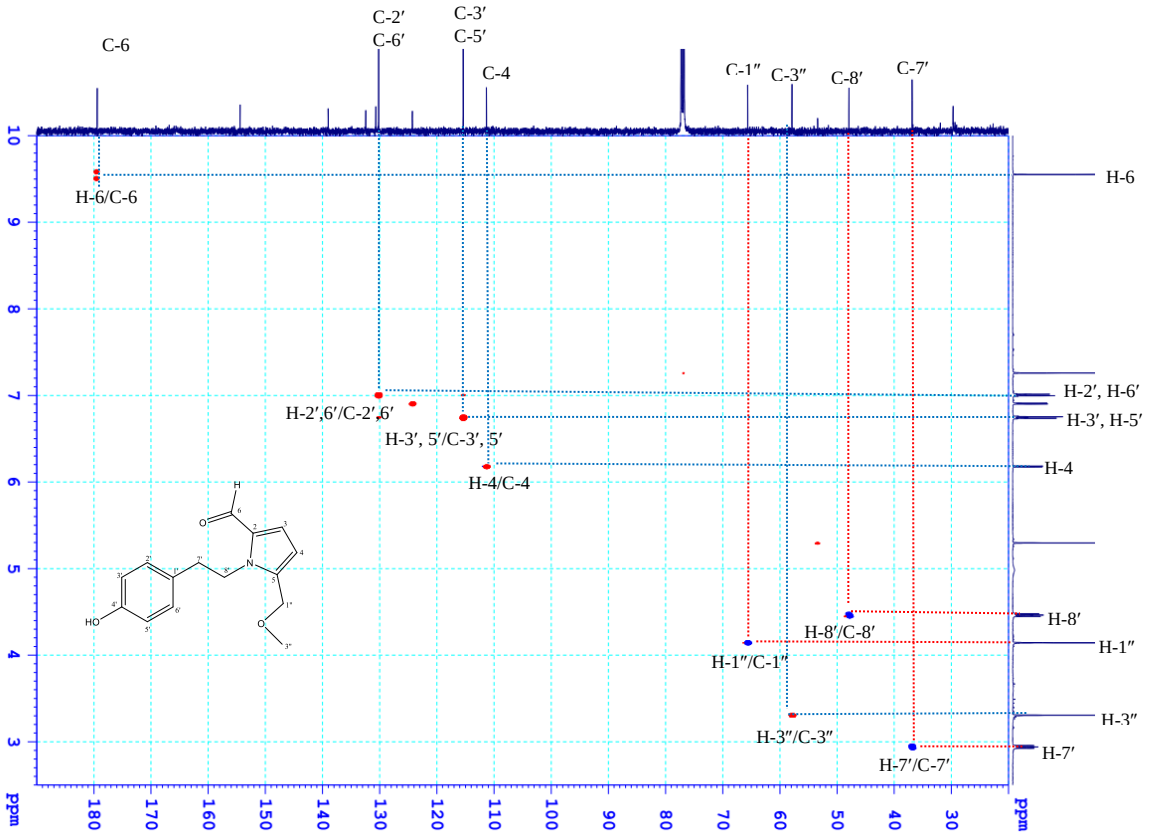

Figure S24: HSQC spectrum of **6** (pyrrolezanthine-6-methyl ether)

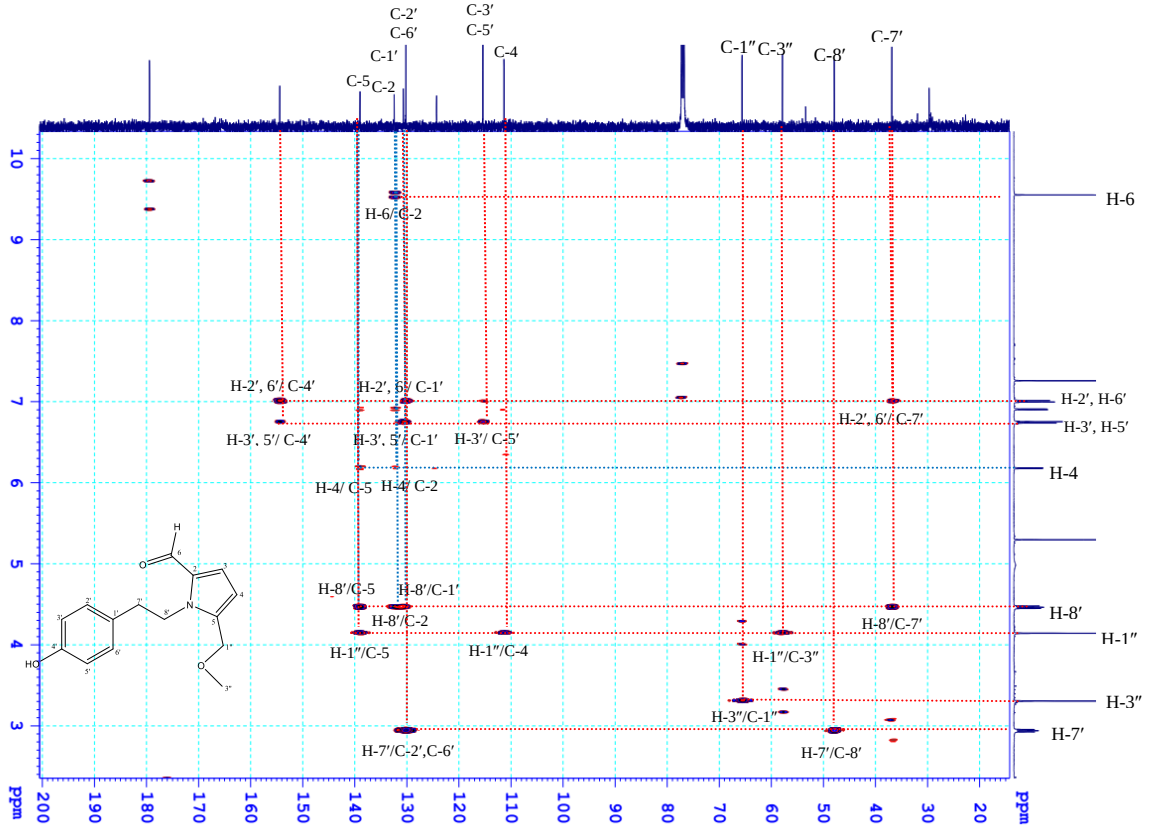

Figure S25: HMBC spectrum of **6** (pyrrolezanthine-6-methyl ether)

