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ORIGINAL PAPER

Synthesis and in vitro and in vivo antitumor/anticancer activity of novel O-Mannich bases of 4,6-diaryl-3,4-dihydropyrimidine-2(1*H*)-ones

K. Venkateshwarlu · G. Chakradar Rao · V. M. Reddy · Y. Narasimha Reddy

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Abstract A new series of [4,6 substituted diaryl-1,6-dihydropyrimidin-2-yl-oxymethyl]-amines **5a–o** have been synthesized by the Mannich condensation on the respective 4,6-diaryl-3,4-dihydropyrimidine-2(1*H*)-ones **4**, in basic medium using formaldehyde along with three secondary amines, viz., dimethylamine, piperidine and morpholine. The dihydropyrimidinones **4** in turn were synthesized by the cyclocondensation of **chalcones 3** with urea. Alternatively, compounds **4** were also prepared directly by one-pot 3-component cyclocondensation reaction starting from acetophenone, benzaldehyde and urea. The structures of all the newly synthesized compounds have been confirmed by their spectral and analytical data. All the O-Mannich bases **5** have been evaluated for their in vitro cytotoxic and antitumor activities, and based on the results, the potent compounds were selected for in vivo activity, as well. Only one compound **5m** of the series has been found to be relatively more effective.

K. Venkateshwarlu · Y. Narasimha Reddy (✉)
Department of Pharmacology and Toxicology, Kakatiya University, Warangal 506 009, A.P., India
e-mail: ynrku@yahoo.co.in

K. Venkateshwarlu
e-mail: venkataindica@yahoo.com

G. Chakradar Rao · V. M. Reddy
Divis of Research Centre, Divis Laboratories Ltd, B-34, Industrial Estate, Hyderabad 500 018, A.P., India

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Introduction

In recent years, pyrimidine derivatives are reported to exhibit a wide range of biological and pharmacological properties, viz., antitubercular [1], calcium channel blockers [2], antibacterial [3], antiviral [4], antifungal [5, 6], antimalarial [7], antihypertensive [8], analgesic and anti-inflammatory [9–11]. Interestingly, pyrimidines are also known to possess antitumor, anticancer and antineoplastic potencies [12, 13]. Similarly, many Mannich bases are also reported to be associated with potent antineoplastic, analgesic, anti-inflammatory and antibacterial properties [14–17]. In fact, the very precursors of dihydropyrimidine derivatives chalcones themselves are also known to possess cytotoxic potency, tumor reducing and anticancer activities [18–21] besides several other useful biological and pharmacological properties.

It is interesting to note from the literature that all the three molecular moieties, viz., chalcone, pyrimidine and the Mannich base (aminomethylamine) are known to be associated with a most useful, cytotoxic, tumor reducing and anticancer potency. Therefore, in continuation of our studies on diaryl-dihydropyrimidinones/thiones [22], it is considered worthwhile to incorporate all these three pharmacophoric moieties into a single molecular framework by synthesizing chalcones, from them dihydropyrimidinones and pyrimidine Mannich bases from them. The dihydropyrimidinones are synthesized by one-pot synthesis directly from the starting materials, viz., ketones, aldehydes and urea. The title compounds diaryl pyrimidinone-

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O-Mannich bases for their possible antitumor/anticancer activity.

Experimental

Chemistry

All the chemicals were of synthetic grade and commercially procured from Sigma Aldrich, Mumbai, India. Melting points were determined by open capillary method, and uncorrected IR spectra were recorded on FTIR (Bruker Alpha-E) by KBr disc method. ^{1}H NMR spectra were recorded at 400 MHz in DMSO-d_6 as solvent and TMS as an internal standard using BRUKER ADVANCE 400 instrument. Mass spectra were recorded on PEP-SCIUX-APIQ pulsar mass spectrophotometer. Elemental analyses were performed on Perkin-Elmer EAL240 elemental analyser.

All the required (*E*)-1,3-diaryl propen-2-ones (chalcones) were prepared in improved yields by both conventional and microwave-assisted method and characterized with the help of their literature data [23, 24] and novel chalcones indicated by * mark.

(*E*)-1,3-diphenylprop-2-en-1-one (3a)

Yield: 84 %; m.p.: 50–52 °C; IR (KBr) cm^{-1}: 3,068, 2,941, 1,658, 1,465; **^{1}H NMR** (δ ppm): 7.35 (*d*, $J = 3.1$ Hz, 1H, H-α), 7.49 (*d*, $J = 3.8$ Hz, 1H, H-β), 7.50–7.90 (m, 10H, Ar–H).

(*E*)-1,3-bis(4-chlorophenyl)prop-2-en-1-one (3b)

Yield: 90 %; m.p.: 94–96 °C; IR (KBr) cm^{-1}: 3,058, 2,945, 1,660, 1,460; **^{1}H NMR** (δppm): 7.42 (*d*, $J = 3.5$ Hz, 1H, H- α), 7.50 (*d*, $J = 4.1$ Hz, 1H, H-β), 7.55–7.69 (m, 8H, Ar–H).

(*E*)-1-(4-chlorophenyl)-3-(4-methoxyphenyl)prop-2-en-1-one (3c)

Yield: 98 %; m.p.: 120–122 °C; IR (KBr) cm^{-1}: 3,062, 2,935, 1,670, 1,440, 790; **^{1}H NMR** (δppm): 3.85 (s, 3H, –OCH_3), 7.45 (*d*, $J = 3.8$ Hz, 1H, H-α), 7.55 (*d*, $J = 3.7$ Hz, 1H, H-β), 7.64–7.99 (m, 8H, Ar–H).

(*E*)-3-(4-methoxyphenyl)-1-(3-nitrophenyl)prop-2-en-1-one*(3d)

Yield: 80 %; m.p.: 145–147 °C; IR (KBr) cm^{-1}: 3,055, 2,920, 1,660, 1,460, 1,545, 1,360; **^{1}H NMR** (δppm): 3.81 (s, 3H, –OCH_3), 7.42 (*d*, $J = 3.7$ Hz, 1H, H-α), 7.51 (*d*, $J = 3.9$ Hz, 1H, H-β), 7.60–7.80 (m, 8H, Ar–H).

