Supplementary Material

A 3D-QSAR study on the antritrypanosomal and cytotoxic activities of steroid alkaloids by comparative molecular field analysis

Charles O. Nnadi,[1,2] Julia B. Althaus,[1] Ngozi J. Nwodo,[2] Thomas J. Schmidt[1,*]

[1] Institute of Pharmaceutical Biology and Phytochemistry (IPBP), University of Münster, PharmaCampus Corrensstraße 48, D-48149, Münster, Germany; E-mail: charles.nnadi@unn.edu.ng

[2] Department of Pharmaceutical and Medicinal Chemistry, Faculty of Pharmaceutical Sciences, University of Nigeria Nsukka, 410001 Enugu State Nigeria; E-mail: ngozi.nwodo@unn.edu.ng

* Correspondence: thomschm@uni-muenster.de; Tel.: +49-251-83-33378

Table S1. Statistical detaily of 3D QSAR CoMFA model for trypanocidal (*Tbr*) activity

Model statistics/*N*	1	2	3	4	5
R^2	0.717	0.920	**0.995**	0.997	0.999
SDEC	0.405	0.216	**0.056**	0.043	0.028
F-values	25.336	51.473	**482.639**	539.039	917.456
Q^2 (SDEP)	0.42 (0.58)	0.67 (0.44)	**0.83 (0.33)**	0.82 (0.32)	0.84 (0.30)
P^2 (SDEP)	0.48 (0.67)	0.69 (0.51)	**0.79 (0.51)**	0.68 (0.52)	0.69 (0.51)

Q^2 = coefficient of determination for leave-one-out cross-validation; N = number of statistical components; R^2 = coefficient of determination for non-cross validated model data; SDEC = standard deviation error in calculation; SDEP = standard deviation error in prediction; F = Fisher value; P^2 = coefficient of dermination for the test set predictions.

Table S2. Statistical details of 3D QSAR CoMFA model for cytotoxic (L6) activity

Model statistics/*N*	1	2	3	4	5
R^2	0.829	**0.940**	0.981	0.994	0.998
SDEC	0.187	**0.111**	0.063	0.034	0.019
F-values	48.562	**70.452**	134.159	302.887	693.318
Q^2 (SDEP)	0.46 (0.33)	**0.64 (0.28)**	0.67 (0.26)	0.70 (0.25)	0.72 (0.24)
P^2 (SDEP)	0.44 (0.49)	**0.59 (0.42)**	0.74 (0.33)	0.78 (0.30)	0.81 (0.28)

Q^2 = coefficient of determination for leave-one-out cross-validation; N = number of statistical components; R^2 = coefficient of determination for non-cross validated model data; SDEC = standard deviation error in calculation; SDEP = standard deviation error in prediction; F = Fisher value; P^2 = coefficient of dermination for the test set predictions.

