Convergent One Pot Synthesis of Novel Thiazolo Pyrimidine and Their Antimicrobial Activities

KEYWORDS
2-amino thiazole, Thiazolo pyrimidines, synthesis, antimicrobial study

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ABSTRACT
One pot three component cyclocondensation of 2-amino-4-phenyl thiazole, ethyl benzoyl acetate and aromatic aldehydes produces Thiazolo Pyrimidine in moderate to good yields. Some of the compounds were screened for anti-bacterial (Staphylococcus aureus, Staphylococcus aureus (MRSA), Streptococcus pyogenes) and antifungal (Aspergillus niger) studies by Agar Diffusion method. Compounds show moderate to good antifungal activity but low antibacterial activity.

INTRODUCTION
Various 2-amino thiazole derivatives are of great importance to chemists as well as biologists as they exhibit a variety of biological activities like antibacterial, antiviral, anti-inflammatory, antiallergy and antihypertensive. The high scientific interest attracted by thiazolo-pyrimidines is due to broad spectrum of biological activities exhibited by these compounds like antihypertensive, antioxidant agents, anti-tumor activity and calcium channel modulation. In 1893, Italian Chemist Pietro Bignelli synthesized 3,4-dihydro-pyrimidin-2(1H)-ones by heating a mixture of aldehyde, β-ketoester and urea in ethanol containing a catalytic amount of HCl. However, this procedure suffers from harsh reaction conditions, long reaction time and frequently low yield. Later on subsequent multistep synthesis furnished some what higher yield but these do not have the simplicity of original one pot protocol.

This paper reports a novel approach to the synthesis of thiazolo-pyrimidines. As thiazolo pyrimidines are classes of fused heterocycles that are of considerable interest because of their wide range of biological activities like anti cancer, phosphate inhibitors, antimicrobial and acetylcholinesterase inhibitors.

Various methods have been reported for the synthesis of thiazolo pyrimidine in literature which are associated with many drawbacks like multistep synthetic root, longer reaction time with drastic conditions, difficult workup, unsatisfactory yields and use of expensive & hazardous chemicals. Hence, here we report herein a general, single step and efficient approach towards the synthesis of thiazolo pyrimidines.

Prompted by the observed biological activities of the above mentioned derivatives and in continuation of our research for thiazolo pyrimidines, a rapid and efficient synthesis of a series of novel thiazolo pyrimidines and evaluation of their antimicrobial potency is being reported.

RESULTS AND DISCUSSION
Synthesis and characterization
In view of the recent emphasis aimed at developing simple, highly efficient and one pot methodologies for the preparation of organic compounds, herein we report convergent one pot cyclocondensation of ethyl benzoyl acetate 1 (0.01 mole), thiazole 2 (0.01 mole), various substituted aromatic aldehydes 3 (0.01 mole) which give thiazolo pyrimidines 4 (Scheme 1). A variety of aromatic aldehydes carrying either electron donating or electron withdrawing substituents reacted very well, giving products in high purity.

Scheme 1: synthesis of thiazolo pyrimidine

The structure of the purified compounds (4a-m) was characterized by elemental analysis, IR, 1H NMR and mass spectral studies. Formation of 4b was confirmed from its spectral data. IR spectra of the compound 4b showed absorption bands at 3282.1 cm⁻¹, 1628 cm⁻¹ and 1537 cm⁻¹ due to NH, C=O and C=N stretching vibrations. The 1H NMR showed broad singlet at 6.87 corresponding to NH proton that got exchanged with D₂O, multiplet at 3.75. Also singlet at 5.61 was observed for aromatic and HA protons. 3 protons of –OCH₃ exchanged with D₂O, quartet for –OCH₃ at m/z 470 which confirms the structure of 4b. The mass spectra of compound 4b showed molecular ion peak M⁺ at m/z 470 which correspond to the molecular formula C₇₇H₇₅N₃O₅S which further give the evidence for the formation of compound 4b. The elemental analysis of compounds (4a-m) was found to be in good agreement with the calculated values (± 0.4%). The characterization data of ethyl-3,7-biphenyl-5-[substituted
phenyl]-8,8a-dihydro-5H-[1,3]thiazolo[3,2-aj]pyrimidine-6-carboxylate (4a-m) are given in experimental section. The structure of 4b was further established via acetylation of compound 4b with acetic anhydride under reflux led to the formation of 8-acetyl-5-[4-methoxyphenyl]-3,7-diphenyl-8,8a-dihydro-5H-thiazolo[3,2-aj]pyrimidine-6-carboxylic acid 5.

