Indian Journal of Chemistry
Vol. 48B, January 2009, pp. 88-96

Synthesis and *in vitro* study of biological activity of heterocyclic N-Mannich bases

T B Shah*[a], A Gupte[b], M R Patel, V S Chaudhari, H Patel & V C Patel

[a]Department of Chemistry

and

[b]N V Patel Science College, Sardar Patel University, Vallabh Vidyanagar 388 120, India

E-mail: vish_ckpatel@yahoo.co.in

Received 22 February 2008; accepted (revised) 19 September 2008

Three series of N-Mannich bases of 3,4-dihydropyrimidine-2(1*H*)-ones(DHPMs) **7a-g, 8a-g,** and **9a-g** have been prepared by Mannich reaction of DHPMs **4a-c** with seven different heterocyclic secondary amino compounds **6a-g** and formaldehyde **5**. The precursors DHPMs **4a-c** have been derived by a Biginelli reaction of three aromatic aldehydes **1a-c** with ethylacetoacetate **2** and urea **3**. The chemical structure of all the three series of N-Mannich bases have been elucidated by elemental analysis and spectral studies (IR, ^{1}H and ^{13}C NMR). They have been assayed *in vitro* for their biological activity against *E. coli* and *B. subtilis* bacterial species and *A. niger* and *C. albicans* fungal microorganisms.

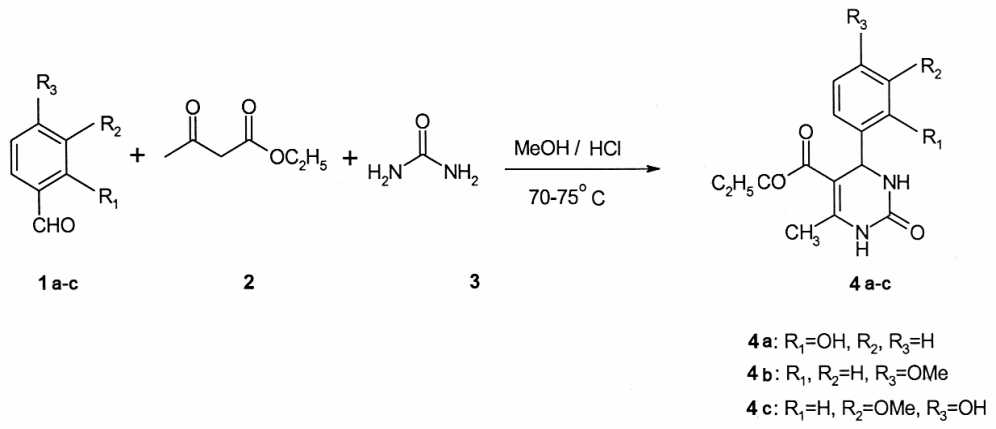
Keywords: Biginelli reaction, dihydropyrimidones, N-Mannich bases, antimicrobial activity

Dihydropyrimidone moieties have been reported to possess diverse pharmacological activities like antiviral, antibacterial and antihypertensive activity and have efficacy as calcium channel modulators and α_{1a}-antagonist[1,2]. In addition to this, the biological activity of some alkaloids isolated recently has been attributed to the dihydropyrimidinone moiety, notably monastrol is the only cell-permeable molecule currently known to specifically inhibit mitotic kinesis E_g5 and therefore considered as a lead for the development of new anticancer drugs[3,4]. Further a considerable amount of work has been reported on the synthesis and pharmacological activity of various Mannich bases for analgesic, antispasmodic, anesthetic and antimicrobial activity as well as intermediates in drug synthesis[5-18]. In this context, literature survey has revealed a number of reports on antimicrobial activity of N-Mannich bases derived from different heterocycles such as pyrrole, pyrazole, benzimidazole, benzotriazole, *etc*[19-25]. R S Verma has carried out Mannich reaction of five membered heterocyclic ring systems with formaldehyde and primary or secondary amines and the resulting compounds have been tested for antibacterial, antifungal, antiviral, anticancer, antileishmanial and antimalarial activity[26]. Keeping in view the importance of these two organic moieties, DHPMs and N-Mannich base in the field of medicine and biology, an attempt has now been made to synthesize novel heterocyclic N-Mannich bases containing both the moieties and to investigate their antimicrobial activity.

