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Synthesis of Ibuprofen Derivatives with Improved Antibacterial Activity

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A new series of ibuprofen derivatives *i.e.*, (S)-ibuprofen amide, (S)-4-(2-(4-isobutylphenyl)propanamido)benzoic acid, (S)-3-(4-isobutylphenyl)butan-2-one compound with morpholine, (S)-2-(4-isobutylphenyl)-N-(4-nitrophenyl)propanamide and (S)-3-hydroxy-5-(2-(4-isobutylphenyl)propylamino benzoic acid have been synthesized by treating commercially available 2-(4-isobutyl-phenyl)propionic acid (ibuprofen) with thionyl chloride to get 2-(4-isobutyl-phenyl)propionyl chloride. Acid chloride was further reacted with different amines to get ibuprofen amides. The structures of all these compounds were confirmed on the basis of their analytical and spectral data. FTIR and elemental analysis were performed to characterize the synthesized compounds. Chemical stability studies revealed that amide derivatives were chemically stable at pH 1.2-6.8. Disc diffusion and micro-dilution assays were used to evaluate the antibacterial potential of synthesized ibuprofen derivatives against Gram positive *i.e.*, *Bacillus subtilis* and Gram negative strains *i.e.*, *Escherichia coli.* The amide derivatives of ibuprofen exhibited stronger inhibitory effect against tested bacteria than ester derivatives. MIC values of all derivatives were in the range of 2-20 mg/mL. Results suggested that amide derivative of ibuprofen has more potential as antimicrobial agent it may find its application against infectious diseases.

Keywords: Ibuprofen derivatives, Microbial assay, Anti-inflammatory, Analgesic.

INTRODUCTION

Ibuprofen (NSAIDs), a member of propionic acid derivatives (2-arylpropionic acids) was first time introduced in 1969 as a superior alternative of aspirin[1]. Having excellent pharmaceutical properties and better tolerating power, it is extensively used to relieve non-rheumatic inflammation, pain, acute arthritis, fever and primary dysmenorrehea[2]. Repeated administrations are inevitable in most cases to achieve good therapeutic effects. Gastrointestinal (GI) ulceration and hemorrhage are very common side effects due to long-term oral administration but very fewer than aspirin[2]. These side effects result from the direct contact attributed to the amalgamation of local irritation produced by the free carboxylic group in the molecular structure of ibuprofen due to inhibition of the cyclooxygenase (COX) in the gastrointestinal tract[3]. Consequently, the development of bioreversible derivatives *i.e.* prodrugs is the need of time to decrease the toxicity induced by NSAIDs.

Accorging to Bundgaard, prodrug is a compound that has ability to undergo biotransformation prior to exhibiting its pharmacological properties[4]. A prodrugs normally comprise of two biologically active agents *i.e.*, carrier and drug so that these prodrugs may have some extra pharmacological properties missing in the parent drug[5]. Thus, these prodrugs exhibit additional therapeutic advantage with lesser side effects as compared to the parent drug[6]. Amide derivatives of ibuprofen are gastroprotective as they shift its enzyme selectivity from COX-1 towards COX-2 by masking the free carboxylic group[7]. The free carboxylic acid of NSAIDs shows critical interactions with glutamine, tyrosine and arginine residues of the cyclooxygenase active site[6]. The shielding effect of this free carboxylic group of the ibuprofen seems to be the primary source of reduced irritation[8]. Amidases responsible of hydrolysis of amide bond are present only in intestine[9]. Amide derivatives of ibuprofen with pharmacological effects, including antifungal, antihelmintic, anti-HIV, antiulcer, cardio tonic, antihypertensssive, antibacterialand neuroleptic are in clinical practice.

In order to obtain more effective chemotherapeutic agents, a variety of reports have been presented on the synthesis and biological evaluation of new ibuprofen derivatives yet no amide derivatives of ibuprofen with aniline, morpholine, 4-nitro

aniline, 4-nitrobenzoic acid and 4-aminosalicylic acid have been reported. The present work was planned to synthesize the different amide derivatives of ibuprofen to improve the antibacterial activity.

