activity with moderate *in vivo* activity in hollow fiber assay. Further structural modifications of this lead, to improve pharmacokinetic parameters may yield potential anticancer compounds.

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Synthesis and Antimicrobial Evaluation of Some Novel Organometallic Compounds Against *Helicobacter pylori*

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Some novel organometallic compounds were prepared by complexing the antibiotics, tetracycline, azithromycin, cefotaxime, cephalexine, and antibacterials, ofloxacin, norfloxacin and gatifloxacin with bismuth citrate. They were characterized by UV, IR, NMR and elemental analysis. Their antibacterial activity against *Helicobacter pylori* and other microorganisms was investigated. Tetracycline-bismuth citrate was found to possess strong activity against *H. pylori* with a lowest inhibitory concentration of 125 mg/l. These complexes exhibited moderate activity against *Escherichia coli, Klebsiella pneumoniae, Bacillus pumilis, Staphylococcus aureus* and *Candida albicans.* The find-

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ings suggest that the activity of these organometallic compounds might be specifically directed against *H. pylori*.

Helicobacter pylori, a Gram-negative, microaerophilic bacterium, is a prevalent human pathogen in the gastric mucosa, which is responsible for gastric and duodenal ulcers. Therefore, drugs that can specifically inhibit *H. pylori* have been included in the category of anti ulcer agents. Although various combinations of antiulcer agents have so far been used in the treatment of ulcers, no single compound or complex from any of the antibiotic and antibacte-

TABLE 1: ANALYTICAL DATA OF SYNTHESIZED COMPOUNDS.

Complex. No	Complex	Empirical Formula	M.P. (°)	Yield (%)	Elementary analysis	
					Calculated	Found
I	Ofloxacin-bismuth citrate	$C_{24}H_{29}N_3O_{11}FBi$	298	82.1	C: 4.62 H: 45.93 N: 6.69	C: 4.60 H: 46.02 N: 6.50
II	Norfloxacin-bismuth citrate	$C_{22}H_{28}N_3O_{10}FBi$	276	78.2	C: 5.04 H: 43.82 N: 7.30	C: 4.99 H: 44.64 N: 7.55
III	Gatifloxacin-bismuth citrate	$C_{25}H_{29}N_3O_{11}FBi$	286	84.7	C: 4.50 H: 46.58 N: 6.52	C: 4.45 H: 47.50 N: 6.37
IV	Tetracycline-bismuth citrate	$C_{28}H_{36}N_2O_{14}Bi$	228	84.7	C:5.09 H: 47.52 N:3.96	C:5.01 H: 48.66 N:3.95
V	Azithromycin-bismuth citrate	$C_{44}H_{66}N_2O_{19}Bi$	305	82.1	C:6.54 H: 52.32 N:1.38	C:6.50 H: 50.30 N:1.35
VI	Cefotaxime-bismuth citrate	$C_{22}H_{27}N_5O_{14}Bi$	210	80.1	C:3.68 H:36.06 N:9.56	C:3.64 H:35.01 N:9.20
VII	Cephalexin-bismuth citrate	$C_{22}H_{26}N_3O_{24}Bi$	235	85.2	C:4.06 H:41.31 N:5.57	C:4.00 H:40.20 N:5.40

