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Synthesis and Antimicrobial Activities of 3-N-Substituted-4,5-bis (3-indolyl)oxazol-2-ones

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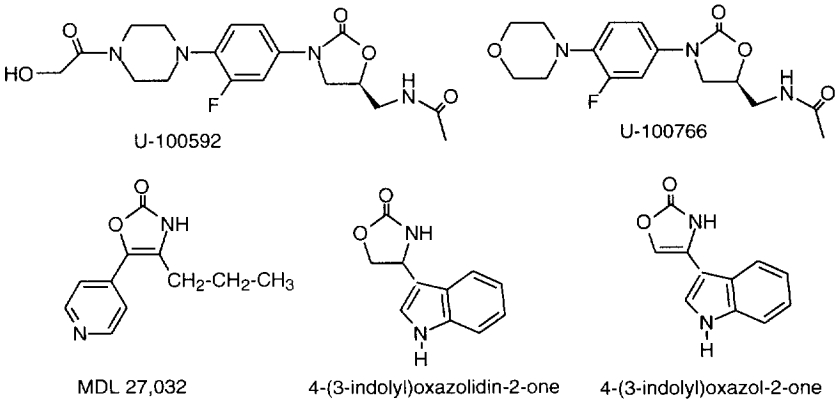
Abstract: A short synthesis of 3-N-substituted-4,5-bis(3-indolyl)oxazol-2-ones is described from bis(3-indolyl)glyoxal. Their *in vitro* antimicrobial activities against different strains of microorganisms are examined. © 1997 Elsevier Science Ltd.

Since the discovery of penicillin, pharmaceutical companies have produced more than one hundred antibacterial agents to combat a wide variety of bacterial infections. However, in the past several years, the rapid emergence of bacterial resistance to antibiotics have been observed. Consequently, there is an urgent need to develop new agents to treat patients infected with these resistant bacteria. Oxazolidin-2-ones as well as oxazol-2-ones are important classes of chemotherapeutic agents useful against many diseases. They can exhibit antimicrobial, antiinflammatory, antimitotic, analgesic, hypotensive and antimetastatic properties. Oxazolone MDL 27,032 was found to have smooth muscle vasorelaxant effects and antimetastatic properties linked to its ability to inhibit protein kinase C (PKC).[1-3] Oxazolidinones have been described as a novel class of synthetic protein synthesis inhibitors with a novel mechanism of action that involves the inhibition of bacterial protein synthesis at a very early stage.[4] N-substituted oxazolidinones U-100592 and U-100766 (Scheme 1) exhibiting antibacterial activity against a wide range of Gram-positive bacteria are currently in clinical development.[5] Oxazolidinones and oxazolones bearing a large variety of substituents have been described. To our knowledge, indole substituents have never been investigated, however the indole ring is present in many biologically active compounds such as PKC inhibitors bis-indolyl maleimides and staurosporine[6-8]
Our aim was to test the influence of indole substituents on this kind of compounds. In a previous work, we reported the synthesis of 4-(3-indolyl)oxazolidin-2-one and 4-(3-indolyl)oxazol-2-one (Scheme 1). The first one had a strong inhibitory effect against several Gram-negative strains and the second one inhibited strongly the growth and sporulation of different *Streptomyces* species.[9] Since these compounds were not PKC inhibitors, other mechanisms than PKC inhibition must be involved.



Scheme 1.

