Supporting Information

Design and Synthesis of Highly Potent and Isoform Selective JNK3 Inhibitors: SAR Studies on Aminopyrazole Derivatives

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1. Chemistry

1.1 Synthesis of 6

A solution of 3-(2-chlorophenyl)-2-oxopropanoic acid (0.22 mmol) in CH₂Cl₂ (1.0 mL) was added EDC (0.33 mmol), HOBt (0.33 mmol) and diisopropyl ethyl amine (0.66 mmol) and stirred for 30 min. Then the 3-(4-amino-1H-pyrazol-1-yl)-N-(6-methylpyridin-3-yl)benzamide **5** (0.2 mmol) was added and the resulting mixture was stirred over night. Water (10 ml) was added to the reaction mixture and extracted with EtOAc (2x10 mL). The solvent was evaporated and the residue was purified by reverse-phase preparative HPLC to give 3-(4-(2-(2-chlorophenyl)acetamido)-1H-pyrazol-1-yl)-N-(6-methylpyridin-3-yl)benzamide **6a** as a white powder.

1.2 Synthesis of 7

A mixture of 3-(4-amino-1H-pyrazol-1-yl)-N-(6-methylpyridin-3-yl)benzamide **5** (0.1 mmol), 5-bromo-2-chloro-1H-benzo[d]imidazole (1.1 eq.) and HCl (4 eq.) in EtOH (1.0 mL) in a sealed tube was heated at 140 °C using microwave for 2 hrs. The solvent was evaporated and the residue was purified by reverse-phase preparative HPLC to give the product 3-(4-((6-fluoro-1H-benzo[d]imidazol-2-yl)amino)-1H-pyrazol-1-yl)-N-(6-methylpyridin-3-yl)benzamide **7** as a white powder.

1.3 Synthesis of 12

A mixture of ethyl 1-(3-((6-methylpyridin-3-yl)carbamoyl)phenyl)-1H-pyrazole-4-carboxylate **10** (5 mmol) and LiOH (2.0 g) was stirred in THF (30 mL)/water (30 mL) at RT for 2-3 hours. The resulting homogenous reaction was acidified with 2M HCl (20 mL) and extracted with EtOAc (2x100 mL). The combined organic extracts were washed with water (100 mL), dried (Na₂SO₄) and evaporated. The crude residue was dried under vacuum and used without further purification.

The 1-(3-((6-methylpyridin-3-yl)carbamoyl)phenyl)-1H-pyrazole-4-carboxylic acid **11** (0.1 mmol) was added HATU (2 eq.) and diisopropyl ethyl amine (2 eq.) in DMF, stirred for 30 min. The amide (0.12 mmol) was added to the mixture and

the resulting mixture was stirred over night. The residue was purified by reverse-phase preparative HPLC to give the product **12**.

1.4 Synthesis of **16**

A mixture of ethyl 3-bromobenzoate **13** (10 mmol), ethyl 3-bromo-4-fluorobenzoate **13** (20 mmol), CuI (2.0 mmol), *trans*-*N,N*-dimethylcyclohexane-1,2-diamine (4.0 mmol) and Cs₂CO₃ (30 mmol) in DMF (20 mL) was purged with argon and stirred for 12 h at 90 °C in a sealed tube. The reaction mixture was cooled, and filtered through a pad of silica gel and rinsed with EtOAc. The resulting solution was concentrated *in vacuo* to yield a crude residue.

A mixture of crude residue (10 mmol) and LiOH (2.0 g) was stirred in THF (30 mL)/water (30 mL) at RT for 2-3 hours. The resulting homogenous reaction was acidified with 2M HCl (20 mL) and extracted with EtOAc (2x100 mL). The combined organic extracts were washed with water (100 mL), dried (Na₂SO₄) and evaporated. The residue was purified by chromatography on silica gel (dichloromethane/methanol) to give the ethyl 4-fluoro-3-(4-nitro-1H-pyrazol-1-yl)benzoate **14**.

After amide formation and Pd/C reduction of intermediate **14**, a urea formation was then applied to intermediate **15** to furnish the final product **16** (The synthetic procedures are the same to inhibitor **8**).

