Synthesis and Antimicrobial Activity of Some New Anilino Benzimidazoles

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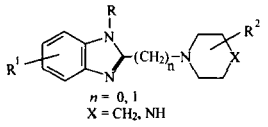
Summary

A series of 2-(anilino or 2,6-dichloroanilino)-1,5(6)-disubstituted-1*H*-benzimidazoles (**1–13**) were prepared by reaction of several 2-chloro- or 2-chloromethyl-1*H*-benzimidazoles with aniline derivatives. The prepared compounds were screened for their *in vitro* antibacterial and antifungal activities. Compounds **2, 8**, and **9** exhibited the best activity.

Introduction

In our previous papers[1,2] we reported the synthesis and antimicrobial evaluation of 2-(substituted-piperidinyl- or



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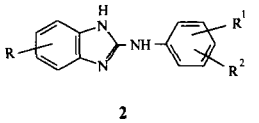

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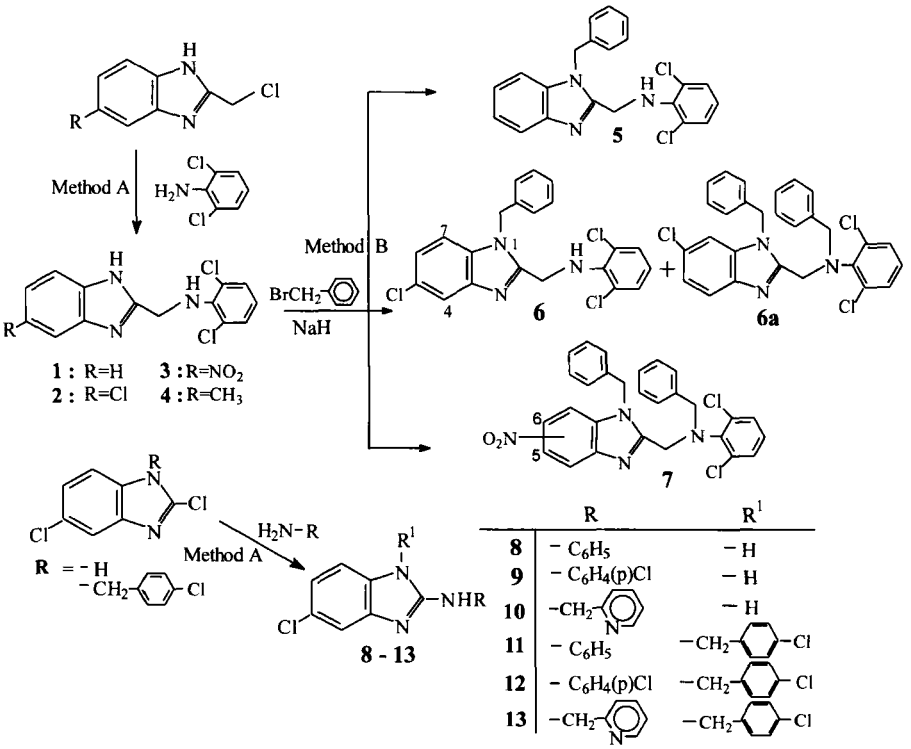
piperazinyl)benzimidazoles **1.** In the context of our work on these derivatives, we found a patent [3] concerning the synthesis of anilinobenzimidazoles **2**, some of which exhibit potent activity against *Staphylococcus aureus*, as determined by the tube dilution method.

This result encouraged us to prepare new derivatives of **2,** and in order to investigate the influence of N^1-alkylation on the antimicrobial activity some N^1-substituted anilinobenzimidazoles were prepared.

Results and Discussion

2-Chloromethyl or 2-chloro-5(6)-substituted-1*H*-benzimidazoles were prepared according to ref.[1]. Substitution of these precursors with anilines or other amines and benzyl bromide gave **1–13** by the methods as A and B (Scheme 1, Table 1). In the final stage of preparing **6** and **7**, due to the tautomerism of benzimidazole, alkylation occurs at the 1-position as well as the 3-position; two regioisomers are formed as a mixture of 1,5- and 1,6-disubstituted 2-(2,6-dichloroanilino)benzimidazoles [4]. The 1,5-isomer was isolated as **6** and the other isomer, **6a**, was also obtained as the *N*-aralkylated product. In the NMR spectra, 4-H appears at lower field strength than 7-H due to the anisotropic effect of the double bond in the imidazole ring[1]. 4-H in **6** resonates at $\delta = 7.69$ as a doublet ($J = 1.45$Hz), well separated from 7-H $\delta = 7.56$ as a doublet ($J = 8.61$Hz). On the other hand, 4-H in **6a** appears at $\delta = 7.66$ (doublet, $J_{4,5} = 8.5$Hz). Since the signal of H-7 overlaps with other signals, this proton does not appear clearly.

