SYNTHESIS AND ANTITUMOR ACTIVITY OF 2-S-SUBSTITUTED PYRIMIDINE DERIVATIVES

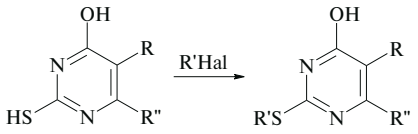
L. A. Grigoryan,[1] M. A. Kaldrikyan,[1] R. G. Melik-Ogandzhanyan,[1] F. G. Arsenyan,[1] G. M. Stepanyan,[1] and B. G. Garibdzhanyan[1]

Translated from Khimiko-Farmatsevticheskii Zhurnal, Vol. 39, No. 9, pp. 18 – 22, September, 2005.

Original article submitted January 27, 2004.

A series of new 2-S-substituted pyrimidine derivatives has been synthesized via the interaction of 2-mercaptopyrimidines with various alkylbromides, chloracetamide, and unsubstituted and substituted benzyl chlorides. The synthesized compounds have been tested with respect to antitumor activity.

Previously, we have synthesized a series of 2-alkylthio-4,5,6-substituted pyrimidines and studied their antitumor activity [1]. The interest in these thio derivatives of pyrimidine is related to their use as inert substituents [2, 3] and to the possibility of readily replacing the substituted groups [4 – 6]. In continuation of the search for new 5-substituted pyrimidines. possessing antitumor activity, we have synthesized a series of new compounds I – XLVIII.



I –XLVIII

By varying the functional groups in pyrimidine and benzene rings, it is possible to follow the effect of the substituents on the antitumor activity of the compounds.

The initial reagents for the synthesis of pyrimidines I – XLVII were thiobarbituric acid and 5-amyl, 5-isoamyl, 5-benzyl, 5-(4-alkoxybenzyl), 5-(3-methyl-4-alkoxybenzyl), and 5-(2-alkoxy-5-acetylbenzyl)pyrimidines [1, 7 – 11]. The alkylating agents were various alkyl bromides, chloracetamide, and unsubstituted and substituted benzyl chlorides. The reactions of these compounds with 2-mercaptopyrimidines in water or methanol in the presence of potassium hydroxide yielded the corresponding S-substituted derivatives I – XLVII. The alkylation of thiobarbituric acid proceeds with a rather low yield (42 – 46%) of target compounds (IX, XXII, XXXIV). In order to increase the product yield, we tried to carry out these reactions in aqueous ethanol solutions, but these attempts were unsuccessful. In order to avoid the formation of O- and N-substituted pyrimidine derivatives, the reactions of 2-mercaptopyrimidines with benzyl chloride were also performed under mild conditions (on standing for 30 – 40 h at room temperature). This led to a significant decrease in the product yield (down to 35%) and still did not exclude the formation of an insignificant amount of O- and N-substituted pyrimidine derivatives.

The purity of the synthesized compounds was checked by TLC; the proposed structures were confirmed by the results of elemental analyses and by the IR, ^{1}H NMR, and mass-spectroscopic data. The mass spectra of compounds II, VII, XI, XV, XXV, and XLIV measured under the conditions of dissociative ionization of molecular ions showed the presence of fragments such as R′, M^+–R′, (M^+–R′)–R, (M^+–R′)–$CH_2C_6H_4OR$, and some others, which was indicative of the splitting of the pyrimidine rings. The formation of such characteristic fragments (in addition to the molecular ions) helps establishing the molecular structures of the synthesized pyrimidines.

EXPERIMENTAL CHEMICAL PART

The TLC analyses were conducted on Silufol UV-254 plates; the spots were visualized by exposure to iodine vapor and under UV irradiation. The IR spectra were recorded on an IR-75 spectrophotometer (Germany). The ^{1}H NMR spectra were measured on a Varian Mercury 300 spectrometer at a working frequency of 300 MHz, using DMSO-d_6 as the solvent and TMS as the internal standard. The mass spectra were recorded on an MX-1321 spectrometer. The samples

[1] Mndzhoyan Institute of Fine Organic Chemistry, National Academy of Sciences of the Republic of Armenia, Yerevan, Armenia.

