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Fluorine-substituted dihydrobicyclomycins: Synthesis and biochemical and biological properties

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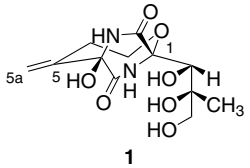
Abstract—Many studies show that selective introduction of fluorine within pharmacological agents leads to improved activities. In this study, we determine the effects of aryl fluorine substitution in 5a-(benzylsulfanyl)-dihydrobicyclomycin (**3**) on the in vitro inhibition of *Escherichia coli* rho-dependent ATPase activity. Compound **3** is an analog of bicyclomycin (**1**), which is the only known selective inhibitor of rho, and **1** and **3** have comparable in vitro inhibitory activities. We demonstrate that aryl fluorine substitution of **3** leads to increase in inhibitory activity but that the beneficial effects due to fluorine were dependent upon the site and number of fluorine substituents. The bioactivities are rationalized in terms of the bond moment created by the aryl fluoride bond within the 5a-aryl dihydrobicyclomycin-rho-binding pocket. Use of this hypothesis led to the design of dihydrobicyclomycin derivatives with superior in vitro rho inhibitory activities.

1. Introduction

Bicyclomycin (**1**) is a structurally unique antibiotic[1,2] with a novel mode of action.[3–7] The molecular target of **1** in *Escherichia coli* is the rho transcription termination factor.[8] In *E. coli*, rho has been estimated to actively terminate transcription in approximately half of the open reading frames.[9] Thus, the rho protein is integral for the expression of many bacterial gene products and without rho the cell loses viability.[10] The rho termination pathway extends to most Gram-negative organisms.[10]



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Rho is a homohexameric RNA/DNA helicase/translocase.[11] mRNA is rho's substrate serving as the recognition element for its binding, the template for its function, and the signal that triggers the hydrolysis of ATP that powers rho along the mRNA.[10–13] Rho binds mRNA at two sites. The primary, tight RNA-binding site is located in the N-terminus and encompasses all six subunits. The secondary RNA site binds mRNA less tightly and spans the N and C subdomains along the central hole within each subunit.

Bicyclomycin inhibits rho function by preventing the secondary site RNA-induced conformational changes necessary for ATP hydrolysis.[5,14] Moreover, the recent X-ray crystallographic images of bicyclomycins bound to rho show that the antibiotic occludes the nucleophilic water molecule required for the conversion of ATP to ADP and P_i.[7]

Here, we examine the effect of fluorine substitution on bicyclomycin bioactivity. Fluorine is found in many pharmacologically active compounds[15,16] including anticancer,[17–19] antiviral,[20–22] antibiotic,[23] cardiovascular,[24] and anesthetic[15,16] agents. No single factor accounts for the beneficial pharmaceutical effects observed with fluorine substitution, in part, because fluorine affects many molecular properties that influence drug function and efficacy (e.g., lipophilicity, bioavailability, chemical stability, metabolic reactivity, and receptor binding).[25]

Keywords: Bicyclomycins; Fluorine substitution; Inhibitory activity; Bond moment.
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We report the synthesis and evaluation of 12 fluorinated derivatives of **1** and document that fluorination can improve the bicyclomycin rho inhibitory activity compared with **1** in an in vitro assay. Our structure–activity relationship (SAR) study revealed a distinctive fluorine substitution pattern for rho inhibitory activity and we provide a rational for this pattern in context of the bicyclomycin-rho inhibition pathway. Support for this hypothesis is gained by the design of bicyclomycin derivatives with superior in vitro rho inhibitory activities.

2. Results and discussion

2.1. Selection of fluorine-substituted compounds and method of assessment

Structurally, **1** can be divided into the C(1) triol group, the [4.2.2]-bicyclic ring system, and the C(5)–C(5a) exomethylene moiety (Fig. 1). We showed that modifications of the C(1) triol group[26,27] and the [4.2.2]-bicyclic ring system[28,29] led to diminished inhibitory activities in in vitro rho functional assays while select changes of the C(5)–C(5a) exomethylene moiety in **1**[30,31] provided compounds with similar or improved inhibitory activities compared with **1**. These findings indicated that the C(1) triol and [4.2.2]-bicyclic ring were essential for bicyclomycin-rho inhibitory activity while the C(5)–C(5a) exomethylene moiety was not. The X-ray crystallographic structures of **1** and 5a-((3-formyl)phenylsulfanyl)-dihydrobicyclomycin[7](**2**) bound to the opened, lock washer conformation of rho showed why this is the case (see Fig. 2 for **2**). We found that **1** and **2** bind to a pocket located adjacent to the ATP and the secondary RNA-binding sites in the C-terminal half of rho. The bicyclomycin-binding pocket (12 Å (deep) × 10 Å (wide)) is formed by the P and R loops, the β7/α8 connector loop of one monomer and helix α12 of the adjacent monomer. The [4.2.2] piperazinedione ring and C(1) triol group dock snuggly into this binding pocket. The C(1) triol group extends into the bottom of the pocket where the hydroxyl groups create a network of hydrogen bonds with the nearby amino acid residues (Glu211, Asp265, and Ser266). The 5a-exomethylene-substituent in **1** projects into the hexameric hole. When the C(5)–C(5a) exomethylene group in **1** is modified to give 5a-substituted dihydrobicyclomycins, such as **2**, the 5a-moiety is positioned along side the center hole and

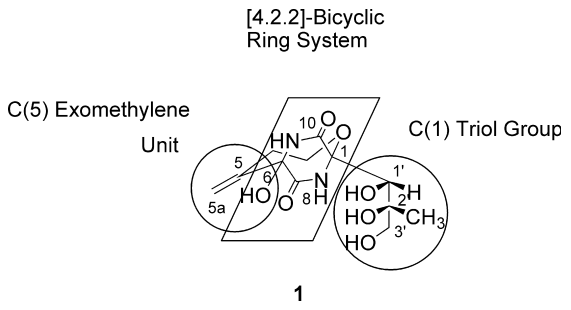

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Figure 1. The three structural sectors of bicyclomycin.

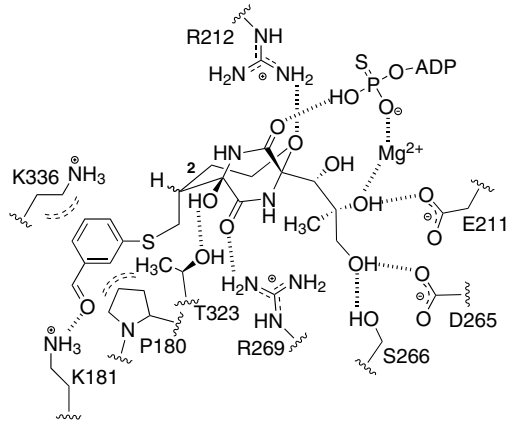
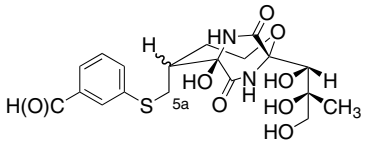


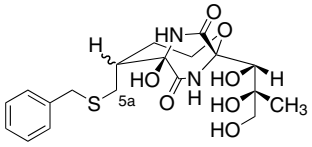
Figure 2. Bicyclomycin **2** contacts when bound to the rho lock washer conformation.

directed to the C terminus. Most important, the 5a-(3-formyl)phenylsulfanyl substituent in **2** is contained within a hydrophobic pocket situated between the Pro180 side chain in one subunit and the aliphatic side chain of Lys336 from the adjacent subunit.



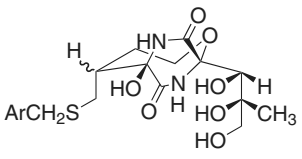
2

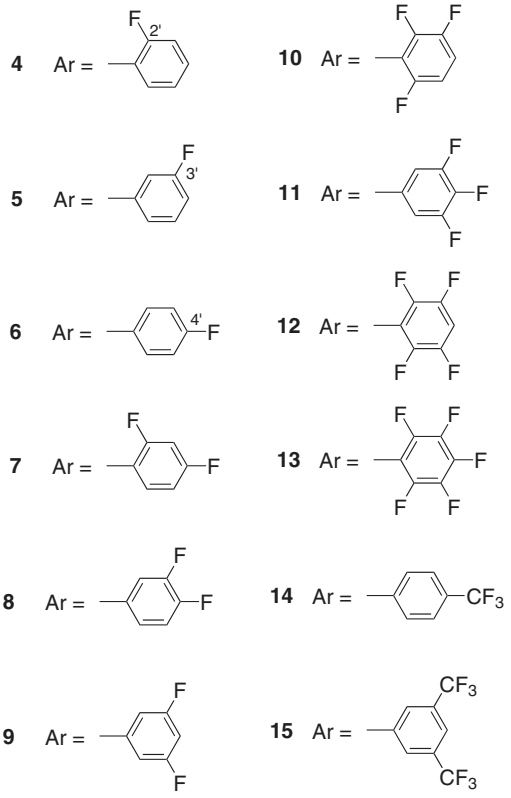
We selected 5a-benzylsulfanyldihydrobicyclomycin (**3**) as our parent compound since an earlier study showed that **3** exhibited inhibitory activity comparable with **1** in the rho poly(C)-dependent ATPase assay.[30] Thus, beginning with **3** we asked whether aromatic fluorine-substitution influenced the dihydrobicyclomycin inhibitory activity. We felt confident that the modest steric changes incurred with fluorine aromatic substitution[25] of **3** would not adversely impact drug binding to rho because we had documented that increases in the size of the appended aromatic ring in **3** gave dihydrobicyclomycins with excellent inhibitory activities.[14] Significantly, **3** is structurally similar to **2** used in the rho X-ray crystallographic study.[7] Distinguishing **3** from **2** is the inclusion of a methylene group between the sulfur atom and the phenyl ring.



3

Ten fluorine-substituted derivatives of **3** were selected for synthesis and evaluation where the fluorine substituent was directly attached to the aromatic ring. They consisted of the mono-fluoro **4**–**6**, di-fluoro **7**–**9**, tri-fluoro **10**, **11**, tetra-fluoro **12**, and penta-fluoro **13** benzyl derivatives. We also prepared the two trifluoromethyl analogs of **3**, **14**, and **15**, to evaluate the effect of alkyl fluorine substitution.





We determined the effect of site-specific inclusion of fluorine within the appended aryl unit in **3** by measuring the inhibitory activity of **4–15** in the rho poly(C)-dependent ATPase assay.[32] This test has proven to be a reliable assay to measure bicyclomycin inhibition of rho.[26–31] We also determined the antimicrobial activity of select fluorine-substituted dihydrobicyclomycins against *E. coli* W3350 using a filter disk assay.[33] Since we did not measure the intracellular concentrations of **4–15** under the assay conditions, we did not use this information in our SAR. Significantly, our use of a rapid, biochemical test to measure the effects of fluorine substitution on bioactivity rather than a cellular-based assay removes several factors (i.e., bioavailability, chemical stability, and metabolic reactivity) previously shown to influence the efficacy of fluorine-substituted compounds.[25]

2.2. Synthesis and structural characterization

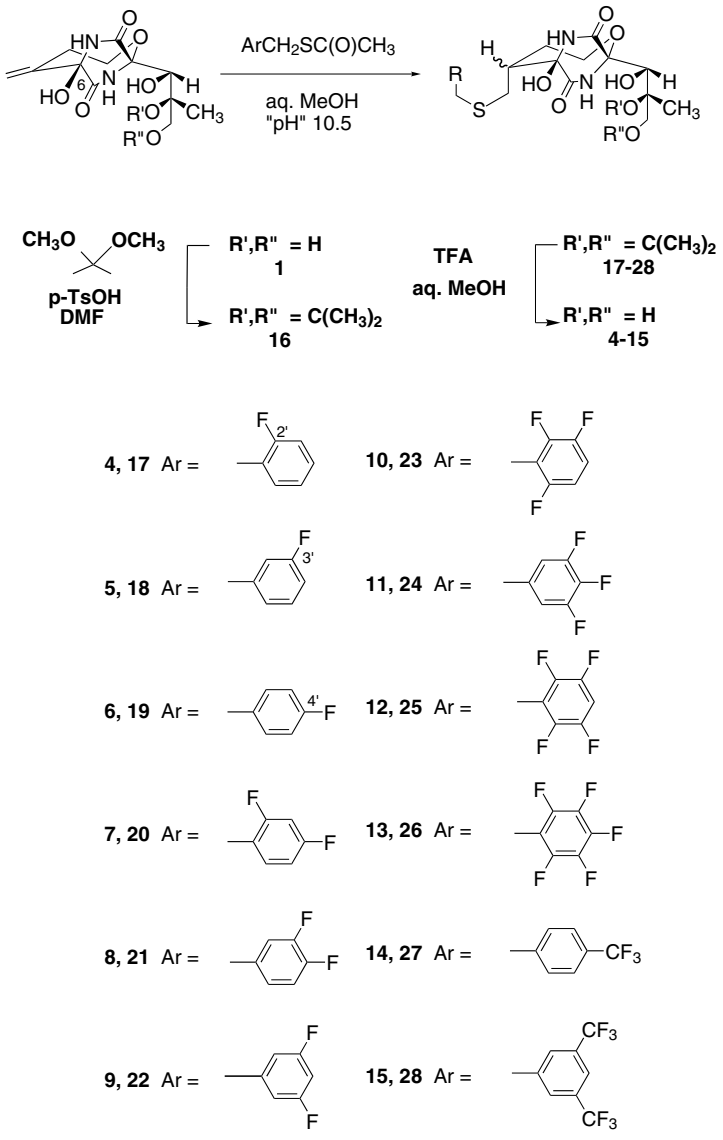
A three-step synthetic sequence was used to prepare **4–15** (Scheme 1). Key to our synthesis was the Michael addition of the incipient thiolate anion to bicyclomycin 2′,3′-acetonide (**16**).[34] The Michael acceptor was the enone generated upon hemiaminal ring opening at C(6) of **16** under basic conditions. In every case but one, we generated the benzylthiolates in situ from the corresponding benzylthiolacetates (i.e., **29**, **30**, and **32–40**) under the conditions of the Michael reaction. The benzylthiolacetates were prepared from commercially available benzyl halides and potassium thioacetate in acetone (Scheme 2).[14] For **6**, we used commercially available 4-fluorobenzenethiol in place of benzylthiolacetate **31** to prepare **19**. TFA removal of the 2′,3′-acetonide group in **17–28** in the final step (Scheme 1) provided the desired **4–15**, respectively, in 33–52% overall yields. The dihydrobicyclomycins were isolated as diastereomeric mixtures (~3:1–10:1). We were not able to separate the isomers by PTLC. For the structurally related 5a-anilinodihydrobicyclomycin, X-ray crystallographic analysis showed that the major isomer corresponded to the C(5)–*S* isomer.[30]

Satisfactory spectral data (IR, ^{1}H NMR, ^{13}C NMR, and MS) were obtained for all new compounds. Distinctive fluorine-coupled ^{13}C NMR signals were observed for all compounds consistent with the fluorine substitution pattern in the aryl unit.

2.3. Biochemical evaluation of 4–15

Table 1 lists the I_{50} values of the fluorinated analogs **4–15** in the rho poly(C)-dependent ATPase assay.[32] Each compound was evaluated against a concurrently run sample of **1** and then the I_{50} values were normalized to the reported I_{50} for **1** (60 μM).[8] Inspection of the data revealed important structure–activity patterns. First, introduction of a fluorine substituent in **3** at either the 3′-(**5**) or the 4′-(**6**) aryl position led to improved inhibitory activity compared with **3** (I_{50} (μM): **3**, 47; **5**, 23; **6**, 42). Second, successive introduction of fluorine substituents in the aromatic ring led to progressive decreases in bioactivity (I_{50} (μM): **5**, 23; **8**, 31; **11**, 50; **12**, 73; **13**, 108). Third, placement of fluorine at the 2′-aryl position provided dihydrobicyclomycins with lower rho inhibitory activities than the corresponding 3′-aryl derivatives (I_{50} (μM): **4**, 60 vs. **5**, 23; **7**, 73 vs. **8**, 31; **10**, 59 vs. **11**, 50). The two trifluoromethyl derivatives of **3**, **14** and **15**, were less active than **3**. Once again, we observed a decrease in inhibitory activity with increasing fluorine content (I_{50} (μM): **14**, 52; **15**, 80). Together, these findings showed that while fluorine substitution of **3** can enhance dihydrobicyclomycin inhibition of rho (i.e., **5**, **6**) the effects of this substitution on **3** bioactivity were finely tuned to the site and the number of fluorine residues.

We attempted to correlate the **4–15**—rho inhibitory activities with various physicochemical parameters associated with fluorobenzenes. Table 1 lists the I_{50} values of **3–15** along with the available lipophilicity constants[35] and the dipole moments for the fluoro-substituted benzene derivatives[36–38] that corresponded to the 5a-benzyl unit. We observed that increases in the fluorine composition of the benzene analog led to increased lipophilicity and, in most cases, an increase in the I_{50} value (decreased rho inhibitory activ-



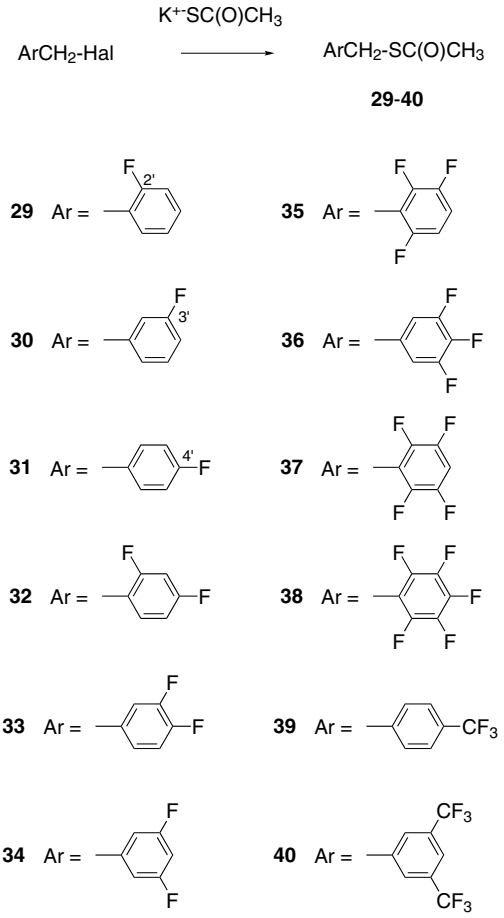
Scheme 1. Preparation of **4–15**.

ity). A similar finding was observed when the inhibitory data were compared with the available lipophilicities for the corresponding fluorine-substituted toluene derivatives (data not shown).[36–38] The only noticeable exceptions to this pattern were **5** and **6**. These findings were surprising in view of the projected hydrophobic pocket[7] for the 5a-benzyl substituent in **3–15** (see Fig. 2 for **2**). These trends suggested that factors other than lipophilicity influenced the bioactivity of dihydrobicyclomycins **3–15**.

