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Original research paper

Metalloantibiotics: Synthesis, characterization and antimicrobial evaluation of bismuth-fluoroquinolone complexes against *Helicobacter pylori*

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Novel organometallic compounds have been prepared by complexing the fluoroquinolones, norfloxacin, ofloxacin, ciprofloxacin, sparfloxacin, lomefloxacin, pefloxacin and gatifloxacin, with bismuth. The complexes were characterized by UV, IR, atomic absorption spectroscopy, elemental analysis, differential scanning calorimetry, thermogravimetric analysis and mass spectrometry. Their antibacterial potential against *Helicobacter pylori* and other microorganisms was investigated. These compounds were found to possess strong activity against *Helicobacter pylori* with a minimum inhibitory concentration of 0.5 mg L^{-1}. They also exhibited moderate activity against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus pumilus* and *Staphylococcus epidermidis*. These bismuth-fluoroquinolone complexes have the potential to be developed as drugs against *H. pylori* related ailments.

Keywords: fluoroquinolone, bismuth, complex, antimicrobial activity, *Helicobacter pylori*

Helicobacter pylori, a Gram-negative microaerophilic bacterium, a prevalent human pathogen in the gastric mucosa, is responsible for gastric and duodenal ulcers (1). In *H. pylori* infections the therapeutic aim is complete eradication of *H. pylori* in the stomach. The current therapy of choice is a triple combination that consists of an acid inhibitor, for example a proton pump inhibitor such as omeprazole, and two antibiotics, such as clarithromycin and amoxicillin. This triple therapy is, however, associated with disadvantages (2). As a result of differing diffusion properties, the three different substances, which should act together, do not reach uniformly the inflammatory foci caused by *H. pylori*. Thus, in order to achieve good healing results, very high doses, which are accompanied by serious side effects, are necessary. It is obvious that a triple therapy has also other great disadvantages in comparison with administration of only one medicament or even of two medicaments (3).

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Metal ions play a key role in the actions of metalloantibiotics and are involved in specific interactions of these antibiotics with proteins, membranes, nucleic acids, and other biomolecules (4). The ligand environment of transition metals (present in very low concentration *in vivo*) can be considerably altered upon administration of a therapeutically effective dose of an antibacterial drug. This change in balance between the metal ion and the ligand may have a profound effect upon the activity of the drug against potentially susceptible bacteria. It has also been reported that transport of organic ligands into cells can be facilitated by the formation of metal complexes (5).

Fluoroquinolones (FLQs) are broad spectrum chemotherapeutic agents widely used for the treatment of numerous diseases (6). They are active against a wide variety of aerobic Gram-negative and Gram-positive bacteria in general, and active specifically against aminoglycoside-resistant *Pseudomonas aeroginosa* and beta-lactamase producing organisms. The mechanism of action of FLQs involves inhibition of bacterial DNA gyrase essential for DNA replication (7). It has been proposed that metal complex intermediates are involved in this process (8). Foroumadi *et al*. (9) reported structure-activity relationship studies on fluoroquinolones as anti-*H. pylori* agents. Transport of quinolones across the bacterial cytoplasmic membrane is strongly pH dependent, peaking at neutral pH. It has been proposed that uncharged quinolone species are responsible for diffusion through cytoplasmic membranes (10) and the presence of metal ions results in higher uptake of quinolones by bacterial cells when compared to the drug alone (11). Therefore, the formation of metal complexes is likely to increase the bioavailability of metal ions or ligands or both.

Use of bismuth salts for the treatment of gastric ulcer has been widely reported in literature (12). According to Vertesy *et al*. (13), bismuth compounds are useful in peptic ulceration in three ways; bismuth protects the stomach wall by making a physical barrier against stomach contents in the ulcer, it has positive biochemical effects on the stomach wall and it has anti-*H. pylori* activity. Antimicrobial activity of bismuth could also be due to interference of iron metabolism, *i.e.*, inhibition of iron uptake, since iron is essential for virulent growth of *H. pylori*. Binding of bismuth to the ferric ion-binding proteins has also been reported by Guo *et al*. (14). It is believed that bismuth in complex form is more stable and better tolerated than bismuth in ionic form.

Interaction of a fluoroquinolone drug with bismuth should result in an uncharged complex. As per these observations (10, 11), an uncharged complex would be better absorbed through various biological membranes and would show higher bioavailability than the charged one. This would lead to enhanced levels of both the fluoroquinolone antibacterials and bismuth ions at the site of action. Earlier, we have reported a preliminary work on bismuth-norfloxacin complex and its antibacterial activity against some Gram-positive and Gram-negative bacteria (15). Here, we present a detailed study on the synthesis, characterization and antibacterial profile of complexes of bismuth with commonly used fluoroquinolone drugs (BFCs).

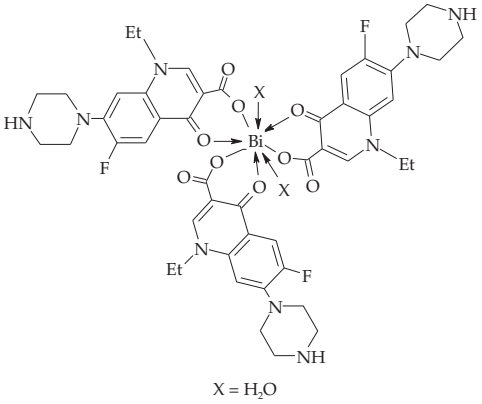

Fig. 1. Representative structure of one of the fluoroquinoline-bismuth complexes (norfloxacin-bismuth complex, **1**).

