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Synthesis and antibacterial properties of newer (2-butyl-5-amino-3-benzofuranyl)-4-methoxy phenyl methanone amino acids

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ABSTRACT

The (2-butyl -5-amino-3-benzofuranyl) 4-methoxy phenyl methanone amino acids have been synthesized by the reaction of (2-butyl -5-amino-3-benzofuranyl) 4-methoxy phenyl methanone with different hydrophobic amino acids were described (DBMY1-9). All the compounds were synthesized by conventional method and characterized by IR, 1H NMR and mass spectral data. The synthesized compounds were tested for antimicrobial activity against Staphylococcus aureus, Escherichia coli and pseudomonas aeruginosa. Among the synthesized compounds (2-butyl -5-amino-3-benzofuranyl) 4-methoxy phenyl methanone amino acids (DBMY-3,7,2) was found the most active derivative against S. aureus, E. coli and P. aeruginosa and The compounds DBMY-2, 3, 4 was found the most active derivative against Candida albicans and Asperigillus fumigatus respectively. The other Compounds exhibited moderate activity against the other test microorganisms.

Keywords: Antimicrobial activity, conventional, Amino Benzofuran, hydrophobic amino acids.

INTRODUCTION

It is an object of this invention to provide new medicinal compounds. This invention relates to new Benzofuran derivatives, their preparation, characterization and biological use. Upon further study of the specification and appended claims, further objects and advantages of this invention will become those skilled in the art. Benzofuran derivatives exhibit various types of physiological activity and enter into medicinal products and this determines the great attention that has been paid to the synthesis of new compounds of this series[1-2].

The Benzofuran derivatives drew a lot of attention on various pharmacological activities like anti-microbia[3-5], anti-inflammatory[6], antioxidant[7], antithyroid[8] and antitumour[9-11] and in the treatment of allergic conditions, asthma, cardiovascular disorders and as cytoprotective agents[12], arrhythmia[13] and Alzheimer diseases[14-15]. Benzofurans have been drawn as promising structural units in the field of medicinal chemistry. Ameridone, Dronedarone, are well known marketed drugs. Owing to the importance and established pharmacological activity of these compounds we directed our attention towards synthesis and study of biological activity of some newer derivatives of Benzofuranamine substituted compounds.

MATERIALS AND METHODS

Thin layer chromatography was used to monitor the completion of the reaction and homogeneity of the synthesised compound. Melting points were determined using a manual Buchi electro thermal apparatus (range 0-300 c) in open capillary tubes and uncorrected. IR spectra in KBr pellets were recorded Perkin-Elmer Spectrum 100 FT IR spectrometer (400 MHZ) in DMSO-d6 /CDCl3 using TMS as an internal standard (chemical shifts are expressed in ppm). The homogeneity of the compounds was checked on silica gel–G coated plates, Hexane, ethyl acetate and Chloroform as the eluent and observed in UV lamp, iodine vapours or KMnO4 spray as developing agents. All the synthesised compounds gave satisfactory elemental analysis.