When the poly(C)-dependent I_{50} values for **3–13** were compared with the molecular dipole moments for the corresponding substituted benzene derivatives,[36–38] we observed no apparent correlation (Table 1). The molecular dipole moment is a resultant of the moments associated with all the bonds in the entire molecule.[39] We found a near 3-fold range in inhibitory activities for the isomeric mono-fluoro dihydrobicyclomycins **4–6** (I_{50} (μM): **4**, 60; **5**, 23; **6**, 42) where the dipole moment for flurobenzene is 1.40 D. Similarly, the I_{50} values for **4–7**, **10**, and **13** ranged from 23 to 108 μM, yet the dipole moments for the corresponding benzene analogs were comparable to one another (1.37–1.51 D). A similar observation was found when we used the molecular dipole moments for the available *meta*-substituted toluene derivatives[36,40,41] in place of the benzene analogs (data not shown). The differential I_{50} values for the mono-fluoro derivatives **4–6** suggested that the bioactivities for this class of compounds were governed by site-specific biomolecular (protein, RNA) interactions.

2.4. Synthesis and biochemical evaluation of the non-fluorinated dihydrobicyclomycins

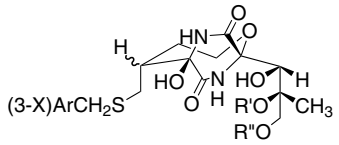
We considered if a defined bond moment within the aryl substituent of dihydrobicyclomycins **4–15** affected the inhibition process. Since **5** was the most active fluorine-substituted dihydrobicyclomycin, we focused on interactions at the 3′-benzyl site. Fluorine substitution



Scheme 2. Preparation of benzylthiolacetates.

at this site may enhance binding of the dihydrobicyclomycin to rho or perturb the rho–RNA interaction necessary for protein function, or both, leading to a loss of activity. To test the importance of this interaction, we prepared 5a-(benzylsulfanyl)-dihydrobicyclomycins that contained either a chloro (**41**), cyano (**42**), methoxy (**43**), or nitro (**44**) substituent at the 3′-benzyl position. Attempts to prepare the corresponding 3′-hydroxy derivative **45** were unsuccessful (data not shown). The rho inhibitory activities of **41**–**44** were compared with those of **3** and **5**. We expected that our choice of aryl substituents would lead to significant differences in the magnitude of the bond moment at the 3′-site in **5**, and **41**–**44**.

Compounds **41**–**44** were synthesized by the same method used for **3**–**15** (Schemes 1 and 2). Thus, we prepared **46**–**49** by treating **16** with benzylthiolacetates **50**–**53**, respectively, under basic conditions, and then the 2′,3′-acetonide group was removed (TFA) to provide dihydrobicyclomycins **41**–**44**. In Table 2, we list the inhibitory activities for **3**, **5**, and **41**–**44** in the poly(C)-dependent ATPase assay, the lipophilicity constants,[35] and the dipole moments[36,42–45] for the corresponding substituted benzene analogs, the Hammett σ_{meta} value[46] for the 3′-aryl substituent, and various atomic or group electronegativity values[47–50] reported for the selected 3′-aryl substituents. The I_{50} values for **41**–**44** ranged from 16 to 86 μM where the 3′-OCH_3 (**43**) and the 3′-NO_2 (**44**)



41 R′, R″ = H, X = Cl

42 R′, R″ = H, X = CN

43 R′, R″ = H, X = OCH_3

44 R′, R″ = H, X = NO_2

45 R′, R″ = H, X = OH

46 R′, R″ = $C(CH_3)_2$, X = Cl

47 R′, R″ = $C(CH_3)_2$, X = CN

48 R′, R″ = $C(CH_3)_2$, X = OCH_3

49 R′, R″ = $C(CH_3)_2$, X = NO_2

(3-X)$ArCH_2SC(O)CH_3$

50 X = Cl

51 X = CN

52 X = OCH_3

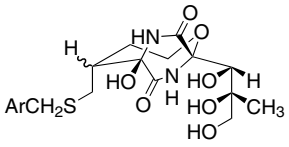
53 X = NO_2

derivatives were the most potent inhibitors (I_{50} ~16 μM) while the 3′-Cl (**41**) and the 3′-CN (**42**) analogs were the weakest inhibitors (I_{50} = 61–86 μM).

In agreement with the I_{50} values for the fluoro-substituted dihydrobicyclomycins **4**–**13**, the bioactivities for **3**, **5**, and **41**–**44** did not correlate with the lipophilicity constants for the corresponding benzene and toluene (data not shown) derivatives (Table 2).[35] For example, we found that the 3′-chloro derivative **41** was nearly 3-fold less active than the 3′-fluoro analog **5** (I_{50} (μM): **5**, 23; **41**, 61), even though chlorobenzene is more lipophilic than fluorobenzene. Similarly, we were not able to correlate the dihydrobicyclomycin inhibitory activities of **3**, **5**, and **41**–**44** with the molecular dipole moment for the corresponding benzene[36] and *meta*-substituted toluene[42–45] (data not shown) derivatives. The I_{50} for 3′-cyano derivative **42** was more than five times higher than the 3′-nitro analog **44** (I_{50} (μM): **42**, 86; **44**, 16), despite the fact that both benzonitrile and nitrobenzene exhibited comparable molecular dipole moments (i.e., μ (D): benzonitrile, 3.98; nitrobenzene, 4.21). Similarly, **5** and **43** displayed enhanced rho inhibitory activity (i.e., I_{50} (μM): **5**, 23; **43**, 17) compared with **41** (I_{50} (μM): **41**, 61), yet the dipole moments of the corresponding aromatic analogs, chlorobenzene, fluorobenzene, and anisole were nearly the same (1.32–1.57 D).

We attempted to correlate the inhibitory activities for **3**, **5**, and **41**–**44** with the Hammett σ_{meta} values derived from the linear free energy relationship that correlates

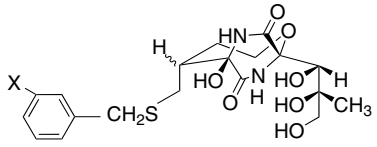
Table 1. Biochemical and biological activities of fluorine-substituted dihydrobicyclomycins **4–15** and physicochemical values of fluorobenzenes



Compound	Ar	Biochemical activity		Lipophilicity (benzene analog) log *P*	Dipole moment (benzene analog) (D)
		I_{50} (μM)[a]			
		Actual (BCM)[b]	Normalized value[c]		
3	H	67 (85)	47	2.13[d]	
4	2-F	73 (73)	60		
5	3-F	29 (77)	23	2.27[d]	1.40[d,e]
6	4-F	50 (71)	42		
7	2,4-Di-F	88 (72)	73	—	1.51[f]
8	3,4-Di-F	44 (85)	31	2.37[d]	2.59[e,f]
9	3,5-Di-F	46 (85)	32	—	—
10	2,3,6-Tri-F	70 (70)	59	—	1.40[f]
11	3,4,5-Tri-F	58 (70)	50	2.41[g]	3.00[f]
12	2,3,5,6-Tetra-F	88 (72)	73	—	2.47[f]
13	2,3,4,5,6-Penta-F	127 (70)	108	2.53[d]	1.42[f]
14	4-CF_3	60 (70)	52	3.01[d]	2.56[h]
15	3,5-Di-CF_3	94 (71)	80	—	1.37[h]

[a] Inhibitory activity measured using the rho poly(C)-dependent ATPase assay.[32] The I_{50} value is the average 50% inhibition concentration determined from duplicate tests. The corresponding value obtained from bicyclomycin in a concurrently run experiment is provided in parentheses.
[b] BCM = bicyclomycin.
[c] Values for dihydrobicyclomycin after bicyclomycin value in a concurrently tested sample was normalized to 60 μM.
[d] Ref. 35.
[e] Ref. 36.
[f] Ref. 37.
[g] Value is for the 2,3,5-trifluorobenzene found in Ref. 35.
[h] Ref. 38.

Table 2. Biochemical and biological properties of 3′-substituted 5a-benzylsulfanyldihydrobicyclomycins and physicochemical values of substituted benzenes



Compound	X	Biochemical activity		Lipophilicity (benzene analog) log *P*	Dipole moment (benzene analog) (D)	σ_m[d]	Electronegativity scales		
		I_{50} (μM)[a]					Marriott[e]	Inamoto[f]	Mullay[g]
		Actual (BCM)[b]	Normalized value[c]						
3	H	67 (85)	47	2.13[h]	0	0	2.00		
5	F	29 (77)	23	2.27[h]	1.40[i]	0.34	0.52	3.10	4.73
41	Cl	90 (88)	61	2.89[h]	1.57[i]	0.37	0.28	2.37	
42	CN	125 (88)	86	1.56[h]	3.98[i]	0.56	0.31	3.20	3.46
43	OCH_3	25 (85)	17	2.11[h]	1.32[i]	0.12	0.44	3.54	4.03
44	NO_2	19 (69)	16	1.85[h]	4.00[i]	0.71	0.40	3.42	4.08
54	COOH	6 (82)	5	0.24[h]	0.56[i]	0.37	0.18	2.82	3.15

[a] Inhibitory activity measured using the rho poly(C)-dependent ATPase assay.[32] The I_{50} value is the average 50% inhibition concentration determined from duplicate tests. The corresponding value obtained from bicyclomycin in a concurrently run experiment is provided in parentheses.
[b] BCM = bicyclomycin.
[c] Value for dihydrobicyclomycin after bicyclomycin value run in a concurrently tested sample was normalized to 60 μM.
[d] Ref. 46.
[e] Ref. 47.
[f] Ref. 48.
[g] Ref. 49.
[h] Ref. 35.
[i] Ref. 36.

the inductive effect of *meta*-aryl substituents on the ionization of benzoic acid derivatives[46] (Table 2). Using the σ_{meta} scale, we found that substituents (F, CN, and NO_2) that promoted ionization ($\sigma_{meta} = 0.56$–0.71) both led to increased (I_{50} (μM): **5**, 23; **44**, 16) and decreased (I_{50} (μM): **42**, 86) dihydrobicyclomycin inhibitory activities compared with that of **3** (I_{50} (μM): **2**, 47). Furthermore, we observed that while the 3′-fluoro (**5**), 3′-methoxy (**43**), and 3′-nitro (**44**) aryl-substituted dihydrobicyclomycins were among the most potent analogs evaluated (I_{50}: ~16–23 μM), the σ_{meta} values for the 3′-fluoro and 3′-methoxy substituents (i.e., **5**, 0.34; **43**, 0.12) were considerably lower than the 3′-nitro moiety **44** (0.71). A similar result was observed when the dihydrobicyclomycin bioactivities were compared with the Taft σ^* values[51] (data not shown).

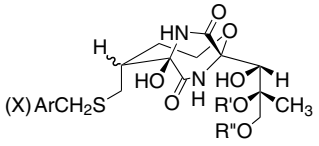
Next, we considered whether the bond moment[39,52] created by the asymmetry of the charge distribution within the 3′-X benzyl bond in **5** and **41**–**44** accounted for the dihydrobicyclomycin's bioactivities. Studies have shown that a qualitative correlation exists between the bond moment values and the differences in electronegativities of the bonded atoms.[39,52] Numerous empirical and theoretical electronegativity scales have been developed to quantify the ability of an atom or functional group to attract electrons.[47–50,53–56] Unfortunately, there is no agreement of which scale provides the best description of electronegativity[53] and efforts have been made to account for the atom's (group's) molecular environment and the effect of multiple bonds on electronegativity.[49] In Table 2, we list the I_{50} values for **3**, **5,** and **41**–**44** versus the atom or group electronegativity values in the Marriott,[47] Inamoto,[48] and Mullay[49,50] scales. We observed a qualitative correlation where atoms or groups with increased electronegativities (higher values) exhibited enhanced (lower) I_{50} values. For example, the Mullay electronegativities for the nitro-, methoxy-, and fluoro-moieties ranged from 0.40 to 0.52 and dihydrobicyclomycins with these 3′-aryl substituents exhibited inhibitory activities that ranged from 16 to 23 μM (I_{50} (μM); **5**, 23; **43**, 17; **44**, 16). Correspondingly, the chloro- and cyano-substituents have lower electronegativities (0.28–0.31) and dihydrobicyclomycins with these 3′-aryl substituents displayed higher I_{50} values (I_{50} (μM); **41**, 61; **42**, 86). Similar results were obtained using the Marriott and Inamoto values, except that the Mullay scale appeared to provide a better correlation for the 3′-CN benzyl analog **42**. This improved fit of the data may reflect Mullay and co-workers inclusion of resonance effects in their assigned value for the cyano group.[49] Noteworthy, none of the scales provided a linear correlation of inhibitory activity with electronegativity.

We also considered if dihydrobicyclomycin **3**, **5**, and **41**–**44** binding to rho was promoted by a hydrogen bond donor interaction from rho to the 3′-aryl substituent. The biochemical I_{50} values for **5** and **41**–**44** do not support this interaction. If the enhanced inhibitory activity of **5** compared with that of **3** is a manifestation of a weak hydrogen bond interaction at the binding site (rho-X-H—(3′-F)Ar-dihydrobicyclomycin), then a similar but stronger interaction would have occurred with either the 3′-methoxy moiety in **43** or the 3′-nitro unit in **44** leading to an increase in inhibitory activity. When the inhibitory activities for the 3′-fluoro (**5**), 3′-methoxy (**43**), and the 3′-nitro (**44**) compounds were compared, we found that they were all similar (I_{50} (μM): **4**, 23; **43**, 17; **44**, 16). Moreover, there are few reports documenting hydrogen bonding to aryl and alkyl fluorides.[57–63]

We concluded that the 3′-X bond moment in 5a-aryl dihydrobicyclomycins was an important structural determinant that affected rho inhibitory activities. Inspection of the X-ray crystallographic image for **2** bound to the lock washer conformation of rho[7] suggested two plausible explanations for the proposed bond moment effect on bioactivity. First, close to the 3′-formyl group in **2** is Lys181. We have reported that incubation of **2** with rho followed by $NaBH_4$ reduction led to reductive amination of Lys181.[64] Thus, a favorable bond moment interaction between the carbonyl unit in **2** and the protonated Lys181 may promote **2** binding to rho prior to imine formation. Dihydrobicyclomycin **2** exhibited an apparent I_{50} of 4 μM in the rho poly(C)-dependent ATPase assay.[64] Similarly, the bond moments created by the 3′-fluorine (**5**), 3′-methoxy (**43**), and 3′-nitro (**44**) groups in these dihydrobicyclomycins may be stabilized by the positively charged Lys181 leading to enhanced rho binding. This hypothesis suggests that the observed increase in bioactivity for select 5a-aryl dihydrobicyclomycins is due to increased dihydrobicyclomycin binding.[12] The second explanation considers the impact of dihydrobicyclomycin **5** and **41**–**44** binding on RNA-mediated ATP catalysis. We have shown that the I_{50} value for **1** decreases in rho functional ATPase assays with RNA substrates that bind less tightly to the secondary site,[12] suggesting that **1** is more able to disrupt RNA-mediated ATP catalysis for weaker-binding RNAs. The aldehyde group in **2** is wedged between Pro180 of one subunit and Lys336 of the adjacent subunit. We have previously shown that Lys336 is part of the extended secondary RNA tracking site that consists of a strip of positively charged amino acid residues that line the central hole on each subunit.[12] A significant bond moment at the 3′-aryl substituent in **3** may electrostatically perturb (weaken) the binding of the RNA phosphate backbone to the secondary site and disrupt ATP catalysis. Since we have not measured the dissociation constants of the dihydrobicyclomycin–rho complexes, we cannot at this time distinguish these two inhibitory pathways.

Our identification of the importance of the 3′-aryl moiety in substituted **3** led us to further test the importance of this site. We rationalized that introduction of a 3′-carboxyl group in **3** to give **54** would lead to the corresponding 3′-carboxylate species under the rho poly(C)-dependent ATPase conditions (pH 7.9).[32] Generation of a discrete negative charge should either enhance dihydrobicyclomycin binding or perturb RNA-binding to the secondary site, or both, leading to a low I_{50} value.

Correspondingly, we predicted that the 4′-carboxyl isomer **55** would have diminished inhibitory activities compared with **54**. Synthesis of **54** and **55** followed the same protocols (Schemes 1 and 2) previously used and required our initial preparation of **56** and **57** from **16** using **58** and **59**, respectively.



54 R′, R″ = H, X = 3′-CO_2H

55 R′, R″ = H, X = 4′-CO_2H

56 R′, R″ = $C(CH_3)_2$, X = 3′-CO_2H

57 R′, R″ = $C(CH_3)_2$, X = 4′-CO_2H

(X)$ArCH_2SC(O)CH_3$

58 X = 3′-CO_2H

59 X = 4′-CO_2H

The I_{50} for **54** was 5 μM (Table 2) making this compound, with **2**,[64] the most potent rho inhibitors discovered to date. In agreement with the proposed site-specific interaction, we found that the 4′-substituted carboxyl derivative **55** was less effective in inhibiting rho poly(C)-ATPase activity (I_{50} (μM): **54**, 5; **55**, 31). This difference in inhibitory activities mirrored those observed for **5** and **6** (I_{50} (μM): **5**, 23; **6**, 42). We were unable to correlate the bioactivity for **54** with published electronegativity tables since most scales provide values for the unionized carboxylic acid residue. For example, in the Mullay electronegativity scale[49] the carboxyl moiety is 3.15, thus indicating that it is less electronegative than a cyano (3.46) substituent.

The antibacterial activities of fluorine-substituted 5a-(benzylsulfanyl)-dihydrobicyclomycins (**5**, **8**, **11**, **12**, and **14**) against W3350 *E. coli* were determined using the filter disk assay.[33] The compounds tested showed no detectable antibiotic activities (MIC > 32 mg/mL). We have observed similar findings for other dihydrobicyclomycins that exhibited pronounced inhibitory activities in rho functional assays.[14,30]

3. Conclusion

Selective fluorine substitution of 5a-benzylsulfanyldihydrobicyclomycin (**3**) has permitted us to probe the microenvironment surrounding the 5a-(benzylsulfanyl)-dihydrobicyclomycin-binding pocket in rho. We documented that incorporation of either an electronegative atom or group at the 3′-aryl site leads to improved rho inhibitory activity and project that this increase in bioactivity stems from either a beneficial bond moment stabilizing interaction with the nearby protonated Lys181 or the disruption of RNA binding to the secondary RNA tracking site in rho, or both. These hypotheses explain why the inhibitory activities were dependent on the site of fluorine substitution within the mono-fluorine dihydrobicyclomycins **4**–**6**. We can also rationalize why decreased inhibitory activities were observed for the multi-fluorine-substituted dihydrobicyclomycins **8**–**13** compared with **5**. The introduction of additional electron-withdrawing fluorine substituents on the aromatic ring in **5** to give **8**–**13** reduces the bond moment at the 3′-benzyl site, and diminishes either the site-specific bond moment stabilizing interaction of the 3′-fluoro substituent with Lys181 or the adverse interaction with the RNA phosphate backbone. Our data set led to the successful prediction that dihydrobicyclomycin **54** would function as a potent rho inhibitor. These findings, which support a site-specific, bond moment interaction between the 3-fluoro moiety in **5** with activated rho, may help explain, in part, the beneficial properties observed for other fluorine-substituted ligands[15–24] and their cognate receptors.

