Identification of Peptide Substrate and Small Molecular Inhibitors of Testis-specific Serine/Threonine Kinase1 (TSSK1) By the Developed Assays

*Leilei Zhang, Yan Yu, Liu Zijie, Li Li, Zeper Abliz, Gang Liu**

*To whom correspondence should be addressed.

Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, 2 Nanwei Rd., Xuanwu Dist, Beijing 100050, China.

Tel: 86-10-63167165

Fax: 86-10-63167165.

E-mail:gangliu27@yahoo.com.

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Supporting Information

1. Protein sequence and purification of TSSK1

1 mddaavlkrr gyllginlge gsyakvksay serlkfnvai kiidrkkapa dflekflpre

61 ieilamlnhc siiktyeife tshgkvyivm elavqgdlle liktrgalhe dearkkfhql

121 slaikychdl dvvhrdlkcd nllldkdfni klsdfsfskr clrddsgrma lsktfcgspa

181 yaapevlqgi pyqpkvydiw slgvilyimv cgsmpyddsn ikkmlriqke hrvnfprskh

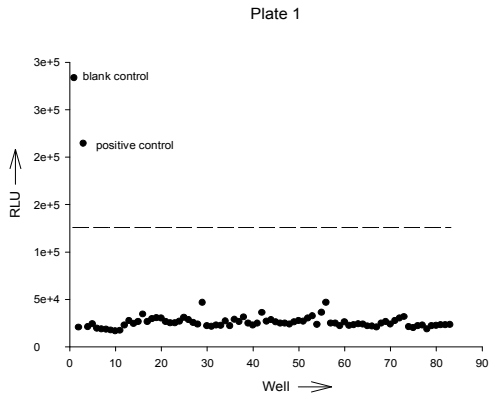
241 ltgeckdliy hmlqpdvnrr lhideilshc wmqpkargsp svainkeges srgteplwtp

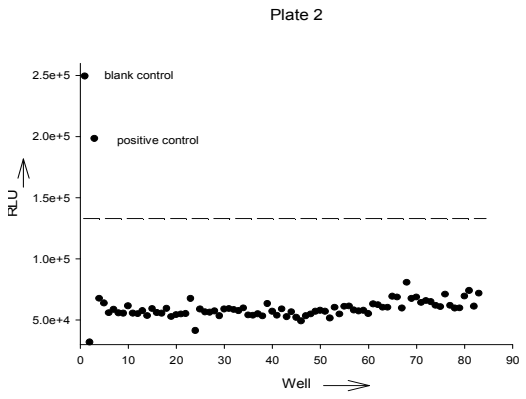
301 epgsdkksat klepegeaqp qaqpetkpeg tamqmsrqse ilgfpskpst meteegppqq

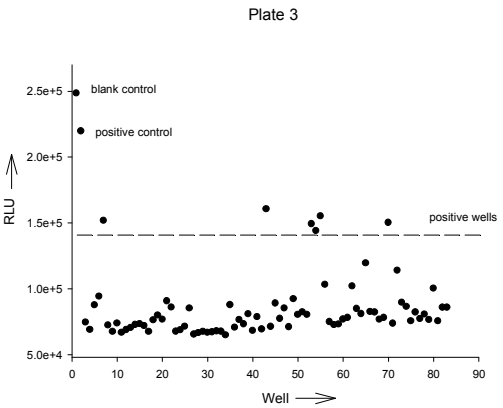
361 ppetraq

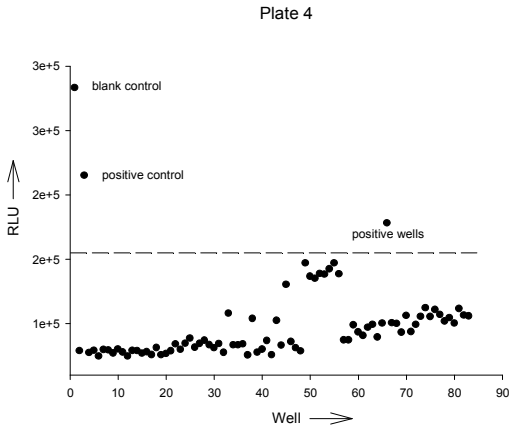
To purify recombinant TSSK1 protein, TSSK1 was inserted into pAcHLT-B (BD Pharmingen, San Diego, USA) to express the target protein as a 6×His fusion protein using the baculovirus expression system. TSSK1 protein was purified from infected Sf9 cells using standard protocol.

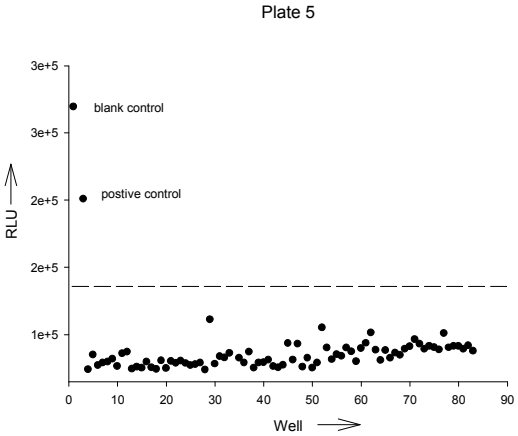
2. Sreening results of 640 single pure compounds by luminescent kinase assay.

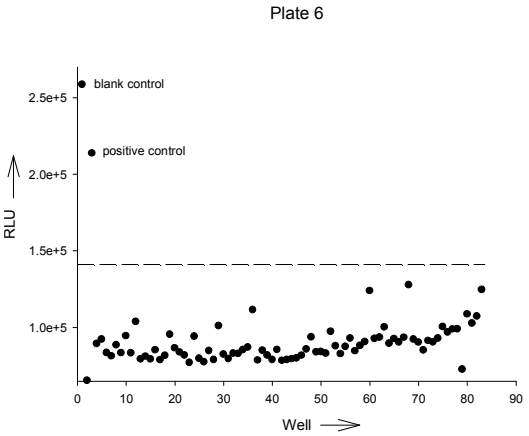












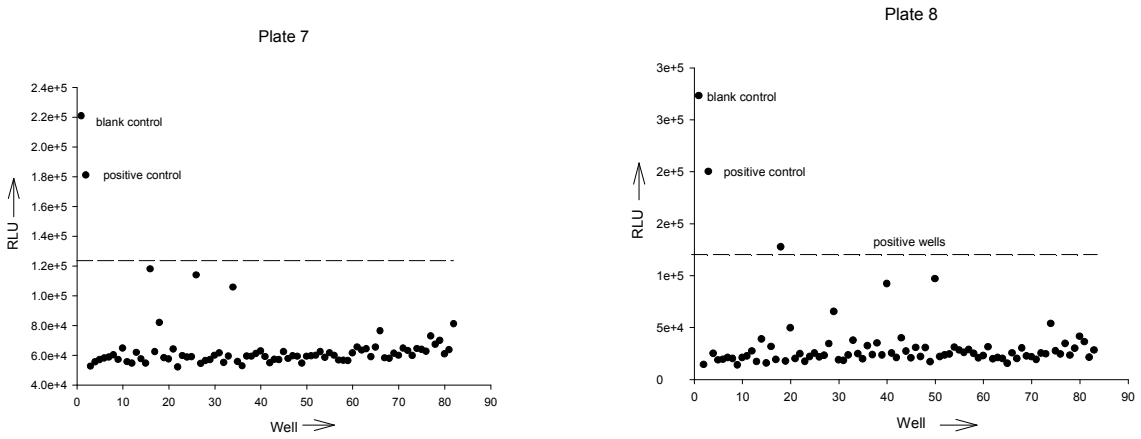


Figure S1. Complete preliminary screening results of 640 pure compounds with initial concentration of 20 μM. Plate represents the inhibition of the average data in duplicated wells for 80 compounds, negative control (without TSSK1) and positive control (staurosporine, 100 μM). The dots above dash line represent positive compounds.

