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Synthesis, Characterization, Antimicrobial Studies and Pharmacological Screening of Some Substituted 1,2,3-triazoles

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A few substituted 1,2,3-triazoles have been synthesized by the 1,3-dipolar cycloaddition of azides with substituted acetylene. The cycloaddition of some amido alkyl azides with dimethylacetylene dicarboxylate furnish 1,4,5-trisubstituted triazoles. Three new 2-(4,5-dicarbomethoxy-1,2,3-triazol-1-yl)-N-(substituted aryl) acetamide were synthesized, represented as IIIa, IIIb and IIIc in which R=OCH_3, CH_3 and H respectively. The products have been characterized by IR, PMR and Mass spectra. All the three compounds were screened for antimicrobial (antifungal and antibacterial) activities. All of these exhibit antimicrobial activities comparable with standard drugs. Among the three, the compound IIIa shows more antimicrobial activities. Therefore, the same compound was taken for detailed pharmacological screening including, analgesic, antiinflammatory, local anaesthetic and antihistaminic activities. The compound answers for analgesic, antiinflammatory and local anaesthetic activities comparable with standard drugs but failed to show any antihistaminic activity.

Pyrazolo [3,4-d] [1,2,3] triazolo-1-carboxamides and 5-alkyl amino pyrazolo [3,4-d] oxazoles[1] were shown to have antifungal activity and N-benzyl-4-phenyl-1,2,3-triazole derivatives[2] showed prostaglandin synthesis inhibition. Triazole derivatives are known to possess a variety of pharmacological properties such as antineoplastic[3], anti-HIV-I[4], antidepressant[5], antiinflammatory[5], anticonvulsant[6], CNS depressant[6], anthelmintic[7], antitubercular[8], antihypertensive[9] and antiviral[10] activity. These observation prompted us to synthesize the title compounds which may have some biological activities.

Treatment of substituted aniline (amino compounds) with chloro acetylchloride gives 2-chloro-N-(substituted aryl) acetamide, compound (I) (Scheme), which reacted with sodium azide to give 2-azido-N- substituted aryl acetamide, compound (II). The compound 2-(4,5-dicarbomethoxy-1,2,3-triazol-1-yl)-N-(substituted aryl) acetamide, compound (III), have been synthesized from 1,3-dipolar cycloaddition of compound (II) with dimethyl acetylene dicarboxylate[11]. The structural assignments of the products are based on their IR[12], PMR and Mass spectral data[13].

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EXPERIMENTAL

All the melting points were taken in open capillary tubes and were uncorrected. IR spectra were recorded using a Brucker IFS 66V FT-IR spectrophotometer. PMR spectra were recorded on a HITACHI, R-600, 60 MHz NMR spectrometer using tetramethylsilane (TMS) as internal standard. Mass spectra were recorded on a Shimadzu QP 5000, Mass spectrometer.

Preparation of 2-chloro-N-(substituted aryl) acetamide (I)

Chloroacetyl chloride (0.1 mol) was dissolved in 50 ml of dry benzene and added dropwise to a well cooled and magnetically stirred benzene solution (100 ml) of the substituted aniline (e.g. anisidine, p-toluidine and aniline) (0.1 mol) and pyridine (0.1 mol). The stirring was continued for 3-6 h and left overnight. The product (I) was recrystallized from benzene and petroleum ether[14].

Preparation of 2-azido-N-(substituted aryl) acetamide (II)

A mixture of the 2-chloro-N-(substituted aryl) acetamide (50 m mol) and sodium (55 m mol) in 50 ml of acetone and 5 ml of dimethyl formamide was stirred magnetically for 3-6 h at 50-60°. The acetone was removed. The product (II) obtained was purified by recrystallization from benzene and petroleum ether[15].

Preparation of 2-(4,5-dicarbomethoxy-1,2,3-triazol-1-yl)-N-(substituted aryl) acetamide (III)

A mixture of 2-azido-N-(substituted aryl) acetamide and dimethyl acetylene dicarboxylate in 15 ml of dry toluene was stirred magnetically at room temperature for 3 h. The solution was concentrated and the product (III) was collected and recrystallized from chloroform and petroleum ether[16]. The completion of the reaction in each step was confirmed by thin layer chromatography (TLC).