TABLE 2: SPECTRAL ANALYSIS DATA

COMPLEX	UV λmax (nm)	IR (KBr) (cm^{-1})	PNMR (CDC_{13}/DMSO-d_6) d(ppm)
I	285	854,1250,1342,1433, 2830, 3010, 3250, 3500.	1.3-1.9 (4H,d), 1.9 (1H:s), 3.5-3.8 (2H,s), 7.2 (2H,d), 7.87 (1H,s), 8.88 (1H,d).
II	276	855,1350,1433,1610,1690,2935, 3020,3452.	1.58 (3H,t), 3.2-3.4 (9H,m), 4.5-5.0 (2H,q), 7.20 (1H,d:J_{HF}-7.0Hz), 7.87 (1H,d:J_{HF}-13.0 Hz), 8.88 (1H, s).
III	298	610,856, 1342,1433,1610,2943, 3020,3452.	1.4-1.5 (4H,d),2.4(1H,s), 3.33 (4H,q): 3.5 (1H,s), 3.97 (6H,t), 6.83 (1H,t), 9.2 (2H,d)
IV	375	858,1334,1620,1670,2600,3100, 3350.	1.72 (1H,s), 3.10 (3H,s), 6.95 (1H,d), 7.20 (1H,d), 7.58 (1H,d).
V	311	858,1130,1340,1435,1521,1730, 2924.	1.3 (4H,q), 3.5 (1H,s), 4.2 (1H,t), 4.4 (3H,t), 4.7 (1H,d).
VI	255	854,1314,1340,1433,1610,2970, 3046,3451.	2.0 (3H,s), 3.33 (2H,q), 3.83 (3H,s), 4.97 (1H,q), 5.60 (7H,d), 6.70 (1H,s), 7.22 (2H,s), 9.47 (1H,d).
VII	277	858, 1340, 1342, 1432, 1620, 1770, 2968,3010.	2.07 (3H,s), 3.30 (2H,q), 4.97 (1H,d:J=4Hz), 5.34 (1H,s), 5.67 (1H,d:J=4Hz), 7.60 (5H,s)

rial derivative is known in the literature[1]. Literature survey revealed that bismuth possesses good antibacterial activity. Therefore, an attempt has been made to synthesize some novel organometallic complexes of bismuth with various classes of antibiotics as well as antibacterials, which could be used as novel antibacterial as well as antiulcer agents.

Melting points were determined by visual melting range apparatus (MR-VIS) and were uncorrected. Purity of the compounds was checked by thin layer chromatography. IR spectra were recorded on a Jasco-FT/IR 5300, PNMR spectra were recorded on a Varian Model EM-360 NMR spectrometer using TMS as internal standard. Elemental analysis was performed using Themofinnagane-EA-112 series

TABLE 3: ANTIMICROBIAL SCREENING DATA OF SYNTHESIZED COMPOUNDS

Comp lex	Microorganisms used											
	E.coli		*K. pnemoniae*		*B.pumilis*		*S.aureus*		*C.albicans*		*H.pylori*	
	Conc# (µg/1)	* Zone	Conc	Zone	Conc	Zone	Conc	Zone	Conc	Zone	Conc	Zone
I	5	30	2.5	20	5	25	5	22	15	18	2	20
II	10	27	5	18	7.5	21	10	24	10	28	2.5	28
III	12.5	35	7.5	35	12.5	28	10	34	12.5	25	2.5	28
IV	5	27	10	19	12.5	25	5	35	15	22	1.25	30
V	12.5	24	5	25	2.5	30	5	18	10	18	1.75	24
VI	7.5	29	5	23	5	21	5	3	12.5	19	3	28
VII	2.5	27	5	31	5	26	5	23	10	20	3.5	22
Sandard (Tetra cycline	24	40	14	38	25	46	28	48	22	50	30	38

Concentration of the synthesized compound in (µg/l) at which considerable zone of inhibition was observed. *Diameter of the zone of inhibition (cm) recorded with the zone reader Campbell electronics.

instrument. Ofloxacin USP, norfloxacin USP, gatifloxacin USP were obtained from Ajanta Pharma Ltd., Mumbai. Cefotaxime, Cephalexin, were obtained from Wokhardt Ltd. Mumbai as gift sample.

Antibiotic-bismuth citrate complexes were prepared using a 2-step process[1]. In step I, bismuth citrate was freshly prepared by the reaction of bismuth nitrate with citric acid in hot water. The product obtained was decanted and washed with 90% ethanol until ethanol left no residue upon evaporation. It was then dried at 60° and formation of bismuth citrate was confirmed by its melting point and by performing various chemical tests for bismuth compounds, as well as for the presence of citrate group. In step II, freshly prepared bismuth citrate (1 mole) was added to the selected drug (1.05 mole) in an alcoholic medium at 0-5° whilst stirring and after the addition, the reaction mixture was heated over a water bath at 40° for 6 to 8 h. The products were collected by filtration under suction, washed with a mixture of alcohol:water (1:1) and dried under vacuum. All the synthesized complexes gave satisfactory elemental analysis. Seven such compounds were synthesized and characterized by UV, IR, and PNMR[2,3]. The spectral analysis data is shown in Table 2.

