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AN EFFICIENT AND FACILE SYNTHESIS OF SOME ETHYL -1-CARBAMOYL-4-(2 OR 4-SUBSTITUTED PHENYL)-6-METHYL-2-OXO-1, 2, 3, 4-TETRAHYDROPYRIMIDINE-5-CARBOXYLATE THROUGH ULTRASOUND ACCELERATED VILSMEIER-HAACK REACTION AND THEIR SCREENING AS ANTIPLATELET & ANTICARDIAC ACTIVITY

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ABSTRACT

An efficient synthesis of some 5-ethoxycarbonyl-4-(2 or 4-substituted phenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-2(1H)-carboxylic acids **(3)** has been reported through ultrasonically assisted Vilsmeier-Haack reaction of their corresponding 5-ethoxycarbonyl-4-(4-substituted phenyl)-6-methyl-3,4-dihydropyrimidin-2(1H)-ones **(1)** using silica gel as catalyst yielding N-1-formyl derivative **(2)**; followed by oxidation with 30% H_2O_2. The N-1 carboxylic acids **(3)** thus obtained were converted to their amide derivatives **(4)** in very good yield by classical reactions. Preliminary pharmacological screening of these novel carboxamides indicated that they possessed significant antiplatelet activity and moderate anticardiac activity.

KEYWORDS

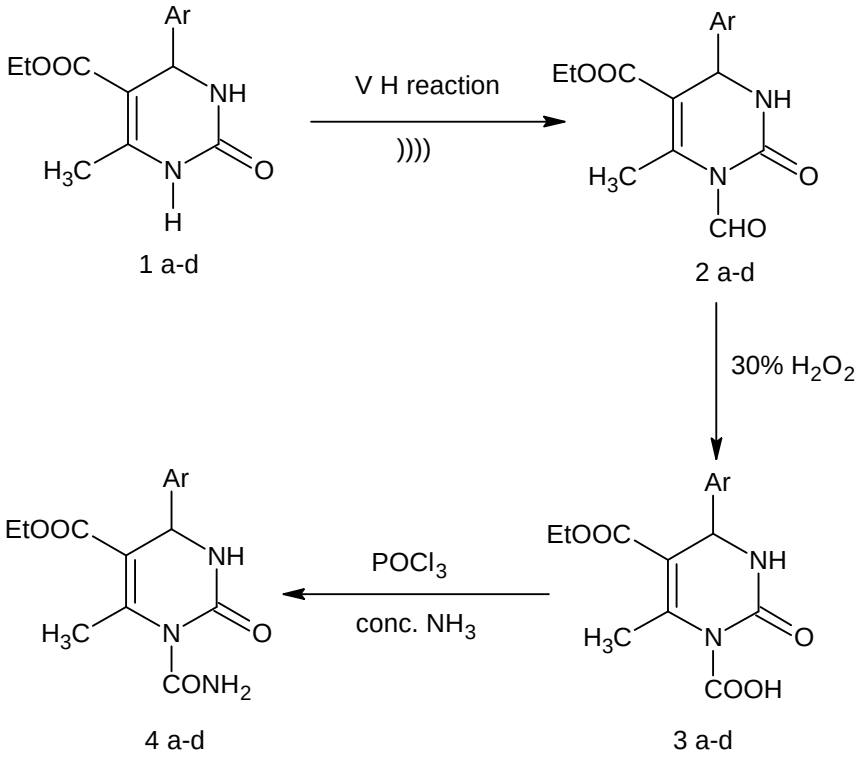
3,4-dihydropyrimidine, Vilsmeier-Haack reagent, silica gel, ultrasonic irradiation

INTRODUCTION

4- aryl-3, 4 dihydropyrimidin-2(1H)-ones (DHPM) and 4- aryl-1, 4 dihydropyrimidin (DHP) are well established medicinally useful class of compounds and have received great attention in recent days, because of their wide range of therapeutic and pharmacological properties. DHPM C-5 amides have also potent activity against hypertension and benign prostative hyperplasia [1a- c]

