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Synthesis of N-(Alkyl or Aryl)-2-(1*H*-benzotriazol-1-yl) -acetamides as selective COX-2 inhibitor

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Abstract:
Microwave assisted organic synthesis (MAOS) has emerged as frontier in pharmaceutical research for synthesis of newer drugs and implementing GREEN chemistry. Arylacetamides are pharmaceutically interesting as they show various biological activities such as analgesic [1], local anesthetic, antiarthritic, antiarrhythmic activities [2], etc. In arylacetamides the nature of aromatic ring and its substituent is primary determinant for its activity. To serve this purpose we have decided to substitute arylacetamide with benzotriazole which may exploit the analgesic potential of newly synthesized derivatives i.e N-(Alkyl or Aryl)-2-(1H-benzotriazol-1-yl)-acetamides and thus it may be helpful in reducing the pain without having side effect of ulcerogenicity.
Key Words: Microwave, N-(Alkyl or Aryl)-2-(1H-benzotriazol-1-yl)-acetamides, ulcerogenicity.

INTRODUCTION:

A major mechanism of action of NSAIDs (nonsteroidal anti-inflammatory drugs) is in lowering prostaglandin biosynthesis through inhibition of cyclooxygenase (COX), which has a dual function, mediation of inflammation and cytoprotection of the stomach and intestine. It was discovered that COX exists in two isoforms, COX-1 and COX-2, which are encoded by two distinct genes. COX-1 is expressed constitutively providing cytoprotection, while COX-2 is transiently upregulated by proinflammatory mediators. This regulated expression suggests that a selective inhibitor of COX-2 may have anti-inflammatory properties and lack the ulcerogenic side effects. This hypothesis has been supported by several studies with selective COX-2 inhibitors, among these reported studies of anti-inflammatory, analgesic agents were 2-(5-Acetyl-3-phenyl-1,3,4-thiadiazol-2-ylidene)-1-(2-benzofuryl)-2-(1*H*-benzotriazolyl)-ethanone [1]. To obtain new anti-inflammatory agents as potent as the commonly used anti-inflammatory drugs and to reduce side effects, such as ulcerogenicity. As Arylacetamide derivatives are non-acidic and shows analgesic activity also the 1*H*-1,2,3-Benzotriazole bearing an N(3)-heterocycle which is azimidobenzene, benzene ring-fused azole compounds have drawn our attention due to their interesting anti-inflammatory and analgesic activities as well as a lack of ulcerogenic effects [5].

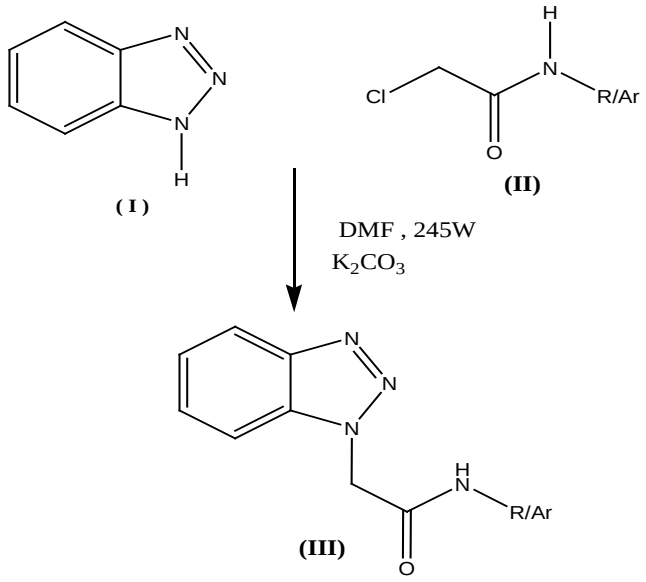
Aryl group of arylacetamide substituted with benzotriazole may exploit the analgesic potential of newly synthesized derivatives i.e N-(Alkyl or Aryl)-2-(1*H*-benzotriazol-1-yl)-acetamides and thus it may be helpful in reducing the pain without having side effect of ulcerogenicity. As it will form an bulkier molecule which will bind to side pocket of COX-2 receptor and the derivatives may show specific COX-2 inhibition.

