

Structure Activity Relationship, Drug Likeness and Evaluation of Antioxidant Activity of Some Mannich Bases of Dihydropyrimidinones

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A series of 21 O- and N-Mannich bases of 3,4-dihydropyrimidinones (**2a-j** and **3a-k**) were synthesized by using microwave irradiation technique by multi-component reaction in two steps. All the compounds were evaluated for their free radical scavenging activity by four methods. Structure activity relationship studies revealed that the compounds **2h**, **2g**, **3h** and **3g** exhibited profound antioxidant properties compared to standard ascorbic acid. Among O- and N-Mannich bases, N-Mannich bases were found to be more potent in scavenging free radicals. The correlation between structure and activities of these compounds with concern to drug likeness profile and other physico-chemical parameters are portrayed and verified experimentally.

Keywords: Antioxidant activity, Dihydropyrimidinones, Drug likeness, Mannich bases, Structure activity relationship.

INTRODUCTION

Excess production of reactive oxygen species (ROS) leads to oxidative stress and damages cell components [1]. ROS that carries a single electron in an atomic orbital are capable enough to interact with various biomolecules [2] thereby leading to several pathological conditions such as Parkinson's, Alzheimer's, cancer, CVS disorders, cirrhosis, *etc.* [3,4].

Antioxidants are stable components that can donate an electron to highly unstable ROS and can neutralize them, thereby preventing their harmful effects [5]. Antioxidants breaks the chain reactions instigated by ROS by donating electron and can works like single oxygen quencher, hydrogen and electron donor, metal chelating agents, a radical scavenger, peroxide decomposer, synergist and as an enzyme inhibitor [6]. Therefore, the scaffolds that hold antioxidant activity are of tremendous importance in the current drug research targeting multiple diseases.

Dihydropyrimidinones (DHPMs) exhibits diverse range of biological activities including anticancer [7], antimicrobial [8], anti-inflammatory [9], antidiabetic [10], analgesic [11], antitubercular [12], antimalarial [13] and antiviral activities

[14]. The biological importance of this pharmacophore has attracted the interest of chemists in drug discovery and research. In the present study, the scope of this pharmacophore is extended by aryl substitution at 4th and 6th positions, which are further substituted by electron donating and electron withdrawing groups at various positions.

Mannich bases of some dihydropyrimidinones (DHPMs) were synthesized by microwave irradiation and screened for antioxidant activity by 2,2-diphenyl-1-picrylhydrazyl (DPPH) method, hydrogen peroxide method, nitric oxide scavenging method and iron chelation method, to establish the structure activity relationships (SAR).

EXPERIMENTAL

The titled compounds were characterized by melting point, IR, ¹H NMR, Mass and elemental analyses. The IR spectra were recorded on Perkin-Elmer FT-IR instrument using KBr discs (Perkin Elmer, USA). ¹H NMR was recorded on a Bruker Avance II 300 instrument (300 MHz) in CDCl₃ solvent and TMS as internal standard. The mass spectra were obtained by electron spray ionization (ESI) on Shimadzu LCMS 2010A spectro-

meter. Microwave irradiation was carried out using an IFB domestic microwave oven. Melting points were measured by melting point apparatus on an open capillary and are uncorrected. Precoated silica gel TLC plates (Silica gel GF₂₅₄) were obtained from E. Merck and the solvent system of 1:1:0.2 ratio of ethyl acetate:*n*-hexane:methanol was employed for TLC and was selected by trial and error method. All the chemicals were procured from S.D. Fine Chemicals Ltd. (Mumbai) and Sigma-Aldrich (Merck), India. The solvents were of laboratory grade.

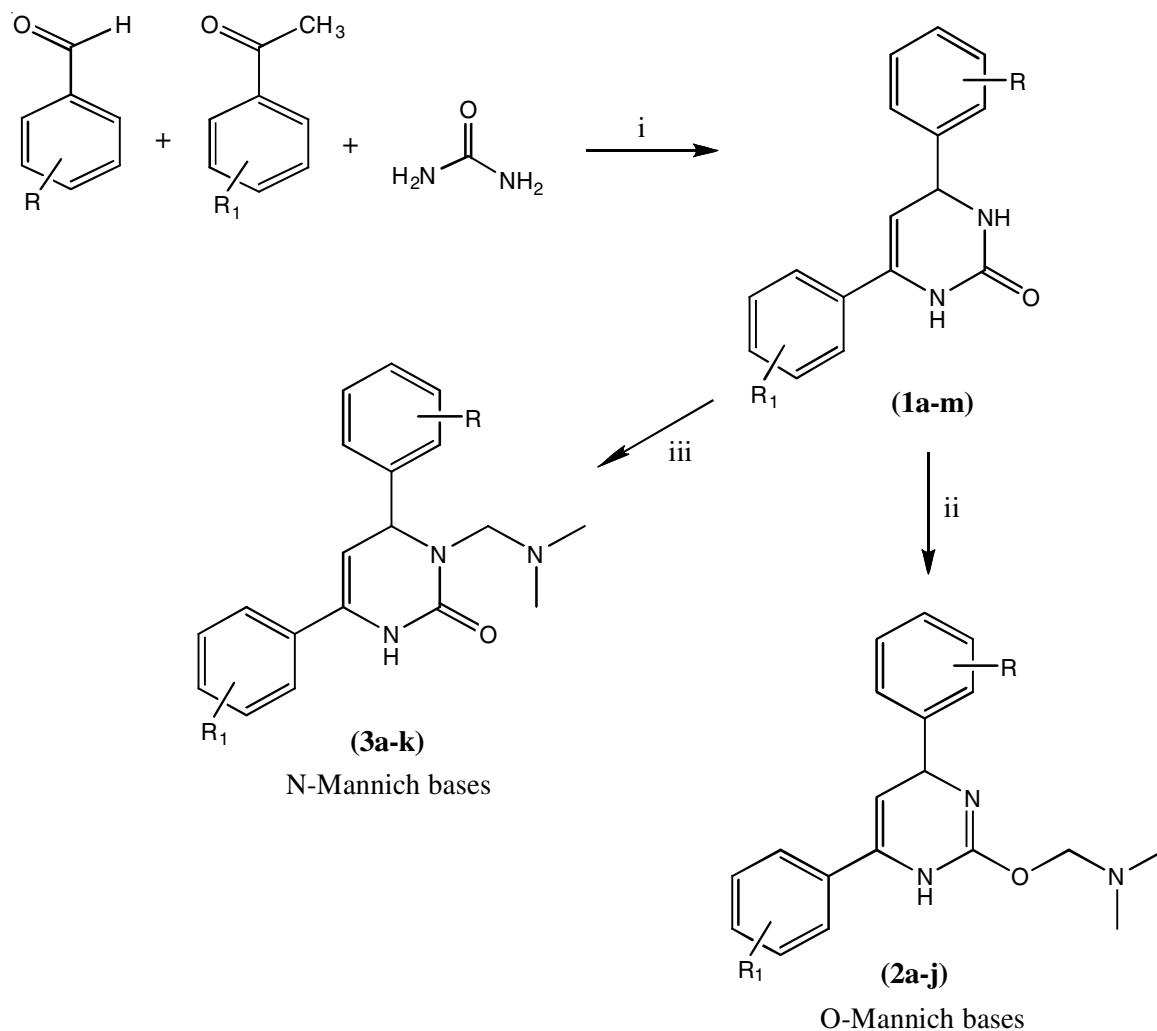
General procedure for the synthesis of 3,4-dihydropyrimidine-2-ones (1): 3,4-Dihydropyrimidinones were synthesized by modification of protocol described by Venkateswarlu *et al.* [15], by replacing catalyst AlCl₃ with *p*-toulene sulphonic acid (PTSA). A mixture of 0.01 mol of appropriately substituted benzaldehydes, acetophenones, urea and PTSA were dissolved in 30 mL of ethanol. The reaction mixture was irradiated in a microwave oven at 60 % power. The reaction was monitored by TLC. After completion of the reaction, contents were poured in ice cold water with continuous stirring. The product formed was filtered and dried. The product obtained was purified by recrystallization from ethanol.