The age-old Vilsmeier-Haack (VH) reagent[2] has been applied for introduction of formyl group and to carry out other reactions like cycloaddition[3], cyclisation[4] and ring annulation[5] has been mostly demonstrated in relation to thermal reactions via ionic reactions. However, scanty reports are available in literature about its deployment under non-traditional conditions e.g. microwave[6] or ultrasound[7] irradiation etc.; which recently have been found to be more advantageous and eco-friendly than the conventional methods as well as to promote new reactions *via* radical pathways. In continuation of our work on green synthesis of 5-ethoxycarbonyl-6-methyl-4-aryl-3,4-dihydropyrimidin-2(1H)-ones by modified Biginelli reaction[8]; it appeared worthwhile to introduce another carboxylic acid group in the DHPM pharmacophore through

ultrasonically VH reaction followed by transformation of the formyl group into carboxylic acid derivatives by conventional route. Thus it appeared rational that aza-anlogs of DHP nucleus possessing two carboxyl groups could also exhibit significant pharmacological action; as earlier evidenced in orally active antihypertensive agent SQ-32926[9]; in analogy to calcium channel blocking activity shown by 4-aryl-1,4-dihydropyridine pharmacophore[10] having two ester groups like nifedipine, amlodipine, etc. Hence, the title compounds **(4)** were synthesized following **Scheme-I** entailing ultrasonically accelerated VH reaction on DHPMs **(1)** to result N-1-formylated products **(2)** in excellent yield; which on subsequent transformations by conventional reactions yielded the target compounds in good amount. The preliminary screening of their pharmacological action was planned in order to throw light on their structure activity relationship.



1 a-d 2 a-d 3 a-d 4 a-d

a, Ar = C_6H_5 ; b, Ar = 4-(OCH_3) C_6H_4; c, Ar = 2-(NO_2) C_6H_4; d, Ar = 2-(Cl) C_6H_4

Scheme I

RESULTS AND DISCUSSION

The required 5-ethoxycarbonyl-6-methyl-4-(4-substituted phenyl)-3, 4-dihydropyrimidin-2(1H)-ones **(1a-d)** were prepared by modified Biginelli reaction in excellent yield following the reported procedure[8] and then subjected to VH reaction under ultrasonic irradiation at 50-55^{0}C for about an hour employing silica gel as catalyst. The formylated DHPMs thus obtained in excellent yield (90-92%) was observed to be a mixture of N-1 formylated product **(2)** as major one along with a trace of its N-3 isomer; as evidenced from the IR spectrum showing peaks at 2785 and

2800 cm-1 (aldehydic –CH), 1715 and 1700 (-C=O) and also supported by ^{1}H NMR spectrum indicating two clear aldehydic peaks at δ 9.1 and 9.3 as well as repetition of all other significant peaks. After two or three fractional crystallizations from ethanol: acetone (4:1); the pure compounds **2(a-d)** were obtained and subjected to oxidation by employing 30% H_2O_2 yielding their corresponding acids **(3a-d)** in around 75% yield; which were not accessible by classical method as to our knowledge. The N-1 carboxylic acids thus obtained could easily be converted to their ammonium salts by conventional method; as indicated from the absence of –COOH peaks in IR and ^{1}H NMR spectrum with appearance of a strong peak at 1585 cm^{-1} ($-CO_2^-$) in IR and at δ 8.1 in ^{1}H NMR spectra. On treatment of acids **3(a-d)** with PCl_5 and $POCl_3$ in CH_3CN; the respective acid chlorides were obtained in quantitative yield as semisolid mass; as evidenced from the shift of strong carboxylate peak at 1696 cm^{-1} to 1776 cm^{-1}. The acid chlorides thus prepared were smoothly converted to amides **4(a-d)** in about 70% yield by reacting with conc. NH_4OH for one hour and isolated as white crystalline product by following routine procedure; which conformed satisfactorily to their spectral (UV, IR, ^{1}H NMR, MS) data as well as elemental analysis.

Table 1.

Physical and spectral data of compounds 1a-d

Preliminary screening of the title acids **(3a-3d)** for antiplatelet[11] and anticardiac[12]