Chemistry:

1*H*-1,2,3-Benzotriazole was synthesized by diazotization of ortho phenylene di amine using glacial acetic acid and sodium nitrate. 2-chloro-N- Alkyl/Aryl acetamide was synthesized by drop wise adding Four equivalents of chloroacetyl chloride over one hour to the aqueous amine solution. Finally N-(Alkyl or Aryl)- 2-(1H-benzotriazol-1-yl)-acetamide derivatives were synthesized by adding 2-chloro-N- Alkyl/Aryl acetamide to 1*H*-1,2,3-Benzotriazole and using anhydrous potassium carbonate as a base.

EXPERIMENTAL:

All chemicals used were of Laboratory Reagent (LR) Grade. The synthesized compounds were characterized by melting point, TLC, FT-IR, GC-MS and NMR. Thin Layer Chromatography was performed using Silica Gel G (Merck Index) coated on glass plates and the spots were visualized by exposure to iodine. Melting points were taken in open glass capillary tubes in liquid paraffin bath and were uncorrected. IR spectra were recorded on FTIR-8400S SHIMADZU spectrophotometer. GC-MS spectra & chromatogram were recorded on GCMS-QP 2010 SHIMADZU instrument. All solvents were distilled before use. All nonaqueous reactions were performed in dry glassware. All microwave reactions were carried on 'Catalyst systems Scientific microwave System' with automatic power setting from 140 watt to 700 watt. The reactions were started for initial 2 min. and monitored by TLC for completion of the reaction. All required chemicals were procured from commercial sources.



1*H*-1,2,3-Benzotriazole(I)

OPD (0.1mole) was dissolved in a mixture of glacial acetic acid (0.2mole) and 30ml of water contained in a 250ml of beaker, it was warmed slightly. Clear solution was cooled to 15^0C , then it was stirred magnetically and added to a solution of sodium nitrate (0.11mole) in 15 ml of water. Reaction mixture became warmed and within 2-3min reached a temperature of about 85°C ,colling was started with the change of colour from deep red to pale brown. Stirring was continued for 15min and then it was chilled in ice water bath for 30min.Pale borown precipitate of benzotriazole **(I)** seperates out which was washed with ice cold water. Recrystalised using boiling water [3].

Yield: 67.90 %

m.p. 96-99°C

2-chloro-*N*- Alkyl/Aryl acetamide(II)

Four equivalents of chloroacetyl chloride was added drop wise over one hour to the aqueous amine solution. Then the solution was left to stir overnight. The desired product was isolated as precipitate after pouring reaction mixture to an ice-cold water. Precipitate was filtered, washed with cold water and dried. Recrystalised using 95% ethanol [4].

***N*-(Alkyl or Aryl)- 2-(1*H*-benzotriazol-1-yl)-acetamide derivatives (III)**

Synthesized 2-chloro-*N*- Alkyl/Aryl acetamide derivative **(I)** (0.02mole) was dissolved in DMF. To it benzotriazole **(II)** (0.02mole) in DMF was added. Anhydrous Potassium carbonate (3g) was added to above reaction mixture. Then the mixture was heated in a microwave at 245watt and completion of reaction was monitored using TLC after every 2min. After completion of reaction, mixture was poured into ice-cold water, precipitate obtained was filtered, dried and recrystalised using 95% ethanol. Melting point and percentage yield was reported.

ANALGESIC ACTIVITY:

Writhing test

The antinociceptive effect is assessed by using the writhing test. In this test pain is induced by injection of an irritating agent such as phenylquinone or acetic acid into the intraperitonial cavity of mice. Intraperitonial administration of acetic acid (0.6%) provokes characteristic stretching behavior which is called writhing [5]. Reduction of number of writhings was considered as antinociception [6].

RESULTS:

The structure, physical properties, yield, R_f values, Melting point, IR,and Mass are presented in Table 1.

IIIa.

IR: 3279.10 (N-H stretch 2° amide), 1681.96 (C=O stretch 2° amide), 1548.89 (N-H bend 2° amide) ,1249.91 (C-N strech 2° amide) , 750.33 (1,2 disubstituted benzene ring) . MS m/z =252 (M^+).

IIIb.

IR: 3275.24 (N-H stretch 2° amide), 1662.69 (C=O stretch 2° amide), 1570.11 (N-H bend 2° amide), 1450.52 (C-N strech 2° amide), 1543.10 (Bending due to combination of C-N strech and N-H bend), 763.84 (Ortho-disubstituted benzene ring). MS m/z =282 (M+).

