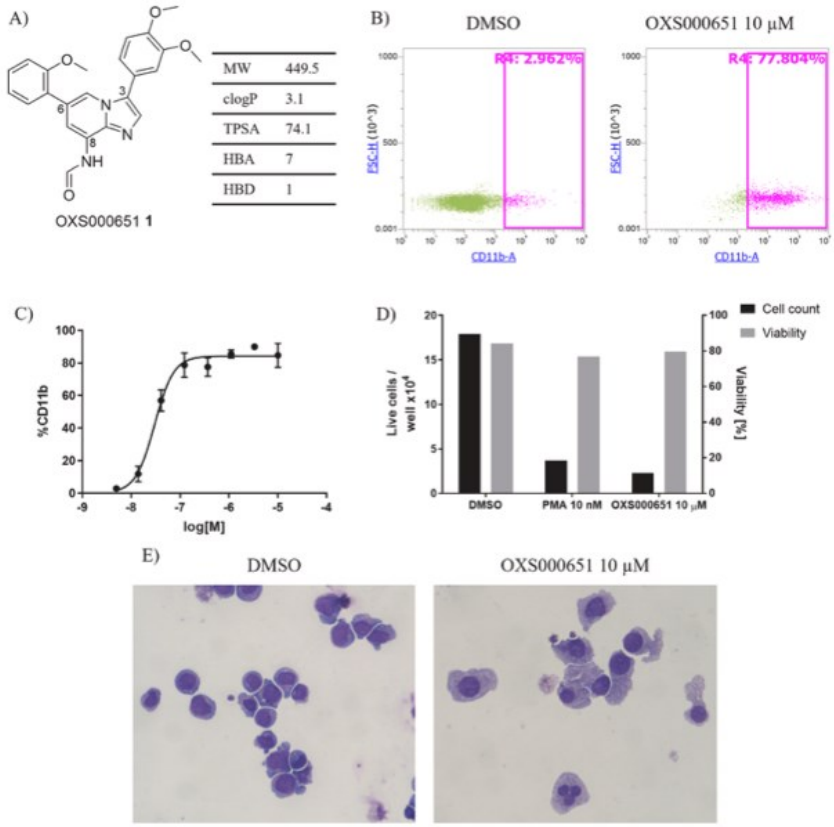
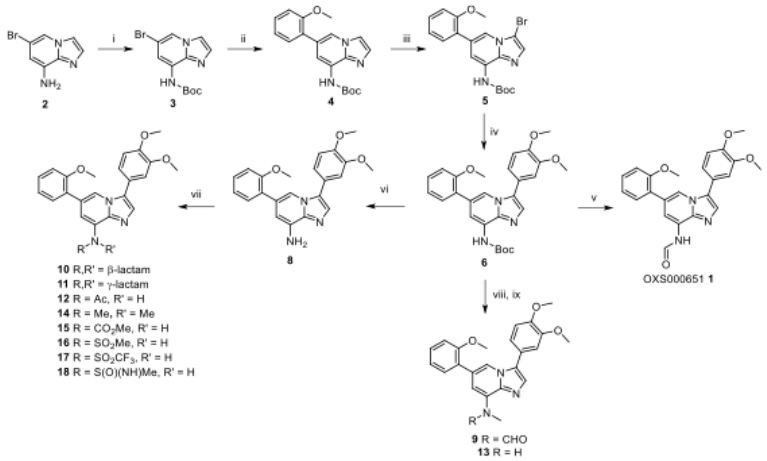
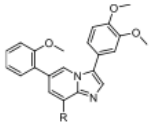


Fig. 1. Profile of OXS000651 in HL-60 cells. (A) Chemical structure and predicted [27] physicochemical properties; (B) Flow cytometry plots representing CD11b expression (yellow channel) of cells treated with either DMSO control or 10 µM OXS000651 for 4 days; (C) Concentration-response curve showing %CD11b response on cells treated with OXS000651 for 4 days, giving EC_{50} = 34 nM (biological triplicates); D) Number of live cells per well and %viability of cells treated with either DMSO control, 10 nM phorbol 12-myristate 13-acetate (PMA) control, or 10 µM OXS000651 over 4 days, determined with acridine orange and DAPI; E) Morphology of HL-60 cells treated with either DMSO control or 10 µM OXS000651.



