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Pyrimidinones as reversible metaphase arresting agents

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Summary — 5-Halo-*N*(1)-substituted 2(1*H*)-pyrimidinones have the ability to cause reversible arrest of mitosis during metaphase. Highly active compounds have a heteroatom (*O*, *S* or *N*) in the β-position of the *N*(1)-carbon chain which is further substituted by an aryl group. *In vitro* data have been provided. It is suggested that reversible metaphase inhibitors can be used as synchronizing agents of cell-cycles by applying them in a sequential manner when a phase-specific cytotoxic drug is used in the treatment of diseases caused by uncontrolled rapidly proliferating cells. The active compounds are prepared from 2-pyrimidinones by alkylation reactions. The key reactants are α-chloroalkyl ethers, sulfides and amides; methods for their syntheses have been described.

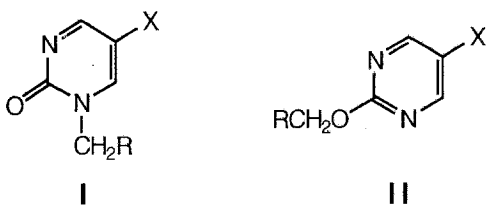
5-chloro-2-pyrimidinones / metaphase / reversible inhibition / cell-cycle synchronization

Introduction

It has been found that certain *N*-1 substituted 2-pyrimidinones **I** will arrest the cell-cycle of mouse and human cells grown in cultures [1, 2]. The *O*-substituted isomers **II** are inactive.The arrest is in the relatively narrow metaphase region. Compounds have been found where the metaphase arrest is reversed when the active compound is removed, and the cells suffer no damage provided that the time of arrest is not longer than the cell-cycling time [1]. Cycling cells of different origin will all be arrested in metaphase. With a rapid release of the block, parasynchronous resumption of the cell-cycle leads to separation of cells into groups due to kinetic differences between the different types of cells. We think that this principle can be applied to achieve differential synchronisation of cycling cells from normal and abnormal tissue in diseases caused by rapidly proliferating cells. Thus sequential timed treatment with a phase-specific cytotoxic drug may be used to increase the kill of abnormal cells after pretreatment with a synchronising agent. Due to the heterogeneity of the abnormal cells, however, it may be easier to spare vital and sensitive cells, such as bone-marrow cells, from destruction. This we hope to achieve by synchronization of their cell cycles so that the bone-marrow cells are largely kept out of the sensitive phase at the time when the phase-specific drug is active against the abnormal cells which are in the sensitive phase.

For the rapid release of the cells, when the arrest is to be lifted we rely on metabolism. The most important reaction seems to be enzymatic oxidation of the 2-pyrimidinone in the 4-position to form the corresponding 1-substituted uracil (Hatlelid *et al*, unpublished results).

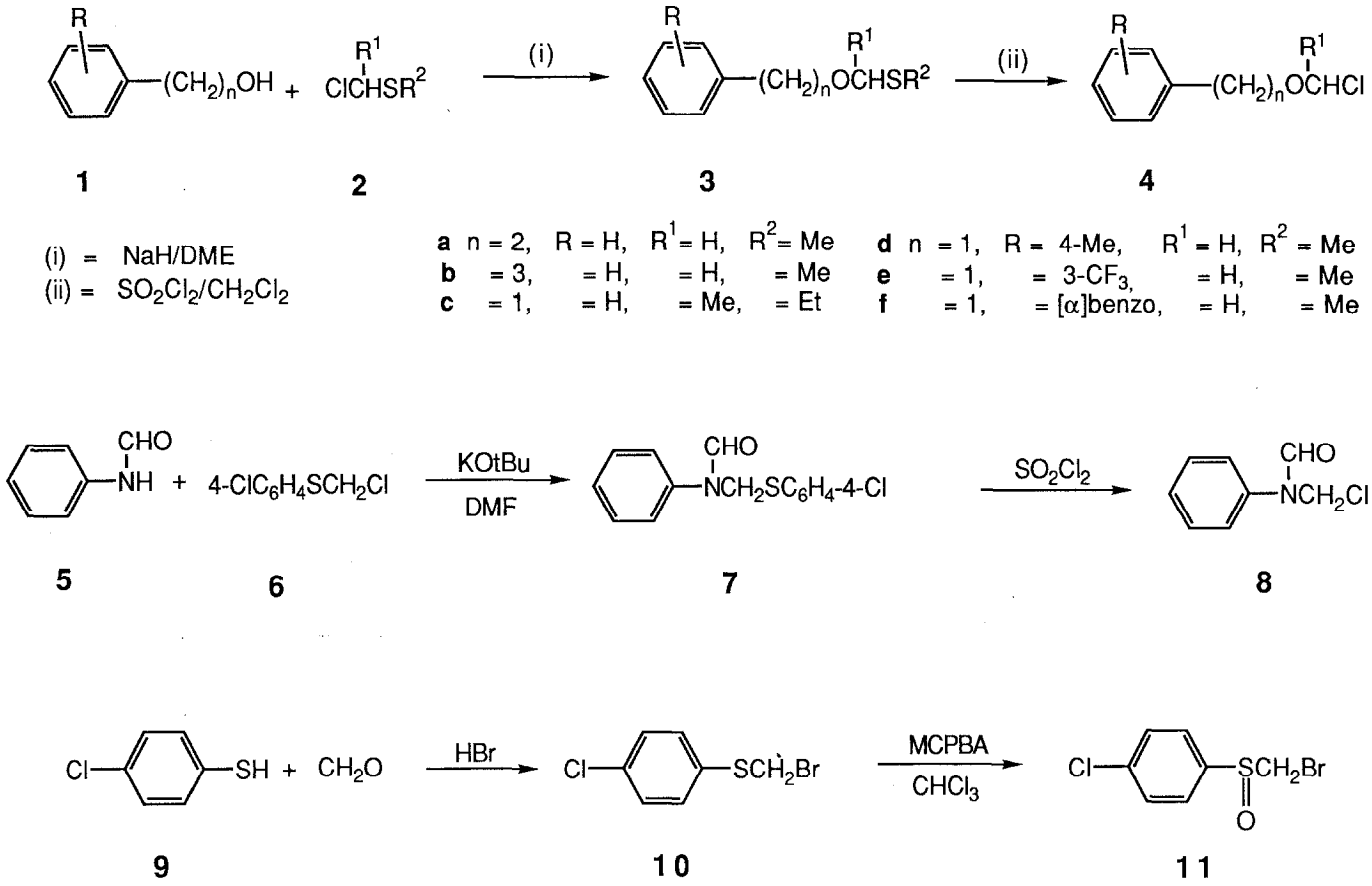
The pyrimidinones **I** are highly polarized and form adducts in the 4(6)-position [3, 4]. The less polarized 2-alkoxy or aryloxy-pyrimidines form adducts less readily and are not active as metaphase arrestors. Adduct formation in the 2-pyrimidinone is dependent on polarization in the ring and can be controlled by the nature of the 5-substituent. It appears that electronic effects, albeit weak, from the 1-substituent may also be transmitted into the ring which has been



I **II**

R = Alk, Alkenyl, Alkynyl, Ar
X = F, Cl, Br, I

Scheme 1.

