METAHALONES, A NEW CLASS OF METAPHASE INHIBITORS

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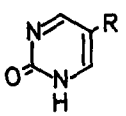
1. Introduction

5-Fluoro- (I) and 5-chloro- (II) pyrimidin-2-one has been shown to exhibit metaphase-arresting activity [1,2].



R = –F (I)
R = –Cl (II)
R = –Br (III)
R = –H (IV)

We have now extended our work on this class of substances and have attempted to establish the structure–activity relationship. The results suggest that the metaphase-inhibitory activity is associated with the 2-oxo group and the 5-halogen, and that certain substituents in other positions are permissable for the retainment of activity. We should like to suggest the name *Metahalones* for this class of inhibitors. *Meta* from metaphase, *hal* from halogen and *one* because the activity is associated with the one-form of the 2-hydroxy group; *O*-substituted derivatives as shown below are inactive whereas *N*-substituted derivates are active.

2. Materials and methods

The substances tested were synthesized by methods described in the references (see table 1,2). All substances were tested for metaphase-arresting activity 6 h after addition to monolayer cultures of the Chang strain of human liver cells. The methods used have been detailed in [1]. The substances were tested over a range of concentrations. The following terms are used for describing the activity of the substances:

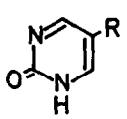
Complete arrest: there were no ana- and telophases and accumulation of cells in metaphase.

Significant metaphase arrest: the arrest was only partial with some ana- and telophases but with a significant increase in the number of cells in metaphase. Approximately 100% increase of that in the control cells was considered significant.

No metaphase arrest: there was no significant difference between the number of cells in metaphase, anaphase and telophase in the treated and controls.

3. Results and discussion

A number of pyrimidin-2-ones with substituents other than halogen in the 5-position were tested. It is seen from table 1 that the 5 substituents, $-CH_3$ (V), $-CH_2OH$ (VI), $-CN$ (VII), $-NO_2$ (VIII) and $-SO_3H$ (IX), did not produce a substance with metaphase-arresting activity at 2–3 mM whereas 5-halogeno-pyrimidin-2-ones (I, II, III) exhibit significant metaphase-arresting activity (table 2). As shown in [1], pyrimidin-2-one (IV) did not exhibit any activity even at 8 mM. Hence the results suggest that the 5-halogen substituent is required for activity.



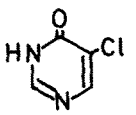
R = $-CH_3$ (V)
R = $-CH_2OH$ (VI)
R = $-CN$ (VII)
R = $-NO_2$ (VIII)
R = $-SO_3H$ (IX)

Furthermore, it is seen from table 1 that the

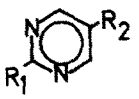
Table 1
Effect of substances on Chang liver cells

Substances	No metaphase arrest at (mM)	
Pyrimidin-2-one (IV)	[1]	8
5-Methylpyrimidin-2-one (V)	[12]	2
5-Hydroxymethylpyrimidin-2-one (VI)	[12]	2
5-Cyanopyrimidin-2-one (VII)	[13]	3
5-Nitropyrimidin-2-one (VIII)	[14]	2
5-Sulfopyrimidin-2-one (IX)	[15]	2
5-Chloropyrimidin-4-one (X)	[17]	2
2-Methoxy-5-fluoropyrimidine (XI)	[20]	3
2-Methoxy-5-chloropyrimidine (XII)	[18]	3
2-Methoxy-5-bromopyrimidine (XIII)	[19]	3
2-Amino-5-chloropyrimidine (XIV)	[16]	2
2-Hydrazino-5-chloropyrimidine (XV)	[16]	3
2-Methylthio-5-chloropyrimidine (XVIII)	[20]	3
2-Methylthio-5-bromopyrimidine (XIX)	[21]	3
5-Chlorouracil (XXV)	[17]	2
1-Propargyl-5-flurouracil (XXVI)	[8]	3
4-Ethoxy-5-fluoropyrimidin-2-one (XXVII)	[3]	3

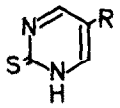
position of the oxygen atom in the pyrimidine ring relative to the halogen atom is critical since 5-chloropyrimidin-4-one (X) is inactive. The one-form of the 2-hydroxy groups is necessary for activity since the methoxy group (XI, XII, XIII) in the 2-position results in inactive substances (table 1).