4. Experimental

4.1. General methods

^1H (300 MHz) and ^{13}C (75 MHz) NMR were recorded on a Varian VXR300 spectrometer. Low-resolution and high-resolution (CI) mass spectral studies were run at the University of Texas at Austin Department of Chemistry by Dr. M. Moini and by Dr. Dana Reed at the University of Minnesota Department of Chemistry. Thin-layer chromatographies were run on precoated silica gel slides (20 × 20 cm; Sigma Z12272-6). Bicyclomycin was purified by silica gel chromatography (3×) using 20% MeOH–$CHCl_3$ as the eluant solvent prior to use in biological experiments. Rho protein was isolated from *E. coli* AR 120 containing the overexpressing plasmid p39-ASE, which has been corrected for the K155E substitution seen in the original p39-AS plasmid.[65] His-tagged wild-type rho was expressed and purified using the pET-14b vector previously described.[66] Rho concentration was measured according to the bicinchoninic acid (BCA) method. Poly(C) concentrations were estimated by determining the average size of the RNA using denaturing PAGE electrophoresis. [γ-^{32}P]ATP was purchased from Perkin Elmer (Boston, MA), and nucleotides were obtained from Sigma. Polyethyleneimine (PEI) thin-layer chromatography (TLC) plates used for ATPase assays were purchased from J.T. Baker, Inc. (Phillipsburg, NJ).

4.2. General procedure 1. Preparation of 5a-substituted dihydrobicyclomycin 2′,3′-acetonides (methods A and B)

To a methanolic solution (3–10 mL) of **16** under Ar was added the substituted benzylthioacetate (3–4 equiv, method A) or the substituted benzylthiol (3–4 equiv, method B) and then the "pH" was adjusted to 10.5 with aqueous 1.0 N NaOH. The mixture was stirred at room temperature (2–72 h) until no starting material remained (TLC analysis). The "pH" of the mixture was adjusted to 7.0 with aqueous 0.1 N HCl. The solvent was removed in vacuo and the residue was redissolved in

MeOH and purified by preparative TLC (10% MeOH–$CHCl_3$) to afford the desired product.

4.3. General procedure 2. Preparation of substituted 5a-benzylsulfanyldihydrobicyclomycins

To a 50% aqueous methanolic solution (2–10 mL) containing the substituted 5a-benzylsulfanyldihydrobicyclomycin 2′,3′-acetonide was added TFA (2–10 drops), and then the solution was stirred at room temperature (2–48 h) until no starting material remained (TLC analysis). The solvent was removed in vacuo and the residue was purified by preparative TLC (10–20% MeOH–$CHCl_3$) to afford the desired product.

4.4. General procedure 3. Preparation of benzylthioacetates 29–30, 32–40, 50–53, 58, and 60

To an acetone solution (10–20 mL) of the desired aromatic benzyl halide under Ar was added potassium thioacetate (1.2 equiv). The mixture was heated to reflux (0.5–4 h) until no starting material remained (TLC analysis). The solvent was removed in vacuo and H_2O (20 mL) was added. The reaction mixture was extracted with CH_2Cl_2 (2×10 mL), washed with brine, dried ($MgSO_4$), and concentrated in vacuo. The product was purified by silica gel column chromatography using 5–20% EtOAc–hexanes.

4.5. 5a-(2-Fluorobenzylsulfanyl)-dihydrobicyclomycin 2′,3′-acetonide (17)

Using General procedure 1(A), **16** (50 mg, 0.15 mmol) and thioacetic acid *S*-(2-fluorobenzyl) ester (**29**) (62 mg, 0.44 mmol) gave **17** as a mixture of diastereomers (~3:1): yield, 49 mg (72%); R_f 0.71 (10% MeOH–$CHCl_3$); FT-IR (KBr) 3303, 2897, 1687, 1403, 1043 cm^{-1}; ^1H NMR (CD_3OD) for the major diastereomer, δ 1.36 (s, 3H, C(2′)CH_3), 1.43 (s, 3H, $C(CH_3)_2$), 1.45 (s, 3H, $C(CH_3)_2$), 1.85–2.37 (m, 3H, C(4)*H*H′, C(4)H*H*′, C(5)H), 2.17 (d, J = 7.7 Hz, 1H, C(5a)*H*H′), 3.16 (d, J = 7.7 Hz, 1H, C(5a)H*H*′), 3.71 (d, J = 8.2 Hz, 1 H, C(3′)*H*H′), 3.69–3.76 (m, 1H, C(3)*H*H′), 3.73 (s, 2H, C(5b)H_2), 3.81–3.95 (m, 1H, C(3)H*H*′), 4.44 (d, J = 8.2 Hz, 1H, C(3′)H*H*′), 4.09 (s, 1H, C(1′)H), 7.03–7.14 (m, 2H, ArH), 7.23–7.30 (m, 1H, ArH), 7.36–7.44 (m, 1H, ArH); ^1H NMR (CD_3OD) for the minor diastereomer, δ 4.08 (s, 1H, C(1′)H), the remaining peaks overlapped with nearby signals and were not detected; the structural assignments were in agreement with the ^1H–^1H COSY experiment; ^{13}C NMR (CD_3OD) for the major diastereomer, 24.1, 26.0, 27.4, 29.3, 29.5, 31.1, 51.5, 62.7, 72.3, 72.4, 82.9, 85.6, 88.0, 110.8, 115.2 (d, J = 21.8 Hz), 124.4 (d, J = 4.5 Hz), 126.4 (d, J = 14.9 Hz), 129.2 (d, J = 8.1 Hz), 131.5 (s), 161.0 (d, J = 243.9 Hz), 167.3, 171.8 ppm; ^{13}C NMR (CD_3OD) for the minor diastereomer, 27.5, 62.6, 82.8, 88.2, 110.9 ppm, the remaining peaks overlapped with nearby signals and were not detected; MS (+ESI) 507 $[M+Na]^+$; M_r (+ESI) 507.1600 $[M+Na]^+$ (calcd for $C_{22}H_{29}FN_2NaO_7S$ 507.1572 $[M+Na]^+$).

4.6. 5a-(3-Fluorobenzylsulfanyl)-dihydrobicyclomycin 2′,3′-acetonide (18)

Using General procedure 1(A), **16** (50 mg, 0.15 mmol) and thioacetic acid *S*-(3-fluorobenzyl) ester (**30**) (62 mg, 0.44 mmol) gave **18** as a mixture of diastereomers (~3:1): yield, 47 mg (70%); R_f 0.73 (10% MeOH–$CHCl_3$); FT-IR (KBr) 3304 (br), 2986, 1681, 1404, 1046 cm^{-1}; ^1H NMR (CD_3OD) for the major diastereomer, δ 1.35 (s, 3H, C(2′)CH_3), 1.41 (s, 3H, $C(CH_3)_2$), 1.44 (s, 3H, $C(CH_3)_2$), 1.83–2.14 (m, 2H, C(4)*H*H′, C(4)H*H*′), 2.06–2.21 (m, 1H, C(5)H), 2.12 (d, J = 11.8 Hz, 1H, C(5a)*H*H′), 3.14 (d, J = 11.8 Hz, 1H, C(5a)H*H*′), 3.69 (s, 2H, C(5b)H_2), 3.70 (d, J = 8.2 Hz, 1H, C(3′)*H*H′), 3.76 (d, J = 8.2 Hz, 1H, C(3)*H*H′), 3.87 (d, J = 8.2 Hz, 1H, C(3)H*H*′), 4.43 (d, J = 8.2 Hz, 1H, C(3′)H*H*′), 4.03 (s, 1H, C(1′)H), 7.02–7.13 (m, 2H, ArH), 7.22–7.29 (m, 1H, ArH), 7.36–7.43 (m, 1H, ArH); ^1H NMR (CD_3OD) for the minor diastereomer, δ 4.08 (s, 1H, C(1′)H), the remaining peaks overlapped with nearby signals and were not detected; the structural assignments were in agreement with the ^1H–^1H COSY experiment; ^{13}C NMR (CD_3OD) for the major diastereomer, 23.1, 26.0, 27.4, 29.6, 30.7, 35.8, 51.4, 63.0, 72.3, 72.4, 82.9, 85.6, 88.0, 110.8, 113.8 (d, J = 21.8 Hz), 115.9 (d, J = 21.7 Hz), 125.2 (d, J = 3.4 Hz), 130.3 (d, J = 8.0 Hz), 142.2 (d, J = 6.9 Hz), 163.4 (d, J = 243.9 Hz, C(3″)), 167.4, 171.0 ppm; ^{13}C NMR (CD_3OD) for the minor diastereomer, 27.6, 72.5, 82.8, 88.2, 111.9, 166.3, 173.0 ppm, the remaining peaks overlapped with nearby signals and were not detected; MS (−CI) 483 $[M-1]^-$; M_r (−CI) 483.160 00 $[M-1]^-$ (calcd for $C_{22}H_{28}FN_2O_7S$ 483.160 13 $[M-1]^-$).

4.7. 5a-(4-Fluorobenzylsulfanyl)-dihydrobicyclomycin 2′,3′-acetonide (19)

Using General procedure 1(B), **16** (50 mg, 0.15 mmol) and 4-fluorobenzenethiol (62 mg, 0.44 mmol) gave **19** as a mixture of diastereomers (~3:1): yield, 45 mg (75%); R_f 0.70 (10% MeOH–$CHCl_3$); FT-IR (KBr) 3368 (br), 2953, 1691, 1406, 1042 cm^{-1}; ^1H NMR (CD_3OD) for the major diastereomer, δ 1.27 (s, 3H, C(2′)CH_3), 1.33 (s, 3H, $C(CH_3)_2$), 1.36 (s, 3H, $C(CH_3)_2$), 1.75–2.20 (m, 2H, C(4)*H*H′, C(4)H*H*′), 1.97–2.12 (m, 1H, C(5)H), 2.03 (d, J = 11.7 Hz, 1H, C(5a)*H*H′), 3.05 (d, J = 11.7 Hz, 1H, C(5a)H*H*′), 3.59 (s, 2H, C(5b)H_2), 3.59–3.71 (m, 1H, C(3)*H*H′), 3.62 (d, J = 8.2 Hz, 1H, C(3′)*H*H′), 3.70–3.84 (m, 1H, C(3)H*H*′), 4.00 (s, 1H, C(1′)H), 4.36 (d, J = 8.2 Hz, 1H, C(3′)H*H*′), 6.89–6.95 (m, 2H, ArH), 7.23–7.28 (m, 2H, ArH); ^1H NMR (CD_3OD) for the minor diastereomer, δ 4.00 (s, 1H, C(1′)H), the remaining peaks overlapped with nearby signals and were not detected; the structural assignments were in agreement with the ^1H–^1H COSY experiment; ^{13}C NMR (CD_3OD) for the major diastereomer, 23.8, 26.0, 27.4, 29.5, 30.6, 35.5, 51.3, 62.7, 72.2, 72.3, 82.9, 85.6, 88.0, 110.8, 115.2 (d, J = 20.0 Hz, C(3″), C(5″)), 131.1 (d, J = 8.0 Hz, C(2″), C(6″)), 135.2 (d, J = 3.4 Hz, C(1″)), 162.4 (d, J = 246.2 Hz, C(4″)), 167.4, 170.8 ppm; ^{13}C NMR (CD_3OD) for the minor diastereomer, 27.5, 35.9, 62.9, 72.4, 82.8, 88.2, 110.9, 166.2, 172.8 ppm, the remaining

peaks overlapped with nearby signals and were not detected; MS (−CI) 483 $[M-1]^-$; M_r (−CI) 483.160 83 $[M-1]^-$ (calcd for $C_{22}H_{28}FN_2O_7S$ 483.160 13 $[M-1]^-$).

4.8. 5a-(2,4-Difluorobenzylsulfanyl)-dihydrobicyclomycin 2′,3′-acetonide (20)

Using General procedure 1(A), **16** (40 mg, 0.12 mmol) and thioacetic acid *S*-(2,4-difluorobenzyl) ester (**32**) (88 mg, 0.44 mmol) gave **20** as a mixture of diastereomers (~3:1): yield, 32 mg (53%); R_f 0.69 (10% MeOH–$CHCl_3$); FT-IR (KBr) 3308 (br), 2950, 1689, 1403, 1041 cm^{-1}; ^1H NMR (CD_3OD) for the major diastereomer, δ 1.28 (s, 3 H, C(2′)CH_3), 1.34 (s, 3H, $C(CH_3)_2$), 1.37 (s, 3H, $C(CH_3)_2$), 1.75–2.28 (m, 3H, C(4)*H*H′, C(4)H*H*′, C(5)H), 2.07 (d, J = 10.2 Hz, 1H, C(5a)*H*H′), 3.09 (d, J = 10.2 Hz, 1H, C(5a)H*H*′), 3.61 (d, J = 8.1 Hz, 1H, C(3′)*H*H′), 3.62 (s, 2H, C(5b)H_2), 3.67–3.89 (m, 2H, C(3)*H*H′, C(3)H*H*′), 4.01 (s, 1H, C(1′)H), 4.36 (d, J = 8.1 Hz, 1H, C(3′)H*H*′), 6.79–6.87 (m, 1H, ArH), 7.29–7.37 (m, 2H, ArH); ^1H NMR (CD_3OD) for the minor diastereomer, δ 3.95 (s, 1H, C(1′)H), the remaining peaks overlapped with nearby signals and were not detected; the structural assignments were in agreement with the ^1H–^1H COSY experiment; ^{13}C NMR (CD_3OD) for the major diastereomer, 23.9, 25.2, 27.8, 28.5, 30.3, 30.8, 51.2, 62.4, 72.0, 72.1, 82.6, 85.4, 87.8, 103.6 (t, J = 26.3 Hz), 110.6, 111.1 (dd, J = 21.0, 4.0 Hz), 122.4 (dd, J = 13.4, 4.3 Hz), 125.3 (dd, J = 6.3, 3.4 Hz), 161.2 (dd, J = 248.2, 12.0 Hz), 162.5 (dd, J = 248.7, 12.0 Hz), 167.1, 170.5 ppm; ^{13}C NMR (CD_3OD) for the minor diastereomer peaks overlapped with nearby signals and were not detected; MS (+CI) 503 $[M+1]^+$; M_r (+CI) 503.165 47 $[M+1]^+$ (calcd for $C_{22}H_{29}F_2N_2O_7S$ 503.166 36 $[M+1]^+$).

4.9. 5a-(3,4-Difluorobenzylsulfanyl)-dihydrobicyclomycin 2′,3′-acetonide (21)

Using General procedure 1(A), **16** (50 mg, 0.15 mmol) and thioacetic acid *S*-(3,4-difluorobenzyl) ester (**33**) (88 mg, 0.44 mmol) gave **21** as a mixture of diastereomers (~3:1): yield, 45 mg (63%); R_f 0.65 (10% MeOH–$CHCl_3$); FT-IR (KBr) 3300 (br), 2932, 1689, 1402, 1049 cm^{-1}; ^1H NMR (CD_3OD) for the major diastereomer, δ 1.35 (s, 3H, C(2′)CH_3), 1.44 (s, 3H, $C(CH_3)_2$), 1.45 (s, 3H, $C(CH_3)_2$), 1.83–2.40 (m, 3H, C(4)*H*H′, C(4)H*H*′, C(5)H), 2.17 (d, J = 11.3 Hz, 1H, C(5a)*H*H′), 3.22 (d, J = 11.3 Hz, 1H, C(5a)H*H*′), 3.65 (d, J = 8.3 Hz, 1H, C(3′)*H*H′), 3.70 (s, 2H, C(5b)H_2), 3.76–3.98 (m, 2H, C(3)*H*H′, C(3)H*H*′), 4.09 (s, 1H, C(1′)H), 4.44 (d, J = 8.3 Hz, 1H, C(3′)H*H*′), 6.23–6.31 (m, 2H, ArH), 7.39 (s, 1H, ArH); ^1H NMR (CD_3OD) for the minor diastereomer, δ 4.08 (s, 1H, C(1′)H), 4.45 (d, J = 7.3 Hz, 1H, C(3′)H*H*′), the remaining peaks overlapped with nearby signals and were not detected; the structural assignments were in agreement with the ^1H–^1H COSY experiment; ^{13}C NMR (CD_3OD) for the major diastereomer, 23.8, 25.7, 27.2, 29.4, 30.3, 34.9, 51.1, 62.4, 72.1, 72.2, 82.6, 85.3, 87.7, 110.6, 117.0 (d, J = 9.2 Hz), 117.8 (d, J = 9.2 Hz), 125.5 (dd, J = 9.2, 3.4 Hz), 136.7 (dd, J = 9.2, 3.4 Hz), 149.5 (dd, J = 246.2, 13.1 Hz), 150.2 (dd, J = 246.2, 12.5 Hz), 167.1, 170.6 ppm; ^{13}C NMR (CD_3OD) for the minor diastereomer, 27.3, 34.8, 82.5, 110.5 ppm, the remaining peaks overlapped with nearby signals and were not detected; MS (+CI) 503 $[M+1]^+$; M_r (+CI) 503.167 89 $[M+1]^+$ (calcd for $C_{22}H_{29}F_2N_2O_7S$ 503.166 36 $[M+1]^+$).

4.10. 5a-(3,5-Difluorobenzylsulfanyl)-dihydrobicyclomycin 2′,3′-acetonide (22)

Using General procedure 1(A), **16** (50 mg, 0.15 mmol) and thioacetic acid *S*-(3,5-difluorobenzyl) ester (**34**) (88 mg, 0.44 mmol) gave **22** as a mixture of diastereomers (~3:1): yield, 44 mg (60%); R_f 0.65 (10% MeOH–$CHCl_3$); FT-IR (KBr) 3303 (br), 2987, 1690, 1402, 1047 cm^{-1}; ^1H NMR (CD_3OD) for the major diastereomer, δ 1.29 (s, 3H, C(2′)CH_3), 1.36 (s, 3H, $C(CH_3)_2$), 1.38 (s, 3H, $C(CH_3)_2$), 1.78–1.90 (m, 1H, C(4)*H*H′), 1.92–2.33 (m, 2H, C(4)H*H*′, C(5)H), 2.07 (d, J = 10.8 Hz, 1H, C(5a)*H*H′), 3.06 (d, J = 10.8 Hz, 1H, C(5a)H*H*′), 3.63 (s, 2H, C(5b)H_2), 3.64 (d, J = 8.3 Hz, 1H, C(3′)*H*H′), 3.68–3.91 (m, 2H, C(3)*H*H′, C(3)H*H*′), 4.03 (s, 1H, C(1′)H), 4.38 (d, J = 8.3 Hz, 1H, C(3′)H*H*′), 6.71–6.77 (m, 2H, ArH), 6.85–6.95 (m, 1H, ArH); ^1H NMR (CD_3OD) for the minor diastereomer, δ 3.91 (s, 1H, C(1′)H), the remaining peaks overlapped with nearby signals and were not detected; the structural assignments were in agreement with the ^1H–^1H COSY experiment; ^{13}C NMR (CD_3OD) for the major diastereomer, 23.6, 25.2, 27.1, 29.4, 30.4, 35.2, 51.1, 62.4, 72.1, 72.3, 82.6, 85.3, 88.0, 102.0 (t, J = 26.4 Hz), 110.7, 111.9 (dd, J = 17.7, 7.7 Hz, 2C), 143.8 (t, J = 9.2 Hz), 163.3 (dd, J = 246.2, 13.4 Hz, C(3″), C(5″)), 167.1, 170.6 ppm; ^{13}C NMR (CD_3OD) for the minor diastereomer peaks overlapped with nearby signals and were not detected; MS (+ESI) 525 $[M+Na]^+$; M_r (+ESI) 525.1464 $[M+Na]^+$ (calcd for $C_{22}H_{28}F_2N_2NaO_7S$ 525.1478 $[M+Na]^+$).

