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Original article

Highly regioselective synthesis of N-3 organophosphorous derivatives of 3,4-dihydropyrimidin-2(1*H*)-ones and their calcium channel binding studies

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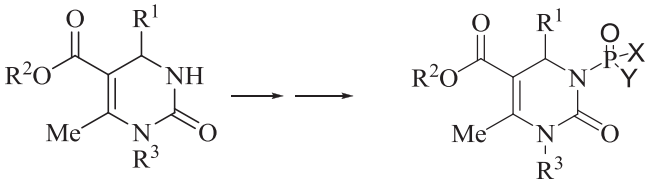
HIGHLIGHTS

- Novel 3,4-dihydropyrimidinones are obtained in highly regioselective manner.
- Organophosphorous compounds are valuable owing to therapeutic potential.
- N3-substituted dihydropyrimidinones are prized for different biological effects.
- Calcium channel binding study has been conducted on new compounds.
- X-Ray shows structure similar to receptor bound conformation similar to nifedipine.

GRAPHICAL ABSTRACT



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ABSTRACT

A series of novel N-3 substituted 3,4-dihydropyrimidin-2(1*H*)-ones derivatives bearing diaminophosphinyl, phosphonate and phosphorous containing heterocycles were obtained from 3,4-dihydropyrimidinones (DHPMs) in a regioselective manner through an efficient reaction protocol, tolerant to substitutional variation at the key diversity positions around the DHPM core. None of the representative compounds screened for calcium channel blocking activity was found to have significant activity compared to nifedipine.

1. Introduction

1,4-dihydropyridine (DHP) derivative nifedipine **1** is a well-known calcium channel blocker (CCB) used for the clinical treatment of cardiovascular diseases such as hypertension, cardiac arrhythmias and angina pectoris [1,2]. Recently, structurally related 3,4-dihydropyrimidin-2(1*H*)-ones (DHPMs), also known as Biginelli compounds [3] have been identified as efficient calcium channel blockers [4,5] as well as α_{1a}-adrenergic receptor antagonists, mitotic kinesin inhibitors, and hepatitis B virus replication suppressors. Several marine natural products containing the DHPM core [6,7] are potent HIV gp-120CD inhibitors.

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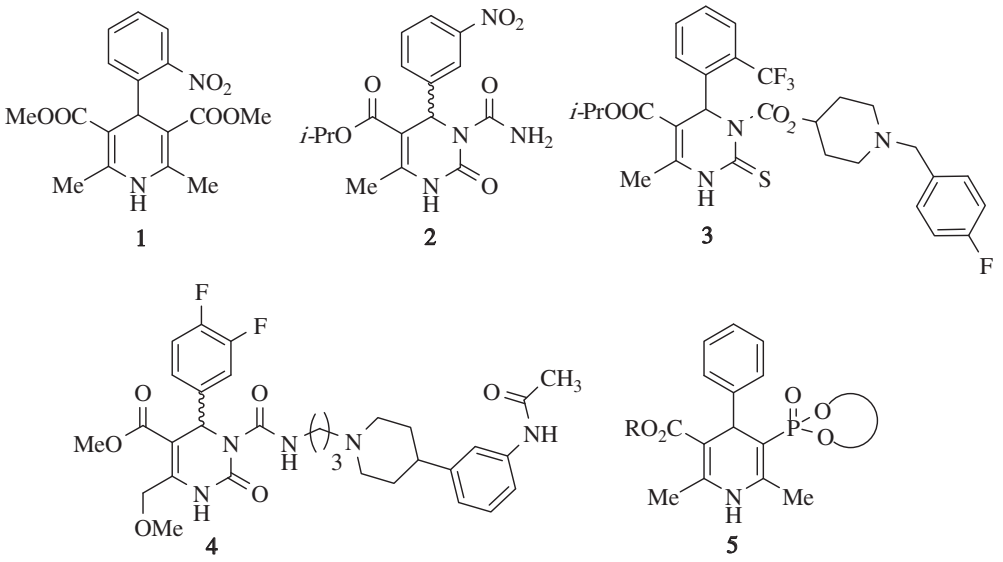
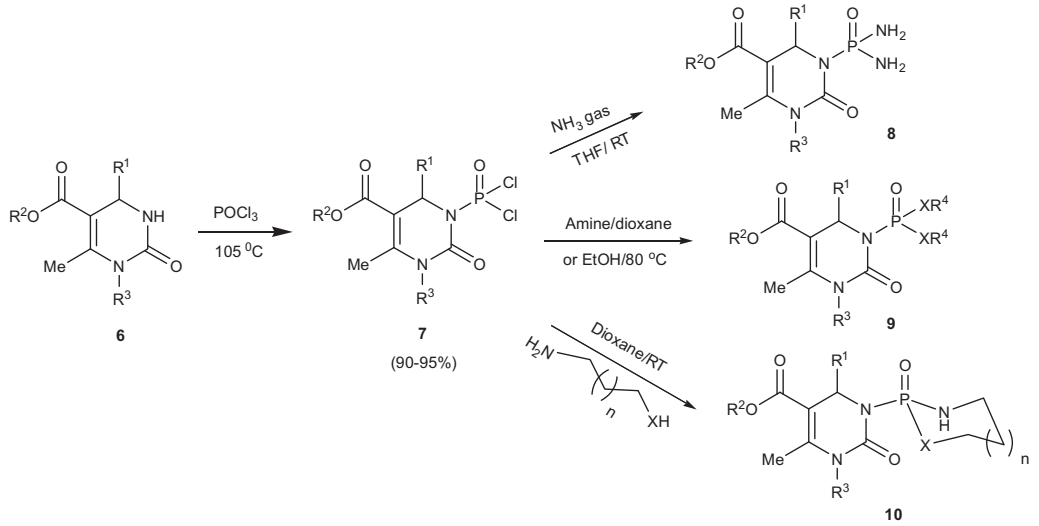


Fig. 1. Biologically important 3,4-dihydropyrimidin-2(1*H*)-ones derivatives.

