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Synthesis of 3-acetoxyazetidin-2-ones and 3-hydroxyazetidin-2-ones with antifungal and antibacterial activity

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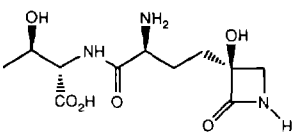
Summary — The synthesis of a series of 3-acetoxyazetidin-2-ones **3a–n** and 3-hydroxyazetidin-2-ones **6a–j** is reported together with the antibacterial and antifungal evaluation of these compounds. An additional series of 3-acetoxyazetidin-2-ones **11a–h** which possess a free carboxylic acid group on the N-1 aryl ring were obtained by treatment of suitably substituted Schiff bases **10a–h** with acetoxyacetyl chloride. The novel bicyclic structures 7-acetoxy-6-phenyl-5-thia-1-azabicyclo[4.2.0]octan-8-one **13** and 7-hydroxy-6-phenyl-5-thia-1-azabicyclo[4.2.0]octan-8-one **14** were also obtained. Many of the compounds displayed antifungal activity in vitro when evaluated against the pathogenic fungi *Cryptococcus neoformans*, *Candida albicans*, *Candida tropicalis*, *Candida parapsilosis*, *Candida glabrata*, and *Trichosporon cutaneum*, while 3-acetoxyazetidin-2-ones **11a–h** containing a free carboxylic acid group on the N-1 aryl ring displayed antibacterial activity against *Staphylococcus aureus*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Klebsiella aerogenes* and *Escherischia coli*

3-acetoxyazetidin-2-one / 3-hydroxyazetidin-2-one / antifungal activity / antibacterial activity

Introduction

In recent years the stereocontrolled synthesis of monocyclic β-lactams has become an area of intense activity as many substituted azetidin-2-ones can be used as synthons for a variety of natural products [1]. We now report the synthesis and antibacterial activity of a series of novel 3-acetoxy and 3-hydroxyazetidin-2-ones. The first naturally occurring 3-hydroxyazetidin-2-one to be reported was Wildfire toxin, tabtoxin **1** [2]. 3-Hydroxyazetidin-2-ones have been prepared by reacting a protected hydroxyacetyl chloride (eg, acetoxyacetyl chloride or benzyloxyacetyl chloride) with imines in the presence of base followed by removal of the protecting groups [3–8]. The stereoselective synthesis of variously substituted 3-hydroxy β-lactams was reported by Cossio and Palomo by annulation of Schiff bases with trimethylsiloxyacetic acid promoted by phenyl dichlorophosphate [9]. 3-Acetoxyazetidin-2-ones have been prepared from 2-acyloxy-3-chlorocarboxamides by anionic activation with cesium fluoride [10].



Tabtoxin 1

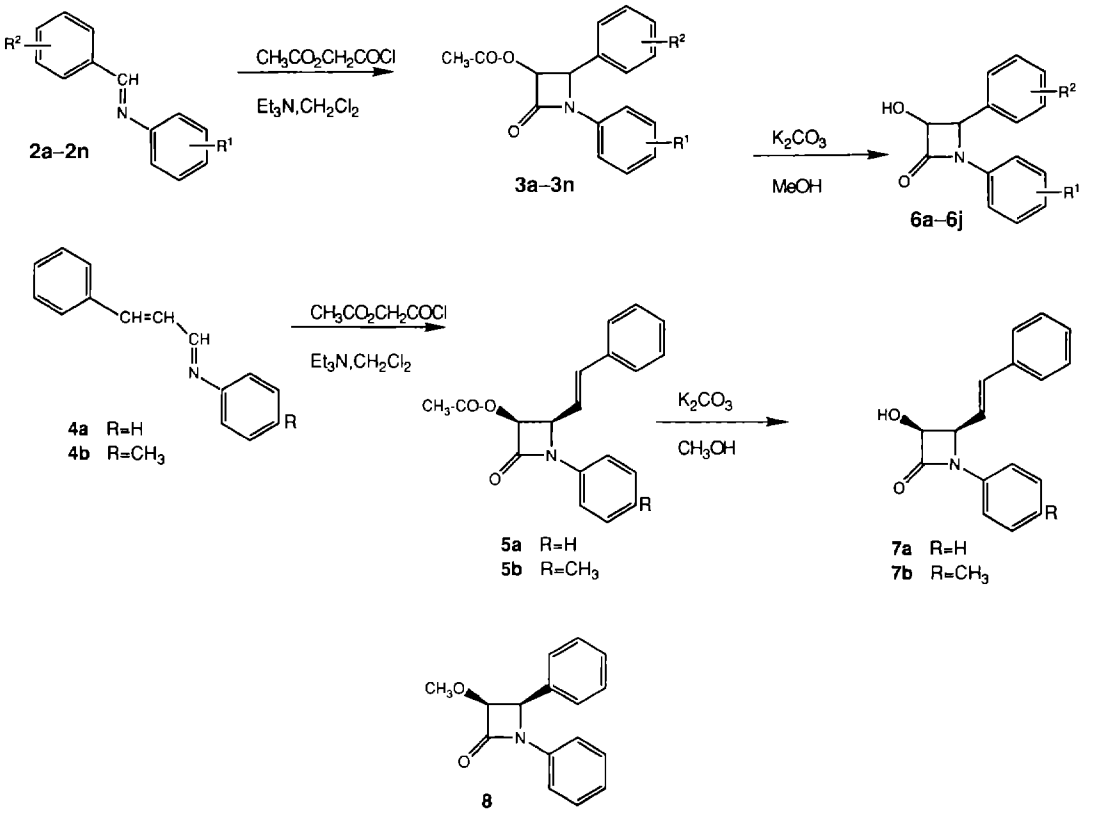
Optically active 3-acetoxyazetidin-2-ones have been prepared by annelation of Schiff bases derived from D-glyceraldehyde acetonide [11, 12], *N,O*-diprotected L-serinal [13] or (S)-2-(*tert*-butyldiphenylsilyloxy)propanal [14] with acetoxyacetyl chloride, while optically active 3-hydroxy-4-alkoxycarbonylazetidin-2-ones have been obtained from L-tartaric acid [15]. Reaction of a chiral carbohydrate-based ketone with an imine to afford a *cis*-3-hydroxy-β-lactam has been reported with good asymmetric induction (70% enantiomeric excess (ee)) [16].

In the present work the preparation and antifungal and antibacterial activity of a series of 3-acetoxy and 3-hydroxy-1,4-diarylazetidin-2-ones and related compounds were investigated. Of particular interest was the preparation of 3-acetoxy-1,4-diarylazetidin-2-ones containing a free carboxylic acid group on the N-1 aryl ring.

Chemistry

The cycloaddition of acetoxyacetyl chloride to the Schiff bases **2a–n** afforded the 3-acetoxy-1,4-diaryl-azetidin-2-ones **3a–n** (scheme 1). The yields, melting point and spectroscopic data (^{1}H-NMR and IR) for compounds **3a–n** are presented in table I. The structures of the products were established from their IR and ^{1}H-NMR spectra. All compounds showed a broad IR absorption band in the region 1750–1765 cm^{-1}, which is indicative of both β-lactam and ester carbonyl absorption. The stereochemical nature of the products was determined by ^{1}H-NMR. Both *cis* and *trans* isomers were isolated in low to moderate yields. Compounds **3a**, **3g** and **3k** were observed to be *cis* products with large coupling constants (J_{cis} = 4.2–5.0 Hz) displayed between H-3 and H-4. Isomer mixtures *(cis/trans)* were obtained for compounds **3f**, **3h** and **3i** while the *trans* isomers were obtained exclusively for β-lactams **3b–e** and **3j**, **3l**, **3m** and **3n**, with small coupling constants between H-3 and H-4 (J_{trans} = 1.2–2.4 Hz). In the case of compound **3h**, which is a *cis/trans* mixture, two sets of coupled doublets (J_{trans} = 1.5 Hz, J_{cis} = 4.8 Hz) are observed together with two signals for the acetoxy methyl group (δ 2.13, 2.21). The *cis/trans* ratio for this product is 0.55:0.45. Guo et al have reported exclusively *cis* products obtained when the Schiff bases benzylidene *p*-anisidine and *p*-methoxybenzylidene-*p*-anisidine are reacted with acetoxyacetyl chloride [3]. The 3-acetoxy-4-styrylazetidin-2-ones **5a** and **5b** were also prepared by reaction of the cinnamylidene Schiff bases **4a** and **4b** with acetoxyacetyl chloride under the appropriate reaction conditions (scheme 1).

