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An Efficient and Green Synthesis of Benzylidene-2-N-(carbothioamido)-6-oxo-1,2,5,6-tetrahydro-1-NH-1,2,4-triazine Derivatives and their Antibacterial Activity Evaluation

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An efficient and green synthesis of (Z)-3-allkyl-5(benzylidene/substituted benzylidene)-2N-(carbothioamido)-6-oxo-1,2,5,6-tetrahydro-1-NH-1,2,4-triazine derivatives have been developed in good yields and tested for their antibacterial 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, 1,2,4-Triazine derivatives, Antibacterial 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], anti-cancer[4], anti-inflamatory[5], analgesic[6] and anti hypertensive[7] activities besides this, triazines were used as herbicides, pesticides and dyes[8,9]. This prompted us to synthesize derivatives of (Z)-3-allkyl-5(benzylidene / substituted benzylidene)-2N-(carbothioamido)-6-oxo-1,2,5,6-tetrahydro-1-NH-1,2,4-triazine derivatives and evaluate them for antibacterial activity.

EXPERIMENTAL

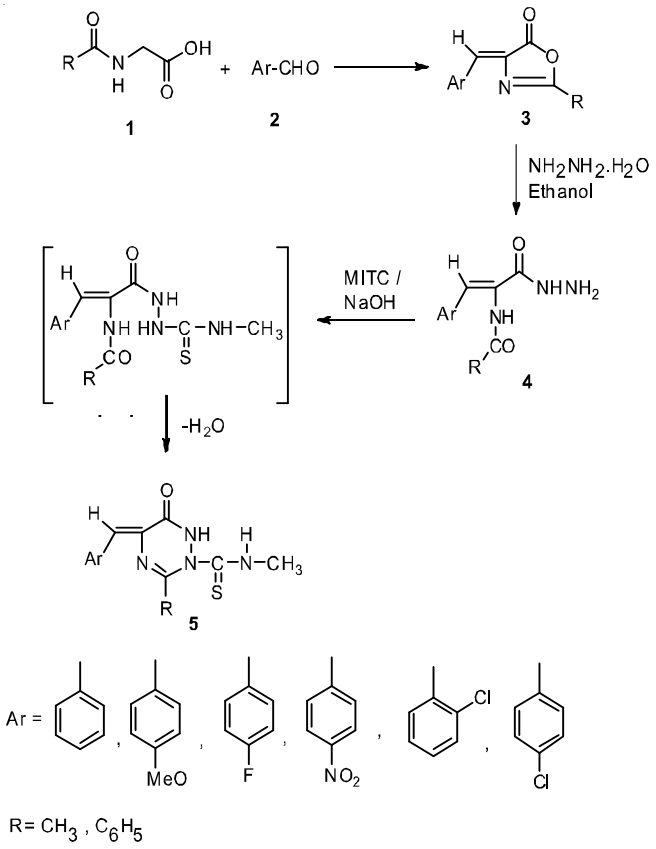
Melting points are uncorrected and taken in open capillary tubes in sulphuric acid bath. TLC was run on silica gel-G and visualization was done using UV light. IR spectra were recorded using Perkin-Elmer 1000 instrument in KBr pellets. ^{1}H NMR spectra were recorded in $CDCl_3$ using TMS as internal 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[11].

Preparation of 3a-3l: A mixture of **1a-1b** (10 mM) and **2a-2f** (10 mM) was added to solution of acetic anhydride and anhydrous sodium acetate for 2 h at 80 °C followed by added ethanol and allowed it in ice for overnight. Separated solid was collected, washed with water (10 mL), dried and recrystallized from ethanol to afford **3a-3l**.

Preparation of 4a-4l: Compounds **3a-3l** (10 mM) was added to hydrazine hydrate (15mM) in ethanol stirred at room temperature for 0.5 h. The deep yellow colour of the solution changed to light yellow. Solid that separated was collected, washed with water (10 mL), dried and recrystallized from ethanol to afford **4a-4l**.