3. LC–MS analysis of the kinase reaction mixture and the mixture solution including internal standard and Pep8-P

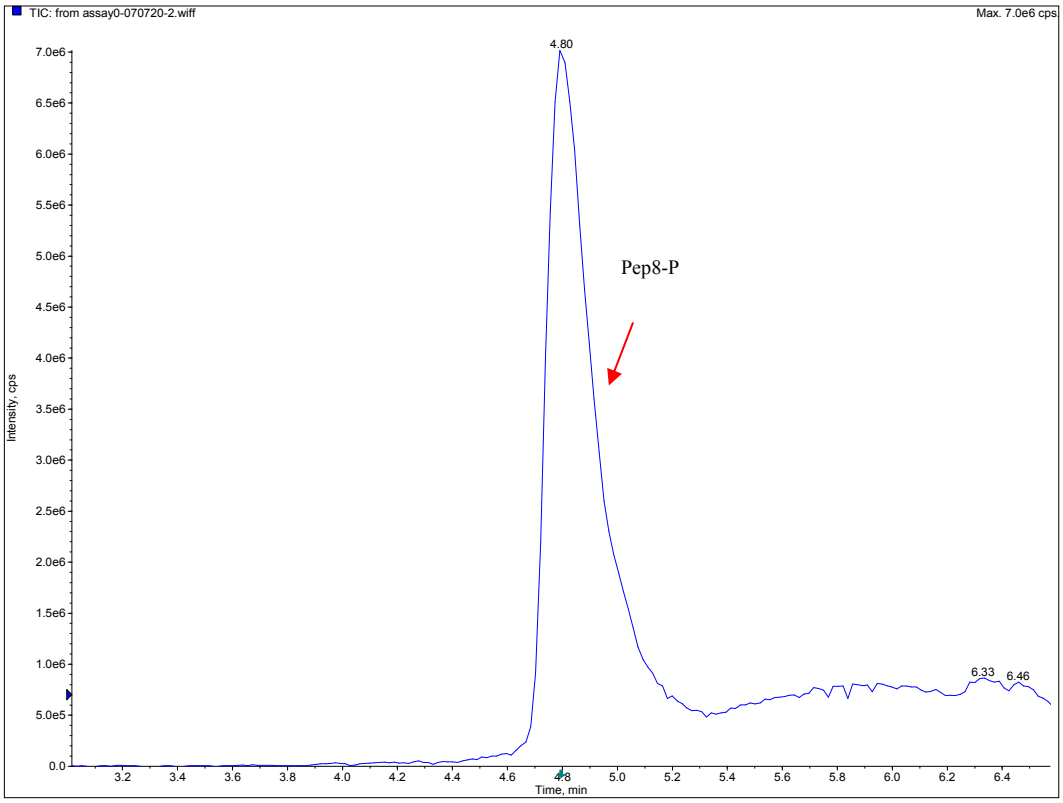


Figure S2. The total ion chromatogram (TIC) of reaction mixture during LC–MS analysis of TSSK1 reaction (the analysis conditions are included in experimental section). Pep8's TIC was ranged from retention time of 5.4 min to 6.4 min that is completely separated with Pep8-P.

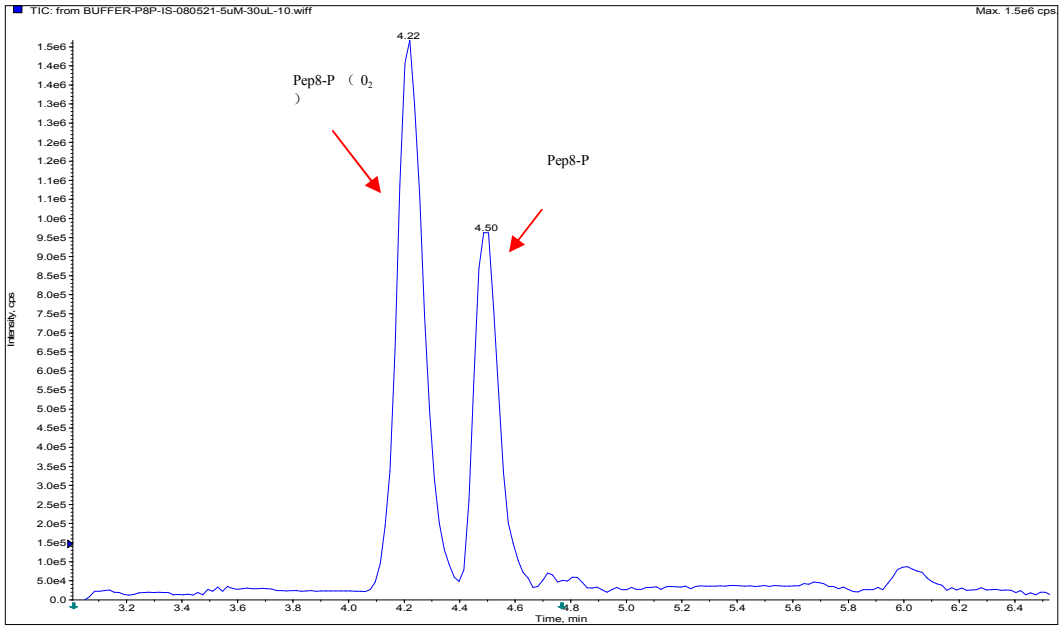


Figure S3. The total ion chromatogram of the mixture solution of internal standard and Pep8-P using the LC–MS analysis system (the analysis conditions are included in experimental section).

4. HPLC and MS analysis of Pep8 , Pep8-P and Pep8-P(O_2)

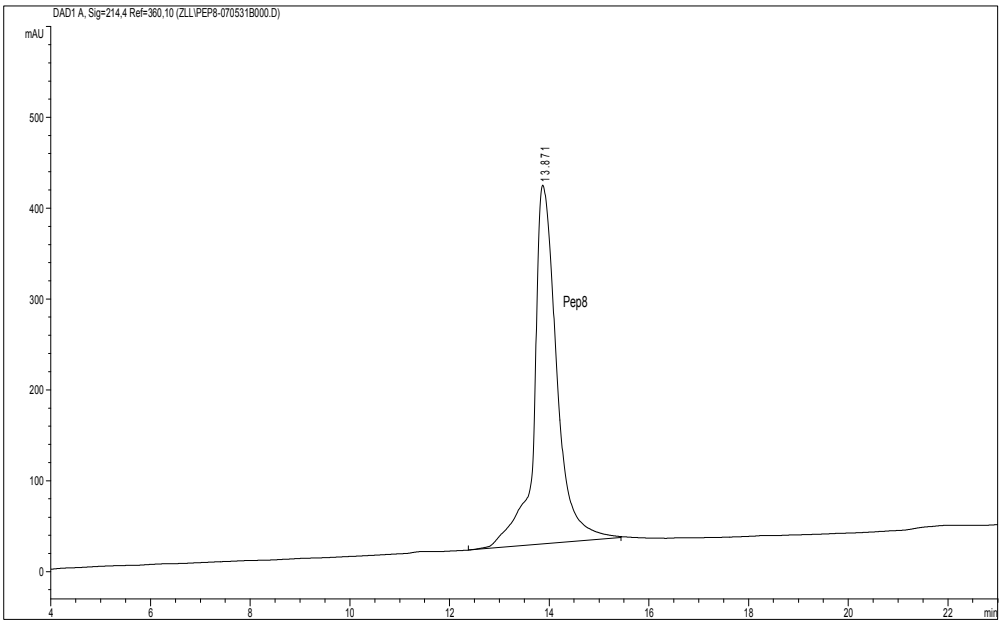


Figure S4. The HPLC spectrum of pep8 (HPLC: Agilent 1100 system; Colum: AGILENT ZORBAX EXTEND-C18 (250 mm × 4.6 mm i.d., 5 μm); Chromatographic Conditions: Mobile Phase A: 0.1%TFA in H_2O; Mobile Phase B: 0.1%TFA in ACN; Gradient: 20-50% B in 25 min steps; Flow: 1.0 mL/min; Detection wavelength: 214 nm)