Table S3. Actual vs predicted pIC_{50} for anti-*Tbr* and L6 cytotoxic activity

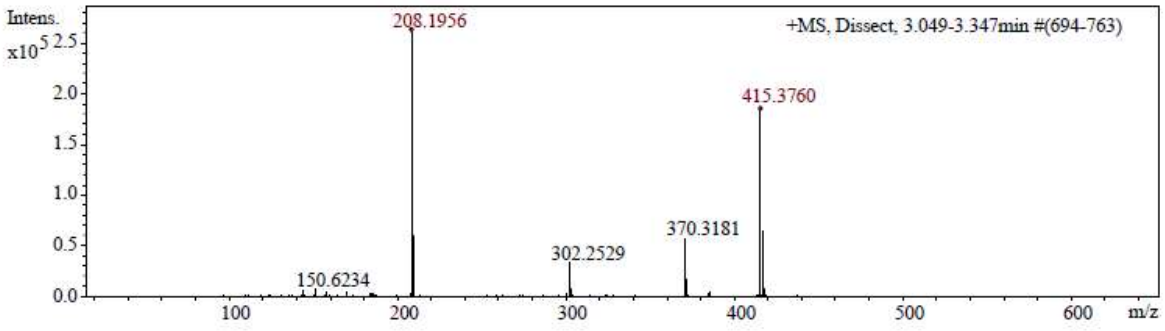
Compounds	pIC_{50} (*Tbr*), 3PC		pIC_{50} (L6), 2PC	
	Actual	**Predicted**	**Actual**	**Predicted**
1	6.3958	6.0267	5.2928	4.7213
2	5.3159	5.9046	4.8135	4.8189
3	7.1249*	6.4687	5.6057*	4.9596
4	6.4320	6.0267	4.7993*	4.7511
5	6.1726*	6.4385	4.7707	4.9136
6	5.9190	6.1137	4.7603	4.7833
7	4.8282*	5.3394	n.t	n.p
8	5.0624	4.9017	4.1816	4.1339
9	4.7568	5.2974	3.8897	4.1848
10	5.1326*	5.3685	4.3800*	4.1369
11	6.7781	6.3490	4.2972	4.3223
12	6.7781*	6.0757	4.5618	4.3017
13	6.3778	6.2482	4.2130	4.1438
14	6.9245	6.7948	4.8444	4.5317
15	5.7807	5.5249	4.0103*	4.4999
16	5.0856	5.1469	3.7426	4.3146
17	4.6798	5.0961	3.9045	4.0640
18	5.4342*	6.1487	4.7233*	4.4447
19	6.7905*	6.4609	4.5047*	4.8203

*Test set compounds; pIC_{50} = -log(IC_{50}) for CoMFA analysis; n.t = not tested; n.p = not predicted

Table S4. ^{1}H and ^{13}C NMR Data of cyclovirobuxein B (600 and 150 MHz, respectively, $CDCl_3$), Data are from [17].

Position	^{1}H-NMR δ(ppm)	mult.	J (Hz)	^{13}C NMR δ (ppm)
1	1,52	*(2H)		31,17
2	1,75	*		20,02
	1,53	*		
3	2,06	dd	3,2; 11,7	71,40
4				41,62
5	1,85	dd	5,49; 14,86	48,78
6	5,62	ddd [dt]	1,2; 1,2; 10,6	127,70
7	5,40	ddd	3,25; 6,1; 10,6	128,38
8	2,53	dd	2,0; 6,0	43,39
9				20,94
10				28,87
11	1,82	dd	5,5; 14,9	24,98
	1,41	ddd	1,57; 4,8; 14,97	
12	1,69	ddd	5; 13,4	32,10
	1,37	ddd	1,5; 5,4 13	
13				45,56
14				49,86
15	1,98	dd	10; 13,4	41,75
	1,26	dd	13,4; 2,1	
16	4,15	ddd	2,1; 7,8; 9,6	78,89
17	1,58	dd	6,7; 10,5	61,59
18	0,91	s (3H)		15,71
19	0,72	d	4,1	18,37**
	-0,21	d	4,1	
20	2,49	dq	10,4; 6,1	59,15
21	1,09	d(3H)	6,1	18,90
28	0,94	s(3H)		18,35**
29	1,05	s(3H)		26,17
30	0,79	s(3H)		16,68
31/32	2,29	s(6H)		44,31
33	2,44	s(3H)		33,89

*chemical shift values extracted from HSQC spectrum due to signal overlap; **signals of C-19 and C-18 assigned with the help of an APT spectrum.



Meas. m/z	I	z	Formula	m/z	err [mDa]	err [ppm]	mSigma	rdb	e¯ Conf
415.3760	182993	1+	C27 H47 N2 O	415.3683	-7.7	-18.5	24.1	5.5	even

Figure S1. +ESI QqTOF mass spectrum of cylcovirobuxein B (**19**)

