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Synthesis and cytotoxicity studies of some furanone derivatives

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ABSTRACT

A series of 2(5H)-Furanone derivatives were prepared starting from 3,4-dibromocrotonolactone, which was obtained from highly functionalized mucobromic acid. Lactone on treatment with various nucleophiles gave 4-substituted-3-bromo-furanone derivatives. These derivatives were tested for their short term cytotoxic activity and three of them were found active. Compound 4-(2-Aminoanilino)-3-bromo-2(*5H*)-furanone 5 was found to be most active against DLA and HeLa cell lines.

KEYWORDS

Mucohalic acid;
3,4-dibromo-5-hydroxy-2(*5H*)-furanone;
2,3-dibromo-3-formyl acrylic acid;
2,3-dibromo-4-oxo-2-butenoic acid.

INTRODUCTION

2(5H)-Furanone derivatives are a large family of heterocycles that include synthetically useful compounds, several natural products and drugs with diverse biological activities ranging from antibiotic, cytotoxic, antitumor and inhibition of cholesterol biosynthesis[1-4] Mucobromic acid or 3,4-dibromo-5-hydroxy-2(*5H*)-furanone is a highly functionalised, inexpensive starting material which can provide a simple and convenient entry to a wide variety of interesting organic compounds. Reactions of mucohalic acid series with dl penicillamine and its methyl ester gave the fused γ-lactam thiazolidines which are structurally related to penicillin's[5]. Mucobromic acid was prepared from furanoic acid by treatment with bromine in 60-70% yield or it may be prepared from furfural with bromine[6,7]. Stable reaction products of mucohalic acid with aromatic and heterocyclic thiols were synthesized and characterized. Under basic conditions the reactions proceeded with the substitution of the chlorine atom(s) by arylthiogroup(s), while in an acidic medium the hydroxyl group at C_5 was substituted. Different types of new sulphur-containing products of di- and tri substitution on the basis of mucochloric acid were obtained[8]. To study the reactivity of the system towards nucleophiles, the first series of reaction was carried out with hydrazine and its derivatives and it was found that pyridazone derivatives were the products[9].

EXPERIMENTAL

Melting points were determined on a Neolab capillary melting point apparatus and are uncorrected. UV spectra were obtained with a Schimadzu 160-A spectrophotometer. IR spectra (KBr) were recorded on a Schimadzu DR8001FT-IR. 1H NMR spectra were obtained on a Jeol GSX 400NBFT-NMR spectrom-

eter operating at 400 MHz. Chemical shifts were reported in δ units (ppm) relative to $(CH_3)_4Si$ as internal standard. Mass spectra were recorded on a Finnigan Mat 8230 mass spectrometer. Elemental analyses (C, H, N) for final compounds were performed on a Carlo Erba Model 1106 analyzer. Thin layer chromatography (TLC) was carried out on Silica gel G plates. The products were separated by column chromatography on silica gel of 60-120 mesh size.

3,4-Dibromo-2(*5H*)-furanone (2)

To a mixture of mucobromic acid in water (1g, 0.0039mol) was added sodium borohydride (0.15g, 0.0018mol) with stirring. The mixture frothed and turned yellow. After stirring for 1h at room temperature it was diluted with 2N HCl (20mL) and cooled. The solid separated was collected, dried and recrystallised from hexane.

Yield 0.65g (65%), mp 89°C; UV(CH_3OH): λmax(ε), 237nm(7000); IR(KBr) ν cm^{-1}: 1790 (C=O), 1610 (C=C), 1215 (C-O); $_1$H NMR (TMS) δppm : 4.92(s, 2H, CH_2); MS: 240 (M^+),242(M+2).

4-Azido-3-bromo-2(*5H*)-furanone (3)

To an ice cold solution of NaN_3 (100mg, .0016mol) in CH_3CN (10mL) was added 3,4-dibromocrotonolactone (200mg,0.0008mol) in CH_3CN (5mL) and stirred overnight at room temperature. It was diluted with water (10mL) and then extracted with ether (50mL). The ether layer was washed with 5% $Na_2S_2O_3$ ad then with water. The ether layer was dried and evaporated to give needle shaped crystals which was purified by crystallization from hexane.

