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SYNTHESIS AND BIOEVLUATION OF SERIES OF 2-(4-BROMOPHENYL)-2-METHYL-N-PHENYLPROPANAMIDE ANALOGUES

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ABSTRACT

A series of six Synthesis of 2-(4-bromophenyl)-2-methyl-N-phenylpropanamide derivatives were synthesized by condensation of substituted aromatic primary amines with 2-(4-bromophenyl)-2-methylpropanoylchloride in the presence of strong base such as triethyl amine in DCM at 40^0C. This compound can be synthesized 2-(4-bromophenyl)-2-methylpropanoylchloride from 2-(4-bromophenyl)-2-methyl propionic acid with thionyl chloride in the presence of DCM at0-10^0C. All the five compounds can be confirmed by advanced spectral data (1HNMR, 13CNMR & LCMS) and the structure of the all compounds can be determined by elemental analysis. All the derivatives were evaluated, by antibacterial activity.

KEYWORDS: 2-(4-bromophenyl)-2-methyl-N-phenylpropanamide analogues, Aromatic primary amines, with 2-(4-bromophenyl)-2-methylpropanoylchloride, 2-(4-bromophenyl)-2-methyl propionic, bioevluation.

1. INTRODUCTION

Amide is a key functional group in organic chemistry and medicinally chemistry for its widespread occurrence in peptide and non-peptide natural products, therapeutic small molecules, and new polymeric materials.[1–4] The most general route for synthesis of amides that involves the activation of the carboxylic function by means the conversion of carboxylic acids into the corresponding acid chlorides.[5–8] Subsequently this reactive derivative is coupled with the appropriate aromatic or aliphatic primary amine to yield the corresponding amide. Alternatively, carboxylic acids, by the use of activating reagents, can be transformed into reactive acylating intermediates such as acyl chlorides which directly react in situ with the suitable amines without their preliminary isolation and purication.[9–12] The important significance of amides has promoted the development of new protocols and reagents based on these approaches and alternative methods for amide bond formation.[13–16]

The direct formation of amides by condensing nonactivated carboxylic acids and amines is extremely attractive because of its low environmental impact. Using transition metal or non-transition metal based catalysis in direct amide preparation, as an alternative to coupling reagents, has been reported.[17–19] The main synthetic catalysts employed for direct amidation are boronic acids and esters together with Lewis acid metal complexes. Boron-based compounds are reported as catalysts promoting the condensation of carboxylic acids and amines in refluxing toluene.[20,21] In addition to amidation reaction protocols by employing boronic acid and ester catalysts were also developed for the formation of dipeptide systems.[22–24]

2. RESULTS AND DISCUSSION

2.1. Chemistry

The target derivatives 4a-e has been successfully synthesized according to Scheme-1.Thesynthesiswascommencedby2-(4-bromophenyl)-2-methyl-N-phenyl propanmide namide derivatives of the synthetically available with aromatic primary amines in methylene dichloride in the presence of a catalytic strong organic base amount 1H-NMR spectra of compounds showed the aromatic protons for the derivatives 4a-e in the range of δ = 6.678-7.846 ppm. Meanwhile, compounds4a-4e showed singlet's at δ = 1.095-1.248 ppm representing three protons of CH_3 group. Singlet's integrated for protons were noticed in the range of δ =10.854 ppm of acid protons and amide protons which were assigned at10.094-11.194 of amide protons. The methoxy protons were observed in the region of δ = 3.694ppm. Additionally, the OH signals disappeared at 9.175ppm and the 13C-NMRspectra of the target compounds4a-4e exhibited signals in the range of δ = 26.78 ppm indicating carbons of the ic CH2. The cyclohexyl carbons for compounds 6o-u were observed in the range of δ = 24.9-56.8 ppm and the aromatic carbons appeared in the range of δ = 129-127 ppm. The molecular weight of the derivatives appeared at (M+2) peaks.

2.2 Antimicrobial Evaluation

The in vitro antibacterial potential activity of the tested compounds 4a-e was estimated against five standard bacterial strains, namely the Gram-negative *Escherichia coli*, *Pseudomona aeruginosa* and the Gram-positive *Staphylococcus aureus* and *Bacillus subtilis*. Meanwhile, the antifungal potential was estimated against two standard fungal strains, namely Candida albicans, A.Ngier. The results are expressed as diameter of the inhibition zone (DIZ) and the minimum inhibition concentration (MIC). Ampicillin (antibacterial) and fluconazole (antifungal) were used as reference drugs, and the antimicrobial results are presented in Tables I and II.

Table-I: Antibacterial activity of the titled compounds 4a-e against E. coli, Pseudomonas aeruginosa, Staphylococcus aureus, and Bacillus subtilis.