Compound **7** (3-(3-aminopropyl)-6-[(4-hydroxyphenyl)methyl]-2,5-piperazinedione):

^{1}H NMR (500 MHz, $CDCl_3$) δ ppm: 1.89 (1H, m, Ha-2′), 1.94 (1H, m, Ha-1′), 2.00 1H, (m, Hb-2′), 2.32 (1H, m, Hb-1′), 2.74 (1H, dd, J = 10.5, 14.5 Hz, Ha-7″), 3.49 (1H, m, Hb-7″), 3.56 (1H, m, Ha-3′), 3.63 (1H, m, Hb-3′), 4.07 (1H, t, J = 7.5 Hz, H-3), 4.21 (1H, br.d, J = 8.0 Hz, H-6), 6.80 (2H, d, J = 8.5 Hz, H-3″, H-5″), 7.07 (2H, d, J = 8.5 Hz, H-2″, H-6″).
^{13}C NMR (125 MHz, $CDCl_3$) δ ppm: 169.5 (C-2), 59.1 (C-3), 165.1 (C-5), 56.2 (C-6), 28.3 (C-1′), 22.5 (C-2′), 45.4 (C-3′), 127.4 (C-1″), 130.3 (C-2″, C-6″), 116.1 (C-3″, C-5″), 155.3 (C-4″), 35.9 (C-7″). [Yusuke Sasaki, Yasuyuki Akutsu, Kenji Suzuki, Shinnobu Sakurada, and Kensuke Kisara (1981). Structure and Analgesic activity relationship of Cyclo-Tyrosyl-Arginyl and Its Three Stereoisomers, *Chem. Pharm. Bull*. **29**(11), 3403-3406.]

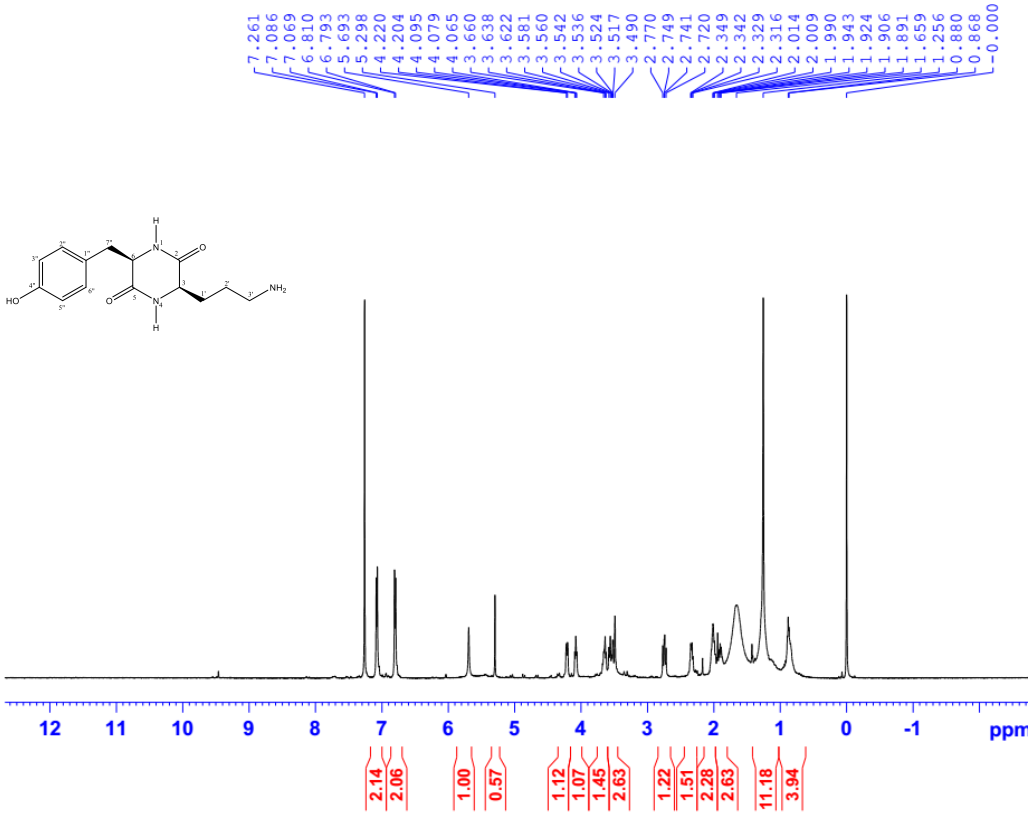

Figure S26: ^{1}H-NMR (500 MHz, CDCl3) spectrum of **7** (3-(3-aminopropyl)-6-[(4-hydroxyphenyl)methyl]-2,5-piperazinedione)

