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Syntheses of substituted 2,4-dioxo-thienopyrimidin-1-acetic acids and their evaluation as aldose reductase inhibitors

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Summary — A series of 2,4-dioxo-thieno[2,3-*d*], [3,2-*d*] and [3,4-*d*]pyrimidin-1-acetic acids (**2**) with a benzyl moiety at the N-3 position were prepared and tested *in vitro* for aldose reductase inhibitory activity against partially purified enzyme from rat lens. Some of these compounds were also evaluated for inhibition of sorbitol accumulation in the sciatic nerve or lens of streptozotocin-induced diabetic rats *in vivo*. Among the synthesized compounds, several showed potent aldose reductase inhibitory activity with IC_{50}s in the 10^{-8} M range. Particularly, the potencies of non-substituted thieno- (**2a** and **2aa**), 5-methylthieno- (**2c**), 5,6-dimethylthieno-(**2g**), 6-isopropylthieno- (**2j** and **2k**), 6-chlorothienopyrimidine (**2q**) and benzothienopyrimidine (**2ac**) analogs were approximately equipotent to FK-366 (**1A**) and Ponalrestat (**1B**) as references. Although most compounds were inactive *in vivo*, 2 compounds, **2k** and **2q**, possessed moderate *in vivo* activity.

2,4-dioxo-thieno[2,3-*d*], [3,2-*d*] and [3,4-*d*]pyrimidin-1-acetic acids / aldose reductase inhibitory activity / sorbitol accumulation inhibition

Introduction

Aldose reductase (AR), the first enzyme in the polyol pathway, catalyses the NADPH-dependent reduction of D-glucose to its corresponding sugar alcohol, D-sorbitol. Under normal physiological conditions AR participates in osmoregulation, but under hyperglycemic conditions it also contributes to the onset and development of severe diabetic complications [1], *ie*, retinopathy, neuropathy, cataract, nephropathy and angiopathy [2, 3]. Experimental evidence indicates that inhibition of AR constitutes a possible approach to the treatment or prevention of certain secondary complications [4]. The development of clinically useful aldose reductase inhibitors (ARIs) has led to the identification of a large number of structurally diverse compounds with AR inhibitory activity [4, 5]. One of these ARIs, FK-366 [6] exhibits potent AR inhibitory activity and prevents the development of diabetic complications in animals. Clinical studies of FK-366 performed in Japan and the United States suggest it to be a promising ARI. FK-366 belongs to the carboxylic acid class of ARIs and bears a quinazolinedione nucleus. Bioisosteric replacement of the benzene ring with a thiophene ring frequently leads to compounds with improved potency and selectivity [7–11], therefore leading us to focus on the preparation of novel carboxylic acid thienopyrimidine derivatives (**2**) that are the thiophene isosteres of FK-366 (**1A**) [6]. In the synthesized compounds, several members of the thieno[2,3-*d*] and [3,2-*d*]pyrimidine series displayed potent inhibitory activity against rat lens AR *in vitro*. Their *in vitro* potency was equivalent to that of FK-366 and Ponalrestat and they moderately reduced sorbitol accumulation in the sciatic nerve and lens of hyperglycemic rats *in vivo*. Here, we describe the preparation and inhibitory activity of substituted 2,4-dioxothienopyrimidin-1-acetic acids (**2**) on rat lens AR *in vitro*, and also describe the lowering effects of some of these compounds on sorbitol accumulation in the sciatic nerve and lens in the streptozotocin-induced diabetic rat [12] in comparison to the carboxylic acids FK-366 (**1A**) [6] and Ponalrestat (**1B**) [13].

Chemistry

The 2,4-dioxothieno[2,3-*d*], [3,2-*d*] and [3,4-*d*]pyrimidin-1-acetic acids (**2**) were prepared using the 5 gen-

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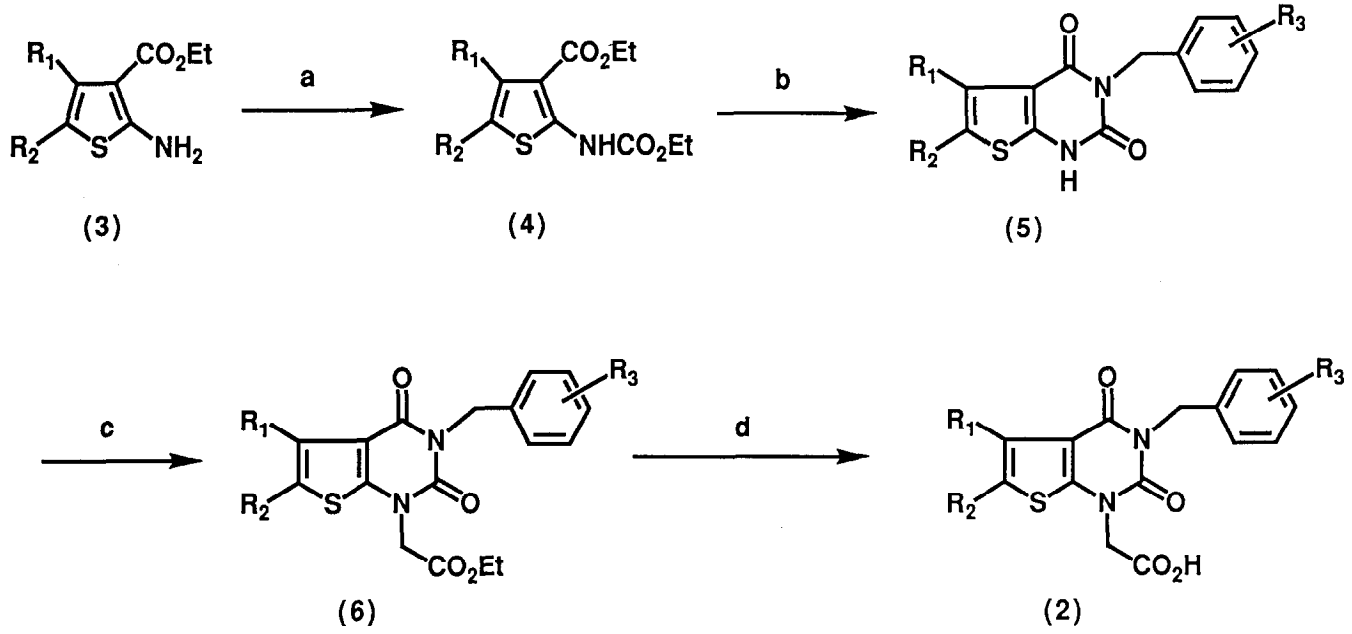
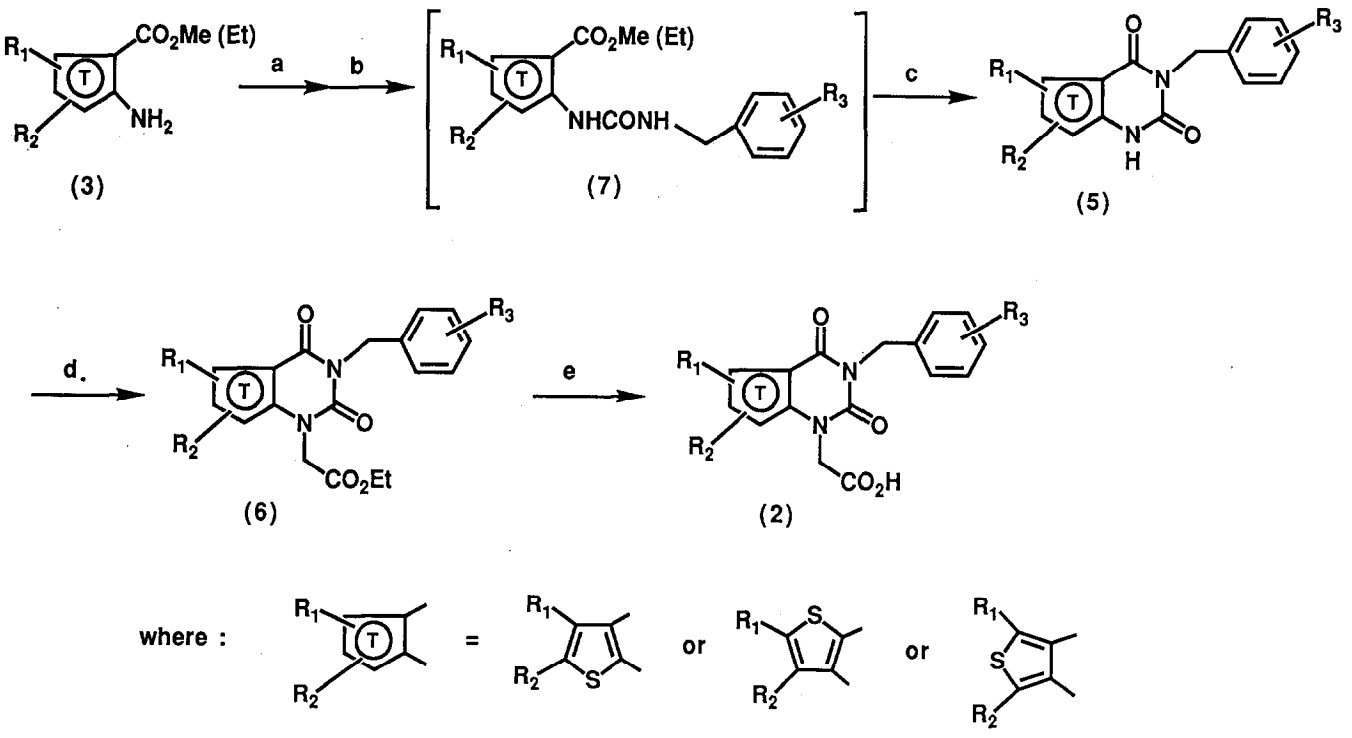
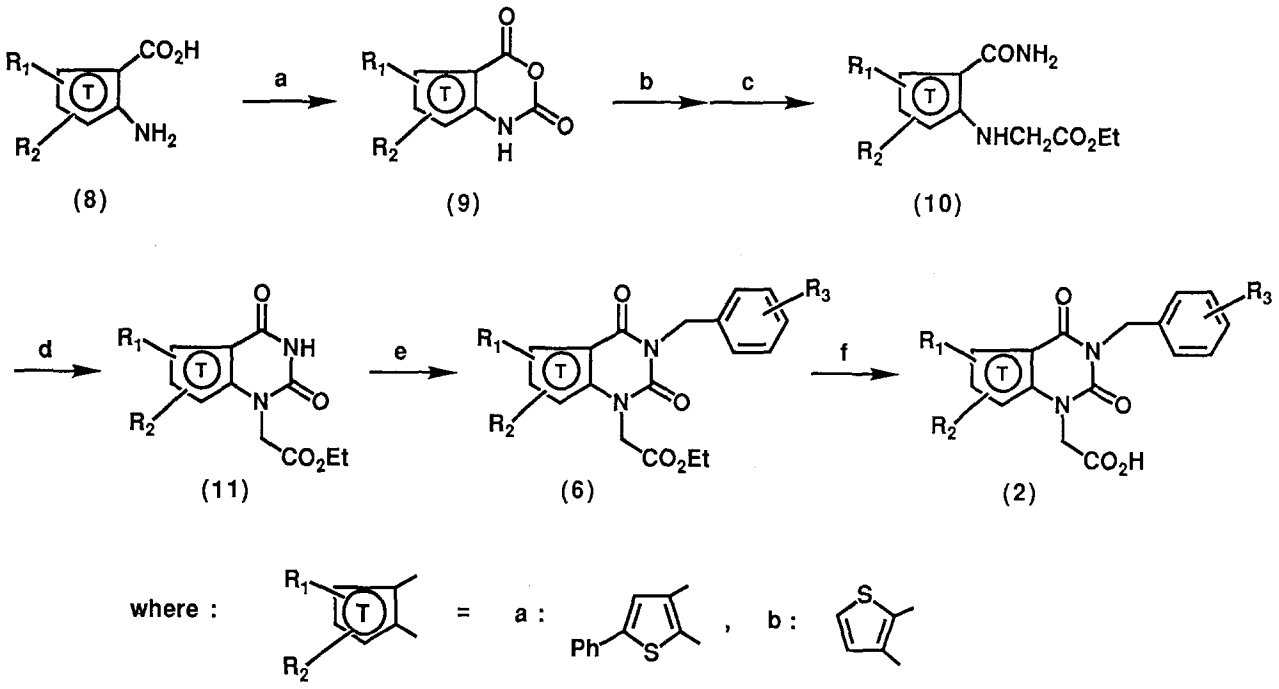