TABLE 1. Yields and Physicochemical Characteristics of Compounds I – XXXIV

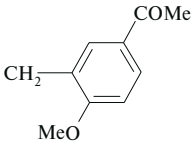
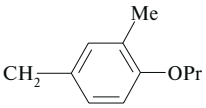
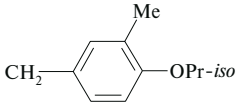
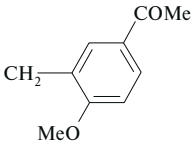
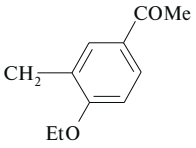
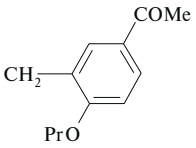
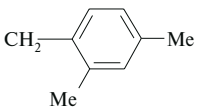
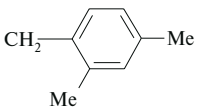
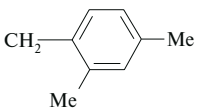
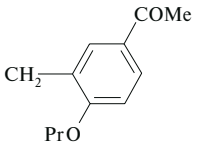
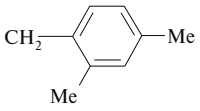
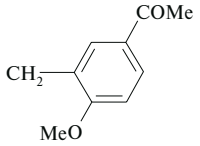
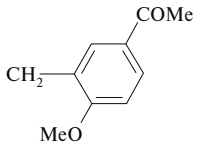
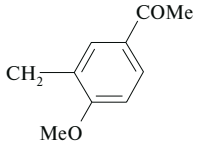
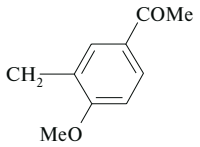
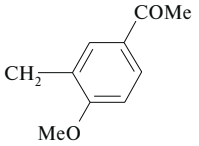
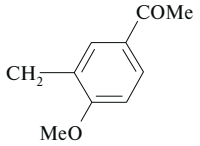
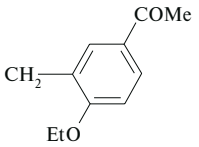
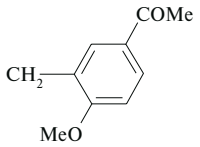
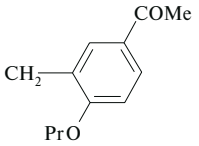
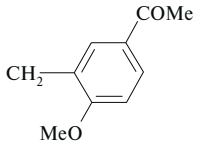
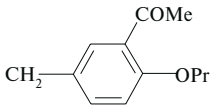
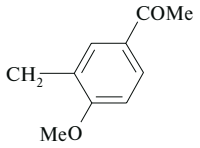
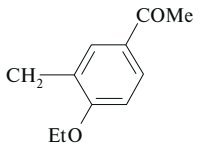
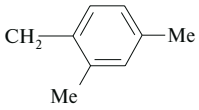
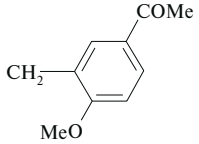
Compound	R	R′	Yield, %	M.p., °C	R_f	Empirical formula
I	CH_2Ph	Bu	53	268 – 270	0.61*	$C_{13}H_{14}N_2O_2S$
II	4-$CH_2C_6H_4O$-*iso*-Bu	Pr	54	188 – 190	0.63*	$C_{18}H_{24}N_2O_3S$
III	4-$CH_2C_6H_4O$-*iso*-Bu	*iso*-Pr	49	160 – 161	0.59*	$C_{18}H_{24}N_2O_3S$
IV	4-$CH_2C_6H_4O$-*iso*-Bu	*iso*-C_5H_{11}	56	248 – 250	0.60*	$C_{20}H_{28}N_2O_3S$
V	CH_2Ph	$CH_2CH{=}CH_2$	70	250 – 251	0.58*	$C_{14}H_{14}N_2O_2S$
VI	4-$CH_2C_6H_4OEt$	$CH_2CH{=}CH_2$	75	226 – 228	0.62*	$C_{16}H_{18}N_3O_3S$
VII	4-$CH_2C_6H_4OPr$	$CH_2CH{=}CH_2$	76	220 – 222	0.65*	$C_{17}H_{20}N_2O_3S$
VIII	4-$CH_2C_6H_4O$-*iso*-Bu	$CH_2CH{=}CH_2$	72	231 – 232	0.66*	$C_{18}H_{22}N_2O_3S$
IX	H	CH_2CONH_2	44	>300	0.53*	$C_6H_7N_3O_3S$
X	4-$CH_2C_6H_4OEt$	CH_2CONH_2	59	207 – 209	0.57*	$C_{15}H_{17}N_3O_4S$
XI		CH_2CONH_2	67	250 – 252	0.56*	$C_{16}H_{17}N_3O_5S$
XII	CH_2Ph	CH_2Ph	72	262 – 264	0.71**	$C_{18}H_{16}N_2O_2S$
XIII	4-$CH_2C_6H_4OMe$	CH_2Ph	69	246 – 247	0.69**	$C_{19}H_{18}N_2O_3S$
XIV	4-$CH_2C_6H_4OEt$	CH_2Ph	67	274 – 275	0.70**	$C_{20}H_{20}N_2O_3S$
XV	4-$CH_2C_6H_4OPr$	CH_2Ph	66	263 – 264	0.75**	$C_{21}H_{22}N_2O_3S$
XVI	4-$CH_2C_6H_4O$-*iso*-Bu	CH_2Ph	64	270 – 272	0.62**	$C_{22}H_{24}N_2O_3S$
XVII		CH_2Ph	65	244 – 245	0.63**	$C_{22}H_{24}N_2O_3S$
XVIII		CH_2Ph	75	224 – 226	0.61**	$C_{22}H_{24}N_2O_3S$
XIX		CH_2Ph	73	266 – 267	0.58**	$C_{21}H_{20}N_2O_4S$
XX		CH_2Ph	70	252 – 253	0.60**	$C_{22}H_{22}N_2O_4S$
XXI		CH_2Ph	82	216 – 218	0.75**	$C_{23}H_{24}N_2O_4S$
XXII	H		46	>300	0.58**	$C_{13}H_{14}N_2O_2S$
XXIII	CH_2Ph		73	110 – 111	0.64***	$C_{20}H_{20}N_2O_2S$
XXIV	4-$CH_2C_6H_4O$-*iso*-Bu		58	227 – 228	0.67***	$C_{24}H_{28}N_2O_3S$