These ^{1}H-NMR findings confirmed the structural elucidation of the **6** and **6a** regioisomers with certainty. In addition the X-ray analysis data of **6a** also support these findings. Fig.1 shows the molecular conformation of **6a** and depicts the numbering scheme. The fractional coordinates and mean temperature factors with estimated standard deviations for



	R	R^1
8	$-C_6H_5$	-H
9	$-C_6H_4$(p)Cl	-H
10	$-CH_2$-(N)	-H
11	$-C_6H_5$	$-CH_2$-(Cl)
12	$-C_6H_4$(p)Cl	$-CH_2$-(Cl)
13	$-CH_2$-(N)	$-CH_2$-(Cl)

Scheme 1. General synthesis of **1–13**.

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Table 1. Yields, physical and spectral data of compounds **1–13.**

No	Yield %	Mp (°C)	^{1}H NMR (δ ppm)	MS (70 eV) *m/z* (%)
1	10.5	142–144	4.7(d,2H,CH_2), 5.6(t,1H,NH), 6.8–7.8 (aromat, 7H), 12 (br.s, imidazole NH)	291(4.3)$M^{+\cdot}$, 256(71.6), 258(21.2), 221(4.5), 131(100)
2	23	139–141 283(HCl)	4.65(d,2H,CH_2), 5.3(t,1H, NH), 6.7–7.5(aromat, 6H), 12(br.s, imidazole NH)	325(5.1)$M^{+\cdot}$, 90(100), 292(62), 165(70.5), 167(24)
3	13	234–236	4.7(d,2H,CH_2), 5.6(t,1H, NH), 6.8–8.5(aromat, 6H), 13(br.s, imidazole NH)	336(3.16) $M^{+\cdot}$, 301(100), 255(31), 176(27.5), 161(52.9), 130(64.9)
4	14.5	244–245	2.55(s,3H,CH_3), 4.9(s,2H, CH_2), 5.9 (s,1H,NH), 6.9–7.8(aromat,6H)	305(2.6)$M^{+\cdot}$, 270(48.2), 145(100), 118(13.4)
5		ref.[5]		
6	16	145–146	4.68(d,2H,C*H*$_2$NH, J = 5.2Hz), 4.91 (t,1H, NH, J = 5.43Hz), 5.44(s,2H,CH_2), 6.8–7.4 (aromat,9H), 7.56(d,1H,H-7, $J_{6,7}$ = 8.61Hz), 7.69 (d,1H,H-4, $J_{4,5}$ = 1.45Hz)	415(0.2)$M^{+\cdot}$, 380(6.4), 288(6.3), 255(8.1) 165(3.5), 91(100), 65(18.38)
6a	11	129	4.38 (s,2H,NC*H*$_2$-), 4.5 (s,2H,C*H*$_2$N), 5.21(s,2H,N^1-C*H*$_2$), 6.75–7.5(aromat, 15H), 7.66(d,1H,H-4, $J_{4,5}$ = 8.5Hz)	506(0.06) $M^{+\cdot}$, 414 (0.03), 256(4.41), 164(4.12), 90.8(100)
7	18.5	120–123.5	4.411 and 4.416(s,2H,NC*H*$_2$-), 4.547 and 4.552 (s,2H,C*H*$_2$N), 5.272 and 5.286(s,2H,N^1-C*H*$_2$), 6.75–8.65(arom. 16H),	CI method was used. 517(21.5) M+1, 391(100), 280(58), 252(76)
8	18	ref.[6] 215–217 212–213	6.85–7.8(aromat, 8H), 8.92 (s,1H, NH), 10.6(s,1H,imidazole NH)	243(32.23)$M^{+\cdot}$, 207(22.59), 152(6.5), 125(5.5), 76.7(100)
9	22	ref[3] 182–184 190–92	7.0–7.6(aromat, 7H)	277(14.26)$M^{+\cdot}$, 124(13.24), 75(100)
10	28	197	4.76(s,2H,NHC*H*$_2$), 6.56(br.s,1H,N*H*CH$_2$), 6.9–8.54(aromat, 7H)	258(30.9)$M^{+\cdot}$, 180(17.66), 168(57.6), 105.1(100)
11	40	199	5.45(s,2H, N^1-C*H*$_2$),6.9–7.8(aromat, 12H), 8.84(s,1H,NH)	367(21.7)$M^{+\cdot}$, 242.4(37.56), 207.4(34.01), 125(100)
12	36	210	5.45(s,2H,N^1-C*H*$_2$), 6.9–7.8(aromat, 11H), 8.87(s,1H,NH)	401(14.34)$M^{+\cdot}$, 276(5.71), 278(3.81), 241.2 (27.33), 125(100)
13	51	180	4.80(d,2H,NHC*H*$_2$), 5.22(s,2H, N^1-CH$_2$), 6.9-8.54(aromat,11H)	383(3.6)$M^{+\cdot}$, 257(8.2), 152(5.15), 125(100)

