Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Suppl 5, 2012

Research Article

SYNTHESIS AND CHARACTERIZATION OF NEW INDOLE DERIVATIVES FOR ANALGESIC ACTIVITY

BLESSI PRIYANKA.K, MAHARAJ.POGULA, SURESH.S, K.THIRUPATHI, SAMMAIAH.G.

Medicinal Chemistry Laboratory, University College of Pharmaceutical Sciences, Kakatiya University, Warangal.

Received: 07 July 2012, Revised and Accepted: 21 Aug 2012

ABSTRACT

A series of indole derivatives were prepared and profiled as antipyretic compounds. All the synthesized derivatives were characterized by means of chromatographic, IR, ^{1}H NMR & MASS spectral analysis. The synthesized derivatives were evaluated for *in vivo* analgesic activity by using Diclofenac sodium as a standard. The compound VIe (R=5-NO_2) showed potent activity when compared to Diclofenac sodium.

Keywords: Isatin derivatives, Benzimidazole, Analgesic.

INTRODUCTION

Indole is the most beneficial heterocyclic nucleus which has gained prominence in medicinal chemistry due to its diverse biological activities such as antimicrobial [1-6], anticancer, antioxidant [7-11], antipyretic, analgesic and anti-inflammatory [12-13] activities. It is interesting to note from literature that benzimidazole [15-21] exhibits spectrum of biological activities in different heterocyclic nuclei like microbial [15-19], anti-inflammatory [20-21].It is therefore thought worthwhile to synthesize some new indole derivatives by incorporating benzimidazole moiety in a single molecular frame work with the hope to posses better antimicrobial, anti-inflammatory and analgesic activities. In recent years, research is being focused on existing molecules and their modifications in order to reduce side effects and to explore their other pharmacological and biological effects.

Chemistry

Melting points of all synthesized compounds were determined by open capillary tubes using Toshniwal & Cintex melting point apparatus, expressed in ^{0}C and are uncorrected. The IR spectra (KBr pellets) were recorded on Elmer Spectrum BX-1 spectrometer for the compounds, ^{1}H NMR spectra were recorded for compounds on AV 300MHz NMR Spectrometer using TMS as an internal standard. The Mass spectra were recorded on LCQ ion Mass spectrometer. The purity of the compounds were checked by Thin Layer Chromatography(TLC) on Merck Silica gel 60 F254 pre coated sheet using Petroleum Ether and Ethyl acetate in 1:1 v/v.

METHODS

I. Synthesis of Indole-2, 3-diones (Isatins, III)

a) Isonitrosoacetanilides (II) – General Procedure:

In a 5 lit. R.B. flask was placed chloral hydrate (0.54 mol) and 1200 ml of water. To this solution, was then added crystallized sodium sulphate (1300 g) followed by a solution of an appropriate aromatic amine in 300 ml of water and concentrated hydrochloric acid (0.52mol). Finally, a solution of hydroxylamine HCl (1.58 mol) in 500 ml of water was then added. The contents of the flask were heated over a wire-gauge by a Meckner burner so that vigorous boiling begins in about 45 minutes. After 1 to 2 minutes of vigorous boiling the reaction was completed. During the heating period itself the crystals of isonitrosoacetanilides starts separating out. Upon cooling under the current of water, the entire product was solidified. It was filtered under suction, air dried and purified by recrystallization from suitable solvent(s).

b) Indole-2,3-diones (III) – General Procedure :

Sulphuric acid (600g, density 1.84, 326 ml) was warmed at 50^{0}C in a one liter RB flask fitted with an efficient mechanical stirrer and to this, finely powdered appropriate isonitrosoacetanilide (II, 0.46 mol) was added at such a rate so as to maintain the temperature between 60^{o}C to 70^{o}C but not higher. External cooling was applied at this stage so that the reaction could be carried out more rapidly. After the addition of isonitroso compound was completed the temperature of the solution was raised to 80^{o}C and maintained at that temperature for 10 minutes to complete the reaction. Then the reaction mixture was cooled to room temperature and poured onto crushed ice (2.5 kg) with stirring. After standing for about half-an-hour, the product separated was filtered, washed several times with small portions of cold water and dried. Purification of the compound was effected by the recrystallization from methanol.

II. Preparation of N-[6-(phenylsulfanyl)-1H-benzimidazol-2-yl] Hydrazine carboxamides (V)

A mixture of *N*-[6-(phenylsulfanyl)-1H-benzimidazol-2-yl] hydrazine carboxamides(V) (IV, 0.1mole) was refluxed with 0.1 mole of hydrazine hydrate 99% (0.2mole) in 20 ml of methanol (0.2mole) for 2 hrs. The reaction mixture was poured onto ice cold water and product separated was collected by filtration, dried and purified with methanol.m.p:180^{0}C.Yield: 90%.