Synthesis of O-Mannich bases (2a-j): A mixture of 0.005 mol of DHPM (dissolved in 10 mL of DMSO), 0.01 mol of 37% formaldehyde, 20 mg of anhydrous K₂CO₃ and 0.005

mol of dimethylamine were taken in a flask and stirred for couple of hours. Then the reaction mixture was irradiated in a microwave oven at 60 % power. Completion of reaction was confirmed by TLC and the product formed was refrigerated for 36 h. The separated product was filtered, washed with cold water and dried. Recrystallization was done from petroleum ether:chloroform (1:1 ratio).

Synthesis of N-Mannich bases (3a-k): A mixture of 0.005 mol of DHPM (dissolved in 10 mL of DMSO), 0.01 mol of 37 % formaldehyde and 0.005 mol of dimethylamine were taken in a flask and stirred for couple of hours. Then the reaction mixture was irradiated in a microwave oven at 60% power. Completion of reaction was confirmed by TLC and the product formed was refrigerated for 36 h. The separated product was filtered, washed with cold water and dried. Recrystallization was done from petroleum ether:chloroform (1:1 ratio) (Scheme-I).

Antioxidant activity: All the titled compounds were dissolved in methanol to make concentrations of 20, 40, 60, 80 and 100 µg/mL. These concentrations were allowed to react with different free radicals in various methods. Antioxidant activity was determined by four different methods *viz.*, DPPH scavenging activity [16], H₂O₂ scavenging activity [17], NO scavenging activity [18] and iron chelation activity [19].



Scheme-I: Synthesis of O- and N-Mannich bases of DHPMs. Reagents and conditions (i) PTSA, ethanol, MW 60% (ii) Dimethylamine, formaldehyde, K₂CO₃, DMSO, MW 60% (iii) Dimethylamine, formaldehyde, DMSO, MW 60 %

Ascorbic acid was used as the standard. Control was prepared by adding all the contents except test compound. The absorbance was measured on UV-visible spectrophotometer (Shimadzu, UV-1800, Japan). All the experiments were carried out in triplicate and the results were expressed as mean $\pm$ standard error mean (SEM). The free radical scavenging activity (FRSA) of the tested samples and standard was calculated as % FRSA in all methods by using following formula:

$$\text{FRSA (\%)} = \frac{A_o - A_t}{A_o} \times 100$$

where, A_o = Absorbance of control, A_t = Absorbance of tested samples. % FRSA was plotted against concentration. IC_{50} values were obtained from the line equation above. Smaller IC_{50} value indicates higher radical scavenging activity.

4-(4-Methoxyphenyl)-6-(3-nitrophenyl)-1,4-dihydropyrimidine-2-yl-oxy-*N,N*-dimethylmethanamine (2a): Colour: pale brownish amorphous solid, m.w. 382, m.p. 205-208 °C, R_f : 0.58 (*n*-hexane:ethylacetate:methanol; 1:1:0.2 for all compounds); IR (KBr, $\nu_{\max}$, cm^{-1}): 3294, 1529, 1220, 1427, 2921, 2821, 1590, 952, 804; ^1H NMR (300 MHz, CDCl_3) δ : 2.56 (s, 6H, 2CH_3), 3.8 (s, 3H, OCH_3), 5.2 (s, 2H, CH_2), 8.6 (s, 1H, NH), 6.8-7.5 (m, 8H, Ar-H), 5.56 (d, 1H, CH), 6.2 (d, 1H, $\text{C}=\text{CH}$, $J = 4.2$ Hz); ESI-MS: Found $[M^+]$ 382; Elemental analyses for calcd. (found) %: C 62.82 (62.79), H 5.75 (5.72), N 14.65 (14.71).

4-(4-Chlorophenyl)-6-(phenyl)-1,4-dihydropyrimidine-2-yl-oxy-*N,N*-dimethylmethanamine (2b): Colour: pale yellowish amorphous solid, m.w. 341, m.p. 218-220 °C, R_f : 0.65; IR (KBr, $\nu_{\max}$, cm^{-1}): 751, 3231, 1195, 1241, 3024, 2914, 1596, 1152, 1082, 816; ^1H NMR (300 MHz, CDCl_3) δ : 2.3 (s, 6H, 2CH_3), 4.8 (s, 2H, CH_2), 8.8 (s, 1H, NH), 6.6-7.3 (m, 9H, Ar-H), 4.5 (d, 1H, CH), 5.6 (d, 1H, $\text{C}=\text{CH}$, $J = 3.0$ Hz); ESI-MS: Found $[M+2]$ 344; Elemental analyses for calcd. (found) %: C 66.66 (66.52), H 5.84 (5.79), N 12.28 (12.34).

4-(Phenyl)-6-(4-chlorophenyl)-1,4-dihydropyrimidine-2-yl-oxy-*N,N*-dimethylmethanamine (2c): Colour: white amorphous solid, m.w. 341, m.p. 228-230 °C, R_f : 0.52; IR (KBr, $\nu_{\max}$, cm^{-1}): 752, 3213, 1083, 1521, 3050, 2778, 1609, 962, 810; ^1H NMR (300 MHz, CDCl_3) δ : 2.6 (s, 6H, 2CH_3), 4.7 (s, 2H, CH_2), 8.2 (s, 1H, NH), 6.4-7.5 (m, 9H, Ar-H), 5.4 (d, 1H, CH), 6.0 (d, 1H, $\text{C}=\text{CH}$, $J = 5.4$ Hz); ESI-MS: Found $[M+1]$ 343; Elemental analyses for calcd. (found) %: C 64.77 (64.72), H 5.68 (5.56), N 15.90 (15.84).