Compound	Diameter of the inhibition zone			
	E. coli	P.aeruginosa	S. aureus	B. subtilis.
4a	07	09	08	11
4b	**17**	**16**	**17**	**19**
4c	**17**	**15**	**17**	**18**
4d	12	10	13	13
4e	09	07	09	10
Ampicillin	20	20	22	22
DMSO	-	-	-	-

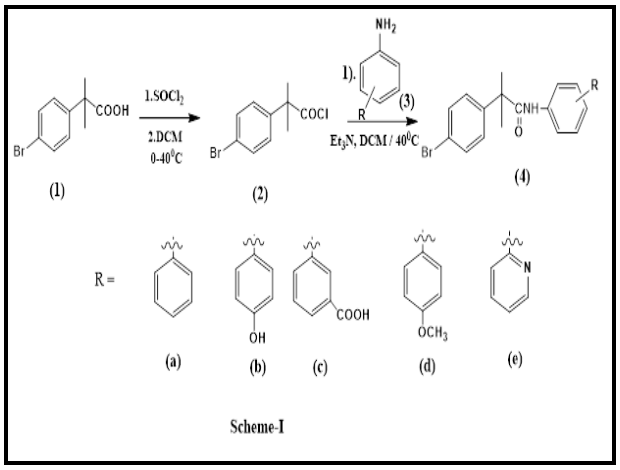
Table-I indicated that the antibacterial activity of the titled derivatives 4a-e in the DIZ against the tested bacterial strains. They exhibited different degrees of potent activities against the tested microorganisms. Most of the investigated compounds 4a-e excellent potent activity in the DIZ assay toward the tested Gram-negative bacterial strains with DIZ values in the range of 15-17 mm. Whereas, their MIC value was around 250 µg/mL which is lower than the MIC values of ampicillin against *E.coli* (250 µg/mL), *P. aeruginosa* (500 µg/mL). In addition, Gram-positive bacteria were less sensitive toward the tested compounds 4a-e than Gram-negative bacteria. Usually, increasing the lipophilic characteristic of the synthesized compound increases the antibacterial activity on Gram-positive bacteria. This can be explained on the bases of the low lipid content of the cell wall of Gram-positive bacteria, which would permit the activity of more lipophilic compounds.

Table-II: Anti-fungal activity of the titled compounds 4a-e against A.Ngier and C.albicans.

Compounds	Zone of inhibition	
	A.Ngier	C.albicans
4a	06	07
4b	**16**	**17**
4c	16	16
4d	10	09
4e	07	08
Fluconazole	20	20

The in *vitro* antifungal activity of the target compounds 4a-e is presented in Table -II. They showed potent activity in vitro antifungal activity against A.Ngier and Candida albicans with an MIC value as low as 2 µg/mL. Most of compounds 4a-e exhibited MIC value of 250 µg/mL being equal to that of the reference drug fluconazole except for compound 4b bearing 4-hydro moiety.

The in vitro antifungal activity of the target compounds 4a-e against most of the tested fungal strains was generally higher than their antibacterial activity. This could be attributed to the presence of the amides functional group ring in their scaffold, which is consistent with the presence of the titled derivatives ring in a number of clinically used antifungal agents.



Scheme-I

3. Experimental

All the chemicals used for the synthesis of title compounds were procured from Himedia Laboratories PVLtd. Ltd., Mumbai. The chemicals were used without further purification. All the melting points were determined in open capillaries and are uncorrected. Thin layer chromatography was performed on microscopic slides coated with silicaGel-G and spots were visualized under UV light. UVspectra were recorded in U.V-1700 Shimadzu spectrophotometer. IR spectra of all the compounds were recorded in KBr on FT-IR 8400 S Shimadzu spectrophotometer using KBr. Mass spectra were obtained using 2010EV LCMS Shimadzu instrument. The 1 H NMR was recorded on Bruker advanced–IINMR-400 MHz instruments using

CDCl3/DMSO-d6as solvent and tetramethylsilane as internal standard, chemical shifts were expressed as δ values (ppm).

3.1.2-(4-bromophenyl)-2-methylpropanoyl chloride
Take clean and dry four neck 50mL RBF. The mixtures of 2-(4-bromophenyl)-2-methyl propionic acid with thionyl chloride in the presence of DCM at0-100C and total arrangement fitted on the magnetic stirrer. The reaction was preceded for 5hrs. The reaction mixture was maintained for 3hrs at 300C.The reaction mixture was monitored by TLC (EtOAc: n-hexane = 5:5). After completion of the reaction, unconsumed thionyl chloride can be evaporated and also solvent will be distilled off. We got final product after completion of the distillation. The pure compounds can also be characterised by advanced spectroscopic data.