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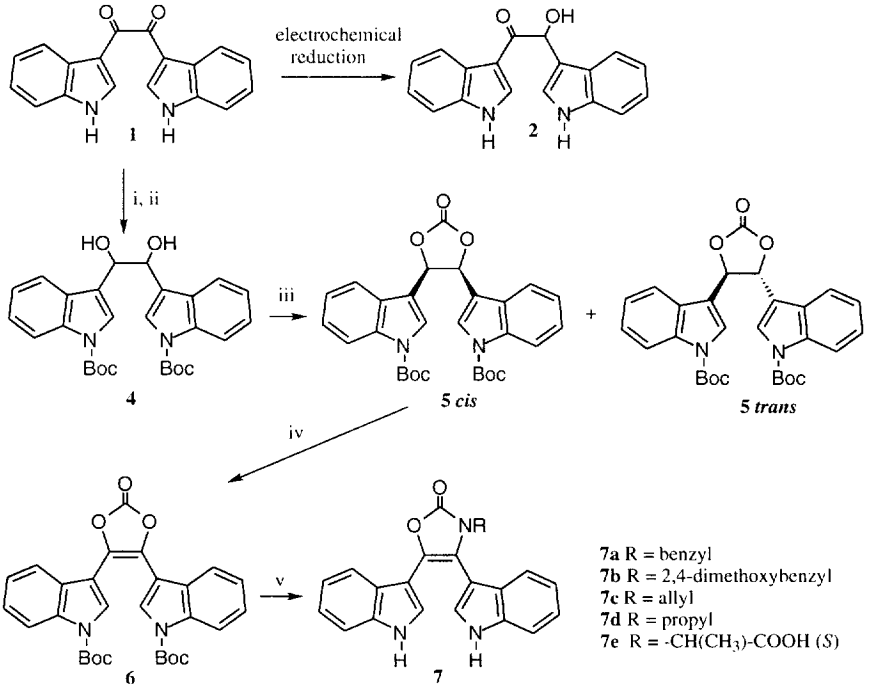
We report here the synthesis and *in vitro* antibacterial activities of 3-N-substituted-4,5-bis(3-indolyl)oxazol-2-ones **7** toward different Gram-positive bacteria (*Bacillus cereus*, *Corynebacterium equi*, *Enterococcus faecalis*, *Streptomyces chartreusis*), a Gram-negative bacterium *Escherichia coli* and a fungus *Candida albicans*.

The most common route to substituted oxazol-2-ones is the treatment of α-hydroxy ketones with potassium cyanate or urethanes.[10] Several attempts to apply this method to the synthesis of bis(indolyl)oxazol-2-ones were carried out. α-Diketone **1** was readily available from commercial 3-indoleglyoxylyl chloride and indolylmagnesium bromide.[11] The electrochemical reduction of **1** led to α-hydroxy ketone **2**[12] but the conversion of **2** to the bis(indolyl)oxazol-2-one failed using either potassium cyanate or ethyl carbamate in different reaction conditions, even when the indole nitrogens were protected with methyl groups.

An alternative route to oxazol-2-ones is the reaction of cyclic carbonates with primary amines *via* an alcohol intermediate.[13,14] Accordingly, carbonate **6** was prepared in four steps from **1** by i) protection of the indole nitrogens with Boc groups, ii) reduction to α-diol using sodium borohydride that lead to a mixture of stereoisomers **4** [15] which could not be separated by chromatography; the diastereomeric ratio determined from ^{1}H NMR spectrum on the signals shifted at 4.47 and 4.64 ppm was 3:1, iii) cyclisation of the isomeric mixture to **5** *cis* and *trans* carbonates[16] using carbonyldiimidazole (CDI), iv) oxidation of the *cis* isomer to carbonate **6**[17] using 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (Scheme 2).

Separation of the individual *cis* and *trans* isomers (**5** ***cis*** and **5** ***trans***) was readily accomplished by silica gel column chromatography using cyclohexane:ethylacetate (9:1) as the eluent mixture.



Reagents and conditions: i) tBuOK, THF, reflux, 1h then $(tBuO_2C)_2O$, reflux, 3h (compound **3**: 93%). ii) $NaBH_4$, EtOH/THF(1:1,v/v), r.t., 5h (100%). iii) CDI, THF, r.t., 12h (cis isomer 51%, trans isomer 13%). iv) DDQ, toluene, reflux, 6h (22%). v) RNH_2, DMF, 0.5h then TFA, 2h (**7a** 17%, **7b** 32%, **7c** 47%, **7d** 12%, **7e** 48%).

Scheme 2.

The reaction of a primary amine with cyclic carbonates leading to oxazol-2-ones requires an unsaturated five membered cyclic carbonate. Only **5** ***cis*** could be oxidized to give **6**.

Reaction with benzylamine, 2,4-dimethoxybenzylamine, allylamine, propylamine and L-alanine at room temperature followed by an acidic work-up provided N-substituted-oxazol-2-ones **7a, 7b, 7c, 7d, 7e**[18] respectively with concomitant deprotection of the indole nitrogens. Bulky amines such as tritylamine and

amines bearing electron withdrawing groups such as *tert*-butyloxycarbonylamine or aminoacetonitrile did not react with carbonate **6**. L-alanine was used in order to enhance the solubility of the oxazolone.
The *in vitro* growth inhibitory effects of compounds **7a-e** against *B. cereus, C. equi, E. faecalis, S. chartreusis, E. coli* and *C. albicans* were examined (Table 1).