4.11. 5a-(2,3,6-Trifluorobenzylsulfanyl)-dihydrobicyclomycin 2′,3′-acetonide (23)

Using General procedure 1(A), **16** (50 mg, 0.15 mmol) and thioacetic acid *S*-(2,3,6–trifluorobenzyl) ester (**35**) (97 mg, 0.44 mmol) gave **23** as a mixture of diastereomers (~3:1): yield, 44 mg (59%); R_f 0.60 (10% MeOH–$CHCl_3$); FT-IR (KBr) 3306 (br), 2988, 1689, 1405, 1046 cm^{-1}; ^1H NMR (CD_3OD) for the major diastereomer, δ 1.30 (s, 3H, C(2′)CH_3), 1.38 (s, 3H, $C(CH_3)_2$), 1.39 (s, 3H, $C(CH_3)_2$), 1.78–1.90 (m, 1H, C(4)*H*H′), 1.92–2.30 (m, 1H, C(4)H*H*′), 2.05–2.33 (m, 1H, C(5)H), 2.20 (d, J = 12.6 Hz, 1H, C(5a)*H*H′), 3.22 (d, J = 12.6 Hz, 1H, C(5a)H*H*′), 3.62 (d, J = 8.4 Hz, 1H, C(3′)*H*H′), 3.67–3.94 (m, 2H, C(3)*H*H′, C(3)H*H*′), 3.72 (s, 2H, C(5b)H_2), 4.01 (s, 1H, C(1′)H), 4.38 (d, J = 8.4 Hz, 1H, C(3′)H*H*′), 6.86–6.93 (m, 1H, ArH), 7.09–7.11 (m, 1H, ArH); ^1H NMR (CD_3OD) for the minor diastereomer, δ 4.02 (s, 1H, C(1′)H), 4.40 (d, J = 8.4 Hz, C(3′)H*H*′), the remaining peaks overlapped with nearby signals and were not detected; the structural assignments were in agreement with the ^1H–^1H COSY experiment; ^{13}C NMR (CD_3OD) for the major diastereomer, 23.7, 26.3, 27.7, 29.9, 30.7, 32.2, 51.9, 63.0, 72.6, 72.6, 83.1, 85.9, 88.3, 109.9 (ddd, J = 24.0, 6.9, 4.6 Hz), 111.1,

114.9 (dd, $J = 20.1$, 10.3 Hz), 117.9 (dd, $J = 21.1$, 13.4 Hz), 147.9 (ddd, $J = 246.1$, 13.8, 3.6 Hz), 149.3 (ddd, $J = 249.1$, 11.8, 7.8 Hz), 157.1 (ddd, $J = 246.2$, 5.7, 2.3 Hz), 167.5, 171.0 ppm; ^{13}C NMR (CD_3OD) for the minor diastereomer, 27.8, 62.9, 72.7, 82.9, 88.5, 111.2, 166.7, 173.1 ppm, the remaining peaks overlapped with nearby signals and were not detected; MS (−CI) 519 $[M-1]^-$; M_r (−CI) 519.141 80 $[M-1]^-$ (calcd for $C_{22}H_{26}F_3N_2O_7S$ 519.141 28 $[M-1]^-$).

4.12. 5a-(3,4,5-Trifluorobenzylsulfanyl)-dihydrobicyclomycin 2′,3′-acetonide (24)

Using General procedure 1(A), **16** (50 mg, 0.15 mmol) and thioacetic acid *S*-(3,4,5-trifluorobenzyl) ester (**36**) (97 mg, 0.44 mmol) gave **24** as a mixture of diastereomers (~3:1): yield, 46 mg (61%); R_f 0.65 (10% MeOH–$CHCl_3$); FT-IR (KBr) 3296 (br), 2988, 1688, 1472, 1043 cm^{-1}; ^{1}H NMR (CD_3OD) for the major diastereomer, δ 1.28 (s, 3H, C(2′)CH_3), 1.33 (s, 3H, $C(CH_3)_2$), 1.36 (s, 3H, $C(CH_3)_2$), 1.79–1.87 (m, 1H, C(4)*H*H′), 1.97–2.14 (m, 1H, C(4)H*H*′), 2.04 (d, $J = 11.5$ Hz, 1H, C(5a)*H*H′), 2.16–2.27 (m, 1H, C(5)H), 2.99 (d, $J = 11.5$ Hz, 1H, C(5a)H*H*′), 3.59 (s, 2H, C(5b)H_2), 3.62 (d, $J = 8.2$ Hz, 1H, C(3′)*H*H′), 3.67–3.74 (m, 1H, C(3)*H*H′), 3.78–3.90 (m, 1H, C(3)H*H*′), 4.02 (s, 1H, C(1′)H), 4.35 (d, $J = 8.2$ Hz, 1H, C(3′)H*H*′), 7.04 (d, $J = 8.2$ Hz, 1H, ArH), 7.09 (d, $J = 8.2$ Hz, 1H, ArH); ^{1}H NMR (CD_3OD) for the minor diastereomer, δ 4.01 (s, 1H, C(1′)H),the remaining peaks overlapped with nearby signals and were not detected; the structural assignments were in agreement with the ^{1}H–^{1}H COSY experiment; ^{13}C NMR (CD_3OD) for the major diastereomer, 23.9, 25.9, 27.4, 29.7, 30.5, 35.0, 51.2, 62.7, 72.3, 72.4, 82.8, 85.5, 88.0, 110.9, 113.5 (dd, $J = 16.0$, 6.8 Hz, C(2″), C(6″)), 135.7–136.7 (m, C(1″)), 138.9 (ddd, $J = 248.5$, 10.3, 3.9 Hz, C(4″)), 150.5 (ddd, $J = 249.6$, 10.3, 3.9 Hz, C(3″), C(5″)), 167.3, 170.8 ppm; ^{13}C NMR (CD_3OD) for the minor diastereomer, 26.0, 27.6, 34.8, 82.7, 85.6, 88.3, 110.9, 166.3, 171.8 ppm, the remaining peaks overlapped with nearby signals and were not detected; MS (+ESI) 543 $[M+Na]^+$; M_r (+ESI) 543.1396 $[M+Na]^+$ (calcd for $C_{22}H_{27}F_3N_2NaO_7S$ 543.1384 $[M+Na]^+$).

4.13. 5a-(2,3,5,6-Tetrafluorobenzylsulfanyl)-dihydrobicyclomycin 2′,3′-acetonide (25)

Using General procedure 1(A), **16** (50 mg, 0.15 mmol) and thioacetic acid *S*-(2,3,5,6–tetrafluorobenzyl) ester (**37**) (104 mg, 0.44 mmol) gave **25** as a mixture of diastereomers (~3:1): yield, 46 mg (59%); R_f 0.63 (10% MeOH–$CHCl_3$); FT-IR (KBr) 3301 (br), 2988, 1691, 1380, 1046 cm^{-1}; ^{1}H NMR (CD_3OD) for the major diastereomer, δ 1.36 (s, 3H, C(2′)CH_3), 1.43 (s, 3H, $C(CH_3)_2$), 1.45 (s, 3H, $C(CH_3)_2$), 1.81–2.00 (m, 1H, C(4)*H*H′), 2.02–2.28 (m, 2H, C(4)H*H*′, C(5)H), 2.16 (d, $J = 8.2$ Hz, 1H, C(5a)*H*H′), 3.08 (d, $J = 8.2$ Hz, 1H, C(5a)H*H*′), 3.67–4.01 (m, 2H, C(3)*H*H′, C(3)H*H*′), 3.71 (d, $J = 8.2$ Hz, 1H, C(3′)*H*H′), 3.73 (s, 2H, C(5b)H_2), 4.10 (s, 1H, C(1′)H), 4.45 (d, $J = 8.2$ Hz, 1H, C(3′)H*H*′), 7.22–7.32 (m, 1H, ArH); ^{1}H NMR (CD_3OD) for the minor diastereomer, δ 3.99 (s, 1H, C(1′)H), the remaining peaks overlapped with nearby signals and were not detected; the structural assignments were in agreement with the ^{1}H–^{1}H COSY experiment; ^{13}C NMR (CD_3OD) for the major diastereomer, 24.0, 26.0, 27.4, 29.7, 28.3, 30.9, 51.3, 62.7, 72.2, 72.3, 82.8, 85.6, 88.0, 110.8, 112.6 (ddd, $J = 19.5$, 4.3, 3.4 Hz), 123.7–123.9 (m), 138.2–139.4 (m), 141.5–142.7 (m), 144.5–145.5 (m), 147.8–148.7 (m), 168.0, 171.3 ppm; ^{13}C NMR (CD_3OD) for the minor diastereomer, 27.5, 82.7, 88.3, 110.9, 166.8, 173.4 ppm, the remaining peaks overlapped with nearby signals and were not detected; MS (−CI) 537 $[M-1]^-$; M_r (−CI) 537.131 81 $[M-1]^-$ (calcd for $C_{22}H_{25}F_4N_2O_7S$ 537.131 86 $[M-1]^-$).

4.14. 5a-(2,3,4,5,6-Pentafluorobenzylsulfanyl)-dihydrobicyclomycin 2′,3′-acetonide (26)

Using General procedure 1(A), **16** (50 mg, 0.15 mmol) and thioacetic acid *S*-(2,3,4,5,6-pentafluorobenzyl) ester (**38**) (111 mg, 0.44 mmol) gave **26** as a mixture of diastereomers (~3:1): yield, 52 mg (65%); R_f 0.61 (10% MeOH–$CHCl_3$); FT-IR (KBr) 3303 (br), 2958, 1688, 1392, 1045 cm^{-1}; ^{1}H NMR (CD_3OD) for the major diastereomer, δ 1.26 (s, 3H, C(2′)CH_3), 1.30 (s, 3H, $C(CH_3)_2$), 1.34 (s, 3H, $C(CH_3)_2$), 1.78–1.85 (m, 1H, C(4)*H*H′), 2.03–2.11 (m, 1H, C(4)H*H*′), 2.07 (d, $J = 10.2$ Hz, 1H, C(5a)*H*H′), 2.11–2.33 (m, 1H, C(5)H), 3.15 (d, $J = 10.2$ Hz, 1H, C(5a)H*H*′), 3.56 (d, $J = 8.1$ Hz, 1H, C(3′)*H*H′), 3.66 (s, 2H, C(5b)H_2), 3.78–3.86 (m, 2H, C(3)*H*H′, C(3)H*H*′), 4.03 (s, 1H, C(1′)H), 4.34 (d, $J = 8.1$ Hz, 1H, C(3′)H*H*′); ^{1}H NMR (CD_3OD) for the minor diastereomer, δ 4.02 (s, 1H, C(1′)H), the remaining peaks overlapped with nearby signals and were not detected; the structural assignments were in agreement with the ^{1}H–^{1}H COSY experiment; ^{13}C NMR (CD_3OD) for the major diastereomer, 23.0, 24.0, 25.8, 27.2, 29.7, 32.0, 51.6, 62.8, 72.1, 72.3, 82.8, 85.5, 88.0, 110.8, 113.9–114.1 (m), 135.9–136.8 (m), 138.9–140.0 (m, 2C), 143.5–147.3 (m, 2C), 167.1, 170.7 ppm; ^{13}C NMR (CD_3OD) for the minor diastereomer, 26.1, 27.8, 29.3, 30.8, 62.6, 82.6, 85.6, 88.1, 110.9, 166.5, 172.8 ppm, the remaining peaks overlapped with nearby signals and were not detected; MS (−CI) 555 $[M-1]^-$; M_r (−CI) 555.121 86 $[M-1]^-$ (calcd for $C_{22}H_{24}F_5N_2O_7S$ 555.122 44 $[M-1]^-$).

4.15. 5a-(4-Trifluoromethylbenzylsulfanyl)-dihydrobicyclomycin 2′,3′-acetonide (27)

Using General procedure 1(A), **16** (40 mg, 0.12 mmol) and thioacetic acid *S*-(4-trifluoromethyl) ester (**39**) (84 mg, 0.36 mmol) gave **27** as a mixture of diastereomers (~3:1): yield, 44 mg (68%); R_f 0.70 (10% MeOH–$CHCl_3$); FT-IR (KBr) 3312 (br), 2948, 1667, 1401, 1043 cm^{-1}; ^{1}H NMR (CD_3OD) for the major diastereomer, δ 1.35 (s, 3H, C(2′)CH_3), 1.42 (s, 3H, $C(CH_3)_2$), 1.44 (s, 3H, $C(CH_3)_2$), 1.82–1.99 (m, 1H, C(4)*H*H′), 2.13–2.17 (m, 1H, C(4)H*H*′), 2.18 (d, $J = 8.3$ Hz, 1H, C(5a)*H*H′), 2.17–2.27 (m, 1H, C(5)H), 3.15 (d, $J = 8.3$ Hz, 1H, C(5a)H*H*′), 3.72 (d, $J = 8.2$ Hz, 1H, C(3′)*H*H′), 3.74–3.82 (m, 2H, C(3)*H*H′, C(3)H*H*′), 3.88 (s, 2H, C(5b)H_2), 4.08 (s, 1H, C(1′)H), 4.26 (d,

J = 8.2 Hz, 1H, C(3′)HH'), 7.45–7.53 (m, 2H, ArH), 7.58–7.68 (m, 2H, ArH); ^{1}H NMR (CD_3OD) for the minor diastereomer, δ 4.07 (s, 1H, C(1′)H), the remaining peaks overlapped with nearby signals and were not detected; the structural assignments were in agreement with the ^{1}H–^{1}H COSY experiment; ^{13}C NMR (CD_3OD) for the major diastereomer, 23.7, 25.7, 27.2, 29.4, 30.5, 35.4, 51.1, 62.4, 72.1, 72.2, 82.6, 85.3, 87.7, 110.5, 123.6-123.7 (m, CF_3), 125.6–125.7 (m), 129.2 (2C), 132.8 (2C), 140.6, 167.0, 170.6 ppm; ^{13}C NMR (CD_3OD) for the minor diastereomer 27.3, 28.6, 28.8, 35.2, 110.7, 166.0, 172.7 ppm, the remaining peaks overlapped with nearby signals and were not detected; MS (−CI) 533 $[M-1]^-$; M_r (−CI) 533.157 67 $[M-1]^-$ (calcd for $C_{23}H_{28}F_3N_2O_7S$ 533.157 93 $[M-1]^-$).

4.16. 5a-(2,5-Bis-trifluoromethylbenzylsulfanyl)-dihydrobicyclomycin 2′,3′-acetonide (28)

Using General procedure 1 (A), **16** (50 mg, 0.15 mmol) and thioacetic acid *S*-(2,5-bis-trifluoromethylbenzyl) ester (**40**) (62 mg, 0.44 mmol) gave **28** as a mixture of diastereomers (∼3:1): yield, 61 mg (70%); R_f 0.50 (10% MeOH–$CHCl_3$); FT-IR (KBr) 3296 (br), 2990, 1697, 1380, 1047 cm^{-1}; ^{1}H NMR (CD_3OD) for the major diastereomer, δ 1.28 (s, 3H, C(2′)CH_3), 1.36 (s, 3H, C(CH_3)$_2$), 1.37 (s, 3H, C(CH_3)$_2$), 1.75–1.96 (m, 1H, C(4)HH′), 2.02–2.24 (m, 2H, C(4)HH', C(5)H), 2.05 (d, J = 13.2 Hz, 1H, C(5a)HH′), 3.02 (d, J = 13.2 Hz, 1H, C(5a)HH'), 3.63 (d, J = 8.2 Hz, 1H, C(3′)HH′), 3.64 (s, 2H, C(5b)H_2), 3.67–3.92 (m, 2H, C(3)HH′, C(3)HH'), 4.02 (s, 1H, C(1′)H), 4.36 (d, J = 8.2 Hz, 1H, C(3′)HH'), 7.75 (s, 1H, ArH), 7.89 (s, 2H, ArH); ^{1}H NMR (CD_3OD) for the minor diastereomer, δ 4.01 (s, 1H, C(1′)H), the remaining peaks overlapped with nearby signals and were not detected; the structural assignments were in agreement with the ^{1}H–^{1}H COSY experiment; ^{13}C NMR (CD_3OD) for the major diastereomer, 24.0, 26.0, 27.4, 29.8, 30.5, 34.8, 51.2, 62.7, 72.3, 72.4, 82.7, 85.6, 88.0, 110.8, 120.8–120.9 (m, 2C, CF_3), 125.6, 129.8–129.9 (m), 131.2–132.6 (m, 2C), 143.0, 167.3, 170.7 ppm; ^{13}C NMR (CD_3OD) for the minor diastereomer, 27.5, 28.5, 29.1, 34.6, 82.7, 88.2, 166.2, 172.8 ppm, the remaining peaks overlapped with nearby signals and were not detected; MS (−CI) 601 $[M-1]^-$; M_r (−CI) 601.145 06 $[M-1]^-$ (calcd for $C_{24}H_{27}F_6N_2O_7S$ 601.144 32 $[M-1]^-$).