EXPERIMENTAL

Materials

FLQs were procured from Alembic Laboratories and Mercury Laboratories, India, as gift samples. Bismuth oxynitrate (BON), dimethyl sulphoxide (DMSO) and ammonia solution were obtained from S.D. Fine chemicals, India. Müller-Hinton agar, type 1, and nutrient broth were obtained from HiMedia Ltd., India. *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 29213), *Bacillus pumilus* (NCTC 8241), *Staphylococcus epidermidis* (ATCC 12228) obtained from Food and Drug Laboratory, India, were used for antimicrobial screening. *H. pylori* cultivation and screening of the complexes was performed on 16 different strains of *H. pylori* (including fluoroquinolone resistant strains) obtained from Hôpital Pellegrin, Place Amélie Raba-Léon, France.

Preparation of BFCs

In a round bottom flask (100 mL), the respective fluoroquinolone drug (FLQ) (3.483 mmol) was dissolved in distilled water (15–20 mL) with addition of conc. hydrochloric acid (2–3 mL). In a separate conical flask (50 mL), bismuth oxynitrate (0.696 mmol) was dissolved in distilled water (2–3 mL) with addition of conc. hydrochloric acid (2–3 mL). BON solution was then added into the above FLQ solution. The resulting mixture was filtered to remove traces of the insoluble residues formed. The reaction mixture was then basified by adding ammonia solution (10 %, *V*/*V*) and was kept at 80–100 °C for about 5–6 h. The reaction mixture was maintained basic by adding ammonia solution from time to time. The product obtained as a precipitate was collected by filtration and washed with a mixture of ethanol/water (50:50) till the filtrate did not show the spot of the respective FLQ in TLC. The product thus obtained was dried under vacuum.

Characterization

Molar absorptivity determination. – Accurately weighed quantity of the compound was dissolved in hydrochloric acid (0.1 mol L^{-1}) and the concentration was adjusted to get

absorbance between 0.2 and 0.8 at its λ_{max}. The absorbance was recorded on a UV spectrophotometer (UV-1700, Shimadzu, Japan). Molar absorptivity for the synthesized compounds was compared with the molar absorptivity of the ligands (respective fluoroquinolones) to get the metal/ligand mole ratio.

Differential scanning calorimetry (DSC). – DSC scans of the synthesized complexes were obtained on a differential scanning calorimeter (Model DSC-60, Shimadzu). The instrument was calibrated using indium as standard. The sample (about 5 mg) was weighed accurately in an aluminium pan and sealed hermetically using a crimper. Thermographs were obtained by heating the encapsulated samples at a constant heating rate of 10 °C min^{-1} under a nitrogen atmosphere. The temperature range for the scan was 30 to 360 °C for all samples. Melting points were defined as being the point of intersection between the base line and the linear section of the ascending endothermic curve (»onset«) (Fig. 2). The conditions of obtaining thermographs for all samples were essentially the same, as described here.

Thermogravimetric analysis (TGA). – Thermal analyses using TGA were conducted on a thermobalance (TGA-7, Perkin-Elmer, Germany). An accurately weighed quantity of the compound was placed into an aluminium cup suspended from an analytical balance

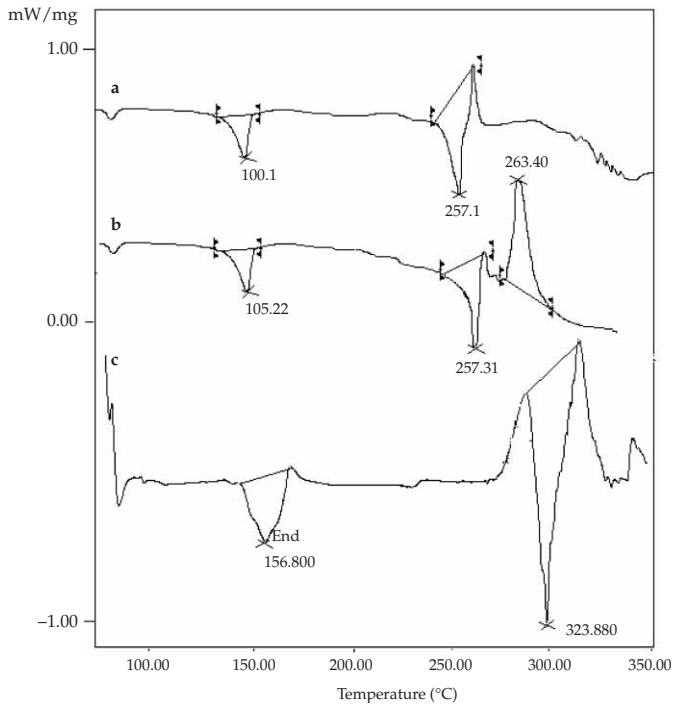

Fig. 2. Representative overlaid DSC thermographs for: a) ciprofloxacin, b) ciprofloxacin and BON in physical admixture, c) ciprofloxacin-bismuth complex (**3**).

