Ilam University of Medical Sciences

Plant Biotechnology Persa

Online ISSN: 2676-7414

Homepage: https://pbp.medilam.ac.ir

Synthesis and In Vitro Antitrypanosomal Evaluation of 2-Hydrazino-4-Thiazolidinones Containing 6-Phenylimidazo[2,1-b] [1,3,4] thiadiazole Moiety

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Article Info

Article type:
Original Article

Article History:
Received: 23 Sep 2025
Revised: 29 Jan 2026
Accepted: 25 Jan 2026
Published Online:

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A B S T R A C T

Objective: A convenient and effective method for the obtaining of a 4-thiazolidinone ring is the [2+3]-cyclocondensation reaction of S,N-binucleophiles with different equivalents of the dielectrophilic synthon [C2]2+. For this purpose, we achieved the synthesis of intermediate N1-methylidenethiosemicarbazones 3a-b starting from 2-amino/allylsulfanyl-5-ethyl-1,3,4-thiadiazoles 1-b via Vilsmeier-Haak formylation reaction following by condensation with thiosemicarbazide in acetic acid medium.

Methods: Organic Synthesis, NMR Spectroscopy, Elemental analysis, Pharmacological screening.

Results: The interaction of intermediate N1-methylidenethiosemicarbazones 3a-b with α-halocarboxylic (monochloroacetic, 2-bromopropionic, 2-bromobutanoic) acids or α-bromo-γ-butyrolactone in acetic acid at the presence of sodium acetate was performed. As results, a series of targeted 2-hydrazino-4-thiazolidinones containing 6-phenylimidazo[2,1-b] [1,3,4] thiadiazole moiety 4a-b and their 5-alkyl substituted derivatives 5a-d and 6a-b were obtained. The structure of the synthesized compounds was confirmed by elemental analysis and NMR spectroscopy. The synthesized compounds 3a-b, 4a-b, 5a-d, and 6a-b were evaluated for their in vitro antitrypanosomal activity against Trypanosoma brucei gambiense (Feo strain).

Conclusion: The results of in vitro screening of antitrypanosomal activity against Trypanosoma brucei gambiense (TBG) allowed us to identify three highly active compounds 4b, 5b and 5c, which exhibited significant trypanocidal activity with a range of IC50 values of 3.7-4.4 μM and were comparable to the reference drug nifurtimox (IC50 = 4.4 μM).

Keywords: 2-Hydrazono-4-thiazolidinones, Imidazo[2,1-b] [1,3,4] thiadiazoles, Heterocyclization Reaction, Spectral Data, Antitrypanosomal Activit

➢ **How to cite this paper**
Lelyukh M, Chaban I, Lysiuk R, Savchenko A, Yelahina N, Martyniuk D, Komarytsya O, Chaban T. Synthesis and In Vitro Antitrypanosomal Evaluation of 2-Hydrazino-4-Thiazolidinones Containing 6-Phenylimidazo[2,1-b] [1,3,4] thiadiazole Moiety. Plant Biotechnology Persa. 2026; 8(1): Proof.

DOI:
Publisher: Ilam University of Medical Sciences

Introduction

Neglected tropical diseases (NTDs) are a group of approximately 20 diseases that affect part of the population in Sub- and Tropical countries [1, 2]. In the past, pharmaceutical industries and governmental agencies have invested in the control, elimination and eradication of such diseases. Among these diseases, Chagas disease (CD) [3] and Human African trypanosomiasis (HAT) [4] are a public health problem, mainly in the countries from the American continent and sub-Saharan African. In humans, HAT is caused by two sub-species of the parasite known as T. brucei gambiense in central and western Africa and T. brucei rhodesiense in eastern and southern Africa [5, 6]. T. brucei gambiense accounts for more than 95% of reported cases [7]. In this context, the search for new therapeutic alternatives against such diseases has been growing in recent years, presenting cysteine proteases as the main strategy to discover new anti-trypanosomal drugs.

One of the promising directions in the field of chemistry and pharmacology of 4-thiazolidinones is the search for potential antiparasitic [8, 9], in particular antitrypanosomal [10-12] agents. The results demonstrated that the trypanocidal effect of substituted 4-thiazolidinones may be associated with the inhibition of several biological targets, including cysteine protease cruzain [13], cruzipain protease [14], and dolicholphosphate mannose synthase [15]. At the same time, highly active 4-thiazolidinone hydrazones are considered as bioisosteres of arylidentiosemicarbazones, which belong to a well-known class of antiprotozoal agents [16, 17].

