Synthesis, Antibacterial and Antitubercular Activities of Some 5H-Thiazolo[3,2-a]pyrimidin-5-ones and Sulfonic Acid Derivatives

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Academic Editor: Jean Jacques Vanden Eynde

Received: 4 August 2015 / Accepted: 3 September 2015 / Published: 10 September 2015

Abstract: A series of 5H-thiazolo[3,2-a]pyrimidin-5-ones were synthesized by the cyclization reactions of S-alkylated derivatives in concentrated H2SO4. Upon treatment of S-alkylated derivatives at different temperatures, intramolecular cyclization to 7-(substituted phenylamino)-5H-thiazolo[3,2-a]pyrimidin-5-ones or sulfonation of cyclized products to sulfonic acid derivatives occurred. The structures of the target compounds were confirmed by IR, 1H-NMR, 13C-NMR and HRMS studies. The compounds were evaluated for their preliminary in vitro antibacterial activity against some Gram-positive and Gram-negative bacteria and screened for antitubercular activity against Mycobacterium tuberculosis by the broth dilution assay method. Some compounds showed good antibacterial and antitubercular activities.

Keywords: thiazolo[3,2-a]pyrimidine; sulfonation; antibacterial; antitubercular
1. Introduction

The rapid development of bacterial drug resistance is growing into a global problem. Consequently, there is a pressing need to develop new antimicrobial drugs with potent activity in order to overcome the bacterial drug resistance. Electron-rich nitrogen heterocycles and sulfur compounds play an important role in diverse biological activities. Thiazolo[3,2-a]pyrimidine nucleus have been consistently regarded as structural analogs of biogenic purine bases and can be considered as potential purine antagonists [1,2]. These heterocyclic systems are the key chemical building blocks for numerous compounds that play important roles in the functioning of biologically active molecules. As one type of those heterocyclic rings, 5H-thiazolo[3,2-a]pyrimidin-5-ones are considered a promising class of bioactive heterocyclic compounds encompassing a diverse range of biological activities such as anti-inflammatory [3,4], antihypertensive [5], antifungal [6], antibiotic [7], antiviral [8], antioxidant [9], antitumor [10,11], anti-HIV [12], calcium channel blocking [13], antitubercular [14], glutamate receptor antagonistic [15], 5-HT2a receptor antagonistic [16] and group II metabotropic glutamate receptor antagonist activities [15]. Those compounds have also been reported as inhibitors of CDC25B phosphatase [17], Bcl-2 family proteins [17], and acetylcholinesterase enzymes [18].

The sulfonic acid group represents a key structural motif in both synthetic and medicinal chemistry. The phosphate functional group can be replaced by sulfonic acid moieties via bioisosteric replacement. These features are functionally interchangeable due to their ability to adopt a negative charge at biological pH values [19]. Many compounds containing sulfonic acid groups are well known as antibacterial [20–23], antifungal [24–28] and antitubercular agents [29]. Additionally, compounds containing sulfonic acid groups are used as dyes [30], and in metal arenesulfonate complexes [31].

It has been found that some 5H-thiazolo[3,2-a]pyrimidin-5-one structural analogues possess potent antimicrobial activity. A literature survey revealed that different halogen-substitution positions on the phenyl ring of thiazolotriazinones (I, Figure 1) result in a wide range of important pharmacological properties [32]. Moreover, aminotriazolothiadiazines (II, Figure 1) showed very good antibacterial and antifungal activities at 6.25 mg/mL concentrations [33]. On the other hand, it was reported that the introduction of a sulfonic acid group might augment the antimicrobial activity [34]. Numerous 4-(1H-benzoimidazol-2-yl)-benzenesulfonic acids (III, Figure 1) were found to be the most effective antibacterial and antifungal compounds. In addition, the role of electron-withdrawing nitro groups in increasing the antimicrobial activity was noted.

![Figure 1. Model compounds with pharmacological activities.](image-url)
Encouraged by the enormous pharmacological importance of 5\(H\)-thiazolo[3,2-\(a\)]pyrimidin-5-ones and the sulfonic acid motif, we focused on applying a scaffold hopping approach and developing a novel series of substituted 5\(H\)-thiazolo[3,2-\(a\)]pyrimidin-5-ones and their sulfonic acid derivatives. These compounds were subsequently evaluated for their in vitro antibacterial and antitubercular activity.

2. Results and Discussion

2.1. Chemistry

The synthesis of the new 5\(H\)-thiazolo[3,2-\(a\)]pyrimidin-5-ones and their derivatives containing a sulfonic acid moiety is summarized in Scheme 1.

\[ \text{Reagents and Conditions: I, } 170 \, ^\circ \text{C, reflux; II, } \text{K}_2\text{CO}_3, \text{ substituted phenacyl halides, DMF; III, } \text{concentrated } \text{H}_2\text{SO}_4, 20 \, ^\circ \text{C or } 80 \, ^\circ \text{C; IV, Fe, NH}_4\text{Cl, } 2:1 \text{ ethanol/water, } 80 \, ^\circ \text{C; V, } \text{concentrated } \text{H}_2\text{SO}_4, 20 \, ^\circ \text{C; VI, Fe, } \text{NH}_4\text{Cl, } 2:1 \text{ ethanol/water, } 80 \, ^\circ \text{C; VII, } \text{concentrated } \text{H}_2\text{SO}_4, 80 \, ^\circ \text{C.} \]