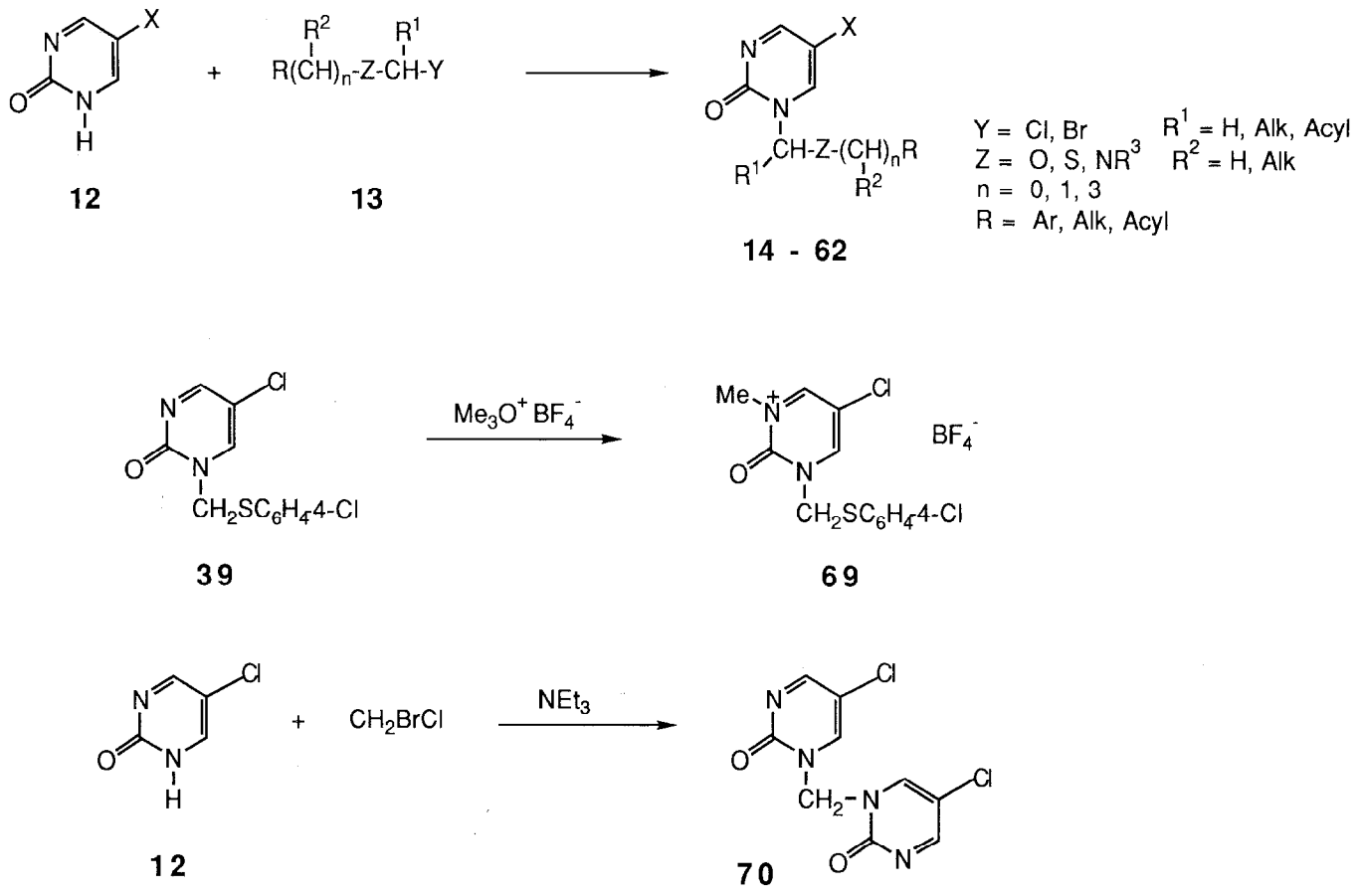


Scheme 2.

demonstrated in adduct-forming reactions between thiols and 2-pyrimidinones as well as in the irreversible carbon–carbon bond formation between the latter and organometallics [3, 4].

Chemistry

In the synthesis of the metaphase arresting pyrimidinones (scheme 3) the substituent in the 1-position is introduced by an alkylation reaction of the 2(1*H*)-pyrimidinone using an α-haloalkyl ether, sulfide or amide. The preparation of the intermediate alkylating agents is shown in scheme 2. For the synthesis of α-haloalkyl ethers we have described a convenient and general method which gives access to α-haloalkyl ethers from alcohols, including unsaturated alcohols and phenolic derivatives, even when these carry electron-donating substituents [5, 6]. The phenol or alcohol is initially alkylated by an α-haloalkyl sulfide and the resultant *O,S*-acetal is cleaved by sulfuryl chloride or bromine to furnish the corresponding α-chloro- or bromoalkyl ether. Alternatively, the sulfide is oxidized to its sulfoxide which is cleaved to the α-chloroalkyl ether by acetyl chloride as the reagent of choice [7]. A sulfenyl halide is the other product from the cleavage reaction of the *O,S*-acetal, and this may be trapped as an adduct with cyclohexene to avoid side-reactions in an unsaturated alkyl derivative or in an electron-rich aromatic derivative. Methylthio derivatives are often used for the chlorination reactions because methanesulfenyl chloride is volatile and can be removed by distillation from the less volatile chloroalkyl ether. In the present work chloromethyl methyl sulfide or α-chloroethyl ethyl sulfide was used for the alkylation of the alcohol **1** and the resultant *O,S*-acetal **3** was cleaved to the α-chloroalkyl ether **4** by sulfuryl chloride. The reactive α-haloalkyl ether **4** may, in simple cases, be isolated by distillation, but the crude α-haloalkyl ether is often used directly without further purification in the alkylation reactions of the pyrimidine.



Scheme 3.

N-Chloromethylation of amides can also be achieved by a similar technique. The amide **5** was initially metallated and reacted with chloromethyl 4-chlorophenyl sulfide. Sulfuryl chloride treatment of the product **7** gave the *N*-chloromethylamide **8**. Cyclohexene was used as trapping agent.

The α-haloalkyl sulfide reactants are available by direct haloalkylation reactions on the thiol or by α-halogenation of sulfides [8, 9]. The bromomethyl sulfide **10** was chemoselectively oxidized by *m*-chloroperbenzoic acid to the corresponding sulfoxide **11** which was used for the alkylation reactions.

The 2-pyrimidinone **12** is metallated before alkylation. The anion is ambident and may react either on the nuclear nitrogen or on the oxygen. With the relatively hard α-haloalkyl ether electrophile [10], significant *O*-alkylation takes place besides the desired *N*-alkylation.The *O*-alkylated product is more lipophilic than the *N*-alkylated product, and was normally removed by extraction of the reaction products by diethyl ether. Alternatively, the isomers were separated by chromatography.

The *N*(3)-nitrogen in the pyrimidinones is of low nucleophilicity. No reaction took place between the pyrimidine **39** and methyl iodide on heating. Trimethyloxonium fluoroborate, a strong methylating agent, gave the desired alkylation and formation of the pyrimidinium derivative **69**.

