Bioorganic & Medicinal Chemistry Letters 22 (2012) 5129–5133

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Synthesis, antitubercular and antimicrobial evaluation of 3-(4-chlorophenyl)-4-substituted pyrazole derivatives

Ravindra B. Pathak [a,b,*], P. T. Chovatia [a,c], H. H. Parekh [a,*]

[a] Department of Chemistry, Saurashtra University, Rajkot, Gujarat, India
[b] Faculty of Natural Sciences, Keele University, Keele, Staffordshire ST5 5BG, UK
[c] Evotech ltd, Milton Park, Abingdon, Oxfordshire OX14 4SA, UK

ARTICLE INFO

Article history:
Received 7 April 2012
Revised 13 May 2012
Accepted 15 May 2012
Available online 24 May 2012

Keywords:
Antimicrobial
Antibacterial
Antitubercular
Antifungal
Hydrazone
Pyrazole
Azetidinone
Thiazolidinone

ABSTRACT

As a part of our research to develop novel antitubercular and antimicrobial agents, a series of 3-(4-chlorophenyl)-4-substituted pyrazoles have been synthesised. These compounds were tested for antitubercular activity in vitro against *Mycobacterium tuberculosis* H37R*v* strain using the BACTEC 460 radiometric system, antifungal activity against a pathogenic strain of fungi and antibacterial activity against gram-positive and gram-negative organisms. Among them tested, many compounds showed good to excellent antimicrobial and antitubercular activity. The results suggest that hydrazones, 2-azetidinones and 4-thiazolidinones bearing a core pyrazole scaffold would be potent antimicrobial and antitubercular agents.

Pyrazoles and their derivatives have attracted much attention due to diverse biological activities.[1,2] Enormous interest in the chemistry of pyrazoles is reflected by the design of new synthetic approaches due to their significant biological and therapeutic value. There has been overwhelming literature reports reflecting the immense biological potential of pyrazoles derivatives as antitumor, anti-HIV, anti-inflammatory and anti-microbial activities.[3]

Over the past few years continuous interest in the synthesis of C_2-substituted thiazolidinone derivatives for their significant activity,[4,5] thiazolidinones derivatives posses verities of bilogical activities like antibacterial,[6] antituberculosis,[7] antifungal,[8] and anticancer[9] has been observed. Azetidinones known[10] as a β-lactams since 1907, possess a four member ring system bearing internal amide linkage. The discovery of an azitidin-2-one ring in penicillin led to development of the compounds bearing this ring system. The powerful activity of penicillin[11,12] and the clinically more useful cephalosporin[13] led chemists and medicinal chemists to investigate the chemistry of the azetidinone ring system. The basic skeleton commonly encountered in β-lactam antibiotics are the penam and cepham and the high electrophilicity of the β-lactam ring system is responsible for the antibiotic activity of these compounds.

After a comprehensive literature survey our focus was to synthesise thiazolidinone and azetidinone derivatives as less toxic, more active antimicrobial and antituberculosis agents. (See Fig. 1)

Our efforts focused on the introduction of chemical diversity in the molecular framework in order to synthesise pharmacologically interesting compounds of different composition. This motivated us to design and synthesise new hydrazone, 2-azetidinones and 4-thiazolidinone templates bearing pyrazole moieties (Scheme 1).

A convenient method for the synthesis of the pyrazole compound was achieved starting from *p*-chloro acetophenone. According to a previously reported method[14] the *p*-chloro acetophenone upon tretment with diethyl oxalate in presence of base afforded ethyl 4-(4-chlorophenyl)-2,4-dioxobutanoate **(2)**. 3-(4-chlorophenyl)-1*H*-pyrazole-5-carbohydrazide **(3)** is previously reported compound,[15] was synthesised by the reaction of **(2)** with hydrazine hydrate under reflux condition. The hydrazone **(4)** was prepared by the condensation of hydrazide **(3)** and the appropriate aromatic aldehyde under reflex.[16] The Staudinger [2+2] cycloaddition reaction[17,18] of the hydrazone with chloroacetyl chloride to afford β-lactam **(5)** with a *trans*-configuration (On the basis of ^{1}H NMR, the coupling constant of the the protons on C3 and C4 is in the range of 2.0–2.4 Hz, indicating *trans*-configuration of the β-lactam). The reaction of the hydrazone **(4)** with mercaptoacetic acid in presence of sodium acetate and galcial acetic acid afforded racemic mixture of the thiazolidin-4-one **(6)**. The yields varied from

* Corresponding authors.
E-mail addresses: dryash_271177@yahoo.co.uk, r.pathak@epsam.keele.ac.uk (R.B. Pathak).

