

Supplementary Methods

Nematode strains. *C. elegans* worms were cultured as described by Brenner (1974) at 20°C unless otherwise noted. *lon-2(e678)*, *egl-1(n1084n3082)*, *ced-1(e1735)*, *ced-3(n717)*, *ced-9(n1950)*, *mek-2(n1989)*, *mrt-2(e2663)*, *rad-5(mn159)*, *rad-51(ok713)*, *cep-1(gk138)*, and *cep-1(w40)* were provided by the Caenorhabditis Genetics Center. *hus-1(op241)* was isolated as described previously¹.

Primers for strain construction.

	<u>Mutated allele (MT)</u>	<u>Wild-type allele (WT)</u>
<i>abl-1(ok171)</i>	EL1/ER1	EL1/42a 5' -gggaatctcttcattctcgg- 3'
<i>egl-1(n1084n3082)</i>	5' -gtacgggatgctgctcagag- 3' /5' -cacacccaacattcacacc-3'	5' -gtagccgatgctgctgatct- 3' /5' -cacacccaacattcacacc-3'
<i>ced-3(n717)</i>	5' -cggcttctttctccacacttgta- 3' /5' -ggcgcacaccccattgcattg- 3'	5' -cggcttctttctccacacttgcta- 3' /5' -ggcgcacaccccattgcattg- 3'
<i>ced-9(n1950)</i>	5' -caatgtccaatgtcttctga- 3' /5' -gcttagagcatattccgggg- 3'	5' -caatgtccaatgtcttctgg- 3' /5' -gcttagagcatattccgggg- 3'
<i>cep-1(gk138)</i>	5' -cagggtgagttggcgtagg- 3' /5' -aattggtacagcgacttcttca- 3'	5' -ttctccacgcatacatcaacaagtctg- 3' /5' -ttcatcgcttctgtagcggtataaa- 3'
<i>mek-2(n1989)</i>	p33a 5' -atgcgattttggatactt- 3, /p34 5' -tgtatagtgggatcctgt- 3'	p33 5' -atgcgattttggatgctc- 3 /p34 5' -tgtatagtgggatcctgt- 3'

Northern blot. Total and polyA-enriched RNA were isolated from wild-type and *abl-1(ok171)* worms using the RNAwiz reagent and the MicroPoly(A)Purist mRNA purification kit, respectively (Ambion). The 684 bp DNA fragment corresponding to *abl-1* cDNA sequence 1-684 was cloned into pcDNA3, which was linearized with the restriction enzyme NotI, and anti-sense RNA probes were generated using SP6 polymerases. Probes were labeled with [γ - 32 P]UTP using Strip-EZ RNA kit (Ambion) according to the manufacturer's instructions.

In situ hybridization. Synchronized worms were washed twice with PBS and transferred to a poly-L-lysine coated 3-well slide. The slide was covered with a cover slip and frozen by placing on dry ice. The cover slip was then quickly peeled off and the slide was immediately immersed in 100% methanol at -20°C for 5 minutes. Re-hydration and dehydration were performed as described by Motohashi and co-workers². The 578 bp DNA fragment corresponding to *abl-1* cDNA 1,631–2,208 was cloned into pcDNA3 linearized with NotI, and anti-sense RNA probes were generated using SP6 polymerases. Probes were labeled with digoxigenin-UTP according to manufacturer's instructions (Boehringer-Mannheim). The 630 bp RNA probe corresponding to the *C. elegans glp-1* cDNA sequence was used as a positive control. The fixed slides were rinsed in water and PBS for 10 minutes and digested with Proteinase K 10 $\mu\text{g}/\text{ml}$ for 10 minutes at room temperature. Pre-hybridization was performed for 30 minutes at 48°C in 50% deionized formamide and 2X SSC. The hybridization solution consisted of 50% deionized formamide (v/v), 10% dextran sulphate (50% stock solution), 2X SSC, 1% SDS and 0.25 mg/ml of herring sperm DNA (10 mg/ml). Hybridization was performed overnight at 48°C applying 10 pmol/L of digoxigenin-labeled riboprobe in 50 μl of hybridization buffer per section under a cover slip. Slides were washed in pre-warmed 2 X SSC for 10 minutes and then incubated in 10% normal sheep serum diluted in the

