Synthesis of new derivatives of pyrimido[5,4-*e*][1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine and their enzyme inhibitory activity assessment on soybean 15-lipoxygenase

Mehdi Bakavoli[a]*, Seyed Mohammad Seyedi[a], Ali Shiri[a], Sattar Saberi[a], Mahmoud Gholami[a] and Hamid Sadeghian[b]

[a]*Department of Chemistry, School of Sciences, Ferdowsi University of Mashhad, 91775-1436 Mashhad, Iran*

[b]*Department of Laboratory Sciences, School of Paramedical Sciences, Mashhad University of Medical Sciences, 91857-63788 Mashhad, Iran*

The synthesis of new derivatives of pyrimido[5,4-*e*][1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine is described. These derivatives have a wide range of medicinal applications. Their inhibitory activity against the enzyme 15-lipoxygenase was also investigated.

Keywords: pyrimidotriazolothiadiazines, triazoles, annulated pyrimidines, 15-lipoxygenase inhibitor, heterocyclisation

Pyrimidine derivatives and heterocyclic annulated pyrimidines continue to attract great interest because of their wide variety of interesting biological activities, such as anticancer,[1] antiviral,[2] antitumor,[3] and anti-inflammatory activities.[4] Moreover, triazoles and especially fused triazoles are also an important class of heterocyclic compounds with antifungal,[5] bactericidal,[5,6] anxiolytic,[7,8] anticonvulsant[9] and antidepressant activities.[10]

Numerous methods for the synthesis of 1,2,4-triazoles have been reported, which includes utilising toxic reagents such as phosphorus oxychloride,[11] lead tetraacetate,[11,12] and bromine[12,13] as well as other oxidative reagents such as chloramine-T,[14] iodobenzene diacetate,[15,16] iron(III) chloride,[17] and $CuCl_2$.[18] The synthesis of 1,2,4-triazoles by an electrochemical method[19] has also been reported. Some triazolothiadiazines have also been reported which possess a broad spectrum of biological activities.[20–22] Keeping this in mind, and due to our recent studies on the enzyme inhibitory activity of pyrimido[5,4-*e*][1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines against 15-lipoxygenase (15-LO), (a main group of the non-haeme, iron-containing proteins which can catalyse hydroperoxidation of polyunsaturated fatty molecules containing a *cis,cis*-1,4-pentadiene structure such as arachidonic and linoleic acid[23]), we considered the synthesis of pyrimidotriazolothiadiazine compounds wherein the biologically active pyrimidine moiety is fused to a potent triazolo[3,4-*b*][1,3,4]thiadiazine ring across the 6,7-positions.

We now describe the synthesis of some new derivatives of tricyclic 3,6-dimethyl-5H-pyrimido[5,4-*e*][1,2,4]triazolo[3,4-*b*][1,3,4] thiadiazines **5a–f** and their enzyme inhibitory activity towards 15-LO.

Results and discussion

5-Bromo-2,4-dichloro-6-methylpyrimidine **1** was prepared according to our previously published method.[24] Treatment of compound **1** with 1-amino-2-mercapto-5-methyl-1,2,4-triazole **2** which was prepared from the reaction of hydrazine hydrate with CS_2 followed by reaction with acetic acid according to the published procedure[25] afforded the intermediate **3**. The facility with which substitution of the C-4 chlorine atom in compound **1** occurs by nucleophilic attack of the sulfur function in the triazole **2** had been established previously using similar conditions.[24] Subsequent reaction of compound **3** with various secondary amines led to the selective replacement of the chlorine atom at the 2-position of the pyrimidine ring and gave the corresponding diheteroaryl sulfide intermediates **4a–f**. The latter compounds subsequently underwent an intramolecular S_NAr reaction in the presence of $NaNH_2$ in boiling acetonitrile to give the desired tricyclic 3,6-dimethyl-5*H*-pyrimido[5,4-*e*][1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazines **5a–f**. (Scheme 1)

