SYNTHESIS OF 1,3,5-TRIAZINE BASED PYRIMIDINES AS POTENT ANTI-BACTERIAL AND ANTI-FUNGAL AGENTS

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ABSTRACT

Objectives: Pyrimidines and 1,3,5-triazines play an important role in many biological processes. 2,4,6-trichloro-1,3,5-triazine (TCT) provides a good opportunity to a chemist in the synthesis of its various derivatives by the replacement of its chlorine atom with bioactive pharmacophores at three different temperatures. Hence, present work was carried out keeping in mind that combination or addition of two or more pharmacophores may enhance the biological activities with wide spectrum of their pharmacological profile. Methods: Chalcone derivatives of [(E)-1-(4-(4,6-bis(pyrimidine-2-ylamino)-1,3,5-triazine-2-ylamino) phenyl)-3(pyridine-2-yl)prop-2-en-1-one & (E)-3-(pyridine-2-yl)-1-(4-(4-(4-(E)-3-(pyridine-2-yl)acryloyl)phenylamino)-6-(pyrimidine-2-ylamino)-1,3,5-triazine-2-ylamino)phenyl)prop-2-en-1-one] were prepared to explore to provide an easy one pot synthesis with urea, thiourea and guanidine to furnish pyrimidine analogues. All the compounds synthesized were characterized elemental analysis and IR, $^1$H NMR, MS data. Conclusion: Compound 2 and 8 has been found to possess good anti-bacterial and anti-fungal activity.

KEYWORDS: Chalcones, s-triazines, urea, thiourea, guanidine nitrate, pyrimidines, anti-bacterial and anti-fungal activities.

INTRODUCTION

The pyrimidine nucleus occurs in a considerable number of natural products of vital importance in living organisms.$^{[1-2]}$ As a structural component of key bio-molecules, the pyrimidine moiety is widely incorporated to design the privileged structures of medicinally potent molecules. Pyrimidine derivatives have been reported to possess a variety of biological
activities such as analgesic,\textsuperscript{[3]} anti-hypertensive,\textsuperscript{[4]} anti-pyretic,\textsuperscript{[5]} anti-viral,\textsuperscript{[6]} anti-inflammatory activities, \textit{etc.}\textsuperscript{[7]} Pyrimidines are also associated with antibiotic, anti-malarial, anti-cancer drugs,\textsuperscript{[8]} CNS depressant,\textsuperscript{[9]} \textit{etc.} s-Triazine or (1,3,5-triazine) derivatives having active 2, 4 and 6 positions have been shown in literature to exhibit impressive pharmacological properties such as anti-cancer, anti-malarial, anti-viral, anti-bacterial, anti-fungal, herbicidal, anti-ulcer, anti-arthritis, hypoglycemic, anti-inflammatory, anti-tubercular activities. Presence of \(\alpha,\beta\)-unsaturated carbonyl compounds in various molecular framework makes the molecule to undergo reactions with the incoming neucleophiles very easily to give different heterocyclic compounds.\textsuperscript{[10]} This property of \(\alpha,\beta\)-unsaturated carbonyl compounds was explored in the present work in order to generate pyrimidine ring with various bidentate nucleophiles such as urea, thiourea and guanidine nitrate.

**MATERIALS AND METHODS**

**Synthesis**

All chemicals and reagents were purchased from commercial sources. Physical data of all the compounds were found to be consistent to the structures assigned to these molecule. Melting points were determined in open glass capillaries and are uncorrected. Completion of the reaction was checked by TLC on silica gel G plates. IR spectra were recorded on KBr (SHIMADZU) FTIR-8400S. \(^1\)H NMR was recorded on model AVANCE II 400 (BRUKER) using CDCl\(_3\) and DMSO-d\(_6\) as solvents and TMS as an internal reference. Chemical shift are expressed in \(\delta\)ppm. Structures of all the compounds were established on the basis of elemental analysis, IR and \(^1\)H NMR, MS mass spectral data.

