Electronic Supplementary Information

"Development of a simple and stability-indicating RP-HPLC method for determining olanzapine and related impurities generated in the preparative process"

Daoping Cui, Yueqing Li, Mingming Lian, Feng Yang and Qingwei Meng*

[a]*State Key Laboratory of Fine Chemicals, School of Pharmaceutical Science and Technology, Dalian University of Technology, No.2 Linggong Road, Ganjingzi District, Dalian, Liaoning Province, 116024, P. R. China;* [b]*Merro Pharmaceutical Companies, No.9 Yingsheng Road, Ganjingzi District, Dalian, Liaoning Province, 116036, P. R. China*

Corresponding author: Qingwei Meng

E-mail: mengqw@dlut.edu.cn

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1. X-ray crystallographic data table of Imp-7 (1-(5-methyl-thionphen-2-yl)-1H-benzimidazol-2(3H)-one)

The recrystallization mother liquor of OLN was concentrated under high vacuum to obtain dark brown crude samples. By column chromatograph (the mobile phase consisting of CH_2Cl_2: methanol: Et_2NH), each fraction was collected and analyzed. The fractions of Imp-7 were concentrated to white powder. Transparent acicular single crystals were obtained by crystallizing from ethanol at room temperature (13~20°C) through natural evaporation.

Table 1 X-ray crystallographic data processing and refinement statistics for Imp-7

Empirical formula	$C_{12}H_{10}N_2OS$
Formula weight	230.28
Temperature(K)	298
Wavelength(Å)	0.71073
Crystal system	Monoclinic
Space group	P2(1)/n
a(Å)	20.459(11)
b(Å)	9.635(5)
c(Å)	23.657(12)
α (°)	90.00
β (°)	111.067(6)
γ (°)	90.00
V ($Å^3$)	4352(4)
Z	16
D_{calcd}(g/cm^{-3})	1.406
Absorption coefficient(mm^{-1})	0.275
F(000)	1920
Crystal size(mm)	0.30×0.16×0.11
θ Range for data collection(°)	2.27-26.71
Limiting indices	$-26 \le h \le 26$, $-7 \le k \le 12$, $-30 \le l \le 25$
Reflections collected/unique	23289/9592 [R_{int}=0.05]
Completeness 2θ= 50.00(%)	0.968
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	9592 / 0 / 577
Goodness of fit on F^2	1.01
Final R indices (I > 2σ(I))	R_1=0.063, wR_2=0.1616
R indices (all data)	R_1=0.1111, wR_2=0.1982
Largest diff. Peak and hole (e $Å^{-3}$)	0.39 and -0.71

Table 2 Fractional atomic coordinates and isotropic or equivalent isotropic displacement parameters ($Å^2$) for compound Imp-7

Atom	*x*	*y*	*z*	U_{iso}*/U_{eq}
S1	0.59800 (5)	0.56901 (8)	0.22074 (3)	0.0528 (2)
O1	0.52039 (10)	0.2539 (2)	0.08870 (8)	0.0477 (5)
N1	0.60523 (12)	0.4277 (2)	0.12298 (9)	0.0369 (5)
C1	0.55805 (14)	0.3429 (3)	0.07914 (11)	0.0368 (7)
N2	0.56367 (12)	0.3778 (2)	0.02535 (9)	0.0392 (6)
H2A	0.5401	0.3403	−0.0089	0.047*
C2	0.61257 (14)	0.4822 (3)	0.03353 (12)	0.0347 (6)
S2	0.09460 (4)	0.17875 (8)	0.21701 (3)	0.0502 (2)
O2	0.01819 (11)	0.4941 (2)	0.08419 (8)	0.0480 (5)
C3	0.63612 (15)	0.5493 (3)	−0.00759 (13)	0.0441 (7)
H3	0.6185	0.5271	−0.0486	0.053*
S3	−0.04842 (4)	0.45151 (9)	0.30116 (3)	0.0430 (2)
O3	−0.01924 (10)	0.2651 (2)	0.42126 (8)	0.0446 (5)
N3	0.10318 (12)	0.3207 (2)	0.11982 (9)	0.0382 (6)
S4	0.44895 (4)	0.29818 (9)	0.29682 (3)	0.0436 (2)
O4	0.47929 (10)	0.4863 (2)	0.41627 (8)	0.0448 (5)
C4	0.68700 (16)	0.6509 (3)	0.01517 (14)	0.0482 (8)
H4	0.7037	0.6978	−0.0112	0.058*
N4	0.06361 (12)	0.3702 (2)	0.02244 (9)	0.0388 (6)
H4A	0.0403	0.4071	−0.0120	0.047*
C5	0.71329 (15)	0.6840 (3)	0.07590 (14)	0.0467 (7)
H5	0.7475	0.7523	0.0896	0.056*
N5	−0.10726 (11)	0.4322 (2)	0.38763 (9)	0.0352 (5)
C6	0.68964 (14)	0.6171 (3)	0.11717 (12)	0.0414 (7)
H6	0.7071	0.6399	0.1581	0.050*
N6	−0.06287 (12)	0.3862 (2)	0.48542 (9)	0.0390 (6)
H6A	−0.0379	0.3506	0.5198	0.047*
N7	0.39151 (11)	0.3181 (2)	0.38404 (9)	0.0340 (5)
C7	0.63923 (14)	0.5155 (3)	0.09473 (11)	0.0345 (6)
N8	0.43782 (11)	0.3651 (2)	0.48167 (9)	0.0361 (6)
H8A	0.4634	0.4008	0.5158	0.043*
C8	0.61318 (14)	0.4240 (3)	0.18450 (11)	0.0378 (7)
C9	0.62711 (14)	0.3131 (3)	0.22200 (11)	0.0404 (6)
H9	0.6364	0.2251	0.2106	0.049*
C10	0.62630 (16)	0.3428 (3)	0.27891 (13)	0.0508 (8)
H10	0.6350	0.2763	0.3092	0.061*
C11	0.61173 (15)	0.4774 (3)	0.28664 (12)	0.0455 (7)
C12	0.60674 (18)	0.5469 (4)	0.34102 (13)	0.0659 (10)
H12C	0.6155	0.4804	0.3731	0.099*
H12B	0.5607	0.5852	0.3313	0.099*
H12A	0.6408	0.6200	0.3537	0.099*

C13	0.05699 (14)	0.4058 (3)	0.07575 (11)	0.0374 (7)
C14	0.11323 (14)	0.2663 (3)	0.03135 (11)	0.0360 (7)
C15	0.13826 (15)	0.2006 (3)	−0.00883 (13)	0.0446 (7)
H15	0.1219	0.2236	−0.0497	0.054*
C16	0.18899 (16)	0.0986 (3)	0.01462 (14)	0.0510 (8)
H16	0.2065	0.0516	−0.0112	0.061*
C17	0.21384 (15)	0.0659 (3)	0.07538 (14)	0.0485 (8)
H17	0.2481	−0.0021	0.0897	0.058*
C18	0.18866 (14)	0.1329 (3)	0.11603 (12)	0.0419 (7)
H18	0.2051	0.1103	0.1570	0.050*
C19	0.13835 (14)	0.2338 (3)	0.09258 (11)	0.0353 (6)
C20	0.11067 (14)	0.3246 (3)	0.18123 (12)	0.0378 (6)
C21	0.12525 (15)	0.4350 (3)	0.21886 (12)	0.0447 (7)
H21	0.1349	0.5230	0.2077	0.054*
C22	0.12435 (16)	0.4039 (3)	0.27656 (13)	0.0491 (8)
H22	0.1331	0.4695	0.3072	0.059*
C23	0.10938 (15)	0.2683 (3)	0.28339 (12)	0.0449 (7)
C24	0.10396 (17)	0.1976 (4)	0.33751 (12)	0.0603 (9)
H24C	0.1133	0.2631	0.3700	0.090*
H24B	0.1375	0.1234	0.3497	0.090*
H24A	0.0576	0.1606	0.3277	0.090*
C25	−0.05825 (14)	0.3507 (3)	0.43132 (11)	0.0343 (6)
C26	−0.11361 (14)	0.4878 (3)	0.47748 (12)	0.0355 (6)
C27	−0.13628 (15)	0.5557 (3)	0.51859 (12)	0.0423 (7)
H27	−0.1173	0.5357	0.5598	0.051*
C28	−0.18835 (15)	0.6547 (3)	0.49612 (13)	0.0455 (8)
H28	−0.2048	0.7016	0.5227	0.055*
C29	−0.21620 (15)	0.6850 (3)	0.43523 (13)	0.0442 (7)
H29	−0.2506	0.7530	0.4217	0.053*
C30	−0.19406 (15)	0.6161 (3)	0.39328 (12)	0.0415 (7)
H30	−0.2134	0.6356	0.3521	0.050*
C31	−0.14196 (14)	0.5177 (3)	0.41604 (11)	0.0327 (6)
C32	−0.11787 (13)	0.4306 (3)	0.32573 (11)	0.0346 (6)
C33	−0.17957 (14)	0.4189 (3)	0.27850 (11)	0.0415 (7)
H33	−0.2228	0.4067	0.2825	0.050*
C34	−0.16990 (16)	0.4275 (3)	0.22232 (12)	0.0484 (8)
H34	−0.2069	0.4215	0.1854	0.058*
C35	−0.10266 (16)	0.4451 (3)	0.22650 (12)	0.0451 (7)
C36	−0.0739 (2)	0.4584 (4)	0.17661 (14)	0.0654 (10)
H36C	−0.1115	0.4531	0.1381	0.098*
H36B	−0.0412	0.3846	0.1798	0.098*
H36A	−0.0505	0.5461	0.1800	0.098*
C37	0.44090 (14)	0.4005 (3)	0.42695 (11)	0.0356 (7)
C38	0.38749 (13)	0.2631 (3)	0.47471 (11)	0.0320 (6)