Preparation of 5a-5l: Equimolar quantities of **4a-4l** (10 mM) and MITC (10 mM) were mixed together in 2N NaOH (20 mL). The mixture was refluxed for 2 h. The completion of the reaction was checked by TLC, then this reaction mixture was cooled to room temperature and poured into ice-cold water (50 mL) and neutralized with 2N HCl solution. Solid separated out which was collected, washed with water (10 mL) and dried. The product was recrystallized from ethanol to obtain **5a-5l**.

Title compounds (Z)-3-allkyl-5(benzylidene/substituted benzylidene)-2N-(carbothioamido)-6-oxo-1,2,5,6-tetrahydro-1-NH-1,2,4-triazine derivatives (**5**) have been synthesized by green approach through synthetic sequence shown in **Scheme-I**. Initially, acetylglycine/benzoyolglycine was reacted with benzaldehydes (**2a-2l**) in the presence of acetic anhydride and anhydrous sodium acetate for 2 h at 80 °C followed by the addition of ethanol and allowing it to stay overnight produced (Z)-4-(benzylidene/substituted benzylidene)-2-(methyl/phenyl)-oxazol-5(4H)-ones(**3a-3l**) (**Scheme-I**) (Table-1). Then **3a-3l** was reacted with hydrazine hydrate at room temperature for 0.5 h to form (Z)-N-(3-hydrazinyl-3-oxo-1-phenylprop-1-en-2-yl)acetamides or benzamides (**4a-4l**) (**Scheme-I**) (Table-2). Finally, **4a-4l** were reacted with methyl isothiocyanate in 20 ml of 2N NaOH for 2 h under reflux condition



Scheme-I

followed by neutralized with 2N HCl solution to give **5a-5l** with 80 % yield. Its structure has been established on the basis of spectral and analytical data. (**Scheme-I**) (Table-3). To test its generality the method has been extended to six other enamides and in the all cases the corresponding (Z)-3-allkyl-5-(benzylidene/substituted benzylidene)-2N-(carbothioamido)-6-oxo-1,2,5,6-tetrahydro-1-NH-1,2,4-triazine derivatives (**5a-5l**) were isolated in good yields. The physico-chemical data of the compounds **5a-5l** are given in Table-4.

(Z)-3-allkyl-5(benzylidene/substituted benzylidene)-2N-(carbothioamido)-6-oxo-1,2,5,6-tetrahydro-1-NH-1,2,4-triazine derivatives 5a-5l.

5a: IR (KBr, ν_{max}, cm^{-1}) : 3471 (broad, -NH-N), 3084 (broad, -NH), 1714 (-C=O), 1270 (C=S); ^{1}H NMR (400 MHz, $CDCl_3$/TMS): δ 2.1 (s, 3H, C-CH_3), δ 2.6 (s, 3H, N-CH_3), δ 6.8 (s, 1H, -NH-CH_3, D_2O exchangeable) 7.4-8.4 (m, 6H, Ar-H and s, 2H, =CH-Ar), 10.6 (s, 1H, -NH, D_2O exchangeable). ^{13}C NMR ($CDCl_3$): δ 24.66 (C-CH_3), 42.94 (N-CH_3), 116.79 (Ar-C=C), 120.14-137.69 (Ar), 147.79 (N-C-CH_3), 149.96 (Ar-C=C), 164.01 (C=S), 177. 70 (O=C-N). Mass: 239 (20 %), 260 (10 %). $M^{+\bullet}1$ = 275.

