Full Paper

Facile One-Pot Synthesis and Antimycobacterial Evaluation of Pyrazolo[3,4-*d*]pyrimidines

Amit Trivedi, Dipti Dodiya, Janak Surani, Samir Jarsania, Hitesh Mathukiya, Naresh Ravat, and Viresh Shah

Chemical Research Laboratory, Department of Chemistry, Saurashtra University, Rajkot, India

The present article describes a facile one-pot synthesis of a series of eight pyrazolo[3,4-*d*]pyrimidines **4a**–**h** which were evaluated for their *in-vitro* antibacterial activity against *Mycobacterium tuberculosis* H37Rv using the Alamar-Blue susceptibility test and the activity expressed as the minimum inhibitory concentration (MIC) in mg/mL. The compounds **4b**, **4c**, **4d,** and **4g** exhibited the best results (1.2 μg/mL) when compared with first-line drugs such as isoniazid (INH) and rifampicin (RIP). Therefore, this class of compounds could be a good starting point to develop new lead compounds in the treatment of multidrug-resistant tuberculosis.

Keywords: Antimycobacterial activity / Antitubercular activity / *Mycobacterium tuberculosis* H37Rv / One-pot synthesis / Pyrazolo[3,4-*d*]pyrimidines

Received: February 19, 2008; accepted: March 27, 2008

DOI 10.1002/ardp.200800027

Introduction

Nowadays, microorganisms resistant to multiple antimicrobial agents are a serious problem worldwide in the fight against infectious diseases, increasing morbidity and mortality with an overall increase in healthcare costs. In this context, tuberculosis (TB) has become again an important public health problem worldwide since the mid-1980s, due to two major factors, the AIDS epidemic and the advent of multidrug-resistant strains (MDR). TB is responsible for 20% of all deaths in adults, and, each year, there are about 8.9 to 9 millions of new cases, of which 15% are children, and 1.7 to 2 millions of deaths, of which 450,000 are children. Globally, the number of TB cases is currently rising at 2% per year with the estimative of 32% of the world population, about two billion people, being infected by latent TB. In the case of patients with AIDS, TB is the most common opportunistic infection and cause of death killing one of every three patients [see: http://www.who.int/tdr/diseases/tb/default.htm.]. Due to the increase of MDR-TB and AIDS cases worldwide and the lack of new drugs nowadays, there is an urgent need for new drugs to fight against this disease.

In this context, pyrazole and pyrimidine derivatives attracted organic chemists very much due to their biological and chemotherapeutic importance. Pyrazolopyrimidines and related fused heterocycles are of interest as potential bioactive molecules. They are known to exhibit pharmacological activities such as CNS depressant [1], neuroleptic [2], and tuberculostatic [3]. Pyrazolo[3,4-*d*]pyrimidines were identified as a general class of adenosine receptors [3, 4]. There is not much difference in the basic structures of pyrazolopyrimidines and purines. Purine-containing molecules are ubiquitous in nature and are found as components of nucleosides, nucleotides, co-factors, and signaling molecules. A significant proportion of any genome codes for proteins that recognize purine-containing ligands (*e.g.*, kinases, DNA and RNA polymerases, ATPases, GTPases, purine receptors). Unsurprisingly, structural analogues of purines have proved attractive templates for drug discovery programs, leading to a number of significant synthetic drugs (*e.g.*, 6-thiopurine for leukaemia [5], acyclovir as an antiviral [6] and allopurinol for treatment of gout [7]). Recently, the purine core has been exploited in the synthesis of protein kinase inhibitors [8, 9, 10, 11] inhibitors of carbohydrate

Correspondence: Dr. Viresh Shah, Chemical Research Laboratory, Department of Chemistry, Saurashtra University, Kalawad Road, 360005 Rajkot, India.
E-mail: drvireshshah@gmail.com
Fax: +91 281-2576802