(X)



$R_1 = -OCH_3$ $R_2 = -F$ (XI)
$R_1 = -OCH_3$ $R_2 = -Cl$ (XII)
$R_1 = -OCH_3$ $R_2 = -Br$ (XIII)
$R_1 = -NH_2$ $R_2 = -Cl$ (XIV)
$R_1 = -NHNH_2$ $R_2 = -Cl$ (XV)



R = –Cl (XVI)
R = –Br (XVII)

Introducing an amino group into the 2-position (XIV) resulted in an inactive molecule (table 2). The 2-hydrazino derivative (XV) was also inactive but toxic.

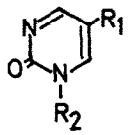
When replacing the oxygen with sulphur (XVI, XVII) substances with significant metaphase-inhibitory activity at 3 mM were obtained (table 2). *S*-Alkylation (XVIII, XIX, formulae not shown) leads to inactive substances (table 1).

Alkylation of the 1-position (XX, XXI, XXII) in the 5-halogenopyrimidin-2-ones results in metaphase arresting substances. Certain substituents such as the

Table 2
Effect of substances on Chang liver cells

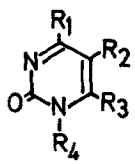
Substances		Complete metaphase arrest at (mM)	Significant metaphase arrest at (mM)
5-Fluoropyrimidin-2-one (I)	[3]	1.75	0.87
5-Chloropyrimidin-2-one (II)	[4]	2.0	0.50
5-Bromopyrimidin-2-one (III)	[5]		3.0
5-Chloropyrimidin-2-thione (XVI)	[6]		3.0
5-Bromopyrimidin-2-thione (XVII)	[8]		3.0
1-Methyl-5-fluoropyrimidin-2-one (XX)	[7]	3.0	1.5
1-Methyl-5-chloropyrimidin-2-one (XXI)	[7]	2.0	1.0
1-Methyl-5-bromopyrimidin-2-one (XXII)	[5]	3.0	1.5
1-Propargyl-5-chloropyrimidin-2-one (XXIII)	[8]	0.18	0.09
1-*p*-Fluorobenzyl-5-chloropyrimidin-2-one (XXIV)	[8]	0.25	0.125
4-Methyl-5-bromopyrimidin-2-one (XXVIII)	[9]	3.0	0.75
4-Methylthio-5-fluoropyrimidin-2-one (XXIX)	[10]		3.0
1,4-Dimethyl-5-bromopyrimidin-2-one (XXX)	[9]	2.0	1.0
4,6-Dimethyl-5-bromopyrimidin-2-one (XXXI)	[11]	1.5	0.75

propargyl (XXIII) and *p*-fluorobenzyl (XXIV) will result in more active substances as compared with the parent compound (table 2).



$R_1 = -F$	$R_2 = -CH_3$	(XX)
$R_1 = -Cl$	$R_2 = -CH_3$	(XXI)
$R_1 = -Br$	$R_2 = -CH_3$	(XXII)
$R_1 = -Cl$	$R_2 = -CH_2-C\equiv CH$	(XXIII)
$R_1 = -Cl$	$R_2 = -CH_2C_6H_4-p-F$	(XXIV)

Substitutions in the 4-position may and may not result in metaphase-arresting substances. 5-Chlorouracil (XXV), 1-propargyl-5-fluorouracil (XXVI), as well as 4-ethoxy-5-fluorouracil (XXVII) were inactive at 3 mM (table 1). On the other hand substituents such as the methyl group (XXVIII) and the methylthio group (XXIX) gave substances with some activity (table 2). Introduction of two methyl groups in the 1,4- or 4,6-position (XXX and XXXI), respectively, gave substances with metaphase-arresting activity (table 2).