Antimicrobial Studies

The antibacterial and antifungal activities of the synthesized compounds were determined. The compounds were taken at a concentration of 5 mg/ml using dimethyl sulphoxide as solvent by cylinder-plate[17] method against Gram positive organisms that include *Bacillus subtilis*, *Bacillus cereus* and *Staphylococcus aureus*, and Gram negative organisms such as *Escherichia coli* and *Salmonella typhi*. The standards used were tetracycline for Gram positive organisms, gentamycin for Gram negative organism and chloramphenicol for *Salmonella typhi*. The standards were tested at a concentration of 100 µg/ml. For antifungal activity the compounds were tested at 5 mg/ml concentration. The method used to study antifungal activity was the disc-plate[18] method against *Candida albicans*. The activity was compared with a well known antifungal drug clotrimazole at 100 µg/ml concentration.

Acute Toxicity-LD_{50} Determination

LD_{50} is the dose that is expected to kill 50% of test animals. It is determined by Karber's method[19]. The test animals used were albino mice. The compound was administered by intraperitoneal route. The LD_{50} of the compound IIIa, determined by this method was found to be 419 mg/kg.

Analgesic Activity : Tail-flick Method[20,21]

Three doses of the compound IIIa given to different groups of albino rats were approximately 5%, 10% and 15% of LD_{50} value which corresponds to 21.5, 43 and 64.5 mg/kg by intraperitoneal route. The time required to withdraw the tail from the radiant heat source was noted every 15 min upto 90 min. This activity was compared with known standard pentazocine lactate administered at 5 mg/kg.

Antiinflammatory Activity : Formalin-Induced Rat Hind Paw Qedema Method[22]

Three doses of the compound IIIa given to different groups of albino rats were approximately 5%, 10% and 15% of LD_{50} value which corresponds to 21.5, 43 and 64.5 mg/kg by the intraperitoneal route. Thirty minutes after the compound administration, each rat in all the groups were injected with 0.1 ml of 3.5% formalin solution in to the right hind paw at the tibiotarsal joint. The thickness of the paw was measured with the help of a plethysmograph at the end of 60, 120, 180, 240 and 300 min. Per cent reduction in oedema was calculated. The

Table I - Physical and Spectral data of Compounds IIIa-c

Compounds	R	Yield %	mp	Formula	IRγ max (KBr) cm^{-1}	PMR($CDCL_3$) (δPPM) Aromatic	Others	Mass Peak m/e
IIIa	4-OCH_3	25.15	140-140	$C_{15}H_{17}N_4O_6$	3193, 1756, 1677, 1614, 1554, 1357, 1284, 832	6.71 to 7.42 (m, 4H)	3.76 (s, 3H, Ar-OCH_3) 3.95 (s, 6H, two-OCH_3) 5.50 (s, 2H, -CH_2-) 8.27 (NH)	83, 97, 111, 129, 149, 171, 183, 201, 211, 227, 257.
IIIb	4-CH_3	60.75	141-142	$C_{15}H_{17}N_4O_5$	3194, 2003, 1741, 1668, 1621, 1555, 1350, 822	7.1 to 7.28 (m, 4H)	2.31 (s, 3H, $ArCH_3$) 4 (s, 6H, two-OCH_3) 5.5 (s, 2H,-CH_2-)	83, 97, 111, 129, 149, 157, 171, 183
IIIc	H	38.01	125-126	$C_{14}H_{15}N_4O_5$	3194, 1741, 1612, 1560, 1350	7.18 to 7.42 (m, 5H)	3.985 (s, 6H, Two-OCH_3) 5.51 (s, 2H, -CH_2-)	97, 111, 129, 143, 157, 171, 183, 257

Table II - Antimicrobial Activity of Compounds IIIa-c

Compound	R	Antibacterial Activity					Antifungal Activity
		Zone of Inhibition (mm)					Zone of Inhibition (mm)
		B. cerus	*S. aureus*	*E. coli*	*B. subtilis*	*S. typhi*	Candida albicans
III a	$-OCH_3$	11.6	12.5	12	-	-	15.3
III b	$-CH_3$	10.2	11.5	11	-	-	13.9
III c	-H	9.2	10.5	10.5	-	-	13.7
Tetracycline	-	15.7	14.5	-	15.3	-	-
Gentamycin	-	-	-	14.5	-	-	-
Chloramphenicol	-	-	-	-	-	14.5	-
Clotrimazole	-	-	-	-	-	-	12.5
DMSO	-	-	-	-	-	-	-