Abbreviations: tuberculosis (TB); multidrug-resistant strains (MDR)

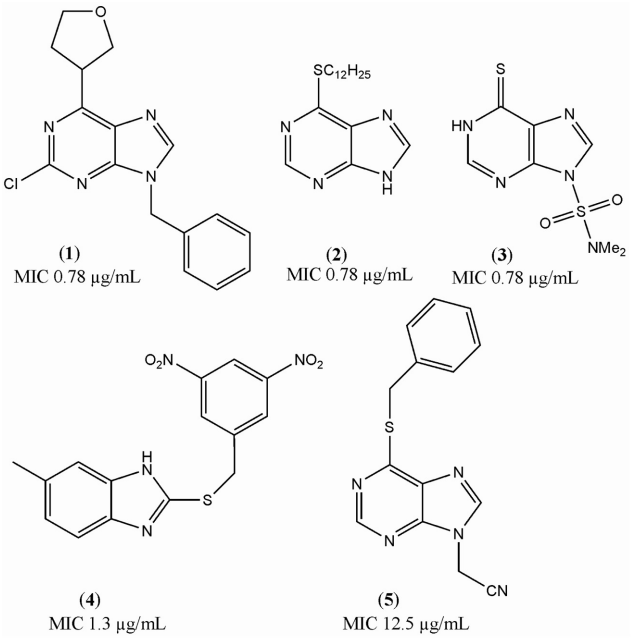


Figure 1. Purine-related anti-mycobacterials: (**1**) [15], (**2**) [16], (**3**) [17], (**4**) [18], (**5**) [19].

[12] and estrogen [13] sulfotransferases, as well as in compounds displaying osteogenesis-inducing activity in stem cells [14]. Compounds of this generic nature **1**–**5** have also been reported to display anti-mycobacterial activity (Fig. 1) [15, 16, 17, 18, and 19].

The isomeric pyrazolo[3,4-*d*]pyrimidine nucleus has attracted comparatively little attention, though it has been exploited in inhibitors of Src protein kinases [20], EGF receptor tyrosine kinases [21], cyclic AMP phosphodiesterases [22], and *Staphylococcus aureus* DNA polymerase III [23]. Interestingly, such a compound reported as an intermediate in the synthesis of prospective HIV replication inhibitors showed modest anti-mycobacterial activity (compound **5**; MIC 12.5 μg/mL) [19]. In addition, few other pyrazolo[3,4-*d*]pyrimidine nucleus has been previously reported to demonstrate anti-mycobacterial activity [24, 25, 26]. These diverse bioactivity results prompted us to explore the pyrazolo[3,4-*d*]pyrimidine template in our search for new antimycobacterial agents, given an encouraging level of potency and straightforward one-pot synthesis of pyrazolo[3,4-*d*]pyrimidines.