Over a century old acid catalyzed three-component Biginelli condensation [3] as well as a plethora of its variants furnish DHPMs in moderate to excellent yields, although rationally designed tailor-made diversification of six (N1, C2, N3, C4, C5 and C6) positions around the DHPM core [8–12] gave access to those DHPMs which could not be procured through the three-component route owing to either inaccessibility of the latter route or non-availability of one of these components. Most of the potent DHPM based calcium channel blockers for example SQ 32926 **2** and SQ 32547 **3** or α_{1a}-adrenergic receptor like SNAP 6201 **4** [5] are substituted at N3 position (Fig. 1). A series of 1,4-dihydropyridine-5-cyclic phosphonate derivatives **5** (Fig. 1) were found to have activities superior or comparable to that of nifedipine **1** [13]. Recently phosphorous heterocycles have received widespread attention due to their ubiquity in biological systems [14,15] and their potential to serve as novel pharmaceuticals [16–19]. In continuation of our interest in N-3 substitution of DHPMs [11,12], we wondered, if an organophosphorus group or heterocyclic moiety at N3 could yield a beneficial cardiovascular effect as well as furnish hitherto unknown DHPM derivatives. In this paper we report a useful and highly regioselective reaction giving access to DHPMs bearing a diaminophosphinyl, phosphonate as well as phosphorous heterocyclic unit at the N-3 position as well as report their calcium channel binding activity.

2. Chemistry

Reaction of DHPM lacking an N3 substituent with phosphorous oxychloride yielding a DHPM bearing a reactive phosphorous oxychloride group at the more basic N3 presented itself as a method of choice owing to ease in availability of reagents as well as operational ease of this as well as its further reactions with various nucleophiles. Thus, when DHPM **6** was treated with $POCl_3$ at 105 °C (Scheme 1), N3-elaborated **7** was obtained in 90–95% yield. Lower stability of **7** at ambient temperature meant that it could only be used in subsequent reaction without purification. Thus, treating **7** *in situ* with ammonia gas dissolved in dry THF for 45 min at room temperature, led to the smooth formation of N3-diaminophosphinyl DHPM **8** in excellent yields, after recrystallization from methanol (Table 1). This fairly simple and efficacious



Scheme 1. Synthesis of N3-substituted 3,4-dihydropyrimidin-2(1*H*)-ones.

Table 1
Synthesis of N3-substituted 3,4-dihydropyrimidin-2(1*H*)-ones.

Entry	Product	R^1	R^2	R^3	R^4/X	Yield (%)
1.	**8a**	C_6H_5	C_2H_5	H	–	95
2.	**8b**	4-$OMeC_6H_4$	C_2H_5	H	–	95
3.	**8c**	3-$NO_2C_6H_4$	$(CH_3)_2CH$	H	–	95
4.	**8d**	C_6H_5	CH_3	H	–	93
5.	**8e**	H	C_2H_5	H	–	85
6.	**8f**	CH_3	C_2H_5	H	–	92
7.	**8g**	CH_3	CH_3	H	–	83
8.	**9a**	C_6H_5	C_2H_5	H	C_4H_9/NH	66
9.	**9b**	C_6H_5	C_2H_5	H	C_6H_5/NH	60
10.	**9c**	C_6H_5	C_2H_5	H	C_2H_5/O	70
11.	**10a**	C_6H_5	C_2H_5	H	-/NH	72
12.	**10b**	CH_3	C_2H_5	H	-/NH	65
13.	**10c**	C_6H_5	C_2H_5	H	-/O	64
14.	**10d**	C_6H_5	C_2H_5	CH_3	-/O	65

reaction served as a general protocol for obtaining a range of regioselectively N-3 substituted DHPMs tolerant to substitutional variation around DHPM core as shown in Table 1. In addition to ^{31}P NMR and spectral data the characteristic feature of the ^{1}H NMR spectrum of C-4 aryl derivatives (**8a**–**8d**) is the presence of doublet corresponding to C4–H, due to P–H coupling.

In analogy with the reactions of **7** with ammonia, reactions of various amines with **7** led to the formation of N3-dialkylamino-phosphinyl substituted DHPMs **9**. Thus, when a solution of appropriate N3-dichlorophosphinyl appended DHPM in dry dioxane was treated with amines such as *n*-butylamine and benzylamine or ethanol (80 °C) (Scheme 1) corresponding N-3 substituted DHPMs **9a**–**9c** were obtained in good yield after purification with column chromatography (entries 8–10, Table 1). Similar reactions with diamines and amino alcohols in dry dioxane at room temperature furnished DHPMs derivatives **10a**–**10d** The scope and limitations of this reaction protocol has been explored by varying substituents at C-4 position as well as ring size of phosphorous containing heterocyclic ring substituted at N-3 position (Table 1). All these novel compounds have been well characterized using spectroscopic techniques and presence of phosphorous in these compounds has been ascertained by recording their ^{31}P NMR spectrum.

3. Result and discussion

3.1. Structural correlation with receptor bound conformation

In order to unambiguously establish the incorporation of the diaminophosphinyl group at N-3 positions of **6** and to establish correlation with proposed receptor bound confirmation with nifedipine as well as DHPM type calcium channel blockers, structure of **8b** was additionally confirmed by single crystal X-ray analysis (Fig. 2) [20].