C39	0.36665 (15)	0.1953 (3)	0.51688 (12)	0.0406 (7)
H39	0.3863	0.2164	0.5579	0.049*
C40	0.31513 (15)	0.0941 (3)	0.49538 (13)	0.0436 (7)
H40	0.3006	0.0452	0.5226	0.052*
C41	0.28516 (15)	0.0649 (3)	0.43416 (13)	0.0430 (7)
H41	0.2503	−0.0024	0.4211	0.052*
C42	0.30596 (14)	0.1340 (3)	0.39174 (12)	0.0391 (7)
H42	0.2857	0.1144	0.3506	0.047*
C43	0.35765 (13)	0.2324 (3)	0.41323 (11)	0.0321 (6)
C44	0.37998 (13)	0.3196 (3)	0.32203 (11)	0.0331 (6)
C45	0.31809 (14)	0.3317 (3)	0.27535 (11)	0.0422 (7)
H45	0.2752	0.3443	0.2799	0.051*
C46	0.32684 (16)	0.3230 (3)	0.21891 (12)	0.0497 (8)
H46	0.2894	0.3285	0.1822	0.060*
C47	0.39350 (17)	0.3060 (3)	0.22224 (12)	0.0468 (8)
C48	0.4223 (2)	0.2909 (4)	0.17202 (14)	0.0700 (11)
H48C	0.3846	0.2973	0.1335	0.105*
H48B	0.4449	0.2024	0.1754	0.105*
H48A	0.4556	0.3635	0.1753	0.105*

Table 3 Bond length (Å,) for compound Imp-7

Bond length	(Å)	Bond length	(Å)
S1—C11	1.724 (3)	C14—C19	1.387 (4)
S1—C8	1.725 (3)	C15—C16	1.392 (4)
O1—C1	1.227 (3)	C15—H15	0.9300
N1—C1	1.398 (3)	C16—C17	1.378 (4)
N1—C8	1.406 (3)	C16—H16	0.9300
N1—C7	1.407 (3)	C17—C18	1.401 (4)
C1—N2	1.361 (3)	C17—H17	0.9300
N2—C2	1.382 (3)	C18—C19	1.379 (4)
N2—H2A	0.8600	C18—H18	0.9300
C2—C7	1.389 (3)	C20—C21	1.350 (4)
C2—C3	1.391 (4)	C21—C22	1.404 (4)
S2—C23	1.721 (3)	C21—H21	0.9300
S2—C20	1.732 (3)	C22—C23	1.365 (4)
O2—C13	1.228 (3)	C22—H22	0.9300
C3—C4	1.389 (4)	C23—C24	1.489 (4)
C3—H3	0.9300	C24—H24C	0.9600
S3—C35	1.716 (3)	C24—H24B	0.9600
S3—C32	1.729 (3)	C24—H24A	0.9600
O3—C25	1.229 (3)	C26—C27	1.384 (4)
N3—C13	1.394 (3)	C26—C31	1.388 (4)
N3—C19	1.402 (3)	C27—C28	1.386 (4)
N3—C20	1.406 (3)	C27—H27	0.9300

S4—C47	1.722 (3)	C28—C29	1.377 (4)
S4—C44	1.730 (3)	C28—H28	0.9300
O4—C37	1.227 (3)	C29—C30	1.398 (4)
C4—C5	1.378 (4)	C29—H29	0.9300
C4—H4	0.9300	C30—C31	1.383 (4)
N4—C13	1.360 (3)	C30—H30	0.9300
N4—C14	1.387 (3)	C32—C33	1.357 (3)
N4—H4A	0.8600	C33—C34	1.415 (4)
C5—C6	1.394 (4)	C33—H33	0.9300
C5—H5	0.9300	C34—C35	1.354 (4)
N5—C25	1.394 (3)	C34—H34	0.9300
N5—C32	1.401 (3)	C35—C36	1.502 (4)
N5—C31	1.406 (3)	C36—H36C	0.9600
C6—C7	1.382 (4)	C36—H36B	0.9600
C6—H6	0.9300	C36—H36A	0.9600
N6—C25	1.360 (3)	C38—C39	1.382 (3)
N6—C26	1.390 (3)	C38—C43	1.392 (3)
N6—H6A	0.8600	C39—C40	1.391 (4)
N7—C37	1.395 (3)	C39—H39	0.9300
N7—C44	1.400 (3)	C40—C41	1.383 (4)
N7—C43	1.408 (3)	C40—H40	0.9300
N8—C37	1.362 (3)	C41—C42	1.392 (4)
N8—C38	1.390 (3)	C41—H41	0.9300
N8—H8A	0.8600	C42—C43	1.375 (4)
C8—C9	1.352 (4)	C42—H42	0.9300
C9—C10	1.382 (4)	C44—C45	1.353 (3)
C9—H9	0.9300	C45—C46	1.412 (4)
C10—C11	1.358 (4)	C45—H45	0.9300
C10—H10	0.9300	C46—C47	1.348 (4)
C11—C12	1.486 (4)	C46—H46	0.9300
C12—H12C	0.9600	C47—C48	1.511 (4)
C12—H12B	0.9600	C48—H48C	0.9600
C12—H12A	0.9600	C48—H48B	0.9600
C14—C15	1.384 (4)	C48—H48A	0.9600

Table 4 Valence angles (°) for compound Imp-7

Valence angles	(°)	Valence angles	(°)
C11—S1—C8	92.00 (14)	C20—C21—H21	123.3
C1—N1—C8	123.0 (2)	C22—C21—H21	123.3
C1—N1—C7	109.1 (2)	C23—C22—C21	113.7 (3)
C8—N1—C7	127.9 (2)	C23—C22—H22	123.2
O1—C1—N2	128.0 (2)	C21—C22—H22	123.2
O1—C1—N1	125.7 (2)	C22—C23—C24	129.0 (3)
N2—C1—N1	106.3 (2)	C22—C23—S2	110.2 (2)
C1—N2—C2	110.5 (2)	C24—C23—S2	120.8 (2)
C1—N2—H2A	124.8	C23—C24—H24C	109.5
C2—N2—H2A	124.8	C23—C24—H24B	109.5
N2—C2—C7	107.9 (2)	H24C—C24—H24B	109.5
N2—C2—C3	131.0 (2)	C23—C24—H24A	109.5
C7—C2—C3	121.1 (3)	H24C—C24—H24A	109.5
C23—S2—C20	92.13 (15)	H24B—C24—H24A	109.5
C4—C3—C2	117.2 (3)	O3—C25—N6	128.3 (2)
C4—C3—H3	121.4	O3—C25—N5	125.3 (2)
C2—C3—H3	121.4	N6—C25—N5	106.4 (2)
C35—S3—C32	92.08 (14)	C27—C26—C31	121.2 (3)
C13—N3—C19	109.2 (2)	C27—C26—N6	131.4 (3)
C13—N3—C20	123.3 (2)	C31—C26—N6	107.4 (2)
C19—N3—C20	127.4 (2)	C26—C27—C28	117.5 (3)
C47—S4—C44	91.68 (14)	C26—C27—H27	121.2
C5—C4—C3	121.6 (3)	C28—C27—H27	121.2
C5—C4—H4	119.2	C29—C28—C27	121.3 (3)
C3—C4—H4	119.2	C29—C28—H28	119.4
C13—N4—C14	110.6 (2)	C27—C28—H28	119.4
C13—N4—H4A	124.7	C28—C29—C30	121.6 (3)
C14—N4—H4A	124.7	C28—C29—H29	119.2
C4—C5—C6	121.3 (3)	C30—C29—H29	119.2
C4—C5—H5	119.3	C31—C30—C29	116.8 (3)
C6—C5—H5	119.3	C31—C30—H30	121.6
C25—N5—C32	124.7 (2)	C29—C30—H30	121.6
C25—N5—C31	109.1 (2)	C30—C31—C26	121.6 (2)
C32—N5—C31	126.2 (2)	C30—C31—N5	131.8 (2)
C7—C6—C5	117.2 (3)	C26—C31—N5	106.6 (2)
C7—C6—H6	121.4	C33—C32—N5	127.6 (2)
C5—C6—H6	121.4	C33—C32—S3	111.5 (2)
C25—N6—C26	110.5 (2)	N5—C32—S3	120.83 (19)
C25—N6—H6A	124.7	C32—C33—C34	111.5 (3)
C26—N6—H6A	124.7	C32—C33—H33	124.3
C37—N7—C44	124.2 (2)	C34—C33—H33	124.3
C37—N7—C43	109.4 (2)	C35—C34—C33	114.8 (3)