Where the usual acetylation took place at secondary NH group (position 8) and ester group at position 6 got hydrolysed to acid group which is established by the downfield appearance of the acid hydrogen at position ð11.67 and further the compound gave brisk effervescence on treatment with aq. NaHCO₃. Disappearance of quartet because of CH₂ group of ethyl at ð3.47 and a triplet because of CH₃ group of ethyl at ð1.06 further proves its structure.

**ANTIMICROBIAL STUDIES**

Some of the compounds were screened for anti-bacterial (Staphylococcus aureus, Staphylococcus aureus (MRSA), Streptococcus pyogenes) and antifungal (Aspergillus niger, Neurospora crassa) studies by Agar Diffusion method. For evaluating antimicrobial activity Gentamycin and Ampho-

tericin were used as the standard drug. The observed minimum inhibitory concentration (MIC) and zone of inhibition at a concentration of 800 μg/ml of some newly synthesized compounds were measured according to various strains (Table 1).

**Table 1: Antimicrobial screening of different compounds against different strains**

| Tested Samples                  | S. Au-reus | MRSA | S. Pyo-gens | A. Niger | N. Crassa |
|--------------------------------|-----------|------|------------|---------|----------|
|                                | A' MIC (µg) | A' MIC (µg) | A' MIC (µg) | A' MIC (µg) | A' MIC (µg) |
| 4b                             | >800       | >800  | >800       | >800     | >800     |
| 4d                             | >800  7    | 400   | 9          | 400  5   | 200 13    |
| 4g                             | 3         | 800   | >800       | >800     | >800     |
| 4h                             | 2          | 800   | >800       | 3        | >800     |
| 4i                             | 2          | 800   | >800       | >800     | >800     |
| 4m                             | 4          | 400   | 7          | 100      | 10 400 5 |
| Gentamycin                     | 34 <25     | 34 <25| 33 <25     |          |          |
| Amphotericin                   |           |       |            | 7        | 100 9 400|

* A = Zone of inhibition in mm. at a concentration of 800 µg/ml

(--) showing no activity.

The compounds ‘4d’ and ‘4m’ show maximum activities against this bacterial and fungal strain. For strain N. Crassa, they show MIC value even less than the standard drug. So compounds ‘4d’ and ‘4m’, having hydroxy group at the para position of the aryl moiety are more active then the compounds bearing other substituents on the aryl moiety. In general compounds exert very low antibacterial activity but moderate to good antifungal activity.

**EXPERIMENTAL Instrumentation**

Melting points were taken in open end capillaries and were uncorrected. The purity of the synthesized compounds was checked by thin layer chromatography on Silica gel G (Merck) plates and spots were located by iodine vapours. ¹H NMR spectra were recorded on BRUKER ADVANCE II 400 (400 MHz) NMR Spectrometer using tetramethyl silane (TMS) as internal standard. All chemical shifts were reported as δ (ppm) values. The IR spectra were recorded on Perkin-Elmer spectrum RX IFT-IR System using KBr pellets. The mass spectra were obtained on a JEOL 5 x 102/DA-6000 mass spectrometer. The elemental analyses were recorded on VARIO MICO CHNS ANALYZER. All the compounds gave satisfactory results within ± 0.4% of theoretical values.

**General Procedure for the synthesis of 2-amino-4-phenyl thiazole(2):** 2-amino-4-phenyl thiazole was prepared as per method reported in literature [18]: m.p. 146°C; IR (KBr, cm⁻¹): 3251.9(NH), 1599.3, 1533(aromatic stretching), 1460(C=N); PMR(400MHz, CDCl₃, δ, ppm): 5.20(s, 2H, NH), 6.711(s, 1H, CH), 7.75-7.25(m, 5H, CH₃).

**General Procedure for the synthesis of ethyl-3,7-biphenyl-5-[substituted phenyl]-8,8a-dihydro-5H-[1,3]thiazolo[3,2-aj]pyrimidine-6-carboxylate(4a-m):** A mixture of ethyl benzoyl acetate (0.01 mole), thiazole 2 (0.01 mole), various substituted aromatic aldehydes 3 (0.01 mole) in absolute ethanol(10ml) with 4-5 drops of hydrochloric acid was heated under reflux for 2-6 hour (Scheme 1). After completion of the reaction (followed by TLC), Removal of the solvent under reduced pressure yielded the product which was recrystallized from ethanol. The structure of the prepared compounds was characterized on the basis of melting point, IR, NMR, Mass and elemental analysis spectra. The spectral data of the prepared compounds are given below:

**Ethyl-3,7-biphenyl-5-[phenyl]-8,8a-dihydro-5H-[1,3]thiazolo[3,2-aj]pyrimidine-6-carboxylate (4a):**

This compound was obtained as off white solid; yield 44%; m.p. 235-237°C; IR (KBr, cm⁻¹): 3276.1, 3103.3, 2975.5, 1631.8, 1537.3, 1492.6; ¹H NMR (DMSO-d₆, δ, ppm): 8.17(s, 1H, NH), 6.72-7.54 (m, 16H, Ar-H & HA), 5.60 (s, 1H, HB), 3.56 (q, 2H, -OCH₂CH), 2.49 (s, 1H, HC), 7.05 (s, 3H, OCH₃); MS: m/z (M⁺) 440; Anal. Calcd. for C₁₇H₁₂N₂O₅S: C 73.55, H 5.45, N 6.36; Found: C 73.81, H 5.46, N 6.33.

**Ethyl-3,7-biphenyl-5-[4-methoxyphenyl]-8,8a-dihydro-5H-[1,3]thiazolo[3,2-aj]pyrimidine-6-carboxylate (4b):**

This compound was obtained as off white solid; yield 40%; m.p. 242-244°C; IR (KBr, cm⁻¹): 3282.1, 3125.1, 2962, 1628, 1537.7; ¹H NMR (DMSO-d₆, δ, ppm): 8.12 (s, 1H, NH), 6.87-7.27 (m, 15H, Ar-H & HA), 5.61 (s, 1H, HB), 3.75 (s, 3H, OCH₃), 3.47 (q, 2H, -OCH₂CH), 2.51 (s, 1H, HC), 1.06 (s, 3H, OCH₃); MS: m/z (M⁺) 470; Anal. Calcd. for C₁₇H₁₄N₂O₇S: C 71.41, H 5.53, N 5.95; Found: C 71.58, H 5.52, N 5.94.

**Ethyl-3,7-biphenyl-5-[2-nitrophenyl]-8,8a-dihydro-5H-[1,3]thiazolo[3,2-aj]pyrimidine-6-carboxylate (4c):**

This compound was obtained as yellow solid, yield 39%; m.p. 182-184°C; IR (KBr, cm⁻¹): 3284, 2922.4, 1640, 1592; ¹H NMR (DMSO-d₆, δ, ppm): 9.10 (s, 1H, NH), 6.82-7.65 (m, 15H, Ar-H & HA), 5.63 (s, 1H, HB), 3.78 (q, 2H, -OCH₂CH), 2.50 (s, 1H, HC), 1.10 (s, 3H, OCH₃); MS: m/z (M⁺) 485; Anal. Calcd. for C₁₉H₁₅N₂O₈S: C 66.74, H 4.74, N 8.65; Found: C 66.90, H 4.72, N 8.64.

**Ethyl-3,7-biphenyl-5-[4-hydroxy-3-methoxyphenyl]-8,8a-dihydro-5H-[1,3]thiazolo[3,2-aj]pyrimidine-6-carboxylate (4d):**

This compound was obtained as green solid, yield 44%; m.p. 215-217°C; IR (KBr, cm⁻¹): 3438.6, 3266.4, 3063.8, 2946.7,
Ethyl-3,7-biphenyl-5-[4-chlorophenyl]-8a,8-dihydro-5H-[1,3]thiazolo[3,2-a]pyrimidine-6-carboxylate (4f): This compound was obtained as light brown solid, yield 42%; m.p. 190-192°C (IR (KBr cm\(^{-1}\)): 3450.2, 3272.4, 3060.5, 2935.2, 1638, 1525.8; \(^1\)H NMR (DMSO-d\(_6\), ppm): 9.10 (s, 1H, OH), 8.14 (s, 1H, NH), 6.80 -7.51 (m, 15H, Ar-H & HA), 5.58 (s, 1H, HB), 3.65 (q, 2H, -OCH\(_2\), 2.57 (s, 1H, HC), 1.15 (t, 3H, OCH\(_3\)) cm\(^{-1}\); MS: m/z (M\(^+\)) 456; Anal. Calcd. for C\(_{26}\)H\(_{26}\)N\(_6\)O\(_5\): C 70.97, H 5.25, N 6.13; Found: C 71.21, H 5.27, N 6.11.

