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Synthesis and pharmacological screening of novel 6-methyl-2-oxo-4-substituted-5-(5-phenyl-1, 3, 4-oxadiazole-2yl)-1, 2, 3, 4-tetrahydropyrimidine

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ABSTRACT

1,3,4-oxadiazole derivatives prepared from DHPM has some antinflammatory and antibacterial activity. Drugs were synthesized and investigated for lipoxygenase inhibitory and *in vitro* antibacterial activity. The target compounds were obtained by synthesis of DHPM using substituted aldehydes and conversion of DHPM to the respective hydrazides followed by treatment with phosphorus oxychloride and dichloroethane to yield corresponding substituted (1, 3, 4-oxadiazole-2-yl)-1, 2, 3, 4-tetrahydropyrimidine-2(1H)-one, (3a-3h). The formation of the product was confirmed by spectroscopic and elemental analysis. Synthesized derivatives exhibited significant lipoxygenase inhibitory and antibacterial activity.

Key words- DHPM, 1, 3, 4-oxadiazole, Lipoxygenase.

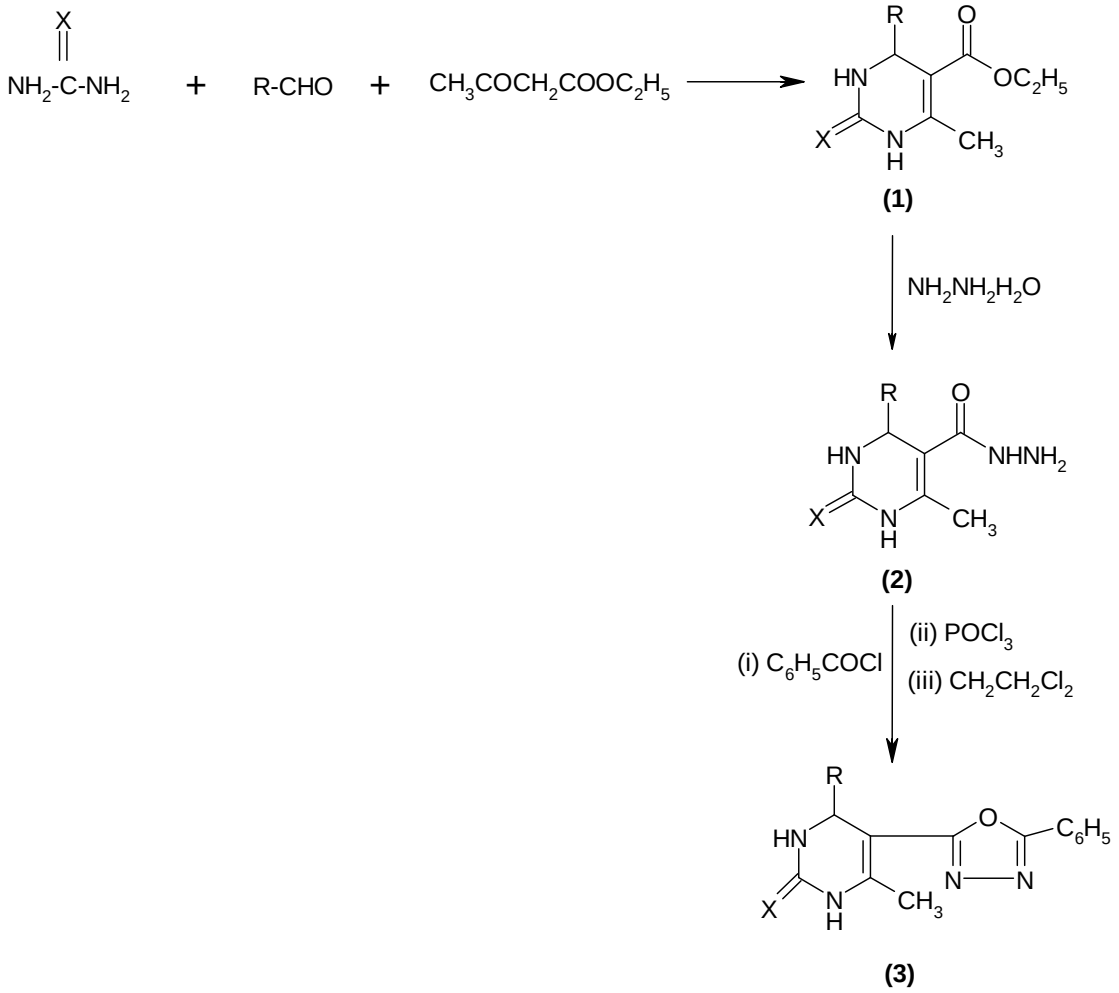
INTRODUCTION

One of the greatest triumphs of modern medicine has been the introduction of a rational system of antimicrobial therapy to combat infectious disease. More recently, appropriately functionalized DHPMs have been emerged as orally active calcium channel blocker, antimicrobial, antibacterial agents. A large number of medicines which have been discovered belong to a class of heterocyclic containing nitrogen, oxygen and sulphur. Biological activity of these heterocyclic compounds has helped the medicinal chemist to plan, organize and improve newer approaches towards the discovery of new drugs.

In view of the general observation that pharmacological activity is invariably associated with a large variety of heterocyclic compounds, the investigation of some heterocyclic such as substituted 1, 3, 4-oxadiazole, has been undertaken. Derivatives of these compounds are reported to posses a wide spectrum of biological properties which include antibacterial, anticancer, anti-inflammatory, antihypertensive, analgesic and antifungal activities. Several derivatives of 5-substituted-1, 3, 4-oxadiazole, synthesized by various authors were reported to possess hypoglycemic, analgesic, anti-inflammatory, anticonvulsant, tranquillizing, muscle relaxant, bronchodilatory & antiemetic activity. Leukotrines derived from lipoxygenase also play an important role in the initiation of inflammation & pain along with prostaglandins.Literature revels that conversion of hydrazide group into corresponding 1, 3, 4-oxadiazole, which show some antiinflammatory and cyclo-oxygenase inhibitory activity of parent drug but also induced lipoxygenase inhibition.

