pharmaceuticals

MDPI

Article

Involvement of serotonergic and dopaminergic systems in *Aloysia gratissima* var. *gratissima* antidepressant-like effect, UPLC-DAD-MS chemical characterization and computational evidence.

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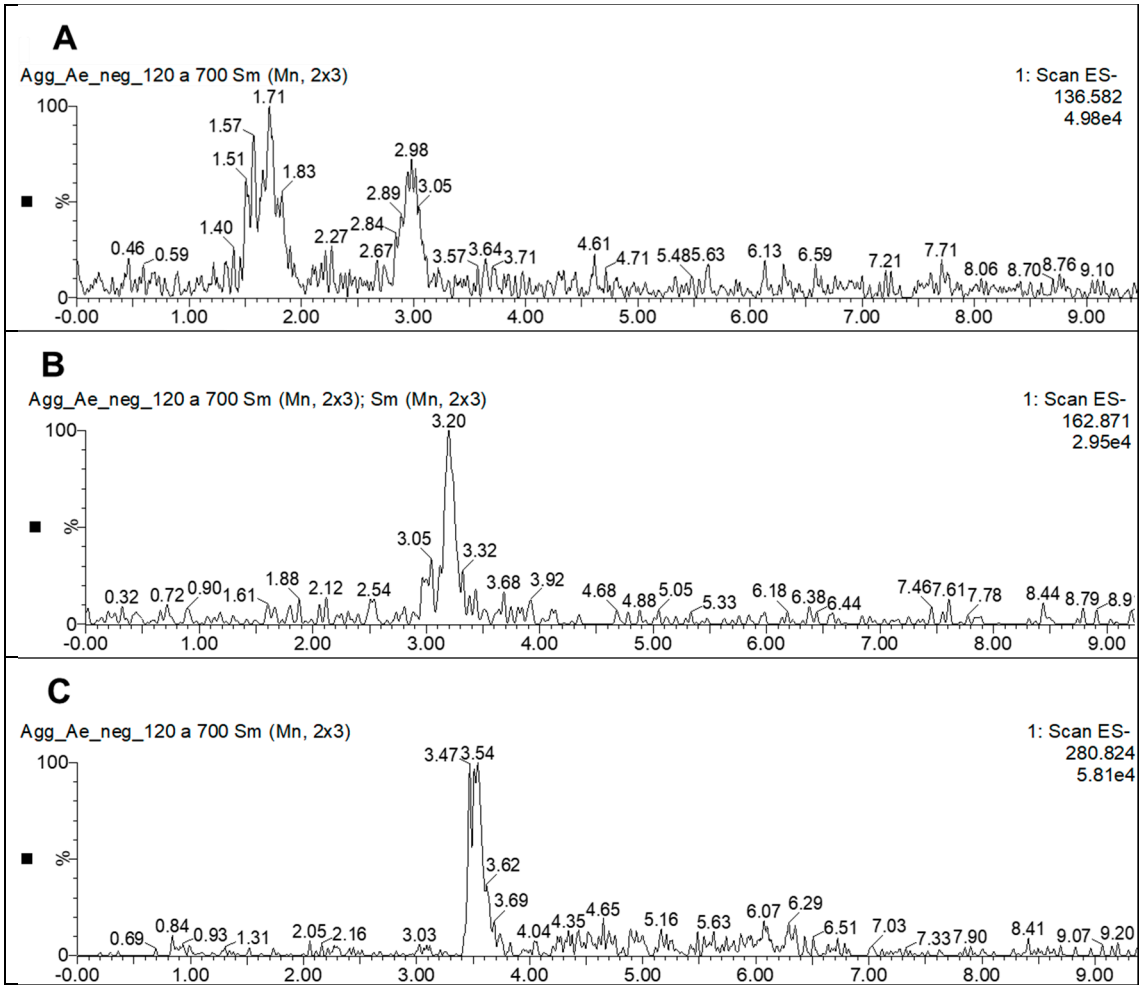
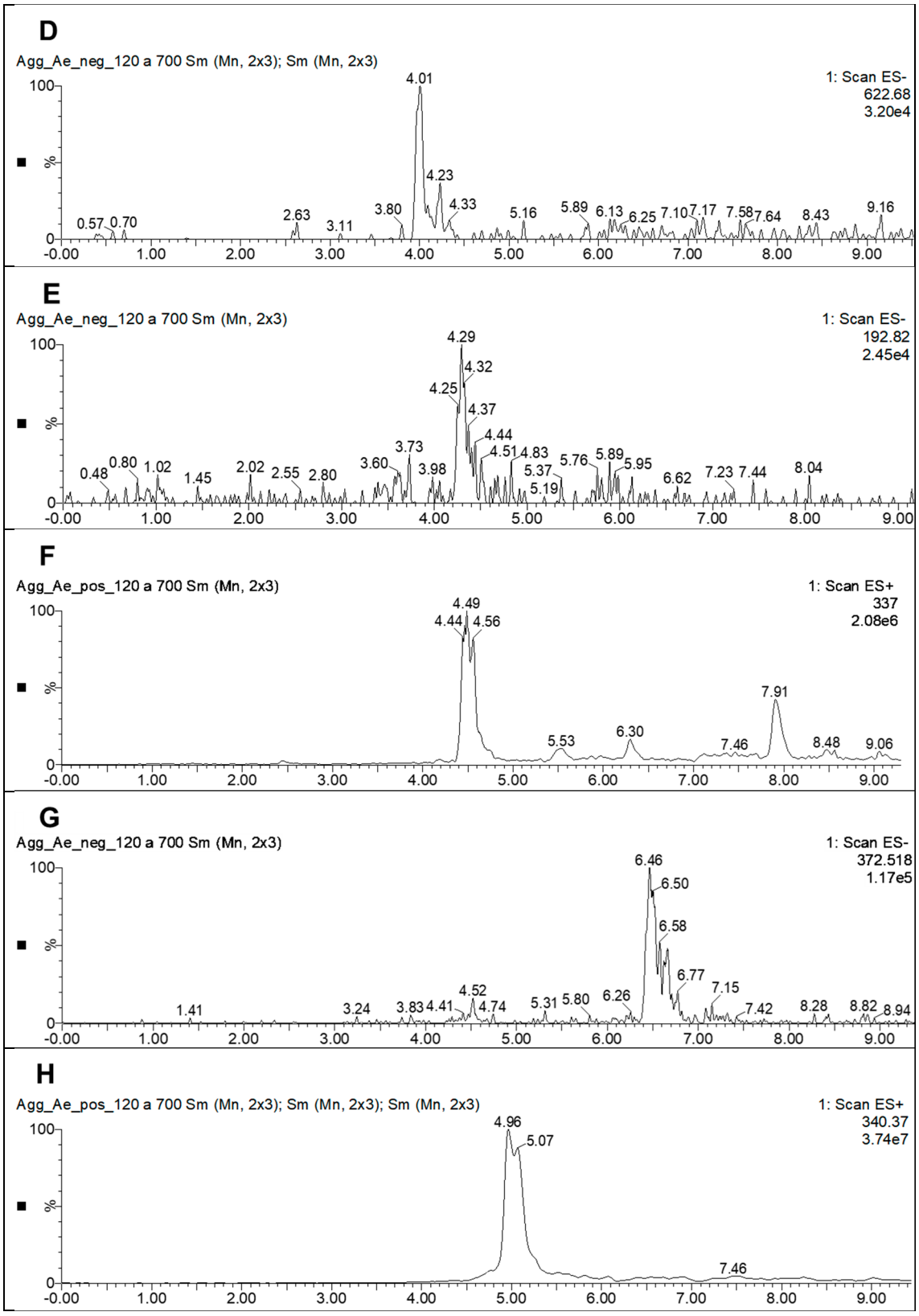
Figure S1. LC-MS Extracted Ion Chromatograms (EIC) of the detected compounds from *A. gratissima* active fractions.