4-(4-Chlorophenyl)-6-(3-nitrophenyl)-1,4-dihydropyrimidine-2-yl-oxy-*N,N*-dimethylmethanamine (2d): Colour: Yellowish amorphous solid, m.w. 386, m.p. 198-201 °C, R_f : 0.71; IR (KBr, $\nu_{\max}$, cm^{-1}): 759, 1514, 3190, 1298, 1242, 3034, 2884, 1630, 978, 806; ^1H NMR (300 MHz, CDCl_3) δ : 2.4 (s, 6H, 2CH_3), 4.9 (s, 2H, CH_2), 8.0 (s, 1H, NH), 7.0-7.8 (m, 8H, Ar-H), 5.2 (d, 1H, CH), 6.1 (d, 1H, $\text{C}=\text{CH}$, $J = 4.7$ Hz); ESI-MS: Found $[M+]$ 387; Elemental analyses for calcd. (found) %: C 58.91 (59.02), H 4.90 (4.96), N 14.47 (14.38).

4-(2-Chlorophenyl)-6-(3-chlorophenyl)-1,4-dihydropyrimidine-2-yl-oxy-*N,N*-dimethylmethanamine (2e): Colour: brownish amorphous solid, m.w. 375, m.p. 234-236 °C, R_f : 0.48; IR (KBr, $\nu_{\max}$, cm^{-1}): 730, 752, 3282, 1230, 1354, 3072, 2926, 1636, 954, 778; ^1H NMR (300 MHz, CDCl_3) δ :

2.1 (s, 6H, 2CH_3), 4.4 (s, 2H, CH_2), 8.8 (s, 1H, NH), 6.8-7.6 (m, 8H, Ar-H), 5.2 (d, 1H, CH), 5.8 (d, 1H, $\text{C}=\text{CH}$, $J = 6.2$ Hz); ESI-MS: Found $[M+2]$ 379; Elemental analyses for calcd. (found) %: C 60.63 (60.54), H 5.05 (5.12), N 11.17 (11.24).

4-(Phenyl)-6-(4-hydroxyphenyl)-1,4-dihydropyrimidine-2-yl-oxy-*N,N*-dimethylmethanamine (2f): Colour: pale brownish amorphous solid, m.w. 323, m.p. 192-194 °C, R_f : 0.55; IR (KBr, $\nu_{\max}$, cm^{-1}): 3500, 3399, 1242, 1320, 3120, 2894, 1608, 942, 754; ^1H NMR (300 MHz, CDCl_3) δ : 2.8 (s, 6H, 2CH_3), 4.0 (s, 2H, CH_2), 8.2 (s, 1H, NH), 6.4-7.7 (m, 9H, Ar-H), 4.2 (s, 1H, OH), 4.9 (d, 1H, CH), 5.4 (d, 1H, $\text{C}=\text{CH}$, $J = 8.3$ Hz); ESI-MS: Found $[M+]$ 323; Elemental analyses for calcd. (found) %: C 70.58 (70.42), H 6.50 (6.44), N 13.00 (12.98).

4-(4-Hydroxyphenyl)-6-(phenyl)-1,4-dihydropyrimidine-2-yl-oxy-*N,N*-dimethylmethanamine (2g): Colour: yellowish amorphous solid, m.w. 323, m.p. 214-216 °C, R_f : 0.74; IR (KBr, $\nu_{\max}$, cm^{-1}): 3422, 3220, 1212, 1268, 3066, 2921, 1606, 982, 748; ^1H NMR (300 MHz, CDCl_3) δ : 2.5 (s, 6H, 2CH_3), 3.8 (s, 2H, CH_2), 9.3 (s, 1H, NH), 4.8 (s, 1H, OH), 6.4-7.1 (m, 9H, Ar-H), 5.1 (d, 1H, CH), 5.6 (d, 1H, $\text{C}=\text{CH}$, $J = 5.6$ Hz); ESI-MS: Found $[M+]$ 323; Elemental analyses for calcd. (found) %: 70.58 (70.46), H 6.50 (6.45), N 13.00 (13.18).

4,6-bis-(4-Hydroxyphenyl)-1,4-dihydropyrimidine-2-yl-oxy-*N,N*-dimethylmethanamine (2h): Colour: brownish crystalline solid, m.w. 339, m.p. 219-221 °C, R_f : 0.44; IR (KBr, $\nu_{\max}$, cm^{-1}): 3395, 3294, 1196, 1344, 3053, 2905, 1588, 944, 786; ^1H NMR (300 MHz, CDCl_3) δ : 2.9 (s, 6H, 2CH_3), 3.6 (s, 2H, CH_2), 8.4 (s, 1H, NH), 6.2 (s, 1H, OH), 6.6 (s, 1H, OH), 7.1-7.9 (m, 8H, Ar-H), 5.4 (d, 1H, CH), 5.7 (d, 1H, $\text{C}=\text{CH}$, $J = 4.3$ Hz); ESI-MS: Found $[M+1]$ 341; Elemental analyses for calcd. (found) %: C 67.25 (67.31), H 6.19 (6.25), N 12.38 (12.41).

4-(Phenyl)-6-(3-nitrophenyl)-1,4-dihydropyrimidine-2-yl-oxy-*N,N*-dimethylmethanamine (2i): Colour: pale yellowish amorphous solid, m.w. 352, m.p. 244-246 °C, R_f : 0.39; IR (KBr, $\nu_{\max}$, cm^{-1}): 3600, 1490, 1206, 1392, 3018, 2788, 1596, 968, 754; ^1H NMR (300 MHz, CDCl_3) δ : 2.6 (s, 6H, 2CH_3), 3.6 (s, 2H, CH_2), 8.4 (s, 1H, NH), 7.1-8.0 (m, 9H, Ar-H), 4.2 (d, 1H, CH), 4.9 (d, 1H, $\text{C}=\text{CH}$, $J = 5.2$ Hz); ESI-MS: Found $[M+]$ 352; Elemental analyses for calcd. (found) %: C 64.77 (64.72), H 5.68 (5.73), N 15.90 (15.87).

