ric flask and diluted to volume with 0.5 M sodium hydroxide solution. The absorbance of the sample solution was recorded at 355 nm. The drug content in the sample was then calculated.

Recovery study was performed by spiking a 10 ng/ml solution of drug and found to be 99.5% and 99.2% in the marketed samples of dolib (Panacea Biotech Ltd.) and rofebax (Ranbaxy Laboratories), respectively.

A yellow color produced in alkaline solution (pH 10-12) of rofecoxib was used to determine the drug in the dosage forms. A linear curve was constructed between the concentration and absorbance and find out the equation of the line, which is $Y=0.02333X+0.002827$ with correlation coefficient of 0.9936. This indicates a good linearity between concentration and absorbance. It also shows that rofecoxib followed Beer-Lambert's Law in the concentration range of 5-40 $\mu g/ml$. The value for molar absorptivity and Sandell's sensitivity were found to be 7.592×10^3 l/mol.cm and 4.5×10^{-4} $\mu g/cm^2/0.001$, respectively.

The proposed method was successfully applied to the analysis of rofecoxib in two different brands of tablets (dolib and rofebax) obtained commercially. The mean recovery was found to be in the range of 0.8% with RSD values less than 0.5%. It was observed that the excipients did not interfere in the determination of rofecoxib. Hence, the proposed method could be used for routine determination of rofecoxib in its dosage forms, as it is cheap, convenient, precise and reproducible.

REFERENCES

1. Jacson, L.M. and Hawkey C.J., **Drugs,** 2000,59, 1207.
2. Hawkey, C.J. **Lancet,** 1999, 353,307.
3. Hawkey, C.J., Jacson, L.M., Harper, S.E., Simon, T.J., Martensen, K. and Lines, C.R., **Aliment. Pharmacol. Ther.,** 2001, 17, 1.
4. Blain, H., Jouzean, J.Y., Nelter, P. and Jeandel, C., **Rev. Med. Internet.,** 2000, 21, 978.
5. Wallence, J.L., **Amer. J. Med.,** 1999, 107, 115.
6. Lefkwith, J.B., **Amer. J. Med.,** 1999, 106, 43S.
7. Lipskey, L.P., Abramson, S.B., Crufford, L., Simon, L. S., and Vande Putte, L.B., **J. Rheum.,** 1998, 25, 2298.
8. Chabez-Eng, C.M., Constnnzer, M.L. and Matuszewskib, K., **J. Chromatogr. Biomed. Sci.,** 2000, 784, 31.
9. Jamali, F. and Satirri, S., **J. Pharm. Sci.,** 2000, 3, 312.

Synthesis and Biological Activity of 2-(3',5'-Dibromo-2'-hydroxyphenyl)-3-aryl-5H/methyl/carboxymethyl-4-Thiazolidinones

A. H. BAPODRA, FATEMA BHARMAL AND HANSA PAREKH*
Department of Chemistry, Saurashtra University, Rajkot-360 005.

Accepted 8 May 2002
Revised 19 April 2002
Received 9 May 2000

With a view to map out the synthesis and antimicrobial activity of series of 2-(3',5'-dibromo-2'-hydroxyphenyl)-3-aryl-5H/methyl/carboxymethyl-4-thiazolidinones (III-a-ss) have been prepared by the cyclocondensation of N-substituted-(3,5-dibromo-2-hydroxy benzylidene)-anilines (II) with α-substituted mercaptoacetic acids and evaluated for their *in vitro* growth inhibiting activity against several microbes. Some of the compounds show significant antimicrobial activity.

4-Thiazolidinones play a vital role owing to their wide range of biological activities and industrial importance[1-4]. Furthermore bromophenols are also known to possess significant biological activities[5-8]. In continuation of our earlier work, we have therefore undertaken synthesis of 4-thiazolidinones incorporating bromophenolic moiety with a view to assessing pharmacological profile of the compounds synthesised.