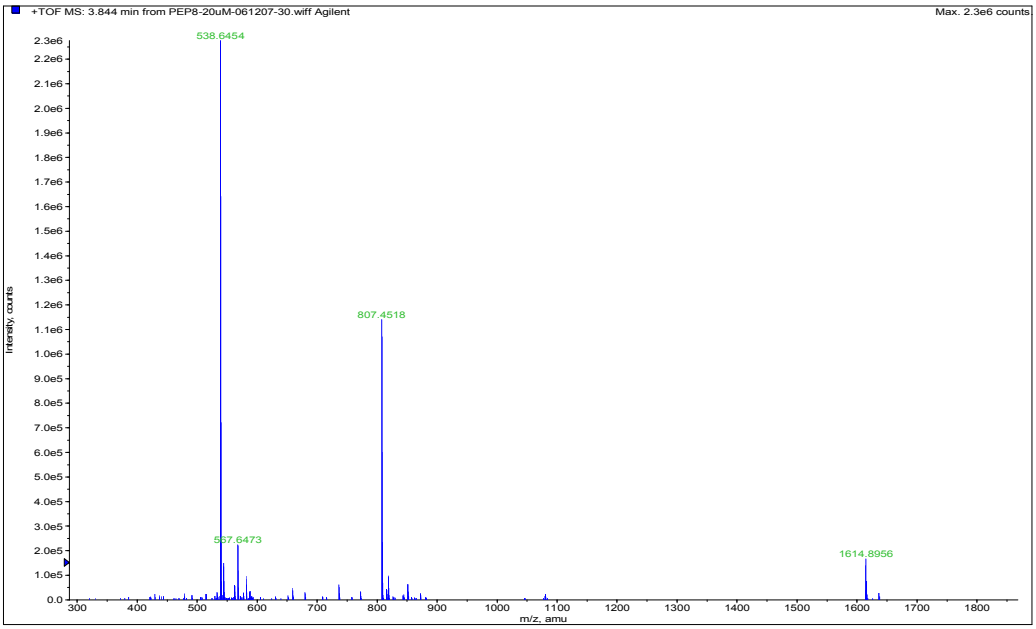


Figure S5. Mass spectrum of Pep8.

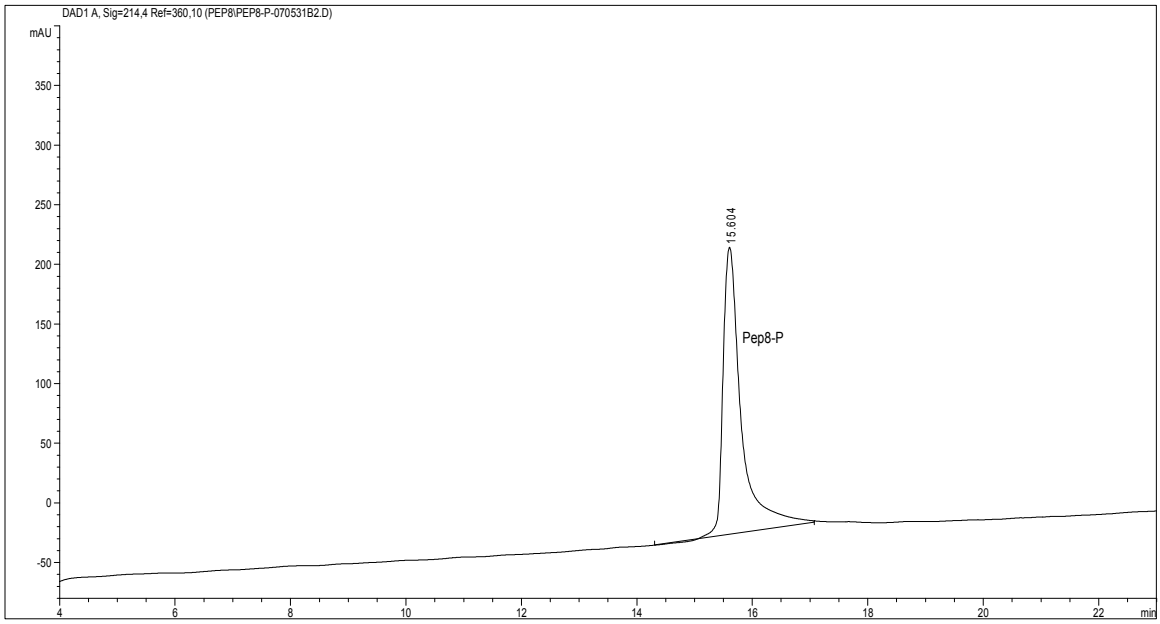


Figure S6. The HPLC profile of pep8-p. (HPLC: Agilent 1100 system; Colum: AGILENT ZORBAX EXTEND-C18 (250 mm × 4.6 mm i.d., 5 μm); Chromatographic Conditions: Mobile Phase A: 0.1%TFA in H_2O; Mobile Phase B: 0.1%TFA in ACN; Gradient: 15-50% B in 25 min steps; Flow: 1.0 mL/min; Detection wavelength: 214 nm)

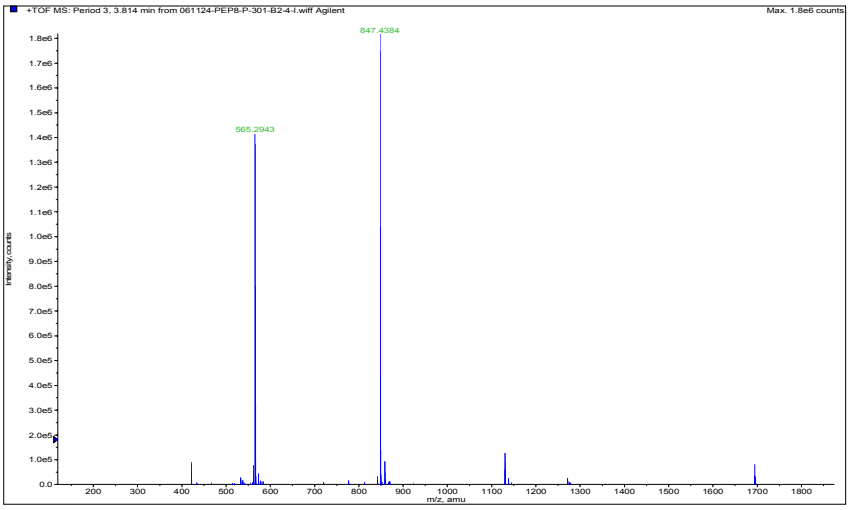


Figure S7. Mass spectrum of pep8-p.

