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Synthesis and in vitro cytotoxicity of 5-substituted 2-cyanoimino-4-imidazodinone and 2-cyanoimino-4-pyrimidinone derivatives

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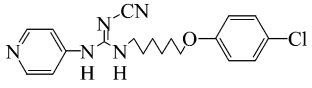
Abstract—A series of 5-substituted 2-cyanoimino-4-imidazodinone and 2-cyanoimino-4-pyrimidinone derivatives were synthesized and their anticancer cytotoxicity were evaluated in in vitro assay. It was found that the bulky aryl functionality in the 5-position of the 2-cyanoimino-4-imidazolidinone compounds was essential for the cytotoxicity of these heterocyclic compounds. Some of the derivatives exhibited modest cytotoxicity against a variety of cancer cell lines. One of the derivatives, [1-[6-(4-chlorophenoxy)hexyl]-5-oxo-4-phenyl-3-(4-pyridyl)tetrahydro-1*H*-2-imidazolyliden]aminomethanenitrile (Compound **11**), exhibited the most potent cytotoxic activity with IC_{50} in the nanomolar range. The cytotoxicity of these derivatives was selection with no apparent toxic effect toward normal fibroblasts.

1. Introduction

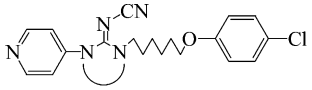
Cancer remains a formidable disease with a high mortality second only to cardiovascular diseases. Various methods and therapies have been developed to treat this disease but all have their limitations. As such, there is an imminent need to develop new anticancer drugs. In the late 1990s, a new anticancer agent based on the pyridyl cyanoguanidine system was discovered.[1,2] The in vivo screening led to the development of CHS 828 which is currently undergoing Phase I clinical trial (Fig. 1).[2] CHS 828 was found to exhibit selective potent cytotoxic effects in human breast and lung cancer cell lines. In nude mice bearing tumor xenografts, CHS 828 administered orally inhibited the growth of MCF-7 breast cancer tumors and caused regression of NYH small cell lung cancer tumors without toxic side effect. Although CHS 828 is a new agent with potent cytotoxic effect against cancer cells, the mechanism of action is still unkown.[3] The main objective of our project is to develop a second-generation drug candidate derived from CHS 828 with the desire to improve its cytotoxicity and/or selectivity. Various modifications of the side chain as well as the pyridinyl ring did not yield compounds with substaintially higher cytotoxicity. Subsequently, we made a drastic change of the parent cyanoguanidine core. We propose to install a bridge connecting the two amino nitrogens and thus, forming a cyclic structure **A** (Fig. 1). The structural rigidity imposed by modification may impart a different biological activity to the molecule. Furthermore, the inserted new bridge may serve as sites for additional derivatization. The execution of the above strategies are described in the following.

2. Results and discussion

A variety of synthetic procedures for cyclic cyanoguanidines have been reported in the literature.[4,5] The most common route is the reaction of *S*,*S*′-dimethyl-*N*-cyano-



CHS 828



A

Figure 1. Structures of CHS 828 and compound **A**.

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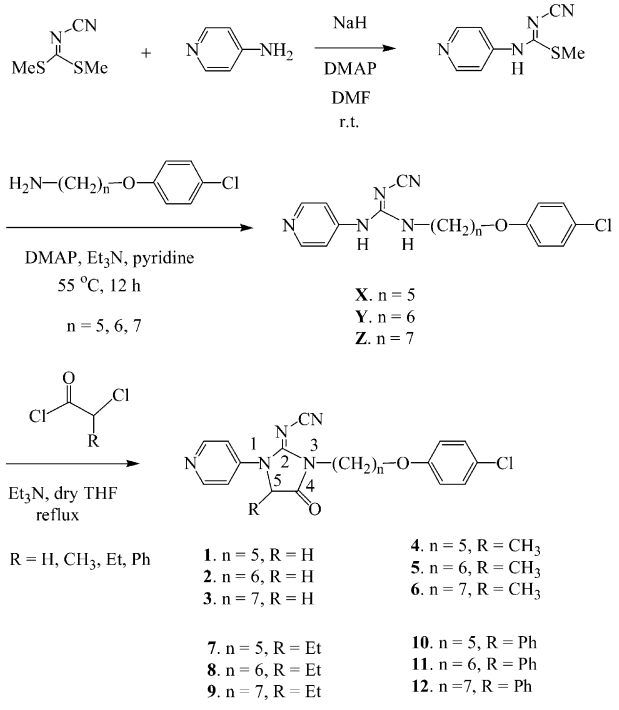
dithio-iminocarbonate with an appropriate primary and secondary diamines.[4] To the best of our knowledge, there is no report concerning the synthesis of the 5-substituted 2-cyanoimino-4-imidazodinone and 2-cyanoimino-4-pyrimidinone compounds via ring closure of the 4-pyridyl cyanoguanidines and 2-substituted chloroacetylchloride.[6] We report here a new and convenient synthesis of the novel anticancer heterocyclic compounds through cyclization of 4-pyridyl cyanoguanidines with 2-substituted chloroacetylchloride (e.g., chloroacetylchloride, 2-chloropropionylchloride, 2-chlorobutyrylchloride, and 2-chloro-2-phenylacetylchloride) (Scheme 1) or 3-chloropropionylchloride (Scheme 3). Preliminary structure–activity relationships against a variety of human cancer cell lines are also reported.