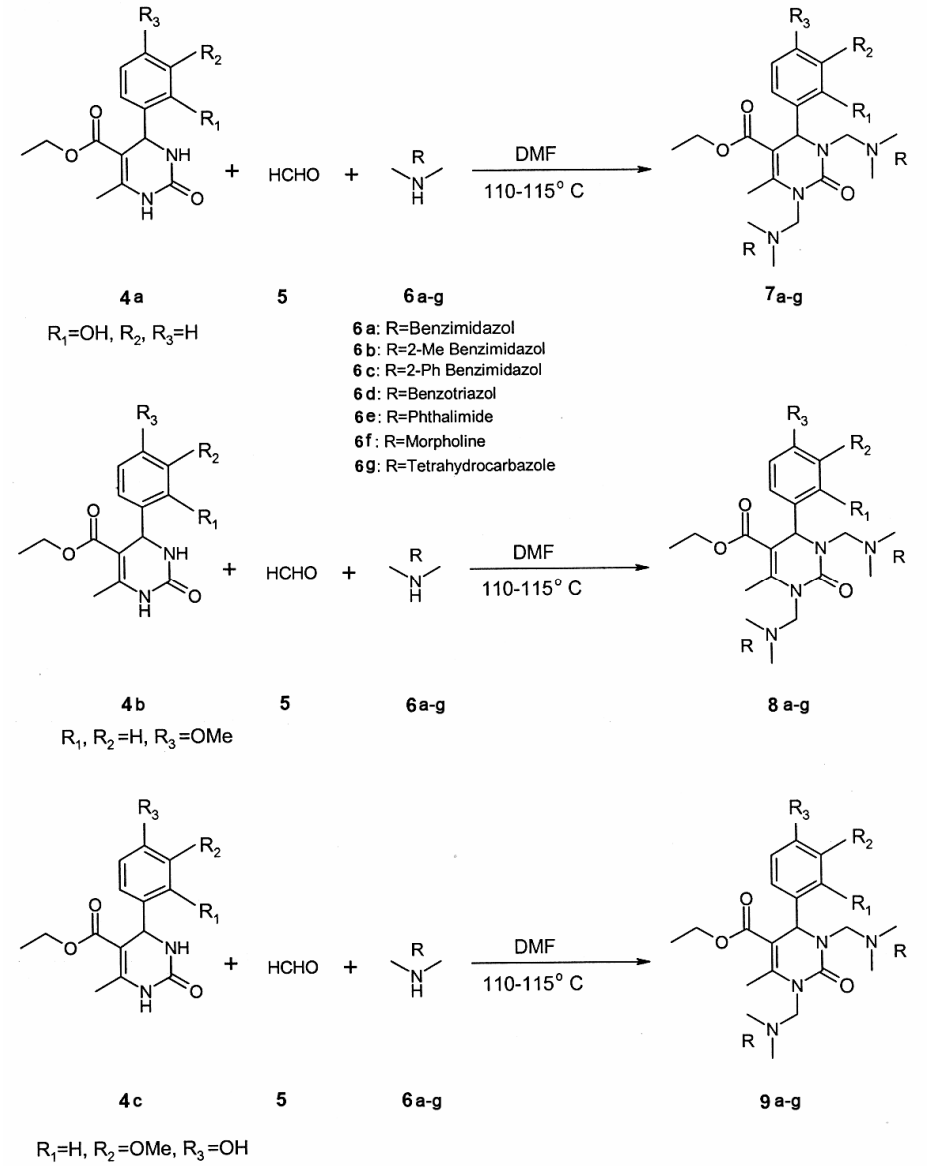
The present paper describes the synthesis of N-Mannich bases of DHPMs and *in vitro* evaluation of their antimicrobial activity against Gram –ve bacteria (*E. coli*), Gram +ve bacteria (*B. Subtilis*), fungi (*A. niger*) and yeast fungi (*C. albican*). The heterocyclic precursor DHPMs **4a-c** were synthesized by Biginelli reaction of aromatic aldehydes **1a-c**, ethyl acetoacetate **2** and urea **3** according to the literature procedure[27] **(Scheme I)**. Three series of N-Mannich bases of DHPMs **(7a-g, 8a-g, 9a-g)** have been synthesized by Mannich reaction of 3,4-Dihydropyrimidin-2(1*H*)-one **4a-c** with seven different heterocyclic secondary amines namely benzimidazole, 2-methyl benzimidazole, 2-benzyl benzimidazole, benzotriazole, phthalimide, morpholine, terahydrocarbazole **6a-g** and formaldehyde **5** (**Scheme II**). All the three DHPMs substrates and their N-Mannich bases have been characterized by elemental and spectral studies. The analytical data of the three series of N-Mannich bases derived from three DHPMs substrates are given in **Table I**.

Result and Discussion

DHPMs precursors **4a-c** synthesized by one pot multicomponent Biginelli reaction were obtained in good yield (≥ 80%). Synthesis of N-Mannich bases based on these DHPMs substrates resulted in three series of N-Mannich bases **7a-g, 8a-g** and **9a-g**,



Scheme I — Synthetic protocol for DHPMs



Scheme II — Synthetic protocol for the heterocyclic N-Mannich bases

70-90% yield. Examination of analytical and spectral data of all the N-Mannich bases **(Table I** and **Table II)** are in good agreement with calculated values based on proposed structure shown in **Scheme II**.

Spectral studies of N-Mannich bases have shown the following characteristic features: The characteristic absorption bands of DHPMs component in IR spectra of N-Mannich bases resemble the pattern observed for parent DHPMs substrate reported in Experimental Section with the exception that the absorption bands at 1550 and 700 cm^{-1} due to secondary (-NH) group of DHPM substrate disappeared in the IR spectra of each of the N-Mannich bases. Two strong bands respectively in the region 2780-2770 and 1430-1420 cm^{-1} are due to –CH stretching and bending vibration of two methylene linkages between DHPMs substrate and two heterocyclic secondary amino components. ^1H NMR spectra of the N-Mannich bases have shown the absence of two (1H, –NH) singlet of secondary amino groups of DHPMs ring systems. This suggest that the hydrogen atom of two secondary amino groups have reacted with formaldehyde and heterocyclic amino compounds to form disubstituted N-Mannich bases. This can be further confirmed from the appearance of two new ^1H NMR signals in the range of δ 4.20-6.10 and 4.80-6.90 values due to (2H, $–CH_2$) of methylene linkage formed between DHPMs and heterocyclic secondary amino compounds. The strong bands in the region of 1750-1690 cm^{-1} represent the –C=O stretching vibration of the conjugated ester. All these inferences support the predicted chemical structure of novel N-Mannich bases as shown in **Scheme II**.

Table I — Analytical characterization data of N-Mannich bases **7a-g, 8a-g** and **9a-g**

Compd	Yield %	m.p. °C	Mol. formula	Found (%) (Calcd) C	H	N
7a	81	186	$C_{30}H_{28}N_6O_4$	67.15 (67.28	5.26 5.18	15.66 15.52)
7b	76	189	$C_{32}H_{32}N_6O_4$	68.07 (67.94	5.71 5.80	14.88 14.66)
7c	81	255	$C_{44}H_{40}N_6O_4$	73.72 (73.51	5.62 5.89	11.72 11.61)
7d	75	215	$C_{28}H_{26}N_8O_4$	62.45 (62.52	4.87 4.78	20.81 20.77)
7e	84	230	$C_{32}H_{26}N_4O_8$	64.64 (64.29	4.41 4.35	9.42 9.15)
7f	76	204	$C_{24}H_{34}N_4O_6$	60.74 (60.44	7.22 7.53	11.81 11.38)
7g	71	222	$C_{40}H_{42}N_4O_4$	74.74 (74.91	6.59 6.35	8.72 8.45)
8a	78	201	$C_{31}H_{30}N_6O_4$	67.62 (67.77	5.49 5.64	15.26 15.42)
8b	82	201	$C_{33}H_{34}N_6O_4$	68.50 (68.33	5.92 5.66	14.52 14.47)
8c	87	195	$C_{45}H_{42}N_6O_4$	73.95 (73.60	5.79 5.56	11.50 11.67)
8d	86	188	$C_{29}H_{28}N_8O_4$	63.03 (62.91	5.11 5.32	20.28 20.33)
8e	71	166	$C_{33}H_{28}N_4O_8$	65.13 (65.27	4.64 4.44	9.21 9.04)
8f	79	215	$C_{25}H_{36}N_4O_6$	61.46 (61.63	7.43 7.59	11.47 11.24)
8g	80	236	$C_{41}H_{44}N_4O_4$	74.97 (74.77	6.75 6.49	8.53 8.17)
9a	77	242	$C_{31}H_{30}N_6O_5$	65.71 (65.62	5.34 5.09	14.83 14.62)
9b	77	235	$C_{33}H_{34}N_6O_5$	66.65 (66.37	5.76 5.54	14.13 14.22)
9c	82	193	$C_{45}H_{42}N_6O_5$	72.37 (72.08	5.67 5.81	11.25 11.13)
9d	83	179	$C_{29}H_{28}N_8O_5$	61.26 (61.11	4.96 4.78	19.71 19.57)
9e	85	196	$C_{33}H_{28}N_4O_9$	63.46 (63.61	4.52 4.25	8.97 8.82)
9f	71	243	$C_{25}H_{36}N_4O_7$	59.51 (59.28	7.19 7.34	11.10 11.27)
9g	80	252	$C_{41}H_{44}N_4O_5$	73.19 (73.00	6.59 6.67	8.33 8.15)