The solid state structure of **8b** (Fig. 2) shows a half-boat type conformation of the dihydropyrimidinone ring. The C-4 aryl ring is perpendicular to the molecular plane and bisects the half-boat conformation of the DHPM ring. The C-5 ester is in *cis* orientation with respect to the C5, C6 double bond of the ring and coplanar with the DHPM ring, so is the N3–P=O link, the P=O oxygen pointing in the direction of C4-H of the DHPM unit. Such conformations drawn close analogy with the proposed receptor bound conformation of DHPMs resulting in the observed calcium channel blocking activity (calcium antagonists) [21].

3.2. Calcium channel binding studies

Representative compounds **8**–**10** were screened for calcium blocking activity based on their ability to relax a membrane

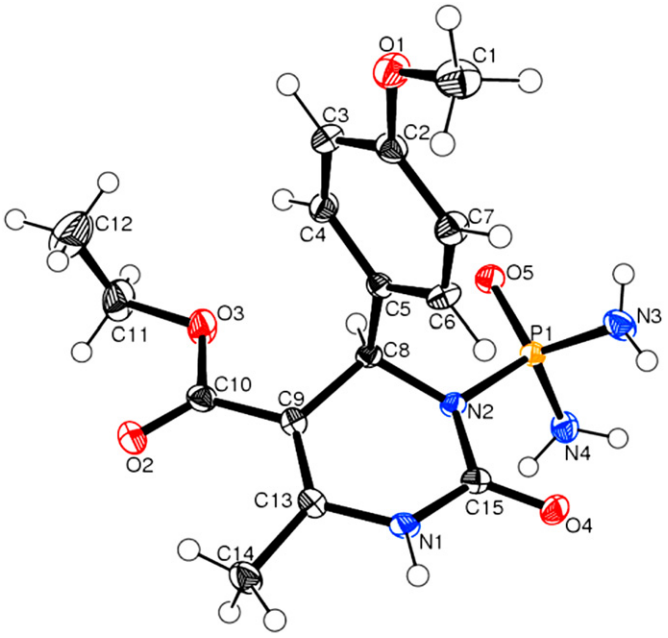


Fig. 2. X-ray crystal structure of **8b** showing the stereoview of the molecule and the numbering scheme used in the structure analysis. (N2, C8, C13 positions may be read as N3, C4 and C6, respectively, following IUPAC nomenclature scheme).

depolarization induced contraction of vascular smooth muscle. For this purpose, swine carotid arteries were obtained from a slaughterhouse and transported to the laboratory in an ice-cold physiological salt solution (PSS) buffered (pH 7.4) with 3-[N-morpholino] propane sulfonic acid (MOPS). The PSS contained, in mM: 140 NaCl, 4.7 KCl, 1.2 $MgSO_4$, 1.6 $CaCl_2$, 1.2 Na_2HPO_4, MOPS, 5 D-glucose, and 0.02 Na_2-ethylenediaminetetraacetic acid (EDTA). Arteries were cleaned of connective tissue, and then dissected free of both intima and adventitia leaving a thin medial layer for experimentation. Intact medial strips of swine carotid artery (7 × 0.7 mm) were suspended between a Grass FT.03 force transducer and a stationary clip in water jacketed organ baths. The strips were equilibrated in PSS at 37 °C, pH 7.4 and bubbled with 100% O_2 for 90–120 min. A passive force of ~2 g was applied to all tissues. The passive force sets the muscles at a length that approximates L_o, the length at which maximal active force is generated. During the equilibration period, tissues were maximally contracted with 110 mM KCl (equimolar substitution for NaCl) several times until similar levels of force were attained.

The equilibrated vascular strips were contracted with 110 mM KCl containing PSS, allowed to achieve a stable level of force and then subjected to the cumulative addition of calcium channel blockers (0.001 μM–10 μM), nifedipine (0.001 μM–10 μM) or DMSO as a vehicle control. Data are presented (Fig. 3) as a percent of the initial maximal response to 110 mM KCl at each dose of compound. The calcium channel blockers were compared against nifedipine **1** for their ability to relax a membrane depolarization induced contraction which is almost exclusively dependent on the influx of extra-cellular calcium [22]. The novel compounds and nifedipine were tested across a concentration range of 0.001 μM–10 μM. Nifedipine completely relaxed the KCl-induced contractions with an IC_{50} of approximately 0.01 μM against 110 mM KCl. In contrast the compounds **8a**–**g**, **9a**–**c** and **10a**–**c** maximally relaxed the KCl-induced contractions by only 0–25% with the relaxation only significant at 10 μM.

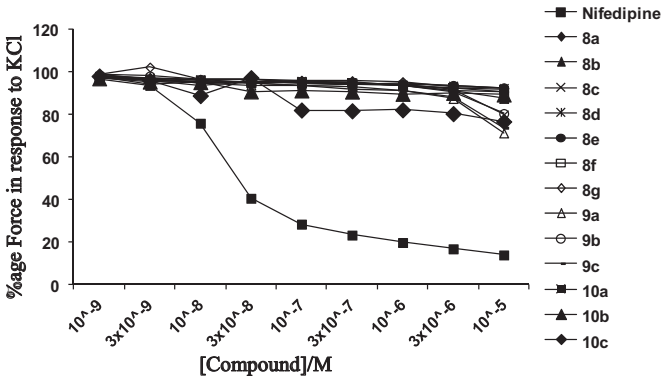


Fig. 3. Medial strips from the swine carotid artery were contracted with 110 mM KCl–PSS and then subjected to the cumulative addition of calcium channel blocker to determine their potential for relaxation. Nifedipine and 13 novel calcium channel blockers (**8a**–**g**, **9a**–**c** and **10a**–**c**) were tested.

