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Synthesis, characterization and antimicrobial activity of mannich bases of ethyl 5-(2-chlorophenyl)-7-methyl-3-oxo-3,5-dihydro-2*H*-thiazolo [3,2-*a*]pyrimidine-6-carboxylate derivatives

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ABSTRACT

Mannich bases of ethyl 5-(2-chlorophenyl)-7-methyl-3-oxo-3,5-dihydro-2H-thiazolo[3,2-a]pyrimidine-6-carboxylate ***(6)*** *were prepared by Mannich reaction respectively with seven different heterocyclic secondary amino compounds* ***(8a-g)*** *and formaldehyde* ***(7)****. Compound* ***(6)*** *were derived by the cyclization reaction of ethyl 4-(2-chlorophenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate* ***(4)*** *with chloroacetic acid* ***(5)*** *in dimethylformamide (DMF). The precursor compound* ***(4)*** *was prepared by a Biginelli reaction of 2-chlorobenzaldehyde* ***(1)*** *with ethylacetoacetate* ***(2)*** *and thiourea* ***(3)****. All the newly synthesized Mannich bases were analyzed with a view to elucidate their chemical structure by elemental analysis and spectral studies (FTIR,* ^{1}H *&* ^{13}C *NMR). They were assayed in vitro for their biological activity against Escherichia coli and Bacillus subtilis bacterial species as well as Aspergillus niger and Candida albicans fungal microorganisms. Evaluation of the title compounds as antimicrobacterial agent indicate that ethyl-5-(2-chlorophenyl)-7-methyl-2-(morpholinomethyl)-3-oxo-3,5-dihydro-2H-thiazolo [3,2-a]pyrimidine-6-carboxylate* ***(9e)*** *have shown promising antimicrobacterial activity against both bacterial and fungal microorganisms and thus could be promising novel drug candidates.*

Keywords: Biginelli reaction, Dihydropyrimidones, thiazolo[3,2-a]pyrimidine, Mannich bases, Antimicrobial activity.

INTRODUCTION

Incorporation of more than one bioactive heterocyclic moiety into a single framework may result into the production of novel heterocycles with enhanced bioactivity [1] as well as annulations of heterocyclic ring often improves biological properties such as potency, selectivity, toxicity and metabolic stability[2]. In these context, a literature survey described a variety of substituted thiazoles and antimetabolite thiazolopyrimidine derivatives that received a great deal of attention for their anticancer,[3] antiviral,[4,5] antiinflammatory[6] and antimicrobial[7] activities. In addition to these, Pyrimidine derivatives have played an important role in the medicinal chemistry [8-10].Thienopyrimidines, formed by the fusion of thiophene moiety with pyrimidine ring, also have been reported to have wide variety of biological activities such as anti- inflammatory [11,12] and antimicrobial activities [13,14]. Combination of thiazolidine template with other heterocycles is a well-known approach for drug-like molecules' build-up, which allows achieving new pharmacological profile, action strengthening or toxicity lowering [15]. For that purpose we used Mannich reaction which consists of an amino alkylation of an acidic proton placed next to a carbonyl functional group with formaldehyde and ammonia or any primary or secondary amine. The final product is a β-amino-carbonyl compound is known as a Mannich base. In the past few decades, Mannichbases [16] of heterocyclic molecules have been grabbing the attention of the synthetic chemists for their wide range of

antimicrobial properties [17,18]. They possess many interesting pharmacological properties. Mannich bases have several biological activities such as anti microbial, cytotoxic, anticancer, analgesic and diuretic activities [19-24].

On the basis of these reports, we have planned to synthesize C-Mannich bases of thiazolo pyrimidine derived from thiazolo[3,2-*a*]pyrimidine as hydrogen active substrate and heterocyclic secondary amines such as benzimidazole, 2-metyl benzimidazole, 2-benzyl benzimidazole, benzotriazole, morpholine, phthalimide, tetrohydrocarbazole with a view to achieve better antimicrobial activity.

MATERIALS AND METHODS

All the solvents and reagents were obtained from commercial sources and were used without further purification. Melting points were determined in open capillaries and were uncorrected. TLC was used to monitor the progress of all reactions and to check the purity of compounds. Heterocyclic secondary amines were used as secondary amino compound in the synthesis of Mannich bases were prepared by method reported in literature [25] except morpholine. They were recrystallized from appropriate solvents and their purity was checked by TLC. While all other chemicals and solvents were used of LR grade without further purification. Elemental analysis for C, H, N content were carried out on Perkin Elmer (USA) 2400, Infra red spectra (KBr pellets) were recorded on Perkin-Elmer spectrum BX series FT-IR spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 400 MHz spectrometer with TMS as an internal standard.

Methods:
2-Chlorobenzaldehyde **(1)** reacts with ethylacetoacetate **(2)** and thiourea **(3)** via Biginelli reaction to give 3,4-dihydropyrimidine-2(*1H*)-thione **(4)** followed by the cyclization reaction with chloroacetic acid **(5)** in DMF gives ethyl 5-(2-chlorophenyl)-7-methyl-3-oxo-3,5-dihydro-2*H*-thiazolo[3,2-*a*]pyrimidine-6-carboxylate **(6)** which upon Mannich reaction with different heterocyclic secondary amines namely benzimidazole, 2-metyl benzimidazole, 2-benzyl benzimidazole, benzotriazole, morpholine, phthalimide, tetrohydrocarbazole **(8a-g)** and formaldehyde **(7)** furnish a series of Mannich bases **(9a-g)**. **Scheme I** shows the synthetic protocol of the final compounds **(9a-g)**.

