

SHORT COMMUNICATION

**A facile synthesis and the antimicrobial activity of some
4-aryltriazoles**

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Abstract: Some 4-aryltriazoles were synthesized and tested for their antibacterial effects against *Bacillus cereus*, *Pseudomonas testosteroni*, *Klebsiella pneumoniae*, *Micrococcus flavus*, and *Citrobacter freundii*. The 4-aryltriazoles were obtained by cyclization of the potassium salt of the appropriately substituted dithiocarbazine acid with aromatic amines. The new synthesized compounds were characterized using infrared and ¹H nuclear magnetic resonance spectroscopy and further supported by mass spectrometry.

Keywords: 4-aryltriazoles, synthesis, antibacterial activity.

INTRODUCTION

Amongst the five-membered nitrogen containing heterocycles, the position of nitrogen atoms at 1, 2 and 4 activates the ring. 1,2,4-Triazole and its derivatives represent one of the most biologically active classes of compounds, possessing a wide spectrum of activities.^{1–4} Many workers have reported different activities of 4-aryltriazoles. Chambers *et al.*⁵ investigated 4-aryltriazoles for use in the treatment of neurodegenerative disease. Pier *et al.*⁶ reported them as irreversible antagonists at the A₃, A₂A adenosine receptor.⁷ Furthermore, many workers reported 4-aryltriazoles as aromatic steroid sulfatase inhibitors,⁸ GSKK-3 inhibitors,⁹ anticancer,¹⁰ fungicidal,¹¹ antibacterial,¹² anti-inflammatory,¹³ PKB (protein kinase B)¹⁴ inhibitors. Triazole derivatives have also been reported as better therapeutic agents.¹⁵

It has also been shown that the antiviral¹⁶ and antibacterial^{17,18} activities of thiourea derivatives are due to the presence of the –NH–C(S)–NH– function in the molecule and that changes in this activity depend on the nature of the substituents. With the aim of synthesizing better therapeutic agents, some new 4-aryltriazole derivatives were investigated. All the synthesized compounds were characterized by

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infrared and ^1H nuclear magnetic resonance spectroscopy. The compounds were screened for their *in vitro* antibacterial activity towards Gram positive and Gram negative bacterial strains.

EXPERIMENTAL

The following compounds were synthesized:

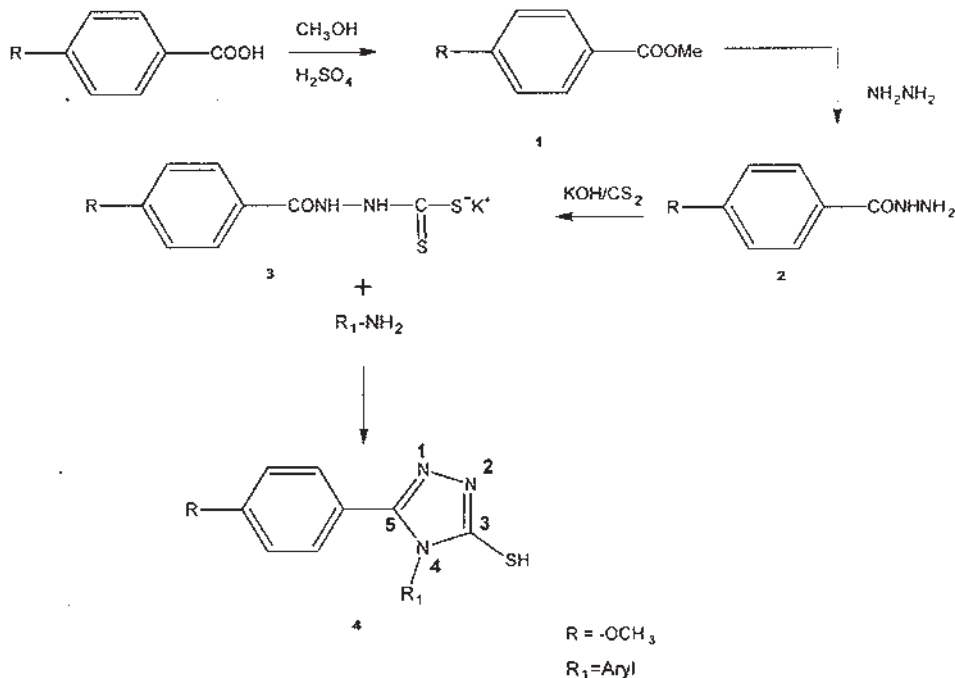
1. HAS-D1: 5-(4-methoxyphenyl)-4-(4-methylphenyl)-4*H*-1,2,4-triazole-3-thiol
2. HAS-D2: 4,5-bis(4-methoxyphenyl)-4*H*-1,2,4-triazole-3-thiol
3. HAS-D3: 4-(4-chlorophenyl)-5-(4-methoxyphenyl)-4*H*-1,2,4-triazole-3-thiol
4. HAS-D4: 5-(4-methoxyphenyl)-4-(2-nitrophenyl)-4*H*-1,2,4-triazole-3-thiol
5. HAS-D5: 5-(4-methoxyphenyl)-4-(3-nitrophenyl)-4*H*-1,2,4-triazole-3-thiol
6. HAS-D6: 4-(2,4-dimethylphenyl)-5-(4-methoxyphenyl)-4*H*-1,2,4-triazole-3-thiol
7. HAS-D7: 4-(2,3-dichlorophenyl)-5-(4-methoxyphenyl)-4*H*-1,2,4-triazole-3-thiol
8. HAS-D8: 5-(4-methoxyphenyl)-4-(2-methylphenyl)-4*H*-1,2,4-triazole-3-thiol
9. HAS-D9: 4-(3-chloro-4-fluorophenyl)-5-(4-methoxyphenyl)-4*H*-1,2,4-triazole-3-thiol
10. HAS-D10: 4-(2-methoxyphenyl)-5-(4-methoxyphenyl)-4*H*-1,2,4-triazole-3-thiol

