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Synthesis, Antitumor and Antibacterial Activities of Certain Substituted Pyrimidines Bearing Benzofuran

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Benzofuran chalcones (2a-i) were prepared by the reaction of 2-acetylbenzofuran (1) with different aromatic aldehydes in the presence of a strong base. Cyclocondensation of benzofuran chalcones with guanidine hydrochloride, thiourea and urea resulted in the formation of various aminopyrimidines (3a-i), thiopyrimidines (4a-i) and hydroxy pyrimidines (5a-i), respectively. The structures of all the compounds (2, 3, 4, 5 a-i) have been established on the basis of analytical and spectral data. All the compounds have been screened for antitumour and antimicrobial activities. Compounds 3b and 3d showed significant antitumour activity. While compounds 4d and 4h showed only moderate activity against *Staphylococcus aureus* at 500 μg/ml, compounds 4h, 4i, 5a, 5b, 5h and 5i showed promising activity against *Candida albicans* at 500 μg/ml concentrations.

The pyrimidine derivatives have been reported to possess a variety of biological activities. Notable among these is the anticancer activity. Benzofuran derivatives have been reported to possess sedative and hypnotic[1], anticonvulsant[2], CNS stimulant[3], antibacterial[4-6] and antifungal activities[7]. In view of these observations it was thought worthwhile synthesizing the title compounds, as they appeared to be highly promising.

Various benzofuran chalcones (2a-i) were synthesized by reacting 2-acetyl benzofuran (1) with different aromatic aldehydes in the presence of an alkali. The benzofuran chalcones were reacted with guanidine hydrochloride, thiourea and urea in presence of sodium hydroxide to get the corresponding aminopyrimidines (3a-i), thiopyrimidines (4a-i) and hydroxy pyrimidines (5a-i). The structures of all the compounds have been established on the basis of analytical and spectral data. All the twenty-five synthesized compounds were screened for antitumour activity by Brine Shrimp bioassay method followed by *in vivo* survival time method using Ehrlich Ascitic Carcinoma. Antibacterial (*Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Escherichia coli and Klebsiella)* and antifungal (*Candida albicans)* activities were also studied.

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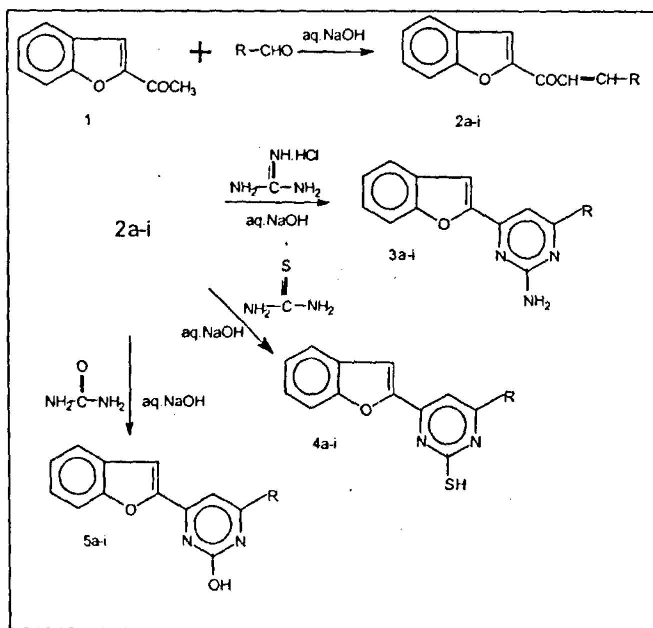
MATERIALS AND METHODS

Melting points were determined in open capillary tubes and are uncorrected. The purity of the synthesized compounds was checked by TLC using silica gel-G glass plate method using ethanol:water (7:3) as eluent and visualized in an iodine chamber. IR spectra were recorded (in KBr) on FTIR 8300 (Shimadzu) spectrophotometer. NMR spectra were recorded on Gemini 200 MHz: S_2 Pul using TMS as internal standard. All the chemicals used were of analytical grade.