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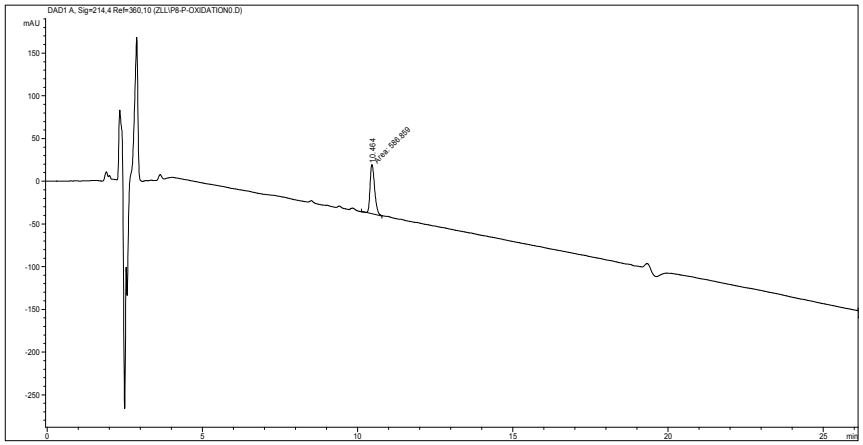


Figure S8. HPLC spectrum of Pep8-P(O2). (HPLC: Agilent 1100 system; Colum: AGILENT ZORBAX EXTEND-C18 (250 mm × 4.6 mm i.d., 5 μm); Chromatographic Conditions: Mobile Phase A: 0.1%TFA in H_2O; Mobile Phase B: 0.1%TFA in ACN; Gradient: 15-50% B in 25 min steps; Flow: 1.0 mL/min; Detection wavelength: 214 nm)

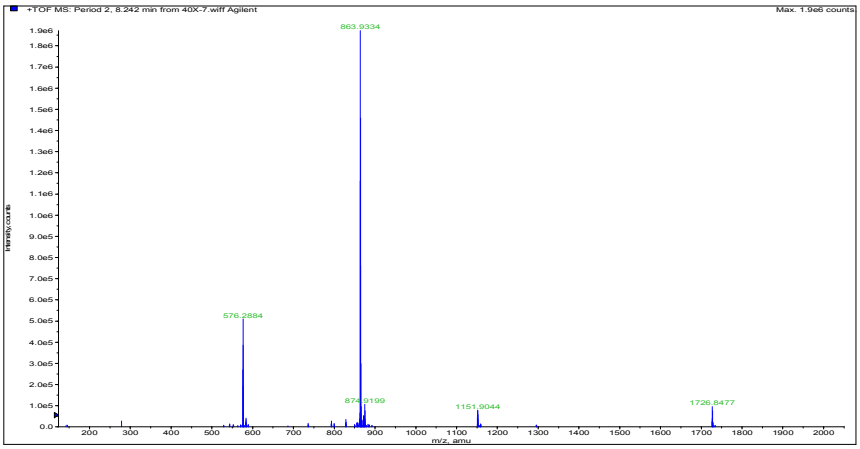


Figure S9. Mass spectrum of the Pep8-P(O_2).

5. Table 1. Peptide library for TSSK1 peptide substrate screening

Code	Peptide sequence	FW		Purity（%）
		Calcd MW.	found $[M+H]^+$ or $[M+2H]^{2+}$	
C9	SIHsFIG	758	759.3	85
D11	KKPsDYG	792	793.3	95
B12	RHSsYPA	815	816.4	73
A1	QEtVE	603	604.2	68
E8	KYKtLRQ	934	782.5	No product
E9	RTTsFAE	809	810.4	90
A2	ISFsQPT	777	778.3	75
A3	LIDsMAN	761	762.3	35
A5	RTQsFSL	836	837.4	51
C10	RKVsKQE	872	873.5	80

F8	PQLsFSD	791	792.4	86
F12	RSPsMPE	801	802.4	43
C12	RTWtLCG	834	835.4	80
D9	RAAsMDN	762	763.4	53
G4	RGFsFVA	781	782.4	90
B10	QGYsFVA	769	770.3	95
A6	IAKtRDA	772	773.4	40
D10	RRMsDEF	938	939.4	75
D2	EDLyKDF	927	928.4	95
E10	ETFsDLW	895	896.3	87
B9	AAPyLKT	761	762.3	84.5
B6	VTKtPPR	796	797.4	46
F1	STLsPIA	686	687.3	77.5
E2	SSLsRER	832	833.5	68
E3	LVDsVAK	729	730.4	76
F4	RSVtLPR	826	827.5	77
D12	PTLsPIP	722	723.3	93
D7	RVYtHEV	901	902.4	88
E11	RTSsFAE	795	796.4	80
G1	SPRtPVsPVK	1065	1066.6	78
G6	QGYsFVA	769	770.3	82
G10	FLRtSCG	781	782.4	58
B2	TMKtFCG	785	786.3	77

D3	RRTtLCG	804	805.4	50
E4	RRRsLLE	927	928.5	80.5
C4	RSRsAPP	768	769.5	63
B5	RQLsSGV	744	745.5	74
D4	RTWtLCG	834	835.4	92
B4	GFLtEyVAT	998	999.4	90
G2	RFSsLHF	891	892.5	91
E5	RRPsYRK	960	961.6	83
B3	GMAsGVA	590	591.2	90
E1	YRYsDTT	903	904.4	83
E12	RSCtWPL	860	861.4	88
F11	TVStQEL	775	776.4	78
A12	EMtGyVA	768	769.3	83
G11	QTPtPTR	798	799.4	64
D1	LLTsPDV	742	743.4	88
C7	NGLLMtP	743	744.3	41
C11	KSGsTTK	706	707.4	53
F2	RFFtRHP	1055	1057.3	No product
G9	RKRsRKE	957	958.6	38
G3	GSVtQsQGS	848	849.3	90
B8	YMsFT	646	647.2	85
A10	PPLsQET	769	770.3	79
F6	RRQsLIE	899	900.5	78

F10	RRVsNAG	757	758.4	63
B1	LPLsQNS	756	757.4	88
F7	TTStFCG	714	715.2	49
G5	PPLsQET	769	770.4	86
D6	FMMtPyVVT	1086	1087.6	83
F3	RSVtLPR	826	827.4	80
A7	FSTtPGG	664	665.3	55
D5	RHDsGLD	797	798.4	89
C8	EMtGyVA	768	769.2	85
A4	VPQtPLH	789	790.4	76
D8	RSRsAPP	768	769.5	40
C2	ARKsTGG	674	675.4	63
B7	LGFtYVA	768	769.4	93
C5	QTPtPTR	798	799.4	53
H2	QEtVE	603	604.2	52
C6	RSPsMPE	801	802.4	30
A11	RGFsFVA	781	782.4	89
H1	GFLtEyVAT	998	999.4	87
E7	LPMsPRT	799	800.4	42
F9	VPQtPLH	789	790.3	81
Pep1	ARKRERTYSFGHHA	1713	858.3	95
Pep2	KKRFSFKKSFKL	1541	772.3	83
Pep3	PLSRTLSVAAKK	1268	636.0	85

Pep4	PLSRTLSVSS	1044	523.4	95
Pep5	GPHRSTPESRAAV	1362	682.0	98
Pep6	LRRASLG	770	386.3	97
Pep7	AAKIQASFRGHMARKK	1797	901.0	90
Pep8	AALVRQMSVAFFFK	1613	807.8	92
Pep9	RRRLSSLRA	1112	557.5	80
Pep10	RKRSRAE	900	451.4	93