Yield 100mg (50%), mp 60°C; UV(CH_3OH): λmax(ε), 268nm(11500); IR(KBr) cm^{-1}: 2131 (N_3), 1750 (C=O),1625 (C=C), 1230 (C-O); $_1$H NMR (TMS) δppm: 5.19(s, 2H, CH_2); MS: 203(M^+), 205(M+2).

3-Amino-3-bromo-2(*5H*)-furanone (4)

A mixture of 2 (150mg, 0.0006mol) and NaN_3 (80mg, 0.0012mol) in CH_3CN (10mL) was refluxed for 4h on a water bath. The mixture was diluted with water (15mL) and extracted with ether (100mL). The ether layer was washed with water, dried and concentrated under reduced pressure and the residue obtained was recrystallised from ethylacetate-hexane mixture (99:1).

Yield 90mg (60%), mp 178°C; Uv(CH_3OH): λmax(ε), 258nm(19560); Ir (KBr) cm^{-1}: 3300,3456 (NH_2), 1750 (C=O), 1649 (C=C); $_1$H NMR (TMS) δppm :4.73(s, 2H, CH_2), 4 (s, 2H, NH_2); MS: 268(M^+), 270(M+2). Anal. Calcd. for $C_4H_4BrNO_2$: C 28.51; H 2.06; N 7.90. Found: C 28.39; H 2.23; N 7.84.

4-(2-Amino)anilino-3-bromo-2(*5H*)-furanone (5)

To a solution of 2(200mg, 0.0008mol) in DMF (1mL) was added o-phenylene diamine (80mg, 0.00075mol) and stirred for 3h. It was poured into crushed ice and the solid separated was filtered, dried and recrystallised from ethyl acetate-hexane mixture (99:1).

Yield 100mg (50%), mp 145°C; UV(CH_3OH): λmax(ε), 267nm(14870), 240nm(13320), ; IR(KBr) cm^{-1}: 3366,3347,3312 (NH and NH_2), 1736 (C=O), 1640 (C=C); 1H NMR (TMS) δppm: 9(s, 1H, NH), 6.45-7.1(m,4H,Ar,), 5.2(s, 2H, CH_2), 4.67(s, 2H, NH_2); MS: 268(M^+), 270(M+2). Anal. Calcd. for $C_{10}H_9BrN_2O_2$: C 44.77; H 3.35; N 10.44. Found: C 44.05; H 3.13; N 10.40.

3-Bromo-4-(2-hydroxy)anilino-2(*5H*)-furanone (6)

To a solution of 3,4-dibromocrotonolactone (500mg, 0.002mol) in CH_3OH (5mL) was added o-aminophenol (200mg, 0.002mol) and two drops of pyridine. The mixture was refluxed for 3h on a water bath and poured over crushed ice. The solid separated was filtered and dried. Further purification was done on a silica column with $CHCl_3$-CH_3OH (99:1) as eluent.

Yield 350mg (70%), mp 171°C; UV(CH_3OH): λmax(ε), 280nm(23000),; IR(KBr) cm^{-1}: 3088,3341 (OH and NH), 1716 (C=O), 1626 (C=C), 1230 (C-O); $_1$H NMR (TMS) δppm :10.01(s, 1H, OH), 8.9(s, 1H, NH), 6.8-7.1(m,4H,Ar,), 4.8 (s, 2H, CH_2),; MS: 269(M^+), 271(M+2). Anal. Calcd. for $C_{10}H_8BrNO_3$: C 44.60; H 2,79; N 5.20. Found: C 44.32; H 2.80; N 5.14.

N,N'-Bis(3-bromocrotonolactonyl) hydrazine (7)

To a solution of hydrazine hydrate (0.2mL.0.004mol) in $CH_3OH$was added 3,4-dibromocrotonolactone (500mg, 0.002mol) and stirred overnight. The reaction mixture was diluted with water (25mL) and extracted with CH_2Cl_2(150mL). The com-

pound was purified by a silica column with CH_2Cl_2 as eluent and further recrystallisation from $CHCl_3$-CH_3OH to give the product.