5b: IR (KBr, ν_{max}, cm^{-1}): 3313 (broad, -NH-N), 3249 (broad, -NH) 1656 (-C=O), 1263 (C=S); ^{1}H NMR (400 MHz, $CDCl_3$/TMS): δ 2.2 (s, 3H, C-CH_3), δ 2.6 (s, 3H, N-CH_3), δ 3(s, 3H, -CH_3), δ 6.8 (s, 1H, -NH, D_2O exchangeable) 7.2-8.3 (m, 5H, Ar-H and s, 2H, =CH-Ar), 10.6 (s, 1H, -NH, D_2O exchangeable). ^{13}C NMR ($CDCl_3$): δ 23.62 (C-CH_3), 43.93 (N-CH_3),

TABLE-1
SYNTHESIS OF **3a-3l** FROM **1a-1b** AND **2a-2f**

Entry	Starting Material used		Product obtained	Time (min)	Yield*	m.p. (°C) [lit. m.p. °C]	m.w.
1	**1a**	2a	3a	120	80	148-150[150-152][11]	187
2	**1a**	2b	3b	120	85	160-162[157-159][12]	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 **4a-4l** FROM **3a-3l** AND HYDRAZINE HYDRATE

Entry	Starting material	Product obtained	Time (min)	Yield*	m.p. (°C) [lit. m.p. °C]	m.w.
1	**3a**	**4a**	60	80	154-156 [156-158][11]	219
2	**3b**	**4b**	60	80	175-179 [176-180][12]	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 **5a-5l** FROM **4a-4l** AND MITC

Entry	Starting material	Product obtained	Time (min)	Yield*	m.p. (°C)	m.w.
1	**4a**	**5a**	120	80	> 220	274
2	**4b**	**5b**	125	80	> 220	304
3	**4c**	**5c**	120	78	> 220	292
4	**4d**	**5d**	130	80	210-212	319
5	**4e**	**5e**	125	75	> 220	308
6	**4f**	**5f**	125	80	190-192	308
7	**4g**	**5g**	125	81	> 220	336
8	**4h**	**5h**	130	80	> 220	366
9	**4i**	**5i**	140	82	216-218	354
10	**4j**	**5j**	120	82	> 220	381
11	**4k**	**5k**	140	81	220-222	370
12	**4l**	**5l**	130	80	160-162	370

* Refers to yields of crude products only

TABLE-4
ELEMENTAL ANALYSIS OF **5a-5l**

Compd. No.	m.f.	m.w.	Elemental analysis (%)		
			C	H	N
5a	$C_{13}H_{14}N_4OS$	274	56.91	5.30	20.42
5b	$C_{14}H_{16}N_4O_2S$	304	56.25	5.30	18.42
5c	$C_{13}H_{13}N_4OSF$	292	53.41	4.48	19.17
5d	$C_{13}H_{13}N_5O_3S$	319	48.89	4.10	21.93
5e	$C_{13}H_{13}N_4OSCl$	308	50.97	4.24	18.42
5f	$C_{13}H_{13}N_4OSCl$	308	50.97	4.24	18.42
5g	$C_{18}H_{16}N_4OS$	336	64.26	4.79	16.65
5h	$C_{19}H_{18}N_4O_2S$	366	62.28	4.95	15.29
5i	$C_{18}H_{15}N_4OSF$	354	61.01	4.27	15.81
5j	$C_{18}H_{15}N_5O_3S$	381	56.68	3.90	18.42
5k	$C_{18}H_{15}N_4OSCl$	370	58.91	4.80	15.42
5l	$C_{18}H_{15}N_4OSCl$	370	58.91	4.80	15.42

53.93 (-OCH_3), 114.29 (Ar-C=C), 124.13-133.65 (Ar), 146.73 (N-C-CH_3), 149.94 (Ar-C=C), 163.31 (C=S), 176. 30 (O=C-N). Mass: 273 (10 %). $M^{+\bullet}1$ = 305.

