10th International Electronic Conference on Synthetic Organic Chemistry (ECSOC-10). 1-30 November 2006. http://www.usc.es/congresos/ecsoc/10/ECSOC10.htm & http://www.mdpi.org/ecsoc-10/

[c010] *Anton V. Dolzhenko, Anna V. Dolzhenko and Wai-Keung Chui*

Synthesis of 2,5,7-triamino[1,2,4]triazolo[1,5-*a*][1,3,5]triazines as potential antifolate agents

Department of Pharmacy, Faculty of Science, National University of Singapore, 18 Science Drive 4, Singapore 117543, Singapore.
E-mails: phada@nus.edu.sg, phacwk@nus.edu.sg

Abstract

The synthesis of 2,5,7-triamino[1,2,4]triazolo[1,5-*a*][1,3,5]triazines was developed. The 3,5-diamino-1,2,4-triazoles were prepared using partial aminolysis of dimethyl *N*-cyanodithiocarbonimidate followed by cyclization of the obtained *N*-substituted *N'*-cyano-*S*-methylisothioureas with hydrazine. The reaction of 3,5-diamino-1,2,4-triazoles with cyanoguanidine was found to afford 2,5,7-triamino[1,2,4]triazolo[1,5-*a*][1,3,5]triazines. The structure of the compounds obtained was established using NMR spectroscopy.

Key words: triazoles, triazines, cyanoguanidine, cyclization.

Introduction

Most of the known antifolate drugs such as methotrexate, trimethoprim, cycloguanil and trimetrexate possess the diamino heterocyclic fragment (**1**) in their molecular structure (Figure 1). The diamino heterocyclic structure (**1**) forms an essential part of the pharmacophore in the molecules of dihydrofolate reductase (DHFR) inhibitors that have found applications for chemotherapy of cancer or infectious diseases [1]. Our group works on the synthesis of fused 1,3,5-triazines as potential inhibitors of DHFR [2-4]. In continuation of these investigations we became interested in the synthesis of 1,2,4-triazolo[1,5-*a*][1,3,5]triazine heterocyclic system, which is the 5-aza- analogue of the purine scaffold [5]. This report describes a new synthesis of 2,5,7-triamino[1,2,4]triazolo[1,5-*a*][1,3,5]triazines (**2**) which contain the pharmacophoric structure (**1**) (Figure 1). In addition, lipophilic aromatic moiety that is located in the side chain as a distal part of the molecule, is also included in the series of compounds synthesized. These are common features for the “nonclassical” DHFR inhibitors. The 1,2,4-triazolo[1,5-*a*][1,3,5]triazine nucleus is linked to the lipophilic aromatic part by amino or alkylamino group.