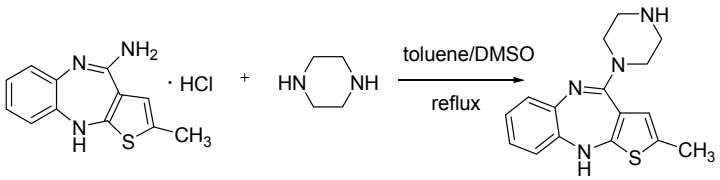
C44—N7—C43	126.4 (2)	C35—C34—H34	122.6
C6—C7—C2	121.5 (2)	C33—C34—H34	122.6
C6—C7—N1	132.2 (2)	C34—C35—C36	128.9 (3)
C2—C7—N1	106.3 (2)	C34—C35—S3	110.1 (2)
C37—N8—C38	110.5 (2)	C36—C35—S3	121.0 (2)
C37—N8—H8A	124.8	C35—C36—H36C	109.5
C38—N8—H8A	124.8	C35—C36—H36B	109.5
C9—C8—N1	128.2 (3)	H36C—C36—H36B	109.5
C9—C8—S1	110.3 (2)	C35—C36—H36A	109.5
N1—C8—S1	121.3 (2)	H36C—C36—H36A	109.5
C8—C9—C10	113.7 (3)	H36B—C36—H36A	109.5
C8—C9—H9	123.1	O4—C37—N8	128.0 (2)
C10—C9—H9	123.1	O4—C37—N7	125.7 (2)
C11—C10—C9	114.1 (3)	N8—C37—N7	106.3 (2)
C11—C10—H10	122.9	C39—C38—N8	131.0 (2)
C9—C10—H10	122.9	C39—C38—C43	121.4 (2)
C10—C11—C12	129.3 (3)	N8—C38—C43	107.6 (2)
C10—C11—S1	109.8 (2)	C38—C39—C40	117.2 (3)
C12—C11—S1	120.8 (3)	C38—C39—H39	121.4
C11—C12—H12C	109.5	C40—C39—H39	121.4
C11—C12—H12B	109.5	C41—C40—C39	121.2 (3)
H12C—C12—H12B	109.5	C41—C40—H40	119.4
C11—C12—H12A	109.5	C39—C40—H40	119.4
H12C—C12—H12A	109.5	C40—C41—C42	121.5 (3)
H12B—C12—H12A	109.5	C40—C41—H41	119.3
O2—C13—N4	127.7 (3)	C42—C41—H41	119.3
O2—C13—N3	126.2 (2)	C43—C42—C41	117.2 (3)
N4—C13—N3	106.2 (2)	C43—C42—H42	121.4
C15—C14—N4	131.1 (2)	C41—C42—H42	121.4
C15—C14—C19	121.6 (3)	C42—C43—C38	121.5 (2)
N4—C14—C19	107.3 (2)	C42—C43—N7	132.2 (2)
C14—C15—C16	117.1 (3)	C38—C43—N7	106.2 (2)
C14—C15—H15	121.4	C45—C44—N7	127.7 (2)
C16—C15—H15	121.4	C45—C44—S4	111.6 (2)
C17—C16—C15	121.3 (3)	N7—C44—S4	120.59 (19)
C17—C16—H16	119.3	C44—C45—C46	111.5 (3)
C15—C16—H16	119.3	C44—C45—H45	124.2
C16—C17—C18	121.5 (3)	C46—C45—H45	124.2
C16—C17—H17	119.3	C47—C46—C45	114.9 (3)
C18—C17—H17	119.3	C47—C46—H46	122.5
C19—C18—C17	117.0 (3)	C45—C46—H46	122.5
C19—C18—H18	121.5	C46—C47—C48	129.7 (3)
C17—C18—H18	121.5	C46—C47—S4	110.2 (2)
C18—C19—C14	121.5 (3)	C48—C47—S4	120.1 (3)

C18—C19—N3	131.9	(2)	C47—C48—H48C	109.5
C14—C19—N3	106.7	(2)	C47—C48—H48B	109.5
C21—C20—N3	128.3	(3)	H48C—C48—H48B	109.5
C21—C20—S2	110.7	(2)	C47—C48—H48A	109.5
N3—C20—S2	120.9	(2)	H48C—C48—H48A	109.5
C20—C21—C22	113.3	(3)	H48B—C48—H48A	109.5

2. Preparation/seperation of Imp-1~ -8

Unless otherwise stated, all commercial reagents and solvents were used without additional purification. Analytical TLC was visualized with UV light at 254nm. Purification of reaction products was carried out by chromatography using silica gel 60 (200-400 mesh).

2.1 Imp-1 (2-methyl-4-(1-piperazinyl)-10*H*-thieno [2, 3-*b*] [1, 5] benzodiazepine)

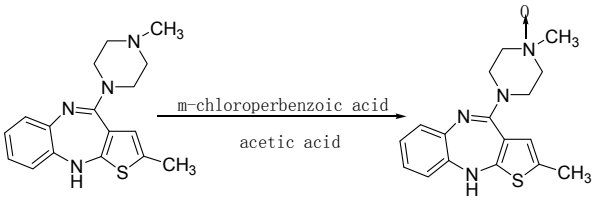


4-amino-2-methyl-10H-thieno[2,3-b][1,5]-benzodiazepine hydrochloride (5.01g, 0.0189mol), piperazine (9.76g, 0.1134mol) were added into a 100ml three-necked flask, refluxed for 5h in the mixed solvent of 10ml toluene and 20ml DMSO. The mixture was cooled to 50°C, 400ml water added. The product was filtered and 4.59g off-white powder was obtained (yield: 81.5%, CDP20100419 ②).[1]

It was purified by crystallizing from ethanol/water (4:1) to get a slightly pink powder.

(M+1)329.0, ^{1}H NMR (400 MHz, DMSO-d_6), δ 7.57 (s, 1H, C-NH-C), 6.81 (dt, 3H, Ar-H), 6.72- 6.59 (m, 1H, Ar-H), 6.34 (s, 1H, C-CH-C on thiophene ring), 3.27 (d, 4H, CH_2-NH-CH_2), 2.83-2.62 (m, 4H, CH_2-N-CH_2), 2.27(s, 3H, S-C-CH_3)

2.2 Imp-2 (2-methyl-4-(N-oxo-4-methyl-1-piperazinyl)-10H-thieno [2, 3-b] [1, 5] benzodiazepine)

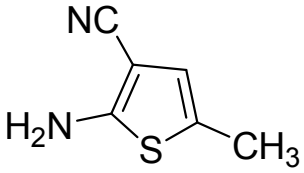


To a mixture of olanzapine (CDP20091228, 5g, 0.016mol) in acetic acid (25ml), *m*-chloroperbenzoic acid (3.99g, 0.016mol) was added and the mixture stirred at 50°C for reaction completion. The reaction mass was concentrated under reduced pressure and the residual mass was dissolved in dichloromethane (25ml). The resultant solution was washed with water, concentrated under reduced pressure and finally purified by column chromatography (CH_2Cl_2: MeOH: Et_2NH) to yield Imp-2 (1.2g, CDP20100421).[1]

(M+1)329.0; ^{1}H NMR (DMSO-d_6), 7.74 (s, 1H, C-NH-C), 6.91-6.75 (m, 3H, Ar-H), 6.75-6.64 (m, 1H, Ar-H), 6.39 (s, 1H, C-CH-C on thiophene ring), 3.78 (d, J =

12 Hz, 2H, CH_2-N-CH_2), 3.58 (t, J = 12 Hz, 2H, CH_2-N-CH_2), 3.40 (s, 2H, CH_2-C-CH_2), 3.12 (s, 3H, N-CH_3, 2.99 (d, 2H, CH_2-C-CH_2), 2.27 (s, 3H, S-C-CH_3)

2.3 Imp-3 (2-amino-5-methylthiophene-3-carbonitrile)



Imp-3

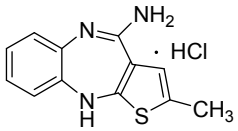
The preparative process was shown in the text (Fig. 1).

A mixture of sulphus (1.61g, 0.05mol), propionaldehyde (3.48g, 0.06mol) and dimethylformamide (10ml) was placed in a 100ml one-necked flask with drooping funnel. Triethylamine (4.21ml, 0.03mol) was added over 30minutes while the temperature was between 5-10°C with an ice-bath. The temperature was increased to 18°C keeping the mixture well stirred. Then a solution of malononitrile (3.35g, 0.05mol) in dimethylformamide (6.60ml) was added drop wise over 70min keeping the temperature about 20°C. The mixture was stirred at 15-20°C for a further 45min, and then sampled for TLC. The mixture was poured onto ice water (150ml) with stirring and the brick-red product precipitated. The solid was isolated by filtration, washed with water and then dried over night in vacuo to give the product (5.30g, yield: 76.8%, CDP20100305).[2]

It was purified by crystallizing from ethanol/water (1:1) to yield 2.82g brown powder.

^{1}H NMR ($CDCl_3$), δ 6.35 (s, 1H, C-CH-C), 4.60 (s, 2H, NH_2), 2.28 (s, 3H, CH_3)

2.4 Imp-4 (4-amino-2-methyl-10H-thieno [2, 3-b] [1, 5]-benzodiazepine hydrochloride)

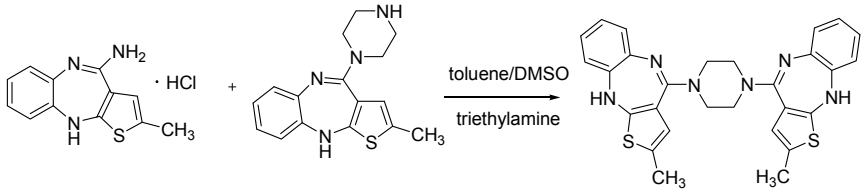


The preparative process was shown in the text (Fig. 1).