4. Conclusion

Thus an efficient protocol using neutral reaction conditions for the regioselective synthesis of N3-substituted DHPM derivatives has been developed. DHPM derivatives bearing a diaminophosphinyl, phosphonate as well as phosphorous containing heterocycles at N-3 position has been synthesized. Among the novel compounds tested the calcium channel blocking activity was less compared to nifedipine in a screen using relaxation of a membrane depolarized vascular tissue as the end-point.

5. Experimental section

5.1. General

All liquid reagents were dried/purified following recommended drying agents and/or distilled over 4 Å molecular sieves. ^{1}H NMR (400 MHz and 300 MHz), ^{13}C NMR (100 MHz and 75 MHz) and ^{31}P (162 MHz and 121 MHz) spectra were recorded in DMSO-d_6 and $CDCl_3$ on a multinuclear Bruker-400 MHz and Jeol FT-AL-300 instruments with chemical shifts being reported in parts per million (δ) relative to internal tetramethylsilane (TMS, δ 0.0, ^{1}H NMR or $CDCl_3$, δ 77.0, ^{13}C NMR). Mass spectra were recorded from Indian Institute of Integrative Medicine (CSIR), Jammu, under electron impact at 70 eV on a Bruker Daltonics Esquire 3000 spectrometer. Elemental analysis was performed on FLASH EA 112 (Thermoelectron Corporation) analyzer at Department of Chemistry, Guru Nanak Dev University, Amritsar and the results are quoted in %. IR recorded on FTIR Shimadzu 8400 Fourier-transform spectrophotometer in the range 400–4000 cm^{-1} using KBr. Melting points were determined in open capillaries and are uncorrected. For monitoring the progress of a reaction and for comparison purpose, thin layer chromatography (TLC) was performed on pre-coated aluminium sheets Merck ($60F_{254}$, 0.2 mm) using an appropriate solvent system. The chromatograms were visualized under UV light. For column chromatography silica gel (60–120 mesh) was employed and eluents were ethyl acetate/hexane mixtures.

5.2. General procedure for the synthesis of N3-dichlorophosphinyl DHPM derivatives **7**

Appropriate DHPM 6 (0.01 mol) was suspended in phosphorous oxychloride (10 ml) and heated at 105 °C for 30 min. Excess phosphorous oxychloride ($POCl_3$) was removed under reduced pressure and last traces of $POCl_3$ were removed through azeotropic distillation with dry benzene to furnish the N3-dichlorophosphinyl DHPM derivatives 7 in 90–95% yield, and were used for subsequent reactions without further purification.

5.3. General procedure for the synthesis of N3-substituted DHPM derivatives **8**

N3-dichlorophosphinyl DHPM derivative 7 (0.5 g) was treated with ammonia gas (evolved by warming 30% aqueous ammonia solution using standard assembly and dried through KOH trap) dissolved in THF at room temperature for 45 min. White precipitates formed in the reaction mixture were filtered and recrystallized from methanol to obtain pure compound. The characteristic data of the compounds is given below.

5.3.1. 5-Ethoxycarbonyl-6-methyl-3-(diaminophosphinyl)-4-phenyl-3,4-dihydropyrimidin-2(1H)-one (**8a**)

White crystalline solid. Rf: 0.4 (methanol:ethyl acetate/10:90). Yield: 95%. M.p. 258–260 °C (methanol). IR (KBr): ν_{max} 1090, 1220, 1615, 1680, 3320 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6, 25 °C): δ 1.17 (t, J = 7.2 Hz, 3H, ester-CH_3), 2.23 (s, 3H, C6-CH_3), 4.02 (br, 2H, D_2O exchangeable, NH_2), 4.04–4.15 (m, 2H, ester-CH_2), 4.33 (br, 2H, D_2O exchangeable, NH_2), 6.09 (d, J = 7.2 Hz, 1H, C4-H), 7.17–7.32 (m, 5H, ArH), 9.62 (br, 1H, D_2O exchangeable, N1-H). ^{13}C NMR (75 MHz, DMSO-d_6, 25 °C) δ 14.2, 17.5, 54.5, 59.6, 102.6, 126.1, 127.0, 128.2, 142.7, 148.1, 153.3 and 165.4. ^{31}P NMR (121 MHz, DMSO-d_6, 25 °C): δ 11.95. Anal. Calcd. for $C_{14}H_{19}N_4O_4P$: C, 49.70; H, 5.62; N, 16.57; Found: C, 49.77; H, 5.93; N, 16.20. MS: m/z 339 (M^++1).

5.3.2. 5-Ethoxycarbonyl-6-methyl-3-(diaminophosphinyl)-4-(4-methoxyphenyl)-3,4-dihydropyrimidin-2(1H)-one (**8b**)

Wite crystalline solid. Rf: 0.2 (methanol:ethyl acetate/10:90). Yield: 95%. M.p. 260–262 °C (methanol). IR (KBr): ν_{max} 1091, 1235, 1631, 1690, 3399 cm^{-1} ^{1}H NMR (300 MHz, DMSO-d_6, 25 °C): δ 1.16 (t, J = 7.2 Hz, 3H, ester-CH_3), 2.23 (s, 3H, C6-CH_3), 3.36 (s, 3H, OCH_3), 3.95 (br, 2H, D_2O exchangeable, NH_2), 4.02–4.10 (m, 2H, ester-CH_2), 4.30 (br, 2H, D_2O exchangeable, NH_2), 6.00 (d, J = 6.9 Hz, 1H, C4-H), 6.80–6.83 (m, 2H, ArH), 7.22 (d, J = 8.7 Hz, 2H, ArH), 9.59 (br, 1H, D_2O exchangeable, N1-H). ^{13}C NMR (75 MHz, DMSO-d_6, 25 °C): δ 14.3, 17.4, 54.2, 55.0, 59.5, 102.7, 113.5, 127.5, 134.7, 147.8, 153.2, 158.3 and 165.3. ^{31}P NMR (121 MHz, DMSO-d_6, 25 °C): δ 12.04. Anal. Calcd. for $C_{15}H_{21}N_4O_5P$: C, 48.91; H, 5.71; N, 15.22; Found: C, 49.19; H, 6.01; N, 15.25. MS: m/z 369 (M^++1).

