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Original paper

Antirhinovirus structure–activity relationships of 6-substituted-9-(4-methylbenzyl)-2-trifluoromethyl-9*H*-purines

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Summary – To evaluate the effect of different 6-substituents on antirhinoviral activity, a series of 6-substituted-9-(4-methylbenzyl)-2-trifluoromethyl-9*H*-purines was synthesized and tested. A matrix map of space adjacent to the 6-position was constructed to facilitate structure–activity analysis. This study provided evidence that a lipophilic pocket exists on the virus capsid surface, which accomodates the methyl group of the 6-methylaminopurines.

Résumé – **Rapports entre structure et activité antirhinovirus des dérivés 6-substitués de 9-(4-méthylbenzyl)-2-trifluorométhyl-9*H*-purines.** *Afin de déterminer l'effet de différents substituants en 6 sur l'activité antivirale, une série de dérivés 6-substitués de 9-(4-méthylbenzyl)-2-trifluorométhyl-9*H*-purines ont été préparés et soumis à des essais relatifs à l'activité antivirale. Une carte matricielle de l'espase contigu à la position 6 a été construite afin de faciliter l'analyse de la structure et de l'activité. L'étude a mis en évidence l'existence à la surface de la capside du virus d'une poche lipophile pouvant accueillir le groupe méthyle des 6-méthylaminopurines.*

benzylpurine / purine / trifluoromethylpurine / antiviral / rhinovirus

Introduction

We previously reported the potent *in vitro* antirhinovirus activity of 9-benzyl-2-substituted-6-dimethylaminopurines [1–3]. One of the most active compounds against rhinovirus type 1B was 6-dimethylamino-9-(4-methylbenzyl)-2-trifluoromethyl-9*H*-purine (**1**, 429U80), which had an IC_{50} of 0.03 μM [2]. Small lipophilic aryl substituents provided compounds with optimum activity [3]. Although **1** and several analogues had potent activity against rhinovirus type 1B, activity against other serotypes was not uniform; the IC_{50}s ranged over 260-fold [2, 3]. To develop an agent with a broader spectrum of antirhinovirus activity, we prepared a series of 6-substituted analogues of **1.** The synthesis and structure–activity relationships (SAR) of these analogues are reported herein.

Chemistry

The compounds in Table I were prepared in one or two steps from the 6-chloro-2-trifluoromethylpurine **24** [2, 4] (Scheme 1). Compounds **2, 3, 8–13, 15–17** and **20–22** were prepared by amination of **24** with the appropriate amine; methoxy derivative **6** was isolated as a side-product in the preparation of **3**. The anion of **2** was alkylated with chloromethyl methyl sulfide or 2-chloroethylamine to give **14** or **18,** respectively. Acylation of **2** with acetic anhydride and 4-dimethylaminopyridine provided amine **23**. Ester **20** was hydrolyzed with sodium hydroxide to give acid **19.**

Biological results and Discussion

The compounds in Table II were tested initially against rhinovirus type 1B in a plaque inhibition assay using monolayers of M-HeLa cells. The 50% inhibitory concentration for most of the compounds was measured with the plaque reduction assay. For compounds **3** and **5**, IC_{50} values could not be determined due to limited solubility; for several others only the percent inhibition at the highest concentration measured is given in parentheses. The assays were performed as described previously [5].

The parent 6-dimethylaminopurine **1** had an IC_{50} = 0.03 μM [2] (Table II). Removal of an *N*-methyl substituent to give **2** resulted in an 18-fold loss in activity. If both *N*-methyl substituents were removed, the resultant 6-aminopurine **3** was over 1000-fold less active. Other 6-substituents such as hydrogen **(4)**, oxo **(5)** and methoxy **(6)** gave compounds devoid of significant antirhinovirus activity. However, the 6-methylthio compound **7** had activity comparable to **2**.