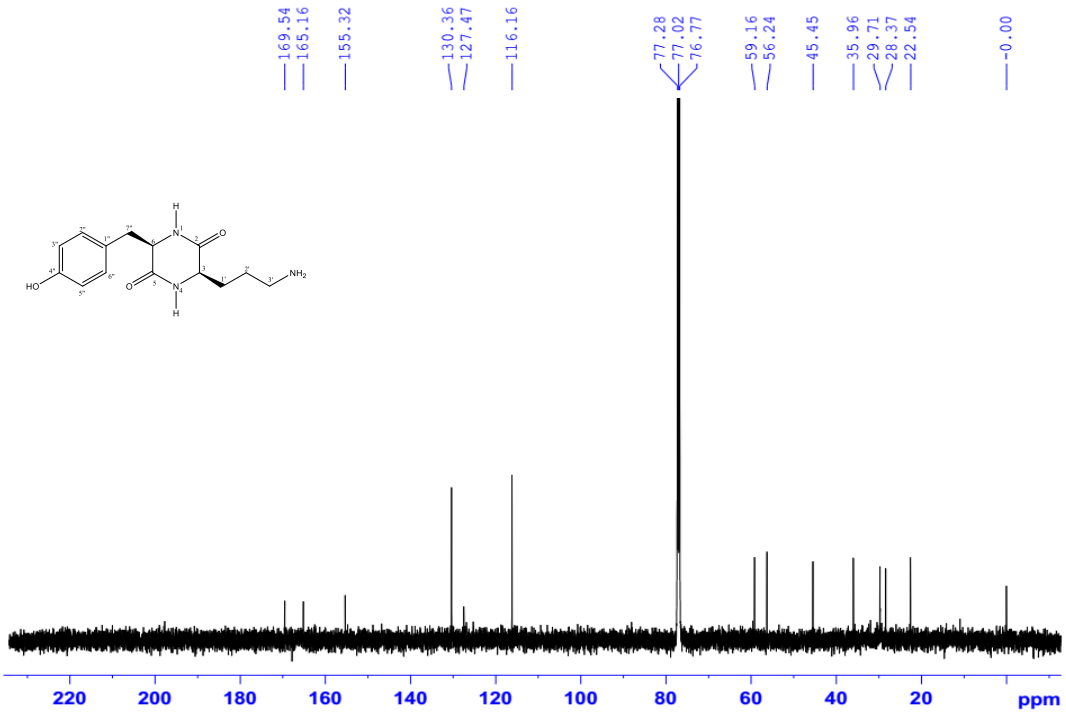

Figure S27: 13C-NMR (125 MHz, CDCl3) spectrum of **7** (3-(3-aminopropyl)-6-[(4-hydroxyphenyl)methyl]-2,5-piperazinedione)

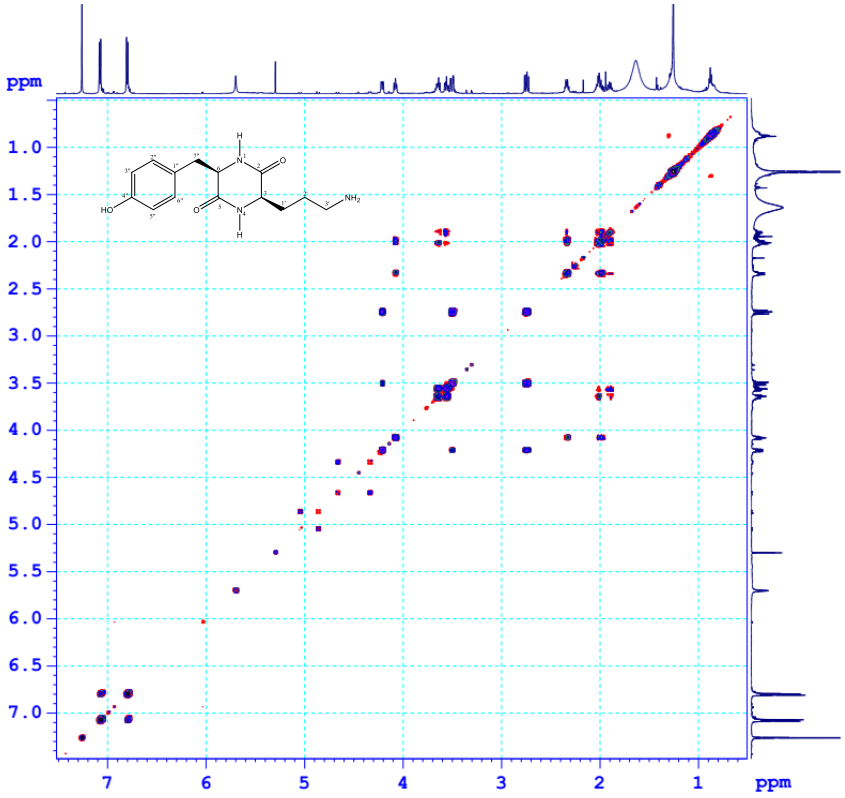

Figure S28: COSY (1H-1H) spectrum of **7** (3-(3-aminopropyl)-6-[(4-hydroxyphenyl)methyl]-2,5-piperazinedione)