5c: IR (KBr, ν_{max}, cm^{-1}): 3445 (broad, -NH), 3051 (broad, -NH), 1724 (-C=O), 1280 (C=S); ^{1}H NMR (400 MHz, $CDCl_3$/TMS): δ 2.4 (s, 3H, C-CH_3), δ 2.8 (s, 3H, N-CH_3), δ 6.6 (s, 1H, -NH, D_2O exchangeable) 7.4-8.4 (m, 5H, Ar-H and s, 2H, =CH-Ar), 10.4 (s, 1H, -NH, D_2O exchangeable). ^{13}C NMR ($CDCl_3$): δ 23.26 (C-CH_3), 42.24 (N-CH_3), 116.59 (Ar-C=C), 123.15-136. 69 (Ar), 144.49 (N-C-CH_3), 148.96 (Ar-C=C), 163.04 (C=S), 174. 60 (O=C-N). Mass: 239 (20 %). $M^{+\bullet}1$ = 293.

5d: IR (KBr, ν_{max}, cm^{-1}): 3283 (broad, -NH), 3251 (broad, -NH), 1726 (-C=O), 1257 (C=S); ^{1}H NMR (400 MHz, $CDCl_3$/TMS): δ 1.8 (s, 3H, C-CH_3), δ 2.3 (s, 3H, N-CH_3), δ 6.6 (s, 1H, -NH, D_2O exchangeable) 7.4-8.4 (m, 5H, Ar-H and s, 2H, =CH-Ar), 10.2 (s, 1H, -NH, D_2O exchangeable). ^{13}C NMR ($CDCl_3$): δ 23.63 (C-CH_3), 41.93 (N-CH_3), 115.39 (Ar-C=C), 121.13-136.62 (Ar), 146.74 (N-C-CH_3), 148.93 (Ar-C=C), 163.04 (C=S), 179.78 (O=C-N). Mass: 273 (10 %). $M^{+\bullet}1$ = 320.

5e: IR (KBr, ν_{max}, cm^{-1}): 3307 (broad, -NH), 3198 (broad, -NH) 1729 (-C=O), 1255 (C=S); ^{1}H NMR (400 MHz, $CDCl_3$/TMS): δ 1.8 (s, 3H, C-CH_3), δ 2.4 (s, 3H, N-CH_3), δ 6.6 (s, 1H, -NH, D_2O exchangeable) 7.4-8.4 (m, 5H, Ar-H and s, 2H, =CH-Ar), 10.2 (s, 1H, -NH, D_2O exchangeable). ^{13}C NMR ($CDCl_3$): δ 23.26 (C-CH_3), 41.93 (N-CH_3), 113.29 (Ar-C=C), 121.24-135.66 (Ar), 146.76 (N-C-CH_3), 148.94 (Ar-C=C), 163.05 (C=S), 174. 60 (O=C-N). $M^{+\bullet}1$ = 309.

5f: IR (KBr, ν_{max}, cm^{-1}): 3300 (broad, -NH), 3280 (broad, -NH), 1710 (-C=O), 1280 (C=S); ^{1}H NMR (400 MHz, $CDCl_3$/TMS): δ 2.4 (s, 3H, C-CH_3), δ 2.6 (s, 3H, N-CH_3), δ 6.8 (s, 1H, -NH, D_2O exchangeable) 7.4-8.4 (m, 6H, Ar-H and s, 2H, =CH-Ar), 10.6 (s, 1H, -NH, D_2O exchangeable). ^{13}C NMR ($CDCl_3$): δ 23.56 (C-CH_3), 41.54 (N-CH_3), 114.69 (Ar-C=C), 122.24-137.65 (Ar), 146.69 (N-C-CH_3), 148.76 (Ar-C=C), 163.21 (C=S), 176. 40 (O=C-N). $M^{+\bullet}1$ = 309.

5g: IR (KBr, ν_{max}, cm^{-1}) : 2989 (broad, -NH), 3460 (broad, -NH), 1720 (-C=O), 1292 (C=S); ^{1}H NMR (400 MHz, $CDCl_3$/TMS): δ 2.3 (s, 3H, N-CH_3), δ 6.4 (s, 1H, -NH, D_2O exchangeable) 7.4-8.4 (m, 11H, Ar-H and s, 2H, =CH-Ar), 10.2 (s, 1H, -NH, D_2O exchangeable). ^{13}C NMR ($CDCl_3$): δ 39.34 (N-CH_3), 113.29 (Ar-C=C), 121.14-143.45 (Ar), 145.65 (N-C-CH_3), 148.46 (Ar-C=C), 162.11 (C=S), 173.20 (O=C-N). Mass: 221 (20 %), 318 (10 %). $M^{+\bullet}1$ = 337.

