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Studies on Anti-*Helicobacter pylori* Agents. Part 1: Benzyloxyisoquinoline Derivatives

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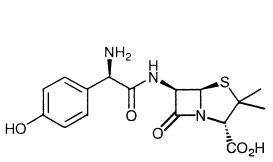
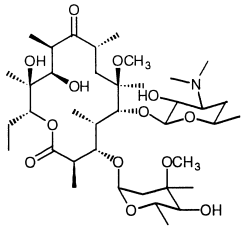
Abstract—The synthesis and optimization of the anti-*Helicobacter pylori* activity of a novel series of benzyloxyisoquinoline derivatives that was discovered by a random screening process, are described. In the in vitro assay, compound **10c** containing a 3-acetamido-2,6-dichlorobenzyl substituent was found to have extremely potent activity against *H. pylori* and no activity against other common bacteria. The anti-*H. pylori* activity of **10c** was superior to that of amoxicillin (AMPC) (**1**) and clarithromycin (CAM) (**2**). However, **10c** did not show in vivo efficacy in a mouse infection model; a feature attributed to the lack of strong bactericidal activity at short contact times.

Introduction

Since its discovery in the gastric mucosa of humans,[1] the relationship between infection with *Helicobacter pylori* bacteria and various peptic diseases has been reported by many investigators. As a result, it is now clear that *H. pylori* is a major causative factor in chronic gastritis and peptic ulcer disease, and that eradication of *H. pylori* results in a dramatic decrease in the recurrence rate in peptic ulcer patients.[2–5] Moreover, the relationship between *H. pylori* infection and malignant diseases is becoming more established, and the World Health Organization (WHO) has stated that *H. pylori* has been proposed as a Group 1 carcinogen in humans.[6] In addition, relationships between *H. pylori* and allergosis or hepatic encephalothy have been noted.[7,8] Hence, the clinical importance of eradication of *H. pylori* has increased significantly and the obvious remedy of treating *H. pylori* infection with antibiotics is attractive, however in practice this has often proven difficult.[9] To date only a small number of multi-drug therapy regimens have attained clinical use,[10] such as a combination of broad-spectrum antibiotics, for example amoxicillin (AMPC, **1**) and clarithromycin (CAM, **2**), an antiprotozoal agent (metronidazole), bactericidal agents (bismuth salts) and proton pump inhibitors, such as omeprazole. Although eradication of *H. pylori* with these multi therapy regimens containing antibacterial agents has shown a reasonable, if somewhat variable response, there remain a number of unsolved problems such as drug resistance,[11,12] side effects[13,14] and non-compliance.[15] As a result, the need for alternative and novel treatments is evident, and has stimulated the search for novel agents that are *H. pylori* specific and suitable for single-therapy treatment.[16–19]



Amoxicillin(AMPC, **1**)



Clarithromycin(CAM, **2**)

Figure 1. Anti-*H. pylori* agents.

At the start of this work, we reasoned that known antimicrobial agents may not be appropriate as suitable chemical seed compounds for this research, since it is very

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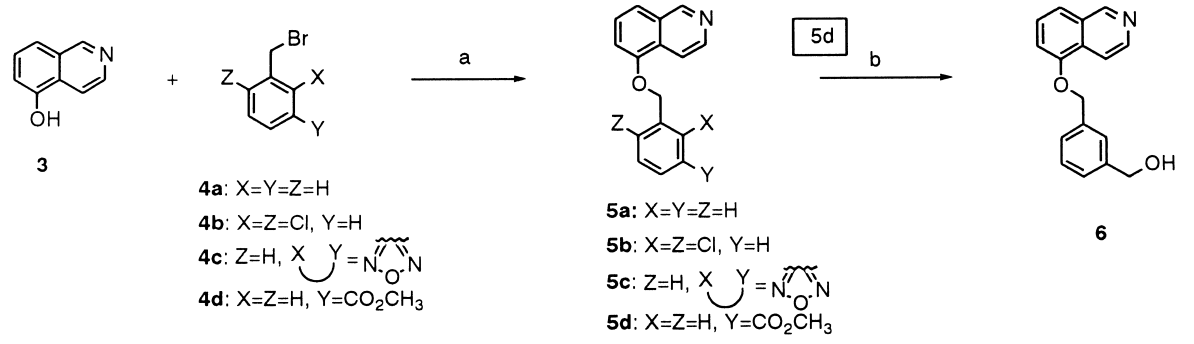
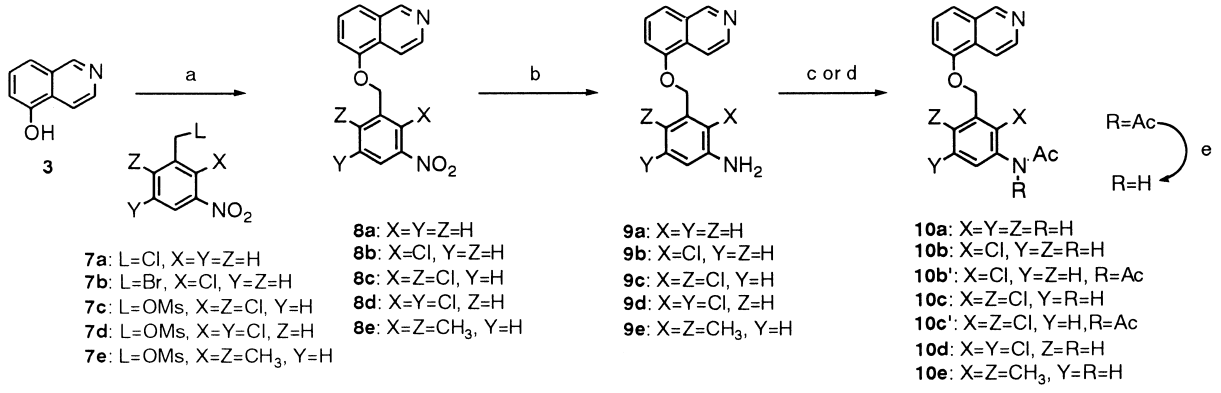
difficult to solve problems such as drug resistance and side effects due to the original antimicrobial activity. Thus, in order to search for a novel chemical seed compound, we carried out screening of the Fujisawa chemical library. As a result of this directed random screening program of various non-antimicrobial derivatives, we discovered that 5-hydroxyisoquinoline **3** possessed weak, *H. pylori* specific activity. During studies to enhance the anti-*H. pylori* activity of this seed compound, we investigated the preparation of a novel series of isoquinoline derivatives. In this paper, we wish to describe the synthesis and anti-*H. pylori* activity of this novel series of compounds.[20]

Chemistry

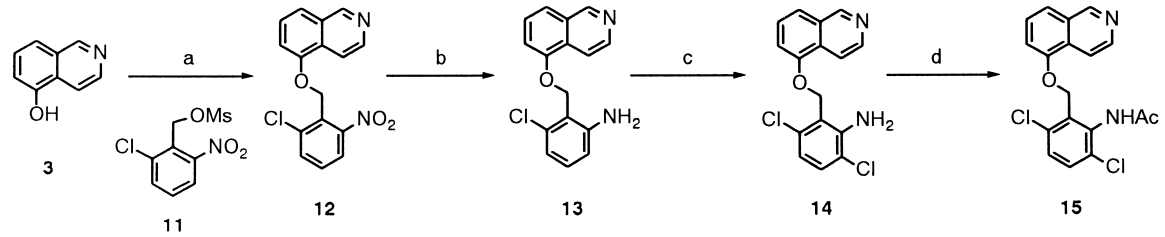
5-Hydroxyisoquinoline derivatives having various *O*-benzyl moieties, but not containing nitrogen substituents, were synthesized by the route shown in Scheme 1. Treatment of commercially available **3** with sodium hydride in DMF at 0°C, followed by addition of benzyl bromides **4a–d** yielded coupled compounds **5a–d** in good yield. Compound **6** having a hydroxymethyl moiety was prepared by LAH reduction of **5d**. The basic synthetic route to 3-acetamidobenzyl derivatives is summarized in Scheme 2. The nitro compounds **8a–c** were synthesized by the same coupling method as in Scheme 1. In the case of **8d,e**, we employed a multistep method without intermediate purification, from the corresponding carboxylic acids **45a,b** (Scheme 8). The other electrophilic benzyl derivatives **7b,c** were obtained by the methods of Uneme[21] and Abe,[22] respectively. Subsequently, compounds **9a–e** having an amino group were prepared by reduction of the nitro group. Since it was possible that the isoquinoline ring and the chloro groups were potentially labile under hydrogenolysis conditions, we opted to employ iron-catalyzed reduction with hydrazine (NH_2NH_2–$FeCl_3$). Subsequent acylation of the amino group (Ac_2O–pyridine) afforded acetamides **10a–e**. Occasionally, acetylation under these conditions afforded substantial



Scheme 1. Reagents: (a) NaH, DMF; (b) LAH, THF.



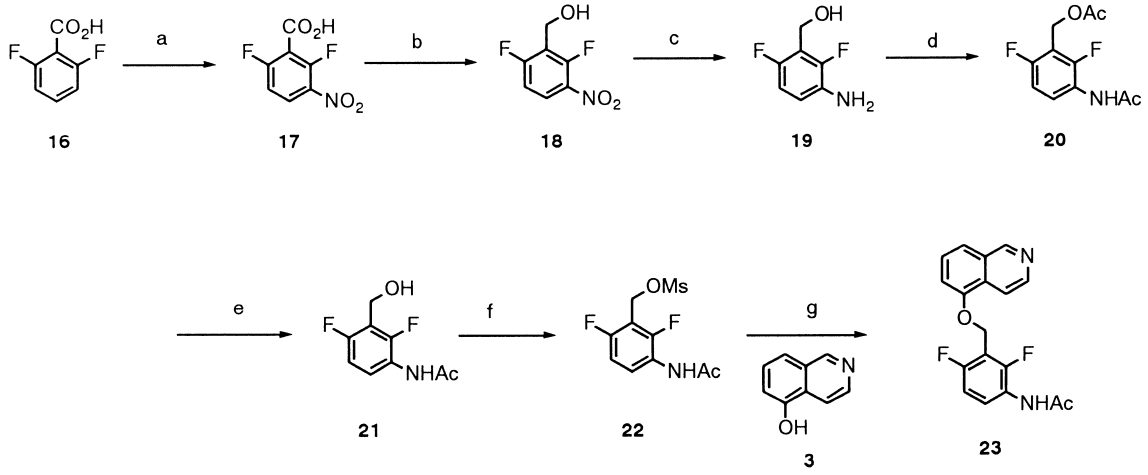
Scheme 2. Reagents: (a) NaH, DMF; (b) NH_2NH_2, $FeCl_3$, H_2O–MeOH; (c) Ac_2O, Py, DMAP; (d) Ac_2O, $ClCH_2CH_2Cl$; (e) pyrrolidine, EtOH.



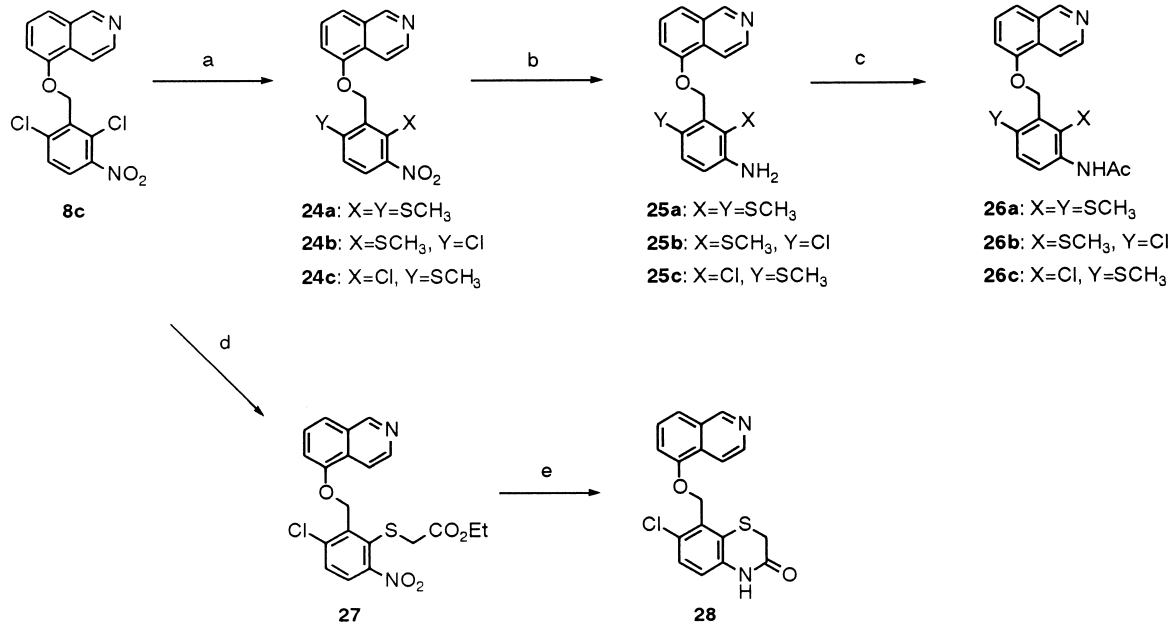
Scheme 3. Reagents: (a) NaH, DMF; (b) NH_2NH_2, $FeCl_3$, H_2O–MeOH; (c) NCS, $CHCl_3$; (d) Ac_2O, $ClCH_2CH_2Cl$.

amounts of di-acylated compound (**10b′**,**c′**) that was readily converted to the mono-acyl derivative by treatment with a secondary amine (pyrrolidine) in ethanol at room temperature. Alternatively, selective mono acylation was achieved in the absence of base (Ac_2O–$ClCH_2CH_2Cl$).

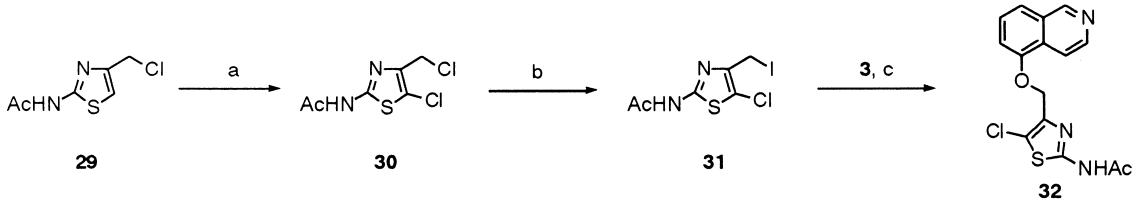
2-Acetamido-3,6-dichlorobenzyl derivative **15** was synthesized by the route shown in Scheme 3. 2-Amino-6-chloro benzyl derivative **13** was synthesized from **3** and the corresponding benzyl mesylate **11**, followed by reduction of the nitro group by similar methods to those in Scheme 2. Treatment of **13** with *N*-chlorosuccinimide (NCS) gave predominantly 2-amino-3,6-dichloro derivative **14**, which was separated by chromatography to give pure compound. Subsequently, acylation of the amino group afforded 2-acetamido-3,6-dichlorobenzyl derivative **15**.



Scheme 4. Reagents: (a) HNO_3; (b) $NaBH_4$, $BF_3{\cdot}Et_2O$, THF; (c) H_2, Pd/C, AcOEt; (d) (1) Ac_2O, Et_3N, $ClCH_2CH_2Cl$, (2) pyrrolidine, EtOH; (e) 1 N-NaOH, MeOH; (f) MsCl, Et_3N, CH_2Cl_2; (g) NaH, DMF.