Results and discussion

The metaphase arrestors have been designed to exert their metaphase arresting effect through reversible adduct formation with thiol functions in proteins such as tubulin, which is the microtubular protein vital for the formation of the spindle apparatus. When the essential thiol groups in tubulin are blocked, mitosis is temporarily stopped, and is only continued when the dissociated pyrimidinone is removed by metabolism.

Thiol groups are more strongly bound in the adduct form than hydroxyl derivatives and amines.

It is important to control the π-electron deficiency of the pyrimidine ring by suitable substitution because the π-electron deficiency will determine the position of the equilibrium between the free heterocycle and its adducts. When the π-electron deficiency is high, the equilibrium is in favor of the adduct form which means that such molecules are toxic to the cell and may have potential use as a phase-specific cytotoxic drug.

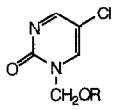
The Chang line of human liver cells was used in testing activity [11]. Each test substance was dissolved in the culture medium at the highest concentration used. In each dilution step the concentration of the test compound was halved. Some of the strongest hydrophobic substances were difficult to dissolve in the medium. These were dissolved in dimethylformamide (DMF) before medium was added. The concentration of DMF was below the concentration toxic to the cells. The metaphase arresting activity was accessed 6 h after addition of the pyrimidinone to monolayer cultures of the Chang strain of human liver cells. The minimum inhibitory concentration refers to complete accumulation of the cells in metaphase with no ana- or telophases.

It is an important requirement that neither the metaphase arrester nor its metabolites are cytotoxic to the bone marrow cell. Information on any hidden effects in reversibly arrested cells in our tests was sought by replating of cells after the reversible arrest. The daughter cells were studied for 4–5 generations after arrest, and in several cases no visible damage to the daughter cells could be detected.

The pyrimidinones can also be regarded as prodrugs for uracil derivatives, such as the family of 5-fluorouracils used in cancer chemotherapy. We therefore included only a limited number of 5-fluoropyrimidinones in our study. In the *in vitro* test for metaphase arrest the 5-chloropyrimidinones were at least as active, and in most cases more active than the corresponding fluoro analogues *(vide infra)*. Without a halogen in the 5-position, the pyrimidinones were inactive in the concentration at which the testing was started.

Saturated aliphatic derivatives of the pyrimidinone **I** in scheme 1 (*eg* R = Pr, X = Cl) cause complete accumulation of the cells in metaphase at 3.0 mM concentration. Activity is generally increased in unsaturated derivatives; by 4 dilution steps, down to 0.18 mM in the propargyl analogue, but not significantly in the allyl analogue [1]. An aryl group in the 1-alkyl substituent potentiates the activity; the 1-benzyl derivative **I** (R = Ph, X = Cl) is active at 0.25 mM [1]. The activity in aralkyl derivatives is further improved when the β-carbon in the 1-alkyl

Table I. Minimum inhibitory concentration (mM) for complete metaphase arrest of Chang liver cells.



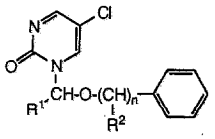
Compound	*R*	*mM*
14	Et	0.125
15 [15]	$CH_2C{\equiv}CH$	0.125
16	CO-*t*-Bu	0.250

substituent is replaced by a carbonyl group such as in phenacyl derivatives [12], or by insertion of a heteroatom [1, 13].

The 1-butyl derivative **I** (R = Pr, X = Cl; scheme 1) shows full arrest in metaphase at 3 mM concentration. The corresponding value for the bioisosteric β-oxa analogue, the ethoxy derivative **14**, was 0.125 mM (table I), which corresponds to 4 dilution steps, increased activity. The propargyl derivative **15** was active at the same concentration level, whereas the pivaloyl ester derivative **16** was 1 dilution step behind.

The activity value of 0.06 mM for the 1-phenoxymethyl derivative **17** is to be compared with 0.5 mM for its 2'-phenylethyl analogue **I** (R = CH_2Ph, X = Cl) which means that bioisosteric substitution by oxygen

Table II. Minimum inhibitory concentration (mM) for complete metaphase arrest of Chang liver cells.



Compound	*n*	R_1	R_2	*mM*
17 [14]	0	H-	–	0.060
18 [3, 16]	1	H	H	0.060
19	2	H	H	0.125
20	3	H	H	0.060
21 [14]	0	Me	–	0.060
22	1	Me	H	0.060
23	1	H	Me	0.060
24* [14]	0	PhCO	–	> 0.5

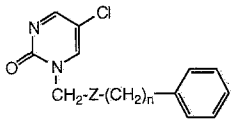
*Substituted in the *para* position by a chlorine.

increased the activity by 4 dilution steps. The length of the alkyl chain between the oxygen and the phenyl ring from 1 to 3 carbon atoms (**17**, **18**, **19**) had little importance. Methyl substitution on either side of the oxygen in the *N*(1)-chain, on the α-carbon (**21**, **22**) or on the γ-carbon (**23**), had no effect on metaphase-arresting activity. A benzoyl group on the α-carbon (**24**) gave a compound which was inactive at 0.5 mM, the highest concentration tested. The low activity was rationalized as due to enolization in **24** towards the α-carbon and subsequent addition of the enolic oxygen into the electrophilic 6-position in the pyrimidine ring. The resultant dihydropyrimidine lost the ability to form adducts with thiols and hence was inactive as a metaphase inhibitor. Compound **24** can also be considered as a phenacyl derivative substituted in the α-position by an aryloxy group. The simple phenacyl derivative (**I** R = COPh) was active at the same level as **17**. These compounds exist in the oxo-form. It seems likely that ether oxygen facilitates enolization. Previously we found that activity was lost in the homologous benzyl ketone (**I** R = $COCH_2Ph$) because enolization towards the benzyl methylene group gave an enol which formed a cyclic adduct with the heterocycle in a similar way, and in this form the compound was inactive as a metaphase inhibitor [1].

Comparison of the activity data for the oxygen and sulfur analogues **17** (table II) and **25** (table III), and the nitrogen analogue **28** (or **29**, **30**) shows that the nature of the β-heteroatom in the 1-substituent appears to have little influence on metaphase inhibitory properties. There was at least a 2-fold reduction in activity after oxidation of the sulfide **39** (table IV) to its sulfoxide **27**. By analogy to the oxygen series, insertion of a methylene carbon between the phenyl group and the sulfur (**26**) or the nitrogen (**31**) gave compounds with the same activity as found for the corresponding phenyl derivatives **25** and **30**. Studies on the reversibility of the metaphase arrest, however, have shown that the sulfur compounds in general are more toxic and may act as enzyme inhibitors for enzymatic oxidation of the pyrimidinone in the 4-position (Hatlelid *et al*, unpublished results). All the amines are acylated. This is because earlier work has shown that simple, basic amino derivatives gave compounds of low activity [1].

The nature of the substituents in the phenyl ring in either the phenyl or benzyl series has little effect on metaphase inhibiting activity. The effect appears to be independent of the heteroatom, and there is no clear difference between the influence from electron donating and electron withdrawing substituents. Exchange of the phenyl ring with the more hydrophobic naphthyl ring gave more active compounds (**44**, **45**), but the naphthyl derivatives suffer from very low water solubility. The activity data for the heterocyclic thienyl (**46**), furyl (**47**) and pyrimidinyl (**48**) analogues are comparable with the data for phenyl derivatives.