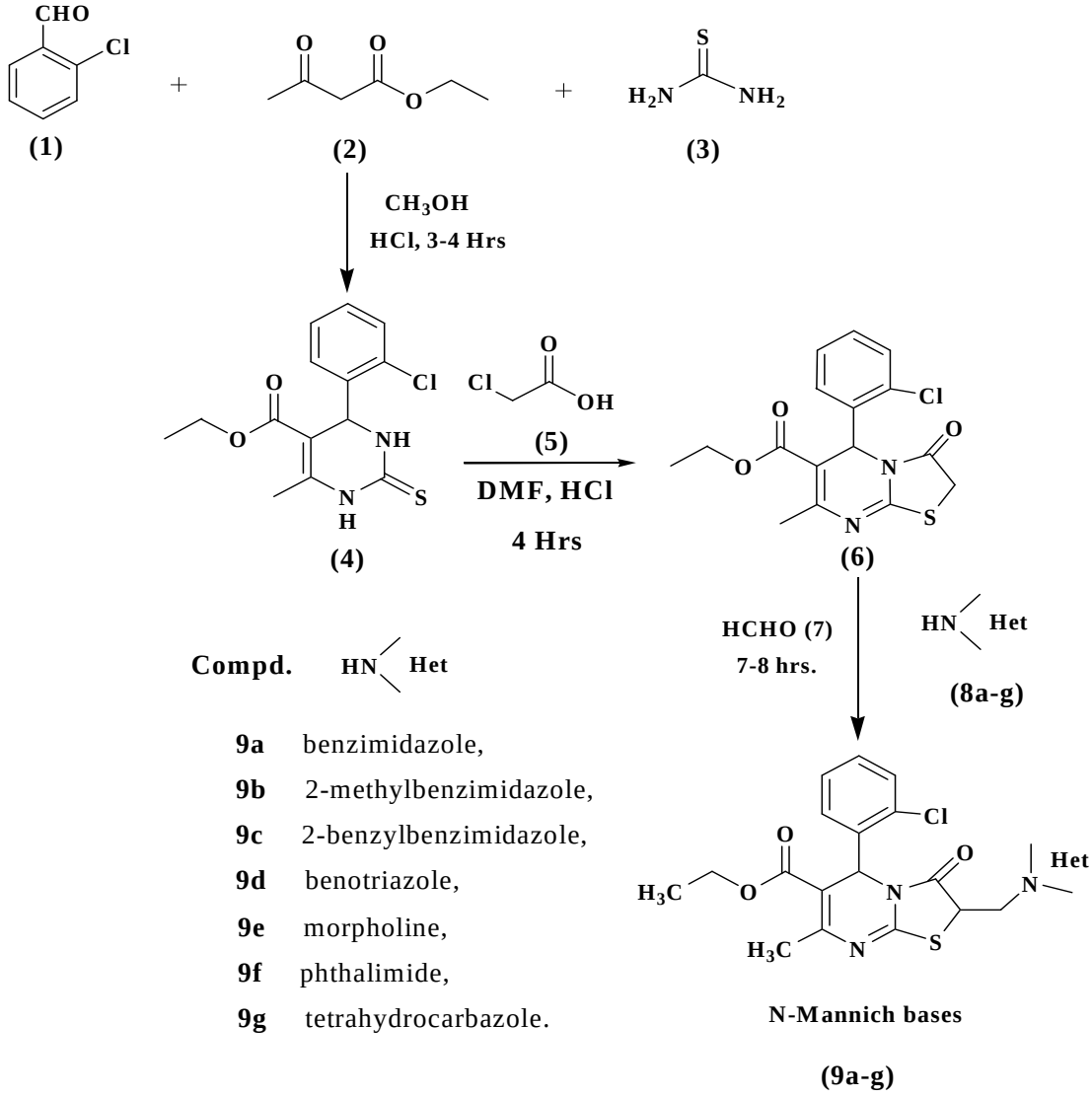
Procedure for the synthesis of ethyl 4-(2-chlorophenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (4):
A mixture of 2-chlorobenzaldehyde **(1)** (0.05 mol), Ethylacetoacetate **(2)** (0.05 mol), and thiourea **(3)** (0.05 mol) and a few drops of HCl as catalyst was refluxed in ethanol for about 1.5 hr. The progress of the reaction was monitored continuously by TLC. After completion of reaction, the resulting mixture was cooled and poured into crushed ice. The solid separated was filtered off and washed several times with water to remove unreacted thiourea. The product was further washed with ether, and purified by recrystallization from methanol.