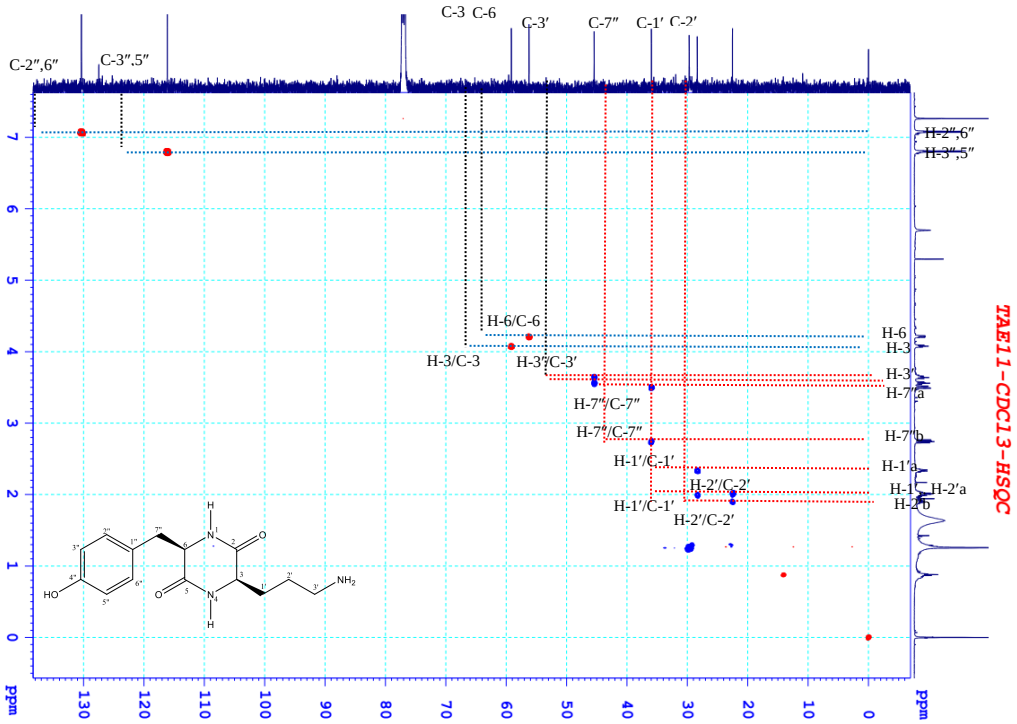

Figure S29: HSQC spectrum of **7** (3-(3-aminopropyl)-6-[(4-hydroxyphenyl)methyl]-2,5-piperazinedione)

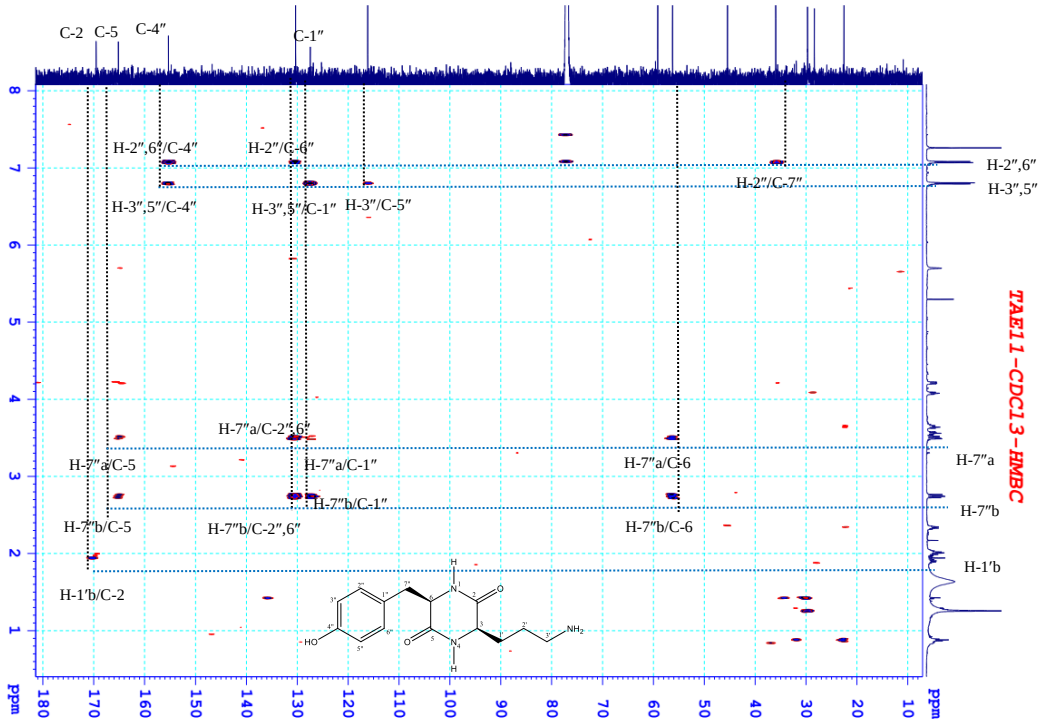

Figure S30: HMBC spectrum of **7** (3-(3-aminopropyl)-6-[(4-hydroxyphenyl)methyl]-2,5-piperazinedione)

Compound **8** (5-methyluracil)

^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 7.23 (1H, d, J = 1.0 Hz, H-6), 1.71 (3H, d, J= 1.5 Hz, H_3-7), 10.55 (1H, s, 1-NH), 10.96 (1H, 3-NH).

^{13}C NMR (125 MHz, DMSO-d_6) δ ppm: 151.5 (C-2), 164.9 (C-4), 107.7 (C-5), 137.7 (C-6), 11.7 (C-7).

[Z.-G. Ding, J.-Y. Zhao, P.-W. Yang, M.-G. Li, R. Huang, X.-L. Cui and M.-L. Wen (2009), ^{1}H and ^{13}C NMR assignments of eight nitrogen containing compounds from *Nocardia alba* sp.nov (YIM 30243T), *Magn. Reson. Chem.* **47**, 366-370].

