

Original Article

Synthesis and Evaluation of Dihydropyrimidine Derivatives as Antiulcer Agents.

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Abstract

Wide range of biological activities is associated with 1, 4-dihydropyrimidines/ pyrimidines, individually or in combination. In view of this, the present study, was an attempt to synthesize a molecules, Ethyl-2 [(substituted thio) 1, 4 dihydro 6 methyl 4phenyl] 5 pyrimidine carboxylate, with improved biological activity, lesser toxicity with undesirable side effects in clinical use. All the synthesized compounds have been characterized by using IR, ¹H NMR and elemental analysis. Further, some compounds were screened for anti-ulcer activity. Compound AT4 has shown maximum anti-ulcer activity as compared to control group.

Keywords: Anti-ulcer Activity, Dihydropyrimidines, Thioethers, tetrahydropyrimidines.

1. Introduction

An ulcer is a discontinuity or break in a bodily membrane that impedes the organ, of which that membrane is a part of, from continuing its normal functions. As many as 70–90% of such ulcers are associated with *Helicobacter pylori*, a spiral-shaped bacterium that lives in the acidic environment of the stomach; however, only 40% of those cases go to a doctor^[1]. Among a wide variety of heterocycles that have been explored for developing pharmaceutically important molecules, pyrimidines^[2] have played an important role in medicinal chemistry. Some of them have received considerable attention as potential anti-hypertensive agents. Moreover, pyrimidines acquired a special place in heterocyclic field because of their diversified activities such as anti-virus, anti-tumor, anti-bacterial agents^[3-6] etc.

Further, dihydropyrimidines (DHPMs; popularly known as Biginelli's compounds) are associated with broad spectrum of biological activities ever since 4-Aryl-1, 4-dihydropyrimidines of nifedipine type were first introduced into clinical medicine in 1975. Even today they are the most potent calcium channel modulators available for the treatment of various cardiovascular diseases^[7]. Several calcium channel blockers including nifedipine are reported with anti-ulcer activity^[8-9]. The combination of an aldehyde, β -keto ester, and urea under acid catalysis to give a dihydropyrimidine was first reported by Pietro Biginelli in 1893. The Biginelli reaction, is a one-pot condensation reaction generates compounds with pharmacological activity, including calcium channel modulation, mitotic kinesin Eg5 inhibition, and antiviral and antibacterial activity. Although the original reaction conditions suffered from poor yields and a limited substrate scope, the recent discovery of dihydropyrimidine biological activity has led to a renewed exploration of the reaction conditions, revealing a variety of compatible solvents, acid catalysts^[10].

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Pyrimidine and its derivatives such as dihydropyrimidine and tetrahydropyrimidine are versatile nitrogen heterocyclic compounds which have long been known as a promising class of biologically active compounds.^[11] It has been reported that many derivatives of dihydropyrimidine shows antiulcer activity. A vast number of dihydropyrimidine derivatives have been synthesized to provide synthetic drugs and to design more effective medicines. Various approaches toward the synthesis of dihydropyrimidine and tetrahydropyrimidine derivatives have been explored during the past years. Some of them have received considerable attention as potential anti-hypertensive agents. Moreover, pyrimidines acquired a special place in heterocyclic field because of their diversified activities such as anti-virus, anti-tumor, anti-bacterial agents etc. Further, dihydropyrimidines are associated with broad spectrum of biological activities. Several calcium channel blockers including nifedipine are reported with anti-ulcer activity. It is thus envisaged that structural analogues of nifedipine may possess anti-ulcer potential. So, in view of these observations it was considered worthwhile to synthesize some dihydropyrimidines and evaluate for antiulcer activity^[12].