IIIc.

IR: 3248.23 (N-H stretch 2° amide), 1662.69 (C=O stretch 2° amide), 1546.96 (N-H bend 2° amide), 1404.22 (C-N strech 2° amide), 746.48 (1,2 disubstituted benzene ring), 871.85 (Para-disubstituted benzene ring), 1550-1490 (NO_2- Asymmetric stretch), 1355-1315 (NO_2- Symmetric stretch). MS m/z =297(M^+).

Table -1: physicochemical properties of the synthesized analogues

Analogues	*Substituent (R)*	*Yield (%)*	*Rf value*	*M.P.(℃)*
IIIa	Phenyl	59.76%	0.69	215-217
IIIa	Methoxy Phenyl	85.60%	0.66	167-169
IIIa	p- Nitro phenyl	96.38%	0.59	242-245
IIIa	p- Methyl phenyl	90.09%	0.48	198-200
IIIa	p-Flouro phenyl	87.06%	0.47	295-297
IIIa	o-Chloro phenyl	70.12%	0.69	178-180
IIIa	Napthalene	87.50%	0.39	230-233

Table 2: Effect of synthesized analogues on number of writhings of mice.

Treatment Group (mg/kg)	*No. of Writhings*	*% Inhibition*
Control	70.33±6.11	-
Aspirin (100)	14.67 ± 3.52*	79.18%
Bt(30)	38.33 ± 8.00*	45.49%
IIIa. (30)	20.0± 10.07*	71.56%
IIIb (30)	22.33± 5.48*	68.24%
IIIc (30)	15.66 ±2.96*	77.73%
IIId (30)	14.67± 4.17*	79.14%
IIIe (30)	12.33± 6.93*	82.46%
IIIf (30)	13.0± 2.30*	81.85%
IIIg (30)	16.33± 3.18*	76.78%

IIId.
IR: 3275.24 (N-H stretch 2° amide), 1680.05 (C=O stretch 2° amide), 1539.25 (N-H bend 2° amide), 1354.07 (C-N strech 2° amide), 817.85 (Para-disubstituted benzene ring), 709.83(1,2 disubstituted benzene ring). MS m/z =266 (M^+).

IIIe.
IR: 3279.10 (N-H stretch 2° amide), 1662.69 (C=O stretch 2° amide), 1546.96 (N-H bend 2° amide), 1354.07 (C-N strech 2° amide), 817.85 (Para-disubstituted benzene ring), 709.83(1,2 disubstituted benzene ring). MS m/z =269 (M^+).

IIIf.
IR: 3285.59 (N-H stretch 2° amide), 1668.48 (C=O stretch 2° amide), 1541.18 (N-H bend 2° amide), 1446.66 (C-N strech 2° amide), 1087.89 (Aryl chloride), 758.05 (Ortho-disubstituted benzene ring). MS m/z =286 (M^+).

IIIg.
IR: 3317.67 (N-H stretch 2° amide), 1689.70 (C=O stretch 2° amide), 1581.68 (N-H bend 2° amide), 1531.53 (C-N strech 2° amide), 752.26 (1,2 disubstituted benzene ring), 856.42 (Meta-disubstituted benzene ring), 767.69 (Meta-disubstituted benzene ring), 628.81 (Meta-disubstituted benzene ring). MS m/z =301(M^+).

ANALGESIC ACTIVITY:

Writhing test [10]

Mice (Swiss Albino) of either sex with weight between 20 to 25 g were used for the study. Groups of five animals each were used for control and treated. Twelve hours before experiments, food was withheld with free access to water. The synthesized arylacetamide derivatives (30 mg/kg p.o.) and aspirin (100 mg/kg p.o.) were given to animal by preparing suspension of compounds in 0.05% sodium carboxymethyl cellulose. After 1 hr acetic acid (0.1 ml/10 g body weight of a 0.6% v/v solution) was injected intraperitoneally to induce pain. Immediately after injection of acetic acid, the animal was isolated in an individual observation chamber. The number of writhing responses (abdominal constriction) was recorded for 20 min. Aspirin was used as standard analgesic drug. Number of writhing responses (abdominal constriction) was recorded for 20 min are tabulated (Table No 2).

