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SYNTHESIS AND ANTI-TUBERCULAR ACTIVITY OF 8-BROMO-4-CHLORO BENZOFURO[3,2-d] PYRIMIDINES

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Abstract
3-amino-5-bromo-1-benzofuran-2-carboxamide (**3**) was prepared by the reaction of 5-bromosalicylonitrile (**2**) with chloroacetamide in dry acetone in presence of anhydrous potassium carbonate to maintain basic condition. The claisen-schmidt condensation of compound (**3**) with various substituted aromatic aldehydes in presence of strong acid in absolute ethanol underwent cyclization and resulted in the formation of 8-bromo-2(phenyl substituted)benzofuro[3,2-*d*]pyrimidin-4(3*H*)-ones (**4a-e**) in good yields. The compounds (**4a-e**) upon treatment with phosphorus oxychloride to gave above titled compounds (**5a-e**). All synthesized compounds were characterised on the basis of IR, [1]H-NMR, mass spectra, physical and analytical studies. Further these compounds were evaluated for their antibacterial, antifungal and anti-tubercular activities.

Keywords: Antibacterial, antifungal and Anti-tubercular activities.

Introduction:

Benzofuro[3,2-d]pyrimidine derivatives are well documented for its antimicrobial, anti-tubercular and pharmacological activities[1-4]. In view of these facts and continuation of our research work on synthesis of biologically and pharmacologically active agents[5]. We wish to report the synthesis, antimicrobial and anti-tubercular activities of 8-bromo-4-chloro benzofuro[3,2-d]pyrimidines (**5a-e**).

Compounds (**5a-e**) were obtained by the reaction of 8-bromo-2(phenyl substituted) benzofuro [3,2-*d*] pyrimidin-4(3*H*)-ones (**4a-e**) with phosphorus oxychloride at reflux temperature.

3-amino-5-bromo-1-benzofuran-2-carboxamide (**3**) was prepared by the condensation of 5-bromosalicylonitrile (**2**) with chloroacetamide. Compound (**3**) upon treatment with aromatic aldehyde in ethanol in presence of hydrochloric acid to gave 8-bromo-2(phenyl substituted)benzofuro[3,2-*d*]pyrimidin-4(3*H*)-ones(**4a-e**) in good yield

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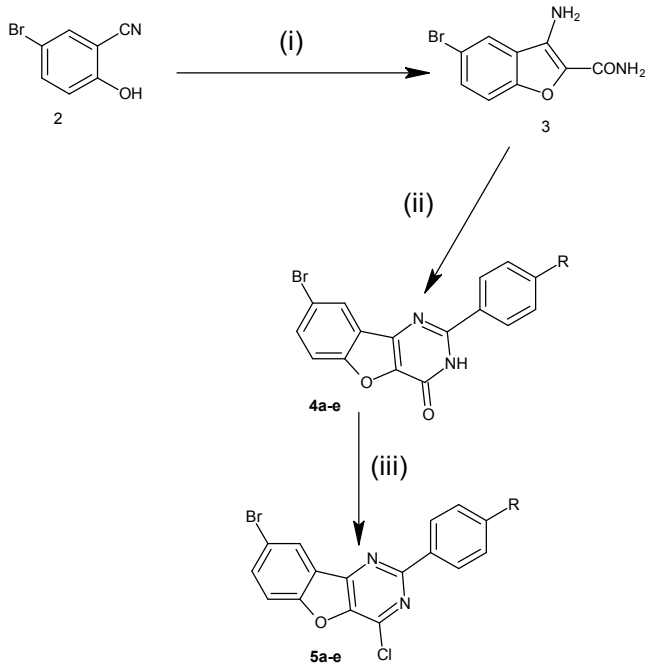

SCHEME-1

Method

(i) $ClCH_2CONH_2$ / Dry acetone / Anhy.K_2CO_3

(ii) R-C$_6$H$_4$-CHO / C_2H_5OH / HCl

(iii) $POCl_3$

Where	R
a	-H
b	$-CH_3$
c	$-OCH_3$
d	$-NO_2$
e	$-N(CH_3)_2$

Material and Methods:

All the reagents were obtained commercially and used after purification. Melting point were taken in open capillaries and are uncorrected. All the compounds were crystallized using suitable solvents. IR spectra were recorded using a FTIR -8400S (SHIMADZU) Spectrophotometer Perkin-Elmer 1000 spectrometer in KBr. ^{1}H-NMR Spectra were recorded on a Bruker 400 MHz Spectrophotometer. The chemical shifts were expressed in δ ppm (δ scale).The mass spectra were recorded with an LCMS-2010A data report Shimadzu Japan and elemental analysis were done with a flash EA 1112 series CHN report thermo Finnegan Silica gel Merck (60-120mesh).