The structural assignment of compounds **5a–f** is based upon spectroscopic and microanalytical data. For example, the ^{1}H NMR spectrum of **5d** showed two singlet peaks at δ 2.44 and 2.50 ppm belonging to methyl groups of the triazole and pyrimidine moieties, respectively. The multiplet signals in the range of δ 3.44–3.63 ppm corresponded to the morpholine ring proton signals. The spectrum of the precursor **4d** showed the NH_2 signals at δ 4.92 ppm which was removed on adding D_2O. However, the ^{1}H NMR spectrum of the cyclised product **5d** did not show this signal and instead an exchangeable broad singlet peak at δ 7.72 ppm confirmed that heterocylisation to **5d** had occurred. The IR spectrum was devoid of the NH_2 absorption bands at ν 3313 and 3137 cm^{-1} of the precursor, but an absorption band at ν 3324 cm^{-1} demonstrated the existence of the NH group in product **5d**. The mass spectrum of **5d** showed a molecular ion signal at *m/z* 304 (M^+) corresponding to the molecular formula $C_{12}H_{14}N_7OS$.

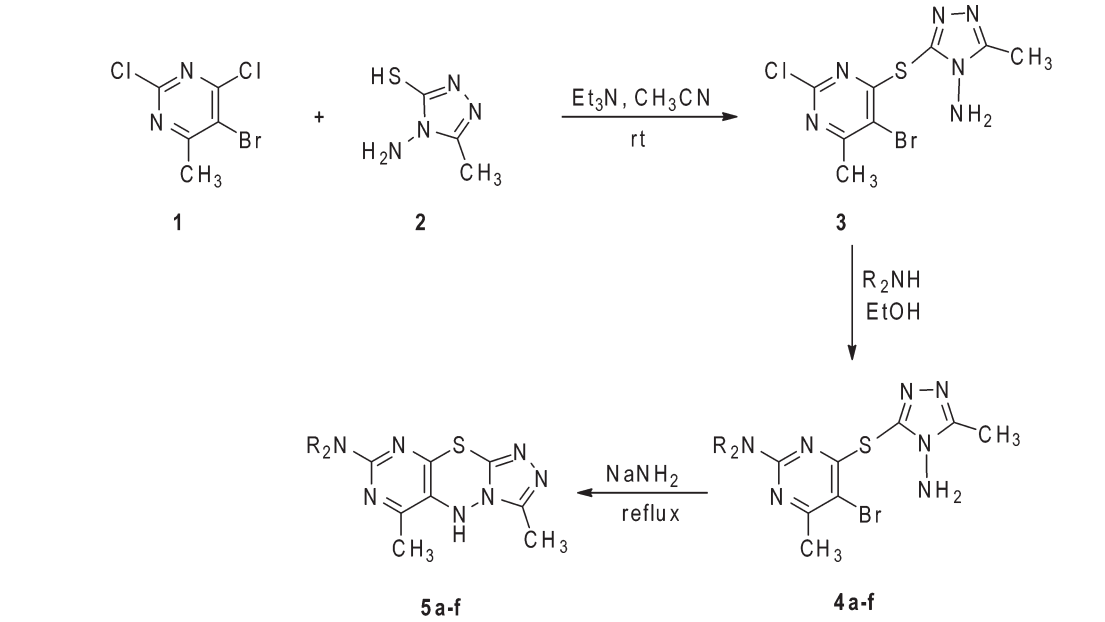
The inhibitory property of compounds **4a–f** and **5a–f** on 15-LO was assessed according to our previously reported procedure.[23] The compounds showed very low inhibitory activity. However, the sulfide **4c** among others showed the best inhibitory activity (IC_{50} = 499 μM). By comparing the results of enzyme inhibitory activity of pyrimido[5,4-*e*][1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine with the results of our previous work on pyrimido[4,5-*b*][1,4]benzothiazines, we suggest that the triazolo moiety by being an electron deficient ring, prohibits the facile oxidation of the sulfur atom which is crucial for the enzyme inhibitory activity. Work is currently in progress in our laboratory with electron rich heterocycles to establish the validity of this proposal.