5h: IR (KBr, ν_{max}, cm^{-1}): 3210 (broad, -NH), 3380 (broad, -NH),1750 (-C=O), 1280 (C=S); ^{1}H NMR (400 MHz, $CDCl_3$/TMS): δ 2.6 (s, 3H, N-CH_3), δ 6.9 (s, 1H, -NH, D_2O exchangeable) 7.4-8.4 (m, 10H, Ar-H and s, 2H, =CH-Ar), 10.3 (s, 1H, -NH, D_2O exchangeable). ^{13}C NMR ($CDCl_3$): δ 36.34 (N-CH_3), 115.35 (Ar-C=C), 120.34-139.65 (Ar), 146.69 (N-C-CH_3), 148.76 (Ar-C=C), 163.21 (C=S), 173.43 (O=C-N). $M^{+\bullet}1$ = 367.

5i: IR (KBr, ν_{max}, cm^{-1}): 3210 (broad, -NH), 3340 (broad, -NH), 1740 (-C=O), 1300 (C=S); ^{1}H NMR (400 MHz, $CDCl_3$/TMS): δ 2.4 (s, 3H, N-CH_3), δ 6.8 (s, 1H, -NH, D_2O exchangeable) 7.4-8.4 (m, 10H, Ar-H and s, 2H, =CH-Ar), 10.6 (s, 1H, -NH, D_2O exchangeable). ^{13}C NMR ($CDCl_3$): 42.54 (N-CH_3), 113.49 (Ar-C=C), 121.24-139.65 (Ar), 146.56 (N-C-CH_3), 147.76 (Ar-C=C), 164.21 (C=S), 175. 40 (O=C-N). $M^{+\bullet}1$ = 355.

5j: IR (KBr, ν_{max}, cm^{-1}): 3210 (broad, -NH), 3330 (broad, -NH), 1740 (-C=O), 1280 (C=S); ^{1}H NMR (400 MHz, $CDCl_3$/TMS): δ 2.6 (s, 3H, N CH_3), δ 6.4 (s, 1H, -NH, D_2O exchangeable) 7.4-8.4 (m, 10H, Ar-H and s, 2H, =CH-Ar), 10.7 (s, 1H, -NH, D_2O exchangeable). ^{13}C NMR ($CDCl_3$): 40.35 (N-CH_3), 113.56 (Ar-C=C), 124.24-142.65 (Ar), 146.68 (N-C-CH_3), 148.74 (Ar-C=C), 162.22 (C=S), 174. 43 (O=C-N). $M^{+\bullet}1$ = 382.

TABLE-5
ANTIBACTERIAL ACTIVITY OF **5a-5l**

S.No.	Compound	*Escherichia coli*	*Providencia aeruginosa*	*Pseudomonas azotogensis*	*Baccilus subtillis*
1	**5a**	12	10	09	10
2	**5b**	11	09	08	12
3	**5c**	18	11	11	18
4	**5d**	19	09	10	19
5	**5e**	17	07	11	18
6	**5f**	18	06	09	18
7	**5g**	09	09	08	11
8	**5h**	19	10	10	10
9	**5i**	17	11	10	17
10	**5j**	08	11	07	10
11	**5k**	17	10	06	18
12	**5l**	19	11	05	18
13	Streptomycin	30	32	28	30