4.17. 5a-(3-Chlorobenzylsulfanyl)-dihydrobicyclomycin 2′,3′-acetonide (46)

Using General procedure 1(A), **16** (50 mg, 0.15 mmol) and thioacetic acid *S*-(3-chlorobenzyl) ester (**50**) (88 mg, 0.44 mmol) gave **46** as a mixture of diastereomers (∼3:1): yield, 42 mg (56%); R_f 0.65 (10% MeOH–$CHCl_3$); FT-IR (KBr) 3297 (br), 2985, 1683, 1404, 1048 cm^{-1}; ^{1}H NMR (CD_3OD) for the major diastereomer, δ 1.36 (s, 3H, C(2′)CH_3), 1.42 (s, 3H, C(CH_3)$_2$), 1.46 (s, 3H, C(CH_3)$_2$), 1.84–2.28 (m, 3H, C(4)HH′, C(4)HH', C(5)H), 2.13 (d, J = 11.1 Hz, 1H, C(5a)HH′), 3.15 (d, J = 11.1 Hz, 1H, C(5a)HH'), 3.69 (s, 2H, C(5b)H_2), 3.70 (d, J = 8.3 Hz, 1H, C(3′)HH′), 3.69–3.78 (m, 1H, C(3)HH′), 3.84–3.93 (m, 1H, C(3)HH'), 3.94 (s, 1H, C(1′)H), 4.44 (d, J = 8.3 Hz, 1H, C(3′)HH'), 7.21–7.32 (m, 3H, ArH), 7.37 (s, 1H, ArH); ^{1}H NMR (CD_3OD) for the minor diastereomer, δ 3.10 (d, J = 9.8 Hz, 1H, C(5a)HH′), 4.09 (s, 1H, C(1′)H), 4.47 (d, J = 8.3 Hz, 1H, C(3′)HH'), the remaining peaks overlapped with nearby signals and were not detected; the structural assignments were in agreement with the ^{1}H–^{1}H COSY experiment; ^{13}C NMR (CD_3OD) for the major diastereomer, 24.1, 26.0, 27.4, 29.5, 30.6, 35.6, 51.4, 62.6, 72.2, 72.3, 82.8, 85.5, 88.0, 110.8, 127.2, 127.7, 129.2, 130.6, 134.3, 141.7, 167.3, 170.7 ppm; ^{13}C NMR (CD_3OD) for the minor diastereomer, 25.9, 27.4, 72.2, 82.7, 85.6, 88.2, 110.9, 127.3, 127.8, 129.5, 130.3, 134.4, 166.2, 172.8 ppm, the remaining peaks overlapped with nearby signals and were not detected; MS (+ESI) 523 $[M+Na]^+$; M_r (+ESI) 523.1305 $[M+Na]^+$ (calcd for $C_{22}H_{29}ClN_2NaO_7S$ 523.1277 $[M+Na]^+$).

4.18. 5a-(3-Cyanobenzylsulfanyl)-dihydrobicyclomycin 2′,3′-acetonide (47)

Using General procedure 1(A), **16** (50 mg, 0.15 mmol) and thioacetic acid *S*-(3-cyanobenzyl) ester (**51**) (84 mg, 0.44 mmol) gave **47** as a mixture of diastereomers (∼3:1): yield, 42 mg (56%); R_f 0.63 (10% MeOH–$CHCl_3$); FT-IR (KBr) 3302 (br), 2986, 1689, 1402, 1047 cm^{-1}; ^{1}H NMR (CD_3OD) for the major diastereomer, δ 1.36 (s, 3H, C(2′)CH_3), 1.42 (s, 3H, C(CH_3)$_2$), 1.44 (s, 3H, C(CH_3)$_2$), 1.83–2.31 (m, 2H, C(4)HH′, C(4)HH'), 2.05–2.31 (m, 1H, C(5)H), 2.12 (d, J = 12.6 Hz, 1H, C(5a)HH′), 3.14 (d, J = 12.6 Hz, 1H, C(5a)HH'), 3.74 (s, 2H, C(5b)H_2), 3.75 (d, J = 8.2 Hz, 1H, C(3′)HH′), 3.78–3.97 (m, 2H, C(3)HH′, C(3)HH'), 4.10 (s, 1H, C(1′)H), 4.44 (d, J = 8.2 Hz, 1H, C(3′)HH'), 7.46–7.74 (m, 3H, ArH), 7.76 (s, 1H, ArH); ^{1}H NMR (CD_3OD) for the minor diastereomer, δ 3.13 (d, J = 9.4 Hz, 1H, C(5a)HH'), 4.06 (s, 1H, C(1′)H), 4.44 (d, J = 7.7 Hz, 1H, C(3′)HH'), the remaining peaks overlapped with nearby signals and were not detected; the structural assignments were in agreement with the ^{1}H–^{1}H COSY experiment; ^{13}C NMR (CD_3OD) for the major diastereomer, 24.5, 26.0, 27.4, 29.6, 30.5, 35.1, 51.2, 62.7, 72.2, 72.3, 82.9, 85.5, 88.0, 110.8, 112.5, 118.8, 129.8, 130.9, 132.8, 134.1, 141.3, 167.4, 171.0 ppm; ^{13}C NMR (CD_3OD) for the minor diastereomer, 27.5, 35.0, 72.2, 82.8, 85.6, 88.2, 111.9, 129.6, 131.0, 132.9, 134.2, 166.2, 172.7 ppm, the remaining peaks overlapped with nearby signals and were not detected; no signals were observed in the ESI and CI MS.

4.19. 5a-(3-Methoxybenzylsulfanyl)-dihydrobicyclomycin 2′,3′-acetonide (48)

Using General procedure 1(A), **16** (50 mg, 0.15 mmol) and thioacetic acid *S*-(3-methoxybenzyl) ester (**52**) (62 mg, 0.44 mmol) gave **48** as a mixture of diastereomers (∼3:1) along with small amount of unreacted **16**. PTLC purification of the product mixture gave **48**: yield, 36 mg (48%); R_f 0.50 (10% MeOH–$CHCl_3$); FT-IR (KBr) 3303 (br), 2986, 1689, 1405, 1047 cm^{-1}; ^{1}H NMR (CD_3OD) for the major diastereomer, δ 1.35 (s, 3H, C(2′)CH_3), 1.42 (s, 3H, C(CH_3)$_2$), 1.44 (s, 3H,

$C(CH_3)_2$), 1.81–2.14 (m, 3H, C(4)*H*H′, C(4)H*H*′, C(5)H), 2.12 (d, J = 10.9 Hz, 1H, C(5a)*H*H′), 3.16 (d, J = 10.9 Hz, 1H, C(5a)H*H*′), 3.63–4.02 (m, 2H, C(3)*H*H′, C(3)H*H*′), 3.69 (s, 2H, C(5b)H_2), 3.70 (d, J = 8.2 Hz, 1H, C(3′)*H*H′), 3.78 (s, 3H, $ArOCH_3$), 4.08 (s, 1H, C(1′)H), 4.42 (d, J = 8.2 Hz, 1H, C(3′)H*H*′), 6.76 (d, J = 8.2 Hz, 1H, ArH), 6.90 (s, 1H, ArH), 6.94 (d, J = 8.2 Hz, 1H, ArH), 7.18 (t, J = 8.2 Hz, 1H, ArH); ^{1}H NMR (CD_3OD) for the minor diastereomer, δ 3.13 (d, J = 9.4 Hz, 1H, C(5a)H*H*′), 4.06 (s, 1H, C(1′)H), 4.44 (d, J = 7.7 Hz, 1H, C(3′)H*H*′), the remaining peaks overlapped with nearby signals and were not detected; the structural assignments were in agreement with the ^{1}H–^{1}H COSY experiment; ^{13}C NMR (CD_3OD) for the major diastereomer, 23.8, 25.8, 27.2, 29.2, 30.4, 36.2, 51.3, 54.6, 62.3, 72.1, 72.2, 82.7, 85.3, 87.7, 110.6, 112.7, 114.4, 121.3, 129.4, 140.5, 160.2, 167.3, 170.5 ppm; ^{13}C NMR (CD_3OD) for the minor diastereomer, 23.7, 25.7, 27.3, 36.1, 62.5, 72.3, 111.7, 114.5, 121.4, 129.5, 160.3, 166.0, 172.6 ppm, the remaining peaks overlapped with nearby signals and were not detected

4.20. 5a-(3-Nitrobenzylsulfanyl)-dihydrobicyclomycin 2′,3′-acetonide (49)

Using General procedure 1(A), **16** (50 mg, 0.15 mmol) and thioacetic acid *S*-(3-nitrobenzyl) ester (**53**) (74 mg, 0.44 mmol) gave **49** as a mixture of diastereomers (~3:1): yield, 31 mg (40%); R_f 0.49 (10% MeOH–$CHCl_3$); FT-IR (KBr) 3302 (br), 2996, 1687, 1402, 1045 cm^{-1}; ^{1}H NMR (CD_3OD) for the major diastereomer, δ 1.32 (s, 3H, C(2′)CH_3), 1.37 (s, 3H, $C(CH_3)_2$), 1.45 (s, 3H, $C(CH_3)_2$), 1.86–2.43 (m, 4H, C(4)*H*H′, C(4)H*H*′, C(5)H, C(5a)*H*H′), 3.08 (d, J = 13.2 Hz, 1H, C(5a)H*H*′), 3.66 (d, J = 9.3 Hz, 1H, C(3′)*H*H′), 3.67–4.02 (m, 2H, C(3)*H*H′, C(3)H*H*′), 3.89 (s, 2H, C(5b)H_2), 4.07 (s, 1H, C(1′)H), 4.12 (d, J = 9.3 Hz, 1H, C(3′)H*H*′), 7.57 (t, J = 8.2 Hz, 1H, ArH), 7.78 (d, J = 8.2 Hz, 1H, ArH), 8.12 (d, J = 8.2 Hz, 1H, ArH), 8.26 (s, 1H, ArH); ^{1}H NMR (CD_3OD) for the minor diastereomer, δ 3.12 (d, J = 9.4 Hz, 1H, C(5a)H*H*′), 4.17 (d, J = 8.8 Hz, 1H, C(3′)H*H*′), the remaining peaks overlapped with nearby signals and were not detected; the structural assignments were in agreement with the ^{1}H–^{1}H COSY experiment; ^{13}C NMR (CD_3OD) for the major diastereomer, 24.9, 26.8, 28.2, 30.5, 31.9, 36.3, 52.3, 63.5, 73.2, 73.3, 83.7, 86.4, 88.8, 111.7, 122.9, 124.8, 130.6, 136.1, 142.7, 149.8, 168.1, 171.5 ppm; ^{13}C NMR (CD_3OD) for the minor diastereomer, 26.5, 27.1, 27.3, 31.9, 62.5, 73.4, 86.4, 89.9, 111.8, 122.8, 142.8, 149.7, 173.6 ppm, the remaining peaks overlapped with nearby signals and were not detected; MS (ESI) 534 $[M+Na]^+$; M_r (+ESI) 534.1538 $[M+Na]^+$ (calcd for $C_{22}H_{29}N_3NaO_9S$ 534.1517 $[M+Na]^+$).

4.21. 5a-(3-Carboxybenzylsulfanyl)-dihydrobicyclomycin 2′,3′-acetonide (56)

Using General procedure 1(A), **16** (50 mg, 0.15 mmol) and thioacetic acid *S*-(3-carboxybenzyl) ester (**58**) (62 mg, 0.44 mmol) gave **56** as a mixture of diastereomers (3:1): yield, 23 mg (35%); R_f 0.33 (30% MeOH–$CHCl_3$); FT-IR (KBr) 3259 (br), 2986, 1697, 1404, 1041 cm^{-1}; ^{1}H NMR (CD_3OD) for the major diastereomer, δ 1.37 (s, 3H, C(2′)CH_3), 1.44 (s, 3H, $C(CH_3)_2$), 1.47 (s, 3H, $C(CH_3)_2$), 1.85-2.18 (m, 4H, C(4)*H*H′, C(4)H*H*′, C(5)H, C(5a)*H*H′), 3.16 (d, J = 10.4 Hz, 1H, C(5a)H*H*′), 3.63 (d, J = 8.2 Hz, 1H, C(3′)*H*H′), 3.70–3.77 (m, 1H, C(3)*H*H′), 3.75 (s, 2H, C(5b)H_2), 3.89–3.96 (m, 1H, C(3)H*H*′), 4.10 (s, 1H, C(1′)H), 4.45 (d, J = 8.2 Hz, 1H, C(3′)H*H*′), 7.30–7.35 (m, 1H, ArH), 7.41 (d, J = 7.7 Hz, 1H, ArH), 7.85 (d, J = 7.7 Hz, 1H, ArH), 8.00 (s, 1H, ArH); ^{1}H NMR (CD_3OD) for the minor diastereomer, δ 3.12 (d, J = 9.4 Hz, 1H, C(5a)H*H*′), 4.09 (s, 1H, C(1′)H), 4.47 (d, J = 6.6 Hz, 1H, C(3′)H*H*′), the remaining peaks overlapped with nearby signals and were not detected; the structural assignments were in agreement with the ^{1}H–^{1}H COSY experiment; ^{13}C NMR (CD_3OD) for the major diastereomer, 23.7, 25.8, 27.2, 29.4, 30.8, 36.2, 51.3, 62.7, 72.2, 72.3, 82.7, 85.3, 87.7, 110.6, 127.7, 127.9, 130.0, 130.9, 138.1, 138.5, 166.7, 170.5, 207.0 ppm; ^{13}C NMR (CD_3OD) for the minor diastereomer, 62.5 ppm, the remaining peaks overlapped with nearby signals and were not detected; MS (ESI) 533 $[M+Na]^+$; M_r (+ESI) 533.1546 $[M+Na]^+$ (calcd for $C_{23}H_{30}N_2NaO_9S$ 533.1564).

4.22. 5a-(2-Fluorobenzylsulfanyl)-dihydrobicyclomycin (4)

Using General procedure 2, **17** (55 mg, 0.07 mmol) gave **4** as a mixture of diastereomers (~3:1): yield, 49 mg (72%); R_f 0.45 (20% MeOH–$CHCl_3$); FT-IR (KBr) 3302 (br), 2951, 1679, 1401, 1044 cm^{-1}; ^{1}H NMR (CD_3OD) for the major diastereomer, δ 1.32 (s, 3H, C(2′)CH_3), 2.02–2.26 (m, 3H, C(4)*H*H′, C(4)H*H*′, C(5)H), 2.21 (d, J = 9.6 Hz, 1H, C(5a)*H*H′), 3.17 (d, J = 9.6 Hz, 1H, C(5a)H*H*′), 3.45 (d, J = 11.5 Hz, 1H, C(3′)*H*H′), 3.65 (d, J = 11.5 Hz, 1H, C(3′)H*H*′), 3.68–3.78 (m, 1H, C(3)*H*H′), 3.72 (s, 2H, C(5b)H_2), 3.87–3.94 (m, 1H, C(3)H*H*′), 4.01 (s, 1H, C(1′)H), 7.02–7.13 (m, 2H, ArH), 7.22–7.29 (m, 1H, ArH), 7.36–7.44 (m, 1H, ArH); ^{1}H NMR (CD_3OD) for the minor diastereomer, δ 3.96 (s, 1 H, C(1′)H), the remaining peaks overlapped with nearby signals and were not detected; the structural assignments were in agreement with the ^{1}H–^{1}H COSY experiment; ^{13}C NMR (CD_3OD) for the major diastereomer, 23.4, 29.2, 29.4, 31.0, 51.4, 62.4, 67.7, 71.3, 77.3, 82.9, 88.5, 115.5 (d, J = 21.8 Hz), 124.4 (d, J = 3.4 Hz), 126.4 (d, J = 14.9 Hz), 129.2 (d, J = 8.0 Hz), 131.5 (d, J = 4.5 Hz), 161.4 (d, J = 246.2 Hz), 168.0, 171.3 ppm; ^{13}C NMR (CD_3OD) for the minor diastereomer, 61.1, 82.7, 88.8 ppm, the remaining peaks overlapped with nearby signals and were not detected; MS (+ESI) 467 $[M+Na]^+$; M_r (+ESI) 467.1281 $[M+Na]^+$ (calcd for $C_{19}H_{25}FN_2NaO_7S$ 467.1259 $[M+Na]^+$).

4.23. 5a-(3-Fluorobenzylsulfanyl)-dihydrobicyclomycin (5)

Using General procedure 2, **18** (30 mg, 0.06 mmol) gave **5** as a mixture of diastereomers (~3:1): yield, 16 mg (59%); R_f 0.43 (20% MeOH–$CHCl_3$); FT-IR (KBr) 3416 (br), 2934, 1697, 1404, 1046 cm^{-1}; ^{1}H NMR (CD_3OD) for the major diastereomer, δ 1.26 (s, 3H,

C(2′)CH_3), 1.84–2.28 (m, 3H, C(4)*H*H′, C(4)H*H*′, C(5)H), 2.10 (d, J = 10.4 Hz, 1H, C(5a)*H*H′), 3.04 (d, J = 10.4 Hz, 1H, C(5a)H*H*′), 3.44 (d, J = 11.5 Hz, 1H, C(3′)*H*H′), 3.55 (d, J = 11.5 Hz, 1H, C(3′)H*H*′), 3.61–3.67 (m, 1H, C(3)*H*H′), 3.63 (s, 2H, C(5b)H_2), 3.80–3.89 (m, 1H, C(3)H*H*′), 3.95 (s, 1H, C(1′)H), 6.84-6.95 (m, 1H, ArH), 7.01–7.09 (m, 2H, ArH), 7.19–7.27 (m, 1H, ArH); ^{1}H NMR (CD_3OD) for the minor diastereomer, δ 3.96 (s, 1H, C(1′)H), the remaining peaks overlapped with nearby signals and were not detected; the structural assignments were in agreement with the ^{1}H–^{1}H COSY experiment; ^{13}C NMR (CD_3OD) for the major diastereomer, 23.4, 29.2, 30.6, 35.8, 51.3, 62.4, 67.7, 71.3, 77.3, 82.9, 88.6, 113.8 (d, J = 21.8 Hz), 115.9 (d, J = 21.8 Hz), 125.1 (d, J = 3.4 Hz), 130.3 (d, J = 8.0 Hz), 142.3 (d, J = 6.9 Hz), 163.4 (d, J = 245.1 Hz), 168.0, 171.3 ppm; ^{13}C NMR (CD_3OD) for the minor diastereomer, 82.7, 88.9 ppm, the remaining peaks overlapped with nearby signals and were not detected; MS (−CI) 443 $[M-1]^-$; M_r (−CI) 443.128 12 $[M-1]^-$ (calcd for $C_{19}H_{24}FN_2O_7S$ 443.128 83 $[M-1]^-$).

4.24. 5a-(4-Fluorobenzylsulfanyl)-dihydrobicyclomycin (6)

Using General procedure 2, **19** (45 mg, 0.09 mmol) gave **6** as a mixture of diastereomers (∼3:1): yield, 25 mg (65%); R_f 0.40 (20% MeOH–$CHCl_3$); FT-IR (KBr) 3304 (br), 2986, 1691, 1404, 1046 cm^{-1};^{1}H NMR (CD_3OD) for the major diastereomer, δ 1.25 (s, 3H, C(2′)CH_3), 1.98–2.00 (m, 2H, C(4)*H*H′, C(4)H*H*′), 2.06 (d, J = 11.5 Hz, 1H, C(5a)*H*H′), 2.15–2.23 (m, 1H, C(5)H), 3.02 (d, J = 11.5 Hz, 1H, C(5a)H*H*′), 3.03 (d, J = 11.7 Hz, 1H, C(3′)*H*H′), 3.56–3.65 (m, 1H, C(3)*H*H′), 3.58 (d, J = 11.7 Hz, 1H, C(3′)H*H*′), 3.60 (s, 2H, C(5b)H_2), 3.72–3.87 (m, 1H, C(3)H*H*′), 3.95 (s, 1H, C(1′)H), 6.91–6.97 (m, 2H, ArH), 7.24–7.30 (m, 2H, ArH); ^{1}H NMR (CD_3OD) for the minor diastereomer, δ 3.94 (s, 1H, C(1′)H), the remaining peaks overlapped with nearby signals and were not detected; the structural assignments were in agreement with the ^{1}H–^{1}H COSY experiment; ^{13}C NMR (CD_3OD) for the major diastereomer, 23.4, 29.2, 30.5, 35.5, 51.3, 62.7, 67.7, 71.3, 77.3, 82.9, 88.5, 115.2 (d, J = 20.0 Hz, C(3″), C(5″)), 130.5 (d, J = 8.0 Hz, C(2″), C(6″)), 135.3 (d, J = 2.3 Hz, C(1″)), 162.4 (d, J = 243.9 Hz, C(4″)), 168.0, 171.3 ppm; ^{13}C NMR (CD_3OD) for the minor diastereomer, 62.6, 72.4, 82.8, 88.8, 166.7, 173.3 ppm, the remaining peaks overlapped with nearby signals and were not detected; MS (−CI) 443 $[M-1]^-$; M_r (−CI) 443.127 96 $[M-1]^-$ (calcd for $C_{19}H_{24}FN_2O_7S$ 443.128 83 $[M-1]^-$).