No	R	Molecular formula	Yield (%)	Mp (^{0}C)	λ_{max} (εmax)	Rf	IR	NMR
1a	C_6H_5	$C_{14}H_{16}O_3N_2$	92	203-04	283	0.63	757-823 (aromatic), 1649 (-C=O), 3114 & 3242 (-NH)	8 (s,1H,-NH), 7.21-7.3 (m,5H,arom), 5.7 (s,1H,NH (D_2O exchang)), 2.3 (s, 3H,-C-CH_3)
1b	4-(OCH_3)C_6H_4	$C_{15}H_{18}O_4N_2$	87	198-99	282.5	0.45	781-790 (aromatic), 1634 (C=O), 2956-3242 (-NH)	9.14 (s,1H), 7.66 (s,1H), 7.14 (d, J=8.4Hz,2H), 6.86 (d, J=8.4Hz,2H)
1c	4-(NO_2)C_6H_4	$C_{14}H_{15}O_5N_3$	83	208-09	282.5	0.71	773-856 (aromatic), 1519 ($-NO_2$), 1648 (-C=O), 2941-2979 (-NH)	9.32 (s,1H), 8.28 (d, J=8.4Hz,2H), 7.89 (s,1H)
1d	2-(Cl)C_6H_4	$C_{14}H_{15}O_3N_2Cl$	90	215-17	280	0.58	3224 (-NH), 3098,1706, 1651 (-C=O)	9.25 (brs,1H), 7.86 (brs,1H), 7.19~7.41(m,4H), 5.15 (s,1H)

compounds **(4a-d)** along with their precursor activity was carried out following the standard

procedures using adrenaline and aspirin as standards. While all these compounds **(2, 3 & 4)** were found to possess significant antiplatelet and moderate anticardiac activity; the title compounds **(4a-d)** were much better in action than their respective precursor carboxylic acids **(3a-d)**. It was also observed that **4a** exhibited higher anticardiac calcium channel blocking activity at 0.8 µg/ml and better antiplatelet activity than the other three (**4b-d)** and hence it deserves elaborate study on its pharmacological profile. Based on these observations; it appeared that substitution at 2 or 4 position in 4-aryl group of DHPM did not enhance anticardiac or antiplatlet activity of the pharmacopore under study.

Experimental Section

Melting points were determined in open capillary tubes on Veego digital automatic heated melting point apparatus and are uncorrected. UV measurements were done on Shimadzu 1700 UV-visible spectrometer and IR spectra were recorded from KBr pellets on Shimadzu 8400S FT-IR spectrometer. ^{1}H-NMR spectra were run in $CDCl_3$ at 300 MHz on Shimadzu FT-NMR spectrometer with TMS as an internal standard (chemical shifts in δ) and mass spectra were recorded on Bruker (ultraflex TOF) spectrophotometer using electron-impact technique at 70eV and only the relevant and prominent mass fragments were considered. All reactions were monitored by TLC using silica gel-G plate (0.2mm) as adsorbent and ethyl acetate: methanol(1:3) was used as eluent unless otherwise stated; while the spots were visualized by iodine vapor or UV lamp. The purity of the compounds was checked by HPLC using C-18 column and mobile phase as acetonitrile: phosphate buffer (80:20) at flow rate 1ml/min. An ultrasonic bath equipped with 40 KHz frequency transducer was used for ultrasonically irradiated reactions. Laboratory grade reagents, solvents and chemicals were used as such.

General procedure for preparation of 5-ethoxycarbonyl-6-methyl-4-(4-substituted phenyl)-3,4-dihydropyrimidin-2(1H)-ones (1a-d)

The title compounds **1a-d** was prepared following the reported procedure of Chatterjee, et.al[8] in 87-92% yield and characterized by comparison of their spectral (UV, IR, ^{1}H NMR, MS) data as well as by mixed melting point with an authentic sample. (Table 1)

General procedure for synthesis of ethyl-1-formyl-4-substituted phenyl-6-methyl-2-oxo-3,4-dihydropyrimidin-5-carboxylic acid (2a-d)

In a typical experiment, a Vilsmeier-Haack adduct prepared from $POCl_3$ (15mmol) and DMF (15mmol) at -2 to -5 ^{0}C was slowly added to a round bottom flask containing the appropriate DHPM (5mmol) in acetonitrile (3ml) and silica gel (0.2g) was kept under sonication at 50-55 ^{0}C for 50 min. After completion of reaction as monitored by TLC (chloroform: cyclohexane: 4:1); the reaction mixture was dissolved in $CHCl_3$ and quenched with 50% sodium thiosulfate solution. The organic layer was separated, washed with water and dried over anhydrous Na_2SO_4 and evaporated under vaccum to obtain a dense liquid; which on trituration with hexane gave the crude product. After two recrystallisations from ethanol : acetone (4:1);it afforded the pure crystalline product.

