Research Article

SYNTHESIS AND ANALGESIC ACTIVITY OF NOVEL PYRIMIDINE DERIVATIVES

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ABSTRACT

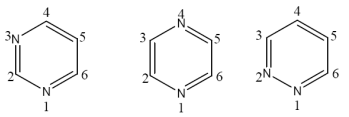
Pyrimidine is an aromatic heterocyclic compound substituted at position 1 and 3 of benzene ring with two nitrogen atoms. Until today they have been derived from natural product. Since, the discovery of 3, 4 – dihydropyrimidin – 2(1H) – ones there is always the problem of low yield with the final product. 2 - imino - 5 - carboxy - 3, 4 - dihydropyrimidines have been discovered but they suffer from the same problem of low yield which can only be overcome with different strategies and techniques but it increases the cost of research due to the use of expensive material and methods. In the present work, less expensive and a simple technique is used which can be performed on laboratory scale and under room temperature conditions. The modification is done and good yield is obtained. In the initial step substitution is done at C - 2 position of 3,4 – dihydropyrimidin – 2(1H) – ones, a compound synthesized by Biginelli reaction followed by treatment with hydrazine hydrate in the presence of acid to give hydrates which leads to modification at C – 5 position followed by condensation with different aromatic aldehydes and ketones to yield 2 -Imino-6-methyl-4-phenyl-1, 2, 3, 4-tetrahydro -pyrimidine-5-carboxylic acid substituted hydrazides. The compounds are characterized by IR, ^{1}H NMR and elemental analysis. All the synthesized compounds are screened for analgesic activity. All the test compounds show good analgesic activity but the compound NA – 10 is found to be most active both centrally and peripherally. The compound is similar in mechanism to morphine as central analgesic and to diclofenac sodium as peripheral analgesic but the exact mechanism is still a mystery.

Keywords: Pyrimidine, Guanidine, Benzaldehyde, Ehylacetoacetate, Acetophenone

INTRODUCTION

Guanidine containing heterocyclic compounds have varied biological applications and one such derivative is pyrimidine found mainly in alkaloids.[1] The biological importance of pyrimidine include anti – cancer, anti – viral, anticonvulsant etc. which encourages the researchers to work upon it. The technique is expensive as they have been derived from natural product and also time – consuming. This agenda is solved by P. Biginelli with the synthesis of 3, 4 – dihydropyrimidin – 2(1H) – one but drawback is still there of being having a low yield. 2 - imino - 5 - carboxy - 3, 4 - dihydropyrimidines have also been synthesized by amminolysis and triazone – protecting strategy.[1] This strategy gives successful product but with low yield and the technique is expensive also. To overcome such problem, pyrimidine derivatives have been synthesized using easily available chemicals with no special conditions or heavy catalyst or other expensive technique thus reducing the cost of research and time has also been saved.

Pyrimidine is a heterocyclic aromatic organic compound similar to benzene and pyridine, containing two nitrogen atoms at positions 1 and 3 of the six-member ring. It is isomeric with two other forms of diazine. The two isomeric forms of pyrimidine are: Pyrazine and Pyridazine.[2]



In the present work, pyrimidine derivatives have been synthesized and screened for analgesic activity by Eddy's hot plate method (central analgesic activity) and Acetic acid induced writhing test(peripheral analgesic activity). All the compounds show good analgesic activity except few like NA – 4, NA – 5 and NA – 7 are inactive while compounds NA – 3 and NA – 2 were poor peripheral analgesics. The outstanding compound with excellent activity as central as well as peripheral analgesic activity is NA – 10. The mystery is still there on its mechanism of action.

MATERIALS AND METHODS

All the solvents and chemicals used were of LR grade and obtained from Qualigens (INDIA) and Rankem Rfcl Limited (INDIA). Melting points were determined by open – ended capillary tube using the Thistle tube apparatus and were uncorrected. Infrared (IR) was recorded for the compounds on Thermo Electron Corporation, USA. MODEL - AVATAR 370 FTIR by using potassium bromide pellets and the value of υ_{max} are reported in cm^{-1}. ^{1}H NMR was recorded on AV – 500 using DMSO – d_6 as solvent and the value was reported in parts per million (ppm) using tetramethylsilane (TMS) as an internal standard. Analgesiometer used in hot plate method is Eddy's Hot plate old Model GPIT 9504. Thin layer chromatography (TLC) was run throughout the reaction to optimize the reaction for purity and completion. Thin layer chromatographic analysis of compounds was performed on the Silica Gel G coated glass plates.