Different aromatic amines were condensed with 3,5-dibromo salicyldehyde to yield respective Schiff's bases (II)

***For correspondence**

in 56-72% yield which were subjected to cyclocondensation with α-substituted mercaptoacetic acids resulting in target cyclised 4-thiazolidinones (IIIa-ss). The structural assignment of the compounds synthesised were based on the elemental analyses, IR, NMR and Mass spectral study. All the compounds were tested for their *in vitro* antimicrobial activity against variety of microbes.

All melting points were taken by open capillary method and are uncorrected. Infrared spectra were determined on a Shimadzu IR-435 spectrophotometer using a potassium bromide technique (υmax incm^{-1}). PMR spectra were recorded on a Hitachi NMR-R 1200 using TMS as internal standard (chemical shifts in δ ppm) and Mass spectra on a Jeol JMS D-300 spectrometer at 70 eV.

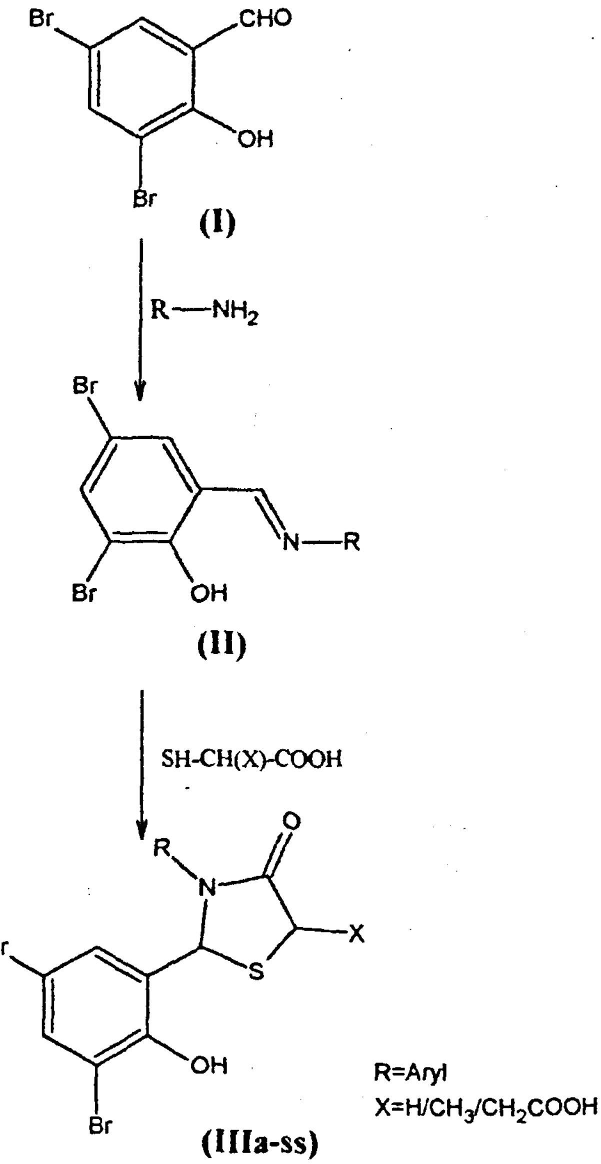
N-2,3-Dimethyl-(3,5-dibromo-2-hydroxy benzylidene)-aniline (II) was prepared by refluxing a mixture of 3,5-dibromo-2-hydroxy benzaldehyde (2.81 g, 0.01 mol) and 2,3-dimethyl aniline (0.43 g, 0.01 mol) in methanol for 9 h. The contents were cooled and product was isolated and crystallised from methanol, m.p. 93°, Yield 69% (Found C, 46.95; H, 3.35; N, 3.63, $C_{15}H_{13}NOBr_2$ requires C, 46.99, H, 3.39, N, 3.66). IR (KBr): 3080 cm^{-1} (Aromatic str.); 3400-3200 (Ar-OH str.); 2960 ($-CH_3$ str); 1620 (R-N = CH - R1); 540 (C-S Str.); PMR: ($CDCl_3$): δ ppm 2.18 (s, 3H, $-C\underline{H}_3$); 6.78 (s, 1H, =C-$\underline{H}$); 6.86-7.8 (m, 5H, Ar-H); 9.1 (s, 1H, -O$\underline{H}$); MS: m/z 382 (m$^+$), 304, 287, 208, 194, 179, 104, 77, 65.

