Bioorganic & Medicinal Chemistry Letters 24 (2014) 1493–1495

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

4,5-Dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidine containing phenothiazines as antitubercular agents

Arif B. Siddiqui [a,*], Amit R. Trivedi [b], Vipul B. Kataria [c], Viresh H. Shah [c]

[a] *Amneal Pharmaceuticals India Pvt Ltd, 882/1-871, Village Rajoda, Tal.: Bavla Dist.: Ahmedabad 382220, Gujarat, India*
[b] *Division of Medicinal Chemistry, Department of Chemistry (DST-FIST Sponsored), Mahatma Gandhi Campus, Maharaja Krishnakumarsinhji Bhavnagar University, Bhavnagar 364002, Gujarat, India*
[c] *Department of Chemistry, Saurashtra University, Kalawad Road, Rajkot 360005, Gujarat, India*

ARTICLE INFO

Article history:
Received 28 August 2013
Revised 1 February 2014
Accepted 4 February 2014
Available online 14 February 2014

Keywords:
Dihydropyrazolo[3,4-*d*]pyrimidine
Phenothiazines
Biginelli multicomponent reaction
Cytotoxicity
Antitubercular activity

ABSTRACT

A series of novel dihydropyrazolo[3,4-*d*]pyrimidine derivatives bearing a phenothiazine nucleus were synthesized in excellent yields via a modified Biginelli multicomponent reaction. The newly synthesized compounds were characterized by IR, ^{1}H NMR, ^{13}C NMR, Mass spectra and elemental analysis followed by antimycobacterial screening. Among all the screened compounds, compound **4g** showed most pronounced activity against *Mycobacterium tuberculosis* (Mtb) with minimum inhibitory concentration (MIC) of 0.02 μg/mL, making it more potent than first line antitubercular drug isoniazid.

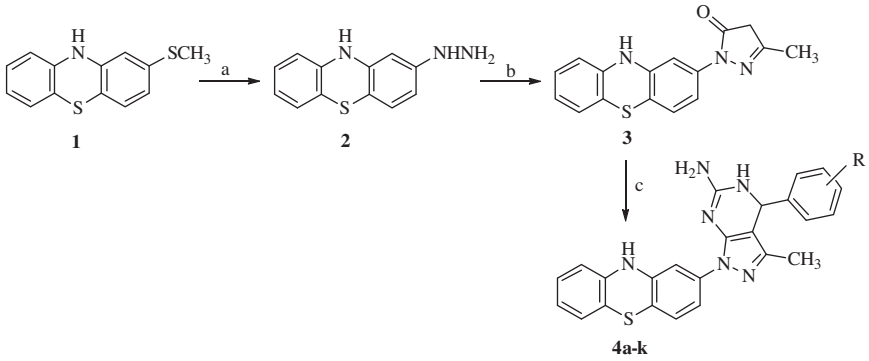
Tuberculosis (TB) is a highly infectious airborne disease caused by the pathogenic bacterium *Mycobacterium tuberculosis* (Mtb).[1] According to the latest World Health Organization (WHO) report, an estimated 8.6 million people developed TB and 1.3 million died from the disease (including 320,000 deaths among HIV-positive people) in 2012.[2] The majority of cases worldwide in 2012 were in the South-East Asia (29%), African (27%) and western Pacific (19%) regions. India and China alone accounted for 26% and 12% of total cases, respectively.[2] The standard antitubercular treatment regimen, termed DOTS (Directly Observed Therapy, Short-course),[3] is based on the co-administration of age-old drugs like isoniazid (INH), rifampin (RMP), ethambutol (EMB), and pyrazinamide (PZA) for the first two months, followed by a prolonged treatment with INH and RMP for additional 4–7 months with no guarantee of complete sterilization from the infection.[4,5] Furthermore, emergence of new virulent forms of TB such as multi drug resistant (MDR-TB) and extremely drug resistant (XDR-TB), and its synergy with human immunodeficiency virus (HIV) has fuelled its epidemic nature.[6–9] These reasons make a compelling case for an urgent need for new and effective antitubercular agents with improved properties such as enhanced activity against MDR strains, reduced toxicity, rapid mycobactericidal mechanism of action and the ability to penetrate host cells and exert antimycobacterial effects in the intracellular environment.[10–12]