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1: Scan ES-
622.68
3.20e4
E
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1: Scan ES-
192.82
2.45e4
F
Agg_Ae_pos_120 a 700 Sm (Mn, 2x3)
1: Scan ES+
337
2.08e6
G
Agg_Ae_neg_120 a 700 Sm (Mn, 2x3)
1: Scan ES-
372.518
1.17e5
H
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1: Scan ES+
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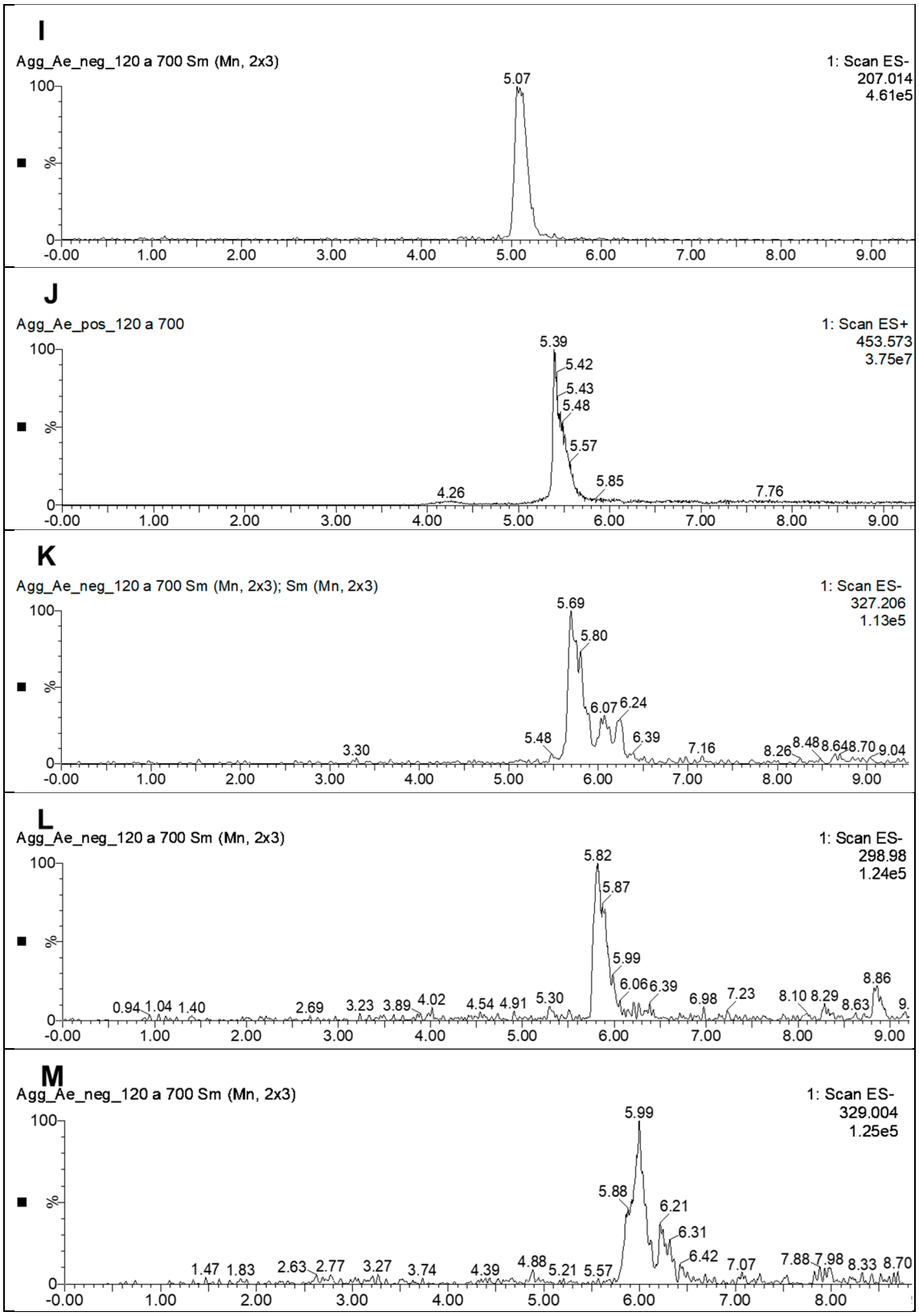
3.74e7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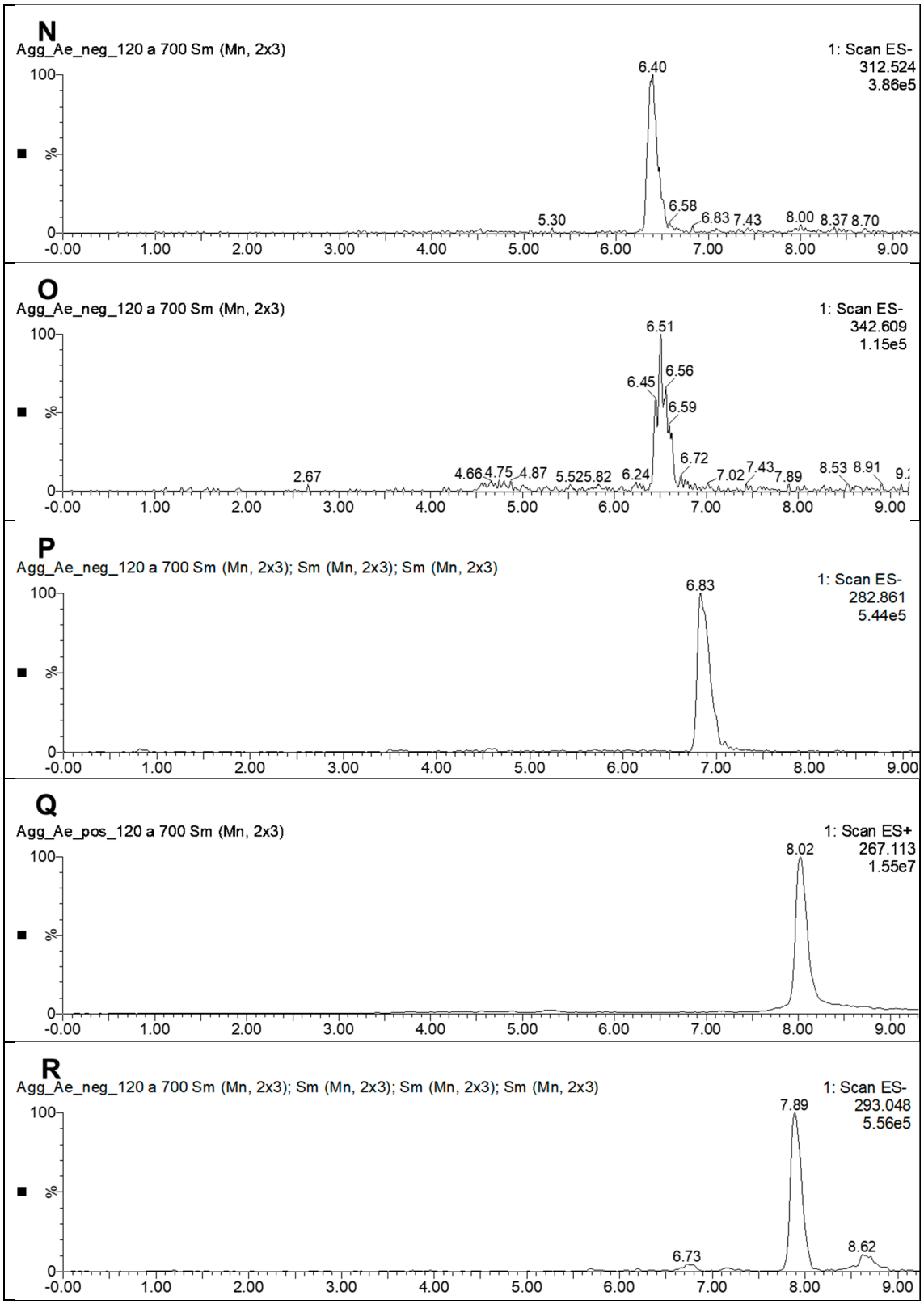
1: Scan ES-
207.014
4.61e5
5.07
J
Agg_Ae_pos_120 a 700
1: Scan ES+
453.573