2-(3',5'-Dibromo-2'-hydroxyphenyl)-3-(2',3'-dimethylphenyl)-5H-4-thiazolidinone (IIIj) was prepared by heating a mixture of thioglycolic acid (0.28 g, 0.01 mol) and N-2,3-dimethyl-(3,5-dibromo-2-hydroxy benzylidene) aniline (3.83 g, 0.01 mol) in an oil-bath at 115-120° for 12 h. The resulting mass was treated with 10% sodium bicarbonate solution and the precipitates were washed with water, dried and crystallised from methanol, m.p. 280°, yield 63%. (Found C, 44.58; H, 3.25; N, 3.01, $C_{17}H_{15}NO_2SBr_2$ requires C, 44.64; H, 3.28; N, 3.06). IR (KBr): 3080 cm^{-1}(Aromatic str.); 2975, 2860 (-CH str.); 3320 (-OH str.); 1680 (C=O str.); 540 (C-Br str.); PMR: ($CDCl_3$): δ ppm 2.1 (s, 3H, $-C\underline{H}_3$); 2.9 (s, 2H, $-C\underline{H}_3$); 5.82 (s, 1H, -C$\underline{H}$-R); 6.8-7.6 (m, 5H, Ar-$\underline{H}$); 9.5 (s, 1H, -O$\underline{H}$); MS: m/z 457 (m$^+$), 376, 361, 345, 331,317, 288.

2-(3',5'-Dibromo-2'-hydroxyphenyl)-3-(2',3'-dimethylphenyl)-5-methyl-4-thiazolidinone (IIIy) was prepared by heating a mixture of thiolactic acid (0.30 g, 0.01 mol) and N-2,3-dimethyl-(3,5-dibromo-2-hydroxy benzylidene) aniline (3.83 g, 0.01 mol) was heated in oil-bath at 115-120° for 12 h. The resulting mass was treated with 10% sodium bicarbonate solution and the precipitates were washed with water, dried and crystallised from methanol, m.p. 102°, yield 64%. (Found C, 45.80; H, 3.58; N, 2.91, $C_{18}H_{17}NO_2SBr_2$ requires C, 45.86; H, 3.61; N, 2.97). IR (KBr): 3300 cm^{-1} (-OH str.); 2950, 2880 ($-CH_3$ str.); 1685 (C=O str.); 660 (C-S-C str.); 550 (C-Br str.): MS: m/z 471 (m+) 411, 331, 304, 209, 194, 105, 77.

2-(3',5'-Dibromo-2'-hydroxyphenyl)-3-(2',3'-

SCHEME - 1



dimethylphenyl)-5-carboxymethyl-4-thiazolidinone (IIInn) was prepared by condensing N-2,3-dimethyl-(3,5-dibromo-2-hydroxy benzylidene) aniline (3.83 g, 0.01 mol) with thiomalic acid (0.42 g, 0.01 mol) in the presence of anhydrous zinc chloride (0.5 g) at 160° for 2 h. Temperature was then raised to 180° for 30 min. The product obtained was dissolved in sodium bicarbonate solution and filtered. The filtrate was reprecipitated with dilute hydrochloric acid and crystallised from methanol, m.p. 88°, Yield 64% (Found C, 44.23; H, 3.27; N, 2.68 : $C_{19}H_{17}NO_4SBr_2$ requires C, 44.27; H, 3.30; N, 2.72). IR (KBr): 3400 cm^{-1} (-OH str.), 3080 (Aromatic str.); 700 (C-S-C str.), 535 (C-Br str.).