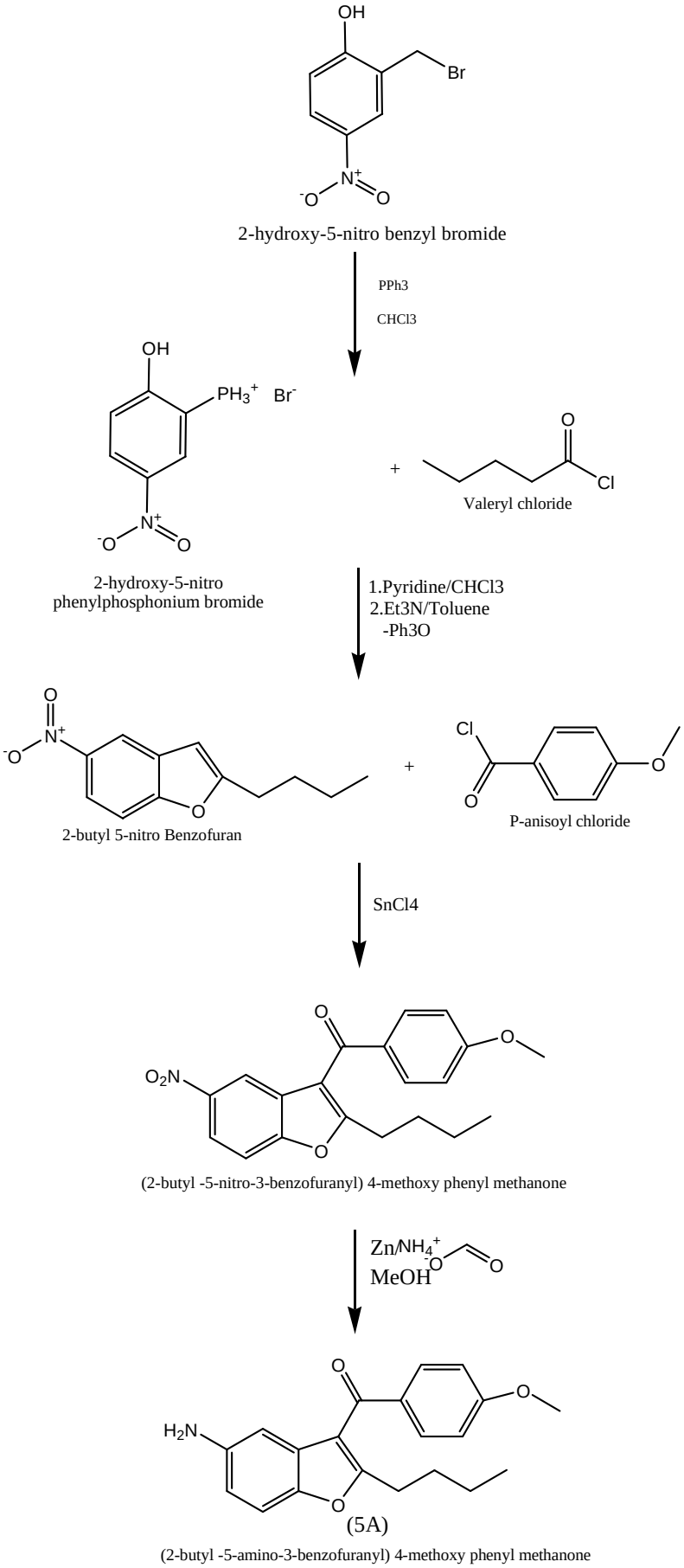
Synthesis of 2-hydroxy-5-nitro phenylphosphonium bromide.
100 g(0.43 mole) of 2-hydroxy 5-nitrobenzyl bromide and 113 g (0.43 mole) of triphenylphosphine are heated at reflux for 0.5 hour in 1600 ml of chloroform. The mixture is allowed to cool and the white precipitate formed is filtered off. The filtrate is evaporated to dryness in a vacuum and the residue is taken up in 500 ml of toluene. The precipitate is filtered off, washed with toluene and the solids formed are pooled and dried in a vacuum at 50 C. In this manner, 210.5 g of 2-hydroxy 5-nitrobenzyl triphenylphosphoium bromide are obtained. Yield: 99.01%

Synthesis of 2-butyl 5-nitro Benzofuran.
48.98 g (0.94 mole) of pentanoyl chloride are added slowly with stirring to a mixture of 200g (0.40 mole) of 2-hydroxy 5-nitrobenzyltriphenylphosphonium, bromide and 120.2g (1.52 mole) of pyridine in 700 ml of chloroform. The mixture is heated at reflux for 2 hours. 2800 ml of toluene is added and 1400 ml of solvents are distilled. 228 g (2.28 moles) of triethylamine are then added and the mixture is heated at reflux for 3 hours. It is allowed to cool, the triphenylphosphine-oxide, formed is filtered off, washed with ethyl acetate and the filtrate is concentrated in a vacuum. The viscous residue obtained is dissolved in acetonitrile and extracted with pentane in a liquid-liquid, extraction apparatus. The solution is stirred over sodium sulphate, filtered and evaporated to dryness. In this manner, crude 2-n-butyl 5- nitrobenzofuran is obtained. Purity [high performance liquid chromatography] (HPLC):97.9% B.p.120-123 C.