2. Materials and Methods

The starting materials were commercially available and purchased from loba chemie, Mumbai. Melting points were measured on a VEEGO Amp-1 melting point apparatus. Thin layer chromatography (TLC, silica gel-G) was used to monitor reactions and check product's homogeneity. The structure of synthesized compounds was determined by spectral analysis. The λ_{\max} of synthesized compound was determined by using Shimadzu model 1700 spectrophotometer. IR spectra were recorded in Bruker spectrometer using ABS technique. ¹H-NMR spectra were recorded on a JEOL GSX 270 MHz spectrometer (tetramethylsilane as internal standard). Splitting patterns are described as singlet (s) and multiplet (m). ¹H NMR spectra on a Bruker's WM 400 FT MHz NMR instrument using CDCl₃ as solvent and TMS as internal reference (chemical shifts in δ ppm).

2. Chemistry

Synthesis of dihydropyrimidine derivatives was done in two steps as shown in scheme 1. In the first step ethyl acetoacetate, thiourea and benzaldehyde aldehydes were stirred for 4 hrs which on standing for 24-36 hr afforded the products 6-methyl-4-(phenyl)-2-thioxo -1, 2, 3, 4-tetrahydropyrimidin-5-carboxylic acid ethyl ester. In second step tetrahydropyrimidine intermediate was refluxed with substituted alkyl resulted in Ethyl-2[(substituted thio) 1, 4 dihydro 6 methyl 4phenyl]5 pyrimidine carboxylate.

2.1. Step-1: General procedure for synthesis of 6-methyl-4-(phenyl)-2-thioxo -1, 2, 3, 4-tetrahydropyrimidin-5-carboxylic acid ethyl ester(1)

Ethyl acetoacetate (0.05mole, 6.4ml), thiourea (0.05 mole, 3.8gm) and benzaldehyde aldehydes (0.05 mole, 5.1ml) ethanol (25ml) as solvent and piperidine (10 ml) as catalyst were mixed together in a conical flask and stirred for 4 hrs which on standing for 24-36 hr afforded the products.

2.2. Step 2

(i) General procedure for synthesis of Dihydropyrimidine derivatives containing alkyl substitution (2a-2b)

A mixture of powdered tetrahydropyrimidine (0.008 mole) substituted alkyl group (0.8 ml, 0.008 mole) and absolute alcohol (10 ml) was refluxed for 5hr. Then the product was allowed to separate at room temperature. The product was filtered under reduced pressure and recrystallised from ethanol.

(ii) General procedure for synthesis of Dihydropyrimidine derivatives containing benzyl substitution (2c-2e)

Tetrahydropyrimidine (0.008 mole) was dissolved in alcohol (5 ml) then substituted benzene (0.008 mole) was added and the mixture was refluxed for 4 hr. The mixture was cooled at room temperature. The solid separated was filtered and recrystallised from ethanol.

Synthesis of Ethyl-2[(chloromethylthio) 1,4dihydro 6 methyl 4phenyl] 5 pyrimidine carboxylate (2a)

Yield 65%; m.p. 127-129^o; λ_{\max} 307 nm; IR (ABS): 3378 cm⁻¹ (N-H), 2939 cm⁻¹ (C-H), 1610 cm⁻¹ (C-C), 1534 cm⁻¹ (N-H), 1472 cm⁻¹ (C=C),

1205 cm^{-1} (C-C(O)-C), 984 cm^{-1} (C-H), 703 cm^{-1} (C-Cl), 1360 cm^{-1} (C-N); ^1H NMR (CDCl_3): δ values 1.3 (S, 2H, R₂CH₂), 1.7 (m, 1H, RCHCR=CR₂), 2.3 (s, 1H, RC=OCHR₂), 2.5-2.6 (t, 1H, R₂NH), 5.2 (d, 1H, R-C=CH), 6.2-7.9 (d, 8H, Ar-H); MS m/z: 325.87 (M + 1)

Synthesis of Ethyl-2[(butyl thio)1, 4dihydro 6 methyl 4phenyl]5 pyrimidine carboxylate (2b)