In our present study was undertaken in which attempts were made to convert the hydrazide of DHPM to corresponding 1, 3, 4-oxadiazole system and to explore the antibacterial and lipoxygenase inhibitory activity *in vitro*.

The scheme for synthesis of Dihydropyrimidines, 1, 3, 4-oxadiazole is depicited below



Scheme 1

MATERIAL AND METHODS

In our present investigation NSAIDS required were obtained from respective manufacturers and enzymes used for lipoxygenase inhibitoy activity were obtained as gift samples from Sigma chemicals Co. USA. Standard samples required for antibacterial activity were procured from respective manufacturers.

EXPERIMENTL

Synthesis of Ethyl-6-methyl-2-oxo-4-substituted phenyl-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylate (1a-1h)

To a mixture of urea (0.15 mole), substituted aldehyde (0.10 mole) and ethylacetoacetate (0.10 mole) in ethanol (75 ml), few drops of concentrated hydrochloric acid was added and heated for 1.5 hrs at 70°C. The reaction

mixture was poured into ice water (100 ml) with stirring and left overnight at room temperature. Filtered and residue dried at room temperature, recrystallised from ethanol. The reaction was monitored by using TLC and IR data.

Compound 1h

IR (KBr) 1645 cm^{-1} (amide C=O), 1714 cm^{-1} (ester C=O), 3246 cm^{-1} (-NH).^{1}H NMR ($DMSO_{d6}$): δ 1.07(t, 3H, CH_3-ester); 2.25 (s, 3H, dihydropyridyl-CH_3); 3.93 (q, 2H, CH_2-ester); 5.15 (d, 1H, dihydropyridyl-CH); 7.2-7.5 (m, 4H, Ar-H) 7.7 (s, 1H, 3-NH); 9.25 (s, 1H, 1-NH), Mass (FAB):237(M+,12%), 189(Base peak 100%).

Synthesis of 6–Methyl–2–oxo–4–substituted phenyl-1, 2, 3, 4 – tetrahydropyrimidine -5-carbohydrazide (2a-2h)

To a hot solution (0.01mole) of compound 1 in ethanol (150 ml), was added hydrazine hydrate (99%, 0.015mole) and the reaction mixture was heated under reflux for 3 hrs. The solvent was removed to possible extent by distillation and the product thus separated was filtered and purified by recrystallization from ethanol to get a colorless crystalline solid. The reaction was monitored by using TLC and IR data.

