Synthesis Novel Fluorinated Cyclic Nanomeric Aza Crown Macrocyclic System Containing 1,2,4-triazine moiety and Ru-complex as Cyclin-dependent kinase 2 (CDK2) inhibitors of tumor cells (Protection of DNA Damage)-Part II

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ABSTRACT

Novel fluorinated 1,5-disubstituted-1,3,5-triazepine-6,7-dione (4) has been obtained from the interaction between 5,6-bis(4-fluorophenyl)-1,2,4-triazine-3-thiol (1) with 2,6-diaminopyridine followed by ring closer reaction with diethyl oxalate. Also, Ru-complex 7 obtained by refluxing of compound 1 with 6-(4-fluorophenyl)-1,2,4-triazine-3,5-diamine (5) to produce compound 6, the later was reacted with RuCl$_3$.xH$_2$O to produce the target 7. Structures of the products deduced from their elemental analysis and spectral measurements. Compounds 3, 4, 6, and 7 were evaluated as CDK2 inhibitors of tumor cells; these compounds exhibited a potential activity against CDK2, where the IC$_{50}$ values were 4.5, 6.8, 4.0, and 5.0 μM respectively in comparison with Olomoucine standard (IC$_{50}$=5.0 μM).

Keywords: Fluorinated aza-crown, Ru-complex, CDK2 inhibitors, 1,2,4-triazine, Nanomeric.

INTRODUCTION

Recently, fluorine substituted heterocyclic nitrogen systems have been incorporated into drug discovery research to improve the physicochemical properties of drugs. Also, replacing hydrogen and other functional groups with fluorinated systems can have a dramatic effect on the modulation of electronic, lipophilic and steric parameters for example, important complexes. 6,7

On the other hand, during the past recent years, a variety of approaches have been taken for many classes of azathio crown macrocycles containing two 1,2,4-triazine rings as bioactive subunits to building new chemotherapy antitumor drugs. 8,9 Various attempts to obtain novel lariat azathia-crown macrocycles containing two heterocyclic rings as well their trivalent metal complexes have been reported. 10-13 Sun et al., 14 prepared for a stable type of ruthenium complexes containing 3-amino-1,2,4-triazino[5,6-f]1,10-phenanthroline and reported their electrochemical and photophysical properties. Moreover, Abdel-Rahman et al., 15 synthesized nanomeric thia-aza and aza-crown compounds and these systems exhibited excellent activity as potential
inhibitors of cyclin-dependent kinases 2 (CDK2) for
tumor cells. Based on these facts, the purpose of the
work is to extend the scope of our previous studies and
to substitute fluorine on various 1,2,4-triazines in
order to evaluate as CDK2 inhibitors of tumor cell.

EXPERIMENTAL

The chemicals were used, purchased from Fluka, Merck and Sigma-Aldrich
companies and used without any purification. The melting points were recorded on
Stuart scientific SMP30 (Bibby, UK) melting point apparatus and
reported as uncorrected. UV-Vis spectra (nm) were recorded on E531 UV
spectrophotometer. FT-IR spectra were recorded on Perkin Elmer Lambda 550
spectrophotometer. FT-IR (ATR, ν, cm⁻¹) was used as
solvent. Microanalysis data (C, H, N) were carried out with a Perkin Elmer 240Q elemental analyzer.

Synthesis of 1,5-disubsitiuted-2,5-dihydro-1H-1,3,5-triazepine-6,7-dione (4)

A mixture of compound 1 (1.50 g, 4.98 mmol) and compound 5 (0.51 g, 2.49 mmol) in
isopropyl alcohol (20 ml) was heated to reflux for 6 h, cooled then poured on to ice. The solid obtained was filtered washed with ethanol and recrystallized from ETOH to give 4 as yellowish solid. Yield 2.24 g, 70%. m.p: 108-110°C. FT-IR (ATR, ν, cm⁻¹): 3290 (NH), 1600 (C=N), 1250 (C-F). ¹H NMR (400 MHz, DMSO-d₆, δ, ppm): 8.80-8.44, 8.20-7.80, 7.42-7.01, 6.80-6.66 (each m, aromatic protons). ¹³C NMR (100 MHz, DMSO-d₆, δ, ppm): 145 (C-F), 141 (C=N), 130-127 (aromatic carbons), 110 (C-N). Anal. Calcd.: C, 63.10; H, 2.89; N, 20.72%.

RESULTS AND DISCUSSION

Due to the highly important of nanomeric heterocyclic systems asaza crown macrocyclic units, the presence work tends to synthesize novel fluorine substituted nanomeric aza crown bearing 1,2,4-triazines as well as their Ru-complex.
Ashraf\textsuperscript{10} reported that the synthesis of membered macrocycles fused with two triazole ring containing nitrogen, oxygen and sulfur (as strong nucleophilic centers) inside the macrocyclic ring as donor atoms. In an attempt to produce more insight into the effect of ligand functionalization on the properties of the ruthenium (II) complex, we describe here the synthesis of cyclic aza-crown systems and their novel ruthenium (II) complexes, were these complexes depend on the nature of the ligands and there is a correlation between their spectroscopic properties and Ru (II)/Ru (III) redox potential.\textsuperscript{14}