General method of synthesis:

2-Acetylbenzofuran (0.01 mol) and aromatic aldehyde (0.01 mol) in ethanol (20 ml) were stirred together for 24 hrs in 10% aqueous sodium hydroxide (8 ml). It was then diluted with water (100 ml) and acidified with concentrated HCl. The product (substituted chalcones) obtained was filtered, washed with water and crystallized from ethanol. IR (KBr) cm^{-1} (2b): 1650 (C=O), 1620 (C=C), 1080 (C-O-C) of



Scheme 1: Synthesis of Aminopyrimidines, Thiopyrimidines and Hydroxypyrimidines bearing Benzofuran moiety.

benzofuran and 2830 ($O-CH_3$ str). H^1 NMR ($CDCl_3$) δ ppm: 6.68-7.00 (m, 9H, Ar-H), 6.60 (d, 1H, -COCH=), 8.60 (d, 1H, =CH-Ar), 3.7 (s, 3H, $Ar-OCH_3$); MS: m/z: 262 (8.4%), M^+ peak.

Chalcone (0.01 mol) and guanidine hydrochloride/thiourea/urea (0.01 mol) in ethanol (50 ml) were refluxed for 6 h in aqueous sodium hydroxide (0.01mol). The reaction mixture was poured into 250 ml water. The product obtained was filtered, washed with water and recrystallised from ethanol (Table 1).

4-(2-Benzofuran)-6-(4'-chloro phenyl)-2-amino pyrimidine (3g):

IR (KBr) cm^{-1}: 3340.27 (N-H Str), 1665.45 (N-H Bend), 1612.80 (C=C), 1550.70 (C=N), 1138.04 (C-O-C), 881.45 (C-N), 822.88, 939.21, 1255.16 (C-H), 743.83 (C-Cl). H^1-NMR ($CDCl_3$) δ ppm: 6.85 - 8.3m (10H; aromatic protons), 6.63s(2H; NH_2).

4-(2-Benzofuran)-6-(4'-methoxy, 3'-hydroxy phenyl)-2-thio pyrimidine (4a):

IR (KBr) cm^{-1}: 3120 (C-H Str, Aromatic), 2590 (S-H), 1593.38 (C=C), 1553 (C=N), 1478.74, 1137.54 (C-O-C), 881.63 (C-N), 787.53 (C-S), 753.94, 727.63 (C-H Def, out of plane, 4 adj H); H^1- NMR ($CDCl_3$) δ ppm: 6.7 - 8.1m (9H; aromatic protons), 7.86d (J=7.7 Hz), 7.76d (J=8.3 Hz), 3.3s (1H; S-H), 2.75s (3;$Ar-OCH_3$), 2.34s (3H; $Ar-CH_3$).

4-(2-Benzofuran)-6-(4'-methoxy, 3'-hydroxy phenyl)-2-Hydroxy pyrimidine (5a):

IR (KBr) cm^{-1}: 2917.85 (O-H enolic), 881.45 (C-N), 1551.16, 811.97 (C=N), 1600.89, 1513.80 (C=C), 740.79, 721.37 (C-H Def, out of plane), 1139.21 (C-O-C), 985.67, 1258.55 (C-H in plane bending asymmetric); H^1- NMR ($CDCl_3$) δ ppm: 8.3s (1H; O-H), 6.6 - 7.9m (10H; aromatic protons), 2.72s (3H;$Ar-OCH_3$), 2.37s (3H; $Ar-CH_3$).