Results and discussion

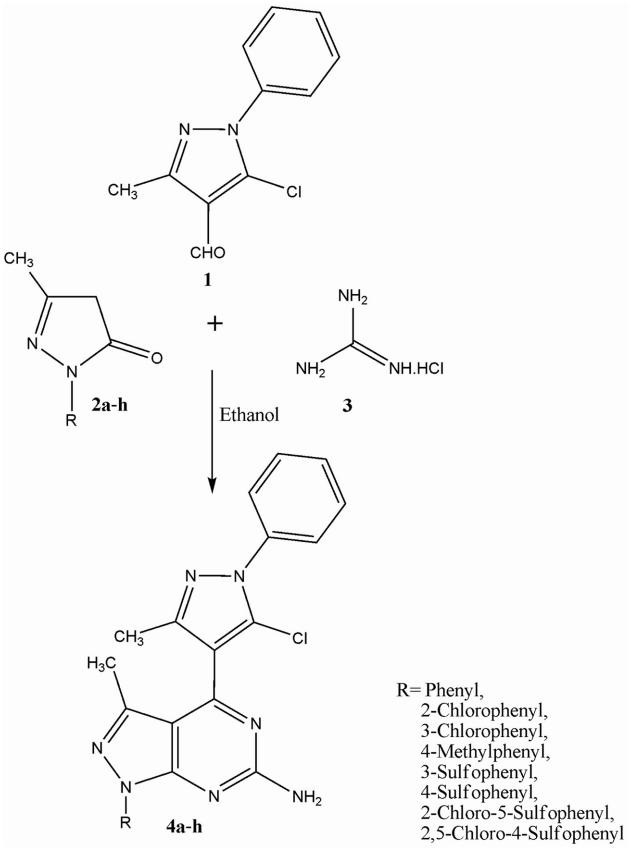
Chemistry

To date, several methods have been developed to synthesize pyrazolo[3,4-*d*]pyrimidine, Yoneda *et al*. [27] used the cycloaddition of azahexatrienes obtained from reaction of an arylaldehyde and a 6-uracil hydrazone. One disadvantage of this approach is the concomitant arylation of the pyrazole moiety. Earlier, this class of compounds was synthesized by fusion of 6-uracil hydrazones at 300°C [28]. Another synthesis reported by Maki *et al.* [28] required the cycloaddition of an arylhydrazone to 6-chloro-5-nitrouracil, which involved several steps. Further, Kanazawa *et al*. [29] synthesized pyrazolo[3,4-*d*]pyrimidines by the reaction of 6-benzylidenehydrazonouracils with N-bromosuccinimide (NBS) in acetic acid under reflux conditions, which yielded triazino- and pyridazino-uracils in addition to the pyrazolo[3,4-*d*]pyrimidines. However, literature survey revealed only a few reports on the synthesis of the parent pyrazolo[3,4-*d*]pyrimidine moiety, which usually requires drastic conditions, long reaction times, and complex pathways.

Therefore, we developed an interest in pyrazolopyrimidines and herein present a simple one-pot synthetic strategy toward their synthesis with complete regiocontrol using readily available starting materials and reagents. Thus, the synthesis of 6-amino-4-(5-chloro-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)-1-(aryl)-4,5-dihydro-3-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidine derivatives **4a**–**h** involved a one-pot reaction between 5-chloro-3-methyl-1-phenyl-1*H*-pyrazole-4-carbaldehyde **1** [30], 3-methyl-1-(4-aryl)-5-pyrazolone **2a**–**h** [31], and guanidine hydrochloride **3** as described in the general procedure; (see Section 4 Experimental), leading to the desired compounds **4a**–**h** in 68–100% yields (Scheme 1). All the compounds were identified by spectral data. In general, IR spectra showed confirmatory absence of C=O peak around 1650 cm^{-1}. The ^{1}H-NMR spectrum showed the amine protons as singlet around δ = 8.912–8.996 ppm. Further, the ^{13}C-NMR spectrum showed the C-NH_2 signals at δ = 163–164.9 ppm. This confirmed the formation of the desired compounds.

Biological screening

The antimycobacterial activities of compounds **4a**–**h** were assessed against *M. tuberculosis* ATTC 2729415 using the microplate Alamar Blue assay (MABA) [32] (Table 1). This method is nontoxic, uses a thermally-stable reagent, and shows good correlation with proportional and BACTEC radiometric methods [33, 34]. Briefly, 200 μL of sterile deionized water was added to all outer-perimeter wells of sterile 96-well plates (falcon, 3072: Becton Dickinson, Lincoln Park, NJ, USA) to minimize evaporation of the medium in the test wells during incubation. The 96-well plates received 100 μL of the Middlebrook 7H9 broth (Difco laboratories, Detroit, MI, USA) and a serial dilution of the compounds **4a**–**h** were made directly on the plate. The final drug concentrations tested were 0.01 to 20.0 μL/



Scheme 1. Synthesis of 6-amino-4-(5-chloro-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)-1-(aryl)-4,5-dihydro-3-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidine derivatives **4a**–**h**.