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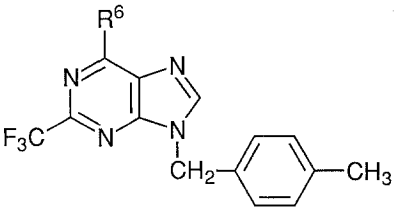
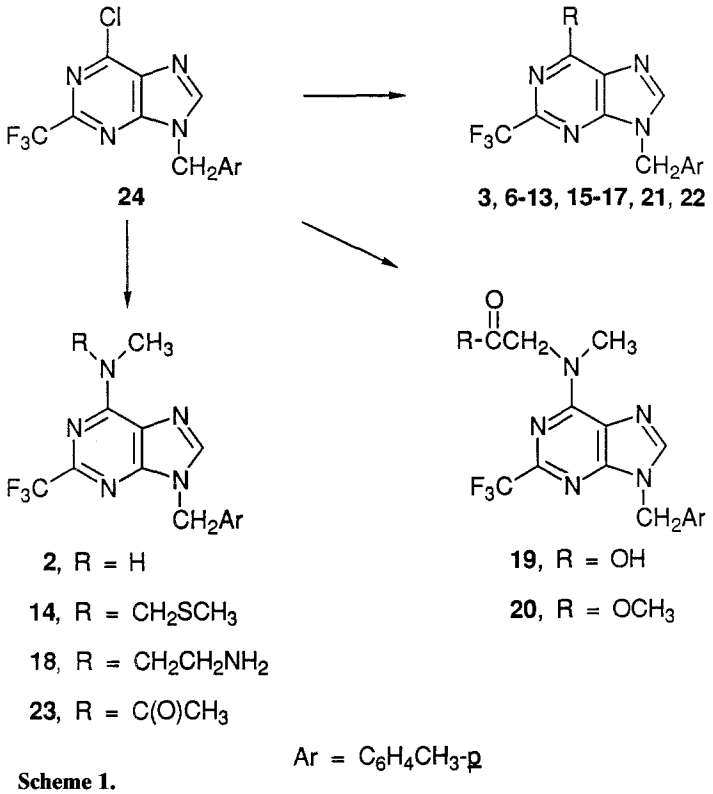
Table I. Physical properties of 6-substituted-9-(4-methylbenzyl)-2-trifluoromethyl-9*H*-purines.



Compd. No.	R	Methods[a]	% Yield	mp (°C)	Formula[b]
2	$NHCH_3$	A	48[c]	188.5–189.5	$C_{15}H_{14}F_3N_5$
3	NH_2	A[d]	30[e]	188 –189	$C_{14}H_{12}F_3N_5$
6	OCH_3	A[f]	47[g]	175.5–176.5	$C_{15}H_{13}F_3N_4O$
7	SCH_3	exptl.	40[e]	151 –152	$C_{15}H_{13}F_3N_4S$
8	$N(CH_3)CH_2CH_3$	A[h]	28[i]	65 – 67	$C_{17}H_{18}F_3N_5$
9	$N(CH_3)CH(CH_3)_2$	A[j]	30[i]	86 – 88	$C_{18}H_{20}F_3N_5$
10	$N(CH_3)C_3H_5$-cyclo	A[j]	55[i]	87.5– 89	$C_{18}H_{18}F_3N_5$
11	$NH-C_3H_5$-cyclo	A[j]	54[c]	115 –116	$C_{17}H_{16}F_3N_5$
12	$N(CH_3)CH_2CH_2CH_3$	A[j]	34[c]	109.5–110.5	$C_{18}H_{20}F_3N_5$
13	$N(CH_3)CH_2CH=CH_2$	A[j]	77[i]	106 –106.5	$C_{18}H_{18}F_3N_5$
14	$N(CH_3)CH_2SCH_3$	B	69[g]	100.5–101.5	$C_{17}H_{18}F_3N_5S$
15	$N(CH_3)CH_2CH_2CH_2CH_3$	A[j]	19[c]	75 – 76.5	$C_{19}H_{22}F_3N_5$
16	$N(CH_3)C_6H_5$	exptl.	33[i]	79 – 80.5	$C_{21}H_{18}F_3N_5$
17	$N(CH_3)CH_2CH_2OH$	A[k]	70[g]	92 – 94	$C_{17}H_{18}F_3N_5O$
18	$N(CH_3)CH_2CH_2NH_2$	B	8	220 –230 (dec)	$C_{17}H_{19}F_3N_6 \cdot HCl \cdot 1/2H_2O$
19	$N(CH_3)CH_2COOH$	exptl.	78[l]	170 –171	$C_{17}H_{16}F_3N_5O_2$
20	$N(CH_3)CH_2COOCH_3$	A[m]	49[g]	114 –115	$C_{18}H_{18}F_3N_5O_2$
21	$N(CH_3)OH$	A[m]	59[e]	175.5–177	$C_{15}H_{14}F_3N_5O$
22	$N(CH_3)OCH_3$	A[m]	73[g]	84 – 85	$C_{16}H_{16}F_3N_5O$
23	$N(CH_3)C(O)CH_3$	exptl.	54[g]	131.5–132.5	$C_{17}H_{16}F_3N_5O$