\text{Scheme 1. Synthesis of 5\(H\)-thiazolo[3,2-\(a\)]pyrimidin-5-one derivatives.}

The 6-substituted anilino-2-thiouracil starting materials 1a–c were synthesized according to known procedures based on the reactions of 6-amino-2-thiouracils with substituted anilines in the presence of aniline hydrochloride at high temperature [35–37]. Thus, the obtained thiopyrimidines 1a–c were nearly quantitatively S-alkylated with the appropriate substituted phenacyl halides in the presence of anhydrous potassium carbonate [36,38]. The products 2a–f could be used in subsequent reactions without further purification. The S-alkylated derivatives are proved to exist in solution largely in the lactam form as indicated from spectroscopic studies. The tautomeric hydrogen was found to favour N3 rather than N1 [39–43] (Scheme 2).
Scheme 2. A plausible mechanism for this selective cyclization

Inspection of the 400-MHz $^1$H-NMR spectra of the S-alkylated derivatives revealed an interesting phenomenon. The $^1$H-NMR spectrum (DMSO-$d_6$) of compound 2a showed the characteristic singlet at $\delta$ 5.02 ppm for the methenyl protons and at $\delta$ 5.46 ppm for the 5-H proton of pyrimidine, the 3-nitrophenyl protons appeared between $\delta$ 8.59–8.61, 8.51–8.53, 7.33–7.35 ppm, the phenyl (anilino) protons between $\delta$ 7.42–7.45, 6.99–7.03, 6.83–6.86 ppm and the NH protons (at the pyrimidine C-6 atom) around $\delta$ 8.73 ppm. The signal was observed at $\delta$ 11.96 due to the N3-H proton. Moreover, the compound 2a seem to exist only partially in the cyclic form. The spectrum of compound 2a had a pair of doublets centered at $\delta$ 3.78 and 3.67 ppm comprising an AB system ($J = 12.3$ Hz) [12] and two singlets at $\delta$ 5.85, 5.24 ppm for OH, but an AB quartet did not fully account for two methylene protons. In most cases tautomerism in these compounds is clearly solvent-dependent [40].

A similar phenomenon is also observed in other $^1$H-NMR spectra of S-alkylated derivatives (see Supporting Information). We interpret this effect as being due to the presence of thermally interconvertible cis-trans geometric isomers and their keto-enol tautomerism [44,45] (Scheme 3).

Scheme 3. Possible interconversion of S-alkylated derivatives.
Cyclization of S-alkylated derivatives 2a–f in concentrated H₂SO₄ represents an interesting case; mixtures of products (5H-thiazolo[3,2-a]pyrimidin-5-ones and sulfonic acid derivatives) were obtained which varied in relative amounts depending upon the reaction conditions (temperature and substituent groups).

Cyclization of 2c and 2d in concentrated H₂SO₄ at room temperature afforded 5H-thiazolo[3,2-a]pyrimidin-5-ones 5a and 5b, respectively, in good yields. The progress of the reaction was monitored by TLC. As can be seen, formation of aromatic sulfonic acids increased markedly with increasing reaction temperature. For example, only a negligible amount of the aromatic sulfonic acid was formed at 20 °C after 72 h. Upon raising the reaction temperature in increments, increasing amounts of the aromatic sulfonic acid were observed in the reaction mixtures by TLC. When the temperature was raised to 80 °C, the reaction with heating for 24 h gives these heteroaromatic sulfonic acid derivatives as the only products in high yield, which is attributable to the relatively weak electron-donating ability and relatively large steric hindrance of the methyl group.

In contrast, when compounds 2a–b, 2e–f were stirred for 72 h at 20 °C, they were converted completely into the monosulfonic acids of thienopyrimidines (TLC monitoring). Attempts to reduce the reaction times by further increasing the temperature from 20 °C to 80 °C are effective. This one-pot procedure shortened the total reaction time from 72 to 24 h.

Theoretically, the intramolecular cyclization of S-alkylated derivatives may afford the two possible isomeric products: 5H-thiazolo[3,2-a]pyrimidin-5-ones and 7H-thiazolo[3,2-a]pyrimidin-7-ones. Formation of these isomers may be explained on the basis of nucleophilicity differences of N1 and N3 position of S-alkylated derivatives. However, in practice cyclization of S-alkylated derivatives 2a–f were found to afford only the corresponding 5H-thiazolo[3,2-a]pyrimidin-5-ones.

The regioselectivity of the cyclization step, maybe due to a difference in the electron density at the N1 and N3 positions of 3,4-dihydropyrimidine-2(1H)-thione. The higher electron density of the N3 atom resulted in exclusive cyclization at this position [46] (Scheme 2). In addition, theoretical computations also reveal that the regioisomers 5-ones resulting from the N3 intramolecular cyclization are more stable and form the major regioisomer [47]. Moreover, 5H-thiazolo[3,2-a]pyrimidin-5-ones were formed as a result of intramolecular cyclization through nucleophilic attack of the pyrimidine N3, onto the phen acyl carbonyl carbon. The selective C2-N3 annulation is due to the steric repulsions between the aryl group at position 6 of the keto sulfides and carbonyl group [40].