1.5 Synthesis of **20**

A mixture of 4-nitro-1H-pyrazole **2** (10 mmol), 1-bromo-3-iodobenzene **17** (20 mmol), CuI (2.0 mmol), *trans*-*N,N*-dimethylcyclohexane-1,2-diamine (4.0 mmol) and Cs₂CO₃ (30 mmol) in DMF (20 mL) was purged with argon and stirred for 12 h at 100 °C in a sealed tube. The reaction mixture was cooled, and filtered through a pad of silica gel and rinsed with EtOAc. The resulting solution was concentrated *in vacuo* to yield a crude residue which was purified by chromatography on silica gel (EtOAc/hexane).

This intermediate was hydrogenated in anhydrous methanol (100 mL) in the presence of 5% Pt-C (1.0 g) under a balloon of hydrogen for 3 hours. The mixture was filtered through a Celite pad and evaporated. The residue was purified by chromatography on silica gel (dichloromethane/methanol) to give the intermediate **18**. Then the 1-chloro-2-isocyanatobenzene (0.12 mmol) was added to intermediate **18** (0.1 mmol) in CH₂Cl₂ (1.0 mL) at RT and stirred for 1 h. The solvent was evaporated and the residue was purified by chromatography on silica gel to give the intermediate **19a**.

Finally, the R₁B(OH)₂ (0.3 mmol) and the intermediates **19a** (0.2 mmol) were dissolved in degassed 4:1 dioxane/H₂O. Pd(PPh₃)₄ (0.02 mmol) and a 2 M solution of K₂CO₃ (0.6 mmol) were added sequentially under argon, and the mixture was heated at 95 °C for 2 h. After being cooled to room temperature, the mixture was diluted with water and extracted

with ethyl acetate (3 × 5 mL). The organic layers were combined, dried over anhydrous Na₂SO₄, and concentrated in vacuo. The residue was then subjected to preparative HPLC to give the target products **20a-20b** as a white powder.

1.5 Synthesis of 21

A mixture of the intermediates **19b** (0.5 mmol) and N₂H₄·xH₂O (3.0 mmol.) was reflux in EtOH (5 mL) at 90 °C for 24 hours. The solvent was evaporated and the residue was purified by chromatography on silica gel to give an intermediate benzimidamide.

A mixture of acetimidamide hydrochloride (0.75 mmol) and 25% solution of sodium methoxide (0.75) in methanol was stirred in EtOH (10 mL) at RT for 30 min, the mixture was filtered. To the filtrate was added the intermediate benzimidamide and the reaction was stirred overnight. The solvent was evaporated and the residue was purified by preparative HPLC to give the target products **21a-21b** as a white powder.

1.6 Synthesis of 22

A solution of 3 intermediates **19c** (0.22 mmol) in CH₂Cl₂ (1.0 mL) was added EDC (0.33 mmol), HOBT (0.33 mmol) and diisopropyl ethyl amine (0.66 mmol) and stirred for 30 min. Then the amine (0.2 mmol) was added and the resulting mixture was stirred overnight. Water (10 ml) was added to the reaction mixture and extracted with EtOAc (2x10 mL). The solvent was evaporated and the residue was purified by reverse-phase preparative HPLC to give products **22** as a white powder.

1.7 Synthesis of 26

A mixture of the **23** (2.0 mmol) and 4-nitro-1H-pyrazole **2** (1.0 mmol.) was added K₂CO₃ (3.0 mmol), and stirred in DMF (5 mL) at 40 °C overnight. The solvent was evaporated and the residue was purified by chromatography on silica gel. This intermediate was hydrogenated in anhydrous methanol (10 mL) in the presence of 5% Pt-C (0.2 mmol) under a balloon of hydrogen for 3 hours. The mixture was filtered through a Celite pad and evaporated. The residue was purified by chromatography on silica gel (dichloromethane/methanol) to give the intermediate **24**.

(The followed synthetic procedures are the same to inhibitor **8** and the final products **26** were purified by reverse-phase preparative HPLC).

1.8 Purity of inhibitors 6, 7, 8, 12, 16, 20, 21, 22 and 26

Table1, Purity of inhibitors

Cmpd	RT (min)	Purity,% 230 nm	Purity,% 254 nm		Cmpd	RT (min)	Purity,% 230 nm	Purity,% 254 nm
6a	2.90	100	100		22a	3.12	98	98
6b	3.05	100	100		22b	3.65	100	100
6c	2.99	100	100		22c	3.68	100	100
6d	2.25	100	100		22d	3.95	98	98