Synthesis of 2-Imino-6-methyl-4-phenyl-1, 2, 3, 4-tetrahydro-pyrimidine-5-carboxylic acid substituted hydrazides is depicted in Scheme 1. The synthesis has been divided into three steps:

Synthesis of 2-Imino-6-methyl-4-phenyl-1, 2, 3, 4-tetrahydro-pyrimidine-5-carboxylic acid ethyl ester (4): This is the first step involved to synthesize the desired compound (4). In this step, guanidine (2.865g, 0.03mol) was weighed and immediately dissolved in methanol (15 ml) taken in a round bottom flask The above solution was first allowed to dissolve and then benzaldehyde (3.048ml, 0.03mol) and ethylacetoacetate (3.087ml, 0.03mol) was added. Then few drops of HCl were added and the resulting solution was refluxed for 3 – 4 hrs at 50 – 60 ºC. After refluxing it was poured in a beaker. $C_{14}H_{17}N_3O_2$; m. p.: 110 – 112^oC; Yield: 73.76%,;R_f value: 0.54

Synthesis of 2-Imino-6-methyl-4-phenyl-1, 2, 3, 4-tetrahydro-pyrimidine-5-carboxylic acid hydrazide (5):

To the intermediate (4) synthesized in the first step, hydrazine hydrate (2.7ml, 0.03mol) was added slowly and few drops of conc. H_2SO_4 were added. The reaction is vigorous evaporate excess of solvent at low temperature. The solution was filtered and the resulting intermediate (5) was dried. $C_{12}H_{15}N_5O$; m. p. - 240 – 241^oC; Yield: 64.19%; R_f value: 0.78

2-Imino-6-methyl-4-phenyl-1, 2, 3, 4-tetrahydro-pyrimidine-5-carboxylic acid substituted hydrazide: [NA – (1 – 7), NA – 10]:

A same experimental condition was followed for all the titled compound. Equimolar quantities of intermediate (5) and derivative of either aldehyde or ketone such as benzaldehyde, 2 – chlorobenzaldehyde, 4 – chlorobenzaldehyde, acetophenone, 2 – chloro acetophenone, 4 – bromobenzaldehyde, 4 – nitroacetophenone, 2 – nitrobenzaldehyde were dissolved in sufficient quantity of methanol and refluxed for 4 – 5 hr at 50 – 60 ºC. The product was immediately poured in a petridish after the completion of reaction. The excess of solvent was allowed to evaporate on a water bath.

2-Imino-6-methyl-4-phenyl-1, 2, 3, 4-tetrahydro-pyrimidine-5-carboxylic acid benzylidene – hydrazide (NA – 1):

$C_{19}H_{17}N_5O$; m. p.: 150 – 151^oC; Yield - 84%; R_f value: 0.859. Elemental Analysis: Calc.: C - 68.45; H - 5.74; N - 21.01; Found: C - 68.35, H – 5.72, N – 20.09. IR(KBr, υ_{max} cm^{-1}): 687(Ar , C–H out of plane) , 766 (C-H out of plane) ,891(C-H out of plane), 1156 (C–N strch) , 1453 (CH_3) , 1562,1609 (NH_2 in plane bend) , 1656 (C=C strch) , 3422 (R_2N-H strch). ^{1}H NMR (δ, ppm): δ – 1.71(s, 3H, CH_3, methyl), δ - 2.0(s, 3H, NH, amine), δ - 4.59(s, 1H, CH, methine), δ – 7.14, 7.06, 7.06, 7.14, 7.07(d, 5H, CH, benzene), δ - 8.0(s, 1H, NH, hydrazide), δ – 8.1(5H,CH,benzylidenimine), 7.62,7.29, 7.29, 7.29, 7.62 (d, 5H, CH, benzylidenimine).

