Bioorganic & Medicinal Chemistry Letters 20 (2010) 3169–3172

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Novel 1,3,5-triphenyl-2-pyrazolines as anti-infective agents

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ARTICLE INFO

Article history:
Received 15 September 2009
Revised 3 March 2010
Accepted 25 March 2010
Available online 29 March 2010

Keywords:
1,3,5-Triphenyl-2-pyrazolines
Alog P
Mycobacterium tuberculosis $H_{37}Rv$
High resolution mass spectrum (HR-MS)

ABSTRACT

Sixteen 1,3,5-triphenyl-2-pyrazolines were synthesized and their anti-infective activities (against *Mycobacterium tuberculosis* $H_{37}Rv$, six bacterial and four fungal strains) were tested. Only compound with SO_2CH_3 in the *para* position of the A-ring was active against the tubercular strain at 100 μg/ml concentration. All compounds showed good anti infective activity against *Escherichia coli* and poor activity against *Staphylococcus aureus*. Compounds **4, 12, 13** and **14** exhibited reasonable activity against all the organisms tested (<0.309 μM except against *S. aureus.* The activity of these compounds correlated with their lipophilic/hydrophilic nature. Compounds **4, 10** and **16** showed very good activity (>88% reduction) against four fungi studied at 2 mg/ml. All these compounds possess halogen substitutions. Compound **11** showed very high activity (>90%) against three fungi. Majority of the compounds showed more than 90% inhibition against one or two fungi. Since pyrazolines are reported to inhibit the activity of p-glycoprotein, they may prevent drug resistance developed by microorganism.

Tuberculosis is one amongst the three highly infectious diseases apart from HIV/AIDS. It is caused by *Mycobacterium tuberculosis*.[1] It is estimated that between 2002 and 2020, approximately a billion people will be newly infected, more than 150 million people will get sick, and 36 million will die of TB.[2]

Gram +ve and Gram −ve bacterial strains are involved in majority of the infectious diseases. Prolonged use of antibiotics is limited due to their side effects and the resistance developed by the microorganism.[3] Among the two bacterial strains, Gram −ve have rich lipopolysaccharides in their outer leaflet preventing the penetration of majority of the drugs through this membrane. This makes them more drug resistant when compared to Gram +ve organisms. Moreover, they also have the ability to exchange their genetic material (DNA) with same as well as different species leading to genetic change (mutation) thereby developing drug resistance.

Extended use of chemotherapeutic agents for the treatment of cancer, antibacterial agents for diseases and the use of immunosuppressive agents after transplantation leads to fungal infection. Individuals with AIDS or malignancies are also susceptible to fungal infection.[4] Resistance has been observed against triazoles and polyenes by fungi including *Candida albicans* and *Aspergillus* species.[5]

Resistance developed by these microbial strains delineates the therapy against tuberculosis and bacterial and fungal diseases. P-Glycoprotein is an ABC transporter family of proteins that is responsible for multidrug resistance. It has an impact on all the above said infectious diseases.[6] It also plays a role in expelling the anticancer agents and make the cancerous cells resistant. American Society for Microbiology (ASM) in association with the Society for Healthcare Epidemiology of America (SHEA) together have launched a program known as Antimicrobial Resistance Prevention Initiative (ARPI) and it is dedicated to explore the epidemiology and mechanisms of resistance by the microorganisms.[5]

So studies that contribute to the design of new potential drugs with diverse mechanism of action and broad spectrum of activity than the current ones that are available for the treatment of infectious diseases and at the same time counteract the resistance developed by bacteria are gaining importance. Since drug resistance due to the over expression of P-glycoprotein is associated with tuberculosis and infectious diseases, designing molecules which posses both P-glycoprotein inhibitory activity and antimicrobial activity will be useful to combat infectious diseases. Hence our study was focused towards the synthesis of novel 1,3,5-triphenyl-2-pyrazolines and to determine their activity against *M. tuberculosis,* Gram positive and Gram negative bacteria and fungi.