In the present work, the hydrolysis of 3-acetoxy-azetidin-2-ones **3a–j**, **5a** and **5b** was carried out using potassium carbonate in methanol and afforded the series of 3-hydroxyazetidin-2-ones **6a–j**, **7a** and **7b** in



Scheme 1. **3a** $R^1 = R^2 = H$; **3b** R^1 = 4-OCH_3, R^2 = 3,4-OCH_2O; **3c** $R^1 = H$, R^2 = 4-Cl; **3d** $R^1 = H$, R^2 = 3,4-OCH_2O; **3e** R^1 = 4-OCH_3, R^2 = 4-F; **3f** R^1 = 4-OCH_3, $R^2 = H$; **3g** R^1 = 4-OCH_3, R^2 = 3,4-$(OCH_3)_2$; **3h** R^1 = 4-Br, R^2 = 2,3-CH=CHCH=CH-; **3i** $R^1 = H$, R^2 = 4-NO_2; **3j** R^1 = 4-CO_2CH_3, R^2 = 4-OCH_3; **3k** R^1 = 4-OCH_3, R^2 = 3,4-OCH_2O; **3l** R^1 = 4-CO_2CH_3, $R^2 = H$; **3m** R^1 = 4-CO_2CH_3, R^2 = 4-CH_3; **3n** R^1 = 4-CO_2CH_3, R^2 = 4-F. **6a** $R^1 = R^2 = H$; **6b** R^1 = 4-OCH_3, R^2 = 3,4-OCH_2O; **6c** $R^1 = H$, R^2 = 4-Cl; **6d** $R^1 = H$, R^2 = 3,4-OCH_2O; **6e** R^1 = 4-OCH_3, R^2 = 4-F; **6f** R^1 = 4-OCH_3, $R^2 = H$; **6g** R^1 = 4-OCH_3, R^2 = 3,4-$(OCH_3)_2$; **6h** R^1 = 4-Br, R^2 = 2,3-CH=CHCH=CH-; **6i** $R^1 = H$, R^2 = 4-NO_2; **6j** R^1 = 4-CO_2CH_3, R^2 = 4-OCH_3.

Table I. Physical data for 3-acetoxyazetidin-2-ones **3a–n**, **5a** and **5b**.

Compound	*Yield (%)*	*Mp (°C)*	*Molecular formula*	*IR υ_{max} (KBr) (cm^{-1})*	*^{1}H-NMR δ ($CDCl_3$)*
3a	42.7	185–187	$C_{17}H_{15}NO_3$	1750 (C=O)	7.88–7.08 (8H, m, aromatic H), 5.93 (1H, d, J_{cis} = 4.94 Hz, H-3), 5.36 (1H, d, J_{cis} = 4.94 Hz, H4), 1.65 (3H, s, -CH_3)
3b	23.0	188–190	$C_{19}H_{17}NO_6$	1758 (C=O)	7.40–6.80 (7H, m, aromatic H), 5 83 (2H, s, OCH_2O), 5.40 (1H, d, J_{trans} = 2.0 Hz, H-3), 5.30 (1H, d, J_{trans} = 2.0 Hz, H-4), 3.62 (3H, s, OCH_3), 2.12 (3H, s, -CH_3)
3c	19.2	168–170	$C_{17}H_{14}ClNO_3$	1758 (C=O)	7.38–6.60 (9H, m, aromatic H), 5.28 (1H, d, J_{trans} = 1.2 Hz, H-3), 4.90 (1H, d, J_{trans} = 1.2 Hz, H-4), 2.12 (3H, s, -CH_3)
3d	69.7	135–136	$C_{18}H_{15}NO_5$	1758 (C=O)	7.30–6.60 (8H, m, aromatic H), 5.92 (2H, s, OCH_2O), 5.30 (1H, d, J_{trans} = 1.2 Hz, H-3), 4.60 (1H, d, J_{trans} = 1.2 Hz, H-4), 2.16 (3H, s, -CH_3)
3e	16.0	136–137	$C_{18}H_{16}FNO_4$	1760 (C=O)	7.35–6.50 (8H, m, aromatic H), 5.30 (1H, d, br, J_{trans} = 2.0 Hz, H-3), 4.60 (1H, d, br, J_{trans} = 2.0 Hz, H-4), 3.70 (3H, s, OCH_3), 2.10 (3H, s, -CH_3)
3f	50.2	158–160 [3]	$C_{18}H_{17}NO_4$	1758 (C=O)	7.50–6.70 (9H, m, aromatic H), 5.92 (H, d, J_{trans} = 1.2 Hz, H-3), 5.90 (H, d, J_{cis} = 4.8 Hz, H-3), 5.86 (H, d, J_{trans} = 1.2 Hz, H-4), 5.30 (H, d, J_{cis} = 4.8 Hz, H-4), 3.85 (1.2H, s, -OCH_3), 3.70 (1.8H, s, -OCH_3), 2.15 (1.1H, s, -CH_3), 2.12 (1.9H, s, -CH_3)
3g	7.5	139–140	$C_{20}H_{21}NO_6$	1758 (C=O)	7.40–6.70 (7H, m, aromatic H), 5.90 (1H, d, J_{cis} = 4.2 Hz, H-3), 5.26 (1H, d, J_{cis} = 4.2 Hz, H-4), 3.87 (3H, s, OCH_3), 3.82 (3H, s, OCH_3), 3.75 (3H, s, OCH_3), 1.75 (3H, s, -CH_3)
3h	10.9	Oil	$C_{20}H_{16}BrNO_3$	1765 (C=O)	8.10–7.25 (11H, m, aromatic H), 6.40 (0.6H, d, J_{cis} = 4.8 Hz, H-3), 6.08 (0.6H, d, J_{cis} = 4.8 Hz, H-4), 5.77 (0.4H, d, J_{trans} = 1.5 Hz, H-3), 5.56 (0.4H, d, J_{trans} = 1.5 Hz, H-4), 2.21 (1.2H, s, -CH_3), 2.13 (0.8H, s, -CH_3)
3i	26.7	160–161	$C_{17}H_{14}N_2O_5$	1758 (C=O)	7.70–7.20 (9H, m, aromatic H), 6.02 (0.7H, d, J_{cis} = 4.8 Hz, H-3), 5.51 (0.7H, d, J_{cis} = 4.8 Hz, H-4), 5.04 (0.3H, d, J_{trans} = 1.2 Hz, H-3), 4.59 (0.3 H, d, J_{trans} = 1.2 Hz, H-4), 2.22 (1.1H, s, CH_3), 1.76 (1.9H, s, CH_3)
3j	20.0	140	$C_{20}H_{19}NO_6$	1718 (-CO_2CH_3) 1765 (C=O)	8.45–6.85 (8H, m, aromatic H), 5.38 (1H, d, J_{trans} = 2.0 Hz, H-3), 4.96 (1H, d, J_{trans} = 2.0 Hz, H-4), 3.86 (3H, s, OCH_3), 3.82 (3H, s, OCH_3), 2.16 (3H, s, -CH_3)
3k	56.7	195–197	$C_{19}H_{17}NO_6$	1750 (C=O)	7.35–6.65 (7H, m, aromatic H), 5.95 (2H, s, -OCH_2O), 5.90 (1H, d, J_{cis} = 5.0 Hz, H-3), 5.21 (1H, J_{cis} = 5.0 Hz, H-4), 3.75 (3H, s, -OCH_3), 1.80 (3H, s, -CH_3)
3l	6.0	122–124	$C_{19}H_{17}NO_5$	1710 (-CO_2CH_3) 1760 (C=O)	8.14–6.48 (9H, m, aromatic H), 5.41 (1H, d, J_{trans} = 2.4 Hz, H-3), 5.02 (1H, d, J_{trans} = 2.4 Hz, H-4), 3.85 (3H, s, OCH_3), 2.19 (3H, s, -CH_3)
3m	11.7	83–87	$C_{20}H_{19}NO_5$	1710 (-CO_2CH_3) 1750 (C=O)	7.85–6.61 (8H, m, aromatic H), 5.40 (1H, d, J_{trans} = 1.2 Hz, H-3), 4.96 (1H, d, J_{trans} = 1.2 Hz, H-4), 3.85 (3H, s, -OCH_3), 2 34 (3H, s, -CH_3), 2.18 (3H, s, -CH_3)
3n	65.0	133–136	$C_{19}H_{16}FNO_5$	1722 (-CO_2CH_3) 1765(C=O)	8.14–6.48 (8H, m, aromatic H), 5.39 (1H, d, J_{trans} = 1.9 Hz, H-3), 5.06 (1H, d, J_{trans} = 1.9Hz, H-4), 3.85 (3H, s, -OCH_3), 2.18 (3H, s, -CH_3)
5a	5.2	151–153	$C_{19}H_{17}NO_3$	1760 (C=O)	7.70–6.83 (11H, m, aromatic H), 6.32–6.18 (1H, m, -CH=C*H*CH-), 5.90 (1H, d, J_{cis} = 5 Hz, H-3), 4.60–5.05 (1H, m, H-4), 2.06 (3H, s, -CH_3)
5b	5.9	144–145	$C_{20}H_{19}NO_3$	1758 (C=O)	7.50–6.80 (10H, m, aromatic H), 5.82–6.31 (1H, m, -CH=C*H*CH-), 5.85 (1H, d, J_{cis} = 5.5 Hz, H-3), 4.42–5.12 (1H, m, H-4), 2.28 (3H, s, -CH_3), 2.03 (3H, s, -CH_3)