Scheme 1. Synthesis of hit compound OXS000651 and C-8 amino derivatives. i. NaHMDS, Boc_2O, THF, rt, 3 h, 67%; ii. 2-methoxyphenylboronic acid, K_3PO_4, $Pd(dppf)Cl_2$, DME/H_2O, 90 °C, o/n, 82%; iii. NBS, THF, rt, 2 h, 66%; iv. 3,4-dimethoxyphenylboronic acid, K_2CO_3, $Pd(dppf)Cl_2$, DME/H_2O, 90 °C, o/n, 98%; v. formic acid, 50 °C, 3 h, 60%; vi. TFA, DCM, rt, 3 h, quant.; vii. 2-bromoacetyl chloride (**10**) or 3-bromopropanoyl chloride (**11**), pyr./DMF, 90 °C, 3 h, then NaH, DMF, rt, 3 h, 24–34%/Ac_2O, DCM, rt, o/n, quant. (**12**)/NaH, MeI, rt, 5.5 h, 50% (**14**)/$ClCO_2Me$, pyr., DCM, rt, 6 h, 78% (**15**)/$MeSO_2Cl$, Et_3N, DCM, rt, 1 h, quant. (**16**)/$(CF_3SO_2)_2O$, Et_3N, DCM, rt, o/n, 22% (**17**)/Ph_3PCl_2, Et_3N, SO_2NHTBS, $CHCl_3$, rt, 2 days, 25% (**18**); viii. NaH, MeI, DMF, rt, 1.5 h, 98%; ix. formic acid, 50 °C, o/n, 34% (**9**)/HCl in dioxane, MeOH, rt, 5 h, 95% (**13**).

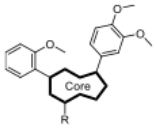
Table 1
Analogues with different C-8 substituents.



Compound	R	EC_{50} [nM][a]	ER in mS9[b] (% without cofactor)[c]	Solubility [μM][d]	clogP[e]	LLE[f]
OXS000651 **1**	-NHCHO	34	1 (28%)	37	3.1	4.4
7	-H	2100	0.36	156	3.8	1.9
8	$-NH_2$	>10000	0.93	38	3.1	n.a.[h]
9	-N(Me)CHO	1100	0.37 (46%)	1	3.4	2.6
10	-N-β-lactam	>10000	n.d.[g]	n.d.	3.7	n.a.
11	-N-γ-lactam	>10000	n.d.	n.d.	3.4	n.a.
12	-NHC(O)Me	3600	1 (1%)	39	3.5	1.9
13	-NHMe	120	0.97 (81%)	8	3.4	3.5
14	$-NMe_2$	>10000	n.d.	n.d.	3.7	n.a.
15	$-NHCO_2Me$	5900	n.d.	n.d.	3.7	1.5
16	$-NHSO_2Me$	950	0.25	43	2.9	3.1
17	$-NHSO_2CF_3$	>10000	n.d.	n.d.	4.9	n.a.
18	-NHS(O)(NH)Me	>10000	n.d.	n.d.	3.4	n.a.
19	$-SO_2Me$	3300	n.d.	n.d.	2.8	2.7
20	-S(O)(NH)Me	4800	n.d.	n.d.	4.8	0.5
21	$-CONH_2$	1900	0.90	12	2.9	2.8

[a] %CD11b response in HL-60 cells;
[b] Extraction ratio (ER)= Cl_{int}/species flow rate (mice: 90 ml/min/kg) in mouse S9 fraction (mS9), high (>0.7), intermediate (0.3–0.7) or low (<0.3);
[c] % compound remaining after 45 min without cofactor addition, only shown if <80%;
[d] Semi-thermodynamic aqueous solubility;
[e] Calculated octanol-water partition coefficient clogP determined using Datawarrior;
[f] Lipophilic efficiency LLE = -LogEC_{50}-cLogP;
[g] Not determined;
[h] Not applicable.

Table 2
Analogues with different core patterns.