Compound 2h

IR (KBr) 1647 cm^{-1} (amide C=O), 3247 cm^{-1} (-NH).^{1}H NMR (DMSO-d_6) δ 2.25 (s, 3H, dihydropyridyl-CH_3); 4.0 (s, 2H, NH_2);5.1(d,1H,-NH);7.22-7.5(m, 4H, Ar-H); 7.77 (s, 2H, 3-NH/CH); 9.25 (s, 1H, 1-NH), Mass (FAB):251(M+,9%), 186(Base peak 100%).

Synthesis of 6 – Methyl-2-oxo – 4 –substituted – 5 - (5- phenyl -1, 3, 4 – oxadiazol -2- yl) -1, 2, 3, 4-tetrahydropyrimidine-2(1*H*)-one (3a-3h)

To a solution of benzoyl chloride (0.01 mole) in dichloroethane (10 ml) and compound 2 (0.01 mole), phosphorous oxychloride (5ml) was added and content were refluxed for 8 hrs on an oil bath. After the reaction, excess of solvent and $POCl_3$ were distilled at reduced pressure. Reaction mass was cooled and poured into ice, left overnight. The product was obtained by filtration and purified by recrystallization from aqueous ethanol. The reaction was monitored by using TLC and IR data. (Table 1)

Compound 3c

IR (KBr) 1689 cm^{-1} (amide C=O), 1026 cm^{-1} (C-O-C), 1603 &1582 cm^{-1} (C=N). ^{1}H NMR (DMSO-d_6) δ 1.28 (s, 6H, CH_3-N-CH_3); δ 2.1 (s, 3H, -CH_3); δ 2.65(s, 1H, -CH); 7.43-7.47 (d, 4H, Ar-H, attached with dihydropyrimidine); 7.55 (s, 1H, 1NH); 7.57 (s, 1H, 1NH); 7.95-8.15 (d, 5H, Ar-H, attached with oxadiazoles); Mass (FAB):305(M+,15%), 188(Base peak 100%).

Melting points of synthesized compounds were determined in sealed capillaries using paraffin thieles tube. IR spectra (KBr disc) were recorded on FTIR-8400s Shimadzu system. Proton Magnetic Resonance spectra (HNMR) were recorded on bruker AC-300F NMR spectrometer (300 MHz) using DMSO-d6 as solvent and Tetramethyl silane (TMS) as internal standard. Mass spectrum was recorded on Jeol SX 102/DA-6000 mass spectrometer/data system using Argon/Xenon (6Kv, 10mA) as FAB gas. The accelerating voltage was 10 kV and the spectra were recorded at room temperature using m-nitro benzyl alcohol (NBA) matrix.

Lipoxygenase inhibitory activity

The lipoxidase activity was determined by the measurements of spectral absorbance of the conjugated hydroperoxidase produced by lipoxidase catalysis. The lipoxygenase inhibitory activity was determined by *in vitro* method. A direct spectrophotometric assay employing increase in absorbance at 234nm as a function of time where soyabean lipoxidase as a representative of 5-lipoxygenase enzyme and linoleic acid as the substrate were used. For calculating the enzyme activity maximum absorbamce. (A) at 234nm /min between 1-3 min interval was noted and the enzyme activity was calculated by formula.(Fig. 1)

$$\text{Enzyme activity (unit mg solid)} = \frac{\text{A at 234/min}}{0.004 \times \text{mg enzyme/3.0 ml reaction mixture}}$$

Study of antimicrobial activity

The synthesized compounds are screened for antibacterial activity for this activity two species were selected, Staphylococcus aureus for gram positive and Eschellia coli for gram negative activities respectively.

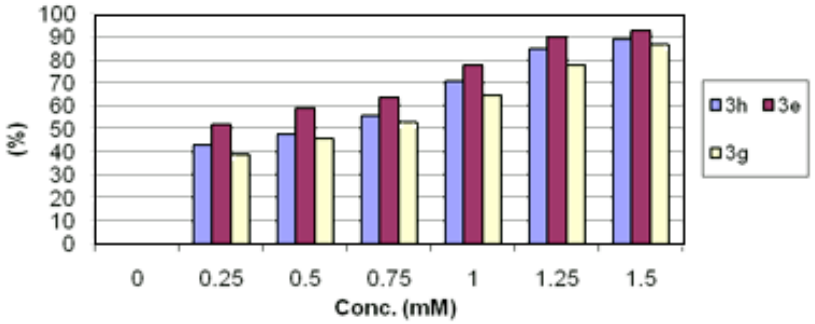
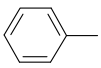
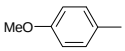
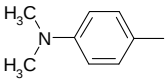
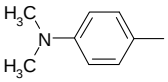
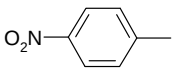
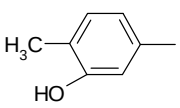
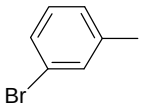


Fig. 1: Lipoxygenase inhibitory activity of synthesized compounds 3e, 3g, 3h.

