

TABLE 2: ESTIMATION OF CIPROFLOXACIN HYDROCHLORIDE IN TABLETS

Dosage form (Brand name)	Labeled amount (mg)	Amount obtained (mg)	% Recovery*	
			Proposed Method	I. P. Method
Tablet 1 (Ciprobid) ^a	250	249.5	99.80	101.2
Tablet 2 (Ciprolet) ^b	250	248.0	98.20	98.65

^aCadila Healthcare; ^bDr. Reddy's Lab. Ltd.; *Each result is the mean of five replicates

ceutical Sciences for providing facilities.

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Synthesis, Antibacterial and AntiHIV Activities of 3-[5-Amino-6-(2,3-Dichloro-Phenyl)-[1,2,4]Triazin-3-yl]-6,8-Dibromo-2-Substituted-3H-Quinazolin-4-one

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A series of novel 2,3-disubstituted/6,8-dibromo-2,3-disubstituted quinazolin-4(3H)-ones have been synthesized by condensing the primary amino group of lamotrigine with benzoxazin-4-one. The structure of the synthesised compounds was elucidated by spectral analysis (IR, NMR and Mass). The compounds synthesized were screened for antibacterial and antiHIV activities against replication of HIV-1 and HIV-2 in acutely infected MT-4 cells. The compounds SPC-I, SPC-I Br and SPC-

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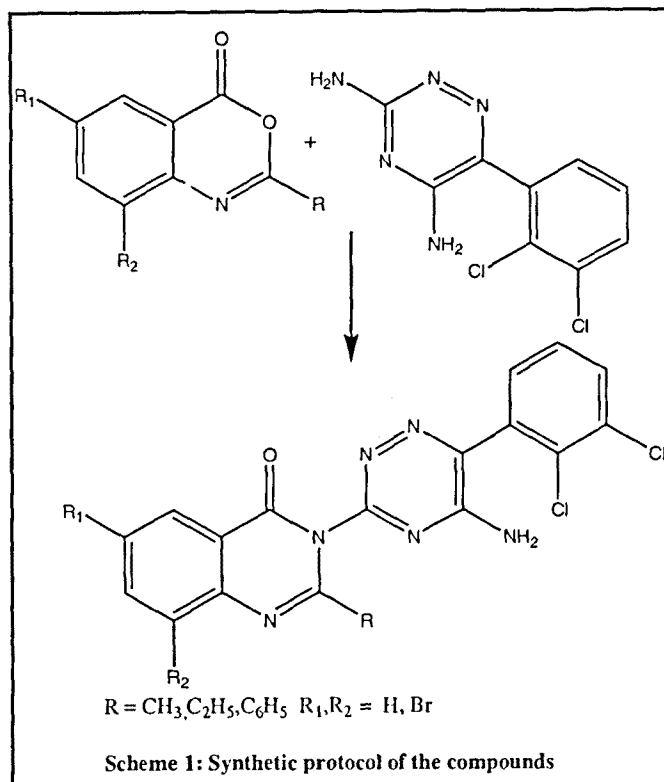
III Br (100 µg/ml) exhibited equivalent antibacterial activity with the standard ciprofloxacin (10 µg/ml) against *salmonella typhi*. All the compounds exhibited cytotoxic properties in the MT-4 lymphocyte cell line,.

Quinazolin-4(3H)-one is a versatile lead molecule for designing potential bioactive agents. 2,3-disubstituted quinazolin-4(3H)-one derivatives have been evaluated for a wide spectrum biological activities such as sedative¹, anti-convulsant², antifungal and antibacterial³⁻⁶, antiHIV^{4,7,8} antiviral^{9,10} and anticancer activities^{11,12}. The objective of the study was to synthesize a series of hitherto unreported 2,3-disubstituted and 6,8-dibromo 2,3-disubstituted quinazolin-4(3H)-ones and these compounds were also evaluated for antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Staphylococcus aureus* by the disc diffusion method and antiHIV activity against the replication of HIV-1 (IIIB) and HIV-2 (ROD) in actually infected MT-4 cells.

Anthranillic acid/3,5-dibromo anthranillic acid on reacts with acetic anhydride, propionic anhydride and benzoyl chloride to form corresponding 2-methyl/-ethyl/-phenyl benzoxazin-4-one by N-acylation followed by dehydrative cyclization mechanism¹³. 2-substituted/6,8-dibromo derivatives of benzoxazin-4-one were condensed with the primary amino group of lamotrigine to afford 2,3-disubstituted/6,8-dibromo-2, 3-disubstituted benzoxazin-4-one (Scheme 1). IR and NMR spectra were consistent with the assigned structure.

Melting points were determined using Thomas melting point apparatus and are uncorrected. The purity was checked by TLC using silica gel G as stationary phase. The structure of the synthesized compounds was elucidated using a Perkin Elmer FT-IR in KBr disc and PMR was taken on a Bruker AMX-(400 MHz) FT-NMR. Mass spectra were obtained on a Varian Atlas CH-7 Mass spectrometer at 70 eV.