4-(4-Hydroxyphenyl)-6-(3-nitrophenyl)-1,4-dihydropyrimidine-2-yl-oxy-*N,N*-dimethylmethanamine (2j): Colour: pale brownish amorphous solid, m.w. 368, m.p. 221-223 °C, R_f : 0.77; IR (KBr, $\nu_{\max}$, cm^{-1}): 3420, 1512, 3178, 1178, 1248, 3096, 2922, 1602, 843, 736; ^1H NMR (300 MHz, CDCl_3) δ : 3.1 (s, 6H, 2CH_3), 3.4 (s, 2H, CH_2), 8.2 (s, 1H, NH), 6.4 (s, 1H, OH), 6.9-7.7 (m, 8H, Ar-H), 5.2 (d, 1H, CH), 5.8 (d, 1H, $\text{C}=\text{CH}$, $J = 8.1$ Hz); ESI-MS: Found $[M+1]$ 369; Elemental analyses for calcd. (found) %: C 61.95 (61.82), H 5.43 (5.38), N 15.21 (15.25).

4-(4-Methoxyphenyl)-6-(4-chlorophenyl)-1,4-dihydropyrimidine-2(1*H*)-one-3-yl-*N,N*-dimethylmethanamine (3a): Colour: white amorphous solid, m.w. 371, m.p. 231-233 °C, R_f : 0.62; IR (KBr, $\nu_{\max}$, cm^{-1}): 743, 1670, 3208, 1220, 1285, 3024, 2916, 1592, 944, 780; ^1H NMR (300 MHz, CDCl_3) δ : 2.3 (s, 6H, 2CH_3), 4.2 (s, 2H, CH_2), 8.1 (s, 1H, NH), 3.3 (O- CH_3), 8.8 (m, 1H, OH), 7.1-7.6 (m, 8H, Ar-H), 4.7 (d, 1H, CH), 5.6 (d, 1H, $\text{C}=\text{CH}$, $J = 4.2$ Hz); ESI-MS: Found $[M+1]$ 373;

Elemental analyses for calcd. (found) %: C 64.51 (64.58), H 5.91 (5.86), N 11.29 (11.22).

4-(4-Chlorophenyl)-6-(phenyl)-1,4-dihydropyrimidine-2(1H)-one-3-yl-N,N-dimethyl methanamine (3b): Colour: pale yellowish amorphous solid, m.w. 341, m.p. 207-209 °C, R_f : 0.68; IR (KBr, $\nu_{\max}$, cm^{-1}): 739, 3380, 1687, 1340, 3012, 2842, 1598, 1152, 810-982; ^1H NMR (300 MHz, CDCl_3) δ : 2.5 (s, 6H, 2CH₃), 3.8 (s, 2H, CH₂), 8.6 (s, 1H, NH), 7.2-7.9 (m, 9H, Ar-H), 4.3 (d, 1H, CH), 5.2 (d, 1H, C=CH, $J=7.4$ Hz); ESI-MS: Found [M+] 342; Elemental analyses for calcd. (found) %: C 66.66 (66.69), H 5.84 (5.88), N 12.28 (12.21).

4-(Phenyl)-6-(4-chlorophenyl)-1,4-dihydropyrimidine-2(1H)-one-3-yl-N,N-dimethylmethanamine (3c): Colour: yellowish crystalline solid, m.w. 341, m.p. 240-242 °C, R_f : 0.46; IR (KBr, $\nu_{\max}$, cm^{-1}): 759, 3388, 1606, 1221, 3096, 2962, 1621, 976, 820; ^1H NMR (300 MHz, CDCl_3) δ : 3.2 (s, 6H, 2CH₃), 3.7 (s, 2H, CH₂), 8.9 (s, 1H, NH), 7.0-7.6 (m, 9H, Ar-H), 4.5 (d, 1H, CH), 5.5 (d, 1H, C=CH, $J=8.5$ Hz); ESI-MS: Found [M+] 343; Elemental analyses for calcd. (found) %: C 66.66 (66.62), H 5.84 (5.81), N 12.28 (12.25).

4,6-bis-Phenyl-1,4-dihydropyrimidine-2(1H)-one-3-yl-N,N-dimethylmethanamine (3d): Colour: pale brownish amorphous solid, m.w. 307, m.p. 215-217 °C, R_f : 0.41; IR (KBr, $\nu_{\max}$, cm^{-1}): 3332, 1676, 1246, 3116, 2930, 1618, 732-926; ^1H NMR (300 MHz, CDCl_3) δ : 2.6 (s, 6H, 2CH₃), 3.4 (s, 2H, CH₂), 7.9 (s, 1H, NH), 6.6-7.2 (m, 10H, Ar-H), 3.9 (d, 1H, CH), 5.6 (d, 1H, C=CH, $J=3.9$ Hz); ESI-MS: Found [M+] 307; Elemental analyses for calcd. (found) %: C 74.26 (74.31), H 6.84 (6.77), N 13.68 (13.64).

4,6-bis-(4-Chlorophenyl)-1,4-dihydropyrimidine-2(1H)-one-3-yl-N,N-dimethyl methanamine (3e): Colour: pale red crystalline solid, m.w. 375, m.p. > 250 °C, R_f : 0.36; IR (KBr, $\nu_{\max}$, cm^{-1}): 747, 753, 3452, 1668, 1326, 3056, 2942, 1609, 945, 792; ^1H NMR (300 MHz, CDCl_3) δ : 2.4 (s, 6H, 2CH₃), 2.9 (s, 2H, CH₂), 9.2 (s, 1H, NH), 7.0-7.7 (m, 8H, Ar-H), 4.8 (d, 1H, CH), 8.1 (d, 1H, C=CH, $J=4.5$ Hz); ESI-MS: Found [M+2] 379; Elemental analyses for calcd. (found) %: C 60.63 (60.60), H 5.05 (5.09), N 11.17 (11.13).

4-(Phenyl)-6-(4-hydroxyphenyl)-1,4-dihydropyrimidine-2(1H)-one-3-yl-N,N-dimethylmethanamine (3f): Colour: pale yellowish amorphous solid, m.w. 323, m.p. 196-198 °C, R_f : 0.34; IR (KBr, $\nu_{\max}$, cm^{-1}): 3462, 3421, 1715, 1358, 3124, 2885, 1614, 916, 765; ^1H NMR (300 MHz, CDCl_3) δ : 2.9 (s, 6H, 2CH₃), 3.6 (s, 2H, CH₂), 9.1 (s, 1H, NH), 5.6 (s, 1H, OH), 7.2-7.8 (m, 9H, Ar-H), 4.4 (d, 1H, CH), 6.3 (d, 1H, C=CH, $J=5.2$ Hz); ESI-MS: Found [M+] 323; Elemental analyses for calcd. (found) %: C 70.58 (70.51), H 6.50 (6.42), N 13.00 (12.57).