$R_1 = -OH$	$R_2 = -Cl$	
$R_3 = -H$	$R_4 = -H$	(XXV)
$R_1 = -OH$	$R_2 = -F$	
$R_3 = -H$	$R_4 = -CH_2-C\equiv CH$	(XXVI)
$R_1 = -OC_2H_5$	$R_2 = -F$	
$R_3 = -H$	$R_4 = -H$	(XXVII)
$R_1 = -CH_3$	$R_2 = -Br$	
$R_3 = -H$	$R_4 = -H$	(XXVIII)
$R_1 = -SCH_3$	$R_2 = -F$	
$R_3 = -H$	$R_4 = -H$	(XXVIII)
$R_1 = -CH_3$	$R_2 = -Br$	
$R_3 = -H$	$R_4 = -CH_3$	(XXX)
$R_1 = -CH_3$	$R_2 = -Br$	
$R_3 = -CH_3$	$R_4 = -H$	(XXXI)

The data presented show that substitution in the 5-position is a prerequisite for activity since pyrimidin-2-one even at 8 mM exhibited no activity. A number of substances with substituents in the 5-position, however, such as, methyl-, hydroxymethyl-, cyano-, nitro- and sulpho-, showed no metaphase arresting activity at the dose level where, i.e., the 5-chloro- and 5-fluoropyrimidin-2-one exhibit complete arrest. These results suggest that the 5-halogen substituent in the pyrimidin-2-one molecule is required for activity. Furthermore, in the case of 1-propargyl-5-chloropyrimidin-2-one (XXIII) which gives complete metaphase arrest at 0.18 mM, the parent substance, i.e., 1-propargylpyrimidin-2-one (8 (at 3 mM), result not included in tables) at about 17-times the above concentration is devoid of metaphase arresting activity. This again underlines the necessity of the 5-halogen substituent.

Another essential feature of the molecule for metaphase-arresting activity is the 2-one or 2-thione structure since *O*- and *S*-methylation or substitution at C-2 with other groups yield inactive substances.

It is seen from table 2 that substituents such as propargyl and *p*-fluorobenzyl will increase the activity more than 1 order of magnitude. In the case of the latter substance, the presence of the bulky *p*-fluorobenzyl group has no adverse effect on the activity. Furthermore, it is interesting to note that the propargyl group itself confers no metaphase-arresting activity to the molecule since 1-propargyl-5-fluorouracil and 1-propargylpyrimidin-2-one exhibit no activity at nearly 20-times higher concentration.

The nature of a substituent in the 4-position seems to be critical since the hydroxy group or the ethoxy group gives molecules with no activity at the 3 mM level. On the other hand, a methyl or a methylthio group in the 4-position does not abolish activity.

Several classes of substances are known to arrest cells in metaphase. The best known are colchicine [22], the vinca alkaloids, vinblastine and vincristine [22], podophyllotoxin [23] and steganacin [24]. Recently, a new metaphase-arresting agent

nocadazole was also reported [25]. All these substances arrest cells in metaphase apparently by condensing with the microtubular protein vital for the formation of the spindle apparatus.

Another metaphase inhibitor, 5-substituted 3-(1-anilinoethylidinyl) pyrrolidine-2,4-dione has also been described [26].

The structure of the metahalones is quite unrelated to the metaphase inhibitors described above. Their structure is also less complex.

The mode of action of the metahalones is not yet known but preliminary data with 5-chloropyrimidin-2-one could suggest that it combines with tubulin [27].

The concentration of the metahalones required to obtain complete metaphase arrest is, however, several order of magnitudes higher than that found for the most-used metaphase inhibitors such as colchinine and the vinca alkaloids. However, the present results indicate that it is possible to increase the activity by suitable substitution in the 5-halogenopyrimidin-2-one molecule. It is also worth mentioning that the activity of the substance seems to depend on the cell system used. For instance, using sea urchin egg, it was found that 5-chloropyrimidin-2-one at 20 μM was sufficient to achieve complete metaphase arrest [28].

Acknowledgements

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