On the other hand, of great interest for the bioorganic and medicinal chemistry is the bicyclic system formed by a thiadiazole ring fused with an imidazole ring – imidazo[2,1-b] [1,3,4] thiadiazole [18, 19]. Thus, imidazo[2,1-b] [1,3,4] thiadiazole derivatives possess a wide spectrum of biological activity, in particular antitumor [20, 21], antituberculosis [22, 23], antimicrobial [24, 25], antifungal [26], antioxidant [27, 28] and anti-inflammatory [28]. Therefore, a directed search for new antitrypanosomal agents among heterocyclic systems containing 2-hydrazono-4-thiazolidinone and pharmacologically attractive 6-arylimidazo[2,1-b] [1,3,4] thiadiazole moiety is promising and justified.

Experimental Part

Materials and methods

All reagents and solvents were of analytical grade, commercially available, and used without further purification or drying. The starting materials, 6-phenylimidazo[2,1-b] [1,3,4] thiadiazoles, were prepared according to previously reported procedures [29, 30].

Melting points were determined using a NAGEMA-K8 polarization microscope equipped with a Boetius heating stage and a digital thermometer (Ama-digit ad 14 th). Reported values are uncorrected.

The ^{1}H NMR spectra were recorded on a Varian Gemini 400 MHz spectrometer in DMSO-d6, with tetramethylsilane (TMS) as the internal standard. Chemical shifts are expressed in ppm on the δ scale.

Elemental analyses (C, H, N) were carried out on an Elementar Vario L cube instrument. The obtained values were within ±0.4% of the theoretical calculations.

Chemistry

General procedure for the synthesis of 2-ethyl/allylsulfanyl substituted 6-phenylimidazo[2,1-b]thiadiazoles 1a-b. A mixture of equimolar amounts of 2-ethyl- or 2-allylsulfanyl-1,3,4-thiadiazole-5-amine and α-bromoacetophenone (0.02 mol each) is refluxed in 100 ml of ethanol for 8 h. The excess solvent is distilled off under reduced pressure, and the corresponding hydrobromide precipitate is filtered off, suspended in water, and an aqueous solution of sodium bicarbonate is added until the free base is isolated. The resulting precipitate of the reaction product is filtered off, washed three times with water, dried, and recrystallized from ethanol or acetonitrile.

2-Ethyl-6-phenylimidazo[2,1-b][1,3,4]thiadiazole (1a). Yield 75%; m.p. = 143–144○C. 1H NMR (400 MHz, DMSO-d6): δH = 8.62 (s, 1H, 5-H), 7.87 (d, 2H, J = 7.2 Hz, arom), 7.41 (t, 2H, J = 7.3 Hz, arom), 7.28 (t, 1H, J = 7.3 Hz, arom), 3.07 (q, 2H, J = 7.5 Hz, CH2CH3), 1.37 (t, 3H, J = 7.5 Hz, CH2CH3). Calcd for C12H11N3S: C, 62.86; H, 4.84; N, 18.32. Found: C, 63.04; H, 4.96; N, 18.48.

2-Allylsulfanyl-6-phenylimidazo[2,1-b][1,3,4]thiadiazole (1b). Yield 73%; m.p. = 111–112○C. 1H NMR (400 MHz, DMSO-d6): δH = 8.68 (s, 1H, 5-H), 7.87 (d, 2H, J = 7.5 Hz, arom), 7.42 (t, 2H, J = 7.4 Hz, arom), 7.28 (t, 1H, J = 7.4 Hz, arom), 6.06-5.92 (m, 1H, –CH=), 5.37 (d, 1H, J = 16.9 Hz, =CH2), 5.23 (d, 1H, J = 10.0 Hz, =CH2), 3.98 (d, 2H, J = 6.8 Hz, SCH2–). Calcd for C13H11N3S2: C, 57.12; H, 4.06; N, 15.37. Found: C, 57.31; H, 4.22; N, 15.53.

General procedure for the synthesis of 2-ethyl/allylsulfanyl-6-phenylimidazo[2,1-b]thiadiazole-5-carbaldehydes 2a-b. The Vilsmeier-Haack reagent is prepared by adding phosphorus oxochloride (3 ml) to absolute dimethylformamide (20 ml) at 0°C and stirring for 5-10 min. Then, 0.01 mol of the corresponding 2-substituted 6-phenylimidazolo[2,1-b][1,3,4]thiadiazole 1a or 1b is added to the prepared reagent and stirred first at 0°C for 30 min, then for 2 h at room temperature and the next 2 h at 60°C. After that, a solution of sodium bicarbonate is added to the reaction mixture, heated to 90°C and stirred for another 2 h, then cooled and poured into water. The reaction product is extracted with chloroform (three times 30 ml), the resulting extracts are combined, washed with water and dried over anhydrous sodium sulfate. The solvent is distilled off under vacuum, and the resulting dry residue is recrystallized from a mixture of toluene and petroleum ether (1:2).