(*E*)-3-(4-methoxyphenyl)-1-phenylprop-2-en-1-one (3e)

Yield: 82 %; m.p.: 60–62 °C; IR (KBr) cm^{-1}: 3,050, 2,935, 1,650, 1,441; **^{1}H NMR** (δppm): 3.87 (s, 3H, –OCH_3), 7.43(*d*, $J = 3.5$ Hz, 1H, H-α), 7.50 (*d*, $J = 3.4$ Hz, 1H, H-β), 7.69–8.04 (m, 8H, Ar–H).

General procedures for synthesis of 4,6-diaryl-3,4-dihydropyrimidin-2(1*H*)-ones (4)

Conventional method

A mixture of appropriate chalcone (0.01 mol), urea (0.012 mol) and potassium hydroxide (1.0 g) was taken in a RB flask and added ethanol (20 ml). The reaction mixture was stirred for 15–20 min followed by heating under reflux for about 6 h (TLC). On completion of the reaction, the reaction mixture was poured onto crushed ice, while stirring well. The solid product obtained was filtered and washed with cold water. It was purified by recrystallization from ethanol.

Conventional, alternative method

A mixture of appropriate chalcone (0.01 mol) and urea (0.01 mol) was taken in a RB flask along with ethanol (20 ml) and stirred with a mechanical stirrer. Then, sodium ethoxide solution (20 % in ethanol) was added and the solution was heated under stirring for 1 h (TLC). Once the reaction was over, the reaction mixture was cooled to room temperature and poured onto crushed ice with stirring. The solid so-obtained was filtered, washed with portions of cold water and dried. The crude solid was purified by recrystallization from ethanol.

One-pot catalyst-mediated method

A mixture of appropriate acetophenone (5.0 mmol), the catalyst aluminum chloride with potassium iodide (0.5 mmol, 10 mol %) and acetonitrile (25 ml) was taken into a dry RB flask. Appropriate benzaldehyde (5.0 mmol) and urea (750 mg; 7.5 mmol) were added to the reaction flask. The reaction mixture was heated under reflux to complete the reaction (TLC). It was cooled to room temperature and poured into ice-cold water with stirring. The precipitated crude product was filtered, washed with portions of cold water and dried. The product was purified by recrystallization from ethanol, yield range: 83–90 %.

Microwave-assisted solvent-free one-pot method

An intimate mixture of appropriate benzaldehyde (5.0 mmol), appropriate acetophenone (5.0 mmol) and urea (0.36 g; 6.0 mmol) were taken into a flame dried RB flask then added the catalyst zinc iodide (0.319 g; 1.0 mmol). The reaction mixture was placed in a microwave oven at 750 Watts for 6–10 min. Once the reaction was over (TLC), cold water was added to the flask and stirred effectively for 45 min. The precipitated product was filtered, washed with cold water and dried. It was purified by recrystallization from ethanol, yield range: 85–96 %.

Adopting the general procedures, the following 4,6-diaryl-3,4-dihydropyrimidin-2(1*H*)-ones were synthesized and reported to be identified based on the literature data [25] and the novel pyrimidinones* characterized with the help of analytical and spectral data:

4,6-diphenyl-3,4-dihydropyrimidin-2(1*H*)-one (4a)

Yield: 85 %; m.p.: 234–236 °C; **IR** (KBr) cm^{-1}: 3,235, 2,940, 1,680, 1,602, 1,410; **^{1}HNMR** (δppm): 3.48 (br, s, 1H, –NH), 5.13 (*d*, $J = 3.6$ Hz, 1H, =CH), 5.18 (*d*, $J = 3.1$ Hz, 1H, –CH), 7.20–7.68 (m, 10H, Ar–H), 8.21 (s, 1H, –NH).

4,6-bis(4-chlorophenyl)-3,4-dihydropyrimidin-2(1*H*)-one* (4b)

Yield: 88 %; m.p.: 251–253 °C; **IR** (KBr) cm^{-1}: 3,228, 2,926, 1,685, 1,605, 1,452, 752; **^{1}HNMR** (δppm): 3.39 (br, s, 1H, –NH), 5.15 (*d*, $J = 3.4$ Hz, 1H, =CH), 5.19 (*d*, $J = 3.4$ Hz, 1H, –CH), 7.25–7.70 (m, 8H, Ar–H), 8.23 (s, 1H, –NH).

6-(4-chlorophenyl)-4-(4-methoxyphenyl)-3,4-dihydropyrimidin-2(1*H*)-one (4c)

Yield: 89 %; m.p.: 262–264 °C; **IR** (KBr) cm^{-1}: 3,245, 2,945, 1,681, 1,603, 1,461, 761; **^{1}HNMR** (δppm): 3.50 (br, s, 1H, –NH), 3.76 (s, 3H, $–OCH_3$), 5.11 (*d*, $J = 3.1$ Hz, 1H, =CH), 5.17 (*d*, $J = 2.9$ Hz, 1H, –CH), 7.29–7.71 (m, 8H, Ar–H), 8.19 (s, 1H, –NH).

4-(4-methoxyphenyl)-6-(3-nitrophenyl)-3,4-dihydropyrimidin-2(1*H*)-one* (4d)

Yield: 91 %; m.p.: 271–273 °C; **IR** (KBr) cm^{-1}: 3,251, 2,930, 1,674, 1,595, 1,450, 1,380; **^{1}HNMR** (δppm): 3.41 (br, s, 1H, –NH), 3.71 (s, 3H, $–OCH_3$), 5.17 (*d*, $J = 2.8$ Hz, 1H, =CH), 5.20 (*d*, $J = 3.3$ Hz, 1H, –CH), 7.26–7.69 (m, 8H, Ar–H), 8.19 (s, 1H, –NH).