buffer (Tris-HCl 2M, NaCl 5M, pH 7.5) and successively in the same buffer with anti-digoxigenin-AP antibody (Boehringer-Mannheim) at a dilution of 1:2000 for 1 hour at room temperature. Visualization was accomplished by nitro-blue tetrazolium 5-bromo-4-chloro-3-indolyl-phosphate. The slides were counterstained with methyl green and mounted.

RACE analysis. For analysis of the 5' end of *C. elegans abl-1*, two rounds of RACE were performed using a RACE kit (Gibco-BRL) following the manufacturer's instructions. Briefly, for the first round, total RNA was reverse transcribed to cDNA using a primer, 5'-GCAATTGTGCAGTCATGTCG-3', derived from sequence within exon 8, and amplified using AAP primer (Gibco-BRL) and 5'-CCAGTGATTCCACTGCCTAG-3' primer derived from sequence within exon 7. Nested PCR amplification subsequently was performed using UAP (Gibco-BRL) and 5'-TATCCAAAGAGTTGTACGGA-3' primers. The second round of RACE was performed using the primer 5'-ATGTGAAGAATGTCTGGATT-3' derived from exon 11 for reverse transcription, AAP and 5'-TCGTTCTCTAACGTTAGACC-3' primers for amplification and UAP and 5'-TGCACCTCGTCGTTCAAGGA-3' primers for nested PCR. PCR products were cloned directly into pCRII-TOPO (Invitrogen) and 30 individual clones for each RACE round were selected and sequenced.

RT-PCR. Two micrograms of total RNA from mixed-stages of wild-type worms were reverse transcribed to cDNA using pd(N₆) random hexamers (Amersham-Pharmacia Biotech). Full-length transcripts of *abl-1* were amplified using the SL1 primer 5'-GGTTTAATTACCCAAGTTTGAG-3' and 5'-TCAGCGATCCACCAGCCTCAT-3' primer containing the TGA stop codon derived from sequence within exon 18. The PCR reactions were performed using *Pfu* high fidelity DNA polymerase (Stratagene) in a

thermal cycler for 30 cycles in a final volume of 50 μ l following the manufacturer's instructions.

Mammalian expression vectors. Epitope-tagged *abl-1A* was generated by PCR using 5'-GGATCCATGGGTCATTCACAT-3'/5'-

CCCGGGTCAGCGATCCACCAGCCTCAT -3'. The resulting PCR product was sequenced and cloned into the mammalian expression vector pCMV2B (Stratagene) digested with BamHI and ApaI restriction enzymes. Retrovirus vectors pLPC and pLPC-h-p53 were kindly provided by Dr. Scott Lowe.

Cell culture and gene transfection. HEK293 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum and 2 mM glutamine at 37°C in 5% CO₂. Transfections were performed using Fugene 6 according to the manufacturer's instructions (Roche). HEK293 cells stably-expressing *C. elegans abl-1A* were generated by transfecting pCMV2B-*abl-1*-Flag vector and selecting with 400 μ g/ml G418 (Gibco). Stably-transfected cells containing the empty vector pCMV2B were generated concomitantly. Protein expression was verified by immunoblotting cell lysates with M2 anti-Flag monoclonal antibody (Sigma) for ABL-1 and anti-human p53 polyclonal antibody (Santa Cruz) for h-p53.