Compound	Core	R	EC_{50} [nM][a]	ER in mS9[b] (% without cofactor)[c]	Solubility [μM][d]	clogP[e]	LLE[f]
30		-NHCHO	60	1 (0.5%)	1	2.6	4.6
31		-NHMe	102	1	39	2.9	4.1
32		$-NMe_2$	2700	n.d.[g]	n.d.	3.1	2.4
33			>10000	0.14	4	2.9	n.a.[h]
34			>10000	0.18	26	2.9	n.a.
35			3500	n.d.	n.d.	2.6	2.9
36			>10000	n.d.	n.d.	3.4	n.a.
37		–	>10000	1 (70%)	1	3.1	n.a.
38		-H	>10000	n.d.	n.d.	3.3	n.a.
39		–	180	n.d.	n.d.	3.3	3.4
40		-H	140	1	200	3.3	3.6

[a] %CD11b response in HL-60 cells;
[b] Extraction ratio (ER)= Cl_{int}/species flow rate (mice: 90 ml/min/kg) in mouse S9 fraction (mS9), high (>0.7), intermediate (0.3–0.7) or low (<0.3);
[c] % compound remaining after 45 min without cofactor addition, only shown if <80%;
[d] Semi-thermodynamic aqueous solubility;
[e] Calculated octanol-water partition coefficient clogP determined using Datawarrior;
[f] Lipophilic ligand efficiency LLE = -LogEC_{50}-cLogP;
[g] Not determined;
[h] Not applicable.

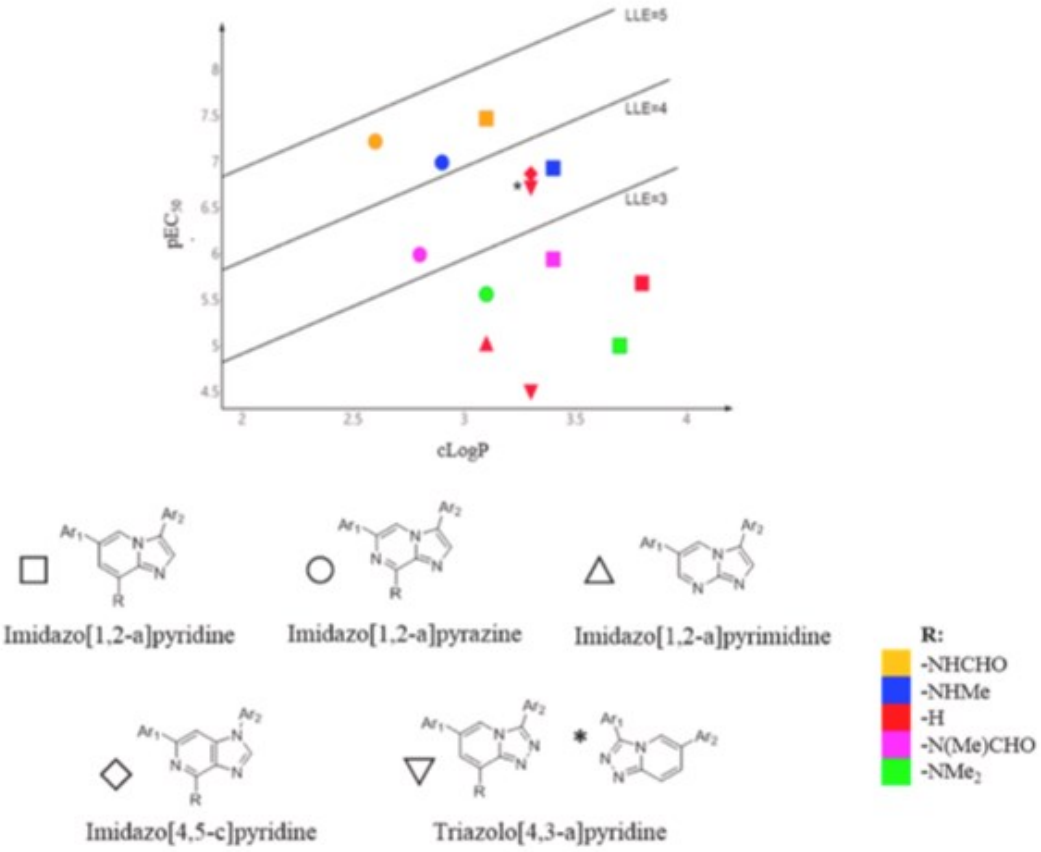
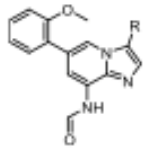
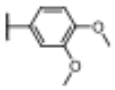
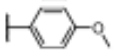
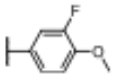
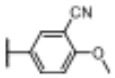
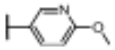
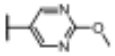
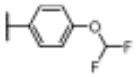
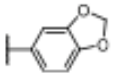
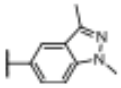
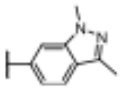
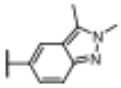
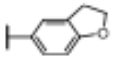
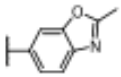
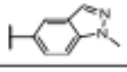