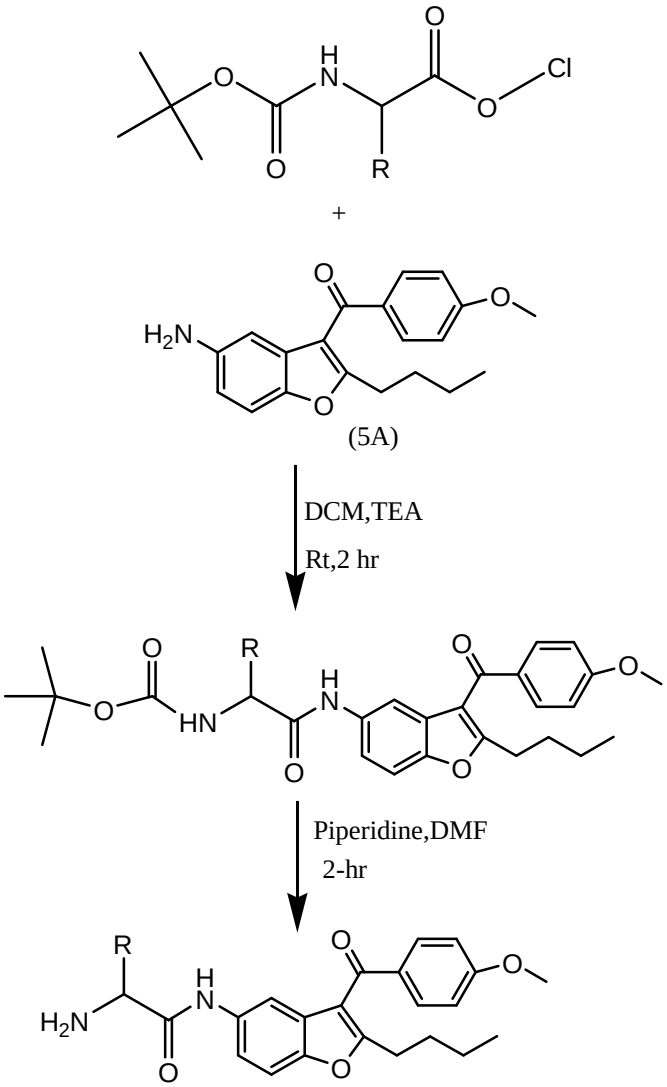
Synthesis of (2-butyl -5-nitro-3-benzofuranyl) 4-methoxy phenyl methanone.
Tin chloride (2.7ml, 23.0mmol) was added drop wise to a stirred solution of N-2-butyl 5-nitrobenzofuran (2.50g, 9.4mmol) and P-anisoyl chloride (3.62g, 10.0mmol) in dichloromethane (20ml) at 0-5 C. The mixture was stirred for 30 min at the same temperature and for 2 hours at room temperature. Water (50ml) was added drop wise to the stirred mixture at 0-5 C. the mixture was extracted with dichloromethane (3X30 ml). the combined organic layers were washed with saturated sodium bicarbonate aqueous solution, dried over sodium sulphate and evaporated .3.45g (65.9%) of (2-butyl -5-nitro-3-benzofuranyl) 4-methoxy phenyl methanone was obtained after purification of the residue by column chromatography on silica gel 60-120(ethyl acetate/hexane 1:3v/v). LC-MS: m/z 354.15 [M+]. IR (KBR): 1720,1545,1465,1370 Cm^{-1}, ^1H NMR($CDCl_3$): δ 0.92-1.01 (3H,t,-CH_3-); δ 1.38-1.40 (2H,m-CH_2-); δ 1.58-1.64 (2H,m,-CH_2-); δ 1.99-2.22 (2H,t,-CH_2-); δ 3.82-3.90 (3H,s,-Ar-OCH_3-); δ 7.02-8.56 (7H,m,-Ar-).Anal. Calcd for $C_{20}H_{19}NO_5$: C, 67.98: H, 5.42: N, 3.96. Found: C, 66.90: H, 5.62: N, 3.68.

