Supporting Information

Discovery of a Potent and Selective Sphingosine Kinase 1 Inhibitor through the Molecular Combination of Chemotype Distinct Screening Hits

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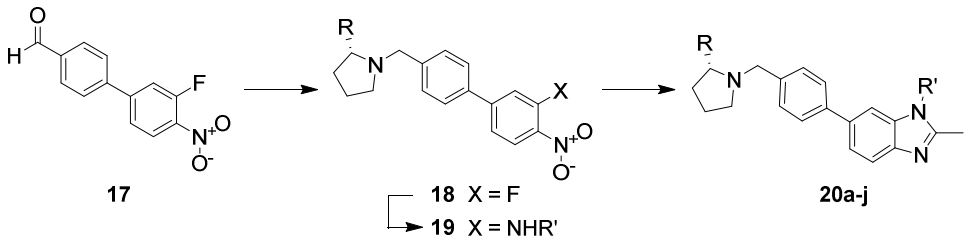
Table of Contents

1. Synthesis and characterization of benzimidazole analogs 20b-j

The following compounds (**20b-j**) were prepared using procedures analogous to that described for compound **20a** in the experimental section. Compounds **20e-j** were prepared in a library format following the same conditions employing compound **18** (R=CH_2OH) with the corresponding amine described and purified by reverse-phase preparative HPLC.



((*R*)-1-(4-(2-methyl-1-(((*S*)-tetrahydrofuran-2-yl)methyl)-1*H*-benzo[d]imidazol-6-yl)benzyl)pyrrolidin-2-yl)methanol (20b). Prepared analogous to **20a** employing (*R*)-2-(hydroxymethyl)pyrrolidine and (*S*)-2-(aminomethyl)tetrahydrofuran. MS (ES+) m/z 406 (M+H). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.78 (s, 1H), 7.64 (d, *J*=8.1 Hz, 2H), 7.55 (d, *J*=8.4 Hz, 1H), 7.42 (d, *J*=9.9 Hz, 1H), 7.39 (d, *J*=8.1 Hz, 2H), 4.46 (br. s., 1H), 4.38 (d, *J*=12.5 Hz, 1H), 4.29–4.14 (m, 3H), 4.08 (d, *J*=13.2 Hz, 1H), 3.76 (q, *J*=7.0 Hz, 1H), 3.61 (q, *J*=7.5 Hz, 1H), 3.53–3.44 (m, 1H), 2.82 (br. s., 1H), 2.63–2.53 (m, 5H), 2.18 (br. s., 1H), 2.04 (qd, *J*=6.3, 12.7 Hz, 1H), 1.91-1.74 (m, 3H), 1.69-1.52 (m, 4H).

((*S*)-1-(4-(2-methyl-1-(((*R*)-tetrahydrofuran-2-yl)methyl)-1*H*-benzo[d]imidazol-6-yl)benzyl)pyrrolidin-2-yl)methanol (20c). Prepared analogous to **20a** employing (*S*)-2-(hydroxymethyl)pyrrolidine and (*R*)-2-(aminomethyl)tetrahydrofuran. MS (ES+) m/z 406 (M+H). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.78 (s, 1H), 7.64 (d, *J*=8.1 Hz, 2H), 7.55 (d, *J*=8.1 Hz, 1H), 7.42 (dd, *J*=8.4, 1.5 Hz, 1H), 7.39 (d, *J*=8.1 Hz, 2H), 4.45 (br. s., 1H), 4.38 (d, *J*=12.1 Hz, 1H), 4.29–4.14 (m, 3H), 4.08 (d, *J*=13.2 Hz, 1H), 3.76 (q, *J*=7.0 Hz, 1H), 3.61 (q, *J*=7.3 Hz,

1H), 3.52–3.44 (m, 1H), 2.82 (br. s., 1H), 2.63–2.53 (m, 5H), 2.23–2.13 (m, 1H), 2.04 (s, 1H), 1.92–1.75 (m, 3H), 1.68–1.52 (m, 4H).

