Green synthesis of (*Z*)-N-5-(benzylidene/substituted benzylidene) 2 benzamido-3-(methyl/phenyl)-6-oxo-1, 2, 5, 6-tetrahydro-1, 2, 4-triazines & their anti-bacterial activity evaluation.

V. Anitha Rani*[1] and Y. Bharathi Kumari[2]

1. Department of Chemistry, Institute of Aeronautical Engineering, Dundigal, Hyderabad
2. Department of Chemistry, Jawaharlal Nehru Technological University Hyderabad College of Engineering, Kukatpally, Hyderabad (A.P), India - 500 085.

Abstract

An efficient one pot green synthesis of (Z)-N-5-(benzylidene/substituted benzylidene) -2-benzamido--3-(methyl/phenyl)-6-oxo-1, 2, 5, 6-tetrahydro-1, 2, 4-triazines has been developed in good yields and tested for their anti-bacterial activities against *Escherichia coli, Providencia aeruginosa*, *Pseudomonas azotogensis* and *Baccilus Subtillis.* Some of the synthesized compounds possess good activity against *Escherichia coli* and *Baccilus Subtillis* compared to standard drug *streptomycin*.

Keywords: Green synthesis, Schiff bases and anti-bacterial activity.

Introduction

1,2,4-Triazines and their derivatives have been widely studied in terms of their synthetic methodologies and reactivity since some of these derivatives were reported to have promising biological activities, including antifungal[1,2], anti HIV[3], anticancer[4], anti-inflamatory[5], analgesic[6], and anti hypertensive[7] activities besides this, triazines were used as herbicides , pesticides and dyes[8,9].

Proton acceptor-donor catalyst L-proline has been playing a vital role in synthetic organic chemistry[10]. L-proline has emerged as an efficient and important catalyst in several transformations such as aldol reactions[10], conjugate addition[11], additions to imines and nitro-alkenes[12].

This prompted us to synthesize derivatives of (Z)-N-5-(benzylidene/substituted benzylidene) -2-benzamido--3-(methyl/phenyl)-6-oxo-1, 2, 5, 6-tetrahydro-1, 2, 4-triazines in presence of L-proline along with their biological evaluation.

Experimental:

Melting points are uncorrected and taken in open capillary tubes in sulphuric acid bath. TLC was run on silica gel – G and visualization was done using UV light. IR spectra were recorded using Perkin – Elmer 1000 instrument in KBr pellets. ^{1}H NMR spectra were recorded in $CDCl_3$ using TMS as internal standard with 400 MHZ spectrometer. Mass spectra were recorded on Agilent-LCMS instrument under CI conditions and given by Q+1 value only. Compound **1** was prepared by literature method[13].

Preparation of (Z)-4-(benzylidene/substituted benzylidene)-2-(methyl/phenyl)-oxazol-5(4H)-ones [3(a-l)]

A mixture of acetylglycine / benzoylglycine (10 mM) and benzaldehydes/Substituted benzaldehydes (10 mM) was added to solution of acetic anhydride and anhydrous sodium acetate for 2 h at 80^{o}C followed by the addition of ethanol and allowed it in ice for overnight. The separated solid was collected, washed with

water (10 ml), dried and recrystallised from ethanol to produce [**3(a-l)**].

Preparation of (Z)-N-[3-hydrazinyl-3-oxo-1-(phenyl/Substituted phenyl)-prop-1-en-2-yl)acetamides or benzamides [4(a-1)]

(Z)-4-(benzylidene/substituted benzylidene)-2-(methyl/phenyl)-oxazol-5(4H)-ones (10 mM) was added to hydrazine hydrate (15mM) in ethanol stirred at RT for 30 min. The deep yellow colour of the solution changed to light yellow. Solid that separated was collected, washed with water (10 ml), dried and recrystallised from ethanol to produce (Z)-N-[3-hydrazinyl-3-oxo-1-(phenyl/Substituted phenyl)-prop-1-en-2-yl)acetamides or benzamides [4(a-1)]

