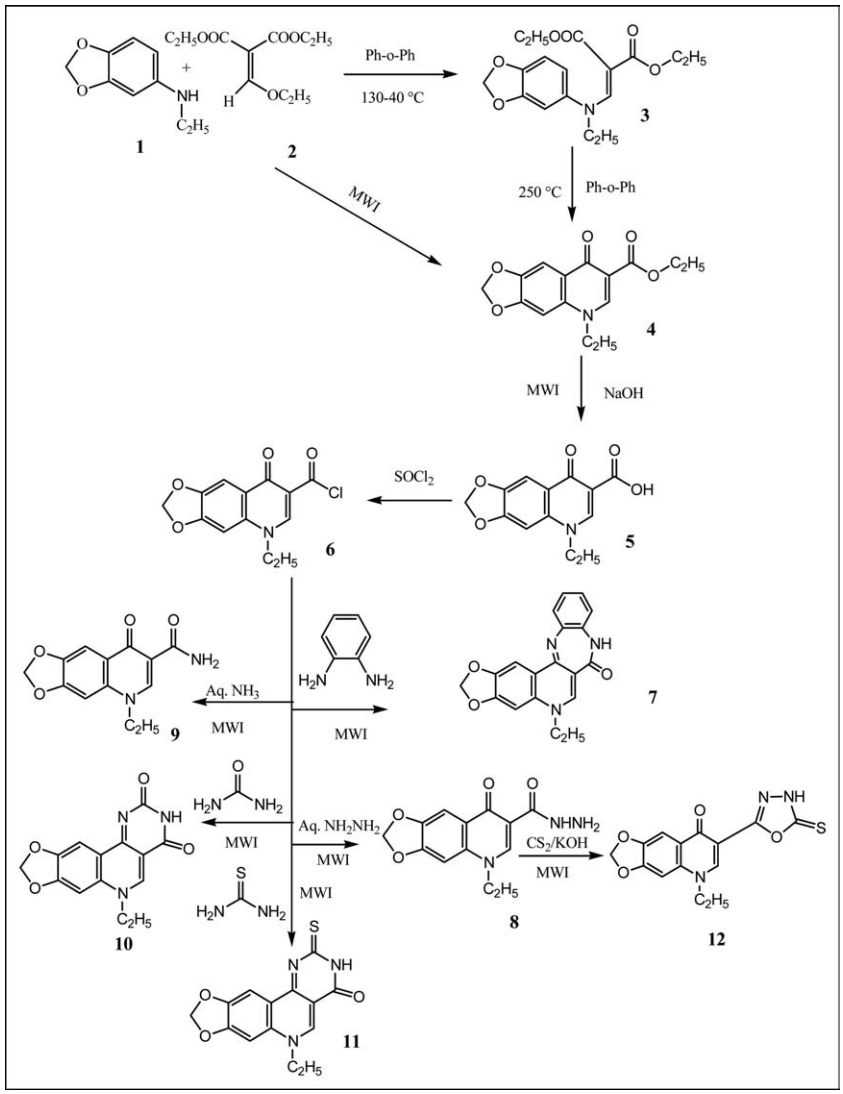
Suhas Pednekar and Anil Kumar Pandey*

Organic Chemistry Research Laboratory, Ramnarain Ruia College, Matunga, Mumbai-400019, Maharashtra, India
*E-mail: pandeyanil20@yahoo.com

Received July 5, 2009
DOI 10.1002/jhet.430
Published online 16 July 2010 in Wiley Online Library (wileyonlinelibrary.com).



The Gould-Jacob type of reaction for the synthesis of ethyl 5-ethyl-8-oxo-5,8-dihydro-[1,3]-dioxolo[4,5-g]quinoline-7-carboxylate **4** has been carried out conventionally by the condensation between *N*-ethyl-3,4-methylenedioxyaniline **1** and diethyl ethoxymethylenemalonate **2** gave the unsaturated ester **3** and thermal cyclization in refluxing diphenyl oxide gave quinolone ethyl ester **4** and the results obtained were compared with single step microwave irradiation under solvent free conditions for the synthesis of **4**. The esters on basic hydrolysis formed free acid **5,** which, upon treatment with thionyl chloride gave the acid chloride **6**. Treatment of acid chloride with *o*-phenylenediamine, hydrazine hydrate, ammonia, urea, and thiourea gave the amides (**7–11**). CS_2 treatment in presence of KOH on **8** gave **12**. We prepared **7–12** derivatives by conventional as well as microwave irradiation. These compounds have been characterized on the basis of IR, ^{1}H NMR, MS, and elemental analysis. All the compounds prepared herein were screened for their antibacterial activity. Compounds **4**, **5** possess promising antibacterial activity and compound **8** showed significant antibacterial activity.