Table 1. Antimycobacterial activities and log P measurements of 6-amino-4-(5-chloro-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)-1-(aryl)-4,5-dihydro-3-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidines **4a**–**h**.

Compound	R	MIC (μg/mL)	Log P[a)]
4a	Phenyl	3.25	4.91
4b	2-chlorophenyl	1.20	5.47
4c	3-chlorophenyl	1.20	5.47
4d	4-methylphenyl	1.20	5.40
4e	3-sulfophenyl	3.25	4.07
4f	4-sulfophenyl	3.25	4.07
4g	2-chloro-4-sulfophenyl	1.20	4.63
4h	2,5-dichloro-4-sulfophenyl	3.25	5.15
RIP		1.0	2.78
INH		0.2	−0.96

[a)] Calculated by http://www.molinspiration.com/

mL. Plates were covered and sealed with parafilm and incubated at 37°C for five days. After this time, 25 μL of a freshly prepared 1 : 1 mixture of Alamar Blue (Accumed International, Westlake, OH, USA) reagent and 10% Tween 80 was added to the plate and incubated for 24 h. A blue color in the well was interpreted as no bacterial growth, and a pink color was scored as growth. The MIC (minimal inhibition concentration) was defined as the lowest drug concentration, which prevented a color change from blue to pink.

It was clearly evident from the antitubercular activity data that all the compounds displayed significant activity. But, compounds **4b**, **4c**, **4d**, and **4g** were found to be particularly active against *Mycobacterium tuberculosis* H37 Rv strain. These results led us to the SAR conclusion that the presence of 2-chloro-, 3-chloro-, 4-methyl- and 2-chloro-5-sulfonyl substituents markedly enhances the biological activity.

Conclusions

In summary, 6-amino-4-(5-chloro-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)-1-(aryl)-4,5-dihydro-3-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidines have been investigated as antimycobacterial agents. In the case of all the evaluated compounds, the best result was the compound **4b**, **4c**, **4d**, and **4g** (1.2 μg/mL) when compared with first line drugs as isoniazid (INH) and rifampin (RIP). It suggests that this class of compounds may be selectively targeted to *M. tuberculosis* growth and could be a good starting point to find new lead compounds.

The authors have declared no conflict of interest.

Experimental

Melting points were determined on a Büchi apparatus (Büchi Labortechnik, Flawil, Switzerland) and are uncorrected. Infrared spectra were recorded on a Thermo Nicolet Nexus 670 spectrometer (Nicolet, Madison, WI, USA) as potassium bromide pellets and frequencies are expressed in cm^{-1}. Mass spectra (CG/MS) were recorded on a Agilent Tecnologies 6890/5972A mass spectrometer (Agilent, Palo Alto, CA, USA). ^{1}H- and ^{13}C-NMR spectra were taken on Varian Mercury VX-BB 300 at 300 and 75 MHz, respectively (Varian, Palo Alto, CA, USA). TMS was used as an inner standard. Microanalyses were recorded on Fisons Instruments EA1110CE CHN analyst (Fisons Instruments, Mainz, Germany). For TLC, plates coated with silica gel were run in chloroform/methanol mixture and spots were developed in ultraviolet.

General procedure for 6-amino-4-(5-chloro-3-methyl-1-phenyl-1*H*-pyrazol-4-yl)-1-(aryl)-4,5-dihydro-3-methyl-1*H*-pyrazolo[3,4-d]pyrimidines *4a–h*

An equimolar mixture of 5-chloro-3-methyl-1-phenyl-1*H*-pyrazole-4-carbaldehyde **1**, 3-methyl-1-(4-aryl)-5-pyrazolone **2a**–**h** and guanidine hydrochloride was heated under reflux condition for 5 h using ethanol as a solvent. The reaction mixture was kept at room temperature for 3 h. The product was filtered, dried, and recrystallized from ethanol.