6. Z' factor determination

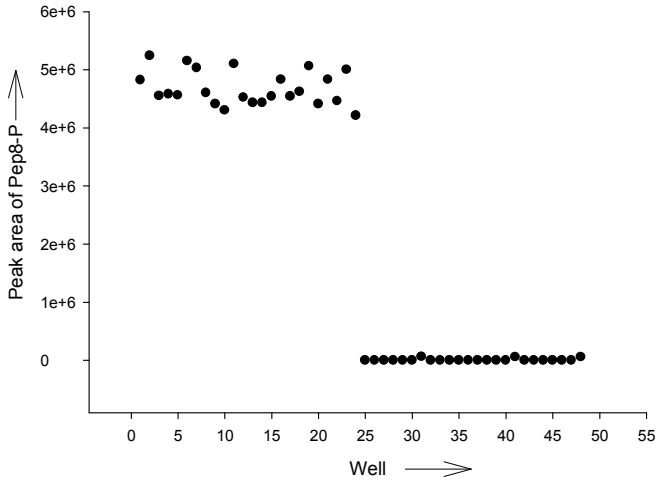
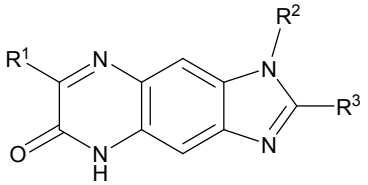
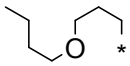
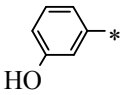
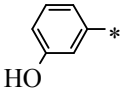
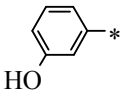
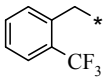
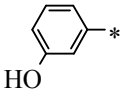
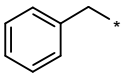
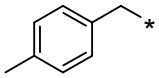
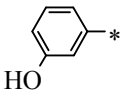
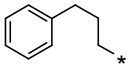
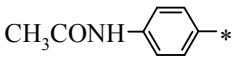
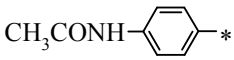
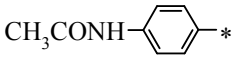
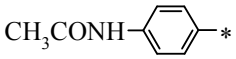
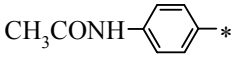
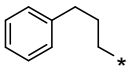
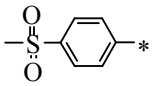
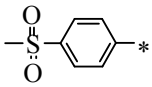
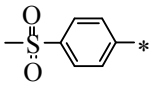
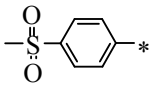
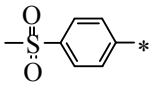
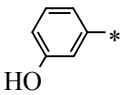
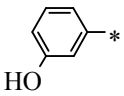
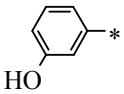


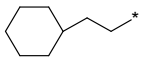
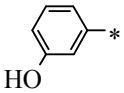
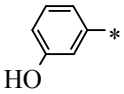
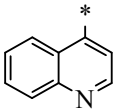
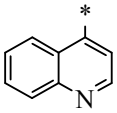
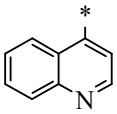
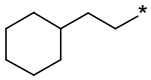
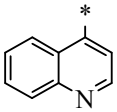
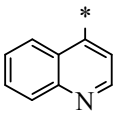
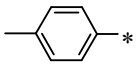
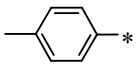
Figure S10. Z' values for MS-based assay.

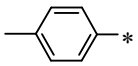
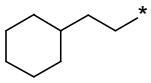
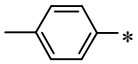
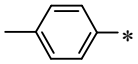
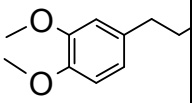
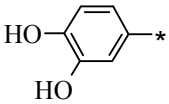
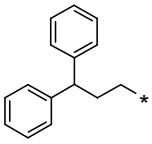
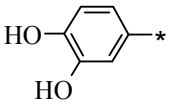
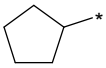
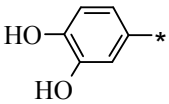
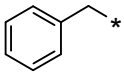
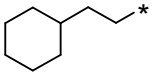
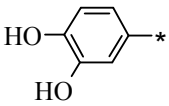
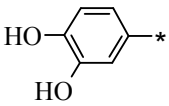
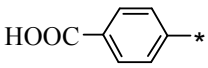
7. Table 2. The structure and their physical data of single compound with 1,2,7-trialky-1H-imidazo[4, 5-g]quinoxalin-6-one

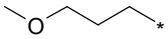
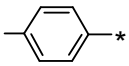
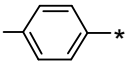
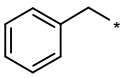
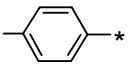
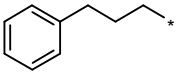
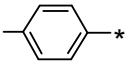


Entry	R^1	R^2	R^3	Formula	HPLC Purity[a] (%)	HRMS $[M + H]^+$	
						found	calcd.
A3-A	H			$C_{22}H_{24}N_4O_3$	99	393.1923	393.1921
A3-B	CH_3	$CH_3(CH_2)_2$		$C_{19}H_{18}N_4O_2$	99	335.1504	335.1502
A3-C	H	$CH_3(CH_2)_4$		$C_{20}H_{20}N_4O_2$	99	349.1661	349.1659
A3-D	$CH_2CH(CH_3)_2$			$C_{27}H_{23}F_3N_4O_2$	97	493.1849	493.1845
A3-E				$C_{30}H_{24}N_4O_2$	99	473.1968	473.1972
E5-A (**16**)	CH_3			$C_{27}H_{25}N_5O_2$	99	452.2083	452.2081
E5-B	CH_3	$CH_3(CH_2)_4$		$C_{23}H_{25}N_5O_2$	99	404.2090	404.2081
E5-C	H	$CH_3CH_2CH_2$		$C_{20}H_{19}N_5O_2$	99	362.1618	362.1611

E5-D	$(CH_3)_2CHCH_2$	$(CH_3)_2CH$		$C_{24}H_{27}N_5O_2$	99	418.2243	418.2237
E5-E	$PhCH_2$	$CH_3(CH_2)_5$		$C_{30}H_{31}N_5O_2$	99	494.2557	494.2550
E9-A	CH_3			$C_{26}H_{24}N_4O_3S$	95	473.1648	473.1641
E9-B	CH_3	$CH_3(CH_2)_4$		$C_{22}H_{24}N_4O_3S$	99	425.1642	425.1641
E9-C	H	$CH_3CH_2CH_2$		$C_{19}H_{18}N_4O_3S$	98	383.1177	383.1172
E9-D	$(CH_3)_2CHCH_2$	$(CH_3)_2CH$		$C_{23}H_{26}N_4O_3S$	90	439.1797	439.1798
E9-E	$PhCH_2$	$CH_3(CH_2)_5$		$C_{29}H_{30}N_4O_3S$	99	515.2111	515.2111
G3-A	CH_3	$C_6H_5CH_2CH_2$		$C_{24}H_{20}N_4O_2$	98	397.1664	397.1659
G3-B	H	$CH_3(CH_2)_5$		$C_{21}H_{22}N_4O_2$	95	363.1820	363.1815
G3-C	H	$CH_3CH_2CH(CH_3)CH_2$		$C_{20}H_{20}N_4O_2$	99	349.1657	349.1659