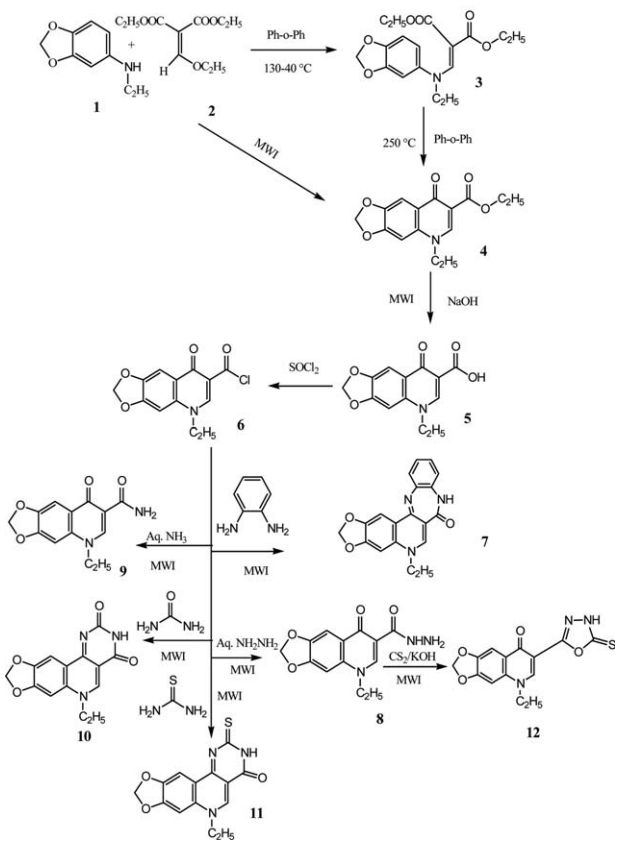
INTRODUCTION

Nalidixic acid and its quinolone analogs for example oxolinic acid, norfloxacin, pefloxacin, ciprofloxacin, and ofloxacin have been used for treatment of various bacterial infections (urology, ophthalmology) [1]. The purpose of this investigation was to provide a novel and more advantageous method that the synthesis of 5-ethyl-8-oxo-5,8-dihydro-[1,3]dioxolo[4,5-g]quinoline-7-carboxylic acid **5** and its chloride **6** and then different derivatives of amides **7–12** by conventional as well as microwaves irradiation and comparative study of both the method have been carried out. The solvent free reactions [2–6] under microwave condition are especially for providing an environmentally benign system. Thus microwave assisted synthesis becomes a part of ''Sustainable chemistry''. Microwave accelerated organic synthesis is an effective and an alternative route proposed during the last decade due to drastic reduction in the reaction time, to minimize cumbersome work-up and better yield [7–10]. We report herein the synthesis of quinolone derivatives and related compounds using conventional as well as microwave methodologies and comparative study of both the method have been carried out. These compounds were evaluated for antibacterial activity *in vitro* and *in vivo* in comparison with nalidixic acid [11–14].