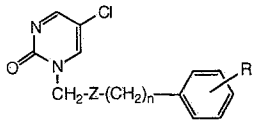
Table III. Minimum inhibitory concentration (mM) for complete metaphase arrest of Chang liver cells.



Compound	*n*	*Z*	*mM*
25 [a][16]	0	S	0.060
26	1	S	0.060
27 [b]	0	SO	>0.125[c]
28	0	NCHO	0.125
29	0	NCO_2Me	0.125
30	0	NCO_2Et	0.060
31 [3]	1	NCO_2Et	0.060

[a]Substituted in the *meta* position by a methyl group; [b]substituted in the *para* position by a chlorine; [c]the compound was not tested at higher concentrations.

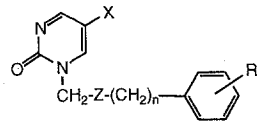
Table IV. Minimum inhibitory concentration (mM) for complete metaphase arrest of Chang liver cells.



Compound	*n*	*Z*	*R*	*mM*
32 [14]	0	*O*	4-Me	0.060
33	1	*O*	4-Me	0.060
34	1	*O*	2-Me	0.030
35 [17]	0	*S*	3-Me	0.060
36	1	*O*	4-OMe	0.030
37 [17]	0	*S*	4-OMe	0.060
38 [14]	0	*O*	4-Cl	0.125
39 [17]	0	*S*	4-Cl	0.125
40 [17]	0	*S*	4-F	0.060
41	1	*O*	3-CF_3	0.060
42 [3]	0	*O*	4-CHO	0.060
43 [3]	0	*O*	4-COMe	0.060
44	1	*O*	1-Naphthyl[a]	0.016
45 [14]	0	*O*	2-Naphthyl[a]	0.030
46	1	*O*	2-Thienyl[a]	0.030
47	1	*O*	2-Furyl[a]	0.060
48 [17]	0	*S*	5-Cl-Pyrimidin-2-yl[a]	0.060

[a]The phenyl ring is exchanged with the naphthyl, thienyl, furyl or pyrimidinyl ring as shown.

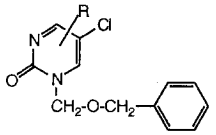
Table V. Minimum inhibitory concentration (mM) for complete metaphase arrest of Chang liver cells.



Compound	*X*	*n*	*Z*	*R*	*mM*
49	F	0	*S*	3-Me	0.060
50 [17]	Cl	0	*S*	3-Me	0.060
51 [17]	Br	0	*S*	3-Me	0.125
52 [16]	CF_3	0	*O*	4-Cl	0.060
53 [14]	Cl	0	*O*	4-Cl	0.125
54 [14]	Br	0	*O*	4-Cl	0.125
55 [14]	I	0	*O*	4-Cl	0.250
56 [16]	CF_3	1	*O*	4-Cl	0.125
57 [3]	Cl	1	*O*	4-Cl	0.030
58	I	1	*O*	4-Cl	0.125
59 [18]	CF_3	0	*O*	4-CHO	0.060
60 [3]	Cl	0	*O*	4-CHO	0.060
61 [18]	CF_3	0	*O*	4-COMe	0.125
62	Cl	0	*O*	4-COMe	0.060

Three series, **49–51**, **52–55** and **56–58** are shown in table V where the halogen substituent in the 5-position has been varied. The activity figures for the fluoro and chloro derivatives **49** and **50** in the sulfur series are the same. In our work we have seen several cases where the activity figures for chloro and fluoro analogues are the same, but also cases where the chloro derivative is the more active compound. Bromo derivatives are generally slightly less active, and activity is further decreased for iodo derivatives in homologous series. The lower activity in bromo and iodo derivatives may be caused by the size of the

Table VI. Minimum inhibitory concentration (mM) for complete metaphase arrest of Chang liver cells.

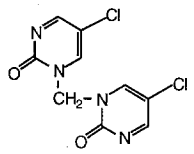


Compound	*R*	*mM*
63 [19]	4-Ph	0.125
64[a]	6-Ph	> 0.250
65 [19]	4-(4-ClC_6H_4)	0.125
66 [19]	4-(4-$MeOC_6H_4$)	0.250
67[a]	4-(2-Thienyl)	> 0.250
68[a]	6-(2-Thienyl)	> 0.250

[a]Rise F, Undheim K; unpublished results.

Table VII. Minimum inhibitory concentration (mM) for complete metaphase arrest of Chang liver cells.

69



70

Compound	*mM*
69	0.250
70	0.500

halogen substituent and non-bonded interaction with the incoming group for adduct formation in the pyrimidine 4-position. Also important is the progressively lower electronegativity from fluorine to iodine which leads to lower electrophilic activation in the 4-position and hence reduced reactivity. The trifluoro group is a strongly electron-withdrawing group and this may adversely affect the reversibility of metaphase inhibition. In general the most interesting compounds are the 5-fluoro and 5-chloro derivatives. The reversibility of the metaphase block which involves enzymatic oxidation in the pyrimidine 4-position leads to the formation of corresponding 5-substituted uracils. Since 5-fluorouracils are cytotoxic agents used in cancer chemotherapy, we have mainly concentrated our study on 5-chloropyrimidinones.

The metaphase arresting activity has been explained as due to reversible adduct formation in the pyrimidine 4-position. The compounds **63**, **65** and **66** in which the 4-position in the parent compound **18** has been blocked by phenyl, 4-chlorophenyl or 4-methoxyphenyl substitution, have the 6-position open for adduct formation. These compounds were active, but less so than the parent compound **18**. The 6-phenyl derivative **64** which has a free 4-position, however, was inactive at the 0.250 mM level where the testing was started. The compounds with a 2'-thienyl substituent in either the 4- or the 6-position (**67**, **68**), were inactive at the test level used.

Compound **69** (scheme 3) is the 3-methyl derivative of **39**. The activity of the former is half that of the parent compound. The lower activity can be explained in part as due to the higher chemical reactivity of the pyrimidinium salt towards nucleophiles such as hydroxy and amino derivatives which will complete with thiols in the formation of adducts with the pyrimidinium derivative. Since the neutral adduct form in equilibrium with the free salt will be chemically

favored, part of the pyrimidine molecules will be locked in adducts not derived from the desired thiols.

The di(1-pyrimidinyl)methane derivative **70** (scheme 3), in which the β-heteroatom is part of a pyrimidine ring, was active at the 0.500 mM level.

In the present work it has been shown that *N*(1)-substituted 5-halo-2-pyrimidinones are active metaphase inhibitors. High activity has been found for derivatives where the *N*(1)-substituent is a carbon chain with a β-heteroatom (*O, S* or *N*) bonded to an aryl or alkaryl group.

Experimental protocols

Chemistry

The mass spectra under electron impact conditions were recorded at 70 eV ionizing current. Isobutane was used for chemical ionizing mass spectra (CI); the spectra are presented as *m/z* (% rel int). The ^{1}H-NMR spectra were recorded at 60 or 300 MHz. The solvent was deuteriochloroform unless otherwise stated.

