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SUPPORTING INFORMATION

Title: Electron-Density Determination of Electrophilic Building Blocks as Model Compounds for Protease Inhibitors

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Table S1: Crystallographic data

chemical formula	$C_{12}H_{12}O_6N_2$
M (g/mol)	280.24
crystal system	triclinic
space group	$P\bar{1}$
Z	4
a (Å)	8.013(2)
b (Å)	13.312(3)
c (Å)	13.652(2)
α (°)	105.984(7)
β (°)	106.137(12)
γ (°)	107.137(9)
V (Å^3)	1231.36
ρ (g/cm^3)	1.51
F(000)	584
μ (mm^{-1})	0.07
crystal size (mm^3)	0.45 × 0.25 × 0.15
colour	red
T (K)	9
λ (Å)	0.560
$\sin\theta/\lambda_{max}$ (Å^{-1})	1.02
max 2θ (°)	69.67
number of collected reflections	163095
number of unique reflections	18463
number of reflections with I > 3σ	15032
completeness (%)	83.9
R_{int} (%)	6.70
spherical refinement	
R(F) (%)	4.08
wR(F^2) (%)	10.44
GooF	1.03
multipole refinement	
ratio reflections / parameters	24.56
R(F) (%)	2.71
wR(F)	2.41
GooF	1.85

Refinement strategy:

The second molecule of the asymmetric unit was completely constrained to the first molecule. In the first molecule the three non-hydrogen atoms C1, C2 and N1 of the aziridine ring were modelled without fitting them to a local symmetry pattern because this ring was examined in detail as electrophilic centre of the protease inhibitor model compound and even small changes in the electron charge distribution at this site are of interest and importance. The atoms C4, C7, C11, C12, O2, O3 and O4 were chemically constrained to the corresponding atoms in the other methoxycarbonyl chain or the aryl group. Local mirror symmetry was applied to the atoms C5 and C6 of the aryl group whereas mm2 symmetry was applied to the ring atoms C3 and C8. Three-fold symmetry was used for the methyl groups (C10) and mirror symmetry for all atoms in the ester groups and the nitro group (C9, O1, O5, O6 and N2). κ was refined, κ' and higher order of κ for non-hydrogen atoms were set to a value of 1.0, all κ values for hydrogen atoms were set to 1.2.

Table S2: Density (ρ in e/Å^3), Laplacian ($\nabla^2\rho$ in e/Å^5) and ellipticity (ε) of bond and ring critical points; theoretical values from geometry optimisations.