RESULTS AND DISCUSSION

The aim of this work was to synthesize 5-ethyl-8-oxo-5, 8-dihydro-[1,3]dioxolo[4,5-g] quinoline -7- carboxylic acid (oxolinic acid) and its chloride **6** and different derivatives of amides. In conventional method, *N*-ethyl methylene dioxyaniline **1** was condensed with EMME **2** at 130–140°C for 1.5–2 h to obtain unsaturated ester **3**, which on thermal cyclization in boiling diphenyl oxide at 250°C for 2–3 h provided quinolone ethyl ester **4** in 70% overall yield on the basis of compound **1**. On the other hand, single step microwave assisted reaction of **1** with EMME **2** without solvent (Method B) provided identical compound **4** within 8–10 min in 88% overall yield (Scheme 1). Thus microwave assisted synthesis quinolone ethyl ester **4** has remarkable advantages over the conventional techniques because of easier work up, better yield, rapid and solvent free cleaner reaction. Compound **4** on hydrolysis gave compound **5**, which upon treatment with thionyl chloride gave acid chloride **6**. Treatment of this acid chloride **6** with *o*-phenylenediamine, hydrazine hydrate, ammonia solution, urea, and thiourea gave the amides **7–11** and Compound **8** when refluxed with ethanol and carbon disulfide in presence of potassium hydroxide yielded the corresponding 5-ethyl-7-(5-thioxo-4, 5-dihydro-[1,3,4] oxadiazol-2-yl)-5-H-[1, 3] dioxolo [4, 5-g] quinolin-8-one **12** by conventional heating as well as under microwave irradiation (Scheme 1). The comparison between conventional and microwave methodologies has been shown in Table 1. Compounds obtained from both methods were identical and were confirmed on the basis of TLC, mp, elemental, and spectral analysis.

Scheme 1



In conclusion, we have developed a simple, fast, solvent free, and high yielding method for the synthesis quinolone derivatives and related compounds, in which compounds **4** and **5** displayed very promising activity as expected (*in vitro* as well as *in vivo* activity better than the standard Nalidixic acid), whereas synthesized novel compound 8 exhibited significant antibacterial activity. Rest of the novel compounds displayed weak antibacterial activity.

EXPERIMENTAL

Melting points were determined in open capillaries on a BÜCHI melting point apparatus B-540 and are uncorrected. Infrared (ir) spectra were recorded on a PerkinElmer paragon 1000 FTIR spectrophotometer (potassium bromide pellets). The ^{1}H NMR spectra were recorded on Varians 400 MHz spectrophotometer in deuteriodimethyl sulfoxide using TMS as internal standard and the chemical shifts are expressed in ppm.

Table 1

A comparison between conventional and microwave assisted synthesis of quinolone derivatives and related compounds.

Compound no.	Conventional (Method A) Time (h)	Yield (%)	Microwave (Method B) Time [c] (min)	Yield (%)	Melting point (°C)
4	3	70	4	88	169–73
5	2	82	4	93	313–14
6	12	86	–	–	245–47
7	10	60	4	87	335–37
8	03	78	4	92	300–02
9	01	77	2	92	288–90
10	03	70	4	88	285–87
11	03	70	4	86	265–68
12	24	71	5	86	260–63

Mass spectral (MS) data were obtained using an Agilent 1100LC/MSD VL system (G1946C) with a 0.4 mL/min flow rate using a binary solvent system of 95:5 metanol/water. Microwave irradiation was carried out in modified microwave oven fitted with a condenser BPL microwave oven, Model BMO 700T, (2450 MHz, 700 W). The purity of compounds was checked by TLC using silica gel G plates.

Synthesis of 5-ethyl-8-oxo-5, 8-dihydro-[1, 3] dioxolo[4, 5-g] quinoline-7-carboxylic acid (5). ***Three step conventional method A.*** *Step 1: 2-[(Benzo[1,3]dioxolo-5-yl-ethyl-amino) methylene]-malonic acid diethyl ester (3).* A mixture of benzo[1,3]dioxol-5yl-ethyl-amine **1** (3.30 g, 0.02 mol), diethyl ethoxy methylenemalonate **2** (4.40 g, 0.02 mol), and diphenyl oxide (10 mL) was heated at 130–140°C for 1.5 h, and the alcohol generated from reaction mixture was allowed to escape. The reaction mixture was keep for further step.

Step 2: Ethyl 5-ethyl-8-oxo-5, 8-dihydro-[1,3]dioxolo [4, 5-g] quinoline-7-carboxylate (4). 2-[(Benzo[1,3]dioxolo-5-yl-ethyl-amino)-methylene]-malonic acid diethyl ester **3** (6.70 g) was dissolved in boiling diphenyl oxide (10 mL) and heated at 250°C for 1.5–2 h. The liberated alcohol was collected in a Dean-Stark trap. Then the mixture was cooled to room temperature, the resulting solid was filtered and washed with petroleum ether and dried *in vacuo*, yielding 4.1 g (70%) of (**4),** mp 169–173°C (ref. [15], mp 172–173°C).