3. Chemistry

The five-membered heterocyclic compounds **1–12** prepared in this study were obtained by the method summarized in Scheme 1. The 4-pyridyl cyanoguanidine compounds **X**, **Y** and **Z** with an appropriate chain length (5–7 carbons) were prepared by coupling the starting 4-amino pyridine with *S*,*S*′-dimethyl *N*-cyano-dithio-iminocarbonate. The key intermediate, *S*-methyl-*N*-cyano-*N*′-pyridylisothiourea, was then coupled with one of primary amine to give compounds **X**, **Y** and **Z** in excellent yields (Scheme 1). In the presence of excess triethylamine, compounds **X**, **Y** and **Z** can undergo cyclization reaction with chloroacetylchloride, 2-chloropropionylchloride, 2-chlorobutyrylchloride, and 2-chloro-2-phenylacetylchloride respectively, to give the desired cyclic compounds **1–3**, **4–6**, **7–9**, **10–12** in 55–80% yield (Scheme 1).[7]



Scheme 1. General synthetic route to 2-cyanoimino-4-imidazodinone derivatives.

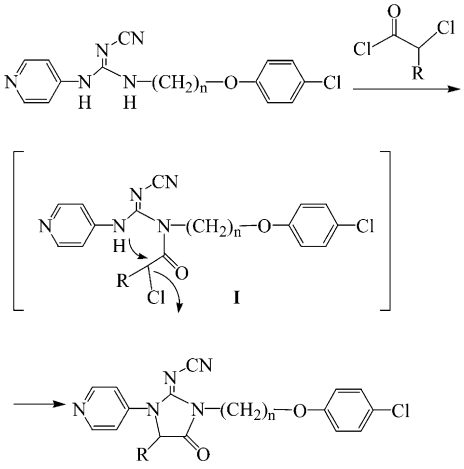
The formation of these heterocyclic compounds **1–12** was explained as follows. After acylation of the 4-pyridyl cyanoguanidines **X**, **Y** and **Z** with 2-substituted chloroacetylchloride, the resulting key intermediate **I** could undergo an intramolecular cyclization to give the desired cyclic compounds **1–12**. The products of the intermolecular substitutions were not observed (Scheme 2).

In a similar manner, 4-pyridyl cyanoguanidine compounds **X**, **Y** and **Z** are allowed to react with 3-chloropropionylchloride via cyclization in the presence of an excess of triethylamine in refluxing 1,4-dioxane to yield novel six-membered heterocyclic compounds **13–15** in 45–50% yield (Scheme 3). All the new cyclic compounds gave satisfactory ^{1}H NMR and ES mass spectra.

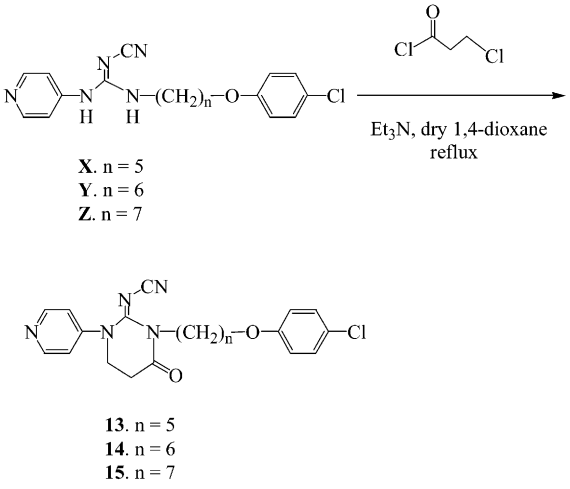
A novel series of cyclic compounds was prepared as described above via a short convergent synthetic Scheme.

4. Bioactivity

The heterocyclic compounds, prepared in this study, were evaluated according to standard protocols for their



Scheme 2. Mechanism of cyclic structure formation.