Synthesis of 3-amino-5-bromo-1-benzofuran-2-carboxamide 3:

To a solution of 5-bromosalicylonitrile (**2**) (0.01 mole) in anhydrous acetone (15ml) and chloroacetamide (0.01 mole) in presence of anhydrous potassium carbonate. The reaction mixture was heated for 8-10 hours, the potassium salt were filtered off. The solvent is removed under reduced pressure gave 3-amino-5-bromo-1-benzofuran-2-carboxamide (**3**) as yellow colored solid and it was crystallized from suitable solvent . m.p 185^{o}C and yield 78%.

3. IR(KBr)cm^{-1}1674 (C=O), 1575,1492 (C=C) and 3326-3199 (NH_2). ^{1}H-NMR (δ ppm) δ 7.20 (s,2H,NH_2), 4.90 (s,2H, $CONH_2$) 7.00-8.00(m, 3H,Ar-H). MS; m/z 255. (M^+100%).

Synthesis of 8-bromo-2(phenyl substituted)benzofuro[3,2-*d*]pyrimidin-4(3*H*)-one 4a- e:

A mixture of 3-amino-5-bromo-1-benzofuran-2-carboxamide (**3**)(0.002 mole) in ethanol (10 ml), aromatic aldehyde (0.002 mole) and catalytic quantity of concentrated hydrochloric acid (0.05 ml). The reaction mixture was heated at reflux temperature for about 4 hrs. Upon cooling the solid separated, collected and crystallized from ethanol(**Table-1**).

4a.IR(KBr)cm^{-1}1700(C=O),1630(C=N), 1600,1550 and 1500(C=C), 3180(NH), 1280(C-N) , 1180(C-O-C) and 850(C-Br). ^{1}H-NMR(δ ppm) δ 4.2(s, 1H, NH), 7.09-7.66(m,8H,Ar-H). MS; m/z 341(M^+100%) and m/z 343(M^{+2}).

4b.IR(KBr)cm^{-1}1700(C=O), 1630(C=N), 1600,1550,1500(C=C), 3240 and 3180(-NH) 3060 (C-H Ar) 2990 and 2850(C-H, CH_3), 1250(C-N), 1180 (C-O-C), 850(C-Br). ^{1}HNMR(δ ppm) 1.4 (s,3H,CH_3), 7.1-7.8(m, 7H,Ar-H) and 5.4(b.p,1H, NH) MS; m/z 355 (M^+100%).

4c.IR(KBr)cm^{-1} 3180(-NH), 3010(C-H, Ar), 2990 and 2860(C-H,CH_3,OCH_3) 1740 and 1680(C=O),1600(C=N), 1600,1500, 1450(C=C), 1270(C-N), 1180(C-O-C) and 850(C-Br).

4d.IR(KBr)cm^{-1}3180(-NH), 1700(C=O),1600(C=N), 1600,1550 and 1500(C=C), 1550 and 1350(-NO_2), 1180(C-O-C) and 850(C-Br). ^{1}H-NMR(δ ppm) 4.3 (s,1H,NH), 7.22-8.00(m, 7H,Ar-H). MS; m/z 386 (M^+100%).

4e.IR(KBr)cm^{-1}3180(-NH), 2960-2850(C-H, -CH_3), 1700(C=O), 1600 (C=N), 1600,1550 and 1500(C=C),1270(C-N),1180(C-O-C) and 850(C-Br). ^{1}H NMR (δ ppm) 2.56 [s,6H,(N-CH_3)$_2$], 5.81(s,1H,NH) 7.09-7.66(m, 7H,Ar-H). MS; m/z 384 (M^+100%), m/z 386(M^{+2}).

Synthesis of 8-bromo-4-chlorobenzofuro[3,2-d]pyrimidine 5a-e.

A mixture of 8-bromo-2(phenyl substituted)benzofuro[3,2-*d*]pyrimidin-4(3*H*)-one (**4a-e)** (0.00032 mole) and phosphorus oxychloride (2 ml) was added and the reaction mixture was heated under reflux for one hrs. The reaction mixture was cooled and poured into crushed ice with stirring. The solid separated was filtered, washed with water and crystallized from Benzene & Petroleum ether,(**Table-1**).