In summary, an interesting fused ring system containing the pyrimido[5,4-*e*][1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine residue was synthesised through the treatment of 5-bromo-2,4-dichloro-6-methylpyrimidine **1** with 1-amino-2-mercapto-5-methyl-1,2,4-triazole **2** which was subsequently reacted with secondary amines and cyclised in presence of $NaNH_2$ in boiling CH_3CN. The inhibitory activity of compounds **4a–f** and **5a–f** on 15-LO has been assessed.

Experimental

Melting points were recorded on an Electrothermal type 9100 melting point apparatus and are uncorrected. The IR spectra were obtained on an Avatar 370 FT-IR Thermo-Nicolet spectrometer. The ^{1}H NMR (100 MHz) spectra were recorded on a Bruker AC 100 spectrometer. The mass spectra were scanned on a Varian Mat CH-7 at 70 eV. Elemental analysis was performed on a Thermo Finnigan Flash EA microanalyser.

* Correspondent. E-mail: mbakavoli@um.ac.ir; mbakavoli@yahoo.com



Compound	4a or 5a	4b or 5b	4c or 5c	4d or 5d	4e or 5e	4f or 5f
R_2N—	N—	N—	Me N—	O N—	Me-N N—	Ph-N N—

Scheme 1

*3-[(5-Bromo-2-chloro-6-methylpyrimidin-4yl)thio]-5-methyl-4*H-*1,2,4-triazol-4-amine* (**3**)*:* To a stirred solution of 5-bromo-2,4-dichloro-6-methylpyrimidine **1** (2.44 g, 10 mmol) and Et_3N (1.6 mL, 13 mmol) in CH_3CN (25 mL), a solution of 1-amino-2-mercapto-5-methyl-1,3,4-triazole **2** (1.31 g, 10 mmol) in CH_3CN (30 mL) was added dropwise over 30 min. The solution was stirred vigorously until the white precipitate is appeared. Stirring was then continued at room temperature for an extra 30 minutes and the resulting solid was filtered and washed with warm water. Yield 95%, m.p. 207–208 °C, ^{1}H NMR (DMSO-d_6) δ 2.47(s, 3H, CH_3), 2.62 (s, 3H, CH_3), 5.73 (br s, 2H, NH_2, D_2O exchangeable); IR (KBr disc) ν 3247, 3149, 2998, 1524, 750 cm^{-1}. MS (*m/z*) 334 (M^+), 336 (M^+ + 2). Anal. Calcd for $C_8H_8BrClN_6S$: C, 28.63; H, 2.40; N, 25.04; S, 9.55. Found: C, 28.53; H, 2.37; N, 24.94; S, 9.46%.

*Synthesis of compounds (***4a–f***); general procedure*

The appropriate secondary amine (12 mmol) was added to a stirred mixture of compound **3** (3.35 g, 10 mmol) in ethanol (30 mL), and the solution was heated under reflux for 6 h. After cooling the solution, water (20 mL) was added and the resulting solid was filtered and recrystallised in ethanol.

*3-[(5-Bromo-6-methyl-2-(pyrrolidin-1-yl)pyrimidin-4-yl)thio]-5-methyl-4*H-*1,2,4-triazol-4-amine* (**4a**): Yield 78%, m.p. = 221–222 °C, ^{1}H NMR ($CDCl_3$) δ 1.88 (m, 4H, $2CH_2$), 2.43 (s, 3H, CH_3), 2.52 (s, 3H, CH_3), 3.11–3.32 (m, 4H, $2CH_2$–N), 5.02 (s, 2H, NH_2, D_2O exchangeable). IR (KBr disc) ν 3284, 3170, 2970, 1564, 770 cm^{-1}; MS (*m/z*) 369 (M^+), 371 (M^+ + 2). Anal. Calcd for $C_{12}H_{16}BrN_7S$: C, 38.93; H, 4.36; N, 26.48; S, 8.66. Found: C, 38.88; H, 4.31; N, 26.42; S, 8.60%.

