Asian Journal of Chemistry Vol. 17, No. 2 (2005), 943–948

Synthesis and Evaluation of Ibuprofen Conjugate with Salicylamide and its Mannich Bases for Analgesic and Antiinflammatory Activities

DEEPA K. NAIR, ASHUTOSH MISHRA† and M.S. VIJAY KANTH*
Department of Pharmaceutical Sciences
Dr. H. S. Gour University, Sagar-470 003, India
E-mail: msvijaykanth@rediffmail.com

Ibuprofen conjugate with salicylamide and its mannich bases were synthesized and characterized using melting point, TLC, elemental analysis, IR spectroscopy, NMR and mass spectroscopy. Further, the compounds were screened for pharmacological activity which indicated that some mannich bases of mutual prodrug of ibuprofen and salicylamide were better than their individual parent compound and its mutual prodrug for its analgesic and anti-inflammatory activities. They also had lesser ulcerogenic index, an indication of lesser toxicity.

Key Words: Ibuprofen, Salicylamide, Analgesic, Mannich base, Antiinflammatory activity.

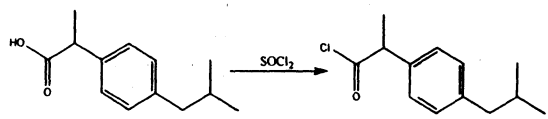
INTRODUCTION

Ibuprofen is a potent non-steroidal antiinflammatory drug. Like other NSAIDs, it also suffers from various side effects such as peptic ulceration along with gastrointestinal bleeding, nausea, vomiting, dizziness, etc.[1] Various researchers[2-5] have attributed these side effects to the free carboxyl group and inhibition of endogenous prostaglandins; therefore they have attempted either to substitute suitably or replace this group by some bioisoteric group or have tried to mask chemically to modify the undesirable effects of this group. The mannich bases of some NSAIDs have been synthesized with the claim to have greater activity and lesser side effects. The survey of literature revealed that some mannich bases of ibuprofen and salicylamide individually have been synthesized with enhanced pharmacological activities with lesser ulcerogenic index[6]. It is also revealed that the conjugates of drug with salicylamide also showed some enhanced activity. In the present study, an attempt towards the concept of drug design through conjugation of two different pharmacophores having similar pharmacological activities has been made by synthesizing and evaluating conjugates of ibuprofen with salicylamide and its mannich bases the prodrugs of a mutual prodrug.

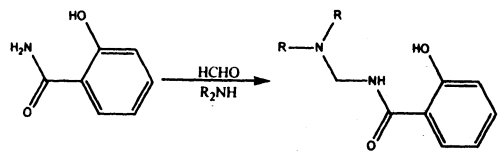
†Principal, Acharya Narendra Dev College of Pharmacy, Bhaban-272 713, India.

EXPERIMENTAL

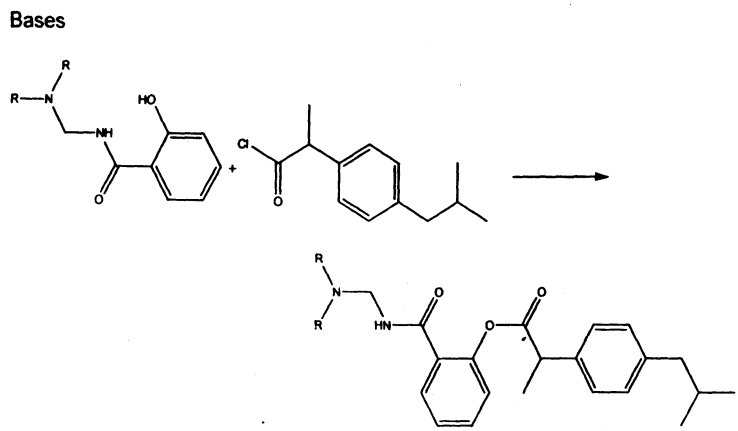
All chemicals used were of general reagent and fine chemicals grade. TLC was performed on silica gel G and KBr phase was used for IR on Shimadzu IR-47 spectrophotometer. Melting points of synthesized compounds were determined by Toshniwal melting point determination apparatus in open capillaries and are uncorrected. ^{1}H NMR spectra were recorded on Bruker at 200 MHz and mass spectra on Perkin-Elmer, U.S.A. using electrospray ionization technique.



Step I. Synthesis of acid chloride of ibuprofen



Step II. Synthesis of mannich bases of salicylamide



R = methyl, ethyl, propyl, butyl and morpholyl.