EXPERIMENTAL

Ibuprofen was purchased from Aldrich (Deisenhofen, Germany). The chemicals used for the research work were analytical grade and purchased from Sigma Aldrich (Steinheim, Germany).

Synthesis of amide derivatives of ibuprofen: Acid chloride is formed by the reaction of commercially obtained 2-(4-isobutyl-phenyl)propionic acid with thionyl chloride to get 2-(4-isobutyl-phenyl)propionyl chloride (Fig. 1). Acid chloride was further reacted with different amines to get ibuprofen amides. The aniline (0.002 mol, 197 µL), morpholine (0.005 mol, 424 µL), 4-aminobenzoic acid (0.9 eq), 4-nitroaniline (0.002 mol, 189 µL) and 4-aminosalicylic acid (0.9 eq) were added to aqueous NaOH (20 mL, 5 %) solution in a sequence. Reaction mixture was cooled followed by the addition of (S)-2-[4-(2-methylpropyl)phenyl]propanoyl chloride. The reaction mixture was stirred on an ice bath for 0.5 h. A white amide derivative was formed as residual which was filter off and dried to constant weight. The crude amide derivatives were crystallized with absolute ethanol synthesized amide prodrugs were characterized with the help of melting point and FTIR data.

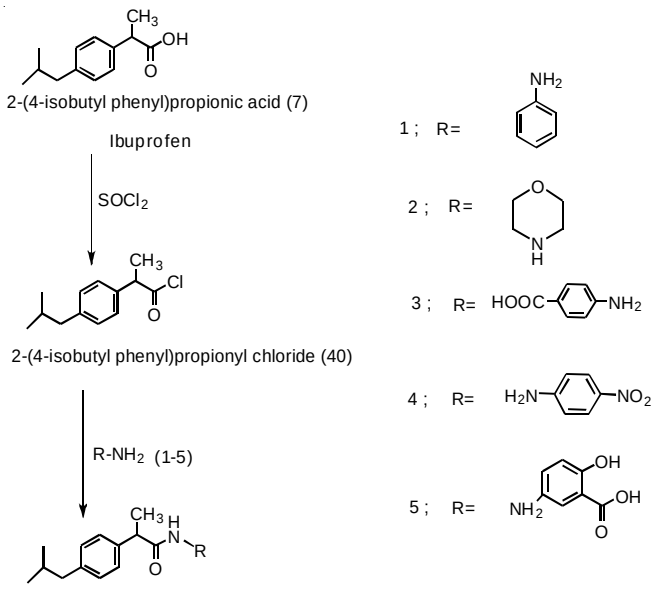


Fig. 1. Preparation of amide derivatives of ibuprofen a) preparation of (S) 2-(4-(2-methylpropyl)phenyl)propanoyl chloride (**1**) Preparation of (S) 2-(4-(2-methylpropyl)phenyl)propanamide (**2**) 4-(2-(4-isobutylphenyl)propanamido benzoic acid (**3**) 2-(4-isobutylphenyl)-N-(4-nitrophenyl)propanamide (**4**) 3-hydroxy-5-(2-(4-isobutylphenyl)propylamino benzoic acid (**5**) 3-(4-isobutylphenyl)butan-2-one compound with morpholine

Antibacterial activity: Urine samples (n = 500) were collected from patients in different wards from Independent University Hospital, Faisalabad. Samples were centrifuged and sediments were cultured primarily on blood agar and MacConkey's agar by spread plate technique. Bacterial colonies having different morphology were selected, purified and identified by their biochemical profiles.