Compounds 3a–f were obtained by one-pot cyclization and sulfonation of 2a–f. These conditions were favorable for the introduction of one sulfonic acid group and avoided undesirable oversulfonation. When compounds 2a–b undergo this one-pot procedure, sulfonation occurs solely at the electronically favored positions which are para to the amino groups to give the compounds 3a–b. For steric reasons, sulfonation of the phenyl ring did not afforded ortho-sulfonic acids. Thus, for compounds 2c–f, two substituents already present of the phenyl ring have a direct effect the introduction of the sulfonic acid group, as there are only two possible sulfonate isomers that can be formed. N-phenyl substitution of products 5H-thiazolo[3,2-a]pyrimidin-5-ones could lead to an amino conjugation effect of the cyclic α,β-unsaturated ketone of 5H-thiazolo[3,2-a]pyrimidin-5-ones (Scheme 2), which simultaneously decreases the conjugation on the phenyl ring. When a third substituent is introduced into the phenyl ring of 5H-thiazolo[3,2-a]pyrimidin-5-ones, both NH and R₁ (electron-donating groups) exert an influence, but the group R₁ whose influence predominates directs the sulfonic acid group to the place it will
occupy. We find that compounds with the new substituent in the ortho-positions relative to R1 are obtained exclusively. Another explanation of this phenomenon might be some steric influence of the 5H-thiazolo[3,2-a]pyrimidin-5-one nucleus. Similar conclusions regarding the regioselectivity of sulfonation of the phenyl ring have also been reported in the literature [48–52].

2.2. Biological Assays

All of the synthesized compounds were evaluated in vitro using a broth micro dilution method to obtain their minimum inhibitory concentration (MIC) values against two Gram-positive bacterial strains: *Staphylococcus aureus* (*S. aureus*), *Bacillus subtilis* (*B. subtilis*); two Gram-negative bacterial strains: *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Mycobacterium smegmatis* (*M. smegmatis*). The MIC values of these compounds were presented in Table 1.

**Table 1. In vitro antibacterial and antitubercular activity.**

| Compd. No. | Antibacterial Activity MIC (µg/mL) | Antitubercular Activity MIC (µg/mL) |
|------------|-----------------------------------|-------------------------------------|
|            | *S. aureus* | *B. subtilis* | *E. coli* | *P. aeruginosa* | *M. smegmatis* |
| 3a         | 200         | 400         | 50      | 100            | -             |
| 3b         | 100         | 200         | 100     | 200            | -             |
| 3c         | 200         | 200         | 100     | 100            | -             |
| 3d         | 200         | 400         | 100     | 100            | -             |
| 3e         | 400         | 800         | 200     | 200            | -             |
| 3f         | 400         | 800         | 100     | 200            | -             |
| 4a         | 400         | 800         | 400     | 400            | 100           |
| 4b         | 400         | 800         | 200     | 400            | 100           |
| 4c         | 800         | -           | 400     | -              | 50            |
| 4d         | -           | -           | 400     | -              | 50            |
| 5a         | 100         | 100         | 100     | 100            | -             |
| 5b         | 50          | 100         | 50      | 50             | -             |
| 6a         | 100         | 100         | 100     | 400            | 800           |
| 6b         | 200         | 200         | 200     | 400            | -             |
| CIP        | 25          | 100         | 25      | 50             | /             |
| RIP        | /           | /           | /       | /              | 25            |

“-”Indicates bacteria is resistant to the compounds at >800 µg/mL. MIC (µg/mL) = lowest concentration to completely inhibit bacterial growth. Reference drugs: CIP, Ciprofloxacin; RIP, Rifampicinn.

Examination of the antibacterial screening data reveals that all the tested compounds display significant antibacterial activity against Gram-negative bacteria and moderate activity against Gram-positive bacteria. In general, compounds having nitro substituents displayed significant inhibitory activity, that was only slightly affected by the nitro substituent being located on the 3- or 4-position of the phenyl group. In addition, compounds without sulfonic group had better antibacterial than the corresponding compounds with sulfonic acid groups, which could be seen from compounds 5a, 5b that possess the highest antibacterial activities. From first examination of the antitubercular activity results, it appears that compounds 4a–d, containing an amino group, show better activity against *M. smegmatis* and compounds 4c, 4d showed the highest activity (MIC 50 µg/mL). This may be due to the influence of the methoxy substituent.
3. Experimental Section

3.1. General Information

Melting points were determined in open capillary tubes with a WRS-1B melting point apparatus (Shanghai Shenguang Instrument Co., Ltd, Shanghai, China) and are uncorrected. IR spectra (KBr) were recorded on a FTIR920 spectrophotometer (Tianjin Tuopu Instrument Co., Ltd., Tianjin, China). The $^1$H- and $^13$C-NMR spectra were obtained from a solution in DMSO-$d_6$ with TMS as internal standard using a 400/101 MHz ($^1$H-$^13$C-) spectrometer (Agilent Technologies, Santa Clara, CA, USA). Mass spectra were acquired from an Agilent 6200 Series TOF and 6500 Series Q-TOF LC/MS System B.05.01. (B5125, Agilent Technologies, Santa Clara, CA, USA).