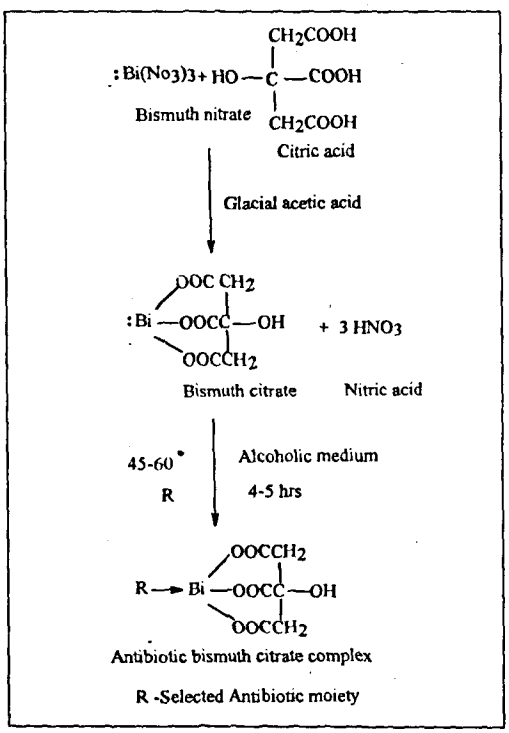

Fig. 1: Synthetic Scheme for the compounds prepared i the present investigation.