moderate yields (scheme 1). Prolonged exposure of the 3-hydroxy-1,4-diphenylazetidin-2-one **3a** product to methanol in this reaction afforded 3-methoxy-1,4-diphenylazetidin-2-one **8** as the sole reaction product. The yield, melting point and relevant spectroscopic data for compounds **6a–j**, **7a**, **7b** are given in table II. The IR spectra showed characteristic β-lactam carbonyl absorbances at 1725–1750 cm^{-1}, while the OH stretching absorbances occurred in the region 3350–3400 cm^{-1}. In the ^{1}H-NMR of compound **6e**, which was typical of the series, H-4 appeared as a doublet (δ 4.62, J_{trans} = 1.2 Hz) which was coupled to H-3 (δ 4.76, J_{trans} = 1.2 Hz). In all compounds, the H-3 signal appeared downfield within the range δ 4.76–6.02 due to the deshielding influence of the C-3 hydroxyl group. From the ^{1}H-NMR spectrum it was seen that compounds **6a**, **7a** and **7b** were *cis* isomers ($J_{3,4}$ = 5.0 Hz, 4.5 Hz, 5.0 Hz, respectively). Compounds **6c**, **6e**, **6f** and **6j** were all obtained as *trans* isomers ($J_{3,4}$ = 1.2–3.6 Hz). For compounds **6b**, **6d**, **6g**, **6h** and **6i** the signals for H-3 and H-4 were broad and hence the stereochemistry could not be deduced. The direct preparation of compound **6g** by reaction of trimethylsiloxyacetic acid with the imine **2g** in the presence of phenyl dichlorophosphate [9] afforded the β-lactam product **6g** in low yield (<10%).

The 3-hydroxyazetidin-2-ones **6a** and **6f** were converted to their corresponding sulfonyl esters **9a–e** by treatment with the appropriate sulfonyl chloride (scheme 2). Compounds **9a–c** were obtained exclusively as *cis* isomers where a large coupling constant ($J_{3,4}$ = 4.8 Hz) was displayed, while compounds **9d**, **9e** were obtained as *trans* isomers exclusively with $J_{3,4}$ = 2.6 Hz.

One of the essential features attributed to the antibacterial activity of the bicyclic β-lactams is the free carboxylic acid group present at C-3 of penicillins (C-4 of cephalosporins and C-2 of carbapenems) [17]. However there has been considerable interest in the antibacterial and antifungal activity of monocyclic β-lactams [18] including those incorporating a free carboxylic acid function [19–22]. In the present work we now report the synthesis of a novel series of 3-acetoxy-1,4-diarylazetidin-2-ones containing a free carboxylic acid function in the N-1 aryl ring. Direct reaction of 4-aminobenzoic acid or 3-aminobenzoic acid with the appropriate aryl aldehyde in ethanol afforded the Schiff bases **10a–h** in good yield (scheme 3). The yields, melting point and relevant spectroscopic data for these Schiff bases are given in table III. The products were characterized by the IR spectrum which showed the imine absorption υ(C=N) at 1585–1600 cm^{-1} and the carboxylic acid absorption υ(COOH) at 1675–1690 cm^{-1}. In the ^{1}H-NMR spectrum, the imine proton C*H*=N appeared at δ 8.40–8.60.

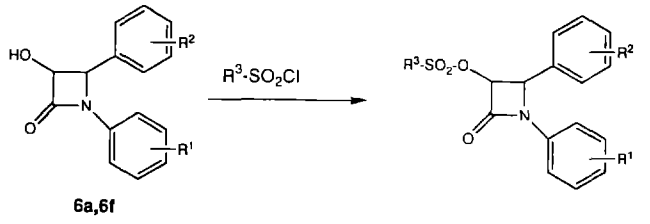
A series of 3-acetoxy-4-aryl-1-(carboxyphenyl)-azetidin-2-ones **11a–h** were prepared by reaction of the Schiff bases **10a–h** with trimethylsilyl chloride to form the silyl esters which were subsequently treated with acetoxyacetyl chloride to afford the β-lactam products (scheme 3). The yield, melting point and relevant spectroscopic data for the 3-acetoxyazetidin-2-ones **11a–h** are given in table IV. A high β-lactam carbonyl absorption frequency was observed for all compounds in the region 1760–1770 cm^{-1}, which is indicative of a highly strained four-membered ring. The carboxylic acid absorbance occurs at 1680–1690 cm^{-1}. The stereochemical nature of the products was determined from the ^{1}H-NMR spectrum to be exclusively *trans* with coupling constants in the region J = 0.6–1.5 Hz exhibited between H-3 and H-4. Purification of the acidic compounds **11a–h** proved difficult. Compounds **11c**, **11e**, **11f** and **11h** gave satisfactory C, H, N microanalytical data. Confirmation of the structures of compounds **11a**, **11b**, **11d** and **11g** was provided by methylation of the carboxylic acid function with diazomethane to afford the corresponding methyl esters **3j**, **3l**, **3m** and **3n**, respectively.

6-Hydroxypenams and 7-hydroxycephems have been utilized as intermediates in the preparation of novel β-lactam antibiotics [23]. The preparation of some related bicyclic compounds was now examined. The novel 7-acetoxy-6-phenylcepham (7-acetoxy-6-phenyl-5-thia-1-azabicyclo[4.2.0]octan-8-one) **13** was prepared in 11.5% yield under mild conditions by the base-catalyzed cycloaddition reaction of acetoxyacetyl chloride with 2-phenyl-5,6-dihydro-4*H*-1,3-thiazine **12** (scheme 4). The IR spectrum of **13** showed the β-lactam and ester carbonyl absorbance as a broad band centered at υ 1765 cm^{-1}. In the ^{1}H-NMR spectrum of **13** a six line signal (doublet of triplets) at δ 3.95–4.28 is assigned to the equatorial proton at C-4 (J_{gem} = 12.63 Hz, J_{eqax} = 2.92 Hz, and J_{eqeq} = 6.65 Hz). The equatorial proton on C-4 resonates at a lower field than any of the other protons on the six-membered ring. This may be attributed to the deshielding effect of the carbonyl group which lies in the same plane as the C-4 equatorial proton [24]. The axial proton at C-4 and the methylene protons at C-2 appear as an unresolved multiplet at δ 2.60–3.30 while the C-3 protons also appear as a multiplet at δ 1.52–2.06. The acetoxy group protons are observed as a singlet δ 1.55. A single isomer product was formed in this reaction. However, NMR spectroscopy did not reveal stereochemistry [25, 26].

Hydrolysis of the 7-acetoxy-6-phenylcepham **13** with potassium carbonate in methanol afforded the 7-hydroxy-6-phenylcepham **14** in 50% yield. The IR spectrum of **14** showed hydroxyl absorbance at 3400 cm^{-1} and β-lactam carbonyl absorption at 1750 cm^{-1}. In the ^{1}H-NMR spectrum of **14** the C-3

Table II. Physical data for 3-hydroxyazetidin-2-ones **6a–j**, **7a** and **7b**.