4-(4-methoxyphenyl)-6-phenyl-3,4-dihydropyrimidin-2(1*H*)-one (4e) [28]

Yield: 86 %; m.p.: 249–251 °C; **IR** (KBr) cm^{-1}: 3,378, 2,935, 1,689, 1,608, 1,405; **^{1}HNMR** (δppm): 3.46 (br, s, 1H, –NH), 3.74 (s, 3H, $–OCH_3$), 5.16 (*d*, $J = 3.0$ Hz, 1H, =CH), 5.22 (*d*, $J = 3.2$ Hz, 1H, –CH), 7.24–7.71 (m, 8H, Ar–H), 8.20 (s, 1H, –NH).

General procedure for synthesis of N-[(4,6-diaryl pyrimidin-2-yloxy)methyl]amines (5)

Dihydropyrimidinone **4** (0.005 mol) was dissolved in dimethyl sulfoxide (25 mL), the contents were stirred with 37 % formaldehyde (0.01 mol), and then anhydrous potassium carbonate (1 g) and appropriate secondary amine (0.005 mol) were added and the solution was stirred continuouslyfor 2 h. The reaction mixture was further refluxed for about 5 h. Completion of reaction was confirmed by TLC and then kept in refrigerator for 48 h, filtered the product, washed with small portions of cold water and dried. The crude product was purified by recrystallization from petroleum ether–chloroform (1:1) mixture.

Making use of the general procedure, the following novel Mannich bases were synthesized and characterized:

4-(((4,6-diphenyl-1,6-dihydropyrimidin-2-yl)oxy)methyl)morpholine (5a)

Yield 69 %, mp 170–172 °C; **IR** (KBr) cm^{-1}: 3,278, 3,050, 2,900, 1,658, 1,596, 1,475, 1,245, 1,090; **^{1}H NMR** (δppm): 2.97 (t, 4H, $2CH_2$), 3.39 (br, s, 1H, –NH), 3.69 (t, 4H, $2CH_2$), 5.01(s, 2H, CH_2), 5.16 (*d*, $J = 3.4$ Hz, 1H, =CH), 5.20 (s, 1H, CH), 7.35–7.86 (m, 10H, Ar–H); **EI-MS**: 349(M^+); Anal. Calcd. For $C_{21}H_{23}N_3O_2$: C, 72.60; H, 6.09; N, 12.10. Found: C,72.59; H,6.07; N,12.08 %.

4,6-diphenyl-2-(piperidin-1-ylmethoxy)-1,6-dihydropyrimidine (5b)

Yield 72 %, mp 180–182 °C; **IR** (KBr) cm^{-1}: 3,227, 3,055, 2,890, 1,660, 1,596, 1,475, 1,248, 1,092; **^{1}H NMR** (δppm): 2.19–2.49 (m, 10H, $5CH_2$), 3.39 (br, s, 1H, –NH), 5.13 (s, 2H, CH_2), 5.16 (*d*, $J = 3.5$ Hz, 1H, =CH), 5.21 (s, 1H, CH), 7.26–7.90 (m, 10H, Ar–H); **EI-MS**: 347(M^+); Anal. Calcd. For $C_{22}H_{25}N_3O$: C, 76.49; H, 6.71; N, 12.16. Found: C,76.45; H,6.66; N,12.14 %.

1-((4,6-diphenyl-1,6-dihydropyrimidin-2-yl)oxy)-*N*,*N*-dimethylmethanamine (5c)

Yield 75 %, mp 162–164 °C; **IR** (KBr) cm^{-1}: 3,235, 3,060, 2,855, 1,665, 1,601, 1,475, 1,246, 1,095; **^{1}H NMR**

(δppm): 2.90 (s, 6H, $2CH_3$), 3.40 (br, s, 1H, –NH), 5.16 (*d*, $J = 3.5$ Hz, 1H, =CH), 5.21 (s, 2H, CH_2), 5.23 (s, 1H, CH), 7.36–7.90 (m, 10H, Ar–H); **EI-MS**: 307(M^+); Anal. Calcd. For $C_{19}H_{21}N_3O$: C, 74.73; H, 6.27; N, 13.76. Found: C,74.71; H,6.24; N,13.70 %.

4-(((4,6-bis(4-chlorophenyl)-1,6-dihydropyrimidin-2-yl)oxy)methyl)morpholine (5d)

Yield 65 %, mp 146–149 °C; **IR** (KBr) cm^{-1}: 3,254, 3,062, 2,860, 1,660, 1,596, 1,475, 1,250, 1,090, 750; **^{1}H NMR** (δppm): 2.87 (t, 4H, $2CH_2$), 3.41 (br, s, 1H, –NH), 3.54 (t, 4H, $2CH_2$), 4.70 (s, 2H, CH_2), 5.18 (*d*, $J = 4.2$ Hz, 1H, =CH), 5.22 (s, 1H, CH), 7.31–8.10 (m, 8H, Ar–H); **EI-MS**: 417(M^+); Anal. Calcd. For $C_{21}H_{21}Cl_2N_3O_2$: C, 60.59; H, 4.60; N, 10.09. Found: C, 60.57; H, 4.58; N, 10.07 %.

4,6-bis(4-chlorophenyl)-2-(piperidin-1-ylmethoxy)-1,6-dihydropyrimidine (5e)

Yield 76 %, mp 163–166 °C; **IR** (KBr) cm^{-1}: 3,248, 3,055, 2,900, 1,666, 1,596, 1,476, 1,252, 1,092, 755; **^{1}H NMR** (δppm): 2.19–2.49 (m, 10H, $5CH_2$), 3.39 (br, s, 1H, –NH), 5.01 (s, 2H, CH_2), 5.16 (*d*, $J = 3.0$ Hz, 1H, =CH), 5.20 (s, 1H, CH), 7.34–8.10 (m, 8H, Ar–H); **EI-MS**: 415(M^+); Anal. Calcd. For $C_{22}H_{23}Cl_2N_3O$: C, 63.77; H, 5.11; N, 10.14. Found: C,63.71; H,5.12; N,10.10 %.