TABLE 1 (Continued)

Compound	R	R′	Yield, %	M.p., °C	R_f	Empirical formula
XXV			65	178 – 179	0.68**	$C_{25}H_{28}N_2O_4S$
XXVI	CH_2Ph		68	150 – 152	0.72*	$C_{21}H_{20}N_2O_4S$
XXVII	4-$CH_2C_6H_4OEt$		53	210 – 211	0.57*	$C_{23}H_{24}N_2O_5S$
XXVIII	4-$CH_2C_6H_4OPr$		55	179 – 181	0.58*	$C_{24}H_{26}N_2O_5S$
XXIX	4-$CH_2C_6H_4O$-*iso*-Bu		65	197 – 198	0.59*	$C_{25}H_{28}N_2O_5S$
XXX			85	116 – 118	0.61**	$C_{24}H_{24}N_2O_6S$
XXXI			81	267 – 268	0.58**	$C_{25}H_{26}N_2O_6S$
XXXII			83	124 – 126	0.63**	$C_{26}H_{28}N_2O_6S$
XXXIII			78	198 – 199	0.60**	$C_{25}H_{27}N_2O_5S$
XXXIV	H		42	>300	0.49**	$C_{15}H_{16}N_2O_4S$

Notes: Benzene – acetone * 2 : 1; ** 1 : 1; *** 1 : 2.