The titled compounds, 3-[5-Amino-6-(2,3-dichlorophenyl)-[1,2,4]triazin-3-yl]-6,8-dibromo-2-substituted-3H-quinazolin-4-one were prepared by the following method. An equimolar (0.01 mol) mixture of 2-substituted-1,3-benzoxazin-4-one and lamotrigine was refluxed for 6 h in 10ml of glacial acetic acid and. The mixture was cooled to room temperature and poured into crushed ice; the solid thus obtained was recrystallized from ethanol. 3-[5-Amino-6-(2,3-dichloro-phenyl)-[1,2,4]triazin-3-yl]-2-methyl-3H-quinazolin-4-one (SPC-I), yield: 68%, mp: 210°, IR (KBr) cm⁻¹: 3300



(NH), 1510 (C=N), 1674 (C=O), 1583 (C=C), 1438 (N=N); PMR (DMSO-d₆) δ ppm: 1.3 (s, 3H, -CH₃), 7.3 (m, 3H, Ar-H), 7.0 (t, 1H, Q-6H) 7.5 (t, 1H, Q-7H), 8.1 (d, 1H, Q-8H), 8.6 (d, 1H, Q-5H); EI- MS (m/e): 399.328.

3-[5-Amino-6-(2,3-dichloro-phenyl)-[1,2,4]triazin-3-yl]-2-ethyl-3H-quinazolin-4-one (SPC-II), yield: 52%, mp: 168°, IR (KBr) cm⁻¹: 3311 (NH), 1678 (C=O), 1509 (C=N), 1581 (C=C), 1442 (N=N); PMR (DMSO-d₆) δ ppm: 1.2 (t, 3H, CH₃), 2.4 (q, 2H, -CH₂) 4.5 (b, 2H, -NH₂), 7.0(t, 1H, Q- 6H), 7.6 (t, 1H, Q-7H); 8.0 (d, 1H, Q-8H) 8.6 (d, 1H, 5H); EI-MS (m/e) : 413.265.

3-[5-Amino-6-(2,3-dichloro-phenyl)-[1,2,4]triazin-3-yl]-2-phenyl-3H-quinazolin-4-one (SPC-III), yield: 86%, mp: 228°. IR (KBr) cm⁻¹: 3297 (NH), 1665 (C=O), 1607 (C=C), 1508 (C=N), 1435 (N=N); PMR (DMSO-d₆) δ ppm: 4.5 (b, 2H, NH₂), 7.4 (m, 7H, Ar-H) 7.0 (t, 1H, Q-6H), 7.6(t, 1H, Q-7H), 8.1 (d, 1H, Q-8H); 8.6 (d, 1H, Q-5H); EI-MS (M/e): 461.309.

TABLE 1: ANTIBACTERIAL ACTIVITY OF THE SYNTHESIZED COMPOUNDS.

Compounds	Zone of Inhibition ^a			
	<i>E. coli</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>S. typhi</i>
SPC-I	11	12	11	18
SPC-II	15	-	-	-
SPC-III	12	-	-	-
SPC-I Br	-	12	-	16
SPC-II Br	-	13	-	-
SPC-III Br	-	11	-	18
Ciprofloxacin	31	10	12	17

^aAverage zone of inhibition measured in mm.

3-[5-Amino-6-(2,3-dichloro-phenyl)-[1,2,4]triazin-3-yl]-6,8-dibromo-2-methyl-3H-quinazolin-4-one (SPC-1 Br), yield: 69%, mp: 130°, IR (KBr) cm^{-1} : 3216 (NH), 1698 (C=O), 1556 (C=N), 1440 (N=N); PMR (DMSO- d_6) δ ppm: 1.3 (s, 3H, -CH₃), 4.8 (b, 2H, -NH₂), 7.3 (m, 3H, Ar-H), 7.8 (t, 1H, Q-7H), 8 (d, 1H, Q-5H); EI- MS (m/e): 557.031.

3-[5-Amino-6-(2,3-dichloro-phenyl)-[1,2,4]triazin-3-yl]-6,8-dibromo-2-ethyl-3H-quinazolin-4-one (SPC-II Br): Yield: 64%, mp: 165°, IR (KBr) cm^{-1} : 3245 (NH), 1672 (C=O), 1508 (C=N), 1442 (N=N); PMR (DMSO- d_6) δ ppm: 1.3 (t, 3H, -CH₃), 2.5 (q, 2H, -CH₂), 6.8 (m, 3H, Ar-H), 7.3 (d, 1H, Q-7H), 8.0 (s, 1H, Q-5H); EI-MS (m/e): 571.057.