0960-894X/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmcl.2012.05.063

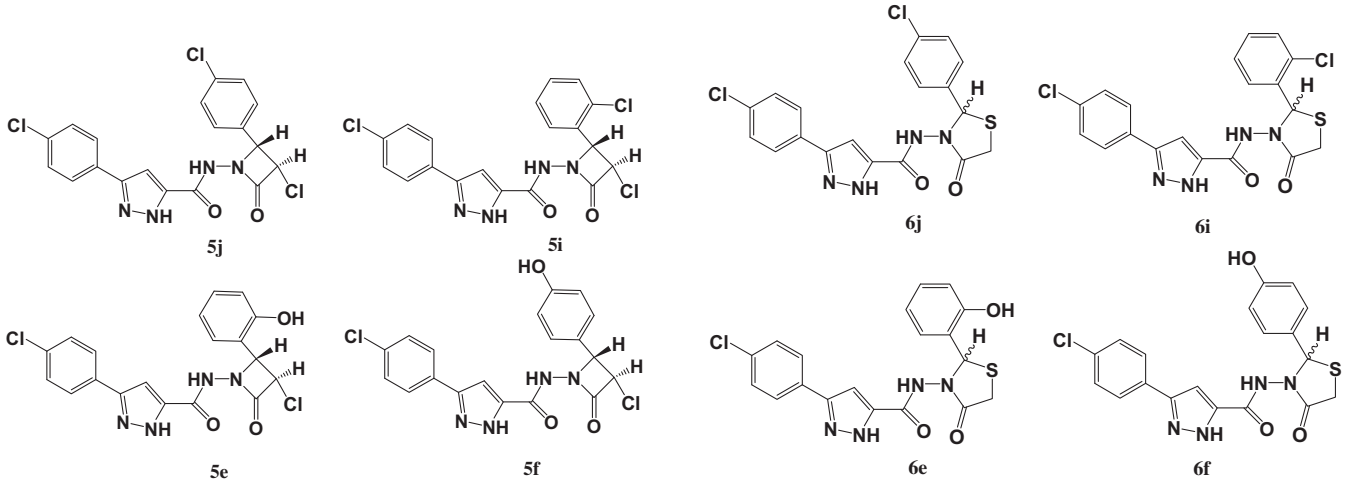
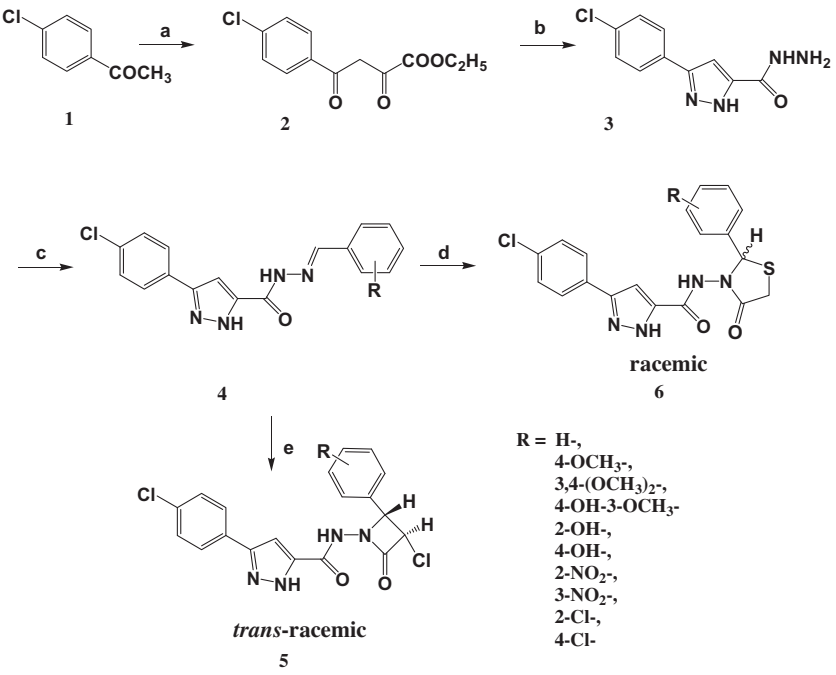


Figure 1. Structures of the most active compounds.