4.25. 5a-(2,4-Difluorobenzylsulfanyl)-dihydrobicyclomycin (7)

Using General procedure 2, **20** (30 mg, 0.06 mmol) gave **7** as a mixture of diastereomers (∼3:1): yield, 17 mg (63%); R_f 0.39 (20% MeOH–$CHCl_3$); FT-IR (KBr) 3381 (br), 2941, 1688, 1401, 1044 cm^{-1}; ^{1}H NMR (CD_3OD) for the major diastereomer, δ 1.27 (s, 3H, C(2′)CH_3), 1.96–2.03 (m, 2H, C(4)*H*H′, C(4)H*H*′), 2.01–2.08 (m, 1H, C(5)H), 2.15 (d, J = 9.3 Hz, 1H, C(5a)*H*H′), 3.08 (d, J = 9.3 Hz, 1H, C(5a)H*H*′), 3.45 (d, J = 11.3 Hz, 1H, C(3′)*H*H′), 3.60 (d, J = 11.3 Hz, 1H, C(3′)H*H*′), 3.64 (s, 2H, C(5b)H_2), 3.65–3.92 (m, 2H, C(3)*H*H′, C(3)H*H*′), 4.00 (s, 1H, C(1′)H), 6.82–6.90 (m, 2H, ArH), 7.32–7.40 (m, 1H, ArH); ^{1}H NMR (CD_3OD) for the minor diastereomer, δ 3.95 (s, 1H, C(1′)H), the remaining peaks overlapped with nearby signals and were not detected; the structural assignments were in agreement with the ^{1}H–^{1}H COSY experiment; ^{13}C NMR (CD_3OD) for the major diastereomer, 23.1, 28.5, 29.4, 30.6, 51.1, 60.9, 67.4, 71.0, 77.1, 82.6, 88.3, 103.6 (t, J = 25.7 Hz), 111.0 (dd, J = 21.2, 3.4 Hz), 122.4 (dd, J = 14.9, 4.0 Hz), 132.2 (dd, J = 5.7, 4.0 Hz), 161.3 (dd, J = 249.1, 12.0 Hz), 162.4 (dd, J = 246.7, 12.0 Hz), 167.8, 171.0 ppm; ^{13}C NMR (CD_3OD) for the minor diastereomer, 28.9, 82.4, 88.6, 167.7, 173.0 ppm, the remaining peaks overlapped with nearby signals and were not detected; MS (+ESI) 485 $[M+Na]^+$; M_r (+ESI) 485.1172 $[M+Na]^+$ (calcd for $C_{19}H_{24}F_2N_2NaO_7S$ 485.1164).

4.26. 5a-(3,4-Difluorobenzylsulfanyl)-dihydrobicyclomycin (8)

Using General procedure 2, **21** (40 mg, 0.08 mmol) gave **8** as a mixture of diastereomers (∼3:1): yield, 20 mg (53%); R_f 0.35 (20% MeOH–$CHCl_3$); FT-IR (KBr) 3401 (br), 2976, 1701, 1401, 1043 cm^{-1}; ^{1}H NMR (CD_3OD) for the major diastereomer, δ 1.25 (s, 3H, C(2′)CH_3), 1.83–2.22 (m, 3H, C(4)*H*H′, C(4)H*H*′, C(5)H), 2.09 (d, J = 8.8 Hz, 1H, C(5a)*H*H′), 2.99 (d, J = 8.8 Hz, 1H, C(5a)H*H*′), 3.43 (d, J = 10.7 Hz, 1H, C(3′)*H*H′), 3.62–3.90 (m, 2H, C(3)*H*H′, C(3)H*H*′), 3.63 (d, J = 10.7 Hz, 1H, C(3′)H*H*′), 3.70 (s, 2H, C(5b)H_2), 3.95 (s, 1H, C(1′)H), 7.07–7.16 (m, 2H, ArH), 7.16–7.23 (m, 1H, ArH); ^{1}H NMR (CD_3OD) for the minor diastereomer, δ 3.94 (s, 1H, C(1′)H), the remaining peaks overlapped with nearby signals and were not detected; the structural assignments were in agreement with the ^{1}H–^{1}H COSY experiment; ^{13}C NMR (CD_3OD) for the major diastereomer, 23.1, 29.0, 30.2, 35.1, 51.0, 61.0, 67.4, 71.0, 77.1, 82.4, 88.3, 117.1 (d, J = 9.2 Hz), 117.9 (d, J = 9.2 Hz), 125.5 (dd, J = 9.2, 3.4 Hz), 136.7 (dd, J = 9.2, 3.4 Hz), 149.6 (dd, J = 248.8, 13.1 Hz), 150.3 (dd, J = 248.2, 12.5 Hz), 167.7, 171.0 ppm; ^{13}C NMR (CD_3OD) for the minor diastereomer peaks overlapped with nearby signals and were not detected; MS (−FAB) 461 $[M-1]^-$; M_r (−FAB) 461.118 14 $[M-1]^-$ (calcd for $C_{19}H_{23}F_2N_2O_7S$ 461.119 41 $[M-1]^-$).

4.27. 5a–(3,5–Difluorobenzylsulfanyl)–dihydrobicyclomycin (9)

Using General procedure 2, **22** (22 mg, 0.04 mmol) gave **9** as a mixture of diastereomers (∼3:1): yield, 12 mg (61%); R_f 0.41 (20% MeOH–$CHCl_3$); FT-IR (KBr) 3301 (br), 2938, 1687, 1392, 1045 cm^{-1}; ^{1}H NMR (CD_3OD) for the major diastereomer, δ 1.25 (s, 3H, C(2′)CH_3), 1.82–1.94 (m, 1H, C(4)*H*H′), 1.95–2.01 (m, 1H, C(4)H*H*′), 2.09 (d, J = 10.9 Hz, 1H, C(5a)*H*H′), 2.11–2.19 (m, 1H, C(5)H), 3.02 (d, J = 10.9 Hz, 1H, C(5a)H*H*′), 3.43 (d, J = 11.0 Hz, 1H, C(3′)*H*H′), 3.61 (s, 2H, C(5b)H_2), 3.63 (d, J = 11.0 Hz, 1H, C(3′)H*H*′), 3.65–3.79 (m, 2H, C(3)*H*H′, C(3)H*H*′), 3.95 (s, 1H, C(1′)H), 6.69–6.77 (m, 1H, ArH), 6.87–6.93 (m, 2H,

ArH); ^{1}H NMR (CD_3OD) for the minor diastereomer, δ 3.62 (s, 2H, C(5b)H), 3.93 (s, 1H, C(1′)H), the remaining peaks overlapped with nearby signals and were not detected; the structural assignments were in agreement with the ^{1}H–^{1}H COSY experiment; ^{13}C NMR (CD_3OD) for the major diastereomer, 23.2, 29.1, 30.1, 35.1, 51.0, 60.9, 67.4, 71.1, 77.1, 82.6, 88.3, 102.0 (t, J = 26.3 Hz, C(4″)), 111.8 (dd, J = 17.6, 7.9 Hz, C(2″), C(6″)), 143.8 (t, J = 9.2 Hz, C(1″)), 163.3 (dd, J = 247.0, 13.1 Hz, C(3″), C(5″)), 167.7, 171.1 ppm; ^{13}C NMR (CD_3OD) for the minor diastereomer peaks overlapped with nearby signals and were not detected; MS (−FAB) 461 [M−1]$^-$; M_r (−FAB) 461.120 73 [M−1]$^-$ (calcd for $C_{19}H_{23}F_2N_2O_7S$ 461.119 41 [M−1]$^-$).

4.28. 5a-(2,3,6-Trifluorobenzylsulfanyl)-dihydrobicyclomycin (10)

Using General procedure 2, **23** (40 mg, 0.08 mmol) gave **10** as a mixture of diastereomers (∼3:1): yield, 23 mg (62%); R_f 0.41 (20% MeOH–$CHCl_3$); FT-IR (KBr) 3384 (br), 2939, 1681, 1406, 1038 cm^{-1}; ^{1}H NMR (CD_3OD) for the major diastereomer, δ 1.27 (s, 3H, C(2′)CH_3), 1.90–2.00 (m, 2H, C(4)*H*H′, C(4)H*H*′), 2.03–2.44 (m, 1H, C(5)H), 2.24 (d, J = 12.6 Hz, 1H, C(5a)*H*H′), 3.18 (d, J = 12.6 Hz, 1H, C(5a)H*H*′), 3.44 (d, J = 11.4 Hz, 1H, C(3′)*H*H′), 3.60 (s, 2H, C(5b)H_2), 3.61–3.93 (m, 2H, C(3)*H*H′, C(3)H*H*′), 3.62 (d, J = 11.4 Hz, 1H, C(3′)H*H*′), 3.96 (s, 1H, C(1′)H), 6.86–6.94 (m, 1H, ArH), 7.09–7.20 (m, 1H, ArH); ^{1}H NMR (CD_3OD) for the minor diastereomer, δ 3.48 (d, J = 11.4 Hz, C(3′)*H*H′), 3.66 (d, J = 11.4 Hz, C(3′)H*H*′), 4.00 (s, 1H, C(1′)H), the remaining peaks overlapped with nearby signals and were not detected; the structural assignments were in agreement with the ^{1}H–^{1}H COSY experiment; ^{13}C NMR (CD_3OD) for the major diastereomer, 23.3, 29.2, 30.8, 31.8, 51.5, 63.0, 67.7, 71.3, 77.3, 82.8, 88.6, 111.2 (ddd, J = 25.2, 4.5, 3.4 Hz), 116.1 (dd, J = 19.5, 9.1 Hz), 117.9 (dd, J = 21.1, 16.8 Hz), 147.7 (ddd, J = 246.7, 12.8, 3.8 Hz), 148.9 (ddd, J = 242.8, 12.7, 7.8 Hz), 156.8 (ddd, J = 243.9, 5.7, 3.4 Hz), 167.9, 171.3 ppm; ^{13}C NMR (CD_3OD) for the minor diastereomer, 62.9, 82.6, 88.7, 167.0, 173.4 ppm, the remaining peaks overlapped with nearby signals and were not detected; MS (−CI) 479 [M−1]$^-$; M_r (−CI) 479.114 24 [M−1]$^-$ (calcd for $C_{19}H_{22}F_3N_2O_7S$ 479.109 98 [M−1]$^-$).

4.29. 5a-(3,4,5-Trifluorobenzylsulfanyl)-dihydrobicyclomycin (11)

Using General procedure 2, **24** (30 mg, 0.06 mmol) gave **11** as a mixture of diastereomers (∼3:1): yield, 18 mg (68%); R_f 0.46 (20% MeOH–$CHCl_3$); FT-IR (KBr) 3302 (br), 2947, 1678, 1391, 1042 cm^{-1}; ^{1}H NMR (CD_3OD) for the major diastereomer, δ 1.26 (s, 3H, C(2′)CH_3), 1.85–2.15 (m, 2H, C(4)*H*H′, C(4)H*H*′), 2.10 (d, J = 10.4 Hz, 1H, C(5a)*H*H′), 2.15–2.25 (m, 1H, C(5)H), 2.98 (d, J = 10.4 Hz, 1H, C(5a)H*H*′), 3.44 (d, J = 11.0 Hz, 1H, C(3′)*H*H′), 3.60 (s, 2H, C(5b)H_2), 3.61–3.93 (m, 3H, C(3)*H*H′, C(3)H*H*′, C(3′)H*H*′), 4.00 (s, 1H, C(1′)H), 7.11–7.21 (m, 2H, ArH); ^{1}H NMR (CD_3OD) for the minor diastereomer, δ 1.22 (s, 1H, C(2′)H), 3.63 (d, J = 11.0 Hz, 1H, C(3′)H*H*′), the remaining peaks overlapped with nearby signals and were not detected; the structural assignments were in agreement with the ^{1}H–^{1}H COSY experiment; ^{13}C NMR (CD_3OD) for the major diastereomer, 23.4, 29.3, 30.3, 34.9, 51.2, 62.7, 67.6, 71.3, 77.3, 82.7, 88.6, 113.4 (dd, J = 15.0, 6.3 Hz, C(2″), C(6″)), 133.7 (dd, J = 11.5, 7.9 Hz, C(1″)), 138.7 (dt, J = 245.5, 17.0 Hz, C(4″)), 151.4 (ddd, J = 243.6, 11.3, 3.4 Hz, C(3″), C(5″)), 167.3, 170.8 ppm; ^{13}C NMR (CD_3OD) for the minor diastereomer, 82.8, 88.7, 166.9 ppm, the remaining peaks overlapped with nearby signals and were not detected; MS (−CI) 479 [M−1]$^-$; M_r (−CI) 479.110 83 [M−1]$^-$ (calcd for $C_{19}H_{22}F_3N_2O_7S$ 479.109 98 [M−1]$^-$).

4.30. 5a-(2,3,5,6-Tetrafluorobenzylsulfanyl)-dihydrobicyclomycin (12)

Using General procedure 2, **25** (40 mg, 0.07 mmol) gave **12** as a mixture of diastereomers (∼3:1): yield, 21 mg (58%); R_f 0.39 (20% MeOH–$CHCl_3$); FT-IR (KBr) 3303 (br), 2958, 1688, 1392, 1045 cm^{-1}; ^{1}H NMR (CD_3OD) for the major diastereomer, δ 1.25 (s, 3H, C(2′)CH_3), 1.85–2.31 (m, 3H, C(4)*H*H′, C(4)H*H*′, C(5)H), 2.13 (d, J = 11.5 Hz, 1H, C(5a)*H*H′), 3.01 (d, J = 11.5 Hz, 1H, C(5a)H*H*′), 3.44 (d, J = 11.5 Hz, 1H, C(3′)*H*H′), 3.59 (d, J = 11.5 Hz, 1H, C(3′)H*H*′), 3.66 (s, 2H, C(5b)H_2), 3.67–3.91 (m, 2H, C(3)*H*H′, C(3)H*H*′), 3.95 (s, 1H, C(1′)H), 7.14–7.23 (m, 1H, ArH); ^{1}H NMR (CD_3OD) for the minor diastereomer, δ 3.01 (d, J = 11.5 Hz, 1H, C(5a)H*H*′), 3.96 (s, 1H, C(1′)H), the remaining peaks overlapped with nearby signals and were not detected; the structural assignments were in agreement with the ^{1}H–^{1}H COSY experiment; ^{13}C NMR (CD_3OD) for the major diastereomer, 23.4, 28.2, 29.3, 30.7, 51.2, 62.4, 67.7, 71.3, 77.3, 82.8, 88.6, 112.5 (dt, J = 19.5, 4.3 Hz), 123.7–123.9 (m), 138.2–142.7 (m, 2C), 144.5–148.7 (m, 2C), 168.0, 171.3 ppm; ^{13}C NMR (CD_3OD) for the minor diastereomer, 82.6, 88.8, 166.9, 173.4 ppm, the remaining peaks overlapped with nearby signals and were not detected; MS (−CI) 497 [M−1]$^-$; M_r (−CI) 497.101 67 [M−1]$^-$ (calcd for $C_{19}H_{21}F_4N_2O_7S$ 497.100 56 [M−1]$^-$).

4.31. 5a-(2,3,4,5,6-Pentafluorobenzylsulfanyl)-dihydrobicyclomycin (13)

Using General procedure 2, **26** (40 mg, 0.07 mmol) gave **13** as a mixture of diastereomers (∼3:1): yield, 28 mg (71%); R_f 0.39 (20% MeOH–$CHCl_3$); FT-IR (KBr) 3301 (br), 2946, 1686, 1391, 1041 cm^{-1}; ^{1}H NMR (CD_3OD) for the major diastereomer, δ 1.25 (s, 3H, C(2′)CH_3), 1.83–2.22 (m, 3H, C(4)*H*H′, C(4)H*H*′, C(5)H), 2.07 (d, J = 12.3 Hz, 1H, C(5a)*H*H′), 3.10 (d, J = 12.3 Hz, 1H, C(5a)H*H*′), 3.44 (d, J = 11.3 Hz, 1H, C(3′)*H*H′), 3.60 (d, J = 11.3 Hz, 1H, C(3′)H*H*′), 3.65–3.92 (m, 2H, C(3)*H*H′, C(3)H*H*′), 3.75 (s, 2H, C(5b)H_2), 3.95 (s, 1H, C(1′)H); ^{1}H NMR (CD_3OD) for the minor diastereomer peaks overlapped with nearby signals and were not detected; the structural assignments were in agreement with the ^{1}H–^{1}H COSY experiment; ^{13}C NMR (CD_3OD) for the major diastereomer, 23.5, 29.4, 30.9, 31.8, 51.7, 61.3, 67.8, 71.5, 77.5, 82.8, 88.8, 113.7–113.9 (m), 137.8–138.8 (m), 138.9–140.0 (m, 2C), 141.5–147.3 (m, 2C), 168.2,

171.4 ppm; ^{13}C NMR (CD_3OD) for the minor diastereomer, 23.0, 62.4, 78.1, 82.7, 88.9, 167.1, 173.4 ppm, the remaining peaks overlapped with nearby signals and were not detected; MS (−CI) 515 $[M-1]^-$; M_r (−CI) 515.092 43 $[M-1]^-$ (calcd for $C_{19}H_{20}F_5N_2O_7S$ 515.091 14 $[M-1]^-$).