*3-[(5-Bromo-6-methyl-2-(piperidin-1-yl)pyrimidin-4-yl)thio]-5-methyl-4*H-*1,2,4-triazol-4-amine* (**4b**): Yield 80%, m.p. 208–210 °C, ^{1}H NMR ($CDCl_3$) δ 1.51–1.67 (m, 6H, $3CH_2$), 2.41 (S, 3H, CH_3), 2.5 (s, 3H, CH_3), 3.41–3.58 (m, 4H, CH_2N), 4.89 (s, 2H, NH_2, D_2O exchangeable); IR (KBr disc) ν 3327, 3133, 2943, 1556, 770 cm^{-1}. MS (*m/z*) 383 (M^+), 385 (M^+ + 2). Anal. Calcd for $C_{13}H_{18}BrN_7S$: C, 40.63; H, 4.72; N, 25.51; S, 8.34. Found: C, 40.58; H, 4.70; N, 25.47; S, 8.29%.

*3-[(5-Bromo-6-methyl-2-(4-methylpiperidin-1-yl)pyrimidin-4-yl)thio]-5-methyl-4*H-*1,2,4-triazol-4-amine* (**4c**): Yield 78%, m.p. 150–152 °C, ^{1}H NMR ($CDCl_3$) δ 0.95 (d, *J* = 9 Hz, 3H, CH_3), 1.45–1.71 (m, 5H, CH and $2CH_2$), 2.38 (s, 3H, CH_3), 2.47 (s, 3H, CH_3), 2.51–2.92 (m, 2H, equatorial hydrogens of CH_2N), 3.12–3.25 (m, 2H, axial hydrogens of CH_2N), 4.89 (s, 2H, NH_2, D_2O exchangeable); IR (KBr disc) ν 3299, 3196, 2990, 1637, 772 cm^{-1}. MS (*m/z*) 397 (M^+), 399 (M^+ + 2). Anal. Calcd for $C_{14}H_{20}BrN_7S$: C, 42.21; H, 5.06; N, 24.61; S, 8.05. Found: C, 42.19; H, 5.01; N, 24.57; S, 7.98%.

*3-[(5-Bromo-6-methyl-2-morpholinopyrimidin-4-yl)thio]-5-methyl-4*H-*1,2,4-triazol-4-amine* (**4d**): Yield 80%, m.p. 232–234 °C, ^{1}H NMR ($CDCl_3$) δ 2.44 (s, 3H, CH_3), 2.50 (s, 3H, CH_3), 3.44–3.49 (m, 4H, CH_2N), 3.58–3.63 (m, 4H, CH_2O), 4.92 (br s, 2H, NH_2, D_2O exchangeable); IR (KBr disc) ν 3313, 3137, 2986, 1597, 771 cm^{-1}. MS (*m/z*) 385 (M^+), 387 (M^+ + 2). Anal. Calcd for $C_{12}H_{16}BrN_7OS$: C, 37.31; H, 4.18; N, 25.38; S, 8.30. Found: C, 37.27; H, 4.16; N, 25.35; S, 8.27%.

*3-[(5-Bromo-6-methyl-2-(4-methylpiperazin-1-yl)pyrimidin-4-yl)thio]-5-methyl-4*H-*1,2,4-triazol-4-amine* (**4e**): Yield 80%, m.p. 205–207 °C, ^{1}H NMR ($CDCl_3$) δ 2.21 (s, 3H, CH_3), 2.31 (br. t, 4H, $2CH_2N$), 2.42 (s, 3H, CH_3), 2.59 (s, 3H, CH_3), 3.51 (br. t, 4H, $2CH_2N$), 4.82 (s, 2H, NH_2, D_2O exchangeable) IR (KBr disc) ν 3276, 3186, 2969, 1548, 771 cm^{-1}. MS (*m/z*) 398 (M^+), 400 (M^+ + 2). Anal. Calcd for $C_{13}H_{19}BrN_8S$: C, 39.10; H, 4.80; N, 28.06; S, 8.03. Found: C, 39.05; H, 4.77; N, 28.01; S, 7.98%.