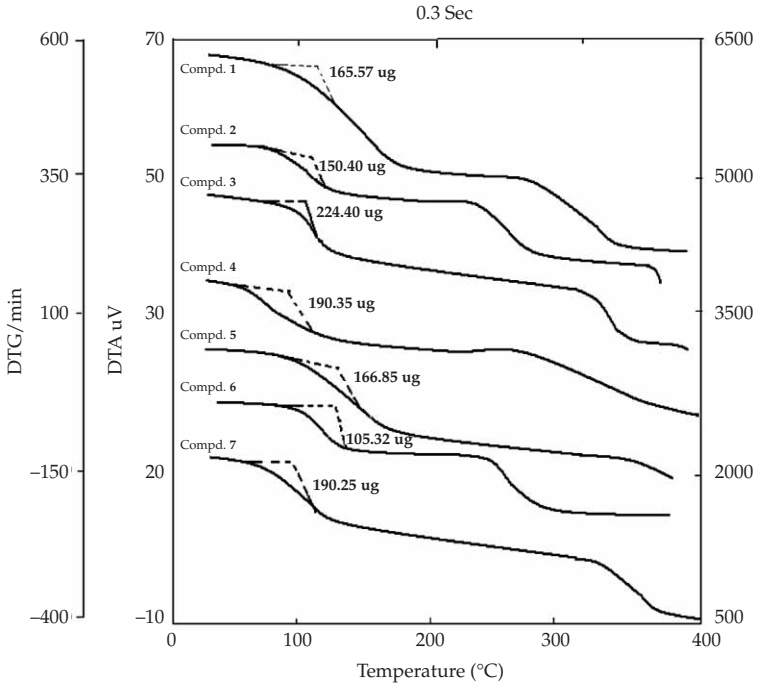

Fig. 3. Overlaid TGA thermographs of: norfloxacin-bismuth complex (**1**), ofloxacin-bismuth complex (**2**), ciprofloxacin-bismuth complex (**3**), sparfloxacin-bismuth complex (**4**), lomefloxacin-bismuth complex (**5**), pefloxacin-bismuth complex and (**6**) gatifloxacin-bismuth complex (**7**).

located outside the furnace chamber. The sample cup was then heated at a heating rate of 10 °C min^{-1}. The temperature range for the scan was 30 to 400 °C (Fig. 3).

Karl-Fischer (KF) aquametry. – A Karl-Fischer titrimeter (Aqua Cal, India) was used for water content determination. Karl-Fischer reagent was calibrated with disodium tartarate dihydrate as standard. An accurately weighed amount of the compound was added to dry methanol in a KF reaction vessel and titrated against Karl-Fischer reagent. Titration was performed in triplicate to get reproducible results.

Atomic absorption spectrophotometry (AAS). – Standard solution of bismuth oxynitrate (equivalent to bismuth 1 mg mL^{-1}) was diluted with hydrochloric acid (0.1 mol L^{-1}) to get concentrations in the range from 9 to 36 μg L^{-1}. The absorbance was recorded on an atomic absorption spectrophotometer (Thermo Finnigan, Italy) using a hollow cathode lamp operating at the mentioned wavelength (223.1 nm) against hydrochloric acid solution (0.1 mol L^{-1}) as blank. Lamp current was adjusted to 10 mA. The absorbance thus measured was plotted against the concentration. The closest sets of readings were regressed to afford a regression equation, which was used to estimate the bismuth content in the synthesized compound. The pressure of air was kept at 103 kPa and acetylene 35 kPa. Readings were recorded in triplicate for all the samples (Table I).

Table I. Physico-chemical data of synthesized compounds

Compd. No.	Name of the complex (BFC)	Molecular formula	M.p. (°C)	Elemental analysis (%)	
				Calculated	Found
1	Norfloxacin-bismuth complex	$Bi(C_{16}H_{17}FN_3O_3)_3 \times 2H_2O$	282–285	C: 48.04 H: 4.62 N: 10.51 Bi: 17.41	C: 48.02 H: 4.07 N: 10.59 Bi: 17.86
2	Ofloxacin-bismuth complex	$Bi(C_{18}H_{19}FN_3O_4)_3 \times 2H_2O$	308–310	C: 48.91 H: 4.64 N: 9.51 Bi: 15.75	C: 48.93 H: 5.01 N: 9.23 Bi: 15.62
3	Ciprofloxacin-bismuth complex	$Bi(C_{17}H_{17}FN_3O_3)_3 \times 2H_2O$	322–324	C: 49.56 H: 4.49 N: 10.20 Bi: 16.90	C: 49.98 H: 4.99 N: 10.02 Bi: 16.52
4	Sparfloxacin-bismuth complex	$Bi(C_{19}H_{21}F_2N_4O_3)_3 \times 2H_2O$	340–343	C: 48.28 H: 4.76 N: 11.84 Bi: 14.72	C: 48.01 H: 4.31 N: 11.65 Bi: 14.27
5	Lomefloxacin-bismuth complex	$Bi(C_{17}H_{18}F_2N_3O_3)_3 \times 2H_2O$	312–315	C: 47.26 H: 4.51 N: 9.73 Bi: 16.12	C: 47.55 H: 4.49 N: 9.65 Bi: 16.89
6	Pefloxacin-bismuth complex	$Bi(C_{17}H_{19}FN_3O_3)_3 \times 2H_2O$	285–288	C: 49.32 H: 4.95 N: 10.15 Bi: 16.82	C: 49.46 H: 4.99 N: 10.53 Bi: 16.56
7	Gatifloxacin-bismuth complex	$Bi(C_{19}H_{21}FN_3O_4)_3 \times 2H_2O$	283–285	C: 50.04 H: 4.94 N: 9.21 Bi: 15.27	C: 49.94 H: 5.01 N: 9.23 Bi: 15.68