Disc diffusion method: The antibacterial activity of the ibuprofen drug derivatives was assessed by disc diffusion method as reported earlier[10]. Briefly, 100 µL of the suspension containing 10^8 colony-forming units (CFU)/mL of bacteria cells were spread on nutrient agar medium. The paper discs (6 mm in diameter) were separately impregnated with 10 µL of drug derivative solution (10 mg/mL) and placed on the agar which had previously been inoculated with the selected test microorganism. Amohicillin (25 µg/dish) and ibuprofen (100 µg/disc) were used as a positive reference while the discs with solvent were used as a negative control. Plates were kept at 4 ºC for 1 h and then incubated at 37 ºC for 24 h. Antibacterial activity was assessed by measuring the diameter of the growth-inhibition zone in millimeters (including disc diameter of 6 mm) for the test organisms comparing to the controls.

Resazurin microtitre-plate assay: The minimum inhibitory concentration (MIC) of the drug derivatives were evaluated by a modified resazurin microtitre-plate assay as reported[11] with modification. Briefly, a volume of 100 L of 100 mg mL^{-1} (w/v) drug derivative and ibuprofen solutions in 10 % dimethyl sulfoxide (v/v) and 10 mg mL^{-1} of standard antibiotic in 10 % DMSO was transferred into the first row of the 96 well plates. To all other wells, 50 µL of nutrient broth was added. Two-fold serial dilutions were performed using a multichannel pipette such that each well had 50 µL of the test material in serially descending concentrations. Thirty microliter of 3.3 time stronger isosensitized broth (3.3x) and 10 µL of resazurin indicator solution (prepared by dissolving 270 mg resazurin tablet in 40 mL of sterile distilled water) were added to each well. Finally, 10 µL of bacterial suspension were added to each well to achieve a concentration of approx 5×10^5 cfu/mL. Each plate was wrapped loosely with cling film to ensure that bacteria did not become dehydrated. Each plate had a set of controls: a column with a amphicillin and ibuprofen as positive control, a column with all solutions with the exception of the test compound, a column with all solutions with the exception of the bacterial solution adding 10 L of nutrient broth instead and a column with 10 % DMSO (v/v) solution as a negative control. The plates were prepared in triplicate and incubated at 37 °C for 24 h. The colour change was then assessed visually. The growth was indicated by colour changes from purple to pink or colourless. The lowest concentration at which colour change appeared was taken as the MIC value.

Statistical analysis: All the experiments were conducted in triplicate and the data are presented as mean values ± standard deviation. Statistical analysis of the data was performed by Analysis of Variance (ANOVA) using STATISTICA 5.5 (Stat Soft Inc, Tulsa, OK, USA) software and a probability value of p - 0.05 was considered to denote a statistical significance difference among mean values.

RESULTS AND DISCUSSION

Free carboxylic group of ibuprofen has been modified into various amide derivatives using different amines such as aniline (0.00216 mol), morpholine (0.00487 mol), 4-nitro aniline (0.00244), 4-nitrobenzoic acid (0.9 eq) and 4-amino-

salicylic acid (0.9 eq). Results depicted in Table-1 indicated structures, moderate to good yields and various physico-chemical characteristic of all derivatives.

Table-2 depicted the characteristics of synthesized amide derivatives of ibuprofen by mass spectroscopy, elemental analysis and IR spectral data. The IR spectra of all the amide derivatives of ibuprofen were showed absorption band at 1200-1000 cm^{-1} due to -C-C stretching, 1257-1250 cm^{-1} due to -C-O stretching, 3417-3200 cm^{-1} due to -C-H stretching, 1710-1045 cm^{-1} due to -C-N stretching and 3070-3050 cm^{-1} due to -N-H stretching. The mass spectra of the amide derivatives of ibuprofen were showed molecular ion peak corresponding to their molecular formula. The mass spectra and elemental analysis were found in harmony with the assigned molecular structures.