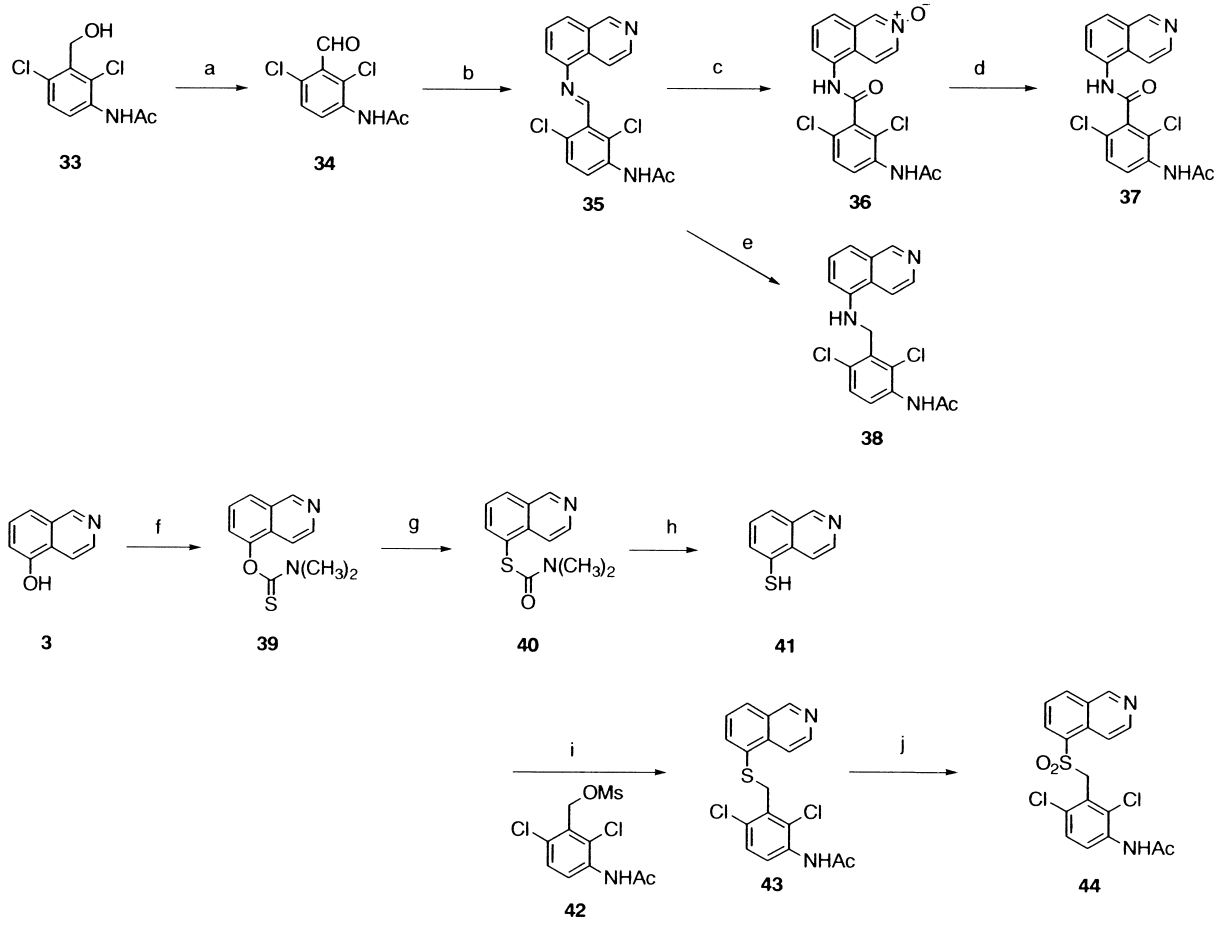


Scheme 5. Reagents: (a) NaSMe, DMF; (b) NH_2NH_2, $FeCl_3$; (c) Ac_2O, $ClCH_2CH_2Cl$; (d) $HSCH_2CO_2Et$, Et_3N, DMF; (e) Fe, NH_4Cl, EtOH–H_2O.

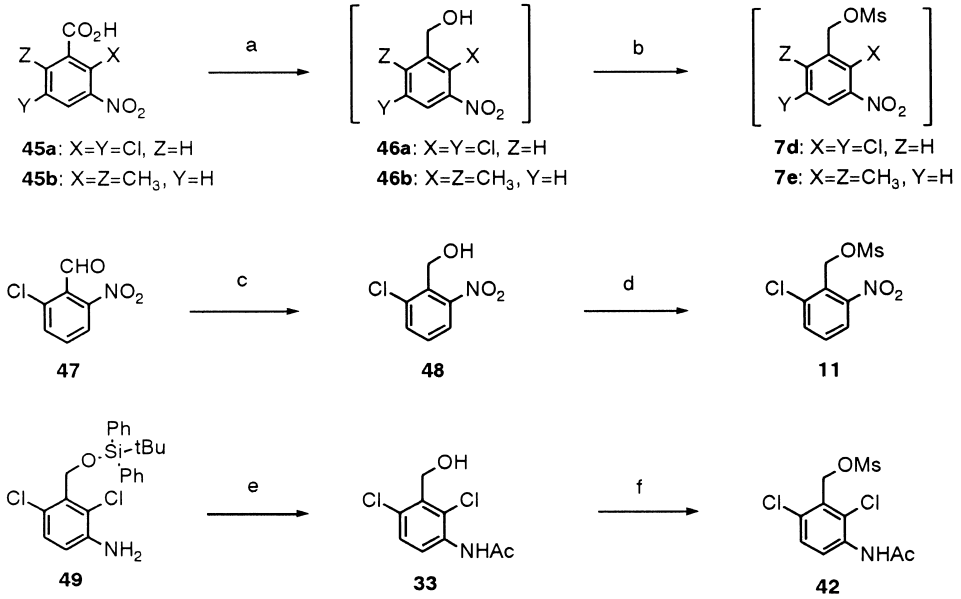


Scheme 6. Reagents: (a) NCS, $CHCl_3$; (b) NaI, acetone; (c) NaH, DMF.

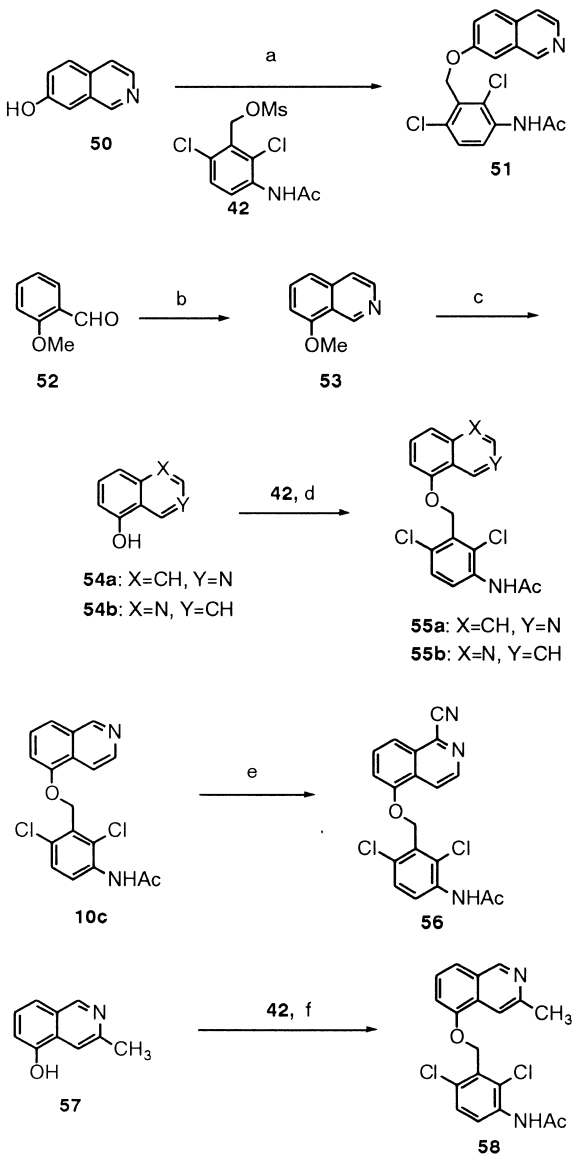
Difluoro derivative **23** was prepared as shown in Scheme 4. After nitration of 2,6-difluorobenzoic acid **16** with nitric acid, reduction with $NaBH_4$–$BF_3{\cdot}Et_2O$ afforded hydroxymethyl derivative **18** and subsequent reduction of the nitro group afforded the corresponding 3-amino-2,6-difluorobenzylalcohol **19**. After *N,O*-diacetylation of **19** with Ac_2O, selective deacetylation with 1 N-NaOH afforded *N*-acetyl derivative **21** which was mesylated to



Scheme 7. Reagents: (a) MnO_2, $CHCl_3$; (b) 5-aminoisoquinoline, EtOH; (c) mCPBA, $CHCl_3$; (d) 10% Pd-C, $NH_4^+HCO_2^-$, MeOH; (e) $NaBH_4$, EtOH; (f) dimenthylthiocarbamoyl chloride, NaH, DMF; (g) Δ; (h) 1 N-NaOH, MeOH; (i) Et_3N, DMF; (j) $KMnO_4$, AcOH, H_2O.



Scheme 8. Reagents: (a) $NaBH_4$, $BF_3{\cdot}Et_2O$, THF; (b) MsCl, Et_3N, AcOEt; (c) $NaBH_4$, EtOH; (d) MsCl, Et_3N, AcOEt–CH_2Cl_2; (e) (1) Ac_2O, Py, DMAP, CH_2Cl_2, (2) TBAF, THF; (f) MsCl, Et_3N, AcOEt–CH_2Cl_2.



Scheme 9. Reagents: (a) NaH, DMF; (b) (1) $(MeO)_2CHCH_2NH_2$, benzene, (2) $ClCO_2Et$, $P(OMe)_3$, THF, (3) $TiCl_4$, CH_2Cl_2; (c) BBr_3, CH_2Cl_2; (d) NaH, DMF; (e) (1) p-TsCl, KCN, CH_2Cl_2–H_2O, (2) DBU, THF; (f) NaH, DMF.

yield the corresponding benzylmesylate **22**. Compound **23** was obtained by coupling reaction of **22** with **3**.

Methylthiolation of the dichloro compound **8c** with sodium thiomethoxide afforded a mixture of 2,6-dimethylthio and two mono-methylthio derivatives **24a**–**c**. After isolation of the three compounds, reduction of the nitro group and acylation afforded **26a**–**c**, respectively. The bicyclic derivative **28** was prepared by reaction of **8c** with ethyl thioglycolate, followed by cyclization using Fe/NH_4Cl conditions to afford cyclized compound directly (Scheme 5).

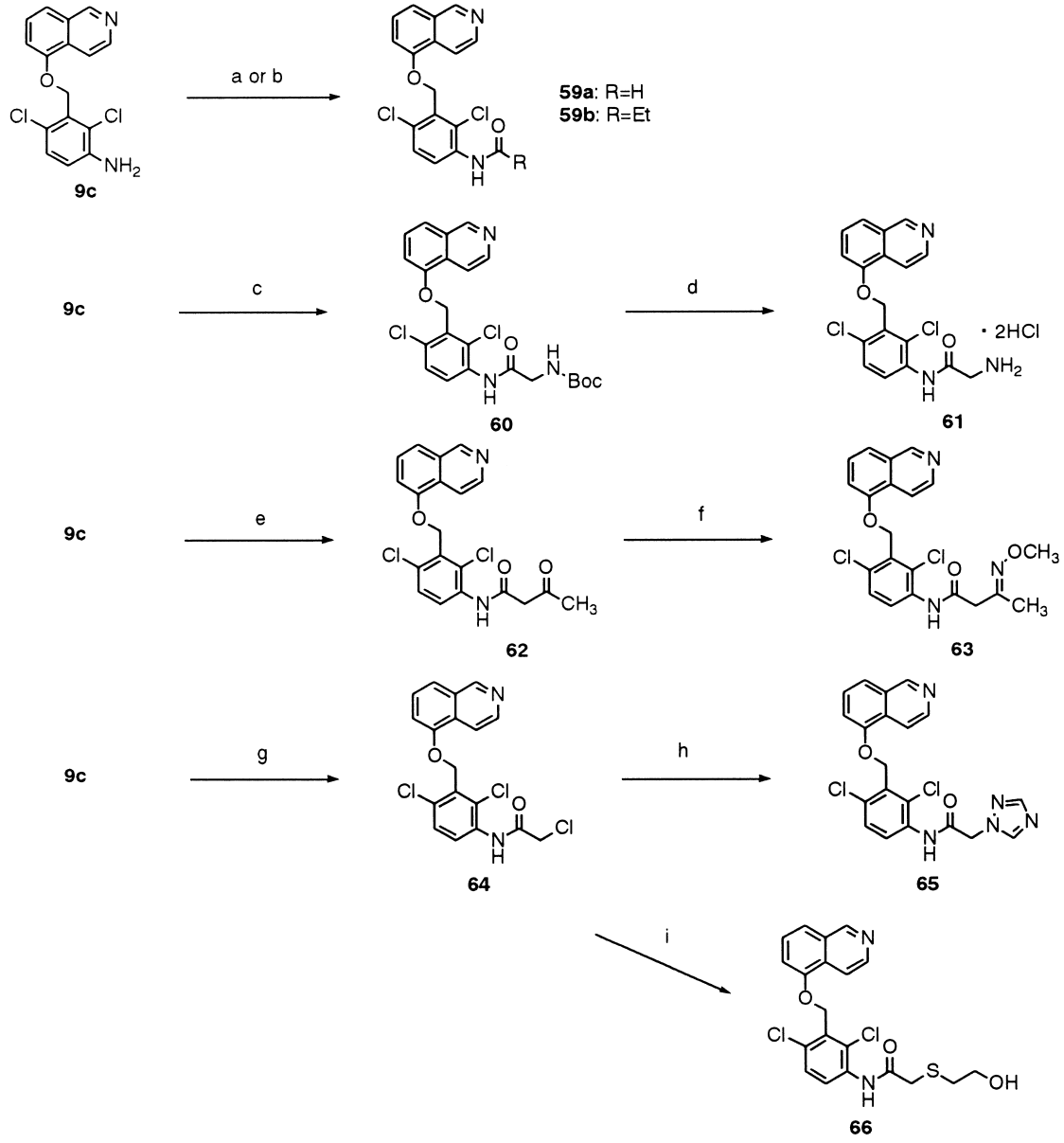
5-{(2-Acetamido-5-chlorothiazol-4-yl)methoxy}isoquinoline **32** was prepared by the route shown in Scheme 6. Treatment of 2-acetamido-4-chloromethylthiazole **29** with NCS gave 5-chloro derivative **30**, which was iodinated with sodium iodide to yield 4-iodomethyl derivative **31**. Subsequent coupling reaction with **3** afforded 2-acetamido-5-chlorothiazole derivative **32**.

Compounds having *N*- or *S*-containing spacer groups (**37**, **38**, **43**, **44**), were prepared as shown in Scheme 7. After oxidation of the hydroxymethyl compound **33** with manganese dioxide to give aldehyde **34**, reaction with 5-aminoisoquinoline afforded imino compound **35**. Subsequent treatment of **35** with mCPBA gave amide derivative **36** which had also undergone isoquinoline *N*-oxidation. Reduction with Pd-C/$NH_4^+HCO_2^-$ then yielded amide spacer derivative **37**. Reduction of imino compound **35** with sodium borohydride gave amino spacer compound **38**. Sulfur-containing spacer groups were obtained via thiol **41**. Treatment of **3** with dimethylthiocarbamoyl chloride gave coupling compound **39**, which underwent thermal rearrangement to yield **40**. After hydrolysis of **40** with sodium hydroxide, reaction of the thiol **41** with benzyl mesylate **42** afforded sulfide compound **43**. MCPBA oxidation afforded the *N*-oxide derivative of sulfone, however **44** was selectively obtained by $KMnO_4$ oxidation of **43**.

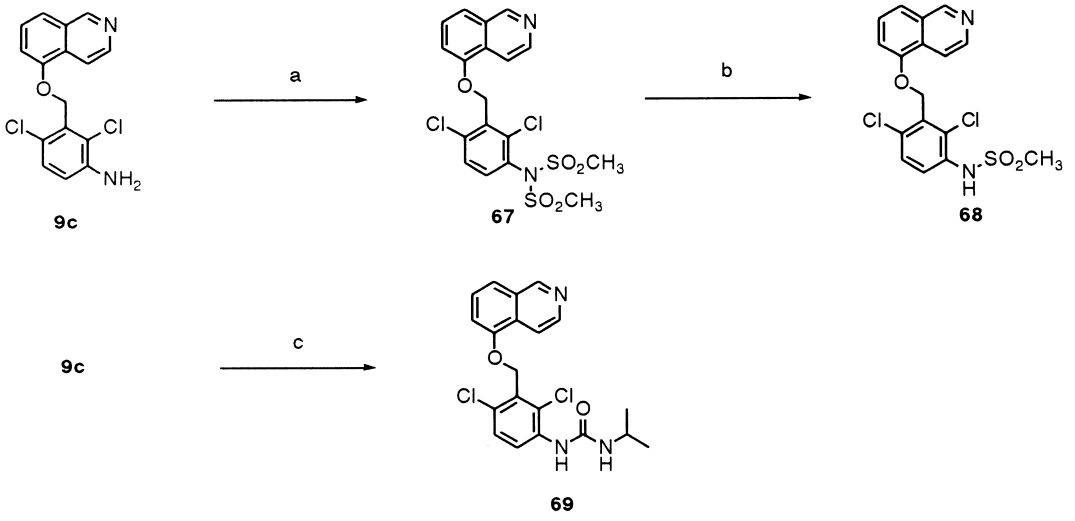
Preparation of the various electrophilic benzyl derivatives employed in coupling reactions with hydroxyisoquinoline were performed according to the routes shown in Scheme 8. Reduction of the corresponding carboxylic acid (**45a**,**b**[23]) or aldehyde **47** with sodium borohydride gave the hydroxymethyl derivatives (**46a**,**b**, **48**), which were mesylated to yield the corresponding benzylmesylate (**7d**,**e**, **11**). 3-Acetamide-2,6-dichlorobenzylmesylate **42** was prepared from *O*-silylated aminobenzylalcohol **49**.[24] Treatment of **49** with Ac_2O/Py, followed by de-silylation using TBAF yielded **33** in good yield and subsequent mesylation afforded **42**.