Scheme 1. Reagents: a) $ClCO_2Et$; b) $NH_2CH_2C_6H_4$-R_3, Δ; c) $BrCH_2CO_2Et$, NaH, DMF; d) OH^-, MeOH-H_2O; *Method A*.



Scheme 2. Reagents: a) trichloromethylchloroformate (TCF), dioxane; b) $NH_2CH_2C_6H_4$-R_3; c) $NaOCH_3$, MeOH; d) $BrCH_2CO_2Et$, NaH, DMF; e) conc HCl, AcOH; *Method B*.



Scheme 3. Reagents: a) $COCl_2$, dioxane; b) $BrCH_2CO_2Et$, NaH, DMF; c) NH_4OH; d) 1,1-carbonyldiimidazole (CDI), Δ; e) $BrCH_2C_6H_4$-R_3, NaH, DMF; f) conc HCl, AcOH; *Method C*.

eral synthetic methods (*Methods A,B,C,D* and *E*) as shown in schemes 1–5, where R_1, R_2 and R_3 represent the appropriate substituents as shown in tables I–III. In the first method (*Method A*) outlined in scheme 1, the amino group of **3** was ethoxycarbonylated with ethyl chlorocarbonate to give **4**. Condensation of **4** with an appropriate benzylamine at a reaction temperature of 230–250°C produced the cyclized thienopyrimidine **5**, which was subsequently subjected to alkylation with ethyl bromoacetate at the 1-position of **5** to give the 1,3-disubstituted thienopyrimidine (**6**), which was then hydrolyzed to the acetic acids **2**. Due to the strenuous high reaction temperatures (> 240°C) required for the cyclization of the carbamate **4** with amines (formation of compound **5**) as shown in scheme 1, the preparation *Method B* as shown in scheme 2 was adapted for most compounds (**2**). Refluxing isomeric aminothiophene carboxylates **3** with trichloromethylchloroformate (TCF) followed by treatment with appropriate benzylamines afforded the corresponding ureas (**7**). Ring closure of **7** with sodium methoxide in methanol proceeded easily to give thienopyrimidines **5** in fair yield. The alkylation of **5** with ethyl bromoacetate in the presence of sodium hydride in DMF produced **6**, followed by hydrolysis to yield the desirable thienopyrimidin-1-acetic acids **2** . Of these compounds **2**, the preparation of 2 compounds (**2o** and **2aa**) was carried out *via* the intermediate thienoxazine dione **9** as shown in scheme 3 *(Method C)*. Treatment of aminothiophene carboxylic acid **8** with excess phosgene gave thieno[2,3-*d*] [1, 3]-oxazine dione **9** in high yield. Alkylation of **9** with ethyl bromoacetate in NaH/DMF and consequent ammonolysis with aqueous ammonia afforded the corresponding glycine anologues **10**. These intermediates were subjected to ring closure by condensation with 1,1-carbonyldiimidazole (CDI) to yield the thienopyrimidines **11**. Alkylation at the 3-position of **11** was accomplished by treatment with appropriate benzyl halides in NaH/DMF to give 1,3-disubstituted thienopyrimidines **6**. Compounds **6** were also subjected to hydrolysis under acidic conditions to yield thienopyrimidin-1-acetic acids **2**. The halogenated compounds on the thiophene ring were prepared by the general synthetic method as shown in scheme 4 (*Method D*). Halogenation at the 2-position of the thiophene ring of **6** with SO_2Cl_2 or *N*-bromosuccimimide (NBS) in CH_2Cl_2 or CCl_4 afforded the halogenated thienopyrimidines, which through hydrolysis gave the corresponding halogenated thienopyrimidin-1-