7	3.92	100	100		22e	3.99	100	100
8a	3.47	100	100		22f	3.82	100	100
8b	3.69	98	98		22g	3.65	100	100
8c	3.05	100	100		22h	3.05	100	100
8d	3.25	98	100		22i	3.45	95	100
8e	3.18	100	100		26a	4.06	98	98
8f	2.90	100	100		26b	2.35	100	100
8g	2.83	100	100		26c	2.34	100	100
8h	2.88	100	100		26d	2.25	100	100
8i	3.12	100	100		26e	3.11	100	100
8j	3.12	100	100		26f	2.76	95	98
8k	2.69	100	100		26g	2.34	100	100
8l	3.78	98	98		26h	2.33	100	100
12a	3.54	100	100		26i	2.88	100	100
12b	3.13	100	100		26j	2.41	100	100
16a	3.62	98	98		26k	2.65	100	100
16b	3.80	98	98		26l	2.75	100	100
16c	3.65	100	100		26m	2.38	100	100
20a	3.91	100	100		26n	2.45	100	100
20b	2.87	98	98		26o	2.68	100	100
21a	3.66	100	100		26p	2.66	95	98
21b	3.76	98	98		26q	2.64	100	100

2. Pharmacology experiment

2.1 Pharmacokinetics

Pharmacokinetics studies were conducted in Sprague Dawley rats. The compound was formulated in a generic formulation at 1 mg/mL (e.g. 10:10:80, DMSO:tween 80: water, v:v:v) and dosed at 0.5 mg/kg intravenous into the femoral vein or 3 mg/kg by oral gavage. Blood was obtained at t=5 min, 15 min, 30 min, 1hr, 2hr, 4hr, 6hr, and 8hr. Blood was collected into EDTA containing tubes and plasma was generated by standard centrifugation methods. All procedures and handling were according to standard operating procedures approved by IAPUC at Scripps Florida. In order to assess in vivo pharmacokinetic parameters an LC-MS/MS bioanalytical method was developed where 25 μ L of plasma was treated with 125 μ L of acetonitrile containing an internal standard in a Millipore Multiscreen Slovinter 0.45 micron low binding PTFE hydrophilic filter plate (#MSRLN0450) and allowed to shake at room temperature for five minute. The plate was then centrifuged for 5 minutes at 4000 rpm in a tabletop centrifuge and the filtrate was collected in a polypropylene capture plate. The filtrate (10 μ L) is injected using an Agilent 1200 HPLC equipped with a Thermo Betasil C18 HPLC column 5 T (50 $\times$ 2.1 mm)

#70105-052130. Mobile Phase A was water with 0.1% formic acid. Mobile phase B was acetonitrile with 0.1% formic acid. Flow rate was 375 μ L/min using a gradient of 90% A/10% B from, 0-0.5 min, ramped to 5%A95%B at 2 min, held at 5%A95%B until 3.0 min, ramped to 90% A/10% B at 4 min, and held at 90% A10% B until 7 min. An API Sciex 4000 equipped with a turbo ion spray source was used for all analytical measurement. MRM methods were developed in position ion mode. Peak areas of the analyte ion were measured against the peak areas of the internal standard. Data was fit using WinNonLin using an IV bolus model.

2.2 P450s inhibition

P450s inhibition for four major isoforms are evaluated using a cocktail inhibition assay where the metabolism of specific marker substrate (CYP1A2 phenacetin demethylation to acetaminophen); CYP2C9, tolbutamide hydroxylation to hydroxytolbutamide; CYP2D6, bufuralol hydroxylation to 4'-Hydroxybufuralol; and CYP3A4, midazolam hydroxylation to 1'-Hydroxymidazolam) in the presence or absence of 10 μ M probe compound is evaluated. The concentration of each marker substrate is approximately its K_m . Conditions were similar to those described by Tesino and Patonay (Testino and Patonay, 2003) except 2C19 was not evaluated as we found that stock solution of the 2C19 probe substrate, omeprazole, had poor stability. Specific inhibitors for each isoform are included in each run to validate the system.

2.3 Solubility

The solubility of compounds was tested at pH 3.5 and pH 7.4. Compounds were inverted for 24 hours in test tubes containing 1-2 mg of compound with 1 mL of pH 5.5 potassium phosphate buffer. The samples were centrifuged and analyzed by HPLC (Agilent 1100 with diode-array detector). Peak area will be compared to a standard of known concentration.