bond/ ring	compound	ρ_{exp}	ρ_{theor}	$\nabla^2\rho_{exp}$	$\nabla^2\rho_{theor}$	ε_{exp}	ε_{theor}
N1 -C1	aziridine **1**	1.762(14)	1.765	-10.2(1)	-13.0	0.45	0.34
O7-C1	oxirane **2**		1.690		-8.9		0.52
N1-C2	aziridine **1**	1.770(14)	1.766	-8.6(1)	-12.2	0.56	0.34
O7-C2	oxirane **2**		1.733		-10.1		0.50
N1-H1N	aziridine **1**	2.135(10)	2.329	-23.6(1)	-40.1	0.03	0.02
C1-C2	aziridine **1**	1.595(12)	1.557	-9.4(1)	-9.3	0.70	0.49
	oxirane **2**		1.644		-11.5		0.27
	olefin **3**		2.253		-23.2		0.31
C1-C3	aziridine **1**	1.815(14)	1.772	-18.1(1)	-15.7	0.09	0.07
	oxirane **2**		1.779		-15.8		0.08
	olefin **3**		1.836		-16.8		0.08
C1-H1	aziridine **1**	1.791(13)	1.869	-18.4(1)	-22.4	0.05	0.03
	oxirane **2**		1.943		-24.2		0.03
C2-C9	aziridine **1**	1.835(11)	1.771	-16.8(1)	-15.8	0.06	0.06
	oxirane **2**		1.745		-15.2		0.08
	olefin **3**		1.762		-15.7		0.03
C2-C11	aziridine **1**	1.804(12)	1.759	-15.6(1)	-15.2	0.07	0.12
	oxirane **2**		1.719		-14.8		0.10
	olefin **3**		1.767		-15.4		0.13
O1-N2	aziridine **1**	3.207(17)	3.318	-9.5(1)	-24.1	0.05	0.11
	oxirane **2**		3.466		-27.7		0.11
	olefin **3**		3.377		-25.5		0.11
O2-N2	aziridine **1**	3.290(22)	3.343	-12.3(1)	-24.8	0.09	0.11
	oxirane **2**		3.464		-27.6		0.11
	olefin **3**		3.361		-25.1		0.11
O3-C9	aziridine **1**	2.814(19)	2.814	-13.6(2)	-4.2	0.13	0.10
	oxirane **2**		2.890		-2.0		0.09
	olefin **3**		2.900		-1.9		0.09
O4-C9	aziridine **1**	2.285(15)	2.071	-34.3(1)	-11.3	0.12	0.01
	oxirane **2**		2.117		-9.7		0.01
	olefin **3**		2.130		-9.5		0.01
O4-C10	aziridine **1**	1.644(11)	1.568	-17.7(1)	-6.7	0.00	0.01
	oxirane **2**		1.531		-7.0		0.03
	olefin **3**		1.521		-7.7		0.03
O5-C11	aziridine **1**	2.794(15)	2.781	-15.5(1)	-5.5	0.14	0.07
	oxirane **2**		2.908		-1.4		0.08
	olefin **3**		2.886		-2.6		0.08
O6-C11	aziridine **1**	2.314(15)	2.130	-35.1(1)	-10.6	0.10	0.01
	oxirane **2**		2.194		-8.2		0.01
	olefin **3**		2.128		-9.4		0.00
O6-C12	aziridine **1**	1.633(11)	1.532	-20.1(1)	-6.5	0.00	0.00
	oxirane **2**		1.501		-7.0		0.03
	olefin **3**		1.524		-7.1		0.03
N2-C8	aziridine **1**	1.779(15)	1.757	-19.8(1)	-16.3	0.18	0.12
	oxirane **2**		1.746		-16.2		0.12
	olefin **3**		1.751		-16.3		0.12
C3-C4	aziridine **1**	2.207(14)	2.060	-24.3(1)	-20.2	0.15	0.19
	oxirane **2**		2.078		-20.7		0.18
	olefin **3**		2.051		-20.2		0.18
C3-C5	aziridine **1**	2.208(11)	2.064	-24.3(1)	-20.4	0.15	0.19
	oxirane **2**		2.110		-21.3		0.20
	olefin **3**		2.103		-21.3		0.19

C4-C7	aziridine **1**	2.119(10)	2.093	-22.4(1)	-20.9	0.17	0.19
	oxirane **2**		2.144		-22.0		0.20
	olefin **3**		2.131		-21.7		0.20
C4-H4	aziridine **1**	1.764(9)	1.928	-17.0(1)	-24.1	0.02	0.02
	oxirane **2**		1.929		-24.1		0.02
	olefin **3**		1.922		-23.8		0.02
C5-C6	aziridine **1**	2.126(10)	2.101	-22.7(1)	-21.0	0.17	0.21
	oxirane **2**		2.079		-20.6		0.20
	olefin **3**		2.140		-22.0		0.20
C5-H5	aziridine **1**	1.765(9)	1.909	-17.0(1)	-23.5	0.02	0.02
	oxirane **2**		1.909		-23.5		0.02
	olefin **3**		1.913		-23.6		0.02
C6-C8	aziridine **1**	2.138(11)	2.084	-21.2(1)	-20.8	0.22	0.21
	oxirane **2**		2.163		-22.5		0.21
	olefin **3**		2.144		-22.1		0.21
C6-H6	aziridine **1**	1.805(9)	1.920	-17.6(1)	-24.0	0.02	0.01
	oxirane **2**		1.920		-24.0		0.01
	olefin **3**		1.920		-24.0		0.01
C7-C8	aziridine **1**	2.159(14)	2.116	-21.9(1)	-21.5	0.22	0.20
	oxirane **2**		2.116		-21.5		0.21
	olefin **3**		2.143		-22.1		0.20
C7-H7	aziridine **1**	1.806(9)	1.923	-17.6(1)	-24.0	0.02	0.01
	oxirane **2**		1.923		-24.0		0.01
	olefin **3**		1.921		-24.0		0.01
C10-H10A	aziridine **1**	1.840(6)	2.014	-18.5(1)	-26.1	0.11	0.04
	oxirane **2**		2.013		-26.0		0.04
	olefin **3**		2.002		-25.7		0.05
C10-H10B	aziridine **1**	1.838(6)	2.008	-18.4(1)	-25.9	0.12	0.04
	oxirane **2**		2.015		-26.1		0.04
	olefin **3**		2.021		-26.2		0.04
C10-H10C	aziridine **1**	1.839(6)	2.014	-18.5(1)	-26.1	0.11	0.04
	oxirane **2**		2.007		-26.0		0.05
	olefin **3**		2.007		-26.0		0.05
C12-H12A	aziridine **1**	1.829(6)	2.017	-18.2(1)	-26.3	0.12	0.04
	oxirane **2**		2.010		-26.06		0.05
	olefin **3**		2.011		-26.09		0.05
C12-H12B	aziridine **1**	1.832(6)	2.014	-18.2(1)	-26.1	0.12	0.04
	oxirane **2**		2.016		-26.1		0.04
	olefin **3**		2.012		-26.0		0.04
C12-H12C	aziridine **1**	1.830(6)	2.014	-18.1(1)	-26.1	0.12	0.04
	oxirane **2**		2.015		-26.1		0.05
	olefin **3**		2.012		-26.0		0.04
phenyl ring	aziridine **1**	0.231(19)	0.149	3.1(1)	3.8		
	oxirane **2**		0.149		3.8		
	olefin **3**		0.147		3.8		
three-membered ring	aziridine **1**	1.416(18)	1.379	6.6(1)	4.8		
	oxirane **2**		1.394		7.4		

