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Background

Ruthenium-based compounds are of interest because of their ability to provide possible alternatives to platinum-based chemotherapeutic agents, especially where the classical platinum drugs are not effective.[1] Moreover; in many cases these complexes show significant selectivity towards cancerous cell promising reduced damage to the healthy tissue. Although the possible cause behind this selectivity of the ruthenium complexes has been debated the most possible reason could be the mimicking ability of the ruthenium complexes to iron in reversible binding to plasma proteins such as transferrin, which is correlated with the overexpressed concentration of receptors for this protein on the surface of cancer cells.[2] Two ruthenium(III)-based drugs, which are currently under clinical trial, KP1019[3] and NAMI-A,[4] have been shown to bind to the iron(III)-binding sites of transferrin.[5]

Recently it has been shown that ruthenium(II)-arene (RAPTA) complexes, which are based on a Ru(II) center with an η^6-coordinated arene, a monodentate P-bound pta ligand, and two chloride ligands, could act as potential anticancer agent.[6] *In vitro* studies on various RAPTA complexes (differing by the substitution pattern of the arene ligand) indicate a greater toxicity toward cancer cells than healthy cells, although the corresponding IC_{50} values are relatively high. Moreover, *in vivo*, studies with [Ru(η^6-C_6H_6)(pta)Cl_2], abbreviated RAPTA-B, and [Ru(η^6-*p*-$C_6H_4Me^iPr$)(pta)Cl_2], abbreviated RAPTA-C, revealed a high activity toward metastatic tumors in combination with very low general toxicity.[7]

On the other hand Curcumin, a naturally occurring pigment in Turmeric and its analogues, are quite interesting multifaceted compounds as it is well known for its biological and pharmacological properties, i.e. antioxidant, antinflammatory and anticarcenogenic nature.[8] Although β-diketones are known to form complexes with almost every metal, few metal complexes of curcumin and its derivatives have been reported,[9] most of which are based on bis M(curc)$_2$, M=VO, Cu(II), Ni(II) and Co(II) or tris M(curc)$_3$ M=Ga(III) and In(III), for evaluation as anticancer agents in vitro and in vivo.[10]

Moreover, phenol compounds were shown to be the only competitive inhibitor with CO_2 as substrate for the main isoform of carbonic anhydrase (CA), that is, human CA II (hCA II).[11] Crystallographic evidence showed that phenol as an inhibitor behaves in a completely unprecedented manner, with its OH moiety hydrogen-bonded to the zinc bound water/hydroxide ion of the enzyme as well as to the NH amide of Thr199.[12] Sulfonamide CAIs, such as acetazolamide, methazolamide, and ethoxzolamide among others, are clinically used drugs as diuretics, antiglaucoma, or anticonvulsant agents for a long period,[13] whereas more recent drug design studies evidenced some other CAIs belonging to the sulfonamide or sulfamate classes as molecules of interest for developing novel therapies for obesity and cancer based on selective inhibition of CA isozymes involved in such pathologies, among the 16 presently known α-CAs in vertebrates.[13a, 13c]

In the present study, keeping all these aspects in mind, we have investigated the scope of the syntheses of new hybrid complexes based on RAPTA-C, RAPTA-T and RAPTA-B and on curcuminoid fragments. We have also conducted in vitro studies on the A2780 and A2780cisR cancer cell lines and the results are reported and discussed here. Furthermore, in the search of non-sulfonamide CAIs belonging to different classes of compounds, and possibly showing selectivity for isozymes of medicinal chemistry interest, we decided to examine curcumin and its ruthenium complexes as carbonic anhydrase inhibitors. These Ru(II)-curcuminate were screened for enzymatic inhibition of the physiologically dominant CA isozymes: hCA I and II and it was envisioned that this strategy could result in the preparation of improved CA inhibitors capable of inhibiting enzyme-mediated tumor growth and prompting cell death via cytotoxic mechanisms.

Results and Discussion

The neutral complexes **1a** – **1c (Fig 1)** were prepared in a one pot reaction by refluxing the appropriate $[Ru(\eta^6\text{-arene})Cl_2]_2$ dimer with two molar equivalents of curcumin in the presence of triethylamine in acetone solution for 3 hours **(scheme 1)**. Removal of the solvent and addition of diethyl ether induced the precipitation of compound **1a**. However, compounds **1b** and **1c** were precipitated in refluxing condition owing to their poor solubility in reaction medium. Compounds **1a** and **1b** were recrystallized from methanol/ether mixture and isolated in moderate to good yield (63 – 68%). However

compound **1c** is poorly soluble in common organic solvent and it was isolated and purified by repeated washing with acetone and diethylether. Compounds **2a** – **2c** were prepared by the treatment of the corresponding precursors **1a** – **1c** with pta and an excess of NH_4BF_4 in acetone/dichloromethane. The mixture was refluxed for 30 minutes to 1 hour and removal of the solvent followed by addition of diethyl ether and washing with ice cold water to remove the excess of pta and fluoroborate salts. Compound **2a** was recrystallized from acetonitrile and **2b** and **2c** from methanol/ether mixture.