Fig. 2. Pairwise analysis of different cores. Lipophilic ligand efficiency LLE = -LogEC$_{50}$-cLogP.

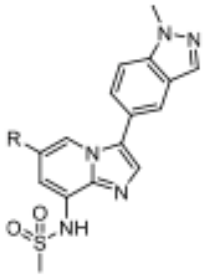
Table 3
Analogues with different C-3 substituents.

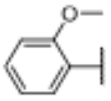
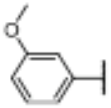
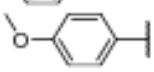
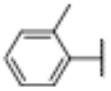
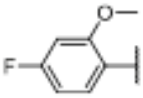
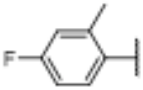
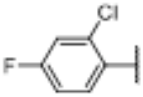
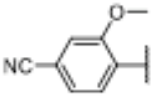
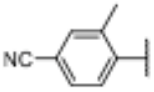
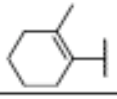


Compound	R	EC_{50} [nM][a]	Solubility [μM][b]	clogP[c]	LLE[d]
OXS000651 **1**		34	37	3.1	4.4
44	-H	>10000	>200	1.5	n.a.[e]
45		>10000	2	3.3	n.a.
46		30	n.d.[f]	3.2	4.3
47		99	n.d.	3.2	3.8
48		>10000	n.d.	3.2	n.a.
49		38	n.d.	3.3	4.1
50		30	2	3.0	4.5
51		700	n.d.	2.6	3.6
52		3500	n.d.	2.0	3.5
53		530	n.d.	3.4	2.9
54		318	n.d.	3.4	3.1
55		63	6	3.1	4.1
56		116	2	3.1	3.8
57		24	4	3.1	4.5
58		11	n.d.	3.5	4.5
59		10	2	3.5	4.5
60		5	2	2.7	5.6

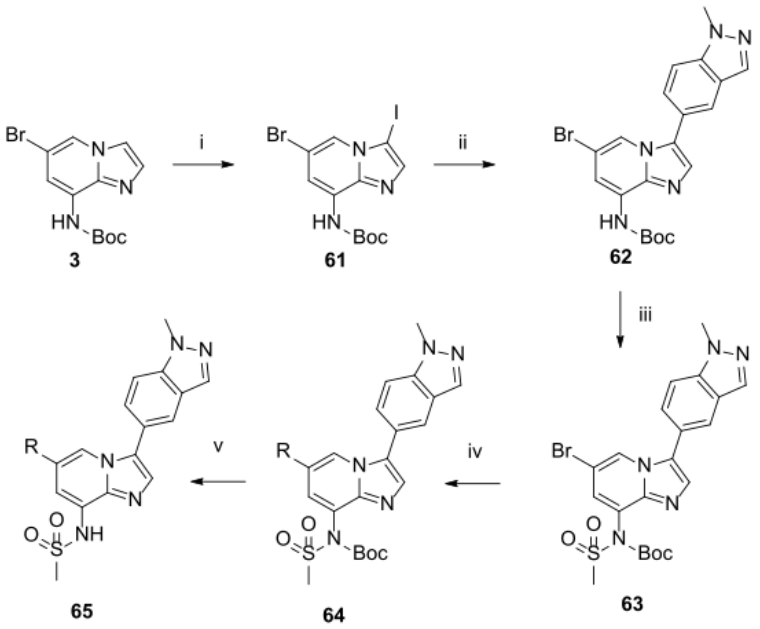
[a] %CD11b response in HL-60 cells;
[b] Semi-thermodynamic aqueous solubility;
[c] Calculated octanol-water partition coefficient clogP determined using Datawarrior;
[d] Lipophilic ligand efficiency LLE = -LogEC_{50}-cLogP;
[e] Not determined;
[f] Not applicable.