Yield 250mg (50%), mp 150°C; UV(CH_3OH): λmax(ε), 320nm(23460), 276nm(22390; Ir (KBr) cm^{-1}: 3300 (NH), 1749 (C=O), 1626 (C=C), 1219 (C-O); $_1$H NMR (TMS) δppm: 8.5(s, 1H, NH), 5.29 (s, 2H, CH_2),; MS: 269(M^+), 271(M+2). Anal. Calcd. for $C_8H_6Br_2N_2O_4$: C 27.27; H 1.70; N 7.95. Found: C 27.9; H 1.65; N 8.01

4-Anilino-3-bromo-2(*5H*)-furanone (8)

To a cold solution of 3,4-dibromocrotonolactone 2 (100mg, 0.0004mol) in absolute ethanol(1mL) was added aniline (0.07mL, 0.0008mol) in 1mL alcohol. The mixture was cooled overnight, when a solid was formed, The solid was separated and recrystallised from $CHCl_3$.

Yield 65mg (65%), mp 81°C; UV(CH_3OH): λmax(ε), 284nm(29000); IR (KBr) cm^{-1}: 3300 (OH), 1790 (C=O), 1600 (C=C), 1259 (C-O); $_1$H NMR (TMS) δppm :9.69 (s, 1H, NH), 7.12-7.45(m,5H,Ar,) 5.12 (s, 2H, CH_2),; MS: 253(M^+), 255(M+2). Anal. Calcd. for $C_{10}H_8BrNO_2$: C 47.43; H 3.16; N 5.53. Found: C 47.22; H 3.10; N 5.63.

3-Bromo-4-N-(pyrrolidino)-2(*5H*)-furanone (9)

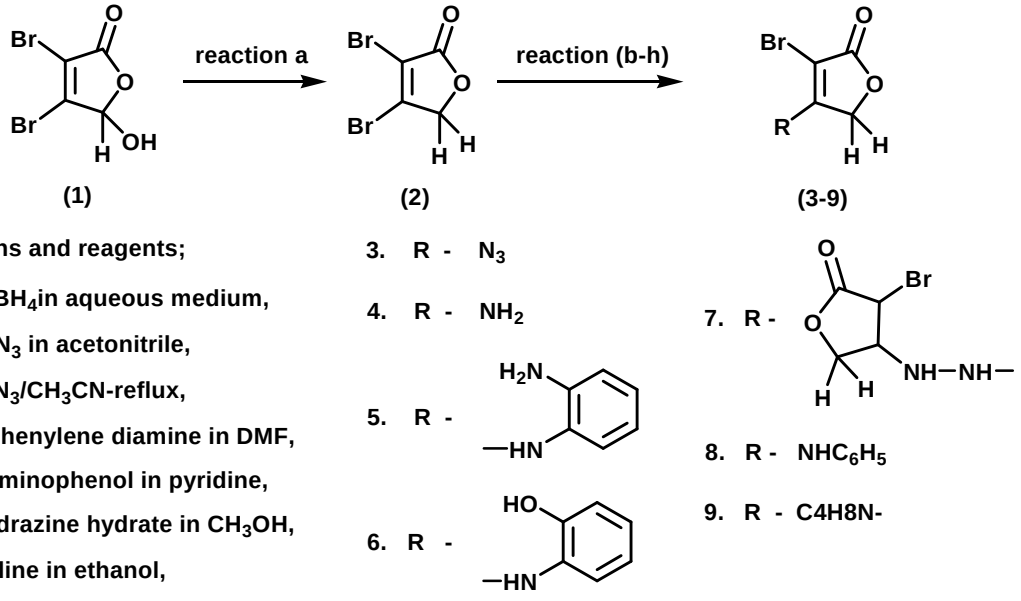
To a solution of 2(500mg, 0.002mol) in THF (3mL) was added pyrrolidine (0.3mL, 0.0042mol) in THF (3mL) The reaction mixture was stirred until the spot of lactone on TLC (methylene chloride) disappeared. The volume was reduced and diluted with water (25mL), extracted with chloroform (100mL). The solvent was removed and the component was separated by silica column using $CHCl_3$-CH_3OH (99:1) mixture as eluent.