Synthesis of (2-butyl -5-amino-3-benzofuranyl) 4-methoxy phenyl methanone (5A).
Dissolved (2-butyl -5-nitro-3-benzofuranyl) 4-methoxy phenyl methanone in 10 ml of methanol added sodium formate and zinc dust .stirred for 2-3 hour until greenish yellow colour. The progress of the reaction was monitored by the TLC. Reaction mixture was distilled out under reduced pressure. The (2-butyl -5-amino-3-benzofuranyl) 4-methoxy phenyl methanone was purified by column chromatography using silica gel 60-120(hexane/ethyl acetate7:3) to remove starting material, followed by (chloroform /methanol 9:1).yield is 90% purity: 98.70%. LC-MS: m/z 324.25 [M+]. IR (KBR): 3450,1720,1465,1370,1290 Cm-1, 1H NMR(CDCl3): δ 1.00-1.20 (3H,t,-CH3-); δ 1.42-1.48 (2H,m-CH2-); δ 1.80-1.86 (2H,m,-CH2-); δ 2.30-2.46 (2H,t,-CH2-); δ 3.79-3.92 (3H,s,-Ar-OCH3-); δ 4.22-4.38 (3H,s,-Ar-NH_2-); δ 6.932-8.77 (7H,m,-Ar-).Anal. Calcd for $C_{20}H_{21}NO_3$: C, 74.28: H, 6.55: N, 4.33. Found: C, 74.00: H, 6.72: N, 4.82.

General procedure for preparation of amide bond formation.
Acid amide coupling and deprotection.
Acid chloride (1mmol) of hydrophobic amino acids (R=alanine, valine, leuccine, isoleucine, proline, methanone, phenylalanine, tryptophan) and (2-butyl -5-amino-3-benzofuranyl) 4-methoxy phenyl methanone (1mmol) was dissolved in dichloromethane (10ml), triethylamine (1mmol) stirred for 2 hours and reaction was monitored by TLC. Added 20ml of piperidine and 3-5ml of dimethyl formamide stirred for two hours at room temperature the reaction mixture was poured to50ml of water product was extracted with chloroform dried with sodium sulphate and distilled crystallized by ethanol. Yield 92% .purity-98.8%.


OH
Br
N+
-O
O
2-hydroxy-5-nitro benzyl bromide
PPh3
CHCl3
OH
PH3+ Br-
N+
-O
O
+
O
Cl
Valeryl chloride
2-hydroxy-5-nitro
phenylphosphonium bromide
1.Pyridine/CHCl3
2.Et3N/Toluene
-Ph3O
O
N+
-O
O
2-butyl 5-nitro Benzofuran
+
Cl
O
O
P-anisoyl chloride
SnCl4
O
O
O2N
O
(2-butyl -5-nitro-3-benzofuranyl) 4-methoxy phenyl methanone
Zn/NH4+
-O
O
MeOH
O
O
H2N
O
(5A)
(2-butyl -5-amino-3-benzofuranyl) 4-methoxy phenyl methanone

Scheme-1



(DBMY1-9)

R= Hydrophobic aminoacids; Glycine, Alanine, Valine, Leuccine, Isoleucine, Proline, Methionine, Phenylalanine, Tryptophan (DBMY1-9 respectively).Scheme-2.

Synthesis of 2-amino-N-3(4-methoxyphenyl, 2-butylBenzofuran-3,5-yl) acetamide. (DBMY-1)

Yield is 74.0% purity: 98.88%. LC-MS: m/z 381.30 [M+]. IR (KBR): 3460,1720, 1650, 1580, 1465, 1370, 1290 Cm-1,^{1}H NMR(DMSO-d6): δ 0.98-1.02 (3H,t,-CH3-); δ 1.30-1.34 (2H,m-CH2-); δ 1.68-1.73 (2H,m,-CH2-); δ 2.22-2.32 (2H,s,-NH2-); δ 2.50-2.56 (2H,t,-CH2-); δ 3.60-3.68 (3H,s,--OCH_3-); δ 7.02-7.98 (7H,m,-Ar-); δ 8.22-8.44 (1H,s,-Ar-NH2-).Anal. Calcd for $C_{22}H_{24}N_2O_4$: C, 69.46: H, 6.36: N, 7.36. Found: C, 69.86: H, 6.00: N, 7.46.