The compounds so synthesised were evaluated for antimicrobial activity using the cup-plate method on the following strains of bacteria *Staphylococcus aureus, Staphylococcus pyogens, Escherichia coli, Klebsiella aerogens* and the fungal strain used for testing was *Aspergillus niger.* The nutrient agar broth was prepared and sterilised under pressure of 15 lb for 1 h. It was then allowed to cool to 40-50° and poured into sterile petridishes (20-25 ml in each petridish) and allowed to set for 1 h. To each petridish, 0.1 ml of culture was injected with the help of micropipette. Thereafter the cups were made by punched into the set agar with a sterile cork borer and scooping out the punched part of

TABLE 1: CHARACTERISATION DATA OF SOME COMPOUNDS OF 2-(3',5'-DIBROMO-2'-HYDROXYPHENYL)-3-ARYL-5H/METHYL/CARBOXYMETHYL-4-THIAZOLIDINONES.

Compd.	R	Yield %	M.P.	Molecular Formula	% of Nitrogen* Calc. Found	Zones of Inhibition in mm				
						S. aure	*S. pyo*	*E. coli*	*K. aero*	*A. niger*
IIIb	4-Arsenophenyl	62	76	$C_{15}H_{12}NO_5SBr_2As$	2.53/2.49	12	**18**	12	13	13
IIIe	2-Chloro-6-methyl phenyl	62	123	$C_{16}H_{12}NO_2SBr_2Cl$	2.93/2.88	13	**19**	12	13	12
IIIf	2,5-Dichlorophenyl	58	89	$C_{15}H_9NO_2SBr_2Cl_2$	2.81/2.74	**19**	13	13	12	11
IIIg	2,6-Dichlorophenyl	74	81	$C_{15}H_9NO_2SBr_2C1_2$	2.81/2.76	**15**	12	12	14	**20**
IIIh	4-N,N-Diethyl aminophenyl	69	85	$C_{19}H_{20}N_2O_2SBr_2$	5.60/5.53	12	**15**	13	13	13
IIIp	4-Antipyrinyl	58	107	$C_{21}H_{19}N_3O_3SBr_2$	7.59/7.52	**15**	11	12	13	13
IIIq	4-Arsenophenyl	64	112	$C_{16}H_{14}NO_5SBr_2As$	2.48/2.43	14	12	13	**17**	14
IIIr	4-Bromophenyl	72	118	$C_{16}H_{12}NO_2SBr_3$	2.68/2.61	11	**18**	13	**15**	13
IIIt	2-Chloro-6-methylphenyl	62	93	$C_{17}H_{14}NO_2SBr_2Cl$	2.85/2.79	12	13	14	13	**19**
IIIu	2,5-Dichlorophenyl	68	107	$C_{16}H_{11}NO_2SBr_2Cl_2$	2.73/2.74	**15**	12	12	12	12
IIIaa	3,5-Dimethylphenyl	66	76	$C_{18}H_{17}NO_2SBr_2$	2.97/2.91	**18**	12	14	13	13
IIIbb	2-Ethylphenyl	71	81	$C_{18}H_{17}NO_2SBr_2$	2.97/2.94	12	11	13	11	**16**
IIIcc	4-Ethylphenyl	61	72	$C_{16}H_{17}NO_2SBr_2$	2.97/2.93	11	11	11	11	**19**
IIIgg	4-Bromophenyl	61	90	$C_{17}H_{12}NO_4SBr_3$	2.47/2.41	**17**	12	12	11	11
IIIjj	2,5-Dichlorophenyl	67	107	$C_{17}H_{11}NO_4SBr_2Cl$	2.52/2.48	12	10	11	**17**	12
IIIll	4-N,N-Diethyl aminophenyl	69	103	$C_{21}H_{22}N_2O_4SBr_2$	5.02/4.98	12	**19**	12	12	13
IIInn	2,3-Dimethylphenyl	64	88	$C_{19}H_{17}NO_4SBr_2$	2.72/2.68	14	13	11	13	**21**
IIIoo	2,4-Dimethylphenyl	68	86	$C_{19}H_{17}NO_4SBr_2$	2.72/2.67	13	11	14	12	**15**
IIIpp	3,5-Dimethylphenyl	62	93	$C_{19}H_{17}NO_4SBr_2$	2.72/2.70	12	13	13	11	**16**
IIIrr	4-Ethylphenyl	72	103	$C_{19}H_{17}NO_4SBr_2$	2. 72/2.68	**18**	13	12	11	11