TABLE 2. Yields and Physicochemical Characteristics of Compounds XXXV – XLVII

Compound	R	R′	Yield, %	M.p., °C	R_f	Empirical formula
XXXV	*iso*-C_5H_{11}	CH_2-CH=CH_2	65	98 – 100	0.62*	$C_{13}H_{20}N_2OS$
XXXVI	C_5H_{11}	CH_2CONH_2	45	188 – 190	0.51***	$C_{12}H_{19}N_2O_2S$
XXXVII	*iso*-C_5H_{11}	CH_2CONH_2	48	198 – 200	0.52**	$C_{12}H_{19}N_2O_2S$
XXXVIII	4-$CH_2C_6H_4OC_3H_{iso}$-C_3H_7	CH_2CONH_2	79	150 – 152	0.55**	$C_{17}H_{21}N_3O_3S$
XXXIX	4-$CH_2C_6H_4OCH_3$	CH_2Ph	60	183 – 184	0.64**	$C_{20}H_{20}N_2O_2S$
XL	4-$CH_2C_6H_4OC_2H_5$	CH_2Ph	62	178 – 179	0.58**	$C_{21}H_{22}N_2O_2S$
XLI	4-$CH_2C_6H_4OC_3H_{iso}$-C_3H_7	CH_2Ph	63	162 – 164	0.63**	$C_{22}H_{24}N_2O_2S$
XLII	4-$CH_2C_6H_4O$-*iso*-C_4H_9	CH_2Ph	80	171 – 172	0.65**	$C_{23}H_{26}N_2O_2S$
XLIII	4-$CH_2C_6H_4OCH_3$		63	172 – 173	0.58**	$C_{22}H_{24}N_2O_2S$
XLIV	4-$CH_2C_6H_4O$-*iso*-C_4H_9	–"–	81	145 – 146	0.55**	$C_{25}H_{30}N_2O_2S$
XLV	*iso*-C_5H_{11}		55	139 – 140	0.68*	$C_{20}H_{26}N_2O_3S$
XLVI	4-$CH_2C_6H_4OCH_3$	–"–	81	178 – 179	0.57**	$C_{23}H_{24}N_2O_4S$
XLVII	4-$CH_2C_6H_4O$-*iso*-C_4H_9	–"–	66	198 – 199	0.62**	$C_{26}H_{30}N_2O_4S$

Notes: Benzene – acetone * 2 : 1; ** 1 : 1; *** 1 : 2.

were introduced directly into the ionization chamber at a temperature 40 – 50°C below the melting point of the corresponding substance and ionized at an electron impact energy of 60 eV.

Alkylthiobarbituric acids (IX, XXII, XXXIV). A mixture of 1.68 g (0.03 mole) of potassium hydroxide, 25 ml of water, 1.44 g (0.01 mole) of 2-mercaptobarbituric acid, and 0.011 mole of chloracetamide or the corresponding substituted benzyl chloride was boiled for 1.5 – 2 h, cooled, and extracted with benzene. The aqueous layer was filtered and acidified with hydrochloric acid to pH 2 – 3. The precipitate was doubly boiled in water and filtered hot (Table 1).

The IR spectra of compounds IX, XXII, and XXXIV contain no absorption bands at 2590 – 2550 cm^{-1} characteristic of the stretching vibrations of SH groups.

2-Alkyl, 2-allyl, 2-acetylamido, 2-benzyl, and 2-substituted benzylthiopyrimidines (I – VIII, X – XXI, XXIII — XXXIII, XXXV – XLVII). To a solution of 0.28 g (0.005 mole) of potassium hydroxide in 30 ml of methanol was added 0.005 mole of 2-mercaptopyrimidine and the mixture was stirred until complete dissolution. To this solution was added 0.0055 mole of the corresponding bromide or alkylchloride and the mixture was heated on a water bath for 30 min, cooled, and diluted with 70 ml water. The precipitated crystals were filtered, washed with water, dried, and purified either by boiling in ethanol or by recrystallization from dioxane (Tables 1 and 2).