3.2. Synthesis

3.2.1. General Procedure for the Synthesis of 1a–c

A mixture of 6-amino-2-thiouracil (50 mmol), an appropriate aniline (100 mmol) together with anilinium chloride (75–100 mmol) was heated at 175 °C for 7–12 h. The warm mixture was diluted with 65% ethanol (200 mL) and cooled. The precipitate was filtered and washed with cold ethanol, which was dissolved in hot 5% NaOH solution, and the filtrate was neutralized with 10% HCl to get more pure product. The solid deposited was filtered, washed with water, dried, and crystallized from a large volume of CH$_3$OH to yield the title compounds.

6-(Phenylamino)-2-thioxo-2,3-dihydropyrimidin-4(1H)-one (1a): White solid; Yield, 79.3%; m.p.: 266.5–267.8 °C (lit. [36,53] 287–288 and 281–283 °C); HRMS (m/z): calcd. for C$_{10}$H$_9$N$_3$OS (neutral M + H) 220.0545, found 220.0553.

2-Thioxo-6-(p-tolylamino)-2,3-dihydropyrimidin-4(1H)-one (1b): White solid; Yield, 91.8%; m.p.: 256.6–257.1 °C (lit. [35] 293–295 °C, decomp., from DMF–H$_2$O); HRMS (m/z): calcd. for C$_{11}$H$_{11}$N$_3$OS (neutral M + H) 234.0701, found 234.0719.

6-((4-Methoxyphenyl)amino)-2-thioxo-2,3-dihydropyrimidin-4(1H)-one (1c): White solid; Yield, 94.1%; m.p.: 269.7–270.1 °C (lit. [35] 284–286 °C, decomp., from DMF–H$_2$O); HRMS (m/z): calcd. for C$_{11}$H$_{11}$N$_3$O$_2$S (neutral M + H) 250.0650, found 250.0661.

3.2.2. General Procedure for the Synthesis of Compounds 2a–f

Anhydrous potassium carbonate (10 mmol) and substituted phenacyl halides (10 mmol) were added in succession to a suspension of 6-substituted-2-thiouracil 1 (12 mmol) in dry N,N-dimethylformamide (10 mL). After stirring for 3 h at room temperature, the mixture was quenched with water (100 mL) and filtered. The residues were purified by crystallization to give compounds 2a–f.

2-((2-(3-Nitrophenyl)-2-oxoethyl)thio)-6-(phenylamino)pyrimidin-4(3H)-one (2a): Yellow solid; Yield, 80.3%; m.p.: 222.3–223.0 °C; HRMS (m/z): calcd. for C$_{18}$H$_{14}$N$_4$O$_4$S (neutral M + H) 383.0814, found 383.0837.
2-((2-(4-Nitrophenyl)-2-oxoethyl)thio)-6-(phenylamino)pyrimidin-4(3H)-one (2b): Yellow solid; Yield, 82.8%; m.p.: 218.3–219.5 °C; HRMS (m/z): calcd. for C_{18}H_{14}N_{4}O_{5}S (neutral M + H) 383.0814, found 383.0837.

2-((2-(3-Nitrophenyl)-2-oxoethyl)thio)-6-(p-tolylamino)pyrimidin-4(3H)-one (2c): Yellow solid; Yield, 83.2%; m.p.: 222.1–224.8 °C; HRMS (m/z): calcd. for C_{19}H_{16}N_{4}O_{5}S (neutral M+H) 397.0971, found 397.0997.

2-((2-(4-Nitrophenyl)-2-oxoethyl)thio)-6-(p-tolylamino)pyrimidin-4(3H)-one (2d): Yellow solid; Yield, 83.9%; M.p.: 227.6–228.6 °C; HRMS (m/z): calcd for C_{19}H_{16}N_{4}O_{5}S (neutral M+H) 397.0971, found 397.0986.

6-((4-Methoxyphenyl)amino)-2-((2-(3-nitrophenyl)-2-oxoethyl)thio)pyrimidin-4(3H)-one (2e): Yellow solid; Yield, 79.6%; m.p.: 217.1–217.3 °C; HRMS (m/z): calcd. for C_{19}H_{16}N_{4}O_{5}S (neutral M + H) 413.0920, found 413.0951.

6-((4-Methoxyphenyl)amino)-2-((2-(4-nitrophenyl)-2-oxoethyl)thio)pyrimidin-4(3H)-one (2f): Yellow solid; Yield, 78.5%; m.p.: 215.8–217.7 °C; HRMS (m/z): calcd. for C_{19}H_{16}N_{4}O_{5}S (neutral M + H) 413.0920, found 413.0948.

3.2.3. General Procedure for the Synthesis of Compounds 3a-f

S-alkylated derivatives 2 (1 mmol) were carefully dissolved in concentrated sulfuric acid (7.5 mL) and heated in an oil bath at 80 °C for 24 h. After cooling, the reaction mixture was carefully poured into ethyl acetate (about 50 mL), to form a precipitate which was collected, washed with ethyl acetate and dried. The crude product was recrystallized from ethyl acetate to give 3a-f.