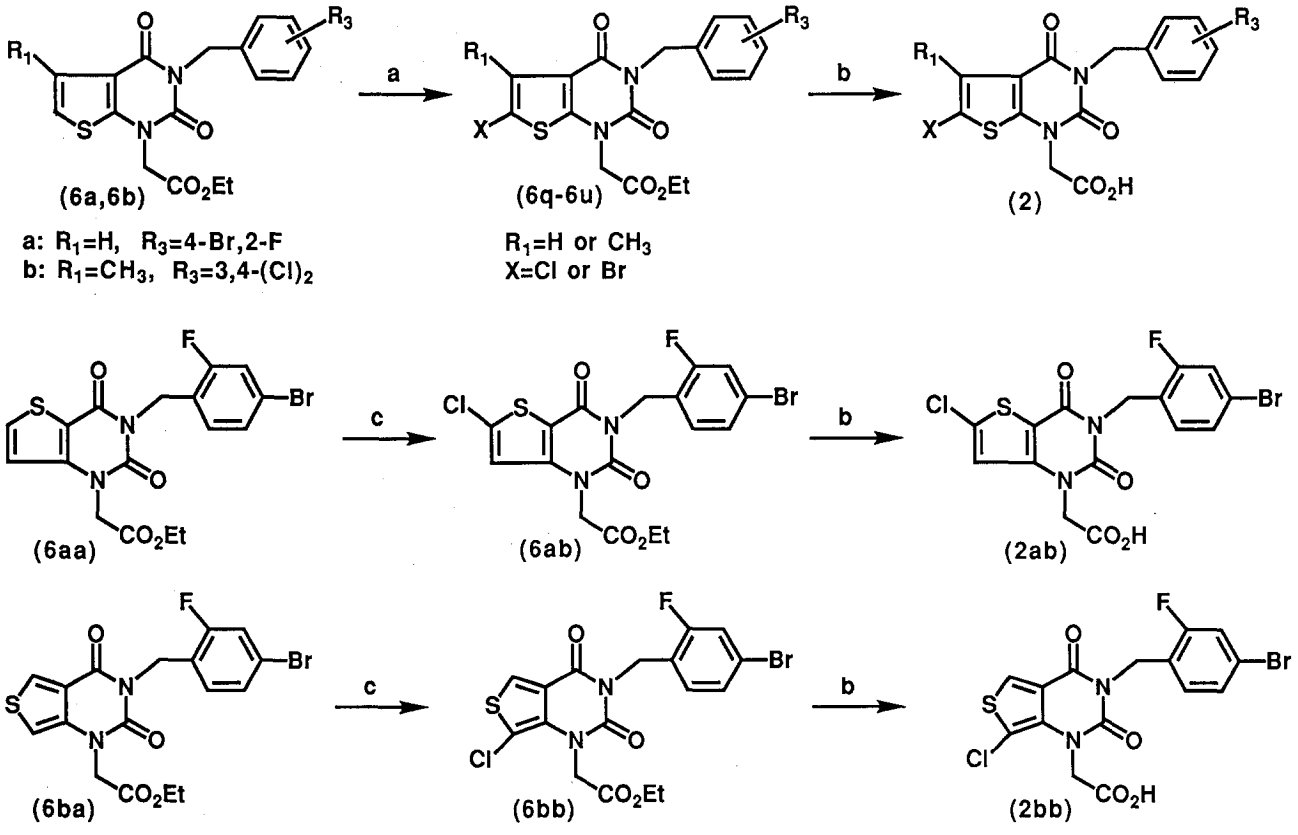
acetic acids **2**. The (4-methylpiperazin-1-yl)methyl, (imidazol-1-yl)methyl or (morphorin-4-yl)methyl substituents at the 6-position of thieno[2,3-*d*]pyrimidines **2** were introduced by the synthetic method, as shown in scheme 5 (*Method E*). Bromination of 6-methyl-thienopyrimidine **6h** was carried out with NBS in the presence of benzoylperoxide in CCl_4 to yield bromomethyl **12**. Subsequent treatment of **12** with appropriate amines gave **6z**, which was hydrolyzed under acidic conditions to afford thienopyrimidines **2y**. The compounds synthesized and their physical properties have been summarized in tables I–III.

Results and discussion

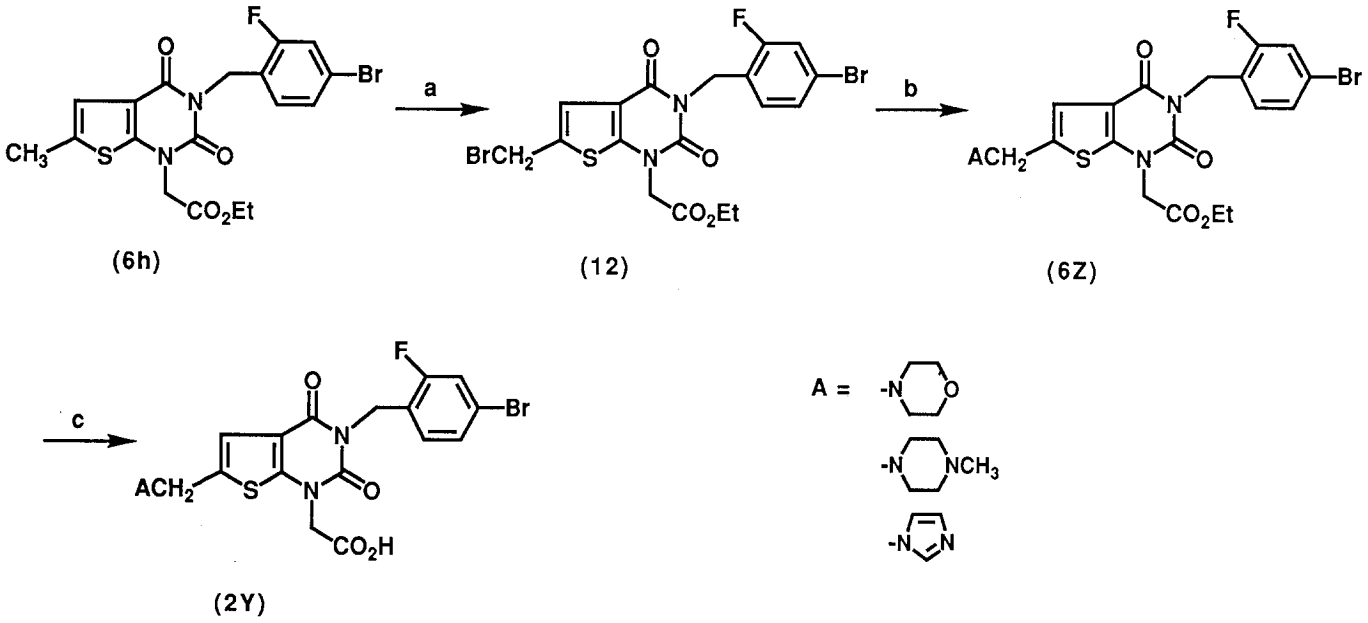
The synthesized compounds were assessed for the inhibitory activity against rat lens AR [14, 15]. Several compounds demonstrating potent *in vitro* inhibitory activity were also evaluated *in vivo* for their ability to reduce sorbitol accumulation in rat sciatic nerve according to Peterson *et al* [16] and Clements *et al* [17]. Moreover, in order to investigate the relation between *in vitro* and *in vivo* activity, the plasma half-lives of compounds **2k** and **2q** were compared in rats with those of FK-366 and Ponalrestat as references. The biological results have been summarized in tables I, IV and figure 1.

A. Thieno[2,3-d]pyrimidine series

As shown in table I, the *in vitro* AR inhibitory activity of the compounds with either no or short alkyl substituents (excluding bulky substituents, such as a *tert*-butyl group) on the thiophene ring of thieno[2,3-*d*]-pyrimidines was equipotent with FK-366 and Ponalrestat with IC_{50}s in the 10^{-8} M range. Compounds **2o–2t**, containing phenyl or cyclopentyl groups or halogen atom on the thiophene ring of thieno[2,3-*d*]-pyrimidine, also retained intrinsic activity. Compound **2x**, containing a bicyclic structure, also showed potent AR inhibitory activity. *In vitro* inhibitory activity

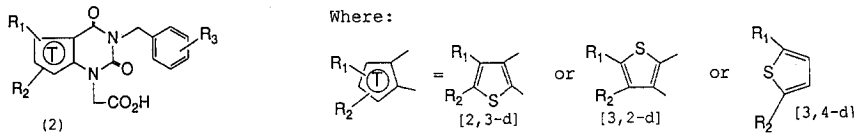


Scheme 4. Reagents: a) SO_2Cl_2 or NBS, CCl_4; b) conc HCl, AcOH; c) SO_2Cl_2, CH_2Cl_2; *Method D*.



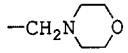
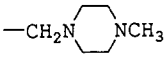
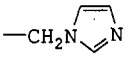
Scheme 5. Reagents: a) NBS, benzoylperoxide, CCl_4; b) AH, benzene; c) conc HCl, AcOH; *Method E.*

Table I. Chemical and biological data of 3-substituted 2,4-dioxo-thienopyrimidin-1-acetic acid **2**.