Table 1. Antimicrobial activities of **7a-e** toward four Gram-positive bacteria *B. cereus, C. equi, E. faecalis, S. chartreusis*, a Gram-negative bacterium *E. coli* and a fungus *C. albicans*.

Compounds	*B. cereus* ATCC 14579	*C. equi IFO 3720*	*E. faecalis ATCC 11700*	*S. chartreusis* NRRL 11407	*E. coli ATCC 11303*	*C. albicans IP 444*
7a	±	+	-	±	-	-
7b	-	nd	nd	-	-	-
7c	±	++	-	++	-	-
7d	±	+	-	++	-	-
7e	+	+	±	++	-	-

The antibacterial activity was determined by the conventional paper disk (Durieux, N°268, 6 mm in diameter) diffusion method. The size of zones of growth inhibition was 9-10 mm(++), 7-8 mm (+).

All of them were inactive toward Gram-negative bacterium *E. coli* and yeast *C. albicans*. Only **7e** exerted growth inhibitory effects against *S. faecalis*. Except **7b**, all of them were active against *B. cereus, C. equi* and *S. chartreusis*. Compound **7e** inhibited very strongly the sporulation of *S. chartreusis* (size of zone of sporulation inhibition = 18 mm). We are now investigating the syntheses and antimicrobial properties of bis(indolyl)oxazol-2-one analogues of **7e** by reaction of carbonate **6** with different natural and non natural amino-acids. Their mode of action which does not involve PKC inhibition remain to be determined.

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15) Data for **4** : IR (KBr) $\nu_{C=O}$ 1740 cm^{-1} ν_{OH} 3500 cm^{-1}. HRMS (EI) calcd. for $C_{28}H_{32}N_2O_6$ 492.2260 found 492.2337. ^1H NMR (400 MHz, acetone-d_6): 1.60 and 1.65 (18H, 2s), 4.47 and 4.64 (2H, 2dd, J = 2.9 and 1.4 Hz), 5.25 and 5.28 (2H, 2dd, J = 2.9 and 1.4 Hz), 7.12 and 7.16 (2H, 2dt, J = 7.4 and 0.9 Hz), 7.25 and 7.27 (2H, t, J = 7.3 and 1.0 Hz), 7.51 and 7.61 (2H, 2s), 7.59 and 7.70 (2H, 2d, J = 7.8 Hz), 8.07 and 8.11 (2H, 2d, J = 8.4 Hz). ^{13}C NMR (100 MHz, acetone-d_6): 27.4 and 27.6, 70.9 and 71.0, 83.2 and 83.3, 114.8, 120.1, 120.5, 122.1, 122.2, 123.9, 124.0 (C tert. arom.), 121.6, 121.8, 129.5, 129.8, 135.5 (C

quat. arom.), 149.3, 149.4 (C=O).

16) Data for **5** ***cis*** : mp. 83-84°C. IR (KBr) $\nu_{C=O}$ 1730, 1740 and 1820 cm^{-1}. HRMS (EI) calcd. for $C_{29}H_{30}N_2O_7$ 518.2053 found 518.2055. ^1H NMR (400 MHz, acetone-d_6): 1.57 (18H, s), 6.65 (2H, s), 7.11 (2H, dt, J = 7.4 and 0.9 Hz), 7.21 (2H, dt, J = 7.3 and 1.1 Hz), 7.50 (2H, d, J = 7.8 Hz), 7.53 (2H, s), 7.95 (2H, d, J = 8.3 Hz). ^{13}C NMR (100 MHz, acetone-d_6): 27.2, 76.5, 83.9, 114.9, 119.5, 122.5, 124.5, 125.1 (C tert. arom.), 114.2, 127.7, 135.1 (C quat. arom.), 148.7, 154.3 (C=O).

5 ***trans*** : mp. 181-184°C. IR (KBr) $\nu_{C=O}$ 1730, 1740 and 1820 cm^{-1}. ^1H NMR (400 MHz, acetone-d_6): 1.65 (18H, s), 6.34 (2H, s), 7.32 (2H, dt, J = 7.3 and 0.8 Hz), 7.41 (2H, t, J = 7.3 and 1.0 Hz), 7.70 (2H, d, J = 7.8 Hz), 8.09 (2H, s), 8.21 (2H, d, J = 8.3 Hz). ^{13}C NMR (100 MHz, acetone-d_6): 27.3, 76.9, 94.9, 115.6, 119.4, 123.2, 125.2, 126.5 (C tert. arom.), 114.7, 127.6, 136.0 (C quat. arom.), 149.1, 153.9 (C=O).