G3-D	$(CH_3)_2CHCH_2$			$C_{27}H_{32}N_4O_2$	98	445.2601	445.2598
G3-E	$PhCH_2$	$CH_3(CH_2)_8$		$C_{31}H_{34}N_4O_2$	99	495.2758	495.2754
G8-A	CH_3	$C_6H_5CH_2CH_2$		$C_{27}H_{21}N_5O$	98	432.1822	432.1818
G8-B	H	$CH_3(CH_2)_5$		$C_{24}H_{23}N_5O$	98	398.1970	398.1975
G8-C	H	$CH_3CH_2CH(CH_3)CH_2$		$C_{23}H_{21}N_5O$	99.5	384.1821	384.1824
G8-D	$(CH_3)_2CHCH_2$			$C_{30}H_{33}N_5O$	97	480.2762	480.2763
G8-E	$PhCH_2$	$CH_3(CH_2)_8$		$C_{34}H_{35}N_5O$	95	530.2917	530.2919
G9-A	CH_3	$C_6H_5CH_2CH_2$		$C_{25}H_{22}N_4O$	98	395.1866	395.1871
G9-B	H	$CH_3(CH_2)_5$		$C_{22}H_{24}N_4O$	96	361.2026	361.2028

G9-C	H	$CH_3CH_2CH(CH_3)CH_2$		$C_{21}H_{22}N_4O$	98	347.1877	347.1871
G9-D	$(CH_3)_2CHCH_2$			$C_{28}H_{34}N_4O$	95	443.2807	443.2810
G9-E	$PhCH_2$	$CH_3(CH_2)_8$		$C_{32}H_{36}N_4O$	92	493.2966	493.2961
D6-A	H			$C_{25}H_{22}N_4O_5$	99.5	459.1662	459.1662
D6-B	H			$C_{30}H_{24}N_4O_3$	98	489.1926	489.1921
D6-C	H			$C_{20}H_{18}N_4O_3$	95	363.1449	363.1451
D6-D				$C_{30}H_{30}N_4O_3$	90	495.2382	495.2390
D6-E (**15**)		CH3(CH2)6		$C_{25}H_{30}N_4O_3$	97	435.2391	435.2390
F3-A	H			$C_{21}H_{20}N_4O_3$	98	377.1608	377.1608

F3-B	H		HOOC 	$C_{20}H_{18}N_4O_4$	97	379.1404	379.1400
F3-C		$CH_3(CH2)_8$	HOOC 	$C_{29}H_{36}N_4O_3$	95	489.2857	489.2860
F3-D			HOOC 	$C_{29}H_{26}N_4O_3$	95	479.2073	479.2077
F3-E (**14**)			HOOC 	$C_{28}H_{26}N_4O_3$	99	467.2080	467.2077

a Purity based on the integration area of the HPLC peaks (the detection wavelengh was UV 254 nm).

Data of ^{1}H-NMR:

A3-A: ^{1}H NMR (300 MHz, DMSO-d6): δ 0.81 (t, J=7.5Hz, 3H), 1.17～1.37(m, 4H), 1.92(t, J=5.7Hz, 2H), 3.14(t, J=6.3Hz, 2H), 3.21(t, J=5.7Hz, 2H), 4.44(t, J=7.2Hz, 2H), 6.96(dd, J=1.8Hz, J=6.3Hz, 1H), 7.19(d, J=2.1Hz, 1H), 7.21(d, J=9.6Hz, 1H), 7.36(t, J=7.8Hz, 1H), 7.49(s, 1H), 8.06(s, 1H), 8.12(s, 1H), 9.82(s, 1H), 12.30(s, 1H)

A3-B: ^{1}H NMR (300 MHz, DMSO-d6): δ 0.75(t, J=7.5Hz, 3H), 1.72(m, 2H), 2.42(s, 3H), 4.30 (m, 2H), 6.96(dd, J=6.6Hz, J=1.5Hz, 1H), 7.16(d, J=1.8Hz, 1H), 7.18(d, J=10.5Hz, 1H), 7.38(t, J=7.8Hz, 1H), 7.47(s,1H), 8.02(s,1H), 9.83(s,1H), 12.20(s, 1H)

A3-C: ^{1}H NMR (300 MHz, DMSO-d6): δ 0.74 (t, J=6.9Hz, 3H), 1.13 (m, 4H), 1.68 (m, 2H), 4.36(m, 2H), 6.97(dd, J=6.3Hz, J=1.8Hz, 1H), 7.17(d, J=5.1Hz, 1H), 7.19(d, J=7.8Hz, 1H), 7.38(t, J=7.5Hz, 1H), 7.50(s, 1H), 8.09(s, 1H), 8.12(s, 1H), 9.84(s, 1H), 12.30(s, 1H)

A3-D: ^{1}H NMR (300 MHz, DMSO-d6): δ 0.90 (d, J=6.6Hz, 6H), 2.20 (m, 1H), 2.63 (d, J=6.9Hz, 2H), 5.74(s, 2H), 6.69(d, J=6.6Hz, 1H), 6.95(m, 2H), 7.07(s, 1H), 7.27(t, J=7.8Hz, 1H), 7.52(m, 2H), 7.57(s, 1H), 7.66（s, 1H）, 7.84(m, 1H), 9.81(s,1H), 12.25 (s, 1H)

A3-E: ^{1}H NMR (300 MHz, DMSO-d6): δ 2.22 (s, 3H), 4.10(s, 2H), 5.58(s, 2H), 6.92(m, 3H), 7.07～7.32(m, 10H), 7.51(s, 1H), 7.74(s, 1H), 9.82(s,1H), 12.29 (s, 1H)

E5-A: ^{1}H NMR (300 MHz, DMSO-d6): δ 2.03 (m, 2H), 2.10(s, 3H), 2.42(s, 3H), 2.56 (t, J=7.5Hz, 2H), 4.35(t, J=6.9Hz, 2H), 7.09～7.25(m, 5H), 7.47(s, 1H), 7.71(dd, J=8.7Hz, J=11.1Hz, 4H), 8.03(s, 1H), 10.24(s, 1H), 12.22(s, 1H)

E5-B: ^{1}H NMR (300 MHz, DMSO-d6): δ 0.74 (t, J=6.9Hz, 3H), 1.14(m, 4H), 1.68(m, 2H), 2.09(s, 3H), 2.42(s, 3H), 4.35(t, J=7.2Hz, 2H), 7.46(s, 1H), 7.76(dd, J=9.0Hz, J=5.7Hz, 4H), 7.99(s, 1H), 10.23(s, 1H), 12.19(s, 1H)

E5-C: ^{1}H NMR (300 MHz, DMSO-d6): δ 0.74 (t, J=7.5Hz, 3H), 1.70(m, 2H), 2.09(s, 3H), 4.34(t, J=7.5Hz, 2H), 7.48(s, 1H), 7.77(dd, J=4.8Hz, J=9.0Hz, 4H), 8.08(s, 1H), 8.10(s, 1H), 10.24(s, 1H), 12.28(s, 1H).

E5-D: ^{1}H NMR (300 MHz, DMSO-d6): δ 0.95 (d, J=6.9Hz, 6H), 1.62(d, J=6.6Hz, 6H), 2.10(s, 3H), 2.28(m, 1H), 2.68(d, J=6.9Hz, 2H), 4.79(m, 1H), 7.49(s, 1H), 7.72(dd, J=8.7Hz, J=8.7Hz, 4H), 8.10(s, 1H), 10.34(s, 1H), 12.21(s, 1H)

E5-E: ^{1}H NMR (300 MHz, DMSO-d6): δ 0.74(t, J=6.6Hz, 3H), 1.11(s, 6H), 1.66(m, 2H), 2.09(s, 3H), 4.14(s, 2H), 4.38(t, J=7.2Hz, 2H), 7.29(m, 5H), 7.48(s, 1H), 7.78(dd, J=6.0Hz, J=9.0Hz, 4H), 8.09(s, 1H), 10.27(s, 1H), 12.31(s, 1H)