[a]Method A: see preparation of **1** in [2].
[b]All compounds were analyzed for C, H, N.
[c]Recrystallized from pentane–ethyl acetate.
[d]Saturated methanolic ammonia with one equivalent of triethylamine was used.
[e]Recrystallized from hexane–ethyl acetate.
[f]Isolated as a side-product from preparation of **3.**
[g]Recrystallized from hexane.
[h]Triethylamine (6 equivalents) and ethylmethylamine prepared from the hydrochloride and 0.5 ml of 1 N sodium hydroxide was used.
[i]Recrystallized from pentane.
[j]Triethylamine (3 equivalents) and 3 equivalents of the appropriate amine were used.
[k]Neat (2-hydroxyethyl)methylamine was used.
[l]Recrystallized from ethanol–water.
[m]Triethylamine (2.2 equivalents) and 1.2 equivalents of the appropriate amine hydrochloride were used.



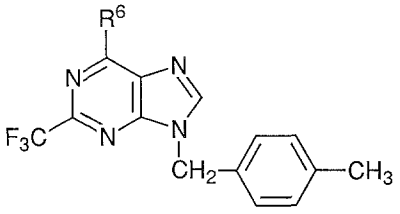
Scheme 1.

Mechanism studies showed that **1** is an inhibitor of virus uncoating, probably *via* binding to a site that stabilizes the virus capsid [6]. This is a highly selective interaction since the structure–activity requirements for optimum activity are very specific [2, 3]. A matrix map [7] of space adjacent to the 6-position of **1** was constructed to facilitate analysis of the SAR of 6-substituted analogues of **1** (Fig. 1).

The 1000-fold difference in inhibitory potency between **1** and **3** may be accounted for if a lipophilic pocket exists on the capsid surface adjacent to the 6-position of the purine. This pocket may accommodate the methyl group of 6-methylaminopurines like **1** and **2** with a marked increase in binding activity. This site may also accommodate the methyl of **7**, but the 6-methoxy group of **6** does not give an optimum interaction. The 18-fold increase in activity of **1** over **2** suggests that the second methyl substituent interacts at another lipophilic site; this is designated as point 1 for purposes of illustration (Fig. 1) in the discussion of structure–activity relationships.

When the size of one of the 6-*N*-methyl groups of **1** was increased to ethyl **(8)**, 2-propyl **(9)** or cyclopropyl **(10)** a 3-fold loss in activity occurred. This suggests that the virus capsid surface at points 2 and 6 is not lipophilic, although this area is able to tolerate a nonpolar hydrocarbon substituent since there is only a minor loss in activity. When the 6-substituent was enlarged to propyl **(12)**, allyl **(13)** or methylthiomethyl **(14)**, a 10-fold loss in activity relative to **1** occurred. Larger substituents at *N*-6 such as butyl **(15)** and phenyl **(16)** gave compounds with higher IC_{50}s. These results suggest there is limited tolerance for increased bulk at points 3, 4, 5 and 10 (Fig. 1).

Table II. Activity of 6-substituted purines against rhinovirus type 1B *in vitro*.



Compound	R_6	IC_{50} (μM)[a, b]
1[c]	$N(CH_3)_2$	0.03
2	$NHCH_3$	0.56
3	NH_2	>40
4[d]	H	11
5[c]	OH (oxo)	>10
6	OCH_3	(–S)
7	SCH_3	0.6
8	$N(CH_3)CH_2CH_3$	0.1
9	$N(CH_3)CH(CH_3)_2$	(46% at 0.1)
10	$N(CH_3)C_3H_5$–cyclo	0.1
11	$NH-C_3H_5$–cyclo	3.58
12	$N(CH_3)CH_2CH_2CH_3$	0.3
13	$N(CH_3)CH_2CH=CH_2$	0.3
14	$N(CH_3)CH_2SCH_3$	0.3
15	$N(CH_3)CH_2CH_2CH_2CH_3$	0.7
16	$N(CH_3)C_6H_5$	1.9
17	$N(CH_3)CH_2CH_2OH$	3.7
18	$N(CH_3)CH_2CH_2NH_2$	(25% at 10)
19	$N(CH_3)CH_2COOH$	20.8
20	$N(CH_3)CH_2COOCH_3$	0.9
21	$N(CH_3)OH$	8.9
22	$N(CH_3)OCH_3$	0.3
23	$N(CH_3)C(O)CH_3$	14.4

[a] The plaque inhibition assay was performed as described in [5].
S = slight toxicity;
– = inactive at 50 μg per disc.
[b] The 50% inhibitory concentration was measured as described in [5].
[c] For synthesis see [2].
[d] For synthesis see [4].