Antimicrobial activity

The result of *in vitro* study of antimicrobial activity of N-Mannich bases against each of the two bacterial species (*E. coli*, *B. subtillis*) and two fungal species (*A. niger, C. albican*) are reported in **Table III**. Compounds **7f** and **9f** have shown excellent antibacterial activity against *E. coli* and *B. subtillis* comparable to that of Streptomycin. They inhibited the bacterial growth upto 98-100% at 500 ppm concentration, whereas **7a,7b, 7e, 8f, 9a** and **9b** were found to have moderate antibacterial activity in the range of 60-96% at 500 ppm concentration and the remaining compounds have poor antibacterial activity. Analogously, **7f, 8f** and **9f** have shown promising antifungal activity and the remaining compounds were moderately active against both the fungal species (*A. niger* and *C. albican).*

Experimental Section

Laboratory grade ethylacetoacetate, urea, different aldehydes and other solvents were used after purifying as and when required. Benzimidazole, 2-methylbenzimidazole, 2-benzylbenzimidazole, benzotriazole, phthalimide and terahydrocarbazole used were synthesized by the literature method[21]. Melting points of Mannich bases were determined by open capillary method and are uncorrected. Elemental

Table II — Spectral characterization data of N-Mannich bases **7a-g**, **8a-g** and **9a-g**

Compd*	^{1}H NMR (δ, ppm)	^{13}C NMR (δ, ppm)
7a	1.20 (t) 3H, -CH_3 of ester group 2.31 (s) 3H, -CH_3 of Pyrimidine ring 4.09 (q) 2H, -CH_2 of ester group 6.13 (s) 2H, -CH_2 of amino methyl bridge 8.00 (s) 1H, -CH of imidazole ring at 2-position 6.95 (s) 1H, H on Pyrimidine ring 9.01(s) 1H, -OH of phenyl ring present in Biginelli compound 7.11-8.10 (m) 12H, Three aromatic rings	116.21(d),154.88(s),130.04(d),120.96(s), 117.71(d),128.42(d),53.9(d),148.85(s), 101.34(s),149.85(s),167.74(s),60.96(t), 13.95(q),17.57(q),43.98(t),47.88(t), 132.32(d),140.73(s),132.66(s),133.4(s), 143.5(d),144.34(s),120.06(d),120.29(d), 107.13(d),122.56(d),110.74(d),122.3(d), 119.8(d),121.00(d)
7b	1.20 (t) 3H, -CH_3 of ester group 2.31 (s) 3H, -CH_3 of Pyrimidine ring 4.08 (q) 2H, -CH_2 of ester group 6.11 (s) 2H, -CH_2 of amino methyl bridge 2.48 (s) 3H, -CH_3 of imidazole ring 6.95 (s) 1H, H on Pyrimidine ring 9.00(s) 1H, -OH of phenyl ring present in Biginelli compound 7.0-7.6 (m) 12H, Three aromatic rings	116.21(d),155.12(s),130.04(d),121.5(s), 117.71(d),128.66(d),53.48(d),148.85(s), 101.34(s),144.98(s),167.74(s),60.96(t), 13.95(q),17.57(q),53.31(t),42.09(t), 147.21(s),65.97(d),68.91(d),68.85(d), 158.39(s),65.97(d),134.89(d),120.47(d), 120.99(d),109.83(d),106.6(d),109.83(d), 134.75(d), 120.47(d),19.9(q),19.22(q)
7c	1.20 (t) 3H, -CH_3 of ester group 2.30 (s) 3H, -CH_3 of Pyrimidine ring 4.00 (q) 2H, -CH_2 of ester group 6.25 (s) 2H, -CH_2 of amino methyl bridge 3.68 (s) 2H, -CH_2 of benzyl group on imidazole ring 6.95 (s) 1H, H on Pyrimidine ring 9.01(s) 1H, -OH of phenyl ring present in Biginelli compound 6.6-7.7 (m)22H, Five aromatic rings	116.21(d),154.88(s),130.04(d),120.96(s), 117.71(d),128.42(d),53.9(d),148.85(s), 101.34(s),149.85(s),167.74(s),60.96(t), 13.95(q),17.57(q),53.65(t),42.44(t), 133.11(s),143.85(s),142.42(s),133.85(s), 147.43(s),156.91(s),107.3(d),124.36(d), 119.82(d),121.6(d),110.92(d),124.09(d), 119.56(d),121.62(d),28.07(t),135.25(s), 28.3(t),135.52(s),128.09(d),128.57(d), 126.03(d),128.09(d),128.57(d),128.8(d), 128.57(d), 126.03(d),128.8(d),128.57(d)
7d	1.20 (t) 3H, -CH_3 of ester group 2.31 (s) 3H, -CH_3 of Pyrimidine ring 4.09 (q) 2H, -CH_2 of ester group 6.56 (s) 2H, -CH_2 of amino methyl bridge 9.00(s) 1H, -OH of phenyl ring present in Biginelli compound 6.85 (s) 1H, H on Pyrimidine ring 6.9-8.0 (m) 12H, Three aromatic ring	116.21(d),154.72(s),130.04(d),121.94(s), 117.71(d),127.26(d),54.2(d),147.95(s), 103.29(s),146.17(s),167.74(s),60.96(t), 13.95(q),17.57(q),53.66(t),42.44(t), 132.75(s),143.77(s),147.38(s),133.49(s), 107.17(d),129.34(d),120.84(d),124.21(d), 120.57(d),124.92(d),110.78(d), 129.07(d)
7e	1.20 (t) 3H, -CH_3 of ester group 2.31 (s) 3H, -CH_3 of Pyrimidine ring 4.09 (q) 2H, -CH_2 of ester group 5.87 (s) 2H, -CH_2 of amino methyl bridge 6.74 (s) 1H, H on Pyrimidine ring 9.00(s) 1H, -OH of phenyl ring present in Biginelli compound 6.9-7.7 (m) 13H, Three aromatic rings	116.21(d),156.2(s),130.04(d),121.96(s), 117.71(d),129.74(d),50.09(d),150.6(s), 101.03(s),141.08(s),167.74(s),60.96(t), 13.95(q),17.57(q),33.51(t),47.00(t), 165.64(s),130.8(s),130.8(s),165.64(s), 165.51(s),131.32(s),165.51(s),131.32(s), 123.65(d),134.58(d),123.65(d),134.58(d), 123.66(d), 134.58(d),123.66(d),134.58(d)
7f	1.20 (t) 3H, -CH_3 of ester group 2.31 (s) 3H, -CH_3 of pyrimidine ring 4.00 (q) 2H, -CH_2 of ester group 4.22 (s) 2H, -CH_2 of aminomethyl bridge 6.66 (s) 1H, H on pyrimidine ring 2.66 (s) 2H, -CH_2 of morpholine ring adjacent to nitrogen 3.56 (s) 2H, -CH_2 of morpholine ring adjacent to oxygen 9.01(s) 1H, -OH of phenyl ring present in Biginelli compound 6.7-7.2 (m)4H, one aromatic rings	116.21(d),153.99(s),130.04(d),120.96(s), 117.71(d),127.52(d),54.34(d),148.85(s), 101.34(s),148.54(s),167.74(s),60.96(t), 13.95(q),17.57(q),62.19(t),61.45(t),63.24(t),58.8(t), 63.24(t),58.8(t),58.8(t),58.8(t), 58.8(t),58.8(t)