E9-A: ^{1}H NMR (600 MHz, DMSO-d6): δ 2.07(m, 2H), 2.43(s, 3H), 2.55(t, J=7.2Hz, 2H), 3.31(s, 3H), 4.37(t, J=8.4Hz, 2H), 7.08～7.23(m, 5H), 7.52(s, 1H), 8.00(d, J=8.4Hz,, 2H), 8.05(d, J=8.4Hz,, 2H), 8.05(d, J=8.4Hz,, 2H), 8.08(s, 1H), 12.23(s, 1H)

E9-B: ^{1}H NMR (300 MHz, DMSO-d6): δ 0.73(t, J=7.2Hz, 3H), 1.12(m, 4H), 1.68(t, J=6.3Hz, 2H), 2.43(s, 3H), 3.32(s, 3H), 4.40(t, J=6.9Hz, 2H), 7.52(s, 1H), 8.08(s, 1H), 8.11(dd, J=5.4Hz, J=8.4Hz, 4H), 12.23(s, 1H)

E9-C: ^{1}H NMR (300 MHz, DMSO-d6): δ 0.73(t, J=7.2Hz, 3H), 1.72(m, 2H), 3.31(s, 3H), 4.40(t, J=7.2Hz, 2H), 7.55(s, 1H), 8.12(dd, J=3.0Hz, J=8.7Hz, 4H), 8.15(s, 1H), 8.21(s, 1H), 12.33(s, 1H)

E9-D: ^{1}H NMR (300 MHz, DMSO-d6): δ 0.96(d, J=6.9Hz, 6H), 1.64(d, J=6.9Hz, 6H), 2.27(m, 1H), 2.69(d, J=7.2Hz, 2H), 3.32(s, 3H), 4.73(m, 1H), 7.52(s, 1H), 7.97(d, J=8.7Hz, 2H), 8.13(d, J=8.4Hz, 2H), 8.16(s, 1H), 12.22(s, 1H)

E9-E: ^{1}H NMR (300 MHz, DMSO-d6): δ 0.72(t, J=6.6Hz, 3H), 1.09(m, 6H), 1.66(m, 2H), 3.32(s, 3H), 4.15(s, 2H), 4.40(t, J=7.2Hz, 2H), 7.18～7.37(m, 5H), 7.53(s, 1H), 8.11(dd, J=5.1Hz, J=8.7Hz, 4H), 8.15(s,1H), 12.31(s, 1H)

G3-A: ^{1}H NMR (300 MHz, DMSO-d6): δ 2.43 (s, 3H), 3.00(t, J=7.2Hz, 2H), 4.54(t, J=7.5Hz, 2H), 6.92～7.02(m, 5H), 7.145(m, 3H), 7.32(t, J=8.1Hz, 1H), 7.46(s, 1H), 8.06(s, 1H), 9.77(s, 1H), 12.20(s, 1H)

G3-B: ^{1}H NMR (300 MHz, DMSO-d6): δ 0.74 (d, J=6.9Hz, 3H), 1.11 (m, 6H), 1.69 (m, 2H), 4.38(t, J=7.5Hz, 2H), 6.99(m, 1H), 7.19(m, 2H), 7.40(t, J=7.8Hz, 1H) 7.51(s, 1H), 8.14(s, 2H), 9.89(s, 1H), 12.33(s, 1H)

G3-C: ^{1}H NMR (300 MHz, DMSO-d6): δ 0.59 (d, J=6.6Hz, 3H), 0.67(t, J=7.2Hz, 3H), 0.90～1.18(m, 2H), 1.74(m, 1H), 4.19～4.41(m, 2H), 6.97(m, 1H), 7.19(m, 2H), 7.37(t, J=7.5Hz, 1H), 7.50(s, 1H), 8.13(s, 2H), 9.84(s, 1H), 12.31(s, 1H)

G3-D: ^{1}H NMR (300 MHz, DMSO-d6): 0.83(m, 1H), 0.95(d, J=6.9Hz, 6H), 1.05(m, 4H), 1.48(m, 8H), 2.26(m, 1H), 2.68(d, J=6.9Hz, 2H), 4.36(t, J=6.9Hz, 2H), 6.96(dd, J=8.4Hz, 1H), 7.16(d, J=11.4Hz, 2H), 7.38(t, J=7.5Hz, 1H), 7.47(s, 1H), 7.99(s, 1H), 9.82(s, 1H), 12.19(s, 1H)

G3-E: ^{1}H NMR (600 MHz, DMSO-d6): δ 0.81(t, J=7.8Hz, 3H), 1.10～1.22(m, 12H), 1.67(m, 2H), 4.15(s, 2H), 4.36(t, J=7.2Hz, 2H), 6.98(dd, J=1.8Hz, J=6.6Hz, 1H), 7.16(m, 1H), 7.20 (m, 2H), 7.29(t, J=7.8Hz, 2H), 7.37(m, 3H), 7.48(s, 1H), 8.08(s, 1H), 9.86(s, 1H), 12.30(s, 1H)

G8-A: ^{1}H NMR (300 MHz, DMSO-d6): δ 2.46 (s, 3H), 2.91(t, J=6.6Hz, 2H), 4.43(t, J=6.6Hz, 2H), 6.64(d, J=6Hz, 2H), 6.98(m, 3H), 7.28(d, J=4.5Hz, 1H), 7.59(m, 3H), 7.83(m, 1H), 8.13(d, J=8Hz, 1H), 8.22(s, 1H), 8.96(d, J=4.2, 1H), 12.28 (s, 1H)

G8-B: ^{1}H NMR (300 MHz, DMSO-d6): δ 0.55 (t, J=6.6Hz，3H), 0.85 (m, 6H), 1.50 (m, 2H), 4.24(t, J=7.2Hz, 2H), 7.62(s, 1H), 7.67(d, J=7.8Hz, 1H), 7.85(m, 3H), 8.18(m, 2H), 8.24(s, 1H), 9.12(d, J=4.5, 1H), 12.37(s, 1H)

G8-C: ^{1}H NMR (300 MHz, DMSO-d6): δ 0.48 (t, J=7.2Hz, 3H), 0.51(d, J=6.3Hz, 3H), 0.76～1.08(m, 2H), 1.56(m, H), 4..04～4.25(m, 2H), 7.62(s, 1H), 7.67(m, 1H), 7.85(m, 3H), 8.18(m, 2H), 8.25(s, 1H), 9.12(d, J=4.5, 1H), 12.37(s, 1H)

G8-D: ^{1}H NMR (300 MHz, DMSO-d6): δ 0.48～0.68(m, 5H), (m, 6H), 0.85(m, 2H), 0.97(d, J=6.9Hz, 6H), 1.12(m, 2H), 1.28～1.45(m, 4H), 2.24～2.31(m, 1H), 2.71(d, J=7.2Hz, 2H), 4.26(t, J=6.9Hz, 2H), 7.59(s, 1H), 7.66(m, 1H), 7.86(m, 3H), 8.15(s, 1H), 8.18(m, 1H), 9.12(d, J=4.5, 1H), 12.28(s, 1H)

G8-E: ^{1}H NMR (300 MHz, DMSO-d6): δ 0.74～1.11(m, 15H), 1.48(m, 2H), 4.17(s, 2H), 4.23(t, J=6.9Hz, 2H), 7.19～7.38(m, 5H), 7.59(s, 1H), 7.64(m, 2H), 7.84(m, 3H), 8.16(m, 1H), 8.18(s, 1H), 9.11(d, J=3.9Hz, 1H), 12.36(s, 1H)