2-Imino-6-methyl-4-phenyl-1, 2, 3, 4-tetrahydro-pyrimidine-5-carboxylic acid (2-chloro- benzylidene)-hydrazide (NA – 2):

$C_{19}H_{16}ClN_5O$; m. p.:250 – 252^oC; Yield –44%; R_f value: 0.903; Elemental Analysis: Calc.: C - 62.04, H - 4.93, Cl - 9.64, N - 19.04; Found: C – 61.84, H – 5.01, Cl – 9.48, N – 20.21; IR(KBr, υ_{max} cm^{-1}): 750 (C-Cl), 875, 969(C-H out of plane), 1062 (C-N strech), 1453 (CH_3), 1484, 1592, 1609 (Ar C-C strch), 1562 (R_2N-H bend) , 3328 (R_2N-H strch); ^{1}H NMR (δ, ppm): δ – 1.71(s, 3H, CH_3, methyl), δ - 2.0(s, 3H, NH, amine), δ - 4.59(s, 1H, CH, methine), δ – 7.14, 7.06, 7.06, 7.14, 7.07(d, 4H, CH, benzene), δ - 8.0(s, 1H, NH, hydrazide), δ – 8.1, 7.6, 7.2, 7.4,7.3(d, 4H,CH, benzylidenimine)

2-Imino-6-methyl-4-phenyl-1, 2, 3, 4-tetrahydro-pyrimidine-5-carboxylic acid (4-chloro-benzylidene)-hydrazide (NA – 3):

$C_{19}H_{16}ClN_5O$; m. p.: 220 – 221^oC; Yield: 71.33%; R_f value: 0.881; Elemental Analysis: Calc.: C - 62.04; H - 4.93; Cl - 9.64; N - 19.04; Found: C – 61.84, H – 5.01, Cl – 9.48, N – 20.21; IR (KBr, cm^{-1}) : 734,969 (C-H out of plane), 750 (C-Cl strch), 1062, 1141, 1219(C-N strch), 1453 (CH_3), 1484, 1594 (Ar-C-C strch), 3328 (R_2N-H strch); ^{1}H NMR (δ, ppm): δ – 1.71(s, 3H, CH_3, methyl), δ - 2.0(s, 3H, NH, amine), δ 4.59(s, 1H, CH, methine), δ – 7.14, 7.06, 7.06, 7.14,7.07(d, 5H, CH, benzene), δ - 8.0(s, 1H, NH, hydrazide), δ - 8.1, 7.62, 7.29, 7.29,7.62(d, 4H,CH, benzylidenimine)

2-Imino-6-methyl-4-phenyl-1, 2, 3, 4-tetrahydro-pyrimidine-5-carboxylic acid (1-phenyl-ethylidene)-hydrazide (NA – 4):

$C_{20}H_{19}N_5O$; m. p.:150 – 152^oC; Yield: 90%;R_f value : 0.901; Elemental Analysis: Calc.: C, 69.14; H, 6.09; N, 20.16; Found: C - 69.11, H – 6.02, N – 20.13;IR (KBr,cm^{-1}): 969 (C-H out of plane), 1062, 1156(C-N strch) , 1453 (CH_3) , 1500,1594(Ar –C-C strch), 1625 (C=N) ,1687(C=O strch), 2953(=C-H strch) , 3078(Ar, C-H strch), 3437(R_2N-H strch); ^{1}H NMR (δ, ppm): δ – 0.9(s, 3H, CH_3, methyl), δ – 1.71(s, 3H, CH_3, methyl), δ - 2.0(s, 3H, NH, amine), δ - 4.59(s, 1H, CH, methine), δ – 7.14, 7.06, 7.06, 7.14, 7.07(d, 5H, CH, benzylidenimine), δ - 7.0(s, 1H, NH, hydrazide), δ – 8.1,7.6, 7.3, 7.3,7.6 (d, 5H,CH, benzylidenimine)

2-Imino-6-methyl-4-phenyl-1, 2, 3, 4-tetrahydro-pyrimidine-5-carboxylic acid [1-(2-chloro-phenyl)-ethylidene]-hydrazide (NA – 5):