Ethyl-3,7-biphenyl-5-[2,3-methylenedioxyphenyl]-8a,8-dihydro-5H-[1,3]thiazolo[3,2-a]pyrimidine-6-carboxylate (4g): This compound was obtained as brown solid. yield 50%; m.p. 210-212°C; IR (KBr cm\(^{-1}\)): 3356.6, 3215, 3085.3, 2924.3, 1627.2, 1595.1; \(^1\)H NMR (DMSO-d\(_6\), ppm): 9.18 (s, 1H, OH), 8.10 (s, 1H, NH), 6.69 -7.35 (m, 15H, Ar-H & HA), 5.62 (s, 1H, HB), 3.56 (q, 2H, -OCH\(_2\), 2.57 (s, 1H, HC), 1.25 (t, 3H, OCH\(_3\)) cm\(^{-1}\); MS: m/z (M\(^+\)) 468; Anal. Calcd. for C\(_{26}\)H\(_{26}\)N\(_6\)O\(_5\): C 70.97, H 5.25, N 5.76; Found: C 69.12, H 4.97, N 5.76.

Ethyl-3,7-biphenyl-5-[4-nitrophenyl]-8a,8-dihydro-5H-[1,3]thiazolo[3,2-a]pyrimidine-6-carboxylate (4h): This compound was obtained as light green solid, yield 68%; m.p. 251-252°C; IR (KBr cm\(^{-1}\)): 3281, 3122.6, 2925, 1632.9, 1539.9, 1349.8; \(^1\)H NMR (DMSO-d\(_6\), ppm): 8.20 (s, 1H, NH), 7.14 -7.50 (m, 15H, Ar-H & HA), 5.78 (s, 1H, HB), 3.52 (q, 2H, -OCH\(_2\), 2.50 (s, 1H, HC), 1.09 (t, 3H, OCH\(_3\)) cm\(^{-1}\); MS: m/z (M\(^+\)) 471; Anal. Calcd. for C\(_{26}\)H\(_{26}\)N\(_6\)O\(_5\): C 67.74, H 4.74, N 5.85; Found: C 67.69, H 4.72, N 8.62.

Ethyl-3,7-biphenyl-5-[2,4-dichlorophenyl]-8a,8-dihydro-5H-[1,3]thiazolo[3,2-a]pyrimidine-6-carboxylate (4i): This compound was obtained as off white solid, yield 32%; m.p. 240-241°C; IR (KBr cm\(^{-1}\)): 3283.6, 3118.4, 2924.4, 1630.6, 1539; \(^1\)H NMR (DMSO-d\(_6\), ppm): 7.59 (s, 1H, NH), 7.10 -7.49 (m, 14H, Ar-H & HA), 5.72s (1H, HB), 3.50 (q, 2H, -OCH\(_2\), 2.56 (s, 1H, HC), 1.12 (t, 3H, OCH\(_3\)) cm\(^{-1}\); MS: m/z (M\(^+\)) 570; Anal. Calcd. for C\(_{30}\)H\(_{29}\)Cl\(_2\)N\(_6\)O\(_5\): C 63.60; H 4.32; N 5.50; Found: C 63.41; H 4.33; N 5.52.

Ethyl-3,7-biphenyl-5-[3-nitrophenyl]-8a,8-dihydro-5H-[1,3]thiazolo[3,2-a]pyrimidine-6-carboxylate (4j): This compound was obtained as yellow solid, yield 36%; m.p. 188-190°C; IR (KBr cm\(^{-1}\)): 3274.6, 3177.7, 2996.8, 1643.8, 1582.6, 1493.1; \(^1\)H NMR (DMSO-d\(_6\), ppm): 9.55 (s, 1H, NH), 7.17 -7.25 (m, 15H, Ar-H & HA), 5.88 (s, 1H, HB), 3.85 (q, 2H, -OCH\(_2\), 2.52 (s, 1H, HC), 1.14 (t, 3H, OCH\(_3\)) cm\(^{-1}\); MS: m/z (M\(^+\)) 485; Anal. Calcd. for C\(_{26}\)H\(_{26}\)N\(_6\)O\(_5\): C 66.74, H 4.74, N 5.86; Found: C 66.93, H 4.72, N 8.62.

Antimicrobial Studies

Antibacterial studies: Some of the synthesized compounds were screened for their antibacterial activity against Staphylococcus aureus, Staphylococcus aureus (MRSA), Streptococcus pyogenes according to the Agar Diffusion Method using dimethylsulfoxide as solvent. The stock cultures of bacteria were revived by inoculating in broth media and grown at 37°C for 18 hrs. The agar plates of the above media were prepared and wells were made in the plate. Each plate was inoculated with 18 h old cultures (100 µl, 10\(^{9}\) cfu) and spread evenly on the plate. After 20 min, the wells were filled with compounds at different concentrations. The control wells with Gentamycin were also prepared. All the plates were incubated at 37°C for 24 h and the diameter of inhibition zone were recorded in Table-1.