Step III. Synthesis of conjugates of ibuprofen with salicylamide and its mannich base.

SCHEME

Step I: Synthesis of acid chloride of ibuprofen: Ibuprofen (0.01 mol) was dissolved in dry benzene (5 mL); to this thionylchloride (0.01 mol) was added gradually with stirring. The reaction mixture was refluxed for 4 h. Thereafter excess of benzene and thionylchloride was removed under reduced pressure and a white crystalline product of acid chloride was collected, m.p. 60–62°C, yield 84%.

Step II: Synthesis of mannich bases of salicylamide: Formaldehyde (0.01 mol) and the respective amine (0.01 mol) were taken in a round-bottom flask along with 3 mL of absolute alcohol and salicylamide (0.01 mol). A small amount of concentrated hydrochloric acid (0.5 mL) was added and refluxed for about 12 h and allowed to cool. Excess of alcohol was distilled off and the remaining solution was kept for cooling at 0°C. The crystals of the compounds were separated, washed with hot water and recrystallized from water/alcohol mixture.

Step III: Synthesis of conjugates of ibuprofen with salicylamide and its mannich bases: The acid chloride of ibuprofen (0.01 mol) was dissolved in freshly distilled and dry pyridine (10 mL) and respective mannich base (0.01 mol) also dissolved in pyridine was added dropwise with shaking. The reaction mixture was allowed to stand overnight and poured into crushed ice to obtain the product. The crude product obtained was filtered, washed, dried and recrystallized from acetone.

Synthesis of 2-methyl-2-(4′-isobutyl phenyl)acetoxyphenyl-2-carboxamide (I): Synthesis was performed as per general procedure but without step II. Yield 64.4%; m.p. 108°C; partition coefficient (phosphate buffer saline (PBS) 7.4/$CHCl_3$): 13.2; TLC (benzene : methanol : ammonia 75 : 25 : 0.25); R_f value: 0.46; % Analysis (Calcd.) Found: C (73.82) 73.64; H (7.12) 6.98; N (4.30) 4.36; IR (KBr, cm^{-1}): 3300, 3200 ν(N—H, primary amide), 3000–2840 ν(C—H), 3020 ν(aromatic C—H), 1740 ν(C=O of ester), 1515 ν(N—H amide II band), 900 ν(1,4-substituted benzene ring), 700 ν(1,2 substituted benzene ring). ^{1}H NMR: δ 1.22 (6H, d, —CH($C\underline{H}_3$)$_2$), δ 1.67 (3H, d, —CH—$C\underline{H}_3$), δ 2.02 (1H, m, —$CH_2C\underline{H}$—($C\underline{H}_3$)$_2$), δ 2.42 (2H, d, —$C\underline{H}_2$—CH—), δ 3.96 (1H, q, $C\underline{H}$—(CH_3)), δ 6.30 (2H, —$CON\underline{H}_2$), δ 7.26 (4H, Ar$\underline{H}$ ibuprofen), δ 7.35–7.96 (4H, Ar$\underline{H}$ salicylamide); Mass: m/z 324.53.

Synthesis of 2-methyl-2-(4′-isobutyl phenyl)acetoxy phenyl-2-(N-dimethyl amino methyl)carboxamide (II): Synthesis was performed as per general procedure using dimethylamine. Yield 56.2%, m.p. 196°C; partition coefficient (PBS 7.4/$CHCl_3$): 19.8; TLC (benzene : methanol : ammonia 75 : 25 : 0.25); R_f value: 0.59; % Analysis (Calcd.) Found: C (72.22) 72.06; H (7.91) 7.72; N (7.32) 7.12; IR (KBr, cm^{-1}): 3300 ν(N—H, secondary amide), 3000–2840 ν(C—H), 3020 ν(aromatic C—H), 1750 ν(C=O of ester), 1640 ν(C=O of amide I band), 1590 ν(N—H amide II band), 900 ν(1,4-substituted benzene ring), 700 ν(1,2-substituted benzene ring). ^{1}H NMR: δ 1.02 (6H, d, —CH($C\underline{H}_3$)$_2$), δ 1.65 (3H, d, —CH—$C\underline{H}_3$), δ 2.11 (1H, m, —$CH_2C\underline{H}$—($C\underline{H}_3$)$_2$), δ 2.16 (6H, m, ($C\underline{H}_3$—CH_2)$_2$), δ 2.54 (2H, d, —$C\underline{H}_2$—CH—), δ 3.84 (1H, q, $C\underline{H}$—(CH_3)), δ 4.15 (2H, d, NH—$C\underline{H}_2$), δ 7.14 (4H, Ar$\underline{H}$ ibuprofen), δ 7.26–7.86 (4H, Ar$\underline{H}$ salicylamide), δ 8.14 (1H, t, $N\underline{H}$—CH_2); Mass: m/z 380.94.