2-Ethyl-6-phenylimidazo[2,1-b][1,3,4]thiadiazole-5-carbaldehyde (2a). Yield 92%; m.p. = 100–101○C. 1H NMR (400 MHz, DMSO-d6): δH = 9.96 (s, 1H, CHO), 7.96-7.93 (m, 2H, arom), 7.53-7.51 (m, 3H, arom), 3.16 (q, 2H, J = 7.5 Hz, CH2CH3), 1.37 (t, 3H, J = 7.5 Hz, CH2CH3). Calcd for C13H11N3OS: C, 60.68; H, 4.31; N, 16.33. Found: C, 60.85; H, 4.46; N, 16.57.

2-Allylsulfanyl-6-phenylimidazo[2,1-b][1,3,4]thiadiazole-5-carbaldehyde (2b). Yield 89%; m.p. = 77–78○C. 1H NMR (400 MHz, DMSO-d6): δH = 9.97 (s, 1H, CHO), 7.96-7.93 (m, 2H, arom), 7.53-7.51 (m, 3H, arom), 6.07-5.94 (m, 1H, –CH=), 5.44 (d, 1H, J = 17.0 Hz, =CH2), 5.25 (d, 1H, J = 10.0 Hz, =CH2), 4.03 (d, 2H, J = 6.9 Hz, SCH2–). Calcd for C14H11N3OS2: C, 55.79; H, 3.68; N, 13.94. Found: C, 55.96; H, 3.81; N, 14.14.

General procedure for the synthesis of N1-[(6-phenylimidazo[2,1-b]thiadiazol-5-

yl)methylidene)-thiosemicarbazones 3a-b. A mixture of equimolar amounts (0.01 mol each) of thiosemicarbazide and the corresponding 6-phenylimidazo[2,1-b][1,3,4]thiadiazole-5-carbaldehyde 2a or 2b in acetic acid (15 ml) heated under reflux for 45 min. After complete cooling of the reaction mixture, the formed precipitate is filtered off, washed sequentially with acetic acid, water and ethanol and recrystallized from acetic acid.

N1-[(2-Ethyl-6-phenylimidazo[2,1-b][1,3,4]thiadiazol-5-yl)methylidene]thiosemicarbazone (2a). Yield 83%; m.p. = 239–240○C. 1H NMR (400 MHz, DMSO-d6): δH = 11.57 (s, 1H, NH), 8.46 (s, 1H, 5-CH=, imidaz), 8.40 (brs, 1H, NH2), 7.71 (d, 2H, J = 7.4 Hz, arom), 7.49 (t, 2H, J = 7.2 Hz, arom), 7.42 (t, 1H, J = 7.2 Hz, arom), 7.18 (brs, 1H, NH2), 3.13 (q, 2H, J = 7.5 Hz, CH2CH3), 1.35 (t, 3H, J = 7.5 Hz, CH2CH3). Calcd for C14H14N6S2: C, 50.89; H, 4.27; N, 25.43. Found: C, 51.12; H, 4.46; N, 25.68.

N1-[(2-Allylsulfanyl-6-phenylimidazo[2,1-b][1,3,4]thiadiazol-5 yl)methylidene]thiosemicarbazone (2b). Yield 79%; m.p. = 159–160○C. 1H NMR (400 MHz, DMSO-d6): δH = 11.58 (s, 1H, NH), 8.46 (s, 1H, 5-CH=, imidaz), 8.44 (brs, 1H, NH2), 7.71 (d, 2H, J = 7.5 Hz, arom), 7.49 (t, 2H, J = 7.3 Hz, arom), 7.42 (t, 1H, J = 7.3 Hz, arom), 7.16 (brs, 1H, NH2), 6.06-5.95 (m, 1H, –CH=), 5.38 (d, 1H, J = 16.9 Hz, =CH2), 5.23 (d, 1H, J = 10.0 Hz, =CH2), 4.00 (d, 2H, J = 6.7 Hz, SCH2–). Calcd for C15H14N6S3: C, 48.11; H, 3.77; N, 22.44. Found: C, 48.34; H, 3.96; N, 22.65.

General procedure for the synthesis of 2-[(6-phenylimidazo[2,1-b]thiadiazol-5-yl)methylidene]-hydrazono-4-thiazolidinones 4a-b and their 5-methyl/ethyl substituted derivatives 5a-d. In a round-bottom flask, 0.001 mol of the corresponding N1-methylidenthiosemicarbazone 3a or 3b, 0.0011 mol of monochloroacetic, 2-bromopropionic or 2-bromobutanoic acid, and 0.001 mol of anhydrous sodium acetate are placed, 10 ml of acetic acid was added and the mixture was refluxed for 2.5 h. The precipitate formed after cooling the reaction mixture was filtered off, washed successively with acetic acid, water and ethanol and recrystallized from a mixture of DMF – acetic acid (1:2)