Similar to our findings, Kumar *et al.*[12] synthesized amide derivatives of ibuprofen by using different aromatic as well as aliphatic amines by masking the carboxylic group. These amide derivatives of ibuprofen have shown better phamacological activity than parent drug and had slighter gastrotoxicity. Prodrug synthesized by coupling ibuprofen with sulfa drugs has ability to overcome the drawback of gastrointestinal irritation and ulceration[13]. Furthermore, Cocco *et al.*[14] reported that ibuprofen belongs to arylpropionic acids family and derivatives of arylpropionic acids as esters and amides may retain the activity of the parent acids and decrease their gastrointestinal toxicity. Transformation of the aryl acetic moiety of ibuprofen to amides furnishes molecules that possess better activity and lower toxicity than the parent drug[15]. Lohade *et al.*[16] also synthesized of amide prodrugs of three NSAIDs (flurbiprofen, ibuprofen and ketoprofen) with different amines *i.e.*, propylamine, iso-propylamine, butylamine, benzylamine & aniline and reported these derivatives were more stable in stomach as amidases that cause the hydrolysis of amide bond are present only in intestine.

All synthesized amide derivatives of ibuprofen were tested *in vitro* qualitatively and quantitatively against the Gram-positive and Gram-negative bacteria by zones of inhibition and MIC (minimum inhibitory concentration) values. A careful

TABLE-1
VARIOUS PHYSICOCHEMICAL PROPERTIES OF THE AMIDE DERIVATIVES OF IBUPROFEN

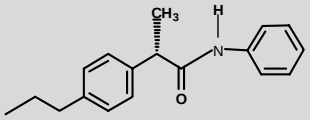
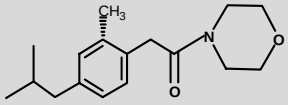
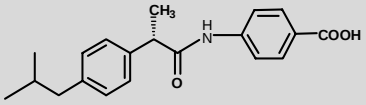
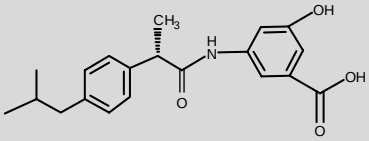
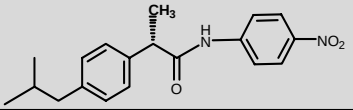
Compd.	Structures	Reaction time (min)	Yield (%)	m.p. (°C)	Colour	Physical state	Solubility
1		60	91.21	183	White	Solid	Ethyl acetate
2		60	70.11	100	White	Solid	Ethyl acetate
3		60	80.1	153	White	Solid	Ethyl acetate
4		60	85.25	155	Yellow	Solid	Ethyl acetate
5		60	46	94	Brown	Solid	Ethyl acetate

TABLE-2
MASS SPECTROSCOPY, ELEMENTAL ANALYSIS AND IR SPECTRAL DATA OF AMIDE DERIVATIVES OF IBUPROFEN

Compd.	m.f.	m.w.	Exact mass	*m/z* values	Elemental analysis				IR absorption (cm^{-1})
					C	H	N	O	
1	$C_{24}H_{39}NO$	357.57	357.30	357.30 (100.0 %), 358.31 (26.4 %), 359.31 (23.6 %)	80.6	10.9	3.92	4.47	C-C = 1157 C-N = 1045, C-H = 3290 N-H = 3070
2	$C_{19}H_{31}NO_2$	305.45	305.24	305.24 (100.0 %), 306.24 (21.0 %), 307.24 (2.5 %)	74.7	10.2	4.59	10.5	C-O-C = 1237, C-C = 1043.5, C-O (stretching) = 1257.3, C-H = 3416.5
3	$C_{12}H_{26}NO_3$	340.44	340.19	340.19 (100.0 %), 341.19 (23.1 %), 342.20 (23.2 %)	74.1	7.70	4.11	14.1	C-C = 1157, C-O = 1257, C-H =3290, N-H = 3070, C-N = 1196
4	$C_{20}H_{25}N_2O_3$	341.42	341.19	341.19 (100.0 %), 342.19 (22.0 %), 343.19 (3.0 %)	70.4	7.38	8.20	14.1	N-H = 3050, C-O = 1250, C-N = 1045, C-H = 3200, C=O = 1705
5	$C_{22}H_{28}NO_4$	370.46	370.20	305.24 (100.0 %), 306.24 (21.0 %), 307.24 (2.5 %)	71.3	7.62	3.78	17.3	C=C = 3090, C-C = 1200, C=O = 1725, C-N= 1710, N-H = 3200