3. Kinase panel profiling in KINOMEscan™ (Ambit)

Table 2, S-score Table for **26n**

Compound	Selectivity Score	Type Number of Hits	Number of Non-Mutant Kinases	Screening Concentration (nM)	Selectivity Score
26n	S(35)	12	395	10000	0.03
26n	S(10)	4	395	10000	0.01
26n	S(1)	2	395	10000	0.005

Table 3, Profiling data for **26n** in Ambit panel screening against 464 kinases.

Gene Symbol	%Ctrl @ 10000nM	Gene Symbol	%Ctrl @ 10000nM	Gene Symbol	%Ctrl @ 10000nM
AAK1	90	BRK	100	CSNK2A1	62
ABL1(E255K)-phosphorylated	49	BRSK1	97	CSNK2A2	79
ABL1(F317I)-nonphosphorylated	92	BRSK2	84	CTK	74
ABL1(F317I)-phosphorylated	88	BTK	91	DAPK1	89
ABL1(F317L)-nonphosphorylated	75	BUB1	76	DAPK2	73
ABL1(F317L)-phosphorylated	58	CAMK1	84	DAPK3	85
ABL1(H396P)-nonphosphorylated	33	CAMK1D	82	DCAMKL1	79
ABL1(H396P)-phosphorylated	64	CAMK1G	89	DCAMKL2	92
ABL1(M351T)-phosphorylated	68	CAMK2A	91	DCAMKL3	98
ABL1(Q252H)-nonphosphorylated	50	CAMK2B	83	DDR1	100
ABL1(Q252H)-phosphorylated	74	CAMK2D	93	DDR2	80
ABL1(T315I)-nonphosphorylated	93	CAMK2G	91	DLK	100
ABL1(T315I)-phosphorylated	55	CAMK4	100	DMPK	86
ABL1(Y253F)-phosphorylated	51	CAMKK1	64	DMPK2	95
ABL1-nonphosphorylated	63	CAMKK2	39	DRAK1	88
ABL1-phosphorylated	72	CASK	90	DRAK2	88
ABL2	80	CDC2L1	100	DYRK1A	42
ACVR1	89	CDC2L2	96	DYRK1B	63
ACVR1B	100	CDC2L5	100	DYRK2	40
ACVR2A	85	CDK11	97	EGFR	86
ACVR2B	89	CDK2	100	EGFR(E746-A750del)	78
ACVRL1	99	CDK3	93	EGFR(G719C)	80
ADCK3	93	CDK4-cyclinD1	54	EGFR(G719S)	97
ADCK4	98	CDK4-cyclinD3	63	EGFR(L747-E749del, A750P)	80
AKT1	99	CDK5	99	EGFR(L747-S752del, P753S)	90
AKT2	100	CDK7	59	EGFR(L747-T751del,Sins)	89
AKT3	100	CDK8	100	EGFR(L858R)	75
ALK	84	CDK9	91	EGFR(L858R, T790M)	100
ALK(C1156Y)	95	CDKL1	48	EGFR(L861Q)	99
ALK(L1196M)	79	CDKL2	90	EGFR(S752-I759del)	100
AMPK-alpha1	98	CDKL3	94	EGFR(T790M)	94
AMPK-alpha2	100	CDKL5	58	EIF2AK1	78
ANKK1	80	CHEK1	93	EPHA1	100
ARK5	94	CHEK2	100	EPHA2	94
ASK1	88	CIT	78	EPHA3	100
ASK2	96	CLK1	35	EPHA4	91
AURKA	87	CLK2	10	EPHA5	92
AURKB	93	CLK3	99	EPHA6	94
AURKC	47	CLK4	24	EPHA7	77
AXL	38	CSF1R	100	EPHA8	75
BIKE	65	CSF1R-autoinhibited	76	EPHB1	86
BLK	91	CSK	87	EPHB2	94
BMPR1A	88	CSNK1A1	58	EPHB3	100
BMPR1B	82	CSNK1A1L	90	EPHB4	98
BMPR2	93	CSNK1D	95	EPHB6	89
BMX	88	CSNK1E	39	ERBB2	93
BRAF	84	CSNK1G1	77	ERBB3	92
BRAF(V600E)	95	CSNK1G2	66	ERBB4	100
		CSNK1G3	82	ERK1	92