**General Procedures**

**Preparation of 4-(4-(4,6-bis(pyrimidin-2-ylamino)-1,3,5-triazin-2-ylamino)phenyl)-6-(pyridine-2-yl)pyrimidin-2-ol (2)**

A mixture of (E)-1-(4-(4,6-bis(pyrimidin-2-ylamino)-1,3,5-triazine-2-ylamino)phenyl)-3-(pyridine-2-yl)prop-2-en-1-one (1) (0.489g, 0.001mol), urea (0.60g, 0.01mol) and 0.2g NaOH in 25ml of 80\% dil. ethanol was refluxed for 10h and then concentrated to half its volume and cooled. Precipitate was filtered and treated with glacial acetic acid (5ml) just enough to dissolve sodium salt of the pyrimidine and refluxed for 15min. The reaction mixture was poured on crushed-ice and the solid obtained was purified by crystallization with DMF-Ethanol (1:9) to give compound (2). Yield :70\%, mp. 272-275\(^\circ\)C. IR (KBr) cm\(^{-1}\): 3488 (O-H str.), 3368 (N-H str.), 3175 (Ar-H str.), 1609 (C=C str.), 1560 (C=N str.), 1180 (C-N str.).
str., 1650 (C=O str.). \(^1\)H NMR (DMSO-d\(_6\)) \(\delta\) ppm: 9.34-8.49 [m, 4H, ArH (pyridine ring)], 8.40-8.45 [m, 6H, ArH (pyrimidine ring)], 6.69-7.54 [dd, 4H, ArH (benzene ring)], 6.52-6.69 [s, 1H, ArH (pyrimidine ring)], 6.58 [s, 3H, NH]. MS (m/z\%): 531(21), 529 (23), 525 (9), 492 (100), 416 (15), 284 (89).

**Preparation of 4-(4-(4,6-bis(pyrimidin-2-ylamino)-1,3,5-triazin-2-ylamino)phenyl)-6-(pyridine-2-yl)pyrimidin-2-thiol (3)**

A mixture of (E)-1-(4-(4,6-bis(pyrimidin-2-ylamino)-1,3,5-triazine-2-ylamino)phenyl)-3-(pyridine-2-yl)prop-2-en-1-one (1) (0.489g, 0.001mol), thiourea (0.76g, 0.01mol) and 0.2g NaOH in 25ml of 80% diluted ethanol was refluxed for 10h and then concentrated to half of its volume and cooled. Precipitate was filtered and residue was treated with glacial acetic acid (5ml) just enough to dissolve sodium salt of the pyrimidine and refluxed for 15min. The reaction mixture was poured on crushed ice and the solid obtained was purified by recrystallization with DMF-Ethanol (1:9) to give compound (3). Yield : 65%, mp. 280-283°C. IR (KBr) cm\(^{-1}\): 3350 (N-H str.), 2800 (Ar-H str.), 2300 (S-H str.), 1584 (C=N str.), 1600 (C=C str.), 1280 (C-N str.), 620 (C=C str.), 1650 (C=O str.), 1600 (C=O str.). \(^1\)H NMR (DMSO-d\(_6\)) \(\delta\) ppm: 12.15 [s, 1H, SH at pyrimidine ring], 9.38-8.32 [m, 4H, ArH (pyridine ring)], 7.70-7.46 [m, 6H, ArH (pyrimidine ring)], 6.79-6.59 [dd, 4H, ArH (benzene ring)], 6.89-6.76 [s, 1H, ArH (pyrimidine ring)], 6.58 [s, 3H, NH]. MS (m/z\%): 548 (1.5), 547 (3), 546 (25), 408 (100), 302 (85), 165 (56).