To stirred slurry of 2-(2-nitroanilino)-5-methylthiophene-3-carbonitrile (1g, 0.00386mol) in ethanol (20ml) at 50°C, a solution of anhydrous stannous chloride (3.48g, 0.0154mol) in hydrochloric acid (15ml) was added dropwise over 10 minutes. The mixture was stirred under reflux for 1h, concentrated under reduced pressure and allowed to crystallize over night at 50°C. The salt was filtered, washed with a small amount of water, dried to give the product (0.89g, yield: 87.3%, CDP20100406).[2]

^{1}H NMR (DMSO-d_6), δ 11.15 (s, 1H, HCl), 9.59 (s, 1H, C-NH-C), 9.12 and 8.89 (s, s, 1H, NH_2), 7.10 (t, 1H, Ar-H), 7.02 (t, 1H, Ar-H), 6.96 (d, 1H, Ar-H), 6.87 (d, 1H, Ar-H), 6.81 (s, 1H, C-CH-C on thiophene ring), 2.26 (s, 3H, CH_3)

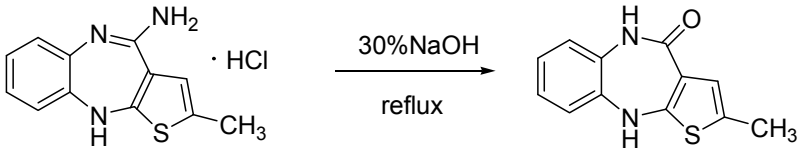
2.5 Imp-5 (bis-2-methyl-4-(1-piperazinyl)-10H-thieno [2, 3-b] [1, 5] benzodiazepine)



A mixture of Imp-1 (1.12g, 0.0037mol), dimethyl sulfoxide 2mL, toluene 8mL and Imp-4 (1.09g, 0.0037mol) was heated to reflux. 2ml triethylamine was added in three equal portions to the reaction mixture at reflux temperature of 102°C and the reaction mass was stirred for reaction completion. The product was filtered, dried, and purified by column chromatography (CH_2Cl_2: methanol: Et_2NH) to give the product (0.8g, CDP20100508).[1]

(M+1)511.2; ^{1}H NMR (DMSO-d_6), δ 7.67(s, 2H, two C-NH-C), 6.84 (m, 6H, two Ar-H), 6.69 (d, 2H, two Ar-H), 6.43(d, 2H, two C-CH-C on thiophene ring), 3.39 (d, 8H, two CH_2-NH-CH_2) (d, 6H, two S-C-CH_3)

2.6 Imp-6 (2-methyl-4-oxo-10H-thieno [2, 3-b] [1, 5] benzodiazepine)



A mixture of Imp-3 (1.00g, 0.0038mol) and 30% aqueous sodium hydroxide (10mL) was heated to reflux at 98°C till the reaction was complete. The reaction mixture was cooled to room temperature and the resulting solid was filtered. The resultant wet cake was dissolved in methanol (20ml) at reflux, treated with carbon (0.2g) and finally water (50ml) was added to precipitate a solid. The isolated solid was filtered, washed with water (100mL) and dried at 70°C to give the product (0.50g, yield: 57.5%, CDP20100419).[2]

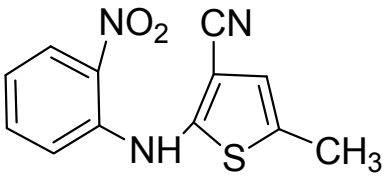
It was purified by column chromatography (CH_2Cl_2: methanol: Et_2NH).

(M+1)231.1; ^{1}H NMR (DMSO-d_6), δ 9.07 (s, 1H, NH-C=O), 8.85 (s, 1H, C-NH-C), 6.95-6.82 (m, 3H, Ar-H), 6.81-6.72 (m, 1H, Ar-H), 6.59 (d, 1H, S-C-CH_3), 2.21 (d, 3H, S-C-CH_3)

2.7 Imp-7 (1-(5-methyl-thionphen-2-yl)-1H-benzimidazol-2(3H)-one)

The isolation of Imp-7 was conducted from the crude sample of OLN crystallization mother liquid by preparative chromatography and fraction collection. The mobile phase used in preparative chromatography was a mixture of CH_2Cl_2, MeOH and Et_2NH. Each collected fraction from preparative chromatography was analyzed by TLC and HPLC. The fractions of the confirmed same compound were pooled together and concentrated under high vacuum on a rota-evaporator.

2.8 Imp-8 (2-(2-nitroanilino)-5-methylthiophene-3-carbonitrile)



Imp-8

The preparative process was shown in the text (Fig. 1).

To stirred slurry of potassium hydride (1.156g, 0.0207mol) in dried DMF (4.44mL), a solution of 2-fluoronitrobenzene (2.04g, 0.0145mol) and 2-amino-5-methylthiophene3-carbonitrile (2.00g, 0.0145mol) in dried DMF (17.78ml) was added dropwise over 30 minutes. The mixture was stirred at 10°C for 2 hours, poured into ice water (125ml). The brick-red product precipated and was extracted into dichloromethane (120ml*3), washed by water (200ml), dried. The solvent was removed under reduced pressure to give Imp-8 (1.64g, yield: 43.6%, CDP20100322).[2]

It was purified by crystallizing from ethanol.

^{1}H NMR ($CDCl_3$), δ 9.62 (s, 1H, C-NH-C), 8.25 (d, 1H, Ar-H), 7.52 (t, 1H, Ar-H), 7.19 (d, 1H, Ar-H), 6.97 (t, 1H, Ar-H), 6.78 (s, 1H,) C-CH-C on thiophene ring, 2.48 (s, 3H, S-C-CH_3)