Ethyl 1-formyl-4-phenyl-6-methyl-2-oxo-3, 4-dihydropyrimidin-5-carboxylate (2a):

Yield 89%; m.p. 173-76^{0}C; IR (KBr, U_{max}): 3238, 3128 (-NH), 3037, 2977, 2755 (aldehyde-CH), 1722 (-C=O), 1700, 1647 (ketone-C=O), 1485, 1276-1080 cm^{-1}; ^{1}H NMR: 9.0 (s, 1H, -CHO), 7.6 (brs, 1H, -NH, D_2O exch), 7.3 (s, 5H, aromatic), 5.5 (s, 1H, -CH), 4.19 (q, 2H, -OCH_2), 1.7 (t, 3H, ester-CH_3), 2.3 (s, 3H, -CH_3); ^{13}C NMR (d_6-DMSO) δ: 171 (-CHO), 166 (-COOEt), 153 (-C=O), 127.5-126.5 (-CH arom), 59 (-CH_2), 49 (-CH); m/z: 288 (M+); Anal. Calcd. C (62.39%) H (5.49%) N (9.52%) Found : C (62.19%) H (5.29%) N (9.62%),

Ethyl-1-formyl-4-(4-methoxyphenyl)-6-methyl-2-oxo-3,4-dihydropyrimidin-5-carboxylate (2b):

Yield 78%; m.p. 211-12^{0}C; IR (KBr, υ_{max}): 3238, 3109 (-NH), 3002, 2981, 1724 (ester-C=O), 1700 (aldehyde-C=O), 1651 (ketone-C=O), 1465, 1512 cm^{-1}; ^{1}H NMR: 8.2 (s, 1H, -CHO), 7.61 (brs, 1H, -NH, D_2O exch), 5.5 (s, 1H, -CH), 4.2 (q, 2H, -OCH_2), 1.72 (s, 3H, -CH_3); ^{13}C NMR (d_6-DMSO) δ: 158 (-C=O), 114, 128, 135, (-CH, arom), 167 (-COOEt), 154 (–C=O), 55 (-OCH_3), 62, 14, 12.2; m/z: 318 (M+), Anal. Calcd. C(60.17%) H(5.50%) N(8.60%), Found: C(60.27%) H(5.40%) N(8.70%).

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Ethyl-1-formyl-4-(2-nitrophenyl)-6-methyl-2-oxo-3,4-dihydropyrimidin-5-carboxylate (2c):

Yield 88%; m.p. 208-10^{0}C; IR (KBr, υ_{max}): 3217, 3116 (-NH), 3047, 3007, 2978, 2788 (aldehyde-CH), 1726 (ester-C=O), 1708 (aldehyde-C=O), 1662 (ketone-C=O), 1224 cm^{-1}; ^{1}H NMR: 9.2 (s, 1H, -CHO), 7.5-7.9 (m, 5H, aromatic), 7.0 (brs, 1H, -NH, D_2O exch), 5.8 (s, 1H, methine-CH), 4.0 (q, 2H, -OCH_2), 2.55 (s, 3H, -CH_3); ^{13}C NMR (d_6-DMSO) δ: 167 (-COOEt), 157 (-C=O), 147, 140.1, 123, 128, 134 (-CH, arom), 154 (–C=O), 44.9 (-CH), 62, 14, 12.2; m/z: 333 (M+), Anal. Calcd. C(53.85%) H(4.64%) N(12.51%), Found . C(53.70%) H(4.50%) N(12.45%).

Ethyl-1-formyl-4-(2-chlorophenyl)-6-methyl-2-oxo-3,4-dihydropyrimidin-5-carboxylate (2d):

Yield 90%; m.p. 210-13^{0}C; IR (KBr, υ_{max}): 3329, 3217 (-NH), 3124, 2996, 2812 (aldehyde-CH), 1720 (ester-C=O), 1698 (aldehyde-C=O), 1660 (ketone-C=O), 1394 cm^{-1}; ^{1}H NMR: 9.1 (s, 1H, -CHO), 6.9(brs, 1H, -NH, D_2O exch), 5.6 (s, 1H, methine-CH), 4.1 (q, 2H, -OCH_2), 2.5 (s, 3H, -CH_3); ^{13}C NMR (d_6-DMSO) δ: 167 (-COOEt), 157.2 (-C=O), 154.3 (–C=O), 132, 128, 126 (-CH, arom), 44 (-CH), 61.9, 14, 12; m/z: 322.07 (M+), Anal. Calcd. C(55.72%) H(4.58%) N(8.48%), Found : C(55.52%) H(4.38%) N(8.68%).