Pyrazolines are useful lead molecules that are found to act as antibacterial, antifungal[7–9] and antitubercular agents.[10,11] Moreover pyrazolines are also tested for their inhibitory activity against P-glycoprotein.[12]

All the chemicals for the synthesis were purchased from Sigma chemicals, Bangalore, India. Control drugs used for the experiments were purchased from Himedia Laboratories India. The synthesized compounds were tested against six bacterial strains namely, *Staphylococcus aureus* NCIM 5021, *Bacillus subtilis* NCIM 2718, *Proteus vulgaris* NCIM 2813, *Escherichia coli* NCIM 2931,

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doi:10.1016/j.bmcl.2010.03.083

Salmonella typhi 2501, and *Enterobacter aerogenes* NCIM 5139 (National Chemical Laboratory, Pune, India), four fungi namely, *Aspergillus flavus* NCIM 594, *Fusarium proliferatum* NCIM1105, *Aspergillus niger* NCIM 596 and *Candida tropicalis* NCIM 3556 (National Chemical Laboratory (NCL), Pune, India) and *M. tuberculosis* $H_{37}Rv$ (Tuberculosis Research Center, Chetpet, Chennai, India). The internal energy, log *P* and *A*log *P* values of the compounds were calculated using Cerius 2® software.

Chalcones were used as starting materials for the synthesis of 1,3,5-triphenyl-2-pyrazolines. The preparation of these chalcones and their characterization were given in our previous research communications.[13,14] The general procedure for the synthesis of sixteen 1,3,5-triphenyl-2-pyrazolines is given in Scheme 1 and it is explained at the Supplementary data.

The antitubercular activity of the synthesized compounds was evaluated against the *M. tuberculosis* $H_{37}Rv$ by LRP (Luciferase reporter phage assay) assay.[13]

Antibacterial activity of these 1,3,5-triphenyl-2-pyrazoline derivatives were determined against the six Gram +ve and Gram −ve strains listed before using the microdilution method reported by Sarker et al. using 96 well plates.[15]

The antifungal activity of these compounds was tested against four fungi mentioned before as per the macrodilution method reported by Kumar et al.[16]

Table 1 lists the sixteen 1,3,5-triphenyl-2-pyrazolines synthesized as per the procedure mentioned before. They were characterized by FT-IR, NMR and Mass spectrum. The FTIR spectra for all the compounds have a C=N stretching band at 1590 cm^{-1} and the NMR spectra have three doublet of doublets for H_X, H_A and H_B.

Spectral data for compounds **6, 9** and **16** are given in Ref. 17 and their ^1H and ^{13}C NMR spectrum, HR-mass spectrum are given in the Supplementary data (see Figs. S1–S9).

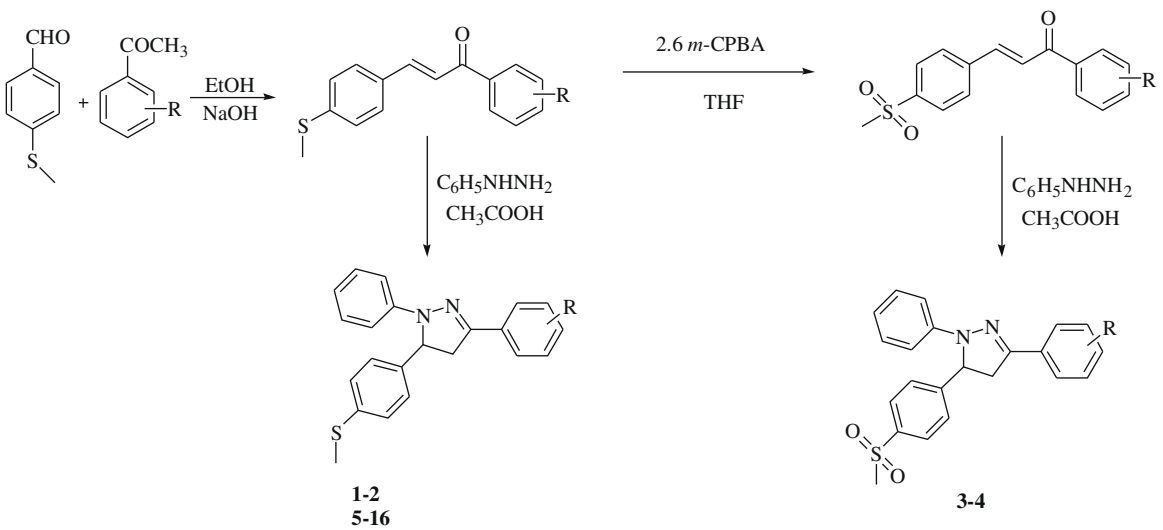
The anti tubercular activity at 50 and 100 μg/ml, anti bacterial activity represented as MIC (minimum inhibitory concentration) and antifungal activity (as % inhibition) of all the compounds are given in Table 1.