3. Spectra of Imp-1~ -8

3.1 Spectra of Imp-1 (Fig. 1- ^{1}H NMR, Fig. 2- MS)

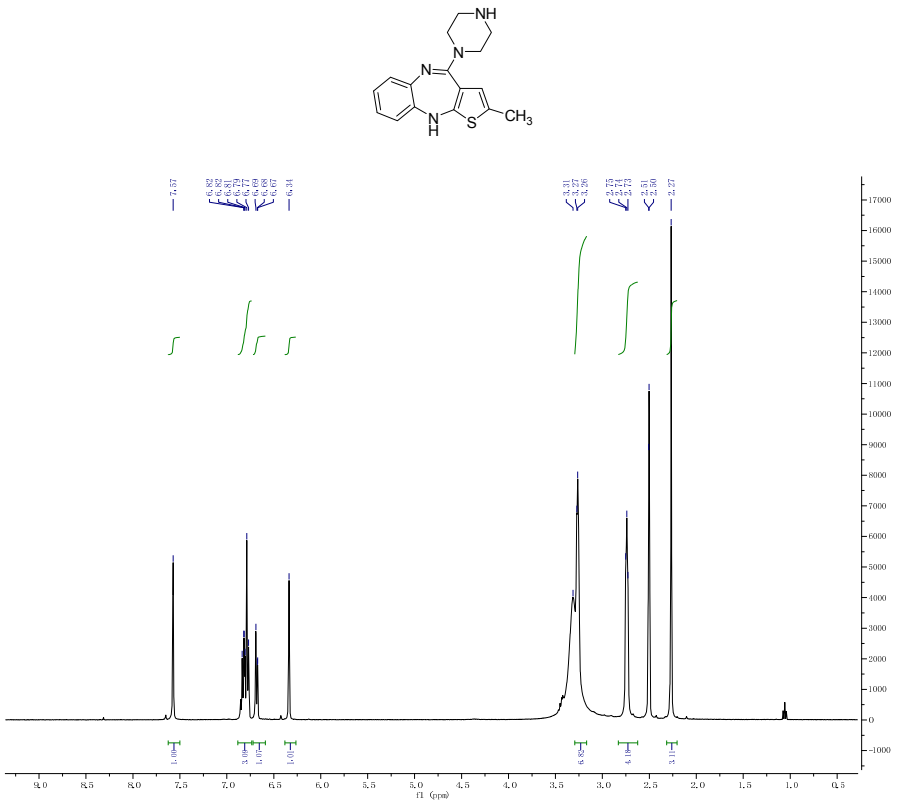


Fig. 1 ^{1}H NMR of Imp-1

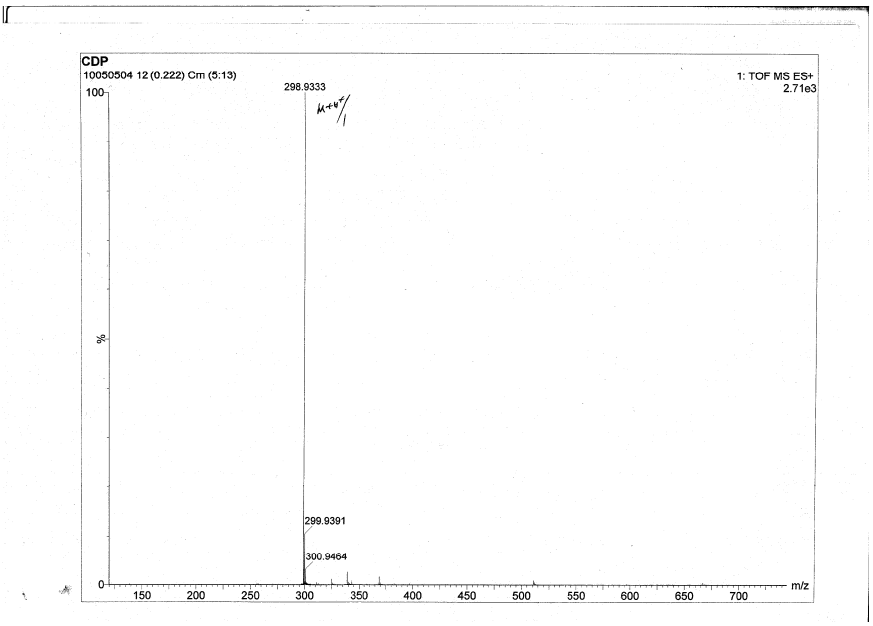


Fig. 2 MS of Imp-1

3.2 Spectra of Imp-2 (Fig. 3- ^{1}H NMR, Fig. 4- MS)

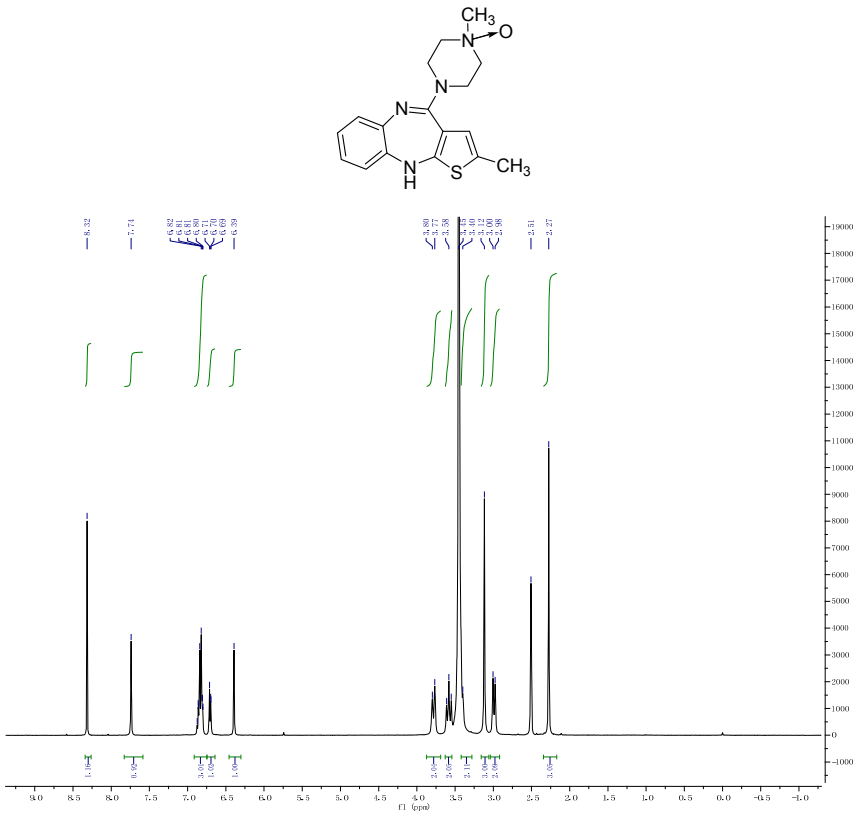


Fig. 3 ^{1}H NMR of Imp-2

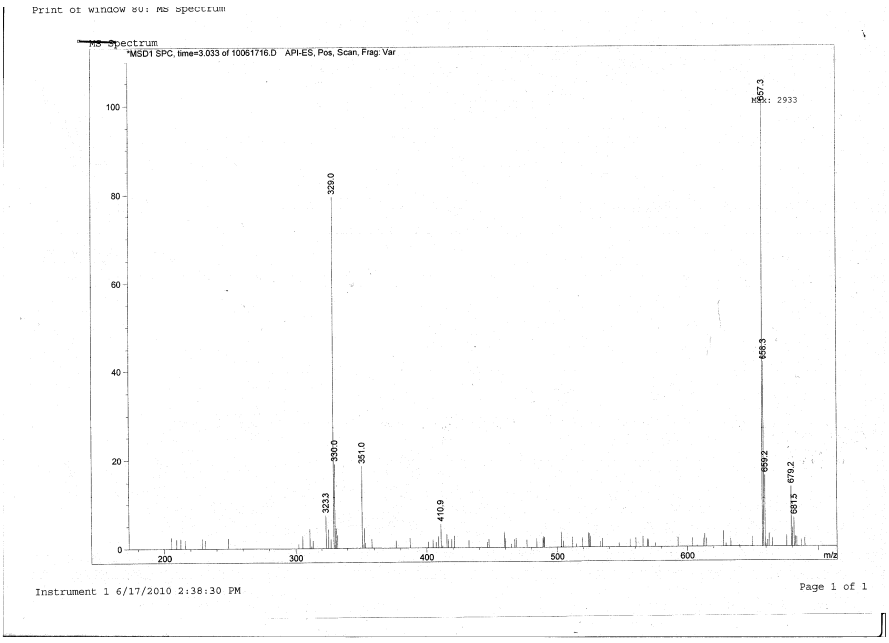


Fig. 4 MS of Imp-2

3.3 Spectra of Imp-3 (Fig. 5- ^{1}H NMR)

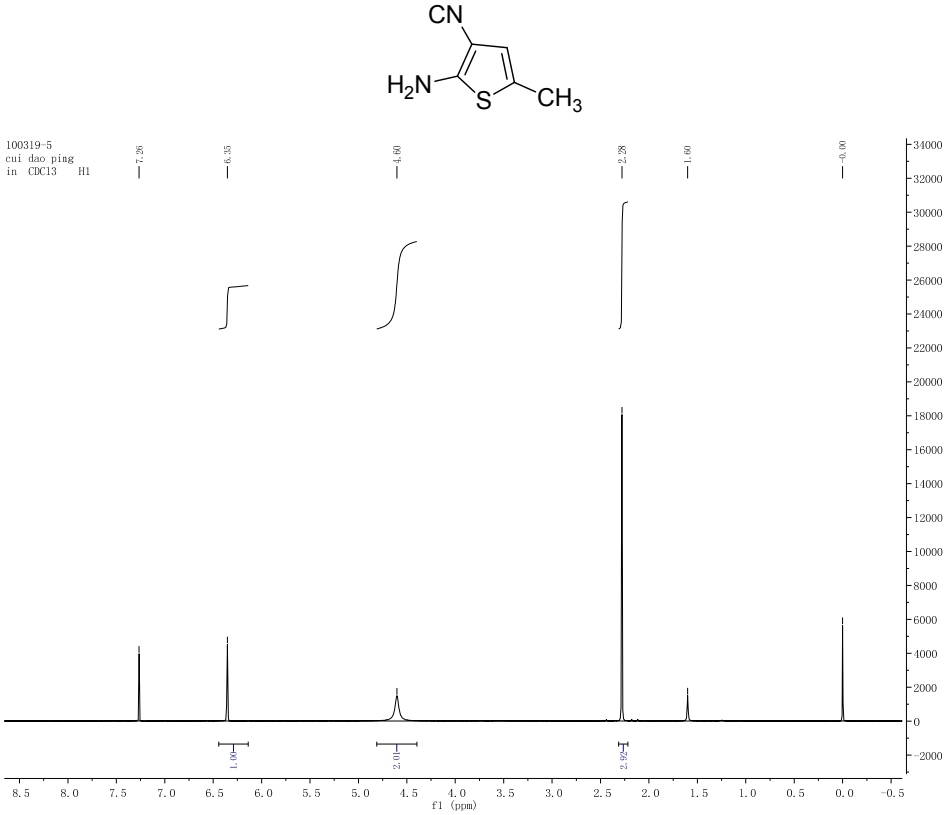
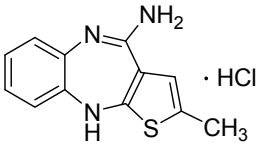