**Preparation of \(N^2\)-(4-(2-amino-6-(pyridine-2-yl)pyrimidin-4-yl)phenyl)-\(N^4\),\(N^6\)-di(pyrimidin-2-yl)-1,3,5-triazine-2,4,6-triamine (4)**

A mixture of (E)-1-(4-(4,6-bis(pyrimidin-2-ylamino)-1,3,5-triazine-2-ylamino)phenyl)-3-(pyridine-2-yl)prop-2-en-1-one (1) (0.489g, 0.001mol) in ethanol (25ml) was added to guanidine nitrate (2.44g, 0.02 mol) and 0.2g NaOH in 25ml of 80% dil. ethanol was refluxed for 7h and then concentrated to half of its volume and cooled. Precipitate was filtered and residue was treated with glacial acetic acid (5 ml.) just enough to dissolve sodium salt of the pyrimidine and refluxed for 15 min. The reaction mixture was poured on crushed ice and the solid obtained was purified by re-crystallization with DMF-Ethanol (1:9) to give flakes like dark coloured crystals of the compound. Yield : 67%, mp. 285-287°C. IR (KBr) cm\(^{-1}\): 3370 (N-H str.), 2965 (Ar-H str.), 1645 (C=C str.), 1563 (C=N str.), 1287(C-N str.). \(^1\)H NMR (DMSO) \(\delta\) ppm: 8.89 [s, 3H, NH\(_2\)], 8.42,8.52 [m, 4H, ArH (pyridine ring)], 8.46 [m, 6H, ArH (pyrimidine ring)], 6.69-7.52 [dd, 4H, ArH (benzene ring)], 6.91[s, 1H, ArH (pyrimidine)].
Preparation of 6,6’-(4,4’-(6-(pyrimidin-2-ylamino)-1,3,5-triazine-2,4-diyl)-bis(azanediyl)-bis(4,1-phenylene)-bis(4-(pyridine-2-yl)pyrimidin-2-ol (6)

A mixture of N (E)-3-(pyridine-2-yl)-1-(4-(4-(E)-3-(pyridine-2-yl)acryloyl) phenylamino)-6-(pyrimidine-2-ylamino)-1,3,5-triazine-2-ylamino)phenyl)prop-2-en-1-one (5) (0.61 g, 0.001 mol), urea (0.22 g, 0.003 mol) and 0.1 g NaOH in 25 ml of 80% dil. ethanol was refluxed for 10 h and then concentrated to half of its volume and cooled. The reaction mixture was filtered and residue was treated with glacial acetic acid (5 ml) just enough to dissolve sodium salt of the pyrimidine and refluxed for 15 min. The reaction mixture was poured on crushed ice and the solid obtained was purified by re-crystallization with DMF-Ethanol (1:9) to give the compound. Yield : 68%, mp. 287-290°C. IR (KBr) cm\(^{-1}\): 3505 (O-H str.), 3350 (N-H str.), 3035 (Ar-H str.), 1650 (C=O str.), 1580 (C=C str.). \(^1\)H NMR (DMSO-\(d_6\)) \(\delta\)ppm: 8.59 [s, 2H, ArH (on pyrimidine ring)], 8.56-8.42 [m, 8H, ArH (pyridine ring)], 7.80 [s, 2H, OH (on pyrimidine ring)], 6.65 [m, 3H, ArH (pyrimidine ring)], 6.69-7.54 [dd, 8H, ArH (benzene ring)], 4.5 [s, 3H, NH]. MS (m/z%) : 701 (35), 700 (26), 699 (40), 698 (30), 520 (56), 480 (65).

Preparation of 6,6’-(4,4’-(6-(pyrimidin-2-ylamino)-1,3,5-triazine-2,4-diyl)-bis(azanediyl) bis(4,1-phenylene)-bis(4-(pyridine-2-yl)pyrimidin-2-thiol (7)