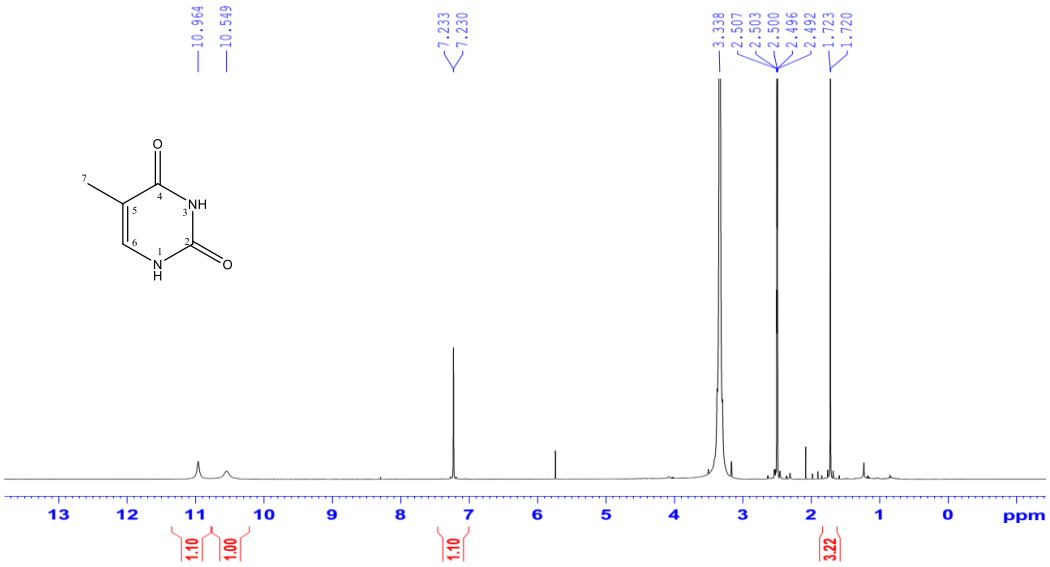

Figure S31: ^{1}H-NMR (500 MHz, DMSO-d_6) spectrum of **8** (5-methyluracil)

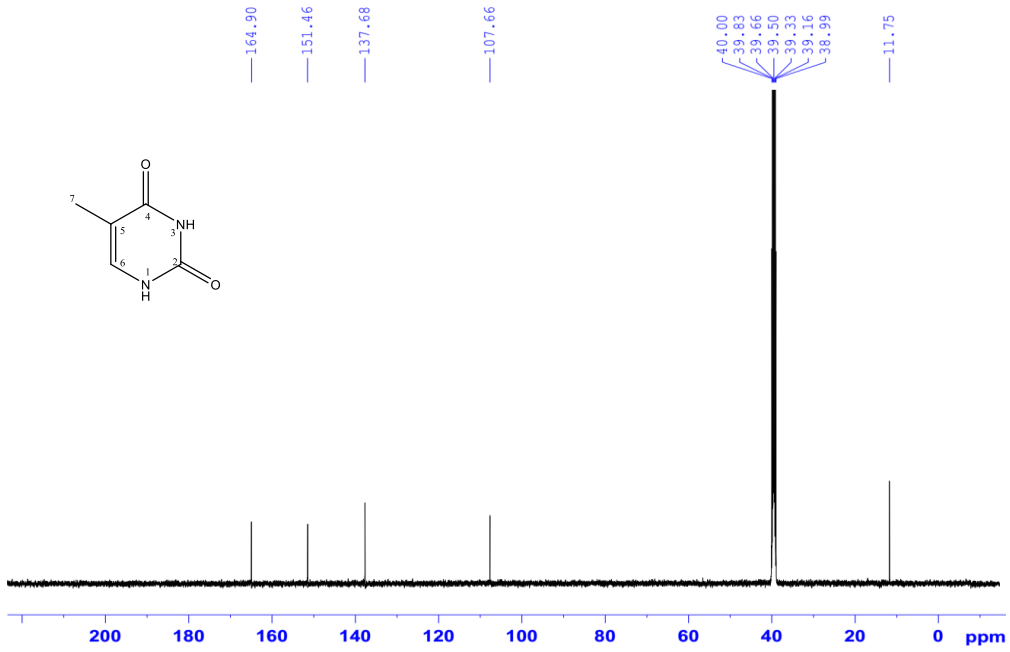

Figure S32: ^{13}C-NMR (125 MHz, DMSO-d_6) spectrum of **8** (5-methyluracil)

Compound **9** (1*H*-indole-3-carboxylic acid)

^{1}H NMR (500 MHz, DMSO-d_6) δ ppm: 7.17 (1H, t, J = 7.5 Hz, H-7), 7.45 (1H, d, J = 7.5 Hz, H-5), 7.14 (1H, t, J = 7.5 Hz, H-6), 7.98 (1H, d, J = 2.0 Hz, H-2), 7.99 (1H, d, J = 7.5 Hz, H-8).

^{13}C NMR (125 MHz, DMSO-d_6) δ ppm: 107.2 (C-2), 125.8 (C-3), 131.8 (C-4), 120.6 (C-5), 120.3 (C-6), 121.8 (C-7), 111.8 (C-8), 136.2 (C-9), 165.5 (C-10).

[M. S. Morales-Rios, J. Espiieira and P. Joseph-Nathan (1987). ^{13}C NMR Spectroscopy of Indole Derivatives, *Magn. Reson.Chem.* **25**, 377-395].

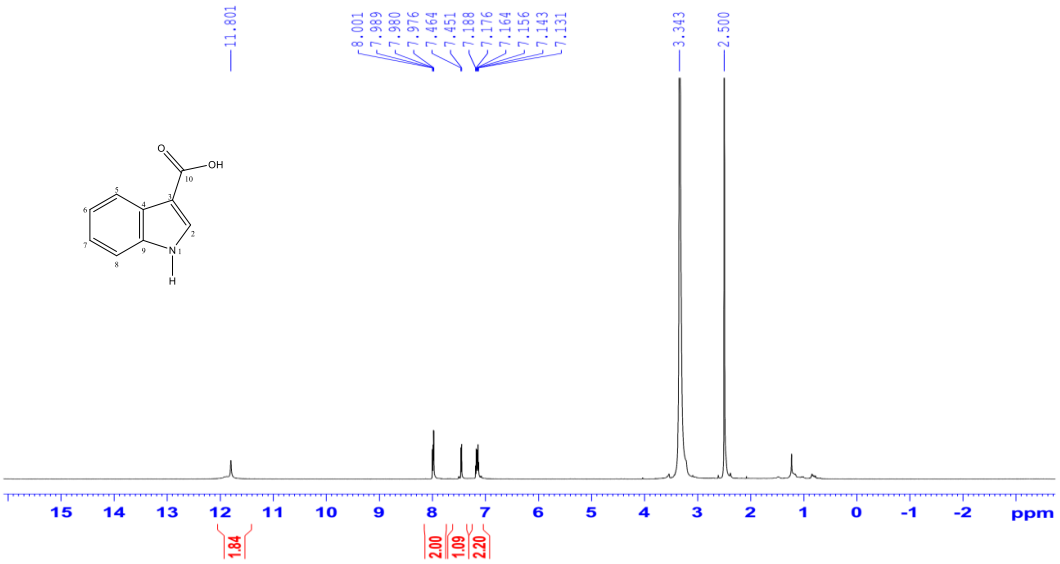

Figure S33: ^{1}H-NMR (500 MHz, DMSO-d_6) spectrum of **9** (1*H*-indole-3-carboxylic acid)