Yield 300mg (60%), mp 135°C; UV(CH_3OH): λmax(ε), 277nm(12360); IR (KBr) cm^{-1}: 1732 (C=O), 1650 (C=C), 1259 (C-O); $_1$H NMR (TMS) δppm: 4.7 (s, 2H, CH_2), 3.3(m,4H, $2CH_2$) 2.1(m, 4H, $2CH_2$),; MS: 231(M^+), 233(M+2). Anal. Calcd. for $C_8H_{10}BrNO_2$: C 41.55; H 4.32; N 6.06. Found: C 42; H 4.46; N 5.60.

DISCUSSION

Here we are reporting some furanone derivatives prepared from 3,4-dibromocrotonolactone (Scheme 1). Mucobromic acid I was reduced with sodium borohydride in aqueous medium to give 3,4-dibromocrotonolactone **(2)**

Reduction of compound **(2)** with NaN_3 in acetonitrile at room temperature gave 4-azido-3-bromo-2(*5H*)-furanone **(3)**. The product gave peaks corresponding to M^+ and M+2 peaks at 203 and 205 respectively in the mass spectrum. NMR gave a singlet corresponding to two hydrogens at δ 5. UV spectrum showed λ_{max} at 268nm and the red shift from that of



Scheme 1

compound **(1)** is due to extended conjugation with N_3 which confirms that the azide group is on the carbon β to the carbonyl group. IR also shows strong azide absorption at 2131cm^{-1}.

The same mixture on refluxing for 3h gave 4-amino-3-bromo-2(*5H*)-furanone **(4)**. Mass spectra gave m/z peaks at 177 and 179 corresponding to M+ and M+2 ions. Ir shows band corresponding to NH_2 and C=C double bond. These data were supported by NMR and elemental analysis.

3,4-Dibromocrotonolactone was stirred with o-phenylene diamine in DMF for 3h to give a solid product which was identified as 4-(2-amino)anilino-3-bromo-2(*5H*)-furanone **(5)**. The compound contains free NH_2 and Br, but no internal cyclisation was taking place even on further heating. Compound **(2)** with o-aminophenol in CH_3OH in presence of pyridine gave a solid in 40% yield. Elemental analysis and other spectral data confirmed the product as 3-bromo-4-(2-hyroxy)anilino-3-bromo-2(*5H*)-furanone **(6)**.

Reaction of hydrazine hydrate and 3,4-dibromocrotonolactone in methanol gave 4-bis(3-bromocrotonolactonyl)hydrazine **(7)**. Reaction of lactone **(2)** with aniline in absolute alcohol gave an off white solid. IR spectra gave bands corresponding to lactone carbonyl and unsaturation. Mass spectra gave molecular ion peak along with M+ 2 peaks. All these suggest the product as 4-Anilino-3-bromo-2(*5H*)-furanone **(8)**. Secondary amines like morpholine, pyrrolidine and piperidine were reacted with the lactone and products were isolated. The product obtained from pyrrolidine alone was stable and it was identified as 4-pyrrolidino-3-bromo-2(*5H*)-furanone **(9)**.

In all these reactions the product formed was by the replacement of halogen β to the carbonyl group. No products were obtained by addition across double bond or replacement of both the halogens. The mechanism for the above reactions can be predicted as addition elimination.

ASSESSMENT OF CYTOTOXICITY

HeLa and DLA cell lines were obtained from NCCS Pune, India and maintained at Laboratory of Tumor Immunology and Functional Genomics, Regional Cancer Centre, Thiruvananthapuram, India. Furanone derivatives were dissolved in DMSO and serially diluted using micropipettes. Cytotoxicity was then assessed using the Trypan Blue Dye exclusion staining and MTS non-radioactive cell proliferation assay. All procedures described were reviewed and approved by the Institutional Animal Ethical Committee.

