Bioorganic & Medicinal Chemistry Letters 20 (2010) 1623–1625

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Novel molecular hybrids of cinnamic acids and guanylhydrazones as potential antitubercular agents

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ARTICLE INFO

Article history:
Received 14 September 2009
Revised 18 December 2009
Accepted 13 January 2010
Available online 20 January 2010

Keywords:
Cinnamic acid
Guanylhydrazone
Phenylacrylamide
Antitubercular

ABSTRACT

In an attempt to identify potential new agents active against tuberculosis, 20 novel phenylacrylamide derivatives incorporating cinnamic acids and guanylhydrazones were synthesized using microwave assisted synthesis. Activity of the synthesized compounds was evaluated using resazurin microtitre plate assay (REMA) against *Mycobacterium tuberculosis* H37Rv. Based on empirical structure–activity relationship data it was observed that both steric and electronic parameters play major role in the activity of this series of compounds. Compound **7s** (2*E*)-*N*-((-2-(3,4-dimethoxybenzylidene) hydrazinyl) (imino) methyl)-3-(4-methoxyphenyl) acrylamide showed MIC of 6.49 μM along with good safety profile of >50-fold in VERO cell line. Thus, this compound could act as a potential lead for further antitubercular studies.

Mycobacterium tuberculosis, a deadly obligate pathogen, is the causative agent of tuberculosis (TB), which remains a leading cause of death worldwide.[1–3] Tuberculosis is difficult to treat due to residence of bacteria within the macrophages and its unusual cell wall barrier. Moreover, multi-drug resistant strains of TB (MDR-TB) and extensively drug resistant tuberculosis (XDR-TB) have emerged recently.[4,5] Hence, there is an urgent need to develop newer anti-mycobacterial agents acting by novel mode of action and with minimal chances of cross resistance to existing drugs. Several researchers continue to work for the identification of newer anti-mycobacterial agents.[6–11]

In an attempt to synthesize and evaluate novel compounds active against TB, herein we report synthesis and evaluation of a series of phenylacrylamides designed by molecular hybridization of *E*-cinnamic acids and guanylhydrazones. Cinnamic acid derivatives are known to have antimicrobial activity. They also show synergism to some first line antitubercular agents.[12–15] Recently, while the current work was under progress, two reports on derivatives of cinnamic acid as antitubercular agents have appeared.[16,17] Guanylhydrazones have been shown to have antimicrobial activity including an interesting Gram-negative bacterial endotoxin lipopolysaccharide (LPS) sequestering activity owing to their cationic nature.[18–20] *M. tuberculosis* contains lipoarabinomannan (LAM), a complex lipid glycoprotein anchored to the cell membrane by phosphatidylinositol which has structural and functional similarity to LPS, including the presence of anionic phosphate groups.[21] Biosynthesis of LAM is known to be a target for several antitubercular agents, including the first line antitubercular agent, ethambutol.[22,23]

In this light, molecules were designed by molecular hybridization of guanylhydrazones and cinnamic acids. The design principle was aimed at combining the synergistic property of cinnamic acid with sequestering activity of guanylhydrazone moiety to get compounds with better antitubercular activity. Yet another objective of the study was to evaluate the effect of steric and electronic parameters on antitubercular activity and to optimize the activity through systematic modification of the substituents on the phenylacrylamide core.

In the present work, the target compounds were synthesized utilizing the reaction sequence as shown in Scheme 1. For the synthesis of desired phenylacrylamide derivatives, guanylhydrazones **3**, required as starting materials were prepared by the microwave assisted reaction of substituted ketones or aldehydes **1**, with guanyl hydrazine hydrochloride **2**. The phenylcinnamates **6**, were prepared by treating phenol **4**, with thionyl chloride and the appropriate cinnamic acid **5**.[16] The reaction of equimolar quantities of guanylhydrazones **3**, with phenylcinnamates **6**, under microwave irradiation in the presence of triethylamine and ethanol as solvent resulted in the formation of the target phenylacrylamide derivatives **7**, Table 1. The microwave assisted synthesis was advantageous over conventional reaction.[24,25] Spectral data (IR, ^{1}H NMR, ^{13}C NMR and MS) of all synthesized compounds were in agreement with the proposed structures.