White solid, mp 166-168^{0}C, Yield 81%, Anal. cacld for $C_{14}H_{15}ClN_2O_2S$ M.W.: 310.5 Calc. C, 54.10; H, 4.86; N, 9.01; found: C, 54.47; H, 4.60; N, 9.10. IR (υ cm^{-1}): benzene ring: 1655, 1620 and 1540 (skeletal vibration), 3140 (C-H stretching), 740 (C-H bending for 1,2-disubstituted ring); aliphatic methyl group (-CH_3): 2920 and 2860 (asymm. and symm. stretching), 1450 (C-H bending); carbonyl of ester group (-C=O): 1255 and 1140 (C-O-C asymm. and symm. stretching), 1720 (C=O stretching); sec. thioamide (NH-C=S-NH): 1275 (C=S stretching), 3450 (N-H stretching), 1575 (N-H bending), 1325 (C-N stretching); functional group: 800 (-C-Cl stretching). ^{1}H NMR: δ 1.2 (3H,t,CH_3 of -OCH_2CH_3), 1.9 (3H,s,CH_3 of pyrimidine ring), 4.2 (2H,q,CH_2 of -OCH_2CH_3), 4.6 (1H,s,H on pyrimidine ring), 6.8-7.3 (4H,m,one aromatic rings), 7.7 (1H,s,NH of pyrimidine ring), 9.1 (1H,s,NH of pyrimidine ring); ^{13}C NMR: 13.9, 16.1, 57.8, 60.9, 111.6, 127.8, 128.1, 129.9, 132.8, 138.5, 147.1, 167.6, 174.1.

Procedure for the synthesis of ethyl 5-(2-chlorophenyl)-7-methyl-3-oxo-3,5-dihydro-2*H*-thiazolo[3,2-*a*]pyrimidine-6-carboxylate (6):
Cyclization of ethyl 4-(2-chlorophenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate **(4)** in to fused thiazolo[3,2-*a*]pyrimidines has been carried out by using chloroacetic acid in dimethylformamide (DMF). A mixture of 4-(2-chlorophenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate **(4)** (0.01 mol) and chloroacetic acid **(5)** (0.011 mol) in DMF was refluxed for 4 hrs. The resulting solution was allowed to stand at room temperature for an hour and cooled to 0°C. The solid separated was filtered off, washed with chilled water and recrystallized from ethanol to yield ethyl 5-(2-chlorophenyl)-7-methyl-3-oxo-3,5-dihydro-2*H*-thiazolo[3,2-*a*]pyrimidine-6-carboxylate **(6)**.

White solid, mp 212-214^{0}C, Yield 67%, Anal. cacld for $C_{16}H_{15}ClN_2O_3S$ M.W.: 350.5 Calc. C, 54.78; H, 4.31; N, 7.99; found: C, 54.67; H, 4.53; N, 8.23. IR (υ cm^{-1}): benzene ring: 1650, 1590 and 1550 (skeletal vibration), 3180 (C-H stretching), 710 (C-H bending for 1,2-disubstituted ring); aliphatic methyl group (-CH_3): 2960 and 2870 (asymm. and symm. stretching), 1425 (C-H bending); carbonyl of ester group (-C=O): 1230 and 1160 (C-O-C

asymm. and symm. stretching), 1760 (C=O stretching); thiazole ring: 1730 (C=O stretching), 1425(ring stretching), 800, 700 and 650 (-CH out of plane bending), 1330 (C-N stretching); functional group: 800 (C-Cl stretching). ^{1}H NMR: δ 1.2 (3H,t,CH_3 of -OCH_2CH_3), 1.9 (3H,s,CH_3 of pyrimidine ring), 4.1 (2H,q,CH_2 of -OCH_2CH_3), 3.9 (2H,q,CH_2 of thiazole ring), 5.8 (1H,s,H on pyrimidine ring), 6.9-7.3 (4H,m,one aromatic rings); ^{13}C NMR: 13.9, 26.1, 32.4, 56.5, 60.9, 118.6, 126.9, 127.0, 127.6, 128.6, 131.5, 137.5, 152.6, 164.1, 172.00, 172.7.



Scheme I: Synthetic routs for the compounds (9a-g)

Procedure for the synthesis of substituted Ethyl 5,7-dimethyl-3-oxo-2,3-dihydro-5*H*-[1,3]-thiazolo[3,2-*a*]pyrimidie-6-carboxylate derivatives (9a-g):

The details regarding preparation of Mannich bases are given in the following section typically from benzimidazole **(8a)** as substrate.

In a three necked flask equipped with a stirrer and dropping funnel, ethanolic solution of ethyl 5-(2-chlorophenyl)-7-methyl-3-oxo-3,5-dihydro-2*H*-thiazolo[3,2-*a*]pyrimidine-6-carboxylate **(6)** and 37% formaldehyde **(7)** were added under stirring. The reaction mixture was stirred at room temperature for half an hour to complete the reaction of formaldehyde to form methylol derivative of **(6)**. To the resulting mixture, a solution of benzimidazole **(8a)** containing catalytic amount of conc. HCl was added dropwise during 15 minutes with stirring during half an hour at room temperature and refluxed for 7-8 hrs. The reaction was monitored continuously by TLC. After completion of reaction, the resulting mixture was cooled to room temperature and poured into crushed ice with continuous stirring. The solid obtained was filtered off, washed thoroughly with hot water, air-dried and recrystallized from appropriate solvent to yield the C-Mannich base **(9a)**.

In a similar manner, Mannich reaction of ethyl 5-(2-chlorophenyl)-7-methyl-3-oxo-3,5-dihydro-2*H*-thiazolo[3,2-*a*]pyrimidine-6-carboxylate **(6)** was further carried out respectively with 2-methyl benzimidazole, 2-benzyl benzimidazole, benzotriazole, morpholine, phthalimide, tetrahydrocarbazole secondary amines **(Scheme I)** using the same procedure to yield other six C-Mannich bases **(9b-g)** forming a series of Mannich bases.