Among the 16 compounds tested compound **3** alone was found to be active against *M. tuberculosis* $H_{37}Rv$ strain (more than 50% reduction in RLU at 100 μg/ml concentration). Other compounds showed less than 50% reduction in RLU and hence they can be considered as inactive at these two concentrations tested. All the 16 compounds exhibited poor activity at 50 μg/ml concentration. The active compound **3**, has a methylsulfonyl group in the A-ring. Other researchers found the importance of sulfonylmethyl substitution towards antibacterial activity.[18] The log *P* (hydrophobicity of the compound) plays an important role in the penetration through *M. tuberculosis* cell wall and its relationship with antitubercular activity is observed by several researchers.[19–21] In this current study only compound **3** has log *P* in between 3 and 5 which is ideal for penetration through mycobacterial cell, while other compounds have more than 5.

Compounds **4, 12, 13** and **14** were found to be active against almost all the bacterial strains tested except *S. aureus*. Most active compounds generally have a halogen in *para* and/or *meta* position in the B-ring (except compound **12**) which is also observed by Ansari et al.[22] They also showed that halogen substitution is more active than methoxy, nitro and hydroxyl substitution in 3′-hydroxyl chalcones. The most active compounds (**4, 12, 13** and **14**) have good correlation between their antibacterial activities and thermodynamic internal energy (correlation coefficient = 0.99) and *A*log *P* (correlation coefficient = −0.87 to −0.99). The later term represents the hydrophobic/lipophilic nature of the molecule. Ansari et al. also observed a correlation between the conformational energy and the lipophilicity of the molecule towards the antibacterial activity.[22] Our previous research communications also give sufficient detail about the hydrophilic/lipophilic balance required for small molecules to penetrate the bacterial cell membrane.[13,14,23] In the case of a slime producing organism (myxobacteria which delineate the antibacterial therapy further), the small molecule has to cross the exopolysacchride which is hydrophobic in nature and then it has to cross the bacterial cell membrane which has a high LPS (lipopolysacchride) content which is hydrophilic in nature. Hence both the hydrophobicity of the compounds and the hydrophobicity of the organism cell surface have to balance for exhibiting antibacterial activity.

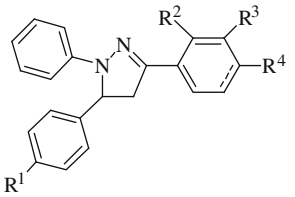
Compounds **4, 10** and **16** were found to be the most active against all the four fungal strains (>88% inhibition). All the three compounds have a halogen (chlorine or fluorine) substitution in the *ortho* or *para* position in their B ring. Earlier Karthikeyan et al. also found that the chloro and fluoro substitution in B-ring favors antibacterial and antifungal activity of 2-pyrzolines.[7]

In conclusion, sixteen 1,3,5 triphenyl-2-pyrazolines were synthesized, characterized and their in vitro anti-infective activities (antimycobacterial, antibacterial and antifungal) were evaluated. Compared to the thiomethyl substitution, methylsulfonyl substitution in the A-ring leads to higher activity, namely compound **3**



Scheme 1. Synthesis of 1,3,5-triphenyl-2-pyrazoline derivatives.