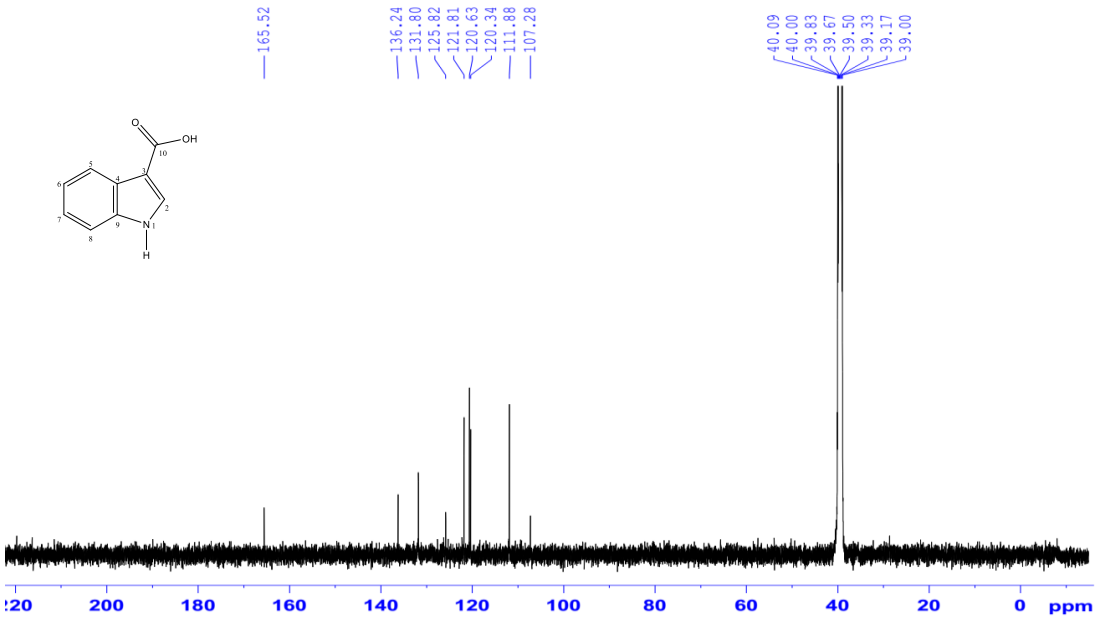

Figure S34: ^{13}C-NMR (125 MHz, DMSO-d_6) spectrum of **9** (1*H*-indole-3-carboxylic acid)

Compound **10** (adenosine): ^{1}H NMR (500 MHz, DMSO-d_6) δ_H ppm: 8.13 (1H, s, H-2), 8.34 (1H, s, H-8), 7.30 (2H, bs, NH_2), 5.87 (1H, d, J = 6.0 Hz, Hα-1'), 4.60 (1H, dd, J = 5.0, 6.0 Hz, Hβ-2'), 4.14 (1H, ddd, J = 3.0, 4.5, 5.0 Hz, H-3′), 3.96 (1H, ddd, J = 3.0, 3.5, 4.5 Hz, H-4′), 3.67 (1H, ddd, J = 3.5, 4.5, 12.0 Hz, H_a-5′), 3.55 (1H, ddd, J = 3.5, 7.0, 12.0 Hz, H_b-5′).
^{13}C NMR (125 MHz, DMSO-d_6) δ_C ppm: 152.42 (C-2), 149.09 (C-4), 119.37 (C-5), 156.17 (C-6), 139.95 (C-8), 87.96 (C-1'), 73.49 (C-2'), 70.68 (C-3'), 85.92 (C-4'), 61.70 (C-5').
[P. Ciuffreda, S. Casati and A. Manzocchi (2007). Spectral Assignments ang Reference data Complete ^{1}H and ^{13}C NMR spectral assignment of α- and β-adenosine, 2'-deoxyadenosine and their acetate derivatives, *Magn. Reson. Chem.* **45**, 781-784.]