Step 3: 5-Ethyl-8-oxo-5, 8-dihydro-[1, 3] dioxolo [4, 5-g] quinoline-7- carboxylic acid (5). This compound was obtained in 82% yield as a white solid, (ref. [16,17]).

Two step microwave assisted method B. *Step 1: Ethyl 5-ethyl-8-oxo-5, 8-dihydro-[1, 3] dioxolo [4, 5-g] quinoline-7-carboxylate (4).* A neat mixture of benzo[1,3]dioxol-5yl-ethyl-amine **1** (3.30 g, 0.02 mol) and diethyl ethoxymethylenemalonate **2** (4.40 g, 0.02 mol) was taken in an open Pyrex tube and subjected to microwave irradiation in domestic microwave oven (BPL, BMO 700T) at an output of about 700 watts for specified time given in (Table 1). Progress of reaction was monitored through TLC at an interval of 45 sec. On completion, the reaction mixture was allowed to cool at room temperature; the resulting solid was filtered and washed with petroleum ether and dried *in vacuo*, to give 5.1 g (88%) of **4**.

mp 169–173°C (ref. [15], mp 172–173°C).

Step 2: 5 - Ethyl-8-oxo-5, 8-dihydro-[1, 3] dioxolo [4, 5-g] quinoline-7- carboxylic acid (5). A neat mixture of ethyl 5-ethyl-8-oxo-5, 8-dihydro-[1, 3] dioxolo [4, 5-g] quinoline -7-carboxylate **4** (4.0 g, 0.014 mol) was dissolved in 20 mL of 10% NaOH. The reaction mixture was subjected to microwave irradiation in Pyrex glass round bottle flask attached with air condenser from outside with special arrangement for 3 min. After cooling at room temperature, the reaction mixture was acidified using concentrated HCl. The resulting precipitated was filtered and washed with water, and recrystallized from aqueous DMF to give 3.4 g (93%) of **5**.

5-Ethyl-8-oxo-5,8-dihydro-[1,3]-dioxolo[4,5-g]quinoline -7-carbonyl chloride (6). This compound was obtained in 86% yield as a light brown solid (ref. [17,18]).

5-Ethyl-5,8-dihydro-[1,3]dioxolo-5,8,13-triaza-benzo[5,6]cyclohepta[1,2-a]naphthalen-7-one(7). *Method A.* To a solution of **6** (0.28 g, 0.001 mol) in 10 mL of xylene was added *o*-phenylenediamine (0.162 g, 0.0015 mol) and the reaction mixture was refluxed for 8–10 h. The liberated water was collected in Dean-Stark trap. After the mixture was cooled to room temperature, the solid precipitated was filtered and recrystallized from ethanol to afford 0.20 g (60%) of 7 as dark yellow solid.

Method B. To a solution of compound **6** (0.28 g, 0.001 mol), *o*-phenylenediamine (0.162 g, 0.0015 mol) in 10 mL of xylene, acidic alumina (2 g) was added. The reaction mixture was stirred well, air dried, and subjected to the MWI intermittently at 30 s intervals for specified time (Table 1). On completion of reaction as monitored by TLC, the product was extracted into ethanol (3 × 5 mL). Removal of solvent under reduced pressure yielded the product, which was recrystalized from ethanol to give 0.29 g (87%) of (**7**) as dark yellow solid.

mp 335–337°C; IR (KBr): 3422, 3218, 2924, 2853, 1676, 1624, 1599, 1555, 1538, 1500, 1473, 1455, 1376, 1299, 1277, 1254, 1202, 1032 cm^{-1}; ^1H NMR (DMSO-d_6): δ 1.39 (t, 3H), 4.70 (q, 2H), 6.43 (s, 2H), 7.08–7.21 (m, 3H) 7.56 (d, d 1H), 7.85 (s, 1H), 8.75 (s, 1H), 9.10 (s, 1H), 10.50 (s, 1H); MS (ESI, *m/z*):334 $[M+H]^+$. Anal.Calcd. For $C_{19}H_{15}N_3O_3$: C, 68.46; H, 4.54; N, 12.61. Found: C, 68.76; H, 4.36; N, 12.54.