Table S3: Experimental atomic charges (Q_{tot}) and atomic volumes (V_{tot}) of the aziridine ring and environment.

atom	Q_{tot} (e)	V_{tot} (Å^3)
O1	-0.26	16.15
O2	-0.27	16.27
O3	-1.17	18.64
O4	-1.08	13.65
O5	-1.15	17.84
O6	-1.13	14.38
N1	-0.89	14.61
N2	0.29	7.98
C1	0.10	9.01
C2	0.11	7.39
C3	0.15	8.89
C4	-0.11	12.27
C5	-0.11	12.55
C6	-0.10	12.06
C7	-0.09	12.89
C8	0.11	9.70
C9	1.67	4.53
C10	0.23	10.22
C11	1.72	5.05
C12	0.21	11.26
H1	0.16	5.53
H4	0.13	6.37
H5	0.13	5.61
H6	0.15	5.35
H7	0.16	6.11
H10A	0.07	6.61
H10B	0.07	5.94
H10C	0.07	5.64
H12A	0.07	6.00
H12B	0.07	6.14
H12C	0.07	6.48
H1N	0.37	3.24
sum	-0.18	304.55
target	0.00	309.61

Syntheses and analytical data of compounds 1, 2, 3, 4b, 5:

1: Dimethyl *p*-nitrobenzylidenemalonate (**3**) (1.73 g, 6.52 mmol) and free *S,S*-diphenylsulfimine (2.00 g, 9.94 mmol) were refluxed in 50 ml dichloromethane for 8 h. The solvent was then removed *in vacuo*, the resulting residue was suspended in a mixture of cyclohexane and ethyl acetate (5:1; R_f = 0.26) and filtered off. Purification by column chromatography (silica gel 60, cyclohexane/ethyl acetate 1:1) yielded 1.58 g (5.54 mmol, 86 %) colourless crystals, which developed a deep-red colouration upon exposure to light, m.p. 128–129 °C. ^{1}H-NMR (400.13 MHz, $CDCl_3$, 300 K): δ = 2.55–2.57 (d, 1 H, *J* = 9.9 Hz, Ar-CHN***H***C(CO_2CH_3)$_2$), 3.51 (s, 3H, -CO_2C***H***$_3$), 3.78–3.81 (d, 1 H, *J* = 9.6 Hz, Ar-C***H***NHC(CO_2CH_3)$_2$), 3.89 (s, 3 H, -CO_2C***H***$_3$), 7.51–7.53 (d, 2 H, *J* = 8.6 Hz, Ar-***H***), 8.16–8.18 (d, 2 H, *J* = 8.8 Hz, Ar-***H***).