*3-[(5-Bromo-6-methyl-2-(4-phenylpiperazin-1-yl)pyrimidin-4-yl)thio]-5-methyl-4*H-*1,2,4-triazol-4-amine* (**4f**): Yield 75%, m.p. 280–282 °C, ^{1}H NMR ($CDCl_3$) δ 2.45 (s, 3H, CH_3), 2.54 (s, 3H, CH_3), 3.10 (br. t, 4H, $2CH_2N$), 3.59 (br. t, 4H, $2CH_2N$), 4.85 (s, 2H, NH_2, D_2O exchangeable), 6.84–6.95 (m, 3H, aromatic), 7.26–7.31 (m, 2H, aromatic). IR (KBr disc) ν 3276, 3219, 2949, 1583, 1546, 1503, 1445, 797 cm^{-1}. MS (*m/z*) 460 (M^+), 462 (M^+ + 2). Anal. Calcd for $C_{18}H_{21}BrN_8S$: C, 46.86; H, 4.59; N, 24.29; S, 6.95. Found: C, 46.83; H, 4.55; N, 24.24; S, 6.91%.

*Synthesis of compounds (***5a–f***); general procedure*

A mixture of each of compounds (**4a–f**) (10 mmol), $NaNH_2$ (30 mmol) in dry acetonitrile (50 mL) was heated under reflux for about 5h the progress of the reaction was monitored by TLC using chloroform: methanol (9:1). The mixture was cooled and the solvent was removed under reduced pressure. Then, a solution of acetic acid (1 mL) in water (20 mL) was added to the residue and the resulting precipitant was filtered off and recrystallised from ethanol.

*3,6-Dimethyl-8-(pyrrolidin-1-yl)-5*H-*pyrimido[5,4-*e*][1,2,4]-triazolo[3,4-*b*][1,3,4]thiadiazine* (**5a**): Yield 60%, m.p. 280–282 °C,

^{1}H NMR ($CDCl_3$) δ 1.81–1.95 (m, 4H, $2CH_2$), 2.39 (s, 3H, CH_3), 2.46 (s, 3H, CH_3), 3.35 (t, 4H, $2CH_2N$), 7.22 (s, 1H, NH, D_2O exchangeable). IR (KBr disc) ν 3268, 3080, 2989, 1617 cm^{-1}. MS (*m/z*) 289 (M^+). Anal. Calcd for $C_{12}H_{15}N_7S$: C, 49.81; H, 5.23; N, 33.88; S, 11.08. Found: C, 49.75; H, 5.20; N, 33.85; S, 11.01%.

*3,6-Dimethyl-8-(piperidin-1-yl)-5*H*-pyrimido[5,4-*e*][1,2,4]-triazolo[3,4-*b*][1,3,4]thiadiazine* (**5b**): Yield 65%, m.p. 235–237 °C, ^{1}H NMR ($CDCl_3$) δ 2.42–2.61 (m, 6H, $3CH_2$), 2.33 (s, 3H, CH_3), 2.39 (s, 3H, CH_3), 3.48–3.54 (m, 4H, CH_2N), 7.63 (s, 1H, NH, D_2O exchangeable). IR (KBr disc) ν 3259, 2932, 1581 cm^{-1}. MS (*m/z*) 303 (M^+). Anal. Calcd for $C_{13}H_{17}N_7S$: C, 51.47; H, 5.65; N, 32.32; S, 10.57. Found: C, 51.43; H, 5.63; N, 32.29; S, 10.55%.