Purity of products

Unless otherwise stated, the products obtained were checked for purity by chromatography (TLC and/or GLC). The GLC analyses of compounds **3** (purity > 90%) were run on a Hewlett–Packard 5710 A GC instrument using a 3% SP-2100 column. The TLC plates (silica, Merck, DC-Alufolien, Kieselgel 60 F 254) with the 2-pyrimidinone samples were developed with 5% methanol in chloroform. Microanalyses were performed by Mikroanalytisches Laboratorium (Kronach, Germany). Analysis of the α-chloroalkyl ethers was limited to spectroscopy because of high chemical reactivity.

General procedure for the preparation of alkylthioalkyl ethers ***3***

Sodium hydride dispersion in liquid paraffin (80%, 22 mmol) and sodium iodide (20 mmol) were added successively to a solution of the phenol or alcohol (20 mmol) in 1,2-dimethoxyethane (20 ml) at 4°C and the mixture stirred for 30 min before the α-chloroalkyl alkyl sulfide (20 mmol) was added. The resultant mixture was allowed to reach ambient temperature and was stirred for 6 h, the volume reduced to 1/3 at reduced pressure, the residue poured onto ice-water, the mixture extracted with diethyl ether, the washed and dried ($MgSO_4$) ether solution evaporated and the residue purified by distillation.

Chloroethyl ethyl sulfide [9]

Methylthiomethyl 2-phenylethyl ether ***3a***
Yield 45%, bp: 65–68°C/0.05 mmHg. ^{1}H-NMR: δ 2.07 (MeS), 2.90 (2H, t, *J* =7 Hz), 3.79 (2H, t, *J* = 7 Hz), 4.62 (OCH_2), 7.27 (Ph).

Methylthiomethyl 3-phenylpropyl ether ***3b***
Yield 40%. ^{1}H-NMR: δ 1.7–2.2, 2.77 and 3.61 ($CH_2CH_2CH_2$, m), 2.20 (SMe), 4.67 (OCH_2), 7.28 (Ph).

Benzyl 1-ethylthioethyl ether ***3c***
^{1}H-NMR: δ 1.28 (Me, t, *J* =7 Hz), 1.60 (Me, d, *J* = 7 Hz), 4.00 (1H, q, *J* = 7 Hz), 4.70 (CH_2), 7.38 (Ph).

4-Methylbenzyl methylthiomethyl ether ***3d***
Yield 72%, bp: 60°C/0.05 mmHg. ^{1}H-NMR: δ 2.18 (SMe), 2.33 (Me), 4.53 (CH_2), 4.64 (SCH_2O), 7.20 (4H, Ar).

Methylthiomethyl 3-trifluoromethylbenzyl ether ***3e***
Yield 68%, bp: 48°C/0.1 mmHg. ^{1}H-NMR: δ 2.15 (MeS), 4.61 (OCH_2S), 4.76 (CH_2), 7.3–7.6 (4H, Ar).

Methylthiomethyl 1-naphthylmethyl ether ***3f***
Yield 50%, bp 105–115°C/0.01 mmHg. ^{1}H-NMR: δ 2.17 (SMe), 4.70 (OCH_2S), 5.01 (CH_2), 7.1–8.2 (naphthyl).

General procedure for the synthesis of α-haloalkyl ethers ***4***

Sulfuryl chloride (2.7 g, 20 mmol) in dry dichloromethane (40 ml) was added dropwise over 10 min at ambient temperature to a solution of the ether **3** (20 mmol) in dry dichloromethane (60 ml). The mixture was stirred for 30 min before the solvent and the sulfenyl chloride, which is the co-product in the reaction, were removed together at reduced pressure. The residue was purified by distillation where possible. The crude product was normally used in the subsequent alkylation reaction.

1-Chloromethyl 2-phenylethyl ether ***4a***
^{1}H-NMR: δ 2.94 (2H, t, *J* =7 Hz), 3.91 (CH_2O), 5.48 ($ClCH_2O$), 7.27 (Ph).

1-Chloromethyl 3-phenylpropyl ether ***4b***
^{1}H-NMR: δ 1.7–2.2 (2H, m), 2.72 (2H, t, *J* = 7Hz), 3.67 (2H, t, *J* = 7 Hz), 5.48 ($ClCH_2O$), 7.20 (Ph).

1-Chloroethyl benzyl ether ***4c***
^{1}H-NMR: δ 1.70 (Me, d, *J* = 7 Hz), 4.55 and 4.95 (CH_2, d, *J* = 12 Hz), 5.75 (CH, q, *J* = 7 Hz), 7.40 (Ph).

Chloromethyl 4-methylbenzyl ether ***4d***
^{1}H-NMR: δ 2.34 (Me), 4.70 (CH_2), 5.48 ($ClCH_2O$), 7.23 (Ar).

Chloromethyl 3-trifluoromethylbenzyl ether ***4e***
Yield 89%, bp: 48–52°C/0.1 mmHg. ^{1}H-NMR: δ 4.75 (CH_2), 5.47 ($ClCH_2O$), 7.2–7.6 (Ar).

Chloromethyl 1-naphthylmethyl ether ***4f***
^{1}H-NMR: δ 5.13 (CH_2), 5.47 ($ClCH_2O$), 7.2–8.3 (naphthyl).

Chloromethyl ethers available by literature methods
The following chloromethyl ethers are available by the literature methods: chloromethyl 1-phenylbenzyl ether [6], chloromethyl 4-phenacyl ether [20], chloromethyl 3-methylbenzyl ether [6], chloromethyl 4-methoxybenzyl ether [6], chloromethyl 4-chlorobenzyl ether [6], chloromethyl 2-thenyl ether [6], chloromethyl 2-furfuryl ether [6].

N-Chloromethylformanilide ***8***
1-Chloromethyl 4-chlorophenyl sulfide [8], (4.05 g, 21 mmol) was added to a mixture of formanilide (2.54 g, 21 mmol) and potassium *t*-butoxide (2.35 g, 21 mmol) in DMF (200 ml). The mixture was stirred at 80°C for 4 h before the solvent was distilled off and the residue dissolved in ether and washed with water (x 5), and the dried ($MgSO_4$) solution evaporated to furnish *N*-[(4-chlorophenyl)thiomethyl]formanilide (**7**); yield 5.37 g (92%), mp: 67°C (Et_2O/light petroleum). ^{1}H-NMR: δ 5.15 (CH_2), 6.9–7.4 (9H, Ar), 8.20 (CHO). This product (4.91, 17.8 mmol) was dissolved in dichloromethane (100 ml) and sulfuryl chloride (2.40 g, 17.8 mmol) in dichloromethane

(25 ml) was added with stirring at 5°C over 30 min. The mixture was stirred for a further 15 min before cyclohexene (1.59 g, 19.3 mmol) in dichloromethane (25 ml) was added. The mixture was evaporated and the residue washed with hexane and dried; yield 1.91 g (61%). ^{1}H-NMR: δ 5.50 (CH_2), 7.34 (Ph), 8.33 (CHO). The product was used without further purification.

N-Chloromethyl urethanes available by published methods
The following *N*-chloromethyl urethanes are available by published methods: *N*-chloromethyl-*N*-methoxycarbonylaniline [21]; *N*-chloromethyl-*N*-ethoxycarbonylaniline [21].