Scheme 1. Reagents and condition: (a) Diethyl oxalate (1.05 equiv), NaOMe (1.05 equiv), THF, rt, 1 h; (b) $NH_2NH_2{\cdot}H_2O$ (2.0 equiv), EtOH, reflux, 6 h; (c) aryl aldehyde (1.05 equiv), MeOH, glacial CH_3COOH, reflux, 6 h; (d) mercaptoacetic acid (1.05 equiv), NaOAc (2.1 equiv), glacial CH_3COOH, reflux, 10 h; (e) chloroacetyl chloride (1.2 equiv), Et_3N (1.2 equiv), DCM, rt, 2 h.

35% to 60% in case of hydrazone **(4)**; for the 2-azetidinones **(5)** the yields varied from 40% to 65% and from 25% to 50% for the 4-thiazolidinones **(6)**. All the compounds structures were confirmed by ^1H, ^{13}C, HR-MS and IR (refer to Supplementary data). All compounds were confirmed to be greater than 95% pure via LC–MS prior to their use in the evaluation of their biological efficacies.

All the compounds were evaluated for in vitro antibacterial activity against *Escherichia coli*, and *Staphylococcus aureus* and antifungal activity against pathogenic fungi. The MIC (minimum inhibitory concentration) values were determined by a comparison with ciprofloxacin (CIP), moxifloxacin (MFX) for antibacterial activity and fluconazole, 5-fluorocytosine for antifungal activity as standard agent. The activities reported as MICs were determined according to NCCLS method.[19–21]

As indicated in Table 1, most of sysnthesised compounds generally showed potent antifungal activity against all pathogenic fungal strains tested. Actully, the activity of compounds **5b**, **5c**, **6d**, **5e**, **6e**, **5f**, **5i**, **5j** and **6j** was superior or comparable to those of 5-fluorocytosine against some strain of fungai. In contrast, the compounds **5a**, **4b**, **4f**, **5g**, **5h** and **6h** did not show significant antifungal activity against all pathogenic fungal strain tested.

The substituents (−OH, −Cl) for the compounds **5** and **6** contribute significantly toward biological potency, Thus, the substituents appear to be an important factor in their antifungal activity. In addition, the compounds containing 2-azetidinone or 4-thiazolidinone showed more potent antifungal activity then the compounds containing hydrazones.

All the compounds were evaluated for in vitro antibacterial activity against *E. coli* ATCC 25922 and *S. aureus* ATCC 29213.

As indicated in Table 2, compounds **4e**, **4f**, **4i**, **4j**, **5e**, **5f**, **5i**, **5j**, **6e**, **6f**, **6i** and **6j** show excellent activity against the strains of bacteria. The substituents (−OH, −Cl) for the compounds **4**, **5** and **6** are

Table 1
In vitro antifungal activity for hydrazones, 2-azetidinones and 4-thiazolidinones