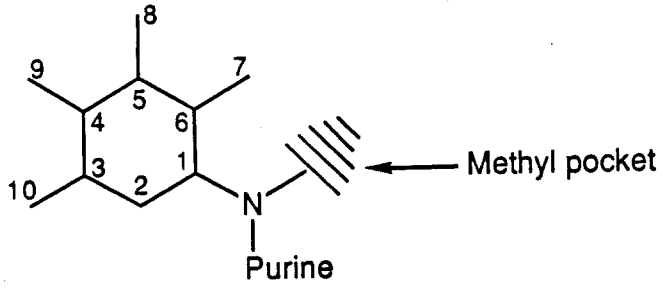


Fig. 1. Map of binding region adjacent to 6-position of 9-benzylpurines.

To further explore the capsid binding region adjacent to the purine 6-position, polar atoms were attached to the 6-amino group. The $HOCH_2CH_2$ **(17)** and $H_2NCH_2CH_2$ **(18)** substituents gave compounds that were over 120- and 330-fold less active than **1.** This suggests that the surface at point 3 into which the polar OH and NH_2 project is lipophilic, or that there is a lack of bulk tolerance [8] for the solvated hydroxyl and amino substituents. This interpretation is further supported by the weak activity of the polar carboxylate analogue **19.** However, the bulky methyl ester **20** was 20-fold more active than **19**, further suggesting that this area is lipophilic and can accommodate some bulk, but the surface repels polar substituents. The nature of point 1 was further probed with the hydroxylamino derivative **21**. This compound, which projects a hydroxyl group to point 1, was 300-fold less active than **1**. However, if the polar hydroxyl was methylated, 30-fold of activity was recouped.

Thus, this mapping study provides evidence that **1** is a potent inhibitor of rhinovirus 1B because of specific interactions by the purine 6-substituent with the virus capsid surface. Structure–activity considerations suggest that a lipophilic pocket accommodates an *N*-methyl group and that additional lipophilic interaction occurs at position 1 to give optimum interaction. Larger lipophilic *N*-6 substituents led to a modest loss in antirhinovirus activity, whereas polar substituents caused a large loss in activity. These results suggest that further improvements in potency will not be found with further variation of the 6-substituent of these compounds.

Experimental protocols

Melting points were taken in capillary tubes on a Mel-Temp block or a Thomas-Hoover Unimelt and were uncorrected. UV spectra were measured on a Unicam SP 800 or Cary 118 UV-vis spectrophotometer. NMR data were recorded on a Varian XL-200, a Varian XL-100-15-FT, a Varian FT-80A, or a Hitachi Perkin–Elmer R-24 spectrometer with tetramethylsilane (TMS) as an internal standard. Mass spectra were obtained from Oneida Research Services, Whitesboro, NY, who used a Finnegan 4500 TFQ mass spectrometer. Each analytical sample had spectral data compatible with its assigned structure and moved as a single spot on thin-layer chromatographs (TLC). TLCs were developed on Whatman 200 micron MK6F plates of silica gel (SG) with fluorescent indicator. Preparative flash chromatography was performed on Silica Gel 60 (40–63 μm, E. Merck No. 9385). All compounds were analyzed for C, H, N and gave combustion values within 0.4% of theoretical. Elemental analyses were performed by Atlantic Microlab, Inc.