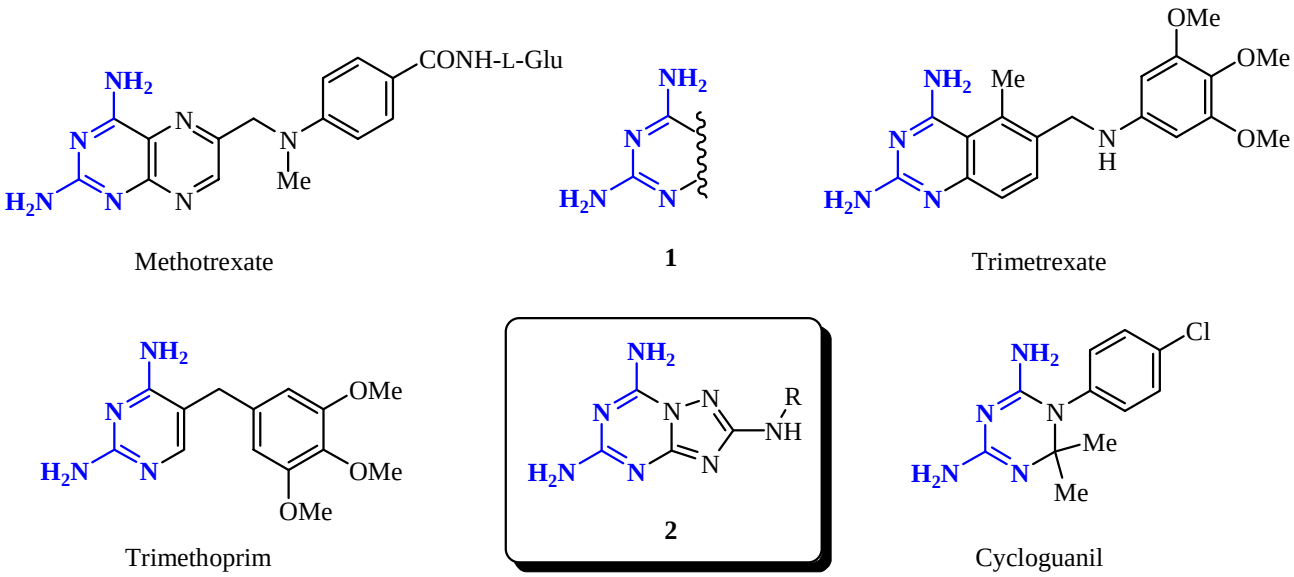
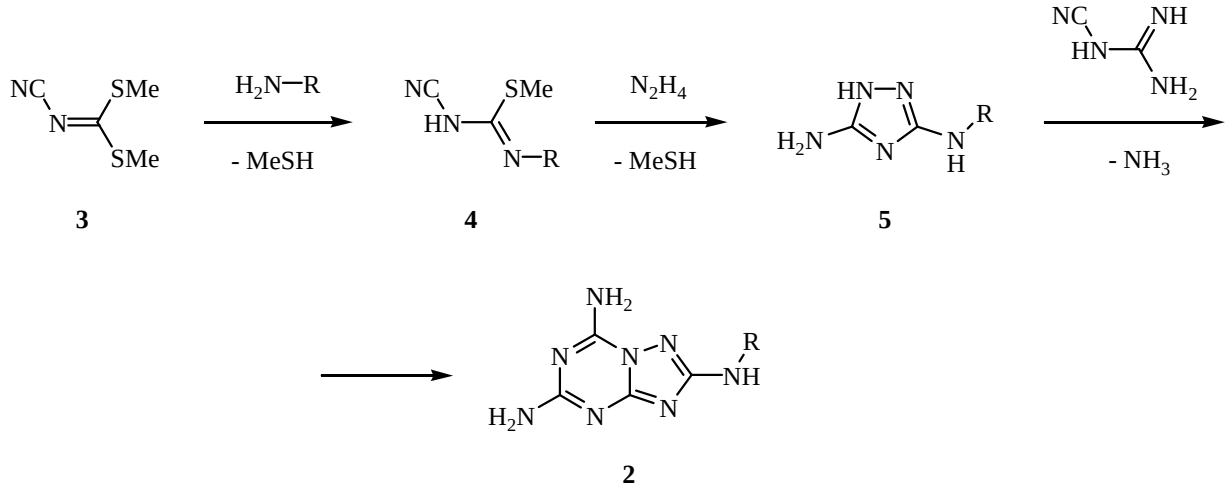
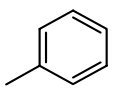
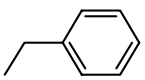
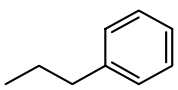
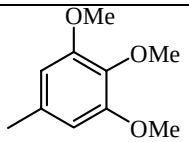
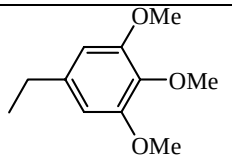


Figure 1

Results and Discussion

The synthesis of 2,5,7-triamino[1,2,4]triazolo[1,5-*a*][1,3,5]triazines (**2**) was realized according to the following scheme:



2, **4** and **5**	**a**	**b**	**c**	**d**	**e**
R					

Scheme

The reaction of dimethyl *N*-cyanodithiocarbonimidate (**3**) with aryl- or arylalkylamines afforded the corresponding *N*-substituted *N'*-cyano-*S*-methylisothioureas (**4**) (Table 1). According to the splitting of signals in ^{1}H NMR spectra of **4**, these compounds were found to exist in the form of tautomer **B** (Figure 2).