2-[(2-Ethyl-6-phenylimidazo[2,1-b][1,3,4]thiadiazol-5-ylmethylidene)hydrazono]thiazolidine-4-one (4a). Yield 77%; m.p. = 257–258○C. 1H NMR (400 MHz, DMSO-d6): δH = 12.03 (s, 1H, NH, thiaz), 8.60 (s, 1H, 5-CH=, imidaz), 7.98 (d, 2H, J = 7.3 Hz, arom), 7.49 (t, 2H, J = 7.4 Hz, arom), 7.41 (t, 1H, J = 7.3 Hz, arom), 3.91 (s, 2H, 5-CH2, thiaz), 3.14 (q, 2H, J = 7.4 Hz, CH2CH3), 1.39 (t, 3H, J = 7.4 Hz, CH2CH3). Calcd for C16H14N6OS2: C, 51.88; H, 3.81; N, 22.69. Found: C, 52.09; H, 3.96; N, 22.93.

2-[(2-Allylsulfanyl-6-phenylimidazo[2,1-b][1,3,4]thiadiazol-5-ylmethylidene)hydrazono]-thiazolidine-4-one (4b). Yield 71%; m.p. = 229–230○C. 1H NMR (400 MHz, DMSO-d6): δH = 12.00 (s, 1H, NH, thiaz), 8.56 (s, 1H, 5-CH=, imidaz), 7.93 (d, 2H, J = 7.3 Hz, arom), 7.50 (t, 2H, J = 7.0 Hz, arom), 7.42 (t, 1H, J = 7.1 Hz, arom), 6.11-5.98 (m, 1H, –CH=), 5.43 (d, 1H, J = 17.3 Hz, =CH2), 5.23 (d, 1H, J = 9.9 Hz, =CH2), 4.07 (d, 2H, J = 6.9 Hz, SCH2–), 3.93 (s, 2H, 5-CH2, thiaz). Calcd for C17H14N6OS3: C, 49.26; H, 3.40; N, 20.27. Found: C, 49.48; H, 3.56; N, 20.48.

2-[(2-Ethyl-6-phenylimidazo[2,1-b][1,3,4]thiadiazol-5-ylmethylidene)hydrazono]-

5-methyl-thiazolidine-4-one (5a). Yield 75%; m.p. = 272–273○C. 1H NMR (400 MHz, DMSO-d6): δH = 11.94 (s, 1H, NH, thiaz), 8.61 (s, 1H, 5-CH=, imidaz), 7.98 (d, 2H, J = 7.5 Hz, arom), 7.50 (t, 2H, J = 7.7 Hz, arom), 7.42 (t, 1H, J = 7.4 Hz, arom), 4.32 (q, 1H, 5-H, thiaz), 3.14 (q, 2H, J = 7.4 Hz, CH2CH3), 1.53 (d, 3H, J = 7.2 Hz, 5-CH3, thiaz), 1.40 (t, 3H, J = 7.4 Hz, CH2CH3). Calcd for C17H16N6OS2: C, 53.11; H, 4.19; N, 21.86. Found: C, 53.34; H, 4.35; N, 22.08.

2-[(2-Ethyl-6-phenylimidazo[2,1-b][1,3,4]thiadiazol-5-ylmethylidene)hydrazono]-5-ethyl-thiazolidine-4-one (5b). Yield 78%; m.p. = 256–257○C. 1H NMR (400 MHz, DMSO-d6): δH = 11.95 (br.s, 1H, NH, thiaz), 8.61 (s, 1H, 5-CH=, imidaz), 7.98 (d, 2H, J = 7.1 Hz, arom), 7.49 (t, 2H, J = 7.6 Hz, arom), 7.42 (t, 1H, J = 7.3 Hz, arom), 4.25 (dd, 1H, J = 3.5 Hz, 4.4 Hz, 5-H, thiaz), 3.14 (q, 2H, J = 7.5 Hz, CH2CH3), 2.05-1.96, 1.87-1.77 (2*m, 2H, 5-CH2CH3, thiaz), 1.40 (t, 3H, J = 7.5 Hz, CH2CH3), 0.98 (t, 3H, J = 7.3 Hz, 5-CH2CH3, thiaz). Calcd for C18H18N6OS2: C, 54.25; H, 4.55; N, 21.09. Found: C, 54.43; H, 4.72; N, 21.32.

2-[(2-Allylsulfanyl-6-phenylimidazo[2,1-b][1,3,4]thiadiazol-5-ylmethylidene)hydrazono]-5-methyl-thiazolidine-4-one (5c). Yield 74%; m.p. = 224–225○C. 1H NMR (400 MHz, DMSO-d6): δH = 11.99 (s, 1H, NH, thiaz), 8.58 (s, 1H, 5-CH=, imidaz), 7.93 (d, 2H, J = 7.1 Hz, arom), 7.51 (t, 2H, J = 7.2 Hz, arom), 7.42 (t, 1H, J = 7.1 Hz, arom), 6.13-5.99 (m, 1H, –CH=), 5.43 (d, 1H, J = 17.4 Hz, =CH2), 5.23 (d, 1H, J = 9.8 Hz, =CH2), 4.24 (q, 1H, J = 7.1 Hz, 5-H, thiaz), 4.08 (d, 2H, J = 6.9 Hz, SCH2–), 1.51 (d, 3H, J = 7.2 Hz, 5-CH3, thiaz). Calcd for C18H16N6OS3: C, 50.45; H, 3.76; N, 19.61. Found: C, 50.68; H, 3.92; N, 19.87.