Table 1
The structure and observed anti-infective activities of 1, 3, 5-triphenyl-2-pyrazolines



Compound No	Substitutions in phenyl ring				Antitubercular activity (% reduction in RLU)		Antibacterial activity (MIC in μM)						Antifungal activity (percentage inhibition at 2 mg/ml concentration)			
	R^1	R^2	R^3	R^4	50 μg/ml	100 μg/ml	*E. coli* NCIM 2931	*P. vulgaris* NCIM 2813	*E. aerogenes* NCIM 5139	*S. aureus* NCIM 5021	*S. typhi* NCIM 2501	*B. subtilis* NCIM 2718	*F. proliferatum* NCIM1105	*C. tropicalis* NCIM 3556	*A. flavus* NCIM 594	*A. niger* NCIM 596
1	SCH_3				0.00	17.65	0.045	0.726	0.363	0.726	0.726	0.363	94.13 ± 0.00	73.11 ± 0.91	30.50 ± 6.13	93.52 ± 0.42
2	SCH_3			Cl	0.00	17.43	0.082	0.660	0.330	0.660	0.330	0.330	72.94 ± 1.79	94.43 ± 0.99	13.11 ± 1.21	92.99 ± 0.34
3	SO_2CH_3				1.70	50.71	0.166	0.332	0.332	0.664	0.332	0.332	97.61 ± 0.47	30.64 ± 0.30	65.85 ± 1.82	47.20 ± 5.03
4	SO_2CH_3			Cl	0.76	15.46	0.152	0.304	0.304	0.608	0.304	0.304	88.26 ± 0.90	95.58 ± 1.05	88.44 ± 1.92	97.54 ± 0.07
5	SCH_3	–O–CH_2–O–			10.59	27.81	0.080	0.322	0.322	0.644	0.322	0.322	81.35 ± 0.06	17.06 ± 0.23	31.19 ± 0.54	71.37 ± 0.00
6	SCH_3			CH_3	0.00	19.26	0.174	0.349	0.349	0.697	0.349	0.349	83.79 ± 1.10	95.30 ± 0.20	38.63 ± 0.24	92.98 ± 2.98
7	SCH_3		NO_2		0.00	5.70	0.160	0.321	0.321	0.642	0.321	0.321	56.86 ± 0.80	45.87 ± 0.00	18.98 ± 1.29	65.16 ± 0.77
8	SCH_3			OEt	36.44	48.35	0.161	0.322	0.322	0.643	0.322	0.322	94.35 ± 0.35	95.38 ± 1.10	38.25 ± 1.86	85.29 ± 1.20
9	SCH_3			OMe	0.00	24.87	0.083	0.334	0.334	0.668	0.334	0.334	65.87 ± 0.76	92.86 ± 0.41	48.37 ± 0.51	81.09 ± 0.82
10	SCH_3			F	0.00	4.75	0.022	0.345	0.345	0.690	0.690	0.172	98.71 ± 0.16	97.41 ± 1.40	94.82 ± 0.16	97.28 ± 1.31
11	SCH_3		OMe		7.45	26.85	0.167	0.334	0.334	0.668	0.334	0.334	96.09 ± 0.51	98.66 ± 0.82	51.53 ± 0.06	90.14 ± 0.41
12	SCH_3		OMe	OMe	32.66	30.30	0.155	0.309	0.155	0.618	0.309	0.309	93.19 ± 0.57	9.12 ± 0.78	63.83 ± 5.72	9.65 ± 0.08
13	SCH_3		Br		8.55	20.17	0.295	0.295	0.295	0.591	0.295	0.295	90.11 ± 2.34	65.14 ± 0.00	91.36 ± 1.01	64.62 ± 0.61
14	SCH_3			Br	0.00	18.55	0.148	0.295	0.295	0.591	0.295	0.295	34.31 ± 3.97	88.13 ± 0.25	28.20 ± 4.05	88.54 ± 1.17
15	SCH_3		NH_2		0.00	13.79	0.173	0.347	0.347	0.694	0.347	0.347	93.56 ± 0.13	86.11 ± 1.00	24.97 ± 3.82	84.37 ± 0.00
16	SCH_3	Cl			0.00	0.00	0.165	0.330	0.165	0.330	0.330	0.330	93.57 ± 0.73	90.96 ± 0.82	88.22 ± 2.12	98.33 ± 1.22