6-Amino-4-(5-chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)-4,5-dihydro-3-methyl-1-phenyl-1H-pyrazolo[3,4-d]pyrimidine ***4a***

Mp. 223–225°C; Yield 71%; IR (cm^{-1}): 3421, 1610, 1599, 1546, 1294, 1066, 756. ^{1}H-NMR ([d_6] DMSO): δ (ppm) = 2.518–2.60 (2 × s, 6H, 2 × CH_3), 7.49–8.18 (m, 10H, H_{arom}), 8.97 (s, 2H, NH_2); ^{13}C-NMR ($CDCl_3$): δ (ppm) = 11.2, 16.2, 109.2, 116.2, 121.4, 126.8, 129.2, 129.9, 140.2, 144.9, 151.2, 163.9, 166.1; MS (m/z) 415. Anal. calcd. for $C_{22}H_{18}ClN_7$ (%): C, 63.54; H, 4.36; Cl, 8.52; N, 23.58. Found (%): C, 63.42; H, 4.25; Cl, 8.41; N, 23.54.

6-Amino-4-(5-chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)-1-(2-chlorophenyl)-4,5-dihydro-3-methyl-1H-pyrazolo[3,4-d]pyrimidine ***4b***

Mp. 239–241°C; Yield 66%; IR (cm^{-1}): 3415, 1616, 1603, 1545, 1289, 1069, 750; ^{1}H-NMR ([d_6] DMSO): δ (ppm) = 2.51–2.59 (2 × s, 6H, 2 × CH_3), 7.47–8.10 (m, 9H, H_{arom}), 8.95 (s, 2H, NH_2); ^{13}C-NMR ($CDCl_3$): δ (ppm) = 11.4, 16.4, 109.6, 115.8, 120.8, 122.1, 126.6, 127.4, 127.9, 128.9, 129.7, 129.9, 130.1, 133.9, 140.2, 143.6, 144.9, 151.2, 164.2, 166.9; MS (m/z) 450. Anal. calcd. for $C_{22}H_{17}Cl_2N_7$ (%): C, 58.68; H, 3.81; Cl, 15.75; N, 21.77. Found (%): C, 58.55; H, 3.70; Cl, 15.61; N, 21.65.

6-Amino-4-(5-chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)-1-(3-chlorophenyl)-4,5-dihydro-3-methyl-1H-pyrazolo[3,4-d]pyrimidine ***4c***

Mp. 207–209°C; Yield 68%; IR (cm^{-1}): 3419, 1614, 1596, 1549, 1299, 1061, 753; ^{1}H-NMR ([d_6] DMSO): δ (ppm) = 2.51–2.57 (2 × s, 6H, 2 × CH_3), 7.46–8.10 (m, 9H, H_{arom}), 8.94 (s, 2H, NH_2); ^{13}C-NMR ($CDCl_3$): δ (ppm) = 11.4, 16.4, 109.2, 115.8, 116.4, 119.4, 120.8, 126.7, 126.8, 129.2, 129.6, 131.2, 135.2, 139.9, 141.7, 144.8, 11.2, 164.4, 166.2; MS (m/z) 450. Anal. calcd. for $C_{22}H_{17}Cl_2N_7$ (%): C, 58.68; H, 3.81; Cl, 15.75; N, 21.77. Found (%): C, 58.56; H, 3.68; Cl, 15.63; N, 21.64.