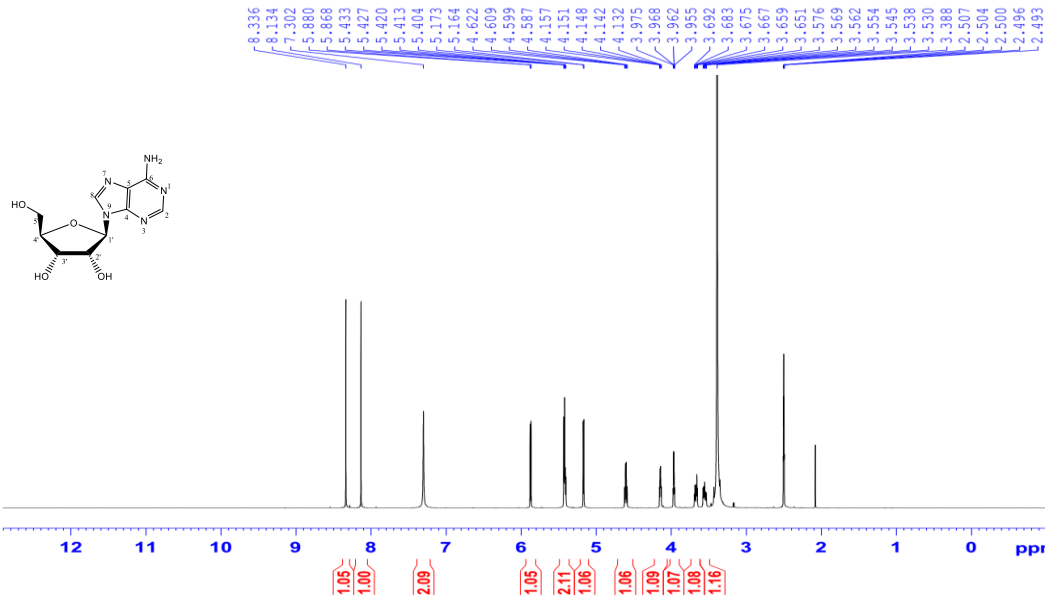

Figure S35: ^{1}H-NMR (500 MHz, DMSO-d_6) spectrum of **10** (adenosine)

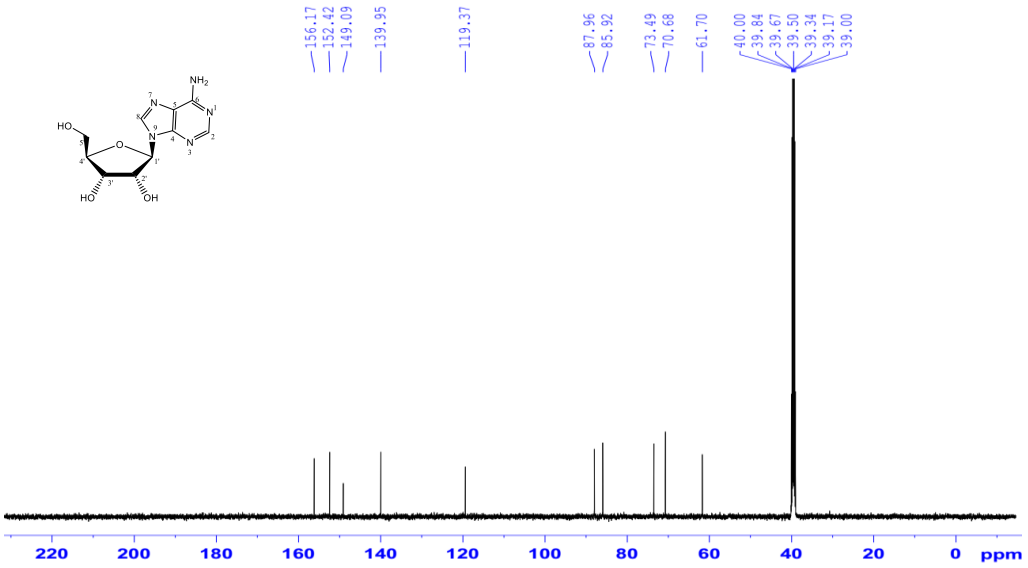

Figure S36: ^{13}C-NMR (125 MHz, DMSO-d_6) spectrum of **10** (adenosine)

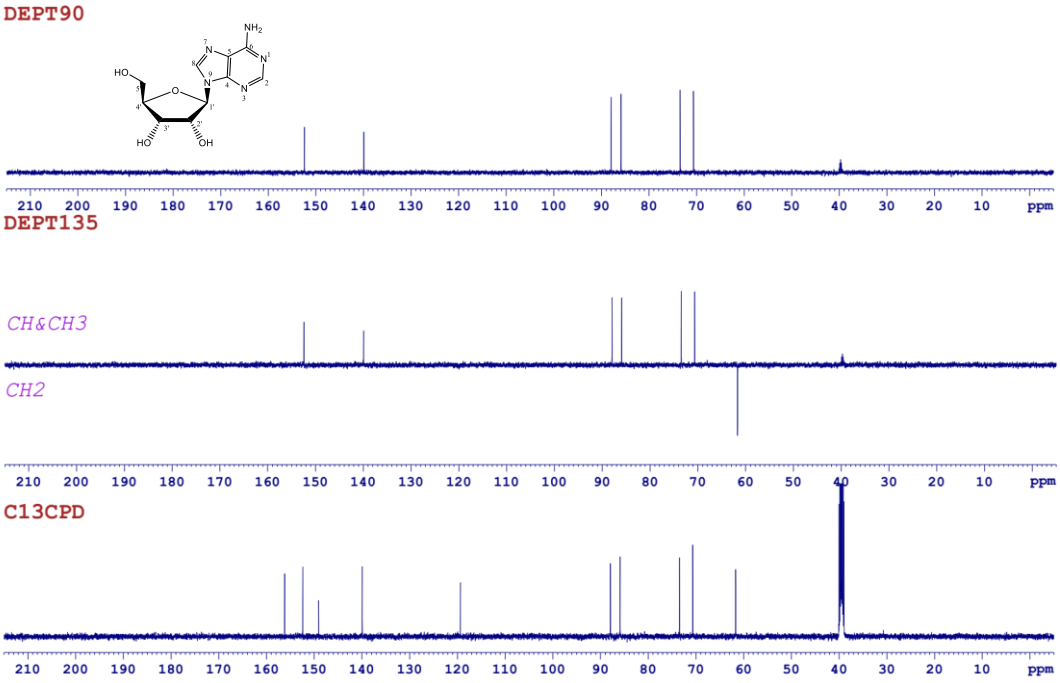

Figure S37: DEPT 90&135 spectrum of **10** (adenosine)

Compound **11** (*p*-hydroxybenzoic acid): ^{1}H NMR (500 MHz, $CDCl_3$+CD_3OD) δ ppm: 6.84 (2H, d, *J* = 8.5 Hz, H-3,H-5), 7.91 (2H, d, *J* = 8.5 Hz, H-2,H-6).