$C_{20}H_{18}ClN_5O$; m. p.: 260 – 262^oC; Yield: 67.84%; R_f value: 0.827; Elemental Analysis: Calc.: C - 62.91; H - 5.28; Cl - 9.28; N - 18.34; Found: C - 62.11, H – 6.02, Cl - 8.89, N – 19.13; IR (KBr, cm^{-1}): 625(C-H bend) ,734,969(C-H out of plane) ,750 (C-Cl) ,1094 (C-N strch) , 1359(C-H) , 1484,1594 (Ar,C=C strch) ,1672 (C=O strch) , 2922(C-H strch) , 3390 (R_2N-H strch); ^{1}H NMR (δ, ppm): δ – 0.9(s, 3H, CH_3, methyl), δ – 1.71(s, 3H, CH_3, methyl), δ - 2.0(s, 3H, NH, amine), δ – 4.59(s, 1H, CH, methine), δ – 7.06, 7.06, 7.14, 7.14, 7.07(d, 5H, CH, benzylidenimine), δ - 7.0(s, 1H, NH, hydrazide),δ - 7.6, 7.2, 7.2, 7.3(d, 4H,CH, benzylidenimine)

2-Imino-6-methyl-4-phenyl-1, 2, 3, 4-tetrahydro-pyrimidine-5-carboxylic acid [1-(4-nitro-phenyl)-ethylidene]-hydrazide (NA – 6):

$C_{20}H_{18}N_6O_3$; m. p.: 200 – 202°C; Yield - 62.85%; R_f value – 0.833; Elemental Analysis: Calc.: C - 61.21; H - 5.14; N - 21.42; Found: C - 60.91; H - 5.19; N - 21.24; IR spectrum show following peaks in cm^{-1}:703,766, 969 (C-H out of plane), 1000 (C-N), 1281 (C=O), 1500 (-NO_2), 1594 (C=C strch), 1672 (C=N), 2922(=C-H strch); ^{1}H NMR (δ, ppm): δ – 0.9(s, 3H, CH_3, methyl), δ – 1.71(s, 3H, CH_3, methyl), δ - 2.0(s, 3H, NH, amine), δ – 4.59(s, 1H, CH, methine), δ – 7.14, 7.06, 7.06, 7.14, 7.07(d, 5H, CH, benzene), δ - 7.0(s, 1H, NH, hydrazide), 7.9, 8.2, 8.2, 7.2 (d, 4H,CH, benzylidenimine).

2-Imino-6-methyl-4-phenyl-1, 2, 3, 4-tetrahydro-pyrimidine-5-carboxylic acid (2-nitro-benzylidene)-hydrazide (NA – 7):

$C_{19}H_{16}N_6O_3$;, m. p.: 200 – 202°C;Yield - 62.85%; R_f value – 0.833; Elemental Analysis: Calc.: C - 60.31; H - 4.79; N - 22.21; Found: C - 60.48; H - 4.81; N - 22.09; IR (KBr, cm^{-1}): 703,969 (C-H out of plane, 1062, 1156(C-N strch), 1359 (N-O sym strch) , 1516 (N-O asym strch) , 1625 (C=N) , 3390 (R_2N-H strch); ^{1}H NMR (δ, ppm): δ – 1.71(s, 3H, CH_3, methyl), δ - 2.0(s, 3H, NH, amine), δ - 4.59(s, 1H, CH, methine), δ – 7.14, 7.06, 7.06, 7.14, 7.07(d, 5H, CH, benzene), δ - 8.0(s, 1H, NH, hydrazide), 8.1, 7.9, 7.7, 7.6, 8.2(d, 15H,CH, benzylidenimine)

2-Imino-6-methyl-4-phenyl-1, 2, 3, 4-tetrahydro-pyrimidine-5-carboxylic acid (4-bromo-benzylidene)-hydrazide (NA – 10):

$C_{19}H_{16}BrN_5O$; m. p.: 210 – 212°C; Yield – 97.4%; R_f value – 0.804; Elemental Analysis: Calc.: C - 55.35; H - 4.40; Br - 19.38; N -16.99; Found: C - 55.32; H - 4.42, Br - 19.34; N - 16.91. IR (KBr, cm^{-1}): 625(C-Br), 875, 984 (C-H out of plane), 1406, 1492(Ar –C-C strch), 1625, 1656 (C=N), 2937 (C-H strch), 3422 (R_2N-H strch). ^{1}H NMR (δ, ppm): δ – 1.71(s, 3H, CH_3, methyl), δ - 2.0(s, 3H, NH, amine), δ - 4.59(s, 1H, CH, methine), δ – 7.14, 7.06, 7.06, 7.14, 7.07(d, 5H, CH, benzene), δ - 8.0(s, 1H, NH, hydrazide), δ - 8.1, 7.5, 7.5, 7.5,7.3 (d, 5H,CH, benzylidenimine)