Analytical and spectral data of synthesized compounds (9a-g):

ethyl 2-((1H-benzo[d]imidazol-1-yl)methyl)-5-(2-chlorophenyl)-7-methyl-3-oxo-3,5-dihydro-2H-thiazolo[3,2-a] pyrimidine-6-carboxylate (9a): White solid, mp 244-246^{0}C, Yield 73%, Anal. cacld for $C_{24}H_{21}ClN_4O_3S$ M.W.: 480.5 Calc. C, 59.93; H, 4.40; N, 11.65; found: C, 59.74; H, 4.28; N, 11.78. IR (υ cm^{-1}): Benzene ring: 3080 (C-H stretching), 720 (C-H bending for 1,2-disubstituted ring); aminomethylene bridge: 2940 and 2800 (-CH_2 aymm. and symm. stretching), 1455 (-CH_2 bending); carbonyl of ester group (-O=C-OC_2H_5): 1245 and 1145 (C-O-C asymm. and symm. stretching), 1750 (-C=O stretching); thiazole ring: 1670 (C=O stretching), 1410 (ring stretching), 1310 (C-N stretching); heterocyclic sec. amine: 1570, 1540 and 1500 (ring stretching), 1145, 1050 (ring breathing), 800 (C-H deformation); 680 (-C-Cl stretching). ^{1}H NMR: δ 2.12 (3H,t,CH_3 of -OCH_2CH_3), 2.32 (3H,s,CH_3 of pyrimidine ring), 4.00 (2H,q,CH_2 of -OCH_2CH_3), 5.32 (2H,d,CH_2 of methylene bridge), 5.75 (1H,q,CH of thiazole ring), 6.12 (1H,s,H on pyrimidine ring), 7.48 (1H,s,H on imidazole ring at 2-position), 7.08-7.88 (8H,m,two aromatic rings); ^{13}C NMR: 13.9, 26.3, 48.9, 55.4, 56.4, 60.9, 110.1, 118.6, 119.1, 121.8, 122.3, 126.9, 127.4, 127.6, 128.6, 131.9, 136.8, 136.9, 143.1, 143.4, 153.3, 167.4, 171.3, 172.0.

ethyl 5-(2-chlorophenyl)-7-methyl-2-((2-methyl-1H-benzo[d]imidazol-1-yl)methyl)-3-oxo-3,5-dihydro-2H-thiazolo [3,2-a]pyrimidine-6-carboxylate (9b): Creamy white solid, mp 146-148^{0}C, Yield 60 %, Anal. cacld for $C_{25}H_{23}ClN_4O_3S$ M.W.: 494.5 Calc. C, 60.66; H, 4.68; N, 11.32; found: C, 60.71; H, 4.29; N, 11.65. IR (υ cm^{-1}): Benzene ring: 3060 (C-H stretching), 770 (C-H bending for 1,2-disubstituted ring); aminomethylene bridge: 2945 and 2890 (-CH_2 aymm. and symm. stretching), 1465 (-CH_2 bending); carbonyl of ester group (-O=C-OC_2H_5): 1275 and 1120 (C-O-C asymm. and symm. stretching), 1780 (-C=O stretching); thiazole ring: 1740 (C=O stretching), 1400 (ring stretching), 1320 (C-N stretching); heterocyclic sec. amine: 1590, 1560 and 1500 (ring stretching), 1120, 1030 (ring breathing), 825 (C-H deformation); 725 (-C-Cl stretching). ^{1}H NMR: δ 2.01 (3H,t,CH_3 of -OCH_2CH_3), 2.47 (3H,s,CH_3 of pyrimidine ring), 2.65 (3H,s,CH_3 of imidazole ring at 2-position), 3.86 (2H,q,CH_2 of -OCH_2CH_3), 4.72 (2H,d,CH_2 of methylene bridge), 5.72 (1H,q,CH of thiazole ring), 6.17 (1H,s,H on pyrimidine ring), 6.93-7.78 (8H,m,two aromatic rings); ^{13}C NMR: 12.6, 13.5, 26.3, 50.0, 53.1, 58.4, 60.9, 109.5, 118.0, 118.6, 120.9, 121.3, 130.2, 130.8, 131.2, 138.0, 139.2, 150.6, 151.8, 151.9, 167.4, 171.4, 172.1.