Table 2. Fractional atomic coordinates and equivalent displacement parameters of non-hydrogen atoms for **6a**. $B_{eq} = (8\pi^2/3)\,\Sigma_i\Sigma_j U_{ij} a_i^* a_j^* a_i a_j$

	x	y	z	B (Å^2)
Cl1	0.6460(2)	0.1325(1)	0.23732(9)	4.77(3)
Cl2	0.9043(2)	0.2064(1)	-0.09691(9)	5.87(4)
Cl3	1.2188(2)	0.0890(1)	0.6375(1)	7.42(4)
N3	1.1645(4)	0.1929(3)	0.2223(2)	3.53(8)
C21	0.7797(5)	0.1623(3)	0.0713(3)	3.1(1)
C8	1.1276(5)	0.2227(3)	0.3839(3)	3.0(1)
N1	1.0606(4)	0.2996(3)	0.3285(2)	2.80(8)
N2	0.8192(4)	0.2817(3)	0.1068(2)	3.07(8)
C9	1.1908(5)	0.1569(3)	0.3174(3)	3.3(1)
C26	0.8170(5)	0.1161(4)	-0.0174(3)	3.7(1)
C2	1.0842(5)	0.2759(3)	0.2326(3)	2.8(1)
C22	0.7044(5)	0.0833(3)	0.1276(3)	3.3(1)
C24	0.7131(6)	-0.0718(4)	0.0135(4)	5.0(1)
C32	0.5541(6)	0.4089(4)	0.1694(3)	4.4(1)
C7	1.1356(6)	0.2058(4)	0.4838(3)	3.9(1)
C4	1.2629(6)	0.0688(4)	0.3504(4)	4.4(1)
C11	1.1004(5)	0.5025(3)	0.3901(3)	3.1(1)
C12	1.2581(6)	0.5236(4)	0.4633(3)	4.5(1)
C23	0.6719(6)	-0.0314(4)	0.1005(4)	4.3(1)
C5	1.2700(6)	0.0491(4)	0.4490(4)	5.0(1)
C31	0.6661(5)	0.4395(3)	0.1054(3)	3.5(1)
C20	1.0106(5)	0.3333(3)	0.1468(3)	3.4(1)
C15	1.1697(7)	0.6983(4)	0.3665(4)	6.4(1)
C25	0.7859(6)	0.0012(4)	-0.0461(4)	4.6(1)
C36	0.7229(6)	0.5534(4)	0.0976(3)	4.5(1)
C33	0.5014(6)	0.4903(4)	0.2244(4)	5.4(1)
C10	0.9773(5)	0.3859(3)	0.3647(3)	3.5(1)
C16	1.05571(6)	0.5907(4)	0.3435(3)	4.6(1)
C6	1.2058(6)	0.1170(4)	0.5127(3)	4.5(1)
C30	0.7218(6)	0.3505(3)	0.0441(3)	4.0(1)
C35	0.6695(6)	0.6347(4)	0.1539(4)	5.3(1)
C13	1.3686(6)	0.6317(4)	0.4859(4)	6.6(2)
C14	1.3247(7)	0.7187(4)	0.4396(5)	7.0(2)
C34	0.5596(6)	0.6035(4)	0.2165(4)	5.7(1)