4-(4-Hydroxyphenyl)-6-(phenyl)-1,4-dihydropyrimidine-2(1H)-one-3-yl-N,N-dimethylmethanamine (3g): Colour: yellowish crystalline solid, m.w. 323, m.p. 237-239 °C, R_f : 0.57; IR (KBr, $\nu_{\max}$, cm^{-1}): 3420, 3232, 1682, 1202, 3174, 2933, 1588, 939, 790; ^1H NMR (300 MHz, CDCl_3) δ : 2.0 (s, 6H, 2CH₃), 4.1 (s, 2H, CH₂), 8.4 (s, 1H, NH), 4.8 (s, 1H, OH), 7.1-8.0 (m, 9H, Ar-H), 4.8 (d, 1H, CH), 5.6 (d, 1H, C=CH, $J=7.1$ Hz); ESI-MS: Found [M+] 323; Elemental analyses for calcd. (found) %: C 70.58 (70.49), H 6.50 (6.53), N 13.00 (12.52).

4,6-bis-(4-Hydroxyphenyl)-1,4-dihydropyrimidine-2(1H)-one-3-yl-N,N-dimethylmethanamine (3h): Colour:

pale brownish amorphous solid, m.w. 339, m.p. 204-206 °C, R_f : 0.37; IR (KBr, $\nu_{\max}$, cm^{-1}): 3390, 3332, 1666, 1224, 3082, 2912, 1594, 980, 726; ^1H NMR (300 MHz, CDCl_3) δ : 1.8 (s, 6H, 2CH₃), 3.7 (s, 2H, CH₂), 8.6 (s, 1H, NH), 5.6 (s, 1H, OH), 5.9 (s, 1H, OH), 6.8-7.6 (m, 8H, Ar-H), 5.1 (d, 1H, CH), 6.2 (d, 1H, C=CH, $J=6.8$ Hz); ESI-MS: Found [M+1] 341; Elemental analyses for calcd. (found) %: C 67.25 (67.34), H 6.19 (6.15), N 12.38 (12.33).

4-(Phenyl)-6-(3-nitrophenyl)-1,4-dihydropyrimidine-2(1H)-one-3-yl-N,N-dimethylmethanamine (3i): Colour: Yellowish amorphous solid, m.w. 352, m.p. 190-192 °C, R_f : 0.79; IR (KBr, $\nu_{\max}$, cm^{-1}): 3510, 1524, 1724, 1222, 3046, 2892, 1576, 954, 786; ^1H NMR (300 MHz, CDCl_3) δ : 3.1 (s, 6H, 2CH₃), 3.6 (s, 2H, CH₂), 8.2 (s, 1H, NH), 6.9-7.7 (m, 9H, Ar-H), 4.4 (d, 1H, CH), 5.6 (d, 1H, C=CH, $J=4.6$ Hz); ESI-MS: Found [M+] 352; Elemental analyses for calcd. (found) %: C 64.77 (64.79), H 5.68 (5.69), N 15.90 (15.89).

4-(4-Hydroxyphenyl)-6-(3-nitrophenyl)-1,4-dihydropyrimidine-2(1H)-one-3-yl-N,N-dimethylmethanamine (3j): Colour: pale brownish crystalline solid, m.w. 368, m.p. 241-243 °C, m.p. 229-231 °C, R_f : 0.82; IR (KBr, $\nu_{\max}$, cm^{-1}): 3395, 1512, 3355, 1706, 1245, 3030, 2972, 1624, 964, 726; ^1H NMR (300 MHz, CDCl_3) δ : 2.5 (s, 6H, 2CH₃), 3.4 (s, 2H, CH₂), 8.0 (s, 1H, NH), 4.9 (s, 1H, OH), 7.0-7.7 (m, 8H, Ar-H), 5.3 (d, 1H, CH), 6.2 (d, 1H, C=CH, $J=8.2$ Hz); ESI-MS: Found [M+1] 369; Elemental analyses for calcd. (found) %: C 61.95 (61.91), H 5.43 (5.45), N 15.21 (15.26).

4-(4-Chlorophenyl)-6-(3-nitrophenyl)-1,4-dihydropyrimidine-2(1H)-one-3-yl-N,N-dimethyl methanamine (3k): Colour: yellowish crystalline solid, m.w. 386, m.p. 241-243 °C, R_f : 0.84; IR (KBr, $\nu_{\max}$, cm^{-1}): 730, 1516, 3291, 1714, 1320, 3008, 2946, 1606, 1240, 972, 740; ^1H NMR (300 MHz, CDCl_3) δ : 2.6 (s, 6H, 2CH₃), 4.2 (s, 2H, CH₂), 8.3 (s, 1H, NH), 6.6-7.4 (m, 8H, Ar-H), 5.0 (d, 1H, CH), 5.8 (d, 1H, C=CH, $J=5.6$ Hz); ESI-MS: Found [M+1] 388; Elemental analyses for calcd. (found) %: C 58.91 (58.82), H 4.90 (4.88), N 14.47 (14.42).

RESULTS AND DISCUSSION

The synthesis of a series of O- and N-Mannich bases of 4,6-diaryl-3,4-dihydropyrimidinones is carried out as outlined in **Scheme-I**. Initially 3,4-DHPMs (**1a-m**) were obtained by modified Biginelli reaction by a multi-component system comprising substituted benzaldehydes, substituted acetophenones and urea in the presence of catalytic amount of *p*-toluene sulphonic acid (PTSA) in ethanol [20] under microwave irradiation. Unlike other lewis acid catalysts, PTSA does not require any anhydrous conditions [21]. Simple distilled ethanol without further drying was employed as solvent.