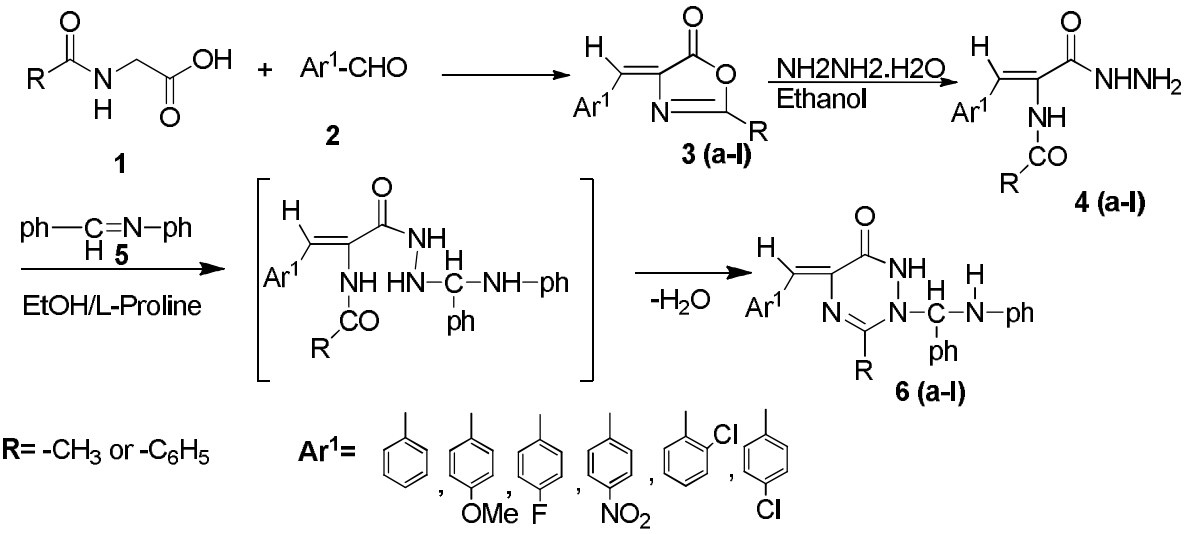
Preparation of (Z)-N-5-(benzylidene/substituted benzylidene) 2-benzamido--3-(methyl/phenyl)-6-oxo-1, 2, 5, 6-tetrahydro-1, 2, 4-triazines [6(a-l)]

Equimolar quantities of (Z)-N-[3-hydrazinyl-3-oxo-1-(phenyl/Substituted phenyl)-prop-1-en-2-yl)acetamides or benzamides (10mM) and Schiff 's base **5** (10mM) were mixed together in 20 ml of EtOH in the presence of L-proline (10mM) as catalyst. The mixture was refluxed for 2 h. The completion of the reaction was monitored by TLC using hexane and ethylacetate as solvent system.The reaction mixture was cooled to room temperature and poured into ice-cold water (50 ml).The solid separated out was collected, washed with water (10 ml) and dried. The product was recrystallised from ethanol to obtain (Z)-N-5-(benzylidene/substituted benzylidene) 2-benzamido--3-(methyl/phenyl)-6-oxo-1, 2, 5, 6-tetrahydro-1, 2, 4-triazines [6(a-l)].

Results and Discussion

Title compounds (Z)-N-5-(benzylidene/substituted benzylidene) 2-benzamido--3-(methyl/phenyl)-6-oxo-1, 2, 5, 6-tetrahydro-1, 2, 4-triazines **[6(a-l)]** have been synthesized by green approach through synthetic sequence shown in **scheme-I**. Initially, acetylglycine / benzoylglycine (1) was made to react with benzaldehydes/Substituted benzaldehydes [2(a-f)] in the presence of acetic anhydride and anhydrous sodium acetate for 2 h at 80^{o}C followed by the addition of ethanol and allowing it to stay overnight produed 4-(benzylidene / substituted benzylidene)-2-(methyl/phenyl)-oxazol-5(4H)-ones [**3(a-l)**], which were made to react with hydrazine hydrate at RT for 30 min to produce (Z)-N-[3-hydrazinyl-3-oxo-1-(phenyl/Substituted phenyl) prop-1-en-2-yl]acetamides or benzamides **[4(a-l)]** Finally, [**4(a-l)**] was made to react with Schiff 's base **5** in the presence of L-proline in ethanol for 2 h under reflux condition to produce [**6(a-l)**] with 80% yield. whose structure has been established on the basis of spectral data. When compared with the conventional methods the synthesis of (Z)-N-5-(benzylidene/substituted benzylidene) 2-benzamido--3-(methyl/phenyl)-6-oxo-1, 2, 5, 6-tetrahydro-1, 2, 4-triazines [**6(a-l)**] in presence of L-proline produced high yields and purities and the time consumed for the reaction is less .

