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SYNTHESIS AND BIOLOGICAL EVALUATION OF NEWER BENZOFURAN DERIVATIVES AS POTENTIAL ANTICANCER AND ANTHELMINTIC AGENTS

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ABSTRACT

In the present study we have made an attempt to synthesize novel benzofuran derivatives and evaluate them as potential therapeutic agents by anticancer screening. First, 2-ethoxy carbomethoxy (1) was prepared from 2-hydroxy acetophenone and ethylchloroacetate, which gave ethyl-3-methyl-2-benzofuran carboxylate (2) on reaction with anhydrous potassium carbonate. Treatment of ethyl-3-methyl-2-benzofuran carboxylate (2) with hydrazine hydrate afforded 3-methyl-2-benzofuran carbohydrazide (3) and then Condensation of 3-methyl-2-benzofuran carbohydrazide (4) with various substituted benzaldehydes led to a novel series of benzofuran derivatives (5a-k). The synthesized compounds were analyzed by physical and analytical data. The synthesized compounds were evaluated for their short-term anticancer activity. The synthesized imidazole derivative possessed significant cytotoxic activity against Ehrlich's Ascites Carcinoma (EAC) cell lines and HEP_2 cell line by SRB assay. The synthesized compounds have shown moderate to good anthelmintic activity.

KEY WORDS: Benzofuran. Schiff's base. Anticancer activity. Anthelmintic activity.

INTRODUCTION

The discovery of new compounds with anticancer activity has become one of the most important goals in medicinal chemistry. One of the most often used classes of chemotherapeutic agents in cancer therapy comprises molecules that interact with DNA, such as groove binders, DNA alkylating substances and intercalators. Moreover, the study of the exact mechanism of action of these agents, as well as, DNA damage of the cancer cells are of

high interest for medicinal chemists and molecular biologists. For example, the intercalation of planar aromatic molecules into the DNA double helix and poisoning of DNA topoisomerases I and/or II are considered to be important in the therapeutic action of many anticancer agents.

Syntheses of heterocyclic compounds from readily available reagents by simple and efficient methods are the major requirements of heterocyclic chemistry. A survey of the pertinent literature reveals that, benzofuran derivatives possess diverse biological activities apart from their synthetic interests. They are reported to exhibit pharmacological activities such as cognitive enhancers. [1] anticancer [2-7] antithrombonic. [8] antifungal [9-11] and anti-inflammatory.[12-14] anticonvulsant activities. [15] They were also found to be useful as anticonvulsant and antidepressant agents and cardiac phosphpodiesterase inhibitors. Some of the bestselling therapies today contain this versatile heterocycle in their core structures. Therefore, it would be difficult to underestimate the importance of benzofuran in the pharmaceutical industry. [16-20]

MATERIAL AND METHOD

The analytical grade chemicals and reagents of SIGMA ALDRICH were used to synthesize all the reported compounds. The melting points were determined in open capillaries and are uncorrected. The temperatures were expressed in ºC and are uncorrected. The IR spectra of compounds were recorded on Perkin-Elmer infrared-283 FTIR spectrometer by KBr pellet technique and are expressed in cm^{-1}. ^{1}H-NMR spectra were recorded on Bruker DRX-300 (300 MHZ, FT NMR) spectrophotometer using TMS as an internal standard, $CDCl_3$ and DMSO-d_6 as solvents. Mass spectrum was obtained using LC-MS (Schimadzu-2010AT) under Electro Spray Ionization (ESI) technique and elemental analysis was performed using Elemental Vario EL III, Carlo-Erba 1108. TLC was performed to monitor the reactions and to determine the purity of the products on a precoated aluminum plates using 10% methanol in chloroform or 20% ethyl acetate in chloroform as a mobile phase.