Synthesis of 5-(4-methoxyphenyl)-4-(4-methylphenyl)-4H-1,2,4-triazole-3-thiol

Synthesis of the ester.¹⁹ A methanolic solution of *p*-methoxybenzoic acid containing few drops of conc. sulfuric acid was refluxed for 6 h. The reaction mixture was then poured onto ice. The product was isolated and treated with saturated sodium bicarbonate solution.

*Synthesis of hydrazide.*¹⁹ An equimolar mixture of ester and hydrazine hydrate was heated for 10 h and poured onto ice. The product was isolated and crystallized from ethanol.

Synthesis of potassium salt. A mixture of hydrazide, KOH and CS_2 in methanol was stirred for 12 h and poured onto ice. The product was isolated from diethyl ether.



Scheme 1. Reaction scheme.

Synthesis of 5-(4-methoxyphenyl)-4-(4-methylphenyl)-4H-1,2,4-triazole-3-thiol. A mixture of the potassium salt and *p*-toluidine was heated up to the evolution of H₂S gas. DMF was added to this mixture and the contents were poured onto ice. The crude product was filtered and crystallized from ethanol, yield 65 %, m.p. 190 °C.

Similarly, the other 4-aryltriazoles were synthesized (Scheme 1).

Spectral data:

IR (KBr) cm⁻¹. HAS-D1: 2948 (C–H str. asym), 1560 (C=C str. ring. ske.), 1656 (C=N str.), 2299 (S–H), HAS-D2: 2940 (C–H str. asym), 1514 (C=C str.), ring. ske.), 1641 (C=N str.), 2337 (S–H), HAS-D3: 2936 (C–H str. asym), 1516 (C=C str. ring. ske.), 1617 (C=N str.), 2302 (S–H), HAS-D4: 2951 (C–H str. asym), 1519 (C=C str. ring. ske.), 1623 (C=N str.), 2308 (S–H), HAS-D5: 2945 (C–H str. asym), 1533 (C=C str. ring. ske.), 1629 (C=N str.), 2322 (S–H), HAS-D6: 2951 (C–H str. asym), 1566 (C=C str. ring. ske.), 1622 (C=N str.), 2301 (S–H), HAS-D7: 2968 (C–H str. asym), 1577 (C=C str. ring. ske.), 1639 (C=N str.), 2309 (S–H), HAS-D8: 2972 (C–H str. asym), 1507 (C=C str. ring. ske.), 1632 (C=N str.), 2299 (S–H), HAS-D9: 2939 (C–H str. asym), 1551 (C=C str. ring. ske.), 1616 (C=N str.), 2302 (S–H), HAS-D10: 2958 (C–H str. asym), 1581 (C=C str. ring. ske.), 1636 (C=N str.), 2323 (S–H).

¹H NMR (DMSO). HAS-D1: 3.78 (3H, *s*, OCH₃), 2.45 (3H, *s*, CH₃), 11.66 (1H, *s*, SH), 6.78 [2H, *d* (*J* = 8.85 Hz), Ar-H], 7.28 [2H, *d* (*J* = 8.22 Hz), Ar-H], 7.25 [2H, *d* (*J* = 9.2.88 Hz), Ar-H], 7.17 [2H, *d* (*J* = 8.10 Hz), Ar-H].