3.75e7
5.39
5.42
5.43
5.48
5.57
5.85
4.26
7.76
K
Agg_Ae_neg_120 a 700 Sm (Mn, 2x3); Sm (Mn, 2x3)
1: Scan ES-
327.206
1.13e5
5.69
5.80
6.07
6.24
6.39
5.48
3.30
7.16
8.26
8.48
8.64
8.70
9.04
L
Agg_Ae_neg_120 a 700 Sm (Mn, 2x3)
1: Scan ES-
298.98
1.24e5
5.82
5.87
5.99
6.06
6.39
8.86
0.94
1.04
1.40
2.69
3.23
3.89
4.02
4.54
4.91
5.30
6.98
7.23
8.10
8.29
8.63
M
Agg_Ae_neg_120 a 700 Sm (Mn, 2x3)
1: Scan ES-
329.004
1.25e5
5.99
5.88
6.21
6.31
6.42
1.47
1.83
2.63
2.77
3.27
3.74
4.39
4.88
5.21
5.57
7.07
7.88
7.98
8.33
8.70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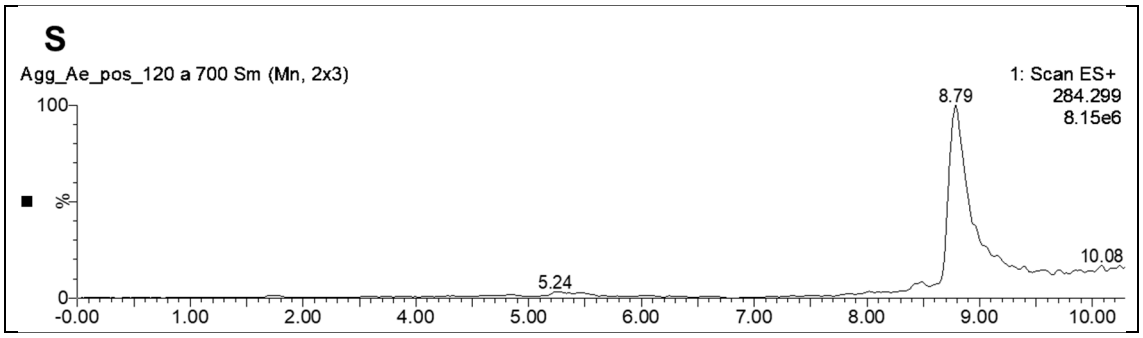