1-((4,6-bis(4-chlorophenyl)-1,6-dihydropyrimidin-2-yl)oxy)-*N*,*N*-dimethylmethanamine (5f)

Yield 73 %, mp 144–148 °C; **IR** (KBr) cm^{-1}: 3,256, 3,057, 2,850, 1,658, 1,596, 1,478, 1,257, 1,094, 750; **^{1}H NMR** (δppm): 2.87 (s, 6H, $2CH_3$), 3.42 (br, s, 1H, –NH), 5.01(s, 2H, CH_2), 5.19 (*d*, $J = 2.6$ Hz, 1H, =CH), 5.21 (s, 1H, CH), 7.34–8.10 (m, 8H, Ar–H); **EI-MS**: 375(M^+); Anal. Calcd. For $C_{19}H_{19}Cl_2N_3O$: C, 60.97; H, 4.58; N, 11.23. Found: C,60.91; H,4.52; N,11.21 %.

4-(((4-(4-chlorophenyl)-6-(4-methoxyphenyl)-1,6-dihydropyrimidin-2-yl)oxy)methyl)morpholine (5 g)

Yield 76 %, mp 168–170 °C; **IR** (KBr) cm^{-1}: 3,245, 3,055, 2,905, 1,654, 1,596, 1,475, 1,259, 1,090, 756; **^{1}H NMR** (δppm): 2.97 (t, 4H, $2CH_2$), 3.47 (br, s, 1H, –NH), 3.69 (t, 4H, $2CH_2$), 3.76 (s. 3H, OCH_3), 5.13 (s, 2H, CH_2), 5.16 (*d*, $J = 4.0$ Hz, 1H, =CH), 5.23(s, 1H, CH) 7.25–8.06 (m, 8H, Ar–H); **EI-MS**: 413(M^+); Anal. Calcd. For $C_{22}H_{24}ClN_3O_3$: C, 64.15; H, 5.38; N, 10.21. Found: C,64.12; H,5.35; N,10.19 %.

4-(4-chlorophenyl)-6-(4-methoxyphenyl)-2-(piperidin-1-ylmethoxy)-1,6-dihydropyrimidine (5 h)

Yield 84 %, mp 148–150 °C; IR (KBr) cm^{-1}: 3,241, 3,058, 2,896, 1,659, 1,601, 1,475, 1,251, 1,092, 760; **^{1}H NMR** (δppm): 2.19–2.49 (m, 10H, $5CH_2$), 3.47 (br, s, 1H, –NH), 3.76 (s, 3H, OCH_3), 5.13 (s, 2H, CH_2), 5.14 (*d*, $J = 3.1$ Hz, 1H, =CH), 5.22 (s, 1H, CH), 7.25–7.96 (m, 8H, Ar–H); **EI-MS**: 411(M^+); Anal. Calcd. For $C_{23}H_{26}ClN_3O_2$: C, 67.39; H, 5.90; N, 10.25. Found: C,67.35; H,5.88; N,10.23 %.

1-((4-(4-chlorophenyl)-6-(4-methoxyphenyl)-1,6-dihydropyrimidin-2-yl)oxy)-*N*,*N*-dimethylmethanamine (5i)

Yield 68 %, mp 169–172 °C; **IR** (KBr) cm^{-1}: 3,246, 3,052, 2,895, 1,658, 1,604, 1,476, 1,255, 1,090, 765; **^{1}H NMR** (δppm): 2.91 (s, 6H, $2CH_3$), 3.44 (br, s, 1H, –NH), 3.75 (s, 3H, OCH_3), 5.01 (s, 2H, CH_2), 5.16 (*d*, $J = 4.2$ Hz, 1H, =CH), 5.23 (s, 1H, CH), 7.30–8.00 (m, 8H, Ar–H); **EI-MS**: 371(M^+); Anal. Calcd. For $C_{20}H_{22}ClN_3O_2$: C, 64.95; H, 5.45; N, 11.36. Found: C,64.91; H,5.43; N,10.32 %.

4-(((6-(4-methoxyphenyl)-4-(3-nitrophenyl)-1,6-dihydropyrimidin-2-yl)oxy)methyl)morpholine (5j)

Yield 73 %, mp 192–194 °C; **IR** (KBr) cm^{-1}: 3,254, 3,058, 2,901, 1,658, 1,596, 1,525, 1,470, 1,350, 1,250, 1,090; **^{1}H NMR** (δppm): 2.97 (t, 4H, $2CH_2$), 3.43 (br, s, 1H, –NH), 3.69 (t, 4H, $2CH_2$), 3.83 (s, 3H, OCH_3), 5.01 (s, 2H, CH_2), 5.18 (*d*, $J = 3.4$ Hz, 1H, =CH), 5.22(s, 1H, CH) 7.25–8.06 (m, 8H, Ar–H); **EI-MS**: 424(M^+); Anal. Calcd. For $C_{22}H_{24}N_4O_5$: C, 62.55; H, 5.25; N, 13.26. Found: C,62.52; H,5.22; N,13.22 %.

6-(4-methoxyphenyl)-4-(3-nitrophenyl)-2-(piperidin-1-ylmethoxy)-1,6-dihydropyrimidine (5 k)

Yield 77 %, mp 181–184 °C; **IR** (KBr) cm^{-1}: 3,241, 3,054, 2,905, 1,652, 1,601, 1,522, 1,472, 1,348, 1,252, 1,091; **^{1}H NMR** (δppm): 2.19–2.49 (m, 10H, $5CH_2$), 3.47 (br, s, 1H, –NH), 3.76 (s, 3H, OCH_3), 5.01 (s, 2H, CH_2), 5.19 (*d*, $J = 3.9$ Hz, 1H, =CH), 5.21 (s, 1H, CH), 7.30–8.00 (m, 8H, Ar–H); **EI-MS**: 422(M^+); Anal. Calcd. For $C_{23}H_{26}N_4O_4$: C, 68.70; H, 5.75; N, 13.33. Found: C,68.68; H,5.72; N,13.30 %.