2-[(2-Allylsulfanyl-6-phenylimidazo[2,1-b][1,3,4]thiadiazol-5-ylmethylidene)hydrazono]-5-ethyl-thiazolidine-4-one (5d). Yield 76%; m.p. = 225–226○C. 1H NMR (400 MHz, DMSO-d6): δH = 11.98 (s, 1H, NH, thiaz), 8.57 (s, 1H, 5-CH=, imidaz), 7.90 (d, 2H, J = 7.5 Hz, arom), 7.48 (t, 2H, J = 7.3 Hz, arom), 7.42 (t, 1H, J = 7.2 Hz, arom), 6.12-6.01 (m, 1H, –CH=), 5.44 (d, 1H, J = 16.9 Hz, =CH2), 5.22 (d, 1H, J = 10.0 Hz, =CH2), 4.24 (dd, 1H, J = 3.7 Hz, 4.1 Hz, 5-H, thiaz), 4.09 (d, 2H, J = 6.4 Hz, SCH2–), 2.03-1.96, 1.82-1.76 (2*m, 2H, 5-CH2CH3, thiaz), 0.96 (t, 3H, J = 7.2 Hz, 5-CH2CH3, thiaz). Calcd for C19H18N6OS3: C, 51.56; H, 4.10; N, 18.99. Found: C, 51.78; H, 4.18; N, 19.21.

General procedure for the synthesis of 5-(β-acetoxyethylene)-2-[(6-phenylimidazo[2,1-b]thiadiazol-5-yl)methylidene]hydrazono-4-thiazolidinones 6a-b. In a round-bottom flask, 0.001 mol of the corresponding N1-methylidenthiosemicarbazone 3a or 3b, 0.0011 mol of α-bromo-γ-butyrolactone, and 0.001 mol of anhydrous sodium acetate are placed, 10 ml of acetic acid was added and the mixture was refluxed for 2.5 h. The precipitate formed after cooling the reaction mixture was filtered off, washed successively with acetic acid, water and ethanol and recrystallized from a mixture of DMF – acetic acid (1:2)

2-[(2-Ethyl-6-phenylimidazo[2,1-b][1,3,4]thiadiazol-5-ylmethylidene)hydrazono]-5-(β-acetoxyethylene)thiazolidine-4-one (6a). Yield 76%; m.p. = 186–187○C. 1H NMR (400 MHz, DMSO-d6): δH = 12.06 (br.s, 1H, NH, thiaz), 8.60 (s, 1H, 5-CH=, imidaz), 7.99 (d, 2H, J = 7.7 Hz, arom), 7.50 (t, 2H, J = 7.6 Hz, arom), 7.41 (t, 1H, J = 7.4 Hz, arom), 4.30 (dd, 1H, J = 3.2 Hz, 4.6 Hz, 5-H, thiaz), 4.25-4.21, 4.17-4.11 (2*m, 2H, 5-AcOCH2CH2,

thiaz), 3.14 (q, 2H, J = 7.4 Hz, CH2CH3), 2.36-2.31, 2.16-2.10 (2*m, 2H, 5-AcOCH2CH2, thiaz), 2.01 (s, 3H, CH3CO), 1.39 (t, 3H, J = 7.4 Hz, CH2CH3). Calcd for C20H20N6O3S2: C, 52.62; H, 4.42; N, 18.41. Found: C, 52.86; H, 4.61; N, 18.67.

2-[(2-Allylsulfanyl-6-phenylimidazo[2,1-b][1,3,4]thiadiazol-5-ylmethylidene)hydrazono]-5-(β-acetoxyethylene)thiazolidine-4-one (6b). Yield 79%; m.p. = 193–194○C. 1H NMR (400 MHz, DMSO-d6): δH = 12.05 (s, 1H, NH, thiaz), 8.58 (s, 1H, 5-CH=, imidaz), 7.92 (d, 2H, J = 6.2 Hz, arom), 7.49 (t, 2H, J = 6.7 Hz, arom), 7.43 (t, 1H, J = 6.6 Hz, arom), 6.08-6.03 (m, 1H, –CH=), 5.43 (d, 1H, J = 17.0 Hz, =CH2), 5.22 (d, 1H, J = 9.8 Hz, =CH2), 4.32 (t, 1H, J = 5.7 Hz, 5-H, thiaz), 4.25-4.20, 4.15-4.12 (2*m, 2H, 5-AcOCH2CH2, thiaz), 4.08 (d, 2H, J = 5.9 Hz, SCH2–), 2.34-2.29, 2.16-2.11 (2*m, 2H, 5-AcOCH2CH2, thiaz), 2.01 (s, 3H, CH3CO). Calcd for C21H20N6O3S3: C, 50.38; H, 4.03; N, 16.79. Found: C, 50.61; H, 4.17; N, 16.97.