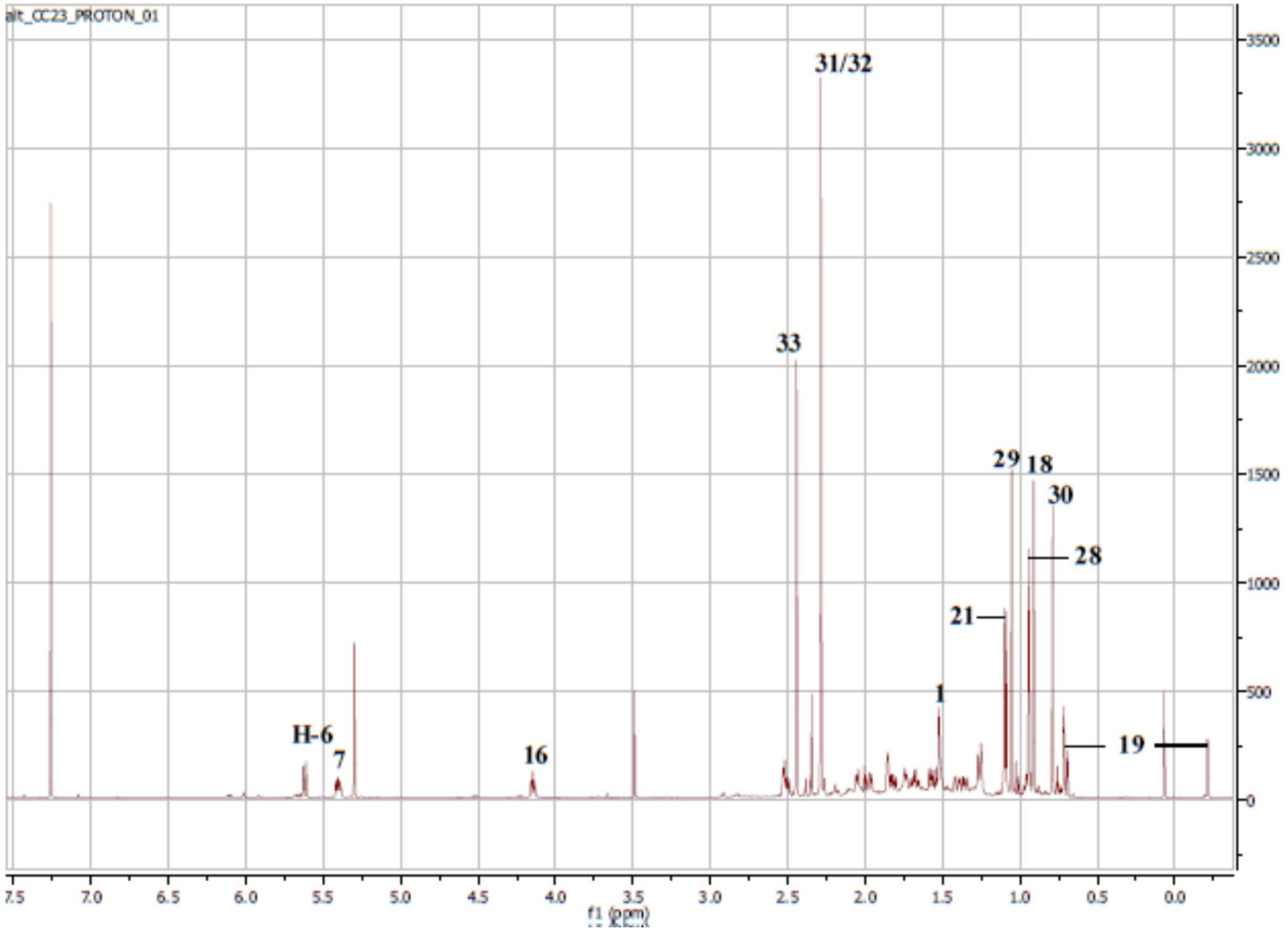


Figure S2. ^{1}H NMR spectrum (600 MHz, $CDCl_3$) of cylcovirobuxein B (**19**)