Benzyl chloromethyl sulfide [22]

1-Bromomethylsulfinyl-4-chlorobenzene ***11***
3-Chloroperbenzoic acid (90%; 3.26 g, 17 mmol) in chloroform (35 ml) was added dropwise with stirring over 1 h at 0°C to a solution of 1-bromomethyl 4-chlorophenyl sulfide [9] (3.56 g, 15 mmol) in chloroform (35 ml). The mixture was stirred at ambient temperature overnight, shaken with 1 M K_2CO_3 aqueous solution, the dried ($MgSO_4$) chloroform solution evaporated and the solid residue recrystallized from chloroform/light petroleum; yield 3.20 g (84%), mp: 100°C. ^{1}H-NMR: δ 4.28 (CH_2), 7.53 (Ar). IR(KBr): 1040–1030 cm^{-1} (SO).

General procedures for the preparation of the pyrimidinones ***14–62***

Method A: Potassium *t*-butoxide (5.0 mmol) was added to a solution of 5-halo-2(1*H*)-pyrimidinone (5.0 mmol) in dry DMF (50 ml). The mixture was stirred at ambient temperature for 20 min before the α-chloroalkyl ether, sulfide or carbamate (5.0 mmol) was added. The resultant mixture was stirred at 60°C for 3 h, the solvent removed at reduced pressure and the residue extracted with chloroform (100 ml), the chloroform solution washed with water (3x) and the dried ($MgSO_4$) solution evaporated. The residual product was an almost pure mixture of the *N*- and *O*-alkylated isomers. The isomers differ by their solubility in diethyl ether, the *O*-alkylated isomer being the more soluble. Extraction of the crude product with diethyl ether largely removed the *O*-alkyl isomer. The *N*-alkyl isomer was further purified by recrystallization.

Method B: The α-chloroalkyl ether, sulfide or carbamate (2 mmol) in dichloromethane (4 ml) was added dropwise with stirring under N_2 to a solution which had been prepared from 5-chloro-2(1*H*)-pyrimidinone (2 mmol) and triethylamine (2 mmol) in dichloromethane (20 ml). The reaction mixture was stirred at ambient temperature for 24 h, aqueous NaCl was then added, the organic layer separated, dried ($MgSO_4$) and evaporated to give the crude product which was a mixture of the *N*- and *O*-alkylated isomers in 80–95% yield. The isomers could be separated by the lower solubility of the *N*-alkylated isomer in diethyl ether or by chromatography. We were only interested in the isolation and purification of the *N*-alkylated isomer.

5-Chloro-1-ethoxymethyl-2(1H)-pyrimidinone ***14***
Compound **14** was prepared by *Method B*; yield 43%; mp: 89°C. Found: C, 44.39; H, 5.02 calc for $C_7H_9ClN_2O_2$: C, 44.57; H, 4.82. ^{1}H-NMR (acetone–d_6): δ 1.15 and 3.67 (EtO), 5.30 (CH_2), 8.22 and 8.53 (H-6 and H-4, J = 3 Hz). MS: 188 (1, *M*), 146 (15), 145 (6), 144 (46), 131 (12), 130 (11), 116 (12), 102 (19), 59 (100).

5-Chloro-1-(pivaloyloxy)methyl-2(1H)-pyrimidinone ***16***
Compound **16** was prepared by *Method B*; yield 57%; mp: 164°C. Mol weight found (MS): 244.0620; calc for $C_{10}H_{13}ClN_2O_3$: 244.0615. ^{1}H-NMR (acetone–d_6): δ 1.18 (*t*-Bu), 5.80 (CH_2), 8.30 and 8.59 (H-6 and H-4, J = 3 Hz). MS: 244 (5, *M*), 216 (2), 214 (7), 145 (4), 143 (12), 133 (8), 131 (30), 130 (6), 57 (100).

5-Chloro-1-(2-phenylethoxy)methyl-2(1H)-pyrimidinone ***19***
Compound **19** was prepared by *Method B*; yield 62%; mp: 123°C. Found: C, 58.87; H, 4.88; calc for $C_{13}H_{13}ClN_2O_2$: C, 58.98; H, 4.96. ^{1}H-NMR: δ 2.91 and 3.87 (CH_2CH_2), 5.23 (NCH_2O), 7.25 (Ph), 7.54 and 8.53 (H-6 and H-4, J = 3 Hz). MS: 234 (3), 145 (3), 144 (2), 143 (7), 131 (2), 116 (5), 105 (3), 104 (100), 91 (11).

5-Chloro-1-(3-phenylpropoxy)methyl-2(1H)-pyrimidinone ***20***
Compound **20** was prepared by *Method B*; yield 56%; mp: 99°C. Mol weight found (MS): 278.0817; calc for $C_{14}H_{15}ClN_2O_2$: 278.0822. ^{1}H-NMR: δ 1.7–2.2, 2.72 and 3.61 ($CH_2CH_2CH_2$), 5.27 (OCH_2), 7.21 (Ph), 7.87 and 8.50 (H-6 and H-4, J = 3 Hz). MS: 148 (23), 133 (5), 131 (16), 119 (14), 118 (74), 117 (24), 92 (15), 91 (100).

1-(1-Benzyloxyethyl)-5-chloro-2(1H)-pyrimidinone ***22***
Compound **22** was prepared by *Method B*; yield 40%; mp: 114°C. Found: C, 58.09; H, 4.98; calc for $C_{13}H_{13}ClN_2O_2$: C, 57.98; H, 4.99. Mol weight found (MS): 264.0671; calc for $C_{13}H_{13}ClN_2O_2$: 264.0666. ^{1}H-NMR: δ 1.50 (CH_3CH, d, J = 6 Hz), 4.50 (CH_2), 6.02 (CH_3CH, q, J = 6 Hz), 7.31 (Ph), 7.85 (H-6, d, J = 3 Hz) and 8.3–8.5 (H-4, br s). MS (CI): 265 (37, M + 1), 233 (20), 224 (11), 221 (60), 220 (16), 148 (10), 135 (37), 133 (26), 131 (78), 91 (100).

5-Chloro-1-(1-phenylethoxy)methyl-2(1H)-pyrimidinone ***23***
Compound **23** was prepared by *Method B*; yield 58%; mp: 139°C. Found: C, 58.62; H, 5.03; calc for $C_{13}H_{13}ClN_2O_2$: C, 58.98; H, 4.95. ^{1}H-NMR: δ 1.40 and 4.63 (CH_3CH), 5.18 (CH_2), 7.0–7.3 (Ph), 7.47 and 8.27 (H-6 and H-4, J = 4 Hz). IR (KBr): 1670 cm^{-1} (CO). MS (CI): 265 (16, M + 1), 237 (13), 235 (43), 173 (8), 171 (8), 144 (21), 133 (14), 131 (44), 105 (100).

1-(Benzylthio)methyl-5-chloro-2(1H)-pyrimidinone ***26***
Compound **26** was prepared by *Method A*; yield 36%; mp: 189°C. Mol weight found (MS): 266.0291; calc for $C_{12}H_{11}ClN_2OS$: 266.0281. ^{1}H-NMR: δ 3.84 (CH_2), 4.90 (NCH_2S), 7.20 (Ph), 7.48 and 8.30 (H-6 and H-4, J = 4 Hz). IR (KBr): 1660 cm^{-1} (CO). MS: 266 (1, *M*), 175 (4), 146 (7), 145 (9), 144 (24), 143 (25), 136 (85), 135 (21), 131 (17), 91 (100).