1-((6-(4-methoxyphenyl)-4-(3-nitrophenyl)-1,6-dihydropyrimidin-2-yl)oxy)-*N*,*N*-dimethylmethanamine (5 l)

Yield 64 %, mp 167–170 °C; **IR** (KBr) cm^{-1}: 3,252, 3,052, 2,908, 1,658, 1,596, 1,525, 1,478, 1,350, 1,258,

1,095; **^{1}H NMR** (δppm): 2.89 (s, 6H, $2CH_3$), 3.51 (br, s, 1H, –NH), 3.76 (s, 3H, OCH_3), 5.02 (s, 2H, CH_2), 5.21 (*d*, $J = 2.8$ Hz, 1H, =CH), 5.24 (s, 1H, CH), 7.50–8.06 (m, 8H, Ar–H); **EI-MS**: 382(M^+); Anal. Calcd. For $C_{20}H_{22}N_4O_4$: C, 63.15; H, 5.30; N, 14.73. Found: C,63.12; H,5.27; N,14.71 %.

4-(((6-(4-methoxyphenyl)-4-phenyl-1,6-dihydropyrimidin-2-yl)oxy)methyl)morpholine (5 m)

Yield 70 %, mp 174–176 °C; **IR** (KBr) cm^{-1}: 3,248, 3,059, 2,910, 1,660, 1,600, 1,475, 1,249, 1,092; **^{1}H NMR** (δppm): 2.97 (t, 4H, $2CH_2$), 3.40 (br, s, 1H, –NH), 3.69 (t, 4H, $2CH_2$), 3.81 (s, 3H, OCH_3), 5.01 (s, 2H, CH_2), 5.11 (*d*, $J = 3.7$ Hz, 1H, =CH), 5.27 (s, 1H, CH), 7.25–8.06 (m, 9H, Ar–H); **EI-MS**: 379(M^+); Anal. Calcd. For $C_{22}H_{25}N_3O_3$: C, 70.01; H, 6.14; N, 11.13. Found: C,70.00; H,6.10; N,11.10 %.

6-(4-methoxyphenyl)-4-phenyl-2-(piperidin-1-ylmethoxy)-1,6-dihydropyrimidine (5n)

Yield 76 %, mp 187–190 °C; **IR** (KBr) cm^{-1}: 3,244, 3,061, 2,905, 1,662, 1,596, 1,470, 1,245, 1,095; **^{1}H NMR** (δppm): 2.19–2.49 (m, 10H, $5CH_2$), 3.52 (br, s, 1H, –NH), 3.76 (s, 3H, OCH_3), 5.03 (s, 2H, CH_2), 5.24 (*d*, $J = 4.2$ Hz, 1H, =CH), 5.23 (s, 1H, CH), 7.25–7.91 (m, 9H, Ar–H); **EI-MS**: 377(M^+); Anal. Calcd. For $C_{23}H_{27}N_3O_2$: C, 73.57; H, 6.71; N, 11.19. Found: C,73.54; H,6.68; N,11.14 %.

1-((6-(4-methoxyphenyl)-4-phenyl-1,6-dihydropyrimidin-2-yl)oxy)-*N*,*N*-dimethylmethanamine (5o)

Yield 65 %, mp 161–164 °C; **IR** (KBr) cm^{-1}: 3,256, 3,055, 2,902, 1,658, 1,596, 1,470, 1,249, 1,091; **^{1}H NMR**(δppm): 2.90 (s, 6H, $2CH_3$), 3.57 (br, s, 1H, –NH), 3.83 (s, 3H, OCH_3), 5.01 (s, 2H, CH_2), 5.23 (*d*, $J = 2.8$ Hz, 1H, =CH), 5.29 (s, 1H, CH), 7.30–8.00 (m, 9H, Ar–H); **EI-MS**: 337(M^+); Anal. Calcd. For $C_{20}H_{23}N_3O_2$: C, 71.62; H, 6.31; N, 12.53. Found: C,71.58; H,6.29; N,12.50 %.

Evaluation of anticancer activity

In vitro anticancer activity

The human cell cultures HeLa (cervical), Ehrlich ascites carcinoma (EAC) & MCF-7 (breast cancer) cell lines were obtained from National Center for Cancer Cell Sciences (NCCS), Pune, India. These cell lines were grown in recommended media supplemented with 10 % FBS, 1 % L-glutamine and 1 % penicillin–streptomycin–amphotericin B in a 5 % CO_2 humidified atmosphere at 37 °C. Cells were seeded in 25 cm^2 tissue culture flasks (Tarsons, India), at 250,000 cells/flask in a total volume of 9 mL. When confluent, all the cells were trypsinized (using Trypsin-EDTA, HiMedia, Mumbai, India) and seeded in 96-well plate (Tarsons, India). The cell suspension of 1×10^5 cells/mL was prepared in complete growth medium. Stock solutions of the compounds were prepared in DMSO. The stock solutions were serially diluted with complete growth medium containing 50 mg/mL of gentamycin to obtain working test solution of required concentrations (having <1 % DMSO). The 100 μL of cell suspension was added to each well of the 96-well plate. The test materials in complete growth medium (100 μL) were added after 24-h incubation to the wells containing cell suspension. After 48 h of treatment with different concentrations of test compounds, the cells were incubated with MTT (2.5 mg/mL) for 2 h. The medium was then removed, and 100 μL of DMSO was added into each well to dissolve formazan crystals, the metabolite of MTT. After thoroughly mixing, the plate was read at 490 nm for optical density that is directly correlated with cell quantity. The cytotoxic effects of the compounds were calculated as percentage inhibition in cell growth as per the formula.

$$\% \text{ Cytotoxicity} = 1 - [(\text{O.D. in sample well}) / (\text{O.D. in control well})] \times 100$$