4.32. 5a-(4-Trifluoromethylbenzylsulfanyl)-dihydrobicyclomycin (14)

Using General procedure 2, **27** (30 mg, 0.06 mmol) gave **14** as a mixture of diastereomers (~3:1); yield, 17 mg (64%); R_f 0.35 (20% MeOH–$CHCl_3$); FT-IR (KBr) 3397 (br), 2939, 1688, 1405, 1125 cm^{-1}; ^{1}H NMR (CD_3OD) for the major diastereomer, δ 1.25 (s, 3H, C(2′)CH_3), 2.00–2.09 (m, 3H, C(4)*H*H′, C(4)H*H*′, C(5)H), 2.10 (d, J = 9.9 Hz, 1H, C(5a)*H*H′), 3.03 (d, J = 9.9 Hz, 1H, C(5a)H*H*′), 3.43 (d, J = 13.7 Hz, 1H, C(3′)*H*H′), 3.56–3.87 (m, 2H, C(3)*H*H′, C(3)H*H*′), 3.58 (d, J = 13.7 Hz, 1H, C(3′)H*H*′), 3.70 (s, 2H, C(5b)H_2), 3.95 (s, 1H, C(1′)H), 7.23–7.29 (m, 2H, ArH), 7.56–7.59 (m, 2H, ArH); ^{1}H NMR (CD_3OD) for the minor diastereomer, the peaks overlapped with nearby signals and were not detected; the structural assignments were in agreement with the ^{1}H–^{1}H COSY experiment; ^{13}C NMR (CD_3OD) for the major diastereomer, 24.0, 29.3, 30.5, 35.6, 51.3, 61.0, 67.7, 71.3, 77.3, 82.9, 88.6, 123.7–123.9 (m, CF_3), 125.8–126.0 (m), 129.4 (2C), 133.1 (2C), 140.9, 168.0, 171.0 ppm; ^{13}C NMR (CD_3OD) for the minor diastereomer, 29.3, 62.3, 71.5, 82.6, 88.8 ppm, the remaining peaks overlapped with nearby signals and were not detected; MS (−CI) 533 $[M-1]^-$; M_r (−CI) 533.157 67 $[M-1]^-$ (calcd for $C_{20}H_{24}F_3N_2O_7S$ 533.156 93 $[M-1]^-$).

4.33. 5a-(2,5-Bis-trifluoromethylbenzylsulfanyl)-dihydrobicyclomycin (15)

Using General procedure 2, **28** (45 mg, 0.07 mmol) gave **15** as a mixture of diastereomers (~3:1); yield, 28 mg (68%); R_f 0.43 (20% MeOH–$CHCl_3$); FT-IR (KBr) 3389 (br), 2939, 1689, 1380, 1043 cm^{-1}; ^{1}H NMR (CD_3OD) for the major diastereomer, δ 1.24 (s, 3H, C(2′)CH_3), 1.87–2.21 (m, 3H, C(4)*H*H′, C(4)H*H*′, C(5)H), 2.10 (d, J = 11.8 Hz, 1H, C(5a)*H*H′), 2.97 (d, J = 11.8 Hz, 1H, C(5a)H*H*′), 3.42 (d, J = 11.5 Hz, 1H, C(3′)*H*H′), 3.58 (d, J = 11.5 Hz, 1H, C(3′)H*H*′), 3.68–3.90 (m, 2H, C(3)*H*H′, C(3)H*H*′), 3.78 (s, 2H, C(5b)H_2), 3.95 (s, 1H, C(1′)H), 7.75 (s, 1H, ArH), 7.89 (s, 2H, ArH); ^{1}H NMR (CD_3OD) for the minor diastereomer, δ 1.21 (s, 3H, C(2′)CH_3), 3.47 (d, J = 11.5 Hz, 1H, C(3′)*H*H′), 3.94 (s, 1H, C(1′)H), the remaining peaks overlapped with nearby signals and were not detected; the structural assignments were in agreement with the ^{1}H–^{1}H COSY experiment; ^{13}C NMR (CD_3OD) for the major diastereomer, 23.4, 29.3, 30.3, 34.7, 51.0, 61.1, 67.6, 71.3, 77.3, 82.7, 88.1, 120.8–120.9 (m, 2C, CF_3), 125.5, 129.8–129.9 (m), 131.2–132.6 (m, 2C), 143.0, 168.0, 171.2 ppm; ^{13}C NMR (CD_3OD) for the minor diastereomer, 29.0, 29.8, 62.4, 71.4, 82.6, 88.8, 166.7, 173.4 ppm, the remaining peaks overlapped with nearby signals and were not detected; MS (−CI) 561 $[M-1]^-$; M_r (−CI) 561.113 77 $[M-1]^-$ (calcd for $C_{21}H_{23}F_6N_2O_7S$ 561.113 02 $[M-1]^-$).

4.34. 5a-(3-Chlorobenzylsulfanyl)-dihydrobicyclomycin (41)

Using General procedure 2, **46** (30 mg, 0.06 mmol) gave **41** as a mixture of diastereomers (~3:1): yield, 14 mg (52%); R_f 0.45 (20% MeOH–$CHCl_3$); FT-IR (KBr) 3259, 1697, 1404, 1041 cm^{-1}; ^{1}H NMR (CD_3OD) for the major diastereomer, δ 1.40 (s, 3H, C(2′)CH_3), 2.01–2.23 (m, 3H, C(4)*H*H′, C(4)H*H*′, C(5)H), 2.14 (d, J = 10.4 Hz, 1H, C(5a)*H*H′), 3.19 (d, J = 10.4 Hz, 1H, C(5a)H*H*′), 3.58 (d, J = 11.5 Hz, 1H, C(3′)*H*H′), 3.73 (d, J = 11.5 Hz, 1H, C(3′)H*H*′), 3.75–3.97 (m, 2H, C(3)*H*H′, C(3)H*H*′), 3.76 (s, 2H, C(5b)H_2), 4.09 (s, 1H, C(1′)H), 7.23–7.35 (m, 3H, ArH), 7.43 (s, 1H, ArH); ^{1}H NMR (CD_3OD) for the minor diastereomer, δ 3.16 (d, J = 14.8 Hz, 1H, C(5a)H*H*′), 3.62 (d, J = 13.2 Hz, 1H, C(3′)*H*H′), 4.10 (s, 1H, C(1′)H), the remaining peaks overlapped with nearby signals and were not detected; the structural assignments were in agreement with the ^{1}H–^{1}H COSY experiment; ^{13}C NMR (CD_3OD) for the major diastereomer, 23.3, 29.2, 30.6, 35.7, 51.3, 61.2, 67.7, 71.4, 77.3, 82.8, 88.5, 127.2, 127.7, 129.2, 130.1, 134.4, 141.7, 168.0, 171.3 ppm; ^{13}C NMR (CD_3OD) for the minor diastereomer, 82.7, 88.9, 127.8 ppm, the remaining peaks overlapped with nearby signals and were not detected; MS (+ESI) 483 $[M+Na]^+$; M_r (+ESI) 483.0985 $[M+Na]^+$ (calcd for $C_{19}H_{25}ClN_2NaO_7S$ 483.0964 $[M+Na]^+$).

4.35. 5a-(3-Cyanobenzylsulfanyl)-dihydrobicyclomycin (42)

Using General procedure 2, **47** (40 mg, 0.08 mmol) gave **42** as a mixture of diastereomers (~3:1): yield, 21 mg (58%); R_f 0.43 (20% MeOH–$CHCl_3$); FT-IR (KBr) 3271, 1697, 1404, 1042 cm^{-1}; ^{1}H NMR (CD_3OD) for the major diastereomer, δ 1.40 (s, 3H, C(2′)CH_3), 1.96–2.37 (m, 3H, C(4)*H*H′, C(4)H*H*′, C(5)H), 2.24 (d, J = 10.4 Hz, 1H, C(5a)*H*H′), 3.14 (d, J = 10.4 Hz, 1H, C(5a)H*H*′), 3.59 (d, J = 11.5 Hz, 1H, C(3′)*H*H′), 3.72–3.91 (m, 1H, C(3)*H*H′), 3.73 (d, J = 11.5 Hz, 1H, C(3′)H*H*′), 3.82 (s, 2H, C(5b)H_2), 3.97–4.05 (m, 1H, C(3)H*H*′), 4.10 (s, 1H, C(1′)H), 7.56 (t, J = 7.7 Hz, 1H, ArH), 7.67 (d, J = 7.7 Hz, 1H, ArH), 7.75 (d, J = 7.7 Hz, 1H, ArH), 7.79 (s, 1H, ArH); ^{1}H NMR (CD_3OD) for the minor diastereomer, δ 3.36 (d, J = 13.2 Hz, 1H, C(5a)H*H*′), 4.09 (s, 1H, C(1′)H), the remaining peaks overlapped with nearby signals and were not detected; the structural assignments were in agreement with the ^{1}H–^{1}H COSY experiment; ^{13}C NMR (CD_3OD) for the major diastereomer, 23.3, 29.3, 30.4, 35.3, 51.2, 61.2, 67.6, 71.4, 77.3, 82.8, 88.5, 112.5, 118.8, 129.7, 130.8, 132.8, 134.1, 141.3, 167.6, 171.1 ppm; ^{13}C NMR (CD_3OD) for the minor diastereomer, 35.2, 71.5, 82.6, 88.8, 134.2, 166.7, 173.2 ppm, the remaining peaks overlapped with nearby signals and were not detected; MS (+ESI) 474 $[M+Na]^+$; M_r (+ESI) 474.1300 $[M+Na]^+$ (calcd for $C_{20}H_{25}N_3NaO_7S$ 474.1306 $[M+Na]^+$).

4.36. 5a-(3-Methoxybenzylsulfanyl)-dihydrobicyclomycin (43)

Using General procedure 2, **48** (35 mg, 0.07 mmol) gave **43** as a mixture of diastereomers (~3:1): yield, 16 mg

(51%); R_f 0.45 (20% MeOH–$CHCl_3$); FT-IR (KBr) 3411, 2926, 1689, 1407, 1042 cm^{-1}; ^1H NMR (CD_3OD) for the major diastereomer, δ 1.32 (s, 3H, C(2′)CH_3), 1.95–2.09 (m, 2H, C(4)*H*H′, C(4)H*H*′), 2.12–2.19 (m, 1H, C(5)H), 2.17 (d, J = 10.9 Hz, 1H, C(5a)*H*H′), 3.14 (d, J = 10.9 Hz, 1H, C(5a)H*H*′), 3.50 (d, J = 11.0 Hz, 1H, C(3′)*H*H′), 3.64 (d, J = 11.0 Hz, 1H, C(3′)H*H*′), 3.62–3.70 (m, 2H, C(3)*H*H′, C(3)H*H*′), 3.66 (s, 2H, C(5b)H_2), 3.83 (s, 3H, 3′ArOCH_3), 4.02 (s, 1H, C(1′)H), 6.78 (d, J = 8.2 Hz, 1H, ArH), 6.89 (s, 1H, ArH), 6.92 (d, J = 8.2 Hz, 1H, ArH), 7.19 (t, J = 8.2 Hz, 1H, ArH); ^1H NMR (CD_3OD) for the minor diastereomer, δ 3.60 (d, J = 11.4 Hz, 1H, C(3′)*H*H′), the remaining peaks overlapped with nearby signals and were not detected; the structural assignments were in agreement with the ^1H–^1H COSY experiment; ^{13}C NMR (CD_3OD) for the major diastereomer, 23.3, 29.5, 30.5, 36.4, 51.3, 54.8, 61.1, 67.7, 71.4, 77.3, 82.9, 88.5, 112.9, 114.6, 121.5, 129.6, 140.8, 160.4, 168.0, 171.2 ppm; ^{13}C NMR (CD_3OD) for the minor diastereomer, 172.6 ppm, the remaining peaks overlapped with nearby signals and were not detected; MS (+ESI) 479 $[M+Na]^+$; M_r (+ESI) 479.1465 $[M+Na]^+$ (calcd for $C_{20}H_{28}N_2NaO_8S$ 479.1459 $[M+Na]^+$).

4.37. 5a-(3-Nitrobenzylsulfanyl)-dihydrobicyclomycin (44)

Using General procedure 2, **49** (20 mg, 0.04 mmol) gave **44** as a mixture of diastereomers (~3:1): yield, 9 mg (48%); R_f 0.41 (20% MeOH–$CHCl_3$); FT-IR (KBr) 3271, 1698, 1401, 1045 cm^{-1}; ^1H NMR (CD_3OD) for the major diastereomer, δ 1.32 (s, 3H, C(2′)CH_3), 2.04–2.33 (m, 3H, C(4)*H*H′, C(4)H*H*′, C(5)H), 2.19 (d, J = 10.4 Hz, 1H, C(5a)*H*H′), 3.08 (d, J = 10.4 Hz, 1H, C(5a)H*H*′), 3.50 (d, J = 11.5 Hz, 1H, C(3′)*H*H′), 3.65 (d, J = 11.5 Hz, 1H, C(3′)H*H*′), 3.72–3.77 (m, 1H, C(3)*H*H′), 3.83 (s, 2H, C(5b)H_2), 3.87–3.97 (m, 1H, C(3)H*H*′), 4.02 (s, 1H, C(1′)H), 7.55 (t, J = 8.2 Hz, 1H, ArH), 7.74–7.79 (m, 1H, ArH), 8.09–8.13 (m, 1H, ArH), 8.23–8.27 (s, 1H, ArH); ^1H NMR (CD_3OD) for the minor diastereomer, δ 3.57 (d, J = 11.4 Hz, 1H, C(3′)*H*H′), 4.01 (s, 1H, C(1′)H), the remaining peaks overlapped with nearby signals and were not detected; the structural assignments were in agreement with the ^1H–^1H COSY experiment; ^{13}C NMR (CD_3OD) for the major diastereomer, 23.4, 29.3, 30.5, 35.3, 51.3, 61.2, 67.7, 71.4, 77.3, 82.8, 88.6, 122.1, 124.0, 129.8, 135.6, 141.9, 148.9, 167.9, 171.2 ppm; ^{13}C NMR (CD_3OD) for the minor diastereomer, 29.8, 35.5, 62.4, 71.5, 82.7, 88.8, 135.7, 141.9, 166.7, 173.2 ppm, the remaining peaks overlapped with nearby signals and were not detected; MS (+ESI) 494 $[M+Na]^+$; M_r (+ESI) 494.1226 $[M+Na]^+$ (calcd for $C_{19}H_{25}N_3NaO_9S$ 494.1204).

4.38. 5a-(3-Carboxybenzylsulfanyl)-dihydrobicyclomycin (54)

Using General procedure 2, **56** (40 mg, 0.08 mmol) gave **54** as a mixture of diastereomers (~10:1): yield, 21 mg (58%); R_f 0.47 (40% MeOH–$CHCl_3$); FT-IR (KBr) 3271, 1697, 1404, 1042 cm^{-1}; ^1H NMR (CD_3OD) for the major diastereomer, δ 1.35 (s, 3H, C(2′)CH_3), 1.91–2.35 (m, 3H, C(4)*H*H′, C(4)H*H*′, C(5)H), 2.20 (d, J = 10.4 Hz, 1H, C(5a)*H*H′), 3.17 (d, J = 10.4 Hz, 1H, C(5a)H*H*′), 3.51 (d, J = 11.5 Hz, 1H, C(3′)*H*H′), 3.65–3.79 (m, 1H, C(3)*H*H′), 3.69 (d, J = 11.5 Hz, 1H, C(3′)H*H*′), 3.76 (s, 2H, C(5b)H_2), 3.83–3.95 (m, 1H, C(3)H*H*′), 4.04 (s, 1H, C(1′)H), 7.31 (t, J = 7.7 Hz, 1H, ArH), 7.42 (d, J = 7.7 Hz, 1H, ArH), 7.84 (d, J = 7.7 Hz, 1H, ArH), 7.94 (s, 1H, ArH); ^1H NMR (CD_3OD) for the minor diastereomer, δ 4.05 (s, 1H, C(1′)H), the remaining peaks overlapped with nearby signals and were not detected; the structural assignments were in agreement with the ^1H–^1H COSY experiment; ^{13}C NMR (CD_3OD) for the major diastereomer, 24.2, 29.9, 30.5, 37.3, 52.2, 62.3, 68.4, 72.3, 78.2, 83.8, 89.3, 117.2, 119.5, 128.9, 129.2, 132.7, 139.8, 163.2, 172.2 ppm, the remaining peaks were not detected; ^{13}C NMR (CD_3OD) for the minor diastereomer, 78.1, 83.7 ppm, the remaining peaks overlapped with nearby signals and were not detected; MS (+ESI) 493 $[M+Na]^+$; M_r (+ESI) 493.1200 $[M+Na]^+$ (calcd for $C_{20}H_{26}N_2NaO_9S$ 493.1251).

4.39. 5a-(4-Carboxybenzylsulfanyl)-dihydrobicyclomycin (55)

Using General procedure 1(A), **16** (50 mg, 0.15 mmol) and thioacetic acid *S*-(4-carboxybenzyl) ester (**59**) (62 mg, 0.44 mmol) gave **57** as a crude mixture (R_f 0.43 (30% MeOH–$CHCl_3$)). The reaction was not purified but immediately deprotected using General procedure 2, to give **55** as a mixture of diastereomers (~10:1): yield, 11 mg (24%); R_f 0.45 (40% MeOH–$CHCl_3$); FT-IR (KBr) 3261, 1698, 1424, 1041 cm^{-1}; ^1H NMR (CD_3OD) for the major diastereomer, δ 1.36 (s, 3H, C(2′)CH_3), 2.02–2.38 (m, 4H, C(4)*H*H′, C(4)H*H*′, C(5)H, C(5a)*H*H′), 3.14–3.18 (m, 1H, C(5a)H*H*′), 3.75–3.84 (m, 4H, C(3′)*H*H′, C(3′)H*H*′, C(3)*H*H′, C(3)H*H*′), 3.76 (s, 2H, C(5b)H_2), 4.04 (s, 1H, C(1′)H), 7.38 (s, 2H, ArH), 7.97 (s, 2H, ArH); ^1H NMR (CD_3OD) for the minor diastereomer, δ 1.20 (s, 3H, C(2′)CH_3), the remaining peaks overlapped with nearby signals and were not detected; the structural assignments were in agreement with the ^1H–^1H COSY experiment; ^{13}C NMR (CD_3OD) for the major diastereomer, 23.0, 28.7, 30.6, 35.8, 50.1, 71.0, 76.9, 82.5, 88.1, 128.3, 128.6, 141.9, 161.2, 170.8 ppm, the remaining peaks were not detected; ^{13}C NMR (CD_3OD) for the minor diastereomer, 82.4 ppm, the remaining peaks overlapped with nearby signals and were not detected; MS (+ESI) 493 $[M+Na]^+$; M_r (+ESI) 493.1248 $[M+Na]^+$ (calcd for $C_{20}H_{26}N_2NaO_9S$ 493.1251).

4.40. Thioacetic acid *S*-(2-fluorobenzyl) ester (29)

Using General procedure 3, 2-fluorobenzyl bromide (500 mg, 2.60 mmol) and potassium thioacetate (362 mg, 3.12 mmol) gave yellow oil **29**: yield, 416 mg (87%); R_f 0.55 (5% EtOAc–hexanes); FT-IR (neat) 1703, 1493, 1234, 1130 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.47 (s, 3H, C(O)CH_3), 4.29 (s, 2H, SCH_2Ar), 7.13–7.23 (m, 2H, ArH), 7.32–7.38 (m, 1H, ArH), 7.47–7.54 (m, 1H, ArH); ^{13}C NMR ($CDCl_3$) 26.1, 29.7, 114.8 (d,

J = 20.6 Hz), 123.7 (d, J = 3.4 Hz), 124.2 (d, J = 14.9 Hz), 128.6 (d, J = 8.0 Hz), 130.5 (d, J = 4.6 Hz), 160.2 (d, J = 247.3 Hz), 194.3 ppm; MS (+ESI) 207 $[M+Na]^+$; M_r (+ESI) 207.0242 $[M+Na]^+$ (calcd for C_9H_9FNaOS 207.0251 $[M+Na]^+$).