Compounds with different isoquinoline ring and quinoline ring isomers, and various substituted derivatives of the 5-substituted type were prepared as shown in Scheme 9. 7-Benzyloxyisoquinoline and 5-benzyloxyquinoline derivatives (**51**, **55b**) were obtained by coupling of commercially available 7-hydroxyisoquinoline and 5-hydroxyquinoline (**50**, **54b**) with the corresponding benzylmesylate **42**. Treatment of *o*-anisaldehyde **52** with $(MeO)_2CHCH_2NH_2$ and $ClCO_2Et/P(OMe)_3$, and subsequently with $TiCl_4$ using the method of Hendrickson,[25] gave 8-methoxyisoquinoline **53**, which was demethylated with BBr_3 to yield 8-hydroxyisoquinoline **54a**. Subsequently, coupling reaction of **54a** with **42** gave 8-benzyloxyisoquinoline derivative **55a**. 1-Cyano-5-benzyloxyisoquinoline derivative **56** was obtained by direct introduction of a cyano group into **10c** with TsCl:KCN:DBU by the method of Boger.[26] 3-Methyl-5-benzyloxyisoquinoline derivative **58** was obtained by coupling reaction of 3-methyl-5-hydroxyisoquinoline **57**[24] with **42**.

Acylation reactions of 5-(3-amino-2,6-dichlorobenzyloxy)isoquinoline **9c** to introduce functional groups are summarized in Scheme 10. Compounds **59a**,**b** were obtained by simple acylation of the amino group using HCO_2H:Ac_2O or $(EtCO)_2O$:Py, respectively. After acylation of **9c** with Boc-Gly:HOBT, deprotection of the *N*-Boc part afforded **61**. Treatment of **9c** with diketene,



Scheme 10. Reagents: (a) HCO_2H, Ac_2O; (b) $(EtCO)_2O$, DMAP, Py; (c) Boc-Gly, HOBT, WSCD-HCl, CH_2Cl_2; (d) HCl, AcOEt; (e) diketene, $ClCH_2CH_2Cl$; (f) NH_2OCH_3·HCl, EtOH; (g) $(ClCH_2CO)_2O$, $ClCH_2CH_2Cl$; (h) 1,2,4-triazole Na-salt, DMF; (i) $HSCH_2CH_2OH$, Et_3N, CH_2Cl, DMF.



Scheme 11. Reagents: (a) MsCl, Et_3N, CH_2Cl_2; (b) 1 N-NaOH; (c) isopropyl isocyanate, Et_3N, EtOH.

followed by addition of *O*-methylhydroxylamine to the intermediate ketone **62** yielded oxime compound **63** as a 1.4:1 mixture of oxime isomers. Treatment of **9c** with chloroacetic anhydride gave chloroacetyl derivative **64**, which then reacted with 1,2,4-triazole Na salt to yield **65**. Treatment of **64** with mercaptoethanol gave **66**.

Compounds with a different bond type were prepared as shown in Scheme 11. Treatment of **9c** with MsCl gave *N,N*-dimesylated compound **67**, which was readily hydrolyzed with sodium hydroxide to yield the mono-mesylated conpound **68**. Ureido compound **69** was obtained by reaction of **9c** with the corresponding isocyanate.

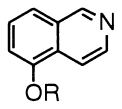
Results and Discussion

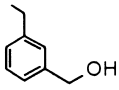
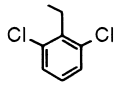
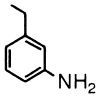
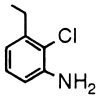
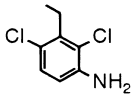
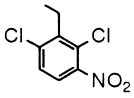
In the search for novel compounds with anti-*H. pylori* activity, we initiated a random screening effort and uncovered 5-hydroxyisoquinoline **3** as a weakly active seed compound. Interestingly, other positional isomers were devoid of activity, leading us to propose that modification of **3** may lead to a selective inhibitor of *H. pylori* growth. It is well known that AMPC (**1**) and CAM (**2**), the antibacterial agents most commonly used in triple therapy against *H. pylori*, display more potent activity against Gram-positive bacteria than against Gram-negative bacteria,[27,28] and furthermore, earlier SAR of inhibitors suggests striking similarities with Gram-positive bacteria.[29,30] Accordingly, even though *H. pylori* is classified as Gram-negative on the basis of bacterial classification, we speculated that introduction of lipophilic substituents to **3** would improve anti-*H. pylori* activity, since increasing lipophilicity generally can lead to more potent activity for antibacterial agents against Gram-positive bacteria.[31,32]

Table 1 shows the results of benzylation of **3**; anti-*H. pylori* activity is expressed as minimum inhibitory concentration value (MIC, μg/mL). Benzyl derivative **5a** showed about 20-fold increased activity compared to **3**. Further introduction of a substituent at the 3-position of the phenyl ring showed interesting possibilities because of the improved activity of **6**. We next attempted to further increase lipophilicity by the introduction of various substituents. 2,6-Dichloro derivative **5b** had slightly improved activity, however benzofuroxan **5c** was even better still, indicating the benefits of nitrogen containing substituents. While amine **9a** did not have improved activity, we found that a combination of amino and chloro groups (**9b**,**c**) was compatible with good activity. On the other hand, a combination of nitro and chloro groups **8c** did not give any improvement.

Next, we introduced an acetyl moiety to the amino group (Table 2), and were surprised to find that **10c**, containing a 3-acetamido-2,6-dichlorobenzyl substituent had remarkably potent in vitro anti-*H. pylori* activity. In contrast, no chloro substituents (**10a**), a monochloro derivative (**10b**), or a regioisomer (**10d**) did not give improved activity. Furthermore, the positional isomer **15**, having a 2-acetamido group showed dramatically decreased activity. Subsequently, we examined other substituents instead of the chloro substituents,

Table 1. Structure–activity relationships: benzyloxyisoquinoline derivatives (1)



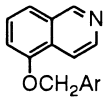
Compound no.	R	MIC (μg/mL)[a]			
		Helicobacter pylori			
		8007	9005	13001	FP1757
3	H	25	50	25	50
5a		1.56	1.56	0.78	1.56
6		0.78	1.56	0.78	0.78
5b		1.56	1.56	0.78	0.78
5c		0.78	0.78	0.39	0.78
9a		1.56	1.56	1.56	1.56
9b		0.39	0.39	0.2	0.78
9c		0.78	0.78	0.78	0.78
8c		1.56	1.56	1.56	1.56

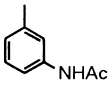
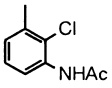
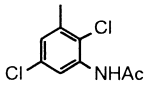
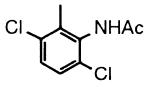
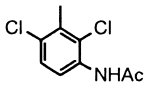
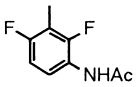
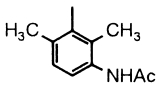
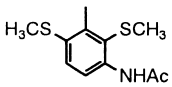
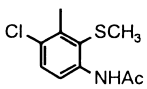
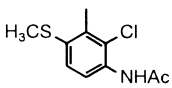
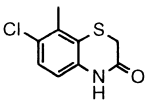
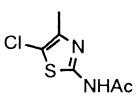
[a] MIC (μg/mL), brucella agar + 7% horse blood, 37°C, 72 h, 10% CO_2, stamp method.

such as 2,6-difluoro, 2,6-dimethyl or 2,6-dimethylthio derivatives (**23**, **10e**, **26a**), but they had decreased activity, and comparison of compounds having methylthio substituent(s) (**26a**–**c**) showed only small differences. These results showed that the anti-*H. pylori* activity of this type is fairly specific in connection with the substitution on the phenyl ring. We also examined a conformationally rigid bicyclic compound (**28**), but it had no anti-*H. pylori* activity at all. Furthermore, the thiazole structure instead of a phenyl ring (**32**) did not have improved activity, but nevertheless was active at < 1 μg/mL.

Table 3 shows the results of investigations into the nature of the spacer group and isoquinoline ring modification. Concerning spacer groups, while amide **37** and sulfur containing spacer groups (**43**, **44**) were not promising, an amino spacer group **38** was equipotent with

Table 2. Structure–activity relationships: benzyloxyisoquinoline derivatives (2)

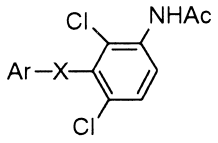


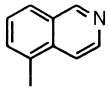
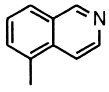
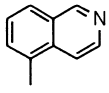
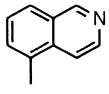
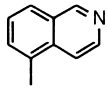
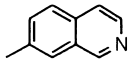
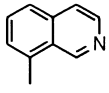
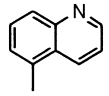
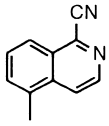
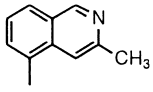
Compound no.	Ar	MIC (μg/mL)[a]			
		Helicobacter pylori			
		8007	9005	13001	FP1757
10a		0.78	0.78	0.78	1.56
10b		0.39	0.39	0.39	0.39
10d		0.39	0.39	0.2	0.39
15		> 12.5	> 12.5	> 12.5	> 12.5
10c		0.025	0.05	0.025	0.0125
23		0.2	0.2	0.2	0.2
10e		0.1	0.2	0.1	0.2
26a		3.13	3.13	1.56	3.13
26b		1.56	1.56	0.78	1.56
26c		6.25	6.25	3.13	6.25
28		> 100	> 100	> 100	> 100
32		0.78	0.78	0.39	0.78
AMPC (**1**)		0.1	0.1	0.025	0.025
CAM (**2**)		0.05	0.1	0.05	0.05

[a] MIC (μg/mL), brucella agar + 7% horse blood, 37°C, 72 h, 10% CO_2, stamp method.

10c. Isoquinoline ring modification, for example, the positional isomers (**51**, **55a**,**b**) had considerably decreased activity. Thus, we found that the activity is fairly specific in connection with the isomeric structure of the (iso)quinoline ring. We also examined a derivative having an electron-withdrawing cyano substituent at the isoquinoline 1-position to moderate basicity (**56**), however this compound showed decreased activity. 3-Methyl-isoquinoline ring substituted derivative **58** had strong anti-*H. pylori* activity comparable to **10c**, however on the basis of ease of synthesis this type had no advantage over the unsubstituted analogue.

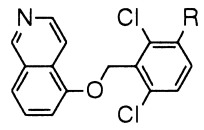
Table 3. Structure–activity relationships: spacer and isoquinoline ring modification

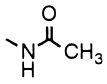
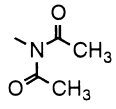
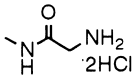
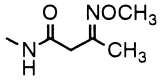
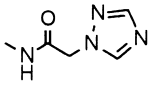
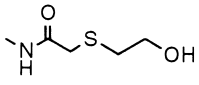
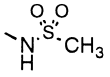
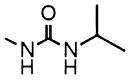


Compound no.	Ar	X	MIC (μg/mL)[a]			
			Helicobacter pylori			
			8007	9005	13001	FP1757
38			0.025	0.025	0.025	0.025
37			6.25	6.25	6.25	12.5
43			> 0.78	> 0.78	> 0.78	> 0.78
44			50	100	50	50
10c			0.025	0.05	0.025	0.0125
51			3.13	3.13	3.13	3.13
55a			6.25	6.25	3.13	6.25
55b			1.56	≧3.13	0.78	≧3.13
56			0.2	0.39	0.2	0.2
58			0.025	0.025	0.025	0.025

[a] MIC (μg/mL), brucella agar + 7% horse blood, 37°C, 72 h, 10% CO_2, stamp method.

Table 4. Structure–activity relationships: optimization of the acyl moiety



Compound no.	R	MIC (μg/mL)[a]			
		Helicobacter pylori			
		8007	9005	13001	FP1757
59a		0.78	1.56	0.78	0.78
10c		0.025	0.05	0.025	0.0125
10c′		0.025	0.05	0.025	0.025
59b		0.05	0.1	0.05	0.1
61		0.78	0.78	0.78	0.78
63		0.05	0.05	0.1	0.1
65		0.39	0.78	0.39	0.39
66		0.1	0.2	0.2	0.1
68		0.39	0.39	0.39	0.39
69		1.56	3.15	1.56	1.56

[a] MIC (μg/mL), brucella agar + 7% horse blood, 37°C, 72 h, 10% CO_2, stamp method.

Next, we attempted modification of the acyl moiety on the phenyl ring to improve the anti-*H. pylori* activity (Table 4). Although simple acyl groups such as formyl **59a** or propionyl **59b** weakened the activity somewhat, a di-acetylated compound **10c′** afforded good activity. However, this di-acetylated derivative **10c′** was relatively unstable chemically, affording mono-acetylated derivative **10c** upon hydrolysis. Other acyl compounds, having an amino (**61**), oxime moiety (**63**), basic heterocycle (**65**) and hydroxy group (**66**) in the side chain, did not have improved activity. Moreover, compounds having other bond types, such as sulfonamide **68** or urea **69** did not give improved activity.

From these data, it is clear that the in vitro anti-*H. pylori* activity of **10c** is highly specific in connection with the structure and is superior to AMPC or CAM. In addition, **10c** has no activity against other common bacteria (Table 5). Therefore, this compound may solve problems resulting from antimicrobial activity against other common bacteria, and express potency as a novel and selective inhibitor of *H. pylori* growth.

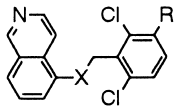
As a preclinical study, we examined the therapeutic effect of **10c** in a mouse infection model in comparison to AMPC or CAM (Table 6). A dosage regimen of three times per day for 4 days at 100 and 10 mg/kg, in *H. pylori* FP1757 infected mouse, followed by assessment of clearance was employed. Unfortunately, at both doses, no clearance was observed at all in comparison to control, despite very potent in vitro activity. To understand the lack of in vivo activity, we considered two possible factors: (1) the compound cannot permeate into mucosa due to high lipophilicity; (2) there is a low concentration of free compound in the stomach because of absorption to food in the stomach. We examined the degree of lipophilicity by measurement of high performance liquid chromatography (HPLC) retention times. Concerning absorption to food, we measured the MIC value in the presence of egg yolk containing agar[33] instead of horse blood. Egg yolk containing agar is thought to be a medium that better reflects the conditions of the stomach. As a result, among various compounds **10c** has fairly high lipophilicity, and a weaker MIC value in egg yolk agar (Table 6). Interestingly, we found that compound **63**, having an amino group in the side chain, showed lower lipophilicity and no reduction in activity in egg yolk, but it was also inactive in the mouse infection model. Additionally, we examined the time-dependence of the bactericidal activity of compound **10c** and AMPC (**1**) (Fig. 2). AMPC displays good bactericidal activity at 6 h in the case of 4MIC, although it is unchanged

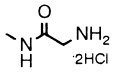
Table 5. Antibacterial activity against other common bacteria

Compound	MIC (μg/mL)[a]				
	S. aureus 209P JC-1	*E. faecalis* 0115	*E. coli* NIHJ JC-2	*S. marcescens* 3013	*M.(B.) catarrhalis* 6014
10c	> 100	> 100	> 100	> 100	> 100
AMPC (**1**)	0.05	0.39	3.13	> 100	0.39
CAM (**2**)	0.1	0.2	100	100	< 0.025

[a] MIC (μg/mL), mueller–hinton agar (difco), 37°C, 18 h, stamp method.