2-Carbethoxy-3 (2H) benzofuranone (2)

A mixture of freshly distilled methyl salicylate 1 (15.2 g), ethyl bromomalonate (24 g) and anhydrous potassium carbonate (30 g) in dry acetone (150 ml) was heated under reflux on a water bath while stiring magnetically for 13 hours. The reaction product was filtered and potassium salts were washed with dry ether until colorless. The dry salt was suspended in water (200 ml) and cooled thoroughly in ice bath. The suspension was carefully acidified

with dilute acid and the 2-Carbethoxy-3 (2H) benzofuranone which separate as colorless solid was collected by filtration. On crystallization from Petroleum ether it was obtained as colourless needles (m.p. 64°C, Yield 14 g).

2-Carbethoxy-3-methoxybenzofuranone (3)

To a solution of 2-Carbethoxy-3 (2H) benzofuranone (1; 10.3 g) in acetone (150 ml), anhydrous K_2CO_3 (25 g) and dimethyl sulphate (8.1 g) were added and the mixture was heated under reflux on a water reflux for 6 hr, cooled and filtered. Removal of solvent under reduced pressure from the filtrate gave thick oil which solidified slowly. It crystallized from Pet. Ether to give 2 as colourless prisms (10.5 g), mp. 61°C.

3-Methoxybenzofuran-2-carbohydrazide (4)

To a solution of 2 (10 g) in ethanol (30 ml), hydrazine hydrate (15 ml, 99%) Was added and the mixture heated under reflux on a water bath for about 4 hr., Most of the ethanol removed under reduced pressure, m cooled and then diluted with water, when a colourless solid separated out. It was filtered and crystallized from benzene to give 3 as colourless needles (6.6 g, 70.5%), mp.110°C.

General synthetic procedure for *N'*-(4-Substituted benzylidene)-3'-methoxybenzofuran-2'-carbohydrazide (5a-k) [Scheme]

Equimolar amounts (0.005 M) of 3-Methoxybenzofuran-2-carbohydrazide **4** and aromatic aldehydes were transferred to a 250 ml flat bottom flask containing 20 ml of ethanol:dioxane mixture (9:1 v/v) and refluxed for 10 h. The reaction mixture was allowed to cool to give product and then recrystallized from ethanol to give substituted benzofurans **(5a-k) .**

RESULTS AND DISCUSSION

The results and discussion pertaining to the synthesis of benzofuran derivative are as follows:

In view of these observations and in continuation of our endeavour to develop better and potent anticancer agents a new series of benzofuran derivatives were synthesized. First, 2-ethoxy carbomethoxy (1) was prepared from 2-hydroxy acetophenone and ethylchloroacetate, which gave ethyl-3-methyl-2-benzofuran carboxylate (2) on reaction with anhydrous potassium carbonate. Treatment of ethyl-3-methyl-2-benzofuran carboxylate (2) with hydrazine hydrate afforded 3-methyl-2-benzofuran carbohydrazide (3) and then Condensation of 3-methyl-2-benzofuran carbohydrazide (4) with various substituted benzaldehydes led to a novel series of benzofuran derivatives **(5a-k)**. On the basis of above facts that the novel series

of synthesized benzofuran derivative may yield compounds with high therapeutic potential. All the synthesized compounds were analyzed by TLC (* Chloroform: Methanol (3:1)), mp, FT-IR, ^{1}H-NMR, ^{13}C-NMR, MASS and elemental analysis.

The FT-IR spectra shows a characteristic peak for -N=CH- between 1561-1590 and for C-O-C between 1101-1137 for compound containing benzofuran ring. The ^{1}H-NMR showed a characteristic peak for OCH_3 between 3.00-3.55 δ ppm and characteristic peak for NH was observed between 9.11-9.32 δ ppm for compound containing hydrazide group.