Fig. 5 ^{1}H NMR of Imp-3

3.4 Spectra of Imp-4 (Fig. 6- ^{1}H NMR)



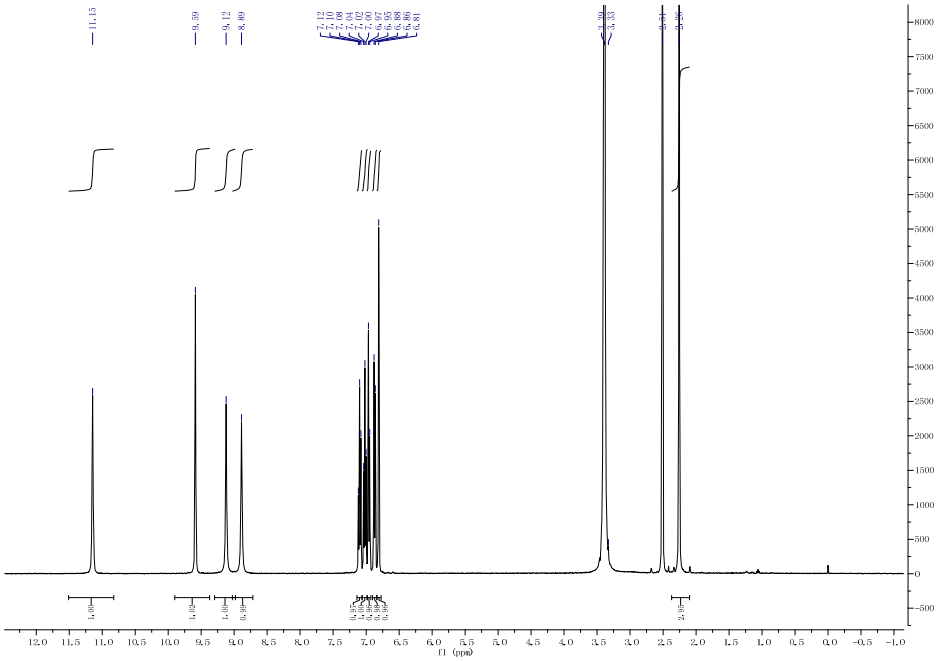


Fig. 6 ^{1}H NMR of Imp-4

3.5 Spectra of Imp-5 (Fig. 7- ^{1}H NMR, Fig. 8- MS)

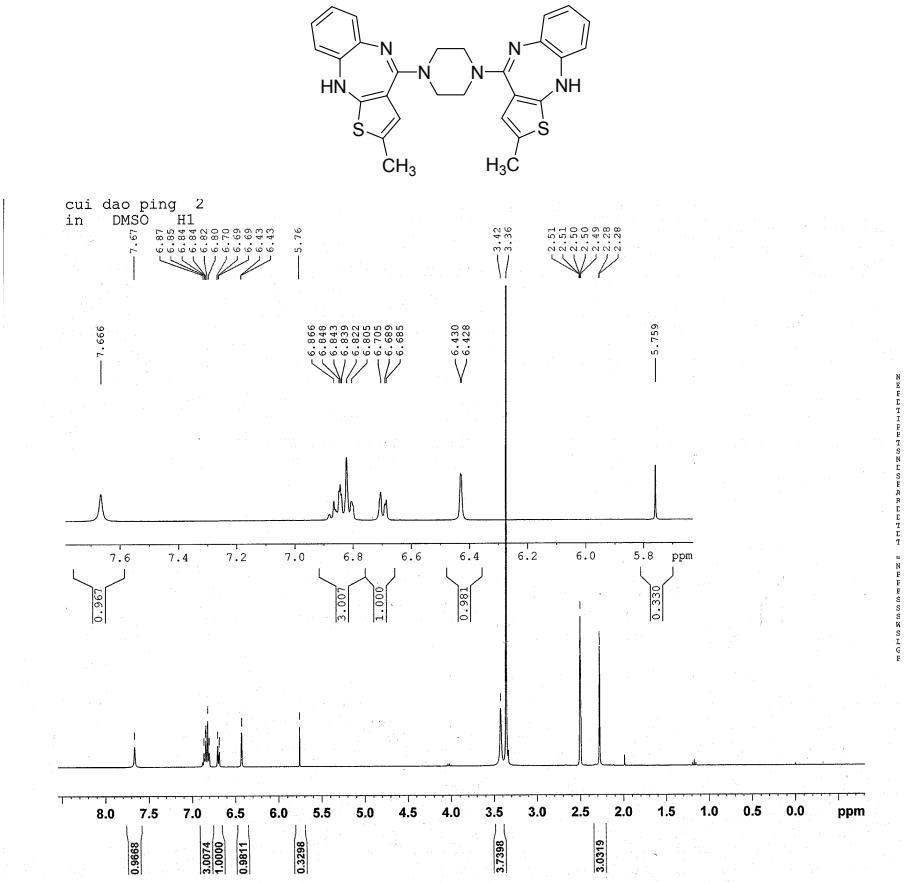


Fig. 7 ^{1}H NMR of Imp-5

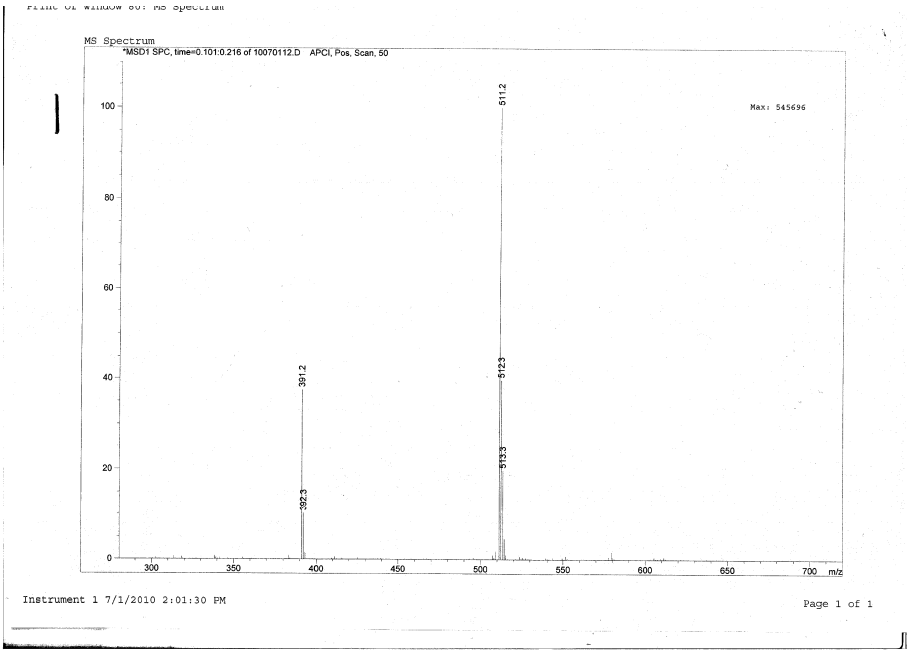


Fig. 8 MS of Imp-5

3.6 Spectra of Imp-6 (Fig. 9- ^{1}H NMR, Fig. 10- MS)

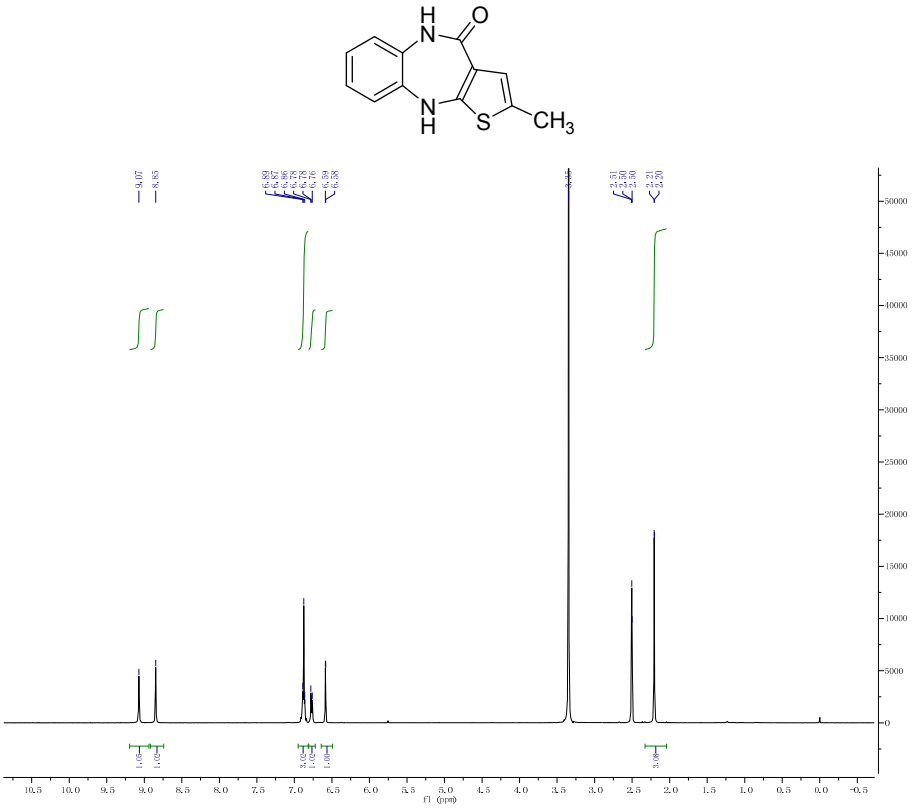


Fig. 9 ^{1}H NMR of Imp-6

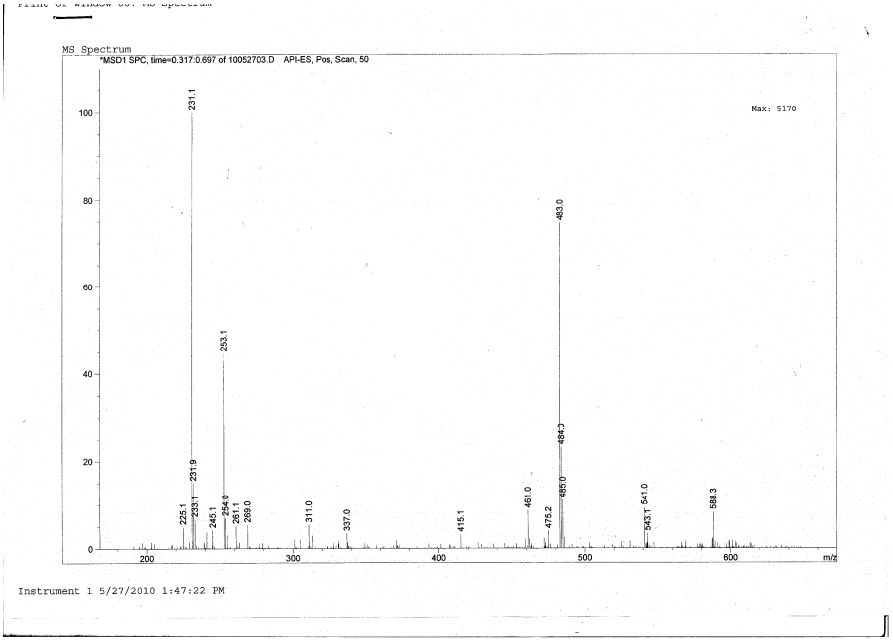
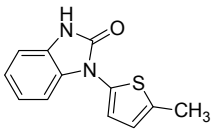