Scheme 3. General synthetic route to 2-cyanoimino-4-pyrimidinone derivatives.

in vitro cytotoxicity against eight human cancer cell lines including cells derived from human gastric cancer (NUGC and HR), human colon cancer (DLD1), human liver cancer (HA22T and HEPG2), human breast cancer (MCF), nasopharyngeal carcinoma (HONE1) and normal fibroblast cells (WI38). All of IC_{50} values were listed in Table 1. Some heterocyclic compounds was observed with significant cytotoxicity against most of the cancer cell lines tested ($IC_{50} = 10$–1000 nM). Normal fibroblasts cells (WI38) were affected to a much lesser extent ($IC_{50} > 10{,}000$ nM). The cyanoguanidine moiety was found to be crucial for the cytotoxic effect of cyclic compounds **1**–**15**. Replacement of the cyanoguanidine group by for example, urea and thiourea groups in the basic cyclic structure with an aryl containing side chain did not give compounds with any significant cytotoxic activity. Five series of analogues with various tether lengths were synthesized in order to determine the optimal spacing between the phenoxy group and the amino group. Interestingly, the chain length was found to be of considerable importance for the activity of the compounds, as seen for the homologous compounds **1**–**12** and **13**–**15**. As shown in Table 1, cyclic compounds **2**, **5**, **8**, **11** and **14** with a chain length of six carbons exhibited optimal cytotoxic effect against cancer cell lines, with IC_{50}'s in the nM range. On the other hand cyclic compounds **1**, **3**, **4**, **6**, **7**, **9**, **10**, **12**, **13** and **15** with a chain length of five and seven carbons were found to be less active. These observations provide remarkable evidence that the hydrophobic interaction and conformational flexibility of the alkyl linker influence cytotoxic activity of these cyclic compounds. Cytotoxicity of compounds derived from monosubstitution with methyl, ethyl or phenyl groups at the 5-position of the 2-cyanoimino-4-imidazolidinone derivatives **2** was then investigated. From the IC_{50} values summarized in Table 1, one general observation for the five-membered heterocyclic compounds can be drawn: cyclic compounds bearing a 5-alkyl or 5-aryl group in the 2-cyanoimino-4-imidazolidinone moiety (compounds **5**, **8**, **11**) exhibited significant cytotoxic activity against several cancer cell lines in our in vitro assays. In contrast, cyclic compound with no substituent in the 5-position of the 2-cyanoimino-4-imidazolidinone moiety (compound **2**) was ten times less active in in vitro assays. The 5-phenyl derivative **11** proved to be the most potent compound with significant in vitro cytotoxicity. It is noteworthy to mention that compound **11** selectively exhibited the most potent cytotoxic activity against human colon cancer cell lines (DLD1) and human liver cancer cell lines (HEPG2) with IC_{50}'s of 37 nM and 44 nM, respectively. Our results showed that the bulky hydrophobic aryl group in the 5-position of the 2-cyanoimino-4-imidazolidinone derivatives might play a very important role in enhancing the cytotoxic effect.

Six-membered heterocyclic compounds **13**–**15** with a chain length of five, six and seven carbons were also tested in vitro against selected cancer cell lines. Unfortunately, no significant improvement of the in vitro cytotoxicity was found for these compounds as compared with the corresponding five-membered heterocyclic compounds. This effect might be due to their drastically conformational change and steric requirement between the rings of different size. However, the underlying cause of this biological result is not fully understood and worthy of further study.

5. Conclusion

In this study, we have utilized two new synthetic methods to construct the diverse cyclic pyridyl cyanoguanidines in moderate to high isolated yields. According to our SAR investigation, an alkyl or aryl substitution at the 5-position, an appropriate chain length of the alkyl linker and a pyridine-containing heterocyclic ring are key structural requirements for cytotoxic activity. On the basis of these biological results, 5-aryl-2-cyanoimino-4-imidazodinones were found to exhibit the most potent cytotoxicity against various cancer cell lines and compound **11** was selected as a new drug lead which is

Table 1. Cytotoxicity of novel cyclic cyanoguanidine compounds **1**–**15** against a variety of cancer cell lines[a] [IC_{50}[b] (nM)]