Scheme-I



Mechanism:

As Schiff's bases (5) in heterocyclisation acts as hydrogen accepters, accepts a proton from acetamides/benzamides[4(a-l)] to from an unstable intermediate[i], which in its enolic from [ii] undergoes cyclocondensation by eliminating one mole of water. The mechanism catalysed by L-proline and ethanol.

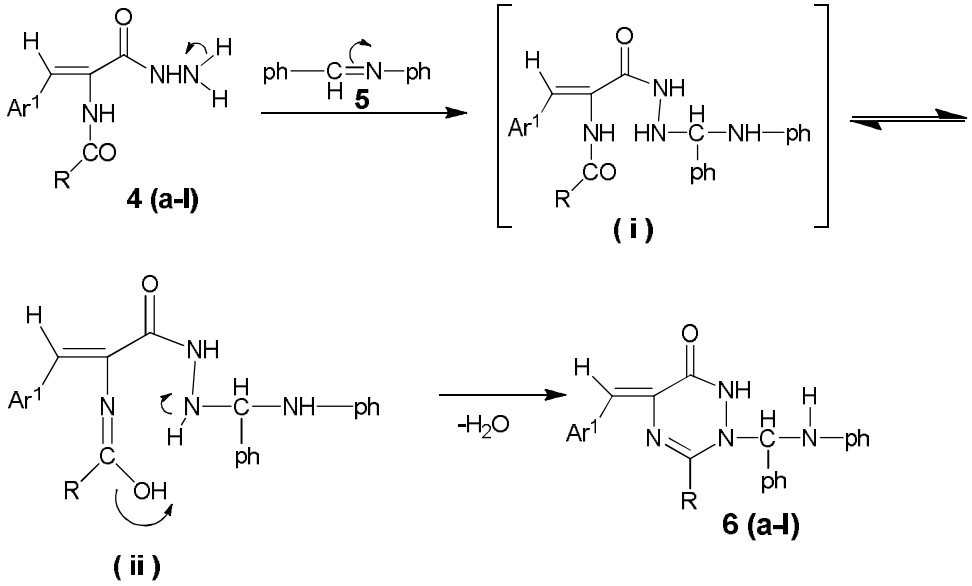


Table-1

Synthesis of [**3(a-l)**] from **1a-1b** and **2a-2f.**

Entry	Starting Material		Product	Time (min)	Yield*	M.P(^{o}C) [lit. M.P ^{o}C]	M. Wt
1	**1a**	**2a**	**3a**	120	80	148-150[150-152][15]	187
2	**1a**	**2b**	**3b**	120	85	160-162[157-159][16]	217
3	**1a**	**2c**	**3c**	130	85	180-182	205
4	**1a**	**2d**	**3d**	125	80	178-180	232
5	**1a**	**2e**	**3e**	125	80	158-160	221
6	**1a**	**2f**	**3f**	125	85	188-190	221
7	**1b**	**2a**	**3g**	130	85	174-176	249
8	**1b**	**2b**	**3h**	140	80	166-168	279
9	**1b**	**2c**	**3i**	120	75	190-192	267
10	**1b**	**2d**	**3j**	140	80	195-197	294
11	**1b**	**2e**	**3k**	130	85	192-194	283
12	**1b**	**2f**	**3l**	150	80	198-200	283

* Refers to yields of crude products only.

Table-2

Synthesis of [**4(a-l)**] from [**3(a-l)**].

Entry	Starting material	Product	Time (min)	Yield*	M.P(°C) [lit. M.P °C]	M. Wt
1	**3a**	**4a**	60	80	154-156 [156-158][15]	219
2	**3b**	**4b**	60	80	175-179 [176-180][16]	249
3	**3c**	**4c**	65	78	208-210	237
4	**3d**	**4d**	60	80	220-222	264
5	**3e**	**4e**	70	75	212-214	253
6	**3f**	**4f**	60	80	> 220	253
7	**3g**	**4g**	65	81	> 220	281
8	**3h**	**4h**	70	80	192-196	311
9	**3i**	**4i**	65	82	210-212	299
10	**3j**	**4j**	70	82	> 220	326
11	**3k**	**4k**	60	81	220-222	315
12	**3l**	**4l**	70	80	> 220	315

* Refers to yields of crude products only.