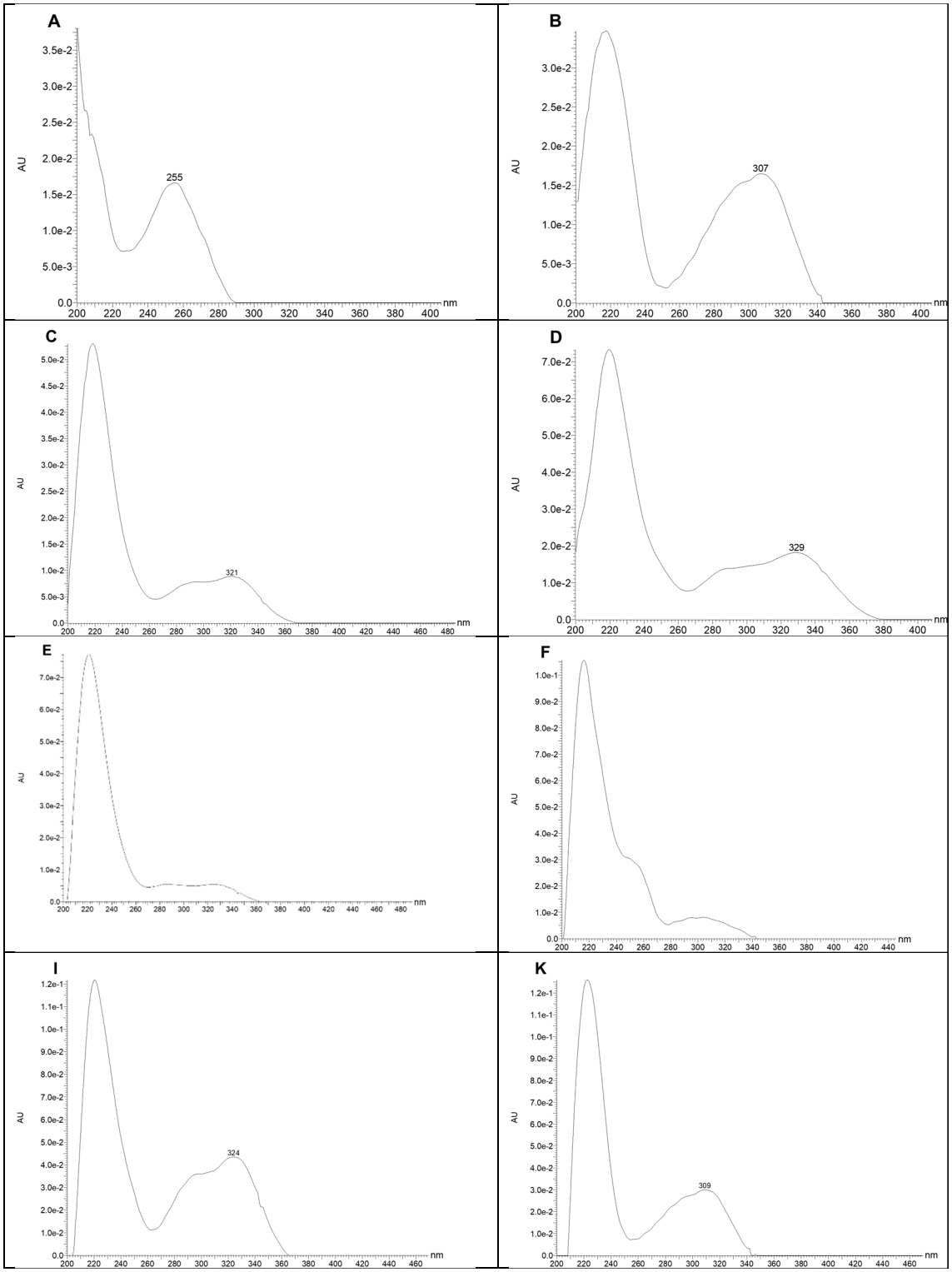
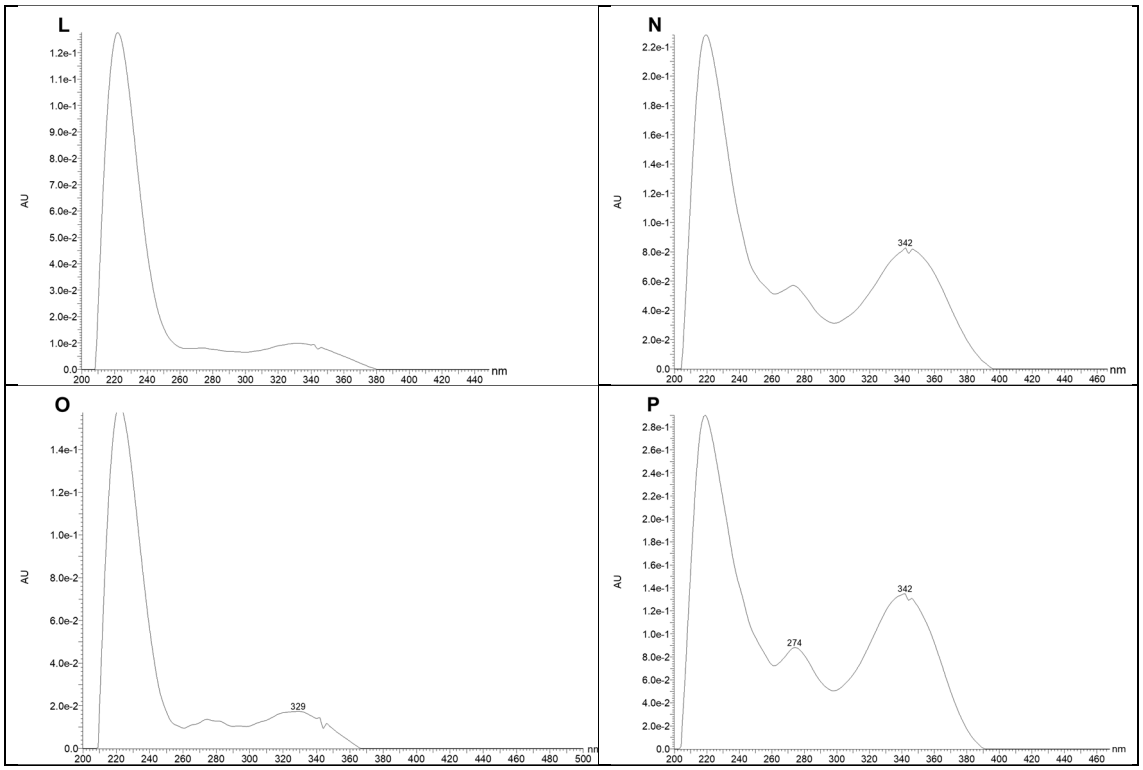
3.86e5
6.40
5.30
6.58
6.83
7.43
8.00
8.37
8.70
O
Agg_Ae_neg_120 a 700 Sm (Mn, 2x3)
1: Scan ES-
342.609
1.15e5
6.51
6.56
6.45
6.59
6.72
2.67
4.66
4.75
4.87
5.52
5.82
6.24
7.02
7.43
7.89
8.53
8.91
P
Agg_Ae_neg_120 a 700 Sm (Mn, 2x3); Sm (Mn, 2x3); Sm (Mn, 2x3)
1: Scan ES-
282.861
5.44e5
6.83
Q
Agg_Ae_pos_120 a 700 Sm (Mn, 2x3)
1: Scan ES+
267.113
1.55e7
8.02
R
Agg_Ae_neg_120 a 700 Sm (Mn, 2x3); Sm (Mn, 2x3); Sm (Mn, 2x3); Sm (Mn, 2x3)
1: Scan ES-
293.048
5.56e5
7.89
6.73
8.62
100
%
0
-0.00
1.00
2.00
3.00
4.00
5.00
6.00
7.00
8.00
9.00