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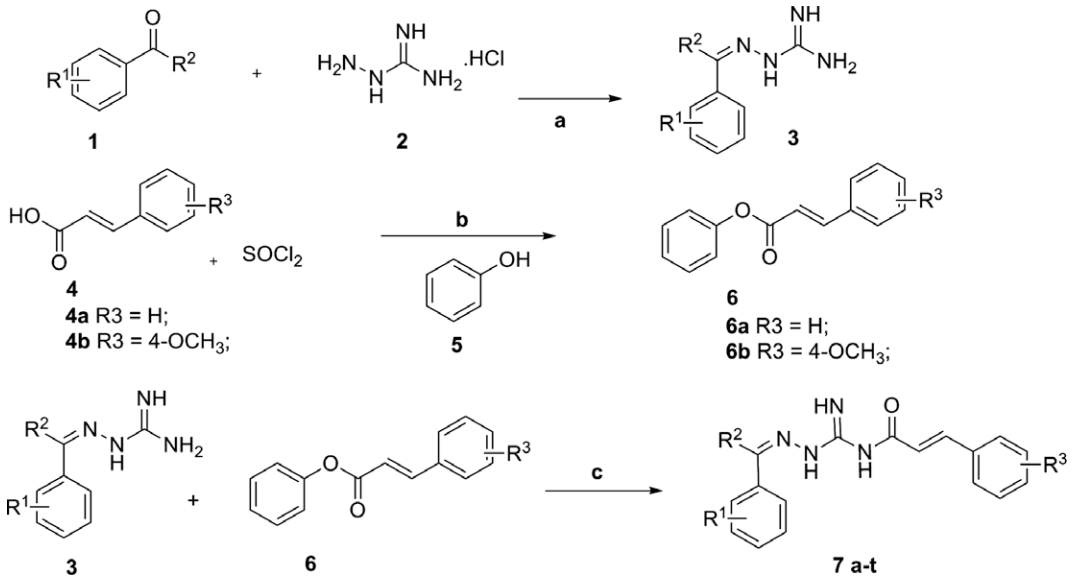
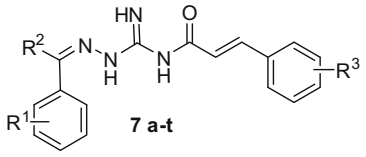
doi:10.1016/j.bmcl.2010.01.031



Scheme 1. Synthetic route for the synthesis of phenylacrylamide derivatives (7**a**–7**t**). Reagents and conditions: (a) EtOH, microwave irradiation 60 W, 15 min, target temperature 100 °C; (b) $SOCl_2$, phenol, reflux; (c) EtOH, triethylamine, microwave irradiation 60 W, 30 min, target temperature 100 °C.

Table 1
In vitro antituberculosis activity of phenylacrylamide derivatives



Compound	R_1	R_2	R_3	MIC (μM)	CC_{50} (μM)
7a	H	H	H	130.0	359.2
7b	H	CH_3	H	160.0	365.6
7c	H	Ph	H	532.0	312.1
7d	H	4-ClPh	H	273.0	327.6
7e	4-Cl	4-ClPh	H	219.5	311.0
7f	4-OH	H	H	40.5	454.1
7g	4-OH	CH_3	H	124.1	465.3
7h	3,4-DiOCH_3	H	H	8.9	368.9
7i	3-Cl	H	H	38.3	367.2
7j	2-Cl	H	H	30.6	413.1
7k	4-Br	CH_3	H	189.5	194.7
7l	4-OCH_3	CH_3	H	36.4	193.2
7m	2-OH	CH_3	H	254.4	201.6
7n	4-F	CH_3	H	46.3	354.6
7o	4-Cl	CH_3	H	18.3	249.4
7p	2,4-DiCl	CH_3	H	17.0	199.9
7q	4-OH	H	OCH_3	9.2	399.0
7r	4-F	H	OCH_3	13.8	405.5
7s	3,4-DiOCH_3	H	OCH_3	6.5	340.0
7t	3,4,5-TriOCH_{33}	H	OCH_3	7.6	308.0
Isoniazid	—	—	—	1.8	5833.5

The synthesized compounds (**7a**–**7t**) were screened against *M. tuberculosis* H37Rv in order to determine the minimum inhibitory concentration (MIC) with Resazurin microtiter assay (REMA).[6–11,26] Homogenous mycobacterial (H37Rv) culture suspension was seeded in microtitre plates at density of 10^5 cells per well in 100 μL of the Middlebrook 7H9 broth (Difco laboratories, Detroit, MI, USA) and the test compounds were serially diluted directly on the plate. The control received equivalent amount of DMSO. The plates were incubated at 37 °C for 7 days. Freshly prepared resazurin dye (0.02%) was added and plates were again incubated for 48 h. MIC is the lowest concentration at which complete inhibition was observed and was determined by visual inspection (color change from blue to pink) (Table 1). Isoniazid was used as the reference drug.