Pharmacological evaluation:

Swiss mice were used for the experiments. They were selected from an inbred colony maintained under controlled conditions of light (10:14 h, light: dark), temperature (23±3°) and humidity (50±5%). Mice were housed in sterile propylene cages containing sterile paddy husk (procured locally) as the bedding material. The animals were fed on autoclaved mice feed and water. Six to eight weeks old female mice, weighing 25±5 g were used. The test compounds were administered intraperitonially at a dose of 100 mg/kg weight in the form of suspension using 2% sodium carboxymethyl cellulose as suspending agent. The experimental dose was selected as 1/10th of the LD_{50} dose calculated using Ghosh[8] method. All the animal experiments were performed according to the protocols and recommendations of the Institutional Animal Ethics Committee. The Mann Whitney U test was applied for median survival time. One way analysis of variance with post hoc Scheffe's test was applied to all other parameters.

Antitumour activity:

Brine Shrimp Bioassay[9,10] was carried out as a preliminary test to screen the drugs for antitumour activity. LC_{50}s and 95 % confidence intervals were determined from the 24 h counts using the probit analysis method described by Finney. From this a total of 5 compounds were selected for *in vivo* animal studies. The antitumour activity was determined by survival time assay method. Ehrlich ascitic carcinoma cells (EAC) were used. EAC was obtained from Cancer Research Institute, Mumbai and was propagated by serial transplantation in Swiss mice in animal house.

Development of EAC:

The mice were used 12 days after tumour transplantation. The ascitic fluid was drawn using an 18 gauge needle into a sterile syringe. An aliquot of tumour fluid was tested for microbial contamination. Tumour viability was determined by tryptan blue exclusion test and cells were counted using haemocytometer or cell counter. The ascitic fluid was suitably diluted in saline to get a concentration of 10^6cells/200μl

TABLE 1: PHYSICAL CONSTANTS OF THE SYNTHESIZED COMPOUNDS

Comp.	Molecular Formula	R	Yield (%)	M. P.(°C)	R_f*
3a	$C_{19}H_{15}O_3N_3$	4-OCH_3, 3-OH-C_6H_3	100.60	69	0.9556
3b	$C_{19}H_{15}O_2N_3$	4-OCH_3- C_6H_4	52.44	98	0.9333
3c	$C_{20}H_{17}O_3N_3$	3,4-Di-OCH_3- C_6H_3	71.54	157	0.8837
3d	$C_{19}H_{15}ON_3$	4-CH_3- C_6H_4	72.35	116	0.8696
3e	$C_{18}H_{12}O_3N_4$	3-NO_2- C_6H_4	38.89	118	0.8936
3f	$C_{20}H_{18}ON_4$	4-N,N-Di-CH_3- C_6H_3	101.11	110	0.8511
3g	$C_{18}H_{12}ON_3Cl$	4-Cl- C_6H_4	62.96	156	0.9302
3h	$C_{18}H_{12}ON_3Br$	4-Br- C_6H_4	45.39	139	0.8511
3i	$C_{16}H_{11}O_2N_3$	2-Furyl	75.45	105	0.8936
4a	$C_{19}H_{14}O_3N_2S$	4-OCH_3,3-OH- C_6H_3	93.10	64	0.9048
4b	$C_{19}H_{14}O_2N_2S$	4-OCH_3- C_6H_4	72.69	130	0.8809
4c	$C_{20}H_{16}O_3N_2S$	3,4-Di-OCH_3- C_6H_3	48.15	139	0.5909
4d	$C_{19}H_{14}ON_2S$	4-CH_3- C_6H_4	46.25	115	0.8478
4f	$C_{20}H_{17}ON_3S$	4-N,N-Di-CH_3- C_6H_3	54.25	150	0.9455
4g	$C_{18}H_{11}ON_2SCl$	4-Cl- C_6H_4	54.62	149	0.9787
4h	$C_{18}H_{11}ON_2SBr$	4-Br- C_6H_4	35.65	163	0.9792
4i	$C_{16}H_{10}O_2N_2S$	2-Furyl	76.07	115	0.9778
5a	$C_{19}H_{14}O_4N_2$	4-OCH_3,3-OH- C_6H_3	69.50	65	0.9556
5b	$C_{19}H_{14}O_3N_2$	4-OCH_3- C_6H_4	56.63	100	0.9792
5c	$C_{20}H_{16}O_4N_2$	3,4-Di-OCH_3- C_6H_3	63.89	120	0.5227
5d	$C_{19}H_{14}O_2N_2$	4-CH_3- C_6H_4	27.50	102	0.9302
5f	$C_{20}H_{17}O_2N_3$	4-N,N-Di-CH_3- C_6H_3	48.39	141	0.8837
5g	$C_{18}H_{11}O_2N_2Cl$	4-Cl- C_6H_4	35.29	158	0.8958
5h	$C_{18}H_{11}O_2N_2Br$	4-Br- C_6H_4	34.79	155	0.8750
5i	$C_{16}H_{10}O_3N_2$	2-Furyl	71.55	109	0.9130