A mixture of N (E)-3-(pyridine-2-yl)-1-(4-(4-(E)-3-(pyridine-2-yl)acryloyl) phenylamino)-6-(pyrimidine-2-ylamino)-1,3,5-triazine-2-ylamino)phenyl)prop-2-en-1-one (5) (0.61 g, 0.001 mol), thiourea (0.22 g, 0.003 mol) and 0.1 g NaOH in 25 ml of 80% dil. ethanol was refluxed for 10 h and then concentrated to half of its volume and cooled. The precipitate was filtered and residue was treated with glacial acetic acid (5 ml) just enough to dissolve sodium salt of the pyrimidine and refluxed for 15 min. The reaction mixture was poured on crushed ice and the solid obtained was purified by re-crystallization with DMF-Ethanol (1:9) to give the compound. Yield : 72%, mp. 292-294°C. IR (KBr) cm\(^{-1}\): 3400 (N-H str.), 3160 (O-H str.), 2864 (Ar-H str.), 1650 (C=O str.), 1580 (C=C str.). \(^1\)H NMR (DMSO-\(d_6\)) \(\delta\)ppm: 12.20 [s, 2H, SH (on pyrimidine)], 9.52 [s, 2H, ArH (pyrimidine ring)], 8.78-8.90 [m, 6H, ArH (pyrimidine)], 8.08-8.65 [m, 8H, ArH (pyridine ring)], 6.41-7.98 [dd, 8H, ArH (benzene ring)], 4.10-4.12 [s, 3H, NH]. MS (m/z%) : 731 (5), 730 (22), 664 (7), 531 (100), 415 (30), 284 (89).
Preparation of N2,N4-bis(4-(2-amino-6-(pyridine-2-yl)pyrimidin-4-yl)phenyl)-N6-(pyrimidin-2-yl)-1,3,5-triazine-2,4,6-triamine (8)

A mixture of N(E)-3-(pyridine-2-yl)-1-(4-(4-(4-E)-3-(pyridine-2-yl)acryloyl)phenylamino)-6-(pyrimidine-2-ylamino)-1,3,5-triazine-2-ylamino)phenyl prop-2-en-1-one (5) (0.61g, 0.001mol) in ethanol (25ml) was added guanidine nitrate (2.44, 0.02mol) and 0.1g NaOH in 25ml of 80% dil. ethanol was refluxed for 7h and then concentrated to half of its volume and cooled. The precipitate was filtered and residue was treated with glacial acetic acid (5ml) just enough to dissolve sodium salt of the pyrimidine and refluxed for 15min. The reaction mixture was poured on crushed ice and the solid obtained was purified by re-crystallization with DMF-Ethanol (1:9) to give flakes like mustard coloured crystals of the compound.

Yield: 64%, mp. 297-299°C. IR (KBr) cm⁻¹: 3450 (N-H str.), 3100 (Ar-H str.), 1665 (C=C str.), 1585 (C=N str.), 1265 (C-N str.). ¹H NMR (DMSO-d₆) δ ppm: 8.80 [s, 2H, NH₂ (on pyrimidine ring)], 8.65-8.70 [m, 3H, ArH (pyrimidine)], 7.99-8.49 [m, 8H, ArH (pyridine)], 7.24-7.65 [m, 8H, ArH (benzene ring)], 4.0 [s, 3H, NH]. MS (m/z%): 698 (11), 697 (46), 696 (15), 502 (55), 412 (100), 185 (87).