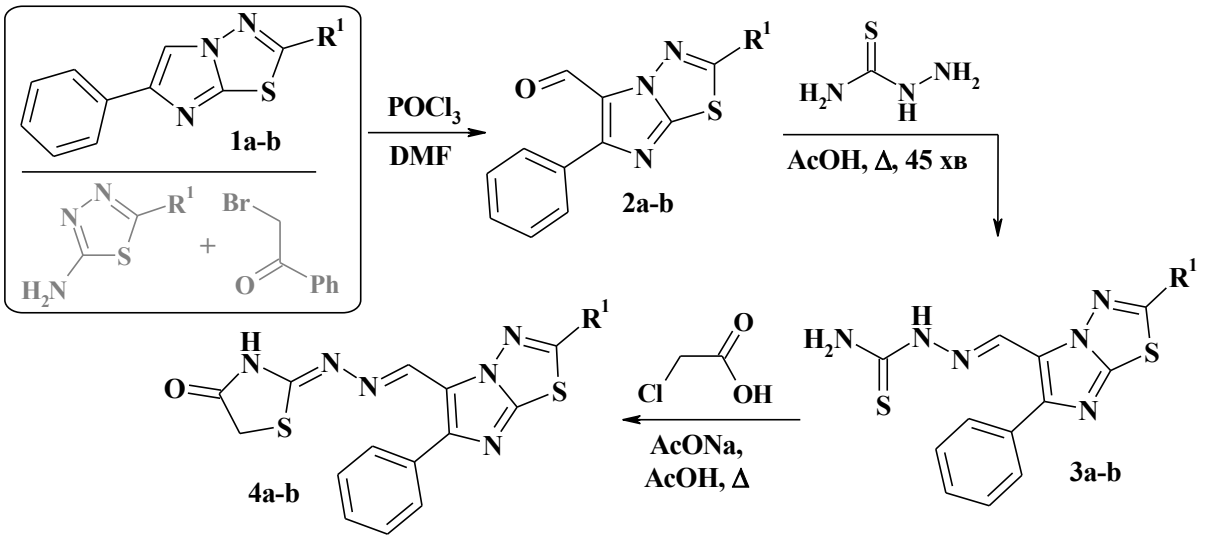
Anti-trypanosomal Activity Assay

Bloodstream forms of T. brucei gambiense (Feo strain) were maintained in HMI-9 medium supplemented with 10% fetal calf serum (FCS) at 37°C in a 5% CO2 atmosphere [31]. Parasites in the logarithmic growth phase were harvested by centrifugation at 3000 x gand immediately used for assays. Drug sensitivity was assessed using the resazurin-based assay, which measures the conversion of the redox-sensitive dye resazurin to a fluorescent product by metabolically active cells, as previously described [32]. Stock solutions of the test compounds were prepared in pure DMSO. Parasites (1 x 104 cells/well) were cultured in 96-well plates in the presence or absence of serial dilutions of the test inhibitors in a final volume of 200 µl. After a 72-h incubation, resazurin solution was added in each well at the final concentration of 45 µM and fluorescence was measured at 530 nm and 590 nm wavelengths after a further 4-h incubation. The percentage of growth inhibition was calculated by comparing the fluorescence intensity of drug-treated parasites with that of untreated controls, with DMSO serving as vehicle control. The IC50 values were derived from dose–response curves over a concentration range of 10–0.625 µg/ml and expressed in micromolar units. Results represent the mean ± SD of three independent experiments.