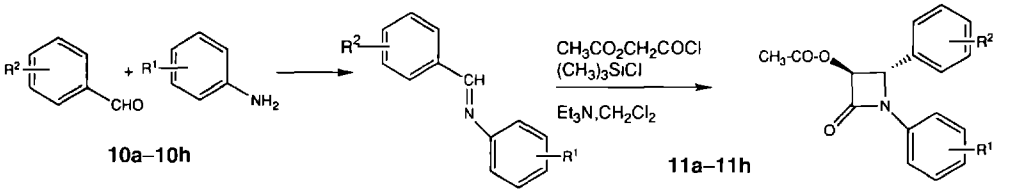
Compound	*Yield (%)*	*Mp (°C)*	*Molecular formula*	*IR υ_{max} (KBr) (cm^{-1})*	*^{1}H-NMR δ (DMSO-d_6)*
6a	57.2	152–154 [8]	$C_{15}H_{13}NO_2$	1730 (C=O) 3400 (OH)	7.80–7.05 (10H, m, aromatic H), 5.65–5.50 (1H, m, H-3), 5.35 (1H, d, J_{cis} = 5.0 Hz, H-4)
6b	52.6	Oil	$C_{17}H_{15}NO_5$	1730 (C=O) 3400 (OH)	7.30–6.75 (7H, m, aromatic H), 5.85 (2H, s, -OCH_2O-), 5.15 (1H, s, br, H-3), 4.65 (1H, s, br, H-4), 3.85 (3H, s, -OCH_3)
6c	58.9	128–129	$C_{15}H_{12}ClNO_2$	1728 (C=O) 3400 (OH)	7.50–7.20 (9H, m, aromatic H), 4.88 (1H, d, J_{trans} = 1.2 Hz, H-3), 4.66 (1H, d, J_{trans} = 1.2 Hz, H-4)
6d	43.7	205–206	$C_{16}H_{13}NO_4$	1728 (C=O) 3400 (OH)	7.42–6.75 (8H, m, aromatic H), 5.90 (2H, s, -OCH_2O-), 4.82 (1H, s, br, H-3), 4.68 (1H, s, br, H-4)
6e	20.1	196	$C_{16}H_{14}FNO_3$	1730 (C=O) 3400 (OH)	7.40–6.68 (8H, m, aromatic H), 4.76 (1H, d, J_{trans} = 1.2 Hz, H-3), 4.62 (1H, d, J_{trans} = 1.2Hz, H-4), 3.68 (3H, s, -OCH_3)
6f	62.3	210–211 [3]	$C_{16}H_{15}NO_3$ M+ 269.1	1725 (C=O) 3400 (OH)	7.30–6.70 (9H, m, aromatic H), 5.52 (1H, d, J_{trans} = 1.2 Hz, H-3), 5.20 (1H, s, br, H-4), 3.70 (3H, s, -OCH_3)
6g	60.0	152–154	$C_{18}H_{19}NO_5$	1730 (C=O) 3400 (OH)	7.40–6.70 (7H, m, aromatic H), 5.28 (1H, s, br, H-3), 5.15 (1H, s, br, H-4), 3.87 (3H, s, OCH_3), 3.82 (3H, s, -OCH_3), 3.75 (3H, s, -OCH_3)
6h	8.4	150–152	$C_{19}H_{14}BrNO_2$	1730 (C=O) 3400 (OH)	7.50–7.22 (11H, m, aromatic H), 6.02 (1H, d, J_{cis} = 5.0 Hz, H-3), 5.30–5.55 (1H, m, J = 1.2 Hz, H-4)
6i	65.9	112–115	$C_{15}H_{12}N_2O_4$	1730 (C=O) 3400 (OH)	8.25–7.18 (9H, m, aromatic H), 5.05 (1H, s, br, H-3), 4.75 (1H, s, br, H-4), 3.99 (1H, s, br, OH exchanges D_2O)
6j	73.8	146–148	$C_{18}H_{17}NO_5$	1710 (-CO_2CH_3) 1750 (C=O) 3400 (OH)	7.85–6.45 (8H, m, aromatic H), 4.85 (1H, d, J_{trans} = 3.6 Hz, H-3), 4.65 (1H, d, J_{trans} = 3.6 Hz, H-4), 3.80 (3H, s, -OCH_3), 3.75 (3H, s, -OCH_3), 4.62 (1H, s, br, OH, exchanges D_2O)
7a	59.7	188–190	$C_{17}H_{15}NO_2$	1725 (C=O) 3350 (OH)	7.70–6.95 (10H, m, aromatic H), 6.70–6.10 (2H, m, -CH=CH-), 5.12 (1H, d, J_{cis} = 4.5 Hz, H-3), 5.00–4.70 (1H, m, H-4)
7b	58.5	193–195	$C_{18}H_{17}NO_2$ M+ 279.1	1725 (C=O) 3350 (OH)	7.50–6.80 (9H, m, aromatic H), 6.70–6.10 (2H, m, -CH=CH-), 5.15 (1H, dd, J_{cis} = 5.0 Hz, H-3), 4.95–4.65 (1H, m, H-4), 1.25 (3H, s, -CH_3)



Scheme 2. **9a** $R^1 = R^2 = H$, $R^3 = 4\text{-}NO_2C_6H_4$; **9b** $R^1 = R^2 = H$, $R^3 = 4\text{-}BrC_6H_4$; **9c** $R^1 = R^2 = H$, $R^3 = CH_3$; **9e** $R^1 = 4\text{-}CO_2CH_3$, $R^2 = 4\text{-}OCH_3$, $R^3 = 4\text{-}CH_3\text{-}C_6H_4$; **9f** $R^1 = 4\text{-}CO_2CH_3$, $R^2 = 4\text{-}OCH_3$, $R^3 = 1$-naphthyl.

protons appeared as a multiplet at δ 1.50–2.06 while the H-4 axial proton and the methylene protons at C-2 occurred as a multiplet at δ 2.56–3.30. The H-4 equatorial proton was again observed as a downfield multiplet δ 3.88–4.26 (J_{gem} = 13.0 Hz, J_{eqeq} = 6.67 Hz, J_{eqax} = 3.0 Hz). H-7 occurred as a broad singlet at δ 4.84–5.10.

7-Acetoxy-6-phenylcepham sulfoxide **15** was prepared by treating the 7-acetoxy-6-phenylcepham **13** with *m*-chloroperbenzoic acid (1 mmol) while the 7-acetoxy-6-phenylcepham sulfone **16** was obtained from **13** using two molar equivalents of *m*-chloroper-



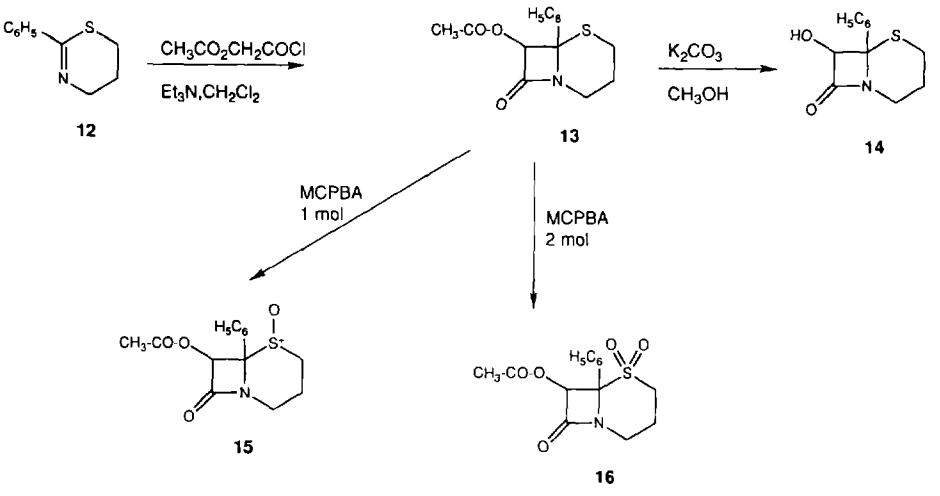
Scheme 3. **10a** R^1 = 4-COOH, R^2 = 4-OCH_3, **10b** R^1 = 4-COOH, R^2 = H; **10c** R^1 = 4-COOH, R^2 = 4-OC_2H_5; **10d** R^1 = 4-COOH, R^2 = 4-CH_3; **10e** R^1 = 4-COOH, R^2 = 3,4-OCH_2O; **10f** R^1 = 4-COOH, R^2 = 4-Br; **10g** R^1 = 4-COOH, R^2 = 4-F; **10h** R^1 = 3-COOH, R^2 = 3,4-OCH_2O. **11a** R^1 = 4-COOH, R^2 = 4-OCH_3; **11b** R^1 = 4-COOH, R^2 = H; **11c** R^1 = 4-COOH, R^2 = 4-OC_2H_5; **11d** R^1 = 4-COOH, R^2 = 4-CH_3; **11e** R^1 = 4-COOH, R^2 = 3,4-OCH_2O; **11f** R^1 = 4-COOH, R^2 = 4-Br; **11g** R^1 = 4-COOH, R^2 = 4-F; **11h** R^1 = 3-COOH, R^2 = 3,4-OCH_2O.