Compounds **7a**–**7t** were synthesized with modifications on R_1, R_2 and R_3 positions on phenylacrylamide core (Table 1). Compounds **7a**–**7e** were synthesized in an attempt to find out optimal substitution at R_2 position. According to biological activity results, the aldehyde derivative **7a** (R_2 = –H) and the acetophenone derivative 7**b** (R_2 = –CH_3) were significantly more potent than the benzophenone derivative **7c** (R_2 = –Ph). The increase in volume or different electronic nature of –H, –CH_3 and –Ph substitutions in **7a**, 7**b** and 7**c** could be the possible reason for this behavior. The substitution –R_2 directly affects the electronic nature of guanylhydrazone bridge which according to our design postulate, is important for activity. Hence, to confirm the reason of this behavior, compounds **7d** (R_1 = H; R_2 = 4-ClPh) and **7e** (R_1 = 4-Cl; R_2 = 4-ClPh) with activating group at *para*-position of phenyl ring were synthesized. Interestingly, compound **7d** was more potent than **7c**. Furthermore, compound **7e** was slightly more potent than **7d**. Thus, at this stage, it can be postulated that decrease in activity in **7c** compared to **7a** can be attributed to combination of steric and electronic factors. Compounds **7f**–**7p** were synthesized with various substitutions at –R_1 position keeping –R_2 as hydrogen or methyl. One compound, **7h** exhibited good MIC of 8.88 μM. Furthermore, the aldehyde derivative **7f** again was more potent than the corresponding ketone derivative **7g,** thus confirming our previous findings and reinforcing the direction of our research. To further improve the activity compounds incorporating a methoxy substitution at cinnamoyl moiety in aldehyde (R_2 = H) derivatives **7q**–**7t** were synthesized. All the four compounds **7q**–**7t** showed good antitubercular activity with MIC <15 μM. Of particular interest one compound **7s** showed MIC of 6.49 μM. The compounds were also evaluated for toxicity in a mammalian VERO cell line (C1008) in 96-well microtitre plates and the CC_{50} values were determined (Table 1).[27] Complete data of percentage cell viability at various concentrations is given in Table 1, Supplementary data. Interestingly, the most potent compound **7s** exhibited good safety profile with selectivity index >50.

To further confirm our design postulate, the MIC's of an guanylhydrazone **3f** ((2)-2-(4-hydroxybenzylidene)hydrazinecarboximidamide), and phenylcinnamate **6a**, were determined. The MIC's obtained for guanylhydrazone **3f**; 1122.4 μM and phenylcin-

namate **6a**; 501.9 μM were ~25- and ~12-fold higher compared to their molecular hybrid **7f** which has MIC of 40.5 μM, thus confirming our design hypothesis. Furthermore, the molecular hybrid **7f** has significantly enhanced activity as compared to cinnamic acid (MIC: 675.0 μM[15]). Thus, these additional evaluation indicate the value of chemical hybridization in this instance.

In summary, using systematic iteration of design, synthesis and evaluation, 20 new compounds based on the molecular framework of cinnamic acids and guanylhydrazones were synthesized and evaluated for their antitubercular activity against *M. tuberculosis* H37Rv. Based on empirical structure–activity relationship data, it was observed that both the steric and the electronic parameters play major role in the activity of this series of compounds. Starting from the initial compounds with MIC of >100 μM, a compound, **7s**, with MIC of 6.49 μM was successfully identified. This compound provides a potential new lead for further studies against tuberculosis. Further studies for synergy with rifampicin are in progress.

Acknowledgements

R.B., J.L. are thankful to University Grand Commission (UGC), India and Ni.R.T., M.K. are thankful to Department of Biotechnology (DBT), India for financial support. This work is funded by Department of Biotechnology, India; Grant No. BT/PR7858/Med/14/1142/2006.

Supplementary data

Supplementary data (detailed experimental procedures and complete data of percentage cell viability at various concentrations) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.01.031.

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