Compd	R_1	R_2	R_3	*Synth method*[a]	*Yield (%)*	*mp (%)*	*Formula*[b]	*RLAR inhibition*[c] IC_{50} $(10^{-8}$ *M)*
[2,3-*d*] Series								
2a	H	H	2-F, 4-Br	B	77	200–202	$C_{15}H_{10}FBrN_2O_4S$	2.0
2b	CH_3	H	3,4-$(Cl)_2$	B	48	>300	$C_{16}H_{12}Cl_2N_2O_4S$	3.0
2c	CH_3	H	2-F, 4-Br	B	87	207–208	$C_{16}H_{12}FBrN_2O_4S$	1.5
2d	$CH(CH_3)_2$	H	2-F, 4-Br	B	84	155–157	$C_{18}H_{16}FBrN_2O_4S$	3.3
2e	CH_3	CH_3	4-Cl	A	61	174–176	$C_{17}H_{15}ClN_2O_4S$	7.5
2f	CH_3	CH_3	2,4-$(Cl)_2$	A	53	266–267	$C_{17}H_{14}Cl_2N_2O_4S$	2.0
2g	CH_3	CH_3	2-F, 4-Br	B	80	233–235	$C_{17}H_{14}FBrN_2O_4S$	2.1
2h	H	CH_3	2-F, 4-Br	B	66	242–243	$C_{16}H_{12}FBrN_2O_4S$	4.1
2i	H	$CH(CH_3)_2$	3,4-$(Cl)_2$	B	61	189–191	$C_{18}H_{16}Cl_2N_2O_4S$	3.0
2j	H	$CH(CH_3)_2$	2,4-$(Cl)_2$	B	44	228–229	$C_{18}H_{16}Cl_2N_2O_4S$	1.7
2k	H	$CH(CH_3)_2$	2-F, 4-Br	B	55	203–205	$C_{18}H_{16}FBrN_2O_4S$	2.2
2l	H	$CH(CH_3)_2$	2,4-$(F)_2$	B	75	194–196	$C_{18}H_{16}F_2N_2O_4S$	10.0
2m	H	$C(CH_3)_3$	2,4-$(Cl)_2$	B	67	195–197	$C_{19}H_{18}Cl_2N_2O_4S$	10.0
2n	H	$C(CH_3)_3$	2-F, 4-Br	B	48	217–218	$C_{19}H_{18}FBrN_2O_4S$	10.0
2o	H	Ph	2-F, 4-Br	C	71	249–251	$C_{21}H_{14}FBrN_2O_4S$	3.4

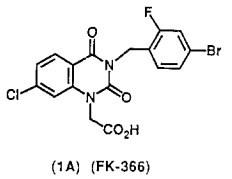
Table I. *(continued).*

Compd	R_1	R_2	R_3	*Synth method*[a]	*Yield (%)*	*mp (%)*	*Formula*[b]	*RLAR inhibition*[c] IC_{50} *(10^{-8} M)*
2p	H		2-F, 4-Br	B	49	160–162	$C_{20}H_{18}FBrN_2O_4S$	3.0
2q	H	Cl	2-F, 4-Br	D	96	221–223	$C_{15}H_9FClBrN_2O_4S$	2.5
2r	H	Br	2-F, 4-Br	D	93	245–247	$C_{15}H_9FBr_2N_2O_4S$	5.6
2s	CH_3	Cl	3.4-$(Cl)_2$	D	19	>300	$C_{16}H_{11}Cl_3N_2O_4S$	2.5
2t	CH_3	Br	3.4-$(Cl)_2$	D	37	>300	$C_{16}H_{11}Cl_2BrN_2O_4S$	2.6
2u			4-CH_3	A	65	253–256	$C_{20}H_{20}N_2O_4S$	26.0
2v			4-OCH_3	A	65	232–234	$C_{20}H_{20}N_2O_5S$	18.0
2w			2,4-$(Cl)_2$	A	63	265–266	$C_{19}H_{16}Cl_2N_2O_4S$	4.2
2x			2-F, 4-Br	B	85	288–290	$C_{19}H_{16}FBrN_2O_4S$	2.3
2ya	H		2-F, 4-Br	E	79	197–199	$C_{20}H_{19}FBrN_3O_5S$ ·HCl·1/5H_2O	12.0
2yb	H		2-F, 4-Br	E	30	282–285	$C_{21}H_{22}FBrN_4O_4S$ ·2(2H_2O)	12.0
2yc	H		2-F, 4-Br	E	92	178–180	$C_{19}H_{14}FBrN_2O_4S$ ·HCl·H_2O	14.0
[3,2-*d*] Series								
2aa	H	H	2-F, 4-Br	C	93	214–216	$C_{15}H_{10}FBrN_2O_4S$	2.0
2ab	Cl	H	2-F, 4-Br	D	92	197–198	$C_{15}H_9FClBrN_2O_4S$	3.0
2ac			2-F, 4-Br	B	74	239–240	$C_{19}H_{12}FBrN_2O_4S$ ·1/2H_2O	1.5
2ad			2-F, 4-Br	B	95	126–128	$C_{19}H_{11}FClBrN_2O_4S$	10.0
2ae			2-F, 4-Br	B	96	270–272	$C_{19}H_{11}FClBrN_2O_4S$	> 100
2af			2-F, 4-Br	B	85	242–243	$C_{19}H_{11}FClBrN_2O_4S$ ·1/2H_2O	> 100
[3,4-*d*] Series								
2ba	H	H	2-F, 4-Br	B	75	200–202	$C_{15}H_{10}FBrN_2O_4S$	40.0
2bb	H	Cl	2-F, 4-Br	D	53	239–241	$C_{15}H_9FClBrN_2O_4S$	> 100
FK-366								1.5
Ponalrestat								1.7

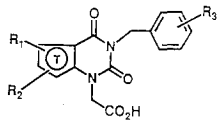
[a]See scheme and *Experimental protocols;* [b]the analysis was within ± 0.4% of the theoretical values; [c]rat lens aldose reductase inhibitory activity.

decreased with the introduction of hydrophobic substituents such as morpholinomethyl, *N*-methylpiperazinomethyl and imidazolylmethyl groups on the thiophene ring as shown in compounds **2ya**, **2yb** and **2yc**. In examining substituents on the benzyl ring at the 3-position of thieno[2,3-*d*]pyrimidine, compounds containing halogen substituents generally displayed potent AR inhibitory activity *in vitro*, especially **2a**, **2c**, **2f**, **2g**, **2j**, **2k**, **2q**, **2s**, **2t** and **2x** containing 2,4- and 3,4-dichloro or 4-bromo-2-fluoro substituents on the benzyl ring. Interestingly, these observations are similar to those for the FK-366 and Ponalrestat series. *In vitro* AR inhibitory activity was significantly influenced by substituents on the benzyl ring at the 3-

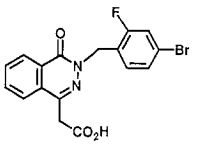
position of thieno[2,3-*d*]pyrimidine. Namely, **2u** and **2v** with an electron-donating group such as methyl or methoxy groups on benzyl ring showed a significant decrease of AR inhibitory activity in comparison with those compounds with an electron-withdrawing group such as halogen, especially dihalogen. From these observations, the substituent at the 3-position of thieno[3,2-*d*]pyrimidines and thieno[3,4-*d*]pyrimidines was focused on the representative 4-bromo-2-fluorobenzyl group with an electron-withdrawing property.



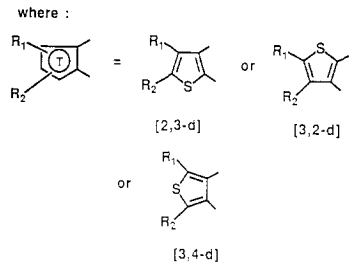
(1A) (FK-366)



(2)



(1B) (Ponalrestat)



B Thieno [3,2-d]pyrimidine series

The *in vitro* AR inhibitory activity for compounds **2aa**, **2ab** and **2ac** of this series was equipotent to that of the thieno[2,3-*d*]pyrimidine series with IC_{50}s in the 1.5–3.0 x 10^{-8} M range. Introduction of a chlorine atom on the benzene ring of benzothieno[3,2-*d*]pyrimidine significantly reduced inhibitory activity.

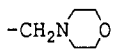
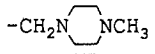
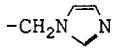
C Thieno[3,4-d]pyrimidine series

Although the structure–activity relationships in this series cannot be fully elucidated due to an inadequate number of synthesized compounds, 2 compounds (**2ba** and **2bb**) failed to show significant AR inhibitory activity *in vitro* in comparison with **2a** and **2aa** in the thieno[2,3-*d*]pyrimidine and thieno[3,2-*d*]pyrimidine series respectively. In *in vivo* evaluations, the compounds **2a**, **2c**, **2g**, **2j**, **2k**, **2q**, **2aa** and **2ac** were compared with FK-366 and Ponalrestat for their ability to reduce galactitol accumulation. Most of the compounds failed to show statistically significant *in vivo* activity, while **2k** and **2q** moderately reduced

Table II. Chemical and biological data of 3-substituted 2,4-dioxo-thienopyrimidin-1-acetic acid ethyl esters **6**.