ethyl 2-((2-benzyl-1H-benzo[d]imidazol-1-yl)methyl)-5-(2-chlorophenyl)-7-methyl-3-oxo-3,5-dihydro-2H-thiazolo [3,2-a]pyrimidine-6-carboxylate (9c): Light brown solid, mp 187-189^{0}C, Yield 57 %, Anal. cacld for $C_{31}H_{27}ClN_4O_3S$ M.W.: 571.5 Calc. C, 65.20; H, 4.77; N, 9.81; found: C, 65.14; H, 4.63; N, 9.49. IR (υ cm^{-1}): Benzene ring: 3040 (C-H stretching), 765 (C-H bending for 1,2-disubstituted ring); aminomethylene bridge: 2900 and 2800 (-CH_2 aymm. and symm. stretching), 1440 (-CH_2 bending); carbonyl of ester group (-O=C-OC_2H_5): 1295 and 1100 (C-O-C asymm. and symm. stretching), 1740 (-C=O stretching); thiazole ring: 1680 (C=O stretching), 1380 (ring stretching), 1320 (C-N stretching); heterocyclic sec. amine: 1580, 1530 and 1500 (ring stretching), 1065, 1010 (ring breathing), 830 (C-H deformation); 700 (-C-Cl stretching). ^{1}H NMR: δ 2.11 (3H,t,CH_3 of -OCH_2CH_3), 2.38 (3H,s,CH_3 of pyrimidine ring), 3.48 (2H,s,CH_2 of benzyl group on imidazole ring), 4.12 (2H,q,CH_2 of -OCH_2CH_3), 4.66 (2H,d,CH_2 of methylene bridge), 5.31 (1H,q,CH of thiazole ring), 6.18 (1H,s,H on pyrimidine ring), 6.79-7.87 (13H,m,three aromatic rings); ^{13}C NMR: 13.9, 26.3, 28.4, 50.0, 53.8, 56.4, 60.9, 109.6, 118.6, 118.7, 120.6, 121.1, 126.0, 126.9, 127.4, 127.6, 128.5, 128.6, 129.4, 130.9, 134.6, 136.9, 139.0, 151.2, 153.3, 157.2, 167.4, 171.3, 172.1.

ethyl 2-((1H-benzo[d][1,2,3]triazol-1-yl)methyl)-5-(2-chlorophenyl)-7-methyl-3-oxo-3,5-dihydro-2H-thiazolo[3,2-a] pyrimidine-6-carboxylate (9d): White solid, mp 240-242^{0}C, Yield 62 %, Anal. cacld for $C_{23}H_{20}ClN_5O_3S$ M.W.: 481.5 Calc. C, 57.32; H, 4.18; N, 14.53; found: C, 57.09; H, 4.27; N, 14.34. IR (υ cm^{-1}): Benzene ring: 3025 (C-H stretching), 825 (C-H bending for 1,2-disubstituted ring); aminomethylene bridge: 3000 and 2910 (-CH_2 aymm. and symm. stretching), 1440 (-CH_2 bending); carbonyl of ester group (-O=C-OC_2H_5): 1275 and 1125 (C-O-C asymm. and symm. stretching), 1750 (-C=O stretching); thiazole ring: 1680 (C=O stretching), 1400 (ring stretching), 1340 (C-N stretching); heterocyclic sec. amine: 1590, 1575 and 1520 (ring stretching), 1170, 1050 (ring breathing), 825 (C-H deformation); 720 (-C-Cl stretching). ^{1}H NMR: δ 2.11 (3H,t,CH_3 of -OCH_2CH_3), 2.32 (3H,s,CH_3 of pyrimidine ring), 3.91 (2H,q,CH_2 of -OCH_2CH_3), 4.81 (2H,d,CH_2 of methylene bridge), 5.20 (1H,q,CH of thiazole ring), 6.62 (1H,s,H on pyrimidine ring), 6.90-8.10 (8H,m,two aromatic rings); ^{13}C NMR: 13.8, 26.2, 48.7, 53.8, 56.4, 60.9, 109.3, 118.6, 118.9, 124.1, 126.3, 126.9, 127.4, 127.6, 128.6, 131.9, 135.6, 136.8, 145.7, 153.3, 167.4, 172.0, 172.3.

ethyl 5-(2-chlorophenyl)-7-methyl-2-(morpholinomethyl)-3-oxo-3,5-dihydro-2H-thiazolo[3,2-a]pyrimidine-6-carboxylate (9e): White solid, mp 234-236^{0}C, Yield 71 %, Anal. cacld for $C_{21}H_{24}ClN_3O_4S$ M.W.: 449.5 Calc. C,

56.06; H, 5.38; N, 9.34; found: C, 55.89; H, 5.75; N, 9.26. IR (υ cm^{-1}): Benzene ring: 3000 (C-H stretching), 725 (C-H bending for 1,2-disubstituted ring); aminomethylene bridge: 2860 and 2800 (-CH_2 aymm. and symm. stretching), 1450 (-CH_2 bending); carbonyl of ester group (-O=C-OC_2H_5): 1250 and 1140 (C-O-C asymm. and symm. stretching), 1750 (-C=O stretching); thiazole ring: 1720 (C=O stretching), 1400 (ring stretching), 1320 (C-N stretching); heterocyclic sec. amine: 1580, 1560 and 1480 (ring stretching), 1140, 1030 (ring breathing), 815 (C-H deformation), 1180 (C-O stretching of morpholine ring); 710 (-C-Cl stretching). ^{1}H NMR: δ 2.07 (3H,t,CH_3 of -OCH_2CH_3), 2.28 (3H,s,CH_3 of pyrimidine ring), 2.52-2.75 (4H,m,CH_2 of morpholine ring adjacent to nitrogen), 2.91 (2H,d,CH_2 of methylene bridge), 3.58-3.89 (4H,m,CH_2 of morpholine ring adjacent to oxygen), 4.16 (2H,q,CH_2 of -OCH_2CH_3), 4.78 (1H,q,CH of thiazole ring), 5.85 (1H,s,H on pyrimidine ring), 7.10-7.74 (4H,m,one aromatic rings); ^{13}C NMR: 13.8, 26.2, 48.1, 51.7, 54.4, 56.4, 60.8, 67.1, 118.5, 126.9, 127.4, 127.6, 128.6, 131.9, 136.8, 153.3, 167.5, 172.1, 172.9.