Scheme-1
Table-1. Physical and analytical data of compounds

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Comp. Codes</th>
<th>Molecular Formula</th>
<th>M.W.</th>
<th>Yield (%)</th>
<th>M.P. (°C)</th>
<th>Elemental Analysis Obs. (Theor.) % of C, H, N, &amp; S</th>
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<tbody>
<tr>
<td>1.</td>
<td>2</td>
<td>C_{26}H_{19}N_{13}O</td>
<td>529.52</td>
<td>70</td>
<td>272-275</td>
<td>C: 58.02 (58.97), H: 3.60 (3.62), N: 33.95 (34.39), S: -</td>
</tr>
<tr>
<td>2.</td>
<td>3</td>
<td>C_{26}H_{19}N_{13}S</td>
<td>545.58</td>
<td>65</td>
<td>280-283°C</td>
<td>C: 62.39 (62.17), H: 4.21 (4.23), N: 15.22 (15.28), S: 05.81 (05.88)</td>
</tr>
<tr>
<td>3.</td>
<td>4</td>
<td>C_{26}H_{20}N_{14}</td>
<td>528.53</td>
<td>67</td>
<td>285-287°C</td>
<td>C: 62.98 (63.50), H: 4.15 (4.18), N: 13.79 (14.01), S: -</td>
</tr>
<tr>
<td>4.</td>
<td>6</td>
<td>C_{37}H_{26}N_{14}O_{2}</td>
<td>698.69</td>
<td>68</td>
<td>287-290°C</td>
<td>C: 65.28 (65.30), H: 4.29 (4.33), N: 14.11 (14.17), S: -</td>
</tr>
<tr>
<td>5.</td>
<td>7</td>
<td>C_{37}H_{26}N_{14}S_{2}</td>
<td>730.83</td>
<td>72</td>
<td>292-294°C</td>
<td>C: 50.11 (50.28), H: 3.89 (3.94), N: 15.27 (15.33), S: 08.73 (08.78)</td>
</tr>
<tr>
<td>6.</td>
<td>8</td>
<td>C_{37}H_{28}N_{16}</td>
<td>696.73</td>
<td>64</td>
<td>287-299°C</td>
<td>C: 61.01 (60.80), H: 4.12 (4.14), N: 15.24 (15.33), S: -</td>
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</table>

**RESULT & DISCUSSION**

Development of methodologies to facilitate the preparation of compound libraries based on the privileged structure is an intense area of research in organic and medicinal chemistry. In this paper, we have reported the preliminary results of our study focused on the direction of
the development of compound libraries from the privileged 1,3,5-triazine nucleus. The synthetic plan depicted in scheme-1 and 2 for the preparation of compound (2-4) and (6-8) proceeded with the intermediates (1, 5). In the present work, various biologically important derivatives of pyrimidine substituted s-triazines are synthesized by the cyclocondensation of corresponding chalcones with urea, thiourea, and guanidine nitrate respectively which furnished their corresponding pyrimidine derivatives of biological and pharmacological importance.

BIOLICAL STUDIES
All the compounds were screened for their antimicrobial activity by the disc diffusion method at 100 µg/ml concentration in DMF against *E. coli* (MTCC 476), *B. subtilis* (MTCC 1272) and antifungal activity against *A. niger* (MTCC 281) and *F. solani* (MTCC 2480). The zone of inhibition and activity index were determined in comparison to the standard drugs “Ciprofloxacin” and “flucanazole. The outcome of this study is presented in tabular form in Table 1. All these compounds were found active against the bacterial and fungal strains.

Table-2. Anti-bacterial and anti-fungal activity of compounds 2-4 & 6-8

<table>
<thead>
<tr>
<th>Comp. Comp. Code</th>
<th>Anti-bacterial Activity</th>
<th>Anti-fungal Activity</th>
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<tr>
<td></td>
<td><em>E. coli</em></td>
<td><em>B. subtilis</em></td>
</tr>
<tr>
<td></td>
<td>Zone of Inhibition (mm)</td>
<td>% activity compared to the standard</td>
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<tr>
<td>2.</td>
<td>11.9</td>
<td>79.33</td>
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<td>3.</td>
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<td>4.</td>
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<tr>
<td>6.</td>
<td>15.9</td>
<td>63.60</td>
</tr>
<tr>
<td>7.</td>
<td>11.9</td>
<td>75.33</td>
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<tr>
<td>8.</td>
<td>16.5</td>
<td>86.84</td>
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</tbody>
</table>

CONCLUSION
In summary, a noteworthy feature of the synthesis is to introduce a one step, clean and practical synthetic route to introduce the pyrimidine ring with bioactive pharmacophores of medicinal utility in s-triazine nucleus from their corresponding chalcone derivatives. Compound 2 and 8 has been found to possess good anti-bacterial and anti-fungal activity.

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REFERENCES