Fig. 10 MS of Imp-6

9. Spectra of Imp-7 (Fig. 11- Tof MS ES$^+$, Fig. 12- ^{1}H NMR, Fig. 13- ^{13}C NMR, Fig. 14- HSQC, Fig. 15- IR)



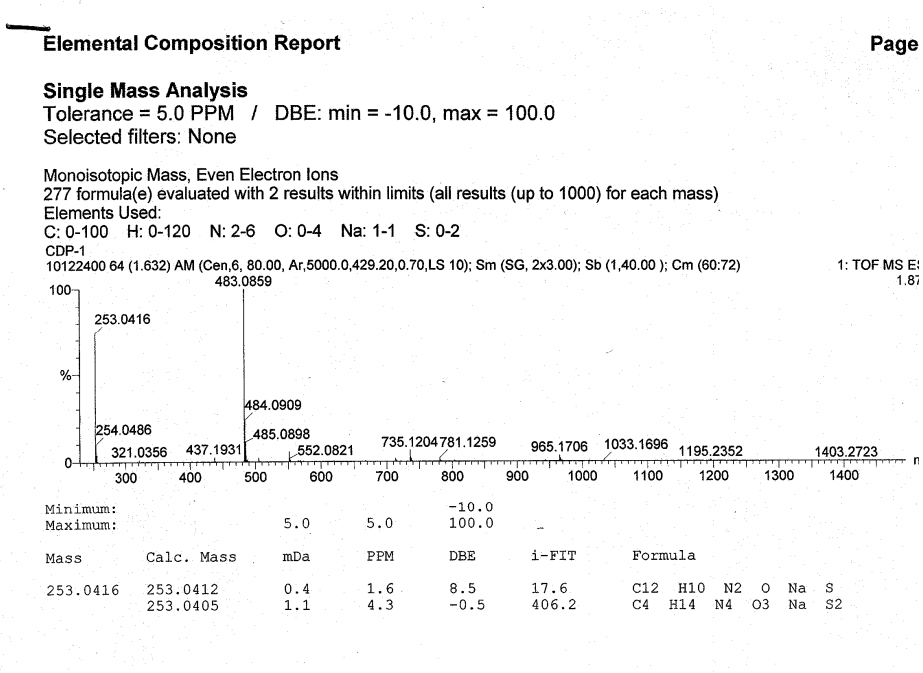


Fig. 11 Tof MS ES$^+$ of Imp-7

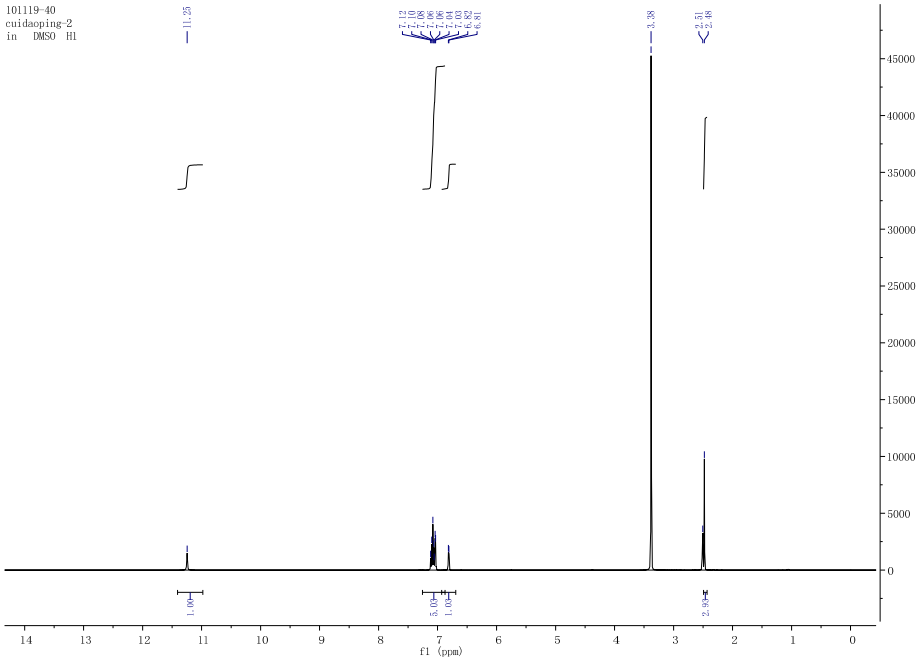


Fig. 12 ^{1}H NMR of Imp-7

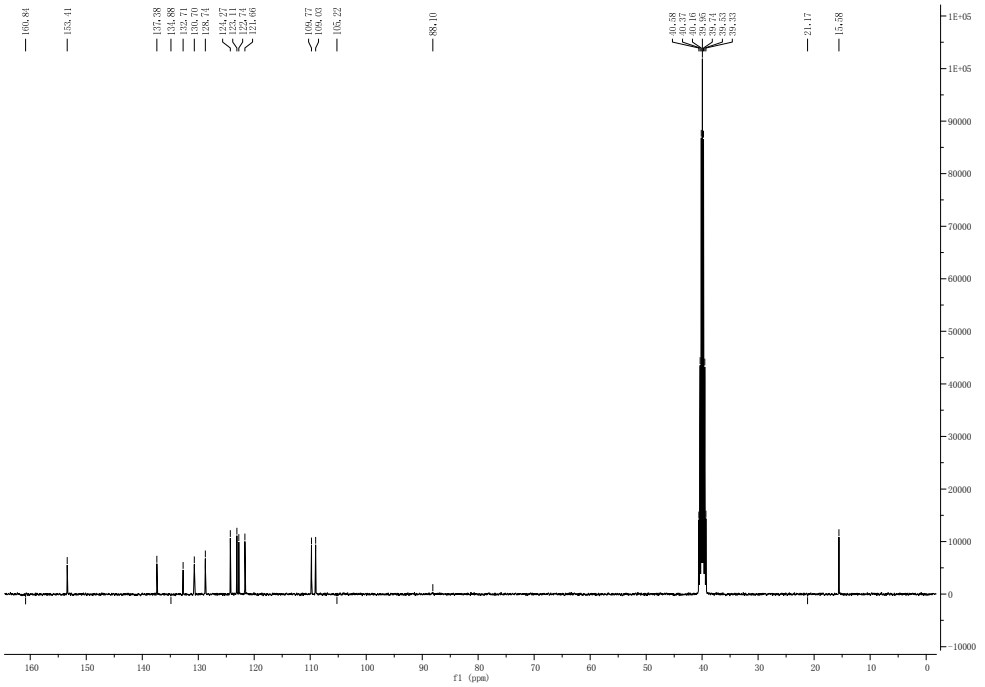


Fig. 13 ^{13}C NMR of Imp-7

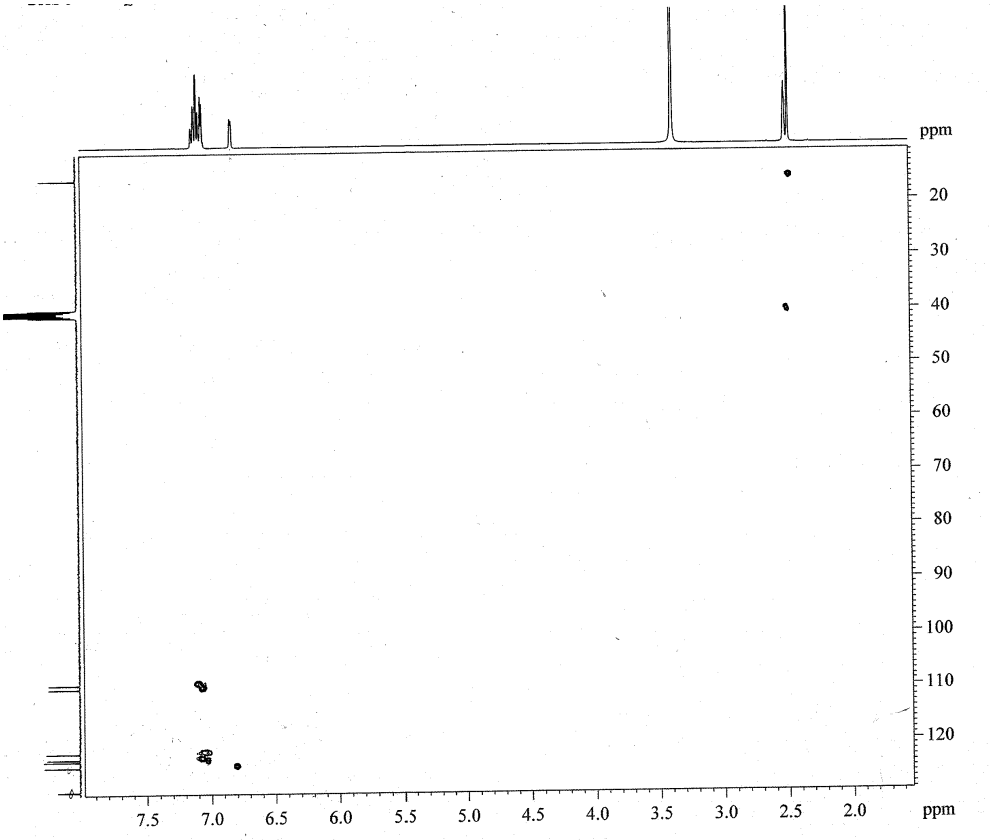


Fig. 14 HSQC of Imp-7

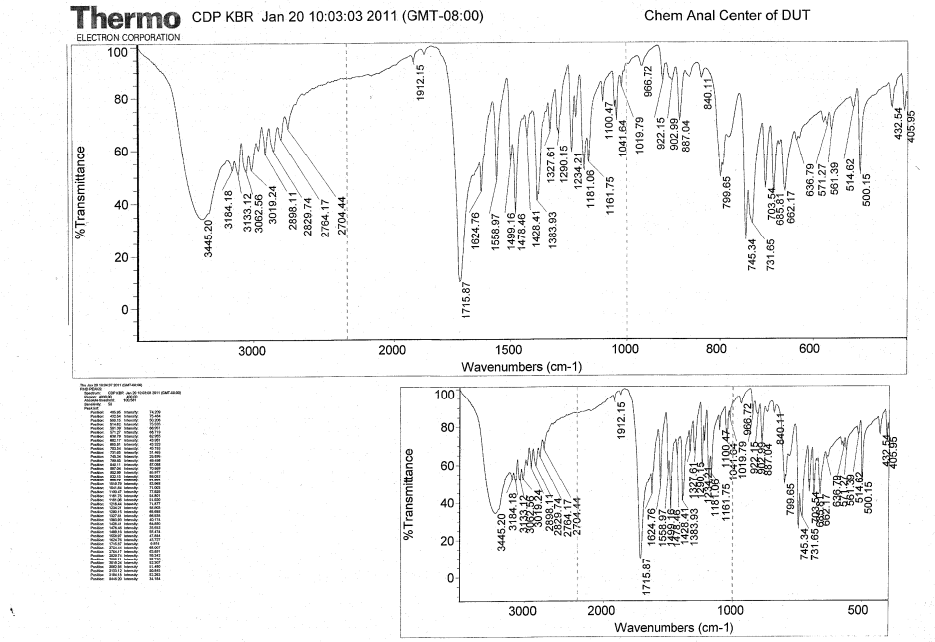
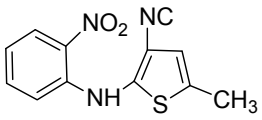