In vivo antitumor activity

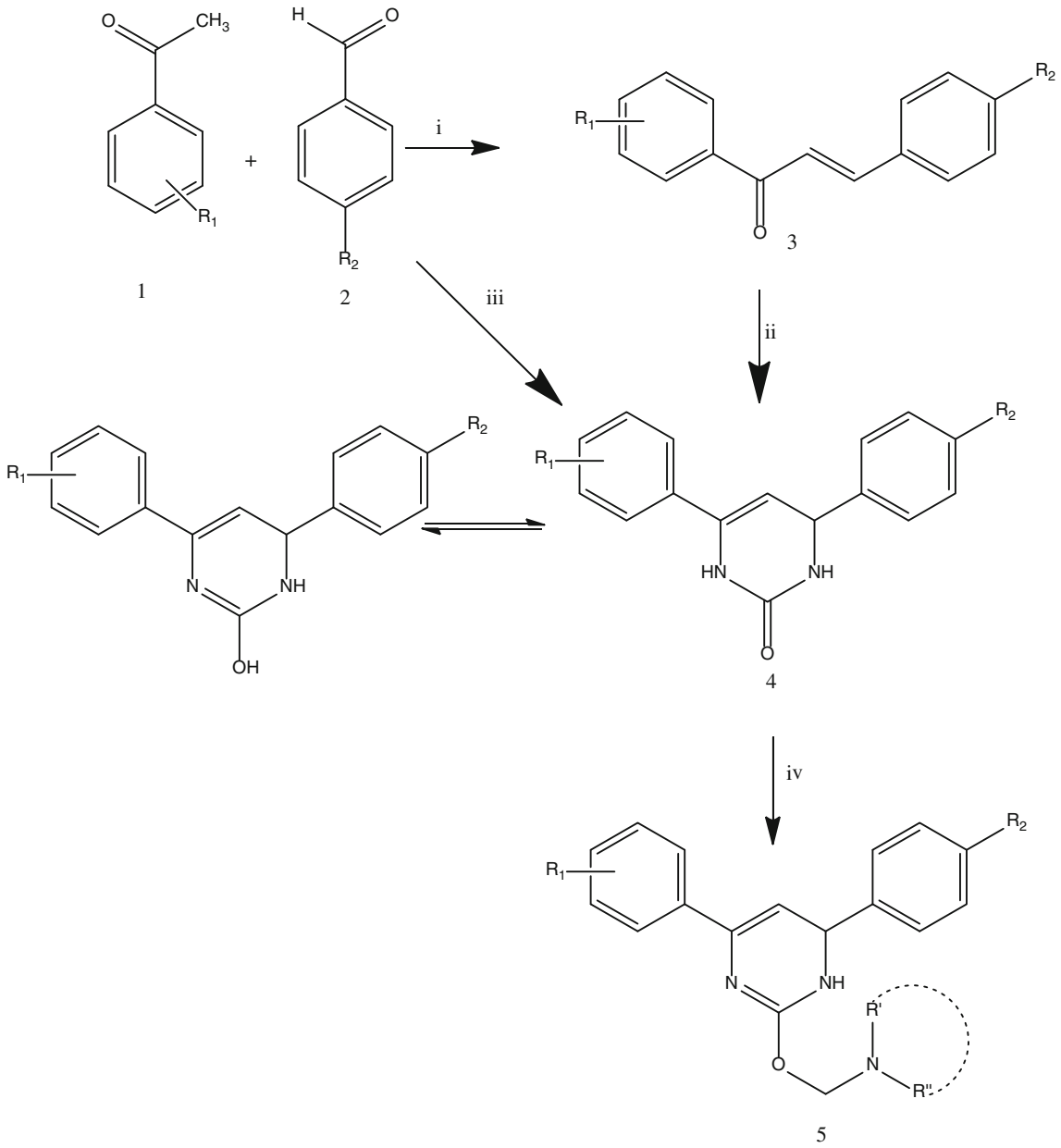
Adult female Swiss albino mice (Mahaveer Enterprises, Hyderabad, India) of 8 weeks old at study start (mean weight in the range of 20–25 g) were selected and housed in polypropylene cages in a room where the congenial temperature was 27 ± 1 °C and 12-h light and dark cycles were maintained. The animals were allowed to acclimatize to the environment for 7 days and supplied with a standard pellet diet and water ad libitum. All procedures using animals were reviewed and approved by the Institutional Animal Ethical Committee, Kakatiya University. The animals were divided into seven groups ($n = 6$). The normal group was not inoculated with tumor cells, while six groups were injected with EAC cells (0.2 mL of 2×10^6 cells/mice) intraperitoneally. This was taken as day '0' and the experimental treatment started 24 h later. From the first day, 100 μL/mouse per day of sterile saline was administered intraperitoneally to the negative control group (EAC-bearing mice). Compounds **5m** and **5n** at doses of 5 mg/kg and 10 mg/kg were administered each day to the treated groups, and the standard drug cisplatin at a dose of 5 mg/kg was administered to each animal from the positive control group. The pharmacological treatment lasted for 9 days. Fourteen days after the

treatment, five mice from each group were killed for the study of antitumor activity. The rest of the animal group was kept to check the mean survival time of EAC-tumor-bearing hosts. The antitumor effect of the compounds was determined from the change in body weight, mean survival time (MST) and percentage increased life span (% ILS). The MST of each group containing five mice was identified by recording the mortality on a daily basis for 30 days, and the % ILS was calculated using the following equations:

$$\mathrm{MST} = (\text{day of the first death} + \text{day of the last death})/2;$$

$$\mathrm{ILS}\ (\%) = [(\text{mean survival time of treated group} \,//\text{mean survival time of control group}) - 1] \times 100.$$

The effect of compounds **5m** and **5n** was also assessed by the determination of the body weight, tumor volume, packed cell volume and viable tumor cell count of EAC-bearing mice by the trypan blue incorporation method.