Table 6. Investigation of therapeutic effect



Compound	X	R	LC retention time[a]	MIC (μg/mL)		b/a	Mouse clearance rate[d]	
				Horse blood[b] (a)	Egg yolk[c] (b)		Dose (mg/kg)	
							100	10
63	O		0.45	0.78	0.78	1.0	0/3	N.T.[e]
10c	O		1.0	0.0125	0.1	8.0	0/5	N.T.
38	NH		1.37	0.025	0.39	15.6	0/3	N.T.
AMPC (**1**)			N.T.	0.025	0.025	1.0	N.T.	3/3
CAM (**2**)			N.T.	0.05	0.1	2.0	3/3	0/3

[a] **10c** = 1.0, 0.1%TFA-H_2O/CH_3CN = 75/25, column: ODS-80TM, flow rate: 1 mL/min, detection: 254 nm.
[b] MIC (μg/mL), *H. pylori* FP1757, brucella agar + 7% horse blood, 37°C, 72 h, 10% CO_2, stamp method.
[c] MIC (μg/mL), *H. pylori* FP1757, brucella agar + 10% egg yolk (raw), 37°C, 72 h, 10% CO_2, stamp method.
[d] Mouse, PO, infection: *H. pylori* FP1757.
[e] NT = not tested.

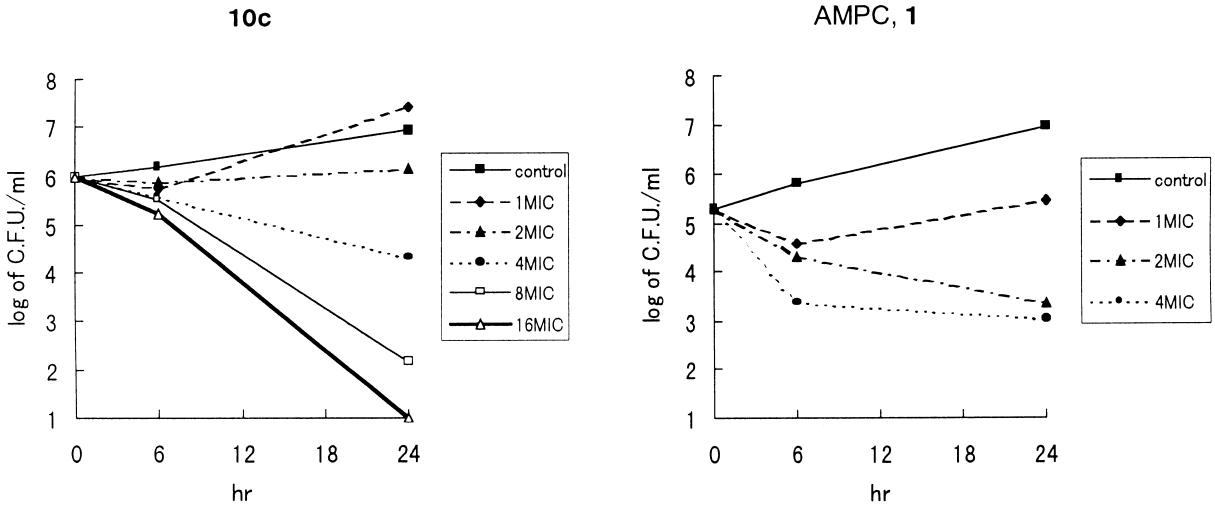


Figure 2. Bactericidal activity against *H. pylori* FP1757. Culture: brucella broth + 2% starch + 3% FBS, 37°C, 10% CO_2.

at 24 h. **10c** expresses poor bactericidal activity at 6 h even at 16MIC. **63** showed the same tendency as **10c** (data not shown). Based on these observations, the poor bactericidal activity of **10c** and other benzyloxyisoquinoline derivatives at early contact times may be the cause of poor in vivo effect. Since in the stomach, mucosa peel off in a short time, we concluded that poor bactericidal activity at early contact times is a major factor for lack of therapeutic effect of **10c** and other benzyloxyisoquinoline derivatives, due to rapid excretion of the drug along with peeling off of mucosa in the stomach.

As part of our search for potent anti-*H. pylori* agents we next investigated a series of compounds having strong bactericidal activity at short contact times, and will describe these results in subsequent publications.

Experimental

Melting points were determined using a Thomas–Hoover capillary melting apparatus and are uncorrected. IR spectra were recorded on a Horiba Spectradesk FT-210 spectrometer as KBr disks, neat or films as indicated. NMR spectra were measured on a Brucker AC200P (^{1}H, 200 MHz) and chemical shifts are expressed in δ ppm with TMS as internal standard. Mass spectra were measured on a Hitachi Model M-1000H mass spectrometer using APCI for ionization. Elemental analyses

were carried out on a Perkin–Elmer 2400 CHN Elemental Analyzer. The analytical HPLC was performed on Hitachi L-6000 instruments using Toso ODS-80TM column (100×4.6 mm) at a flow rate of 1.0 mL/min and an injection volume of 5 μL. Reagents and solvents were used as obtained from commercial suppliers without further purification. Column chromatography was performed using silica-gel, and reaction progress was determined by TLC analysis on silica-gel coated glass plates. Visualization was with UV light (254 nm) or iodine.

5-Benzyloxyisoquinoline (5a). A solution of 5-hydroxyisoquinoline (10 g, 69 mmol) in 150 mL of *N,N*-dimethylformamide (DMF) was cooled to 0–5°C and treated with 60% sodium hydride (2.60 g) over 5 min. After 30 min, benzylbromide (10.1 g, 59 mmol) was added dropwise over 5 min. After a further 1.5 h, the reaction was quenched with brine and extracted with AcOEt (2×). The combined organic layers were washed with 1 N-sodium hydroxide solution (5×), brine, dried over magnesium sulfate and evaporated under reduced pressure. The resulting brown oil was purified by silica-gel chromatography (eluent AcOEt:hexane, 1:1) to give 11.1 g of **5a** (80%) as a purple colored solid: mp 58–62°C; IR (KBr) 1626, 1581 cm^{-1}; ^1H NMR (DMSO-d_6) δ 5.35 (s, 2H), 7.32–7.49 (m, 4H), 7.56–7.71 (m, 4H), 8.00 (d, 1H, J = 5.8 Hz), 8.52 (d, 1H, J = 5.8 Hz), 9.29 (s, 1H); MS m/z 236 (MH^+).

Preparation of **5b**–**d**,**8a**–**c**,**12**, **23**, **32**, **51**, **55a**,**b**, **58** was carried out by a similar method to that described for **5a**.

5-(2,6-Dichlorobenzyloxy)isoquinoline (5b). Yield 24%; mp 112–113°C (from CH_2Cl_2–isopropyl ether (IPE):hexane); IR (KBr) 1583, 1566 cm^{-1}; ^1H NMR (DMSO-d_6) δ 5.48 (s, 2H), 7.50–7.59 (m, 2H), 7.60–7.81 (m, 5H), 8.46 (d, 1H, J = 5.8 Hz), 9.29 (s, 1H); MS m/z 304 (MH^+).

5-(3-Benzofuroxanylmethoxy)isoquinoline (5c). Yield 71%; mp 179–180°C; IR (KBr) 1628, 1585 cm^{-1}; ^1H NMR (DMSO-d_6) δ 5.74 (s, 2H), 7.47 (d, 1H, J = 7.5 Hz), 7.60–7.76 (m, 3H), 7.84 (d, 1H, J = 6.6 Hz), 8.01–8.09 (m, 2H), 8.52 (d, 1H, J = 5.8 Hz), 9.30 (s, 1H); MS m/z 278 (MH^+).

5-(3-Methoxycarbonyl)benzyloxyisoquinoline (5d). Yield 57%; mp 86–90°C; IR (KBr) 1713 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.88 (s, 3H), 5.44 (s, 2H), 7.34 (d, 1H, J = 7.4 Hz), 7.57–7.73 (m, 3H), 7.87 (d, 1H, J = 7.7 Hz), 7.95–8.01 (m, 2H), 8.15 (s, 1H), 8.53 (d, 1H, J = 5.8 Hz), 9.30 (s, 1H); MS m/z 294 (MH^+).

5-(3-Nitrobenzyloxy)isoquinoline (8a). Yield 100%; ^1H NMR ($CDCl_3$) δ 5.36 (s, 2H), 7.06 (d, 1H, J = 6.7 Hz), 7.47–7.66 (m, 3H), 7.87 (d, 1H, J = 7.7 Hz), 8.07 (d, 1H, J = 5.8 Hz), 8.24 (d, 1H, J = 8.1 Hz), 8.40 (s, 1H), 8.57 (d, 1H, J = 5.8 Hz), 9.24 (s, 1H); MS m/z 281 (MH^+).

5-(2-Chloro-3-nitrobenzyloxy)isoquinoline (8b). Yield 98%; IR (KBr) 1627, 1583 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.44 (s, 2H), 7.09 (d, 1H, J = 7.4 Hz), 7.44–7.57 (m, 3H), 7.84 (d, 1H, J = 8.0 Hz), 7.92 (d, 1H, J = 8.5 Hz), 8.10 (d, 1H, J = 5.9 Hz), 8.58 (d, 1H, J = 5.9 Hz), 9.25 (s, 1H); MS m/z 315 (MH^+).

5-(2,6-Dichloro-3-nitrobenzyloxy)isoquinoline (8c). Yield 82%; mp 119–120°C (from AcOEt:hexane); ^1H NMR ($CDCl_3$) δ 5.54 (s, 2H), 7.22 (d, 1H, J = 7.2 Hz), 7.53–7.66 (m, 3H), 7.84 (d, 1H, J = 8.7 Hz), 7.92 (d, 1H, J = 5.9 Hz), 8.50 (d, 1H, J = 5.9 Hz), 9.23 (s, 1H); MS m/z 349 (MH^+).

5-(2-Chloro-6-nitrobenzyloxy)isoquinoline (12). Yield 80%; IR (KBr) 1673, 1627, 1583 cm^{-1}; ^1H NMR (DMSO-d_6) δ 5.57 (s, 2H), 7.41 (d, 1H, J = 6.8 Hz), 7.60–7.78 (m, 4H), 7.96–8.05 (m, 2H), 8.48 (d, 1H, J = 5.8 Hz), 9.29 (s, 1H); MS m/z 315 (MH^+).

5-(3-Acetamido-2,6-difluorobenzyloxy)isoquinoline (23). Yield 34%; mp 195–198°C; IR (KBr) 1703 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.09 (s, 3H), 5.28 (s, 2H), 7.14–7.23 (m, 1H), 7.48 (d, 1H, J = 6.3 Hz), 7.61–7.97 (m, 4H), 8.47 (d, 1H, J = 5.8 Hz), 9.28 (s, 1H), 9.83 (s, 1H); MS m/z 329 (MH^+).

5-{(2-Acetamido-5-chlorothiazol-4-yl)methoxy}isoquinoline (32). Yield 8%; mp 240–242°C (dec.); IR (KBr) 1678 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.15 (s, 3H), 5.28 (s, 2H), 7.38 (d, 1H, J = 7.4 Hz), 7.57–7.73 (m, 2H), 7.89 (d, 1H, J = 5.8 Hz), 8.49 (d, 1H, J = 5.8 Hz), 9.28 (s, 1H), 12.52 (s, 1H); MS m/z 334 (MH^+).

7-(3-Acetamido-2,6-dichlorobenzyloxy)isoquinoline (51). Yield 4%; mp >158°C (dec.) (from CH_2Cl_2:hexane); IR (KBr) 1662, 1629, 1587 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.12 (s, 3H), 5.43 (s, 2H), 7.47 (d, 1H, J = 8.8 Hz), 7.57 (d, 1H, J = 8.8 Hz), 7.80–7.84 (m, 3H), 7.93 (d, 1H, J = 9.1 Hz), 8.41 (d, 1H, J = 5.7 Hz), 9.25 (s, 1H), 9.71 (s, 1H); MS m/z 363 (MH^+).

8-(3-Acetamido-2,6-dichlorobenzyloxy)isoquinoline (55a). Yield 25%; mp 166–170°C (from CH_2Cl_2:hexane); IR (KBr) 1662, 1627 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.13 (s, 3H), 5.53 (s, 2H), 7.41 (d, 3H, J = 7.6 Hz), 7.55–7.88 (m, 5H), 8.51 (d, 1H, J = 5.6 Hz), 9.33 (s, 1H), 9.71 (s, 1H); MS m/z 361 (MH^+).

5-(3-Acetamido-2,6-dichlorobenzyloxy)quinoline (55b). Yield 20%; mp 197–200°C; IR (KBr) 1666, 1591 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.13 (s, 3H), 5.49 (s, 2H), 7.35 (d, 1H, J = 7.2 Hz), 7.45 (dd, 1H, J = 4.6, 8.4 Hz), 7.50–7.88 (m, 4H), 8.37 (d, 1H, J = 8.4 Hz), 8.89 (dd, 1H, J = 1.7, 4.3 Hz), 9.72 (s, 1H); MS m/z 361 (MH^+).

5-(3-Acetamido-2,6-dichlorobenzyloxy)-3-methylisoquinoline (58). Yield 18%; mp 223–225°C; IR (KBr) 3278, 1668 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.13 (s, 3H), 2.56 (s, 3H), 5.46 (s, 2H), 7.43–7.61 (m, 4H), 7.68 (d, 1H, J = 7.9 Hz), 7.85 (d, 1H, J = 8.8 Hz), 9.19 (s, 1H), 9.73 (s, 1H); MS m/z 375 (MH^+).

5-(3-Hydroxymethylbenzyloxy)isoquinoline (6). To an ice-cooled mixture of lithium aluminium hydride (427 mg, 11.3 mmol) in tetrahydrofuran (THF) (30 mL) was added a solution of **5d** (3.0 g, 10.2 mmol) in THF (20 mL) dropwise over 30 min. After a further 1.5 h,

excess reagent was destroyed by dropwise addition of a saturated aqueous solution of Rochelle's salt. The mixture was then filtered and evaporated under reduced pressure. The residue was purified by column chromatography over silica-gel (eluent AcOEt) and then recrystallised from CH_2Cl_2:methanol:IPE to give 1.34 g of **6** (49%) as a white powder: mp 124–126°C; IR (KBr) 3213, 1585 cm^{-1}; ^1H NMR (DMSO-d_6) δ 4.54 (d, 2H, J = 5.7 Hz), 5.23 (t, 1H, J = 5.7 Hz), 5.34 (s, 2H), 7.29–7.42 (m, 4H), 7.52 (s, 1H), 7.56–7.71 (m, 2H), 7.99 (d, 1H, J = 5.8 Hz), 8.52 (d, 1H, J = 5.8 Hz), 9.28 (s, 1H); MS m/z 266 (MH^+).

5-(3-Aminobenzyloxy)isoquinoline (9a). A suspension of **8a** (5.3 g, 18.9 mmol), iron(III) chloride (360 mg) and carbon powder (360 mg) in a mixture of water and methanol (2:8, 200 mL) was treated with hydrazine monohydrate (7.01 g, 140 mmol) and stirred under reflux for 4 h. The mixture was filtered and evaporated under reduced pressure. To the obtained residue was added saturated sodium hydrogen carbonate and the mixture extracted with AcOEt (3×). The combined extracts were washed with brine, dried over magnesium sulfate and evaporated under reduced pressure. The resulting brown oil was purified by silica-gel chromatography (eluent AcOEt:hexane, 1:1) to give 4.0 g of **9a** (84%): mp 175–176°C; IR (KBr) 1629, 1585 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.41 (s, 2H), 6.66 (dd, 1H, J = 2.0, 7.9 Hz), 6.81 (s, 1H), 6.86 (d, 1H, J = 7.6 Hz), 7.02 (dd, 1H, J = 1.2, 7.2 Hz), 7.15–7.54 (m, 3H), 8.08 (d, 1H, J = 5.8 Hz), 8.52 (d, 1H, J = 5.8 Hz), 9.20 (s, 1H); MS m/z 251 (MH^+).

Preparation of **9b–e**, **13**, **25a–c** was carried out by a similar method to that described for **9a**.

5-(3-Amino-2-chlorobenzyloxy)isoquinoline (9b). Yield 43%; mp 166–168°C; IR (KBr) 1625, 1581 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.33 (s, 2H), 6.80 (dd, 1H, J = 1.6, 7.8 Hz), 7.01 (d, 1H, J = 6.0 Hz), 7.02–7.16 (m, 2H), 7.50–7.63 (m, 2H), 8.18 (d, 1H, J = 5.9 Hz), 8.54 (d, 1H, J = 5.9 Hz), 9.25 (s, 1H); MS m/z 285 (MH^+).