analysis of the data was presented with standard antibiotic *i.e.*, amphicilin and parent drug *i.e.*, ibuprofen. Table-3 reveals the antimicrobial activity of amide derivatives of ibuprofen, reference antibiotic and parent drug by disc diffusion assays. The data indicated that the amide derivatives of ibuprofen exhibited strong bactericidal effect at all tested concentrations and displayed a high degree of antimicrobial activity on the both Gram-positive and Gram-negative bacteria.

TABLE-3
ANTIBACTERIAL ACTIVITY OF
IBUPROFEN AND ITS DERIVATIVES

Compounds	*Bacillus subtilis*		*E. coli*	
	Inhibition zone (mm)	MIC (mg/mL)	Inhibition zone (mm)	MIC (mg/mL)
$C_{24}H_{39}NO$	38 ± 2.0	1.5 ± 0.5	20 ± 1.0	3.5 ± 1.5
$C_{19}H_{31}NO_2$	38 ± 2.1	1.5 ± 0.6	0	Not tested
$C_{12}H_{26}NO_3$	12 ± 0.9	10.5 ± 0.8	24 ± 0.9	17.0 ± 1.1
$C_{20}H_{25}N_2O_3$	0	Not tested	20 ± 1.5	11.0 ±1.5
$C_{22}H_{28}NO_4$	36 ± 2.5	2.5 ± 0.3	14 ± 0.5	10.0 ±1.00
Amphicillin	18 ± 1.0	9.0 ± 1.1	12 ± 0.6	16.0 ± 1.1
Ibuprofen	15 ± 0.9	14.0 ± 1.0	11 ± 0.5	21.0 ± 1.4

The antimicrobial data in Table-3 clearly showed that amide derivatives are by far most active derivatives of ibuprofen. Compound **1** and compound **2** exhibited excellent antibacterial activity *i.e.*, 38 mm and 38 mm against *Bacillus subtilis* as compared to reference drugs *i.e.*, 18 mm and 15 mm. Compound **3** showed promising antibacterial activity against *E. coli*. Amide derivatives had strong bactericidal effect in comparison to amphicillin (reference antibiotic) and parent drug.

As the structural activity relationship is concerned, antibacterial activity of amide derivatives of ibuprofen is much higher than parent drug[9] indicating that antibacterial activity may be increased due to masking of free carboxylate. When these amide derivatives of ibuprofen are compared to parent ibuprofen, these new amides exhibited a comparable or improved antibacterial activity and a lower ulcerogenic effect. The acidic nature of ibuprofen may be reduced due to amide derivatization by which greater gastric tolerability at physiological pH and promising anti-inflammatory, analgesic and antibacterial properties even as compared to other members of NSAIDs[17]. Derivatives are better in action as compared to the parent drug and have less side-effects[18-22]. Less dose is required due to less protein binding of the prodrugs as it increases its availability for hydrolysis in plasma[23-25].

Conclusion

Several amide derivatives of ibuprofen are synthesized in a convenient synthetic route. The approach showed significant advantages. These results established the significance of searching of old drugs as a safer template to built new drug candidates. It can be concluded that this class of amide derivatives of ibuprofen are expected to be safer and holds promise towards search to develop agents with improved pharmacological activity. This *in vitro* activity needs to extend for *in vivo* test and further studies are demanding to confirm our results.