Table 4
Analogues with different C-6 substituents.



Compound	R	EC_{50} [nM][a]	ER in mS9[b]	Solubility [μM][c]	clogP[d]	LLE[e]
66		24	0.07	18	2.4	5.2
67		310	n.d.[f]	n.d.	2.4	4.1
68		3000	n.d.	n.d.	2.4	3.1
69		30	0.07	13	2.8	4.7
70		27	0.09	12	2.5	5.1
OXS007464 **71**		36	0.09	21	2.9	4.5
72		57	0.05	9	3.1	4.1
73		710	n.d.	n.d.	2.5	3.6
74		180	0.06	2	2.2	4.5
75		321	0.06	26	2.6	3.9
76		45	0.27	26	2.6	4.7

[a] %CD11b response in HL-60 cells;
[b] Extraction ratio (ER)= Cl_{int}/species flow rate (mice: 90 ml/min/kg) in mouse S9 fraction (mS9), high (>0.7), intermediate (0.3–0.7) or low (<0.3);
[c] Semi-thermodynamic aqueous solubility;
[d] Calculated octanol-water partition coefficient clogP determined using Datawarrior;
[e] Lipophilic ligand efficiency LLE = -LogEC_{50}-cLogP;
[f] Not determined.



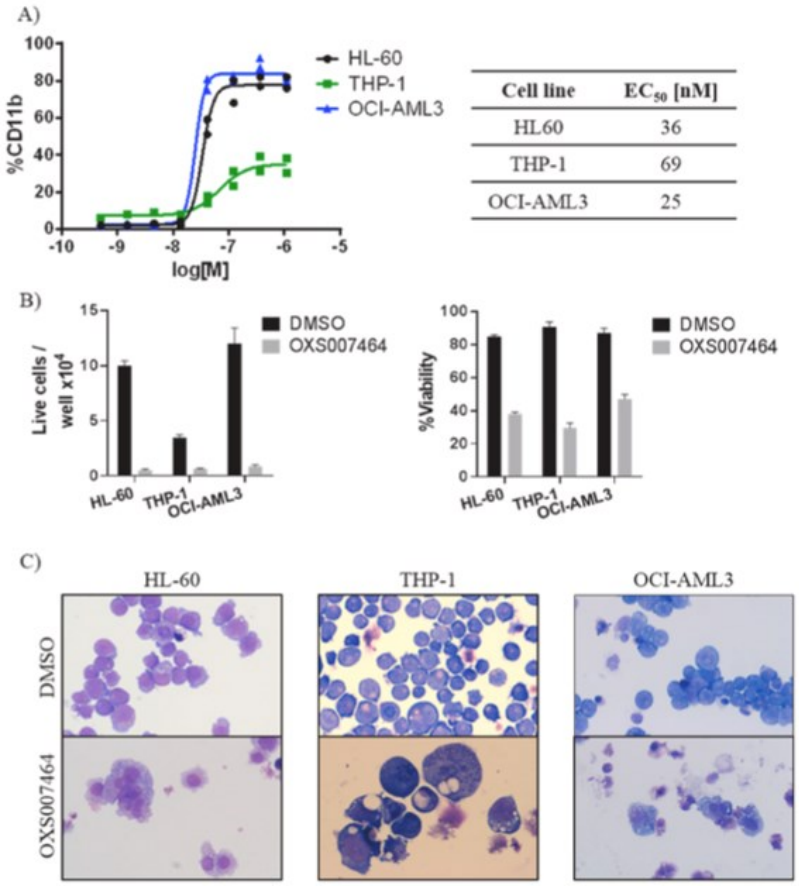
Scheme 4. Synthesis of C-3 derivatives. i. NIS, THF, rt, o/n, 78%; ii. (1-methylindazol-5-yl)boronic acid, K_2CO_3, $Pd(dppf)Cl_2$, dioxane/H_2O, 80 °C, 4 h, 68%; iii. NaH, $MeSO_2Cl$, THF, rt, o/n, 81%; iv. boronic acid or pinacol ester, K_3PO_4, $Pd(dppf)Cl_2$, DME/H_2O, 90 °C, o/n; v. TFA, DCM, rt, o/n.