#Compounds: (A) Hydroxybenzoic acid and isomer; (B) Coumaric acid; (C) caffeoyl tartronic acid; (D) Verbascoside; (E) Ferulic acid; (F) Hydroxymethylhoffmanniaketone and isomer; (G) Methylsudachitin; (H) Unknown; (I) Methyl ferulic acid; (J) Unknown; (K) Coumaroyl derivative; (L) Methoxyapigenin; (M) Hydroxy-di-O-methylluteolin; (N) Dihydroxy-dimethoxyflavone; (O) Dihydroxy-trimethoxy flavone; (P) Methyl apigenin; (Q) Unknown; (R) Oxooctadeca-dienoic acid; (S) Unknown.

Figure S2. Characteristic UV spectra of the major compounds tentatively identified in *A. gratissima* var. *gratissima*.





#Compounds: (A) Hydroxybenzoic acid and isomer; (B) Coumaric acid; (C) caffeoyl tartronic acid; (D) Verbascoside; (E) Ferulic acid; (F) Hydroxymethylhoffmanniaketone; (I) Methyl ferulic acid; (K) Coumaroyl derivative; (L) Methoxyapigenin; (N) Dihydroxy-dimethoxyflavone; (O) Dihydroxy-trimethoxy flavone; (P) Methyl apigenin.