Contd —

Table II — Spectral characterization data of N-Mannich bases **7a-g**, **8a-g** and **9a-g** — *Contd*

Compd*	^{1}H NMR (δ, ppm)	^{13}C NMR (δ, ppm)
7g	1.10 (t) 3H, -CH_3 of ester group 2.30 (s) 3H, -CH_3 of pyrimidine ring 4.02 (q) 2H, -CH_2 of ester group 6.15 (s)2H, -CH_2 of aminomethyl bridge 6.96 (s) 1H, H on pyrimidine ring 9.01(s) 1H, -OH of phenyl ring present in Biginelli compound 2.64 (q) 2H, -CH_2 of carbazole ring adjacent to double bond 1.89 (q) 2H, -CH_2 of carbazole ring 7.0-7.6 (m)12H, Three aromatic rings	116.21(d),154.76(s),130.04(d),121.44(s), 117.71(d),127.30(d),55.26(d),148.85(s), 100.36(s),149.85(s),167.74(s),60.96(t), 13.95(q),17.57(q),57.2(t),42.89(t),132.91(s),93.09(s), 137.7(s),125.51(s),135.57(s), 93.96(s),138.04(s),128.94(s),111.7(d), 121.26(d),118.49(d),119.09(d),21.38(t), 22.99(t),22.25(t),22.99(t),20.45(t),22.99(t), 22.25(t),22.99(t),108.12(d),121.53(d), 118.75(d), 118.37(d)
+8a	1.21 (t) 3H, -CH_3 of ester group 2.31 (s) 3H, -CH_3 of pyrimidine ring 4.10 (q) 2H, -CH_2 of ester group 6.13 (s) 2H, -CH_2 of aminomethyl bridge 8.03 (s) 1H, -CH of imidazole ring at 2-position 7.01 (s) 1H, H on pyrimidine ring 3.75 (s) 3H, -OCH_3 of phenyl ring present in Biginelli compound 7.04-8.03 (m)12H, Three aromatic rings	113.21(d),132.03(d),160.15(s),126.32(s), 113.22(d),132.03(d),61.46(d),148.85(s), 100.36(s),149.85(s),167.74(s),60.96(t), 13.95(q),17.57(q),47.88(t),43.98(t), 132.66(s),140.73(s),132.32(d),133.4(s), 144.34(s),143.5(d),110.74(d),122.3(d), 119.8(d),121.0(d),107.13(d),122.56(d), 120.06(d),120.29(d),55.2(q)
+8b	1.27 (t) 3H, -CH_3 of ester group 2.32 (s) 3H, -CH_3 of pyrimidine ring 4.17 (q) 2H, -CH_2 of ester group 6.11 (s) 2H, -CH_2 of aminomethyl bridge 2.48 (s) 3H, -CH_3 of imidazole ring 7.00 (s) 1H, H on pyrimidine ring 3.78 (s) 3H, -OCH_3 of phenyl ring present in Biginelli compound 7.1-7.6 (m)12H, Three aromatic rings	113.21(d),132.03(d),160.15(s),126.32(s), 113.22(d),132.03(d),61.46(d),148.85(s), 100.36(s),149.85(s),167.74(s),60.96(t), 13.95(q),17.57(q),45.68(t),41.77(t),132.5(s),143.24(s), 140.47(s),133.24(s),146.85(s), 151.65(s),107.17(d),124.59(d),119.69(d), 121.83(d),110.79(d),124.33(d),119.43(d), 121.86(d),55.2(q),13.77(q), 13.09(q)
+8c	1.20 (t) 3H, -CH_3 of ester group 2.33 (s) 3H, -CH_3 of pyrimidine ring 4.17 (q) 2H, -CH_2 of ester group 6.11 (s) 2H, -CH_2 of aminomethyl bridge 3.66 (s) 2H, -CH_2 of benzyl group on imidazole ring 7.01 (s) 1H, H on pyrimidine ring 3.74 (s) 3H, -OCH_3 of phenyl ring present in Biginelli compound 7.1-7.8 (m)22H, Five aromatic rings	113.22(d),132.02(d),160.15(s),126.32(s), 113.22(d),132.02(d),61.46(d),148.85(s), 100.36(s),149.85(s),167.74(s),60.96(t), 13.95(q),17.57(q),53.65(t),42.44(t), 133.11(s),143.85(s),142.42(s),133.85(s), 147.43(s),156.91(s),107.3(d),124.36(d), 119.82(d),121.6(d),110.92(d),124.09(d), 119.56(d),121.62(d),55.2(q),28.3(t), 28.07(t),135.52(s),135.25(s),128.09(d), 128.57(d),126.03(d),128.09(d),128.57(d), 128.57(d),126.03(d),128.8(d),128.57(d), 128.8(d)
+8d	1.27 (t) 3H, -CH_3 of ester group 2.29 (s) 3H, -CH_3 of pyrimidine ring 4.11 (q) 2H, -CH_2 of ester group 6.47 (s) 2H, -CH_2 of aminomethyl bridge 3.76 (s) 3H, -OCH_3 of phenyl ring present in Biginelli compound 6.85 (s) 1H, H on pyrimidine ring 6.9-8.0 (m)12H, Three aromatic rings	113.22(d),132.87(d),160.15(s),127.14(s), 113.22(d),130.87(d),61.76(d),147.96(s), 102.36(s),146.17(s),167.74(s),60.96(t), 13.95(q),17.57(q),53.66(t),42.44(t), 132.75(s),143.77(s),133.49(s),147.38(s), 107.17(d),129.34(d),120.84(d),124.21(d), 110.78(d),129.07(d),120.57(d),124.92(d), 55.2(q)
+8e	1.20 (t) 3H, -CH_3 of ester group 2.34 (s) 3H, -CH_3 of pyrimidine ring 4.16 (q) 2H, -CH_2 of ester group 5.87 (s) 2H, -CH_2 of aminomethyl bridge 6.74 (s) 1H, H on pyrimidine ring 3.74 (s) 3H, -OCH_3 of phenyl ring present in Biginelli compound 7.2-7.7 (m)12H, Three aromatic rings	113.21(d),133.35(d),160.15(s),124.73(s), 113.22(d),133.35(d),57.38(d),150.06(s), 100.06(s),141.09(s),167.74(s),60.96(t), 13.95(q),17.57(q),33.51(t),47.00(t), 165.64(s),130.8(s),130.8(s),165.64(s), 165.51(s),131.32(s),165.51(s),131.32(s), 55.2(q),123.65(d),134.58(d),123.65(d), 134.58(d),123.66(d),134.58(d),123.66(d),134.58(d)