5-Chloro-1-(4-chlorophenylsulfinyl)methyl-2(1H)-pyrimidinone ***27***
Potassium *t*-butoxide (4 mmol) in DMF (10 ml) was added to a solution of 5-chloro-2(1*H*)pyrimidinone (4 mmol) in DMF (40 ml). The mixture was stirred at ambient temperature for 10 min before 1-bromomethylsulfinyl-4-chlorobenzene (4 mmol) was added. The resultant mixture was stirred at 60°C for 2 d, the solvent distilled off at reduced pressure, the residue triturated with water, the insoluble material dried and washed with a little chloroform, recrystallized from DMSO, the crystalline material washed with a little methanol and finally with water; yield 0.30 g (25%), mp: 262°C. Found: C, 43.73; H, 2.68; calc for $C_{11}H_8Cl_2N_2O_2S$: C, 43.59; H, 2.67. ^{1}H-NMR (DMSO–d_6): δ 5.12 (CH_2), 7.60 (Ph, br s), 8.19 and 8.62 (H-6 and H-4, J =4 Hz). IR(KBr): 1670 (CO), 1060 cm^{-1} (SO). MS: 302 (0.5, *M*), 159 (6), 145 (33), 143 (100).

5-Chloro-1-(N-formyl-N-phenylamino)methyl-2(1H)-pyrimidinone ***28***
Compound **28** was prepared by *Method B*; yield 50%; mp: 201°C (EtOH). Found: C, 54.61; H, 3.86; calc for $C_{12}H_{10}ClN_3O_2$: C, 54.65; H, 3.79. ^{1}H-NMR (DMSO–d_6): δ 5.68 (CH_2), 7.32 (Ph), 8.05–8.65 (CHO, H-6 and H-4). IR (KBr): 1660 (CO), 1680 cm^{-1} (CO). MS: 263 (1, *M*), 143 (7), 134 (41), 116 (6), 107 (6), 106 (100), 105 (57), 77 (41).

5-Chloro-1-(N-methoxycarbonyl-N-phenylamino)methyl-2(1H)-pyrimidinone ***29***
Compound **29** was prepared by *Method B*; yield 63%; mp: 222°C. Found: C, 53.09; H, 4.24; calc for $C_{13}H_{12}ClN_3O_3$: C, 53.15; H, 4.13. ^{1}H-NMR: δ 3.82 (Me), 5.77 (NCH_2), 7.1–7.5 (Ph), 8.25 and 8.70 (H-6 and H-4, J = 3 Hz). MS: 293 (13, *M*), 165 (10), 164 (100), 143 (12), 120 (31), 105 (11), 104 (11), 77 (19).

5-Chloro-1-(N-ethoxycarbonyl-N-phenylamino)methyl-2(1H)-pyrimidinone ***30***
Compound **30** was prepared by *Method A*; yield 65%; mp: 174°C Found: C, 54.60; H, 4.59; calc for $C_{14}H_{14}ClN_3O_3$: C, 54.63; H, 4.59. ^{1}H-NMR: δ 1.18 and 4.20 (Et), 5.62 (CH_2), 7.0–7.4 (Ph), 8.10 and 8.46 (H-6 and H-4, J = 4 Hz). IR (KBr): 1720 (CO ester), 1680 cm^{-1} (CO). MS: 307 (*M*), 178 (71), 143 (15), 134 (25), 106 (100), 78 (25).

5-Chloro-1-(4-methylbenzyloxy)methyl-2(1H)-pyrimidinone ***33***
Compound **33** was prepared by *Method B*; yield 49%; mp: 150°C. Found: C, 58.54; H, 4.77; calc for $C_{13}H_{13}ClN_2O_2$: C, 58.78; H, 4.96. ^{1}H-NMR (DMSO–d_6): δ 2.27 (Me), 4.58 (CH_2), 5.29 (NCH_2), 7.17 (Ph), 8.41 and 8.60 (H-6 and H-4, J = 4 Hz). MS: 236 (3), 234 (9), 146 (10), 144 (31), 119 (5), 106 (10), 105 (100), 77 (12).

5-Chloro-1-(2-methylbenzyloxy)methyl-2(1H)-pyrimidinone ***34***
Compound **34** was prepared by *Method B*; yield 50%; mp: 88°C. Found: C, 58.47; H, 4.92; calc for $C_{13}H_{13}ClN_2O_2$: C, 58.98; H, 4.94. ^{1}H-NMR: δ 2.25 (Me), 4.63 (CH_2), 5.30 (CH_2O), 7.00 (Ph), 7.60 and 8.37 (H-6 and H-4, J = 4 Hz). IR (KBr): 1770 cm^{-1} (CO). MS: 264 (0.2, *M*), 146 (14), 144 (44), 132 (6), 130 (21), 119 (5), 116 (4), 106 (9), 105 (100).

5-Chloro-1-(4-methoxybenzyloxy)methyl-2(1H)-pyrimidinone ***36***
Compound **36** was prepared by *Method B*; yield 52%; mp: 118°C. Found: C, 55.44; H, 4.64; calc for $C_{13}H_{13}ClN_2O_3$: C, 55.60; H, 4.67. ^{1}H-NMR: δ 3.77 (OMe), 4.57 (CH_2), 5.27 (CH_2O), 6.6–7.3 (Ph), 7.63 and 8.35 (H-6 and H-4, J = 4 Hz). IR (KBr): 1670 cm^{-1} (CO). MS(CI): 281 (3, M + 1), 253 (4), 251 (13), 146 (5), 144 (15), 122 (9), 121 (100).

5-Chloro-1-(2-trifluoromethylbenzyloxy)methyl-2(1H)-pyrimidinone ***41***
Compound **41** was prepared by *Method B*; yield 52%; mp: 95°C. Found: C, 49.15; H, 3.01; calc for $C_{13}H_{10}ClF_3N_2O_2$: C, 48.99; H, 3.16. ^{1}H-NMR: δ 4.77 (CH_2), 5.42 (CH_2O), 7.3–7.7 (Ph), 7.82 and 8.55 (H-6 and H-4, J = 4 Hz). IR (KBr): 1660 cm^{-1} (CO). MS: 318 (0.2, *M*), 288 (5), 269 (3), 146 (30), 160 (9), 159 (100), 146 (11), 144 (34).

5-Chloro-1-(1-naphthylmethyloxy)methyl-2(1H)-pyrimidinone ***44***
Compound **44** was prepared by *Method B*; yield 33%. ^{1}H-NMR: δ 5.05 (CH_2), 5.26 (CH_2O), 7.2–8.0 (H-6 and naph), 8.20 (H-4, J = 4 Hz). MS: 300 (0.1, *M*), 170 (5), 169 (10), 157 (4), 156 (3), 155 (14), 147 (4), 144 (100), 139 (23).