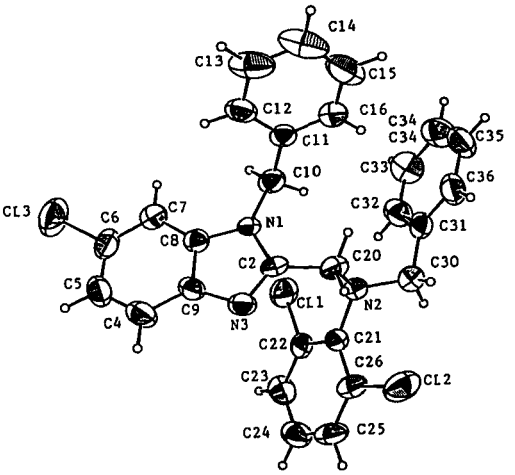

Fig. 1. Numbering scheme with thermal ellipsoids drawn at the 50% probability level of compound **6a**. H atoms are shown as small circles with arbitrary radii.

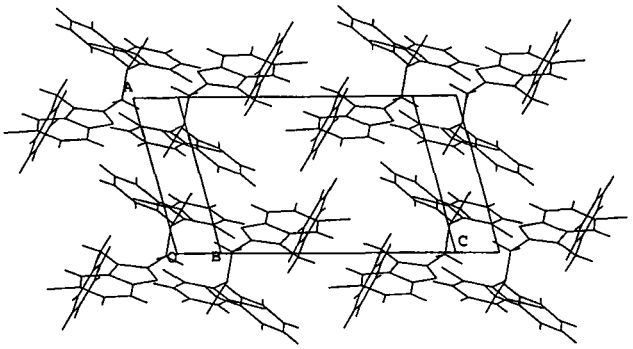

Fig 2. View of the packing arrangement along the *b* axis.

non-hydrogen atoms are listed in Table 2 and selected geometric parameters are given in Table 3.

X-ray structure analysis of **6a** revealed that the molecule is bent almost orthogonally at the methylene which connects the phenyl ring and the benzimidazole ring system. The torsion angle C2-N1-C10-C11 is -81.1(5)° and the dihedral angle between the benzimidazole moiety and the phenyl at N1 is 105.1 (1)°. In 1-(phenylmethyl)-2-(4-methoxyphenyl-

Table 3. Selected bond lengths (Å), bond angles and torsion angles (°) of **6a**

Cl1	C22	1.741(5)	C8	N1	1.378(5)				
Cl2	C26	1.744(5)	N1	C2	1.368(5)				
Cl3	C6	1.749(5)	N1	C10	1.452(6)				
N3	C9	1.393(5)	N2	C20	1.469(5)				
N3	C2	1.312(6)	N2	C30	1.469(6)				
C21	N2	1.436(5)	C2	C20	1.495(6)				
C9	N3	C2	104.3(3)	N3	C2	C20	123.9(4)		
C8	N1	C2	106.2(3)	N1	C2	C20	122.3(4)		
C20	N2	C30	114.9(3)	C13	C6	C7	118.4(4)		
C12	C26	C21	119.4(3)	C11	C22	C21	118.6(3)		
N3	C2	N1	113.6(3)	N2	C20	C2	110.4(3)		
C9	N3	C2	N1	1.9(5)	C30	N2	C20	C2	162.8(3)
C22	C21	C26	C12	177.7(3)	C21	N2	C30	C31	140.3(4)
C8	N1	C2	C20	173.4(4)	C11	C22	C23	C24	178.6(4)
C2	N1	C10	C11	-81.1(5)	C4	C5	C6	Cl3	-179.7(4)

methyl)-1*H*-benzimidazole-5-carboxylic acid and 1,2-di-(phenyl-methyl)-1*H*-benzimidazole-5-carboxylic acid this dihedral angle is 88.5(1) and 95.0(2)°, respectively[7]. The bond lengths N1-C2 [1.368(5)Å] and N3-C2 [1.312(6)Å] are in agreement with the values reported in 2-(*o*-methoxyphenoxy)-1-methylbenzimidazole [N1-C2 is 1.360(2), N3-C2 is 1.295(2)Å][8].