III. General procedure for the preparation of 2(2-oxo-1, 2-dihydro-indole-3-ylidine) - N-[6- (phenylsulfanyl)-1H-benzimidazol -2-yl] Hydrazine carboxamides (VI)

A mixture of isatin (III, 0.01mole) and N-[6-(phenylsulfanyl)-1H-benzimidazol-2-yl] hydrazine carboxamide (V , 0.01mole) in 20 ml methanol containing traces of acetic acid was refluxed for 6 hrs. The solvent was evaporated; the residue was poured onto crushed ice. The product obtained was filtered, washed with cold water, dried and purified by dry column chromatography.

Adopting this procedure 10 isatin derivatives were prepared.

Spectral Data

1) CompoundVI (a): 2(2-oxo-1,2-dihydro-indole-3-ylidine)-N-[6(phenylsulfanyl)-1H benzimidazole-2-yl] hydrazine carboxamide: Yield 85%, m.p.285-286.^{1}H NMR (300 MHz, DMSO): δ [ppm]: 6.9-7.8 (m, 12H, Ar-H),8.2(S,1H,benzimidazoleN-H),8.6(S,1H,indoleN-H),10.7 (S, 1H, NH) and 11.6(S,1H,CONH).MASS: The molecular ion was observed at 429(m+1),the sodium bounded peak was observed at 451(m+Na): IR (KBr) cm^{-1}: 3228(NH), 1734.49 (C = O), 1522.40 (C=N).
2) CompoundVI (b): 2(5-Methyl-2-oxo-1, 2-dihydro-indole-3-ylidine)-N-[6(phenylsulfanyl)-1H-benzimidazole-2-yl] hydrazine carboxamide: Yield 84%, m.p.273-274. ^{1}H NMR(300MHz,DMSO): δ [ppm]:0.9(t,3H,CH_3),1.69 (m,2H,CH_2),6.9-7.8 (m, 11H,Ar-H), 8.2(S,1H,benzimidazole N-H),8.6 (S,1H,indole N-H),10.7 (S, 1H, NH) and11.6((S, 1H, CONH).MASS : The molecular ion was observed at 443(m+1),the sodium bounded peak was observed at 465 (m+Na): IR (KBr) cm^{-1}: 3169.99 (NH),1734.49 (C = O), 1522.44(C=N).