5-Ethyl-8-oxo-5,8-dihydro-[1,3]dioxolo[4,5-g] quinoline -7-carbohydrazide (8). *Method A.* To a solution of **6** (0.42 g, 0.0015 mol) dissolved in 10 mL of ethanol was added hydrazine hydrate (2.5 mL). The mixture was stirred for 1 h at room temperature, and then poured into water. The precipitate was collected by filtration and recrystallized from ethanol to give 0.32 g (78%) of **8** as yellow solid.

Method B. To a solution of compound **6** (0.42 g, 0.0015 mol) and hydrazine hydrate (2.5 mL) in ethanol (10 mL) acidic alumina (2 g) was added. The reaction mixture was stirred well, air dried, and subjected to MWI intermittently at 30 s intervals for specified time (Table 1). On completion of

reaction, as monitored by TLC the product was extracted into ethanol (3 × 5 mL). Removal of solvent under reduced pressure yielded the product, which was recrystallized from ethanol to give 0.38 g (92%) of **8** as yellow solid.

mp 300–302°C; IR (KBr): 3400, 3245, 2986, 1647, 1618, 1592, 1506, 1474, 1375, 1269, 1256, 1238, 1197, 1038 cm^{-1}; MS (ESI, *m/z*):276 $[M+H]^+$. Anal. Calcd. For $C_{13}H_{13}N_3O_4$: C, 56.72; H, 4.76; N, 15.27. Found: C, 56.87; H, 4.56; N, 15.54.

5-Ethyl-8-oxo-5,8-dihydro-[1,3]dioxolo[4,5-g] quinoline-7-carboxamide (9). *Method A*. This compound was obtained in 77% yield as a white solid (ref. [19]).

Method B. To a solution of **6** (0.28 g, 0.001 mol), ammonia solution (5 mL) in ethanol, basic alumina (2 g) was added. The reaction mixture was stirred well, air dried, and subjected to MWI intermittently at 30 s intervals for specified time (Table 1). On completion of reaction, as monitored by TLC the product was extracted into ethanol (3 × 5 mL). Removal of solvent under reduced pressure yielded the product which was recrystallized from ethanol to give 0.24 g (92%) of **9** as a white solid. mp 334–336°C; (lit. ref. [19] 336°C).

6-Ethyl-6H-8, 10-dioxa-1, 3, 6 triaza-cyclopenta [b] phenanthrene-2, 4-dione (10). *Method A*. A mixture of **6** (0.28 g, 0.001 mol), urea (0.1 g, 0.002 mol), 5% aq. KOH (2 mL) and methanol (10 mL) was refluxed for 2 h and cooled. The precipitate obtained after dilution with water was filtered, washed with water, and recrystallized from methanol to give 0.20 g (70%) of **10** as a white solid.

Method B. To a solution of compound **6** (0.28 g, 0.001 mol), urea (0.1 g, 0.002 mol) in methanol (10 mL), 5% aq KOH (2 mL), and basic alumina 2 g was added. The reaction mixture was stirred well, air dried, and subjected to MWI intermittently at 30 s intervals for specified time (Table 1). On completion of reaction, as monitored by TLC the product was extracted into ethanol (3 × 5 mL). Removal of solvent under reduced pressure yielded the product which was recrystallized from methanol to give 0.25 g (88%) of **10** as a white solid.

mp 285–287°C; IR (KBr): 3431, 2926,2853,1721, 1685, 1658, 1604, 1574, 1557, 1508, 1472, 1416, 1335, 1292, 1251, 1216, 1181, 1114, 1084,1024 cm^{-1}; ^1H NMR (DMSO-d_6): δ 1.42 (t, 3H), 4.66 (q, 2H), 6.35 (s, 2H), 7.74 (s, 1H), 8.26 (s, 1H), 9.11 (s, 1H), 15.62 (s, 1H); MS (ESI, *m/z*):286 $[M+H]^+$. Anal.Calcd. For $C_{14}H_{11}N_3O_4$: C, 58.95; H, 3.89; N, 14.73. Found: C, 58.85; H, 3.95; N, 14.85.