White solid, Yield- 93%, m.p-202^{0}C, Rf-0.55(EtOAc: n-hexane-6:4); 1HNMR (400MHz, CDCl3)ppm:7.547-7.362(m,4H,Ar-H),1.123(s,6H,2.CH3);13CNMR(100MHz,CDCl3) ppm: 179.25,134.47,130.78,128.64, 123.75, 27.02.LCMS (m/z): 261.52(M+2); Molecular formulae: C 10H10 Br Cl Elemental analysis: Calculated: C-45.92,H-3.85; Obtained: C- 45.88, H-3.83.

Synthesis of 2-(4-bromophenyl)-2-methyl-N-phenylpropanamide derivatives
Take clean and dry four neck 50mL RBF. The mixtures of 2-(4-bromophenyl)-2-methylpropanoyl chloride with substituted aromatic primary amines was introduced in the four neck RBF and also catalyst amount of strong base triethyl amines with DCM added in a RBF and total arrangement fitted on the magnetic stirrer. The reaction was proceeded 3 hrs. at reflux . The reaction mixture was maintained for 3hrs at 30^{0}C. The reaction mixture was monitored by TLC (EtOAc: n-hexane = 5:5). After completion of the reaction. The reaction mixture was taken ethylacetae and addition with dilute HCl. The separation of organic layer and washed with distilled water two times and separate ethylacetae layer and distilled off under vacuumed. We got final product after completion of the distillation. The pure compounds can also be characterised by advanced spectroscopic data.

1). 2-(4-bromophenyl)-2-methyl-N-phenylpropanamide (4a)
Pale red solid; Yield- 87%; m.p – 247-249^{0}C; Rf- 0.45 (EtOAc: n-hexane = 4:6); 1HNMR (400MHz, CDCl3) ppm:10.143 (s,1H,NH-amide), 7.852-7.274 (m, 8H, Ar-H),1.157 (s,6H,2.CH3);13CNMR (100MHz,CDCl3) ppm:178.48,138.27,134.35,131.86,130.77,129.38, 128.69,125.47,123.56,45.92,28.32.LCMS (m/z): 319.24(M+2); Molecular formulae: C 16H16 Br N; Elemental analysis : Calculated : C-60.39, H-5.07, N-4.40; Obtained : C- 60.31 ,H-5.05 ,N-4.48.

2). 2-(4-bromophenyl)-N-(4-hydroxyphenyl)-2-methylpropanamide (4b)
Pale red solid; Yield- 89%; m.p – 229-231^{0}C; Rf- 0.50 (EtOAc: n-hexane = 4:6); 1HNMR (400MHz, CDCl3) ppm: 10.094 (s,1H,NHamide), 9.175 (s ,1H,-OH), 7.810-7.724 (m,2H,Ar-H),6.845-6.676(m,2H,Ar-H),1.095(s,6H,2.CH3); 13CNMR(100MHz,CDCl3)ppm: 176.25,152.74,134.33,130.09,129.55,128.85,124.54,122. 04,118.57,48.24,27.56.LCMS (m/z): 335.21 (M+2); Molecular formulae: $C_{16}H_{16}BrNO_2$.Elementalanalysis: Calculated: C- 57.50, H- 4.83, N- 4.19; Obtained: C-57.42, H- 4.81, N- 4.27.

3). 2-(4-bromophenyl)-N-(4-methoxyphenyl)-2-methylpropanamide (4c)
Pale red solid; Yield- 90%; m.p – 256-258^{0}C; Rf- 0.50 (EtOAc: n-hexane = 5:5); ^{1}HNMR (400MHz, $CDCl_3$) ppm: 10.198 (s, 1H, NH-amide), 7.784-7.354 (m, 5H,Ar-H),, 6.852-6.732(m,2H,Ar-H),3.694(s,3H,OCH3), 1.248(s,6H, 2.CH_3); 13CNMR (100MHz,$CDCl_3$)ppm:176.77,155.57,134.58,132.09,131. 44,129.72,128.94, 124.61,118.59, 56.49,48.18,26.77;LCMS(m/z):349.12(M+2); Molecular formulae: C17 H18 Br N O2.Elemental analysis: Calculated: C-58.57, H- 4.84, N- 2.96; Obtained: C-58.51, H- 4.82, N- 3.09.

4). 3-(2-(4-bromophenyl)-2-methylpropanamido) benzoic acid (4d)
Dark red solid; Yield- 88%; %; m.p – 264-2660C; Rf-0.45 (EtOAc: n-hexane = 4:6); ^{1}HNMR (400MHz, CDCl3) ppm: 10.854 (s, 1H,-Acid), 10.457 (s, 1H , NH-amide) ,8.218 (s, 1H, Ar-H), 7.947-7.645 (m, 5H,Ar-H), 1.356 (s, 6H, 2.CH3);^{13}CNMR (100MHz, CDCl3) ppm:178.74,167.18,139.75,136.83,132.19,130.68,129.06 ,128.74,128.15, 127.67,123.09, 46.15,26.04; LCMS (m/z): 363.33 (M+2); Molecular formulae: C17 H16 Br N O3.Elemental analysis: Calculated: C-56.37, H- 4.45, N- 3.87; Obtained: C- 56.31, H- 4.43, N- 3.96.