Anticancer activity of the synthesized compounds was evaluated by determining the percentage growth inhibition of Erlich's Ascites Carcinoma (EAC) cells by tryphan blue dye exclusion technique. Compounds **5a, 5c, 5d, 5f, 5i, and 5j** showed good anticancer activity with CTC_{50} (cytotoxic concentration). The CTC_{50} values of the newly synthesized benzofurans are shown in **Table 1.** Presence of CONH group is essential for anticancer activity in benzofuran derivative. Presence of Phenolic and Chlorine group in compound **5c**, **5d** and **5i** significantly affect activity due to the binding capability to the cytoplasmic hormone receptors. Compound **5a** containing nitro group in its structure decreases the melting temperature of DNA in EAC cells and thereby showing significant activity**.**

Anthelmintic activity of the synthesized compounds was carried out against species of earthworms *M. konkanensis* at 4mg/ml concentration. All the compounds showed moderate to good activity at 100mg in tween 80 (0.5%) and distilled water. Comparison of anthelmintic data (Table 2) revealed that derivative **5a, 5b, 5c, 5f and 5h** possessed higher activity against *M. konkanensis* species in comparison to standard Mebendazole.

The analytical and spectral data of final compounds are given in the following text (5a-k).

***N'*-(4-Nitrobenzylidene)-3'-methoxybenzofuran-2'-carbohydrazide (5a)**

Pale yellow needles; IR (KBr) cm^{-1}: 3355 (N-H), 3068 (Ar C-H), 1693 (C=O), 1571 (C=N), 1452 (NO_2), 1271 (C-N), 1110 (C-O-C); ^{1}H-NMR ($CDCl_3$-d_6, ppm) δ: 3.12 (s, 3H, OCH_3), 6.81-8.01 (m, 8H, Ar-H), 9.15 (s, 1H, NH), 10.48 (s, 1H, =CH); ^{13}C-NMR δ : 56.5, 111.0, 120.9, 121.7 (2C), 122.9, 124.1, 127.6, 130.9 (2C), 130.8, 140.1, 142.8, 150.1, 151.9, 158.2, 167.7; MS (m/z): 339.10, 339.85 $(M+H)^{+}$; Calcd. for $C_{17}H_{13}N_3O_5$: C, 60.18; H, 3.86; N, 12.38; O, 23.58; Found: C, 60.23; H, 3.84; N, 12.51; O, 23.53.

***N'*-(4-Methoxybenzylidene)-3'-methoxybenzofuran-2'-carbohydrazide (5b)**

White amorphous solid; (KBr) cm^{-1}: 3347 (N-H), 3055 (Ar C-H), 1666 (C=O), 1580 (C=N), 1278 (C-N), 1120 (C-O-C); ^{1}H-NMR ($CDCl_3$-d_6, ppm) δ: 3..06 (s, 3H, OCH_3), 3.55 (s, 3H, OCH_3), 6.75-7.90 (m, 8H, Ar-H), 9.21 (s, 1H, NH), 10.41 (s, 1H, =CH); ^{13}C-NMR δ : 56.4, 56.9, 111.1, 114.9 (2C), 120.5, 122.6, 123.9, 126.6, 129.0, 129.9 (2C), 131.7, 142.6, 152.0, 157.9, 163.7, 166.6; MS (m/z): 324.10, 324.72 $(M+H)^+$; Calcd. for $C_{18}H_{16}N_2O_4$: C, 66.66; H, 4.97; N, 8.64; O, 19.73; Found: C, 66.61; H, 5.00; N, 8.68; O, 19.70.

***N'*-(4-Hydroxybenzylidene)-3'-methoxybenzofuran-2'-carbohydrazide (5c)**

Pale white crystal; IR (KBr) cm^{-1}: 3414 (O-H), 3346 (N-H), 3053 (Ar C-H), 1675 (C=O), 1580 (C=N), 1263 (C-N), 1118 (C-O-C); ^{1}H-NMR ($CDCl_3$-d_6, ppm) δ: 3.12 (s, 3H, OCH_3), 6.91-8.05 (m, 8H, Ar-H), 9.12 (s, 1H, NH), 9.71 (s, 1H, OH), 10.01 ppm (s, 1H, =CH); ^{13}C-NMR δ : 57.6, 110.9, 116.4 (2C), 120.8, 122.9, 124.1, 125.8, 128.9, 130.1 (2C), 130.6, 142.0, 152.1, 158.9, 161.1, 166.9; MS (m/z): 310.12, 310.60 $(M+H)^+$; Calcd. for $C_{17}H_{14}N_2O_4$: C, 65.80; H, 4.55; N, 9.03; O, 20.62; Found: C, 65.85; H, 4.56; N, 9.10; O, 20.73.