Figure S3. Results obtained in the redocking analysis. (a) 5-HT$_{1A}$ (PDB: 7E2Y). (b) 5HT$_{2A}$ (PDB: 8UWL). (c) 5-HT$_3$ (PDB: 6Y1Z). (d) D$_2$R (PDB: 8IRS).

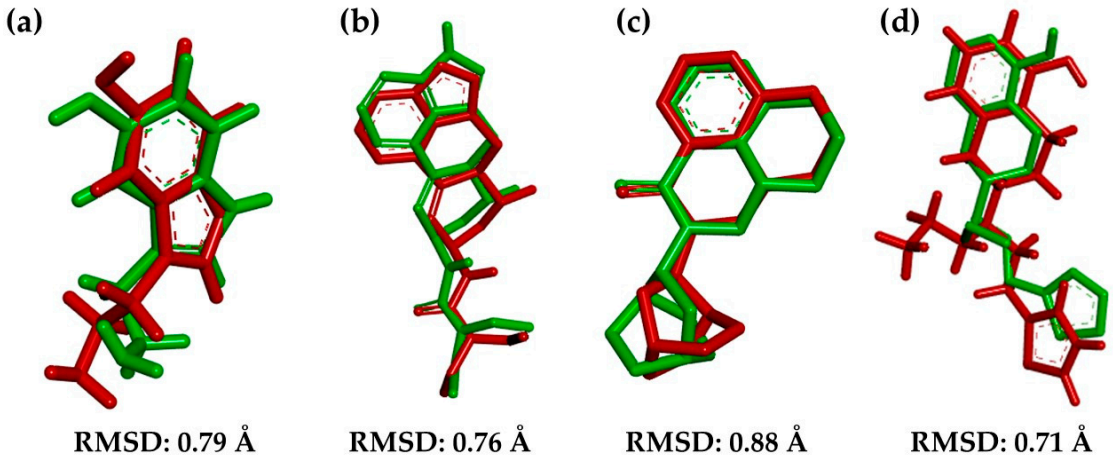


Figure S4. Tridimensional representation of complexes with the highest binding affinity and standard inhibitors. (a) 5-HT_{1A} receptor binding to 13-Oxooctadecadienoic acid (green), Way100635 (yellow). (b) 5-HT_{2A} receptor binding to Ferulic acid (red), Ketanserin (yellow). (c) 5-HT_3 receptor binding to Coumaric acid (orange), Ondansetron (yellow). (d) D_2R receptor binding to Ferulic acid (blue), Haloperidol (yellow).

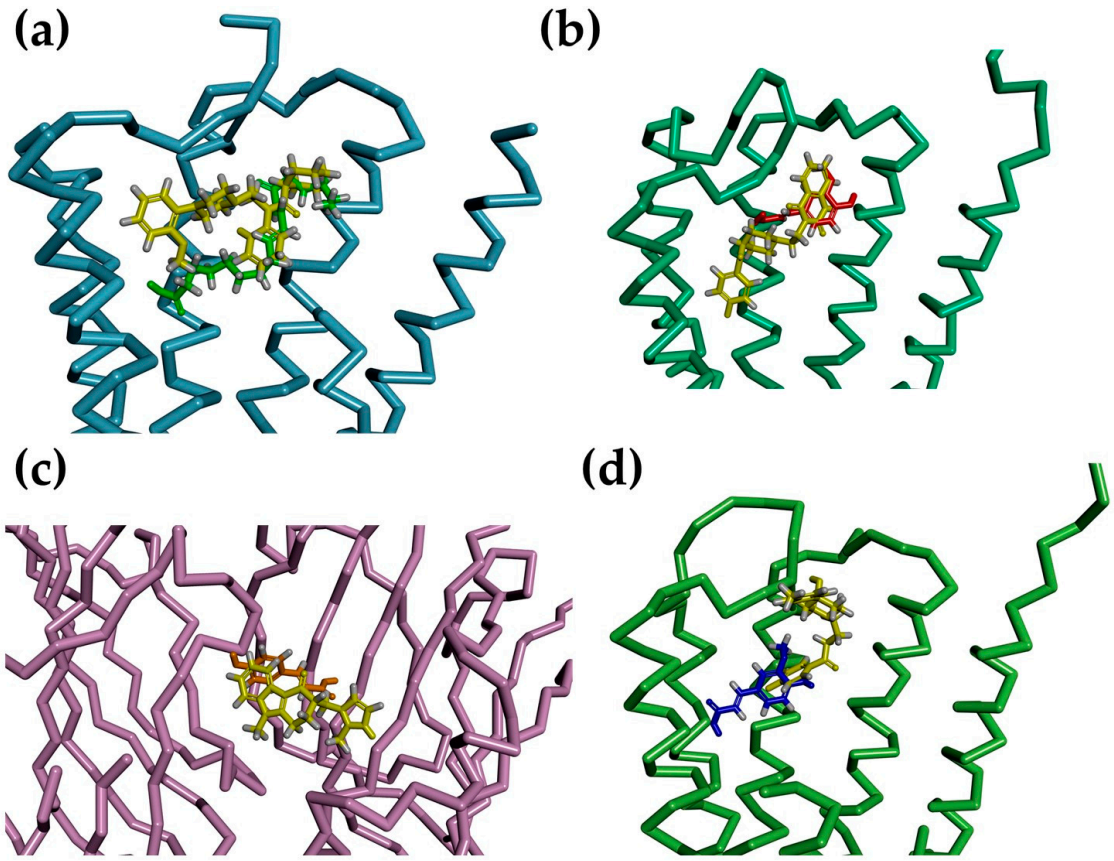


Figure S5. Druggable site identified in receptors. (**a**) 5-HT$_{1A}$ receptor. (**b**) 5-HT$_{2A}$ receptor. (**c**) 5-HT$_{3}$ receptor. (**d**) D$_{2}$R receptor.