Synthesis of 2-methyl-2-(4′-isobutyl phenyl) acetoxy phenyl-2-(N-diethyl-amino methyl)carboxamide (III): Synthesis was performed as per general procedure using diethylamine. Yield 68 %, m.p. 186°C; partition coefficient (PBS 7.4/$CHCl_3$): 20.7; TLC (benzene : methanol : ammonia 75 : 25 : 0.25); R_f Value: 0.60; % Analysis (Calcd.) Found: C (73.14) 73.24; H (8.35) 8.19; N (6.82) 6.67; IR (KBr, cm^{-1}): 3400 ν(N—H, secondary amide), 3030 ν(aromatic C—H),

3000–2840 ν(C—H), 1740 ν(C=O of ester), 1640 ν(C=O of amide I band), 1600 ν(N—H amide II band), 900 ν(1,4-substituted benzene ring), 750 ν(1,2-substituted benzene ring). ^{1}H NMR: δ 1.02 (6H, d, —CH($C\underline{H}_3$)$_2$), δ 1.26 (6H, m, ($C\underline{H}_3$—CH_2)$_2$), δ 1.65 (3H, d, —CH—$C\underline{H}_3$), δ 2.11 (1H, m, —$CH_2C\underline{H}$—($C\underline{H}_3$)$_2$), δ 2.40 (4H, m, (CH_3—$C\underline{H}_2$)$_2$N), δ 2.54 (2H, d, —$C\underline{H}_2$—$C\underline{H}$—), δ 3.84 (1H, q, $C\underline{H}$—(CH_3)), δ 4.15 (2H, d, NH—$C\underline{H}_2$), δ 7.14 (4H, Ar$\underline{H}$ ibuprofen), δ 7.26–7.86 (4H, Ar$\underline{H}$ salicylamide), δ 8.14 (1H, t, $N\underline{H}$—CH_2); Mass: m/z 409.31.

Synthesis of 2-methyl-2-(4′-isobutyl phenyl)acetoxy phenyl-2-(N-dipropylamino methyl)carboxamide (IV): Synthesis was performed as per general procedure using dipropylamine. Yield 52%, m.p. 182°C; partition coefficient (PBS 7.4/$CHCl_3$): 24.0; TLC (benzene : methanol : ammonia 75 : 25 : 0.25); R_f Value: 0.72; % Analysis (Calcd.) Found: C (73.94) 73.69; H (8.73) 8.51; N (6.39) 6.14; IR (KBr, cm^{-1}): 3400 ν(N—H, secondary amide), 3000–2840 ν(C—H), 3040 ν(aromatic C—H), 1740 ν(C=O of ester), 1640 ν(C=O of amide I band), 1600 ν(N—H of amide II band), 900 ν(1,4-substituted benzene ring), 750 ν(1,2-substituted benzene ring). ^{1}H NMR: δ 0.92 (6H, m, ($C\underline{H}_3$—CH_2)$_2$), δ 1.02 (6H, d, —CH($C\underline{H}_3$)$_2$), δ 1.26 (4H, m, ($CH_3C\underline{H}_2CH_2$)$_2$), δ 1.65 (3H, d, —CH—$C\underline{H}_3$), δ 2.11 (1H, m, —$CH_2C\underline{H}$—($C\underline{H}_3$)$_2$), δ 2.32 (4H, m, (CH_3—CH_2—$C\underline{H}_2$)$_2$), δ 2.56 (2H, d, —$C\underline{H}_2$—CH—), δ 3.62 (1H, q, $C\underline{H}$—(CH_3)), δ 4.16 (2H, d, NH—$C\underline{H}_2$), δ 7.16 (4H, Ar$\underline{H}$ ibuprofen), δ 7.24–7.56 (4H, Ar$\underline{H}$ salicylamide), δ 8.04 (1H, t, $N\underline{H}$—CH_2); Mass: m/z 437.12.