4-((3-(3-Nitrophenyl)-5-oxo-5H-thiazolo[3,2-a]pyrimidin-7-yl)amino)benzenesulfonic acid (3a): Yellow solid; Yield, 91.7%; m.p.: (decomp.) 257.6 °C; IR (v/cm⁻¹): 3398, 3099, 1570, 1520, 1345, 1192, 1129, 1043, 821, 734; ¹H-NMR δ 9.50 (s, 1H), 8.33–8.22 (m, 2H), 7.90 (dt, J = 7.8, 1.4 Hz, 1H), 7.67 (t, J = 7.8 Hz, 1H), 7.62–7.54 (m, 2H), 7.47–7.33 (m, 3H), 5.38 (d, J = 1.2 Hz, 1H); ¹³C-NMR δ 164.73, 159.54, 158.98, 146.90, 143.13, 140.14, 136.07, 135.48, 134.13, 128.87, 126.82, 124.42, 123.39, 119.89, 111.26, 82.66; HRMS (m/z): calcd. for C_{18}H_{12}N_{4}O_{5}S (neutral M + H) 445.0277, found 445.0310.

4-((3-(4-Nitrophenyl)-5-oxo-5H-thiazolo[3,2-a]pyrimidin-7-yl)amino)benzenesulfonic acid (3b): Yellow solid; Yield, 92.4%; m.p.: (decomp.) 210.0 °C; IR (v/cm⁻¹): 3442, 3284, 3109, 1671, 1568, 1520, 1350, 1232, 1134, 1031, 1000, 836, 750; ¹H-NMR δ 9.50 (s, 1H), 8.31–8.13 (m, 2H), 7.76–7.67 (m, 2H), 7.60–7.53 (m, 2H), 7.48–7.32 (m, 3H), 5.38 (s, 1H); ¹³C-NMR δ 164.73, 159.36, 158.98, 147.33, 143.29, 140.02, 139.02, 135.68, 130.74, 126.81, 122.49, 119.93, 111.75, 82.52; HRMS (m/z): calcd. for C_{18}H_{12}N_{4}O_{5}S (neutral M + H) 445.0277, found 445.0313.

2-Methyl-5-((3-(3-nitrophenyl)-5-oxo-5H-thiazolo[3,2-a]pyrimidin-7-yl)amino)benzenesulfonic acid (3c): Yellow solid; Yield, 90.6%; m.p.: (decomp.) 244.5–249.8 °C; IR (v/cm⁻¹): 3430, 3267, 3089, 1642, 1588, 1527, 1347, 1218, 1162, 1086, 1019, 810; ¹H-NMR δ 9.36 (s, 1H), 8.30–8.27 (m, 2H), 7.90 (dt,
J = 7.8, 1.4 Hz, 1H), 7.68 (q, J = 7.6 Hz, 1H), 7.63–7.44 (m, 2H), 7.37 (s, 1H), 7.19 (dd, J = 8.2, 2.1 Hz, 1H), 5.44 (s, 1H), 2.29 (s, 3H); 13C-NMR δ 164.42, 159.74, 158.13, 146.91, 137.31, 136.05, 135.39, 134.05, 133.54, 131.69, 130.50, 128.86, 128.09, 124.37, 123.37, 120.84, 111.17, 81.98, 20.82; HRMS (m/z): calcd. for C19H14NaO6S2 (neutral M + H) 459.0433, found 458.9985.

2-Methyl-5-((3-(4-nitrophenyl)-5-oxo-5H-thiazolo[3,2-a]pyrimidin-7-yl)amino)benzenesulfonic acid (3d): Yellow solid; Yield, 88.9%; m.p.: (decomp.) 263.7 °C; IR (νmax/cm⁻¹): 3366, 3110, 2956, 1653, 1601, 1515, 1345, 1176, 1091, 1026, 822; 1H-NMR δ 9.37 (s, 1H), 8.32 (d, J = 8.6 Hz, 1H), 8.22 (d, J = 8.4 Hz, 2H), 7.71 (d, J = 8.4 Hz, 2H), 7.55 (d, J = 6.1 Hz, 1H), 7.38 (s, 1H), 7.20 (d, J = 8.4 Hz, 1H), 5.45 (s, 1H), 2.29 (s, 3H); 13C-NMR δ 164.44, 159.60, 158.13, 147.31, 138.93, 135.60, 133.51, 131.73, 130.71, 128.10, 126.76, 124.61, 122.50, 120.83, 111.72, 81.91, 21.45; HRMS (m/z): calcd. for C19H14NaO6S2 (neutral M + H) 459.0433, found 459.0463.

2-Methoxy-5-((3-(3-nitrophenyl)-5-oxo-5H-thiazolo[3,2-a]pyrimidin-7-yl)amino)benzenesulfonic acid (3e): Yellow solid; Yield, 78.5%; m.p.: (decomp.) 290.3–293.4 °C; IR (νmax/cm⁻¹): 3398, 3096, 1673, 1618, 1570, 1526, 1496, 1448, 1347, 1194, 1091, 1034, 812; 1H-NMR δ 9.24 (s, 1H), 8.32–8.21 (m, 2H), 7.89 (dd, J = 7.8, 1.4 Hz, 1H), 7.72–7.61 (m, 2H), 7.44–7.36 (m, 1H), 7.33 (d, J = 1.2 Hz, 1H), 6.99 (dd, J = 8.8, 1.2 Hz, 1H), 5.17 (d, J = 1.2 Hz, 1H), 3.77 (d, J = 1.3 Hz, 3H); 13C-NMR δ 164.68, 159.87, 159.55, 153.12, 146.93, 136.38, 136.13, 135.50, 134.25, 131.19, 128.86, 124.43, 124.34, 123.37, 123.26, 112.92, 110.70, 80.90, 56.25; HRMS (m/z): calcd. for C19H14NaO6S2 (neutral M + H) 475.0382, found 475.0422.