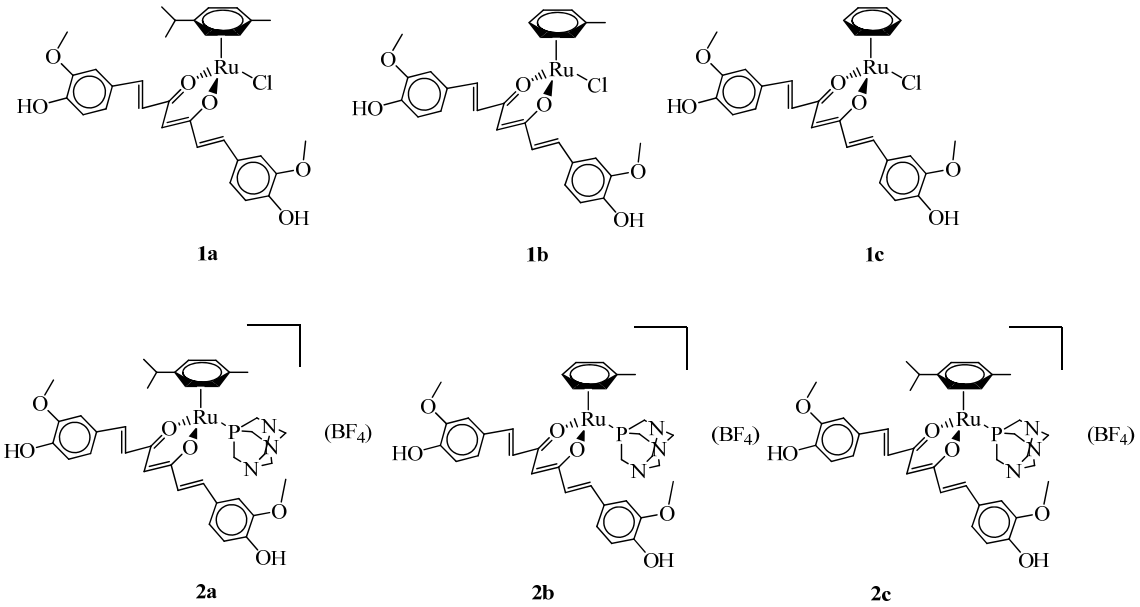
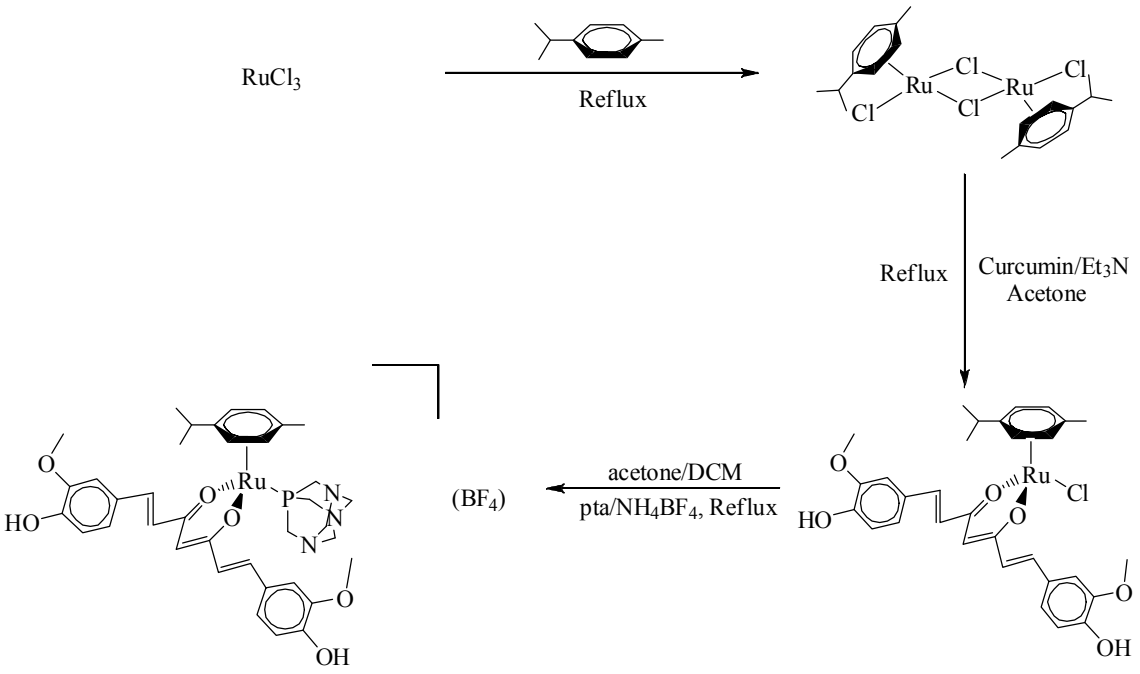