5a.IR(KBr)cm^{-1}3040(C-H),1580-1620(C=N),1565,1530(C=C),1250(C-N),1180,1062(C-O-C), 850(C-Br) and 659(C-Cl). ^{1}HNMR(δ ppm) 7.2-8.6(m, 8H,Ar-H) MS; m/z 360 (M^+100%).

5b.IR(KBr)cm^{-1} 3020(C-H, Ar), 2960(C-H, CH_3),1618(C=N),1565,1530(C=C), 1250(C-N),850(C-Br) and 659(C-Cl). ^{1}H-NMR(δ ppm) 7.2-8.4(m, 7H,Ar-H) and 1.28(s, 3H, CH_3) MS; m/z 374 (M^+100%) and m/z 376(M^{+2}).

5c.IR(KBr)cm^{-11}3020(C-H, Ar), 2960,2840(C-H,OCH_3),1615(C=N),1565,1530(C=C), 1250 (C-N),1250(C-N), 850(C-Br) and 659(C-Cl).

5d.IR(KBr)cm^{-1} 3000(C-H), 1620(C=N), 1452(C=C), 1520 and 1350(-NO_2), 1280(C-N), 850(C-Br) and 659(C-Cl). ^{1}H-NMR(δ ppm)7.2-8.6(m, 7H,Ar-H) MS; m/z 405 (M^+100%), m/z 407(M^{+2}), m/z 373, m/z 169 and m/z 116 or 117.

5e.IR(KBr)cm^{-1}3040(C-H, Ar),2960,2840[C-H,$(CH_3)_2$],1614(C=N),1565,1530(C=C), 1250(C-N), 1180 and 1062(C-O-C), 850(C-Br) and 659(C-Cl). 1H-NMR(δ ppm) 2..62 [s,6H, $N(CH_3)_2$], 6.8-8.4(m, 7H Ar-H). MS; m/z 402(M^+100%), m/z 77 and m/z 93.

Results and Discussion:

Antibacterial Activity:

All the synthesized compounds (**4a-e)** and (**5a-e)** were screened for their *in vitro* antibacterial activity. The method employed was cup-plate diffusion method[6]. The organisms used were *S.aureus* and *E.coli*. Ciprofloxacin was used as a standard drug, All compounds and standard drug used at the concentration of 100µg/0.1ml in dimethylformamide. The zone of inhibition was measured after 24 hrs of incubation at optimum temperature.

Compounds (**4a**), (**4c**), (**5a**) and (**5c**) exhibited high activity against *S.aureus* and *E.coli.* Compounds (**4e**), (**4d**), (**5e**) and (**5d**) exhibited moderate activity against both micro-organism when compared with standard drug. The remaining compounds displayed weak antibacterial activity against *S.aureus* and *E.coli*. The results are summarized in (**Table-2**).

Antifungal Activity:

Compounds(**4a-e**) and (**5a-e**) were tested for their fungicidal activity by cup-plate diffusion method[6] against *C.albicans* and *A.niger* at the concentration of 100µg/0.1ml in dimethylformamide. Griseofulvin was used as standard drug. The zone of inhibition was measured after 24 hrs of incubation at optimum temperature. Compounds (**4e**), (**4a**), (**5e**) and (**5b**) exhibited significant fungicidal activity against *C.albicans* and

A.niger while compounds (**4c**) and (**5c**) exhibited moderate activity against both fungi. The remaining compounds (**4d**), (**4b**), (**5d**) and (**5b**) displayed weak activity against *C.albicans* and *A.niger* when compared with standard drug Griseofulvin. The results are summarized in (**Table-2**).

Anti-tubercular Activity:

Compounds (**4a-e**) and (**5a-e**) were evaluated for anti-tubercular activity using Microplate Alamar Blue Assay(MABA)[7] method against the organism *M.tuberculi* H_{37} R_V. The test compounds were tested at a concentration of 0.2, 0.4, 0.8, 1.6, 3.12, 6.25, 12.5, 25, 50 and 100µg/ml against streptomycin used as a positive control drug at the concentration of 6.25µg/ml. The results are summarized in (**Table-3**).

The compounds (**4a**), (**4c**), (**5a**), and (**5c**) exhibited significant activity against the *M.tuberculi* H_{37} R_V. The compounds (**4e**), (**4d**), (**5e**) and (**5d**) exhibited moderate activity against the *M.tuberculi* H_{37} R_V. The remaining compounds of the series (**4b**) and (**5b**) exhibited weak activity against the *M.tuberculi* H_{37} R_V when compared with the standards drugs.