3) CompoundVI(C): 2(5-Bromo-2-oxo-1, 2-dihydro-indole-3-ylidine)-N [6(phenylsulfanyl)-1H-benzimidazole-2-yl] hydrazine carboxamide: Yield: 86%, m.p.271-272. ^{1}H NMR (300 MHz, DMSO): δ [ppm]: 6.9-7.8 (m, 11H, Ar-H), 8.4(S, 1H, benzimidazole N-H), 8.8(S, 1H, indole N-H) 10.9 (S, 1H, N-H) and 11.6(S, 1H, CONH). MASS: The molecular ion was observed at 508(m+1).The sodium bounded peak was observed at 530 (m+Na): IR (KBr) cm^{-1}: 3169.99 (NH), 1735.26 (C = O), 1527. 19 (C=N).
4) CompoundVI (D): 2(5-chloro-2-oxo-1, 2-dihydro-indole-3-ylidine)-N-[6-(phenylsulfanyl)-1H-benzimidazole-2-yl] hydrazine carboxamide: Yield: 81%, m.p.269-270. ^{1}H NMR (300 MHz, DMSO): δ [ppm]: 6.9-7.8 (m, 12H, Ar-H), 8.4(S, 1H,benzimidazole N-H),8.8(S,1H, indole N-H) 10.9 (S, 1H, NH) and 11.6 (S,1H,CONH).MASS : The molecular ion was observed at 464(m+1)The sodium bounded peak was observed at 486(m+Na): IR (KBr) cm^{-1}: 3228 (NH),1734.49 (C = O), 1522.40(C=N).
5) Compound VI (E): 2(5-Carboxy-2-oxo-1, 2-dihydro-indole-3-ylidine)-N-[6(phenylsulfanyl)-1H-benzimidazole-2-yl] hydrazine carboxamide: Yield: 84%, m.p.265-287. ^{1}H NMR (300 MHz, DMSO): δ [ppm]: 6.9-7.8 (m, 12H, Ar-H), 8.3(S, 1H, benzimidazole N-H), 8.7 (S, 1H, N-H) 10.8 (S, 1H, N-H) and11.6(S,1H,CONH).MASS : The molecular ion was observed at 473(m+1).The sodium bounded peak was observed at 495 (m+Na): IR (KBr) cm^{-1}: 3169.99(NH), 1735.26 (C = O), 1527.19(C=N).
6) Compound VI (f): 2(5-Nitro-(2-oxo-1, 2-dihydro-indole-3-ylidine)-N-[6(Phenylsulfanyl)-1H-benzimidazole-2-yl] hydrazine carboxamide: Yield 83%, m.p 268-270. ^{1}H NMR (300MHz, DMSO): δ [ppm]: 6.9-7.8 (m, 12H, Ar-H), 8.3 (S, 1H, benzimidazole N-H), 8.7(S, 1H,indole N-H),10.8 (S, 1H, N-H) and 11.6(S,1H,CONH). MASS: The molecular ion was observed at 464(m+1), the sodium bounded peak was observed at 486(m+Na): IR (KBr) cm^{-1}: 3228 (NH), 1734.49 (C = O), 1522.40(C=N).
7) CompoundVI (g):2(7-Nitro-2-oxo-1, 2-dihydro-indole-3-ylidine)-N-[6(phenylsulfanyl)-1H-benzimidazole-yl] hydrazine carboxamide: Yield 87%, m.p.267-269. ^{1}H NMR (300 MHz,DMSO): δ [ppm]: 6.9-7.8 (m, 12H, Ar-H), 8.3(S, 1H, benzimidazole N-H), 8.7(S, 1H,Indole N-H), 10.8 (S, 1H, N-H) and 11.6(S, 1H, CONH).MASS: The molecular ionwas observed at 486(m+1). The sodium bounded peak was observed at 451(m+Na): IR(KBr) cm^{-1}: 3345.10 (NH), 1625.26 (C = O), 1323.73(C=N).
8) Compound VI (h): 2(7-Carbomethoxy-2-oxo-1, 2-dihydro-indole-3-ylidine)-N-[6(Phenylsulfanyl)-1H-benzimidazole-2-yl] hydrazine carboxamide: Yield: 88%, m.p.265-267. ^{1}H NMR (300 MHz, DMSO): δ [ppm]: 6.9-7.8 (m, 12H, Ar-H), 8.2(S, 1H, benzimidazole N-H), 8.6(S, 1H, indole N-H), 10.7 (S, 1H, NH) and 11.6(S, 1H, CONH). MASS: The molecular ion was observed at 487(m+1), the sodium bounded peak was observed at 509(m+Na): IR (KBr) cm^{-1}: 3291.22 (NH), 1734.49 (C = O), 1617.45(C=N).
9) Compound VI (i): 2(7-Chloro-2-oxo-1, 2-dihydro-indole-3-ylidine)-N- [6(Phenylsulfanyl)-1H-benzimidazole-2-yl] hydrazine carboxamide: Yield: 84%, m.p.269-270. ^{1}H NMR (300 MHz, DMSO): δ [ppm]: 6.9-7.8 (m, 11H, Ar-H), 8.3(S, 1H, benzimidazole N-H), 8.7(S, 1H, indole N-H) 10.8 (S, 1H, N-H) and 11.6(S, 1H, CONH).MASS: The molecular ion was observed at 464(m+1). The sodium bounded peak was observed at 486(m+Na): IR (KBr) cm^{-1}: 3169.69 (NH), 1734.49 (C = O), 1527.19(C = N).

Experimental Procedure

Analgesic activity by using Eddy's Hot plate method

All the experiments were carried out using male Albino mice (25-30g). On arrival, these animals were placed at random in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature 24 ± 2 ^{o}C and relative humidity 30-70%. A 12:12 light: day cycle was followed; all animals were allowed free access to water with standard commercial chaw pellets. All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics Committee.

Each group of four (n=4) mices were selected for the present study. One group served as control and received the vehicle and another group received the standard drug Diclofenac sodium (2.5mg/kg, i.p.).The drug concentration of 10mg in 25 ml DMSO (30%) was administered i.p to the other groups. The mice were placed on Eddy's hot plate kept at a temperature of 55 ± 0.5 ^{o}C for a maximum time of 15sec. Reaction time was recorded when the animals licked their fore-and hind paws and jumped at 0,15,30,60,90 minutes respectively. The relative potencies of Diclofenac sodium were determined. In the statistical analysis all the data was analyzed by using student *t*- test (Table-I). P values <0.05 were considered as statistically significant. Synthesized compounds (R= H, 5-CH_3, 5-NO_2, 5-Cl, 5-Br) were tested for analgesic activity by using Diclofenac Sodium as a standard. At the first 15th minute all the compounds exhibited potent analgesic activity when compared to the standard. At the 30th minute all the test compounds retained the analgesic activity. At the 60th, 90th minute the test compounds exhibited reduction in the analgesic activity. Compound (R= 5-NO_2) showed potent analgesic activity among the tested compounds when compared to standard Diclofenac sodium. Compound (R= 5-CH_3) showed less analgesic activity, and compounds (R= H, 5-Cl, 5-Br) showed moderate analgesic activity when compared to standard Diclofenac Sodium.