Method B. 6-(N-Methyl-1-(methylthio)methylamino)-9-(4-methylbenzyl)-2-trifluoromethyl-9H-purine ***14***

Compound **2** (1.50 g, 4.67 mmol) was added in portions to a stirred mixture of sodium hydride (60.2% oil dispersion) (0.279 g, 7.00 mmol) and dry dimethylformamide (25 ml). After 30 min chloromethyl methyl sulfide (0.45 ml, 5.14 mmol) was added dropwise. After 1 h, the reaction was poured into an ice-water slurry (100 ml), and the pH of the mixture was adjusted to 4.5 with acetic acid. The mixture was extracted with ether (2 × 100 ml). The combined ether extract was washed with water (2 × 100 ml) and dried with sodium sulfate. The solution was spin evaporated *in vacuo* to give 1.62 g of crude product. The solids were dissolved in ethyl acetate, and the solution was added to 12 g of silica gel 60 wetted with ethyl acetate–hexane (1:3). The column was eluted with acetate–hexane (1:3), and the appropriate fractions were combined and spin evaporated *in vacuo* to give 1.40 g (79%) of **14.** Recrystallization of the solid from hexane gave 1.23 g (69%) of analytically pure **14**, mp: 100.5–101.5°C; TLC-SG, ethyl acetate–cyclohexane, one spot with $R_f = 0.44$; UV (pH 1) λ_{max} 283 nm; (pH 7) λ_{max} 281.5 nm; (pH 13) λ_{max} 282.5 nm; NMR (DMSO–d_6): δ 8.51 (s, 1H, H–8), 7.19 (AB quartet, 4H, ArH), 5.44 (br s, 2H, SCH_2), 5.39 (s, 2H, CH_2Ar), 3.45 (br s, 3H,NCH_3), 2.25 (s, 3H, $ArCH_3$), 2.08 (s, 3H, SCH_3); MS: *m/e* 381 (M^+), 334 (M^+-SCH_3), 230 ($M^+-(SCH_3$ and $C_8H_8)$), 105 ($C_8H_9^+$). Anal. ($C_{17}H_{18}F_3N_5S$) C, H, N.

9-(4-Methylbenzyl)-6-methylthio-2-trifluoromethyl-9H-purine ***7***

A mixture of **24** (2.00 g, 6.12 mmol), thiourea (0.536 g, 7.04 mmol) and propanol (40 ml) was refluxed for 22 h. The solvent was removed by spin evaporation *in vacuo*. The residual solids were triturated in water (20 ml), then collected by suction filtration. Recrystallization from ethanol–water gave material that was characterized as a purinyl sulfide dimer. A mixture of the purinyl sulfide (1.50 g, 2.44 mmol), 1 N sodium hydroxide (4.65 ml, 4.65 mmol), methyl iodide (1.16 ml, 18.6 mmol) and ethanol (30 ml) was vigorously stirred for 3 h at ambient temperature. The excess ethanol was removed by spin evaporation *in vacuo,* and the paste was treated with water (30 ml). The mixture was stirred for several min, and the solids were collected by suction filtration. The solids were treated with ethanol (100 ml) and 10 g of silica gel 60. The solvent was removed by spin evaporation *in vacuo*, and the residual solids were added to a column (5 cm × 18 cm) of silica gel 60 wetted with ethyl acetate–hexane (1:2). The column was eluted with ethyl acetate–hexane (1:2) and fifteen 60-ml fractions were collected. The fractions (10–13) containing product were combined and spin-evaporated *in vacuo* to give 0.635 g (77%) of crude material. Recrystallization of the solids from hexane–ethyl acetate gave 0.332 g (40%) of analytically pure **7,** mp 151–152°C; TLC-SG, CH_2Cl_2, one spot with $R_f = 0.32$; UV (pH 1) λ_{max} 294; (pH 7) λ_{max} 293.5; (pH 13) λ_{max} 294.5; NMR (DMSO–d_6): δ 8.77 (s, 1H, H–8), 7.20 (AB quartet, 4H, ArH), 5.48 (s, 2H, CH_2), 2.72 (s, 3H, SCH_3), 2.26 (s, 3H, $ArCH_3$); MS: *m/e* 338 (M^+), 323 (M^+-CH_3), 319 (M^+–F), 233 ($M^+-C_8H_9$), 105 ($C_8H_9^+$). Anal. ($C_{15}H_{13}F_3N_4S$) C, H, N.