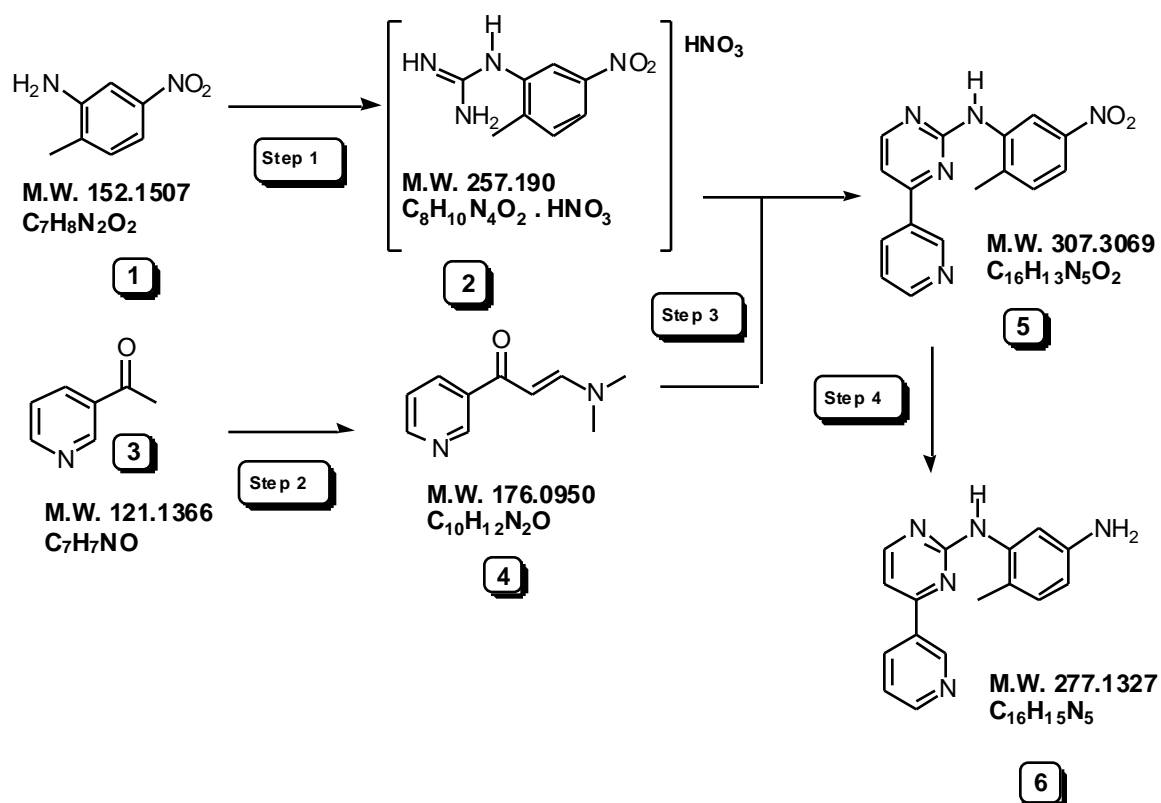
Ionizing radiation and apoptosis measurements. 1×10^6 HEK293 cells stably-expressing ABL-1 were plated onto 6-well plate and after 24 hours were transiently-transfected with 1.25 μ g pLPC or pLPC-h-p53 using Lipofectamine Reagent (Invitrogen) according to the manufacturer's instructions. After 48 hours, cells were irradiated at 0–20 Gy using a Cs¹³⁷ source, harvested 36 hours post-irradiation, fixed in 10% formalin, and resuspended in 50 μ l of 42 μ M bis-benzimide (Sigma) in PBS. >400

cells were examined for apoptosis using Zeiss Axioplan 2 with a fluorescein filter as described by Gulbins *et al*³.