6-Amino-4-(5-chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)-1-(4-methylphenyl)-4,5-dihydro-3-methyl-1H-pyrazolo[3,4-d]pyrimidine ***4d***

Mp. 219–221°C; Yield 65%; IR (cm^{-1}): 3422, 1616, 1589, 1555, 1368, 1292, 1063, 1112, 750; ^{1}H-NMR ([d_6] DMSO): δ (ppm) = 2.53–2.99 (3 × s, 6H, 3 × CH_3), 7.46–8.11 (m, 10H, H_{arom}), 8.91 (s, 2H, NH_2); ^{13}C-NMR ($CDCl_3$): δ (ppm) = 11.4, 15.9, 24.8, 109.2, 116.4, 120.6, 120.8, 126.6, 129.2, 129.6, 129.9, 136.4, 137.2, 140.2, 145.1, 150.9, 164.1; MS (m/z) 429. Anal. calcd. for $C_{23}H_{20}ClN_7$ (%): C, 64.26; H, 4.69; Cl, 8.25; N, 22.81. Found (%): C, 64.22; H, 4.66; Cl, 8.23; N, 22.69.

6-Amino-4-(5-chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)-1-(3-sulfophenyl)-4,5-dihydro-3-methyl-1H-pyrazolo[3,4-d]pyrimidine ***4e***

Mp. 201–203°C; Yield 61%; IR (cm^{-1}): 3426, 1619, 1602, 1552, 1360, 1296, 1108, 1069, 761; ^{1}H-NMR ([d_6] DMSO): δ (ppm) = 2.53–2.64 (2 × s, 6H, 2 × CH_3), 7.49–8.23 (m, 10H, H_{arom}), 8.99 (s, 2H, NH_2), 12.38 (s, 1H, OH); ^{13}C-NMR ($CDCl_3$): δ (ppm) = 11.3, 16.2, 109.2, 116.1, 117.2, 120.8, 124.8, 125.8, 126.9, 129.2, 129.9, 131.2, 140.2, 141.8, 144.9, 150.9, 151.9, 163.5, 166.2; MS (m/z) 495. Anal. calcd. for $C_{22}H_{18}ClN_7O_3S$ (%): C, 53.28; H, 3.66; Cl, 7.15; N, 19.77; O, 9.68; S, 6.47. Found (%): C, 53.17; H, 3.54; Cl, 7.02; N, 19.66; O, 9.56; S, 6.33.

6-Amino-4-(5-chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)-1-(4-sulfophenyl)-4,5-dihydro-3-methyl-1H-pyrazolo[3,4-d]pyrimidine ***4f***

Mp. 229–231°C; Yield 63%; IR (cm^{-1}): 3425, 1612, 1602, 1541, 1368, 1299, 1113, 1061, 752; ^{1}H-NMR ([d_6] DMSO): δ (ppm) = 2.53–2.65 (2 × s, 6H, 2 × CH_3), 5.12 (s, 1H, CH), 7.50–8.25 (m, 9H, H_{arom}), 8.99 (s, 2H, NH_2), 12.39 (s, 1H, OH); ^{13}C-NMR ($CDCl_3$): δ (ppm) = 11.4, 16.3, 109.4, 116, 121.2, 121.9, 126.6, 129.2, 129.8, 130.2, 140.2, 143.9, 144.9, 147.8, 151.2, 163.6, 165.9; MS (m/z) 495. Anal. calcd. for $C_{22}H_{18}ClN_7O_3S$ (%): C, 53.28; H, 3.66; Cl, 7.15; N, 19.77; O, 9.68; S, 6.47. Found (%): C, 53.16; H, 3.55; Cl, 7.04; N, 19.63; O, 9.54; S, 6.37.

6-Amino-4-(5-chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)-1-(2-chloro-5-sulfophenyl)-4,5-dihydro-3-methyl-1H-pyrazolo[3,4-d]pyrimidine ***4g***