***N'*-(4-Chlorobenzylidene)-3'-methoxybenzofuran-2'-carbohydrazide (5d)**

Bright yellow amorphous solid; IR (KBr) cm^{-1}: 3344 (N-H), 3051 (Ar C-H), 1680 (C=O), 1590 (C=N), 1266 (C-N), 1101 (C-O-C), 680 (C-Cl); ^{1}H-NMR ($CDCl_3$-d_6, ppm) δ: 3.19 (s, 3H, OCH_3), 6.85-7.99 (m, 8H, Ar-H), 9.20 (s, 1H, NH), 10.41 (s, 1H, =CH); ^{13}C-NMR δ : 56.7, 110.9, 120.7, 123.8, 124.1, 127.9, 129.5 (2C), 130.1 , 130.8, 131.2, 136.1, 143.6, 151.8, 158.1, 160.3, 166.8; MS (m/z): 328.10, 328.67 $(M+H)^+$; Calcd. for $C_{17}H_{13}ClN_2O_3$: C, 62.11; H, 3.99; Cl, 10.78; N, 8.52; O, 14.60; Found: C, 62.19; H, 4.06; Cl, 10.71; N, 8.56; O, 14.63.

***N'*-(3-Nitrobenzylidene)-3'-methoxybenzofuran-2'-carbohydrazide (5e)**

Yellow crystal; IR (KBr) cm^{-1}: 3342 (N-H), 3047 (Ar C-H), 1677 (C=O), 1585 (C=N), 1460 (NO_2), 1271 (C-N), 1121 (C-O-C); ^{1}H-NMR ($CDCl_3$-d_6, ppm) δ: 3.09 (s, 3H, OCH_3), 6.92-8.10 (m, 8H, Ar-H), 9.30 (s, 1H, NH), 10.43 (s, 1H, =CH); ^{13}C-NMR δ : 57.3, 110.9, 121.2, 123.8, 122.9, 123.8, 124.3, 128.9, 129.1, 130.7, 134.5, 135.1, 142.6, 148.6, 152.2, 157.9, 167.3; MS (m/z): 339.12, 324.69 $(M+H)^+$; Calcd. for $C_{17}H_{13}N_3O_5$: C, 60.18; H, 3.86; N, 12.38; O, 23.58; Found: C, 60.23; H, 3.99; N, 12.34; O, 23.63.

***N'*-(2-Nitrobenzylidene)-3'-methoxybenzofuran-2'-carbohydrazide (5f)**

Brown amorphous solid; IR (KBr) cm^{-1}: 3346 (N-H), 3049 (Ar C-H), 1671 (C=O), 1578 (C=N), 1466 (NO_2), 1268 (C-N), 1125 (C-O-C); ^{1}H-NMR ($CDCl_3$-d_6, ppm) δ: 3.16 (s, 3H, OCH_3), 6.83-8.03 (m, 8H, Ar-H), 9.19 (s, 1H, NH), 10.45 (s, 1H, =CH). ^{13}C-NMR δ : 56.7,

111.4, 120.8, 121.5, 123.9, 124.6, 126.7, 128.0, 129.9, 130.8, 132.6, 134.7, 142.7, 150.1, 152.2, 158.9, 166.9; MS (m/z): 339.13, 339.76 $(M+H)^+$; Calcd. for $C_{17}H_{13}N_3O_5$: C, 60.18; H, 3.86; N, 12.38; O, 23.58; Found: C, 60.21; H, 3.92; N, 12.37; O, 23.63.