Figure 1



Scheme 1

The ^{31}P NMR spectra of **2a** – **2c** consist of one single peak in the region typical for such complexes (from δ = -26.49 ppm for **1c** to -28.60 ppm for **1a**) indicating the presence of the desired product. The ^{1}H NMR spetra of the compounds **1a** – **1c** and **2a** - **2c** all show signals that can be attributed to coordinated arene ring and curcumin moiety. Moreover compounds **2a** - **2c** also show the typical peaks for the coordinated pta ligand. The most distinctive feature for the *p*-cymene ligand is the septet between δ 2.8 and 2.9, is due to the CH proton of isopropyl group. The pta ligand gives rise to two singlet resonances. The one at higher frequency between δ 4.48 and 4.58 may be assigned to the CH_2 protons in the nitrogen heterocycle and the one at lower frequency between δ 4.23 and 4.27 corresponds to the CH_2 protons in the phosphorus-nitrogen heterocycle.

The ESI mass spectra of **1a** – **1c** provide peaks corresponding to the cation $[Ru(\eta^6\text{-arene})(Pcurc)]^+$ with loss of chloride from the parent neutral complex. Compounds **2a** – **2c** show typical peaks corresponding to the parent cation $[Ru(\eta^6\text{-arene})(Pcurc)(pta)]^+$.

In vitro evaluation

Complexes **1a**, **1b**, **2a** and **2b** were evaluated in a comparative in vitro MTT cell viability assay with two cancer cell lines, viz. A2780 human ovarian cancer cells and

the cisplatin resistant A2780cisR variant. The IC_{50} values obtained are listed in Table 1. The complexes (except **2b)** are moderately cytotoxic towards both the cell line although they are more effective in the A2780 cell line. Compound **2b** was found to almost inactive in both the cell lines. Among them the pta complex **1b** was found to be the most cytotoxic. However, no clear correlation has been established between the pta complexes and their precursors with respect to cytotoxicity. Nevertheless it should be noted that while RAPTA-C is scarcely cytotoxic, it is highly effective in vivo, displaying good activity against both metastatic and primary tumors, albeit at high doses. Thus while the greater cytotoxicity of **1a**, **2a** and **1b** may lead to activity against different tumors, they are likely to be applicable at much lower doses than RAPTA-C, which is very interesting from a pharmacological point of view.

Table 1. IC_{50} values for compounds **1a**, **1b, 2a** and **2b.**

Compound	A2780 IC_{50} [μM]	A2780cisR IC_{50} [μM]
1a	70	79
1b	48	64
2a	19	34
2b	>150	>150

Carbonic anhydrase inhibition studies

CA inhibition values for complexes **1a**, **1b**, **2a** and **2b** and curcumin (Table 2) were obtained via assaying the CA hydration of CO_2. It has been found that the organometallic complexes are weaker inhibitors of CA compared with pure curcumin. The complexes inhibited CA in low micromolar concentrations. The correlations between the structure of the complexes and their activity are currently under investigation. However, it should be noted that the chloro complexes are better inhibitors of CA compared to the pta-compounds.

Table 2. Carbonic anhydrase inhibition of the ruthenium(II) complexes and curcumin.

Compound	K_i (nM)[a]	
	hCA I[b]	hCA II[b]

Curcumin	4.0	3.8
1a	7.0	5.3
1b	7.7	6.7
2a	10.0	7.4
2b	11.0	8.9

[a] Errors within the range of ±5–10% of the reported value. The results are the average of the values obtained in three separate experiments.

[b] Human (cloned) isozymes, by the CO_2 hydration method.

Conclusions

Synthesis of new hybrid complexes (**1a-1c** and **2a-2c**) based on RAPTA-C, RAPTA-T and RAPTA-B with curcumin and pta has been carried out. These compounds have been characterized by ^{1}H, ^{13}C and ^{31}P (where applicable) NMR spetroscopy, elemental analyses and mass spectrometry. Preliminary biological studies of these compounds on the A2780 and A2780cisR cancer cell lines reveal moderate activity. Screening for enzymatic inhibition of the physiologically dominant CA isozymes show that the compounds are weak inhibitors of CA compared to curcumin. The complexes inhibited CA in low micromolar concentrations.

Impact

Contribution to the socio-economic development of the Developing Countries or emerging and transition economies by transfer of knowledge and human capacity building

The researcher is an Indian national where cancer is a major problem as 2.5 million cases are estimated at any given time. As the researcher returning to India with a

permanent position, it has been an important learning experience for him to interact with one of the leading groups working on the synthesis of the targeted organometallic anticancer drug and their mode of action. There is no doubt that on his return, he can now utilize the acquired skills, in this area towards the development of superior and more effective antimetastasis drugs, vital for an economically developing country like India where forefront research in this field is not very common.

Use

The results obtained in this project provide an opening towards the estimation of possibility of using ruthenium(II)-curcuminate complexes as possible anticancer drugs. As the project has been ended abruptly due to the return of the fellow to India with a permanent position in a leading educational research institute it has been foreseen that it will open a fruitful collaboration between coordinator and IIF research fellow to investigate this area in depth in the near future.

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