G9-A: ^{1}H NMR (300 MHz, DMSO-d6): δ 2.40(s, 3H), 2.43(s, 3H), 3.02(t, J=7.5Hz, 2H), 4.56(t, J=6.9Hz, 2H), 6.94(m, 2H), 7.14(m, 3H), 7.33(d, J=8.1Hz, 2H), 7.48(d, J=8.1Hz, 2H), 7.47(s, 1H), 8.10(s, 1H), 12.22(s, 1H)

G9-B: ^{1}H NMR (300 MHz, DMSO-d6): δ 0.73(t, J=6.3Hz, 3H), 1.10(m, 6H), 1.66(m, 2H), 2.42(s, 3H), 4.38(t, J=7.5Hz, 2H), 7.41(d, J=7.8Hz, 2H), 7.50(s, 1H), 7.70(d, J=7.8Hz, 2H), 8.12(s, 2H), 12.31(s, 1H)

G9-C: ^{1}H NMR (300 MHz, DMSO-d6): δ 0.59(d, J=6.6Hz, 3H), 0.66(t, J=7.8Hz, 3H), 0.91～1.15(m, 2H), 1.73(m, 1H), 2.42(s, 3H), 4.25～4.42(m, 2H), 7.41(d, J=7.8Hz, 2H), 7.52(s, 1H), 7.74(d, J=8.4Hz, 2H), 8.14(d, J=1.2Hz, 1H), 8.19(s, H), 12.33(s, 1H)

G9-D: ^{1}H NMR (300 MHz, DMSO-d6): δ0.83(m, 1H), 0.95(d, J=6.6Hz, 6H), 1.04(m, 4H), 1.53(m, 8H), 2.26(m, 1H), 2.68(d, J=6.9Hz, 2H), 4.36(t, J=6.9Hz, 2H), 7.40(d, J=7.8Hz, 2H), 7.47(s, 1H), 7.68(d, J=8.1Hz, 2H), 7.99(s, 1H), 12.20(s, 1H)

G9-E: ^{1}H NMR (300 MHz, DMSO-d6): δ 0.80(t, J=7.5Hz, 3H), 1.07(m, 12H), 1.63(m, 2H), 2.40(s, 3H), 4.14(s, 2H), 4.35(t, J=7.2Hz, 2H), 7.20～7.40(m, 5H） 7.39(d, J=8.4Hz, 2H), 7.47(s, 1H), 7.68(d, J=8.1Hz, 2H), 8.04(s, 1H), 12.28(s, 1H)

D6-A: ^{1}H NMR (300 MHz, DMSO-d6): δ 2.92 (t, J=6.6Hz, 2H), 3.59(s, 3H), 3.65(s, 3H), 4.55(t, J=6.9Hz, 2H), 6.48(d, J=8.1Hz, 1H), 6.55(s, 1H), 6.70(d, J=8.1Hz, 1H), 6.86(d, J=8.1Hz, 1H), 6.95(dd, J=1.8Hz, J=6.3Hz, 1H), 7.11(d, J=1.8Hz, 1H) 7.43(s, 1H), 8.08(s, 1H), 8.10(s, 1H), 9.31(s, 1H), 9.54(s, 1H), 12.28(s, 1H)

D6-B: ^{1}H NMR (600 MHz, DMSO-d6): δ 2.52 (dd, J=8.4Hz, J=7.8Hz, 2H), 4.08(t, J=7.8Hz, H), 4.20(t, J=7.8Hz, 2H), 6.80(d, J=7.8Hz, 1H), 6.92(dd, J=6.0Hz, J=1.8Hz, 1H), 7.16(m, 3H), 7.25(m, 8H), 7.44(s, 1H), 7.91(s, 1H), 8.10(s, 1H), 9.37(s, 1H), 9.55(s, 1H), 12.28(s, 1H)

D6-C: ^{1}H NMR (600 MHz, DMSO-d6): δ 1.22(m, 4H), 1.71(m, 2H), 2.24(m, 2H), 4.99(m, 1H), 6.91(d, J=8.4Hz, 1H), 7.00(d, J=6.0Hz, 1H), 7.10(d, J=1.8Hz, 1H), 7.47(s, 1H), 7.89(s, 1H), 8.10(s, 1H)

D6-D: ^{1}H NMR (600 MHz, DMSO-d6): δ0.83(m, 1H), 1.05(m, 4H), 1.48(m, 8H), 2.26(m, 1H), 4.33(t, J=6.6Hz, 2H), 6.90(d, J=8.4Hz, 1H), 7.07(dd, J=6.6Hz, J=1.8Hz, 1H), 7.19(d, J=2.1Hz, 1H), 7.43(s, 1H), 7.93(s, 1H), 9.36(s, 1H), 9.54(s, 1H),12.26(s, 1H)

D6-E: ^{1}H NMR (300 MHz, DMSO-d6): δ 0.79 (t, J=7.2Hz, 3H), 1.51(m, 8H), 1.24(d, J=6.9Hz, 6H), 1.69(m, 2H), 3.48(m, 1H), 4.33(t, J=6.6Hz, 2H), 6.90(d, J=8.1Hz, 1H), 7.09(dd, J=8.1Hz, J=1.8Hz, 1H) 7.19(d, J=2.1Hz, 1H), 7.43(s, 1H), 7.99(s, 1H), 9.37(s, 1H), 9.55(s, 1H), 12.19(s, 1H)

F3-A: ^{1}H NMR (300 MHz, DMSO-d6): δ 0.57(d, J=6.9Hz, 3H), 0.65(t, J=7.5Hz, 3H), 1.02(m, 2H), 1.69(m, 1H), 4.35(m, 2H), 7.54(s, 1H), 7.97(d, J=8.1Hz, 2H), 8.12(d, J=8.4Hz, 2H), 8.13(s, 1H), 8.17(s, 1H), 12.31(s, 1H), 13.26(brs, 1H)

F3-B: ^{1}H NMR (300 MHz, DMSO-d6): δ 1.94(m, 2H), 3.04(s, 3H), 3.18(t, J=5.7Hz, 2H), 4.47(t, J=6.6Hz, 2H), 7.54(s, 1H), 7.97(d, J=8.4Hz, 2H), 8.13(d, J=6.9Hz, 4H), 8.14(s, 2H), 12.32(s, 1H), 13.23(brs, 1H)

F3-C: ^{1}H NMR (600 MHz, DMSO-d6): δ0.80(t, J=7.2Hz, 3H), 0.96(d, J=6.6Hz, 6H), 1.08(m, 12H), 1.65(m, 2H), 2.27(m, 1H), 2.69(d, J=7.2Hz, 2H), 4.41(t, J=7.8Hz, 2H), 7.51(s, 1H), 7.94(d, J=8.4Hz, 2H), 8.10(s, 1H), 8.12(d, J=8.4Hz, 2H), 12.21(s, 1H), 13.23(brs, 1H)

F3-D: ^{1}H NMR (300 MHz, DMSO-d6): δ 1.41(m, 4H), 1.88(m, 4H), 2.34(m, 2H), 4.15(s, 2H), 4.29(m, 1H), 7.28(m, 5H), 7.51(s, 1H), 7.82(d, J=8.4Hz, 2H), 8.13(d, J=8.4Hz, 2H), 8.19(s, H), 12.29(s, 1H), 13.27(brs, 1H)

F3-E: ^{1}H NMR (300 MHz, DMSO-d6): δ 1.25(d, J=6.9Hz, 6H), 2.04(m, 2H), 2.56(t, J=7.8Hz, 2H), 3.50(m, 1H), 4.38(t, J=7.5Hz, 2H), 7.16(m, 5H), 7.51(s, 1H), 7.85(d, J=8.7Hz, 2H), 8.04(d, J=8.4Hz, 2H), 8.08(s, H), 12.22(s, 1H), 13.23(brs, 1H)