Scheme 1: Synthetic scheme of titled compound

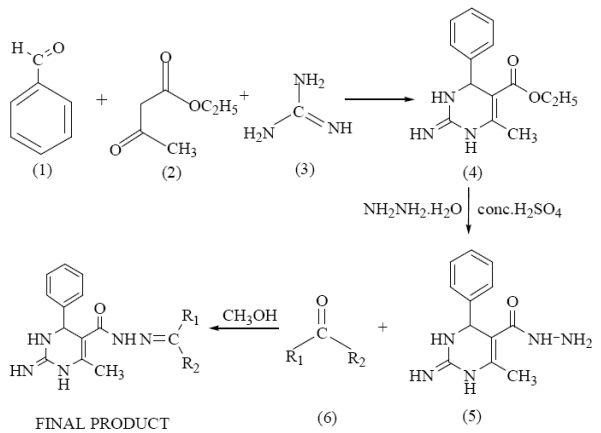


Table 1: Table of substituent at R_1 and R_2 position

COMPOUND CODE	R_1	R_2
NA – 1	- H	-C_6H_5
NA – 2	- H	2 – ClC_6H_4
NA – 3	- H	4 - ClC_6H_4
NA – 4	-CH_3	-C_6H_5
NA – 5	-CH_3	4 - ClC_6H_4
NA – 6	-CH_3	4 – $NO_2C_6H_4$
NA – 7	-H	4 – $NO_2C_6H_4$
NA - 10	- H	4 – BrC_6H_4

Pharmacological screening

The titled compounds were evaluated for their central as well as peripheral analgesic activity. The animals used were Swiss male albino mice weighing about 22 – 25 kg. The animal should have free access to food and water *ad libitum*, kept in large spacious cages and temperature of 22 ± 1°C with 12h light dark cycle should be maintained.[3] Pethidine – 5mg/kg (hot – plate method)[4] and Diclofenac sodium (20mg/kg) as standard drug.[5]

Hot plate method

The experiment was carried out using Analgesiometer Eddy's Hot plate old Model GPIT 9504.The mice were divided into 10 groups of six animals each. The temperature was maintained at 55 ± 1° C. The reaction time was measured prior to administration of synthesized compounds and drug administration (0 min). Group 1 was kept as normal control. The synthesized compounds were injected subcutaneously to mice of groups 2 - 9 at a dose of 5mg/kg b.w. Mice of group 10 served as standard and were treated with Pethidine hydrochloride 5mg/kg b.w. The reaction time was again measured at 0, 30, 60, 90 min. after the treatment. To avoid tissue damage to the mice paws, cut-off time for the response to the thermal stimulus was set at 60s. The increase in the reaction time against control was calculated[3].

Acetic acid induced writhing test

The experiment was performed using 10 groups of 6 mice each. The first group served as control and received only vehicle & the groups 2 - 9 received the tested compounds. The last groups received standard drug such as Diclofenac sodium at a dose of 20 mg/kg b.w.[5]. After 30 min, each mice was administered 0.7% of an aqueous solution of acetic acid (10ml/kg) and the mice were then kept in transparent boxes for observation. The numbers of writhes were counted for 20min after acetic acid injection. The number of writhes in each treated groups was compared to that of control group.[6] The number of writhes was recorded and the percentage protection was calculated using the formula:

% protection = (control mean- treated mean/control mean) ×100 [7]

Statistical analysis

The statistical analysis was performed by ANOVA followed by Dunnett's test for the multiple comparison of test compound as compared to control. The Institutional Animal Ethic Committee approved the protocol of the animal studies.