Scheme 1 Synthesis of novel O-Mannich bases of 4,6-diaryl-3,4-dihydropyrimidine-2(1*H*)-ones, **5a-o**

Table 1 Physical data of chalcones (3a–3e) and dihydropyrimidin-2(1H)-ones (4a–4e)

Code	R1	R2	Yield, %		M.P, °C	
			Reported [28, 29]	Obtained	Reported	Obtained
3a	H	H	85.3	80.2	48–50	50–52
3b	4-Cl	4-Cl	90.3	86.1	96–98	95–97
3c	4-Cl	4-OCH_3	98.4	96	118–120	116–118
3d	3-NO_2	4-OCH_3	–	85	–	98–100
3e	H	4-OCH_3	82.8	80	60–63	60–62
4a	H	H	94	89.5	245–246	240–242
4b	4-Cl	4-Cl	–	78.6	–	260–264
4c	4-Cl	4-OCH_3	–	84.2	–	236–238
4d	3-NO_2	4-OCH_3	–	90.1	–	271–273
4e	H	4-OCH_3	87	85.4	259–261	260–262

Results and discussion

Synthesis of compounds **3-5**

Different (*E*)-1,3-diaryl-prope-2-en-1-ones (**3** chalcones) were prepared by the Claisen–Schmidt condensation of acetophenones **1** with different benzaldehydes **2**, in equimolar ratio and in the presence of a base, under two different experimental conditions, viz., conventional and microwave assisted. Though the relative yields of chalcones obtained from both the methods were almost in the same range, there was a considerably drastic decrease in the reaction time in the MWA method. The chalcones **3** were then cyclocondensed with urea in the presence of a base, KOH [26] or NaOEt [27] by usual conventional heating method to get the corresponding 4,6-diaryl-3,4-dihydropyrimidin-2(1*H*)-ones **4**. Alternatively, compounds **4** were also synthesized by one-pot synthesis, through a 3-component cyclocondensation reaction, directly from acetophenone, benzaldehyde and urea using a Lewis acid catalyst. This one-pot synthesis was also carried out under two experimental conditions: conventional heating [28, 29] and microwave irradiation [30]. One-pot synthesis methods, in general, have resulted in better yields. Each of the synthesized dihydropyrimidinones **4** was subjected to the Mannich condensation by facile method using formaldehyde and appropriate secondary amine, in the presence of K_2CO_3 and DMSO as the solvent, to get the corresponding O-Mannich bases **5** (Scheme 1).

All the synthesized chalcones **3**, dihydropyrimidinones **4** and their O-Mannich bases **5** were purified. Among these, the known compounds were identified with the help of their literature data and the new compounds were characterized with the help of their analytical and spectral data (Table 1).

Antitumor activity

The newly synthesized *N*-[(4, 6-substituted diaryl-1,6-dihydropyrimidin-2-yl-oxy)methyl]-amines **5a-o** were evaluated for in vitro anticancer activity against human cell lines following the procedures described in the literature [31, 32].The tumor cell line panel consisted of HeLa, EAC and MCF-7 cells. Cisplatin (DDP) was used as a standard drug. The results are presented in Table 2. IC_{50} values were based on dose–response curves. IC_{50} value is defined as the concentration corresponding to 50 % growth inhibition. As shown in Table 2, some of the newly synthesized compounds showed good activity against the tumor cell lines. All the compounds showed a moderate to good anticancer activity against three different cell lines, and they were not selective toward any particular cell line.

Since the compounds **5m** and **5n** showed better in vitro cytotoxic properties, they were also evaluated for their in vivo antitumor activity in EAC-bearing mice with the help of liquid tumor model. The effect of the compounds **5m** to **5n,** in two different doses—5 mg/kg and 10 mg/kg—on body weight, mean survival time, % increase in life span, tumor volume, packed cell volume, tumor cell count(viable cells) was studied, and the results are presented in Table 3. The compound **5 m** was observed to exhibit a significant antitumor activity ($P < 0.001$) at both the doses, by decreasing the body weight of EAC-bearing mice, whereas the compound **5m** could show higher activity only at 10 mg/kg dose. The compound **5m** significantly increased the mean survival time and decreased the tumor volume, packed cell volume and viable tumor cell count at both the doses, whereas **5n** in higher dose (10 mg/kg) increased the mean survival time, decreased the tumor volume, packed cell volume and viable tumor cell count. On day 14, the biochemical and hematological parameters were evaluated. Hemoglobin level, erythrocytes

Table 2 Anticancer activity of novel *N*-[(4,6-substituted diaryl-1,6-dihydropyrimidin-2yl-oxy)methyl]-amines (5a-o) on human cancer cell lines

Compound	R_1	R_2	-N-R'R"	**log P**	HeLa	EAC	MCF-7
5a	H	H	Morpholino	4.56	70.6 ± 2.3	89.7 ± 2.8	90.8 ± 2.5
5b	H	H	Piperdino	5.69	78.5 ± 2.5	95.6 ± 2.3	99.2 ± 2.6
5c	H	H	Dimethylamino	4.96	60.2 ± 3.2	78.3 ± 3.3	82.4 ± 3.5
5d	4-Cl	4-Cl	Morpholino	5.67	30.5 ± 2.4	49.2 ± 2.3	51.6 ± 2.5
5e	4-Cl	4-Cl	Piperdino	6.81	55.3 ± 2.7	63.2 ± 2.5	67.2 ± 2.3
5f	4-Cl	4-Cl	Dimethylamino	6.07	50.2 ± 3.4	69.1 ± 3.2	68.2 ± 3.1
5g	4-Cl	4-OCH_3	Morpholino	4.99	30.2 ± 2.1	34.3 ± 1.5	33.3 ± 2.3
5h	4-Cl	4-OCH_3	Piperdino	6.12	35.3 ± 1.6	51.2 ± 1.2	58.1 ± 2.2
5i	4-Cl	4-OCH_3	Dimethylamino	5.39	38.4 ± 1.5	55.3 ± 1.2	50.2 ± 1.6
5j	3-NO_2	4-OCH_3	Morpholino	1.85	35.4 ± 3.4	32.3 ± 2.3	39.5 ± 2.5
5k	3-NO_2	4-OCH_3	Piperdino	3.30	45.3 ± 2.8	43.2 ± 3.5	46.3 ± 3.1
5l	3-NO_2	4-OCH_3	Dimethylamino	2.65	58.2 ± 2.6	55.3 ± 2.3	60.1 ± 2.5
5m	H	4-OCH_3	Morpholino	4.43	23.1 ± 1.2	21.2 ± 1.5	22.2 ± 1.6
5n	H	4-OCH_3	Piperdino	5.56	25.2 ± 1.5	23.4 ± 1.8	24.2 ± 1.5
5o	H	4-OCH_3	Dimethylamino	4.83	30.5 ± 2.1	29.4 ± 1.8	28.5 ± 2.1
Cisplatin	–	–	–	–	4.1 ± 0.1	4.6 ± 0.2	4.3 ± 0.1