4.41. Thioacetic acid *S*-(3-fluorobenzyl) ester (30)

Using General procedure 3, 3-fluorobenzyl bromide (500 mg, 2.6 mmol) and potassium thioacetate (362 mg, 3.12 mmol) gave yellow oil **30**: yield, 397 mg (83%); R_f 0.45 (5% EtOAc–hexanes); FT-IR (neat) 1692, 1483, 1234, 1130 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.48 (s, 3H, $C(O)CH_3$), 4.23 (s, 2H, SCH_2Ar), 7.03–7.21 (m, 3H, ArH), 7.34–7.42 (m, 1H, ArH); ^{13}C NMR ($CDCl_3$) 29.7, 32.3, 113.6 (d, J = 20.1 Hz), 115.1 (d, J = 21.9 Hz), 123.9 (d, J = 3.4 Hz), 129.5 (d, J = 8.0 Hz), 139.7 (d, J = 6.9 Hz), 162.2 (d, J = 246.2 Hz), 194.1 ppm; MS (+CI) 202 $[M+NH_4]^+$; M_r (+CI) 202.0711 $[M+NH_4]^+$ (calcd for $C_9H_{13}FNOS$ 202.0702 $[M+NH_4]^+$).

4.42. Thioacetic acid *S*-(2,4-difluorobenzyl) ester (32)

Using General procedure 3, 2,4-difluorobenzyl bromide (207 mg, 1 mmol) and potassium thioacetate (137 mg, 1.2 mmol) gave yellow oil **32**: yield, 147 mg (73%); R_f 0.40 (5% EtOAc–hexanes); FT-IR (neat) 1696, 1508, 1130 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.36 (s, 3H, $C(O)CH_3$), 4.07 (s, 2H, SCH_2Ar), 6.62–6.72 (m, 2H, ArH), 7.01–7.11 (m, 1H, ArH); ^{13}C NMR ($CDCl_3$) 26.6, 30.6, 104.2 (t, J = 25.2 Hz), 111.7 (dd, J = 21.2, 4.0 Hz), 121.4 (dd, J = 15.2, 3.7 Hz), 132.2 (dd, J = 9.7, 5.2 Hz), 161.2 (dd, J = 250.2, 12.0 Hz), 162.7 (dd, J = 248.7, 11.5 Hz), 195.2 ppm; MS (+CI) 220 $[M+NH_4]^+$; M_r (+CI) 220.0608 $[M+NH_4]^+$ (calcd for $C_9H_{12}F_2NOS$ 220.0608 $[M+NH_4]^+$).

4.43. Thioacetic acid *S*-(3,4-difluorobenzyl) ester (33)

Using General procedure 3, 3,4-difluorobenzyl bromide (207 mg, 1 mmol) and potassium thioacetate (137 mg, 1.2 mmol) gave yellow oil **33**: yield, 163 mg (81%); R_f 0.41 (5% EtOAc–hexanes); FT-IR (neat) 1704, 1347, 1118 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.36 (s, 3H, $C(O)CH_3$), 4.05 (s, 2H, SCH_2Ar), 7.00–7.01 (m, 1H, ArH), 7.06–7.08 (m, 1H, ArH), 7.11–7.15 (m, 1H, ArH); ^{13}C NMR ($CDCl_3$) 30.7, 32.8, 117.7 (d, J = 17.2 Hz), 118.2 (d, J = 17.2 Hz), 125.3 (dd, J = 6.3, 3.4 Hz), 135.4 (dd, J = 5.7, 4.0 Hz), 150.3 (dd, J = 247.9, 12.6 Hz), 150.6 (dd, J = 248.4, 12.6 Hz), 195.1 ppm; MS (+CI) 220 $[M+NH_4]^+$; M_r (+CI) 220.0606 $[M+NH_4]^+$ (calcd for $C_9H_{12}F_2NOS$ 220.0608 $[M+NH_4]^+$).

4.44. Thioacetic acid *S*-(3,5-difluorobenzyl) ester (34)

Using General procedure 3, 3,5-difluorobenzyl bromide (207 mg, 1 mmol) and potassium thioacetate (137 mg, 1.2 mmol) gave yellow oil **34**: yield, 146 mg (72%); R_f 0.40 (5% EtOAc–hexanes); FT-IR (neat) 1697, 1456, 1323, 1153 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.37 (s, 3H, $C(O)CH_3$), 4.01 (s, 2H, SCH_2Ar), 6.65–6.72 (m, 1H, ArH), 6.81–6.84 (m, 2H, ArH); ^{13}C NMR ($CDCl_3$) 30.7, 33.1, 103.2 (t, J = 25.2 Hz, C(4)), 112.7 (dd, J = 15.9, 8.0 Hz, C(2), C(6)), 142.2 (t, J = 9.1 Hz, C(1)), 163.4 (dd, J = 248.7, 12.9 Hz, C(3), C(5)), 194.9 ppm; MS (+CI) 220 $[M+NH_4]^+$; M_r (+CI) 220.0607 $[M+NH_4]^+$ (calcd for $C_9H_{12}F_2NOS$ 202.0608 $[M+NH_4]^+$).

4.45. Thioacetic acid *S*-(2,3,6-trifluorobenzyl) ester (35)

Using General procedure 3, 2,3,6-trifluorobenzyl bromide (500 mg, 2.20 mmol) and potassium thioacetate (301 mg, 2.60 mmol) gave yellow oil **35**: yield, 339 mg (70%); R_f 0.40 (10% EtOAc–hexanes); FT-IR (neat) 1682, 1433, 1344, 1125 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.43 (s, 3H, $C(O)CH_3$), 4.28 (s, 2H, SCH_2Ar), 6.86–6.94 (m, 1H, ArH), 7.08–7.20 (m, 1H, ArH); ^{13}C NMR ($CDCl_3$) 20.6, 30.1, 109.9 (ddd, J = 24.0, 6.9, 4.6 Hz), 114.9 (dd, J = 21.0, 16.4 Hz), 115.3 (dd, J = 19.4, 11.3 Hz), 146.4 (ddd, J = 245.1, 12.6, 3.4 Hz), 148.1 (ddd, J = 251.9, 13.7, 8.0 Hz), 155.8 (ddd, J = 246.2, 5.7, 2.3 Hz), 193.9 ppm; MS (+ESI) 243 $[M+Na]^+$; M_r (+ESI) 243.0081 $[M+Na]^+$ (calcd for $C_9H_7F_3NaOS$ 243.0062 $[M+Na]^+$).

4.46. Thioacetic acid *S*-(3,4,5-trifluorobenzyl) ester (36)

Using General procedure 3, 3,4,5-trifluorobenzyl bromide (500 mg, 2.20 mmol) and potassium thioacetate (301 mg, 2.60 mmol) gave yellow solid **36**: yield, 315 mg (65%); mp 132 °C; R_f 0.40 (10% EtOAc–hexanes); FT-IR (KBr) 1696, 1443, 1324, 1118 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.49 (s, 3H, $C(O)CH_3$), 4.14 (s, 2H, SCH_2Ar), 7.03–7.08 (m, 2H, ArH); ^{13}C NMR ($CDCl_3$) 29.7, 31.8, 112.4 (dd, J = 14.9, 6.9 Hz, 2C), 133.9 (dd, J = 12.6, 8.0 Hz), 138.5 (dt, J = 251.9, 14.9 Hz), 150.5 (ddd, J = 249.6, 10.3, 3.9 Hz), 193.9 ppm; MS (+CI) 238 $[M+NH_4]^+$; M_r (+CI) 238.0503 $[M+NH_4]^+$ (calcd for $C_9H_{11}F_3NOS$ 243.0513 $[M+NH_4]^+$).

4.47. Thioacetic acid *S*-(2,3,5,6-tetrafluorobenzyl) ester (37)

Using General procedure 3, 2,3,5,6-tetrafluorobenzyl bromide (500 mg, 1.90 mmol) and potassium thioacetate (259 mg, 2.60 mmol) gave yellow oil **37**: yield, 272 mg (60%); R_f 0.50 (10% EtOAc–hexanes); FT-IR (neat) 1703, 1463, 1324, 1115 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.38 (s, 3H, $C(O)CH_3$), 4.08 (s, 2H, SCH_2Ar), 7.00–7.09 (m, 1H, ArH); ^{13}C NMR ($CDCl_3$) 25.3, 29.7, 111.5 (dt, J = 20.6, 3.5 Hz), 121.1–121.4 (m), 137.7–138.5 (m), 143.6–148.0 (m), 193.8 ppm; no signal was detected by either CI or ESI MS.

4.48. Thioacetic acid *S*-(2,3,4,5,6-pentafluorobenzyl) ester (38)

Using General procedure 3, 2,3,4,5,6-pentafluorobenzyl bromide (206 mg, 1.00 mmol) and potassium thioacetate (136 mg, 1.20 mmol) gave yellow oil **38**: yield, 153 mg (60%); R_f 0.50 (10% EtOAc–hexanes); FT-IR (neat) 1698, 1463, 1344, 1123 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.39 (s, 3H, $C(O)CH_3$), 4.22 (s, 2H, SCH_2Ar); ^{13}C NMR ($CDCl_3$) 19.5, 29.4, 110.9 (ddd, J = 17.2, 17.2, 3.4 Hz), 135.1–138.6 (m), 138.7–142.1 (m, 2C), 142.8–146.5 (m,

2C), 192.7 ppm; MS (+CI) 274 $[M+NH_4]^+$; M_r (+CI) 274.0319 $[M+NH_4]^+$ (calcd for $C_9H_9F_4NOS$ 274.0325 $[M+NH_4]^+$).

4.49. Thioacetic acid *S*-(4-trifluoromethylbenzyl) ester (39)

Using General procedure 3, 4-trifluoromethylbenzyl bromide (239 mg, 1.00 mmol) and potassium thioacetate (136 mg, 1.20 mmol) gave brown oil **39**: yield, 166 mg (71%); R_f 0.30 (5% EtOAc–hexanes); FT-IR (neat) 1701, 1463, 1324, 1118 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.36 (s, 3H, $C(O)CH_3$), 4.14 (s, 2H, SCH_2Ar), 7.49–7.54 (m, 4 H); ^{13}C NMR ($CDCl_3$) 29.1, 32.0, 123.2–123.4 (m, CF_3), 124.7–124.9 (m), 128.4 (2C), 129.5-130.7 (m, 2C), 131.6, 193.5 ppm; MS (+CI) 252 $[M+NH_4]^+$; M_r (+CI) 252.0655 $[M+NH_4]^+$ (calcd for $C_{10}H_{13}F_3NOS$ 252.0607 $[M+NH_4]^+$).

4.50. Thioacetic acid *S*-(3,5-bis-trifluoromethylbenzyl) ester (40)

Using General procedure 3, 3,5-bis-trifluoromethyl benzyl bromide (307 mg, 1.00 mmol) and potassium thioacetate (136 mg, 1.20 mmol) gave brown oil **40**: yield, 220 mg (73%); R_f 0.27 (5% EtOAc–hexanes); FT-IR (neat) 1704, 1452, 1334, 1123 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.51 (s, 3H, $C(O)CH_3$), 4.33 (s, 2H, SCH_2Ar), 7.90 (s, 3H, ArH); ^{13}C NMR ($CDCl_3$) 29.6, 32.0, 120.6–120.9 (m, 2C, CF_3), 124.5, 128.5–128.6 (m), 130.7–132.0 (m, 2C), 140.2, 193.7 ppm; MS (+CI) 320 $[M+NH_4]^+$; M_r (+CI) 320.0545 $[M+NH_4]^+$ (calcd for $C_{11}H_{12}F_6NOS$ 320.0544 $[M+NH_4]^+$).

4.51. Thioacetic acid *S*-(3-chlorobenzyl) ester (50)

Using General procedure 3, 3-chlorobenzyl bromide (205 mg, 1.0 mmol) and potassium thioacetate (137 mg, 1.2 mmol) gave brown oil **50**: yield, 176 mg (88%); R_f 0.55 (5% EtOAc–hexanes); FT-IR (neat) 1691, 1425, 1234, 1129 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.49 (s, 3H, $C(O)CH_3$), 4.21 (s, 2H, SCH_2Ar), 7.34–7.42 (m, 4H, ArH); ^{13}C NMR ($CDCl_3$) 29.8, 32.3, 126.5, 126.9, 128.4, 129.3, 133.8, 139.3, 194.2 ppm; MS (+CI) 218 $[M+NH_4]^+$; M_r (+CI) 218.0422 $[M+NH_4]^+$ (calcd for $C_9H_{13}ClNOS$ 218.0406 $[M+NH_4]^+$).

4.52. Thioacetic acid *S*-(3-cyanobenzyl) ester (51)

Using General procedure 3, 3-cyanobenzyl bromide (392 mg, 2.0 mmol) and potassium thioacetate (374 mg, 2.4 mmol) gave brown oil **51**: yield, 143 mg (75%); R_f 0.53 (5% EtOAc–hexanes); FT-IR (neat) 1697, 1426, 1234, 1131 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.47 (s, 3H, $C(O)CH_3$), 4.21 (s, 2H, SCH_2Ar), 7.51–7.71 (m, 4H, ArH); ^{13}C NMR ($CDCl_3$) 29.7, 31.9, 112.1, 118.0, 128.3, 130.3, 131.7, 132.7, 139.0, 193.8 ppm; MS (+ESI) 214 $[M+Na]^+$; M_r (+ESI) 214.0290 $[M+Na]^+$ (calcd for $C_{10}H_9NNaOS$ 214.0297 $[M+Na]^+$).

4.53. Thioacetic acid *S*-(3-methoxybenzyl) ester (52)

Using General procedure 3, 3-methoxybenzyl bromide (402 mg, 2.0 mmol) and potassium thioacetate (374 mg, 2.4 mmol) gave brown oil **52**: yield, 317 mg (81%); R_f 0.65 (5% EtOAc–hexanes); FT-IR (neat) 1689, 1450, 1265, 1110 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.24 (s, 3H, $C(O)CH_3$), 3.70 (s, 3H, OCH_3), 4.00 (s, 2H, SCH_2Ar), 6.66–6.70 (m, 1H, ArH), 6.74–6.78 (m, 2H, ArH), 7.08–7.13 (m, 1H, ArH); ^{13}C NMR ($CDCl_3$) 29.7, 32.9, 54.6, 112.3, 113.8, 120.5, 129.1, 138.6, 159.2, 194.4 ppm; MS (+ESI) 219 $[M+Na]^+$; M_r (+ESI) 219.0460 $[M+Na]^+$ (calcd for $C_{10}H_{12}NaO_2S$ 219.0451 $[M+Na]^+$).

4.54. Thioacetic acid *S*-(3-carboxybenzyl) ester (58)

Using General procedure 3, 3-chloromethylbenzoic acid (340 mg, 2.0 mmol) and potassium thioacetate (274 mg, 2.4 mmol) gave white solid **58**: yield, 257 mg (58%); mp 143–145 °C; R_f 0.45 (1:20:50 AcOH–$CHCl_3$-THF); FT-IR (KBr) 1689, 1413, 1299, 1128 cm^{-1}; ^1H NMR (CD_3OD) δ 2.45 (s, 3H, $C(O)CH_3$), 4.27 (s, 2H, SCH_2Ar), 7.48 (t, J = 7.5 Hz, 1H), 7.60 (d, J = 7.5 Hz, 1 H), 8.01 (d, J = 7.5 Hz, 1H), 8.07 (s, 1 H); ^{13}C NMR (CD_3OD) 29.9, 32.7, 127.8, 128.9, 129.5, 130.9, 143.7, 168.7, 195.3 ppm; MS (+ESI) 233 $[M+Na]^+$; M_r (+ESI) 233.0252 $[M+Na]^+$ (calcd for $C_{10}H_{10}NaO_3S$ 233.0243).

4.55. Thioacetic acid *S*-(4-carboxybenzyl) ester (59)

Using General procedure 3, 4-chloromethylbenzoic acid (85 mg, 0.50 mmol) and potassium thioacetate (68 mg, 0.6 mmol) gave white solid **59**: yield, 132 mg (50%); mp 155–158 °C; R_f 0.43 (1:20:50 AcOH–$CHCl_3$-THF); FT-IR (KBr) 1691, 1421, 1287, 1121 cm^{-1}; ^1H NMR (CD_3OD) δ 2.34 (s, 3H, $C(O)CH_3$), 4.16 (s, 2H, SCH_2Ar), 7.37 (s, 2 H), 7.90 (s, 2 H); ^{13}C NMR ($CDCl_3$) δ 30.2, 33.8, 129.9, 131.1, 131.2, 144.8, 169.9, 196.3 ppm; MS (+ESI) 233 $[M+Na]^+$; M_r (+ESI) 233.0251 $[M+Na]^+$ (calcd for $C_{10}H_{10}NaO_3S$ 233.0243).

4.56. Inhibitory properties of bicyclomycin and bicyclomycin derivatives in the poly(C)-dependent ATPase assay[32]

The ability of wild-type rho to hydrolyze [γ-^{32}P]ATP was assayed in 100 μL reactions containing ATPase buffer (40 mM Tris–HCl, pH 7.9, 50 mM KCl, and 12 mM $MgCl_2$), 250 μM ATP, 0.5 μCi [γ-^{32}P]ATP, 300 nM poly(C), and 200 nM rho based on monomer. Reactions were preincubated at 32 °C for 90 s prior to the addition of ATP. Aliquots (1 μL) were removed at various times (15, 30, 45, 60, and 75 s) during the reaction and spotted onto PEI-TLC plates. [γ-^{32}P]ATP and $^{32}P_i$ were separated by chromatography on PEI-TLC plates using 0.75 M KH_2PO_4, pH 3.5, as the mobile phase. The developed PEI-TLC plates were used to expose PhosphorImager Plates (Fuji and Molecular Dynamics) (3 h), scanned on a Storm 860 PC PhosphorImager, and analyzed using Molecular Dynamics's ImageQuant 5.0. The initial rates of reactions were determined by plotting the amount of ATP hydrolyzed versus time. Relative percent activities were calculated from the initial velocities. Each assay was performed in duplicate and the results were averaged.

4.57. Inhibitory properties of bicyclomycin and bicyclomycin derivatives in the antimicrobial assay[33]

Aliquots (200 μL) from suspensions of overnight LB broth cultures (*E. coli* W3350) were diluted into LB broth (2 mL). The suspension was poured onto 15 mL volume LB agar plates. The solution was gently rocked to distribute the cells evenly over the plates surface, and any excess cell solution was removed by pipet. The plates were incubated at 37 °C (30 min) and an antibiotic-assay disk (0.25 in. diameter) containing 20 μL to the test compound (1, 2, 4, 8, 16, and 32 mg/mL in 50–100% DMSO) was placed on the agar surface. The plates were incubated at 37 °C (18 h). Data plots of the zone of inhibited bacterial growth (cm^3) versus $\log(1000 \times C)$, where C is the concentration of the test compound (mg/mL), yielded linear slopes to provide the minimal inhibitory concentrations (MIC) for **1**. The dihydrobicyclomycin derivatives showed no zones of growth inhibition. Each assay was performed in duplicate and the results were averaged.

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