*solvent system- Ethanol: Water(7:3)

of tumour cell suspension. This was injected intraperitoneally to obtain ascitic tumour.

EAC response:

The mice were weighed on the day of tumour inoculation. The drug treatment was started 24 hours later and administered daily for seven days. The mice were weighed on every third day. Tumour response was assessed on the basis of median survival time (MST) and percent treated/control. Percent treated = MST of treated group/MST of con-

TABLE 2: EAC RESPONSE DATA FOR THE *IN VIVO* ANIMAL STUDIES.

Group	Compound	DOSE (mg/kg)	M S T (days)	Percent Treated / Control
Standard	Cisplatin	3.5	24	150
Group I (Control)	2 % SCMC	—	15.0±1.43*	—
Group II	3b	100	20.5±1.76*	137
Group III	3d	100	18.5±2.08*	123
Group IV	3i	100	16.5±1.06*	110
Group V	4i	100	15.5±1.73*	103
Group VI	5i	100	15.5±1.80*	103

*Each value is mean ± SE

trol group X 100. An enhancement of life span by 125 or more over that of control was considered as effective antitumour response[11]. The results are presented in Table 2.

Antimicrobial activity:

The antibacterial activity[12] of the test compounds was tested against *S. aureus, B. subtilis, P. aeruginosa, E. coli, and Klebsiella* using Mueller-Hinton agar medium. The antifungal activity of the test compounds was tested against *C. albicans* using Sabourad dextrose agar medium. A sterile cotton swab was dipped into the suspension of microorganism (matched to McFarland barium sulphate standard) and the sterilized (autoclaved at 120° for 30 min) medium (40-50°) was inoculated by even streaking, dried and wells of diameter (6 mm) were punched in the agar plate with a sterile cork borer. The drug solution was added to these wells with a micropipette. After incubation at 37° for 24 h, the zone of inhibition was measured using mm scale. Ampicillin (1000 units), gentamycin (1000 units) and amphotericin B (100 units) were used as standards for antibacterial and antifungal activity respectively. The observed zones of inhibition are presented in Table 3.

RESULTS AND DISCUSSION

All the synthesized compounds were in conformity with the structures envisaged and were characterized by spectral data. All the compounds were screened for preliminary antitumour studies (Brine Shrimp Analysis) and on the basis of which five active compounds were selected for further animal studies. Table 2 indicates that compounds 3b, 3d, 3i are effective in increasing the life span of tumour bearing mice . While the MST treated/control for 3b was 137, the value for 3d was 123 and are closer to the significant value 125. These values indicate that the compounds 3b and 3d have effective antitumour response. In case of compounds 3i, 4i and 5i, there is no significant increase in the life span of tumour bearing mice.