***N'*-[4-(Dimethylamino) benzylidene]-3'-methoxybenzofuran-2'-carbohydrazide (5g)**

Off white crystal; IR (KBr) cm^{-1}: 3356 (N-H), 3062 (Ar C-H), 1662 (C=O), 1561 (C=N), 1271 (C-N), 1116 (C-O-C); ^{1}H-NMR ($CDCl_3$-d_6, ppm) δ: 3.07 (s, 3H, OCH_3), 3.65 (s, 6H, CH_3), 6.92-8.12 (m, 8H, Ar-H), 9.23 (s, 1H, NH), 10.51 (s, 1H, =CH-).^{13}C-NMR δ : 40.7 (2C), 56.8, 111.7, 114.0 (2C), 120.7, 122.9 (2C), 124.1, 128.9, 129.8 (2C), 131.7, 142.9, 152.0, 152.9, 158.1, 166.4; MS (m/z): 324.10, 324.72 $(M+H)^+$; Calcd. for $C_{19}H_{19}N_3O_3$: C, 67.64; H, 5.68; N, 12.46; O, 14.23 Found: C, 67.61; H, 5.71; N, 12.41; O, 14.30.

***N'*-(4-Fluorobenzylidene)-3'-methoxybenzofuran-2'-carbohydrazide (5h)**

Redish white crystal; IR (KBr) cm^{-1}: 3339 (N-H), 3049 (Ar C-H), 1671 (C=O), 1577 (C=N), 1251 (C-N), 1127 (C-O-C), 1110 (C-F); ^{1}H-NMR ($CDCl_3$-d_6, ppm) δ: 3.14 (s, 3H, OCH_3), 6.84- 8.09 (m, 8H, Ar-H), 9.31 (s, 1H, NH), 10.21 (s, 1H, =CH). ^{13}C-NMR δ : 57.6, 110.9, 115.1 (2C), 120.6, 123.8, 124.2, 128.9, 128.8, 130.1 (2C), 131.7, 142.8, 152.6, 158.1, 164.9, 167.3; MS (m/z): 312.15, 312.81 $(M+H)^+$; Calcd. for $C_{17}H_{13}FN_2O_3$: C, 65.38; H, 4.20; F, 6.08; N, 8.97; O, 15.37; Found: C, 65.42; H, 4.18; F, 6.09; N, 9.10; O, 15.41.

***N'*-(3-Chlorobenzylidene)-3'-methoxybenzofuran-2'-carbohydrazide (5i)**

Cream colour crystal; IR (KBr) cm^{-1}: 3341 (N-H), 3060 (Ar C-H), 1662 (C=O), 1565 (C=N), 1263 (C-N), 1127 (C-O-C), 691 (C-Cl); ^{1}H-NMR ($CDCl_3$-d_6, ppm) δ: 3.00 (s, 3H, OCH_3), 6.81-7.99 (m, 8H, Ar-H), 9.11 (s, 1H, NH), 10.58 (s, 1H, =CH); ^{13}C-NMR δ : 56.7, 111.0, 121.8, 122.8, 124.2, 126.9, 128.0, 129.9, 130.1, 130.8 (2C), 134.0, 135.5, 142.5, 152.8, 158.1, 167.7; MS (m/z): 328.07, 328.89 $(M+H)^+$; Calcd. for $C_{17}H_{13}ClN_2O_3$: C, 62.11; H, 3.99; Cl, 10.78; N, 8.52; O, 14.60; Found: C, 62.06; H, 4.10; Cl, 10.73; N, 8.50; O, 14.70.