RESULTS AND DISCUSSION

The titled compounds have been synthesized and screened for analgesic activity by hot plate method and acetic acid induced writhing test. The results are given in table below:

Hot plate method

From the results of hot plate method it is clearly concluded that the compound NA – 10 is centrally most active while the compound NA – 1 was found to occupy a position next to NA – 10 as a good analgesic. The compound NA – 2 was found to be inactive and rest of the compounds showed moderate analgesic activity. It is structurally related to opioid analgesics as it possess aromatic ring for Vander Waal interaction, an amine group to form ionic bond and two lone pair of electrons to form hydrogen bond at the receptor site thus its structural similarity show that it binds at μ receptor site. The compounds were found to be significant at $p < 0.001$.

The weak analgesic activity of other compounds may be due to difference in the lipophilic character of substituents at C – 19 position. (Table 2)

Acetic acid induced writhing test

The compound NA – 10 and NA – 4 were comparable in potency but even then the potency of NA – 10 was more. It has been reported that acetic acid induces the release of endogenous mediators, such as PGE_2 (prostaglandin E_2) and $PGF_{2\alpha}$ in peritoneal fluids as well as lipooxygenase, indirectly, which stimulate the nociceptive neurons sensitive to analgesics. However, the result of the acetic acid induced writhing response strongly suggested a partial involvement of inhibition of lipooxygenase and/or cyclooxygenase in peripheral tissues, thereby reducing PGE2 synthesis and interfering with the mechanism of transduction in primary afferent nociceptor. When the structure has been correlated it has been found that aromatic ring and presence of electron withdrawing substituent on aromatic ring make the compound highly lipophilic. NA – 4 and NA – 10 showed good peripheral activity as compared to standard (Diclofenac sodium). While rest shows moderate activity and compound NA – 2 and NA – 3 were found to be inactive. All the compounds were found to be significant at $p < 0.001$. (Table 3)

Table 2: Hot plate method

Group	Dose (mg/kg)	0 min	30 min	60 min	90 min
CONTROL	20	4.467 ± 0.097	4.633 ± 0.097	4.833 ± 0.092	5.050 ± 0.081
NA – 1	20	4.400 ± 0.094	7.870 ± 0.503*	9.700 ± 0.114*	10.10 ± 0.096*
NA - 2	20	4.483 ± 0.085	7.200 ± 0.114*	8.150 ± 0.081*	6.660 ± 0.253*
NA – 3	20	4.483± 0.092	7.616 ± 0.081*	10.00 ± 0.094*	7.500 ± 0.322*
NA – 4	20	4.450 ± 0.096	7.050 ± 0.677*	7.430 ± 0.080*	9.000 ± 0.105*
NA – 5	20	4.480 ± 0.096	6.270 ± 0.110*	6.550 ± 0.070*	8.150 ± 0.081*
NA – 6	20	4.450 ± 0.123	7.150 ± 0.107*	9.700 ± 0.081*	8.150 ± 0.087*
NA – 7	20	4.430 ± 0.092	6.120 ± 0.096*	7.616 ± 0.087*	8.160 ± 0.101*
NA – 10	20	4.417 ± 0.096	8.150 ± 0.110*	8.380 ± 0.085*	12.88 ± 0.085*
Pethidine[#]	5	4.467 ± 0.097	10.18 ± 0.099*	11.58 ± 0.656*	15.87 ± 0.125*

[#]Standard = 5 mg/kg b.w.; Values represent mean ± SEM of 6 mice each group; Significant at *P <0.001(Dunnett's test); Dose of test drug = 20mg/kg b.w.

Table 3: Acetic acid induced writhing test

Group	Dose (mg/kg)	No. of Writhings (20 min) [MEAN± SEM]	% Analgesic Activity
CONTROL	20	40.50 ± 0.255	-------
NA – 1	20	30.17 ± 0.216*	25.5
NA - 2	20	36.00 ± 0.236*	11.11
NA – 3	20	35.50 ± 0.255*	12.34
NA – 4	20	16.00 ± 0.236*	60.49
NA – 5	20	31.83 ± 0.269*	21.51
NA – 6	20	23.83 ± 0.302*	41.16
NA – 7	20	30.00 ± 0.296*	25.92
NA – 10	20	15.50 ± 0.255*	61.73
Diclofenac Sodium[#]	20	14.17 ± 0.302*	65.00

[#] Standard = 5 mg/kg b.w.; Values represent mean ± SEM of 6 mice each group
Significant Standard at *P < 0.001(Dunnett's test); Dose of test drug = 20mg/kg b.w.

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