Elemental analysis. – Elemental composition (CHN) was determined using Thermo Finnigan apparatus. Calculated and found values for the elements of synthesized complexes are shown in Table I.

Fourier transform infrared spectroscopy (FT-IR). – FT-IR spectra (Fig. 4) of the complexes were obtained on an FT-IR spectrophotometer (FT-IR-8300, Shimadzu) using KBr pellets. Pellets were prepared by grinding the complexes with KBr in a ratio of 2 mg of crystals to 100 mg of KBr and then applying a pressure of 1 MPa in a die-punch.

Statistical analysis (ANOVA). – Microbiological testing results were analyzed statistically by Tukey's multiple comparison test.

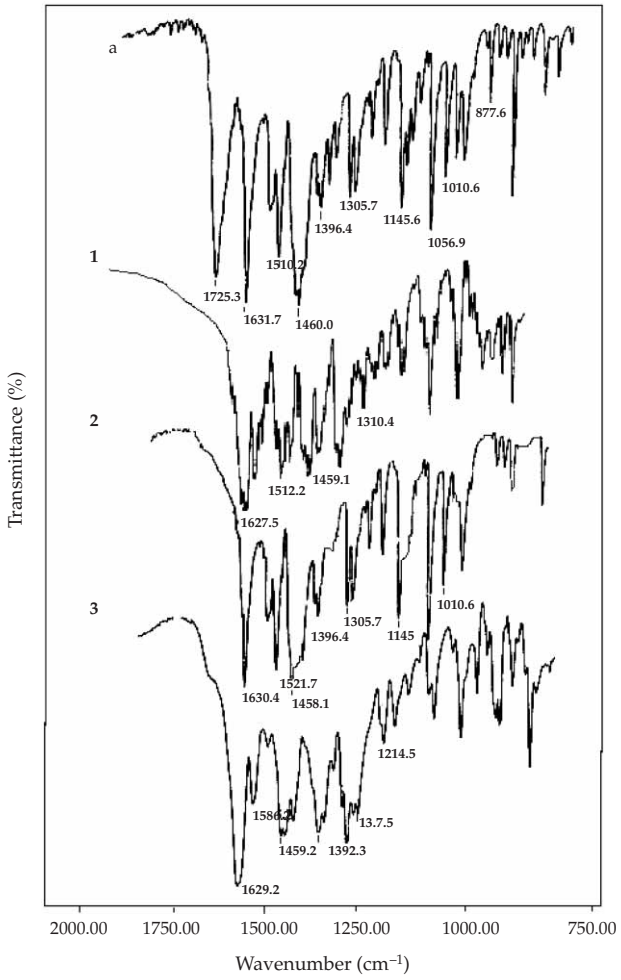

Fig. 4. Representative overlaid IR spectra of: ciprofloxacin (**a**), norfloxacin-bismuth complex (**1**), ofloxacin-bismuth complex (**2**) and ciprofloxacin-bismuth complex (**3**).

Testing against H. pylori *and other microorganisms*

Minimum inhibitory concentration (*MIC*) of FLQs as well as of the synthesized compounds (bismuth fluoroquinolone complexes) (Tables II and III) was determined by the agar diffusion method using Müller-Hinton agar as described of in the guidelines the National Committee for Clinical Laboratory Standards (16), in triplicate. Samples were initially dissolved in DMSO at concentrations between 0.0125 and 128 mg L^{-1}. Isolates were grown for 24 h in nutrient broth to provide turbidity of approximately 10^9 cfu mL^{-1}. Bacterial suspensions were diluted with soft agar-containing tubes at 45–50 °C. These soft agar tubes were then poured over the Müller-Hinton agar type 1 plates previously pre-

Table II. MIC *values and inhibition zone diameters of synthesized complexes*

Compd.	Microorganism							
	E. coli		*S. aureus*		*S. epidermidis*		*B. pumilus*	
	MIC (mg L^{-1})[b]	Zone (mm)	*MIC* (mg L^{-1})[b]	Zone (mm)	*MIC* (mg L^{-1})[b]	Zone (mm)	*MIC* (mg L^{-1})[b]	Zone (mm)
1	0.35	15	0.45	14	0.25	9	0.05	13
Standard[a]	0.35	13	0.50	14	0.30	13	0.25	15
2	0.25	8	0.125	13	0.25	12	0.045	11
Standard[a]	0.30	10	0.25	10	0.35	12	0.05	12
3	0.05	15	0.30	14	0.25	15	0.125	12
Standard[a]	0.125	13	0.35	12	0.35	12	0.20	10
4	0.45	12	0.125	10	0.125	10	0.35	10
Standard[a]	0.50	14	0.25	12	0.20	10	0.40	12
5	0.20	14	0.35	14	0.30	16	0.35	18
Standard[a]	0.25	15	0.40	16	0.40	16	0.45	20
6	0.25	14	0.20	16	0.125	16	0.34	18
Standard[a]	0.35	14	0.35	12	0.25	14	0.45	16
7	0.25	12	0.20	16	0.25	12	0.35	8
Standard[a]	0.35	14	0.25	16	0.35	12	0.50	10
p-value[c]	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05