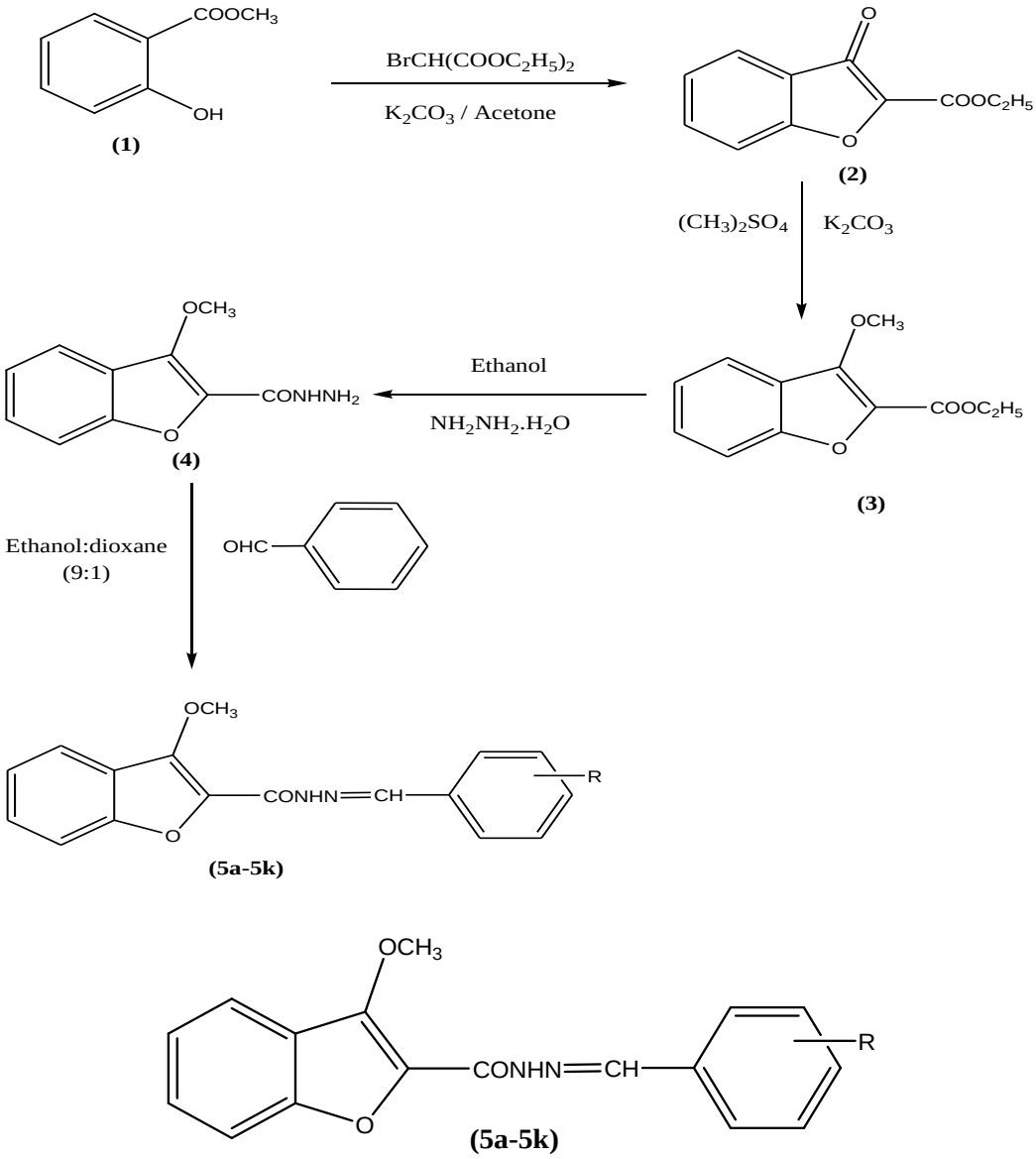
***N'*-(2-Chlorobenzylidene)-3'-methoxybenzofuran-2'-carbohydrazide (5j)**