5-(3-Amino-2,6-dichlorobenzyloxy)isoquinoline (9c). Yield 98%; mp 184–185°C; ^1H NMR (DMSO-d_6) δ 5.40 (s, 2H), 5.70 (s, 2H), 6.92 (d, 1H, J = 9.0 Hz), 7.25 (d, 1H, J = 9.0 Hz), 7.49 (d, 1H, J = 7.3 Hz), 7.61–7.75 (m, 2H), 7.81 (d, 1H, J = 5.8 Hz), 8.45 (d, 1H, J = 5.8 Hz), 9.29 (s, 1H); MS m/z 319 (MH^+).

5-(3-Amino-2,5-dichlorobenzyloxy)isoquinoline (9d). Yield 68%; mp 160–162°C; IR (KBr) 1629, 1591 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.25 (s, 2H), 5.27 (s, 2H), 6.79 (d, 1H, J = 2.4 Hz), 7.01–7.08 (m, 2H), 7.47–7.61 (m, 2H), 8.10 (d, 1H, J = 5.9 Hz), 8.57 (d, 1H, J = 5.9 Hz), 9.24 (s, 1H); MS m/z 319 (MH^+).

5-(3-Amino-2,6-dimethylbenzyloxy)isoquinoline (9e). Yield 75%; IR (KBr) 1625, 1581 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.08 (s, 3H), 2.23 (s, 3H), 4.72 (s, 2H), 5.22 (s, 2H), 6.14 (d, 1H, J = 8.0 Hz), 6.83 (d, 1H, J = 8.0 Hz), 7.47 (dd, 1H, J = 1.9, 6.7 Hz), 7.61–7.75 (m, 2H), 7.79 (d, 1H, J = 5.8 Hz), 8.44 (d, 1H, J = 5.8 Hz), 9.28 (s, 1H); MS m/z 279 (MH^+).

5-(2-Amino-6-chlorobenzyloxy)isoquinoline (13). Yield 80%; IR (KBr) 1629, 1600 cm^{-1}; ^1H NMR (DMSO-d_6) δ 5.34 (s, 3H), 5.64 (s, 2H), 6.64–6.72 (m, 2H), 7.09 (t, 1H, J = 8.0 Hz), 7.45 (dd, 1H, J = 1.7, 7.0 Hz), 7.59–7.71 (m, 2H), 7.87 (d, 1H, J = 5.8 Hz), 8.45 (d, 1H, J = 5.8 Hz), 9.27 (d, 1H, J = 0.7 Hz); MS m/z 285 (MH^+).

5-(3-Amino-2,6-bis-methylthio-benzyloxy)isoquinoline (25a). Yield 100%; ^1H NMR (DMSO-d_6) δ 2.16 (s, 3H), 2.30 (s, 3H), 5.65 (s, 2H), 5.74 (brs, 2H), 6.89 (d, 1H, J = 8.5 Hz), 7.35 (d, 1H, J = 8.5 Hz), 7.50–7.53 (m, 1H), 7.61–7.73 (m, 2H), 7.76 (d, 1H, J = 5.8 Hz), 8.42 (d, 1H, J = 5.8 Hz), 9.27 (s, 1H); MS m/z 343 (MH^+)

5-(5-Amino-2-chloro-6-methylthio)benzyloxyisoquinoline (25b). Yield 41%; mp 167–169°C; ^1H NMR (DMSO-d_6) δ 2.18 (s, 3H), 5.54 (s, 2H), 5.78 (s, 2H), 6.89 (d, 1H, J = 8.8 Hz), 7.26 (d, 1H, J = 8.8 Hz), 7.49–7.52 (m, 1H), 7.61–7.79 (m, 3H), 8.44 (d, 1H, J = 5.8 Hz), 9.28 (s, 1H); MS m/z 331 (MH^+).

5-(3-Amino-2-chloro-6-methylthio)benzyloxyisoquinoline (25c). Yield 100%; ^1H NMR (DMSO-d_6) δ 2.32 (s, 3H), 5.49 (s, 2H), 5.63 (brs, 2H), 6.91 (d, 1H, J = 8.5 Hz), 7.31 (d, 1H, J = 8.5 Hz), 7.49–7.52 (m, 1H), 7.61–7.73 (m, 2H), 7.79 (d, 1H, J = 5.8 Hz), 8.44 (d, 1H, J = 5.8 Hz), 9.28 (s, 1H); MS m/z 331 (MH^+).

5-(3-Acetamidobenzyloxy)isoquinoline (10a). A solution of **9a** (0.50 g, 1.99 mmol) and dimethylaminopyridine (2.5 mg) in a mixture of pyridine (2.5 mL), acetic anhydride (2.71 g, 26 mmol) and CH_2Cl_2 (2.5 mL) was allowed to stand at room temperature for 20 h. The solution was evaporated to dryness, and water and AcOEt were added to the residue. The mixture was extracted with AcOEt, washed with brine, dried over magnesium sulfate and evaporated under reduced pressure. The resulting oil was purified by silica-gel chromatography (eluent AcOEt) to give 510 mg of **10a** (87%): mp 109–110°C (from CH_2Cl_2:IPE:hexane); IR (KBr) 1668, 1622, 1591 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.18 (s, 3H), 5.29 (s, 2H), 7.02 (dd, 1H, J = 1.4, 7.0 Hz), 7.19–7.62 (m, 5H), 7.69 (s, 1H), 8.03 (d, 1H, J = 5.8 Hz), 8.46 (d, 1H, J = 5.8 Hz), 9.18 (s, 1H); MS m/z 293 (MH^+).

Preparation of **59b** was carried out by a similar method to that described for **10a**.

5-{2,6-Dichloro-3-(*N*-propionylamino)benzyloxy}isoquinoline (59b). Yield 60%; mp 192.5–194°C (from AcOEt); IR (KBr) 1657 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.10 (t, 3H, J = 7.5 Hz), 2.43 (q, 2H, J = 7.5 Hz), 5.50 (s, 2H), 7.50–7.87 (m, 6H), 8.45 (d, 1H, J = 5.7 Hz), 9.29 (s, 1H), 9.64 (s, 1H); MS m/z 375 (MH^+).

5-{2-Chloro-3-(*N*,*N*-diacetylamino)benzyloxy}isoquinoline (10b′). A solution of **9b** (0.50 g, 1.75 mmol) and dimethylaminopyridine (2.5 mg) in a mixture of pyridine (2.5 mL), acetic anhydride (2.71 g, 26 mmol) and CH_2Cl_2 (2.5 mL) was allowed to stand at room temperature for 20 h. The solution was evaporated to dryness, and water and AcOEt were added to the residue. The mixture was extracted with AcOEt, washed with water and brine, dried over magnesium sulfate and evaporated under reduced

pressure. The obtained solid was recrystallized from a mixture of AcOEt and hexane to give 363 mg of **10b′** (56%): mp 155–157°C; IR (KBr) 1718, 1627, 1585 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.34 (s, 6H), 5.42 (s, 2H), 7.18 (d, 1H, $J=7.4$ Hz), 7.28 (d, 1H, $J=8.6$ Hz), 7.45–7.70 (m, 3H), 7.78 (d, 1H, $J=7.0$ Hz), 8.19 (d, 1H, $J=5.8$ Hz), 8.56 (d, 1H, $J=5.8$ Hz), 9.29 (s, 1H); MS m/z 369 (MH^+).

5-(3-Acetamido-2,6-dichlorobenzyloxy)isoquinoline (10c), 5-{3-(*N*,*N*-Diacetylamino)-2,6-dichlorobenzyloxy}isoquinoline (10c′). A solution of **9c** (1.05 g, 3.29 mmol) in a 1:1:1 mixture of pyridine:CH_2Cl_2:acetic anhydride (15 mL) was treated with 4-dimethylaminopyridine (5 mg) and stirred for 18 h at room temperature. Ice-cooled saturated sodium hydrogen carbonate solution was then added and the mixture stirred 30 min then evaporated under reduced pressure. The residue was taken up in AcOEt then washed with saturated sodium hydrogen carbonate solution (2×), water (3×), brine, dried over magnesium sulfate and evaporated under reduced pressure. AcOEt (100 mL) was added to the residue and the solid removed by filtration to give 75 mg of **10c** (6%) as a white solid. The filtrate was evaporated under reduced pressure and column chromatographed on silica-gel (eluent AcOEt:hexane) to give an additional 600 mg of **10c** (51%) and 200 mg of **10c′** (15%). **10c**: mp 219–220°C (from CH_2Cl_2:hexane); IR (KBr) 1697 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.27 (s, 3H), 5.48 (s, 2H), 7.20–7.26 (m, 1H), 7.43 (d, 1H, $J=9.0$ Hz), 7.51–7.63 (m, 2H), 7.72 (brs, 1H), 7.94 (d, 1H, $J=5.8$ Hz), 8.42–8.50 (m, 2H), 9.22 (s, 1H); MS m/z 361 (MH^+). **10c′**: ^1H NMR ($CDCl_3$) δ 2.33 (s, 6H), 5.51 (s, 2H), 7.21–7.31 (m, 2H), 7.52–7.64 (m, 3H), 7.92 (d, 1H, $J=6.0$ Hz), 8.49 (d, 1H, $J=6.0$ Hz), 9.22 (s, 1H); MS m/z 403 (MH^+).

5-(3-Acetamido-2,5-dichlorobenzyloxy)isoquinoline (10d). To a solution of **9d** (256 mg, 0.80 mmol) in 1,2-dichloroethane (6 mL) was added acetic anhydride (1.1 g, 10.6 mmol), then stirred at 75°C for 2.5 h, and cooled to room temperature. The mixture was poured into saturated sodium hydrogen carbonate, stirred at room temperature for 30 min, and extracted with AcOEt (3×). The combined extracts were washed with brine, dried over magnesium sulfate and evaporated under reduced pressure. The residual solid was recrystallized from a mixture of CH_2Cl_2 and hexane to give 140 mg of **10d** (48%): mp 217–220°C; IR (KBr) 1668, 1585 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.29 (s, 3H), 5.31 (s, 2H), 7.11 (d, 1H, $J=7.4$ Hz), 7.39 (d, 1H, $J=2.3$ Hz), 7.52–7.73 (m, 2H), 8.14 (d, 1H, $J=5.8$ Hz), 8.51 (s, 1H), 8.57 (d, 1H, $J=5.8$ Hz), 9.28 (s, 1H); MS m/z 361 (MH^+).

Preparation of **10c**,**e**, **15**, **26a**–**c** was carried out by a similar method to that described for **10d**.

5-(3-Acetamido-2,6-dichlorobenzyloxy)isoquinoline (10c). Yield 95%; mp 219–220°C (from CH_2Cl_2:hexane); IR (KBr) 1697 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.27 (s, 3H), 5.48 (s, 2H), 7.20–7.26 (m, 1H), 7.43 (d, 1H, $J=9.0$ Hz), 7.51–7.63 (m, 2H), 7.72 (brs, 1H), 7.94 (d, 1H, $J=5.8$ Hz), 8.42–8.50 (m, 2H), 9.22 (s, 1H); MS m/z 361 (MH^+).

5-(3-Acetamido-2,6-dimethylbenzyloxy)isoquinoline (10e). Yield 91%; mp 214–215°C (from CH_2Cl_2:hexane); IR (KBr) 1658, 1585, 1529 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.05 (s, 3H), 2.21 (s, 3H), 2.36 (s, 3H), 5.30 (s, 2H), 7.10 (d, 1H, $J=8.1$ Hz), 7.26 (d, 1H, $J=8.1$ Hz), 7.50 (dd, 1H, $J=0.9$, 6.9 Hz), 7.62–7.79 (m, 3H), 8.43 (d, 1H, $J=5.8$ Hz), 9.28 (s, 1H), 9.39 (s, 1H); MS m/z 321 (MH^+).

5-(2-Acetamido-3,6-dichlorobenzyloxy)isoquinoline (15). Yield 36%; mp 104–106°C; IR (KBr) 1684, 1630, 1585 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.97 (s, 3H), 5.28 (s, 2H), 7.42 (d, 1H, $J=7.3$ Hz), 7.56–7.73 (m, 4H), 7.82 (d, 1H, $J=5.8$ Hz), 8.45 (d, 1H, $J=5.8$ Hz), 9.27 (s, 1H), 9.98 (s, 1H); MS m/z 361 (MH^+).

5-(3-Acetamido-2,6-bis-methylthiobenzyloxy)isoquinoline (26a). Yield 80%; mp 210–212°C (from $CHCl_3$:IPE: hexane); IR (KBr) 1655 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.15 (s, 3H), 2.24 (s, 3H), 2.46 (s, 3H), 5.66 (s, 2H), 7.48–7.54 (m, 2H), 7.62–7.79 (m, 3H), 7.92 (d, 1H, $J=8.7$ Hz), 8.43 (d, 1H, $J=5.8$ Hz), 9.28 (s, 1H), 9.46 (s, 1H); MS m/z 385 (MH^+).

5-(5-Acetamido-2-chloro-6-methylthio)benzyloxyisoquinoline (26b). Yield 69%; mp 196–198°C (from $CHCl_3$: IPE); IR (KBr) 1664 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.18 (s, 3H), 2.26 (s, 3H), 5.63 (s, 2H), 7.52–7.72 (m, 4H), 7.77 (d, 1H, $J=6.0$ Hz), 7.99 (d, 1H, $J=8.8$ Hz), 8.44 (d, 1H, $J=5.8$ Hz), 9.29 (s, 1H), 9.53 (s, 1H); MS m/z 373 (MH^+).

5-(3-Acetamido-2-chloro-6-methylthio)benzyloxyisoquinoline (26c). Yield 89%; mp 211–214°C (from $CHCl_3$:IPE: hexane); IR (KBr) 1676 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.11 (s, 3H), 2.49 (s, 3H), 5.49 (s, 2H), 7.49 (d, 1H, $J=8.7$ Hz), 7.50–7.53 (m, 1H), 7.61–7.80 (m, 4H), 8.44 (d, 1H, $J=5.8$ Hz), 9.28 (s, 1H), 9.61 (s, 1H); MS m/z 373 (MH^+).

5-(3-Acetamido-2-chlorobenzyloxy)isoquinoline (10b). To a solution of **10b′** (187 mg, 0.51 mmol) in ethanol (2 mL) was added pyrrolidine (36 mg, 0.51 mmol) at room temperature. After stirring at room temperature for 5 min, the solution was poured into water, extracted with CH_2Cl_2, dried over magnesium sulfate and evaporated under reduced pressure. The residual solid was recrystallized from a mixture of CH_2Cl_2, hexane and IPE to give 118 mg of **10b** (71%): mp 196–198°C; IR (KBr) 1662, 1583, 1537 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.11 (s, 3H), 5.41 (s, 2H), 7.37–7.75 (m, 6H), 7.98 (d, 1H, $J=5.8$ Hz), 8.51 (d, 1H, $J=5.8$ Hz), 9.29 (s, 1H), 9.62 (s, 1H); MS m/z 327 (MH^+).

5-(2-Amino-3,6-dichlorobenzyloxy)isoquinoline (14). To a solution of **13** (200 mg, 0.70 mmol) in $CHCl_3$ (2 mL) was added *N*-chlorosuccinimide (95 mg, 0.71 mmol) and then stirred under reflux for 3.5 h. The obtained suspension was filtered and evaporated under reduced pressure. The residue was purified by silica-gel chromatography (eluent AcOEt:IPE) to give 146 mg of **14** (65%) as a solid: ^1H NMR (DMSO-d_6) δ 5.41 (s, 2H), 5.83 (s, 2H), 6.74 (d, 1H, $J=8.5$ Hz), 7.33 (d, 1H, $J=8.5$ Hz), 7.44 (d, 1H, $J=4.5$ Hz), 7.60–7.73 (m, 2H), 7.84 (d, 1H, $J=5.8$ Hz), 8.45 (d, 1H, $J=5.8$ Hz), 9.27 (s, 1H); MS m/z 319 (MH^+).

2,6-Difluoro-3-nitrobenzoic acid (17). Fuming nitric acid (39 g, 620 mmol) was cooled to −50°C and treated with 2,6-difluorobenzoic acid (5.0 g, 31.6 mmol), added

portionwise over 15 min keeping the temperature at −40 to −50°C. The mixture was then warmed to 0°C over 30 min. After a further 30 min at 0°C the reaction was quenched with water and extracted with AcOEt (2×). The combined organic extracts were washed with brine (2×), dried over magnesium sulfate and evaporated under reduced pressure to give 6.4 g of **17** (100%) as a solid: ^{1}H NMR (DMSO-d_6) δ 7.44–7.54 (m, 1H), 8.33–8.45 (m, 1H).