*3,6-Dimethyl-8-(4-methylpiperidin-1-yl)-5*H*-pyrimido[5,4-*e*][1,2,4]-triazolo[3,4-*b*][1,3,4]thiadiazine* (**5c**): Yield 57%, m.p. 170–171 °C, ^{1}H NMR ($CDCl_3$) δ 0.93 (d, J = 8 Hz, 3H, CH_3), 1.47–1.71 (m, 5H, CH and $2CH_2$), 2.33 (s, 3H, CH_3), 2.39 (s, 3H, CH_3), 2.55–2.91 (m, 2H, equatorial hydrogens of CH_2N), 3.23–3.41 (m, 2H, axial hydrogens of CH_2N), 7.96 (br s, 1H, NH, D_2O exchangeable); IR (KBr disc) 3335, 2949, 1579 cm^{-1}. MS (*m/z*) 317 (M^+). Anal. Calcd for $C_{14}H_{19}N_7S$: C, 52.98; H, 6.03; N, 30.89; S, 10.10. Found: C, 52.95; H, 6.01; N, 30.84; S, 10.04%.

*4-(3,6-Dimethyl-5*H*-pyrimido[5,4-*e*][1,2,4]triazolo[3,4-*b*][1,3,4]-thiadiazin-8-yl)morpholine* (**5d**): Yield 70%, m.p. 220–222 °C, ^{1}H NMR ($CDCl_3$) δ 2.36 (s, 3H, CH_3), 2.45 (s, 3H, CH_3), 3.52–3.59 (m, 4H, CH_2N), 3.63–3.73 (m, 4H, CH_2O), 7.72 (br s, 1H, NH, D_2O exchangeable); IR (KBr disc) ν 3325, 2961, 1617 cm^{-1}; MS (*m/z*) 305 (M^+). Anal. Calcd for $C_{12}H_{15}N_7OS$: C, 47.20; H, 4.95; N, 32.11; S, 10.50. Found: C, 47.17; H, 4.90; N, 32.05; S, 10.45%.

*3,6-Dimethyl-8-(4-methylpiperazin-1-yl)-5*H*-pyrimido[5,4-*e*][1,2,4]-triazolo[3,4-*b*][1,3,4]thiadiazine* (**5e**): Yield 65%, m.p. 250–253 °C, ^{1}H NMR ($CDCl_3$) δ 2.22 (s, 3H, CH_3), 2.33 (br. t, 4H, $2CH_2N$), 2.44 (s, 3H, CH_3), 2.58 (s, 3H, CH_3), 3.52 (br. t, 4H, $2CH_2N$), 7.66 (s, 1H, NH, D_2O exchangeable) IR (KBr disc) ν 3247, 2967, 1545 cm^{-1}; MS (*m/z*) 318 (M^+). Anal. Calcd for $C_{13}H_{18}N_8S$: C, 49.04; H, 5.70; N, 35.19; S, 10.07. Found: C, 49.01; H, 5.68; N, 35.16; S, 10.04%.

*3,6-Dimethyl-8-(4-phenylpiperazin-1-yl)-5*H*-pyrimido[5,4-*e*][1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazine* (**5f**): Yield 65%, m.p. 310–313 °C, ^{1}H NMR ($CDCl_3$) δ 2.36 (s, 3H, CH_3), 2.45 (s, 3H, CH_3), 3.12 (br. t, 4H, $2CH_2N$), 3.72 (br. t, 4H, $2CH_2N$), 6.88–6.95 (m, 3H, aromatic), 7.25–7.35 (m, 2H, aromatic), 7.88 (s, 1H, NH, D_2O exchangeable); IR (KBr disc) ν 3245, 2954, 1585 cm^{-1}. MS (*m/z*) 380 (M^+). Anal. Calcd for $C_{18}H_{20}N_8S$: C, 56.82; H, 5.30; N, 29.45; S, 8.43. Found: C, 56.78; H, 5.27; N, 29.41; S, 8.40%.