White crystal; IR (KBr) cm^{-1}: 3348 (N-H), 3058 (Ar C-H), 1660 (C=O), 1570 (C=N), 1271 (C-N), 1118 (C-O-C), 680 (C-Cl); ^{1}H-NMR ($CDCl_3$-d_6, ppm) δ: 3.02 (s, 3H, OCH_3), 6.79-7.95 (m, 8H, Ar-H), 9.23 (s, 1H, NH), 10.36 (s, 1H, =CH); ^{13}C-NMR δ : 57.3, 111.1, 120.2, 122.8, 124.1, 127.7, 128.1, 129.7, 129.9, 131.7, 132.0, 133.4, 134.7, 142.8, 153.0, 158.1, 167.7; MS (m/z): 328.11, 328.79 $(M+H)^+$; Calcd. for $C_{17}H_{13}ClN_2O_3$: C, 65.29; H, 4.19; Cl, 11.34; N, 8.96; O, 19.73; Found: C, 65.26; H, 4.16; Cl, 11.40; N, 9.01; O, 19.69.

***N'*-(3-Methoxybenzylidene)-3'-methoxybenzofuran-2'-carbohydrazide (5k)**

White amorphous solid; IR (KBr) cm^{-1}: 3351 (N-H), 3045 (Ar C-H), 1567 (C=N), 1672 (C=O), 1277 (C-N), 1137 (C-O-C); ^{1}H-NMR ($CDCl_3$-d_6, ppm) δ: 3.11 (s, 3H, OCH_3), 3.61 (s, 3H, OCH_3), 6.92-8.08 (m, 8H, Ar-H), 9.32 (s, 1H, NH), 10.38 (s, 1H, =CH); ^{13}C-NMR δ : 56.2, 56.8, 111.3, 112.9, 116.1, 120.8, 121.1, 123.9, 123.9, 128.8, 130.2, 131.7, 135.0, 143.6, 152.2, 159.0, 161.1, 166.7; MS (m/z): 324.09, 324.72 $(M+H)^+$; Calcd. for $C_{18}H_{16}N_2O_4$: C, 66.66; H, 4.97; N, 8.64; O, 19.73; Found: C, 66.60; H, 5.00; N, 8.61; O, 19.76.

Scheme



Tab1. Data of all synthesized benzofuran derivative compounds screened against EAC cell line and HEP_2 cell line.

Compound No.	R	m.p. (°C)	R_f^* value	% Yield	CTC_{50} Value	
					EAC cell	HEP_2 cell
5a	*p*- NO_2	240-241	0.68	67.2	35.5 µg/ ml	32.65 µg/ ml
5b	*p*-OCH_3	220-222	0.65	70.2	472 µg/ml	460 µg/ml
5c	*p*-OH	233-234	0.70	69.7	33.50µg/ml	31.25µg/ml
5d	*p*-Cl	260-261	0.63	67.2	33.75µg/ml	31.75µg/ml
5e	*m*-NO_2	238-239	0.71	72.1	255 µg/ ml	250 µg/ ml
5f	*o*-NO_2	242-245	0.67	73.3	43 µg/ ml	40 µg/ ml
5g	*p*-$N(CH_3)_2$	247-248	0.63	62.8	280µg/ml	270 µg/ ml
5h	*p*-F	243-244	0.61	61.2	365 µg/ ml	360 µg/ ml
5i	*m*-Cl	257-255	0.68	74.3	34 µg/ ml	32.75µg/ml
5j	*o*-Cl	256-258	0.78	68.3	49 µg/ ml	45 µg/ ml
5k	*m*-OCH_3	225-228	0.65	71.4	478 µg/ ml	465 µg/ ml

CTC_{50} = the cytotoxic concentration (which inhibited 50% of total cells).