6-(N-Methylanilino)-9-(4-methylbenzyl)-2-trifluoromethyl-9H-purine ***16***

A mixture of **24** (1.50 g, 4.59 mmol), *N*-methylaniline (1.52 g, 13.8 mmol), triethylamine (1.40 g, 13.8 mmol) and ethanol (15 ml) was refluxed with stirring for 3.5 h. The reaction was cooled, and the volatiles were spin evaporated *in vacuo* to give an oil that was partitioned between ethyl acetate–water (50 ml:50 ml). The phases were separated, and the organic phase was washed with 0.1 N hydrochloric acid (3 × 50 ml), then dried with magnesium sulfate. The organic solution was concentrated with reduced pressure to an oil (1.50 g) that was dissolved in ethyl acetate. Silica gel 60 (10 g) was added to the solution. This mixture was spin–evaporated *in vacuo,* and the residual solids were introduced on a column (3 × 16 cm) of silica gel 60 wetted with ethyl acetate–hexane (1:15). The column was eluted with ethyl acetate–hexane (1:15) using the flash chromatography technique. The appropriate fractions were combined and concentrated under reduced pressure to give 0.892 g (49%) of **16** as an oil. Crystallization from pentane gave 0.601 g (33%) of a white solid which was analytically pure, mp: 79–80.5°C. Anal. ($C_{21}H_{18}F_3N_5$) C, H, N.

9-(4-Methylbenzyl)-6-(N-methylcarboxymethylamino)-2-trifluoromethyl-9H-purine ***19***

A solution containing **20** (0.450 g, 1.14 mmol), 1 N sodium hydroxide (10 ml, 10 mmol) and methanol (30 ml) was stirred at ambient temperature for 18 h. The excess solvent was spin evaporated *in vacuo*, and 1 N

hydrochloric acid (25 ml) was added to the residue. After several min of stirring, the solids were collected by suction filtration. Recrystallization of the solids from ethanol–water gave 0.340 g (78%) of analytically pure **19**, mp: 170–171°C, TLC-SG, methanol–dichloromethane (1:9), one spot with R_f = 0.37; UV (pH 1) λ_{max} 273.5; (pH 7) λ_{max} 275; (pH 13) λ_{max} 275; NMR (DMSO–d_6): δ 12.8 (br s, 1H, CO_2H), 8.46 (pseudo d[a], 1H, H–8), 7.17 (AB, 4H, ArH), 5.37 (s, 2H, CH_2Ar), 5.03 and 4.39 (2 s[a], 2H, $CH_2C(O)$), 3.76 and 3.31 (s and br s, 3H, NCH_3), 2.24 (s, $ArCH_3$) ([a]attributed to syn and anti rotomers due to restricted rotation about C–N band); MS: *m/e* 379 (M^+), 360 (M^+-F), 335 (M^+-CO_2), 230 ($M^+-(CO_2$ and $C_8H_9)$), 105 ($C_8H_9^+$). Anal. ($C_{17}H_{16}F_3N_5O_2$) C, H, N.

6-(N-Methylacetamido)-9-(4-methylbenzyl)-2-trifluoromethyl-9H-purine ***23***

A mixture of **2** (0.500 g, 1.56 mmol), 4-dimethylaminopyridine (0.150 g, 1.23 mmol) and acetic anhydride (25 ml) was refluxed for 64 h. The reaction was poured into water (200 ml), and the aqueous mixture was extracted with ether (3 × 250 ml). The combined ether extract was reduced to 200 ml by evaporation under reduced pressure and washed with water (3 × 10 ml). The ether extract was dried with sodium sulfate, and the volatiles were removed by spin evaporation *in vacuo*. The residual solids were dissolved with ethyl acetate, and the solution was treated with 3 g of silica gel 60. The solvent was removed by spin evaporation *in vacuo*, and the residual solids were added to a column (3.5 cm × 18 cm) of silica gel 60 wetted with ethyl acetate–hexane (3:2). The column was eluted with the latter solvent, and 25-ml fractions were collected. The fractions containing product (13–23) were combined and spin-evaporated *in vacuo* to give a solid residue. Recrystallization of the solids from hexane gave 0.307 g (54%) of analytically pure **23**, mp 131.5–132.5°C; TLC-SG, ethyl acetate–cyclohexane (3:2), R_f = 0.26; UV (pH 1) λ_{max} 281; (pH 7) λ_{max} 280.5 (pH 13) λ_{max} 269; NMR (DMSO–d_6): δ 8.92 (s, 1H, H–8), 7.25 (AB q, 4H, ArH), 5.52 (s, 2H, CH_2), 3.58 (s, 3H, NCH_3), 2.31 (s, 3H, $COCH_3$), 2.27 (s, 3H, $ArCH_3$); MS: *m/e* 363 (M^+), 321 (M^+-CH_2O), 216 ($M^+-(CH_2O$ and $C_8H_9)$), 105 ($C_8H_9^+$). Anal. ($C_{17}H_{16}F_3N_5O$) C, H, N.

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