((*S*)-1-(4-(2-methyl-1-(((*S*)-tetrahydrofuran-2-yl)methyl)-1*H*-benzo[d]imidazol-6-yl)benzyl)pyrrolidin-2-yl)methanol (20d). Prepared analogous to **20a** employing (*S*)-2-(hydroxymethyl)pyrrolidine and (*S*)-2-(aminomethyl)tetrahydrofuran. MS (ES+) m/z 406 (M+H). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.78 (s, 1H), 7.64 (d, *J*=8.1 Hz, 2H), 7.55 (d, *J*=8.1 Hz, 1H), 7.42 (dd, *J*=8.4, 1.1 Hz, 1H), 7.39 (d, *J*=8.1 Hz, 2H), 4.45 (br. s., 1H), 4.38 (d, *J*=12.1 Hz, 1H), 4.29–4.15 (m, 3H), 4.08 (d, *J*=13.2 Hz, 1H), 3.76 (q, *J*=7.0 Hz, 1H), 3.61 (q, *J*=7.5 Hz, 1H), 3.52–3.45 (m, 1H), 2.82 (br. s., 1H), 2.63–2.53 (m, 5H), 2.18 (s., 1H), 2.10–1.99 (m, 1H), 1.92–1.74 (m, 3H), 1.69-1.53 (m, 4H).

(*R*)-(1-(4-(1-(2-cyclobutylethyl)-2-methyl-1*H*-benzo[d]imidazol-6-yl)benzyl)pyrrolidin-2-yl)methanol (20e). Prepared analogous to **20a** employing (*R*)-2-(hydroxymethyl)pyrrolidine and 2-cyclobutylethan-1-amine. MS (ES+) m/z 404 (M+H).

(*R*)-(1-(4-(1-isopentyl-2-methyl-1*H*-benzo[d]imidazol-6-yl)benzyl)pyrrolidin-2-yl)methanol (20f). Prepared analogous to **20a** employing (*R*)-2-(hydroxymethyl)pyrrolidine and 3-methylbutan-1-amine. MS (ES+) m/z 392 (M+H).

(*R*)-(1-(4-(1-(cyclopentylmethyl)-2-methyl-1*H*-benzo[d]imidazol-6-yl)benzyl)pyrrolidin-2-yl)methanol (20g). Prepared analogous to **20a** employing (*R*)-2-(hydroxymethyl)pyrrolidine and cyclopentylmethanamine. MS (ES+) m/z 404 (M+H).

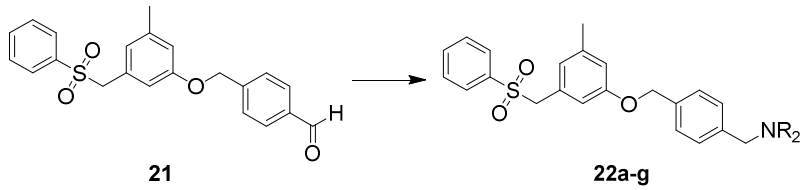
(*R*)-(1-(4-(1-benzyl-2-methyl-1*H*-benzo[d]imidazol-6-yl)benzyl)pyrrolidin-2-yl)methanol (20h). Prepared analogous to **20a** employing (*R*)-2-(hydroxymethyl)pyrrolidine and benzylamine. MS (ES+) m/z 412 (M+H).

(*R*)-(1-(4-(1-Isobutyl-2-methyl-1*H*-benzo[d]imidazol-6-yl)benzyl)pyrrolidin-2-yl)methanol (20i). Prepared analogous to **20a** employing (*R*)-2-(hydroxymethyl)pyrrolidine and 2-methylpropan-1-amine. MS (ES+) m/z 377 (M+H).

(*R*)-2-methyl-6-(4-(pyrrolidin-1-ylmethyl)phenyl)-1-((tetrahydrofuran-2-yl)methyl)-1*H*-benzo[d]imidazole (20j). Prepared analogous to **20a** employing pyrrolidine and (*R*)-2-(aminomethyl)tetrahydrofuran. MS (ES+) m/z 376 (M+H).

2. Synthesis and characterization of arylether amine analogs 22b-g

The following compounds (**22b-g**) were prepared using procedures analogous to that described for compound **22a** in the experimental section employing the corresponding amine described.