Table III. Physical data for Schiff bases **10a–h**.

Compound	*Yield (%)*	*Mp (°C)*	*Molecular formula*	*IR υ_{max} (KBr) (cm^{-1})*	*^{1}H-NMR δ (DMSO-d_6)*
10a	39.2	199–200 [20]	$C_{15}H_{13}NO_3$	1598 (C=N) 1685 (COOH)	8.60 (1H, s, -CH=N-), 8.23–7.03 (8H, m, aromatic H), 3.90 (3H, s, OCH_3)
10b	66.6	191–192 [33]	$C_{14}H_{11}NO_2$	1600 (C=N) 1690 (COOH)	8.48 (1H, s, -CH=N-), 8.20–7.13 (9H, m, aromatic H)
10c	50.8	215	$C_{16}H_{15}NO_3$	1600 (C=N) 1685 (COOH)	8.45 (1H, s,-C*H*=N-), 8.02–6.75 (8H, m, aromatic, H), 3.96 (2H, q, J = 6.6 Hz, $-OCH_2CH_3$), 1.22 (3H, t, J = 6.6 Hz, $-OCH_2CH_3$)
10d	70.6	235–236	$C_{15}H_{13}NO_2$	1598 (C=N) 1680 (COOH)	12.30 (1H, s, br, COOH), 8.40 (1H, s, -C*H*=N-), 8.20–7.10 (8H, m, aromatic H), 2.40 (3H, s, $-CH_3$)
10e	73.2	245–246	$C_{15}H_{11}NO_4$	1585 (C=N) 1675 (COOH)	12.77 (1H, s, br, COOH), 8.53 (1H, s, -C*H*=N-), 8.15–6.95 (7H, m, aromatic H), 6.18 (2H, s, $-OCH_2O$-)
10f	68.2	186–188	$C_{14}H_{10}BrNO_2$	1590 (C=N) 1680 (COOH)	8.50 (1H, s, -C*H*=N-), 8.05–6.94 (8H, m, aromatic H)
10g	61.2	193–194	$C_{14}H_{10}FNO_2$	1590 (C=N) 1680 (COOH)	8.52 (1H, s, -C*H*=N-), 8.08–7.08 (8H, m, aromatic H)
10h	70.0	241–243	$C_{15}H_{11}NO_4$	1600 (C=N) 1690 (COOH)	8.52 (1H, s, -C*H*=N-), 7.70–6.80 (7H, m, aromatic H), 6.20 (2H, s, $-OCH_2O$-)

Table IV. Physical data for 3-acetoxyazetidin-2-ones **11a–h**.

Compound	*Yield (%)*	*Mp (°C)*	*Molecular formula*	*IR υ_{max} (KBr) (cm^{-1})*	*^{1}H-NMR δ (DMSO-d_6)*
11a	19.3	Oil	$C_{19}H_{17}NO_6$	1680 (COOH) 1765 (C=O)	8.10–6.70 (8H, m, aromatic H, 5.30 (1H, s, brm H-3), 4.90 (1H, s, br, H-4), 3.65 (3H, s, -OCH_3), 2.18 (3H, s, -CH_3)
11b	4.8	184–185	$C_{18}H_{15}NO_5$	1690 (COOH) 1760 (C=O)	8.00–6.85 (9H, m, aromatic H), 5.32 (1H, d, J_{trans} = 1.2 Hz, H-3), 4.92 (1H, d, J_{trans} = 1.2 Hz, H-4), 2.08 (3H, s, -CH_3)
11c	17.9	64–66	$C_{20}H_{19}NO_6$	1680 (COOH) 1760 (C=O)	7.90–6.30 (8H, m, aromatic H), 5.42 (1H, d, J_{trans} = 1.2 Hz, H-3), 4.95 (1H, d, J_{trans} = 1.2 Hz, H-4), 3.32 (2H, q, J = 6 Hz, -OCH_2CH$_3$), 2.10 (3H, s, -CH_3), 1.18 (3H, t, J = 6 Hz, -OCH$_2$CH_3)
11d	25.3	175–177	$C_{19}H_{17}NO_5$	1680 (COOH) 1760 (C=O)	8.10–6.80 (8H, m, aromatic H), 5.45 (1H, d, J_{trans} = 1.5 Hz, H-3), 5.32 (1H, d, J_{trans} = 1.5 Hz, H-4), 2.16 (3H, s, -CH_3), 2.05 (3H, s, CH_3)
11e	31.9	147–148	$C_{19}H_{15}NO_7$	1690 (COOH) 1760 (C=O)	8.14–7.14 (7H, m, aromatic H), 5.95 (2H, s, -OCH_2O-), 5.38 (1H, d, J_{trans} = 1.2 Hz, H-3), 4.90 (1H, d, J_{trans} = 1.2 Hz, H-4, 2.16 (3H, s, -CH_3)
11f	78.1	130	$C_{18}H_{14}BrNO_5$	1690 (COOH) 1765 (C=O)	8.10–7.10 (8H, m, aromatic H), 5.35 (1H, d, J_{trans} = 1.2 Hz, H-3), 4.98 (1H, d, J_{trans} = 1.2 Hz, H-4), 2.20 (3H, s, -CH_3)
11g	17.9	174–176	$C_{18}H_{14}FNO_5$	1680 (COOH) 1770 (C=O)	8.15–6.50 (8H, m, aromatic H), 5.35 (1H, d, J_{trans} = 1.6 Hz, H-3), 4.98 (1H, d, J_{trans} = 1.6 Hz, H-4), 2.18 (3H, s, -CH_3)
11h	20.1	155–157	$C_{19}H_{15}NO_7$	1690 (COOH) 1760 (C=O)	8 10–6.80 (8H, m, aromatic H), 5.90 (2H, s, -OCH_2O-), 4.90 (1H, s, br, H-3), 4.70 (1H, s, br, H-4), 2.16 (3H, s, -CH_3)



Scheme 4.

benzoic acid. The IR spectrum of both compounds **15** and **16** shows the β-lactam carbonyl absorbance at 1780 cm^{-1}. In the ^1H-NMR spectrum H-7 is observed as a singlet at δ 6.20 in **15** and 6.45 in **16** thus displaying a downfield shift when compared with H-7 in **13** at δ 5.70 and at δ 4.92 in **14**.

Results and discussion

Antifungal activity

Monocyclic and bicyclic β-lactams have previously been investigated for antifungal activity. A series of 4-aryloxy and 4-arylthioazetidin-2-ones, unsubstituted at C-3 were synthesized and found to show antifungal activity when evaluated against various strains of *Microsporon*, *Trichophyton* and *Candida*, and also plant pathogenic fungi, eg, *Phycomycetes* [22, 34]. A significant difference in activity between the enantiomers of 4-phenylthioazetidin-2-one indicated possible correlation between the 4S configuration of the β-lactam and antifungal activity.

A number of 3-halo and 3-alkylazetidin-2-ones have been reported to be effective against plant pathogenic fungi, eg, *Piricularia oryzae* [35, 36]. A series of cephalosporin derivatives including sodium 7-(*N*-benzyldithiocarbamoylacetamido)cephalosporinate were active against *Trichophyton mentagrophytes*, *Microsporum canis*, *Cryptococcus neoformans* and *Histoplasma capsulatum* [37]. A group of clavam derivatives isolated from *Streptomyces clavuligerus* were reported to exhibit activity against a number of fungi particularly fungal plant pathogens [38].