(high antimycobacterial activity) and **4** (high activity against bacterial and fungal strains) are found to be more active than the rest. Among the 16 compounds tested for antimycobacterial activity by luciferase reporter phage assay only compound **3** was found to be active and rest were inactive even at 100 μg/ml concentration. This shows the importance of correlation between the hydrophobicity of the compound and their antitubercular activity. Compounds **4, 12, 13** and **14** are found to be more active against all bacterial strains tested except *S. aureus*. Compounds **4, 10, 11** and **16** were generally more active against the four fungal strains at 2 mg/ml concentration. These compounds show the importance of a halogen substitution for antibacterial and antifungal activities. Two of the important physico-chemical parameters (conformational energy and *A*log *P*) are correlated with these activities. These studies indicate that small molecules with pyrazoline as template could be designed which may exhibit a broad range of anti-infective properties. The synthetic procedure is also simple which makes it an attractive lead template.

Acknowledgements

Authors thank Dr. Moni (SAIF, IIT) for providing spectral analysis.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.03.083.

References and notes

1. Madison, B. M. *Biotech. Histochem.* **2001**, *76*, 119.
2. Mativandlela, S. P. N.; Lall, N.; Meyer, J. J. M. *S. Afr. J. Bot.* **2006**, *72*, 232.
3. Malmvall, B. E.; Molstad, S.; Darelid, J.; Hiselius, A.; Larsson, L.; Swanberg, J.; Abom, P. E. *Qual. Manag. Health Care* **2007**, *16*, 60.
4. Frosco, M.; Barrett, J. F. *Expert Opin. Invest. Drugs* **1998**, *7*, 175.
5. Moellering, R. C.; Graybill, J. R.; McGowan, J. R., Jr.; Corey, L. *Am. J. Infect. Control* **2007**, *35*, S1.
6. Marquez, B. *Biochimie* **2005**, *87*, 1137.
7. Karthikeyan, M. S.; Holla, B. S.; Kumari, N. S. *Eur. J. Med. Chem.* **2007**, *42*, 30.
8. Padmavathi, V.; Thriveni, P.; Reddy, G. S.; Deepti, D. *Eur. J. Med. Chem.* **2008**, *43*, 917.
9. Chimenti, F.; Bizzarri, B.; Manna, F.; Bolasco, A.; Secci, D.; Chimenti, P.; Granese, A.; Rivanera, D.; Lilli, D.; Scaltrito, M. M.; Brenciaglia, M. I. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 603.
10. Ali, M. A.; Shaharyar, M.; Siddiqui, A. A. *Eur. J. Med. Chem.* **2007**, *42*, 268.
11. Shaharyar, M.; Siddiqui, A. A.; Ali, M. A.; Sriram, D.; Yogeeswari, P. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3947.
12. Manna, F.; Chimenti, F.; Fioravanti, R.; Bolasco, A.; Secci, D.; Chimenti, P.; Ferlini, C.; Scambia, G. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4632.
13. Sivakumar, P. M.; Seenivasan, S. P.; Kumar, V.; Doble, M. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1695.