Compd	R_1	R_2	R_3	*Synth method*[a]	*Yield (%)*	*mp (°C)*	*Formula*[b]
[2,3-*d*] Series							
6a	H	H	2-F, 4-Br	B	63	117–119	$C_{17}H_{14}FBrN_2O_4S$
6b	CH_3	H	3,4-$(Cl)_2$	B	46	124–126	$C_{18}H_{16}Cl_2N_2O_4S$
6c	CH_3	H	2-F, 4-Br	B	87	153–154	$C_{18}H_{16}FBrN_2O_4S$
6d	$CH(CH_3)_2$	H	2-F, 4-Br	B	98	148–149	$C_{20}H_{20}FBrN_2O_4S$
6e	CH_3	CH_3	4-Cl	A	92	151–152	$C_{19}H_{19}ClN_2O_4S$
6f	CH_3	CH_3	2,4-$(Cl)_2$	A	96	150–151	$C_{19}H_{18}Cl_2N_2O_4S$
6g	CH_3	CH_3	2-F, 4-Br	B	88	144–146	$C_{19}H_{18}FBrN_2O_4S$
6h	H	CH_3	2-F, 4-Br	B	58	145–147	$C_{18}H_{16}FBrN_2O_4S$
6i	H	$CH(CH_3)_2$	3,4-$(Cl)_2$	B	89	91–92	$C_{20}H_{20}Cl_2N_2O_4S$
6j	H	$CH(CH_3)_2$	3,4-$(Cl)_2$	B	61	146–148	$C_{20}H_{20}Cl_2N_2O_4S$
6k	H	$CH(CH_3)_2$	2-F, 4-Br	B	88	99–101	$C_{20}H_{20}FBrN_2O_4S$
6l	H	$CH(CH_3)_2$	2,4-$(F)_2$	B	50	111–112	$C_{20}H_{20}F_2N_2O_4S$
6m	H	$C(CH_3)_3$	2,4-$(Cl)_2$	B	68	115–116	$C_{21}H_{22}Cl_2N_2O_4S$
6n	H	$C(CH_3)_3$	2-F, 4-Br	B	90	137–139	$C_{21}H_{22}FBrN_2O_4S$
6o	H	Ph	2-F, 4-Br	C	70	234–235	$C_{23}H_{18}FBrN_2O_4S$

Table II. *(Continued.)*

Compd	R_1	R_2	R_3	*Synth method*[a]	*Yield (%)*	*mp (°C)*	*Formula*[b]
6n	H	$C(CH_3)_3$	2-F, 4-Br	B	90	137–139	$C_{21}H_{22}FBrN_2O_4S$
6o	H	Ph	2-F, 4-Br	C	70	234–235	$C_{23}H_{18}FBrN_2O_4S$
6p	H		2-F, 4-Br	B	83	96–97	$C_{22}H_{22}FBrN_2O_4S$
6q	H	Cl	$3,4\text{-}(Br)_2$	D	73	167–168	$C_{17}H_{13}ClFBr_2N_2O_4S$
6r	H	Cl	2-F, 4-Br	D	93	133–134	$C_{17}H_{13}FClBrN_2O_4S$
6s	H	Br	2-F, 4-Br	D	62	169–170	$C_{17}H_{13}FBr_2N_2O_4S$
6t	CH_3	Cl	$3,4\text{-}(Cl)_2$	D	65	142–145	$C_{18}H_{15}Cl_3N_2O_4S$
6u	CH_3	Br	$3,4\text{-}(Cl)_2$	D	85	166–167	$C_{18}H_{15}Cl_2BrN_2O_4S$
6v			$4\text{-}CH_3$	A	65	147–149	$C_{22}H_{24}N_2O_4S$
6w			$4\text{-}OCH_3$	A	60	153–154	$C_{22}H_{24}N_2O_5S$
6x			$2,4\text{-}(Cl)_2$	A	66	169–170	$C_{21}H_{20}Cl_2N_2O_4S$
6y			2-F, 4-Br	B	60	141–143	$C_{21}H_{20}FBrN_2O_4S$
6za	H		2-F, 4-Br	E	42	178–180	$C_{22}H_{23}FBrN_3O_5S \cdot 2H_2O$
6zb	H		2-F, 4-Br	E	31	105–106	$C_{23}H_{26}FBrN_4O_4S$
6zc	H		2-F, 4-Br	E	46	141–142	$C_{21}H_{18}FBrN_4O_4S$
[3,2-*d*] Series							
6aa	H	H	2-F, 4-Br	C	45	162–163	$C_{17}H_{14}FBrN_2O_4S$
6ab	Cl	H	2-F, 4-Br	D	39	125–126	$C_{17}H_{13}FClBrN_2O_4S$
6ac			2-F, 4-Br	B	82	201–203	$C_{21}H_{14}FBrN_2O_4S$
6ad			2-F, 4-Br	B	25	136–138	$C_{21}H_{15}FClBrN_2O_4S$
6ae			2-F, 4-Br	B	50	175–176	$C_{21}H_{15}FClBrN_2O_4S$
6af			2-F, 4-Br	B	63	212–213	$C_{21}H_{15}FClBrN_2O_4S$
[3,4-*d*] Series							
6ba	H	H	2-F, 4-Br	B	81	149–150	$C_{17}H_{14}FBrN_2O_4S$
6bb	H	Cl	2-F, 4-Br	D	51	171–173	$C_{17}H_{13}FClBrN_2O_4S$

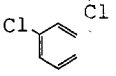
[a, b]See table I.

sorbitol accumulation in the rat sciatic nerve or lens, however, this reduction was less compared to that observed with FK-366 or Ponalrestat. Examination of plasma concentrations of **2k**, **2q**, FK-366 and Ponalrestat after 10 mg/kg *po* administration in rat as shown in figure 1 indicated that all 4 compounds displayed essentially equal C_{max} but reduced plasma half-lives for compounds **2k** and **2q** (3.1 and 6.1 μg/ml at 24 h, respectively) compared to FK-366 and Ponalrestat (11.2 and 7.8 μg/ml at 24 h, respectively). Though the data on plasma concentration for other compounds have not been shown here, the plasma concentration of all compounds subjected to *in vivo* examination was measured in the same manner as that for **2k** and **2q**, and all compounds with poor *in vivo* activity resulted in low C_{max} in plasma. These observations suggest that the *in vivo* activity is proportional to the plasma half-life of these ARIs.

Conclusion

Based on the bioisosteric replacement of the benzene ring on the quinazolidine nucleus with a thiophene ring, a series of thienopyrimidines have been synthesized and evaluated both *in vitro* and *in vivo* for their ability to inhibit aldose reductase *in vitro* and to reduce sorbitol accumulation in sciatic nerve and lens *in vivo*. Although a number of *in vitro* active ARIs have been obtained, none possess *in vivo* inhibitory activity equivalent to that of FK-366 or Ponalrestat. The search for potent *in vivo* active ARIs continues.

Table III. Chemical and biological data of 3-substituted thienopyrimidine-2,4 (1*H*, 3*H*)-diones **5**.