17) Data for **6** : mp 172-173°C. IR (KBr) $\nu_{C=O}$ 1740 and 1830 cm^{-1}. HRMS (EI) calcd. for $C_{29}H_{28}N_2O_7$ 516.1896 found 516.1897 ^1H NMR (400 MHz, acetone-d_6): 1.63 (18H, s), 7.24 (2H, dt, J = 7.3 and 0.9 Hz), 7.41 (2H, dt, J = 7.3 and 1.1 Hz), 7.57 (2H, dd, J = 7.8 and 1.0 Hz), 7.97 (2H, s), 8.22 (2H, d, J = 8.4 Hz). ^{13}C NMR (100 MHz, acetone-d_6): 27.3, 84.8, 115.4, 120.8, 123.4, 125.4, 126.4 (C tert. arom.), 106.1, 127.0, 135.2 (C quat. arom.), 148.8, 151.8 (C=O).

18) Data for **7a** : mp 243-245°C. IR (KBr) $\nu_{C=O}$ 1720 cm^{-1} ν_{NH} 3300–3400 cm^{-1}. HRMS (EI) calcd. for $C_{26}H_{19}N_3O_2$ 405.1477 found 405.1478. ^1H NMR (400 MHz, acetone-d_6): 4.75 (2H, s), 6.95 (1H, dt, J = 7.4 and 0.9 Hz), 7.00 (1H, dt, J = 7.4 and 0.9 Hz), 7.07 (4H, m), 7.18 (4H, m), 7.34 (2H, pt, J = 7.4 Hz), 7.40 (1H, d, J = 2.1 Hz), 7.51 (1H, d, J = 8.2 Hz), 7.62 (1H, d, J = 8.2 Hz), 10.45 (1H, s, N_{indole}-H), 10.70 (1H, s, N_{indole}-H). ^{13}C NMR (100 MHz, acetone-d_6): 45.1, 111.5, 111.9, 119.2, 119.8, 120.1, 120.6, 122.0, 122.3,123.0, 123.1, 127.1, 127.2, 128.1 (C tert. arom.), 101.7, 104.4, 115.0, 124.9, 127.1, 134.0, 136.0, 136.3, 137.5 (C quat. arom.), 155.0 (C=O).

7b : mp 96-98°C. IR (KBr) $\nu_{C=O}$ 1740 cm^{-1}, ν_{NH} 3300–3400 cm^{-1}. HRMS (EI) calcd. for $C_{28}H_{23}N_3O_4$ 465.1688 found 465.1682. ^1H NMR (400 MHz, acetone-d_6): 3.50 (3H, s), 3.70 (3H, s), 4.75 (2H, s), 6.35 (1H, d, J = 2.3 Hz), 6.41 (1H, d,J = 2.3 Hz), 6.41 (1H, dd, J = 2.3 and 8.1 Hz), 6.96 (1H, dt, J = 8.1 and 0.9 Hz), 6.97 (1H, dt, J = 8.1 and 0.9 Hz), 7.02 (1H, d, J = 8.1 Hz), 7.05 (1H, d, J = 2.7 Hz), 7.09 (1H, dt, J = 8.1 and 1.0 Hz), 7.14 (1H, dt, J = 8.1 and 1.1 Hz), 7.30 (1H, d, J = 8.1 Hz), 7.36 (1H, d, J = 8.1 Hz), 7.37 (1H, d, J = 3.2 Hz), 7.49 (1H, d, J = 8.1 Hz), 7.66 (1H, d, J = 8.1 Hz), 10.42 (1H, s, N_{indole}-H), 10.55 (1H, s, N_{indole}-H). ^{13}C NMR (100 MHz, acetone-d_6): 39.7 (CH_2), 54.6, 54.7 (OCH_3), 97.9, 104.3, 111.5, 111.8, 119.2, 119.7, 120.0, 120.6, 122.0, 122.1, 122.9, 126.9, 128.0 (C tert. arom.), 101.9, 104.6, 114.6, 117.5, 124.9, 127.0, 133.8, 136.2, 136.4, 157.5, 160.4 (C quat. arom.), 155.1 (C=O).