The titled compounds, O- and N-Mannich bases of DHPMs (**2a-j** and **3a-k**, respectively) were synthesized by taking equimolar quantity of DHPMs obtained in step 1 (**1a-m**), 37 % formaldehyde, dimethylamine and either with or without K_2CO_3 under microwave irradiation [14]. All the compounds were obtained in good yield (60-94 %). They were further purified by recrystallization and column chromatography. The physical data of the synthesized compounds is shown in Table-1 in which only compound **2a** was reported by Venkateswarlu *et al.* [15] and all others are new molecules. To study the effect

TABLE-1
PHYSICAL DATA OF SYNTHESIZED
COMPOUNDS (2a-j AND 3a-k)

Compd.	R	R ₁	Reaction time (s)	Yield (%)	
				Reported	Obtained*
2a	4-OCH ₃	3-NO ₂	135	64 ^a	85
2b	4-Cl	H	225	—	60
2c	-H	4-Cl	260	—	74
2d	4-Cl	3-NO ₂	340	—	81
2e	2-Cl	3-Cl	200	—	72
2f	-H	4-OH	160	—	65
2g	4-OH	H	220	—	79
2h	4-OH	4-OH	310	—	68
2i	-H	3-NO ₂	380	—	82
2j	4-OH	3-NO ₂	280	—	76
3a	4-OCH ₃	4-Cl	200	—	81
3b	4-Cl	H	280	—	75
3c	-H	4-Cl	170	—	68
3d	-H	H	200	—	80
3e	4-Cl	4-Cl	290	—	64
3f	-H	4-OH	400	—	77
3g	4-OH	H	320	—	86
3h	4-OH	4-OH	340	—	94
3i	-H	3-NO ₂	380	—	88
3j	4-OH	3-NO ₂	190	—	62
3k	4-Cl	3-NO ₂	220	—	74

^a[Ref. 15]

of substitution on antioxidant activity, a series of Mannich bases were prepared by substituting the phenyl rings present at 4th and 6th positions of DHPMs with various electron donating and electron withdrawing groups. Regioselectivity was achieved by employing weak base like K₂CO₃.

Antioxidant activity

DPPH scavenging activity: Till date, antioxidant activity of substituted DHPMs are unexplored. In the present study, efforts have been laid down to evaluate their radical scavenging potency. As presented in Table-2, O- and N-Mannich bases of DHPMs (2a-j and 3a-k, respectively) with IC₅₀ values in the range of 0.13-0.19 μM showed higher DPPH radical scavenging activities compared to standard ascorbic acid. This can be attributed to the H⁺ donating capacity of hydroxyl aryl moieties of DHPMs.

Structural activity relationship (SAR) studies revealed that the phenolic group is essential for radical scavenging activity, as the compounds with phenolic hydroxyl groups **3h**, **2h**, **3f**, **3g**, **2g** and **2f** exhibited profound activity. This could be attributed to the stability of phenoxide ion formed in the due course. It is well established that phenolic hydroxyl group is responsible for antioxidant activity [22]. In compounds **3h** and **2h**, two hydroxyl groups are present on phenyl rings (one on each) at *p*-position, therefore the radical ion generated from the abstraction of either H atom (from either of hydroxyl groups) by DPPH would be stabilized by other hydroxyl group and *vice-versa*. Moreover, an additional stability can be attributed by the formation of phenoxide ion. Wherein, the phenoxide ion is in conjugation with dihydropyrimidine ring enhancing its stability. Thus, compounds **3h** and **2h** with two hydroxyl groups at *p*-position on phenyl rings exhibited highest activity. The compounds **2g**, **2f**, **3g** and **3f** exhibited less activity com-

TABLE-2
ANTIOXIDANT ACTIVITY (IC₅₀, μM*) OF
MANNICH BASES (2a-j AND 3a-k)

Compd.	DPPH scavenging activity	H ₂ O ₂ scavenging activity	NO scavenging activity	Iron chelation activity
2a	0.27±0.043	0.24±0.087	0.16±0.091	0.15±0.044
2b	0.33±0.023	0.31±0.052	0.29±0.065	0.38±0.053
2c	0.36±0.081	0.32±0.042	0.28±0.045	0.36±0.078
2d	0.44±0.029	0.42±0.081	0.34±0.076	0.21±0.049
2e	0.40±0.062	0.39±0.066	0.37±0.023	0.42±0.092
2f	0.19±0.084	0.17±0.023	0.20±0.096	0.27±0.084
2g	0.16±0.045	0.20±0.036	0.18±0.054	0.24±0.073
2h	0.13±0.074	0.15±0.021	0.17±0.032	0.23±0.082
2i	0.29±0.059	0.35±0.032	0.26±0.046	0.20±0.068
2j	0.23±0.042	0.27±0.056	0.25±0.038	0.18±0.026
3a	0.24±0.056	0.27±0.087	0.26±0.091	0.27±0.044
3b	0.35±0.069	0.36±0.052	0.37±0.065	0.35±0.053
3c	0.37±0.091	0.34±0.042	0.38±0.045	0.41±0.078
3d	0.26±0.051	0.33±0.081	0.39±0.076	0.51±0.049
3e	0.45±0.072	0.39±0.066	0.45±0.023	0.47±0.092
3f	0.15±0.036	0.22±0.023	0.29±0.096	0.25±0.084
3g	0.16±0.024	0.19±0.036	0.24±0.054	0.22±0.073
3h	0.12±0.038	0.16±0.021	0.19±0.032	0.17±0.042
3i	0.29±0.073	0.44±0.032	0.35±0.046	0.20±0.068
3j	0.19±0.045	0.30±0.056	0.33±0.038	0.16±0.026
3k	0.51±0.064	0.37±0.046	0.41±0.027	0.29±0.058

*IC₅₀ values were expressed as Mean ± SEM (standard error mean).

pared to compounds **3h** and **2h** due to the presence of single hydroxyl groups. However, these compounds exhibited better antioxidant activity compared to unsubstituted ones (**3d**) and the compounds substituted with electron withdrawing groups such as nitro and chloro groups (**2i**, **2d**, **3e**, **3k**). The compounds **2j** and **3j** substituted with one hydroxyl group at *p*-position on one phenyl ring and with nitro group at *m*-position in other phenyl ring exhibited lower activity compared to compounds with single hydroxyl group such as compounds **2g**, **2f**, **3g** and **3f**. The negative mesomeric effect of *m*-nitro group could be the reason for this. Compounds **2a** and **3a** exhibited moderate to higher activity compared to compounds **2i** and **3i**, respectively. This may be due to predominant stabilization by electron donating methoxy group counteracting the electron withdrawing effect of chloro and nitro groups on the other ring. The compounds **2b**, **2c**, **3b** and **3c** are less active compared to compounds **2i** and **3i**. These four compounds have one chloro substitution on either of the rings destabilizing the radical formed by virtue of its electronegativity. The same effect is responsible for the least scavenging effects of compounds **2d**, **2e**, **3e** and **3k**. The compound **2e** exhibits better activity than compound **2d** in O-Mannich bases (similarly compound **3e** exhibits more activity than compound **3k** in N-Mannich bases). The compounds **2e** and **3e** contains one chlorine group on both phenyl rings. Among these, compound **2e** exhibits better activity than compound **3e**. In compound **3e**, two chlorine groups are present in *p*-positions of two phenyl rings, which would strongly destabilizes the rings, whereas in compound **2e**, the two chlorine groups occupy *m*- and *o*- positions of the two phenyl rings. However, variation in *o*-, *m*- and *p*-positions has shown very little effect on scavenging. The least scavenging ability of compounds **2d** and **3k** is attributed to the presence

of strong electron withdrawing groups chlorine and nitro on one phenyl ring each. In both compounds, chlorine is present at *p*-position and nitro in *m*-position. Both the groups strongly destabilize the rings and are responsible for least activity. Alternatively, compound **3d** with unsubstituted phenyl rings exhibited better activity than the compounds substituted with electron withdrawing groups (like compounds **3e**, **3b**, **3k**, *etc.*) but showed lesser activity compared to compounds with electron donating groups (such as compounds **3h**, **3g** and **3a**) for obvious reasons mentioned above.