Yield 79% ; m.p. 132-134⁰; λ_{max} 306 nm; IR (ABS): 3274 cm^{-1} (N-H), 2940 cm^{-1} (C-H), 1613 cm^{-1} (C=C), 1473 cm^{-1} (C=C), 1402 cm^{-1} (C-C(O)-C), 1206 cm^{-1} (C-H); ^1H NMR (CDCl_3): δ values, 1.2 (s, 3H, R-CH₃), 1.5 (d, 2H, R₂CH₂), 1.61 (m, 1H, R₃CH), 1.65-1.7 (s, 1H, RCHCR=CR₂), 2.9 (s, 1H, RCCH), 5.1 (s, 1H, RC=CH), 7.3 (d, 8H, Ar-H), (7.5 s, 8H, Ar-H); MS m/z: 38.57 (M + 1)

Synthesis of Ethyl-2[(chloro benzyl thio) 1,4dihydro 6 methyl 4phenyl] 5 pyrimidine carboxylate (2c)

Yield 72%; m.p. 139-141⁰; λ_{max} 306 nm ;IR (ABS): 3377 cm^{-1} (N-H), 2849 cm^{-1} (C-H), 1611 cm^{-1} (C=C), 1534 cm^{-1} (N-H), 1474 cm^{-1} (C=C), 1375 cm^{-1} (CH₃), 1325 cm^{-1} (C-N), 1205 cm^{-1} (C-C(O)-C), 743 cm^{-1} (C-Cl); ^1H NMR (CDCl_3): δ values 1.3 (t, 1H, CH), 2.6 (d, 1H, Ar-NH), 3.4 (s, 1H, CH), 4.3 (s, 2H, CH₂), 7.2-7.9 (d, Ar-CH, 8H); MS m/z: 403.94 (M + 1)

Synthesis of ethyl-2[(2benzyl thio) 1,4dihydro 6 methyl 4phenyl] 5 pyrimidine carboxylate (2d)

Yield 63%; m.p. 132-134⁰; λ_{max} 306 nm ;IR (ABS): 2939 cm^{-1} C-H, 1611 cm^{-1} (C=C), 1474 cm^{-1} (C=C), 1205 cm^{-1} (C-C(O)-C), 984 cm^{-1} (C-H), 702 cm^{-1} (C-H); ^1H NMR (CDCl_3): δ values. 1.2 (s, 3H, CH₃), 1.3 (t, 2H, CH₂), 1.7 (S, 1H, R-CH-CR=CR₂), 2.6 (S, 1H, Ar-NH), 3.4 (S, 1H, CH), 4.3 (d, 3H, CH₃), 7.2-7.9 (d, 8H, Ar-H); MS m/z: 369.49 (M + 1)

Synthesis of Ethyl-2[(amino benzylethio) 1,4dihydro 6 methyl 4phenyl] 5 pyrimidine carboxylate (2e)

Yield 75%; m.p. 123-125⁰; λ_{max} 292 nm; IR (ABS): 3376 cm^{-1} (N-H), 1617 cm^{-1} (C=C), 1409 cm^{-1} (C-H), 1205 cm^{-1} (C-C(O)-C), 1309 cm^{-1} (C-N), 1461 (C=C); ^1H NMR (CDCl_3): δ values, 1.3 (t, 3H, CH₃), 1.7 (d, 3H, CH₃), 2.5-2.6 (d, 2H, C-NH₂), 3.4 (s, CH, 1H), 4.9 (t, 2H, CH₂), 6.2-7.9 (d, 8H, Ar-H); MS m/z: 371.49 (M + 1)