The synthesis of WB-BC-20 and WB-BC-15

1. Preparation of N-(5-amino-2-methylphenyl)-4-(3-pyridyl)-2-pyrimidine-amine

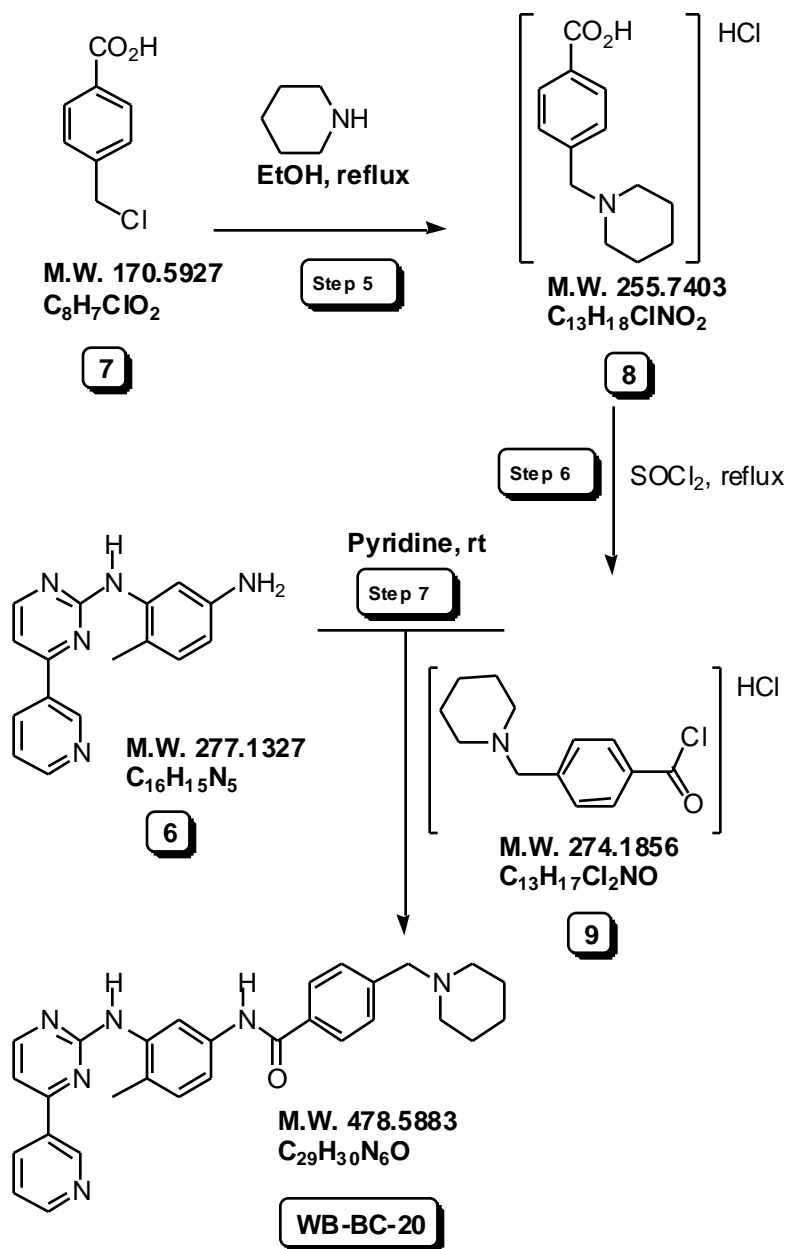
Preparation of compound **6**, N-(5-amino-2-methylphenyl)-4-(3-pyridyl)-2-pyrimidine-amine, was adapted from the US Patent 5521184 (1996) titled: Pyrimidine Derivatives and processes for the preparation thereof by Jurg Zimmerman.



In brief, 9.1ml (0.13 mol) of 65% nitric acid was added in a dropwise fashion over a 15 minute period to a suspension of 20.0 grams (0.13 mol) of 2-amino-4-nitrotoluene **1** in 50