2-Methoxy-5-((3-(4-nitrophenyl)-5-oxo-5H-thiazolo[3,2-a]pyrimidin-7-yl)amino)benzenesulfonic acid (3f): Yellow solid; Yield, 80.4%; m.p.: (decomp.) 272.1 °C; IR (νmax/cm⁻¹): 3373, 3088, 2947, 1709, 1632, 1606, 1570, 1490, 1233, 1200, 1088, 1023, 853; 1H-NMR δ 9.25 (s, 1H), 8.21 (d, J = 8.4 Hz, 2H), 7.70 (d, J = 8.9 Hz, 3H), 7.39 (d, J = 10.5 Hz, 1H), 7.34 (d, J = 0.7 Hz, 1H), 6.99 (d, J = 8.7 Hz, 1H), 5.17 (s, 1H), 3.75 (s, 3H); 13C-NMR δ 164.69, 159.86, 159.41, 153.25, 147.31, 139.12, 136.25, 135.71, 130.74, 126.95, 125.35, 124.73, 123.32, 113.00, 111.26, 80.78, 56.16; HRMS (m/z): calcd. for C19H14NaO6S2 (neutral M + H) 475.0382, found 475.0424.

3.2.4. General Procedure for the Synthesis of Compounds 4a–d

A suspension of nitro compounds 3a–f (3.53 mmol) in ethanol (60 mL) and water (30 mL) was treated with ammonium chloride (3.53 mmol) and iron powder (17.65 mmol). After being stirred at 80 °C for 2 h, the mixture was diluted with ethanol (40 mL) and filtered through diatomaceous earth (Celite®) while hot. The filtrant was washed with hot ethanol, and the filtrate was concentrated. The crude product was purified by column chromatography on silica gel using petroleum CH2Cl2/CH3OH as eluent to afford the pure products.

4-((3-(3-Aminophenyl)-5-oxo-5H-thiazolo[3,2-a]pyrimidin-7-yl)amino)benzenesulfonic acid (4a): Gray solid; Yield, 62.7%; m.p.: (decomp.) 257.6 °C; IR (νmax/cm⁻¹): 3457, 3263, 3093, 2998, 1669, 1592, 1568, 1498, 1446, 1391, 1321, 1187, 1121, 1030, 818, 710; 1H-NMR δ 9.42 (s, 1H), 7.60–7.54 (m, 2H), 7.40 (d, J = 8.2 Hz, 2H), 7.07–6.97 (m, 2H), 6.65–6.54 (m, 3H), 5.50 (s, 2H), 5.35 (s, 1H); 13C-NMR δ
164.95, 159.21, 158.72, 147.10, 142.91, 140.29, 138.80, 133.27, 128.06, 126.78, 119.78, 117.85, 115.48, 114.68, 108.44, 82.72; HRMS (m/z): calcd. for C_{18}H_{14}NaO_{5}S_{2} (neutral M + H) 415.0535, found 415.0524.

4-((3-(4-Aminophenyl))-5-oxo-5H-thiazolo[3,2-a]pyrimidin-7-yl)amino)benzenesulfonic acid (4b): Gray solid; Yield, 65.8%; m.p.: (decomp.) 246.8 °C; IR (ν_{max}/cm^{-1}): 3397, 3100, 1676, 1625, 1568, 1506, 1453, 1328, 1270, 1189, 1124, 1038, 830, 712; ¹H-NMR δ 9.40 (s, 1H), 7.58 (d, J = 7.9 Hz, 2H), 7.40 (d, J = 7.6 Hz, 2H), 7.05 (d, J = 7.9 Hz, 2H), 6.91 (s, 1H), 6.52 (d, J = 8.0 Hz, 2H), 5.44 (s, 2H), 5.35 (s, 1H); ¹³C-NMR δ 165.04, 159.62, 158.56, 149.26, 142.70, 140.42, 139.50, 130.46, 126.81, 119.87, 119.69, 112.56, 106.51, 82.88; HRMS (m/z): calcd. for C_{18}H_{14}NaO_{5}S_{2} (neutral M + H) 415.0535, found 415.0545.