Contd —

Table II — Spectral characterization data of N-Mannich bases **7a-g**, **8a-g** and **9a-g** — *Contd*

Compd*	^{1}H NMR (δ, ppm)	^{13}C NMR (δ, ppm)
$^+$8f	1.12 (t) 3H, -CH_3 of ester group 2.35 (s) 3H, -CH_3 of pyrimidine ring 4.08 (q) 2H, -CH_2 of ester group 4.22 (s) 2H, -CH_2 of aminomethyl bridge 6.69 (s) 1H, H on pyrimidine ring 2.66 (s) 2H, -CH_2 of morpholine ring adjacent to nitrogen 3.56 (s) 2H, -CH_2 of morpholine ring adjacent to oxygen 3.75 (s) 3H, -OCH_3 of phenyl ring present in Biginelli compound 6.7-7.3 (m)4H, one aromatic rings	113.22(d),131.14(d),160.15(s),129.93(s), 113.22(d),131.14(d),61.9(d),148.85(s), 100.36(s),148.54(s),167.74(s),60.96(t), 13.95(q),17.57(q),62.19(t),55.2(q),61.54(t),58.8(t), 63.24(t),58.8(t),63.24(t),58.8(t), 58.8(t),58.8(t),58.8(t)
$^+$8g	1.18 (t) 3H, -CH_3 of ester group 2.36 (s) 3H, -CH_3 of pyrimidine ring 4.07 (q) 2H, -CH_2 of ester group 6.02 (s) 2H, -CH_2 of aminomethyl bridge 6.98 (s) 1H, H on pyrimidine ring 3.75 (s) 3H, -OCH_3 of phenyl ring present in Biginelli compound 2.64 (q) 2H, -CH_2 of carbazole ring adjacent to double bond 1.89 (q) 2H, -CH_2 of carbazole ring 7.1-7.6 (m)12H, Three aromatic rings	113.22(d),130.91(d),160.15(s),130.47(s), 113.22(d),130.91(d),62.61(d),148.85(s), 100.36(s),146.34(s),167.74(s),60.96(t), 13.95(q),17.57(q),57.2(t),55.2(q),42.89(t), 137.3(s),125.51(s),132.91(s),93.09(s), 93.96(s),135.57(s),128.97(s),138.04(s), 118.49(d),119.09(d),111.78(d),121.26(d), 22.25(t),22.99(t),21.38(t),22.99(t), 108.12(d),121.58(d),118.75(d),118.37(d), 20.42(t), 22.99(t),22.25(t),22.99(t)
9a	1.27 (t) 3H, -CH_3 of ester group 2.29 (s) 3H, -CH_3 of pyrimidine ring 4.17 (q) 2H, -CH_2 of ester group 6.26 (s) 2H, -CH_2 of aminomethyl bridge 8.03 (s) 1H, -CH of imidazole ring at 2-position 7.10 (s) 1H, H on pyrimidine ring 4.87 (s) 1H, -OH of phenyl ring present in Biginelli compound 1.07 (s) 3H, -OCH_3 of phenyl ring present in Biginelli compound 6.93-8.00 (m)11H, Three aromatic rings	146.72(s),111.33(d),146.28(s),127.12(s), 114.54(d),123.94(d),62.65(d),148.85(s), 100.36(s),149.85(s),167.74(s),60.96(t), 13.95(q),17.57(q),47.88(t),43.98(t), 132.66(s),140.73(s),132.32(d),133.4(s), 144.34(s),143.5(d),107.13(d),122.56(d), 120.06(d),120.29(d),110.74(d),122.3(d), 119.8(d),121(d),55.9(q)
9b	1.27 (t) 3H, -CH_3 of ester group 2.29 (s) 3H, -CH_3 of pyrimidine ring 4.09 (q) 2H, -CH_2 of ester group 6.11 (s) 2H, -CH_2 of aminomethyl bridge 2.48 (s) 3H, -CH_3 of imidazole ring 7.03 (s) 1H, H on pyrimidine ring 4.87 (s) 1H, -OH of phenyl ring present in Biginelli compound 1.22 (s) 3H, -OCH_3 of phenyl ring present in Biginelli compound 6.8-7.6 (m)11H, Three aromatic rings	146.72(s),111.33(d),146.28(s),127.12(s), 114.54(d),123.94(d),62.65(d),148.85(s), 100.36(s),149.85(s),167.74(s),60.96(t), 13.95(q),17.57(q),45.68(t),41.77(t),132.5(s),143.24(s), 140.47(s),133.24(s),146.85(s), 151.65(s),107.17(d),124.59(d),119.69(d), 121.83(d),110.79(d),124.33(d),119.43(d), 121.86(d),55.9(q),13.77(q), 13.09(q)
9c	1.20 (t) 3H, -CH_3 of ester group 2.29 (s) 3H, -CH_3 of pyrimidine ring 4.09 (q) 2H, -CH_2 of ester group 6.11 (s) 2H, -CH_2 of aminomethyl bridge 3.66 (s) 2H, -CH_2 of benzyl group on imidazole ring 7.05 (s) 1H, H on pyrimidine ring 4.87 (s) 1H, -OH of phenyl ring present in Biginelli compound 1.14 (s) 3H, -OCH_3 of phenyl ring present in Biginelli compound 7.2-7.7 (m)21H, Five aromatic rings	146.72(s),111.33(d),146.28(s),127.12(s), 114.54(d),123.94(d),62.65(d),148.85(s), 100.36(s),149.85(s),167.74(s),60.96(t), 13.95(q),17.57(q),53.65(t),42.44(t), 133.11(s),143.85(s),142.42(s),133.85(s), 147.43(s),156.391(s),107.3(d),124.36(d), 119.82(d),121.6(d),110.92(d),124.09(d), 119.56(d),121.62(d),28.07(t),135.25(s), 28.3(t),135.52(s),128.09(d),128.57(d), 126.03(d),128.09(d),128.57(d),128.8(d), 128.57(d),126.03(d),128.8(d),128.57(d), 55.9(q)
9d	1.27 (t) 3H, -CH_3 of ester group 2.30 (s) 3H, -CH_3 of pyrimidine ring 4.09 (q) 2H, -CH_2 of ester group 6.56 (s) 2H, -CH_2 of aminomethyl bridge 4.87 (s) 1H, -OH of phenyl ring present in Biginelli compound 1.22 (s) 3H, -OCH_3 of phenyl ring present in Biginelli compound 6.91 (s) 1H, H on pyrimidine ring 6.9-8.0 (m)11H, Three aromatic rings	146.72(s),110.18(d),146.28(s),127.95(s), 114.54(d),122.79(d),62.65(d),147.96(s), 102.31(s),146.17(s),167.74(s),60.96(t), 13.95(q),17.57(q),53.66(t),42.44(t), 132.75(s),143.77(s),133.49(s),147.38(s), 107.17(d),129.34(d),120.84(d),124.21(d), 110.78(d),129.07(d),120.57(d),124.98(d), 55.9(q)