Phenothiazines are important classes of compounds which have increasingly attracted attention, owing to their remarkable biological and pharmacological properties, such as antibacterial, antifungal, anticancer, antiviral, anti-inflammatory, antimalarial, antifilarial, trypanocidal, anticonvulsant, analgesic, immunosuppressive and multidrug resistance reversal.[13] These activities are the results of the actions exerted by phenothiazines on biological systems via the interaction of the pharmacophoric substituent (in some cases of strict length), via the interaction multicyclic ring system (π–π interaction, intercalation in DNA) and via the lipophilic character permitting the penetration through the biological membranes to reach its site of action.[13] Further, Phenothiazines have been shown to exhibit in vitro and in vivo activity against Mtb and multidrug-resistant Mtb. Some of the phenothiazine derived antipsychotic agents such as chlorpromazine, trifluoperazine (TPZ) and thioridazine are found to be effective inhibitors of Mtb.[14–20] Phenothiazines are predicted to target the genetically validated respiratory chain component type II NADH:quinone oxidoreductase (Ndh).[21]

On the other hand, Pyrazolopyrimidines and related fused heterocyclic ring systems which structurally resemble purines are of great interest as they are reported to encompass an array of pharmacological activities such as antiviral,[22] anticoccidials,[23] antimicrobial,[24] antitumor,[25] CNS agents,[26] tuberculostatic,[27–29]

* Corresponding author. Tel.: +91 9723558040.
E-mail address: drarifsiddiqui2013@gmail.com (A.B. Siddiqui).

http://dx.doi.org/10.1016/j.bmcl.2014.02.012
0960-894X/



Scheme 1. Synthetic protocol of title compounds **4a–k**. Reagents and conditions: (a) NH_2NH_2, reflux; (b) ethyl acetoacetate, sodium ethoxide, reflux; (c) guanidine hydrochloride, aldehyde (R-CHO), P_2O_5, EtOH, reflux.

antileishmanial,[30] antiinflammatory[31] and cardiovascular activities.[32] Prompted by above mentioned observations and in continuation of our previous work,[33,34] we report herein the synthesis of some novel structural hybrids by combining phenothiazine and dihydropyrazolo[3,4-*d*]pyrimidine pharmacophoric units in single molecular platform in order to investigate their in vitro antitubercular activity.

The reaction sequences employed for synthesis of 3-methyl-1-(10*H*-phenothiazin-2-yl)-4-phenyl-6-amino-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidines (**4a–k**) are outlined in Scheme 1. Initially, synthesis of intermediates **2** and **3** were accomplished according to our previously published methods.[33,34] Reaction of 3-methyl-1-(10*H*-phenothiazin-8-yl)-1*H*-pyrazol-5-(4*H*)-one (**3**), an appropriate aldehyde, guanidine hydrochloride and phosphorus pentoxide under reflux conditions afforded the desired compounds **4a–k**. The yields of the products were obtained in the range of 75–90%. Designed series of molecules **4a–k** were characterized by IR, ^{1}H NMR, ^{13}C NMR and Mass spectrometry techniques before evaluating for in vitro antitubercular screening. In the ^{1}H NMR spectra of **4a–k** a sharp peak representing the methine proton of the pyrimidine was observed in the range of δ = 4.90–5.05 ppm confirming the formation of the dihydropyrazolo[3,4-*d*]pyrimidine nucleus.

All the newly synthesized compounds **4a–k** were initially screened for their in vitro antimycobacterial activity at 6.25 μg/mL against *Mycobacterium tuberculosis* H_{37}Rv strain by the Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF) in BACTEC 12B medium using the microplate alamar blue assay.[35] The results of the antitubercular studies are presented in Table 1. Compounds exhibiting ⩾90% inhibition in the initial screening were tested at and below 6.25 μg/mL using 2-fold dilution to determine the actual MIC. In preliminary screening, five compounds (**4b**, **4e**, **4g**, **4h**, **4j**) inhibited Mtb with 90–100%. In the secondary level, one compound (**4g**) inhibited Mtb with MIC of <1 μg/mL and three compounds (**4b**, **4e**, **4j**) with MIC of <3 μg/mL when compared to isoniazid (MIC: 0.03 μg/mL). Among all the compounds, 4-(4-chlorophenyl)-3-methyl-1-(10*H*-phenothiazin-2-yl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-amine (**4g**) was found to be the most active compound in vitro with MIC of 0.02 μg/mL against Mtb. The activity was considerably affected by substitutions at the phenyl ring of the dihydropyrazolo[3,4-*d*]pyrimidine nucleus. It was observed that in a group of compounds having electron-withdrawing halogen and nitro group on the *ortho* and *para* positions of the phenyl ring were influencing the antitubercular activity significantly (**4b**, **4e**, **4g**, **4h**, **4j**), other substituents on phenyl ring decreased the antitubercular activity, alkoxy group remarkably reduced the potency of the test compound (**4k**).