21 **22a-g**

(*S*)-(1-(4-((3-Methyl-5-((phenylsulfonyl)methyl)phenoxy)methyl)benzyl)pyrrolidin-2-yl)methanol (22b). (*S*)-pyrrolidin-2-ylmethanol. MS (ES+) *m/z* 466 (M+H). ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.80–7.69 (m, 3H), 7.66–7.53 (m, 2H), 7.32 (s, 4H), 6.79 (s, 1H), 6.53 (d, J=7.5 Hz, 2H), 4.90 (s, 2H), 4.57 (s, 2H), 4.43 (br. s., 1H), 4.05 (d, J=13.4 Hz, 1H), 3.51–3.41 (m, 1H), 3.31–3.21 (m, 2H), 2.85–2.71 (m, 1H), 2.63–2.55 (m, 1H), 2.27–2.06 (m, 4H), 1.93–1.75 (m, 1H), 1.68–1.48 (m, 3H).

(*S*)-1-(4-((3-Methyl-5-((phenylsulfonyl)methyl)phenoxy)methyl)benzyl)pyrrolidin-3-ol (22c). (*S*)-pyrrolidin-3-ol. MS (ES+) *m/z* 452 (M+H). ^{1}H NMR (400 MHz, CD_3OD) δ 7.65–7.61 (m, 3H), 7.52–7.48 (m, 6H), 6.73 (s, 1H), 6.56 (s, 1H), 6.42 (s, 1H), 4.99 (s, 2H), 4.50 (s, 1H), 4.45-4.20 (m, 4H), 3.62–3.33 (m, 2H), 3.26-3.10 (m, 2H), 2.39–2.29 (m, 0.5 H), 2.14 (s, 3H), 2.10–1.92 (m, 1.5H).

(*R*)-1-(4-((3-Methyl-5-((phenylsulfonyl)methyl)phenoxy)methyl)benzyl)pyrrolidin-3-ol (22d). (*R*)-pyrrolidin-3-ol. MS (ES+) *m/z* 452 (M+H). ^{1}H NMR(400 MHz, CD_3OD) δ 7.66–7.61 (m, 3H), 7.51–7.47 (m, 6H), 6.73 (s, 1H), 6.55 (s, 1H), 6.42 (s, 1H), 4.97 (s, 2H), 4.47 (m, 1H), 4.36 (s, 2H), 4.29–4.22 (m, 2H), 3.42–3.40 (m, 1H), 3.12–3.09 (m, 2H), 2.22–2.14 (m, 2H), 2.14 (s, 3H), 1.97–1.95 (m, 2H).

(3*R*,4*R*)-1-(4-((3-Methyl-5-((phenylsulfonyl)methyl)phenoxy)methyl)benzyl)pyrrolidine-3,4-diol (22e). (3*R*,4*R*)-pyrrolidine-3,4-diol. MS (ES+) *m/z* 468 (M+H). ^{1}H NMR (400 MHz, CD_3OD) δ 7.68–7.65 (m, 3H), 7.55–7.50 (m, 6H), 6.77 (s, 1H), 6.59 (s, 1H), 6.47 (s, 1H), 5.01 (s, 2H), 4.40 (s, 2H), 4.25 (dd, J=20.0, 11.0 Hz, 1H), 4.21–4.20 (m, 3H), 3.52–3.49 (m, 2H), 3.14-3.11 (d, J=11.6 Hz, 2H), 2.19 (s, 3H).

(3*R*,4*S*)-1-(4-((3-methyl-5-((phenylsulfonyl)methyl)phenoxy)methyl)benzyl)pyrrolidine-3,4-diol (22f). (3*R*,4*S*)-pyrrolidine-3,4-diol. MS (ES+) *m/z* 468 (M+H). ^{1}H NMR (400 MHz, CD_3OD) δ 7.70–7.65 (m, 3H), 7.55–7.49 (m, 6H), 6.77 (s, 1H), 6.59 (s, 1H), 6.47 (s, 1H), 5.00 (s, 2H), 4.40 (s, 2H), 4.28–4.27 (t, J=2.0 Hz, 2H), 4.23 (s, 2H), 3.36–3.35 (dd, J=12.0, 4.8 Hz, 2H), 3.14–3.10 (dd, J=12.0, 4.0 Hz, 2H), 2.19 (s,3H).