ethyl 5-(2-chlorophenyl)-2-((1,3-dioxoisoindolin-2-yl)methyl)-7-methyl-3-oxo-3,5-dihydro-2H-thiazolo[3,2-a] pyrimidine-6-carboxylate (9f): Milky white solid, mp 250-252^{0}C, Yield 56 %, Anal. cacld for $C_{25}H_{20}ClN_3O_5S$ M.W.: 509.5 Calc. C, 58.88; H, 3.95; N, 8.24; found: C, 58.38; H, 4.13; N, 8.49. IR (υ cm^{-1}): Benzene ring: 3100 (C-H stretching), 795 (C-H bending for 1,2-disubstituted ring); aminomethylene bridge: 2950 and 2850 (-CH_2 aymm. and symm. stretching), 1475 (-CH_2 bending); carbonyl of ester group (-O=C-OC_2H_5): 1260 and 1150 (C-O-C symm. and symm. stretching), 1750 (-C=O stretching); thiazole ring: 1700 (C=O stretching), 1370 (ring stretching), 1340 (C-N stretching); heterocyclic sec. amine: 1575, 1540 and 1525 (ring stretching), 1075, 1025 (ring breathing), 795 (C-H deformation), 1640 (C=O stretching of phthalimide); 730 (-C-Cl stretching). ^{1}H NMR: δ 2.12 (3H,t,CH_3 of -OCH_2CH_3), 2.38 (3H,s,CH_3 of pyrimidine ring), 4.18 (2H,q,CH_2 of -OCH_2CH_3), 4.86 (2H,d,CH_2 of methylene bridge), 5.46 (1H,q,CH of thiazole ring), 5.99 (1H,s,H on pyrimidine ring), 7.08-7.73 (8H,m,two aromatic rings); ^{13}C NMR: 13.9, 26.3, 37.2, 48.6, 56.4, 61.0, 118.6, 123.0, 126.9, 127.4, 127.7, 128.4, 131.9, 131.9, 133.1, 136.8, 153.3, 167.2, 167.4, 168.9, 172.2.

ethyl 5-(2-chlorophenyl)-2-((3,4-dihydro-1H-carbazol-9(2H)-yl)methyl)-7-methyl-3-oxo-3,5-dihydro-2H-thiazolo [3,2-a]pyrimidine-6-carboxylate (9g): White solid, mp 145-147^{0}C, Yield 65 %, Anal. cacld for $C_{29}H_{28}ClN_3O_3S$ M.W.: 533.5 Calc. C, 65.22; H, 5.28; N, 7.87; found: C, 65.41; H, 5.57; N, 7.54. IR (υ cm^{-1}): Benzene ring: 3020 (C-H stretching), 790 (C-H bending for 1,2-disubstituted ring); aminomethylene bridge: 2940 and 2900 (-CH_2 aymm. and symm. stretching), 1440 (-CH_2 bending); carbonyl of ester group (-O=C-OC_2H_5): 1250 and 1150 (C-O-C asymm. and symm. stretching), 1725 (-C=O stretching); thiazole ring: 1650 (C=O stretching), 1400(ring stretching), 1350 (C-N stretching); heterocyclic sec. amine: 1585, 1560 and 1500 (ring stretching), 1110, 1010 (ring breathing), 790 (C-H deformation); 740 (-C-Cl stretching). ^{1}H NMR: δ 1.84-2.08 (4H,m,-CH_2 of carbazole ring), 1.71 (3H,t,CH_3 of -OCH_2CH_3), 2.30 (3H,s,CH_3 of pyrimidine ring), 2.40-2.77 (4H,m,-CH_2 of carbazole ring adjacent to double bond), 3.78 (2H,q,CH_2 of -OCH_2CH_3), 4.28 (2H,d,CH_2 of methylene bridge), 5.33 (1H,q,CH of thiazole ring), 6.12 (1H,s,H on pyrimidine ring), 6.80-7.79 (8H,m,two aromatic rings); ^{13}C NMR: 13.7, 21.5, 22.5, 23.0, 26.4, 51.9, 54.4, 56.4, 60.8, 96.7, 108.0, 118.2, 118.4, 118.6, 120.7, 126.8, 127.5, 127.6, 128.4, 128.6, 131.9, 133.9, 136.9, 141.4, 153.3, 167.5, 172.1, 172.4.

Biological Evaluation

Antimicrobial activity

The antimicrobial activity of newly synthesized compounds was determined using agar cup disc method [26,27]. Antibacterial activity was carried out against *Escherichia coli* and *Bacillus subtilis* as while Antifungal activity was carried out against *Aspergillus niger* and *Candida albicans* using sabouraud's dextrose agar medium **(Table I)**. Streptomycin and Imidil was used as standard drugs for antibacterial and antifungal activity respectively. DMSO was used as solvent control. The compounds were tested at a 500 µg/ml concentration against fungal strains.

Antibacterial activity:

A test tube containing sterile melted soft agar (2% in distilled Water, 6.0 ml) was cooled to 50^{0}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 five minutes. The cup-borer was sterilized by dipping into absolute ethanol and flaming it and then allowed to cool it down. With the help of sterile cup-borer, four cups in the agar were marked. Three cups were filled with 0.1 m1 of test sample solutions of 500 ppm concentrations of three different test compounds and one was filled with 0.1 ml of DMSO solvent as control. Then test sample was allowed to diffuse for 1 hour in refrigerator at 4-5^{0}C. The plates were incubated in upright position at 37^{0}C for 24 hrs and on the next day the zone of inhibition of surrounding each cap was observed. Same experiment was performed using standard antibiotics for gram positive and gram negative bacteria.