Compound	R	Antifungal activity MIC[a] (μg/mL)			
		C. krusei[b]	*C. neoformans*[b]	*A. niger*[b]	*A. flavus*[b]
4a	H—	12.5	1.6	50.0	3.2
5a	″	100.0	50.0	25.0	100.0
6a	″	50.0	12.5	25.0	12.5
4b	4-OCH_3—	100.0	50.0	12.5	100.0
5b	″	12.5	1.6	3.2	12.5
6b	″	50.0	12.5	3.2	25.0
4c	3,4-$(OCH_3)_2$—	50.0	12.5	25.0	12.5
5c	″	12.5	3.2	12.5	6.3
6c	″	12.5	50.0	6.3	12.5
4d	4-OH-3-OCH_3—	12.5	1.6	25.0	12.5
5d	″	1.6	12.5	3.2	25.0
6d	″	12.5	1.6	1.6	12.5
4e	2-OH—	50.0	25.0	100.0	6.3
5e	″	6.3	1.6	12.5	12.5
6e	″	12.5	6.3	6.3	12.5
4f	4-OH—	100.0	50.0	12.5	50.0
5f	″	25.0	3.2	1.6	12.5
6f	″	50.0	1.6	1.6	12.5
4g	2-NO_2—	100.0	1.6	3.2	25.0
5g	″	12.5	50.0	100.0	50.0
6g	″	25.0	3.2	50.0	50.0
4h	3-NO_2—	25.0	1.6	25.0	12.5
5h	″	100.0	50.0	12.5	50.0
6h	″	50.0	100.0	25.0	100.0
4i	2-Cl—	12.5	3.2	12.5	25.0
5i	″	1.6	6.3	12.5	12.5
6i	″	12.5	1.6	25.0	50.0
4j	4-Cl—	12.5	1.6	12.5	25.0
5j	″	6.3	1.6	6.3	12.5
6j	″	25.0	12.5	12.5	6.3
Fluconazole	—	25.0	6.3	6.3	25
5-FC[c]	—	6.3	6.3	6.3	25

[a] The MIC value is defined as lowest concentration of the antifungal agent exhibiting no fungal growth. MIC values were read after 1 day for *C. krusei* and *C. neoformans*, and 2 days for *A. niger, and A. flavus* in 37 °C. The inoculum sizes contained approximately 1×10^5 cells/mL. Culture media tested were the modified Sabouraud dextrose broth. The final concentration of antifungal agents was between 0.2 and 100 μg/mL.
[b] Fungi tested: *C. krusei* Berkout KCCM 11655, *Cryptococcus neoformans* KCCM 50564, *Aspergillus niger* KCTC 1231, and *Aspergillus flavus* KCCM 11899.
[c] 5-FC: 5-fluorocytosine.

important for biological potency. In contrast, the introduction of nitro phenyl or phenyl group in compounds **4a**, **4g**, **4h**, **5a**, **5g** and **5h** resulted in poor or no antibacterial activity. Although, many of the compounds showed potent antibacterial activity.

All the compounds were tested for antitubercular activity against *Mycobacterium tuberculosis* H37Rv strain. Employing the BACTEC 460 TB system, all compounds underwent an initial in vitro screening to determine their activities against the *M. tuberculosis* H37Rv strain. The MIC values were determined by a comparison with rifampin (RIP) as standard agent. Antitubercular activity was determined using the modified BACTEC 460 system in which stock solution as test molecules were prepared in dimethylsulfoxide (DMSO) at 1 mg/ml concentration and sterilised by passage through 0.22 wm PFTE filters.[22,23]

As seen from data of Table 2, most of sysnthesised compounds generally possess very good antimycobacterial activity, with MIC values in the range of 0.35–3.15 μg/mL, although the **4a**, **4g** and **4h** are devoid of such an activity. Best activity against *M. tuberculosis* H37Rv was observed for the 2-OH substituted azetidinone derivative **5e**, which is only slightly less potent than the standard drug rifampin, followed by the 3,4-$(OCH_3)_2$ substituted hydrazone derivative **4c**, the 2 and 4-OH substituted thiazolidinone derivatives **6e** and **6f**, the 2-Cl substituted hydrazone and thiazolidinone derivatives **4i** and **6i**, as well as the 4-OCH_3 substituted hydrazone and thiazolidinone derivatives **4b** and **6b**, 4-OH-3-OCH_3 substituted derivatives **4d**, **5d** and **6d**, 4-OH substituted azetidinone derivative **5f**, and 2-Cl substituted azetidinone derivative **5i** (which all possess MIC values under 1 μg/mL).

The compounds **5a**, **5b**, **5c**, **6c**, **4e**, **4f**, **4j**, **5j** and **6j** are exhibited slightly less biological potency. In contrast, 2 or 3-nitro phenyl substituted compounds exhibited poor or no, if any, antimycobacterial activity, while the compounds **4a** and **6a** exhibited lack of activity.

In general, the substituents (−OH, −Cl, −OCH_3) are important for biological potency, Thus, the substituents appear to be an important factor in their antimycobacterial activity.