Tab2. Data of all synthesized benzofuran derivative compounds screened against species of earthworms *M. konkanensis.*

Compound No.	*M. konkanensis*	
	Mean paralyzing time (min)	Mean death time (min)
5a	15.50 ± 0.50	22.33 ± 1.52
5b	14.16 ± 1.25	23.16 ± 0.76
5c	15.37 ± 0.57	24.33 ± 0.57
5d	26.66 ± 0.57	33.66 ± 0.57
5e	32.66 ± 0.57	39.16 ± 0.76
5f	16.83 ± 0.76	23.33 ± 0.57
5g	38.16 ± 1.75	45.66 ± 0.57
5h	15.33 ± 0.57	22.33 ± 0.57
5i	29.16 ± 1.25	38.66 ± 0.57
5j	22.00 ± 1.00	28.33 ± 0.57
5k	26.00 ± 1.00	32.00 ± 1.00
Control	-	-
Mebendazole	18.22 ± 1.00	25.37 ± 1.00

ANTICANCER STUDIES BY EAC CELL LINE METHOD

Anticancer activities of the synthesized compounds were assessed by determining the percentage inhibition of EAC cells by tryphan blue dye exclusion technique according to the standard procedure [21-24]. We checked anticancer activity of all the synthesized compounds at the concentration of 500, 250, 125, 62.5, 31.25 µg/ml. The percentage growth inhibition was calculated by using the following formula:

% Growth inhibition = [(Total cells – Live cells) × 100]/Total cells.

The CTC_{50} values were calculated by plotting the graph between concentration versus percentage growth inhibition and by bisecting concentration at the 50% growth inhibition. The synthesized benzofuran derivatives and their CTC_{50} values are as shown in **Table 1.**

ANTICANCER STUDIES BY SRB ASSAY

The study was done in HEP_2 cell line. SRB is a bright pink aminoxanthine dye with two sulphonic groups. Under mild acidic conditions, SRB binds to protein basic amino acid residues in trichloroacetic acid (TCA) fixed cells to provide a sensitive index of cellular protein content that is linear over a cell density range of at least two orders of magnitude. Colour development in SRB assay is rapid, stable and visible. The developed color can be measured over a broad range of visible wavelength in 96 well plate readers. When TCA-fixed, SRB stained samples are air dried. They can be stored indefinitely without deterioration. The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 X 10^5 cells/ml using medium containing 10% new born calf serum. To each well of the 96 well microtitreplate, 0.1 ml of the diluted suspension (approximately 10,000 cells) was added. After 24 hours, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once and µL of different drug concentrations was added to the cells in microtitre plates. The plates were then incubated at 37º for 3 days in 5% CO_2 atmosphere, and microscopic examination was carried out and observations recorded every 24 hours. After 72 hours, 25 µL of 50% trichloroacetic acid was added to the wells gently such that it forms a thin layer over the drug dilutions to form a over all concentration of 10%. The plates were incubated at 4ºC for one h. The plates were flicked and washed five times with tap water to remove traces of medium, drug and serum and were then air dried. The air dried plates were stained with Sulforhodamine B, a protein binding dye for 30 minutes. The unbound dye was then removed by rapidly washing four tomes with 1% acetic acid. The plates were then air dried. 100 µL of 10 Mm tris base was then added to the wells to solubilize the dye. The plates were shaken vigorously for 5 minutes. The absorbance was measured using microplate readed at a wavelength at 540 nm. The percentage growth inhibition was calculated. [25-26] The results are given in *table 1.*

ANTHELMINTIC STUDIES

Anthelmintic activity studies were carried out against species of earthworms *M. konkanensis* at 2 mg ml^{-1} concentration using Garg and Atal method. Suspensions of samples were prepared by triturating synthesized compounds (100 mg) with Tween 80 (0.5%) and distilled

water and the resulting mixtures were stirred using a mechanical stirrer for 30 min. The suspensions were diluted to contain 0.2% w/v of the test samples. Suspension of reference drug, mebendazole, was prepared with the same concentration in a similar way. Three sets of five earthworms of almost similar sizes (2 inch in length) were placed in Petri plates of 4 inch diameter containing 50 ml of suspension of test sample and reference drug at RT. Another set of five earthworms was kept as control in 50 ml suspension of distilled water and Tween 80 (0.5%). The paralyzing and death times were noted and their mean was calculated for triplicate sets. The death time was ascertained by placing the earthworms in warm water (50 °C) which stimulated the movement, if the worm was alive. The results are given in **table 2.**

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