In the present work, a number of the β-lactam compounds synthesised were evaluated as antifungal agents against the pathogenic fungi *C neoformans*, *Candida albicans*, *Candida parapsilosis*, *Candida tropicalis*, *Candida glabrata* and *Trichosporon cutaneum*. The MIC values obtained in this procedure are displayed in table V. Of the initial 3-acetoxyazetidin-2-ones examined 3-acetoxy-4-(4-nitrophenyl)-1-phenylazetidin-2-one **3i** proved to be the most effective, having MIC ≤ 18.4 μM against *C neoformans*, *C albicans*, *C parapsilosis*, *C tropicalis* and *C glabrata*. The 3-acetoxy(4-fluorophenyl)azetidin-2-one **3n** was effective against *C tropicalis* and *T cutaneum* (MIC ≤ 8.4 μM). Of the 3-hydroxyazetidin-2-ones examined, 3-hydroxy-1-(4-methoxyphenyl)-4-phenylazetidin-2-one **6f** showed good activity against *C neoformans* (MIC ≤ 11.15 μM) and *C glabrata* (MIC ≤ 22.3 μM) while compound **6a** was effective against *C parapsilosis* and *C tropicalis* (MIC ≤ 25.09 μM). Of the 3-sulfonyloxyazetidin-2-ones examined, compounds **9b** and **9e** showed MIC ≤ 3.27 and 2.90 μM respectively when evaluated against *C neoformans* and *C glabrata*, while compounds **9a**, **9b** and **9c** were effective against *T cutaneum* (MIC ≤ 7.05, 6.54, 9.46 μM respectively). Of the 3-acetoxyazetidin-2-ones containing a free carboxylic acid in the N-1 aryl ring only compound **11b** proved effective, having MIC ≤ 18.4 μM against *C glabrata*. The oxidized cepham type compounds **15** and **16** proved to be effective antifungal agents displaying MIC ≤ 19.41 μM against all organisms tested for the sulfone compound **16**, and MIC ≤ 20.47 μM for all organisms (except *T cutaneum*) for the sulfoxide compound **15**.

Antibacterial activity

A number of the compounds prepared were evaluated for antibacterial activity against the following organisms *Staphylococcus aureus*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Klebsiella aerogenes*, *Escherischia coli* and *Streptococcus faecalis*, using a radial growth assay procedure. The 3-acetoxyazetidin-2-ones **3a–n**, 3-hydroxyazetidin-2-ones **6a–j** and the cephams **13–16** were inactive at a concentration of 3.20–4.18 mM. The 3-acetoxy-1,4-diarylazetidin-2-ones **11a–h** containing a free carboxylic acid function displayed antibacterial activity against *S aureus*, *P vulgaris*, *P aeruginosa*, *B subtilis*, *K aerogenes* and *E coli* at concentrations in the range of 2.48–3.08 mM, with zones of inhibition of ≤ 9 mm in diameter. These results are displayed in table VI. On further investigation, compounds **11c** and **11h** showed inhibition of *S aureus* at a concentration of 0.054 mM and compounds **11b** and **11c** showed inhibition of *E coli* at concentrations of 0.062 and 0.054 mM respectively. These results are consistent with reported antibacterial activity for monocyclic β-lactams containing a free aryl carboxylic acid substituent [19–22].

Conclusion

A group of 3-acetoxyazetidin-2-ones containing a free carboxylic acid group on the N-1 aryl ring showed moderate antibacterial activity. It has also been demonstrated that a number of monocyclic and bicyclic β-lactam derivatives displayed useful antifungal activity when evaluated against a number of pathogenic fungi. All of the active products contain an oxygen function at C-3 of the β-lactam ring (OH, $OCOCH_3$, OSO_2R). Antifungal activity was observed for the monocyclic β-lactams having both *cis* and *trans* stereochemistry for H-3 and H-4. Further studies of these compounds, particularly preparation of additional derivatives with C-3 oxygen-containing substituents, together with an investigation of the stereochemical requirement of monocyclic β-lactams at C-4 for antifungal activity are in progress.

Table V. MIC values (μM) of 3-acetoxyazetidin-2-ones and related compounds against *C neoformans, C albicans, C parapsilosis, C tropicalis, C glabrata* and *T cutaneum*.

Compound	*C neoformans (ah27/a)*	*C albicans (sh/t54)*	*C parapsilosis (ah/t42)*	*C tropicalis (cp/k92)*	*C glabrata (t/91k)*	*T cutaneum (jk/92)*
3a	177.93	355.87	177.93	88.96	177.93	44.48
3d	158.84	153.84	76.92	153.84	38.46	38.46
3f	80.38	321.52	80.38	40.19	40.19	80.38
3i	18 40	18 40	18.40	18.40	18.40	38.34
3j	33.87	16.26	33.87	33.87	133.87	33.87
31	73.74	147.49	17.69	17.69	17.69	73.74
3m	70.82	141.64	70.82	35.41	141.64	283.28
3n	35.01	35.01	35.01	8.40	16.80	8.40
6a	52.29	52.29	25.09	25.09	52.29	104.58
6f	11.15	46.45	46.45	46.45	22.30	46.45
6h	153.86	153.86	307.72	38.46	38.46	38.46
7b	5.37	44.80	21.50	21.50	89.60	44.80
9a	14.08	14.08	14.08	7 04	14.08	7.04
9b	3.27	13.08	13.08	27.25	3.27	6.54
9c	18.92	18.92	39.41	9.46	39.41	9.46
9e	2.90	24.17	24.17	11.60	1.54	24.17
11b	153.83	76.91	153.83	307.66	18.46	153.83
11e	135.50	135.50	67.75	135.50	33.87	67.75
13	90.25	45.12	361.01	90.25	45.12	361.01
14	53.19	53 19	106.38	106.38	53 19	53.19
15	5.11	20.47	5.11	20.47	20.47	42.64
16	19.41	19 41	19.41	9.70	4.85	19.41
AMT-B	13.52	13.52	13.52	13 52	6.49	6.49

Table VI. Antibacterial activity of 3-acetoxyazetidin-2-ones **11a–h** (MIC values, mM).

Test organism	***11a***	***11b***	***11c***	***11d***	***11e***	***11f***	***11g***	***11h***
S aureus	2.82	3.08	0.054	2.95	2.71	2.48	2.92	0.054
P vulgaris	2.82	3.08	2.71	2.95	2.71	2.48	2.92	2.71
P aeruginosa	2.82	3.08	2.71	2.95	2.71	2.48	2.92	2.71
B subtilis	2.82	3.08	2.71	2.95	2.71	2.48	2.92	2.71
K aerogenes	2.82	3.08	2.71	2.95	2.71	2.48	2.92	2.71
E coli	2.82	0.062	0.054	2.95	2.71	2.48	2.92	2.71

Experimental section

Mps were determined on a Gallenkamp melting point apparatus and were not corrected. IR spectra were recorded on a Perkin-Elmer SP3-300 spectrometer and a Perkin-Elmer FTIR-1710 spectrometer. ^{1}H-NMR spectra (60 MHz) were obtained on a Perkin-Elmer R12B spectrometer and ^{1}H-NMR spectra (300 MHz) were obtained on a Bruker 300 MSL spectrometer using tetramethylsilane as internal standard. Mass spectra were measured with a VG Micromass 7070H spectrometer. Separations by column chromatography were carried out using Riedel–de Haën Kieselgel S (0.063–0.2 mm) and TLC was carried out on Riedel–de Haën pre-coated plates (silica gel 60, F254). Elemental analyses are within ±0.4% of the theoretical values except where indicated.

Elemental analysis of new compounds was carried out by I O'Brien, Microanalysis Laboratory, University College Dublin. All compounds are within ±0.4% of the theoretical value except where indicated for compounds **3i**, **3k**, **6c**, **11h** and **15**. High resolution mass spectral analysis of new compounds was carried out by DG Watson, Department of Pharmaceutical Sciences, Royal College University of Strathclyde, UK.

Chemistry

Acetoxyacetyl chloride

A mixture of glycolic acid (0.374 mol) and acetyl chloride (1.430 mol) was refluxed for 3 h. The mixture was evaporated under reduced pressure to remove excess acetyl chloride, and the residue was refluxed for 2 h with thionyl chloride (0.491 mol). The title product was obtained by distillation under reduced pressure (68%) (bp 58–61 °C, 19 mmHg, lit [27] bp 51 °C/14 mmHg), IR υ_{max} ($CHCl_3$), 1755 (C=O) cm^{-1}. ^{1}H-NMR δ ($CDCl_3$), 4.90 (2H, s, OCH_2^-), 2.18 (3H, s, $-CH_3$).

General preparation of 3-acetoxy-1, 4-diarylazetidin-2-ones ***3a–n***, ***5a*** *and* ***5b***

A solution of acetoxyacetyl chloride (7.30 mmol) in dry dichloromethane (12 mL) was added dropwise over 1 h at room temperature to a mixture of the appropriately substituted Schiff base (8.76 mmol) and triethylamine (17.52 mmol) in dry dichloromethane (18.2 mL). The reaction mixture was stirred for an additional hour at room temperature and was then washed with water (2 x 50 mL). The organic layer was dried (Na_2SO_4). Evaporation of the solvent and purification of the residue by column chromatography (eluent dichloromethane/diethyl ether, 9:1) afforded the required product.