5k: IR (KBr, ν_{max}, cm^{-1}): 3210 (broad, -NH), 3350 (broad, -NH), 1720 (-C=O), 1270 (C=S); ^1H NMR (400 MHz, $CDCl_3$/TMS): δ 2.5 (s, 3H, N CH_3), δ 6.8 (s, 1H, -NH, D_2O exchangeable) 7.4-8.4 (m, 10H, Ar-H and s, 2H, =CH-Ar), 10.6 (s, 1H, -NH, D_2O exchangeable). ^{13}C NMR ($CDCl_3$): 42.44 (N-CH_3), 115.65 (Ar-C=C), 120.24-139.65 (Ar), 146.60 (N-C-CH_3), 148.70 (Ar-C=C), 163.20 (C=S), 176.49 (O=C-N). $M^{+\bullet}1$ = 371.

5l: IR (KBr, ν_{max}, cm^{-1}): 3200 (broad, -NH), 3353 (broad, -NH) 1750 (-C=O), 1290 (C=S); ^1H NMR (400 MHz, $CDCl_3$/TMS): δ 2.3 (s, 3H, -CH_3), δ 6.5 (s, 1H, -NH, D_2O exchangeable) 7.4-8.4 (m, 10H, Ar-H and s, 2H, =CH-Ar), 10.4 (s, 1H, -NH, D_2O exchangeable). ^{13}C NMR ($CDCl_3$): δ 23.56 (C-CH_3), 41.54 (N-CH_3), 114.69 (Ar-C=C), 122.24-137.65 (Ar), 146.69 (N-C-CH_3), 148.76 (Ar-C=C), 163.21 (C=S), 176. 40 (O=C-N). $M^{+\bullet}1$ = 371.

RESULTS AND DISCUSSION

***in vitro* antibacterial activity:** The synthesized compounds **5a-5l** 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[10]. The petriplates were sterilized using an autoclave at 120 °C for 0.5 h. 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 °C 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-5, it is clear that compounds **5c**, **5d**, **5e**, **5f**, **5i**, **5k** and **5l** 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-5).

Conclusion

Green process for the preparation of moderate anti-biological compounds **5a-5l** has been developed with excellent yields and the evaluation of their anti-microbiological acitivity is encouraging.

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REFERENCES

1. M. Kidwai, Y. Goel and R. Kumar, *Indian J. Chem*., **37B**, 174 (1998).
2. B.S. Holla, R. Gonsalves, B.S. Rao, S. Shenoy and H.N. Gopalakrishna, *Farmaco*, **56**, 899 (2001).
3. R.M. Abdel-Rahman, J.M. Morsy, F. Hanafy and H.A. Amene, *Pharmazie*, **54**, 347 (1999).
4. M.W. Partridge and M.F.G. Stevens, *J. Chem. Soc. C*, 1127 (1966).
5. Z.K. Abd El-Samii, *J. Chem. Technol. Biotechnol*, **53**, 143 (1992).
6. M.P. Hay, F.B. Pruijn, S.A. Gamage, H.D.S. Liyanage, M.S. Kovacs, A.V. Patterson, W.R. Wilson, J.M. Brown and W.A. Denny, *J. Med. Chem*., **47**, 475 (2004).
7. W.P. Heilman, R.D. Heilman, J.A. Scozzie, R.J. Wayner, J.M. Gullo and Z.S. Ariyan, *J. Med. Chem*., **22**, 671 (1979).
8. J.G. Erickson, The Chemistry of Heterocyclic Compounds, Interscience, New York (1956).
9. R.L. Jones and J.R. Kershaw, *Rev. Pure Appl. Chem*, **21**, 23 (1971).
10. G.J. Collee, G.A. Fraser, P.B. Marmion and A. Simmons, Practical Medical Microbiology, Churchill Livingstone: Edinburgh, edn 14, vol. 11, p. 163 (1996).
11. S.S. Tiwari and R.K. Satsangi, *J. Indian Chem. Soc*., **56**, 627 (1979).
12. E.J. Budovskii, C. Chang, N. Kochetkov, *Z. Obschchikim*., **31**, 1297 (1961).