14. Sivakumar, P. M.; Priya, S.; Doble, M. *Chem. Biol. Drug Des.* **2009**, *73*, 403.
15. Sarker, S. D.; Nahar, L.; Kumarasamy, Y. *Methods* **2007**, *42*, 321.
16. Kumar, K. A.; Singh, S. K.; Kumar, B. S.; Doble, M. *CEJC* **2007**, *5*, 880.
17. *Spectral data for compound* **6**: Yield: 81%,^{1}H NMR (500 MHz, $CDCl_3$): δ 2.36 (s, 3H), 2.44 (s, 3H), 3.08 (dd, *J* = 7, 17 Hz, 1H), 3.78 (dd, *J* = 12, 17 Hz, 1H), 5.19 (dd, *J* = 7.5, 12.5 Hz, 1H), 6.76 (t, *J* = 7.5 Hz, 1H), 7.05 (d, *J* = 8.5 Hz, 2H), 7.15–7.24 (m, 8H), 7.60 (d, *J* = 8 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 15.82, 21.40, 43.65, 64.07, 113.37, 119.03, 125.72, 126.48, 127.26, 128.90, 129.27, 129.93, 137.65, 138.73, 139.60, 144.97, 146.95. HR-MS (*m*/*z*) for molecular formula $C_{23}H_{23}N_2S$: calcd = 359.1582, found = 359.1576. *Spectral data for compound* **9**: Yield: 81%, ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.44 (s, 3H), 3.07 (dd, *J* = 7.5, 20 Hz, 1H), 3.78 (dd, *J* = 12.5, 17 Hz, 1H), 3.81 (s, 3H), 5.17 (dd, *J* = 7.5, 12.5 Hz, 1H), 6.76 (t, *J* = 7.5 Hz, 1H), 6.90 (d, *J* = 8.5 Hz, 2H), 7.05 (d, *J* = 8 Hz, 2H), 7.15–7.25 (m, 6H), 7.65 (d, *J* = 9 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 15.82, 43.77, 55.35, 64.10, 113.29, 114.03, 118.90, 125.49, 126.48, 127.22, 127.25, 128.88, 137.61, 139.65, 145.14, 146.78, 160.16. HR-MS (*m*/*z*) for molecular formula $C_{23}H_{23}N_2OS$: calcd = 375.1531, found = 375.1541. *Spectral data for compound* **16**: Yield: 81%, ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.45 (s, 3H), 3.32 (dd, *J* = 7.5, 17.5 Hz, 1H), 4.02 (dd, *J* = 12, 17.5 Hz, 1H), 5.24 (dd, *J* = 7.5, 12 Hz, 1H), 6.80 (t, *J* = 7.5 Hz, 1H), 7.05 (d, *J* = 8.5 Hz, 2H), 7.16–7.29 (m, 8H), 7.38 (d, *J* = 8 Hz, 1H), 7.83 (dd, *J* = 1.5, 8 Hz, 1H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 15.78, 46.21, 64.59, 113.63, 119.54, 126.48, 126.77, 127.23, 128.94, 129.42, 130.02, 130.84, 131.68, 132.20, 137.78, 139.11, 144.58, 145.97. HR-MS (*m*/*z*) for molecular formula $C_{22}H_{20}N_2SCl$: calcd = 379.1036, found = 379.1040.
18. Oh, C.; Lee, C.; Lee, J.; Cho, J. *Arch. Pharm. Pharm. Med. Chem.* **2003**, *336*, 504.
19. Ragno, R.; Marshall, G. R.; Santo, R. D.; Costi, R.; Massa, S.; Rompei, R.; Artico, M. *Bioorg. Med. Chem.* **2000**, *8*, 1423.
20. Matyk, J.; Waisser, K.; Drazkova, K.; Kunes, J.; Klimesova, V.; Palat, K. J.; Kaustova, J. *IL Farmaco* **2005**, *60*, 399.
21. Klimesova, V.; Palat, K.; Waisser, K.; Klimes, J. *Int. J. Pharm.* **2000**, *207*, 1.
22. Ansari, F. L.; Baseer, M.; Iftikhar, F.; Kulsoom, S.; Ullah, A.; Nazir, S.; Shaukat, A.; Haq, I.; Mirza, B. *ARKIVOC* **2009**, *x*, 318.
23. (a) Sivakumar, P. M.; Sheshayan, G.; Doble, M. *Chem. Biol. Drug Des.* **2008**, *72*, 303; Sivakumar, P. M.; Geetha Babu, S. K.; Doble, M. *Chem. Biol. Drug Des.* **2008**, *71*, 447.