Conclusion:

The aim of present research work is to synthesize certain halogen substituted benzofuran pyrimidine derivatives of biological interest. Among all the synthesized compounds some of them showed good antimicrobial, anti-inflammatory, analgesic and anti-tubercular activities when compared with standard drugs. Thus an attempt has been made to study their antimicrobial, anti-inflammatory, analgesic and anti-tubercular activities at different concentration and SAR discuss.

Table -1 Physical Characterization of synthesized compounds (4a-e) and (5a-e)

Comps Code	R	S	M.P (^{0}C)	Yield (%)	Mol. For	Mol. Wt	Elemental Analysis Found(calculated)%		
							C	H	N
4a	H	A	254	67	$C_{16}H_9BrN_2O_2$	340	56.33 (56.30)	2.66 (2.68)	8.21 (8.19)
4b	$-CH_3$	A	170	60	$C_{17}H_{11}BrN_2O_2$	355	57.49 (57.46)	3.17 (3.15)	7.89 (7.88)
4c	$-OCH_3$	A	265	73	$C_{17}H_{11}BrN_2O_3$	371	55.01 (55.02	2.99 (2.96)	7.55 (7.58)
4d	$-NO_2$	A	210	68	$C_{16}H_8BrN_3O_4$	386	49.77 (49.79)	2.09 (2.06)	10.88 (10.84)
4e	$-N(CH_3)_2$	A	185	72	$C_{18}H_{14}BrN_3O_2$	385	56.27 (56.26)	3.67 (3.65)	10.94 (10.91)
5a	H	B	180	75	$C_{16}H_8BrClN_2O$	360	53.44 (53.41)	2.24 (2.27)	7.79 (7.81)
5b	$-CH_3$	B	141	70	$C_{17}H_{10}BrClN_2O$	376	54.65	2.70	7.50

							(54.68)	(2.71)	(7.49)
5c	$-OCH_3$	B	175	68	$C_{17}H_{10}BrClN_2O_2$	389	52.40 (52.38)	2.59 (2.61)	7.19 (7.20)
5d	$-NO_2$	B	194	69	$C_{16}H_7BrClN_3O_3$	405	47.50 (47.49)	1.74 (1.72)	10.39 (10.41)
5e	$-N(CH_3)_2$	B	254	60	$C_{18}H_{13}BrClN_3O$	402	53.69 (53.71)	3.25 (3.22)	10.44 (10.46)

S=Solvent for crystallization A=Ethanol B=Benzene & Petroleum Ether.

Table: 2 Antimicrobial activity data of synthesized compounds (4a-e) and (5a-e).

Sl. No.	Comp. Code.	R	Diameter of Zone of inhibition in mm*			
			Antibacterial		Antifungal	
			S.aureus	*E.coli*	*C. albicans*	*A. niger*
1	4a	H	21	21	19	21
2	4b	$-CH_3$	14	13	14	15
3	4c	$-OCH_3$	20	22	17	16
4	4d	$-NO_2$	18	17	13	12
5	4e	$-N(CH_3)_2$	16	18	20	21
6	5a	H	22	21	19	22
7	5b	$-CH_3$	15	14	12	14
8	5c	$-OCH_3$	20	21	16	17
9	5d	$-NO_2$	17	19	14	13
10	5e	$-N(CH_3)_2$	18	19	21	20
Ciprofloxacin			24	23	-	-
Griseofulvin			-	-	25	24
Control DMF			6	6	6	6

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Table -3
ANTI-TUBERCULAR ACTIVITY STUDIES OF SYNTHESIZED COMPOUNDS (4a-e) AND (5a-e).