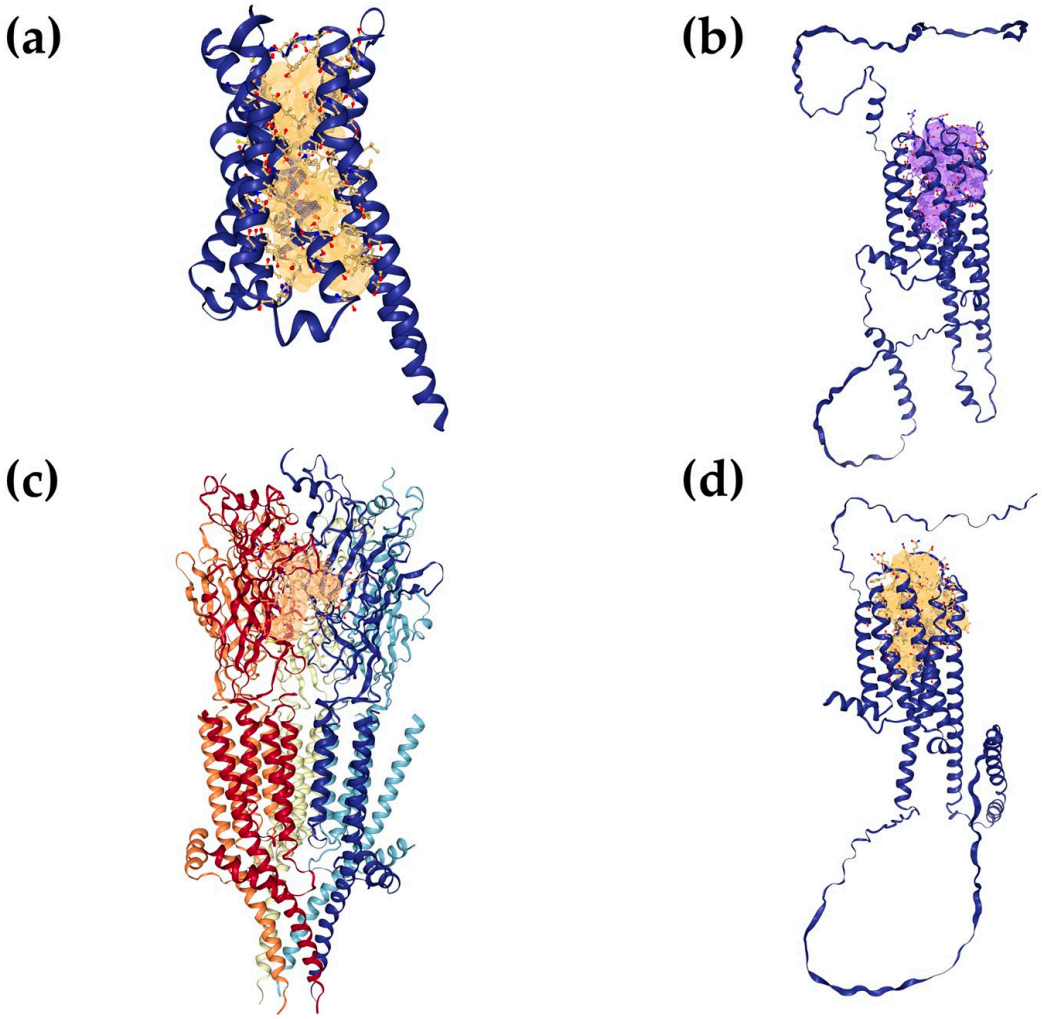


Table S1. Compounds selected for *in silico* analysis.

Compounds*	PubChem Compounds†	PubChem CID
Hydroxybenzoic acid	4-Hydroxybenzoic Acid	135
Hydroxybenzoic acid 2	3-Hydroxybenzoic Acid	7420
Coumaric acid	Coumaric acid	637542
2-O-caffeoyl-hydroxymalonic acid (caffeoyl tartronic acid)	2-O-caffeoyl-hydroxymalonic acid (caffeoyl tartronic acid)	131752962
Verbascoside	Verbascoside	5281800
Ferulic acid	Ferulic acid	445858
Hydroxymethylhoffmanniaketone	Hydroxymethylhoffmanniaketone	162954573
Methylsudachitin	Methylsudachitin	181092
Methylferulic acid	Methylferulic acid	717531
Methoxyapigenin[(a)]	3'-Methoxyapigenin	5280666
	6-Methoxyapigenin	5281628
	3-Methoxyapigenin	5280862
	8-Methoxyapigenin	5322078
Hydroxy-di-O-methylluteolin	Hydroxy-di-O-methylluteolin	5320945
Dihydroxy-dimethoxyflavone	Dihydroxy-dimethoxyflavone	123885531
Dihydroxy-trimethoxy flavone	Dihydroxy-trimethoxyflavone	91248359
Methyl apigenin	Methylapigenin	15661821
Hydroxymethylhoffmanniaketone isomer	Hydroxymethylhoffmanniaketone isomer	101394717
Oxooctadecadienoic acid[(b)]	Oxooctadecadienoic acid	71407514
	13-Oxooctadecadienoic acid	160205
	(6E,8Z)-5-oxooctadecadienoic acid	71751402

*Identified in this study by UPLC-ESI-MS/MS, †Compounds selected for *in silico* analysis, [(a)]Methoxyapigenin isomers detected in database, [(b)] Oxooctadeca-dienoic acid isomers detected in database.