General preparation of 1,4-diaryl-3-hydroxyazetidin-2-ones ***6a–j***, ***7a*** *and* ***7b***

Potassium carbonate (1.0 mmol) in water (0.2 mL) was added to a solution of the appropriately substituted 3-acetoxy-1,4-diarylazetidin-2-one (1.0 mmol) in methanol (10 mL). The reaction mixture was stirred for 1 h at room temperature and was then partitioned between water and dichloromethane. The organic phase was dried (Na_2SO_4). Removal of the solvent afforded the crude product which crystallized from dichloromethane to afford the required β-lactam.

3-Methoxy-1,4-diphenylazetidin-2-one ***8***

Potassium carbonate (0.1 mmol) in water (0.2 mL), was added to a solution of 3-acetoxy-1,4-diphenylazetidin-2-one (1.0 mmol) in methanol (10 mL). The reaction mixture was stirred for 20 h at room temperature and was then partitioned between water and dichloromethane. The organic layer was dried (Na_2SO_4) and the solvent was evaporated to afford the title compound which crystallized from methanol (70.2%), mp 143–145 °C (lit [28] mp 141–142 °C). υ_{max} (KBr), 1735 cm^{-1}. ^{1}H-NMR δ ($CDCl_3$), 7.48–6.42 (10H, m, aromatic H), 4.90 (1H, d, $J_{3,4cis}$ = 5Hz, H-3), 4.48 (1H, d, $J_{4,3cis}$ = 5 Hz, H-4), 3.75 (3H, s, OCH_3).

General preparation of 3-(arylsulfonyloxy)-1,4-diarylazetidin-2-ones ***9a***, ***9d*** *and* ***9e***

To a solution of the appropriately substituted 1,4-diaryl-3-hydroxyazetidin-2-one **6a** and **6f** (0.84 mmol) in pyridine (6 mL) at 0 °C was added the appropriately substituted sulfonyl chloride (1.75 mmol). The solution was allowed to stand at 0 °C for 24 h, then poured onto ice water and extracted with ethyl acetate (2 x 15 mL). The organic layer was washed successively with dilute sulphuric acid (2 x 8 mL), water (2 x 8 mL) and dried (Na_2SO_4). Evaporation of the solvent and purification of the residue by column chromatography (eluent: chloroform) yielded the required product

1,4-Diphenyl-3-(4-nitrophenylsulfonyloxy)azetidin-2-one ***9a***. The product was obtained as a yellow powder (45%), mp 211 °C. IR υ_{max} (KBr), 1768 (C=O), 1195 (SO_2) cm^{-1}. ^{1}H-NMR δ (pyridine-d_5), 8.50–7.00 (14H, m, aromatic H), 6.56 (1H, d, $J_{3,4cis}$ = 4.8 Hz, H-3), 5.67 (1H, d, $J_{4,3cis}$ = 4.8 Hz, H-4). Elemental analysis found: C, 63.90; H, 4.00, N, 6.96. $C_{21}H_{16}N_2O_6S$ requires C, 64.28; H, 4.08; N, 7.14%.

1-(4-Methoxycarbonylphenyl)-4-(4-methoxyphenyl)-3-(4-methylphenylsulfonyloxy)azetidin-2-one ***9d***. The product was obtained as a yellow powder (79.5%), mp 105–107 °C. IR υ_{max} (KBr), 1744 (C=O), 1710 (CO_2CH_3), 1370, 1180 (SO_2) cm^{-1}. ^{1}H-NMR δ (pyridine-d_5), 7.90–6.30 (12H, m, aromatic H), 5.25 (1H, d, $J_{3,4trans}$ = 2.6 Hz, H-3), 4.95 (1H, d, $J_{4,3trans}$ = 2.6 Hz, H-4), 3.68 (3H, s, $-OCH_3$), 3.56 (3H, s, $-OCH_3$), 2.40 (3H, s, $-CH_3$). Elemental analysis found: C, 62.01, H, 4.69; N, 2.80. $C_{25}H_{23}NO_7S$ requires C, 62.37; H, 4.78, N, 2.91%.

1-(4-Methoxycarbonylphenyl)-4-(4-methoxyphenyl)-3-(1-naphthylsulfonyloxy)azetidin-2-one ***9e***. The product was obtained as a yellow powder (13.6%), mp 180 °C. IR υ_{max} (KBr), 1745 (C=O), 1710 ($-CO_2CH_3$), 1350, 1180 ($-SO_2$) cm^{-1}. ^{1}H-NMR δ (pyridine-d_5), 7.95–6.20 (15H, m, aromatic H), 5.27 (1H, d, $J_{3,4trans}$ = 2.6 Hz, H-3), 4.89 (1H, d, $J_{4,3trans}$ = 2.6 Hz, H-4), 3.65 (3H, s, $-OCH_3$), 3.52 (3H, s, $-OCH_3$). Elemental analysis found: C, 64.69; H, 4.47; N, 2.86. $C_{28}H_{23}NO_7S$ requires C, 64.99; H, 4.44; N, 2.70%.

3-(4-Bromophenylsulfonyloxy)-1,4-diphenylazetidin-2-one ***9b***

1,4-Diphenyl-3-hydroxyazetidin-2-one (0.65 mmol) was dissolved in chloroform (3 mL) containing *N,N*-dimethylformamide (0.15 mL) and to this stirred solution was added *p*-bromophenylsulfonyl chloride (0.99 mmol) and triethylamine (1.24 mmol). After 24 h the reaction mixture was diluted with chloroform (20 mL), washed with dilute $NaHCO_3$ (15 mL), water (15 mL), dried (Na_2SO_4) and the solvent evaporated. The crude product was purified by column chromatography (eluent: chloroform) to afford the title compound as a colorless powder (55%), mp 222 °C. IR υ_{max} (KBr), 1768 (C=O), 1195 (SO_2) cm^{-1}. ^{1}H-NMR δ (pyridine-d_5), 7.72–7.05 (14H, m, aromatic H), 5.85 (1H, d, $J_{3,4cis}$ = 4.8 Hz, H-3), 5.32 (1H, d, $J_{4,3cis}$ = 4.8 Hz, H-4). Elemental analysis found: C, 55.25, H, 3.44; N, 3.06. $C_{21}H_{16}BrNO_4S$ requires C, 55.03; H, 3.49; N, 3.05%

1,4-Diphenyl-3-methylsulfonyloxyazetidin-2-one ***9c***

Following the above procedure with methanesulfonyl chloride, the pure product was isolated as a colorless powder (60%),

mp 210 °C. IR υ_{max} (KBr), 1768 (C=O), 1195 (SO_2) cm^{-1}. ^{1}H-NMR δ (pyridine-d_5), 7.42–7.20 (10H, m, aromatic H), 5.87 (1H, d, $J_{3,4cis}$ = 4.8 Hz, H-3), 5.40 (1H, d, $J_{4,3cis}$ = 4.8 Hz, H-4), 2.76 (3H, s, -CH_3). Elemental analysis found: C, 60.23; H, 4.71; N, 4.12. $C_{16}H_{15}NO_4S$ requires C, 60.56; H, 4.73, N, 4.41%.

General preparation of Schiff bases ***10a–h***

A solution of the appropriately substituted aryl aldehyde (0.1 mol) and 4-aminobenzoic acid (0.1 mmol) or 3-aminobenzoic acid (0.1 mol) in ethanol (50 mL) was heated for 30 min on a boiling water bath. The reaction mixture was then reduced to 10 mL in vacuo, and on standing the Schiff base crystallized. The crude product was then filtered and recrystallized twice from ethanol.

General preparation of 3-acetoxy-1,4-diarylazetidin-2-ones ***11a–h***

Triethylamine (12 mmol) was added to a solution of the appropriately substituted Schiff base **10a–h** (4 mmol) in dry dichloromethane (20 mL). Trimethylsilyl chloride (4.40 mmol) was added dropwise and the resulting mixture was stirred for 30 min. Acetoxyacetyl chloride (4.40 mmol) in dichloromethane (20 mL) was added dropwise over 1 h under anhydrous conditions, and the solution was then refluxed for 2 h and stirred overnight. The solution was washed with water (2 x 20 mL) and the organic layer was dried (Na_2SO_4) and the solvent evaporated to yield the crude product which was purified by column chromatography (eluent:dichloromethane/diethyl ether, 90:10 to 10:90). The products were then recrystallized from methanol.