Fluorine substituted aza-crown system 4 has been obtained from the reaction of 5,6-di(4\textsuperscript{'}-fluorophenyl)-1,2,4-triazin-3-thion (1) with 2,6-diamino pyridine (2) in refluxed isopropyl alcohol to give 2,6-diheteroaryl aminopyridine 3. Ring closure reaction of 3 with diethyl oxalate in boiling THF led to the direct formation of 4 (Scheme 1). The FT-IR spectrum of 4 was recorded the presence of carbonyl group ~1700 cm\textsuperscript{-1} with lack of tow NH groups also exhibited a vibrational band at 1250 cm\textsuperscript{-1} for the presence of C-F. The \textsuperscript{1}H NMR spectrum was not showed any resonated signals above 8.80 ppm. Additionally the \textsuperscript{13}C NMR spectrum was showed resonated signals at 0180-160 ppm of two C=O carbons and 140 ppm for carbon–fluorine.

The interaction between 5,6-di(4\textsuperscript{'}-fluorophenyl)-3-mercapto-1,2,4-triazine (1) with 3,5-diamino-6-(4\textsuperscript{'}-fluorophenyl)-1,2,4-triazine (2) (by 2:1 mol) in refluxing DMF afforded N\textsubscript{3},N\textsubscript{5}-bis(5,6-bis(4-fluorophenyl)-1,2,4-triazin-3-yl)-6-(4-fluorophenyl)-1,2,4-triazine-3,5-diamine (6) (Scheme 2). It is interesting that the treatment of two-equivalent amounts of compound 6 with $\text{RuCl}_3 \cdot \text{xH}_2\text{O}$ in warming N-dimethylaniline, gave a new type of complex 7 (Scheme 2).
The FT-IR spectrum of compound 6 was recorded vibrational bands of NH groups at 3330, 3250 cm$^{-1}$, while compound 7 showed a lack of NH functional groups. FT-IR spectra of both 5 and 6 exhibited v at 1250 cm$^{-1}$ for C-F presence. On the other hand, the $^1$H NMR spectrum of 6 showed only resonated signals at $\delta$ 8.9 and 8.5 ppm attribute to NH protons of amino-1,2,4-triazines. Also, that of 7 showed a lack of NH, which confirmed that metal-complex formed. $^1$H NMR spectrum of 7 was showed a slight downfield in comparison to free ligand 6 as a result of coordination to the metal Ru ion, which acts as an electron acceptor from the heteroatoms (N) of ligand to Ru. The UV absorption of Ru-complex 7 was showed a band at 2000-380 nm was attributed to intraligand ($\pi-\pi^*$) transition, while that at >400 nm assigned to metal-ligand charge-transfer transition. Besides, magnetic measurements and electronic spectra of Ru-complex 7 formed in the range 4.00–4.25 BM at room temperature, which confirmed that structure.

Finally, the mass spectral study of compound 4 showed the molecular ion and the base peak at 700 and 214 m/z, respectively, which established the stability and the structural formula.

The Cyclin-dependent kinase 2 inhibition activity
Fluorine incorporation on key positions plays a significant role in altering the physicochemical and biological characteristics of organic chemistry. Thus, fluorine increases binding affinity, reduces plasma protein binding leading to a higher free fraction of the drug and increases cell penetration.$^{19}$ Based on these valuable observations, and in part of our containing efforts in drug development, the present work describes an attempt towards the synthesis of fluorine substituted cyclic nanomeric aza-oxo crown macrocyclic and their Ru-complexes systems as Cyclin-dependent kinase 2 inhibition of tumor cells.$^{20,22}$ One of the attempts to control the tumor cells, is Cyclin-dependent-kinases 2 evaluations.

The new fluorinated aza-oxo systems and their Ru-complexes have been evaluated to inhibit the activity of Cyclin-dependent kinase 2 in a biochemical assay. The inhibitory concentration ($IC_{50}$) values were obtained according to the reported method.$^{23,24}$ Olomoucine used as a standard, and the results data reported in Table 1.

The results obtained reveal that compounds 7, 6, 3, and 4 show very significant CDK2 inhibitory activity. Compound 7 found to be active such as Olomoucine ($IC_{50}= 5.0 \mu M$), while the other tested compounds less activity than the standard compound ($IC_{50}= 4.0-6.8 \mu M$).

Table 1: Cyclin-dependent kinase 2 inhibiting activity of fluorinated compounds 3-7

| Compounds | $IC_{50}$ ± SD (μM) |
|-----------|---------------------|
| 3         | 4.5 ± 2.5           |
| 4         | 6.8 ± 1.0           |
| 6         | 4.0 ± 2.8           |
| 7         | 5.0 ± 1.7           |
| Olomoucine| 5.0 ± 1.0           |

CONCLUSION

Simple methods to obtain fluorine substituted cyclic nanomeric aza-oxo crown macrocyclic systems bearing 1,2,4-triazine moiety have been reported, and stable Ru-complexes, also produced. Cyclin-dependent kinase 2 inhibitors of tumor cells also investigated, were the Ru-complex 7 showed potential inhibitory activity as the reference standard. This work is required in order to explore the scope and limitation of its biocidal effects.

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Conflicts of Interest
The authors declare no conflict of interest.
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