Table 1

N-Substituted *N'*-cyano-*S*-methylisothioureas (**4**)

Compound	Method	mp, ºC	Solvent	Yield, %	mp, ºC [lit.]
4a	A	198-200	MeOH	85	199-200 [6]
4b	A	159-161	MeOH	88	157-158 [7]
4c	A	174	MeOH	90	172-175 [8]
4d	B	172	*i*-PrOH	82	-
4e	A	198-200	MeOH	90	-

Table 2

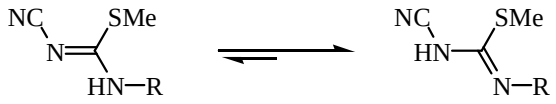
^{1}H NMR spectra of *N*-substituted *N'*-cyano-*S*-methylisothioureas (**4**) (300 MHz, DMSO-d_6) δ, ppm

Compound	SMe	NH	R
4a	s, 2.70	s, 10.16	7.25 (1H, tt, *J* = 6.8, 1.8 Hz, H-4), 7.36-7.48 (4H, m, H-2, H-3, H-5 and H-6)
4b	s, 2.62	s, 8.89	4.49 (2H, s, CH_2), 7.23-7.40 (5H, m, Ph)
4c	s, 2.52	s, 8.42	2.83 (2H, t, *J* = 7.3 Hz, PhCH_2), 3.50 (2H, t, *J* = 7.3 Hz, NCH_2), 7.19-7.35 (5H, m, Ph)
4d	s, 2.67	s, 10.09	3.66 (3H, s, OMe), 3.76 (6H, s, 2 OMe), 6.80 (2H, s, H-2 and H-6)
4e	s, 2.63	s, 8.81	3.64 (3H, s, OMe), 3.77 (6H, s, 2 OMe), 4.42 (2H, s, CH_2), 6.63 (2H, s, H-2 and H-6)

Table 3

^{13}C NMR spectra of *N*-substituted *N'*-cyano-*S*-methylisothioureas (**4**) (75 MHz, DMSO-d_6) δ, ppm

Compound	SMe	C≡N	N=*C*(SMe)N	R
4a	14.7	114.7	170.2	124.1 (C-2 and C-6), 123.3 (C-4), 128.7 (C-3 and C-5), 137.1 (C-1)
4b	14.0	115.6	170.2	46.1 (*C*H_2), 127.1 (C-4), 127.2 (C-2 and C-6), 128.3 (C-3 and C-5), 137.4 (C-1)
4c	13.9	115.8	169.7	33.9 (*C*H_2), 44.3 (*C*H_2), 126.3 (C-4), 128.3 (C-2 and C-6), 128.7 (C-3 and C-5), 138.5 (C-1)
4d	14.7	114.7	170.2	55.9 (2 OMe), 60.0 (OMe), 102.3 (C-2 and C-6), 132.9 (C-1), 135.8 (C-4), 152.6 (C-3 and C-5)
4e	14.1	115.7	170.1	46.4 (CH_2), 55.8 (2 OMe), 59.9 (OMe), 104.9 (C-2 and C-6), 132.9 (C-1), 136.6 (C-4), 152.8 (C-3 and C-5)



A **B**

Figure 2

The reaction of *N*-substituted *N'*-cyano-*S*-methylisothioureas (**4**) with hydrazine resulted in the formation of 3,5-diamino-1,2,4-triazoles (**5**) (Table 4). Annular tautomerism is possible in the prepared triazoles (**5**). Theoretically, they may be present in three tautomeric forms (**A**, **B** and **C**) (Figure 3). Analysis of spectral data (Tables 5 and 6) indicted that compounds **5** existed predominantly in the forms of **A** and **B**, whereas **C** was found to be disfavored.

Table 4

3,5-Diamino-1,2,4-triazoles (**5**)

Compound	Method	mp, ºC	Solvent	Yield, %	mp, ºC [lit.]
5a	A	161-162	H_2O	92	161-162 [9]
5b	A	151-152	*i*-PrOH	94	148-149 [9]
5c	A	118-119	H_2O	89	117-119 [10]
5d	B	246-247	H_2O	93	-
5e	B	167-169	EtOH	96	-

Table 5

^{1}H NMR spectra of 3,5-diamino-1,2,4-triazoles (**5**) (300 MHz, DMSO-d_6) δ, ppm