Antiulcer activity

All the animal studies conducted were approved by the Institutional Animal Ethical Committee (Registration no. 778/03/C/CPCSEA, dated: 30/9/03), VNS Institute of Pharmacy, Bhopal. Anti-ulcer activity of synthesized compounds has been done by using ethanol induced ulcer model. In this method, Albino rat of either sex taken randomly with weights between 150- 200g and were divided into following 7 groups of 6 animals each. The first group served as control (without antiulcer drug administration) second group served as standard and received omeprazole (20mg/kg). The animals of third, fourth, fifth, sixth and seventh groups were given the synthesized compounds equivalent to 20mg/kg orally. The animals were fasted for 36 hrs before administration of ethanol. The above drugs were administered to animals orally by anaesthetic needle. Omeprazole for Standard group, CMC suspension for control and synthesized compounds for test groups was administered 60 minutes prior to the ethanol injection. After 60 minutes of test compound administration, 1ml/200gm of ethanol (90%) was administered. After 1 hr the animals were sacrificed and ulcer index and percentage ulcer inhibition was determined and reported in table 2.

Results and Discussion

The melting point of all compounds was observed different from ingredients melting point which was confirmed the synthesis of product. The purity of synthesized compounds was checked by observing single spot on TLC plate. All synthesized compounds was gave only single spot. It means all synthesized compounds were obtained in pure form. The melting point and R_f value of all synthesized compounds are given in Table 1. The UV λ_{max} of synthesized compounds was observed at range between 292-307 nm. The IR spectra of synthesized compounds showed characteristic absorption peak for the functional group present in compounds. The stretching vibrations for 3378 cm^{-1} (N-H), 2939 (C-H), 1472 cm^{-1} (C=C) showed for amine, alkanes, alkene for functionality respectively present in the synthesized compounds. The ^1H NMR spectrum of synthesized compound 2a and 2b explained the presence of Ar-H group at δ 6.2-

7.9 and δ 7.5 respectively. The compound 2c 2d and 2e explained the presence of AR-NH at δ 2.5- 2.6 respectively.

Ethanol induced Ulcers model was used to evaluate the anti-ulcer effect of synthesized compounds. The order of anti-ulcer activity of the synthesized compounds is as follows:

AT4>AT2>AT3>AT1>AT5

Synthesized compound AT4 and standard drug Omeprazole showed 35.03 and 58.18% inhibition of ulcer respectively. Compound AT4 showed maximum activity as compared to other compounds. As we know that the synthesized compounds are dihydropyrimidine derivatives containing ester group and amino benzene attached to it.