Among DPHM series, the above observations clearly depicted that phenyl rings substituted with electron donating groups is a prerequisite for the scavenging of free radicals along with the DHPM pharmacophore, but alone it is insufficient for radical scavenging activity. Comparatively N-Mannich bases exhibited slightly better activity than O-Mannich bases. N-Mannich bases might add additional stability to the ring systems as two methyl groups present on 'N' are strongly electron donating in nature. Furthermore, one more hydroxyl group (as ketone oxygen at 2nd position of DHPMs can exist in enol form) is added to the DHPM ring system in N-Mannich bases. This hydroxyl moiety may also participate in radical scavenging as well as ring stabilization, whereas the same is not possible in O-Mannich bases.

H₂O₂ scavenging activity: Among the synthesized compounds, **2h** and **3h** exhibited highest activity in this method also for the same reasons mentioned above. The compounds substituted with electron donating groups such as hydroxyl, methoxy (**2f**, **2g**, **3f**, **3g**, **2a** and **3a**) showed moderate to good activity. The compounds with electron withdrawing groups such as chloro and nitro at various positions (**2b**, **2c**, **3b**, **3c**, **2i**, **2e**, **3i**, **3e** and **3k**) exhibited poor activity. This may be due to its inability to donate a hydrogen atom to quench the peroxide radical as discussed above.

NO scavenging activity: Targeting the NO production is a promising strategy in search of novel potent drugs against inflammatory related degenerative diseases [23]. In terms of NO inhibitory activity, Mannich bases of DHPMs substituted with hydroxyl groups displayed excellent activity (**3h**, **3g**, **2h** and **2g**). In this case, it seems to be that presence of hydroxyl group in *p*-position is a prerequisite. Further, the presence of methoxy group at *p*-position of phenyl ring (**2a** and **3a**) also displayed better activity. This may be due to the strong electron donating nature of the methoxy groups. But in the absence of electron donating groups, the effect of electron withdrawing groups is predominant as seen in compounds **2b**, **2c**, **2d**, **2e**, **3c**, **3k**, **3e**. Optimum activity of compounds **2i** and **3j** is attributed to the presence of hydroxyl group on phenyl ring at *p*-position, inspite of having electron withdrawing groups.

Iron chelation activity: Accumulation of unmetabolized excess of free elemental iron is toxic and builds up various ROS *via* Fenton reaction [24]. Therefore, the synthesized compounds were also evaluated for their iron chelation efficiency. Among all the compounds, **2a** and **3j** exhibited excellent metal chelation capacity. This may be due to the involvement of oxygens (of nitro group at *m*-position on phenyl ring) by forming coordinate covalent bonds with metallic iron. Further the metal chelation activity may be enhanced with the electron donating

nature of methoxy and hydroxyl groups (at *p*-position on other phenyl ring) by stabilizing the ring system. SAR studies reveal that all molecules containing nitro group such as **2i**, **2j**, **2d**, **3i**, **3k** exhibited better activity compared to others. The compounds **2h** and **3h** were also found to exhibit good activity due to the presence of hydroxyl group at *p*-position on phenyl ring. Compounds containing neither nitro group nor hydroxyl group exhibited poor activity due to lack of iron chelating atoms. The presence of chlorine in most of these compounds may decrease the iron chelation activity by virtue of its electron negativity.

Drug likeness: The physico-chemical properties of all the synthesized compounds **2a-j** and **3a-k** have been calculated *in silico* and presented in Table-3. Total polar surface area (TPSA) [25] is directly correlated with the transport of molecules across membranes. All the compounds showed TPSA values within the range of limit (< 140 Å). The clogP value is a legitimate measure to determine their hydrophilicity. Compounds with low clogP (< 5) values possess good permeability, there by enhanced bioavailability. All the O- and N-Mannich bases (**2a-j** and **3a-k**) exhibited acceptable clogP value in the range of 0.53-3.46. Interestingly compound **3h**, with excellent antioxidant activity was found to contain least clogP value. Other molecular descriptors such as molecular weight, hydrogen bond donor /acceptors and the number of rotatable bonds (ROTB), were found to be in acceptable range and follow the Lipinski's rule of five. The number of ROTB were also found in between 4-6 (≥ 6) [26]. In a nutshell, *in silico* evaluation of the physico-chemical parameters portrays the drug likeness of synthesized compounds.

TABLE-3
PHYSICO-CHEMICAL PROPERTIES OF SYNTHESIZED
O-MANNICH (**2a-j**) AND N-MANNICH BASES (**3a-k**)

Compd.	Physico-chemical properties					
	MW	TPSA	HBA	HBD	ROTB	cLogP
2a	382.16	36.86	4	1	5	2.59
2b	341.13	36.86	4	1	5	3.31
2c	341.13	36.86	4	1	5	3.31
2d	386.11	80	4	1	6	2.89
2e	375.09	36.86	4	1	5	3.46
2f	323.16	57.09	5	2	5	2.65
2g	323.16	57.09	5	2	5	2.65
2h	339.16	77.32	6	3	5	1.94
2i	352.15	80	4	1	6	2.95
2j	368.15	100.23	5	2	6	2.23
3a	371.52	35.58	4	1	4	1.19
3b	341.13	35.58	4	1	4	1.91
3c	341.13	35.58	4	1	4	1.91
3d	307.17	35.58	4	1	4	1.96
3e	375.09	35.58	4	1	4	1.85
3f	323.16	55.81	5	2	4	1.25
3g	323.16	55.81	5	2	4	1.25
3h	339.16	76.04	6	3	4	0.53
3i	352.15	78.72	4	1	5	1.54
3j	368.15	98.95	5	2	5	0.83
3k	386.11	78.72	4	1	5	1.49

MW = Molecular weight; TPSA = Total polar surface area; HBA = Hydrogen bond acceptor; HBD = Hydrogen bond donor; ROTB = Number of rotatable bonds.