2: A solution of dimethyl *p*-nitrobenzylidenemalonate (**3**) (1.33 g, 5.00 mmol) in acetonitrile was added to neutral aluminium oxide (aluminium oxide 90 active, neutral, activity level I). Then, 3.36 ml aqueous sodium hypochlorite solution (25 %) were added slowly at room temperature. After stirring the mixture for 2 h the solution was filtered off and the solvent was removed *in vacuo*. Again an equal amount (see above) neutral aluminium oxide and acetonitrile was added, as well as sodium hypochlorite solution. This step was repeated until the reaction was completed. Purification by extraction with dichloromethane yielded 509 mg (1.81 mmol, 36 %) white crystals, m.p. 131–133 °C. ^{1}H-NMR (400.13 MHz, $CDCl_3$, TMS, 300 K): δ = 3.52 (s, 3 H, -CO_2C***H***$_3$), 3.83 (s, 3 H, -CO_2C***H***$_3$), 4.59 (s, 1 H, -C***H***OC(CO_2CH_3)$_2$),7.45–7.48 (m, 2 H, Ph-***H***), 8.15–8.17 (m, 2 H, Ph-***H***). ^{13}C-NMR (100.62 MHz, $CDCl_3$, 300 K): δ = 52.9 (-CO_2***C***H_3), 53.8 (-CO_2***C***H_3), 61.0 (-***C***HOC(CO_2CH_3)$_2$), 63.0 (-CHO***C***(CO_2CH_3)$_2$), 123.7 (C3-Ph), 127.3 (C2-Ph), 139.0 (C1-Ph), 148.5 (C4-Ph), 163.3 (-***C***O_2CH_3), 165.1 (-***C***O_2CH_3). ESI MS, m/z (%): 304 (100) [M^+ + Na], 281 (51) [M^+ + 1].

3: *p*-Nitrobenzaldehyde (5.00 g, 33.1 mmol) and dimethyl malonate (4.37 g, 33.1 mmol) were solved in 50.0 ml toluene, piperidine (235 mg, 0.27 ml, 3.31 mmol) was added and the solution was refluxed for about 4 h. When no more water could be collected in the water trap, the mixture was allowed to cool down. The toluene layer was washed with half-saturated sodium chloride solution, dried with sodium sulphate and the solvent was removed in vacuo. Recrystallisation in ethanol yielded 6.18 g (23.3 mmol, 70%) yellow crystals, m.p. 136–137 °C. ^{1}H-NMR (400.13 MHz, $CDCl_3$, 300 K): δ = 3.83 (s, 3 H, -$CO_2C\underline{\boldsymbol{H}}_3$), 3.87 (s, 3 H, -$CO_2C\underline{\boldsymbol{H}}_3$), 7.56–7.58 (d, 2 H, *J* = 8.6 Hz, Ph-H), 7.79 (s, 1 H, -C$\underline{\boldsymbol{H}}$C(-$CO_2CH_3)_2$), 8.22–8.24 (d, 2 H, *J* = 8.6 Hz, Ph-$\underline{\boldsymbol{H}}$).

4b: The synthesis of the open-chain compound **4b** was carried out corresponding to the reaction of **1** (1 mmol) with acetic anhydride described previously.[32] ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm) = 1.23 (t, 6 H, *J* = 7.3 Hz), 2.48 (m, 2 H), 2.64 (m, 1 H), 2.96 (m, 1 H), 3.67 (s, 3 H), 3.76 (s, 3 H), 4.22 (s, 1 H), 7.56 (s, 1 H), 7.70 (d, 2 H, *J* = 8.5 Hz), 8.26 (d, 2 H, *J* = 8.5 Hz); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm) = 8.67, 8.85, 26.45, 27.19, 53.02, 53.07, 58.98, 79.34, 124.00, 127.65, 141.91, 148.51, 165.52, 166.22, 172.62, 173.97; MS (EI, 70 eV) *m/z* (%): 410.4 (1.3) [M^+], 57.1 (100). Yield: 246 mg, 60%.

5: 0.75 mmol aziridine **1** and an equimolar amount of benzyl mercaptane were heated in 10 ml toluene at 80°C for 6 h. After removal of the solvent the reaction mixture was chromatographed on silica gel 60, eluent cyclohexane: ethyl acetate 4:1. ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm) = 2.94 (m, 1 H, NH), 3.55 (d, 1 H, *J* = 14 Hz, -S-CH_2), 3.73 (s, 3 H), 3.77 (s, 3 H), 3.81 (d, 1 H, *J* = 14 Hz, -S-CH_2), 4.28 (d, 1 H, *J* = 9 Hz), 4.89 (d, 1 H, *J* = 7 Hz), 7.25 (m, 5 H), 7.56 (d, 2 H), 8.17 (d, 2 H) (*J* = 8.5 Hz, each); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm) = 53.02, 53.11, 53.27, 65.15, 74.38, 123.91, 127.04, 128.11, 129.69, 130.49, 134.55, 141.90, 148.50, 165.05, 166.25; MS (FAB, 4-nitrobenzyl alcohol) *m/z* (%): 404.5 (20) [M^+], 154.1 (100). Yield: 61 mg, 20%.