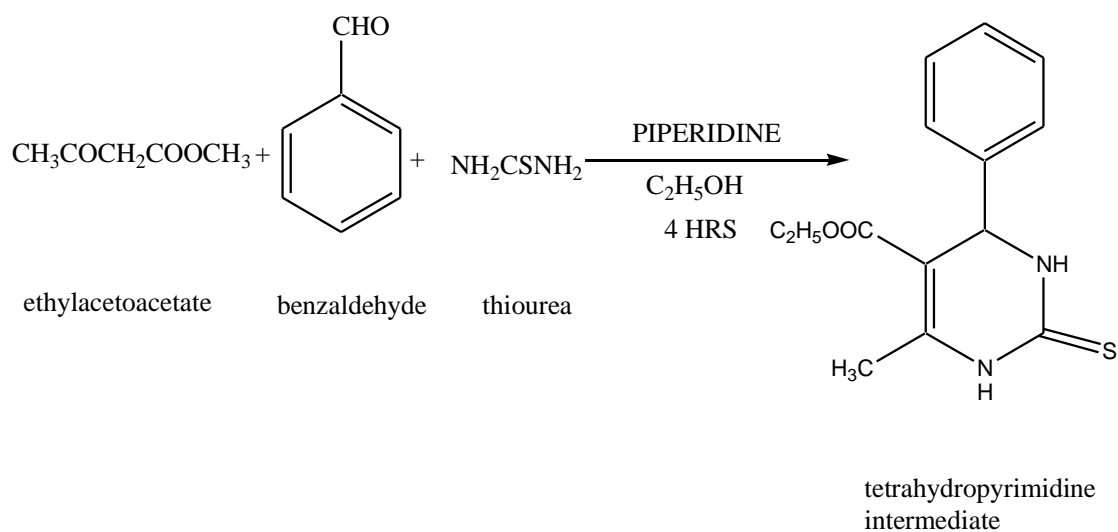
Conclusion

Dihydropyrimidine derivatives were synthesized and characterized for their structure elucidation. Various chemical and spectral data supported the structure of the compounds thought of. The synthesized compounds showed significant antiulcer activity. Synthesized compounds are somewhat structurally similar to nifedipine as both moieties containing nitrogen containing heterocyclic ring, a ester group attached to it at second position. On the basis of above discussion, we can say that the presence of the nitrogen containing heterocyclic ring, ester moiety attached to the ring and benzyl groups are necessary for the antiulcer activity.

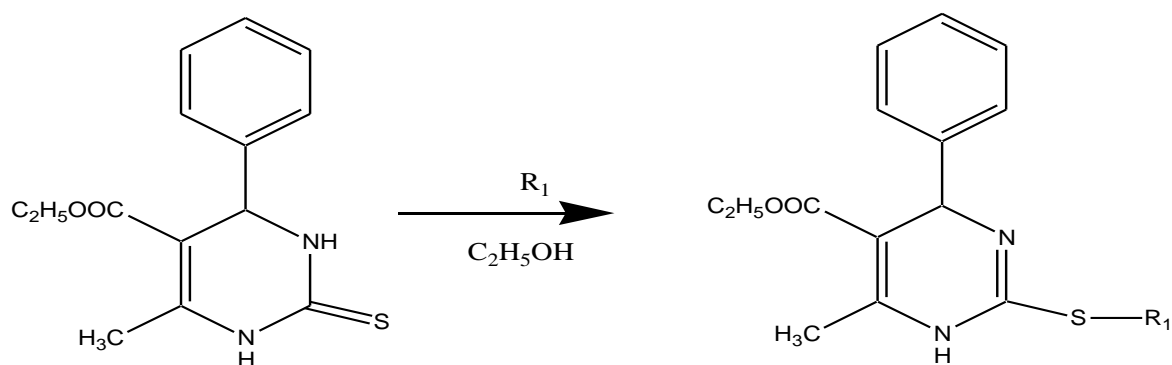
Thus, it may be concluded that benzyl substitution of DHPMs may have significant anti-ulcer activity. Further studies are needed to find out its mechanism of action and to synthesize more substituted DHPMs for characterization of a lead compound.

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Scheme of Synthesis- Step 1



Scheme of Synthesis- Step 2

Table 1. Physical constants of different dihydropyrimidine derivatives

Compound Code	R	M.P. (°C)	Yield (%)	R _f value	Mol. Formula (mol. Wt.)
2a	-CH ₂ Cl	127-129°	65	0.78	324.87
2b	-C ₄ H ₉	132-134°	79	0.62	377.57
2c	-C ₆ H ₄ Cl	139-141°	72	0.71	402.94
2d	-C ₆ H ₅	132-134°	63	0.75	368.49
2e	-C ₆ H ₄ NH ₂	123-125°	75	0.64	370.49

Table 2. Showing percentage inhibition of different synthesized compounds.

S. No.	Groups	Treatment	Dose	Ulcer Index	% ulcer inhibition
1.	Group 1	Control cmc	1ml/kg	10.875±0.115	-
2.	Group 2	Omeprazole	20mg/kg	4.55 ±0.25	58.18 %
3.	Group 3	AT1	20mg/kg	8.25 ±0.24	21.09 %
4.	Group 4	AT2	20mg/kg	7.49 ±0.025	29.83 %
5.	Group 5	AT3	20mg/kg	7.75 ±0.05	27.97 %
6.	Group 6	AT4	20mg/kg	6.69 ±0.3	35.03 %
7.	Group 7	AT5	20mg/kg	8.74 ±0.25	19.14 %

(Values are represent mean ± SEM; n= 6 albino rats per groups; *P<0.05 as compared with control group)

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