Conclusion

Compounds **3h**, **3g**, **2h** and **2g** exhibited better radical scavenging activity in all methods except iron chelation ability. Structure activity relationship studies revealed the effect of electron donating and electron withdrawing groups on antioxidant activity. N-Mannich bases exhibited superior scavenging activity over O-Mannich bases. *in silico* Prediction of physico-chemical parameters revealed the drug likeness of synthesized compounds. The present studies revealed that substitution of dihydropyrimidinones (DHPMs) at the specified positions will improve their antioxidant properties. This adds a top up antioxidant property to DHPMs that have proven anti-inflammatory, anti-cancer, neuro protective activities. It is believed that the results in this paper will be of immediate utility to many scientists who are working on drug repurposing.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

- V. Lobo, A. Patil, A. Phatak and N. Chandra, *Pharmacogn. Rev.*, **4**, 118 (2010); <https://doi.org/10.4103/0973-7847.70902>.
- M. Valko, M. Izakovic, M. Mazur, C.J. Rhodes and J. Telser, *J. Mol. Cell. Biochem.*, **266**, 37 (2004); <https://doi.org/10.1023/B:MCBI.0000049134.69131.89>.
- B. Mistry, R.V. Patel, Y.-S. Keum and D.H. Kim, *Saudi J. Biol. Sci.*, **24**, 36 (2017); <https://doi.org/10.1016/j.sjbs.2015.09.005>.
- K. de la Vega-Hernández, M. Antuch, O. Cuesta-Rubio, Y. Núñez-Figueroa and G.L. Pardo-Andreu, *J. Inorg. Biochem.*, **170**, 134 (2017); <https://doi.org/10.1016/j.jinorgbio.2017.02.019>.
- J.M. McCord, *Am. J. Med.*, **108**, 652 (2000); [https://doi.org/10.1016/S0002-9343\(00\)00412-5](https://doi.org/10.1016/S0002-9343(00)00412-5).
- C.A. Rice-Evans and A.T. Diplock, *Free Radic. Biol. Med.*, **15**, 77 (1993); [https://doi.org/10.1016/0891-5849\(93\)90127-G](https://doi.org/10.1016/0891-5849(93)90127-G).
- S.A. Haque, T.P. Hasaka, A.D. Brooks, P. Lobanov and P.W. Baas, *Cell Motil. Cytoskeleton*, **58**, 10 (2004); <https://doi.org/10.1002/cm.10176>.
- R.H. Tale, A.H. Rodge, G.D. Hatnapure and A.P. Keche, *Bioorg. Med. Chem. Lett.*, **21**, 4648 (2011); <https://doi.org/10.1016/j.bmcl.2011.03.062>.
- C.A. Winter, E.A. Risley and G.W. Nuss, *Exp. Biol. Med.*, **111**, 544 (1962); <https://doi.org/10.3181/00379727-111-27849>.
- M.R. Bhosle, A.R. Deshmukh, S. Pal, A.K. Srivastava and R.A. Mane, *Bioorg. Med. Chem. Lett.*, **25**, 2442 (2015); <https://doi.org/10.1016/j.bmcl.2015.03.068>.
- S.M. Sondhi, N. Singh, M. Johar and A. Kumar, *Bioorg. Med. Chem.*, **13**, 6158 (2005); <https://doi.org/10.1016/j.bmc.2005.06.063>.
- A.R. Trivedi, V.R. Bhuva, B.H. Dholariya, D.K. Dodiya, V.B. Kataria and V.H. Shah, *Bioorg. Med. Chem. Lett.*, **20**, 6100 (2010); <https://doi.org/10.1016/j.bmcl.2010.08.046>.
- S. Fatima, A. Sharma, R. Saxena, R. Tripathi, S.K. Shukla, S.K. Pandey, R. Tripathi and R.P. Tripathi, *Eur. J. Med. Chem.*, **55**, 195 (2012); <https://doi.org/10.1016/j.ejmech.2012.07.018>.
- R.R. Alavala, U. Kulandaivelu, P. Bonagiri, S. Boyapati, V. Jayaprakash and A.T. Subramaniam, *Antimicrobial Agents*, **13**, 154 (2015); <https://doi.org/10.2174/221135251302151029111113>.
- K. Venkateshwarlu, G. Chakradar Rao, V.M. Reddy and Y. Narasimha Reddy, *J. Iran. Chem. Soc.*, **11**, 1619 (2014); <https://doi.org/10.1007/s13738-014-0438-2>.
- P.K. Sahu, *Eur. J. Med. Chem.*, **121**, 510 (2016); <https://doi.org/10.1016/j.ejmech.2016.05.037>.
- L. Ismaili, A. Nadaradjane, L. Nicod, C. Guyon, A. Xicluna, J. Robert and B. Refouvet, *Eur. J. Med. Chem.*, **43**, 1270 (2008); <https://doi.org/10.1016/j.ejmech.2007.07.012>.
- B. Ramesh and C.M. Bhalgat, *Eur. J. Med. Chem.*, **46**, 1882 (2011); <https://doi.org/10.1016/j.ejmech.2011.02.052>.
- E.V. Buravlev, O.G. Shevchenko, A.A. Anisimov and K.Y. Suponitsky, *Eur. J. Med. Chem.*, **152**, 10 (2018); <https://doi.org/10.1016/j.ejmech.2018.04.022>.
- B.R. Prashantha Kumar, G. Sankar, R.B. Nasir Baig and S. Chandrashekar, *Eur. J. Med. Chem.*, **44**, 4192 (2009); <https://doi.org/10.1016/j.ejmech.2009.05.014>.
- R. Mamidala and A. Vema, *Int. J. Pharm. Biol. Sci.*, **7**, 94 (2017).
- K.I. Priyadarsini, D.K. Maity, G.H. Naik, M.S. Kumar, M.K. Unnikrishnan, J.G. Satav and H. Mohan, *Free Radic. Biol. Med.*, **35**, 475 (2003); [https://doi.org/10.1016/S0891-5849\(03\)00325-3](https://doi.org/10.1016/S0891-5849(03)00325-3).
- C. Bogdan, *Nat. Immunol.*, **2**, 907 (2001); <https://doi.org/10.1038/ni1001-907>.
- K.N. Lokesh, Channarayappa, M. Venkataranganna, G.G. Raj, H. Patil and H. Dave, *J. Trace Elem. Med. Biol.*, **45**, 114 (2018); <https://doi.org/10.1016/j.jtemb.2017.10.001>.
- P. Ertl, B. Rohde and P. Selzer, *J. Med. Chem.*, **43**, 3714 (2000); <https://doi.org/10.1021/jm000942e>.
- F.D. Veber, S.R. Johnson, H.-Y. Cheng, B.R. Smith, K.W. Ward and K.D. Kopple, *J. Med. Chem.*, **45**, 2615 (2002); <https://doi.org/10.1021/jm020017n>.