Synthesis of 2-methyl-2-(4′-isobutyl phenyl)acetoxy phenyl-2-(N-dibutylamino methyl)carboxamide (V): Synthesis was performed as per general procedure using dibutylamine. Yield 63.6 %, m.p. 172°C; partition coefficient (PBS 7.4/CHCl3): 25.32; TLC (benzene : methanol : ammonia 75 : 25 : 0.25); R_f Value: 0.56; % Analysis (Calcd.) Found: C (74.64) 74.24; H (9.07) 9.24; N (6.00) 5.86; IR (KBr, cm^{-1}): 3400 ν(N—H, secondary amide), 3000–2840 ν(C—H), 3030 ν(aromatic C—H), 1750 ν(C=O of ester), 1640 ν(C=O of amide I band), 1590 ν(N—H of amide II band), 900 ν(1,4-substituted benzene ring), 750 ν(1,2-substituted benzene ring). ^{1}H NMR: δ 0.92 (6H, m, ($C\underline{H}_3$—CH_2CH_2)$_2$), δ 0.99 (6H, d, —CH($C\underline{H}_3$)$_2$), δ 1.26 (4H, m, ($CH_3C\underline{H}_2CH_2CH_2$)$_2$), δ 1.48 (4H, m, ($CH_3$—$CH_2$—$C\underline{H}_2CH_2$)$_2$), δ 1.65 (3H, d, —CH—$C\underline{H}_3$), δ 2.11 (1H, m, —$CH_2C\underline{H}$—($CH_3$)$_2$), δ 2.38 (4H, m, ($CH_3$—$CH_2$—$CH_2C\underline{H}_2$)$_2$), δ 2.56 (2H, d, —$C\underline{H}_2$—CH—), δ 3.62 (1H, q, $C\underline{H}$—(CH_3)), δ 4.17 (2H, d, NH—CH_2), δ 7.16 (4H, Ar$\underline{H}$ ibuprofen), δ 7.24–7.66 (4H, Ar$\underline{H}$ salicylamide), δ 8.0 (1H, t, $N\underline{H}$—CH_2); Mass: m/z 465.21.

Synthesis of 2-methyl-2-(4′-isobutyl phenyl)acetoxy phenyl-2-(N-morpholino)carboxamide (VI): Synthesis was performed as per general procedure using morpholine. Yield 52 %, m.p. 180°C; partition coefficient (PBS 7.4/$CHCl_3$): 21.73, TLC (benzene : methanol : ammonia 75 : 25 : 0.25); R_f value: 0.70; % Analysis (Calcd.) Found: C (70.73) 70.54; H (7.6) 7.24; N (6.6) 6.51; IR (KBr, cm^{-1}): 3400 ν(N-H, secondary amide), 3000–2840 ν(C—H), 3030 ν(aromatic C—H), 1750 ν(C=O of ester), 1640 ν(C=O of amide I band), 1580 ν(N—H of amide II band), 1115 ν(C—O—C alkyl ether moiety), 900 ν(1,4-substituted benzene ring), 750 ν(1,2-substituted benzene ring). ^{1}H NMR: δ 0.99 (6H, d, —CH($C\underline{H}_3$)$_2$), δ 1.65 (3H, d, —CH—$C\underline{H}_3$), δ 2.11 (1H, m, —$CH_2C\underline{H}$—(CH_3)$_2$),

δ 2.41 (4H, t, $C\underline{H}_2$-morpholyl), δ 2.56 (2H, d, —$C\underline{H}_2$—CH—), δ 3.62 (1H, q, $C\underline{H}$—(CH_3)), δ 3.76 (4H, t, $C\underline{H}_2$-morpholyl), δ 4.17 (2H, d, NH—$C\underline{H}_2$), δ 7.14 (4H, Ar$\underline{H}$ ibuprofen), δ 7.22–7.46 (4H, Ar$\underline{H}$ salicylamide), δ 8.01 (1H, t, N$\underline{H}$—CH_2); Mass: m/z 423.04.

Analgesic activity: The analgesic activity of synthesized compounds was determined by tail flick method[7] using analgesiometer. Albino rats weighing between 150–200 g were randomly distributed in test and standard group of six animals. The screening was done at room temperature (35 ± 1°C). The compounds and standard were orally administered at doses 10 mg/kg body weight. The rat was placed in rat folder through which tail of rat protruded out. Current was adjusted so that more than 90% of rats gave tail flick response within five seconds. Test compounds were injected to respective groups through intraperitonial route. Pain threshold was measured after one hour of administration of test compounds.

Antiinflammatory activity: Antiinflammatory activity was evaluated by carrageenan induced rat paw edema method of Winter *et al.*[8] Albino rats of either sex weighing between 150–200 g were randomly distributed in control and experimental group of six animals. At zero hour the test compounds and standard were administered orally at doses equimolar to standard. 1 h after this treatment edema was induced in hind paw of rat of injection of 0.1 mL of 1% carageenan in distilled water into plantar tissues of paw. The initial paw volume was measured by plethysmometer with 30 s of the injection. The relative increase in paw edema was found by remeasuring the paw volume after 3 h of carageenan injection.