Table III - Analgesic and Antiinflammatory Activity of Compound IIIa

Compound	Dose mg/kg	Analgesic Activity		Antiinflammatory Activity	
		% Reaction Time		% Reduction in oedema	
		After 30 min	After 1 h	After 1 h	After 3 h
IIIa	21.5	71.01	81.13	75	100
	43	70.59	79.59	55.55	75
	64.5	61.53	72.22	55.55	75
Pentazocine	5	70.01	75.99	-	-
Ibuprofen	216.0	-	-	100	100
DMSO	-	-	-	-	-

activity was compared with known standard ibuprofen[26] (216 mg/kg).

Local Anaesthetic Activity

Infiltration Anaesthesia : Guinea Pig Wheal Method[23,24]

Five minutes after giving the compound IIIa at 0.5% and 1% concentration and standard, 1% lignocaine hydrochloride intradermally to guinea pigs, the sensitivity of the area was tested by pricking with a needle, six times lightly on the skin at the site of injection. Failure to twitch upon pricking was considered as a negative response. The test was repeated at 5 min interval for a period of 20 min and after 30, 45 and 60 min. The total number of negative responses out of 42 was noted.

Antihistaminic Activity

Guinea pigs were placed in an aerosol chamber and aerosols were introduced by means of a compressor. The aerosol contained 2% histamine. Animals exposed to a bronchoconstrictor aerosol, showed progressive signs of difficulty in breathing. The time taken to show this effect was noted. As soon as this sign was observed, the animals were removed and placed in fresh air. The test substance and the antihistamine were given by intraperitoneal route, 20 min before aerosol challenge to different

Table IV - Local Anaesthetic Activity of Compound IIIa

Compound	Concentration Injected (0.1 ml)	Total No. of Negative Responses out of 42
IIIa	0.5%	40
	1%	38
Lignocaine Hcl.	1%	40
DMSO	-	-

groups of guinea pigs[25] and time required to show the sign of difficulty in breathing was noted.

RESULTS AND DISCUSSION

Three 2-(4,5-dicarbomethoxy-1,2,3-triazol-l-yl)-N-(substituted aryl) acetamides were prepared by the 1,3-dipolar cycloaddition of azides with dimethyl acetylene dicarboxylate. They were purified by recrystallization. The structure of the product were assigned on the basis of IR, PMR and Mass spectral values (Table 1). All the three compounds were subjected to antimicrobial testing. Compound IIIa showed more antibacterial and antifungal activity (Table 2) when compared to the other two compounds. The activity of the compound may be due to the methoxy substituent on the 1,2,3-triazole ring.

Acute toxicity studies were carried out to find out the LD_{50} of the compound IIIa, 2-(4,5-dicarbomethoxy-1,2,3-triazol-1-yl)-N-(4-methoxy phenyl) acetamide. LD_{50} was found to be 419 mg/kg by Karber's method. This compound was used for further pharmacological screening. The analgesic activity was evaluated by the tail-flick method using pentazocine lactate as standard. In case of the evaluation of analgesic activity, per cent reaction time was calculated, and it was increased with time. This showed that the compound possessed analgesic activity comparable to that of the standard drug (Table 3). The antiinflammatory activity was performed by formalin-induced rat hind paw oedema method using ibuprofen as a standard. In case of antiinflammatory activity, per cent reduction in oedema was calculated and it was also found to increase with time. This showed that the compound possessed antiinflammatory activity (Table 3) which is also comparable to the standard drug.

The local anaesthetic activity was evaluated using the infiltration anaesthesia method also known as guinea pig wheal method with lignocaine hydrochloride as a standard. The local anaesthetic effect of the compound IIIa at 0.5% concentration showed 40 negative responses. The standard drug lignocaine hydrochloride at 1% concentration also showed 40 negative responses. This result showed that the compound IIIa possessed good local anaesthetic effect (Table 4).

Finally, antihistaminitic activity was carried out using pheniramine maleate as a standard drug. The time required to show the sign of difficulty in breathing after pre-treatment with the compound IIIa was not found to be prolonged. This showed that the compound failed to possess antihistaminic activity.

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