Compd Code	R	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml	0.4 µg/ml	0.2 µg/ml
4a	H	S	S	S	S	S	S	S	R	R	R
4b	$-CH_3$	S	S	S	R	R	R	R	R	R	R
4c	$-OCH_3$	S	S	S	S	S	S	S	R	R	R
4d	$-NO_2$	S	S	S	S	S	R	R	R	R	R
4e	$-N(CH_3)_2$	S	S	S	S	R	R	R	R	R	R
5a	H	S	S	S	S	S	S	S	R	R	R
5b	$-CH_3$	S	S	S	R	R	R	R	R	R	R
5c	$-OCH_3$	S	S	S	S	S	S	S	R	R	R
5d	$-NO_2$	S	S	S	S	S	S	R	R	R	R
5e	$-N(CH_3)_2$	S	S	S	S	R	R	R	R	R	R
Std. Drug Streptomycin	-	S	S	S	S	S	S	S	S	S	S

S=Sensitive.
R=Resistant

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Figure-1 Graphical representation of antibacterial activity of synthesiszed compounds (4a-e) & (5a-e)

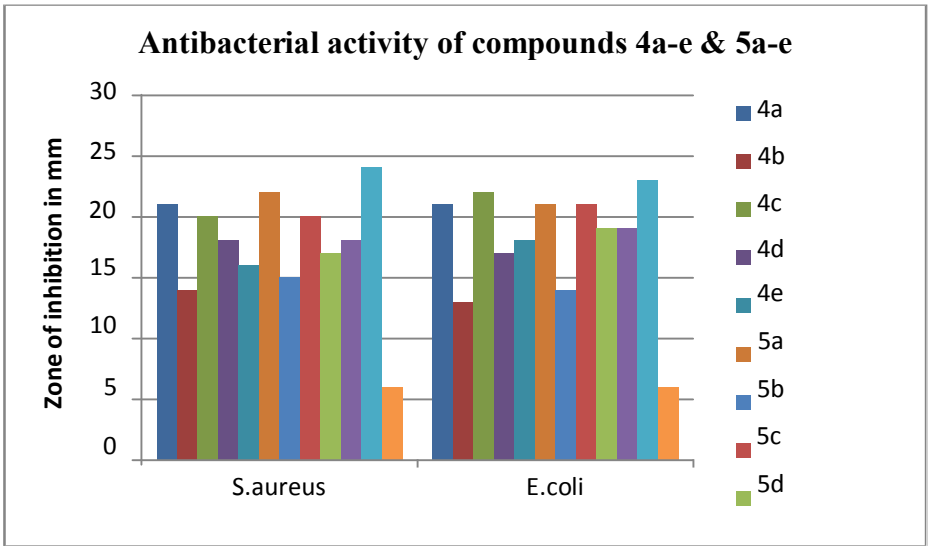
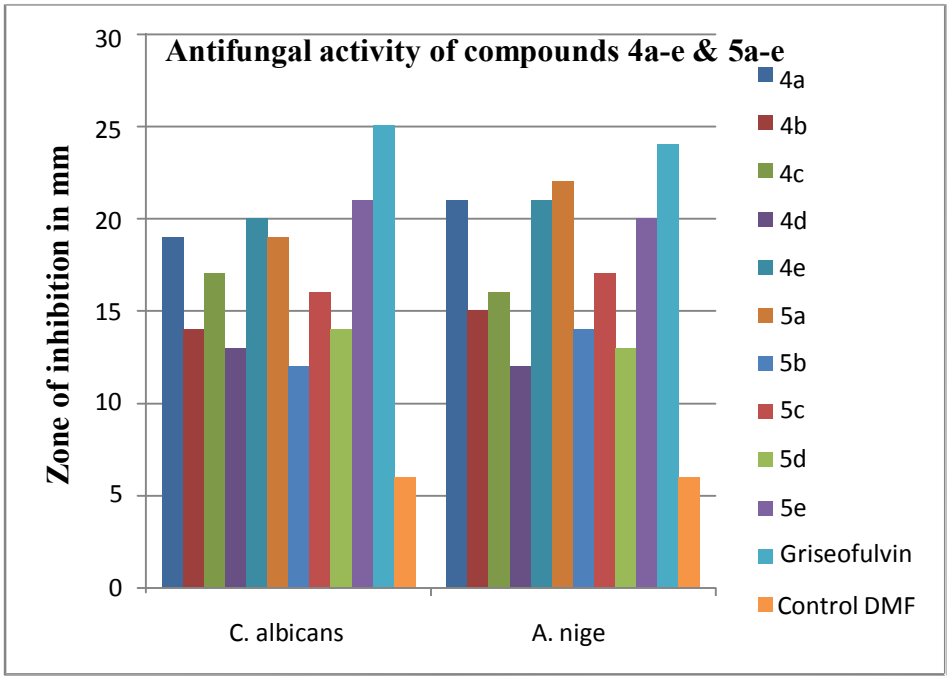


Figure-2 Graphical representation of antifungal activity of synthesiszed compounds(4a-e)&(5a-e)



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