Mp. 215–217°C; Yield 58%; IR (cm^{-1}): 3419, 1623, 1591, 1562, 1362, 1290, 1104, 1060, 762; ^{1}H-NMR ([d_6] DMSO): δ (ppm) = 2.56–2.89 (2 × s, 6H, 2 × CH_3), 7.51–8.32 (m, 8H, H_{arom}), 8.99 (s, 2H, NH_2), 12.39 (s, 1H, OH); ^{13}C-NMR ($CDCl_3$): δ (ppm) = 11.4, 16.6, 108.8, 116.4, 118.4, 120.6, 126.8, 127.4, 129.2, 129.8, 131.2, 139.2, 140.2, 144.4, 144.9, 149.6, 150.8, 164.8, 166.4; MS (m/z) 530. Anal. calcd. for $C_{22}H_{17}Cl_2N_7O_3S$ (%): C, 49.82; H, 3.23; Cl, 13.37; N, 18.49; O, 9.05; S, 6.05. Found (%): C, 49.71; H, 3.11; Cl, 13.25; N, 18.35; O, 8.94; S, 5.93.

6-Amino-4-(5-chloro-3-methyl-1-phenyl-1H-pyrazol-4-yl)-1-(2,5-dichloro-4-sulfophenyl)-4,5-dihydro-3-methyl-1H-pyrazolo[3,4-d]pyrimidine ***4h***

Mp. 246–248°C; Yield 56%; IR (cm^{-1}): 3409, 1610, 1595, 1556, 1367, 1289, 1113, 1058, 759; ^{1}H-NMR ([d_6] DMSO): δ (ppm) = 2.57–2.99 (2 × s, 6H, 2 × CH_3), 7.59–8.44 (m, 7H, H_{arom}), 8.99 (s, 2H, NH_2), 12.38 (s, 1H, OH); ^{13}C-NMR ($CDCl_3$): δ (ppm) = 11.5, 16.9, 109.1, 116.4, 120.9, 126.4, 126.8, 129.2, 130.1, 136.9, 140.2, 143.1, 144.2, 150.6, 150.9, 164.9, 166.2; MS (m/z) 564. Anal. calcd. for $C_{22}H_{16}Cl_6N_7O_3S$ (%): C, 49.82; H, 3.23; Cl, 13.37; N, 18.49; O, 9.05; S, 6.05. Found (%): C, 49.70; H, 3.10; Cl, 13.25; N, 18.34; O, 8.94; S, 5.91.

References

[1] R. Filler, *Chem. Technol.* **1974**, *4*, 752–757.

[2] M. M. Ghorab, Z. H. Ismail, S. M. Abdel-Gawad, A. Abdel Aziem, *Heteroatom Chemistry* **2004**, *15*, 57–62.

[3] L. P. Davies, D. J. Brown, S. C. Chow, G. A. R. Johnston, *Neurosci. Lett.* **2004**, *41*, 189–193.

[4] L. P. Davies, S. C. Chow, J. H. Skerritt, D. J. Brown, G. A. R. Johnston, *Life Sci.* **1984**, *34*, 2117–212.

[5] J. H. Burchenal, M. L. Murphy, R. R. Ellison, M. P. Sykes, *et al., Blood* **1953**, *8*, 965–999.

[6] G. B. Elion, *J. Med. Virol.* **1993**, *41*, 2–6.

[7] R. W. Rundles, *Arch. Intern. Med.* **1985**, *145*, 1492–1503.

[8] G. Chiosis, M. N. Timaul, B. Lucas, P. N. Munster, *et al.*, *Chem. Biol.* **2001**, *8*, 289–299.

[9] C. M. Crews, R. Mohan, *Curr. Opin. Chem. Biol.* **2000**, *4*, 47 – 53.

[10] M. Knockaert, N. Gray, E. Damiens, Y.-T. Chang, *et al.*, *Chem. Biol.* **2000**, *7*, 411 – 422.

[11] S. A. Laufer, D. M. Domeyer, T. R. Scior, W. Albrecht, D. R. Hauser, *J. Med. Chem.* **2005**, *48*, 710 – 722.

[12] J. I. Armstrong, A. R. Portley, Y.-T. Chang, D. M. Nierengarten, *et al.*, *Angew. Chem. Int. Ed. Engl.* **2000**, *39*, 1303 – 1306.