Table 1

Compound-3

S. No.	R1	X	% yield	m.p. (°C)	Molecular Formula
3a		O	72%	198-202	$C_{14}H_{16}O_3N_2$
3b		O	78%	114-116	$C_{15}H_{18}O_4N_2$
3c		O	71%	113-115	$C_{16}H_{21}O_3N_3$
3d		S	69%	115-117	$C_{16}H_{21}O_2N_3S$
3e		O	72%	119-121	$C_{14}H_{15}O_5N_3$
3f		O	64%	112-114	$C_{14}H_{16}O_4N_2$
3g		O	68%	130-132	$C_{15}H_{18}O_5N_2$
3h		O	71%	126-128	$C_{14}H_{15}O_3N_2Br$

Antibacterial activity

The antibacterial activities are performed by cup and plate method (diffusion technique) fresh cultures of bacteria are obtained by inoculating bacteria into peptone water and incubated at 37°±2° C for 18-24 hours in BOD incubator. This culture is mixed with nutrient agar 20% (High Media) and poured into Petri dishes aseptically. After solidification of the media five bores are made at equal distance by using sterile steel cork borer (8 mm diameter), different concentrations of standard drug and synthesized compounds are introduced into these cups. Dimethyl formamide (DMF) is used as a control.

Preparation of standard and test drug solutions

The standard drugs and synthesized compounds were dissolved in minimum quantity of Dimethyl Formamide (DMF) and the volume is adjusted with distilled water to get 50µg/ml and 100µg/ml concentrations. Procaine penicillin and streptomycin are used as standard drugs. These plates were placed in a refrigerator at 8-10°C for 2hrs for proper diffusion of drug in to the media. After cold incubation, the petri plates were transferred to incubator and maintained at 37°±2°C for 18-24 hours. After the incubation period, the petriplates were observed for antimicrobial activity by measuring zone of inhibition using venire scale. The results of the synthesized compounds were compared with standard drugs. (Table 2)

Table 2 :Antibacterial activity of synthesized oxadiazole

S. No.	Name of the compounds	Meanzone of inhibition(in mm) *Staphylococcus aureus* (+ve)		*Escherichia coli* (-ve)	
		50µg/ml	100µg/ml	50µg/ml	100µg/ml
1.	Procain penicillin	20	24	-	-
2.	Streptomycin	-	-	19	23
3.	3a	08	11	09	12
4.	3b	08	12	13	15
5.	3c	08	13	09	13
6.	3d	08	10	08	10
7.	3e	14	15	10	12
8.	3f	08	11	08	11
9.	3g	13	16	11	14
10.	3h	10	12	12	14

<9mm- resistant ,10-14mm-intermediate <14mm susceptible

RESULTS AND DISCUSSION

Anti-bacterial activity

Synthesis and Antimicrobial activity of 6-methyl-2-oxo-4-substituted-5-(5- phenyl-1,3,4-oxadiazol-2-yl)-3,4-dihydropyrimidine was tested for the antibacterial activity against bacteria, the tested compounds 3e,3g,3h showed promising antibacterial activity against gram +ve *(Staphylococcus aureus)* and 3b, 3e, 3g,3h showed promising antibacterial activity against gram-ve *(E. coli),* compared to standard drugs procaine penicillin and streptomycin respectively .

Lipoxygenase inhibitory activity

The substituted 1, 3, 4-oxadiazole derivatives were evaluated for in vitro activity in model of 5-lipoxyenase enzyme. All the derivatives were found to exhibit lipoxygenase inhibition with 3e, 3g, and 3h showing maximum inhibition of 52%, 39%, and 43% respectively at 0.25mM concentration.

CONCLUSION

Antibacterial activity of oxadiazoles were studied out against standard strain of *Staphylococcus aureus* (gram +ve) and *E. coli* (gram –ve).Compound No. 3e,3g,3h showed promising antibacterial activity against gram +ve and 3b, 3e, 3g,3h showed promising antibacterial activity against gram –ve. Also 3e, 3g, 3h, showed promising lipoxygenase inhibitory activity at 0.25mM conc.

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