Ulcerogenic activity: The ulcerogenic activity was determined by the method of Hitchen *et al.*[9] taking ibuprofen as standard. The test compounds and standard were administered orally, as a suspension of 2% gum acacia suspension. Albino rats were divided into seven groups having six animals in each group. The dose (10 mg/kg body weight) was given daily for six days by oral route, water was given *ad libitum*. Rats were sacrificed on the seventh day; their stomachs were removed, opened along the greater curvature and washed slowly with saline. The inner surface was examined for ulceration under 20X magnification. All ulcers 0.5 mm were counted and per compound average number of ulcers were determined.

RESULTS AND DISCUSSION

The synthesized compounds were purified by recrystallization process using acetone and melting point determination, TLC and IR studies along with nitrogen estimation confirmed their purity. The compounds were also subjected to partition coefficient studies. All compounds have greater value of partition coefficient than the parent drug ibuprofen. Greater value obviously indicates high lipophilicity. The increase in lipophilicity may probably be due to increase in number of carbon atoms in the compound.

All the synthesized compounds have shown remarkable analgesic activity (Table-1). The compounds containing morpholine group, diethyl group and dimethyl group were shown to possess better activity as compared to that of standard. The compounds containing propyl and butyl group have shown results comparable with standard. From the above observations it could be inferred that more work could be carried out with other amines to obtain superior analgesics from the parent.

The compounds synthesized have shown antiinflammatory activity superior to the parent compound *i.e.*, ibuprofen, with the exception of the salicyl and propyl conjugates (Table-1). The sequence of the increase in activity being butyl, methyl, ethyl and morpholine and contrary to this the propyl analogue showing activity inferior to the standard, it is difficult to relate the activity with reference to alterations in the size of the moiety introduced. However, these observations suggest that a series of heterocyclic moieties be synthesized and screened for these activities. As such the analgesic as well as the antiinflammatory activity have been found enhanced in morpholine, ethyl, methyl and butyl analogues. The ulcerogenic activity (Table-1) being decreased in all the synthesized compounds, it may be stated therefrom that a detailed study in this respect is likely to give useful findings.

TABLE-1
ANALGESIC, ANTIINFLAMMATORY AND ULCEROGENIC ACTIVITY OF SYNTHESIZED COMPOUNDS

Compounds	Analgesic activity mean test latency sec. (after 1 h)	Anti-inflammatory activity % inhibition of edema at 4 h	Ulcerogenic Index
Control	4.25	—	—
Standard	7.25	41.55	2.50
Compound I	7.50	40.25	1.50
Compound II	8.15	50.64	0.38
Compound III	8.25	53.24	0.03
Compound IV	7.40	40.25	0.03
Compound V	7.75	48.05	0.19
Compound VI	8.50	53.24	0.26

ACKNOWLEDGEMENTS

The paper is dedicated to late Prof J.G. Asthana's aura behind the work. Authors are thankful to the Head, Department of Pharmaceutical Sciences, Dr. H.S. Gour University, Sagar for providing necessary facilities. The author, DKN is grateful to UGC and the authors AM and MSVK are grateful to CSIR, New Delhi for financial support.

REFERENCES

1. G. Singh and P. Sharma, *Indian J. Pharm. Sci.*, **56**, 69 (1994).
2. H. Bundgaard, Design of Prodrugs, Elsevier, Amsterdam, p. 1 (1985).
3. V.J. Stella, T.J. Mikkelson and J.D. Pipkin, In: R.L. Juliano, (Ed.), Drug Delivery System, Oxford University Press, Oxford, p.113 (1980).
4. A. Fukuhara, T. Imai and M. Otagiri, *Chirality*, **8**, 494 (1996).
5. N. Mork and H. Bundgaard, *Pharm. Res.*, **9**, 492 (1992).
6. N. Kawathekar and S.C. Chaturvedi, *The Eastern Pharmacist*, 117 (1997).
7. F.E. D'Amour and D.L. Smith, *J. Pharmacol. Exp. Ther.*, **72**, 74 (1941).
8. C.A. Winter, E.A. Risley and G.W. Nuss, *Proc. Soc. Expt. Biol. Med.*, **111**, 544 (1962).
9. J.T. Hitchen, S. Goldstein, A. Sanbucca and T. Shemano, *Pharmacologists*, **9**, 242 (1967).

(*Received*: 8 June 2004; *Accepted*: 24 December 2004) AJC-4031