Results with standard antibiotics: ampicillin (22-26 mm); chloramphenicol (21-28 mm); norfloxacin (21-27 mm); greseofulvin (24 mm). * All the compounds gave satisfactory C,H and halogen analyses.

agar. The diameter of each cup was 10 mm. To these cups, 50 μl of the test solution was added with the help of a sterile micro pipette. The test solution was prepared by dissolving 5 mg compound in 5 ml of DMF. The drug solution was allowed to diffuse for about 2 h. The plates were incubated at 37° for 24 h and zones of inhibition in mm were recorded. Standard drugs used for testing were ampicillin, chloramphenicol, norfloxacin and greseofulvin. Results showing the highest activity of the compounds by cup plate method are given in Table 1.

All the compounds synthesised were screened for *in vitro* antimicrobial activity against Gram positive bacteria, *S. aureus* and *S. pyogens* and Gram negative bacteria, *E. coli* and *K. aerogens*. The fungal strain used for testing was *A. niger*. The standard drugs used for comparison were ampicillin, chloramphenicol, norfloxacin and greseofulvin. Compounds IIIb, IIIe, IIIf, IIIr, IIIy, IIIaa, IIIII, IIIrr displayed highest activity against above microbes. The other compounds show mild to moderate activity against these organisms. Table 1 represents the activity observed against the microbes investigated.

ACKNOWLEDGEMENTS

The authors thank Prof. A. R. Parikh, Head, Department of Chemistry, Saurashtra University, Rajkot for providing necessary facilities and encouragement.

REFERENCES

1. Shahsafi, M.A., Meshkata, M.H. and Parekh, H., **Indian J. Chem.**, 1987, 26, 803.
2. Shyam, R. and Tiwari, I.C., **Bull Chem. Soc., Jpn.**, 1977, 50, 524.
3. Patel, C.L. and Parekh, H.H., **J. Indian Chem. Soc.**, 1988, 65, 574.
4. Husain, M.I., Amir, M. and Singh, E., **Indian J. Chem.**, 1987, 26, 251.
5. Makhsumov, A.G., Normatov, F.A. and Ergashev, M.S., **Chem. Abstr.**, 1991, 115, 207591.
6. Belliotti. T.R. and Connor. D.T., **Chem. Abstr.**, 1988, 108, 131808.
7. Tevlon, J.M., **Chem. Abstr.**, 1983, 99, 105246.
8. Michaely, W.J., **Chem. Abstr.**, 1991, 115, 135695.

Spectrophotometric Determination of Lamivudine and Stavudine

D. G. SANKAR*, M. V. V. N. REDDY, J. M. RAJENDRA KUMAR AND T. K. MURTHY
Pharmaceutical Analysis Division, Department of Pharmaceutical Sciences,
Andhra University, Visakhapatnam-530 003.

Accepted 8 May 2002
Revised 23 April 2002
Received 12 September 2001

A simple, sensitive spectrophotometric method has been developed for the determination of lamivudine and stavudine in pure and its pharmaceutical formulations. Lamivudine and stavudine give green coloured chromogens with 3-methyl-2-benzothiozolinone hydrazone hydrocloride and ferric chloride with absorption maximum at 660 nm and 630 nm, respectively. The chromogens obey Beer's law in the concentration ranges of 2.5 to 40 μg/ml and 2.5 to 12.5 μg/ml, respectively for lamivudine and stavudine.

Lamivudine (LMD) and stavudine (SVD) are antiHIV drugs. LMD is chemically 4-amino-1-[2-(hydroxy methyl)-1,3-oxathiolan-5-yl]-2-pyrimidinone[1] and it acts by inhibiting nucleoside reverse transcriptase, which selectively inhibits HIV1 replication[2]. SVD is chemically 2,3-didehyro-3-deoxy thymidine[1], which acts as a competitive inhibitor of deoxythymidine tri phosphate and incorporation causes termination of DNA chain elongation[2]. A few HPLC methods for

---

***For correspondence**