Synthesis of 2-amino-N (4-methoxyphenyl, 2-butylBenzofuran-3,5-yl) propanamide. (DBMY-2)

Yield is 72.2% purity: 97.98%. LC-MS: m/z 395.40 [M+]. IR (KBR): 3550,1720, 1650, 1580, 1465, 1380, 1290 Cm-1,^{1}H NMR(DMSO-d6): δ 0.98-1.22 (3H,t,-CH3-); δ 1.30-1.32 (2H,m-CH2-); δ 1.66-1.68 (2H,m,-CH2-); δ 2.02-2.26 (2H,s,-NH2-); δ 2.50-2.56 (2H,t,-CH2-); δ 3.73-3.86 (3H,s,--OCH_3-); δ 6.90-8.02 (7H,m,-Ar-); δ 8.23-8.42 (1H,s,-Ar-NH2-).Anal. Calcd for $C_{23}H_{26}N_2O_4$ C, 70.03: H, 6.64: N, 7.10.Found: C, 70.33: H, 6.94: N, 7.40.

Synthesis of 2-amino-N (4-methoxyphenyl, 2-butylBenzofuran-3,5-yl)-3-methylbutanamide. (DBMY-3)

Yield is 69.44% purity: 98.08%. LC-MS: m/z 423.80 [M+]. IR (KBR): 3520,1720, 1650, 1580, 1465, 1380, 1370, 1290 Cm^{-1},^{1}H NMR(DMSO-d6): δ 0.98-1.00 (3H,t,-CH3-); δ 1.00-1.23 (3H,t,-CH3-); δ 1.30-1.32 (2H,m-CH3-); δ 1.66-1.68 (2H,m,-CH2-); δ 2.2-2.4 (2H,s,-NH2-); δ 2.30-2.32 (1H,m,-CH-); δ 2.40-2.42 (2H,m,-CH2-); δ 3.60-3.64 (1H,m,-CH_2-); δ 3.72-3.74 (3H,s,--OCH_3-); δ 7.00-8.32 (7H,m,-Ar-); δ 8.44-8.63 (1H,s,-Ar-NH2-).Anal. Calcd for $C_{25}H_{30}N_2O_4$ C, 71.07; H, 7.16: N, 6.63: Found: C, 71.03: H, 7.30: N, 6.62.

Synthesis of 2-amino-N (4-methoxyphenyl, 2-butylBenzofuran-3,5-yl)-4-methylpentanamide. (DBMY-4)
Yield is 67.99% purity: 97.56%. LC-MS: m/z 437.24 [M+]. IR (KBR): 3560,1720, 1650, 1580, 1465, 1380, 1370, 1290, Cm^{-1},^{1}H NMR(DMSO-d6): δ 0.98-1.00 (3H,t,-CH3-); δ 1.00-1.22 (3H,t,-CH3-); δ 1.00-1.22 (3H,t,-CH3-); δ 1.70-1.82 (2H,m-CH2-); δ 1.80-1.85 (1H,m,-CH-); δ 1.88-1.90 (2H,m,-CH2-); δ 2.42-2.60 (2H,s,-NH2-); δ 2.80-2.92 (2H,m,-CH2-); δ 3.72-3.86 (1H,m,-CH-); δ 3.86-3.93 (3H,s,--Ar-OCH_3-); δ 7.23-8.87 (7H,m,-Ar-); δ 8.24-8.28 (1H,s,-Ar-NH2-).Anal. Calcd for $C_{26}H_{32}N_2O_4$ C, 71.53: H, 7.39: N, 6.42: Found: C, 71.60: H, 7.45: N, 6.65.

Synthesis of 2-amino-N (4-methoxyphenyl, 2-butylBenzofuran-3,5-yl)-3-methylpentanamide. (DBMY-5)
Yield is 63.66% purity: 98.70%. LC-MS: m/z 437.40 [M+]. IR (KBR): 3560,1720, 1650, 1580, 1465, 1380, 1370, 1290,780 Cm-1,1H NMR(DMSO-d6): δ 0.96-1.00 (3H,t,-CH3-); δ 1.00-1.22 (3H,t,-CH3-); δ 1.00-1.22 (3H,t,-CH3-); 1.33-1.42 (3H,m,-CH3-); δ 1.72-1.78 (2H,m-CH2-); δ 1.82-1.86 (1H,m,-CH_2-); δ 2.02-2.32 (2H,s,-NH2-); δ 2.32-2.43 (1H,m,-CH-); δ 2.68-2.82 (2H,m,-CH_2-); δ 3.63-3.68 (2H,m,-CH-); δ 3.86-3.93 (3H,s,--Ar-OCH3-); δ 7.26-8.92 (7H,m,-Ar-); δ 8.32-8.34 (1H,s,-Ar-NH2-).Anal. Calcd for $C_{26}H_{32}N_2O_4$ C, 71.53: H, 7.39: N, 6.42: Found: C, 71.62: H, 7.65: N, 6.05.