Compound	NH_2	NHR	N-3(5)H	R
5a	br.s, 5.83	s, 8.58	s, 11.12	6.71 (1H, t, *J* = 7.2 Hz, H-4'), 7.15 (2H, t, *J* = 7.7 Hz, H-3' and H-5'), 7.49 (2H, d, *J* = 7.9 Hz, H-2' and H-6')
5b	br.s, 5.37	br.s, 6.15	-	4.23 (2H, d, *J* = 6.4 Hz, CH_2), 7.18 (1H, tt, *J* = 6.7, 1.9 Hz, H-4'), 7.23-7.34 (4H, m, H-2', H-3', H-5' and H-6')
5c	br.s, 5.27	br.s, 5.56	br.s, 10.74	2.78 (2H, t, *J* = 7.5 Hz, PhCH_2), 3.23 (2H, dt, *J* = 7.0, 7.5 Hz, NHCH_2), 7.15-7.32 (5H, m, Ph)
5d	br.s, 5.92	s, 8.56	s, 11.21	3.58 (3H, s, OMe), 3.72 (6H, s, 2 OMe), 6.91 (2H, s, H-2' and H-6')
5e	br.s, 5.55	br.s, 5.94	br.s, 10.82	3.62 (3H, s, OMe), 3.72 (6H, s, 2 OMe), 4.16 (2H, d, *J* = 6.4 Hz, CH_2), 6.65 (2H, s, H-2' and H-6')

Table 6

^{13}C NMR spectra of 3,5-diamino-1,2,4-triazoles (**5**) (75 MHz, DMSO-d_6) δ, ppm

Compound	C-3 and C-5	R
5a	155.3 and 157.8	115.4 (C-2' and C-6'), 118.1 (C-4'), 128.3 (C-3' and C-5'), 142.5 (C-1')
5b	157.7 and 160.0	46.1 (*C*H$_2$), 126.3 (C-4'), 127.0 (C-2' and C-6'), 127.9 (C-3' and C-5'), 140.9 (C-1')
5c	158.1 and 159.8	35.5 (*C*H$_2$), 44.4 (*C*H$_2$), 125.8 (C-4'), 128.1 (C-2' and C-6'), 128.5 (C-3' and C-5'), 139.9 (C-1')
5d	155.3 and 157.9	55.5 (2 OMe), 60.0 (OMe), 93.5 (C-2' and C-6'), 130.1 (C-4'), 138.8 (C-1'), 152.8 (C-3' and C-5')
5e	156.3 and 161.3	46.4 (*C*H$_2$), 55.6 (2 OMe), 59.8 (OMe), 104.4 (C-2' and C-6'), 135.9 (C-4'), 136.6 (C-1'), 152.5 (C-3' and C-5')

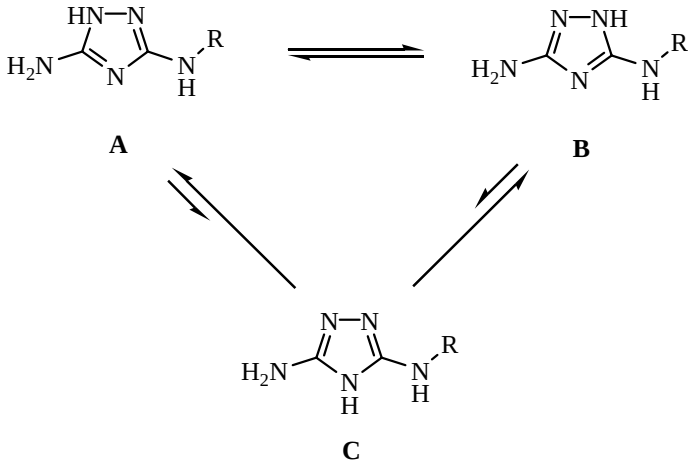
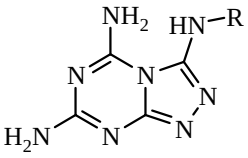
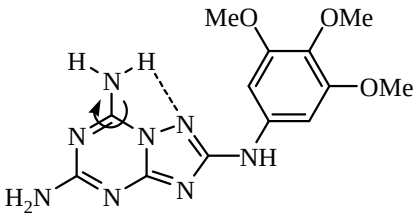


Figure 3

The (3+3) heterocyclization of 5-amino-1,2,4-triazoles (**5**) using cyanoguanidine as the triatomic building block was applied for the synthesis of 2,5,7-triamino[1,2,4]triazolo[1,5-*a*][1,3,5]triazines (**2**). The reaction proceeded with the liberation of ammonia and required high temperature (reflux in DMF) or acidic (HCl) catalysis in aqueous medium. The theoretically possible formation of isomeric 1,2,4-triazolo[4,3-*a*][1,3,5]triazines (**9**) (Figure 4) was excluded based on NMR spectral data (Tables 8 and 9). The 7-amino group of **2** appeared in the ^{1}H NMR spectra as one or two broad signals. That can be explained by the coplanarity of the 7-amino group with the 1,2,4-triazolo[4,3-*a*][1,3,5]triazine nucleus, stabilized with intramolecular hydrogen bonding N-H…N-1, and consequent deshielding of one of the protons. This type of stabilization would not be possible in the case of [4,3-*a*] ring junction. The free energy of activation ($G^‡$) of the rotation across (C-7)-NH_2 bond at the coalescence temperature was estimated for **2d** (Figure 4).