Having identified a good number of active antimycobacterial compounds, the next step was to examine the toxicity of the drug candidates. Compounds exhibiting relatively low MICs were tested for cytotoxicity (IC_{50}) in VERO cells and a selectivity index (SI), defined as IC_{50}: MIC was calculated. The compounds **4j** and **4b** were somewhat more toxic than **4e**, **4g** and **4h**. Generally

Table 1
In vitro antitubercular screening data of compounds **4a–k** against *M. tuberculosis* H_{37}Rv

Sr. No.	R	% Inhibition	MIC[a] (μg/mL)	IC_{50}[b] VERO cells (μg/mL)	SI[c] (SI = IC_{50}/MIC)
4a	H	78	n.d.	n.d.	n.d.
4b	2-OH	92	2.23	7.8	3.5
4c	3-OH	57	n.d.	n.d.	n.d.
4d	4-OH	67	n.d.	n.d.	n.d.
4e	2-Cl	95	1.73	>10	5.8
4f	3-Cl	56	n.d.	n.d.	n.d.
4g	4-Cl	100	0.02	>10	>500
4h	2-NO_2	94	1.73	>10	5.8
4i	3-NO_2	73	n.d.	n.d.	n.d.
4j	4-NO_2	95	1.73	9.1	5.2
4k	4-OCH_3	42	n.d.	n.d.	n.d.
Isoniazid	—	—	0.03[d]	>1000	>40,000[e]

[a] Minimum inhibitory concentration against H_{37}Rv strain of *M. tuberculosis* (μg/mL).
[b] Measurement of cytotoxicity in VERO cells: 50% inhibitory concentrations (μg/mL).
[c] Selectivity index (in vitro): IC_{50} in VERO cells/MIC against *M. tuberculosis*.
[d] Data from TAACF.[37]
[e] Data from literature.[38] The activity criterion for this assay is an SI >10.

compound with an MIC $\leqslant$6.25 μg/mL and SI $\geqslant$10 are interesting compounds, and an MIC $\leqslant$1 μg/mL in a novel compound class may be considered an excellent lead,[36] which makes 3-methyl-4-(4-nitrophenyl)-1-(10*H*-phenothiazin-2-yl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-amine (**4g**) very promising antimycobacterial compound.

In conclusion, we have conveniently synthesized a series of novel dihydropyrazolo[3,4-*d*]pyrimidine derivatives bearing a phenothiazine nucleus and evaluated for their in vitro antitubercular activity with anticipation of generating new structural leads. The results indicate that the preparative pathway elaborated by us to the novel dihydropyrazolo[3,4-*d*]pyrimidine having phenothiazine nucleus may open a relatively facile route to a new class of antitubercular compounds.

Acknowledgments

The authors thank the Professor and Head, Department of Chemistry, Saurashtra University, Rajkot for providing research facilities. Authors are thankful to CDRI Lucknow for spectral and analytical data. A.R.T. is highly grateful to UGC for Dr. D.S. Kothari Post-Doctoral Fellowship.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.02.012.