Short-term invitro cytotoxicity of furanone derivatives using trypan blue

This dye exclusion test is used to determine the number of viable cells present in a cell suspension. For Trypan Blue staining, HeLa/DLA cell lines were incubated with varying concentrations of the furanone derivatives starting from 2000 μg/mL to 0.98μg/mL for 3 hours and 1% Trypan Blue was added for a minute and counted. Viable cells exclude dye while non-viable cells are blue colored. Cytotoxicity is determined by calculating percentage cell deaths.

Determination of long term invitro cytotoxicty by furanone derivatives in DLA (HeLa) cells using MTS assay

MTS assay uses the soluble tetrazolium salt, MTS, which is versatile and advantages over other cytotoxicity assays due to the solubility of the MTS formazan product in culture medium. The measurement of the absorbance of the formazan can be carried out using 96 well microplates at 490nm. The assay measures dehydrogenase enzyme activity found in metabolically active cells. Since the production of formazan is proportional to the number of living cells; the intensity of the produced color is a good indication of the viability of the cells.

DLA (HeLa) cells (5000 cells / well), control 1 (medium only) and control 2 (medium + cells) were seeded in triplicates in 96 well microtitre plates and incubated for attachment at 37°C in 5%CO_2 incubator for 12 hours. After 12 hours, 100 μl of different concentrations of the four active furanone derivatives (concentration ranging from 2000 μg/ml to 0.98 μg/ml) were added to each well excluding the control wells. The plates were then incubated at 37°C in 5% CO_2 incubator for 48 hours. 20 μL of MTS-PMS solution was added and incubated in dark for another 4 hours and absorbances were recorded at 490 nm using ELISA plate reader. IC_{50} values of the four furanone derivatives were calculated as per MTS assay.

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RESULTS

Effect of furanone derivatives on short term *invitro* cytotoxicity

Results of short term cytotoxicity indicated 4 out of 9 compounds in 12 concentrations showed cytotoxic activity against DLA as well as HeLa cell lines in about 3 hours. The cytotoxicity was more severe in DLA cells which brought about 85% of the cell death by Compound **(5)** followed by compound **(8)** 65%, then compound **(3)** 55% and compound **(6)** 45%. In the case of HeLa cells, the cytotoxicity was observed in the order **5**(70%) > **8**(57%) > **3**(56%)> **6**(44%).

Effect of furanone derivatives on long term *invitro* cytotoxicity

Results of long term cytotoxicity of Furanone derivatives by MTS assay confirmed cytotoxicity of these 4 compounds in the order **(5)** > **(8)** > **(3)** > **(6)** in both the cell lines. Compound **(5)** showed the maximum cytotoxic ability in DLA as well as HeLa cell lines in all the doses tested. The IC_{50} values obtained against DLA cells for **(5)** (62.5µg/mL) was the lowest and **(6)** (1350 µg/mL) was the highest, whereas IC_{50} values against HeLa for **(5)** (225 µg/mL) was the lowest and **(6)** (1450 µg/mL) was the highest (TABLE 1).

TABLE 1 : IC_{50} values as per MTS assay

Compound	DLA	HeLa
5	62 µg/mL	225 µg/mL
8	125 µg/mL	500 µg/mL
3	750 µg/mL	1000 µg/mL
6	1350 µg/mL	1450 µg/mL

CONCLUSION

Starting from mucobromic acid a series of furanone derivatives have been prepared and characterized. All the compounds were screened for invitro cytotoxicity and compounds **(5)**, **(8)**, **(3)**, and **(6)** were active. 4-(2-Amino)anilino-3-bromo-2(*5H*)-furanone **(5)** was found to be most active against HeLa and DLA cell lines. According to the review of Lee et al.[11] the electrophilic binding of the reactive unsaturated lactones to cellular nucleophiles such as enzymes or DNA may be proposed as the mechanism of their cytotoxic action[12]. Thus the derivatives **(5)**, **(8)**, **(3)** and **(6)** are considered as agents with antitumour activity and can therefore be suggested for further invitro and invivo screening.

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