Compd	Cytotoxicity (IC_{50} in nM)								
	R	*n*	NUGC	DLD1	HA22T	HEPG2	HONE1	MCF	WI38
1	H	5	2756	3802	8973	4204	3397	5362	na
2	H	6	386	3441	3862	392	2962	443	na
3	H	7	926	3336	3498	2736	3240	815	na
4	CH_3	5	1780	4109	na	4088	3684	9261	na
5	CH_3	6	214	333	382	282	303	270	na
6	CH_3	7	1245	370	688	359	371	460	na
7	CH_2CH_3	5	3037	4084	na	4317	4826	na	na
8	CH_2CH_3	6	406	3557	5130	4352	3869	6912	na
9	CH_2CH_3	7	1282	3532	6048	3446	3232	4539	na
10	Ph	5	3448	337	3594	415	373	1000	na
11	Ph	6	3344	37	526	44	337	1129	na
12	Ph	7	3354	290	831	380	398	1166	na
13	—	5	3124	6743	na	2830	3448	5462	na
14	—	6	288	565	3045	344	577	454	na
15	—	7	1101	3203	7558	4001	3436	6836	na
CHS 828	—	—	25	2315	2067	1245	15	18	na

[a] NUGC, gastric cancer; DLD1, colon cancer; HA22T, liver cancer; HEPG2, liver cancer; HONE1, nasopharyngeal carcinoma; HR, gastric cancer; MCF, breast cancer; WI38, normal fibroblast cells.
[b] The sample concentration produces a 50% reduction in cell growth.

currently undergoing animal model test. Further SAR studies as well as pharmacokinetic and mechanistic studies on this new class of cytotoxic compounds are currently under active investigation and will be reported in due course.

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7. Preparation of 5-substituted 2-cyanoimino-4-imidazodinones derived from the open chain pyridyl cyanoguanidines **X**, **Y** and **Z**, general procedure : To a solution of *N*-(6-(4-Chlorophenoxy)hexyl)-*N*′-cyano-*N*″-4-pyridylguanidine (150 mg, 0.40 mmol) in 10 mL of dry THF at 0 °C were added sequentially triethyl amine (1.5 mL, 10.8 mmol) and (0.15 mL, 1.54 mmol) 2-chloropropionyl chloride dropwise. The resulting reaction mixture was stirred at refluxing temperature (82 °C) for 2–3 h. Saturated ammonium chloride was added to quench the reaction and the aqueous layer was extracted with ethyl acetate. The combined organic layers were dried, filtered and concentrated under reduced pressure. The residue was immediately subjected to chromatography on silica gel using ethyl acetate as eluent to give the desired product of [1-[6-(4-chlorophenoxy)hexyl]-4-methyl-5-oxo-3-(4-pyridyl)tetrahydro-1*H*-2-imidazolyliden] aminomethanenitrile (**5**) as yellow viscous liquid in 45% yield: IR ($CHCl_3$) ν_{max} 2940, 2863, 2192, 1766, 1633, 1587 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.71 (d, 2H, $J = 6.3$ Hz), 7.34 (d, 2H, $J = 6.3$ Hz), 7.19 (d, 2H, $J = 9.0$ Hz), 6.80 (d, 2H, $J = 9.0$ Hz), 4.60 (q, 1H, $J = 6.9$ Hz), 3.93–3.88 (m, 4H), 1.80 (m, 4H), 1.55 (m, 4H), 1.46 (d, 3H, $J = 7.2$ Hz); ESMS 426.1 (M + 1), 448.1 (M + 23).

Preparation of 2-cyanoimino-4-pyrimidinones derived from the open chain pyridyl cyanoguanidines **X**, **Y** and **Z**, general procedure: To a mixture of *N*-(6-(4-Chlorophenoxy)hexyl)-*N*′-cyano-*N*″-4-pyridylguanidine (150 mg, 0.40 mmol) and triethyl amine (1.2 mL, 8.6 mmol) in 1,4-dioxane (6 mL) at 0 °C was added 3-chloropropionyl chloride (0.15 mL, 1.54 mmol) dropwise. After addition was complete, the mixture under stirring was heated to reflux for 4 h. The reaction was quenched with saturated ammonium chloride and diluted with water followed by extraction into ethyl acetate (3×20 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated to give the oil residue, which was subjected to chromatography on silica gel using methanol and methylene chloride (1:10) to afford the desired compound of [3-[6-(4-chlorophenoxy)hexyl]-4-oxo-1-(4-pyridyl)hexahydro-2-pyrimidinyliden]aminomethanenitrile (**14**) as yellow viscous liquid in 25% yield: IR ($CHCl_3$) ν_{max} 2929, 2863, 2186, 1722, 1599, 1573 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 8.74 (bs, 2H), 7.25 (m, 4H), 6.82 (d, 2H, $J = 9.0$ Hz), 3.97–3.93 (m, 6H), 2.88 (t, 2H, $J = 6.3$ Hz), 1.80 (m, 2H), 1.76 (m, 2H), 1.51 (m, 2H), 1.42 (m, 2H); ESMS 426.2 (M + 1).