Received 11 July 2012; accepted 3 December 2012
Paper 1201407 doi: 10.3184/174751912X13549029453945
Published online: 15 January 2013

References

1 C.R. Petrie, H.B. Cottam, P.A. McKernan, R.K. Robins and G.R. Revankar, *J. Med. Chem.*, 1985, **28**, 1010.
2 M.M. Gineinah, M.A. El-Sherbeny, M.N. Nasr and A.R. Maarouf, *Arch. Pharm.*, 2002, **335**, 556.
3 P.G. Baraldi, M.G. Pavani, M.C. Nuņez, P. Brigidi, B. Vitali, R. Gambari and R. Romagnoli, *Bioorg. Med. Chem.*, 2002, **10**, 449.
4 S.M. Sondhi, M. Johar, S. Rajvanshi, S.G. Dastidar, R. Shukla, R. Raghubir and J.W. Lown, *Aus. J. Chem.*, 2001, **54**, 69.
5 S. El-Hawash, N. Habib and N. Fanaki, *Pharmazie*, 1999, **54**, 808.
6 D. Brown and Y. Iwai, *Aus. J. Chem.*, 1979, **32**, 2727.
7 G. Tarzia, E. Occelli, E. Toja, D. Barone, N. Corsico, L. Gallico and F. Luzzani, *J. Med. Chem.*, 1988, **31**, 1115.
8 R.I. Trust and J.D. Albright, *US Patent* 4 242 515, 1980 (*Chem. Abstr.* 1981, 94:139815d).
9 G. Tarzia, E. Occelli and D. Barone, *Farmaco*, 1989, **44**, 3.
10 R. Sarges, H.R. Howard, R.G. Browne, L.A. Lebel, P.A. Seymour and B.K. Koe, *J. Med. Chem.*, 1990, **33**, 2240.
11 J.D. Bower and F.P. Doyle, *J. Chem. Soc.*, 1957, 727.
12 A. Pollak and M. Tisler, *Tetrahedron*, 1966**,** **22**, 2073.
13 M.S. Gibson, *Tetrahedron*, 1963, **19**, 1587.
14 P. Bourgeois, R. Cantegril, A. Chene, J. Gelin, J. Mortier and J. Moyroud, *Synth. Commun.*, 1993, **23**, 3195.
15 A.K. Sadana, Y. Mirza, K.R. Aneja and O. Prakash, *Eur. J. Med. Chem.*, 2003, **38**, 533.
16 D. Kumar, V.G. Kondapalli, S. Chandra, D. Harmeet, S.R. Vajja and S. Rajender, *Green Chem*., 2004, **6**, 156.
17 A.S. Shawali, H.M. Hassaneen and N.K. Shurrab, *Tetrahedron*, 2008, **64**, 10339.
18 M. Ciesielski, D. Pufky and M. Doring, *Tetrahedron*, 2005, **61**, 5942.
19 S. Crljenak, I. Tabakovic, D. Jeremic and I. Gaon, *Acta Chem. Scand.*, 1983, **B37**, 527.
20 A. Hassan, *Phosphorus, Sulfur, Silicon*, 2009, **184**, 2759.
21 R. Miao, J. Wei,; M. Lv, Y. Cai, Y. Du, X. Hui and Q. Wang, *Eur. J. Med. Chem.*, 2011, **46**, 5000.
22 O. Prakash, D.K. Aneja, K. Hussain, P. Lohan, P. Ranjan, S. Arora, C. Sharma and K.R. Aneja, *Eur. J. Med. Chem.*, 2011, **46**, 5065.
23 M. Bakavoli, M. Nikpour, M. Rahimizadeh, M.R. Saberi and H. Sadeghian, *Bioorg. Med. Chem.*, 2007, **15**, 2120.
24 M. Bakavoli, M. Nikpour and M. Rahimizadeh, *J. Heterocycl. Chem.*, 2006, **43**, 1327.
25 L.F. Audrieth, E.S. Scott and P.S. Kippur, *J. Org. Chem.*, 1954, **19**, 733.