References and notes

1. Zumla, A.; George, A.; Sharma, V.; Herbert, N. *Lancet* **2013**, *382*, 1765.
2. World Health Organization *Global Tuberculosis Report*; WHO, 2013.
3. http://www.who.int/tb/dots/whatisdots/en/index.html (accessed January 2014).
4. Blumberg, H. M.; Burman, W. J.; Chaisson, R. E.; Daley, C. L.; Etkind, S. C.; Friedman, L. N.; Fujiwara, P.; Grzemska, M.; Hopewell, P. C.; Iseman, M. D.; Jasmer, R. M.; Koppaka, V.; Menzies, R. I.; O'Brien, R. J.; Reves, R. R.; Reichman, L. B.; Simone, P. M.; Starke, J. R.; Vernon, A. A. *Am. J. Respir. Crit. Care Med.* **2003**, *167*, 603.
5. Ormerod, L. P. *Thorax* **1999**, *54*, S42.
6. Abubakar, I.; Zignol, M.; Falzon, D.; Raviglione, M.; Ditiu, L.; Masham, S.; Adetifa, I.; Ford, N.; Cox, H.; Lawn, S. D.; Marais, B. J.; McHugh, T. D.; Mwaba, P.; Bates, M.; Lipman, M.; Zijenah, L.; Logan, S.; McNerney, R.; Zumla, A.; Sarda, K.; Nahid, P.; Hoelscher, M.; Pletschette, M.; Memish, Z. A.; Kim, P.; Hafner, R.; Cole, S.; Migliori, G. B.; Maeurer, M.; Schito, M.; Zumla, A. *Lancet Infect. Dis.* **2013**, *13*, 529.
7. Lawn, S. D.; Mwaba, P.; Bates, M.; Piatek, A.; Alexander, H.; Marais, B. J.; Cuevas, L. E.; McHugh, T. D.; Zijenah, L.; Kapata, N.; Abubakar, I.; McNerney, R.; Hoelscher, M.; Memish, Z. A.; Migliori, G. B.; Kim, P.; Maeurer, M.; Schito, M.; Zumla, A. *Lancet Infect. Dis.* **2013**, *13*, 349.
8. Prabowo, S.; Gröschel, M.; Schmidt, E. L.; Skrahina, A.; Mihaescu, T.; Hastürk, S.; Mitrofanov, R.; Pimkina, E.; Visontai, I.; Jong, B.; Stanford, J.; Cardona, P.-J.; Kaufmann, S. E.; Werf, T. *Med. Microbiol. Immunol.* **2013**, *202*, 95.
9. Falzon, D.; Jaramillo, E.; Schunemann, H. J.; Arentz, M.; Bauer, M.; Bayona, J.; Blanc, L.; Caminero, J. A.; Daley, C. L.; Duncombe, C.; Fitzpatrick, C.; Gebhard, A.; Getahun, H.; Henkens, M.; Holtz, T. H.; Keravec, J.; Keshavjee, S.; Khan, A. J.; Kulier, R.; Leimane, V.; Lienhardt, C.; Lu, C.; Mariandyshev, A.; Migliori, G. B.; Mirzayev, F.; Mitnick, C. D.; Nunn, P.; Nwagboniwe, G.; Oxlade, O.; Palmero, D.; Pavlinac, P.; Quelapio, M. I.; Raviglione, M. C.; Rich, M. L.; Royce, S.; Rusch-Gerdes, S.; Salakaia, A.; Sarin, R.; Sculier, D.; Varaine, F.; Vitoria, M.; Walson, J. L.; Wares, F.; Weyer, K.; White, R. A.; Zignol, M. *Eur. Respir. J.* **2011**, *38*, 516.
10. Palomino, J. C.; Martin, A. *J. Antimicrob. Chemother.* **2013**, *68*, 275.
11. Nguyen, L.; Jacobs, M. R. *Exp. Rev. Anti Infect. Ther.* **2012**, *10*, 959.
12. Nzila, A.; Ma, Z.; Chibale, K. *Future Med. Chem.* **2011**, *3*, 1413.
13. Pluta, K.; Morak-Młodawska, B.; Jeleń, M. *Eur. J. Med. Chem.* **2011**, *46*, 3179.
14. Wainwright, M.; Amaral, L.; Kristiansen, J. E. *J. Open Pharmacol.* **2012**, *2*.
15. Pluta, K.; Mlodawska, M. B.; Jelen, M. *Eur. J. Med. Chem.* **2011**, *46*, 3179.