ml of absolute ethanol. Upon completion of this addition, a solution of 8.32 grams (0.198 mol) of cyanamide in 8.3 ml of water was added in a dropwise fashion over a 5 minute period to give a deep brown colored solution. This was then refluxed for 24 hours. Upon completion, the solution was cooled to 0°C to give a precipitate which was collected and washed with four 100 ml portions of ethanol:diethyl ether (1:1) to give 2-methyl-5-nitrophenyl-guanidine nitrate 2. The 2-methyl-5-nitrophenyl-guanidine nitrate 2 was added to a solution of 170 grams (0.96 mol) of 3-(dimethylamino-1-3-pyridyl)-2-propene-1-one 4 in 2000 ml of isopropanol. 4 was prepared by the treatment of 5 grams of 3 3-acetylpyridine with 50 ml of dimethyl foramide diethyl acetal for 1 hour at 110°C, which was then cooled down to room temperature and the solution concentrated under reduced pressure via the rotary evaporator to give a orange colored solid which was recrystallized to give 4. This was then followed by the addition of 42.5 grams (1.06 mol) of sodium hydroxide and the resulting suspension refluxed for 12 hours. Once completed, the solution was cooled down to 0°C and the resulting precipitate filtered and sequentially washed with 2000 ml of isopropanol followed by three 400 ml portions of methanol and dried to give N-(2-methyl-5-nitrophenyl)-4-(3-pyridyl)-2-pyrimidine-amine 5. 5 was dissolved into 7150 ml of ethyl acetate to which was added 14.3 grams of 10% palladium on carbon and placed under an atmosphere of hydrogen at atmospheric pressure for 10 hours. At the end of this period of time, the palladium on carbon was filtered from the reaction mixture using a Whatman 0.45 mm PTFE disposable filter funnel to give a brilliant yellow colored filtrate. The ethyl acetate was removed under reduced pressure via the rotary evaporator to give a yellow crystalline solid which was recrystallized from dichloromethane to give 8.911 grams of N-(5-amino-2-methylphenyl)-4-(3-pyridyl)-2-pyrimidine-amine 6, a yield of 69.1%.

2. Preparation of N-{5-[4-(4-(piperidinomethyl)benzoylamido]-2-methylphenyl)}-4-(3-pyridyl)-2-pyrimidine-amine (WB-BC-20)

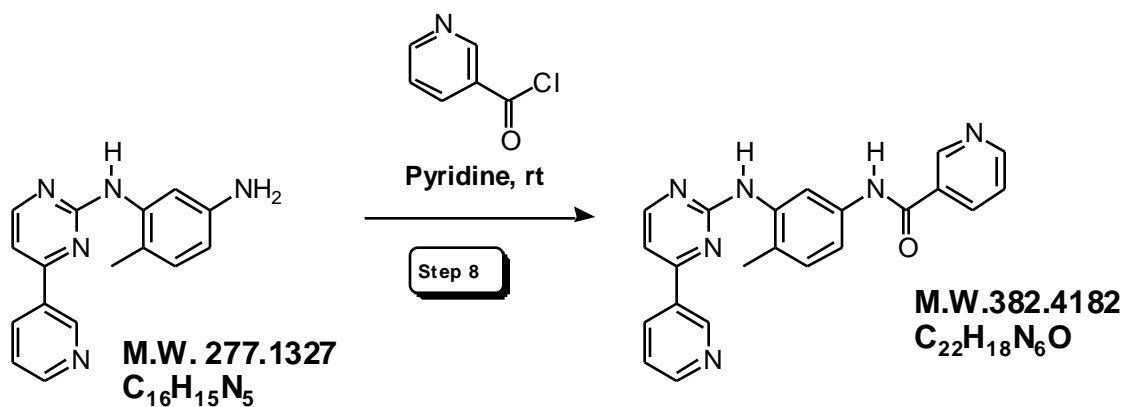


Preparation of 4-(piperidinomethyl)benzoyl chloride hydrochloride **9** is described in the US Patent 4,623,486 (1986) titled: [4-substituted benzyloxy]-N-substituted-2H-1,2-benzothiazine-3-carboxamide-1,1-dioxides having anti-arthritis activity by Joseph G.