Antifungal studies: The antifungal screening studies of some compounds were performed by the standard Agar diffusion Method. Aspergillus niger, Neurospora crassa were used as test organisms.

Media Used: Potato Dextrose Agar (PDA). 250 g of peeled potato were boiled for 20 min and squeezed and filtered. To this filtrate 20 g of dextrose was added and the volume was made up to 1000ml by distilled water. Initially, the stock cultures were revived by inoculating in broth media and grown at 27°C for 48 hrs. The agar plates of the above media were prepared and wells were made in the plate. Each plate was inoculated with 48 h old cultures (100 µl, 10\(^{9}\) cfu) and spread evenly on the plate. After 20 min, the wells were filled with compounds at different concentrations. The control plates were made up to 1000ml by distilled water. Initially, the stock cultures were revived by inoculating in broth media and grown at 27°C for 48 hrs. The agar plates of the above media were prepared and wells were made in the plate. Each plate was inoculated with 48 h old cultures (100 µl, 10\(^{9}\) cfu) and spread evenly on the plate. After 20 min, the wells were filled with compounds at different concentrations. The control
with antibiotic were also prepared. All the plates were incubated at 27°C for 48 h and the diameter of inhibition zone were recorded in Table-1.

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REFERENCE
1. Siddiqui, H.L.; Muhammad, Z.R.; Naveed, A.; George, W.W. and Paul, D., Chemical & Pharmaceutical Bulletin. 55(7), 2007, 1014-1017.
2. Ghaemmaghami, S.; Barnaby, C.H.M.; Adam, R.R.; Stanley, B.P., Journal of Virology, 84(7), 2010, 3408-3412.
3. Venugopala, K. N. and Jayasree, B.S., Ind. J. Heterocyclic Chem. 12, 2003, 307-10.
4. Karl, D.H.; Friedrich, K.H. and James T.O., J. Med. Chem. 26, 1983, 1158-1163.
5. William, C.P.; Harriet, W.H.; Michael, D.T.; Michael, J.R.; David, G.T.Jr.; Cleo, J.C.C.; Annette, M.D.; Sylvester, R.K.; Ila, S.; Steinbaugh, B.A. and Batly, B.L.; Painchaud, C.A.; Rapundalo, S.T.; Michniewich, B.M.; Olson, S.C.J., J. Med. Chem. 35, 1992, 2562-2572.
6. Groves, G. J.; Dzwonczyk, S.; McMullen, D. M.; Normadnian, C. S.; Steph, P.G. and Moreland, S. J., Cardiovasc Pharmacol. 26, 1995, 289-294.
7. Stefani, H. A.; Oliveira, C. B.; Almeida, R. B.; Pereira, C. M. P.; Braga, R. C.; Cella, R.; Borges, V. C.; Savagnago, L. and Nogueira, C. W., Eur. J. Med. Chem. 41, 2006, 513-518.
8. Rusowsky, D.; Canto, R.F.S.; Sanches, S.A.A.; and O’Cear M.G.M et al, Bioorg. Chem., 34, 2006, 173-182.
9. Atwal, K. S.; Rosvnyak, G. C.; Kinball, S. D.; Floyd, D. M.; Moreland, S.; Swanson, B. N.; Gougoutas, J. Z.; Schwartz, J.; Smiley, K. M. and Malley, M. F., J. Med. Chem. 33, 1990, 2629-2635.
10. Biglarelli, P. Gazz. Chim. Ital. 23, 1893, 360-416.
11. Atwal, K.S.; O’Reilly, B.C.; Gougoutas, J.Z. and Malley, M.F., Heterocycles. 26, 1987, 1189-1192.
12. Hammam, A.G.; Sharaf, M.A. and Abdel Hafez, N.A., Indian J. Chem. 40B, 2001, 213-221.
13. Kolb, S.; Mandesert, O.; Godeard, M.L.; Jullien, D.; Villoiteux, B.O.; Ducournon B.; Garbay C. and Braud E., Chem Med Chem. 4, 2009, 633-648.
14. El-Emany, T.I. and Abdel-Mohsen, S.A., Phosphorus Sulfur, 181, 2006, 2459-2474.
15. Zhu, H.; Chen, L.; Zhang, L.; Liu, S.; Wan, D.C.C.; Lin, H. and Hu C., ARKIVOC xiii, 2008, 266-277.
16. Kurbanova, M.M., Russian J. Org. Chem. 42, 2006, 1871-1872.
17. Bansal, M.; Kaur, R. and Kaur B., Heterocyclic Communications. 15(6), 2009, 417-421.