5). 2-(4-bromophenyl)-2-methyl-N-(pyridin-2-yl) propane amide (4e)
Red solid; Yield- 89%; m.p – 248-250^{0}C; Rf- 0.45 (EtOAc: n-hexane = 3:7); 1HNMR (400MHz, CDCl3) ppm: 11.196(s, 1H, NH), 8.246 (s, 1H, pyridine), 7.946-7.746 (m, 5H, Ar-H), 7.365-7.216 (m, 3H, Ar-H), 1.462 (s, 6H, 2.CH_3).13CNMR(100MHz,CDCl3) ppm:178.46,150.15,145.27,136.56,134.33,132.18,130.75 ,129.55,126.76,118.36, 46.16,27.38.LCMS (m/z): 320.19(M+2), Molecular formulae: C15 H15 Br N2 Elemental analysis : Calculated : C- 56.44, H- 4.74, N-8.78; Obtained: C- 56.35, H- 4.72, ,N- 8.85.

3.2. Antimicrobial Evaluation
3.3.1. Materials
Dimethyl sulphoxide (100%) was used to dissolve the standards ampicillin, fluconazole, and the tested compounds 4a-e to give an initial concentration of 1 mg/mL.

Organisms

All the tested strains bacterial and fungal strains in this study were provided from procured commercially. Bacterial strains were divided into Gram-positive bacteria namely S. aureus (ATCC 25451), B.subtilis (ATCC7733). E.coli (ATCC 15956). P. aeruginosa (ATCC 20845) as negative strains. Candida Albicans (ATCC 66019), A.Ngier (ATCC10547) as fungal strains. All these microorganisms were precultured on nutrient agar (Oxoid) and PDA (potato dextrose agar, Oxoid). Then, 0.5McFarland turbidity of each microbial suspension was prepared in 5 mL nutrient broth tubes for the antibacterial assays (Agar well diffusion technique and MIC test).

Agar Well Diffusion Technique

The antimicrobial activity of the tested derivatives was determined using the agar well diffusion method. The prepared microbial suspensions were loaded on the surface of Mueller Hinton (Oxoid) plates using a sterile cotton swab. The agar surface was perforated with a sterile cork borer (6mm), and 100 µL of each compound (1000 µg/mL) were transferred into each well correspondingly. Plates were incubated aerobically for 24 hat 37 ◦C. Diameters of the inhibition zones were measured around each well and were recorded in mm as average of triplicate trials. DMSO was used as a negative control, while ampicillin and fluconazole were used as positive controls.

MIC Test

The minimum inhibitory concentration of the newly synthesized derivatives in the current study against the microbial strains was determined using the micro-broth dilution assay (MIC). The concentrations of the extracts used for MICs ranged from 100% (1mg/mL) to 0.25%. In brief, 100 µL of Mueller–Hinton broth (MH) was first loaded in polystyrene sterile flat-bottom 96-well plates; then, 100 µL of each tested derivatives in study was added, and two fold dilutions were performed until the 0.25% concentration is reached and subsequently, 50 µLof each of the bacterial suspensions was loaded respectively. The first column of the 96-well plate was loaded with only with Mueller Hinton broth; the bacterial suspension was a positive control and the last column was the negative control consisting of the medium broth (MH) and the extract correspondingly. The lowest concentration of compounds where no visible bacterial growth was observed and recorded after 24 h of incubation was considered as the MIC. The experiments were repeated intriplicate.

4. CONCLUSIONS

A series of 2-(4-bromophenyl)-2-methyl-N-phenylpropanamide derivatives molecular hybrids 4a-e was synthesized and their structures were confirmed by various spectroscopic tools. Their *in vitro* antibacterial evaluation indicated that they possess excellent antibacterial activity against most of the tested Gram-negative strains with DIZ values in the range of 17-18 mm and MICvalues around 250 µg/mL. Meanwhile, their antifungal evaluation showed higher activity especially against Candida tropicalis with MIC value as low as 2 µg/mL for most of the tested compounds. Moreover, compound 2-(4-bromophenyl)-2-methyl-N-phenylpropanamide derivatives 4b bearing hydroxy group revealed a potent growth inhibition zone of 17 mm in the DIZ assay and MICvalues of 2 µg/mL against Candida albicans.

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