5.3.3. 5-Isopropoxycarbonyl-6-methyl-3-(diaminophosphinyl)-4-(3-nitrophenyl)-3,4-dihydropyrimidin-2(1H)-one (**8c**)

White crystalline solid. Rf: 0.5 (methanol:ethyl acetate/10:90). Yield: 95%. M.p. 268–270 °C (methanol). IR (KBr): ν_{max} 1090, 1225, 1525, 1635, 1680, 3320 cm^{-1} ^{1}H NMR (300 MHz, DMSO-d_6, 25 °C): δ 1.12 (d, J = 6.3 Hz, 3H, ester-CH_3), 1.21 (d, J = 6.3 Hz, 3H, ester-CH_3), 2.24 (s, 3H, C6-CH_3), 4.08 (br, 2H, D_2O exchangeable, NH_2), 4.41 (br, 2H, D_2O exchangeable, NH_2), 4.87–4.95 (m, 1H, ester-CH), 6.12 (d, J = 6.6 Hz, 1H, C4-H), 7.59 (t, J = 7.8 Hz, 1H, ArH), 7.74 (d, J = 7.8 Hz, 1H, ArH), 8.08–8.14 (m, 2H, ArH), 9.86 (br, 1H, D_2O exchangeable, N1-H). ^{13}C NMR (75 MHz, DMSO-d_6, 25 °C): δ 17.5, 21.6, 54.8, 67.1, 101.8, 121.1, 122.2, 130.0, 132.9, 145.2, 147.7, 148.6, 152.7 and 164.5. ^{31}P NMR (121 MHz, DMSO-d_6, 25 °C): δ 11.90. Anal. Calcd. for $C_{15}H_{20}N_5O_6P$: C, 45.34; H, 5.04; N, 17.63; Found: C, 45.19; H, 5.22; N, 17.43. MS: m/z 398 (M^++1).

5.3.4. 5-Methoxycarbonyl-6-methyl-3-(diaminophosphinyl)-4-phenyl-3,4-dihydropyrimidin-2(1H)-one (**8d**)

White crystalline solid. Rf: 0.7 (methanol:ethyl acetate/20:80). Yield: 93%. M.p. 253–255 °C (methanol). IR (KBr): ν_{max} 1024, 1237,

1551, 1631, 1698, 3317 cm^{-1} ^1H NMR (300 MHz, DMSO-d_6, 25 °C): δ 2.23 (s, 3H, C6-CH_3), 3.62 (s, 3H, OCH_3), 4.06 (br, 2H, D_2O exchangeable, NH_2), 4.36 (br, 2H, D_2O exchangeable, NH_2), 6.09 (d, J = 7.2 Hz, 1H, C4-H), 7.20–7.29 (m, 5H, ArH), 9.67 (br, 1H, D_2O exchangeable, NH). ^{13}C NMR (75 MHz, DMSO-d_6, 25 °C): δ 17.6, 51.3, 54.7, 102.5, 126.3, 127.3, 128.2, 142.5, 148.7, 152.4 and 166.0. ^{31}P NMR (121 MHz, DMSO-d_6, 25 °C): δ 11.93. Anal. Calcd. for $C_{13}H_{17}N_4O_4P$: C, 48.15; H, 5.25; N, 17.28; Found: C, 48.42; H, 5.46; N, 16.96. MS: *m/z* 325 (M^++1).

5.3.5. 5-Ethoxycarbonyl-6-methyl-3-(diaminophosphinyl)-3,4-dihydropyrimidin-2(1H)-one (**8e**)

White crystalline solid. Rf: 0.3 (methanol:ethyl acetate/15:85). Yield: 85%. M.p. 260–262 °C (methanol). IR (KBr): ν_{max} 1096, 1291, 1658, 1710, 1741, 3398 cm^{-1} ^1H NMR (300 MHz, DMSO-d_6, 25 °C): δ 1.19 (t, J = 7.2 Hz, 3H, ester-CH_3), 2.16 (s, 3H, C6-CH_3), 4.07 (q, J = 6.9 Hz, 2H, ester-CH_2), 4.19 (br, 4H, D_2O exchangeable, 2 × NH_2), 5.73 (s, 2H, C4-CH_2), 9.89 (br, 1H, D_2O exchangeable, N1-H). ^{13}C NMR (75 MHz, DMSO-d_6, 25 °C): δ 14.2, 16.9, 42.1, 59.3, 97.0, 148.1, 153.4 and 165.0. ^{31}P NMR (121 MHz, DMSO-d_6, 25 °C): δ 12.87. Anal. Calcd. for $C_8H_{15}N_4O_4P$: C, 36.64; H, 5.72; N, 21.37; Found: C, 36.68; H, 6.00; N, 20.89. MS: *m/z* 263 (M^++1).