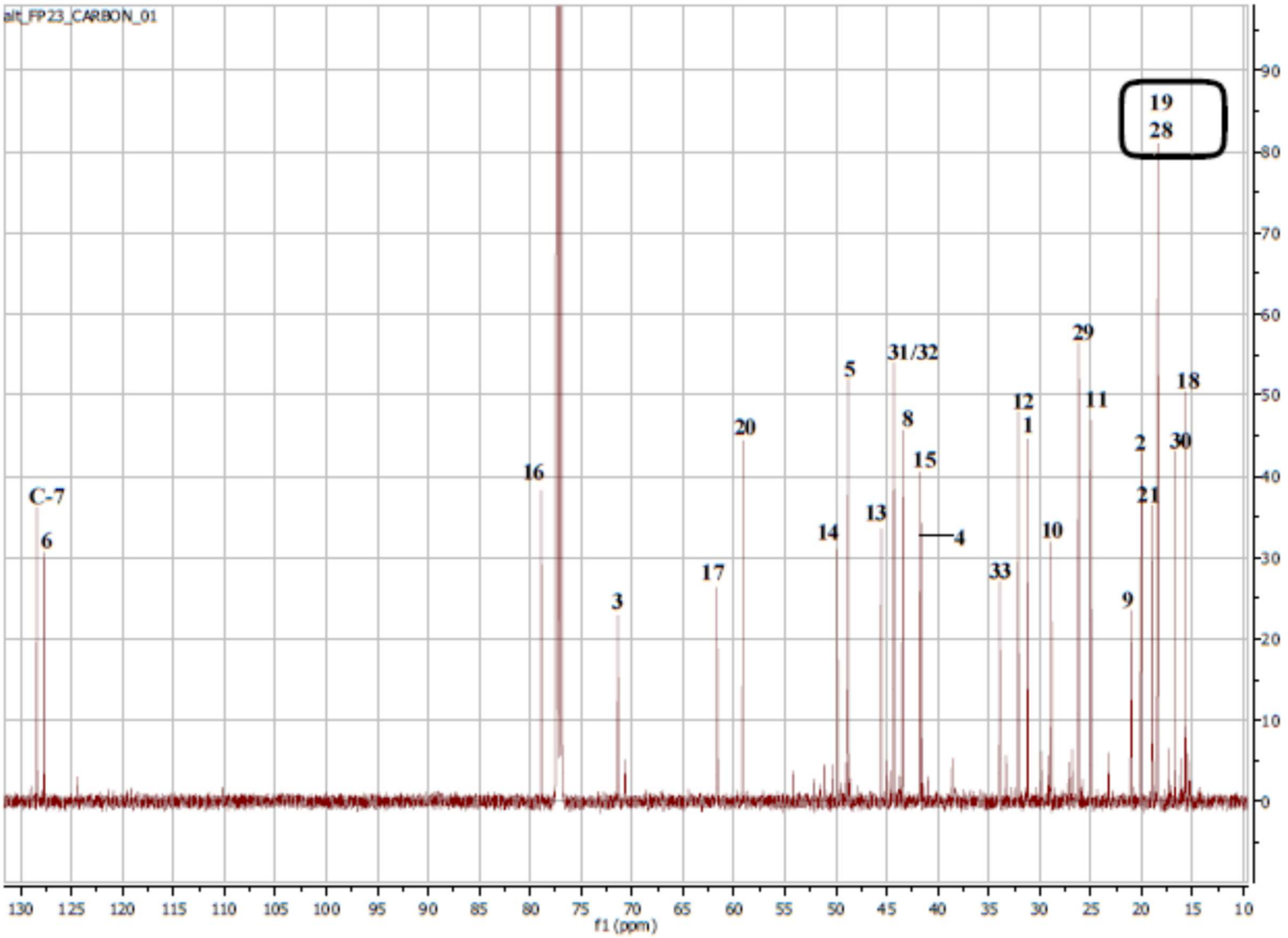


Figure S3. ^{13}C NMR spectrum (150 MHz, $CDCl_3$) of cylcovirobuxein B (**19**)