3-[5-Amino-6-(2,3-dichloro-phenyl)-[1,2,4]triazin-3-yl]-6,8-dibromo-2-phenyl-3H-quinazolin-4-one (SPC-III Br): yield: 71%, mp: 158°, IR (KBr) cm^{-1} : 3222 (NH), 1541 (C=N), 1655 (C=O), 1443 (N=N); PMR (DMSO- d_6) δ ppm: 4.5 (s, 2H, -NH₂), 7.6 (m, 7H, Ar-H), 7.8 (t, 1H, Q-7H), 8.0 (d, 1H, Q-5H); EI MS (m/e): 619.101.

The compounds were screened for antibacterial activity against *E. coli*, *P. aeruginosa*, *S. typhi* and *S. aureus*, by the disc diffusion method¹⁴ at 100 $\mu\text{g/ml}$ in dimethylformamide using nutrient agar medium. The results were evaluated by measuring average zone of inhibition in mm and compared with standard ciprofloxacin at 10 $\mu\text{g/ml}$ concentration. The results are presented in Table 1. The compounds SPC-I, SPC-I Br and SPC-III Br (100 $\mu\text{g/ml}$) exhibited equivalent antibacterial activity with the standard ciprofloxacin (10 $\mu\text{g/ml}$) against *S. typhi*.

The compounds were tested for antiHIV activity against the replication of HIV-1 (III_B) and HIV-2 (ROD) in MT-4 cells¹⁵.

The cells were grown and maintained in RPMI 1640 medium supplemented with 10% heat-inactivated Fetal Calf Serum (FCS), 2 mM-glutamine, 0.1% sodium bicarbonate and 20 $\mu\text{g/ml}$ gentamicin (culture medium). HIV-1 (HTLV-III_B/LAI) and HIV-2 (LAV-2_{ROD}) were used in all experiments. The virus strains were propagated in MT-4 cells. Titer of virus stock was determined in MT-4 cells and the virus stock was stored at -70° until used.

The inhibitory effects of the compounds on HIV-1 and HIV-2 replication were monitored by inhibition of virus-induced cytopathic effect in MT-4 cells and were estimated by the MTT method. Briefly, 50 μl of HIV-1 and HIV-2 (100-300 CCID₅₀) were added to a flat-bottomed microtiter tray with 50 μl of medium containing various concentrations of the test compounds. MT-4 cells were added at a final concentration of 6×10^5 cells/ml. After 5 d of incubation, at 37° the numbers of viable cells were determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method. Cytotoxicity of the compounds for mock-infected MT-4 cells was also assessed by the MTT method.

None of the compounds exhibited antiHIV effect and all the compounds displayed cytotoxic properties in the lymphocyte cell line (MT-4 cells). The 50% effective concentration (EC₅₀) values of the synthesized compounds against the replication of HIV-1 and HIV-2 in acutely infected MT-4 cells were become higher than the cytotoxic concentration (CC₅₀).

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UV and Visible Spectrophotometric Analysis of Pioglitazone Hydrochloride in Bulk and Tablets

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Two simple, accurate and economical spectrophotometric methods in ultraviolet and visible region were developed for the determination of pioglitazone hydrochloride in bulk drug and in pharmaceutical formulation. In method A pioglitazone hydrochloride showed λ_{max} at 269 nm in 0.2 N sulphuric acid solution, showing linearity in the concentration range of 10–60 $\mu\text{g/ml}$ whereas in method B pioglitazone hydrochloride was reacted with diazotized sulphanilic acid in an alkaline medium. Yellowish orange coloured chromogen showed λ_{max} at 420 nm, showing linearity in the concentration range of 10–50 $\mu\text{g/ml}$. The results of analysis have been validated statistically and recovery studies confirmed the accuracy of the proposed method.

Pioglitazone hydrochloride (PGH) is the newest class of oral antidiabetic drug and, chemically, it is [1(A)-5[[4-[2-(5-ethyl-2-pyridinyl) ethoxy] phenyl] methyl]-2,4] thiazolidine dione mono hydrochloride. It is not official in any of the pharmacopoeia. Literature survey revealed very few analytical methods which include HPLC^{1,2}. But there is no evidence in literature for estimation of this drug by UV and visible spectrophotometric methods.

Spectral and absorbance measurements were made on a Chemito Spectra Scan 2600 UV/Vis spectrophotometer

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for estimation in UV and on a Perkin Elmer Lamda 19 UV/Vis/NIR spectrophotometer for estimation in visible range by using 1-cm quartz cells. Gift samples of pioglitazone hydrochloride were obtained from Zydus Cadila Healthcare Ltd., Ahmedabad and Macro Laboratories., Bangalore. All the reagents used were of analytical grade. Tablets of two different brands (Pionorm, Macro Labs Ltd. and Piozone, Nicholas Piramal India Ltd.) containing 15 mg of PGH were procured from the local pharmacy.

For the UV Method, standard drug solution of PGH was prepared by dissolving 10 mg drug in 25 ml of 0.2 N sulphuric acid to get a concentration of 400 $\mu\text{g/ml}$. To construct Beer's