2,6-Difluoro-3-nitrobenzylalcohol (18). An ice-cooled suspension of sodium borohydride (4.53 g, 120 mmol) in THF (200 mL) was treated dropwise with **17** (12.8 g, 63 mmol) in THF (60 mL) over 30 min. Boron trifluoride etherate (24.15 g, 170 mmol) was then added dropwise over 30 min. The solution was then stirred 15 h at room temperature then quenched with 1 N-hydrochloric acid and extracted with ethyl acetate (3×). The combined organic layers were washed with water (2×) and brine and dried over magnesium sulfate and evaporated under reduced pressure to give 11.25 g of **18** (94%) as a solid: ^{1}H NMR (DMSO-d_6) δ 4.57 (d, 2H, J = 5.0 Hz), 5.52 (t, 1H, J = 5.0 Hz), 7.32–7.42 (m, 1H), 8.17–8.33 (m, 1H).

3-Amino-2,6-difluorobenzylalcohol (19). A solution of **18** (2.0 g, 10.6 mmol) in AcOEt (20 mL) was treated with wet 10% palladium on carbon (500 mg) then exposed to hydrogen at atmospheric pressure. After 4 h the mixture was filtered through Celite and the filtrate evaporated under reduced pressure to give 1.68 g of **19** (100%) as a solid: ^{1}H NMR (DMSO-d_6) δ 4.42–4.47 (m, 2H), 4.92 (brs, 2H), 5.09 (t, 1H, J = 5.7 Hz), 6.62–6.80 (m, 2H); MS m/z 160 (MH^+).

3-Acetamido-2,6-difluorobenzylalcohol-*O*-acetate (20). A solution of **19** (1.68 g, 10.5 mmol) in 1,2-dichloroethane (20 mL) was treated with acetic anhydride (5.4 g, 53 mmol) and heated 1 h at 70°C. Triethylamine (2.18 g, 21.5 mmol) was added and the solution heated a further 1.5 h then evaporated under reduced pressure to give a solid residue. The solid was dissolved in ethanol (50 mL) and treated with pyrrolidine (10 mL). After 15 min, the reaction mixture was diluted with AcOEt, washed with 1 N-hydrochloric acid (2×), brine (1×), saturated sodium hydrogen carbonate (2×), water (1×), dried over magnesium sulfate then evaporated under reduced pressure and the residue purified by silica-gel chromatography (eluent AcOEt) and then recrystallized from CH_2Cl_2:IPE to give 1.08 g of **20** (42%) as a white crystalline solid: IR (KBr) 3340, 1722, 1693 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.03 (s, 3H), 2.07 (s, 3H), 5.14 (s, 2H), 7.07–7.16 (m, 1H), 7.78–7.90 (m, 1H), 9.77 (s, 1H); MS m/z 244 (MH^+).

3-Acetamido-2,6-difluorobenzylalcohol (21). A solution of **20** (617 mg, 2.5 mmol) in methanol (10 mL) was treated with 1 N-sodium hydroxide solution (3.0 mL). After 20 h at room temperature, 1 N-hydrochloric acid was added and the mixture extracted with AcOEt (5×). The combined organic layers were dried over magnesium sulfate and evaporated under reduced to give 500 mg of **21** (98%) as a white powder: IR (KBr) 1680 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.07 (s, 3H), 4.50 (s, 2H), 6.98–7.07 (m, 1H), 7.67–7.79 (m, 1H), 9.70 (s, 1H); MS m/z 202 (MH^+).

3-Acetamido-2,6-difluorobenzyl methanesulfonate (22). A solution of **21** (362 mg, 1.79 mmol) and triethylamine (237 mg, 2.34 mmol) in CH_2Cl_2 (10 mL) was cooled to 0°C and treated dropwise with methanesulfonyl chloride (226 mg, 1.98 mmol). After 30 min the reaction was diluted with AcOEt, washed with water (3×), dried over magnesium sulfate and evaporated under reduced pressure to give 520 mg of **22** (100%) as a white solid: ^{1}H NMR ($CDCl_3$) δ 2.23 (s, 3H), 3.05 (s, 3H), 5.34 (t, 2H, J = 1.2 Hz), 6.95 (ddd, 1H, J = 1.9, 9.0, 9.0 Hz), 7.40 (brs, 1H), 8.26–8.38 (m, 1H); MS m/z 280 (MH^+).

Preparation of **11**, **42** was carried out by a similar method to that described for **22**.

2-Chloro-6-nitrobenzyl methanesulfonate (11). Yield 100%; ^{1}H NMR ($CDCl_3$) δ 3.09 (s, 3H), 5.63 (s, 2H), 7.55 (t, 1H, J = 8.1 Hz), 7.75 (dd, 1H, J = 1.3, 8.1 Hz), 7.88 (dd, 1H, J = 1.3, 8.1 Hz); MS m/z 266 (MH^+).

3-Acetamido-2,6-dichlorobenzyl methanesulfonate (42). Yield 96%; ^{1}H NMR ($CDCl_3$) δ 2.27 (s, 3H), 3.09 (s, 3H), 5.53 (s, 2H), 7.39 (d, 1H, J = 9.0 Hz), 7.65 (brs, 1H), 8.45 (d, 1H, J = 9.0 Hz); MS m/z 312 (MH^+).

5-(2,6-Bis-methylthio-3-nitrobenzyloxy)isoquinoline (24a), 5-(2-chloro-6-methylthio-5-nitrobenzyloxy)isoquinoline (24b) and 5-(2-chloro-6-methylthio 3-nitrobenzyloxy)isoquinoline (24c). A solution of **8c** (2.0 g, 5.73 mmol) in DMF (20 mL) was treated with 95% sodium thiomethoxide (465 mg, 6.30 mmol) at 0°C for 1.5 h then diluted with AcOEt, washed with saturated sodium chloride solution, water (5×), saturated sodium chloride solution, dried over magnesium sulfate and evaporated under reduced pressure. The residue was column chromatographed on silica-gel chromatography, eluting with AcOEt:hexane, to give 1.25 g of **24b** (60%), 248 mg of **24a** (12%), and finally 137 mg of **24c** (7%) as off white solids. **24a**: ^{1}H NMR (DMSO-d_6) δ 2.39 (s, 3H), 2.56 (s, 3H), 5.67 (s, 2H), 7.54–7.81 (m, 5H), 7.98 (d, 1H, J = 8.6 Hz), 8.45 (d, 1H, J = 5.8 Hz), 9.29 (s, 1H); MS m/z 373 (MH^+). **24b**: ^{1}H NMR (DMSO-d_6) δ 2.41 (s, 3H), 5.67 (s, 2H), 7.51–7.75 (m, 4H), 7.89–7.95 (m, 1H), 8.07 (d, 1H, J = 8.7 Hz), 8.45 (d, 1H, J = 5.8 Hz), 9.30 (s, 1H); MS m/z 361 (MH^+). **24c**: ^{1}H NMR (DMSO-d_6) δ 2.60 (s, 3H), 5.52 (s, 2H), 7.51–7.81 (m, 5H), 8.15 (d, 1H, J = 8.8 Hz), 8.46 (d, 1H, J = 5.8 Hz), 9.29 (s, 1H); MS m/z 361 (MH^+).

5-(2-Chloro-6-ethoxycarbonylmethylthio-5-nitrobenzyloxy)isoquinoline (27). A solution of **8c** (1.27 g, 3.62 mmol) in DMF (15 mL) was cooled to 0°C and treated with ethyl thioglycolate (457 mg, 3.80 mmol) followed by triethylamine (403 mg) dropwise. After 3 h the reaction was diluted with AcOEt, washed with water (4×), saturated sodium chloride solution, dried over magnesium sulfate, evaporated under reduced pressure and chromatographed over silica-gel (eluent AcOEt:hexane) to give 943 mg of 27 (60%) as a white

solid: IR (KBr) 1738, 1541 cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.97 (t, 3H, $J=7.1$ Hz), 3.76 (s, 2H), 3.88 (q, 2H, $J=7.1$ Hz), 5.68 (s, 2H), 7.53 (d, 1H, $J=7.3$ Hz), 7.64–7.81 (m, 3H), 7.97 (d, 1H, $J=8.7$ Hz), 8.10 (d, 1H, $J=8.7$ Hz), 8.46 (d, 1H, $J=5.9$ Hz), 9.30 (s, 1H); MS m/z 433 (MH^+).

5-{(7-Chloro-2*H*-1,4-benzothiazin-3(4*H*)-one-8-yl)methoxy}isoquinoline (28). A mixture of **27** (935 mg, 2.16 mmol), iron powder (600 mg) and ammonium chloride (81 mg) in a mixture of ethanol (28 mL) and water (3 mL) was refluxed for 4 h, cooled and concentrated under reduced pressure. Water and CH_2Cl_2 were added to the residue, stirred 5 min then filtered. The mixture was extracted with AcOEt and the organic layer was washed with saturated sodium chloride solution (1×), water (2×), dried over magnesium sulfate, evaporated under reduced pressure and chromatographed over silica-gel (eluent AcOEt: hexane) to give 300 mg of **28** (39%) as an off-white solid: mp 265–267°C; IR (KBr) 1686 cm^{-1}; ^1H NMR (DMSO-d_6) δ 3.42 (s, 2H), 5.45 (s, 2H), 7.08 (d, 1H, $J=8.7$ Hz), 7.43 (d, 1H, $J=8.6$ Hz), 7.50 (d, 1H, $J=7.5$ Hz), 7.61–7.76 (m, 2H), 7.81 (d, 1H, $J=5.8$ Hz), 8.46 (d, 1H, $J=5.8$ Hz), 9.29 (s, 1H), 10.79 (s, 1H); MS m/z 357 (MH^+).

2-Acetamido-5-chloro-4-chloromethylthiazole (30). A solution of 2-acetamido-4-chloromethylthiazole (**29**, 4.76 g, 24.9 mmol) and *N*-chlorosuccinimide (3.34 g, 25 mmol) in $CHCl_3$ (200 mL) was heated to reflux for 1.5 h, cooled to room temperature, filtered and the filtrate evaporated to dryness under reduced pressure. Water was added to the residue and the solid filtered, washed thoroughly with water and dried. The solid was recrystallized from benzene to give 2.0 g of **30** (36%) as a light brown powder: ^1H NMR (DMSO-d_6) δ 2.15 (s, 3H), 4.68 (s, 2H), 12.52 (s, 1H); MS m/z 225 (MH^+).

2-Acetamido-5-chloro-4-iodomethylthiazole (31). A solution of **30** (1.0 g, 4.44 mmol) in acetone (20 mL) was treated with sodium iodide (1.33 g, 8.87 mmol) and then stirred 2 h at room temperature. The mixture was diluted with AcOEt, washed with saturated sodium thiosulfate solution (1×), water (2×), dried over magnesium sulfate and evaporated under reduced pressure to give 1.3 g of **31** (92%): ^1H NMR (DMSO-d_6) δ 2.14 (s, 3H), 4.43 (s, 2H), 12.49 (s, 1H); MS m/z 317 (MH^+).

3-Acetamido-2,6-dichlorobenzaldehyde (34). A solution of **33** (5.0 g, 21.4 mmol) in $CHCl_3$ (50 mL) was treated with manganese dioxide (18.8 g, 216 mmol) and stirred under reflux for 3 h. The reaction mixture was filtered and evaporated under reduced pressure to give 4.34 g of **34** (87%) as a white solid: IR (KBr) 1689, 1585 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.28 (s, 3H), 7.40 (d, 1H, $J=9.0$ Hz), 7.78 (s, 1H), 8.58 (d, 1H, $J=9.0$ Hz), 10.47 (s, 1H); MS m/z 232 (MH^+).

***N*-(3-Acetamido-2,6-dichlorobenzylidene)-5-aminoisoquinoline (35).** A solution of **34** (2.0 g, 8.62 mmol) and 5-aminoisoquinoline (1.24 g, 8.60 mmol) in ethanol (50 mL) was stirred at room temperature for 5 days and filtered. The obtained solid was dried in a dessicator to give 2.48 g of **35** (81%) as a pale brown solid: mp 200–202°C; IR (KBr) 1660, 1629, 1583 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.15 (s, 3H), 7.52 (d, 1H, $J=7.5$ Hz), 7.64 (d, 1H, $J=8.8$ Hz), 7.77 (dd, 1H, $J=7.5$, 7.5 Hz), 7.88 (d, 1H, $J=8.8$ Hz), 8.04 (d, 1H, $J=5.8$ Hz), 8.09 (d, 1H, $J=7.5$ Hz), 8.58 (d, 1H, $J=5.8$ Hz), 8.90 (s, 1H), 9.38 (s, 1H), 9.80 (s, 1H); MS m/z 358 (MH^+).

5-(3-Acetamido-2,6-dichlorobenzamido)isoquinoline *N*-oxide (36). A solution of **35** (2.0 g, 5.59 mmol) in $CHCl_3$ (40 mL) was treated with 3-chloroperbenzoic acid (3.48 g, 16 mmol) and stirred under reflux for 4 h. The mixture was quenched by saturated aqueous sodium bicarbonate solution and filtered. The obtained powder was washed with water, AcOEt and CH_2Cl_2, and dried under reduced pressure to give 1.21 g of **36** (56%) as a brown powder: IR (KBr) 1672, 1635, 1525 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.15 (s, 3H), 7.59 (d, 1H, $J=8.8$ Hz), 7.70–7.90 (m, 4H), 8.04 (d, 1H, $J=7.4$ Hz), 8.26 (dd, 1H, $J=1.8$, 7.4 Hz), 9.01 (d, 1H, $J=1.5$ Hz), 9.77 (s, 1H), 11.00 (s, 1H); MS m/z 390 (MH^+).

5-(3-Acetamido-2,6-dichlorobenzamido)isoquinoline (37). To a solution of **36** (50 mg, 0.13 mmol) and ammonium formate (40 mg) in methanol (5 mL) was added 10% palladium:carbon (10 mg) and the mixture stirred at room temperature for 4 h. The mixture was filtered and evaporated under reduced pressure. The obtained residue was chromatographed over silica-gel (eluent AcOEt) to give 35 mg of **37** (72%) as a white solid: mp 240–245°C; IR (KBr) 1666, 1591, 1533 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.15 (s, 3H), 7.60 (d, 1H, $J=8.8$ Hz), 7.70–7.90 (m, 2H), 8.00–8.10 (m, 3H), 8.58 (d, 1H, $J=6.0$ Hz), 9.37 (s, 1H), 9.77 (s, 1H), 10.97 (s, 1H); MS m/z 374 (MH^+).

5-{(3-Acetamido-2,6-dichlorobenzyl)amino}isoquinoline (38). A suspension of **35** (3.15 g, 8.80 mmol) in ethanol (30 mL) was treated with sodium borohydride (0.36 g, 9.52 mmol) at 0°C. After 14 h at room temperature, the reaction was quenched with water (300 mL) and extracted with AcOEt (2×). The combined organic layers were washed with brine, dried over magnesium sulfate and evaporated under reduced pressure to give a crude solid that was then recrystallized from a mixture of methanol, CH_2Cl_2 and hexane to give 2.76 g of **38** (87%) as a white solid: mp 210–212°C; IR (KBr) 1687, 1585 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.12 (s, 3H), 4.59 (d, 2H, $J=4.3$ Hz), 6.48 (t, 1H, $J=4.3$ Hz), 6.88 (d, 1H, $J=7.4$ Hz), 7.31 (d, 1H, $J=8.0$ Hz), 7.45–7.52 (m, 2H), 7.78 (d, 1H, $J=8.8$ Hz), 8.14 (d, 1H, $J=6.0$ Hz), 8.35 (d, 1H, $J=6.0$ Hz), 9.13 (s, 1H), 9.62 (s, 1H); MS m/z 360 (MH^+).