Fig. 15 IR of Imp-7

3.8 Spectra of Imp-8 (Fig. 16- ^{1}H NMR)



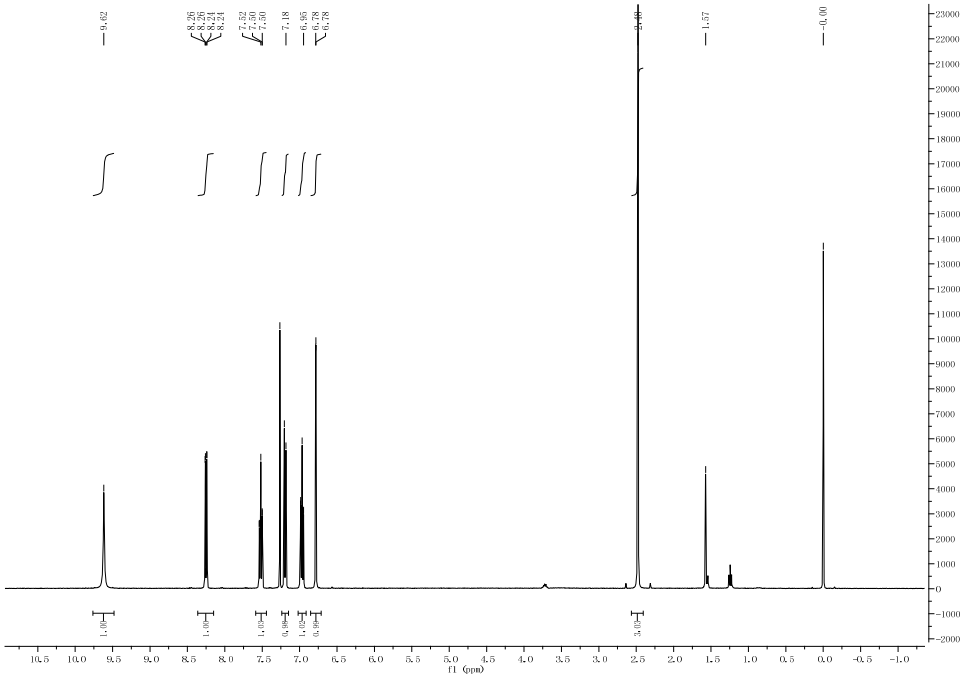


Fig. 16 ^{1}H NMR of Imp-8

4. Linear analysis of OLN and impurities

The method linearity was evaluated from 50μg/ml to 320μg/ml for OLN and from 0.05/0.1μg/ml to 1.0μg/ml for impurities. An example chromatogram of linear experiment was presented in Fig. 17.

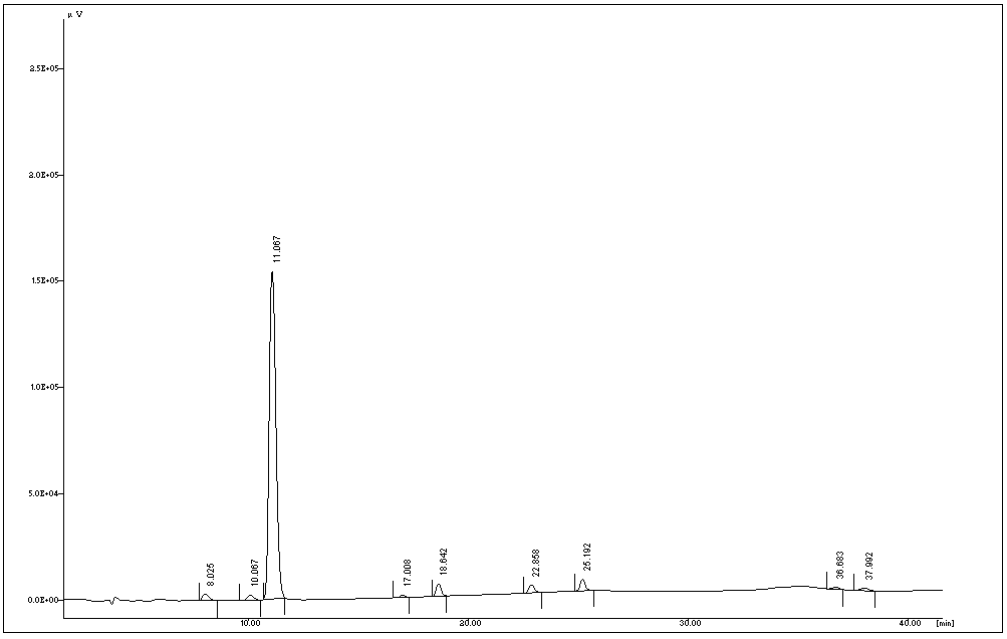
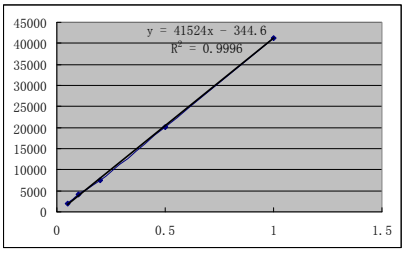
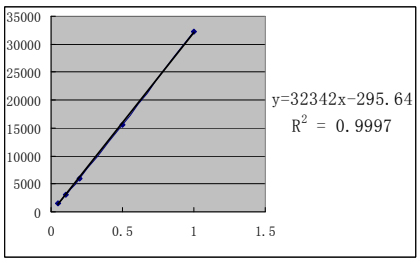


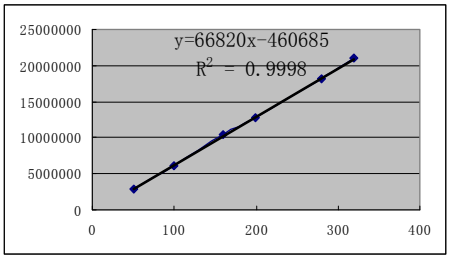
Fig. 17 A chromatogram of linear experiment (OLN: 50μg/ml, each impurity: 1.0μg/ml)

The linear equation of each impurity was established between the concentration and peak area, where y represented the concentration and x represented peak area. The results were listed in Table 5.

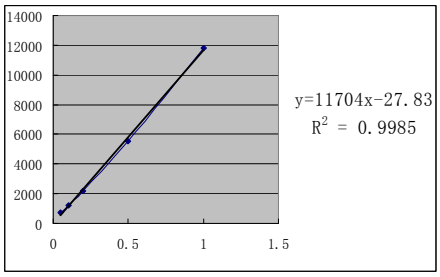
Table 5 Linear data for OLN and impurities

Compounds	Linear analysis
Imp-1 (0.05μg/ml~1.0μg/ml)	
Imp-2 (0.05μg/ml~1.0μg/ml)	

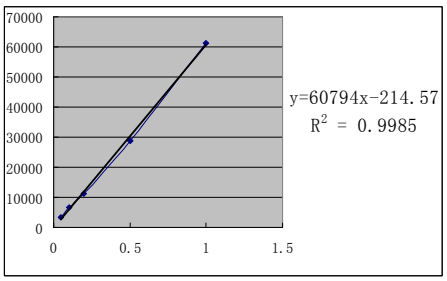
OLN
(50μg/ml~320μg/ml)



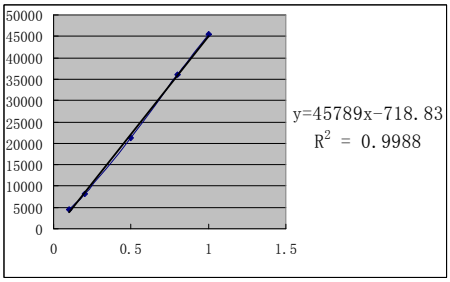
Imp-3
(0.05μg/ml~1.0μg/ml)



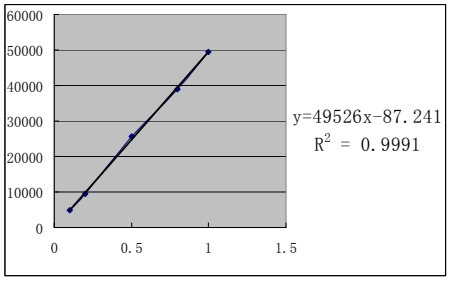
Imp-4
(0.05μg/ml~1.0μg/ml)

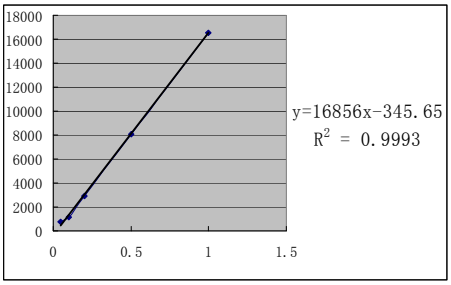
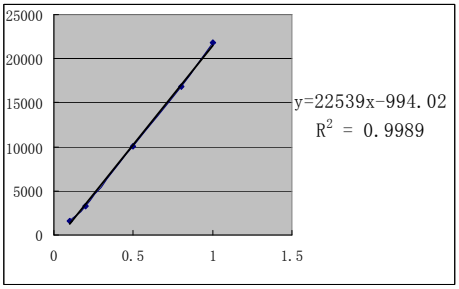


Imp-5
(0.1μg/ml~1.0μg/ml)



Imp-6
(0.1μg/ml~1.0μg/ml)



Imp-7 (0.05μg/ml~1.0μg/ml)	
Imp-8 (0.1μg/ml~1.0μg/ml)	

5. References

1 T. Poornachander, K. Ramesh and B. Chandrasekhar. ARKIVOC, 2008, **11**, 195-201.

2 K. Jiban, F. Chakrabarti and M. Terrence. US: 5229382, 1993.