[a] The standard is the respective fluoroquinolone drug.
[b] Mean of 3 observations. Standard error not bigger than 10 % of the mean value.
[c] *p*-value obtained by Tukey's multiple comparison test. *MIC* values of synthetised compounds compared with *MIC* values of respective FLQs.

pared and allowed to solidify under laminar flow for 15 min. Bores were drawn with the help of a sterile borer (6 mm diameter). Sterile pipettes (0.2 mL) were used in aseptic conditions to add compounds into the bores. Sample solution (0.15 mL) was added to each bore. The same volume of the control (DMSO) was also added into one bore in each plate. Within 15 min after the addition of compounds into the bores, the plates were placed in an incubator at 37 °C. After 24 h of incubation, the plates were examined and the diameter of zones of complete inhibition were measured with a zone diameter measuring scale (HiMedia, India) (Table II).

Isolates of *H. pylori* were directly obtained from patients suffering from dyspepsia and were identified by techniques such as the Gram-negative staining and urease positive test. Cultivation and susceptibility testing of *H. pylori* was performed using Müller-Hinton type 2 agar (BioMerieux, France) with 10 % sheep blood. Bacterial inoculum was prepared from a suspension of approximately 10^9 cfu mL^{-1} in brucella broth from a 48 h agar plate.

RESULTS AND DISCUSSION

The synthesized complexes (**1–7**, Table I) were characterized on the basis of analytical data obtained for individual compounds. The complexes are amorphous, white to buff-colored, insoluble in water and sparingly soluble in common organic solvents such as methanol, ethanol, acetone, acetonitrile, ether, chloroform, pyridine and benzene. The complexes were found to be stable in distilled water at neutral pH. DSC thermographs for individual fluoroquinolone drugs, complexes (**1–7**) and physical admixtures of fluoroquionolone drugs with bismuth oxynitrate were obtained. Representative overlaid thermographs for ciprofloxacin and its complex (**3**) are shown in Fig. 2. Endothermic peaks for individual fluoroquinolone drugs in the pure form and in a physical admixture with bismuth oxynitrate corresponded to the reported melting points of fluoroquinolone drugs. Bismuth oxynitrate also offered consistently an exothermic peak at its reported melting point. But, complexes **1–7** offered endothermic peaks at much higher temperatures than the peaks of individual fluoroquinolones and bismuth oxynitrate [*e.g.*, 323.88 °C for bismuth-ciprofloxacin complex (**3**) compared to 257.1 °C for ciprofloxacin and 263.40 °C for bismuth oxynitrate (Fig. 2)]. This clearly indicated that some chemical interaction had taken place between the fluroquinolone drug and bismuth ion. Endothermic peaks between 100 and 160 °C were also observed for loss of water in the DSC thermographs of all complexes.

IR spectra of FLQs showed two characteristic peaks at about 1725 cm^{-1} (C=O of –COOH) and 1630 cm^{-1} (4-oxo). BFCs showed the absence of a peak at 1725 cm^{-1} and emergence of additional peaks at about 1520 and 1455 cm^{-1} due to symmetrical and asymmetrical carboxylate stretching vibrations, while the other peak at about 1630 cm^{-1} was retained in all complexes. The spectra obtained are very similar to the spectrum reported for the copper-ciprofloxacin complex (17) wherein copper is chelated to the fluoroquinolone moiety via a Cu-O bond through the carboxylate anion and O → Cu bond through the 4-oxo group. It was speculated that the same type of chelation had taken place between the fluoroquinolone drug and bismuth.

Quantitative UV spectrometry indicated the existence of a 1:3 molar ratio of metal to FLQs. Bismuth can show a co-ordination number up to ten in various complexes. Three fluoroquinolone molecules would afford a co-ordination number of six, filling the primary bismuth ion valency of three with three carboxylate anions, thereby turning the complexes to neutral molecules. DSC thermographs also showed the presence of water molecules in the complexes, so the samples were submitted to thermogravimetric analysis. TGA data offered water contents ranging from 2.63 to 3.19 % for the BFCs representing two water molecules with each complex molecule. Final confirmation of water contents in the complexes as dihydrates was obtained by KF aquametry. Quantification of bismuth contents by atomic absorption spectrometry and elemental analyses (C, H, N) of the complexes confirmed the structures of the complexes wherein three fluoroquinolones were bonded to one bismuth ion. A representative structure for bismuth-norfloxacin complex (**1**) is shown in Fig. 1. Molecular mass of this complex was further confirmed by obtaining its mass spectrum [in the electrospray ionization mode where molecules are not lost in co-ordinated water; *m/z* 1200.05 (m^+)].