***O*-5-Isoquinolyl dimethylthiocarbamate (39).** A solution of **3** (5.0 g, 34 mmol) in DMF (50 mL) was cooled to 0–5°C and treated with 60% sodium hydride (1.36 g) and stirred 15 min. Dimethylthiocarbamoyl chloride (4.22 g, 34 mmol) was then added and the solution stirred for 10 min at 0–5°C and 30 min at room temperature then at 60°C for 1 h. The solution was cooled, diluted with 1 N-sodium hydroxide solution and extracted with AcOEt (3×). The combined organic layers were washed with 1 N-sodium hydroxide (3×), brine (2×), water (3×) then brine, and dried over magnesium sulfate and evaporated under reduced pressure. The obtained residue was

chromatographed over silica-gel (eluent AcOEt:hexane) to give 6.0 g of **39** (76%) as a yellow powder: ^{1}H NMR (DMSO-d_6) δ 3.44 (s, 3H), 3.49 (s, 3H), 7.52 (d, 1H, J = 6.7 Hz), 7.65–7.76 (m, 2H), 8.06 (d, 1H, J = 8.2 Hz), 8.54 (d, 1H, J = 5.9 Hz), 9.40 (s, 1H); MS m/z 233 (MH^+).

***S*-5-Isoquinolyl dimethylthiocarbamate (40). 39** (5.69 g, 24.4 mmol) was placed on a preheated oil bath at 210–220°C and heated for 5 h and cooled to room temperature. The solid residue was chromatographed over silica-gel (eluent AcOEt) to give 3.1 g of **40** (55%) as a yellow solid, along with 1.58 g of recovered **39**: ^{1}H NMR ($CDCl_3$) δ 3.03, 3.09, 3.23, 3.27 (each s, total 6H), 7.50–7.66 (m, 1H), 7.96–7.99 (m, 1H), 8.04–8.08 (m, 2H), 8.60 (d, 1H, J = 5.9 Hz), 9.27 (s, 1H); MS m/z 233 (MH^+).

Isoquinoline-5-thiol (41). A solution of **40** (1.0 g, 4.29 mmol) in methanol (20 mL) was treated with 1 N-sodium hydroxide solution (21.5 mL) and the solution warmed to reflux for 1 h. The solution was cooled then evaporated to dryness under reduced pressure. Water was added to the residue and the aqueous solution extracted with $CHCl_3$. The aqueous layer was then evaporated partially to remove organic solvent then adjusted to pH 5.8–6.0 with acetic acid. The precipitate was removed by filtration, washed with water then dried to give 619 mg of **41** (89%) as an orange powder: ^{1}H NMR (DMSO-d_6) δ 5.85–6.60 (brs, 1H), 7.54–7.61 (m, 1H), 7.87–8.00 (m, 3H), 8.57 (d, 1H, J = 6.0 Hz), 9.33 (s, 1H); MS m/z 162 (MH^+).

5-(3-Acetamido-2,6-dichlorobenzylthio)isoquinoline (43). A solution of **41** (315 mg, 1.94 mmol) in DMF (3 mL) was treated with triethylamine (297 mg) followed by **42** (611 mg, 1.96 mmol) in DMF (2 mL) at room temperature. After 30 min the solution was diluted with AcOEt and washed with brine (1×), 0.5 N-sodium hydroxide solution (3×), water (2×), then brine (1×) and dried over magnesium sulfate. After evaporation under reduced pressure the residue was purified by silica-gel chromatography (eluent AcOEt) to give 551 mg of **43** (75%) as a white powder: mp 161–163°C; IR (KBr) 1664 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.10 (s, 3H), 4.44 (s, 2H), 7.36 (d, 1H, J = 8.8 Hz), 7.61–7.68 (m, 2H), 7.91 (d, 1H, J = 7.2 Hz), 8.04 (d, 1H, J = 5.9 Hz), 8.13 (d, 1H, J = 7.8 Hz), 8.54 (d, 1H, J = 5.9 Hz), 9.35 (s, 1H), 9.58 (s, 1H); MS m/z 377 (MH^+).

5-(3-Acetamido-2,6-dichlorobenzylsulfonyl)isoquinoline (44). A solution of **43** (215 mg, 0.57 mmol) in acetic acid (8 mL) and water (2 mL) at 0–5°C was treated dropwise with potassium permanganate (180 mg, 1.14 mmol) in water (5 mL) over 15 min. After a further 30 min, sufficient 30% hydrogen peroxide solution was added to dissolve the precipitated manganese dioxide and the reaction quenched with saturated sodium hydrogen carbonate solution. The mixture was extracted with AcOEt and the organic layer was washed with saturated sodium hydrogen carbonate solution (3×), brine (1×), dried over magnesium sulfate and evaporated under reduced pressure. The residue was recrystallized from $CHCl_3$:IPE to give 177 mg of **44** (76%) as a light-yellow powder: mp 244–245°C; IR (KBr) 1693 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.09 (s, 3H), 5.09 (s, 2H), 7.35 (d, 1H, J = 8.8 Hz), 7.75 (d, 1H, J = 8.8 Hz), 7.89 (t, 1H, J = 7.8 Hz), 8.31–8.36 (m, 2H), 8.60 (d, 1H, J = 8.2 Hz), 8.68 (d, 1H, J = 6.0 Hz), 9.53 (s, 1H), 9.61 (s, 1H); MS m/z 409 (MH^+).

5-(2,5-Dichloro-3-nitrobenzyloxy)isoquinoline (8d). To a suspension of sodium borohydride (910 mg, 24 mmol) in THF (30 mL) was added dropwise a solution of 2,5-dichloro-3-nitrobenzoic acid (**45a**, 3.0 g, 12.7 mmol) in THF (50 mL) at 0°C. After stirring at 0°C for 10 min, to the mixture was added boron trifluoride etherate (4.87 g, 34 mmol) over 5 min. The obtained suspension was stirred at 0°C for 10 min and at room temperature for 1 h, then poured into a mixture of CH_2Cl_2 (300 mL), water (300 mL) and sodium hydrogen carbonate (3.85 g) and stirred at room temperature overnight. The mixture was extracted with CH_2Cl_2 (2×), washed with brine, dried over magnesium sulfate and evaporated under reduced pressure to give 2.93 g of hydroxy derivative. To a solution of the obtained oil in AcOEt (30 mL) were added triethylamine (2.29 mL) and mesylchloride (1.75 g, 15 mmol) at 0°C, and stirred at room temperature for 30 min. The mixture was added to water, extracted with AcOEt, washed with water (2×) and brine (1×), dried over magnesium sulfate, and evaporated under reduced pressure to give 4.07 g of mesylate as an oil. A solution of **3** (2.18 g, 15 mmol) in DMF (40 mL) was cooled to 0°C, treated with 60% sodium hydride (660 mg) and stirred for 15 min at 0°C. To the mixture was added a solution of the former mesyl derivative in DMF at 0°C. After stirring at room temperature for 1 h, the reaction was quenched with 0.5 N-sodium hydroxide solution and extracted with AcOEt (2×). The combined extracts was washed with 1 N-sodium hydroxide solution and brine, dried over magnesium sulfate and evaporated under reduced pressure. The residue was column chromatographed on silica gel to give 870 mg of **8d** (20%) as a solid: ^{1}H NMR ($CDCl_3$) δ 5.40 (s, 2H), 7.07 (d, 1H, J = 7.5 Hz), 7.50–7.68 (m, 2H), 7.84 (s, 1H), 7.91 (s, 1H), 8.10 (d, 1H, J = 5.8 Hz), 8.62 (d, 1H, J = 5.8 Hz), 9.25 (s, 1H); MS m/z 349 (MH^+).

Preparation of **8e** was carried out by a similar method to that described for **8d**.

5-(2,6-Dimethyl-3-nitrobenzyloxy)isoquinoline (8e). Yield 52%; ^{1}H NMR (DMSO-d_6) δ 2.45 (s, 3H), 2.49 (s, 3H), 5.39 (s, 2H), 7.41 (d, 1H, J = 8.4 Hz), 7.52 (dd, 1H, J = 1.5, 7.1 Hz), 7.64–7.88 (m, 4H), 8.45 (d, 1H, J = 5.8 Hz), 9.30 (s, 1H); MS m/z 309 (MH^+).

2-Chloro-6-nitrobenzylalcohol (48). To a suspension of sodium borohydride (150 mg, 3.97 mmol) in ethanol (10 mL) was added 2-chloro-6-nitrobenzaldehyde (**47**, 1.0 g, 5.38 mmol) at 0°C and stirred at room temperature for 30 min. The reaction was quenched with water and extracted with AcOEt (3×). The combined extracts were washed with brine, dried over magnesium sulfate and evaporated under reduced pressure to give 1.0 g of **48** (99%) as a solid: IR (KBr) 1531 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 2.67 (t, 1H, J = 7.4 Hz), 4.93 (d, 2H, J = 7.4 Hz), 7.43 (t, 1H, J = 8.1 Hz), 7.71 (dd, 1H, J = 1.2, 8.1 Hz), 7.82 (dd, 1H, J = 1.2, 8.1 Hz).

3-Acetamido-2,6-dichlorobenzylalcohol (33). A solution of **49** (10 g, 23 mmol) in CH_2Cl_2:pyridine:acetic anhydride (1:1:1, 30 mL) was treated with 4-dimethylaminopyridine (5 mg) then stirred for 5 h at room temperature. Ice was added and the mixture stirred for 15 min, then saturated sodium hydrogen carbonate solution was added and the mixture stirred a further 15 min. The mixture was diluted with AcOEt, washed with saturated sodium hydrogen carbonate solution (2×), 1 N-hydrochloric acid (4×), dried over magnesium sulfate and evaporated under reduced pressure. The residue was dissolved in THF (100 mL), treated with 1 M-tetrabutylammonium fluoride in THF solution (51 mL) and the solution stirred for 62 h at room temperature. The solution was quenched with 1 N-hydrochloric acid and extracted with AcOEt (5×) and the combined organic extracts were dried over magnesium sulfate then evaporated under reduced pressure. The residue was column chromatographed on silica gel (eluent AcOEt:hexane) to give 4.43 g of **33** (82%): ^{1}H NMR (DMSO-d_6) δ 2.10 (s, 3H), 4.70 (d, 2H, J = 5.3 Hz), 5.24 (t, 1H, J = 5.3 Hz), 7.42 (d, 1H, J = 8.8 Hz), 7.67 (d, 1H, J = 8.8 Hz), 9.57 (s, 1H); MS m/z 234 (MH^+).

8-Methoxyisoquinoline (53). A solution of 2-methoxybenzaldehyde (**52**, 2.0 g, 14.7 mmol) and 2,2-dimethoxyethylamine (1.54 g, 14.7 mmol) in benzene (50 mL) was refluxed for 5 h. The water was removed azeotropically. The mixture was evaporated and dissolved in THF (20 mL) then ethyl chloroformate (1.59 g, 14.7 mmol) was added at −10°C. The mixture was warmed to room temperature and treated with trimethyl phosphite (2.1 mL) and stirred at room temperature over 3 days and evaporated under reduced pressure. The obtained oil was dissolved in CH_2Cl_2 (20 mL) and then treated with titanium tetrachloride (9.7 mL) at 0°C, and then the mixture was refluxed for 1 h, quenched with 30% sodium hydroxide aqueous solution (50 mL) at 0°C and filtered. The residue was washed with CH_2Cl_2. The organic layer was extracted with 3 N-hydrochloric acid. The combined extracts were treated with CH_2Cl_2 and neutralized with 30% sodium hydroxide. The mixture was extracted with CH_2Cl_2. The combined extracts were dried over magnesium sulfate, evaporated under reduced pressure and chromatographed on silica-gel (eluent methanol:CH_2Cl_2) to give 1.3 g of **53** (55%) as a solid: ^{1}H NMR (DMSO-d_6) δ 4.02 (s, 3H), 7.12 (d, 1H, J = 7.7 Hz), 7.50 (d, 1H, J = 8.2 Hz), 7.71 (t, 1H, J = 8.0 Hz), 7.78 (d, 1H, J = 5.7 Hz), 8.52 (d, 1H, J = 5.7 Hz), 9.49 (s, 1H); MS m/z 160 (MH^+).

8-Hydroxyisoquinoline (54a). To a 1.0 M solution of boron tribromide in CH_2Cl_2 (6.28 mL) was added **53** (199 mg, 1.25 mmol) at room temperature. After refluxing for 1 h, the mixture was quenched and neutralized with sodium hydroxide and evaporated under reduced pressure. The obtained residue was washed with methanol. After the combined organic layer was evaporated, the residue was purified on a silica-gel column (eluent methanol:CH_2Cl_2) to give 151 mg of **54a** (83%) as a solid: ^{1}H NMR (DMSO-d_6) δ 6.98 (d, 1H, J = 7.6 Hz), 7.35 (d, 1H, J = 8.2 Hz), 7.57 (dd, 1H, J = 7.6, 8.2 Hz), 7.70 (d, 1H, J = 5.6 Hz), 8.45 (d, 1H, J = 5.6 Hz), 9.45 (s, 1H), 10.68 (s, 1H); MS m/z 146 (MH^+).

5-(3-Acetamido-2,6-dichlorobenzyloxy)-1-cyanoisoquinoline (56). A solution of **10c** (400 mg, 1.11 mmol) and *p*-toluenesulfonyl chloride (253 mg, 1.33 mmol) in CH_2Cl_2 (30 mL) was treated with a solution of potassium cyanide (216 mg, 3.32 mmol) in water (10 mL) and the mixture stirred vigorously for 18 h at room temperature. The mixture was diluted with water and extracted with CH_2Cl_2 (2×). The combined organic layers were dried over magnesium sulfate and concentrated under reduced pressure to give a solid that was dissolved in THF (30 mL) and treated with DBU (202 mg, 1.33 mmol). After 1 h the reaction was quenched with saturated ammonium chloride solution and extracted with AcOEt. The organic layer was washed with water and brine, and dried over magnesium sulfate and evaporated under reduced pressure. The residue was chromatographed on silica gel (eluent AcOE:hexane) to give 210 mg of **56** (49%) as a white solid: mp 205–206°C; IR (KBr) 2230, 1664 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.13 (s, 3H), 5.55 (s, 2H), 7.60 (d, 1H, J = 8.9 Hz), 7.71 (d, 1H, J = 7.2 Hz), 7.84–7.97 (m, 3H), 8.17 (d, 1H, J = 5.7 Hz), 8.64 (d, 1H, J = 5.7 Hz), 9.73 (s, 1H); MS m/z 386 (MH^+).

5-{2,6-Dichloro-3-(*N*-formylamino)benzyloxy}isoquinoline (59a). To acetic anhydride (1.60 g, 15.7 mmol) was added formic acid (1.44 g, 31.3 mmol) dropwise over 10 min and the solution warmed at 45°C for 1 h then cooled to 0–5°C. **9c** (1.0 g, 3.13 mmol) was then added to the solution. After 30 min the yellow solution was stirred at room temperature for 15 min then quenched with AcOEt:THF:saturated sodium hydrogen carbonate solution and stirred for 30 min. The organic layer was separated, washed with saturated sodium hydrogen carbonate and brine, and dried over magnesium sulfate and evaporated under reduced pressure to give 1.02 g of **59a** (94%) as a white powder: mp 215–217°C; IR (KBr) 1701 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 5.49 (s, 2H), 7.50–7.81 (m, 5H), 8.25 (d, 1H, J = 8.9 Hz), 8.41–8.47 (m, 2H), 9.30 (s, 1H), 10.12 (s, 1H); MS m/z 347 (MH^+).

5-{3-(*t*-Butoxycarbonylglycylamino)-2,6-dichlorobenzyloxy}isoquinoline (60). A solution of **9c** (2.0 g, 6.27 mmol), boc-glycine (2.2 g, 12.6 mmol) and 1-hydroxybenzotriazole (1.69 g) in CH_2Cl_2 (20 mL) was treated with *N*-ethyl-*N*′-3-dimethylaminopropylcarbodiimide hydrochloride (2.64 g) and the solution stirred 7 days at room temperature. The reaction was quenched with water and extracted with AcOEt. The organic layer was washed with water (3×), dried over magnesium sulfate, concentrated under reduced pressure and chromatographed over silica-gel (eluent $CHCl_3$:methanol) to give 2.7 g of **60** (90%) as a solid: mp 199–200°C (from $CHCl_3$:hexane); IR (KBr) 3379, 3159, 1703 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.40 (s, 9H), 3.82 (d, 2H, J = 5.9 Hz), 5.50 (s, 2H), 7.26 (brt, 1H), 7.51–7.81 (m, 5H), 7.99 (d, 1H, J = 8.9 Hz), 8.45 (d, 1H, J = 5.8 Hz), 9.30 (s, 1H), 9.59 (s, 1H)

5-(2,6-Dichloro-3-glycylaminobenzyloxy)isoquinoline dihydrochloride (61). A solution of **60** (1.0 g, 2.1 mmol) in AcOEt (45 mL) was treated with 4 N-hydrogen chloride in AcOEt (18 mL) and the mixture was stirred for 14 h at room temperature then filtered and dried to give 900 mg of **61** (100%) as a solid: mp 200–205°C (dec.); IR (KBr)

1703 cm^{-1}; ^1H NMR (D_2O) 4.19 (s, 2H), 5.57 (s, 2H), 7.49 (d, 1H, $J = 8.8$ Hz), 7.71–7.80 (m, 2H), 7.90–8.02 (m, 2H), 8.34–8.43 (m, 2H), 9.55 (s, 1H); MS m/z 376 (MH^+, free).