Contd —

Table II — Spectral characterization data of N-Mannich bases **7a-g**, **8a-g** and **9a-g** — *Contd*

Compd*	^{1}H NMR (δ, ppm)	^{13}C NMR (δ, ppm)
9e	1.20 (t) 3H, -CH_3 of ester group 2.31 (s) 3H, -CH_3 of pyrimidine ring 4.17 (q) 2H, -CH_2 of ester group 5.87 (s) 2H, -CH_2 of aminomethyl bridge 6.69 (s) 1H, H on pyrimidine ring 6.39 (s) 1H, -OH of phenyl ring present in Biginelli compound 3.78 (s) 3H, -OCH_3 of phenyl ring present in Biginelli compound 7.0-7.7 (m)11H, Three aromatic rings	146.72(s),112.54(d),146.28(s),130.21(s), 114.54(d),125.26(d),58.57(d),150.6(s), 100.03(s),141.09(s),167.74(s),60.96(t), 13.95(q),17.57(q),33.51(t),47.00(t), 165.64(s),130.8(s),130.8(s),165.64(s), 165.51(s),131.32(s),165.51(s),131.32(s), 123.65(d),134.58(d),123.65(d),134.58(d), 123.66(d),134.58(d),123.66(d),134.58(d), 55.9(q)
9f	1.20 (t) 3H, -CH_3 of ester group 2.31 (s) 3H, -CH_3 of pyrimidine ring 4.2 (q) 2H, -CH_2 of ester group 4.05 (s) 2H, -CH_2 of aminomethyl bridge 6.69 (s) 1H, H on pyrimidine ring 2.30 (s) 2H, -CH_2 of morpholine ring adjacent to nitrogen 3.78 (s) 2H, -CH_2 of morpholine ring adjacent to oxygen 6.39 (s) 1H, -OH of phenyl ring present in Biginelli compound 3.78 (s) 3H, -OCH_3 of phenyl ring present in Biginelli compound 6.7-7.0 (m) 3H, one aromatic rings	146.72(s),110.44(d),146.28(s),130.74(s), 114.54(d),123.04(d),63.09(d),148.85(s), 100.36(s),148.54(s),167.74(s),60.96(t), 13.95(q),17.57(q),62.19(t),61.54(t),58.8(t), 63.24(t),58.8(t),63.24(t),58.8(t),58.8(t), 58.8(t),58.8(t),55.9(q)
9g	1.20 (t) 3H, -CH_3 of ester group 2.31 (s) 3H, -CH_3 of pyrimidine ring 4.09 (q) 2H, -CH_2 of ester group 6.02 (s) 2H, -CH_2 of aminomethyl bridge 6.92 (s) 1H, H on pyrimidine ring 6.39 (s) 1H, -OH of phenyl ring present in Biginelli compound 3.78 (s) 3H, -OCH_3 of phenyl ring present in Biginelli compound 2.66 (q) 2H, -CH_2 of carbazole ring adjacent to double bond 1.90 (q) 2H, -CH_2 of carbazole ring 6.8-7.6 (m)11H, Three aromatic rings	146.72(s),110.33(d),146.28(s),126.6(s), 114.54(d),122.82(d),63.8(d),148.85(s), 100.36(s),146.31(s),167.74(s),60.96(t), 13.95(q),17.57(q),57.2(t),42.89(t),137.3(s), 125.51(s),132.91(s),93.09(s),93.96(s), 135.57(s),128.79(s),138.04(s),118.46(d), 119.09(d),111.78(d),121.26(d),22.25(t), 22.99(t),21.38(t),22.99(t),108.12(d), 121.53(d),118.75(d),118.37(d),20.45(t), 22.99(t),22.25(t),22.99(t), 55.9(q)