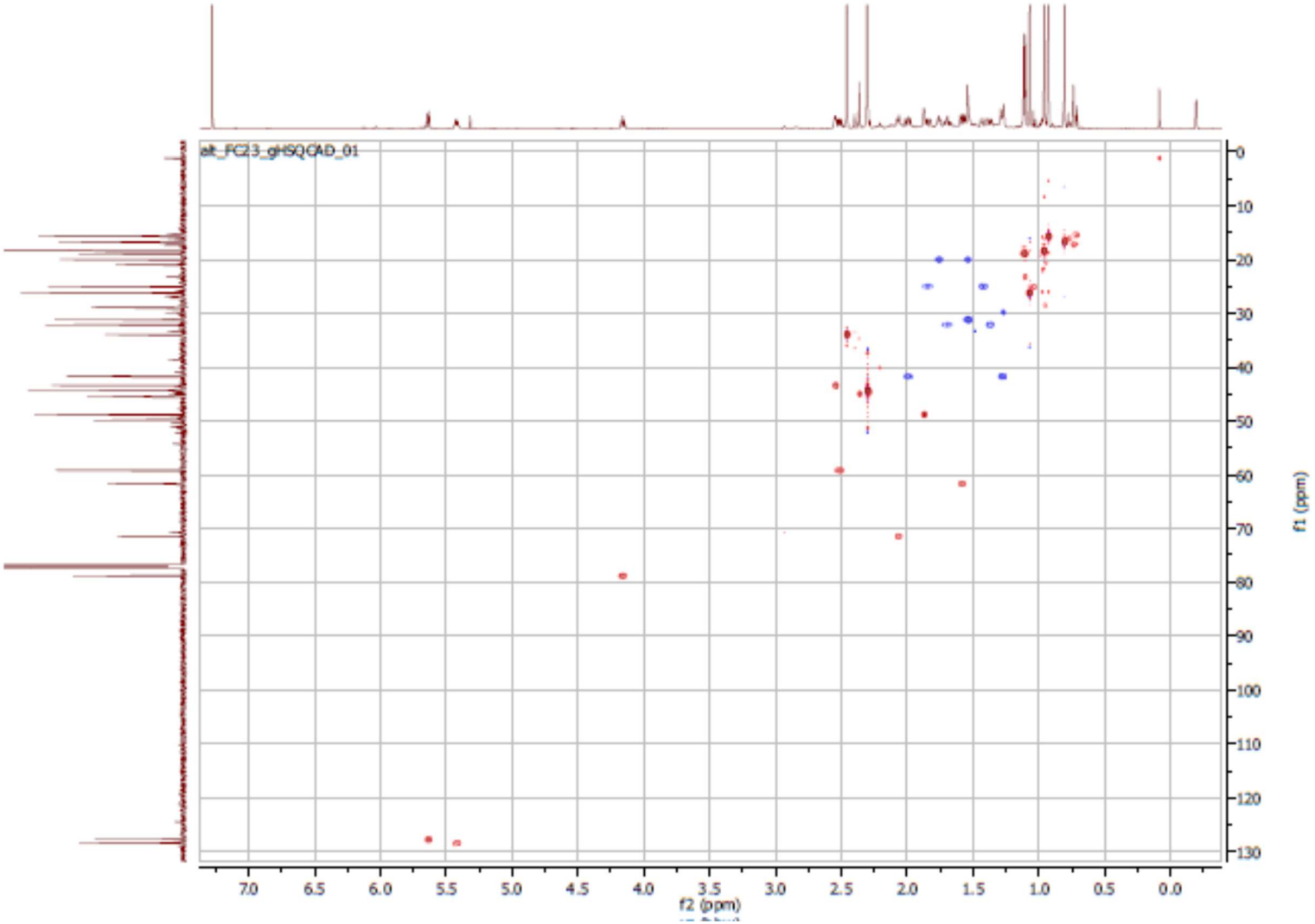


Figure S4. $^{1}H/^{13}C$ HSQC spectrum (600 MHz, $CDCl_3$) of cylcovirobuxein B (**19**)