Gene Symbol	%Ctrl @ 10000nM	Gene Symbol	%Ctrl @ 10000nM	Gene Symbol	%Ctrl @ 10000nM
ERK2	95	IRAK1	63	MEK1	79
ERK3	100	IRAK3	100	MEK2	69
ERK4	100	IRAK4	95	MEK3	17
ERK5	98	ITK	85	MEK4	86
ERK8	97	JAK1(JH1domain-catalytic)	86	MEK5	73
ERN1	79	JAK1(JH2domain-pseudokinase)	83	MEK6	100
FAK	100	JAK2(JH1domain-catalytic)	80	MELK	46
FER	100	JAK3(JH1domain-catalytic)	77	MERTK	60
FES	99	JNK1	2.9	MET	76
FGFR1	85	JNK2	0.3	MET(M1250T)	90
FGFR2	99	JNK3	0.1	MET(Y1235D)	95
FGFR3	94	KIT	90	MINK	69
FGFR3(G697C)	99	KIT(A829P)	74	MKK7	74
FGFR4	97	KIT(D816H)	79	MKNK1	75
FGR	97	KIT(D816V)	96	MKNK2	96
FLT1	81	KIT(L576P)	99	MLCK	93
FLT3	78	KIT(V559D)	96	MLK1	93
FLT3(D835H)	90	KIT(V559D,T670I)	100	MLK2	91
FLT3(D835Y)	96	KIT(V559D,V654A)	100	MLK3	100
FLT3(ITD)	71	KIT-autoinhibited	77	MRCKA	100
FLT3(K663Q)	95	LATS1	83	MRCKB	100
FLT3(N841I)	74	LATS2	94	MST1	91
FLT3(R834Q)	88	LCK	92	MST1R	100
FLT3-autoinhibited	72	LIMK1	92	MST2	81
FLT4	95	LIMK2	100	MST3	90
FRK	100	LKB1	100	MST4	82
FYN	80	LOK	100	MTOR	87
GAK	82	LRRK2	80	MUSK	100
GCN2(Kin.Dom.2,S808G)	68	LRRK2(G2019S)	100	MYLK	62
GRK1	68	LTK	82	MYLK2	95
GRK4	92	LYN	89	MYLK4	84
GRK7	61	LZK	87	MYO3A	96
GSK3A	55	MAK	88	MYO3B	95
GSK3B	24	MAP3K1	88	NDR1	71
HASPIN	16	MAP3K15	84	NDR2	100
HCK	97	MAP3K2	83	NEK1	100
HIPK1	46	MAP3K3	86	NEK10	78
HIPK2	25	MAP3K4	100	NEK11	91
HIPK3	28	MAP4K2	59	NEK2	95
HIPK4	100	MAP4K3	96	NEK3	81
HPK1	90	MAP4K4	79	NEK4	89
HUNK	68	MAP4K5	82	NEK5	94
ICK	23	MAPKAPK2	90	NEK6	96
IGF1R	96	MAPKAPK5	58	NEK7	100
IKK-alpha	100	MARK1	81	NEK9	97
IKK-beta	96	MARK2	93	NIK	99
IKK-epsilon	92	MARK3	80	NIM1	97
INSR	100	MARK4	78	NLK	100
INSRR	100	MAST1	87	OSR1	84