*All compounds exhibited the characteristic IR bands at 1710-1730 cm^{-1} (carbonyl group of aromatic ring) and 3340-3350 cm^{-1} (-OH stretch freq.)
$^+$**8a-g** do not give –OH stretch freq.

analysis for C, H, N content were carried out on Perkin Elmer 2400 (USA) instrument. Infrared absorption spectra (IR) were scanned on a Nicolet-400 D FTIR spectrophotometer using KBr pellets and ^{1}H NMR spectra were scanned in $CDCl_3$ on Bruker AC-90 MHz FT-NMR instrument using TMS as an internal standard.

General procedure for the synthesis of DHPMs: 4a, 4b and 4c

Preparation of 5-acetyl-4-(2-hydroxy-phenyl)-6-methyl-3,4-dihydro-1*H*-pyrimidin-2-one, 4a: A mixture of aromatic aldehyde **1a-c** (0.05 mole), ethyl acetoacetate **2** (0.05 mole), and urea **3** (0.05 mole) and a few drops of HCl as catalyst was refluxed in methanol for about 1.5 hr. The progress of the reaction was monitored continuously by TLC. After completion of reaction, the resulting mixture was cooled to RT and poured into crushed ice. The solid thus separated was filtered off and washed with water several times to remove unreacted urea followed by ether. The product was dried and further purified by recrystallization from methanol.

4a: Yield 81%, m.p. 200°C (dec.). Found: C, 60.8; H, 5.8; N, 10.1. $C_{14}H_{16}N_2O_4$ requires C, 60.8; H, 5.8; N, 10.1%. IR(KBr): 1708 (>C=O), 1499 (-NH), 3349 cm^{-1} (-OH); ^{1}H NMR: δ 1.20 (3H,t,CH_3,-OCH_2CH_3), 2.51 (3H,s,-CH_3), 4.02 (2H,q,CH_2,-OCH_2CH_3), 5.29 (1H,s,H of Pyrimidine-ring), 7.25 (1H,s,-NH), 9.14 (1H,s,-NH), 8.16 (1H,s,-OH), 6.82-7.25 (4H,m,ArH). In a similar manner, the other two DHPMs **4b** and **4c** were synthesized by using anisaldehyde and vanillin respectively.

4b: Yield 79%, m.p. 199°C (dec.). Found: C, 62.0; H, 6.2; N, 9.6. $C_{14}H_{16}N_2O_4$ requires C, 62.1; H, 6.2; N, 9.6%. IR(KBr): 1709 (>C=O), 1460 cm^{-1} (-NH); ^{1}H NMR: δ 1.08 (3H,t,CH_3,-OCH_2CH_3), 2.24 (3H,s, -CH_3), 4.00 (2H,q,CH_2,-OCH_2CH_3), 5.10 (1H,s,H of Pyrimidine-ring), 7.69 (1H,s,-NH), 9.14 (1H,s,-NH), 3.72 (3H,s,-OCH_3), 6.86-7.16 (4H,m,ArH).

Table III — *In vitro* antimicrobial activity of N-Mannich bases **7a-g, 8a-g** and **9a-g**

Compd	Antimicrobial activity, zone of inhibition in percentage			
	E. coli	*B. subtilis*	*A. niger*	*C. albicans*
7a	78.57	60.00	42.85	53.33
7b	96.42	72.00	42.85	50.00
7c	-	68.13	-	-
7d	67.85	48.00	39.28	40.00
7e	96.42	96.00	53.57	33.33
7f	100	96.18	67.85	73.33
7g	35.71	56.00	-	56.00
8a	50.00	60.10	50.00	53.33
8b	78.57	-	46.42	56.66
8c	64.28	-	53.57	50.69
8d	-	54.75	-	-
8e	-	28.14	-	22.86
8f	89.28	76.00	78.57	70.37
8g	50.00	41.15	50.00	60.00
9a	85.71	68.10	-	53.33
9b	82.14	76.00	78.57	-
9c	-	60.00	50.43	-
9d	64.28	-	42.85	-
9e	75.21	-	-	70.00
9f	100	96.58	96.42	73.36
9g	-	48.00	-	46.66
Streptomycin*	100	100	-	-
Imidil*	-	-	100	100

*Commercial drug

4c: Yield 84%, m.p. 215°C (dec.). Found: C, 52.7; H, 5.8; N, 9.0. $C_{14}H_{16}N_2O_4$ requires C, 52.8; H, 5.9; N, 9.1%. IR(KBr): 1705 (>C=O), 1467 (-NH), 3349 cm^{-1} (-OH); ^1H NMR: δ 1.11 (3H,t,CH_3,-OCH_2CH_3), 2.24 (3H,s,-CH_3), 4.00 (2H,q,CH_2;-OCH_2CH_3), 5.07 (1H,s,H of Pyrimidine-ring), 8.92 (1H,s,-NH), 9.15 (1H,s,-NH), 9.14 (1H,s,-OH), 3.72 (3H,-OCH_3), 6.86-7.16 (3H,m,ArH).

General procedure for the synthesis of novel N-Mannich bases: 7a-g, 8a-g and 9a-g

Preparation of ethyl 1,3-bis-benzimidazol-1-yl-methyl-4-(2-hydroxy-phenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydro-5-pyrimidinecarboxylate 7a-g. To a solution of DHPM **4a** (0.1 mole) in DMF, formaldehyde **5** (0.2 mole) were added under stirring. The reaction mixture was stirred at RT for 0.5 hr to complete the reaction of formaldehyde and to yield methylol derivative of **4a**. To this, a solution of **6a** (0.2 mole) in DMF was added dropwise and refluxed for 2 hr. The reaction mixture was poured into ice cold water and filtered off and washed with hot water. Finally, it was dried and purified by recrystallization from chloroform to give **7a**.