^{13}C NMR (125 MHz, $CDCl_3$+CD_3OD) δ ppm: 132.54 (C-2, C-6), 122.08 (C-1), 115.56 (C-3, C-5), 162.31 (C-4), 169.63 (C-7).

[T. Jianwen, B. Pawe, L. Jikai, S. Bernd, S. Ales, H. Klaus (2004). Universally occurring phenylpropanoid and species-specific indolic metabolites in infected and uninfected *Arabidopsis thaliana* roots and leaves, *Phytochemistry*. **65**, 691-699.].

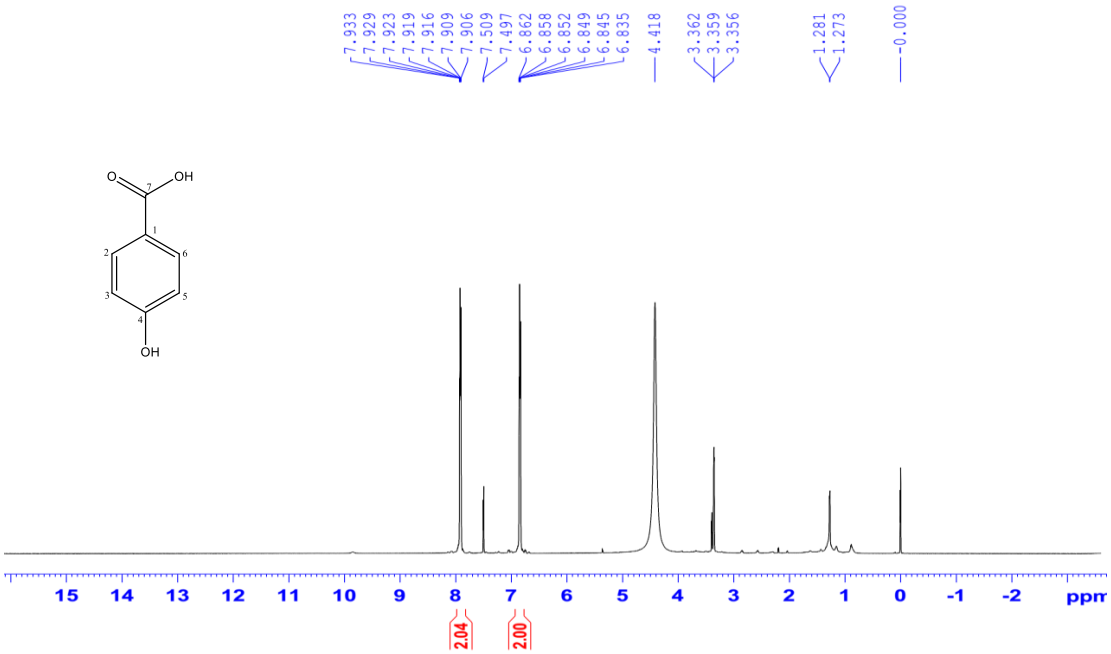

Figure S38: ^{1}H-NMR (500 MHz, $CDCl_3$+CD_3OD) spectrum of **11** (*p*-hydroxybenzoic acid)

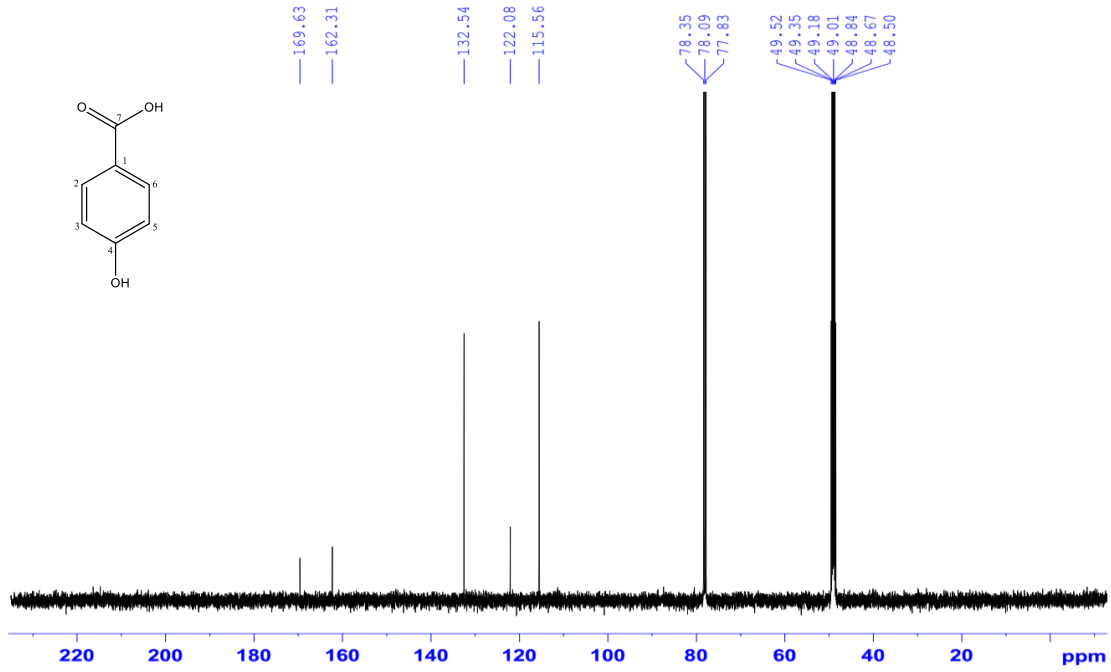

Figure S39: ^{13}C-NMR (125 MHz, $CDCl_3$+CD_3OD) spectrum of **11** (*p*-hydroxybenzoic acid)