Mass. HAS-D1: *m/z* 136, 154, 165, 224, 284, 298.

Antibacterial activity

Test microorganisms. The bacterial strains studied were identified strains and were obtained from the National Chemical Laboratory (NCL), Pune, India. The investigated microorganisms were *Bacillus cereus*, *Pseudomonas testosteroni*, *Klebsiella pneumoniae*, *Micrococcus flavus* and *Citrobacter freundii*.

Preparation of the test compound solutions. The different concentrations, *i.e.*, 20, 10 and 1 mg/L were prepared in DMSO for all the synthesized compounds.

Preparation of the plates and microbiological assays. A loopful of the given test strain was inoculated in 20 mL of N-broth (Nutrient Broth). To activate the given bacterial strain, the N-broth was incubated for 24 h in an incubator at 37 °C. The agar well diffusion method was used for the antibacterial assay. Molten agar (Mueller Hinton Agar No. 2, 28–30 mL) was added into a 100 mm diameter Petri dish. Care should be taken to avoid air bubbles during inoculation and pouring. To maintain sterile condition, all these procedures were done in a laminar air flow. The media were allowed to solidify. After solidification of the media, a well was made in the plates with the help of a cup-borer (0.85 cm), which was filled with a solution of a synthesized 4-aryltriazole derivative in DMSO.

The antibacterial activities of these synthesized compounds were determined by the inhibition zone formed by these compounds against the particular test bacterial strain.

RESULTS AND DISCUSSION

The physical data of the synthesized compounds are recorded in Table I.

The antibacterial activity of all the compounds in DMSO against the above mentioned bacteria is shown in Fig. 1. It can be observed that HAS-D6 showed the maximum activity against *B. cereus*, followed by HAS-D2, HAS-D4, HAS-D7, HAS-D8 and HAS-D9, which showed the same maximum activity against *B. cereus* whereas HAS-D1, HAS-D3, HAS-D5 and HAS-D10 showed no activity at all. The different activities of the synthesized compounds arise because of the different groups present. HAS-D6 contains the 2,4-dimethylphenyl group whereas

HAS-D2 contains the *p*-methoxyphenyl group. Thus, 2,4-dimethylphenyl and *p*-methoxyphenyl groups affect *B. cereus* in DMSO.

TABLE I. Physical constants of the synthesized compounds

| | R ₁ | Code | M.wt./g mol ⁻¹ | M.f. | R _f [*] value | M.p./°C | Yield/% |
|----|--|---------|---------------------------|---|-----------------------------------|---------|---------|
| 1 | 4-CH ₃ -C ₆ H ₄ | HAS-D1 | 297.38 | C ₁₆ H ₁₅ ON ₃ S | 0.63 | 190 | 66 |
| 2 | 4-OCH ₃ -C ₆ H ₄ | HAS-D2 | 313.38 | C ₁₆ H ₁₅ O ₂ N ₃ S | 0.59 | 196 | 79 |
| 3 | 4-Cl-C ₆ H ₄ | HAS-D3 | 317.80 | C ₁₆ H ₁₂ OCIN ₃ S | 0.78 | 160 | 81 |
| 4 | 2-NO ₂ -C ₆ H ₄ | HAS-D4 | 328.35 | C ₁₅ H ₁₂ O ₂ N ₄ S | 0.65 | 240 | 59 |
| 5 | 3-NO ₂ -C ₆ H ₄ | HAS-D5 | 328.35 | C ₁₅ H ₁₂ O ₃ N ₄ S | 0.45 | 260 | 77 |
| 6 | 2,4-(CH ₃) ₂ -C ₆ H ₃ | HAS-D6 | 311.40 | C ₁₇ H ₁₇ ON ₃ S | 0.51 | 162 | 74 |
| 7 | 2,3-Cl ₂ -C ₆ H ₃ | HAS-D7 | 352.24 | C ₁₅ H ₁₁ OCl ₂ N ₃ S | 0.70 | 169 | 83 |
| 8 | 2-CH ₃ -C ₆ H ₄ | HAS-D8 | 297.38 | C ₁₆ H ₁₅ ON ₃ S | 0.66 | 166 | 78 |
| 9 | 3-Cl,4-F-C ₆ H ₃ | HAS-D9 | 335.79 | C ₁₅ H ₁₁ OCIFN ₃ S | 0.61 | 135 | 64 |
| 10 | 2-OCH ₃ -C ₆ H ₄ | HAS-D10 | 313.38 | C ₁₆ H ₁₅ O ₂ N ₃ S | 0.55 | 110 | 68 |