5-(3-Acetylmethylacetamido-2,6-dichlorobenzyloxy)isoquinoline (62). A solution containing **9c** (1.0 g, 3.13 mmol), diketene (395 mg, 4.7 mmol) and 1,2-dichloroethane (20 mL) was heated for 8 h at 70°C then allowed to stand for 3 days at room temperature. The reaction mixture was diluted with AcOEt, washed with saturated sodium hydrogen carbonate solution (2×), water (1×), saturated sodium chloride solution, dried over magnesium sulfate and concentrated under reduced pressure. The residue was chromatographed over silica-gel (eluent AcOEt) to give 500 mg of **62** (40%) as a yellow solid: mp 136–140°C (from CH_2Cl_2:IPE); IR (KBr) 1713, 1687 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.22 (s, 3H), 3.70 (s, 2H), 5.50 (s, 2H), 7.50–7.81 (m, 5H), 7.97 (d, 1H, $J = 8.8$ Hz), 8.45 (d, 1H, $J = 5.8$ Hz), 9.29 (s, 1H), 9.94 (s, 1H); MS m/z 403 (MH^+).

5-{2,6-Dichloro-3-(3-methoxyimino-1-butanoylamino)-benzyloxy}isoquinoline (63). A suspension of **62** (109 mg, 0.27 mmol) in ethanol (3 mL) was treated with *O*-methylhydroxylamine hydrochloride (113 mg, 1.35 mmol), followed, after 5 min by pyridine (0.5 mL). After 30 min stirring at room temperature, the mixture was diluted with AcOEt, washed with saturated sodium chloride solution, dried over magnesium sulfate and concentrated under reduced pressure. The residue was chromatographed over silica-gel (eluent AcOEt) to give 80 mg of **63** (69%) as an off-white powder (1.4:1 of E/Z isomers): mp 169–173°C; IR (KBr) 3319, 1698, 1668 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.86 and 1.91 (each s, 3H total), 3.32 and 3.51 (each s, 2H total), 3.76 and 3.78 (each s, 3H total), 5.50 (s, 2H), 7.50–7.88 (m, 6H), 8.45 (d, 1H, $J = 5.8$ Hz), 9.29 (s, 1H), 9.82 and 9.93 (each s, 1H total); MS m/z 432 (MH^+).

5-{(2,6-Dichloro-3-chloroacetylamino)benzyloxy}isoquinoline (64). A solution of **9c** (600 mg, 1.88 mmol) in 1,2-dichloroethane (10 mL) was treated with chloroacetic anhydride (450 mg, 2.63 mmol) and then stirred for 1 h at room temperature, quenched with saturated sodium hydrogen carbonate solution and stirred for 15 min. The mixture was extracted with AcOEt and the organic layer was washed with saturated sodium hydrogen carbonate solution (2×), saturated sodium chloride solution, dried over magnesium sulfate and concentrated under reduced pressure to give 741 mg of **64** (100%) as a white solid: mp 275–280°C; IR (KBr) 3250, 1668 cm^{-1}; ^1H NMR (DMSO-d_6) δ 4.42 (s, 2H), 5.50 (s, 2H), 7.53 (d, 1H, $J = 7.2$ Hz), 7.62–7.89 (m, 5H), 8.45 (d, 1H, $J = 5.8$ Hz), 9.30 (s, 1H), 10.09 (s, 1H); MS m/z 395 (MH^+).

5-{2,6-Dichloro-3-(1,2,4-triazol-1-yl-acetylamino)benzyloxy}isoquinoline (65). A solution of **64** (100 mg, 0.25 mmol) in DMF (1.0 mL) was treated with 90% 1,2,4-triazole sodium salt (51 mg, 0.56 mmol) and the solution stirred for 30 min at room temperature then diluted with 4:1 AcOEt:THF and washed with saturated sodium chloride solution (4×), filtered, dried over magnesium sulfate and concentrated under reduced pressure to give a white solid. Crystallization from methanol: CH_2Cl_2:IPE gave 92 mg of 65 (85%) as a white powder: mp 235°C (dec.); IR (KBr) 1674 cm^{-1}; ^1H NMR (DMSO-d_6) δ 5.28 (s, 2H), 5.51 (s, 2H), 7.52 (d, 1H, $J = 7.2$ Hz), 7.60–7.81 (m, 4H), 7.88 (d, 1H, $J = 8.8$ Hz), 8.02 (s, 1H), 8.45 (d, 1H, $J = 5.8$ Hz), 8.57 (s, 1H), 9.30 (s, 1H), 10.20 (s, 1H); MS m/z 428 (MH^+).

5-{2,6-Dichloro-3-(2-hydroxyethylthioacetylamino)benzyloxy}isoquinoline (66). A solution of **64** (300 mg, 0.76 mmol) in CH_2Cl_2 (10 mL) was treated with triethylamine (99 mg) followed by 2-mercaptoethanol (62 mg, 0.80 mmol). After 20 h at room temperature, triethylamine (73 mg) and 2-mercaptoethanol (28 mg, 0.36 mmol) were added and stirring continued for a further 4 h. The reaction was diluted with AcOEt, washed with water (3×), saturated sodium chloride solution, dried over magnesium sulfate, concentrated under reduced pressure and chromatographed over silica-gel (eluent AcOEt) to give 210 mg of **66** (63%) as a white solid: mp 164–166°C; IR (KBr) 1678, 1659, 1589, 1525 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.73 (t, 2H, $J = 6.7$ Hz), 3.47 (s, 2H), 3.59 (q, 2H, $J = 6.5$ Hz), 4.85 (t, 1H, $J = 5.4$ Hz), 5.50 (s, 2H), 7.52 (d, 1H, $J = 7.2$ Hz), 7.60–7.81 (m, 4H), 7.93 (d, 1H, $J = 8.8$ Hz), 8.45 (d, 1H, $J = 5.8$ Hz), 9.29 (s, 1H), 9.86 (s, 1H); MS m/z 437 (MH^+).

5-(3-Bismethanesulfonylamino-2,6-dichlorobenzyloxy)isoquinoline (67). A solution of **9c** (319 mg, 1.0 mmol) and triethylamine (348 mg, 3.44 mmol) in CH_2Cl_2 (15 mL) was cooled to 0°C and treated with methanesulfonyl chloride (135 mg, 1.18 mmol) dropwise. After 1.5 h at room temperature, the reaction was recooled to 0°C, treated with a further 138 mg of methanesulfonyl chloride then stirred for 2 h at room temperature, diluted with AcOEt, washed successively with saturated sodium hydrogen carbonate, water and brine, and dried over magnesium sulfate and evaporated under reduced pressure. The residue was chromatographed over silica-gel (eluent AcOEt:hexane) to give 304 mg of **67** (64%) as a white solid: mp >80°C (not sharp-melting); IR (KBr) 1628, 1585, 1495, 1454 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.52 (s, 6H), 5.51 (s, 2H), 7.19–7.23 (m, 1H), 7.42–7.65 (m, 4H), 7.95 (d, 1H, $J = 5.9$ Hz), 8.50 (d, 1H, $J = 5.9$ Hz), 9.23 (s, 1H); MS m/z 475 (MH^+).

5-(2,6-Dichloro-3-methanesulfonamidobenzyloxy)isoquinoline (68). A solution of **67** (250 mg, 0.53 mmol) in THF (5 mL) was treated with 1 N-sodium hydroxide solution (1 mL). After 1 h the reaction was quenched with pH 6.86 buffer solution and extracted with CH_2Cl_2 (3×). The combined organic layers were dried over magnesium sulfate, evaporated under reduced pressure and chromatographed over silica-gel (eluent AcOEt:hexane) to give 174 mg of **68** (83%) as a white powder: mp 215–217°C; IR (KBr) 1632, 1591 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.07 (s, 3H), 5.49 (s, 2H), 7.10 (brs, 1H), 7.21–7.28 (m, 1H), 7.45–7.65 (m, 3H), 7.73 (d, 1H, $J = 8.9$ Hz), 7.93 (d, 1H, $J = 5.9$ Hz), 8.49 (d, 1H, $J = 5.9$ Hz), 9.23 (s, 1H); MS m/z 397 (MH^+).

5-{2,6-Dichloro-3-(*N'*-isopropylureido)benzyloxy}isoquinoline (69). A solution of **9c** (200 mg, 0.63 mmol) in 1,2-dichloroethane (4 mL) was treated with isopropyl isocyanate (433 mg, 5.09 mmol). After 18 h under reflux, the solution was cooled to room temperature and then a

further isopropyl isocyanate (433 mg, 5.09 mmol) and ethanol (4 mL) were added and the mixture stirred at 70°C for a further 30 h. The mixture was filtered and washed with CH_2Cl_2 (2×), and the combined filtrates were dried over magnesium sulfate and concentrated under reduced pressure to give 143 mg of **69** (56%) as a white powder: mp 236–238°C; IR (KBr) 3359, 3275, 1643 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.11 (d, 6H, J = 6.5 Hz), 3.72–3.82 (m, 1H), 5.46 (s, 2H), 7.04 (d, 1H, J = 7.3 Hz), 7.47–7.52 (m, 2H), 7.62–7.81 (m, 3H), 8.11 (s, 1H), 8.31 (d, 1H, J = 9.0 Hz), 8.45 (d, 1H, J = 5.8 Hz), 9.29 (s, 1H); MS m/z 404 (MH^+).

Biological methods

MIC; MICs were determined by the agar dilution method. *H. pylori* cell suspensions (0.5 McFarland) were prepared from cells grown on Brucella agar containing 2% starch and 3% fetal bovine serum (FBS) under 10% CO_2, 37°C for 72 h. Ten fold dilutions of these suspensions were inoculated on Brucella agar plates containing 7% defibrinated horse blood and serial dilutions of test compounds. MICs were read after 72 h incubation under 10% CO_2, 37°C.

Elemental analyses table

Compounds	Formula	Analysis (%): C	H	H
5a	$C_{16}H_{13}NO$	81.68 (81.39)	5.57 (5.51)	5.95 (5.88)
5b	$C_{16}H_{11}Cl_2NO$	63.18 (63.10)	3.64 (3.39)	4.61 (4.54)
5c	$C_{16}H_{11}N_3O_2 \cdot 0.12H_2O$	68.77 (68.39)	4.05 (3.65)	15.04 (14.81)
5d	$C_{18}H_{15}NO_3 \cdot 0.25H_2O$	72.59 (72.71)	5.24 (4.99)	4.70 (4.71)
6	$C_{17}H_{15}NO_2$	76.96 (76.71)	5.70 (5.64)	5.28 (5.22)
8c	$C_{16}H_{10}Cl_2N_2O_3 \cdot 0.5H_2O$	53.65 (53.86)	3.10 (2.75)	7.82 (7.74)
9a	$C_{16}H_{14}N_2O \cdot 0.1H_2O$	76.23 (76.24)	5.64 (5.50)	11.11 (10.95)
9b	$C_{16}H_{13}ClN_2O \cdot 0.1H_2O$	67.07 (66.98)	4.61 (4.62)	9.78 (9.70)
9c	$C_{16}H_{12}Cl_2N_2O$	60.21 (60.26)	3.79 (3.72)	8.78 (8.71)
9d	$C_{16}H_{12}Cl_2N_2O \cdot 0.2H_2O$	59.54 (59.42)	3.87 (3.52)	8.68 (8.51)
10a	$C_{18}H_{16}N_2O_2 \cdot H_2O$	69.61 (69.53)	5.85 (5.81)	9.03 (8.99)
10b	$C_{18}H_{15}ClN_2O_2 \cdot 0.1H_2O$	65.80 (65.73)	4.66 (4.40)	8.52 (8.40)
10b′	$C_{20}H_{17}ClN_2O_3 \cdot 0.5H_2O$	63.58 (63.85)	4.80 (4.71)	7.41 (7.40)
10c	$C_{18}H_{14}Cl_2N_2O_2$	59.85 (59.70)	3.91 (3.63)	7.75 (7.52)
10d	$C_{18}H_{14}Cl_2N_2O_2 \cdot 0.25H_2O$	59.06 (59.06)	3.93 (3.72)	7.66 (7.55)
10e	$C_{20}H_{20}N_2O_2 \cdot 0.5H_2O$	72.92 (72.96)	6.12 (6.22)	8.50 (8.46)
23	$C_{18}H_{14}F_2N_2O_2 \cdot 0.5H_2O$	62.43 (62.02)	4.66 (4.22)	8.09 (7.74)
26a	$C_{20}H_{20}N_2O_2S_2 \cdot 0.3H_2O$	61.61 (61.69)	5.33 (5.10)	7.18 (6.95)
26b	$C_{19}H_{17}ClN_2O_2S \cdot 0.2H_2O$	60.62 (60.70)	4.66 (4.48)	7.44 (7.31)
28	$C_{18}H_{13}ClN_2O_2S$	60.59 (60.60)	3.67 (3.81)	7.85 (7.52)
32	$C_{15}H_{12}ClN_3O_2S$	53.97 (53.52)	3.62 (3.76)	12.59 (12.15)
35	$C_{18}H_{13}Cl_2N_3O$	60.48 (60.35)	3.57 (3.66)	11.64 (11.73)
37	$C_{18}H_{13}Cl_2N_3O_2$	57.77 (57.47)	3.50 (3.45)	11.23 (11.00)
38	$C_{18}H_{15}Cl_2N_3O$	60.01 (59.93)	4.20 (4.12)	11.66 (11.52)
43	$C_{18}H_{14}Cl_2N_2OS$	57.30 (56.98)	3.74 (3.47)	7.42 (7.28)
44	$C_{18}H_{14}Cl_2N_2O_3S \cdot 0.45CHCl_3$	47.86 (47.84)	3.15 (3.29)	6.05 (5.98)
55a	$C_{18}H_{14}Cl_2N_2O_2$	59.85 (59.70)	3.91 (3.80)	7.76 (7.64)
55b	$C_{18}H_{14}Cl_2N_2O_2 \cdot 0.1H_2O$	57.01 (56.89)	4.25 (4.52)	7.39 (6.97)
56	$C_{19}H_{13}Cl_2N_3O_2$	59.08 (58.68)	3.39 (3.29)	10.88 (10.44)
58	$C_{19}H_{16}Cl_2N_2O_2 \cdot 0.1H_2O$	58.03 (58.37)	4.61 (4.50)	7.12 (6.83)
59a	$C_{17}H_{12}Cl_2N_2O_2$	58.81 (58.50)	3.48 (3.32)	8.07 (7.78)
59b	$C_{19}H_{16}Cl_2N_2O_2$	60.81 (60.41)	4.30 (4.06)	7.47 (7.26)
61	$C_{18}H_{17}Cl_4N_3O_2 \cdot H_2O$	46.30 (46.55)	4.10 (4.30)	8.99 (8.77)
62	$C_{20}H_{16}Cl_2N_2O_3$	59.57 (59.55)	4.00 (3.67)	6.95 (6.44)
63	$C_{21}H_{19}Cl_2N_3O_3$	58.34 (58.39)	4.43 (4.25)	9.72 (9.59)
64	$C_{18}H_{13}Cl_2N_2O_2$	54.64 (54.61)	3.31 (3.09)	7.08 (6.85)
67	$C_{18}H_{16}Cl_2N_2O_5S_2$	45.48 (45.90)	3.39 (3.38)	5.89 (5.76)
68	$C_{17}H_{14}Cl_2N_2O_3S$	50.82 (50.50)	3.64 (3.05)	6.97 (6.78)

Therapeutic efficacy in a mouse model; ICR mouse infected with 10^8 CFU of *H. pylori* FP1757 were orally treated with drugs three times per day for 4 days. The number of viable organisms in the gastric mucosa 1 day after final treatment were grown on a Brucella agar plate containing 3% horse serum, 2% starch, Skirrow's antibiotics, 10 μg/mL of nalidixic acid and 30 μg/mL of bacitracin.

Bactericidal effect; Bactericidal activity against *H. pylori* was evaluated by monitoring viable cell counts in Brucella broth containing 2% starch and 3% FBS.

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