Procedure for *Antifungal activity:*
A test tube containing sterilized melted soft agar (2% in distilled Water, 6.0 ml) was cooled to 40°C and inoculated with 0.2 mL suspension of the test culture, mixed thoroughly and poured in the Petri dish containing sterilized sabouraud's dextrose agar medium and allowed to solidify. The cup-borer was sterilized by dipping into absolute ethanol and flaming it and then allowed to cool it down. With the help of sterilized cup-borer, four cups in the agar were made. Three cups were filled with 0.1 ml of test compound solutions of 500 ppm concentrations of three different test compounds and one was filled with 0.1 ml of DMF solvent as control. Then test sample was allowed to diffuse for 1 hour in refrigerator at 4-5°C. The plates were incubated in upright position at 37°C for 48 hrs and after two day the zone of inhibition of surrounding each cup was observed. Same experiment was performed using standard antibiotics for fungi.

After incubation, the zone of inhibition was measured with the help of a scale to the nearest millimetre. All the experiments were conducted in duplicate for each test sample. The average zone of inhibition was noted.

Table I: Antifungal activity of the synthesized compounds (9a-g)

Compounds	Zone of inhibition (in %age) at 500 µg/ml concentration			
	Bacterial species		Fungal species	
	Escherichia coli	*Bacillus subtilis*	*Aspergillus niger*	*Candida albicans*
9a	64	54	47	37
9b	46	50	40	33
9c	50	39	43	47
9d	43	36	33	43
9e	68	57	57	53
9f	46	43	43	33
9g	54	39	37	40
Streptomycin	100	100	--	--
Imidil	--	--	100	100
Control (DMSO)	00	00	00	00

RESULTS AND DISCUSSION

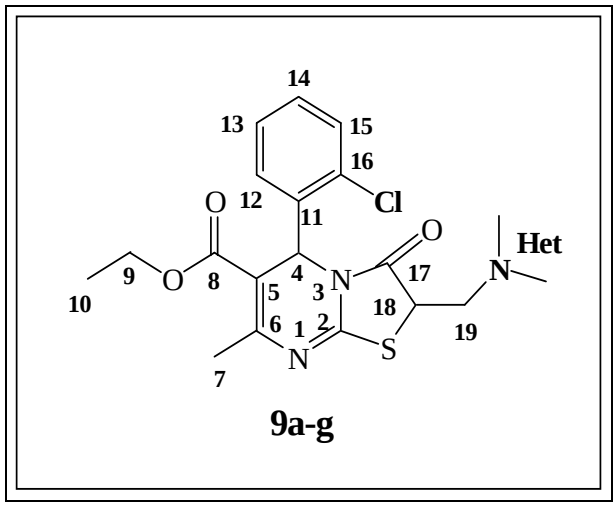
Chemistry
Assignments of the products **(9a-g)** were based on elemental analysis, IR, ^{1}H and ^{13}C NMR spectral studies.

The synthetic route followed for the preparation of ethyl 4-(2-chlorophenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate **(4)**, ethyl 5-(2-chlorophenyl)-7-methyl-3-oxo-3,5-dihydro-2*H*-thiazolo[3,2-*a*]pyrimidine-6-carboxylate **(6)** and ethyl 5-(2-chlorophenyl)-7-methyl-3-oxo-3,5-dihydro-2*H*-thiazolo[3,2-*a*]pyrimidine-6-carboxylate **(9a-g)** are outlined in **Scheme I**. The reaction of ethyl 4-(2-chlorophenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate **(4)** with chloroacetic acid **(5)** gives ethyl 5-(2-chlorophenyl)-7-methyl-3-oxo-3,5-dihydro-2*H*-thiazolo[3,2-*a*]pyrimidine-6-carboxylate **(6)** with the yield of 63-78%. For the synthesis of title compounds **(9a-g)**, the optimum reaction conditions were established by changing molar ratio of reactants, solvent and acidity level. It was observed that the most suitable molar ratios of thiazolopyrimidine, heterocyclic secondary amine and formaldehyde were 1:1:1 and the most suitable reaction medium was ethanol containing conc. HCl. The reaction yields were between 60-75% while the reaction time period was almost same (~7-8 hr) except that of morpholine was increased to 8.5 hrs. All the title compounds **(9a-g)** are new and analyzed for their purity by m.p., TLC. The structures of **(9a-g)** were established on the basis of analytical data and spectral data (FT-IR, ^{1}H and ^{13}C NMR). Examination of analytical data of C, H and N content of final compounds are in good agreement with calculated values based on proposed structure **(Scheme I)**.