16. Shwetha, S.; Pandeya, S. N.; Anupam, V.; Deepika, Y. *Int. J. Res. Ayurveda Pharm.* **2011**, *2*, 1130.
17. Agarwal, P.; Dubey, S.; Mirza, Z.; Pathak, A. K. *Int. J. Pharm. Biomed. Res.* **2013**, *4*, 114.
18. Madrid, P. B.; Polgar, W. E.; Tolla, L.; Tanga, M. J. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3014.
19. Amaral, L.; Kristiansen, J. E. *Int. J. Antimicrob. Agents* **2000**, *14*, 173.
20. Amaral, L.; Kristiansen, J. E.; Lorian, V. *J. Antimicrob. Chemother.* **1992**, *30*, 556.
21. Warman, A. J.; Rito, T. S.; Fisher, N. E.; Moss, D. M.; Berry, N. G.; O'Neill, P. M.; Ward, S. A.; Biagini, G. A. *J. Antimicrob. Chemother.* **2013**, *68*, 869.
22. El-Bendary, E.; Badria, F. *Arch. Pharm.* **2000**, *333*, 99.
23. Rideout, J. L.; Krenitsky, T. A.; Koszalka, G. W.; Cohn, N. K.; Chao, E. Y.; Elion, G. B.; Latter, V. S.; Williams, R. B. *J. Med. Chem.* **1982**, *25*, 1040.
24. Ali, A.; Taylor, G. E.; Ellsworth, K.; Harris, G.; Painter, R.; Lynn, L.; Young, K. *J. Med. Chem.* **2003**, *46*, 1824.
25. Angelucci, A.; Schenone, S.; Gravina, G. L.; Muzi, P.; Festuccia, C.; Vicentini, C.; Botta, M.; Bologna, M. *Eur. J. Cancer* **2006**, *42*, 2838.
26. Chen, Y. L., United States Patent: 2000; Vol. 6051578.
27. Trivedi, A.; Dodiya, D.; Surani, J.; Jarsania, S.; Mathukiya, H.; Ravat, N.; Shah, V. *Arch. Pharm.* **2008**, *341*, 435.
28. Trivedi, A.; Vaghasiya, S.; Dholariya, B.; Dodiya, D.; Shah, V. *J. Enzyme Inhib. Med. Chem.* **2010**, *25*, 893.
29. Trivedi, A. R.; Dholariya, B. H.; Vakhariya, C. P.; Dodiya, D. K.; Ram, H. K.; Kataria, V. B.; Siddiqui, A. B.; Shah, V. H. *Med. Chem. Res.* **2012**, *21*, 1887.
30. Marr, J. J.; Berens, R. L. *Mol. Biochem. Parasitol.* **1983**, *7*, 339.
31. Nugent, R. A.; Dunn, C. J.; Staite, N. D.; Murphy, M. J.; Schlachter, S. T.; Aspar, D. G.; Shields, S. K.; Galinet, L. A. *Phosphorus, Sulfur Silicon Relat. Elem.* **1996**, *109*, 229.
32. Guccione, S.; Modica, M.; Longmore, J.; Shaw, D.; Barretta, G. U.; Santagati, A.; Santagati, M.; Russo, F. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 59.
33. Trivedi, A. R.; Siddiqui, A. B.; Shah, V. H. *ARKIVOC* **2008**, *2*, 210.
34. Trivedi, A.; Dholariya, B.; Vakhariya, C.; Dodiya, D.; Ram, H.; Kataria, V.; Siddiqui, A.; Shah, V. *Med. Chem. Res.* **2012**, *21*, 1887.
35. Collins, L.; Franzblau, S. G. *Antimicrob. Agents Chemother.* **1997**, *41*, 1004.
36. icente, E.; Perez-Silanes, S.; Lima, L. M.; Ancizu, S.; Burguete, A.; Solano, B.; Villar, R.; Aldana, I.; Monge, A. *Bioorg. Med. Chem.* **2009**, *17*, 385.
37. Orme, I. *Antimicrob. Agents Chemother.* **2001**, *45*, 1943.
38. Hearn, M. J.; Cynamon, M. H.; Chen, M. F.; Coppins, R.; Davis, J.; Joo-On Kang, H.; Noble, A.; Tu-Sekine, B.; Terrot, M. S.; Trombino, D.; Thai, M.; Webster, E. R.; Wilson, R. *Eur. J. Med. Chem.* **2009**, *44*, 4169.