Synthesis of N (4-methoxyphenyl, 2-butylBenzofuran-3,5-yl) pyrrolidine-5-carboxamide. (DBMY-6)
Yield is 65.27% purity: 97.90%. LC-MS: m/z 421.82 [M+]. IR (KBR): 3560,1720, 1650, 1580, 1465, 1380, 1370, 1290, Cm-1,1H NMR(DMSO-d6): δ 0.96-1.00 (3H,t,-CH3-); δ 1.32-1.62 (2H,m,-CH3-); δ 1.62-1.85 (4H,m,-CH2,CH2-); δ 2.22-2.30 (1H,m,-NH-); 2.42-2.58 (2H,m,-CH2-); δ 2.72-2.85 (2H,m-CH2-); δ 3.00-3.22 (2H,m,-CH2-); δ 3.42-3.56 (2H,m,-CH2-); δ 3.80-3.82 (3H,s,--Ar-OCH3-); δ 7.70-8.60 (7H,m,-Ar-); δ 8.80-8.96 (1H,s,-Ar-NH2-).Anal. Calcd for $C_{25}H_{28}N_2O_4$ C, 71.41: H, 6.71: N, 6.66: Found: C, 71.55: H, 6.60: N, 6.90.

Synthesis of 2-amino-N(4-methoxyphenyl,2-butylBenzofuran-3,5-yl)-4-(methylthio) butanamide. (DBMY-7)
Yield is 62.52% purity: 98.20%. LC-MS: m/z 456.09 [M+]. IR (KBR): 3560,1720, 1650, 1580, 1465, 1380, 1370, 1290, Cm-1,1H NMR(DMSO-d6): δ 0.90-1.00 (3H,t,-CH3-); δ 1.30-1.34 (2H,m,-CH2-); δ 1.68-1.70 (2H,m,-CH2-); δ 2.22-2.32 (1H,s,-NH2-); 2.40-2.46 (3H,s,-CH3-); δ 2.60-2.68 (2H,m-CH2-); δ 2.80-2.84 (2H,m,-CH2-); δ 3.60-3.74 (1H,m,-CH-); δ 3.92-4.02 (3H,s,--Ar-OCH3-); δ 7.52-8.96 (7H,m,-Ar-); δ 8.22-8.38 (1H,s,-Ar-NH2-).Anal. Calcd for $C_{25}H_{30}N_2O_4S$ C, 66.05: H, 6.65: N, 6.16: Found: C, 66.15: H, 6.42: N, 6.28.

Synthesis of 2-amino-N (4-methoxyphenyl, 2-butylBenzofuran-3,5-yl)-4-phenylpropanamide. (DBMY-8)
Yield is 75.50% purity: 98.60%. LC-MS: m/z 471.80 [M+]. IR (KBR): 3560,1720, 1650, 1580, 1465, 1380, 1370, 1290, Cm-1,1H NMR(DMSO-d6): δ 0.89-0.96 (3H,t,-CH3-); δ 1.28-1.32 (2H,m,-CH2-); δ 1.56-1.62 (2H,m,-CH2-); δ 2.22-2.32 (1H,s,-NH2-); 2.55-2.62 (2H,m,-CH2-); δ 3.42-3.56 (2H,m-CH2-); δ 3.82-3.95 (3H,s,--Ar-OCH3-); δ4.2-4.28 (1H,m-CH-); δ 6.9-8.28 (12H,m,-Ar-); δ 8.40-8.46 (1H,s,-NH-).Anal. Calcd for $C_{29}H_{30}N_2O_4$ C, 74.02; H, 6.43; N, 5.95: Found: C, 74.23: H, 6.65: N, 6.05.