7c : mp 102-105°C. IR (KBr) $\nu_{C=O}$ 1740 cm^{-1}, ν_{NH} 3250–3400 cm^{-1}. HRMS (EI) calcd. for $C_{22}H_{17}N_3O_2$ 355.1320 found 355.1315. ^1H NMR (400 MHz, acetone-d_6): 4.09 (2H, m), 4.98 (2H, m), 5.72 (1H, m), 6.92 (1H, dt, J = 7.0 and 0.9 Hz), 7.00 (1H, dt, J = 7.2 and 0.9 Hz), 7.03 (1H, d, J = 2.6 Hz), 7.05 (1H, dt, J = 7.0 and 1.0 Hz), 7.15 (1H, dt, J = 7.9 and 1.2 Hz), 7.34 (1H, d, J = 8.0 Hz), 7.37 (1H, d, J = 8.1 Hz), 7.51 (1H, d, J = 8.3 Hz), 7.59 (1H, d, J = 2.6 Hz), 7.60 (1H, d, J = 8.0 Hz), 10.30 (1H, s, N_{indole}-H), 10.80 (1H, s, N_{indole}-H). ^{13}C NMR (100 MHz, acetone-d_6): 43.8, 101.7, 104.5, 114.3, 124.9, 126.9, 133.9, 136.2, 136.5 (C quat. arom.), 116.0 (CH_2 of allyl group), 111.6, 112.0, 119.2, 119.7, 120.3, 120.8, 122.0, 122.3, 122.8, 127.1, 133.2 (C tert. arom. + CH of allyl group), 154.7 (C=O).

7d : mp 93-94°C. IR (KBr) $\nu_{C=O}$ 1740 cm^{-1}, ν_{NH} 3220–3450 cm^{-1}. HRMS (FAB$^+$) calcd. for $C_{22}H_{19}N_3O_2$ 357.1477 found 357.1481. ^1H NMR (400 MHz, acetone-d_6): 0.74 (3H, t, J = 7.4 Hz), 1.48 (2H, m), 3.48 (2H, t, J = 7.2 Hz), 6.95 (1H, dt, J = 8.0 and 1.0 Hz), 7.04 (1H, d, J = 2.8 Hz), 7.09 (1H, dt, J = 7.5 and 1.0 Hz), 7.19 (1H, dt, J = 7.0 and 1.0 Hz), 7.36 (1H, d, J = 8.3 Hz), 7.44 (1H, d, J = 8.2 Hz), 7.56 (1H, d, J = 8.2 Hz), 7.63 (1H, d, J = 8.3 Hz), 7.68 (1H, d, J = 2.6 Hz), 10.35 (1H, s, N_{indole}-H), 10.80 (1H, s, N_{indole}-H). ^{13}C NMR (100 MHz, acetone-d_6): 10.3 (CH_3), 21.3 (CH_2), 43.4 (NCH_3), 101.9, 104.6, 113.9, 125.0, 129.5, 133.8, 136.2, 136.6 (C quat. arom.), 111.7, 112.1, 119.1, 119.7, 120.2, 120.6, 121.8, 122.0, 122.3, 127.1 (C tert. arom.), 154.9 (C =O).

7e : mp 138-140°C. $[\alpha]_D^{25}$ = -60 (c: 0.2%, DMSO). IR (KBr) $\nu_{C=O}$ 1730, 1740 cm^{-1}, $\nu_{NH, OH}$ 3100–3600 cm^{-1}. HRMS (FAB$^+$) calcd. for $C_{22}H_{18}N_3O_4$ (M+H$^+$) 388.1297 found 388.1294. ^1H NMR (400 MHz, DMSO-d_6): 1.49 (3H, d, J = 6.7 Hz), 4.25 (1H, m), 6.95 (1H, t, J = 7.6 Hz), 7.04 (1H, t, J = 7.5 Hz), 7.05 (1H, s), 7.10 (1H, t, J = 7.4 Hz), 7.20 (1H, t, J = 7.7 Hz), 7.37 (1H, d, J = 7.8 Hz), 7.40 (1H, d, J = 6.5 Hz), 7.45 (1H, d, J = 8.0 Hz), 7.54 (1H, d, J = 8.2 Hz), 7.66 (1H, d, J = 2.1 Hz), 11.40 (1H, s, N_{indole}-H), 11.85 (1H, s, N_{indole}-H). ^{13}C NMR (100 MHz, DMSO-d_6): 15.0 (CH_3), 50.1 (CH), 100.1, 103.8, 119.3, 121.5, 124.3, 126.7, 135.7, 136.1 (C quat.), 111.8, 112.2, 118.6, 119.5, 119.9, 120.0, 121.7, 121.9, 123.2, 128.2 (C tert. arom.), 159.0, 159.2 (C=O).

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