Compd	R_1	R_2	R_3	*Synth method*[a]	*Yield (%)*	*mp (°C)*	*Formula*[b]
[2,3-*d*] Series							
5a	H	H	2-F, 4-Br	B	71	290–291	$C_{13}H_8FBrN_2O_2S$
5b	CH_3	H	3,4-$(Cl)_2$	B	70	299–300	$C_{14}H_{10}Cl_2N_2O_4S$
5c	CH_3	H	2-F, 4-Br	B	66	296–298	$C_{14}H_{10}FBrN_2O_2S$
5d	$CH(CH_3)_2$	H	2-F, 4-Br	B	52	271–273	$C_{16}H_{14}FBrN_2O_2S$
5e	CH_3	CH_3	4-Cl	A	68	291–292	$C_{15}H_{13}ClN_2O_2S$
5f	CH_3	CH_3	2,4-$(Cl)_2$	A	70	275–276	$C_{15}H_{12}Cl_2N_2O_2S$
5g	CH_3	CH_3	2-F, 4-Br	B	47	258–259	$C_{15}H_{12}FBrN_2O_2S$
5h	H	CH_3	2-F, 4-Br	B	55	304–305	$C_{14}H_{10}FBrN_2O_2S$
5i	H	-$CH(CH_3)_2$	3,4-$(Cl)_2$	B	43	253–254	$C_{16}H_{14}Cl_2N_2O_2S$
5j	H	$CH(CH_3)_2$	2,4-$(Cl)_2$	B	47	223–225	$C_{16}H_{14}Cl_2N_2O_2S$
5k	H	$CH(CH_3)_2$	2-F, 4-Br	B	76	227–228	$C_{16}H_{14}FBrN_2O_2S$
5l	H	$CH(CH_3)_2$	2,4-$(F)_2$	B	64	219–221	$C_{16}H_{14}F_2N_2O_2S$
5m	H	$C(CH_3)_3$	2,4-$(Cl)_2$	B	43	264–265	$C_{17}H_{16}Cl_2N_2O_2S$
5n	H	$C(CH_3)_3$	2-F, 4-Br	B	14	240–241	$C_{17}H_{16}FBrN_2O_2S$
5o	H		2-F, 4-Br	B	69	257–259	$C_{18}H_{16}FBrN_2O_2S$
5p			4-CH_3	A	40	240–241	$C_{18}H_{18}N_2O_2S$
5q			4-OCH_3	A	29	230–232	$C_{18}H_{18}N_2O_3S$
5r			2,4-$(Cl)_2$	A	45	255–256	$C_{17}H_{14}Cl_2N_2O_2S$
5s			2-F, 4-Br	B	49	228–230	$C_{17}H_{14}FBrN_2O_2S$
[3,2-*d*] Series							
5aa			2-F, 4-Br	B	90	> 300	$C_{17}H_{10}FBrN_2O_2S$
5ab			2-F, 4-Br	B	78	267–268	$C_{17}H_9FClBrN_2O_2S$
5ac			2-F, 4-Br	B	96	> 300	$C_{17}H_9FClBrN_2O_2S$
5ad			2-F, 4-Br	B	59	> 300	$C_{17}H_9FClBrN_2O_2S$
[3,2-*d*] Series							
5ba	H	H	2-F, 4-Br	B	32	> 300	$C_{13}H_{18}FBrN_2O_2S$

[a, b]See table I.

Table IV. Biological data *(in vivo)* of thienopyrimidin-1-acetic acids **2**.

Compd	*Dose* (*mg/kg*)	*Reduction (%) of sorbitol accumulation*[a] *Sciatic nerve*	*Lens*
2a	20	33.4**	NT[b]
2c	20	12.6	15.2
2g	20	30.0**[c]	NT
2j	20	14.0	NT
2k	20	60.1**	60.0**
2q	20	73.6***	31.7*
2aa	20	15.7	NT
2ac	20	21.4*	NT
FK-366	10	85.7***	NT
	20	90.4***	91.8***
Ponalrestat	10	65.6***	43.4**

[a]Reduction of sorbitol accumulation in the sciatic nerves and lens on the streptozotocin (70 mg/kg) induced diabetic rats (n = 7). Compounds were orally administered once a day for 4 d; [b]NT: not tested; [c]statistically significant at $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

Experimental protocols

Melting points were obtained on a Yanagimoto micromelting apparatus and are uncorrected. ^{1}H-NMR spectra were recorded on a JEOL JMN-FX 100 spectrometer using tetramethylsilane (TMS) as an internal standard. Chemical shifts are expressed as δ values (ppm). Elemental analyses were carried out with a Yanagimoto CHN Corder MT-2.

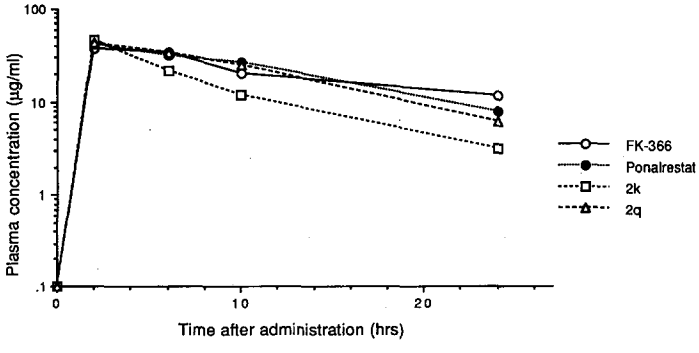


Fig 1. Plasma concentrations of **2k** and **2q** in comparison with that of FK-366 and Ponalrestat after a single oral administration in rats (10 mg/kg) (mean ± SD of 2 or 3 rats).

Starting materials

Ethyl 2-aminothiophene-3-carboxylate [18], ethyl 2-amino-5-methylthiophene-3-carboxylate [19], ethyl 2-amino-4,5-dimethylthiophene-3-carboxylate [19], ethyl 2-amino-5-isopropylthiophene-3-carboxylate [20], ethyl 2-amino-4,5,6,7-tetrahydrobenzothiophene-3-carboxylate [19], ethyl 2-amino-5-phenylthiophene-3-carboxylate [20], methyl 3-aminothiophene-4-carboxylate [21], methyl 3-aminothiophene-4-carboxylate [21], methyl 3-aminobenzothiophene-2-carboxylate[22], methyl 3-amino-4-chlorobenzothiophene-2-carboxylate [22] and methyl 3-amino-6-chlorobenzothiophene-2-carboxylate [22] were prepared according to literature procedures. The following aminothiophene carboxylates were prepared in the same manner as described in the literature [18, 19, 22].

Ethyl 2-amino-5-tert-butylthiophene-3-carboxylate
Yield: 23%; bp: 120–121°C/0.012 mmHg. ^{1}H-NMR ($CDCl_3$): 1.25 (s, 9H), 1.28 (t, 3H), 4.25 (q, 2H), 5.20–6.00 (brs, 2H) and 6.60 (s, 1H).

Ethyl 2-amino-5-cyclopentylthiophene-3-carboxylate
Yield: 75%; bp: 148–150°C/0.022 mmHg. ^{1}H-NMR ($CDCl_3$): 1.33 (t, 3H), 1.45–1.80 (m, 6H), 1.90–2.10 (m, 2H), 2.90–3.10(m, 1H), 4.25 (q, 2H), 5.60–5.90 (brs, 2H) and 6.64 (s, 1H).

Ethyl 2-amino-4-isopropylthiophene-3-carboxylate
Yield: 61%; bp: 115–118°C/0.019 mmHg. ^{1}H-NMR ($CDCl_3$): 1.17 (d, 6H), 1.33 (t, 3H), 3.10–3.60 (m, 1H), 4.28 (q, 2H), 5.80 (s, 1H) and 6.00–6.20 (brs, 2H).

Methyl 3-amino-5-chlorobenzothiophene-2-carboxylate
Yield:70%; mp: 182–183°C. Anal calcd for $C_{10}H_8ClNO_2S$: C, 49.69; H, 3.34; N, 5.80; found: C, 49.95; H, 3.28; N, 5.81.

2-Amino-5-phenylthiophene-3-carboxylic acid ***8a***
Yield: 75%; mp: 175–177°C. Anal calcd for $C_{11}H_9NO_2S$; C, 60.26; H, 4.14; N, 6.39; found: C, 60.53; H, 4.16; N, 6.37.

3-Aminothiophene-2-carboxylic acid ***8b***
Yield: 30%; mp: 88–89°C. Anal calcd for $C_5H_5NO_2S$; C, 41.95; H, 3.52; N, 9.78; found: C, 41.91; H, 3.60; N, 9.74.

4-Bromo-2-fluorobenzylamine
This was prepared from 4-bromo-2-fluorobenzylbromide [23] according to the method of Calat *et al* [24]. Yield: 66%; bp: 61–64°C/0.35 mmHg. ^{1}H-NMR ($CDCl_3$): 3.86 (s, 2H) and 7.10–7.40 (s, 3H). All other aminothiophene carboxylates, benzylamines and benzylhalides were commercially available.

Chemistry

Method A

Ethyl 4,5-Dimethyl-2-ethoxycarbonylaminothiophene-3-carboxylate ***4a.*** A mixture of ethyl 2-amino-4,5-dimethylthiophene-3-carboxylate (3.15 g, 15.8 mmol) and ethyl chloroformate (40 ml) was refluxed for 3 h. After cooling, the reaction mixture was evaporated under reduced pressure and the residue recrystallized from ethanol to give **4a** (3.7 g, 86%), mp: 68–69°C. Anal calcd for $C_{12}H_{17}NO_4S$; C, 53.12; H, 6.32; N, 5.16; found: C, 53.49; H, 6.10; N, 5.17.

*Ethyl 2-ethoxycarbonylamino-4,5,6,7-tetrahydrobenzothiophene-3-carboxylate **4b**.* The title compound was prepared in a similar manner to that described for **4a**. Yield: 67%; mp: 65–66°C. ^{1}H-NMR ($CDCl_3$): 1.30 (t, 3H), 1.40 (t, 3H), 1.67–1.93 (m, 4H), 2.60–3.00 (m, 4H), 4.28 (q, 2H), 4.32 (q, 2H) and 10.40 (brs, 1H).