Table 5

Physicochemical and *in vitro* ADME properties of OXS0007464.

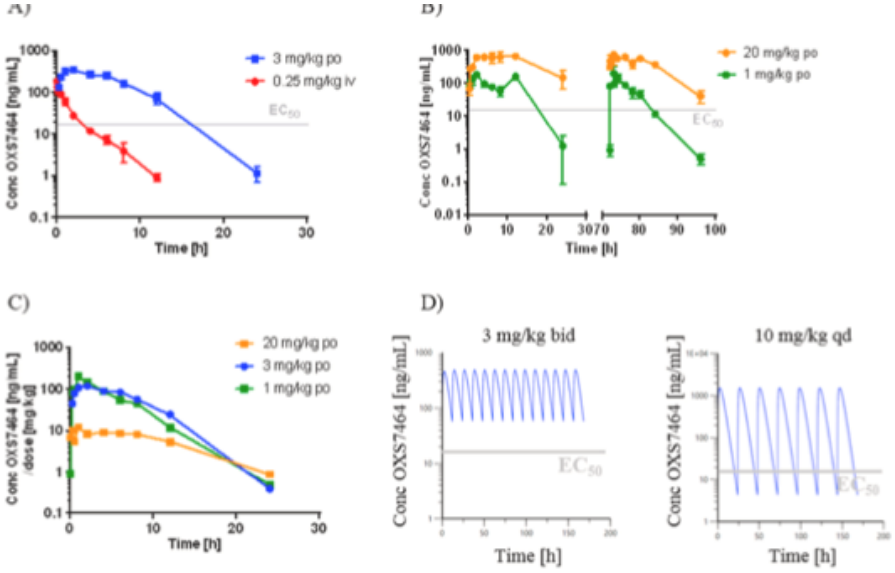
Parameter	Measured value
Solubility, pH = 7	21 μM
Solubility, pH = 1	>100 μM
Extraction ratio, mS9	0.09
Extraction ratio, mHep	0.29
Caco-2 P_{app}A-B [efflux ratio]	16×10^{-6} cm/s [0.9]
mPPB[a]	99.5%

[a] Mouse plasma protein binding



Cell line	EC_{50} [nM]
HL60	36
THP-1	69
OCI-AML3	25

Fig. 3. *In vitro* profile of OXS007464 in three representative cell lines. (A) Concentration-response curves of %CD11b on cells treated with OXS007464 for 4 days and the resulting EC_{50} values; B) Number of live cells per well and %viability of cells treated with either DMSO control or 123 nM OXS007464 over 4 days, determined with acridine orange and DAPI; C) Morphology of HL-60 cells treated with either DMSO control or OXS007464 (10 μM for HL-60, 123 nM for THP-1 and OCI-AML3).



E)

Parameter	3 mg/kg single dose	20 mg/kg repeat dose		1 mg/kg repeat dose	
		Day 1	Day 4	Day 1	Day 4
$T_{1/2}$ (h)	2.2		4.1		2.5
Cl_{obs}/F (mL/min/kg)	16				
Vz/F (L/kg)	3.1				
F (%)	100				
$AUC_{0\text{-}8h}$ (ng/mL·h)	3062	4461	4367	845	731
$AUC_{0\text{-}8h}$/dose (ng/mL·h /(mg/kg))	1021	223	218	845	731
C_{max} (ng/mL)	360	677	784	187	204
C_{max}/dose (ng/mL/(mg/kg))	120	34	39	187	204

Fig. 4. Pharmacokinetic profile of OXS007464 in male CD-1 mice. (A) Blood concentration over 24 h after a single dose at 3 mg/kg po and 0.25 mg/kg iv; (B) Blood concentration at days 1 and 4 after repeat dosing at 1 mg/kg and 20 mg/kg po once daily; (C) Dose-normalised blood concentration over time of the three PK studies (day 4 data shown for the 1 mg/kg and 20 mg/kg repeat dose studies); (D) Simulated exposure at 3 mg/kg bid and 10 mg/kg qd; (E) Pharmacokinetic parameters of OXS007464 determined in the 3 mg/kg single dose, 20 mg/kg repeat dose and 1 mg/kg repeat dose studies.