9



2d

$G^{\ddagger}_{301}$ = 61.0 kJ mol^{-1}

Figure 4

Table 7

2,5,7-Triamino[1,2,4]triazolo[1,5-*a*][1,3,5]triazines (**2**)

Compound	Method	mp, ºC	Solvent	Yield, %
2a	A	359-360	DMF	55
2b	A	288-290	DMF / H_20	68
2c	A	298	DMF / H_20	65
2d	B	300-301	EtOH / H_2O	54
2e	A	246-248	DMF / H_20	62

Table 5

^{1}H NMR spectra of 2,5,7-triamino[1,2,4]triazolo[1,5-*a*][1,3,5]triazines (**2**)

(300 MHz, DMSO-d_6) δ, ppm

Compound	(C-2)-NH	(C-5)-NH_2	(C-7)-NH_2	R
2a	s, 9.45	br.s, 7.83	s, 6.77	6.86 (1H, t, *J* = 7.4 Hz, H-4'), 7.25 (2H, t, *J* = 7.9 Hz, H-3' and H-5'), 7.73 (2H, d, *J* = 7.9 Hz, H-2' and H-6')
2b	t, 6.62, *J* = 6.6 Hz	br.s, 7.67	s, 6.62	4.41 (2H, d, *J* = 6.6 Hz, NHCH_2), 7.21 (1H, t, *J* = 7.0 Hz, H-4'), 7.26-7.39 (4H, m, H-2', H-3', H-5' and H-6')
2c	t, 6.46, *J* = 5.7 Hz	br.s, 7.55	s, 6.61	2.86 (2H, t, *J* = 7.3 Hz, PhCH_2), 3.39 (2H, dt, *J* = 7.3, 7.2 Hz, NHCH_2), 7.16-7.33 (5H, m, Ph)
2d	s, 9.29	br.s, 7.43, br.s, 8.03	s, 6.77	3.60 (3H, s, OMe), 3.78 (6H, s, 2 OMe), 7.01 (2H, s, H-2 and H-6)
2e	t, 6.87, *J* = 6.4 Hz	br.s, 7.58	s, 6.61	3.62 (3H, s, OMe), 3.75 (6H, s, 2 OMe), 4.33 (2H, d, *J* = 6.4 Hz, NHCH_2), 6.70 (2H, s, H-2 and H-6)

Table 9

^{13}C NMR spectra of 2,5,7-triamino[1,2,4]triazolo[1,5-*a*][1,3,5]triazines (**2**)

(75 MHz, DMSO-d_6) δ, ppm

Compound	C-2, C-5, C-7 and C-8a	R
2a	149.7, 157.5, 161.4 and 162.5	116.6 (C-2' and C-6'), 119.8 (C-4'), 128.5 (C-3' and C-5'), 141.0 (C-1')
2b	149.4, 158.5, 162.2 and 165.5	45.4 (CH_2), 126.4 (C-4'), 127.0 (C-2' and C-6'), 128.0 (C-3' and C-5'), 140.5 (C-1')
2c	149.4, 158.4, 162.2 and 165.3	35.2 (CH_2), 43.9 (CH_2), 125.8 (C-4'), 128.1 (C-2' and C-6'), 128.6 (C-3' and C-5'), 139.8 (C-1')
2d	149.7, 157.6, 161.5 and 162.4	55.7 (2 OMe), 60.0 (OMe), 94.9 (C-2' and C-6'), 131.2 (C-4'), 137.2 (C-1'), 152.8 (C-3' and C-5')
2e	149.4, 158.4, 162.2 and 165.3	45.7 (CH_2), 55.7 (2 OMe), 59.8 (OMe), 104.5 (C-2' and C-6'), 136.0, 136.1 (C-1' and C-4'), 152.6 (C-3' and C-5')

Conclusion

A simple 3-step method for the preparation of hitherto unknown 2,5,7-triamino[1,2,4]triazolo[1,5-*a*][1,3,5]triazines (**2**) as potential DHFR inhibitors was successfully developed.

Experimental procedures

Melting points (uncorrected) were determined on a Gallenkamp melting point apparatus. NMR spectra were recorded on a Bruker DPX-300 spectrometer, using DMSO-d_6 as a solvent and TMS as an internal reference.