General method for synthesis of 5-ethoxycarbonyl-4-phenyl-6-methyl-2-oxo-3,4-dihydropyrimidin-1(2H)-carboxylate acid (3a)

To a cooled solution of **2a** (2.88g, 10mmole) in acetonitrile (15ml) was added 30% H_2O_2 (2ml) and the reaction mixture was stirred at room temperature for 2 hour. After the reaction was over as indicated by TLC (ethyl acetate: toluene 3: 2); water (25ml) was added and it was extracted with $CHCl_3$ (15ml×2). The combined organic extract was washed with 3% sodium bisulfite solution followed by 5% sodium bicarbonate (10ml×2) and finally with water. The combined aqueous and alkaline extract was cooled and acidified with dil. HCl to get crude solid (2.38g) which on two recrystallisations from aqueous methanol yielded pure crystalline acid **3a** (2.25g, 75%) melting at 211-13^{0}C. Rf: 0.51 (ethyl acetate : toluene 3 : 2); IR (KBr, υ_{max}): 3360 (-OH), 3250 (-NH), 3040, 1728 (ester-C=O), 1696 (acid-C=O), 1648 (ketone-C=O), 762, 695 cm^{-1} ; ^{1}H NMR ($CDCl_3$) δ: 8.6 (s,1H,D_2O exch, -COOH), 7.5 (m, 5H, arom), 7.3 (s, 1H, -NH, D_2O exch), 5.3 (s, 1H, -CH), 4 (q, 2h, -OCH_2CH_3), 1.17 (t, 3H, -OCH_2CH_3); ^{13}C NMR (d_6-DMSO) δ: 167 (-COOEt), 160.5 (-COOH), 154.6 (-C=O), 126-28 (-CH arom), 49.3 (-CH); m/z: 304 (M+), Anal. Calcd. C (59.11%) H (5.20%) N (9.11%), Found : C(59.21%) H (5.35%) N (9.21%).

To a stirring solution of acid **3a** (1.5g, 5mmol) in CH_3OH (5ml) and ammonium acetate (1g) at 15-18^{0}C; conc. NH_3 solution (10ml) was added at a slow rate; and the stirring was continued for about 3 hr maintaining temperature around 20-22^{0}C. After evaporation of the solvent under vaccum; it afforded semisolid mass, which was triturated with n-hexane and kept overnight in freeze to get the crystalline solid melting at 285-89^{0}C. The ammonium salt of **3a** thus obtained almost in quantitative yield, also gave satisfactory spectral (UV, IR, ^{1}H NMR & MS) data conforming to its assigned structure.

5-ethoxycarbonyl-4-(4-methoxyphenyl)-6-methyl-2-oxo-3,4-dihydropyrimidin-1(2H)-carboxylic acid (3b)

By following identical procedure as mentioned above, the acid **3b** was prepared from **2b** to get 72% yield, mp: 282–85^{0}C; IR (KBr, υ_{max}): 3390 (-OH), 3238 (-NH), 3025, 1726 (ester-C=O), 1649 (ketone-C=O) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ: 9.1 (s,1H,D_2O exch, -COOH), 7.3 (s, 1H, -NH, D_2O exch), 5.3 (s, 1H, -CH), 4.1 (q, 2H, -OCH_2CH_3), 2.75 (s, 3H, -CH_3), 1.3 (t, 3H, -OCH_2CH_3); ^{13}C

NMR (d_6-DMSO) δ: 166.8 (-COOEt), 160 (-COOH), 154 (-C=O), 106 (-CH), 55.6 (-CH_3); m/z: 334 (M+), Anal. Calcd. C (57.28%) H(5.53%) N(8.18%), Found: C(57.18%) H(5.43%) N (8.28%).