REFERENCES

1. G. Cretu, M. Ionica, A.F. Danet, H.A. Enein, R. Macovei and M. Buleandra, *Acta. Chromatogr.*, **15**, 315 (2005).
2. S.K. Mohamed, M.R. Albayati, S.H.H. Younes and M.G. Abed-Alkareem, *Chem. Sci. J.*, 97 (2013).
3. X. Zhao, D. Wei, Q. Song and M. Zhang, *Prep. Biochem. Biotechnol.*, **37**, 27 (2007).
4. H. Bundgaard, Design of Prodrugs, Elsevier, New York, pp. 49-68 (1988).
5. N. Mehta, S. Aggarwal, S. Thareja, P. Malla, M. Misra, T.R. Bhardwaj and M. Kumar, *Int. J. Chem. Technol.*, **2**, 233 (2010).
6. C.S. Larsen and J. Ostergaard, Design and Application of Prodrugs, Textbook of Drug Design and Discovery, Taylor and Francis Inc. New York, edn 3, pp. 410-58 (2002).
7. L. Peikova and B. Tsvetkova, *Pharm.*, **60**, 37 (2013).
8. M.A.G. Uluda, C. Burcu, A.A. Duygu, E. Nilufer, Y.O. Guler and B. Erden, *Turk. J. Chem.*, **35**, 427 (2011).
9. A.I. Hussain, F. Anwar, S.A.S. Chatha, A. Jabbar, S. Mahboob and P.S. Nigam, *Braz. J. Microbiol.*, **41**, 1070 (2010).
10. S.D. Sarker, L. Nahar and Y. Kumarasamy, *Methods*, **42**, 321 (2007).
11. Y.S. Kwon, W.G. Choi, W.J. Kim, W.K. Kim, M.J. Kim, W.H. Kang and C.M. Kim, *Arch. Pharm. Res.*, **25**, 154 (2002).
12. S. Kumar, D.K. Tyagi and A. Gupta, *J. Pharm. Sci. Res.*, **2**, 369 (2010).
13. G.M. Nazeruddin and S.B. Suryawanshi, *J. Chem. Pharm. Res.*, **4**, 508 (2010).
14. M.T. Cocco, C. Congiu, V. Onnis, M. Morelli and O. Cauli, *Eur. J. Med. Chem.*, **38**, 513 (2003).
15. M. Rooseboom, J.N. Commandeur and N.P. Vermeulen, *Pharmacol. Rev.*, **56**, 53 (2004).
16. A.A. Lohade, P. Jain and K.R. Iyer, *Indian J. Pharm. Educ. Res.*, **43**, 140 (2009).
17. A.K. Bansal, R.K. Khar, R. Dubey and A.K. Sharma, *Drug Dev. Ind. Pharm.*, **27**, 63 (2001).
18. A. Rasheed and C.K. Kumar, *Pharm. Res.*, **1**, 47 (2009).
19. J.C. Chen and S.W. Tsai, *Biotechnol. Prog.*, **16**, 986 (2000).
20. M.S.Y. Khan and M. Akhter, *Eur. J. Med. Chem.*, **40**, 371 (2005).
21. N.M. Mahfouz, F.A. Omar and T. Aboul-Fadl, *Eur. J. Med. Chem.*, **34**, 551(1999).
22. V.R. Shanbhag, A.M. Crider, R. Gokhale, A. Harpalani and R.M. Dick, *J. Pharm. Sci.*, **81**, 149 (1992).
23. A. Rasheed and C.K. Ashok Kumar, *J. Enzyme Inhib. Med. Chem.*, **25**, 804 (2010).
24. B.S. Selinsky, K. Gupta, C.T. Sharkey and P.J. Loll, *Biochemistry*, **40**, 5172 (2001).
25. R. Gupta, M. Singh, M. Ajmal, V. Singh and A. Bisht, *Int. J. Res. Pharm. Chem.*, **1**, 2231 (2011).