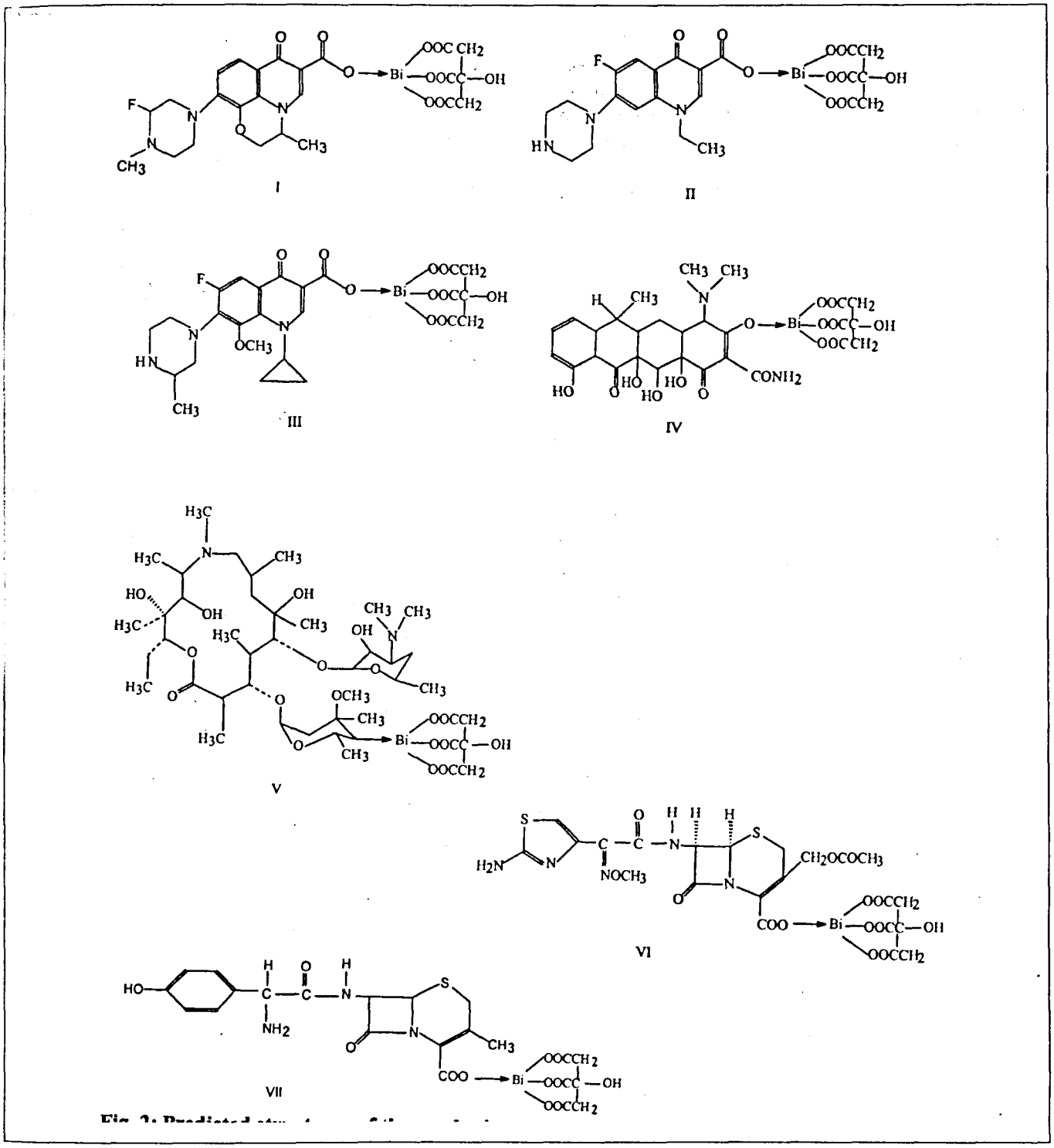

Fig. 2: Predicted structures of the synthesized compounds

Complex I is ofloxacin-bismuth citrate, II is norfloxacin-bismuth citrate, III is gatifloxacin bismuth citrate, IV is tetracycline-bismuth citrate, V is azithromycin-bismuth citrate, VI is cefotaxime-bismuth citrate and VII is cephalexin-bismuth citrate.

H. Pylori culturing and screening of the synthesized organometallic complexes against *H. pylori*[4-8] reference strains were carried out in collaboration with Swami Prakash Anand Ayurvedic Research Centre (SPARC), Mumbai. The culturing and susceptibility testing of *H. pylori* was performed without blood additives by using cyclodextrins as described previously in the literature[9]. The complexes were dissolved in the medium with the help of small volumes dimethyl sulfoxide or N,N-dimethyl formamide (E. Merck, Mumbai). The following microorganisms were tested by agar diffusion methods as reported in USP, with Mullar-Hinton media (HiMedia Ltd.). *E. coli, K. pnemoniae, S. aureus, B. pumilis* and *C. albicans*. The minimum inhibitory concentration (MIC) was determined as the lowest concentration required to prevent visible growth and was compared to the values obtained with the standard, tetracycline.

Appropriate molecules with antibiotic properties were reacted with bismuth salts of an organic tricarboxalic acid, namely bismuth citrate resulting in the organo-bismuth citrate complexes with 80-85% efficiencies (Table 1). These complexes which were amorphous are represented by the structural formulae I to VII and were thoroughly characterized by TLC, IR, UV/Vis, PNMR spectra and by elemental analysis (Table 2). The antibiotics and antibacterials employed in the series were ofloxacin, norfloxacin, tetracycline, azithromycin, cefotaxime and cephalexin. From the homogeneity of the complexes and spectral and analytical data obtained, it became evident that a 1:1 complex of the antibiotic/antibacterial and bismuth citrate was formed which was quite similar to that of ranitidine-bismuth citrate complex reported by Mealy and Castner[10]. The melting points of these complexes are very high and did not completely dissolve in most common solvents tested.

All the compounds of the present investigation, represented by structure I to VII have been found to exhibit antibacterial *in vitro* efficiency against *H. pylori* and also against several other microorganisms. The MICs of these organometallic complexes are shown in Table 3. The MICs of the complexes were within a range comparable to potent single substances. It can be observed that the organometallic complex IV (tetracycline-bismuth citrate) and complex V (azithromycin-bismuth citrate) were found to exhibit much higher activities than complex VI (cefotaxime-bismuth citrate) and complex VII (cephalexin-bismuth citrate) against *H. pylori*. It is therefore evident that a tetracycline antibiotic or a macrolide antibiotic like azithromycin might be much more essential as they posses activity of the highest order as determined by our *in vitro* studies. These complexes on the other hand inhibit the growth of other microorganisms to a much lesser degree as seen from Table 2, which indicates specificity towards *H. pylori*.