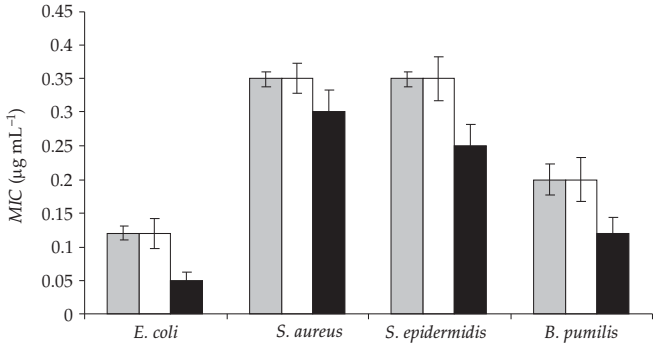

Fig. 5. Representative bar graphs comparing the *MIC* values of ciprofloxacin (gray), physical admixture of ciprofloxacin and bismuth oxynitrate (colouress), and ciprofloxacin-bismuth complex (**3**) (black) (mean ± SD, n = 3).

Antimicrobial evaluation of BFCs

Antibacterial activity evaluation was carried out for the synthesized complexes as well as for the parent drugs. Table II and Fig. 5 show the minimum inhibitory concentrations for bismuth-fluoroquinolone complexes. FLQs alone were used as standards for antimicrobial evaluation of BFCs against various Gram positive and Gram negative mi-

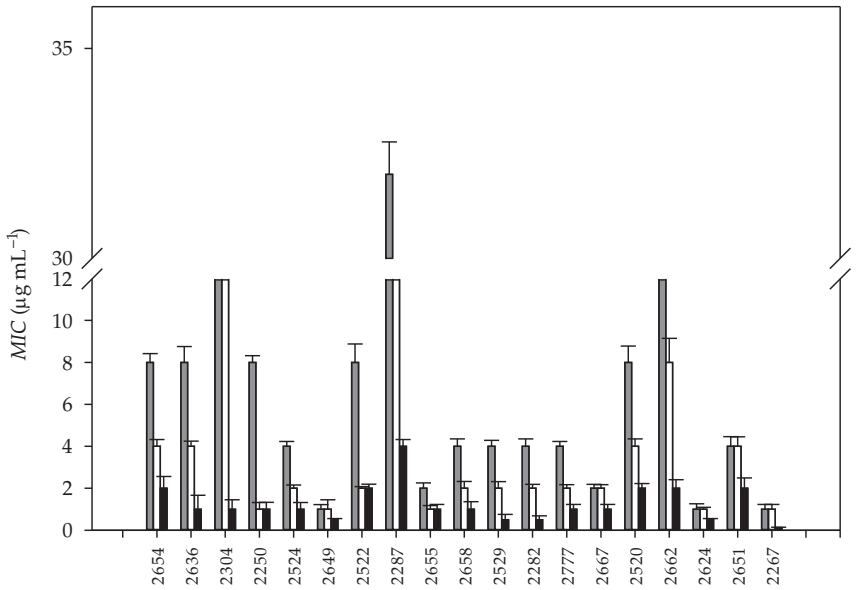

Fig. 6. Representative bar graphs comparing the *MIC* values of ciprofloxacin (gray), physical admixture of ciprofloxacin and bismuth oxynitrate (colourless), and ciprofloxacin-bismuth complex (**3**) (black) against *H. pylori* strains (mean ± SD, n = 3).

Table III. MICs *for the synthesized compounds against 16 different strains of* H. pylori

Compd.	*MIC* (mg L^{-1}) for strain No. of *H. pylori*																		
	2654	2636	**2304**[a]	2250	2524	2649	2522	**2287**[a]	2655	2658	2529	2282	2777	2667	2520	**2662**[a]	2624	2651	2267
1	1.0	1.0	**1.0**	1.0	1.0	0.5	2.0	**1.0**	1.0	1.0	0.5	2.0	0.5	0.5	2.0	**1.0**	0.5	0.5	0.12
Standard[b]	4.0	8.0	16.0	4.0	2.0	1.0	8.0	16.0	2.0	4.0	4.0	4.0	2.0	2.0	8.0	16.0	1.0	0.5	1.0
2	2.0	1.0	**1.0**	1.0	1.0	0.5	2.0	**4.0**	1.0	1.0	0.5	0.5	1.0	1.0	2.0	**2.0**	0.5	2.0	0.12
Standard[b]	8.0	8.0	16.0	8.0	4.0	1.0	8.0	32.0	2.0	4.0	4.0	4.0	4.0	2.0	8.0	16.0	1.0	4.0	1.0
3	2.0	1.0	**2.0**	1.0	1.0	0.5	2.0	**2.0**	1.0	1.0	0.5	0.5	1.0	1.0	2.0	**1.0**	0.5	0.5	0.12
Standard[b]	4.0	4.0	16.0	2.0	4.0	1.0	8.0	16.0	2.0	4.0	4.0	40.	2.0	2.0	8.0	16.0	1.0	0.5	1.0
4	0.5	0.5	**1.0**	1.0	1.0	1.0	1.0	**2.0**	0.5	0.5	1.0	1.0	1.0	1.0	0.5	**2.0**	0.5	0.5	0.5
Standard[b]	2.0	2.0	16.0	4.0	2.0	2.0	4.0	16.0	2.0	2.0	4.0	2.0	8.0	2.0	2.0	16.0	2.0	2.0	8.0
5	0.5	0.5	**2.0**	0.2	1.0	1.0	1.0	**1.0**	0.2	0.5	0.5	1.0	1.0	0.5	0.5	**2.0**	0.2	0.5	0.5
Standard[b]	1.0	1.0	8.0	1.0	2.0	1.0	2.0	4.0	0.5	1.0	1.0	2.0	2.0	1.0	1.0	4.0	0.5	1.0	1.0
6	1.0	1.0	**2.0**	0.5	2.0	0.5	2.0	**1.0**	0.2	1.0	1.0	2.0	1.0	1.0	2.0	**1.0**	0.5	0.5	1.0
Standard[b]	2.0	4.0	16.0	1.0	8.0	4.0	8.0	16.0	2.0	2.0	4.0	8.0	4.0	2.0	8.0	16.0	1.0	2.0	2.0
7	2.0	0.5	**2.0**	1.0	1.0	0.5	1.0	**1.0**	1.0	1.0	1.0	0.5	1.0	1.0	2.0	**1.0**	0.5	0.5	0.12
Standard[b]	8.0	4.0	16.0	2.0	4.0	1.0	8.0	16.0	4.0	4.0	2.0	4.0	2.0	2.0	8.0	32.0	1.0	0.5	2.0