All the compounds were screened for antibacterial and antifungal activity at a concentration of 500 µg/ml and the results are shown in table 3. Among aminopyrimidines, the relatively higher activity of compound 3f against *S. aureus, P. aeruginosa and C. albicans* may be attributed to 4-dimethyl aminophenyl moiety. Among Thiopyrimidines, componds 4a, 4f, 4g and 4h showed a moderate activity against *P. aeruginosa*. Compound 4i showed highest activity against *C. albicans* as compared to standard. The activity of 4i might be due to furyl moiety. Among Hydroxypyrimidines compound 5g showed weak activity against *Klebsiella* and compounds 5a,5b, 5h and 5i showed highest activity against *C. albicans*. Here once again, the furyl moiety in 5i and 4-methoxy, 3-hydroxy phenyl moiety in 5a might be responsible for antifungal activity.From the data, it is clear that the thiopyrimidines and hydroxypyrimidines, if suitably substituted are likely to show good antifungal activity.

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TABLE 3: ANTIMICROBIAL ACTIVITY OF THE SYNTHESIZED COMPOUNDS

Comp.	Organisms used				
	E. coli	*S. aureus*	*P. aeruginosa*	*Klebsiella*	*C. albicans*
3a	-	-	-	-	-
3b	-	-	-	-	-
3c	-	-	-	-	-
3d	-	-	-	-	-
3e	-	-	-	-	-
3f	-	08	14	-	09
3g	-	-	12	-	-
3h	-	-	10	-	-
3i	-	-	13	-	-
4a	-	-	13	-	-
4b	-	-	12	-	-
4c	-	-	10	-	-
4d	-	11	12	-	08
4f	-	-	13	-	-
4g	-	-	15	-	-
4h	-	10	13	-	15
4i	-	-	12	-	23
5a	-	-	11	-	18
5b	10	-	12	-	16
5c	11	-	11	-	-
5d	-	10	10	-	-
5f	-	08	10	09	10
5g	-	08	11	10	12
5h	-	10	11	-	18
5i	-	11	10	-	20
Standard	20	16	18	15	13

Zone of inhibition was measured in mm. Standard for *Staphylococcus aureus* is Ampicillin. Standard for others is Gentamycin. Standard for antifungal study is Amphotericin B. — Resistant.

REFERENCES

1. Nasef M., El-Naem S.J.A., and Ei-Shabrawy O. A., **Egypt J. Pharm. Sci.**, 1992, 33-34, 463, through **Chem. Abst.**, 1994, 120, 323143q.
2. Habele, Riebli P.; **EP** 1985, 130, 151 through **Chem. Abst.**,1985, 103, 160521.
3. Sonnatag C., Klutchko S. and Shavel J(Jr), **U. S.**, 1972, 3, 649, 644, through **Chem. Abst.**, 1977, 77, 5439.
4. Klutchko S., Shovel J(Jr) and Van Strandtmann M., **U. S.**, 1973, 3, 780, 0660, through **Chem. Abst.**, 1974, 80, 82931.
5. Grinev N., Zotova S. A., and Golobova T.M., **Khim. Farm. Zh.**, 1985, 19, 227, through **Chem. Abst.**,1986, 104, 68700.
6. Elliot C. and Anthony V.M., **Ger. Offen. DE.**, 1986, 3, 620, 354, through **Chem. Abst.**,1987, 107, 7063j.
7. Pesteellini V., Ghelardoni M. and Ortolani C., **EP** 1986, 257, 171 through **Chem. Abst.**, 1988, 109, 65205.
8. Ghosh, M. N., In: Fundamentals of Experimental Pharmacology, Ed II., Scientific Book Agency, Kolkata, 1984,153.
9. Meyer H., Ferrigni N.R., Putnam J.F., Jacobson I.B., Nichole D.E. and Mc Laughlin J.I., **Planta Medica**,1982, 45, 31.
10. Ferrigni N.R., and Mc Laughlin J.I., **J. Nat. Products**, 1984, 47, 347.
11. Chitnis M.P., Bhatia K.G., Phatak M.K and Kesava Rao K.V., **Indian J. Expt. Biol.**, 1980, 18, 6.
12. Gillespie, S. H., In; Medical Microbiology-Illustrated., Butterworth Heinemann, London, 1994, 234.