RESULTS AND DISCUSSION

All the compounds were analyzed by means of chromatographic, ^{1}H NMR, IR and MASS spectral analysis. Compounds (R= H, 5-CH_3, 5-NO_2, 5-Cl, 5-Br) were evaluated for their analgesic activity by using Diclofenac sodium as a standard. Compound (R= 5-NO_2) showed potent analgesic activity when compared to Diclofenac sodium.Compound (R= 5-CH_3) showed less analgesic activity and compounds (R=H, 5-Cl, 5-Br) showed moderate analgesic activity when compared to Diclofenac sodium as a standard.

Table: Analgesic activity of 2(2-oxo-1, 2-dihydro-indol-3-ylidine) - N-[6-(phenylsulfanyl)-1H-benzimidazol-2-yl] hydrazine carboxamides (VI):

Control (DMSO)	Standard (Diclofenac)	T_1(R=H)	T_2(R=5NO_2)	T_3 (R=5CH_3)	T_4(R=5-Cl)	T_5(R=5Br)
6	14	9	12	11	9	7
5	13	12	11	9	12	9
8	13	13	13	6	10	8
4	12	8	10	8	11	7
Pvalue	0.0003	0.0176	0.0017	0.087	0.0044	0.0871

REFERENCES

1. A.K. Padhy, S.K. Sahu, P.K. Panda, D.M. Kar and P.K. Misro, *Indian Journal of Chemistry,* 43B, (2004), 97.
2. M.M. Gupta, S. Raman, Vikas, S.N. Srivastava, *Asian Journal of Chemistry,* 16(2),(2004), 779-783.
3. M. Ajitha, K. Rajnarayana and M. Sarangapani, *Pharmazie,* 57(12), (2002), 796.
4. V. Alagarsamy, R.Venkatesh perumal*. Indian Journal of Heterocyclic Chemistry,* 11, (2002), 327.
5. R.M. Abdel Rahman, Z.E.I. Gendy and M.B. Mahmoud, *Indian Journal of.Chemistry,*29B, (1990), 352.

6. S.N. Pandeya, D. Sriram, G. Nath and E. De Clercq, *Drug Research,* 50(I),(2000), 55.
7. G.Sammaiah and M.Sarangapani, *Asian Journal of Chemistry*, 20,(2008), 75-80.
8. G.Sammaiah and M.Sarangapani, *International Journal of Chemical sciences*,5(1), (2007), 181-188.
9. G.Sammaiah and M.Sarangapani, *Asian Journal of Chemistry*, 20(1), (2008), 282-286.
10. G.Sammaiah and M.Sarangapani, *International Journal of Chemical Sciences,* 6(2), (2008), 503- 508.
11. G.Sammaiah and M.Sarangapani, *International Journal of Chemical Sci*ences, 5(3), (2007), 1429-1435.
12. S.K. Sridhar and A. Ramesh, *Indian Journal of Chemistry***,** 41B, (2002), 668.
13. Suroor A. Khan, Anees A. Siddiqui and Shibeer Bhatt, *Asian Journal of Chemistry,* 14(2),(2002), 1117.
14. S.K. Sridhar and A. Ramesh, *Indian Drugs,* 38(4), (2001), 174.
15. N.M. Goudgaon, V. Dhondiba and A. Vijayalaxmi, *Indian Journal of Heterocyclic*
16. *Chemistry***,**13, (2004), 271.
17. Ashutosh K. Bhatt, Hasanali G. Karadia, Palak R. Shah, Manisha P.Parmar and Hitesh D. Patel, *Indian Journal of Heterocyclic Chemistry,* 13, (2004), 281.
18. Vinay Prabha Sharma, *Indian Journal of Heterocyclic Chemistry,* 13, (2003), 171.
19. Ashutosh K. Bhatt, Hasanali Karadiya, Palak R. Shah, Manisha P.Parmae and H.D. Patel, *Indian Journal of Heterocyclic Chemistry***,** 13, (2003), 187.
20. V.Sundari and R. Valliappan, *Indian Journal of Heterocyclic Chemistry***,** 14, (2004), 47.
21. Kulkarni, Manohar V. Patil and D. Vemana, *Arch. Pharm.,* 314(6), (1981), 440.
22. Saehney, Vir, Dharam Gupta, Asha, *Indian Journal of Chemistry, Sec-B,* 29B(12),
23. (1990),1107.
24. U. Grafe and L. Radics, *Journal of Antibiotics,* 39, (1986), 162. (b) U. Grafe, W. Schade and W. Fleck, *Ger (East) DD,* 241, (1986), 749.