[a] Fluoroquinolone resistant strains.
[b] The standard is the respective fluoroquinolone drug.

croorganisms. In order to see the effect of bismuth ion on antimicrobial action of FLQs, physical admixtures of FLQs along with BON were also evaluated for antimicrobial properties. It was observed that BON did not affect the antimicrobial potency of FLQs significantly, while BFCs showed a signifficant increase in antimicrobial potency over FLQs against the four used bacterial strains. The *MIC*s for FLQs as well as for the admixtures (BON plus FLQs) were found to be nearly the same, while the *MIC*s for BFCs were significantly lower ($p < 0.05$, Turkey's test) than the *MIC*s of FLQs. This clearly indicated that the complexation of FLQs with bismuth resulted in a significant increase in the potency of the synthesized complexes (**1–7**). Bismuth-ciprofloxacin complex (**3**) was found to be most potent against *E. coli* with *MIC* of 0.05 mg L^{-1}, bismuth-ofloxacin complex (**2**) against *S. aureus* with *MIC* of 0.125 mg L^{-1}, bismuth-sparfloxacin complex (**I**) against *S. epidermidis* with *MIC* of 0.125 mg L^{-1} and bismuth-ofloxacin complex (**2**) against *B. pumilus* with *MIC* of 0.045 mg L^{-1}.

All the synthesized bismuth-fluoroquinolone complexes were also evaluated for their anti-*H. pylori* activity. Anti-*H. pylori* activity for the bismuth-fluoroquinolone complexes is represented in Table III (Fig. 6). All the synthesized compounds were found to be more potent against all the used strains of *H. pylori* than the parent FLQs. Interestingly, these complexes (**1-7**) also showed high potency against some fluoroquinolone-resistant strains of *H. pylori* (strain No. 2287, 2304 and 2662) with *MIC* of 1 to 4 mg L^{-1}) (Table III). The results were found to be significantly different ($p < 0.005$) and the standard error was found to be in the range of 0.0122–0.875 for *MIC*s of the synthesized compounds.

CONCLUSIONS

Seven bismuth-fluoroquinolone complexes were successfully synthesized, characterized on the basis of instrumental, spectral and elemental analyses and evaluated microbiologically against some Gram negative and Gram positive bacteria, and *H. pylori*. The synthesized compounds showed higher potency (lower *MIC*) compared to the ligands (FLQs). *MIC* for FLQs and their physical admixtures with bismuth oxynitrate were the same or nearly the same but significantly different and higher than that for BFCs. This clearly indicates that FLQs in complexed form with bismuth are more potent. The synthesized compounds were also found to be active against some of the fluoroquinolone--resistant strains (strain No. 2304, 2287 and 2662) of *H. pylori*.

Based upon the above discussion, it can be concluded that bismuth-FLQ complexes could have applications in treatments of ulcers caused by *H. pylori*, drug resistant strains of *H. pylori* and conjunctivitis caused by bacterial eye infections.