2-((4-((3-methyl-5-((phenylsulfonyl)methyl)phenoxy)methyl)benzyl)amino)ethan-1-ol (22g). 2-aminoethan-1-ol. MS (ES+) *m/z* 426 (M+H). 1H NMR(400 MHz, CD_3OD) δ 7.65–7.61 (m, 3H), 7.52–7.47 (m, 6H), 6.75 (s, 1H), 6.55 (s, 1H), 6.43 (s, 1H), 4.99 (s, 2H), 4.38 (s, 2H), 4.25–4.10 (m, 4H), 3.39–3.30 (m, 2H), 2.15 (s, 3H).

3. Kinase selectivity data for compounds 12, 20a and 22a

Table S1. Effect of 10 μM compound on the activity of a panel of human protein and lipid kinases assessed through the University of Dundee Kinase Consortium or Invitrogen[a]

	% Inhibition, 10 μM compound		
Kinase	**12**	**20a**	**22a**
AKT1	-7	-2	-13
AKT2	-3	3	2
AMPK	-2	-6	1
AURKA	-17	0	-15
AURKB	16	3	13
CAMK1	-1	-3	0
CAMK2A	9	16	3
CAMKK2	10	-3	6
CDK2_CyclinA	-9	-8	-8
CHEK1	16	16	14
CHEK2	-8	-1	7
CHKA	0	-1	0
CSK	-2	1	-2
CSNK1A1	5	-8	5
Csnk1d	-6	9	-8
CSNK2A1	3	-3	1
CSNK2A2	8	-8	8
DGKA	5	2	4
Dyrk1a	6	11	2
DYRK2	-1	12	-4
EEF2K	-15	15	10
EGFR	-1	-2	2
EPHA2	2	-17	7
ERBB4	18	12	3
FGFR1	4	46	2
GSK3B	-5	4	-7

	% Inhibition, 10 μM compound		
Kinase	**12**	**20a**	**22a**
IKBKB	2	8	8
INSR	6	-2	6
IRAK4	-11	14	1
JAK3	-1	41	-6
KDR	3	1	7
LCK	20	7	23
MAP3K11	3	-15	8
MAP3K9	5	4	48
MAP4K2	4	3	-3
MAP4K4	-10	-2	-9
MAPK1	-2	3	-3
MAPK12	-18	11	7
MAPK13	-3	13	-2
MAPK14	0	1	0
MAPK15	0	2	-4
MAPK8	-1	6	-3
MAPK9	-7	0	-16
MAPKAPK2	-3	10	-2
MAPKAPK5	-8	3	-2
MARK1	-2	-3	-1
MARK3	7	0	-1
MET	3	-73	6
MINK1	-3	11	4
MKK1	2	11	0
MKNK1	-24	-11	-16
MKNK2	-1	2	-3
MST4	-10	24	-11
MYLK	15	4	-6
MYLK2	-3	0	-5
NEK2	1	ND	-11

	% Inhibition, 10 μM compound		
Kinase	**12**	**20a**	**22a**
NEK6	-11	8	-9
NTRK1	23	-13	19
NUAK1	7	6	16
PAK4	-11	4	-7
PBK	2	-4	0
PDPK1	-5	28	1
PI4KA	3	17	1
PI4KB	0	6	0
PIK3CA	-17	-26	1
PIK3CG	22	70	3
PIM1	-1	14	0
PIM2	-8	19	-6
PIM3	18	-4	-5
PIP4K2A	-1	-4	-1
PKN2	-18	2	-2
PLK1	2	7	4
PRKACA	-2	-2	-8
PRKCA	-24	1	4
PRKCB2	0	7	-10
PRKD1	-6	11	-9
ROCK1	-7	-4	2
ROCK2	-11	0	1
Rps6ka1	6	-5	-1
RPS6KA3	3	5	-27
RPS6KA5	40	6	-3
RPS6KB1	-7	-3	-15
SGK1	-11	7	-11
SRC	12	-3	16
SRPK1	-12	14	-9
STK11	-7	7	-2

	% Inhibition, 10 μM compound		
Kinase	**12**	**20a**	**22a**
STK3	-9	-17	-11
TAOK2	-10	-8	-9
TEK	0	5	-2
TTK	-9	0	-19

[a]ND, not determined.