5.3.6. 5-Ethoxycarbonyl-4,6-dimethyl-3-(diaminophosphinyl)-3,4-dihydropyrimidin-2(1H)-one (**8f**)

White crystalline solid. Rf: 0.3 (methanol:ethyl acetate/20:80). Yield: 92%. M.p. 265–267 °C (methanol). IR (KBr): ν_{max} 1029, 1245, 1411, 1635, 1696, 3401 cm^{-1} ^1H NMR (400 MHz, DMSO-d_6, 25 °C): δ 0.91 (d, J = 6.4 Hz, 3H, C4-CH_3), 0.98 (t, J = 7.0 Hz, 3H, ester-CH_3), 1.96 (s, 3H, C6-CH_3), 3.82–3.92 (m, 2H, ester-CH_2), 3.95 (br, 2H, exchanged with D_2O, NH_2), 3.99 (br, 2H, exchanged with D_2O, NH_2), 4.71 (t, J = 6.2 Hz, 1H, C4-H), 9.28 (br, 1H, exchanged with D_2O, NH). ^{13}C NMR (100 MHz, DMSO-d_6, 25 °C): δ 14.3, 17.3, 21.1, 48.6, 59.3, 103.2, 147.2, 153.0 and 165.0. ^{31}P NMR (162 MHz, DMSO-d_6, 25 °C): δ 12.02. Anal. Calcd. for $C_9H_{17}N_4O_4P$: C, 39.13; H, 6.16; N, 20.29; Found: C, 38.92; H, 6.44; N, 19.94. MS: *m/z* 277 (M^++1).

5.3.7. 5-Methoxycarbonyl-4,6-dimethyl-3-(diaminophosphinyl)-3,4-dihydropyrimidin-2(1H)-one (**8g**)

White crystalline solid. Rf: 0.2 (methanol:ethyl acetate/20:80). Yield: 83%. M.p. 272–274 °C (methanol). IR (KBr): ν_{max} 1080, 1212, 1487, 1642, 1703, 3431 cm^{-1} ^1H NMR (300 MHz, DMSO-d_6, 25 °C): δ 1.11 (d, J = 6.6 Hz, 3H, C4-CH_3), 2.16 (s, 3H, C6-CH_3), 3.61 (s, 3H, OCH_3), 4.16 (br, 4H, D_2O exchangeable, 2 × NH_2), 4.93 (t, J = 6.3 Hz, 1H, C4-H), 9.48 (br, 1H, D_2O exchangeable, NH). ^{13}C NMR (75 MHz, DMSO-d_6, 25 °C): δ 17.3, 21.1, 48.6, 51.0, 103.1, 147.5, 153.1 and 165.5. ^{31}P NMR (121 MHz, DMSO-d_6, 25 °C): δ 11.93. Anal. Calcd. for $C_8H_{15}N_4O_4P$: C, 36.64; H, 5.72; N, 21.37; Found: C, 36.52; H, 5.92; N, 21.13. MS: *m/z* 263 (M^++1).

5.4. General procedure for the synthesis of N3-substituted DHPM derivatives **9**

To a stirred solution of appropriate 7 (1 equiv.) in dry dioxane (10 ml) appropriate amine (1.5 equiv.) was added and resulting solution was stirred at room temperature for 2–4 h. After completion of reaction (TLC), dioxane was removed under reduced pressure and residue was dissolved in ethylacetate (20–30 ml) and washed with water (2 × 30 ml). Organic phase was dried over anhydrous sodium sulphate and compounds 9a-b were obtained through column chromatography using hexane and ethylacetate as eluents. The characteristic data of the compounds is given below.

5.4.1. 5-Ethoxycarbonyl-6-methyl-3-[bis(butylamino)phosphinyl]-3,4-dihydropyrimidin-2(1H)-one (**9a**)

White crystalline solid. Rf: 0.7 (methanol:ethyl acetate/10:90). Yield: 66%. M.p. 115–117 °C (dichloromethane/hexane). IR (KBr): ν_{max} 1023, 1235, 1407, 1634, 1697, 3356 cm^{-1} ^1H NMR (400 MHz, $CDCl_3$, 25 °C): δ 0.76 (t, J = 7.2 Hz, 3H, CH_3), 0.88 (t, J = 7.2 Hz, 3H, CH_3), 1.03–1.16 (m, 4H, 2 × CH_2), 1.19 (t, J = 7.0 Hz, 3H, ester-CH_3), 1.29–1.36 (m, 2H, CH_2), 1.41–1.46 (m, 2H, CH_2), 2.38 (s, 3H, C6-CH_3), 2.48–2.58 (m, 2H, CH_2), 2.75 (br, 1H, D_2O exchangeable, NH), 2.90–2.98 (m, 2H, CH_2), 3.06 (br, 1H, D_2O exchangeable, NH), 4.05–4.14 (m, 2H, ester-CH_2), 6.12 (d, J = 6.0 Hz, 1H, C4-H), 7.23–7.32 (m, 3H, ArH), 7.46–7.48 (m, 2H, ArH), 8.44 (br, 1H, D_2O exchangeable, NH). ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ 13.6, 13.7, 14.1, 17.9, 19.6, 19.8, 33.7, 33.8, 40.0, 40.6, 56.9, 60.2, 104.2, 127.5, 127.9, 128.4, 142.5, 145.6, 154.2 and 165.2. ^{31}P NMR (162 MHz, $CDCl_3$, 25 °C): δ 12.21; Anal. Calcd. for $C_{22}H_{35}N_4O_4P$: C, 58.67; H, 7.78; N, 12.44; Found: C, 58.30; H, 7.50; N, 12.22. MS: *m/z* 473 (M^++23).