EXPERIMENTAL BIOLOGICAL PART

The antitumor activity of the synthesized compounds was studied by conventional methods [12, 13] using rats and mice inoculated with sarcomas 45 and 37. In view of poor solubility in water, all the tested compounds were intraperitoneally injected as suspensions in 0.5% carboxymethylcellulose solution. The injections were made in a single daily dose over a period of eight (rats) or six (mice) days. The therapeutic effect was assessed the next day after the last drug injection and evaluated as percentage tumor growth inhibition (TGI) relative to that in the untreated control. The experimental data were statistically processed using the Student – Fisher method. The differences from untreated control were considered as statistically significant for $p \le 0.05$.

It was established that all derivatives of 2-acetamidothio-6-oxypyrimidines (X, XI, XXXVII, XXXVIII) exhibited a weak antitumor activity with TGI = 40 – 47% ($p \le 0.05$). Among the analogs with 2-methoxy-5-acetylbenzylthio radicals, only the derivatives with 5-(4-methoxybenzyl) and 5-benzyl fragments (XXVI, XLVI) produced reliable therapeutic action (TGI = 40 – 46%, $p \le 0.05$). A comparable antitumor activity was observed for benzylthiopyrimidines with 5-(4-methoxybenzyl) (XIII), 5-(4-isobutoxybenzyl) (XVI), and 5-(4-isopropoxybenzyl) (XLI) radicals (TGI = 43 – 47%, $p \le 0.05$) and the analogs of

TABLE 3. ^{1}H NMR Spectra of the Synthesized Compounds (Chemical Shifts δ, ppm)

Compound	CH_3	CH_2	OCH_2	OCH_3 (s)	SCH_2 (s)	Ar (m)	OH (bs)	$COCH_3$	CH (m)	$=CH_2$	CH=	NH_2 (bs)
II	0.9 s; 1.07 s	1.4 q; 3.58 s	4.0 d	–	4.2	6.9 – 7.3	11.3	–	2.0	–	–	–
VIII	1.00 s	3.45 s	3.65 s	–	3.75	6.6 – 7.2	11.3	–	2.05	5.25 m	5.9 m	–
X	1.35 t	3.5 s	3.9 q	–	4.0	6.7 – 7.2	11.7	–	–	–	–	6.8
XVI	1.05 s	3.65 s	3.5 s	–	4.36	6.7 – 7.1	10.4	–	2.05	–	–	–
XVIII	1.3 s; 2.1 s	3.5 s	–	–	4.4	6.8 – 7.4	11.0	–	4.42	–	–	–
XIX	–	3.6 s	–	3.9	4.2	6.9 – 7.8	11.2	2.45 s	–	–	–	–
XII	–	3.6 s	–	–	4.4	7 – 7.4	11.4	–	–	–	–	–
XX	1.0 t	3.7 s	4.0 q	–	4.3	7.2 – 7.9	11.3	2.40 s	–	–	–	–
XXI	1.1 t	1.9 q; 3.6 s	4.05 t	–	4.4	6.8 – 7.78	11.4	2.45 s	–	–	–	–
XXV	1.05 t; 2.3 m	1.9 q; 4.05 d	3.6 t	–	4.35	6.9 – 7.8	10.7	–	–	–	–	–
XXVI	–	3.6 s	3.95 s	–	4.4	7.0 – 8.3	11.3	2.5 s	–	–	–	–
XXIX	1.05 s	3.5 s	3.66 s	3.97	3.76	6.6 – 8.2	11.45	2.4 s	2.0	–	–	–
XXXI	1.45 t	3.6 s	4.2 t	4.0	4.4	6.8 – 8.2	12.0	2.4 s	–	–	–	–
XXXII	1.1 t	1.9 q	4.1 t	4.0	4.2	6.9 – 8.2	11.5	4.5	–	–	–	–
XXXIII	1.2 t; 3.8 s	1.9 q; 3.5 s	3.8 t	3.9	4.4	6.6 – 8.2	11.5	2.1 s	–	–	–	–
XXXIV	1.3 t	–	3.8 q	–	3.5	7.0 – 7.3	11.3	2.5 s	–	–	7.5 s	–
XXXVII	1.3 s; 2.2 s	3.65 s	–	–	3.7	6.8 – 7.4	12.5	–	4.5	–	–	6.7
XXXIX	2.2 s	3.6 s	–	3.75	4.0	6.7 – 7.5	12.2	–	–	–	–	–
XL	1.4 t; 2.2 s	3.7 s	3.9 q	–	4.3	6.7 – 7.4	12.4	–	–	–	–	–
XLIII	2.1 m	3.7 s	–	3.8	4.2 s	6.7 – 7.2	12.2	–	–	–	–	–
XLVI	2.5 s	4.25 s	–	3.97; 3.7	3.65	6.65 – 8.1	12.2	2.3	–	–	–	–