Methylation of 3-acetoxy-1,4-diarylazetidin-2-ones ***11a, 11b, 11d, 11g***

The 3-acetoxy-4-aryl-1-(4-carboxyphenyl)azetidin-2-ones **11a**, **11b**, **11d** and **11g** were dissolved in methanol (5 mL), and a solution of diazomethane in diethyl ether was added in aliquots of 1–2 mL. The formation of the methyl ester was monitored by tlc until the reaction had gone to completion. The mixture was then washed with water, dried (Na_2SO_4) and the solvent evaporated. The residue was purified by column chromatography over silica gel (eluent:dichloromethane) to afford the products **3j**, **3l**, **3m** and **3n**, respectively, which crystallized from ethanol.

7-Acetoxy-6-phenyl-5-thia-1-azabicyclo[4.2.0]octan-8-one ***13***

A solution of acetoxyacetyl chloride (7.3 mmol) in dry dichloromethane (12 mL) was added dropwise over 1 h at room temperature to a mixture of 2-phenyl-5,6-dihydro-4*H*-1,3-thiazine **12** [29] (8.76 mmol) and triethylamine (14.6 mmol) in dry dichloromethane (18.2 mL). The reaction mixture was stirred for an additional hour at room temperature and was then washed with water (2 x 50 mL). The organic layer was dried (Na_2SO_4). Removal of the solvent, and purification by column chromatography (eluent *n*-hexane/ethyl acetate, 3:1) yielded the title product as a colourless crystal (11.5%), mp 92–93 °C. υ_{max} 1765 (C=O), cm^{-1}. ^{1}H-NMR δ ($CDCl_3$), 7.60–7.21 (5H, m, aromatic H), 5.70 (1H, s, H-7), 4.28–3.95 (1H, m, H-4 eq), 3.30–2.60 (3H, m, H-4ax, H-2), 2.06–1.52 (2H, m, H-3), 1.55 (3H, s, CH_3CO). Elemental analysis found: C, 60.42; H, 5.58; N, 4.90. $C_{14}H_{15}NO_3S$ requires C, 60.64, H, 5.41, N, 5.05%.

7-Hydroxy-6-phenyl-5-thia-1-azabicyclo[4.2.0]octan-8-one ***14***

Potassium carbonate (0.1 mmol) in water (0.2 mL) was added to a solution of 7-acetoxy-6-phenyl-5-thia-1-azabicyclo[4.2.0]-octan-one **13** (1.0 mmol) in methanol (10 mL). The reaction mixture was stirred for 1 h at room temperature. The residue was then partitioned between water and dichloromethane. The organic layer was dried (Na_2SO_4). Removal of the solvent and purification of the residual oil by column chromatography (eluent:hexane/ethyl acetate, 3:1), yielded the title compound as colorless crystals (50.1%), mp 150–151 °C IR υ_{max} (KBr), 3400 (OH), 1750 (C=O) cm^{-1}. ^{1}H-NMR δ ($CDCl_3$), 7.60–7.20 (5H, m, aromatic H), 5.10–4.84 (1H, m, H-7), 4.26–3.88 (1H, m, H-4_{eq}), 3.30–2.56 (3H, m, H-4_{ax}, H-2), 2.06–1.50 (2H, m, H-3). Elemental analysis found C, 61.53; H, 5.56; H, 5.89. $C_{12}H_{13}NO_2S$ requires C, 61.27; H, 5.53; N, 5.95%.

7-Acetoxy-6-phenyl-5-thia-1-azabicyclo[4.2.0]octan-8-one-1-sulfoxide ***15***

A solution of 7-acetoxy-6-phenyl-5-thia-1-azabicyclo[4.2.0]-octan-8-one **13** (0.36 mmol) in dry dichloromethane (3.5 mL) was treated under stirring with *m*-chloroperbenzoic acid (0.37 mmol) at 20 °C. After 24 h, a solution of $NaHSO_4$ was added to the mixture to destroy the excess peracid. The mixture was concentrated under reduced pressure to yield a residue which was partitioned between ethyl acetate and water. The organic layer was separated, and washed successively with $NaHCO_3$ (5%, 10 mL), water, and dried (Na_2SO_4) and evaporated to afford the crude product which was purified by column chromatography (eluent:hexane/ethyl acetate, 3:2) to give the title compound as colorless crystals (15.2%, mp 165–166 °C). IR υ_{max} ($CHCl_3$), 1780 (C=O), 1075, 1050 (S=O) cm^{-1}. ^{1}H-NMR δ (DMSO-d_6), 7.54–7.12 (5H, m, aromatic H), 6.20 (1H, s, H-7), 4.30–3.80 (1H, m, H-4_{eq}), 3.35–2.15 (3H, m, H-4_{ax}, H-2), 2.09–1.54 (2H, m, H-3), 1.55 (3H, s, -CH_3). Elemental analysis found: C, 56.57; H, 5.25, H, 4.85. $C_{14}H_{15}NO_4S$ requires C, 57.14; H, 5.44; N, 4.76%.

7-Acetoxy-6-phenyl-5-thia-1-azabicyclo[4.3.0]octan-8-one-1-sulfone ***16***

A solution of 7-acetoxy-6-phenyl-5-thia-1-azabicyclo[4.2.0]-octan-8-one **13** (0.36 mmol), in dry dichloromethane (3.5 mL) was treated while stirring with *m*-chloroperbenzoic acid (0.72 mmol) at 20 °C as described for compound **15** above. The crude product was purified by column chromatography (eluent:hexane/ethyl acetate, 3:2) to afford the title compound as colorless crystals (11.7%), mp 125–127 °C. IR υ_{max} (KBr), 1780 (C=O), 1320, 1140 (SO_2) cm^{-1}. ^{1}H-NMR δ (DMSO-d_6), 7.60–7.25 (5H, m, aromatic H), 6.45 (1H, s, H-7), 4.38–3.95 (1H, m, H-4_{eq}), 3.60–2.80 (3H, m, H-4_{ax}, H-2), 2.10–1.55 (2H, m, H-3), 1.70 (3H, s, -CH_3). Elemental analysis found C, 53.53; H, 4.82; N, 4.26. $C_{14}H_{15}NO_5S$ requires C, 54.36; H, 4.85; N, 4.53%.

Antimycotic activity

The in vitro antimycotic activity of tested compounds was determined against a series of yeasts and fungi and has been evaluated through the minimum inhibitory concentration (MIC) according to the method of progressive double dilutions in liquid Casitone medium [30].

The yeasts were originally clinical isolates obtained from Kuwait, and were typed by conventional methods [31]. The cells were maintained by a periodic subculture on malt agar (Oxoid) slants. MIC determinations were performed by a micro-titre technique on freshly subcultured 2-day-old cells from slants. One (1 mm) loopful of the freshly cultured cells was suspended in sterile distilled water (10 mL). The seeding rate was adjusted by successive dilution of this stock solution with sterile distilled water until the optical density reading of the solution is 0.05 (530 nm) [32]; 25 μL of this diluted cell

suspension was then added to the wells in the autotray-microtitre plate already containing liquid Casitone medium (Difco: 20%, 0.1 mL). The compounds were then added to the wells in the concentration range of 0.1–100 μg/mL diluted with sterile distilled water from a 1 mg/mL stock solution of the pure compounds in DMSO (0.1 mL), with the highest content of DMSO in any well at 2.5%.

The cells were incubated at 30 °C for 24 h (for *Candida* spp and *T cutaneum*) and 48 h (for *C neoformans* strains and *C glabrata*) and the MIC reading recorded at 24 and 48 h respectively using a colony reader compared to control cultures incubated on the same plate at the same conditions above, and in duplicate sets.

Antibacterial activity

Test organisms and culture media: *E coli*, *K aerogenes*, *B subtilis* (NCTC 10400), *P vulgaris* (NCTC 6435), *P aeruginosa* (NCTC 6749), *S faecalis* and *S aureus* were cultivated in nutrient agar and nutrient broth (Oxoid). Cultures without identification number of source are from the collection of the School of Pharmacy, Trinity College, Dublin. Each 24-h-old culture (10 μL) was added and mixed with Oxoid nutrient broth (10 mL). The resulting mixture (3 mL) was poured and swirled onto a Petri dish containing the set nutrient agar. A solution of each compound to be tested was initially prepared at a concentration of 1 mg/mL in analar dichloromethane. Ampicillin was used as the standard antibiotic. The resultant solution (10 μL) was dispensed on Whatman No 1 filter paper discs of diameter 5 mm and allowed to air dry. Four treated discs were placed on each agar plate and the plates were incubated at 37 °C for 24 h. Zones of inhibition were then measured and recorded.

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