5.4.2. 5-Ethoxycarbonyl-6-methyl-3-[bis(benzylamino) phosphinyl]-3,4-dihydropyrimidin-2(1H)-one (**9b**)

White crystalline solid. Rf: 0.7 (methanol:ethyl acetate/10:90). Yield: 60%. M.p. 183–185 °C (dichloromethane/hexane). IR (KBr): ν_{max} 1086, 1233, 1634, 1700, 3374 cm^{-1} ^1H NMR (300 MHz, $CDCl_3$, 25 °C): δ 1.22 (t, J = 6.9 Hz, 3H, ester-CH_3), 2.29 (s, 3H, C6-CH_3), 3.11 (br, 1H, D_2O exchangeable, NH), 3.56 (t, J = 7.2 Hz, 1H, D_2O exchangeable, NH), 3.68–3.85 (m, 2H, CH_2), 4.08–4.19 (m, 4H, ester-CH_2 and CH_2), 6.13 (d, J = 6.0 Hz, 1H, C4-H), 6.71 (br, 1H, D_2O exchangeable, NH), 7.01–7.04 (m, 2H, ArH), 7.16–7.31 (m, 11H, ArH), 7.43–7.46 (m, 2H, ArH). ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C) δ 14.2, 18.8, 29.7, 55.8, 60.1, 101.5, 126.7, 128.0, 128.8, 143.7, 146.2, 153.3 and 165.7. ^{31}P NMR (162 MHz, $CDCl_3$, 25 °C): δ 11.85. Anal. Calcd. for $C_{28}H_{31}N_4O_4P$: C, 64.86; H, 5.98; N, 10.81; Found: C, 64.66; H, 6.32; N, 10.52. MS: m/z 517 (M^--1).

5.4.3. 5-Ethoxycarbonyl-6-methyl-3-(diethylphosphonate)-4-phenyl-3,4-dihydropyrimidin-2(1H)-one (**9c**)

Compound 7 (R^1 = Ph, R^2 = Et, R^3 = H)was treated with ethanol at 80 °C for 3 h and after completion of reaction (TLC) solvent was removed under reduced pressure. Purification of the residue by column chromatography using hexane and ethyl-acetate as eluents resulted in the isolation of compound 9c as a white crystalline solid. Rf: 0.8 (methanol:ethyl acetate/20:80). Yield: 70%. M.p. 112–114 °C (dichloromethane/hexane). IR (KBr): ν_{max} 1024, 1243, 1390, 1636, 1704, 3236 cm^{-1} ^1H NMR (300 MHz, $CDCl_3$, 25 °C): δ 1.07 (t, J = 6.9 Hz, 3H, ester-CH_3), 1.22 (t, J = 7.2 Hz, 3H, CH_3), 1.32 (t, J = 7.2 Hz, 3H, CH_3), 2.38 (s, 3H, C6-CH_3), 3.52–3.67 (m, 1H, CHH), 3.72–3.82 (m, 1H, CHH), 4.07–4.26 (m, 4H, 2 × CH_2), 6.08 (d, J = 6.9 Hz, 1H, C4-H), 7.26–7.31 (m, 3H, ArH), 7.40–7.46 (m, 2H, ArH), 7.69 (br, 1H, D_2O exchangeable, NH). ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ 14.1, 15.9, 17.9, 58.2, 60.3, 63.7, 64.4, 104.1, 127.2, 128.1, 128.5, 141.9, 145.8, 153.0 and 165.0. ^{31}P NMR (162 MHz, $CDCl_3$, 25 °C): δ −0.53. Anal. Calcd. for $C_{18}H_{25}N_2O_6P$: C, 54.54; H, 6.31; N, 7.07.; Found: C, 54.20; H, 6.52; N, 6.80. MS: *m/z* 419 (M^++23).

5.5. General procedure for the synthesis of N3-substituted DHPM derivatives bearing phosphorous containing heterocycles **10**

To a stirred solution of appropriate 7 (1 equiv.) in dry dioxane (10 ml) an appropriate diamine or amino alcohols (1.5 equiv.) was added and the resulting solution was stirred at room temperature for 2–3 h. After completion of reaction (TLC) dioxane was removed under reduced pressure and residue was dissolved in ethylacetate (20–30 ml) and washed with water

(2 × 30 ml). Organic phase was dried over anhydrous sodium sulphate and compound 10 was obtained through crystallization with methanol. The characteristic data of the compounds is given below.

*5.5.1. 2-[5-ethoxycarbonyl-6-methyl-4-phenyl-3,4-dihydropyrimidin-2(1H)-one]-3-yl-1,3,4,5,6,7-hexahydro [1–3] diazaphosphepine-2-oxide (**10a**)*

White crystalline solid. Rf: 0.3 (methanol:ethyl acetate/10:90). Yield: 72%. M.p. 218–220 °C (methanol). IR (KBr): ν_{max} 1029, 1228, 1390, 1641, 1690, 3369 cm^{-1} ^{1}H NMR (300 MHz, $CDCl_3$, 25 °C): δ 1.23 (t, J = 7.2 Hz, 3H, ester-CH_3), 1.60–1.62 (m, 4H, 2 × CH_2), 2.39 (s, 3H, C6-CH_3), 3.03–3.12 (m, 3H, CH_2 and D_2O exchangeable, NH), 3.24–3.32 (m, 2H, CH_2), 3.71–3.73 (m, 1H, D_2O exchangeable, NH), 4.09–4.19 (m, 2H, ester-CH_2), 6.25 (d, J = 7.5 Hz, 1H, C4-H), 7.22–7.31 (m, 3H, ArH), 7.40–7.43 (m, 2H, ArH), 7.87 (br, 1H, D_2O exchangeable, NH). ^{13}C NMR (75 MHz, $CDCl_3$, 25 °C): δ 14.2, 16.0, 30.1, 34.6, 39.0, 111.0, 126.1, 126.9, 128.0, 144.0, 148.3, 158.0 and 166.9. ^{31}P NMR (121 MHz, DMSO-d_6, 25 °C): δ 16.83; Anal. Calcd. for $C_{18}H_{25}N_4O_4P$: C, 55.10; H, 6.38; N, 14.28. Found: C, 54.82; H, 6.20; N, 14.40. MS: m/z 393 (M^++1).