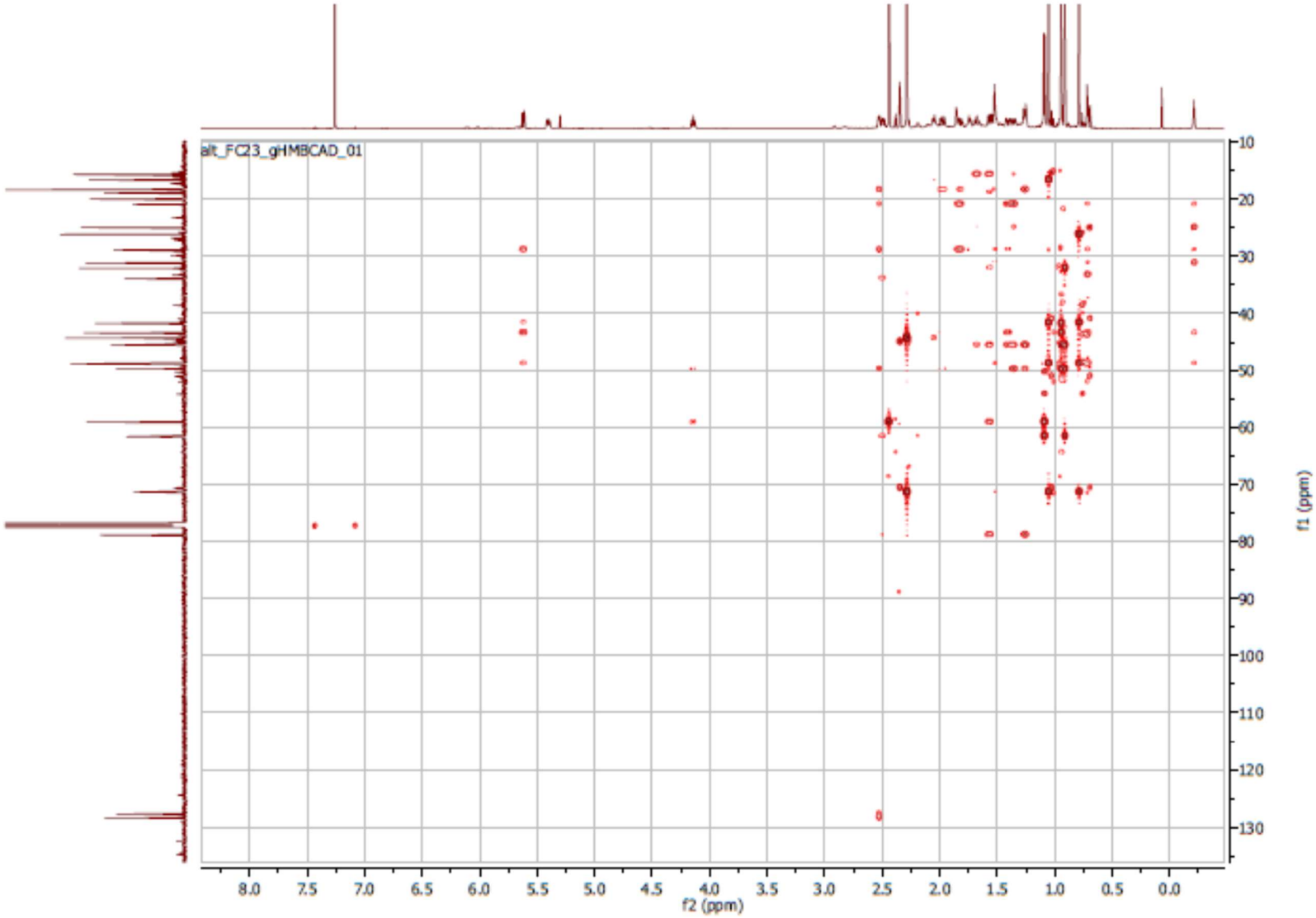


Figure S5. $^{1}H/^{13}C$ HMBC spectrum (600 MHz, $CDCl_3$) of cylcovirobuxein B (**19**)