2-alkylthio-4,6-dioxopyrimidines with ethyl (I), propyl (II), and isopropyl (III) radicals (TGI = 38 – 42%, $p \le 0.05$). The derivatives of benzylpyrimidine with 2-allylthio radicals (V, VI, VIII) did not exhibit reliable antitumor activity on the models studied.

A comparative analysis of the therapeutic action of 2-S-substituted pyrimidines did not reveal any significant correlation between the chemical structure of the derivatives and their antitumor activity.

REFERENCES

1. A. A. Aroyan, M. A. Kaldrikyan, and L. A. Grigoryan, *Arm. Khim. Zh*., **22**(4), 341 – 345 (1969).
2. H. C. Koppel, R. H. Springer, C. C. Chong, and R. K. Robins, *J. Org. Chem*., **27**, 181 – 184 (1962).
3. A. A. Aroyan, M. A. Kaldrikyan, and L. A. Grigoryan, *Arm. Khim. Zh*., **22**(5), 401 – 405 (1969); **32**(12), 1020 – 1025 (1973).
4. R. Hull, B. J. Lovele, H. T. Openshauw, and A. R. Todd, *J. Chem. Soc*., 41 – 44 (1947); F. H. S. Curd and F. L. Rose, *J. Chem*., 343 – 345 (1946); Swiss Patent No. 345, **426** (1962) [*Chem. Abstr*., **58**, 3443e (1963)].
5. M. A. Kaldrikyan, L. A. Grigoryan, and A. A. Aroyan, *Arm. Khim. Zh*., **25**(8), 678 – 682 (1972).
6. J. G. Naim and H. Tiekelmann, *J. Org. Chem*., **25**, 1127 – 1129 (1960).
7. A. A. Aroyan, M. A. Kaldrikyan, and R. G. Melik-Ogandzhanyan, *Arm. Khim. Zh*., **20**(1), 61 – 67 (1967).
8. L. A. Grigoryan, M. A. Kaldrikyan, and A. A. Aroyan, *Arm. Khim. Zh*., **27**(11), 969 – 973 (1974).
9. A. A. Aroyan and M. S. Kramer, *Arm. Khim. Zh*., **20**(3), 218 – 225 (1967).
10. M. A. Kaldrikyan, A. V. Khekoyan, B. T. Garibdzhanyan, and G. M. Stepanyan, *Arm. Khim. Zh*., **34**(4), 318 – 323 (1981).
11. M. A. Kaldrikyan, L. A. Grigoryan, V. A. Gaboyan, et al., *Khim.-Farm. Zh*., **34**(10), 9 – 12 (2000).
12. G. N. Pershin, *Methods of Experimental Chemotherapy* [in Russian], Medgiz, Moscow (1971).
13. Z. P. Sof'ina, A. B. Syrkin, et al. (eds.), *Experimental Evaluation of Antitumor Drugs in the USSR and USA* [in Russian], Meditsina, Moscow (1980).