6-Ethyl – 2- thioxo-2, 6 dihydro-3H-8, 10-dioxa-1, 3, 6 triaza-cyclopenta [b] phenanthrene - 4-one (11). *Method A*. A mixture of **6** (0.28 g, 0.001 mol), thiourea (0.15 g, 0.002 mol), 5% aq. KOH (2 mL), and methanol (10 mL) was refluxed for 2 h and cooled. The precipitate obtained after dilution with water was filtered, washed with water, and recrystallized from methanol to give 0.21 g (70%) of **11** as a white solid.

Method B. To a solution of **6** (0.28 g, 0.001 mol), thiourea (0.15 g, 0.002 mol) in methanol (10 mL), 5% aq KOH 2 mL, and basic alumina 2 g was added. The reaction mixture was stirred well; air dried, and subjected to MWI intermittently at 30 s intervals or specified time (Table 1).On completion of reaction, as monitored by TLC, the product was extracted into ethanol (3 × 5 mL). Removal of solvent under reduced pressure yielded the product, which was recrystallized from methanol to give 0.26 g (86%) of **11** as a white solid.

mp 265–268°C; IR (KBr): 3401, 3061, 2363, 1708, 1629, 1553, 1499, 1471, 1433, 1396, 1370, 1272, 1250, 1221, 1166, 1037, 1017 cm^{-1}; ^1H NMR (DMSO-d_6): δ 1.42 (t, 3H), 4.66 (q, 2H), 6.35 (s, 2H), 7.74 (s, 1H), 8.26 (s, 1H), 9.11 (s, 1H), 15.62 (s,1H); MS (ESI, *m/z*):302$[M+H]^+$.Anal.Calcd. For $C_{14}H_{11}N_3O_3S$: C, 55.80; H, 3.68; N, 13.95. Found: C, 55.70; H, 3.88; N, 14.05.

5-Ethyl -7-(5-thioxo-4, 5-dihydro-[1, 3, 4] oxadiazol-2-yl)-5-H-[1, 3] di-oxolo [4, 5-g] quinolin-8-one (12). *Method A*. A mixture of **8** (0.30 g, 0.0011 mol), CS_2 (0.68 g, 0.009 mol), and KOH (0.22 g, 0.004 mol) in ethanol 30 mL was heated under reflux for 10 h, cooled to room temperature, and diluted with water (4 mL) was heated under reflux until the evolution of H_2S had ceased. The reaction mixture was cooled to room temperature, and poured into water and acidification with HCl. The resulting precipitated was filtered and washed with water and recrystallized from methanol to give 0.25 g (71%) of **12**.

Method B. To a solution of (**8**) (0.30 g, 0.0011 mol), CS_2 (0.68 g, 0.009 mol) and KOH (0.22 g, 0.004 mol) in ethanol 20 mL dissolved in water (4 mL) were added. The reaction mixture was subjected to microwave irradiation in a Pyrex glass round bottle flask attached with water condenser from outside with special arrangement for 4 min (Table 1). On completion of reaction, as monitored by TLC the reaction mixture was cooled to room temperature and poured into water and acidification with HCl. The resulting precipitated was filtered and washed with water and recrystallized from methanol to give 0.3 g (86%) of **12**.

mp 260–263°C; IR (KBr): 3442, 3171, 3050, 2901, 1650,1621, 1595, 1519, 1483, 1392, 1269, 1255, 1230, 1197,1039 cm^{-1}; ^1H NMR (DMSO-d_6): δ 1.35 (t, 3H), 4.40 (q, 2H), 6.19 (s, 2H), 7.48 (s, 1H), 7.52 (s, 1H) 8.58 (s, 1H), 11.26 (s, 1H); MS (ESI, *m/z*):318 $[M+H]^+$. Anal.Calcd. for $C_{14}H_{11}N_3O_4S$: C, 52.99; H, 3.49; N, 13.24. Found: C, 52.88; H, 3.36; N, 13.54.

Caution: Although we did not have any accident in this work, it is highly recommended that the reaction should be performed in an efficient hood.