*5.5.2. 5-Ethoxycarbonyl-4,6-dimethyl-3-(1,3,2-diazaphosphorinane-2-oxide)-3,4-dihydropyrimidin-2(1H)-one (**10b**)*

White crystalline solid. Rf: 0.5 (methanol:ethyl acetate/20:80). Yield: 65%. M.p. 233–235 °C (methanol). IR (KBr): ν_{max} 1010, 1242, 1313, 1635, 1699, 3353 cm^{-1} ^{1}H NMR (400 MHz, DMSO-d_6, 25 °C): δ 0.87 (d, J = 6.5 Hz, 3H, C4-CH_3), 0.94 (t, J = 7.0 Hz, 3H, ester-CH_3), 1.65–1.72 (m, 2H, CH_2), 1.94 (s, 3H, C6-CH_3), 2.64 (t, J = 7.5 Hz, 4H, 2 × CH_2), 3.81–3.88 (m, 2H, ester-CH_2), 4.28 (br, 1H, D_2O exchangeable, NH), 4.56 (br, 1H, D_2O exchangeable, NH), 4.61 (q, J = 6.3 Hz, 1H, C4-H), 9.24 (br, 1H, D_2O exchangeable, N1-H). ^{13}C NMR (100 MHz, DMSO-d_6, 25 °C): δ 14.3, 17.2, 21.0, 25.0, 41.6, 49.0, 59.3, 103.2, 147.2, 153.5 and 165.0. ^{31}P NMR (162 MHz, DMSO-d_6, 25 °C): δ 4.81. Anal. Calcd. for $C_{12}H_{21}N_4O_4P$: C, 45.57; H, 6.64; N, 17.72; Found: C, 45.54; H, 6.51; N, 17.40. MS: m/z 317 (M^++1).

*5.5.3. 5-Ethoxycarbonyl-6-methyl-3-(1,3,2-oxazaphosphino-2-yl)-4-phenyl-3,4-dihydropyrimidin-2(1H)-one (**10c**)*

White crystalline solid. Rf: 0.2 (methanol:ethyl acetate/10:90). Yield: 65%. M.p. 208–210 °C (methanol). IR (KBr): ν_{max} 1019, 1247, 1365, 1635, 1705, 3333 cm^{-1} ^{1}H NMR (400 MHz, DMSO-d_6, 25 °C): δ 1.16 (t, J = 7.0 Hz, 3H, ester-CH_3), 1.72–1.84 (m, 2H, CH_2), 2.28 (s, 3H, C6-CH_3), 3.04–3.15 (m, 1H, CHH), 3.29–3.34 (m, 1H, CHH), 4.05–4.14 (m, 2H, ester-CH_2), 4.16–4.28 (m, 1H, CHH), 4.54–4.56 (m, 1H, CHH), 5.45–5.48 (m, 1H, D_2O exchangeable, NH), 6.07 (d, J = 7.5 Hz, 1H, C4-H), 7.20–7.32 (m, 5H, ArH). ^{13}C NMR (100 MHz, DMSO-d_6, 25 °C): δ 14.2, 17.4, 25.4, 41.2, 55.9, 59.8, 69.4, 102.7, 126.1, 127.3, 128.4, 142.1, 148.1, 153.3 and 165.2. ^{31}P NMR (162 MHz, DMSO-d_6, 25 °C): δ −0.94. Anal. Calcd. for $C_{17}H_{22}N_3O_5P$: C, 53.83; H, 5.80; N, 11.08. Found: C, 53.52; H, 5.63; N, 10.82. MS: m/z 402 (M^++23).

*5.5.4. 5-Ethoxycarbonyl-1,6-dimethyl-3-(1,3,2-oxazaphosphino-2-yl)-4-phenyl-3,4-dihydropyrimidin-2(1H)-one (**10d**)*

White crystalline solid. Rf: 0.4 (methanol:ethyl acetate/20:80). Yield: 65%. M.p. 213–215 °C (methanol). IR (KBr): ν_{max} 1015, 1225, 1345, 1665, 1710, 3343 cm^{-1} ^{1}H NMR (400 MHz, DMSO-d_6, 25 °C): δ 1.18 (t, J = 7.0 Hz, 3H, ester-CH_3), 1.74–1.87 (m, 2H, CH_2), 2.50 (s, 3H, C6-CH_3), 3.07–3.13 (m, 4H, N1-CH_3 and CHH), 3.28–3.34 (m, 1H, CHH), 4.13–4.18 (m, 2H, ester-CH_2), 4.21–4.29 (m, 1H, CHH), 4.58–4.64 (m, 1H, CHH), 5.45–5.48 (m, 1H, D_2O exchangeable, NH), 6.13 (d, J = 8.0 Hz, 1H, C4-H), 7.22–7.33 (m, 5H, ArH). ^{13}C NMR (100 MHz, DMSO-d_6, 25 °C): δ 14.1, 15.7, 25.3, 30.5, 41.1, 54.4, 59.9, 69.4, 106.2, 125.8, 127.3, 128.3, 141.2, 150.2, 153.8 and 165.2. Anal. Calcd. for $C_{18}H_{24}N_3O_5P$: C, 54.96; H, 6.10; N, 10.68; Found: C, 54.66; H, 6.21; N, 10.40. MS: m/z 416 (M^++23).

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