Intramolecular hydrogen bonds exist between C10 and N2 with C10...N2 distance 3.497(5)Å and the angle at H 102 123.01(24)°, as well as an intermolecular hydrogen bond involving the C25 as acceptor and the N3 atom of benzimidazole as donor, C25...N3 3.352(6)Å, angle at H25 148.76 (31)°. Fig 2 shows a projection of the unit cell contents on the ac plane.

Two regioisomers were also formed in the case of **7**, but chromatographic separation proved to be impossible. As expected, in its NMR spectra all the methylene protons appear as doublets due to very little differences in their chemical shift values (See Table 1).

Their ratio was estimated from the integrals of the methylene NMR signals as almost 50%. In order to prepare other N^1-substituted benzimidazoles selective preparation method was chosen. The 2,5-dichloro and 2,5-dichloro-1-(*p*-chlorophenylmethyl)-1*H*-benzimidazoles were prepared via

Table 4. Reaction conditions, antibacterial and antifungal activity[a] of **1–13**.

No	Formula	Reaction time	Reaction temp. °C	Purification procedure	Method	*b* *C.a.*	*c* *S.a.*	*d* *E. c.*
1	$C_{14}H_{11}Cl_2N_3$	7	120	$CHCl_3$ (flash chrom.)	A	10	16	*
2	$C_{14}H_{10}Cl_3N_3 \cdot HCl \cdot 0.5H_2O$	3	120	$CHCl_3$ (flash chrom.)	A	15	21	9
3	$C_{14}H_{10}Cl_2N_4O_2$	7	120	$CHCl_3$ (flash chrom.)	A	*	11	*
4	$C_{15}H_{13}Cl_2N_3 \cdot HCl$	17	120	$CHCl_3$ (flash chrom.) then recrystallization (ethanolic HCl)	A	14	10	*
5						*	*	*
6	$C_{21}H_{16}Cl_3N_3$	2.5	60	(flash chrom.) EtOAc : *n*-Hexane (1:2)	B	10	*	*
6a	$C_{28}H_{22}Cl_3N_3$	2.5	60	(flash chrom.) EtOAc : *n*-Hexane (1:2)	B	*	*	*
7	$C_{28}H_{22}Cl_2N_4O_2$	2.5	60	(flash chrom.) $CHCl_3$: *n*-Hexane (2:1)	B	*	9	*
8	$C_{13}H_{10}ClN_3$	20	120	(flash chrom.) $CHCl_3$: *n*-Hexane (3:1)	A	17	10	18
9	$C_{13}H_9Cl_2N_3$	20	120	(flash chrom.) $CHCl_3$: *n*-Hexane (3:1)	A	21	34	11
10	$C_{13}H_{11}ClN_4 \cdot 0.5HOH$	4	120	(flash chrom.) $CHCl_3$: isopropanol (10:1)	A	12	13	15
11	$C_{20}H_{15}Cl_2N_3$	12	120	(flash chrom.) EtOAc : *n*-Hexane (2:10)	A	*	*	*
12	$C_{20}H_{14}Cl_3N_3$	12	120	(flash chrom.) EtOAc : *n*-Hexane (3:10)	A	*	*	*
13	$C_{20}H_{16}Cl_2N_4$	8	120	(flash chrom.) EtOAc : *n*-Hexane (3:10)	A	*	15	*
F						16	–	–
K						30	–	–
A						–	29	22

[a] Growth-inhibition zone diameter (mm); [b] *Candida albicans*; [c] *Staphylococcus aureus*; [d] *Escherichia coli*; * No activity; [F] Fluconazole; [K] Ketoconazole; [A] Ampicillin.

the several steps as previously reported by us[2]. Some physical and spectral data of **1–13** are shown in Table 1.

Compounds **1–13** were evaluated for their in vitro antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* by the agar diffusion method (Table 4). Among **1–4**, compound **2** shows the large growth inhibition zone, this may to indicate that, introduction of chlorine atom at the 5(6) position of the benzimidazole increase the in vitro antimicrobial activity in this series of compounds. As seen in Table 4, compounds **8** and **9** exhibited the greatest activity. In particular, compound **9** has comparable activity against *C. albicans* to Fluconazole and a better result against *S. aureus* than Ampicillin. It appears that when the compounds **8**, **9**, and **10** were substituted with a *p*-chlorobenzyl group at the N′ atom, both antifungal and antibacterial activities were decreased.