Spectral characterization:
In case of ethyl 4-(2-chlorophenyl)-6-methyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate **(4)**,the characteristic vibration bands observed to thioamide C=S **(4)** are in the region 1420-1260 cm-1,C-N stretching vibration bands and C-O-C stretching of ester group are exhibited in the region 1330-1300 cm^{-1} and 1300-1250 cm^{-1} respectively. Analogously, in the ^{1}H NMR spectrum, showed two singlets at δ 2.1integrating for three protons of C_7. The C_4-proton of pyrimidine appeared as singlet at δ 5.3 ppm integrating for one proton. The N_1 and N_3-proton appeared as a singlet at δ 7.8 and 9.1 ppm respectively. The aromatic protons were observed as multiplets in the region δ 7.2 to 7.4 ppm. Signals observed at δ 1.1 ppm (t, 3H, C_{10}) and δ 4.0 ppm (q, 2H, C_9) for ester group and in case of ^{13}C NMR spectra, characteristic chemical shifts of ester carbon atoms observed at 14.4 ppm (C-10) and 54.6 ppm (C-9). Pyrimidine ring carbons C-4, C-5 and C-6 at δ 59.1, 114.4 and 149.1 ppm, aromatic carbons C-11 to C-16 at δ 129.2, 132.7, 135.4 and 137.1 ppm, carbonyl carbons C-2 at δ 195.2 and thiocarbonyl carbon C-8 at δ 177.3 ppm.

Evidences for the formation of Thiazole ring:
IR spectra of parent **(6)** showed all the characteristic absorption bands except the disappearance of a broad band at 3125 cm^{-1} due to secondary –NH of **(4)** as both of these two groups have participated in cyclization to form thiazolopyrimidine fused ring. Besides this, the characteristic absorption bands of thiazole ring systems are observed at 1425-1375 cm^{-1} (ring stretching), 900-650 cm^{-1} (C-H out of plane bending, several bands), 1730 and 1670 cm^{-1} (C=O stretching). Comparison of ^{1}H NMR spectra of **(6)** with that of **(4)** reveals presence of a singlet at δ 3.9 ppm integrating for 2H of C_{18} and disappearance of two singlets at δ 7.7 and 9.2 ppm due to 1H of N_1 and N_3. This has been further confirmed by two chemical shifts at δ 32.2 and 172.4 ppm for C-18 ($-CH_2$) and C-17 (C=O) respectively in ^{13}C NMR spectrum of **(6)**.

Evidences for the formation C-Mannich bases:
The general structure of substituted thiazolo[3,2-*a*]pyrimidine **(9a-g)** is



9a-g

- Title compounds **(9a-g)** consist of parent **(6)** linked with amino methylene bridge to heterocyclic secondary amino component. The IR spectra showed the disappearance of characteristic absorption band at 3200-3100 cm^{-1} of secondary -NH stretching of heterocyclic secondary amine support the formation of aminomethylene bridge between carbon atom of the thiazolopyrimidine ring and nitrogen atom of the heterocyclic secondary amine in C-Mannich bases.
- Comparison of ^{1}H NMR spectra of the C-Mannich bases with its parent **(6)** have shown the absence of singlet at δ 3.9 ppm (2H, $-CH_2$ of thiazole ring) suggested that the 1H of carbon atom (C_{18}) of thiazolopyrimidine reacted with formaldehyde and heterocyclic secondary amine to yield the corresponding C-Mannich base.
- Besides this, the presence of triplet and doublet at δ 4.4-4.8 and 4.7-5.3 ppm due to 1H of C_{18} and 2H of C_{19} of thiazole ring and methylene bridge of C-Mannich bases respectively except that of **(9e)** because of morpholine as aliphatic secondary amine support the formation of C-Mannich bases. This is further confirmed by the appearance of new signals at δ 53.1-55.4 ppm for the C-19 ^{13}C NMR spectrum of **(9a-g)**.

Antifungal activity
Newly synthesized substituted Ethyl 5,7-dimethyl-3-oxo-2,3-dihydro-5*H*-[1,3]-thiazolo[3,2-*a*]pyrimidie-6-carboxylate derivatives **(9a-g)** were tested for the antimicrobial activity against *Escherichia coli* and *Bacillus subtilis* as antibacterial agents while Antifungal activity was carried out against *Aspergillus niger* and *Candida albicans* as antifungal agents using sabouraud's dextrose agar medium. Streptomycin and Imidil was used as standard drugs for antibacterial and antifungal activity respectively. The antimicrobial activity data revealed that all the synthesized compounds were having promising antifungal activity against all the fungal species and these may be due to the presence of the -Cl group on aromatic ring. Among all the synthesized compounds **(9a-g)**, Mannich bases derived from benzimidazole **(8a)** and morpholine **(8e)** as heterocyclic secondary amine are excellent antimicrobial agents. A compound **(9a)** and **(9c)** shows a moderately active against *Aspergillus niger* and *Candida albicans* respectively while the remaining compound shows poor antimicrobial activity.

CONCLUSION

This study reports the synthesis, characterization and antimicrobial activity of new series of Mannich bases of ethyl 5-(2-chlorophenyl)-7-methyl-3-oxo-3,5-dihydro-2*H*-thiazolo [3,2-*a*]pyrimidine-6-carboxylate derivatives. All the compounds were tested for antimicrobial activity against *Escherichia coli* and *Bacillus subtilis* as antibacterial

agents while Antifungal activity was carried out against *Aspergillus niger* and *Candida albicans* as antifungal agents using streptomycin and imidil which were used as standard drugs for antibacterial and antifungal activity respectively. The screening results show excellent to moderate against microbial species, paving the way for new Mannich bases to be used in various bacterial and fungal infections and may be drug candidates for other pharmacological activities.

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