In an analogous way, the remaining two series of N-Mannich bases **8a-g, 9a-g** have been prepared by Mannich reaction of DHPMs **4b** and **4c** with the seven heterocyclic secondary amines **6a-g** and formaldehyde **5**. The analytical data and spectral studies of these three series of N-Mannich bases are furnished in **Table I** and **Table II**.

Antimicrobial Activity

Sterile and calibrated apparatus were used as and when required. 24 hr old culture suspensions of different organisms (*E. coli*, *B. subtillis*, *A. niger, C. albican*) were obtained from the Faculty of Science, N V Patel Science College, Pure and Applied Chemistry, Sardar Patel University, Vallabh Vidyanagar, Gujarat, India. The antimicrobial activity was investigated against Gram –ve bacteria (*E. Coli*), Gram +ve bacteria (*B. Subtilis*), fungi (*A. Niger*) and yeast fungi (*C. albican*) by Auger cup borer method using Streptomycin and Imidil as a standard for bacterial and fungal culture respectively. The solution of N-Mannich bases were prepared in DMSO and tested. The Minimum Inhibitory Concentration (MIC) study was carried out at different concentrations such as 50, 100, 200, 300, 400, 500 and 1000 ppm, but the zone of inhibition between 500 and 1000 ppm were similar so 500 ppm was selected as MIC. A test tube containing sterile melted soft agar (2% in distilled water, 6.0 mL) was maintained at 50°C and inoculated with 0.2 mL suspension of the test culture, mixed thoroughly and poured in the Petri dish containing sterile nutrient agar medium (Nutrient Plates) and allowed to solidify for 5 min. The cup-borer was sterilized by dipping into absolute ethanol and flaming it and then allowed to cool down. With the help of sterile cup-borer, three cups in the agar were marked and were injected with 0.1 mL of respective test sample solution of concentration 500 ppm in DMSO solvent, 0.1 mL standard drug streptomycin (500 ppm) solution in distilled water and 0.1 mL of DMSO, respectively. Then the test sample was allowed to diffuse for 1 hr in refrigerator at 4-5°C. The plates were incubated in upright position at 37°C for 24 hr and on the next day the zone of inhibition surrounding each cup was observed. The data for antimicrobial activity in terms of zone of inhibition of N-Mannich bases are shown in **Table III**.

Conclusion

The novel heterocyclic N-Mannich bases of **7g, 8g, 9g** showed poor activity against both the bacterial species as well as fungal species and **7a, 7b, 7c, 7d, 7e, 8a, 8b, 8c, 8d, 8e, 9a, 9b, 9c, 9d, 9e** were

moderately active. On the other hand, compounds **7f, 8f, 9f** have revealed promising activity against both the species as comparable to standard drug. Finally, it appears that the promising activity of dihydropyri-midin-2(1*H*)-ones based N-Mannich bases may prove advantageous for their pharmacological applications.

Acknowledgement

The authors are thankful to the Head, Department of Chemistry, S P University for providing research facilities and to N V Patel Science Collage, S P University, Vallabh Vidyanagar for providing microanalysis.

References

1 (a) Rovnyak G C, Kimball S D, Bayer B, Cucinotta G, Di Marco J D, Gougoutas J, Hedberg A, Malley M, McCarthy J P & James P, *J Med Chem,* 38, **1995**, 119; (b) Awal K S, Rovnyak G C, Kimball S D, Floyed D M, Moreland S, Swanson B N, Gougoutas J Z, Schwartz J, Smillie K M & Malley M F, *J Med Chem*, 33, **1990**, 2629; (c) Atwal K S, Swanson B N, Unger S E, Floyd D M, Moreland S, Hedberg A & O'Reilly B C, *J Med Chem* 34, **1991**, 806.
2 Kappe C O, *Eur J Med Chem*, 35, **2000**, 1043.
3 Snider B B & Shi Z, *J Org Chem,* 58, **1993**, 3828.
4 (a) Ayer M, Kapoor T M, Haggarty S J & Mitchison T, *J Science,* 286, **1999,** 971; (b) Kappe C O & Shishkin V, *Tetrahedron*, 56, **2000**, 1859.
5 Denton J J, Turner R, Neir W B & Lawson V A, *J Am Chem Soc*, 71, **1949,** 2048.
6 Burckhalter J P & Johnson S H, *J Am Chem Soc*, 73, **1951,** 4835.
7 Mannich C & Lammering D, *Chem Ber*, 55, **1922,** 3510.
8 Levvy G A & Nisbet H B, *J Chem Soc,* **1938,**1053.
9 Blanton C D & Nobles W L, *J Pharm Sci*, 51, **1962,** 878.
10 Rogers F C & Nobles W L, *J Pharm Sci,* 51, **1962,** 273.
11 Nobles W L & Burckhalter J H, *J Pharm Sci*, 47, **1958,** 77.
12 Caldwell H C & Nobles W L, *J Am Pharm Assn*, *Sci Ed*, 43, **1954,** 644.
13 Hellmann H & Loschman I, *Chem Ber*, 87, **1954,** 1684.
14 Moore M B & Rapela R T, *J Am Chem Soc*, 68, **1946,** 1657.
15 Atkinson R O, *J Chem Soc*, **1954,** 1329.
16 *Ger Pat*, 575, **1954,** 114.
17 Swaminathan S & Narsimhan K, *Ber*, 99, **1966,** 889.
18 Burekhulter J H, Wells J N & Mayer W J, *Tetrahedron Lett*, **1964**, 1353.
19 Bryant G & Heisey L V, *J Am Chem Soc*, 68, **1946,** 2486.
20 Parmar S S, Mishra R S, Chaudheri A & Gupta T K, *J Pharm Sci*, 61 (8), **1972,** 1322.
21 El-Marry A H, Fahmy H H & Abdelwahed S H, *Molecules,* 5, **2000,** 1429.
22 Tramontini M, *Synthesis,* 12, **1973,** 707.
23 Pascal G, Giron C & Froissant J, *Fr Pat Appl*, **1987**, 2593181.
24 Dianov V M, Chikaeva I G & Timirkhanova G A, *Khim-Farm Zh*, 28 (80), **1994,** 21.
25 Maskovski M D, *Drugs* (in Russian), *Minisk,* **1987,** 359, 381, 483.
26 Varma R S, *J Indian Chem Soc*, 81, **2004,** 627.
27 Manjula A, Rao B V & Neelakantam P, *Synth Commun*, 34, **2004**, 2665.