Lombardino. This was performed exactly as stated within the patent. To a suspension of 17.06 grams (0.1 mol) of 4-(chloromethyl)benzoic acid **7** in 150 ml of absolute ethanol under argon at room temperature was added a solution of 37.47 grams (0.44 mole) of piperidine in 50 ml of anhydrous ethanol in a dropwise fashion over 15 minutes. Once completed, the resulting solution was brought to reflux for 16 hours. Once completed, the resulting solution was cooled to room temperature and the solvents removed under reduced pressure via the rotary evaporator to give a thick solid. This was then partitioned between 200 ml of diethyl ether and 200 ml of 3 N sodium hydroxide. The separated aqueous layer was washed with two additional 200 ml portions of diethyl ether, after which the aqueous layer was cooled down to 0°C and acidified with concentrated hydrochloric acid to give a thick solid mass. This was then vacuum filtered and dried under house vacuum (15mmHg) to give a solid which was then titrated with 150 ml of boiling isopropanol followed by filtration to give 8.64 grams of 4-(piperidinomethyl) benzoic acid hydrochloride **8** (33.8% yield). This was then directly used in the next step which consisted of treatment of 8.64 grams (0.034 mol) of 4-(piperidinomethyl)benzoic acid hydrochloride with 55 ml of thionyl chloride (89.65 grams, 0.753 mol) under argon to give a tan colored suspension, which was then refluxed for 3 hours. Once completed, the suspension was cooled to room temperature and the thionyl chloride removed under reduced pressure via the rotary evaporator to give a light brown solid. This solid was washed with anhydrous benzene followed by anhydrous dichloromethane to give a white solid 4-(piperidinomethyl)benzoyl chloride hydrochloride **9**. Treatment of 0.5 grams (1.8mmol) of N-(5-amino-2-methylphenyl)-4-(3-pyridyl)-2-pyrimidine-amine **6** with 0.5936 grams (2.2mmole-1.2 equivalents) of 4-(piperidinomethyl)benzoyl chloride hydrochloride **9** in 20 ml of pyridine was at room temperature for 24 hours. Once completed, the pyridine was removed under reduced

pressure via the rotary evaporator to give a yellow oil. This was partitioned between 100 ml of 10% NH₄OH/sat. sodium chloride and 10% methanol in dichloromethane. The layers were separated and the organic layer concentrated under reduced pressure via the rotary evaporator to a yellow solid mass. This was taken up into 5% methanol (7N NH₃) in dichloromethane and subjected to chromatotron chromatography eluting with the same solvent system to give 0.6174 grams N-{5-[4-(4-(piperidinomethyl)benzoylamido]-2-methylphenyl)-4-(3-pyridyl)-2-pyrimidine-amine as a white crystalline compound in 71.5% yield.

3. Preparation of N-(5-nicotinamide-2-methylphenyl)-4-(3-pyridyl)-2-pyrimidine-amine (WB-BC-15)



Treatment of 0.5 grams (1.8 mmol) of N-(5-amino-2-methylphenyl)-4-(3-pyridyl)-2-pyrimidine-amine **6** with 0.3854 grams (2.2 mmol-1.2 equivalents) of nicotinoyl chloride hydrochloride in 20 ml of pyridine was at room temperature for 24 hours. Once completed, the pyridine was removed under reduced pressure via the rotary evaporator to give a yellow oil. This was partitioned between 100 ml of 10% NH₄OH/sat. sodium chloride and 10% methanol in dichloromethane. The layers were separated and the

organic layer concentrated under reduced pressure via the rotary evaporator to a yellow solid mass. This was taken up into 5% methanol (7N NH₃) in dichloromethane and subjected to chromatotron chromatography eluting with the same solvent system to give 0.4177 grams N-(5-nicotinamide-2-methylphenyl)-4-(3-pyridyl)-2-pyrimidine-amine as a white crystalline compound in 60.5% yield.

Supplementary References

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4. Wisniewski, D. et al. Characterization of potent inhibitors of the Bcr-Abl and the c-kit receptor tyrosine kinases. *Cancer Res* **62**, 4244-4255 (2002).