*3-(4-Chlorobenzyl)-5,6-dimethylthieno[2,3-d]pyrimidine-2,4(1H, 3H)-dione **5e**.* A mixture of **4a** (1.0 g, 3.7 mmol) and 4-chlorobenzylamine (1.0 g, 7.1 mmol) was heated to 230–240°C for 8 h. After cooling, the crude solid was recrystallized from a mixture of ethanol and dimethylformamide (DMF) to yield **5e** (0.8 g, 68%), mp: 291–292°C. ^{1}H-NMR(DMSO–d_6): 2.25 (s, 3H), 2.26 (s, 3H), 4.99 (s, 2H), 7.20–7.45 (m, 4H) and 11.00 (brs, 1H). Compounds **5f** and **5p–5r** were prepared from the corresponding thiophene carboxylate **4** and benzylamine in a similar manner to that described for **5e**. The physical data have been listed in table III.

*Ethyl 3-(4-chlorobenzyl)-5,6-dimethyl-2,4-dioxo-thieno[2,3-d]pyrimidin-1-acetate **6e**.* Compound **5e** (0.6 g, 2.1 mmol) was slowly added to a suspension of NaH (60% in oil, 0.1 g, 2.5 mmol) at 0–5°C. After stirring for 0.5 h at rt, ethyl bromoacetate (0.4 g, 3.2 mmol) was added dropwise and the mixture was stirred for 17 h at the same temperature. The reaction mixture was then evaporated under reduced pressure, and a mixture of ice-water and diluted HCl was added to the residue. The resulting precipitate was collected and recrystallized from ethanol to give **6e** (0.7 g, 92.0%), mp: 151–152°C. ^{1}H-NMR ($CDCl_3$): 1.19 (t, 3H), 2.30 (s, 6H), 4.16 (q, 2H), 4.73 (s, 2H), 5.04 (s, 2H) and 7.20–7.50 (m, 4H). Compounds **6f** and **6v–6x** were prepared in a similar manner to that described for **6e**. The physical data have been listed in table II.

*3-(4-Chlorobenzyl)-5,6-dimethyl-2,4-dioxo-thieno[2,3-d]pyrimidin-1-acetic acid **2e**.* A solution of **6e** (0.7 g, 1.7 mmol) and 4 N–NaOH (2 ml) in MeOH (30 ml) was heated at 60°C for 0.5 h. The reaction mixture was evaporated under reduced pressure and a mixture of ice-water and diluted HCl was added to the residue. The resulting precipitate was collected and recrystallized from methanol to give **2e** (0.4 g, 61%), mp: 174–176°C. ^{1}H-NMR(DMSO–d_6): 2.29 (s, 6H), 4.62 (s, 2H), 5.04 (s, 2H) and 7.15–7.45 (m, 4H). Compounds **2f** and **2u–2w** were prepared in a similar manner to that described for **2e**. The physical data have been listed in table I.

Method B

*3-(4-Bromo-2-fluorobenzyl)-thieno[2,3-d]pyrimidine-2,4-(1H, 3H)-dione **5a**.* Trichloromethylchloroformate (TCF) (12 ml) was added dropwise to a solution of ethyl 2-aminothiophene-3-carboxylate (7.5 g, 43.8 mmol) in dioxane (60 ml) at 5°C. The reaction mixture was stirred for 1 h at rt, heated to 60–70°C for 4 h, and then evaporated under reduced pressure. The resulting residue was dissolved in Et_2O (80 ml) and filtered. The filtrate was added to a solution of 4-bromo-2-fluorobenzylamine (10.0 g, 49.0 mmol) in dioxane (50 ml) at rt, after which the reaction mixture was stirred for 12 h at rt and then evaporated under reduced pressure. The residue was recrystallized from ethanol to yield compound **7a**.

*1-(4-Bromo-2-fluorobenzyl)-3-(3-ethoxycarbonyl-thiophen-2-yl)urea (**7a**).* Compound **7**a (13.2 g, 75%), mp: 158–159°C. ^{1}H-NMR ($CDCl_3$): 1.30 (t, 3H), 4.20–4.50 (m, 4H), 6.79 (d, 1H), 7.06 (d, 1H), 7.20–7.65 (m, 3H), 8.44 (t, 1H) and 10.25 (brs, 1H). The obtained urea (12.65 g, 31.5 mmol) was added to a solution of Na (1.45 g, 63 mmol) in methanol (300 ml) and the reaction mixture refluxed for 2 h and then evaporated under reduced pressure. The residue was dissolved in water (80 ml) and acidified (pH = 1) with diluted HCl. The resulting precipitate was collected and recrystallized from ethanol to yield **5a** (7.9 g, 71%), mp: 290–291°C. ^{1}H-NMR (DMSO–d_6): 5.03 (s, 2H), 7.00–7.65 (m, 5H) and 12.35 (brs, 1H). Compounds **5b–5d**, **5g–5o**, **5s**, **5aa–5ad** and **5ba** were prepared in a similar manner to that described for **5a**. The physical data have been listed in table III.

*Ethyl 3-(4-bromo-2-fluorobenzyl)-2,4-dioxo-thieno[2,3-d]pyrimidin-1-acetate **6a**.* The title compound was prepared for **5a** and ethyl bromoacetate in a similar manner to that described for **6e**. Yield: 63%, mp: 117–119°C. ^{1}H-NMR ($CDCl_3$): 1.19 (t, 3H), 4.18 (q, 2H), 4.80 (s, 2H), 5.09 (s, 2H) and 7.00–7.65 (m, 5H). Compounds **6b–6d**, **6g–6n**, **6p**, **6y**, **6ac–6af** and **6ba** were prepared in a similar manner to that described for **6a**. The physical data have been listed in table II.

*3-(4-Bromo-2-fluorobenzyl)-2,4-dioxo-thieno[2,3-d]pyrimidin-1-acetic acid **2a**.* A solution of **6a** (3.0 g, 6.8 mmol), conc HCl (20 ml) in AcOH (50 ml) was refluxed for 6 h. After cooling, the resulting precipitate was collected, washed with water and recrystallized from 50% ethanol to give **2a** (2.2 g, 77%), mp: 200–202°C. ^{1}H-NMR (DMSO–d_6): 4.70 (s, 2H), 5.09 (s, 2H), 7.00–7.65 (m, 5H) and 13.50 (brs, 1H). Compounds **2b–2d**, **2g–2n**, **2p**, **2x**, **2ac–2af** and **2ba** were prepared in a similar manner to that described for **2a**. The physical data have been listed in table I.

Method C

*6-Phenyl-2H-thieno[2,3-d][1,3]oxazine-2,4-(1H)-dione **9a**.* 15 ml phosgene (2.7 M solution in CCl_4) was added dropwise to a solution of **8a** (5.0 g, 22.8 mmol) in dioxane (50 ml) at 5–10°C. The reaction mixture was refluxed for 4 h and then evaporated under reduced pressure. The residue was recrystallized from a mixture of ethanol and acetone to yield **9a** (5.0 g, 89%), mp: 225–227°C. Anal calcd for $C_{12}H_7NO_3S$: C, 58.77; H, 2.88; N, 5.71; found: C, 59.04; H, 2.84; N, 5.73. Compound **9b** was prepared from **8b** in a similar manner to that described for **9a**; yield: 90%, mp: 204°C. Anal calcd for $C_6H_3NO_3S$: C, 42.60; H, 1.79; N, 8.28; found: C, 42.51; H, 1.85; N, 8.00.

*2-Ethoxycarbonylmethylamino-5-phenyl-thiophene-3-carboxamide **10a**.* A solution of **9a** (5.0 g, 4.1 mmol) in DMF (1 ml) was added dropwise to a suspension of NaH (60% in oil, 1.2 g) in DMF (40 ml) under ice-cooling. The reaction mixture was stirred for 0.5 h at rt and ethyl bromoacetate (4.0 g, 24 mmol) was then added to the mixture at 10°C. After the reaction mixture had been stirred for 2 h at rt, 28% aqueous ammonia (15 ml) was added dropwise at 5°C and stirring was continued for 0.5 h at same temperature. The reaction mixture was then poured into a mixture of ice and diluted HCl, and extracted with ethyl acetate (50 ml x 2). The organic layer was dried over sodium sulfate and evaporated under reduced pressure. The obtained residue was recrystallized from ethanol to yield **10g** (3.0 g, 48%), mp: 155–156°C. Anal calcd for $C_{15}H_{16}N_2O_3S$: C, 59.19; H, 5.30; N, 9.20; found: C, 59.28; H, 5.22; N, 9.10. Compound **10b** was prepared in a similar manner to that described for **10a;** yield: 44%, mp: 135–136°C. Anal calcd for $C_9H_{12}N_2O_3S$: C, 47.36; H, 5.30; N, 12.27; found: C, 47.28; H, 5.36; N, 12.27.