Results

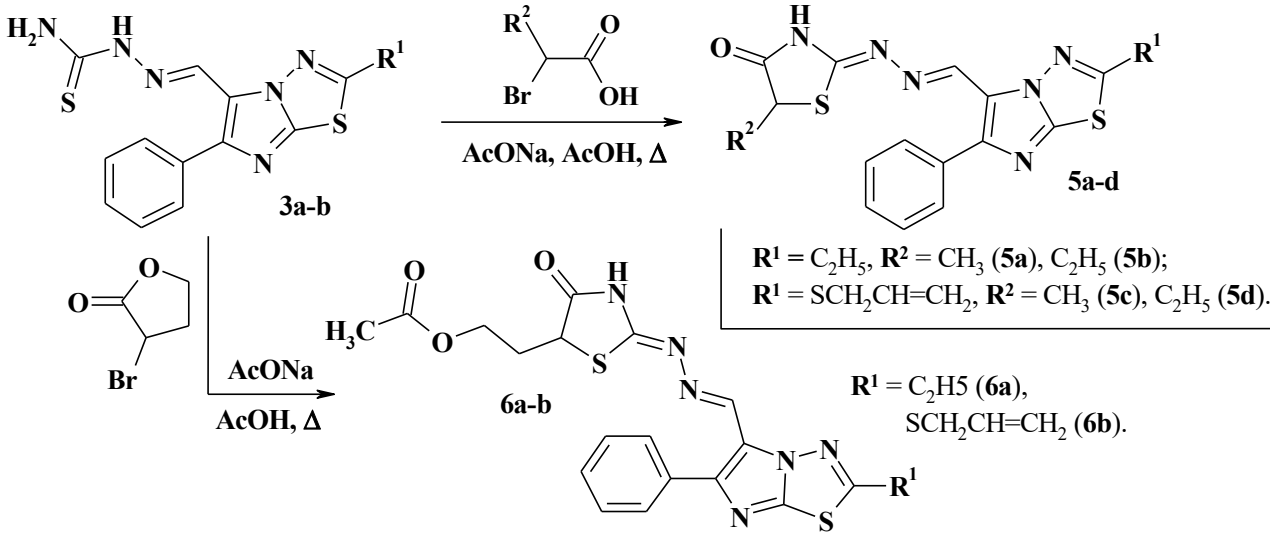
Chemistry

Following the reaction of the starting 2-amino/allylsulfanyl-5-ethyl-1,3,4-thiadiazoles with α-bromoacetophenone the corresponding 2-ethyl/allylsulfanyl 6-arylimidazo[2,1-b][1,3,4]thiadiazoles 1a-b were obtained in a good yields. The formylation of compounds 1a-b under Vilsmeier-Haak reaction conditions afforded the respective 6-phenylmidazo[2,1-b][1,3,4]thiadiazole-5-carbaldehydes 2a-b. Further condensation of carbaldehydes 2a-b with thiosemicarbazide in acetic acid medium under the reflux leads to the formation of N1-(6-phenylimidazo[2,1-b][1,3,4]thiadiazol-5-ylmethylidene)thiosemicarbazones 3a-b as intermediate reagents for further synthetic studies. Thus, further interaction of thiosemicarbazones 3a-b with monochloroacetic acid in refluxing acetic acid at the presence of sodium acetate (Scheme 1) afforded the target 2-methylidenehydrazino-4-thiazolidinones containing imidazo[2,1-b][1,3,4]thiadiazole moiety 4a-b.



Scheme 1. Synthesis of N1-(6-Phenylimidazo[2,1-b][1,3,4]thiadiazol-5-ylmethylidene)-thiosemicarbazones and Their Modification into Imidazo[2,1-b][1,3,4]thiadiazole Containing 2-Hydrazino-4-thiazolidinones

In addition, modification of N1-methylidenthiosemicarbazones 3a-b via interaction with 2-bromopropionic or 2-bromobutanoic acids at the presence of sodium acetate in refluxing acetic acid (Scheme 2) allowed to obtain a series of 5-methyl/ethyl substituted 2-methylidenehydrazino-4-thiazolidinones 5a-d containing a 6-phenylimidazo[2,1-b][1,3,4]thiadiazole moiety. Instead, the interaction of compounds 3a-b with α-bromo-γ-butyrolactone under similar conditions leads to the formation of 5-(β-acetoxyethyl)-2-(6-phenylimidazo[2,1-b][1,3,4]thiadiazol-5-ylmethylidene)hydrazono-4-thiazolidinones 6a-b:



Scheme 2. Synthesis of 5-Methyl/ethyl/ β-acetoxyethyl Substituted 2-Methylidenehydrazino-4-thiazolidinones Containing a 6-Phenylimidazo[2,1-b][1,3,4]thiadiazole Moiety

In vitro Evaluation of Antitrypanosomal Activity

Ten synthesized compounds (3a-b, 4a-b, 5a-d, and 6a-b) were initially screened at fixed concentrations of 50, 10, and 1 µg/ml. Growth inhibition was quantified by Alamar Blue fluorescence relative to untreated controls. Compounds demonstrating >40–50% inhibition at 10 µg/ml were selected for IC50 determination (Table 1). Nifurtimox, clinically used in combination with eflornithine for the treatment of gambiense human African trypanosomiasis [33], was included as a reference drug.

Table 1. Antitrypanosomal Activity of Synthesized 6-Phenylimidazo[2,1-b][1,3,4]thiadiazole Substituted 2-Methylidenehydrazino-4-thiazolidinones and Their 5-Alkyl Derivatives.