5-((3-(3-Aminophenyl))-5-oxo-5H-thiazolo[3,2-a]pyrimidin-7-yl)amino)-2-methoxybenzenesulfonic acid (4c): Yellow solid; Yield, 58.9%; m.p.: (decomp.) 181.5 °C; IR (ν_{max}/cm^{-1}): 3368, 3279, 1661, 1585, 1500, 1436, 1192, 1083, 1028, 815, 700; ¹H-NMR δ 9.11 (s, 1H), 7.66 (s, 1H), 7.34 (d, J = 8.3 Hz, 1H), 6.96 (d, J = 6.9 Hz, 3H), 6.53 (q, J = 8.4, 7.1 Hz, 3H), 5.14 (s, 2H), 5.11 (s, 1H), 3.73 (s, 3H); ¹³C-NMR δ 164.88, 159.62, 159.26, 153.19, 147.89, 138.90, 133.27, 131.18, 128.02, 124.42, 123.32, 117.12, 115.06, 114.22, 112.95, 107.72, 80.80, 56.16; HRMS (m/z): calcd. for C_{19}H_{16}NaO_{5}S (neutral M + H) 445.0640, found 445.0627.

5-((3-(4-Aminophenyl))-5-oxo-5H-thiazolo[3,2-a]pyrimidin-7-yl)amino)-2-methoxybenzenesulfonic acid (4d): Yellow solid; Yield, 56.8%; m.p.: (decomp.) 288.6 °C; IR (ν_{max}/cm^{-1}): 3344, 3030, 2971, 1686, 1618, 1567, 1489, 1440, 1333, 1274, 1194, 1087, 877, 818; ¹H-NMR δ 9.12 (s, 1H), 7.67 (d, J = 2.8 Hz, 1H), 7.39 (dd, J = 8.7, 2.8 Hz, 1H), 7.01 (dd, J = 24.2, 8.5 Hz, 3H), 6.85 (s, 1H), 6.52 (d, J = 8.3 Hz, 2H), 5.43 (s, 2H), 5.14 (s, 1H), 3.76 (s, 3H); ¹³C-NMR δ 164.99, 159.64, 159.46, 152.94, 149.03, 139.45, 136.18, 131.37, 130.42, 124.35, 123.06, 120.13, 112.90, 112.67, 105.97, 81.05, 56.23; HRMS (m/z): calcd. for C_{19}H_{16}NaO_{5}S (neutral M + H) 445.0640, found 445.0646.

3.2.5. General Procedure for the Synthesis of Compounds 5a–b

S-alkylated derivatives 2 (1 mmol) were carefully dissolved in concentrated sulfuric acid (7.5 mL) and stirred at 20 °C for 72 h. After cooling, it was carefully poured into water (about 50 mL), precipitation which was collected, washed with cold water and dried. The crude product was recrystallized from ethyl acetate to give products.

3-(4-Nitrophenyl)-7-(p-tolylamino)-5H-thiazolo[3,2-a]pyrimidin-5-one (5a): Yellow solid; Yield, 94.5%; m.p.:143.2–144.9 °C; IR (ν_{max}/cm^{-1}): 3389, 3107, 1662, 1609, 1568, 1518, 1343, 1134, 1022, 807, 736; ¹H-NMR δ 9.31 (s, 1H), 8.32–8.22 (m, 2H), 7.89 (dq, J = 7.6, 1.4 Hz, 1H), 7.67 (td, J = 7.9, 1.4 Hz, 1H), 7.35 (d, J = 1.6 Hz, 1H), 7.34–7.29 (m, 2H), 7.17 (d, J = 7.9 Hz, 2H), 5.28 (d, J = 1.5 Hz, 1H), 2.29 (s, 3H); ¹³C-NMR δ 164.65, 159.52, 146.89, 142.41, 137.22, 136.03, 135.48, 134.16, 132.71, 129.87, 128.86, 124.40, 123.36, 121.68, 110.87, 81.61, 20.90; HRMS (m/z): calcd. for C_{19}H_{14}N_{2}O_{5}S (neutral M + H) 379.0865, found 379.0892.

3-(4-Nitrophenyl)-7-(p-tolylamino)-5H-thiazolo[3,2-a]pyrimidin-5-one (5b): Yellow solid; Yield, 96.4%; m.p.: 143.8–145.1 °C; IR (ν_{max}/cm^{-1}): 3297, 3105, 2923, 1664, 1604, 1515, 1345, 1197, 1091, 820; ¹H-NMR δ 9.33 (s, 1H), 8.22 (d, J = 8.4 Hz, 2H), 7.69 (d, J = 8.2 Hz, 2H), 7.36 (s, 1H), 7.31 (d, J = 8.0 Hz, ...
2H), 7.17 (d, J = 8.0 Hz, 2H), 5.28 (s, 1H), 2.29 (s, 3H); "C-NMR δ 164.66, 159.39, 159.37, 147.31, 139.04, 137.18, 135.68, 132.76, 130.71, 129.87, 122.49, 121.71, 111.42, 81.52, 20.90; HRMS (m/z): calcd. for C19H16N4O2S (neutral M + H) 379.0865, found 379.0897.

3.2.6. General Procedure for the Synthesis of Compounds 6a–b

A suspension of nitro compound 5a–b (3.53 mmol) in ethanol (60 mL) and water (30 mL) was treated with ammonium chloride (3.53 mmol) and iron powder (17.65 mmol). After being stirred at 80 °C for 2 h, the mixture was diluted with ethanol (40 mL) and filtered through diatomaceous earth (Celit®) while hot. The filtrant was washed with hot ethanol, and the filtrate was concentrated. The combined extracts were washed with brine and dried (Na2SO4), filtered and concentrated to provide the products.