5-ethoxycarbonyl-4-(2-nitrophenyl)-6-methyl-2-oxo-3,4-dihydropyrimidin-1(2H)-carboxylic acid (3c)

The procedure as mentioned above was carried out for **2c** to prepare the acid **3c** obtaining 71% yield, mp: 286–88^{0}C; IR (KBr, υ_{max}): 3360 (-OH), 3250 (-NH), 3040, 2988, 1728 (ester-C=O), 1696 (acid-C=O), 1648 (ketone-C=O), 762, 695; ^{1}H NMR (DMSO) δ: 8.9 (s, 1H, -COOH), 7.4-7.9 (m, 5H, arom), 6.1 (br, 1H, -NH, D_2O exch), 5.75 (s, 1H, -CH), 3.9 (q, 2H, -OCH_2), 2.1 (s, 3H, -CH_3), 0.9 (t, 3H, -CH_2CH_3); ^{13}C NMR (d_6-DMSO) δ: 167.5 (-COOEt), 161 (-COOH), 154.8 (-C=O), 147, 140, 123, 127, 134 (-CH, arom), 44 (-CH); m/z: 349 (M+), Anal. Calcd. C(51.58%) H(4.33%) N(12.03%), Found : C(51.45%) H(4.25%) N(12.13%).

5-ethoxycarbonyl-4-(2-chlorophenyl)-6-methyl-2-oxo-3,4-dihydropyrimidin-1(2H)-carboxylic acid (3d)

Following the procedure as mentioned above, the acid **3d** was prepared to get 69% yield, mp: 292–94^{0}C; IR (KBr, υ_{max}): 3360 (-OH), 3250 (-NH), 3040, 2988, 1728 (ester-C=O), 1696 (acid-C=O), 1648 (ketone-C=O), 762, 695; ^{1}H NMR (DMSO) δ: 8.9 (s, 1H, -COOH), 7.4-7.9 (m, 5H, arom), 6.1 (br, 1H, -NH, D_2O exch), 5.75 (s, 1H, -CH), 3.9 (q, 2H, -OCH_2), 2.1 (s, 3H, -CH3), 0.9 (t, 3H, -CH_2CH_3); ^{13}C NMR (d_6-DMSO) δ: 167.5 (-COOEt), 161 (-COOH), 154.8 (-C=O), 147, 140, 123, 127, 134 (-CH, arom), 44 (-CH); m/z: 338 (M+); Anal. Calcd. C(53.19%) H(4.46%) N(8.27%); Found : C(53.39%) H(4.35%) N(8.25%)

General procedure for preparation of Ethyl-1-carbamoyl-4-phenyl-6-methyl-2-oxo-1, 2, 3, 4-tetrahydropyrimidine-5-carboxylates (4a)

A mixture of PCl_5 (2g) and $POCl_3$ (3ml) was added to a stirring cooled solution of the acid **3a** (10mmol) in acetonitrile (15ml); and the reaction mixture was kept under stirring for 2hr till the reaction was complete (tlc.); which was also indicated by the disappearance of the –COOH group peak in IR with the appearance of a strong –C=O peak at 1670-80 cm^{-1}. The acid chloride thus prepared was reacted with conc. NH_3 solution (10ml) at room temperature for 1 hr when it became hazy. On evaporation of the solvent under vaccum; it afforded white crystalline product (3.1g), which on trituration with ether gave the pure amide **4a** in 70% yield melting at 275-78^{0}C with colour change. Rf: 0.49 ; IR (KBr, υ_{max}): 3230, 3075 (-NH), 2980, 1675 (amide I), 1635 (amide II) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ: 6.1 (s, 1H, D_2O exch, -NH_2), 5.9 (s, 1H, -NH, D_2O exch), 5.6 (s, 1H, -CH), 4.0 (q, 2H, -OCH_2CH_3), 3.6 (s, 3H, -CH_3), 1.2 (t, 3H, -OCH_2CH_3); ^{13}C NMR (d_6-DMSO) δ: 155.8 (-$CONH_2$), 154.3 (-C=O), 49 (-CH), 167 (-COOEt), 126, 128 (-CH, arom); m/z: 302 (M+); Anal. Calcd. C (59.30%) H(5.45%) N(13.75%); Found: C(59.25%) H(5.30%) N(13.55%).

Ethyl-1-carbamoyl-4-(4-methoxyphenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylates (4b)

Yield 65%, mp: 280-83^{0}C; Rf: 0.41; IR (KBr, υ_{max}): 3225, 3180 (-NH), 3025, 2917, 2870, 1695 (amide I), 1660 (amide II) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ: 6.0 (s, 1H, D_2O exch, -NH_2), 5.8 (s, 1H, -NH, D_2O exch), 5.5 (s, 1H, -CH), 4.1 (q, 2H, -OCH_2CH_3), 3.73 (s, 3H, -CH_3), 1.3 (t, 3H, -OCH_2CH_3); ^{13}C NMR (d_6-DMSO) δ: 155.6 (-$CONH_2$), 154.3 (-C=O), 49 (-CH), 167 (-COOEt), 55.8 (-OCH_3); m/z: 335 (M+); Anal. Calcd. C (57.65%) H(5.75%) N(12.61%); Found: C(57.70%) H(5.80%) N(12.25%).