5-Chloro-1-(2-thenyloxy)methyl-2(1H)-pyrimidinone ***46***
Compound **46** was prepared by *Method B*; yield 57%; mp: 132°C. Found: C, 46.57; H, 3.45; calc for $C_{10}H_9ClN_2O_2S$: C, 46.79; H, 3.53. ^{1}H-NMR: δ 4.80 (CH_2), 5.38 (CH_2O), 6.7–7.2 (3H, thiophene), 7.63 and 8.35 (H-6 and H-4, J = 4 Hz). IR (KBr): 1660 cm^{-1} (CO). MS(CI): 257 (7, M + 1), 229 (11), 228 (4), 227 (31), 146 (6), 145 (3), 144 (20), 97 (100).

5-Chloro-1-(2-furfuryloxy)methyl-2(1H)-pyrimidinone ***47***
Compound **47** was prepared by *Method B*; yield 54%; mp: 100°C. Found: C, 49.81; H, 3.93; calc for $C_{10}H_9ClN_2O_3$: C, 49.90; H, 3.78. ^{1}H-NMR: δ 4.61 (CH_2), 5.30 (OCH_2), 6.2–7.4 and 7.2–7.3 (3H, furyl), 7.73 and 8.34 (H-6 and H-4, J = 4 Hz). IR (KBr): 1660 cm^{-1} (CO). MS(CI): 241 (16, M + 1), 213 (12), 211 (36), 146 (5), 144 (16), 82 (6), 81 (100).

5-Fluoro-1-(3-methylphenylthio)methyl-2(1H)-pyrimidinone ***49***
Compound **49** was prepared by *Method B*; yield 25%; mp: 124°C. Found: C, 57.42; H, 4.63; calc for $C_{12}H_{11}FN_2OS$: C, 56.59; H, 4.43. ^{1}H-NMR (DMSO–d_6): δ 2.28 (Me), 5.17 (CH_2), 7.2 (Ph), 8.05 and 8.40 (H-6 and H-4, J = 3 Hz). IR (KBr): 1670 cm^{-1} (CO). MS: 250 (8, *M*), 137 (11), 136 (36), 135 (10), 128 (7), 127 (100), 100 (34), 91 (9).

1-(4-Chlorobenzyloxy)methyl-5-iodo-2(1H)-pyrimidinone ***58***
Compound **58** was prepared by *Method B*; yield 45%; mp: 168°C (acetone). Found: C, 38.39; H, 2.62; calc for $C_{12}H_{10}ClN_2O$: C, 38.27; H, 2.68. ^{1}H-NMR: δ 4.66 (CH_2), 5.35 (CH_2O), 7.31 (Ph), 7.71 and 8.61 (H-6 and H-4, J = 3 Hz). MS: 346 (3), 237 (5), 236 (78), 219 (2), 206 (7), 166 (3), 125 (100), 109 (9).

1-(4-Acetylphenoxy)methyl-5-chloro-2(1H)-pyrimidinone ***62***
Compound **62** was prepared by *Method B*; yield 100%; mp: 182°C (acetone). Found: C, 56.38; H, 4.15; calc for $C_{13}H_{11}ClN_2O_3$: C, 56.02; H, 3 99. ^{1}H-NMR: δ 1.50 (Me), 5.82 (CH_2), 7.0 and 7.8 (H-6 and H-4, J = 3 Hz). MS: 278 (7, *M*), 145 (31), 143 (100), 136 (18), 121 (33), 116 (22), 93 (15).

Preparation of compounds ***69*** *and* ***70***

5-Chloro-1-(4-chlorophenylsulfenyl)methyl-3-methyl-2-oxo-1,2-dihydropyrimidinium tetrafluoroborate ***69***
Trimethyloxonium tetrafluoroborate (0.29 g, 2.0 mmol) was added to a solution of 5-chloro-1-(4-chlorophenylsulfenyl)-methyl-2(1*H*)-pyrimidinone **39** (0.57 g, 2.0 mmol) in dry dichloromethane (30 ml). The mixture was stirred under N_2 at ambient temperature for 3 h before the solvent was evaporated; yield 95 %; mp; 55–60°C. ^{1}H-NMR (CD_3CN): δ 3.80 (NMe), 5.35 (CH_2S), 7.44 (4 H, Ar), 8.30 and 8.88 (H-6 and H-4, J=3Hz). IR (KBr): 1750 cm^{-1} (CO).

1-(5-Chloro-2(1H)-pyrimidinon-1-yl)methyl-5-chloro-2(1H)-pyrimidinone ***70***
Triethylamine (5.1 ml, 36 ml) was added to a suspension of 5-chloro-2(1*H*)-pyrimidinone hydrochloride [14] (3.0 g, 18 mmol) in bromochloromethane (30 ml). The mixture was heated under reflux for 3 h, then cooled and filtered. Solid material was washed with water followed by acetone; yield 1.25 g (51%); mp: > 260 °C. Found: C, 39.53; H, 2.12; calc for $C_9H_6Cl_2N_4O_2$: C 39.58; H 2.22. ^{1}H-NMR (TFA): δ 6.32 (CH_2), 8.7–9.4 (4 H, m, pyr).

Pharmacology

Screening for metaphase arrest

The Chang line of human cells was used. This line, established in 1954 [11], was obtained in 1962 from T Gustafson, The Wenner–Gren Institute for Experimental Biology, University of Stockholm, Sweden, and the cells have since been routinely cultured at the Department of Tissue Culture, Institute for Cancer Research, The Norwegian Radium Hospital.

An E2a culture medium [23], containing human serum (20 %) and horse serum (10 %) was used. The cells which were to be used in the tests were cultured in a medium without any added antibiotic. Each test substance was dissolved in the medium to be used at the maximum concentration for the testing. When compounds were difficult to dissolve in the medium they were initially dissolved in DMF and the solution mixed with the medium. The concentration of DMF was below the concen-tration of DMF toxic to the cells. The medium solutions thus prepared were filtered through a 0.22-μm Millipore bacterial filter and diluted with medium to give the desired concen-tration of the compound for testing. The pH of the medium was adjusted to 7.2. All manipulations of the cells were carried out at 37°C, and all solutions which were added to the cells had this temperature.

At the start of an experiment, cells from stock cultures after trypsinization were suspended in the medium in a concentration of 100 000 cells per ml. One ml of the suspension was added to each of a series of Leighton tubes (16 x 83 mm; Bellco Glass Inc, USA which contained a glass strip (35 x 10.5 mm; Bellco Glass Inc) fastened with a chick plasm-embryo extract clot to the flat wall.

The tubes were placed horizontally and kept at 37°C for 24 h. During this time the cells fastened to the underlayer (the glass strips) and went into exponential growth.The medium was then replaced by new medium. After incubation for another 24 h the strips were covered by an almost confluent sheet of cells in exponential growth.

The medium was now replaced with a medium containing the test substances in the desired concentrations, and the tubes reincubated in a stationary horizontal position. After incubation for 6 h the strips were removed from the tubes and placed in Carnoy 6: 3: 1 fixative and the cells on the strip stained in Boehmer's haematoxylin.

The slides were coded before counting. 1000 cells were counted on each slide and the cells assigned to one of the following categories: prophase, metaphase, anaphase, telophase and interphase. Abnormal mitotic configurations (multipolar cells, etc) and morphological changes were noted. The results given in the tables are the averages of results from 5 slides in each experiment.

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