Ranitidine-bismuth citrate complex reported by Mealy and Castner[10] was found to be active against *H. pylori*. Thiopthalide compounds reported by Dekker *et al.*[11] appeared to be specifically active against *H. pylori*, since these compounds did not exhibit antibacterial activity against other microorganisms. The extracts of the stem bark of the East African plant *Terminalia spinosa* were found to be active against *H. pylori* by Fabry *et al.*[12]. Although various combinations of antiulcer agents have so far been used in the treatment of ulcers, no single compound or complex from any of the antibiotic and antibacterial selected in the present investigation has been reported in the literature. Ranitidine-bismuth citrate is also from a heterocyclic moiety and not from an antibiotic or antibacterial.

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Spectrofluorimetric Estimation of Cefdinir in Formulation

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A simple, accurate and sensitive spectrofluorimetric procedure was developed for the estimation of cefdinir containing heterocyclic fused ring structure, in 1 M sodium hydroxide at 95° for 1 h, which shows strong fluorescence having excitation and emission wavelength 262 and 530 nm, respectively. Linear relationship for the fluorescence intensity was obtained in the range of 0.2–1 μg/ml. The method was statistically validated and was applied successfully to determine the cefdinir in pharmaceutical dosage form.

Cefdinir[1,2] is an extended-spectrum, semi-synthetic cephalosporin antibiotic for oral administration. Chemically it is [6R-[6α,7β(z)]-7-[(2- aminothiazolyl) (hydroxyimino) acetyl] amino] -3-ethenyl-8-oxo-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylic acid (fig. 1). It is not official in any pharmacopoeia. Literature survey revealed that a microbiological assay[3] and HPLC[4] methods have been reported for the estimation of cefdinir from biological fluids. Therefore, it was thought necessary to develop a specific, simple, precise and accurate spectrofluorimetric method for the estimation of cefdinir in its pharmaceutical dosage form.

Generally fluorescence occurs because of transition from first excited singlet state to ground state by emission of light[5,6]. Cefdinir consists of a heterocyclic fused ring structure, which is responsible for fluorescent behaviour[7] in alkaline medium at 95° for 1 h[8]. This fluorophore showed an excitation wavelength at 262 nm with an emission wavelength at 530 nm.

Pure authentic sample of cefdinir was procured from Unichem Laboratories Ltd., Mumbai and 300 mg capsules Sefdin and Cefdiel were purchased from Thulasi Pharmacies India (Pvt.) Ltd., Coimbatore. All chemicals used in this investigation were of AR grade. Triple distilled water was used in this study.

A Jasco model FP/750 fluorimeter with single quartz cell of 1 cm path length was used to measure fluorescence intensity of the resulting solutions. Buffer pH was measured and adjusted using a digital pH meter (Elico make) and a constant temperature water bath (Labtronics make) were also used in the study. Phosphate buffer of pH 7 was used for the preparation of solutions .The buffer was prepared by mixing 61.1ml of dibasic sodium hydrogen phosphate solution (0.95 %w/v solution of Na_2HPO_4 in water) with 38.9 ml of monobasic potassium dihydrogen phosphate solution (0.9 %w/v of KH_2PO_4 in water).

As cefdinir was poorly soluble in other solvents like hydrochloric acid, acetic acid, ammonium hydroxide, acetate buffer, and phostate buffer of pH 3, 4, 5 and 6, phosphate buffer of pH 7 was taken as the solvent of choice. Before developing the method the instrumental parameters like excitation bandwidth, emission bandwidth and response time were optimized at 5 nm, 5 nm and 0.02 s, respectively, as other bandwidth and response time such as 5 nm, 10 nm, 20 nm and 0.02, 0.05, 0.1, 0.25, 0.5, 1, 2 and 8 s have already been installed in the fluorimeter. Apart from these, different parameters like concentration and volume of sodium hydroxide, heating temperature, heating time

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