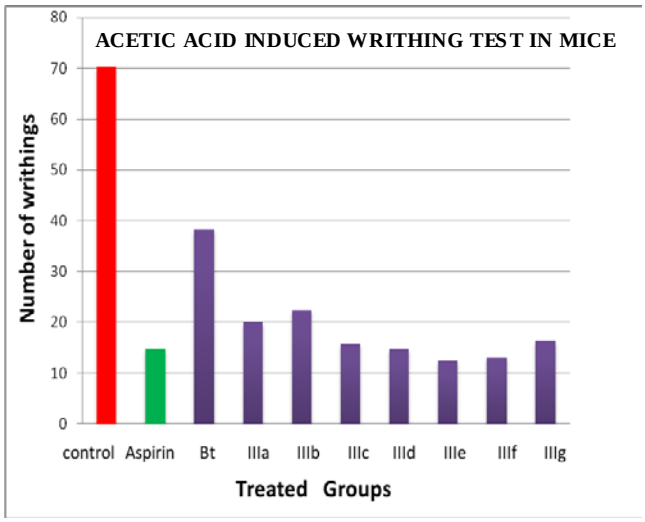


Figure 1: Effect of N-(Alkyl or Aryl)-2-(1*H*-benzotriazol-1-yl) acetamide derivatives on acetic acid induced writhing test in mice. The observations are represented as mean ± SEM (n=5). *p<0.05, as compared to control. All data is subjected to one way ANOVA followed by Dunnett's multiple comparison test. Vertical line represents SEM.

DISCUSSION:

The synthesis of 1*H*-1,2,3-Benzotriazole was carried out by diazotization of Orthophenylene diamine (OPD). In which the nitrosation of primary amine group of OPD was carried out using sodium nitrate and dilute acid which leads to the formation of diazonium cation.

The 2-chloro-N- Alkyl/Aryl acetamides were used as an intermediates for synthesis of N-(Alkyl or Aryl)-2-(1*H*-benzotriazol-1-yl)-acetamide. The synthesis of 2-chloro-N-Alkyl/Aryl acetamide was carried out by nucleophilic substitution reaction of chloroacetyl chloride and various alkyl or aryl amines without using any kind of base. In this reaction instead of using excess of amine we used four equivilent of chloroacetyl chloride, which was added dropwise over 1 hr to the aqueous amine solution. The solution was left to stir overnight and the desired product was isolated as a precipitate after adding the reaction mixture in ice cold water. In this synthesis it was observed that, for aliphatic amines after pouring reaction mixture into ice cold water it was getting dissolved in to water and thus product was not separated. Thus for aliphatic amine we have used ethyl chloroacetate instead of chloroacetyl chloride as side product formed in this reaction is ethanol and not HCl , final product gets precipitated out in the reaction flask without adding it into ice cold water.

The N-(Alkyl or Aryl)-2-(1*H*-benzotriazol-1-yl)-acetamide derivatives were synthesized by nucleophilic substitution reaction of Benzotriazole and 2-chloro-N- Alkyl/Aryl acetamides using potassium carbonate as a base in presence of DMF as a solvent.

The structures of all the synthesized compounds were supported by chemical test for chloride and amide. While conformation of structure was done by spectral data like FTIR, GC-MS.

All N-(Alkyl or Aryl)-2-(1*H*-benzotriazol-1-yl)-acetamide derivatives were screened for peripheral analgesic activity by using acetic acid induced writhing test in mice. The results indicate that structures with highest activity are IIIe and IIIf as compared to aspirin. These two compounds showed more percent inhibition i.e. 82.46% and 81.85% respectively than aspirin (79.18%), used as reference standard. This shows that IIIe and IIIf are more potent than aspirin. Similarly IIId also showed interesting percent inhibition (79.14%) with value almost similar to that of aspirin. Other derivatives Bt, IIIa, IIIb, IIIc and IIIg are considered to have moderate activity.

The highest peripheral analgesic activity shown by IIIe and IIIf may be attributed to electron withdrawing group.

CONCLUSION:

According to the experimental work done and results we conclude that, in aryl acetamide Ar group replaced with 1*H*-1,2,3-Benzotriazole moiety, resulting acetamide derivatives of benzotriazole showed analgesic activity. During pharmacological evaluation, it is found that Compounds Bt, IIIa, IIIb, IIIc and IIIg produced significant antihypertensive activity comparable to aspirin. Further structural activity relationship studies have to be carried out on these compounds to get a better analgesic activity.

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