Table S2. Values of the dissociation constant (Kd_{calc}) calculated from the ΔG values.

Compounds	Kd_{calc} (μM)			
	5-HT$_{1a}$	**5-HT$_{2a}$**	**5-HT$_3$**	**D$_2$R**
4-Hydroxybenzoic acid	112.74	69.28	36.19	81.49
3-Hydroxybenzoic acid	132.61	81.49	30.77	132.61
Coumaric acid	30.77	58.89	9.88	30.77
Ferulic acid	30.77	36.19	11.62	26.16
Methylferulic acid	50.07	58.89	30.77	112.74
Oxooctadecadienoic acid	50.07	69.28	58.89	298.59
13-Oxooctadecadienoic acid	18.91	253.85	30.77	571.57
(6E,8Z)-5-oxooctadecadienoic acid	30.77	413.12	18.91	183.48
Standard inhibitors	0.20[a]	1.19[b]	0.45[c]	4.39[d]

[a]Way100635, [b]Ketanserin, [c]Ondansetron, [d]Haloperidol.

Table S3. Druggable site properties identified in receptors.

Receptor	Volume (Å³)	Surface (Å²)	Depth (Å)	Druggable score	Residues
5-HT$_{1A}$	2244.85	2391.51	26.69	0.82	Ala71, Leu74, Ile75, Ser77, Leu78, Asp82, Val85, Leu90, Ala93, Ala94, Tyr96, Gln97, Phe112, Ile113, Asp116, Val117, Cys119, Cys120, Thr121, Ser123, Ile124, Trp125, His126, Leu127, Cys128, Ala129, Ile130, Ala131, Leu132, Asp133, Arg134, Tyr135, Ile138, Arg148, Ala153, Leu156, Ile157, Leu159, Thr160, Ile163, Ile167, Cys187, Thr188, Ile189, Ser190, Lys191, Tyr195, Thr196, Ser199, Thr200, Ala203, Pro207, Leu208, Leu210, Met211, Leu214, Tyr215, Ile218, Thr343, Lys345, Thr346, Leu347, Ile349, Ile350, Met351, Thr353, Phe354, Leu356, Cys357, Trp358, Leu359, Pro360, Phe361, Phe362, Val364, Ala365, Leu368, Pro369, Met377, Gly382, Ala383, Ile385, Asn386, Trp387, Leu388, Gly389, Tyr390, Asn392, Ser393, Leu395, Asn396, Ile399, Tyr400, Ala401, Phe403, Asn404
5-HT$_{2A}$	2063.11	1954.87	34.5	0.99	Leu116, Ala119, Asp120, Leu123, Met128, Val130, Ser131, Thr134, Ile135, Tyr139, Arg140, Trp141, Trp151, Ile152, Asp155, Val156, Phe158, Ser159, Thr160, Ser162, Ile163, Leu166, Gly205, Ile206, Ser207, Met208, Pro209, Ile210, Lys223, Glu224, Ser226, Cys227, Leu228, Leu229, Ala230, Asp231, Asp232, Phe234, Val235, Ile237, Gly238, Ser239, Val241, Ala242, Phe243, Ile327, Val328, Leu331, Phe332, Met335, Trp336, Phe339, Phe340, Asn343, Ala346, Val347, Cys349, Lys350, Cys353, Asn354, Glu355, Asn356, Ile358, Gly359, Leu362, Asn363, Val366, Trp367, Gly369, Tyr370, Leu371, Ser372, Ser373, Ala374, Val375, Asn376, Val379
5-HT$_3$	937.34	959.03	23.78	0.82	Leu58(chain A), Thr59(A), Thr60(A), Asn101(A), Glu102(A), Leu131(A), Val132(A), Thr133(A), Ala134(A), Cys135(A), Ser136(A), Cys149(A), Ser150(A), Leu151(A), Thr152(A), Thr154(A), Ser155(A), Trp156(A), Leu157(A), His158(A), Thr159(A), Ile160(A), Ile163(A), Asn164(A), Ile165(A), Phe199(A), Ile201(A), Ser206(A), Tyr207(A), Ala208(A), Glu209(A), Lys211(A), Val214(A), Ile44(E), Tyr46(chain E), Trp63(E), Tyr64(E), Arg65(E), Tyr126(E), Lys127(E), Arg169(E), Asp177(E), Ser179(E), Ile180(E), Ile182(E)

D_2R	2224.56	2422.61	39.29	0.81	Tyr37, Leu41, Ile48, Leu76, Ala79, Asp80, Val83, Val87, Trp90, Val91, Leu94, Glu95, Gly98, Glu99, Trp100, Lys101, Phe102, Ser103, Arg104, Cys107, Asp108, Phe110, Val111, Thr112, Asp114, Val115, Met117, Cys118, Thr119, Ser121, Ile122, Leu125, Pro169, Leu170, Gly173, Leu174, Asn175, Asn176, Thr177, Asp178, Gln179, Asn180, Glu181, Cys182, Ile183, Ile184, Ala185, Asn186, Phe189, Val190, Val191, Ser193, Ser194, Ile195, Ser197, Phe198, Tyr199, Val379, Val382, Phe383, Cys386, Trp387, Pro389, Phe390, Phe391, Ile392, HIS394, Ile395, Leu396, Val407, Tyr409, Ser410, Ala411, Phe412, Thr413, Trp414, Leu415, Gly416, Tyr417, Val418, Asn419, Ser420, Val422, Asn423, Ile426