3-(3-Aminophenyl)-7-(p-tolylamino)-5H-thiazolo[3,2-a]pyrimidin-5-one (6a): Yellow solid; Yield, 96.5%; m.p. 220.9–221.7 °C; IR (νmax/cm⁻¹): 3541, 3384, 3111, 3025, 1664, 1615, 1570, 1515, 1400, 1324, 1271, 1190, 1126, 1020, 810, 738; "H-NMR δ 9.22 (s, 1H), 7.30 (d, J = 8.0 Hz, 2H), 7.16 (d, J = 8.1 Hz, 2H), 7.05–6.94 (m, 2H), 6.63–6.49 (m, 3H), 5.28 (s, 2H), 5.25 (d, J = 1.2 Hz, 1H), 2.28 (s, 3H); "C-NMR δ 164.66, 159.52, 159.40, 146.90, 137.23, 136.03, 135.48, 132.44, 130.44, 129.87, 128.86, 124.41, 123.37, 121.69, 110.87, 81.61, 20.90; HRMS (m/z): calcd. for C19H16N4OS (neutral M + H) 349.1123, found 349.1112.

3-(4-Aminophenyl)-7-(p-tolylamino)-5H-thiazolo[3,2-a]pyrimidin-5-one (6b): Brown solid; Yield, 78%; m.p.: 241.2–242.8 °C; IR (νmax/cm⁻¹): 3469, 3378, 3107, 3021, 1659, 1613, 1563, 1510, 1395, 1323, 1268, 1184, 1122, 1017, 819; "H-NMR δ 9.19 (s, 1H), 7.30 (d, J = 8.0 Hz, 2H), 7.15 (d, J = 8.1 Hz, 2H), 7.08–7.01 (m, 2H), 6.87 (d, J = 1.2 Hz, 1H), 6.57–6.45 (m, 2H), 5.34 (s, 2H), 5.25 (d, J = 1.2 Hz, 1H), 2.28 (s, 3H); "C-NMR δ 164.98, 159.62, 159.02, 149.31, 139.51, 137.41, 132.44, 130.44, 129.82, 121.53, 119.89, 112.51, 106.09, 81.80, 20.89; HRMS (m/z): calcd. for C19H16N4OS (neutral M + H) 349.1123, found 349.1128.

3.3. Bioassays

The standard strains were the obtained from National Center for Medical Culture Collection and China General Microbiological Culture Collection Center. The antibacterial and antitubercular activity of the synthesized compounds was performed by broth micro dilution method against the following standard bacterial strains: *Escherichia coli* [CMCC (B) 44102], *Pseudomonas aeruginosa* [CMCC (B) 10104], *Staphylococcus aureus* [CMCC (B) 26003], *Bacillus subtilis* [CMCC (B) 63501] and *M. smegmatis* [CGMCC 1.2621].

The antibacterial and antitubercular activities of the synthesized compounds were tested by the broth micro dilution method. The 2-fold diluted compounds in Mueller Hinton broth were dispensed into 96-well microtiter plates (200 µL/well), and then an aliquot of 5 × 10⁵ colony forming units (cfu)/mL of bacterial culture was added to each well (200 µL/well) to final concentrations in a range of 1–800 µg/mL. After incubating at 37 °C for 24 h, the lowest concentration without any colony growth was recorded as the MIC value. The tested compounds and reference drugs were dissolved in MeOH to
get a solution and MeOH showed no inhibition zones. The resulting values were compared with the value for a reference control (ciprofloxacin in a range of 3.125–200 μg/mL was used as a reference for antibacterial activity, and rifampicin in a range of 3.125–100 μg/mL was used as a reference for antitubercular activity) under the same conditions.

4. Conclusions

In summary, a series of 7-(substituted phenylamino)-5H-thiazolo[3,2-a]pyrimidin-5-ones and sulfonated cyclized products were designed, synthesized and evaluated for antibacterial and antitubercular activities in this study. An efficient synthetic method led to 5H-thiazolo[3,2-a]pyrimidin-5-ones or the corresponding sulfonic acid derivatives at different temperatures in high yield and purity. During our extensive literature survey it was found that N3 of substituted pyrimidines was the cyclization site when S-alkylated derivatives was utilized to give 5H-thiazolo[3,2-a]pyrimidin-5-ones. Our results reveal that compounds having nitro substituents displayed significant antibacterial inhibitory activity, while compounds containing the amino group, show better activity against M. smegmatis. Further structural modification could be performed to improve the bioactivity. We believe that these compounds can be developed into potential class of antimicrobial and antitubercular agents.

Supplementary Material

Supplementary materials can be accessed at: http://www.mdpi.com/1420-3049/20/09/16419/s1.

Acknowledgments

The authors thank the Liaoning Medical University Principal Fund (No. XZJJ20130104-03) for the financial support of this research.

Author Contributions

D.C. conceived and designed the experiments; D.C., Z.Z.H., Y.C., L.J.Z., Y.X.W. and X.Q.L. performed the experiments; D.C., Y.C. and X.J.Y. analyzed the data; D.C. wrote the paper. All authors read and approved the final manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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*Sample Availability:* Samples of the compounds 1a–c, 2a–f, 5a–b, 6a–b are available from the authors.

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