[13] D. E. Verdugo, M. T. Cancilla, X. Ge, N. S. Gray, *et al.*, *J. Med. Chem.* **2001**, *44*, 2683 – 2686.

[14] X. Wu, S. Ding, Q. Ding, N. S. Gray, P. G. Schultz, *J. Am. Chem. Soc.* **2002**, *124*, 14521 – 14520.

[15] L. L. Gundersen, J. Nissen-Meyer, B. Spilsberg, *J. Med. Chem.* **2002**, *45*, 1383 – 1386.

[16] A. Scozzafava, A. Mastrolorenzo, C. T. Supuran, *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1675 – 1678.

[17] V. Klimešová, J. Kocí, K. Waisser, J. Kaustová, *Farmaco* **2002**, *57*, 259 – 265.

[18] A. K. Pathak, V. Pathak, L. E. Seitz, W. J. Suling, R. C. Reynolds, *J. Med. Chem.* **2004**, *47*, 273 – 276.

[19] O. Moukha-Chafiq, M. L. Taha, H. B. Lazrek, J.-L. Barascut, J.-L. Imbach, *C. R. Acad. Sci. IIc: Chimie* **2000**, *3*, 639 – 641.

[20] J. H. Hanke, J. P. Gardner, R. L. Dow, P. S. Changelian, *et al.*, *J. Biol. Chem.* **1996**, *271*, 695 – 701.

[21] P. Traxler, P. Furet, *Pharmacol. Ther.* **1999**, *82*, 195 – 206.

[22] M. R. Bergman, B. J. Holycross, *J. Pharmacol. Exp. Ther.* **1996**, *279*, 247 – 254.

[23] A. Ali, G. E. Taylor, K. Ellsworth, G. Harris, *et al.*, *J. Med. Chem.* **2003**, *46*, 1824 – 1830.

[24] J. O. Moukha-Chafiq, M. L. Taha, H. B. Lazrek, C. Pannecouque, *et al.*, *Nucleosides Nucleotides Nucleic Acids* **2001**, *20*, 1797 – 1810.

[25] O. Moukha-Chafiq, M. L. Taha, H. B. Lazrek, J. J. Vasseur, C. E. De, *Nucleosides Nucleotides Nucleic Acids* **2002**, *21*, 165 – 176.

[26] M. L. Taha, O. Moukha-Chafiq, H. B. Lazrek, J. J. Vasseur, J. L. Imbach, *Nucleosides Nucleotides Nucleic Acids* **2001**, *20*, 955 – 958.

[27] F. Yoneda, M. Higuchi, T. Nagamatsu, *J. Am. Chem. Soc.* **1974**, *96*, 5607 – 5608.

[28] Y. Maki, K. Izuka, M. Suzuki, *J. Chem. Soc. Chem. Commun.* **1971**, *1*, 1442 – 1443.

[29] H. Kanazawa, S. Nishigaki, K. Singa, *J. Heterocycl. Chem.* **1984**, *21*, 969 – 974.

[30] H. Xiao, G. Ouyang, X. Sun, X. Yao, *et al.*, *Hecheng Huaxue* **2005**, *13,* 600 – 602.

[31] A. Kimata, H. Fukuuchi, S. Ohta, T. Suzuki, N. Miyata, *J. Med. Chem.* **2007**, *50*, 5053 – 5056.

[32] S. G. Franzblau, R. S. Witzig, J. C. McLaughlin, P. Torres, *et al., J. Clin. Microbiol.* **1998**, *36*, 362 – 366.

[33] J. D. Vanitha, C. N. Paramasivan, *Diagn. Microbiol. Infect. Dis.* **2004**, *49*, 179 – 182.

[34] R. S. Reis, I. Neves, S. L. S. Lourenco, L. S. Fonseca, M. C. S. Lourenço, *J. Clin. Microbiol.* **2004**, *42*, 2247 – 2248.