* Acetone: benzene = 0.5 : 9.5 for 1, 2, 5, 6, 7 and 9; Methanol: hexane = 3 : 7 for 3, 4, 8 and 10

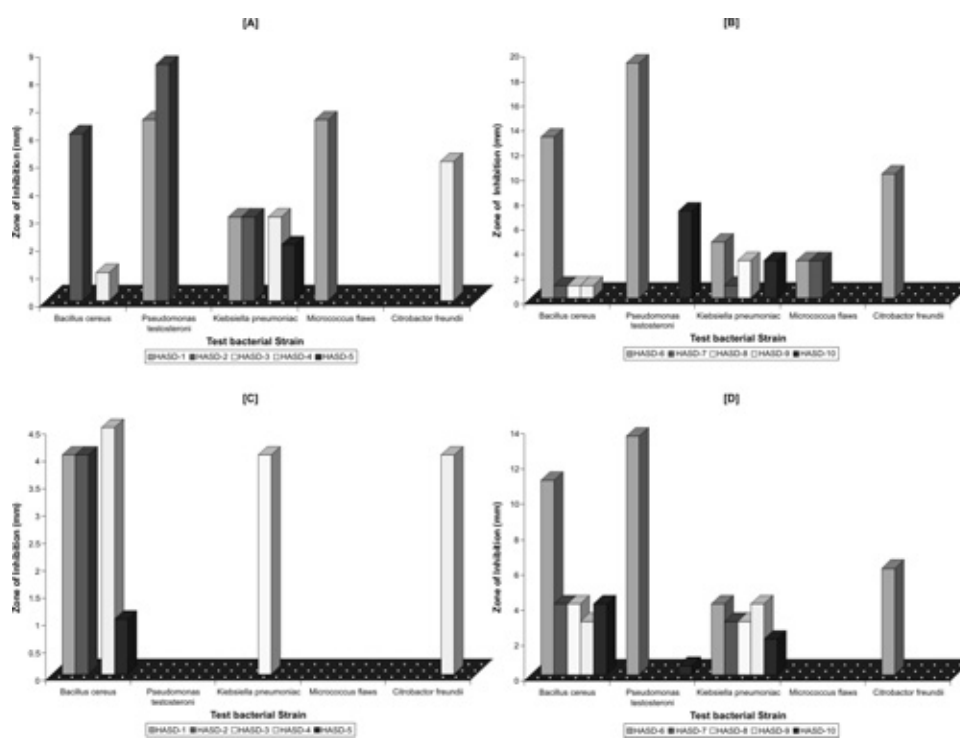


Figure 1. Antibacterial activity of compounds HAS-D1 to HAS-D10.

Against *P. testosteroni*, again HAS-D6 showed the maximum activity, followed by HAS-D2. However, against this bacteria, HAS-D1 and HAS-D10 also showed activity. HAS-D10 and HAS-D1 contain the *o*-methoxyphenyl and *p*-methylphenyl group, respectively, which were found to be effective against *P. testosteroni*. The other compounds showed no effect at all.

HAS-D6 also showed the maximum activity against *K. pneumoniae*. HAS-D3 and HAS-D9 showed no activity at all against this bacterium, while the other compounds showed some activity.

Against *M. flavus*, HAS-D1 showed the maximum activity, followed by HAS-D6 and HAS-D7. All other compounds had no effect on *M. flavus*.

Antibacterial activity against *C. freundii* was observed only with HAS-D6 and HAS-D4. The other compounds had no effect at all.

Thus, the substitute groups present in different compounds have different effects against different bacteria. It is observed from the above findings that the 2,4-dimethylphenyl group present in HAS-D6 was not effective against the bacteria studied in the present investigation.

The most susceptible bacteria is *K. pneumoniae* and the most resistant is *C. freundii*.

ИЗВОД

ЈЕДНОСТАВНА СИНТЕЗА И АНТИМИКРОБНА АКТИВНОСТ НЕКИХ 4-АРИЛТРИАЗОЛА

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Синтетисани су неки 4-арилтриазоли и испитано је њихово антибактеријско дејство на *Bacillus cereus*, *Pseudomonas testosteroni*, *Klebsiella pneumoniae*, *Micrococcus flavus* и *Citrobacter freundii*. 4-Арилтриазоли су добијени циклизацијом калијумових соли подебно супституисаних дитиокарбазинских киселина с ароматичним аминима. Новосинтезиована једињења су окарактерисана применом инфрацрвене, ¹H NMR спектроскопије и масене спектрометрије.

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