*Synthesis of N-substituted N'-cyano-S-methylisothioureas (**4**).*

Method A. A mixture of appropriate amine (30 mmole) and dimethyl *N*-cyanodithiocarbonimidate (**3**) (4.4 g, 30 mmole) in 60 ml of chloroform was stirred at room temperature for 1 h and then heated at 40°C for another 1-4 h. The chloroform and liberated methylthiol were removed under vacuum and the resulting solid was recrystallized.

Method B. A mixture of 3,4,5-trimethoxyaniline (3.7 g, 20 mmole) and dimethyl *N*-cyanimidodithiocarbonate (**3**) (2.9 g, 20 mmole) in 10 ml of ethanol was stirred at room temperature for 10 min and then heated under reflux for 4 h. After cooling, the product was filtered, washed with ethanol, dried and recrystallized from 2-propanol.

*Synthesis of 3,5-diamino-1,2,4-triazoles (**5**).*

Method A. To a stirred suspension of *N*-substituted *N*'-cyano-*S*-methylisothioureas (**4a-c**) (10 mmole) in 15 ml of ethanol, hydrazine hydrate (98%, 1.0 ml, 20 mmole) was added dropwise with

stirring at room temperature within 5 min. The reaction mixture was heated under reflux for 3 h and the solvent and the liberated methylthiol were removed under vacuum. The solid obtained was recrystallized.

Method B. To a stirred suspension of *N*-substituted *N*'-cyano-*S*-methylisothioureas (**4d,e**) (10 mmole) in 15 ml of ethanol, hydrazine hydrate (98%,1.0 ml, 20 mmole) was added dropwise with stirring at room temperature within 2-5 min. The reaction mixture was heated under reflux for 2-4 h. After cooling, the product was filtered, washed with ethanol, dried and recrystallized.

*Synthesis of of 2,5,7-triamino[1,2,4]triazolo[1,5-a][1,3,5]triazines (**2**).*

Method A. To a stirred solution of appropriate 3,5-diamino-1,2,4-triazoles (**5a-c,e**) (5.0 mmole) and 0.5 ml (5.0 mmole) of conc. HCl in 10 ml of water, cyanoguanidine (0.5 g, 6 mmole) was added and the resulting solution was heated under reflux for 7-24 h. After cooling, the product was filtered, washed with cold water, dried and recrystallized.

Method B. To a stirred solution of appropriate 3,5-diamino-1,2,4-triazoles (**5d**) (5.0 mmole) in 5 ml of DMF cyanoguanidine (0.5 g, 6 mmole) of was added and the resulting solution was heated under reflux for 4 h. After cooling, ice-cold water was added to the reaction mixture. The resulting solid was filtered, washed with cold water, dried and recrystallized from aqueous ethanol.

References

[1] I.M. Kompis, K. Islam and R.L. Then, *Chem. Rev.*, 2005, **105**(2), 593-620.

[2] A.V. Dolzhenko and W.K. Chui, *J. Heterocyclic Chem.*, 2006, **43**(1), 95-100.

[3] A.V. Dolzhenko, W.K. Chui and A.V. Dolzhenko, *ECSOC-9*, Nov. 1-30, 2005 C012/1-C012/6.

[4] A.V. Dolzhenko, W.K. Chui, A.V. Dolzhenko and L.W. Chan, *J. Fluorine Chem.*, 2005, **126**(5), 759-763.

[5] A.V. Dolzhenko, A.V. Dolzhenko and W.K. Chui, *Heterocycles*, 2006, **68**(8), 1723-1759.

[6] M. Yokoyama, M. Kurauchi and T. Imamoto, *Tetrahedron Lett.*, 1981, **22**(24), 2285-2288.

[7] J.S. Davidson, *Chem. Ind. (London)*, 1965, (48), 1977-1978.

[8] S. Seshadri, N.M. Sanghavi, R.V. Naik, S.R. Tawate, M.N. Trivedi, M.A. Fruitwala, *Indian J. Chem*., 1993, **32B**(6), 688-692.

[9] J. Reiter, L. Pongo, T. Somorai and P. Dvortsak, *J. Heterocyclic Chem.*, 1986, **23**(2), 401-408.

[10] B. Blank, D.M. Nichols and P.D. Vaidya, *J. Med. Chem.*, 1972, **15**(6), 694-696.