According to the literature survey,[24,25] a study of the azole compounds was found to interact with the target enzyme cytochrome P450-dependent sterol 14-α demethylase in the ergosterol biosynthesis pathway, through computer modelling of drug-enzyme complexes. With this in mind, we assume that the pyrazole ring (compounds **4a**–**j**, **5a**–**j** and **6a**–**j**) may take a position perpendicular to the porphyrine plane, with a ring nitrogen atom coordinate to the haem iron. Presumably, the distance between the nitrogen of the pyrazole ring and the haem iron is most suitable to form a complex.

Moreover, the substituted phenyl group of the compounds might orientate itself in a hydrophobic binding cleft, above the haem ring. This subsite includes the side chains of the ceiling residues. The phenyl ring may be the large enough to extend the number of contacts in the binding site. The 4-chlorophenyl ring, common to all inhibitors, is stabilized by favourable dispersion forces with the range of side chains. However, it should be noted that this is a speculative mechanism of action of these pyrazoles, as we don't have any computational approach/Molecular modelling results to validate our suggested mode of action. In conclusion,

Table 2
In vitro antibacterial and antitubercular activity for hydrazones, 2-azetidinones and 4-thiazolidinones

Compound	R	Antibacterial activity MIC[b] (μg/mL)		Antitubercular activity MIC[b] (μg/mL)
		S. aureus[a]	*E. coli*[a]	H37Rv
4a	H—	4	1	>5
5a	〃	0.51	0.48	1.25
6a	〃	0.91	0.28	3.15
4b	4-OCH_3—	0.18	0.01	0.85
5b	〃	0.0025	0.060	1.2
6b	〃	0.5	0.04	0.65
4c	3,4-$(OCH_3)_2$—	0.25	4	0.37
5c	〃	0.05	0.5	1.15
6c	〃	0.06	0.2	1.2
4d	4-OH-3-OCH_3—	0.12	0.017	0.55
5d	〃	0.125	0.025	0.6
6d	〃	0.15	0.05	0.65
4e	2-OH—	0.40	0.015	1.1
5e	〃	0.125	0.019	0.35
6e	〃	0.06	0.028	0.5
4f	4-OH—	0.35	0.012	1.1
5f	〃	0.04	0.010	0.6
6f	〃	0.125	0.017	0.39
4g	2-NO_2—	8	8	>5
5g	〃	6	0.5	1.9
6g	〃	2	0.1	4
4h	3-NO_2—	1.5	0.13	>5
5h	〃	1.2	0.33	1.99
5h	〃	8	0.6	2
4i	2-Cl—	0.21	0.014	0.36
5i	〃	0.034	0.031	0.55
6i	〃	0.02	0.048	0.5
4j	4-Cl—	0.12	0.012	1.1
5j	〃	0.28	0.010	1
6j	〃	0.048	0.022	1.15
CIP[b]	—	0.25	0.02	—
MFX[b]	—	0.06	0.015	—
RIP[b]	—	—	—	0.125

[a] Bacteria tested: *Strphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922.
[b] CIP: Ciprofloxacin, MFX: Moxifloxacin, RIP: Rifampin, MIC: minimum inhibitory concentration.

the synthesis and biological evaluation of a series of 3-(4-cholorophenyl)-4-substituted pyrazole derivatives as antimicrobial and antitubercular agent has been achieved. Most importently, the azetidinone and thiazolidinone substituted pyrazole scaffold lead to molecules with potent antimicrobial and antimycobacterial activity. Interestingly, the antibacterial evaluation indicates more promising results vs *E.coli* than against *S. aureus*. The results suggest that hydroxy and chlorophenyl azetidinone and thiazolidinone series would be promising leads for the further devlopment of antimicrobial and antitubercular agents. However, because of the end of funding to this project the most active compounds are not tested for cytotoxicity, but it is likely some of these compounds may display cytotoxicity.

Acknowledgments

The authors are thankful to Prof. H. H. Parekh, Head of the Department, Department of Chemistry, Saurashtra University, Rajkot, for insightful discussion and providing research facilities with financial support. Authors are also thankful to Dr. Cecil Kwong, TAACF, Alabama, USA for antitubercular screening.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.05.063.