REFERENCES

1. R. Nagarajan, S. N. Mehta, S. R. Naik and W. Fabry, Preparation and activity of some novel organo-metallic complexes against *Helicobacter pylori*, *Arzneim. Forsch/Drug Res.* **49** (1999) 951–954.
2. I. Muhammad, I. Javed, I. Shahid and I. Nazia, *In vitro* antibacterial studies of ciprofloxacin-imines and their complexes with Cu (II), Ni (II), Co (II) and Zn (II), *Turk. J. Biol*. **31** (2007) 67–72.
3. S. Nakajima, D. Y. Graham, T. Hattori and T. Bamba, Strategy for treatment of *Helicobacter pylori* infection in adults II. Practical Policy in 2000, *Curr. Pharm. Design* **15** (2000) 1515–1529; DOI: 10.2174/1381612003399013.
4. L. J. Ming, Structure and function of »metalloantibiotics«, *Med. Res. Rev.* **23** (2003) 697–762; DOI: 10.1002/med.10052.
5. Z. H. Chohan, C. T. Supuran and A. Scozzafava, Metal binding and antibacterial activity of ciprofloxacin complexes, *J. Enz. Inhib. Med. Chem.* **20** (2005) 303–307; DOI: 10.1080/14756360310001624948.
6. A. J. Schaeffer, The expanding role of fluoroquinolones, *Dis. Mon.* **49** (2003) 129–147; DOI: 10.1016/S0011-5029(03)90005-8.
7. J. Strahilevitz, A. Robicsek and D. C. Hooper, Role of the extended alpha4 domain of Staphylococcus aureus gyrase A protein in determining low sensitivity to quinolones, *Antimicrob. Agents Chemother.* **50** (2006) 600–606; DOI: 10.1128/AAC.50.2.600-606.2006.
8. Y. Dinh, Bacterial topoisomerase I as a target for discovery of antibacterial compounds, *Nucl. Acids Res.* **37** (2009) 731–737; DOI: 10.1093/nar/gkn936.
9. A. Foroumadi, M. Safavi, S. Emami, F. Siavoshi, S. Najjari, F. A. Safari and A. Shafiee, Structure-activity relationship study of a series of N-substituted piperazinyl-fluoroquinolones as anti-*Helicobacter pylori* agents, *Med. Chem.* **4** (2008) 498–502; DOI: 10.2174/157340608785700162.
10. M. Zupancic, I. Turel, P. Bukovec, A. White and D. J. Williams, Synthesis and characterization of two novel zinc(II) complexes with ciprofloxacin. Crystal structure of $[C_{17}H_{19}N_3O_3F]_2 \cdot [ZnCl_4] \cdot 2H_2O$, *Croat. Chem. Acta* **74** (2001) 61–74.
11. T. Mach, P. Neves, E. Spiga, H. Weingart, M. Winterhalter, P. Ruggerone, M. Ceccarelli and P. Gameiro, Facilitated permeation of antibiotics across membrane channels – Interaction of the quinolone moxifloxacin with the Omp F channel, *J. Am. Chem. Soc.* **40** (2008) 13301–13309.
12. J. Clitherow, *Preparation of Ranitidine Salts and Oral Compositions Containing them*, U.K. Pat. GB 2,220,937, Jan 24, 1990; ref. *Chem. Abstr.* **113** (1990) 65266n.

13. L. Vertesy, M. Astrid and K. Michael, *Bismuth Salts of Moenomycin-Like Antibiotic Preparation, Use and Pharmaceuticals Containing Such Salts for Treatment of Stomach Disorders*, Eur. Pat. 864,579, Sep 16, 1998; ref. *Chem. Abstr.* **129** (1998) 216854m.
14. M. Guo, I. Harvey, W. Yang, L. Coghill, A. John, T. A. Ross and P. J. Sadler, Synergistic anion and metal binding to the ferric ion-binding protein from *Neisseria gonorrhoeae*, *J. Biol. Chem.* **278** (2003) 2490–2502.
15. A. R. Shaikh, R. Giridhar and M. R. Yadav, Bismuth-norfloxacin complex: Synthesis, physicochemical and antimicrobial evaluation, *Int. J. Pharm.* **332** (2007) 24–30; DOI: 10.1016/j.ijpharm.2006.11.037.
16. National Committee for Clinical Laboratory Standards, Methods for Antimicrobial Testing of Anaerobic Bacteria, *Approved standard M11-A3*, 2nd ed., Villanova (PA): National Committee for Clinical Laboratory Standards 1993.
17. I. Turel, I. Leban and N. Bukovec, Synthesis, characterization, and crystal structure of a copper(II) complex with quinolone family member (ciprofloxacin); bis (1)-cyclopropyl-6-fluoro-1,4--dihydro-4-oxo-7-piperazin-1-yl-quinoline-3-carboxylate copper(II) chloride hexahydrate, *J. Inorg. Biochem.* **56** (1994) 273–282.

S A Ž E T A K

Metaloantibiotici: Sinteza, karakterizacija i antimikrobno djelovanje kompleksa fluorokinolona s bizmutom na *Helicobacter pylori*

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Komplesacijom fluorokinolona, norfloksacina, ofloksacina, ciprofloksacina, sparfloksacina, lomefloksacina, pefloksacina i gatifloksacina s bizmutom pripravljeni su novi organometalni spojevi. Dobiveni kompleksi karakterizirani su UV, IR i atomskom apsorpcijskom spektroskopijom, elementarnom analizom, diferencijalnom pretražnom kalorimetrijom, termogravimetrijskom analizom i spektrometrijom masa. Nadalje, ispitivan je njihov učinak na *Helicobacter pylori* i druge mikroorganizme. Ispitivani spojevi pokazuju snažno djelovanje na *Helicobacter pylori* u koncentraciji 0,5 mg L^{-1} te umjereno djelovanje na *Escherichia coli*, *Staphylococcus aureus*, *Bacillus pumilus* i *Staphylococcus epidermidis*. Kompleksi fluorokinolona s bizmutom potencijalni su lijekovi za infekcije uzrokovane *H. pylori*.

Ključne riječi: fluorokinolon, bizmut, kompleks, antimikrobno djelovanje, *Helicobacter pylory*

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