Experimental

Chemistry

Melting points were determined with a Büchi SMP-20 melting point apparatus and are uncorrected. All the instrumental analyses were performed by Tubitak (Instrumental Analysis Lab., Ankara) with a Bruker AC 400 NMR spectrophotometer (the ^{1}H NMR spectra of **1–6a** and **6–13** were recorded in [D$_6$]DMSO and CDCl$_3$, respectively) and VG Platform II mass spectrometer. Microanalyses were performed on a Leco CHNS 932 analyzer and satisfactory results ±0.4% of calculated values (C,H,N) were obtained. For the chromatographic analyses Merck Silica Gel 60 (230–400 mesh ASTM) was used. The HCl salts of the **2** and **4** were prepared by using ethanolic HCl. 2-Chloromethyl-1*H*-benzimidazole[1], 2-chloromethyl-5(6)-chloro-1*H*-benzimidazole[1], 2-chloromethyl-5(6)-methyl-1*H*-benzimidazole[1], 2-chloromethyl-5(6)-nitro-1*H*-benzimidazole[1], 2,5-dichloro-1*H*-benzimidazole[2], 2,5-dichloro-1-(*p*-chlorophenylmethyl)-1*H*-benzimidazole[2] were synthesized according to the literature.

Method A

A mixture of related 2-chloro- or 2-chloromethyl benzimidazoles (5 mmol) and anilines (7.5 mmol) or other amines was stirred and heated. The reaction mixture was made alkaline with dilute Na_2CO_3 solution, extracted with AcOEt, washed with water, dried over Na_2SO_4 and evaporated. Reaction conditions are given in Table 4.

Method B

To a solution of related benzimidazoles (2 mmol) in 0.5 ml DMF was added NaH (3 mmol, 50% dispersion in oil) and and the mixture wasstirred. Benzyl bromide (2 mmol) in 1 ml DMF was then added dropwise. Water was subsequently added and the xixture extracted with $CHCl_3$. The organic layer was washed with water and dried over Na_2SO_4 and evaporated.

Antimicrobial Activity Technique

A paper disk (8 mm in diameter) was soaked in a 2000 µg/ml solution of the test compound in propylene glycol (propylene glycol as a blank has no inhibition zone), and placed on an agar plate containing fungi or bacteria cells, which was incubated 37 °C for 24 h. The diameter of the growth inhibition zone around the paper disk was measured.

X-ray Structure Determination of **6a**

Crystal data: $C_{28}H_{22}Cl_3N_3$, M_r = 506.87, triclinic, space group P1, a = 7.860(0), b = 12.179(1), c = 13.607(0)Å, α = 95.19(1), β = 101.62(1), γ = 102.39(0)°, V = 1233.9(2) Å^3, Z = 2, d_c = 1.36 g cm^{-3}, λ(MoKα) = 0.71073Å, μ = 0.39 cm^{-1}, T = 296 K, F(000) = 524.

Intensity data collected on an Enraf-Nonius CAD-4 diffractometer with graphite monochromator. A colorless plate shaped crystal with the dimensions 0.08 × 0.28 × 0.36 mm was used. Cell constants refined by least squares refinement, using the setting angles of 24 reflections in the range $8 < \theta < 18°$. Intensities were measured using ω - 2θ scan technique up to θ_{max} = 23.75°. A total of 3923 reflections were collected, of which 3739 were unique. 2051 reflections [$I > \sigma(I)$] were used in the refinements. The structure was solved using direct methods in SIR and refined on F by full-matrix least squares with MolEN[9]. All H atoms were geometrically located 0.95 Å from their parent atoms and included in refinement using a riding model; displacement parameters were fixed at 1.3 U_{eq} of the parent atom. Full-matrix least squares refinement minimized the function $\Sigma\omega$ ($|F_o| - |F_c|)^2$. Final refinements converged with R = 0.054 and ωR = 0.047 for 2051 reflections and 307 parameters. Min. and max. peaks in the difference Fourier map were 0.00 and 1.21 eÅ^{-3} respectively.

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