Gene Symbol	%Ctrl @ 10000nM	Gene Symbol	%Ctrl @ 10000nM	Gene Symbol	%Ctrl @ 10000nM
p38-alpha	85	PKN2	100	SBK1	91
p38-beta	98	PKNB(M.tuberculosis)	88	SGK	100
p38-delta	91	PLK1	90	Sgk110	92
p38-gamma	75	PLK2	69	SGK2	63
PAK1	89	PLK3	67	SGK3	87
PAK2	94	PLK4	78	SIK	100
PAK3	74	PRKCD	95	SIK2	100
PAK4	75	PRKCE	92	SLK	84
PAK6	92	PRKCH	95	SNARK	93
PAK7	100	PRKCI	81	SNRK	79
PCTK1	89	PRKCQ	84	SRC	97
PCTK2	94	PRKD1	91	SRMS	80
PCTK3	84	PRKD2	76	SRPK1	63
PDGFRA	100	PRKD3	94	SRPK2	80
PDGFRB	87	PRKG1	89	SRPK3	85
PDPK1	89	PRKG2	82	STK16	82
PFCDPK1(P.falciparum)	83	PRKR	81	STK33	70
PFPK5(P.falciparum)	82	PRKX	100	STK35	97
PFTAIRE2	98	PRP4	81	STK36	78
PFTK1	93	PYK2	98	STK39	100
PHKG1	98	QSK	84	SYK	92
PHKG2	86	RAF1	87	TAK1	76
PIK3C2B	97	RET	76	TAOK1	72
PIK3C2G	85	RET(M918T)	85	TAOK2	78
PIK3CA	97	RET(V804L)	85	TAOK3	100
PIK3CA(C420R)	80	RET(V804M)	95	TBK1	75
PIK3CA(E542K)	91	RIOK1	43	TEC	88
PIK3CA(E545A)	67	RIOK2	69	TESK1	85
PIK3CA(E545K)	79	RIOK3	53	TGFBR1	83
PIK3CA(H1047L)	77	RIPK1	98	TGFBR2	97
PIK3CA(H1047Y)	97	RIPK2	96	TIE1	99
PIK3CA(I800L)	100	RIPK4	87	TIE2	100
PIK3CA(M1043I)	84	RIPK5	75	TLK1	100
PIK3CA(Q546K)	87	ROCK1	78	TLK2	100
PIK3CB	66	ROCK2	88	TNIK	98
PIK3CD	67	ROS1	99	TNK1	100
PIK3CG	84	RPS6KA4(Kin.Dom.1-N-terminal)	82	TNK2	100
PIK4CB	78	RPS6KA4(Kin.Dom.2-C-terminal)	67	TNNI3K	96
PIM1	40	RPS6KA5(Kin.Dom.1-N-terminal)	98	TRKA	86
PIM2	84	RPS6KA5(Kin.Dom.2-C-terminal)	100	TRKB	68
PIM3	59	RSK1(Kin.Dom.1-N-terminal)	95	TRKC	94
PIP5K1A	100	RSK1(Kin.Dom.2-C-terminal)	98	TRPM6	81
PIP5K1C	92	RSK2(Kin.Dom.1-N-terminal)	68	TSSK1B	100
PIP5K2B	78	RSK2(Kin.Dom.2-C-terminal)	88	TTK	44
PIP5K2C	38	RSK3(Kin.Dom.1-N-terminal)	95	TXK	91
PKAC-alpha	87	RSK3(Kin.Dom.2-C-terminal)	82	TYK2(JH1domain-catalytic)	87
PKAC-beta	82	RSK4(Kin.Dom.1-N-terminal)	77	TYK2(JH2domain-pseudokinase)	83
PKMYT1	100	RSK4(Kin.Dom.2-C-terminal)	89	TYRO3	97
PKN1	95	S6K1	87	ULK1	81

Gene Symbol	%Ctrl @ 10000nM
ULK2	95
ULK3	74
VEGFR2	85
VRK2	55
WEE1	92
WEE2	100
WNK1	76
WNK3	96
YANK1	79
YANK2	86
YANK3	100
YES	100
YSK1	100
YSK4	4.5
ZAK	89
ZAP70	69

%Ctrl Legend

$0 \leq x < 1$	$.1 \leq x < 1$	$1 \leq x < 10$	$10 \leq x < 35$	$x \geq 35$
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Table 4. Data collection and refinement statistics

Data collection		JNK3/SR12055 complex
Space group		P2 ₁ 2 ₁ 2 ₁
Wavelength (Å)		0.97856
Resolution (Å)		59.24-1.79 (2.00-1.79)
Total reflections		171,287
Unique reflections		39,652 (11,135)
Average I/σ(I)		17.1 (3.2)
R _{merge} ^a		0.049 (0.424)
Redundancy		4.3 (4.3)
Completeness (%) ^b		99.5 (99.8)
Refinement		
Resolution range (Å)		59.24-1.79 (1.84-1.79)
No. of reflections in working set		37,442 (2,703)
No. of reflections in test set		1,933 (166)
^a R _{merge} =	No. of protein atoms	2,776
^b R _{cryst} =	No. of water molecules	353
^c R _{free}	No. of heteroatoms	411
excluded	R _{cryst} ^b	19.49 (22.27)
resolution	R _{free} ^c	22.66 (23.38)
Values in		
resolution		
	R.m.s. bond length (Å)	0.010
	R.m.s. bond angle (°)	1.05
	Average B value (Å ²)	39.42

$\sum | \langle I \rangle - I | / \sum \langle I \rangle$.
 $\sum | |F_o| - |F_c| | / \sum |F_o|$.
 calculated with 5% of all reflections
 from refinement stages using high-
 data.
 parentheses refer to the highest
 shells.