Ethyl-1-carbamoyl-4-(4-nitrophenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylates (4c)

Yield 67%, mp: 292-94^{0}C; Rf: 0.43 ; IR (KBr, υ_{max}): 3218, 3160 (-NH), 3015, 2934, 2850, 1675 (amide I), 1665 (amide II) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ: 6.1 (s, 1H, D_2O exch, -NH_2), 5.7 (s, 1H, -NH, D_2O exch), 5.6 (s, 1H, -CH), 4.2 (q, 2H, -OCH_2CH_3), 3.75 (s, 3H, -CH_3), 1.3 (t, 3H, -

OCH_2CH_3); ^{13}C NMR (d_6-DMSO) δ: 155.6 (-$CONH_2$), 154.3 (-C=O), 49 (-CH), 167 (-COOEt), 55.8 (-OCH_3); m/z: 348 (M+); Anal. Calcd. C(57.65%) H(5.75%) N(12.61%); Found: C(57.72%) H(5.60%) N(12.55%).

Ethyl-1-carbamoyl-4-(4-chlorophenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylates (4d)

Yield: 68%; mp: 310-13^{0}C; Rf: 0.45 ; IR (KBr, υ_{max}): 3225, 3180 (-NH), 3035, 2914, 2865, 1675 (amide I), 1665 (amide II) cm^{-1}; ^1H NMR ($CDCl_3$) δ: 6.2 (s, 1H, D_2O exch, -NH_2), 5.8 (s, 1H, -NH, D_2O exch), 5.7 (s, 1H, -CH), 4.4 (q, 2H, -OCH_2CH_3), 3.65 (s, 3H, -CH_3), 1.4 (t, 3H, -OCH_2CH_3); ^{13}C NMR (d_6-DMSO) δ: 158.6 (-$CONH_2$), 155.3 (-C=O), 49 (-CH), 170 (-COOEt), 56.8 (-OCH_3); m/z: 337 (M+); Anal. Calcd. C (53.65%), H(4.75%), N(12.44%); Found: C(53.72%), H(4.60%), N(12.54%).

Pharmacological screening

The title compounds **(4a & 4b)** were screened for antiplatelet activity by following standard procedure using aspirin as standard and the cardiac activity was determined using isolated frog heart experiment[13,14] as given below in the department of pharmacology; Dr. D. Y. Patil Institute of Pharmaceutical Sciences & Research. The animal facility of the institute is approved by CPCSEA and the experimental protocols for the same have been approved by the Institutional Animal Ethics Committee.

Antiplatelet activity[11]

Healthy male Albino mice (300-350gm) were selected. 1ml of blood withdrawn through retro-orbital route from each mouse under light ether anesthesia was collected in epindroff tubes containing sodium citrate (3.8%). Blood sample was subjected to centrifugation at 1500 - 2000 rpm at room temperature for 10 min. Supernatant fluid containing platelet rich plasma (PRP) was collected and remaining liquid discarded. PPP was collected and used as blank and the absorbance of PPP vs. PRP was measured. Then 2µM of agonist (adrenaline) was added to the PRP that caused platelet aggregation and due to this the absorbance of the solution decreased. The solution of DHPM derivatives under test **(4a-d)** in polyethylene glycol 400 (0.5mg/ml, 1.0mg/ml and 1.5mg/ml) was added to the PRP containing the agonist. Then the absorbance of the samples was checked at five different λmax values.

Cardiac activity[12, 13]

Cardiac activity of **4a to 4d** was checked on healthy frogs of the *Rana tigriana* species. The frog was pithed and heart was exposed. The heart was isolated and cannulated with the syme's cannula. The apex of the ventricle was pinned and it was connected to the force transducer. The flow rate of the ringer solution was kept constant at 10-12 drops/ minute by means of flow adjusting screw. The heart was allowed to stabilize for about 15 min prior to administration of test drug. The suspension of the test compound of 2 & 3 series was prepared in distilled water containing tween-80, while in case of the title compounds (4) the solution was made directly in distilled water. The frog heart was allowed to equilibrate and the test compounds were added in the syme's cannula and its effect was recorded.

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