***In vitro* antibacterial activity**. Minimal inhibitory concentrations (MICs) were determined by means of an agar dilution method, using Mueller Hinton agar plates containing a series of twofold dilutions of drug. Overnight cultures in Mueller Hinton broth were used for precultures of tested strains. MICs were determined after overnight incubation at 37°C, with an inoculum equivalent to 1:100 dilution of an 18 h culture in Mueller Hinton broth (about10^8 cells/mL). Each inoculum was seeded onto agar plates by using an inoculum-replicating apparatus and transferred by a 0.005 mL (about 10^4 cells) calibrated loop. The MIC was the lowest drug concentration that inhibited the development of visible growth on agar plate.

The *in vivo* antibacterial activities of drugs were determined in the systemic infections of mice with bacteria. Ten female Swiss albino mice weighing 18 to 22 g were used for each dose level. Microorganisms grown on Mueller Hinton agar plates were suspended in physiological saline solution. Mice were intraperitoneally challenged with bacteria. Mice infected with *Escherichia coli* ML 4707 were treated at 1 h after infection. Mice infected with *Klebsiella pneumoniae* ML 4730 and *Proteus morganii* ML 4731 were treated, respectively, at 1, 6, and 24 h and then immediately and at 3 h after infection. Drugs to be tested were administered PO. The total number of surviving mice was recorded 1 week after infection, and the

Table 2

Biological activity.

Compound no.	MIC (μg/mL)	ED_{50}(PO) (mg/kg bw)
4	16	30
5	0.5	4.7
7	256	>50
8	<4	15
9	>512	>50
10	256	>50
11	256	>50
12	256	>50
Nalidixic acid	4.0	12.5

amount of a single dose (milligrams per kilogram body weight) that gave protection to 50% of the infected mice was estimated as ED_{50} (Table 2).

REFERENCES AND NOTES

[1] (a) Lesher, G. Y.; Froelich, J. E.; Gruett, M. D.; Bailey, J. H.; Brundage, R. P. J Med Pharma Chem 1962, 5, 1063; (b) Albrecht, R. Prog Drug Res 1977, 21, 9; (c) Radl, S. Cesk Farm 1987, 36, 180.

[2] Loppy, A.; Petit, A.; Hamelin, J.; Texier-Boulet, F.; Jacquault, P.; Mathe, D. Synthesis 1998, 1213.

[3] Varma, R. S Pure Appl Chem 2001, 73, 193.

[4] Harmonised Integrated Classification System for Human Health and Environmental Hazards of Chemical Substances and Mixtures; OECD Series on Testing and Assessment Number 33; ENV/JM/MONO(2001)6; OECD: Paris, 2001.

[5] Oussaid, I.; Thach, N.; Loupy, A. Tetrahedron Lett 1997, 38, 2451.

[6] Tanaka, K.; Toda, F. Chem Rev 2000, 100, 1025.

[7] Bose, A. K.; Manhas, M. S.; Ghosh, M.; Raju, V. S.; Tabei, K.; Urbanczyk-Lipokowska, Z. Heterocycles 1990, 30, 741.

[8] Caddick, S. Tetrahedron 1995, 51, 10403.

[9] Lidstrom, P.; Tierney, J.; Wathey, B.; Westman, J. Tetrahedron 2001, 57, 9225.

[10] Kappe, C. O. Angew Chem Int Ed Engl 2004, 43, 6250.

[11] CLSI. CLSI document M2-A7, Wayne, PA, 2001.

[12] Turner, F. J.; Ringel, S. M.; Martin, J. F.; Storino, P. J.; Daly, J. M.; Schwartz, B. S. Antimicrob Agents Chemother (Bethesda) 1967, 7, 475.

[13] Nagate, T.; Kurashige, S.; Mitsuhashi, S. Antimicrob Agents Chemother 1980, 17, 203.

[14] Litchfield, J. T.; Wilcoxon, F. J Pharmacol 1948, 92, 99.

[15] Agui, H.; Mitani, T.; Nakashita, M.; Nakagome, T. J Hetrocycl Chem 1971, 8, 357.

[16] Kaminsky, D.; Meltzer, R. I. U.S Pat. 3,172,811 (1965).

[17] Kaminsky, D.; Meltzer, R. I. J Med Chem 1968, 11, 160.

[18] Loubinoux, B.; Colin, J. L.; Thomas, V. Eur J Med Chem 1991, 26(4), 461.

[19] Agui, H.; Nakagome, T. J Hetrocyclic Chem 1976, 13, 765.