Compound	R1	R2	IC50		IC50	
			µg/ml	SD	µM	SD
3a	C2H5	–	6.9	0.2	20.9	0.6
3b	SCH2CH=CH2	–	2.3	0.1	6.1	0.3
4a	C2H5	–	6.7	0.8	18.0	2.3
4b	SCH2CH=CH2	–	1.6	0.3	3.9	0.7
5a	C2H5	CH3	>10	-	>26	-
5b	C2H5	C2H5	1.5	0.1	3.7	0.1
5c	SCH2CH=CH2	CH3	1.9	0.3	4.4	0,7
5d	SCH2CH=CH2	C2H5	9.1	2.1	20.6	4.7
6a	C2H5	CH3COCH2CH2	5.4	0.9	11.8	2.0
6b	SCH2CH=CH2	CH3COCH2CH2	2.9	0.3	5.7	0.6
Nifurtimox	–	–	–	–	4.4	0.7

Discussion

The structures of the synthesized compounds were confirmed by ^{1}H NMR spectroscopy and elemental analysis. Spectroscopic data obtained for all new derivatives were consistent with the proposed structures. In particular, the proton in position 5 of 6-phenylimidazo[2,1-b][1,3,4]thiadiazoles 1a-b appears as a singlet at δ ~ 8.68-8.62 ppm, which together with the absence of signals from the NH2 group of the starting amino derivatives reliably confirms the course of the cyclization and the formation of the target compounds. A feature of the spectral pattern for 5-formyl derivatives 2a-b, which allows to reliably interpret the successful completion of the formylation reaction, is the absence of the C5-H proton signal and the presence of a singlet at 9.97-9.96 ppm, which corresponds to the aldehyde group –CHO.

The protons of the methylene group at position 5 of the thiazolidine ring in the NMR spectra of the 5-unsubstituted derivatives 4a-b forms a singlet at δ ~ 3.93-3.91 ppm. The methyl group in the NMR spectra of 5-methyl-2-hydrazonothiazolidin-4-ones 5a and 5c forms a three-proton doublet in the

range of δ ~ 1.53-1.51 ppm. Instead, the ethyl group in position 5 of the thiazolidine ring in the NMR spectra of compounds 5b and 5d corresponds to a three-proton triplet at δ ~ 0.98-0.96 ppm and two multiplets in the range of δ ~ 2.05-1.76 ppm. The β-acetoxyethyl fragment in the NMR spectra of compounds 6a and 6b resonates as a three-proton singlet at δ ~ 2.01 ppm, formed by the protons of the acetyl group CH3CO, and four multiplets with an integrated intensity of one proton for each in the range of δ ~ 4.25-4.11 ppm (OCH2CH2) and δ ~ 2.36-2.10 ppm (OCH2CH2).

Among the tested compounds, the thiazolidinone-imidazo[2,1-b]thiadiazole hybrids 4b, 5b, and 5c exhibited the most potent trypanocidal activity, with IC50 values ranging from 3.7 to 4.4 μM, which was comparable or better than that of the reference drug nifurtimox (IC50 = 4.4 μM). Compound 5a did not show an inhibitory effect on the growth of parasites in the tested conditions. Considering that the best activity was shown by both the 5-unsubstituted derivative 4b and compounds with various substituents (5b, R2 = C2H5; 5c, R2 = CH3), it can be assumed that the nature of the substituent at position 5 of the thiazolidine ring does not play a critical role in the manifestation of antitrypanosomal activity. Structure-activity analysis revealed that the presence of an S-allyl substituent at position 2 of the imidazo[2,1-b][1,3,4]thiadiazole core was more favorable for trypanocidal activity compared with the corresponding ethyl group.

Conclusion

A series of 2-hydrazino-4-thiazolidinones incorporated 6-phenylimidazo[2,1-b][1,3,4]thiadiazole moiety and their 5-alkyl substituted derivatives were synthesized by reaction of N1-(6-phenylimidazo[2,1-b][1,3,4]thiadiazol-5-ylmethylidene)-thiosemicarbazones with (monochloroacetic, 2-bromopropionic, 2-bromobutanoic) acids or α-bromo-γ-butyrolactone in acetic acid at the presence of sodium acetate. The synthesized compounds were evaluated for their antitrypanosomal activity against T. brucei gambience (Feo strain). Trypanocidal activity assay of the synthesized compounds has allowed us to identify thiazolidinone- imidazo[2,1-b]thiadiazole hybrids 4b, 5b and 5c, which were found to be the most active derivatives, with a range of IC50 values of 3.7-4.4 μM. It was established that the presence of S-allyl group in position 2 of the imidazo[2,1-b][1,3,4]thiadiazole core is more optimal in terms of the manifestation of the trypanocidal effect than the ethyl one.

Funding

This research received no external funding.

Acknowledgements

Gratitude is extended to the Armed Forces of Ukraine for enabling the conduct of the research and to the editorial board of the journal for providing the opportunity to publish the results

Conflicts of Interest

The authors declare no conflict of interest.

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