Table-3

Synthesis of [**6(a-l)**] from [**4(a-l)**] and **5.**

entry	Starting material	Product	Time (min)	Yield*	M.P(°C)	M. Wt
1	**4a**	**6a**	120	80	> 230	382
2	**4b**	**6b**	125	80	> 230	412
3	**4c**	**6c**	120	78	> 230	400
4	**4d**	**6d**	130	80	.>230	427
5	**4e**	**6e**	125	75	180-182	416

6	**4f**	**6f**	125	80	170-172	416
7	**4g**	**6g**	125	81	192-194	478
8	**4h**	**6h**	130	80	> 230	474
9	**4i**	**6i**	140	82	>230	462
10	**4j**	**6j**	120	82	>230	489
11	**4k**	**6k**	140	81	225-227	478
12	**4l**	**6l**	130	80	192-194	478

* Refers to yields of crude products only

Spectral Analysis of the title compounds:

(Z)-N-5-(benzylidene/substituted benzylidene)-2-benzamido-3-(methyl/phenyl)-6-oxo-1, 2, 5, 6-tetrahydro-1, 2, 4-triazines [6(a-l)]:

6a: IR (KBr) cm^{-1}: 3360 (broad, -NH-N), 3313 (broad, -NH), 2197(Ar), 1680 (-C=O); ^{1}H- NMR (400MHz, DMSO-d_6/TMS): δ 2.9 (s, 3H, N-C**H**$_3$), 3.6 (s, 1H, -C**H**), 5.3 (s, 1H, -N**H**-CH**, D$_2$O exchangeable**) 7.2-8.8 (m, 16H, Ar-**H**), 8.9(s, 1H, =C**H**-Ar **), 11.2 (s, 1H, -NH, D$_2$O exchangeable**).

6b: IR (KBr) cm^{-1} : 3310 (broad, -NH-N), 3244 (broad, -NH) 1659 (-C=O); ^{1}H- NMR (400MHz, DMSO-d_6/TMS): δ 2.9 (s, 3H, N-C**H**$_3$), 3.5 (s, 1H, -C**H**), 3.9 (s, 3H, -C**H**$_3$), 5.3 (s, 1H, -N**H**-CH**, D$_2$O exchangeable**) 7.0-8.4 (m, 15H, Ar-**H**) 8.5(s, 1H, =C**H**-Ar **), 11.1 (s, 1H, -NH, D$_2$O exchangeable).**

6c: IR (KBr) cm^{-1}: 3440 (broad, -NH), 3250 (broad, -NH), 1710 (-C=O); ^{1}H- NMR (400MHz, DMSO-d_6/TMS): δ 2.8 (s, 3H, N-C**H**$_3$), 3.5 (s, 1H, -C**H**), 5.3 (s, 1H, -N**H**-CH**, D$_2$O exchangeable**) 7.0-8.4 (m, 15H, Ar-**H**) 8.5(s, 1H, =C**H**-Ar **), 11.2 (s, 1H, -NH, D$_2$O exchangeable).**

6d: IR (KBr) cm^{-1}: 3480 (broad, -NH), 3250 (broad, -NH), 1720 (-C=O); ^{1}H- NMR (400MHz, DMSO-d_6/TMS): δ 2.9 (s, 3H, N-C**H**$_3$), 3.5 (s, 1H, -C**H**), 5.3 (s, 1H, -N**H**-CH**, D$_2$O exchangeable**) 7.0-8.4 (m, 15H, Ar-**H**) 8.5(s, 1H, =C**H**-Ar **), 11.1 (s, 1H, -NH, D$_2$O exchangeable).**

6e: IR (KBr) cm^{-1}: 3322 (broad, -NH), 3304 (broad, -NH) 1720 (-C=O); ^{1}H- NMR (400MHz, DMSO-d_6/TMS): δ 2.7 (s, 3H, N-C**H**$_3$), 3.4 (s, 1H, -C**H**), 5.7 (s, 1H, -N**H**-CH**, D$_2$O exchangeable**) 7.0-8.4 (m, 15H, Ar-**H**) 8.5(s, 1H, =C**H**-Ar **), 11.2 (s, 1H, -NH, D$_2$O exchangeable).**