Synthesis of 2-amino-N (4-methoxy phenyl, 2-butyl Benzofuran-3,5-yl)-3-(1H-indol-2-yl)propan amide. (DBMY-9)
Yield is 78.90% purity: 97.50%. LC-MS: m/z 509.62 [M+]. IR (KBR): 3560,1720, 1650, 1580, 1465, 1380, 1370, 1290, Cm-1,1H NMR(DMSO-d6): δ 0.89-0.96 (3H,t,-CH3-); δ 1.28-1.32 (2H,m,-CH2-); δ 1.56-1.62 (2H,m,-CH2-); δ 2.22-2.32 (1H,s,-NH2-); δ 2.55-2.62 (2H,m,-CH2-); δ 3.42-3.56 (2H,m-CH2-); δ 3.82-3.95 (3H,s,--Ar-OCH3-); δ4.2-4.28 (1H,m-CH-); δ6.2-6.26 (2H,m-CH2-); δ 6.52-7.98 (11H,m,-Ar-); δ 8.28-8.32 (1H,s,-NH-); δ 9.8-9.86 (1H,s,-NH-).Anal. Calcd for $C_{31}H_{31}N_3O_4$ C, 73.06; H, 6.13; N, 8.25: Found: C, 73.44: H, 6.32: N, 8.12.

RESULTS AND DISCUSSION

Antibacterial activity
All the synthesized compounds were screened for their antibacterial activity against

Staphylococcus aureus, Escherichia coli and pseudomonas aeruginosa. The activity was carried out using cup plate method [16-18] the zone of inhibition was measured in mm. DMF was used as a vehicle and Ciprofloxacin as standard drug for comparison. The compounds were tested at 10 mg/ml concentration and each well was loaded with 50 µg/ml among the synthesized compounds DBMY-3, 7, 2 was found the most active derivative against S. aureus, E. coli and P. aeruginosa respectively. The other Compounds exhibited moderate activity against the other test microorganisms. The zones of inhibition are presented in Table -1.

Table - 1 Antibacterial activity of the compounds

compound	*E.coli*		*S.aureus*		*P.aeruginosa*	
	zone of inhibition (mm)	% inhibition (mm)	zone of inhibition (mm)	% inhibition (mm)	zone of inhibition (mm)	% inhibition (mm)
DBMY-1	15	66.6	18	81.8	10	40.3
DBMY-2	18	81.8	22	88.0	15	66.6
DBMY-3	20	90.9	24	96.0	18	75.0
DBMY-4	17	80.5	15	66.6	16	72.7
DBMY-5	12	50.0	16	72.7	NS	NS
DBMY-6	10	40.3	08	33.3	12	54.5
DBMY-7	18	81.8	16	72.7	20	90.9
DBMY-8	10	40.3	14	56.2	08	36.3
DBMY-9	NS	NS	NS	NS	NS	NS
Ciprofloxacin	22	100	25	100	24	100

Antifungal activity

The synthesized compounds were evaluated invitro for antifungal activity by using standard agar disc diffusion method [16-18] against Candida albicans and Asperigillus fumigatus. DMF was used as a vehicle. The compounds were tested at 10 mg/ml concentration and each well was Loaded with 50 µg/ml of the sample. Flucanozole was used standard drug. The synthesized compounds DBMY-2, 3, 4 was found the most active derivative against Candida albicans and Asperigillus fumigatus respectively. The other Compounds exhibited moderate activity against the other test microorganisms. The zones of inhibition are presented in Table -2.

Table – 2 Antifungal activities of the compounds

Si No.	Compound No.	Presence/Absence of growth Candida albicans						Presence/Absence of growth Asperigillus fumigatus					
Dilutions		I	II	III	IV	V	VI	I	II	III	IV	V	VI
01	DBMY1	-	+	+	-	+	+	-	-	-	+	+	+
02	DBMY2	-	-	-	-	-	+	-	-	-	-	-	-
03	DBMY3	-	+	-	-	-	-	-	-	-	-	-	-
04	DBMY4	-	-	-	-	-	-	-	-	+	-	-	-
05	DBMY5	+	+	+	+	+	+	-	+	+	+	+	-
06	DBMY6	-	+	+	+	+	+	-	+	+	+	+	-
07	DBMY7	-	-	-	-	-	+	-	-	-	-	+	+
08	DBMY8	-	+	+	+	+	+	-	+	+	+	+	+
09	**FLUCANOZOLE**	-	-	-	-	-	-	-	-	-	-	-	-

'+' indicate presence of growth, '-'indicates absence of growth.

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