References and notes

1. Fomun, Z. T.; Ifeadike, P. N. *J. Heterocycl. Chem.* **1985**, *22*(6), 1611.
2. Ronald, E. O. *J. Pharm. Sci.* **1968**, *57*(4), 537.
3. Turan-Zitouni, G.; Sivaci, M.; Kilic, F. S.; Erol, K. *Eur. J. Med. Chem.* **2001**, *36*, 685.
4. Hutchinson, I.; Jennings, S. S.; Vishnuvajjala, B. R.; Westwell, A. D.; Stevens, M. F. G. *J. Med. Chem.* **2002**, *45*, 744.
5. Quiroga, J.; Hernandez, P.; Insuassty, B. R.; Abonia, R.; Cobo, J. *J. Chem. Soc., Perkin Trans. 1* **2002**, 555.
6. Bonde, C. G.; Gaikwad, N. *J. Bioorg. Med. Chem.* **2004**, *12*, 2151.
7. Kucukguzel, S. G.; Oruc, E. E.; Rolls, S.; Sahin, F.; Ozbek, A. *Eur. J. Med. Chem.* **2002**, *37*, 197.
8. Cesure, N.; Cesur, Z.; Ergenc, N.; Uzun, M.; Kiraz, M.; Kasimoglu, O.; Kaya, D. *Arch. Pharm.* **1994**, 271.
9. Bhatt, J. J.; Shah, B. R.; Shah, H. P.; Trivedi, P. B. *Ind. J. Chem.* **1994**, *33B*, 189.
10. Staudinger, H. *Ann. Chim. (Rome)* **1908**, *356*, 51.
11. Clark, H. T.; Johnson, J. R.; Robinson, R. *The chemistry of penicillins*; Princeton University Press: New York, 1949.
12. Abraham, E. P. *Q. Rev.* **1967**, *21*, 231.
13. Summes, P. G. *Chem. Rev.* **1976**, *76*, 113.
14. Thomas, S. G.; Wenis, E.; Lee, J. *J. Org. Chem.* **1961**, *26*, 1514.
15. Xia, Y.; Dong, Z. W.; Zhao, B. X.; Ge, X.; Meng, N.; Shin, D. S.; Mio, J. Y. *Bioorg. Med. Chem.* **2007**, *15*, 6893.
16. Jin, L.; Chen, J.; Song, B.; Chen, Z.; Yang, S.; Li, Q.; Hu, D.; Xu, R. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5036.
17. Georg, G. I.; Wu, Z. *Tetrahedron Lett.* **1994**, *35*(3), 381.
18. Thomas, R. C. In *Recent Progress in the Chemical Synthesis of Antibiotics*; Luckacs, G., Ohno, M., Eds.; Springer: Berlin, 1990; p 533.
19. The MIC assays were performed in accordance with the NCCLS guidelines; Methods for dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically 5th ed.; NCCLS Document M7–A5; NCCLS, January 2000; 20.
20. Performance Standards for Antimicrobial Susceptibility Testing: Eleventh Informational Supplement; NCCLS Document M100–S11; NCCLS, January 2001; 21, 1.

21. Amsterdam, D. *Susceptibility Testing of Antimicrobials in Liquid Media. In Antibiotics in Laboratory Medicine*, 4th ed.; Williams & Wilkins: Baltimore, MD, 1996. p. 52–111.
22. Collins, L.; Franzblau, S. G. *Antimicrob. Agents Chemother.* **1997**, *1004*, 41.
23. Siddiqi, S. H. BACTEC 460 TB system, Product and Procedure Manual Revision. D. Becton Dickinson Microbiology System, Sparks, MD, 1995.
24. Zampieri, D.; Mamolo, M. G.; Vio, L.; Banfi, E.; Scialino, G.; Fermeglia, M.; Ferrone, M.; Pricl, S. *Bioorg. Med. Chem.* **2007**, *15*, 7444.
25. Zampieri, D.; Mamolo, M. G.; Vio, L.; Banfi, E.; Scialino, G.; Fermeglia, M.; Ferrone, M.; Pricl, S. *J. Antimicrob. Chemother.* **2006**, *58*, 76.