Ethyl 6-phenyl-2,4-dioxo-thieno[2,3-d]pyrimidin-1-acetate ***11a***. A mixture of **10a** (2.8 g, 9.2 mmol) and 1,1-carbonyldiimidazole (CDI) (3.1 g, 19.1 mmol) was heated to 120–130°C for 2 h. Dioxane (30 ml) was then added and the mixture refluxed for 1.5 h. After cooling, the reaction mixture was evaporated under reduced pressure and the residue was recrystallized from a mixture of ethanol and acetone to give **11a** (2.3 g, 76%), mp: 267°C. Anal calcd for $C_{16}H_{14}N_2O_4S$; C, 58.17; H, 4.27; N, 8.48; found: C, 58.24; H, 4.25; N, 8.76. Compound **11b** was prepared in a similar manner to that described for **11a**; yield: 80%, mp: 253–254°C. Anal calcd for $C_{10}H_{10}N_2O_4S$; C, 47.24; H, 3.96; N, 11.02; found: C, 47.38; H, 3.99; N, 10.98.

Ethyl 3-(4-bromo-2-fluorobenzyl)-2,4-dioxo-6-phenyl-thieno[2,3-d]pyrimidin-1-acetate ***6o***. A solution of **11a** (2.0 g, 6.1 mmol) in DMF (10 ml) was added dropwise to a suspension of NaH (60% in oil, 0.4 g) in DMF (40 ml) under ice-cooling. After stirring for 1 h at room temperature, the mixture was cooled to 5°C and a solution of 4-bromo-2-fluorobenzylbromide (1.9 g, 7.1 mmol) was added dropwise. The reaction mixture was then stirred for 16 h at rt, evaporated under reduced pressure, and poured into a mixture of ice and diluted HCl. The resulting precipitate was collected and recrystallized from dioxane to yield **6o** (2.2 g, 70%). Compound **6aa** was prepared in a similar manner to that described for **6o**. The physical data have been listed in table II.

3-(4-Bromo-2-fluorobenzyl)-2,4-dioxo-6-phenyl-thieno[2,3-d]pyrimidin-1-acetic acid ***2o***. The title compound was prepared from **6o** by a similar manner to that described for **2a**. Compound **2aa** was also prepared in a similar manner to that described for **2a**. The physical data have been listed in table I.

Method D

Ethyl 3-(4-bromo-2-fluorobenzyl)-6-chloro-2,4-dioxo-thieno[2,3-d]pyrimidin-1-acetate ***6r***. Sulfuryl chloride (0.41 ml, 5.0 mmol) was added dropwise to a solution of **6a** (2.2 g, 5.0 mmol) in CCl_4 (60 ml) at rt. The reaction mixture was stirred for 4 h at 60°C and evaporated under reduced pressure to give a crude oil which was dissolved in Et_2O and then triturated with *n*-hexane. The resulting precipitate was collected and recrystallized from isopropyl ether to give **6r** (2.1 g, 93%), mp: 133–134°C. ^{1}H-NMR($CDCl_3$): 1.21 (t, 3H), 4.26 (q, 2H), 4.59 (s, 2H), 5.21 (s, 2H), 7.20 (s, 2H) and 7.00–7.40 (m, 3H). Compounds **6q** and **6t** were prepared in a similar manner to that described for **6r**, while compounds **6s** and **6u** were prepared using NBS instead of SO_2Cl_2 and then worked up in a similar manner to that described for **6r**. Compounds **6ab** and **6bb** were prepared using CH_2Cl_2 instead of CCl_4 as a solvent and worked up in a similar manner to that described for **6r**. **6ab**: ^{1}H-NMR($CDCl_3$): 1.20 (t, 3H), 4.20 (q, 2H), 5.20 (s, 2H), 6.90–7.35 (m, 3H) and 7.50 (s, 1H). **6bb**: ^{1}H-NMR($CDCl_3$): 1.29 (t, 3H), 4.27 (q, 2H), 5.13 (s, 2H), 5.24 (s, 2H), 6.80–7.10 (m, 1H) and 8.14 (s, 1H). The physical data have been listed in table II.

3-(4-Bromo-2-fluorobenzyl)-6-chloro-2,4-dioxo-thieno[2,3-d]pyrimidin-1-acetic acid ***2q***. The title compound was prepared from **6r** in a similar manner to that described for **2a**. Compounds **2r–2t** were also prepared by a similar manner to that described for **2a**.

Method E

Ethyl 3-(4-bromo-2-fluorobenzyl)-6-bromomethyl-2,4-dioxo-thieno[2,3-d]pyrimidin-1-acetate ***12***. A mixture of **6h** (0.88 g, 1.9 mmol), NBS (0.38 g, 2.1 mmol) and benzoyl peroxide (75% wet with H_2O, 0.062 g) in CCl_4 (20 ml) was stirred for 6 h at 40°C. After cooling, the reaction mixture was filtered and evaporated under reduced pressure to give an oil which was purified by silica gel chromatography with a mixture of chloroform/ether (50:1) to yield **12** (0.35 g, 34%). ^{1}H-NMR ($CDCl_3$): 1.28 (t, 3H), 4.20 (q, 2H), 4.56 (s, 2H), 4.70 (s, 2H), 5.26 (s, 2H) and 6.89–7.43 (m, 4H).

Ethyl 3-(4-bromo-2-fluorobenzyl)-2,4-dioxo-6-(morphorin-1-yl)methyl-thieno[2,3-d]pyrimidin-1-acetate ***6za.*** A solution of morphorine (0.96 g, 11.2 mmol) in dioxane (5 ml) was added dropwise to a solution of **12** (2.0 g, 3.7 mmol) in dioxane (20 ml) under ice-cooling. The reaction mixture was stirred for 4 h at 80°C and evaporated under reduced pressure to give an oil which was extracted with chloroform (50 ml x 2), washed with water and dried over sodium sulfate. The organic layer was evaporated under reduced pressure and the residue was purified by silica gel chromatography with a mixture of chloroform/ether (4:1) to yield **6za** (0.84 g, 42%), mp: 178–180°C. Compounds **6zb–6zc** were prepared in a similar manner to that described for **6za**. The physical data have been listed in table II.

3-(4-Bromo-2-fluorobenzyl)-2,4-dioxo-6-(morphorin-1-yl)methyl-thieno[2,3-d]pyrimidin-1-acetic acid ***2ya***. The title compound was prepared from **6za** in a similar manner to that described for **2a**. The obtained crude product was recrystallized from ethanol to give **2ya**. Compounds **2yb** and **2yc** were also prepared in a similar manner to that described for **2a**. The physical data have been listed in table I.

Pharmacology

Inhibition of AR in vitro [14]

The preparation of AR and determination of IC_{50} activity were carried out as previously described [14]. AR activity was assayed by spectrophotometrically following the 340-nm oxidation of reduced nicotinamide adenine dinucleotide phosphate (NADPH) to NADP using DL-glyceraldehyde as substrate. The reaction mixture contained 0.1 M phosphate buffer (pH = 6.2), 0.25 mM NADPH, 1.5 mM DL-glyceraldehyde and the enzyme in a total vol of 1 ml. Inhibitor effects on enzymatic activity were determined by adding the inhibitors to the reaction mixture in the desired concentration. The % inhibition for each inhibitor was calculated by comparing the reaction rate of the solution containing the inhibitor with that of control. IC_{50} values were obtained by graphic estimation from the log concentration–response curves.

Inhibition of sorbitol accumulation in vivo [16, 17]

Six-wk-old male Wistar rats were rendered diabetic by an *iv* injection of streptozotocin (70 mg/kg body weight). One wk after injection, rats with plasma glucose levels > 400 mg/dl were grouped into either control or experimental groups, the latter being given a test compound (10 or 20 mg/kg) in suspension in 0.5% methyl cellulose orally once per d for 4 d. All rats were kept in identical cages and had free access to laboratory chow and water. Eighteen h after the final administration of the compound, all rats were anesthetized with ether and the sciatic nerves removed. Sorbitol was extracted from the sciatic nerve by the method of Peterson *et al* [16] and measured enzymatically by the method of Clements *et al* [17]. Sorbitol content was compared with that obtained for the control group given vehicle only.

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