[a] Anticancer activity as IC_{50} (μg/mL) for each cell line is the concentration of compound which reduced by 50 % the optical density of treated cells with respect to untreated cells using the MTT assay

[b] Data represent the mean values of three independent determinations

Table 3 Anticancer activity of *N*-[(4,6-substituted diaryl-1,6-dihydropyrimidin-2yl-oxy)methyl]-amines **5m** and **5n** on EAC-bearing mice

Parameter	EAC control ($5x10^6$ cells)	Cisplatin (5 mg/kg)	5 m		5n	
			(5 mg/kg)	(10 mg/kg)	(5 mg/kg)	(10 mg/kg)
Body weight ± SEM	12.2 ± 0.5	3.9 ± 0.3***	9.1 ± 0.5***	7.2 ± 0.3***	11.5 ± 0.2^{ns}	10.5 ± 0.4*
Mean survival time days ± SEM	13.4 ± 0.3	28.4 ± 0.4***	16.5 ± 0.5***	20.2 ± 0.4***	14.5 ± 0.3^{ns}	18.0 ± 0.3***
% Increased in life span (% ILS)	–	115.43	27.5	80.2	9.8	30.5
Tumor volume (ml ± SEM)	12.6 ± 0.8	2.8 ± 0.3***	6.8 ± 0.5***	5.1 ± 0.5***	11.3 ± 1.2^{ns}	8.9 ± 0.3**
Packed cell volume (ml ± SEM)	2.6 ± 0.3	0.2 ± 0.1***	1.7 ± 0.5^{ns}	0.9 ± 0.2**	2.2 ± 0.5^{ns}	1.2 ± 0.3*
Viable tumor cell count ($\times$ 10^7cells/ml)	6.2 ± 0.5	0.2 ± 0.04***	4.5 ± 0.5*	3.8 ± 0.3***	6.0 ± 0.5^{ns}	4.3 ± 0.2*

$n = 6$ and all values expressed as mean ± SEM, *ns* non-significant, compared with tumor control, *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$,

Table 4 Effect of *N*-[(4,6-substituted diaryl-1,6-dihydropyrimidin-2yl-oxy)methyl]-amines **5 m** and **5n** on biochemical and hematological parameters in EAC-bearing mice

Treatment	Hemoglobin (gm %)	RBC(million/mm^3)	WBC(10^3cells/mm^3)	Lymphocytes (%)	Neutrophils (%)	Monocytes (%)
Normal	13.5 ± 0.5	4.2 ± 0.2	6.9 ± 0.1	70.2 ± 1.2	30 ± 1.5	0.9 ± 0.1
Tumor control	5.8 ± 0.5	2.9 ± 0.1	20.3 ± 0.4	24 ± 0.5	73 ± 1.2	3.2 ± 0.5
Cisplatin	11.4 ± 1.0***	4.2 ± 0.1***	9.2 ± 0.2***	64 ± 0.8***	31 ± 1.2***	1.3 ± 0.1***
5m (5 mg/kg)	7.8 ± 0.6^{ns}	3.1 ± 0.1^{ns}	18.5 ± 0.2***	32 ± 0.8***	56 ± 0.7***	2 ± 0.2*
5m (10 mg/kg)	9.5 ± 1.2**	3.9 ± 0.1***	14.1 ± 0.3***	48 ± 0.7***	40 ± 0.9***	1.5 ± 0.1**
5n (5 mg/kg)	5.8 ± 0.7^{ns}	2.9 ± 0.3^{ns}	20.1 ± 0.3^{ns}	27 ± 0.5^{ns}	68 ± 0.3**	2.3 ± 0.4^{ns}
5n (10 mg/kg)	6.9 ± 0.6^{ns}	3.4 ± 0.1^{ns}	15.1 ± 0.2***	39 ± 0.8***	52 ± 0.7***	1.8 ± 0.4*

$n = 6$ and all values expressed as mean ± SEM, *ns* non-significant, compared to tumor control, *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$,

and leukocytes counts were compared with EAC control group, standard drug (cisplatin)-treated groups and test groups (injected with the compounds **5m** and **5n**). Results are presented in Table 4. Perusal of the table indicates clearly that the test compounds **5m** and **5n** have significantly decreased ascetic fluid volume as compared to the EAC control. The observed results could indicate macrophage activation and inhibition of vascular permeability, in comparison with the tumor control. The compounds **5m** and **5n** increased the hemoglobin and RBC levels when compared with the tumor control, **5m** increased these levels more significantly than **5n**. The compounds **5m** and **5n** decreased the WBC levels when compared with the tumor control; once again **5m** decreased these levels more effectively in comparison with **5n.** Another positive feature in favor of antitumor activity was treatment with the **5m** and **5n** brought back the differential leukocyte count to normal levels. This indicates that the test compounds possess protective action on hemopoietic system. The present results are in agreement with that of earlier reports [33, 34]. The above results from in vivo cytotoxic studies suggest that the compound **5m** was relatively more active (Table 4).

Conclusion

Synthetically, one-pot synthesis of 4,6-diaryl-3,4-dihydropyrimidine-2(1*H*)-ones was proved to be the best both yield and purity wise. The O-Mannich bases of 4,6-diaryl-3,4-dihydropyrimidine-2(1*H*)-ones were synthesized by a facile method. Pharmacologically, one of the compounds **5m** from the present series was found to possess promising antitumor activity, while protecting the hemopoietic system.

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