6f: IR (KBr) cm^{-1}: 3334 (broad, -NH), 3283 (broad, -NH), 1712 (-C=O); ^{1}H- NMR (400MHz, DMSO-d_6/TMS): δ 2.8 (s, 3H, N-C**H**$_3$), 3.5 (s, 1H, -C**H**), 5.5 (s, 1H, -N**H**-CH**, D$_2$O exchangeable**) 7.2-8.4 (m, 15H, Ar-**H**) 8.5(s, 1H, =C**H**-Ar **), 11.2 (s, 1H, -NH, D$_2$O exchangeable).**

6g: IR (KBr) cm^{-1} : 3380 (broad, -NH), 3360 (broad, -NH), 1710 (-C=O); ^{1}H- NMR (400MHz, DMSO-d_6/TMS): δ 3.5 (s, 1H, -C**H**), 5.4 (s, 1H, -N**H**-CH$_3$**, D$_2$O exchangeable**) 7.2-8.6 (m, 21H, Ar-**H**) 8.7(s, 1H, =C**H**-Ar **), 11.1** (s, 1H, **-NH, D$_2$O exchangeable).**

6h: IR (KBr) cm^{-1} : 3313 (broad, -NH), 3302 (broad, -NH), 1700 (-C=O); ^{1}H- NMR (400MHz, DMSO-d_6/TMS): δ 3.5 (s, 1H, -C**H**), 3.9 (s, 3H, -C**H**$_3$), 5.7 (s, 1H, -N**H**-CH**, D$_2$O exchangeable**) 7.0-8.4 (m, 20H, Ar-**H**) 8.5(s, 1H, =C**H**-Ar **), 11.0 (s, 1H, -NH, D$_2$O exchangeable).**

6i: IR (KBr) cm^{-1}: 3342 (broad, -NH), 3330 (broad, -NH), 1722 (-C=O); ^{1}H- NMR (400MHz, DMSO-d_6/TMS): δ 3.5 (s, 1H, -C**H**), 5.0 (s, 1H, -N**H**-CH**, D_2O exchangeable**) 7.2-8.4 (m, 20H, Ar-**H**) 8.5(s, 1H, =C**H**-Ar **),** 11.2 (s, 1H, **-NH, D_2O exchangeable).**

6j: IR (KBr) cm^{-1}: 3340 (broad, -NH), 3320 (broad, -NH), 1700 (-C=O); ^{1}H- NMR (400MHz, DMSO-d_6/TMS): δ 3.2 (s, 1H, -C**H**), 5.3 (s, 1H, -N**H**-CH**, D_2O exchangeable**) 7.1-8.4 (m, 20H, Ar-**H**) 8.5(s, 1H, =C**H**-Ar **),** 11.1 (s, 1H, **-NH, D_2O exchangeable).**

6k: IR (KBr) cm^{-1} : 3413 (broad, -NH), 3352 (broad, -NH), 1722 (-C=O); ^{1}H- NMR (400MHz, DMSO-d_6/TMS): δ 3.3 (s, 1H, -C**H**), 5.3 (s, 1H, -N**H**-CH**, D_2O exchangeable**) 7.2-8.4 (m, 20H, Ar-**H**) 8.5(s, 1H, =C**H**-Ar **),** 11.1 (s, 1H, **-NH, D_2O exchangeable).**

6l: IR (KBr) cm^{-1} : 3402 (broad, -NH), 3352 (broad, -NH) 1702 (-C=O); ^{1}H- NMR (400MHz, DMSO-d_6/TMS): δ 3.4 (s, 1H, -C**H**), 5.5 (s, 1H, -N**H**-CH**, D_2O exchangeable**) 7.0-8.4 (m, 20H, Ar-**H**) 8.5(s, 1H, =C**H**-Ar **),** 11.1 (s, 1H, **-NH, D_2O exchangeable).**

In Vitro Anti-bacterial activity:

The synthesized compounds [**6(a-l)**] were screened for their in vitro antibacterial activity aganist *Escherichia coli* (NCIM 2065*), Providencia aeruginosa* (NCIM 2200), *Pseudomonas azotogensis* (NCIM 2075) and *Baccilus Subtillis* (NCIM 2063). Antibacterial activity of compounds was evaluated using agar-well diffusion method[14]. The petriplates were sterilized using an autoclave at 120 ^oC for 30 min. A petri-dish of 100 mm diameter was filled with 50 ml of freshly prepared Nutrient Agar media and allowed to solidify. Different bacterial species were inoculated on to the medium by streak plate method. The plates were incubated at 30^oC temperature and zone of inhibition was measured after 24 h. The standard used for determining the antibacterial activity is streptomycin. The compounds were dissolved in DMF and activity described at 100 µg/ml level. From the data presented in **Table 4**, it is clear that compounds **6c, 6d**, **6e, 6f, 6i, 6k and 6l** possess good activity against *Escherichia coli* and *Baccilus Subtillis*. Other compounds exhibited moderate antibacterial activity against *E.coli*. However, all compounds showed moderate activity against, *Providencia aeruginosa* and *Pseudomonas azotogensis.* **(Table-4).**

Table-4

Anti-bacterial activity of [**6(a-l)**]**.**

S.No.	Compound	*Escherichia coli*	*Providencia aeruginosa*	*Pseudomonas azotogensis*	*Baccilus subtillis*
1	**6a**	11	12	05	12
2	**6b**	10	06	07	13
3	**6c**	20	12	10	19
4	**6d**	22	10	11	20
5	**6e**	22	09	13	22
6	**6f**	24	09	06	23
7	**6g**	07	12	08	13
8	**6h**	21	11	11	15
9	**6i**	20	12	11	19
10	**6j**	08	10	08	19

11	**6k**	15	11	05	16
12	**6l**	23	13	08	23
13	**Streptomycin**	**30**	**32**	**28**	**30**

Conclusion

Green process for the preparation of the title compounds [**6(a-l)**] has been developed with excellent yields, high purities and minimum reaction times. The evaluation of their biological acitivity is encouraging.

Acknowledgement

The Authors are very thankful to the authorities of **Department of Chemistry, Institute of Aeronautical Engineering, Dundigal, Hyderabad** for providing laboratory facilities.

References:

1. M. Kidwai, Y. Goel, R. Kumar, *Indian J. chem*., 37B, 174 (1998).
2. B. S. Holla, R. Gonsalves, B. S. Rao, S. Shenoy, H. N. *Gopalakrishna, Farmanco,* 56, 899 (2001).
3. R. M. A. Rahman, J. M. Morsy, F. Hanafy, H. A. Amene, *Pharmazie*, 54, 347 (1999).
4. M. W. Partridge, M. F. G. Stevens, *J. chem. Soc*.,1127 (1966).
5. E. I. Abd, Z. K. Samii, *J-Technol.Biote-chanol*., 53, 143 (1992).
6. M. P. Hay, F. B. Prujin, S. A. Gamage, H. D. Liyanage, W. R. Wilson, *J. Med .chem*., 47, 475 (2004).
7. W. P. Heilman, R. D. Heilman, J. A. Scozzie, R. J. Wayner, J. M. Gullo, Z. S. Ariyan, *J. Med. chem.,* 22, 671, (1979).
8. J. G. Erickson, *Chem.. Heterocycle. Comp*., 10, 44 (1956).
9. R. L. Jones, J. R. Kershaw, *Rev. Pure Appl. Chem*., 21, 23 (1971).
10. B. List, R. A. Lerner, C. F. Barbas, *J. Am. Chem. Soc.* 122, 2395 (2000).
11. M. Yamaguchi, N. Yokota. *J . Chem. Soc. Chem. Commun*. 1088 (1991).
12. Y. Y. Peng, Q. P. Ding. *Tetrahedron Lett*. 44. 3871 (2003)
13. S. S. Tiwari, R. K. Satsangi, *J. Indian. Chem. Soc.,* 56, 627 (1979).
14. G. J. Collee, G. A. Fraser, P. B. Marmion, A. Simmons, *Practical Medical Microbiology,14thEd.; Churchill Livingstone: Edinburgh,* 11, 163, (1996).
15. S. S. Tiwri, R. K. Satsangi, *J. Indian. Chem. Soc* .56, 627 (1979).
16. E. J. Budovskii, C. Chang, N. Kochetkov, *Zur.obschchikim*, 31, 1297 (1961).