Applied Organometallic Chemistry (1989) 3 491–497

0268–2605/89/03604491/$03.50

Assessment of the *in vitro* broad-spectrum antiviral activity of some selected antitumor metallocene and metallocenium complexes

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Received 15 March 1989 *Accepted 26 July 1989*

Six neutral metallocenes and six metallocenium salts, all of which have demonstrated antiproliferative properties, were evaluated for their *in vitro* broad-spectrum antiviral properties and cytotoxicities. The metallocenes include the compounds (η-$C_5H_5)_2MCl_2$, where M = Ti, V, Mo, Zr and Hf, and (η-$C_5H_5)_2$Tibis(hydrogen maleinate), whereas the metallocenium complexes include the three ferrocenium salts, (η-$C_5H_5)_2Fe^+X^-$, where X^- = trichloroacetate, tetrachloroferrate(III) and picrate, and three recently discovered antitumor titanocenium complexes, i.e. [(η-$C_5H_5)_2Ti(CH_3CN)Cl^+$] [$FeCl_4^-$], [(η-$C_5H_5)_2$Ti(2,2′-bipyridyl)$^{2+}$][$CF_3SO_3^-]_2$, and [(η-$C_5H_5)_2$Ti(*N*-methyl-*o*-aminothiophenolate$^+$] [I^-]. These 12 species were evaluated against DNA viruses (herpes simplex virus type 1, type 2 and vaccinia virus), and RNA viruses [vesicular stomatitis virus, Coxsackie virus B4, Sindbis virus, Semliki forest virus, parainfluenza virus type 3 and human immunodeficiency virus type 1 (HIV-1), the etiologic agent of AIDS]. In the case of HIV-1, the complexes were evaluated for their ability to inhibit HIV-associated reverse transcriptase activity and HIV-1 induced cytopathogenicity in human T-lymphocyte MT4 cells. Selectivity indexes [ratio of the minimum cytotoxic concentration (dose) to the minimum (antiviral) inhibitory concentration (dose)] were determined for all complexes and viruses. In general, the netural metallocenes and the ferrocenium salts were only marginally active towards some specific viruses. However, [(η-$C_5H_5)_2$Ti(bipy)$_2^{2+}$][$CF_3SO_3^-]_2$ was active towards the DNA viruses at a concentration that was ten times lower than the cytotoxicity threshold. (η-$C_5H_5)_2VCl_2$ was weakly inhibitory towards HIV reverse transcriptase. All species were ineffective in inhibiting HIV-induced cytopathogenicity in human T-lymphocyte MT4 cells.

Keywords: Metallocenes, metallocenium complexes, antiviral activity, DNA viruses, RNA viruses, HIV-1

INTRODUCTION

This discovery of the antitumor activity of cisplatin [*cis*-$PtCl_2(NH_3)_2$] in 1969 by Rosenberg and coworkers[1] and subsequent developments in this area in the past 20 years have helped alter, to a large degree, the bias against inorganic compounds as clinically useful chemotherapeutic agents. In addition to cisplatin and other platinum-containing complexes, a number of inorganic complexes of other platinum-group metals have been shown to exhibit cytostatic properties although none have yet proven useful in clinical trials. A third group of antitumor active compounds encompass several main-group elements, most notably gallium nitrate,[2] a number of organogermanium complexes,[2] and some organotin(IV) species.[3] Finally, several metallocene and metallocenium complexes have been shown to possess tumor-inhibiting efficacy. These include: (a) the neutral metallocene dichlorides, (η-$C_5H_5)_2MCl_2$ (**1**) and derivatives such as titanocene bis(hydrogen maleinate)

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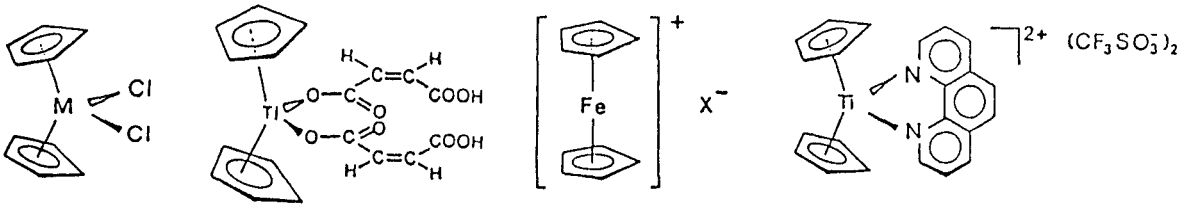

[1] [2] [3] [4]

(**2**); (b) ferrocenium complexes, $[(\eta\text{-}C_5H_5)_2Fe^+]X^-$, where the anions X^- include trichloroacetate ($Cl_3CO_2^-$), picrate (2,4,6- $(NO_2)_3C_6H_2O^-$), and tetrachloroferrate(III) ($FeCl_4^-$) (**3**);[4,5] and (c) some recently discovered titanocenium complexes of the type $[(\eta\text{-}C_5H_5)_2TiXL]Y$ or $[(\eta\text{-}C_5H_5)_2Ti](Y)_2$, where X is an anionic ligand, L is a neutral mono- or bi-dentate Lewis base and Y is a non-coordinated anion (**4**).[6]

During the past few years, attention has been focused on the potential antiviral efficacy of various inorganic and organometallic antitumor agents. In particular, it has been shown that cisplatin exhibits antiherpetic activity both *in vitro* and *in vivo*.[7] Also, several other second-generation platinum-containing antitumor agents[7] as well as some diorganotin species[8] are weakly active against herpes simplex virus types 1 and 2 *in vitro*. Since the antitumor metallocene and metallocenium complexes mentioned above represent a class of agents whose antiproliferative properties distinguish them from the other groups of inorganic antitumor agents, it was of interest to examine the broad-spectrum antiviral activity of some representative examples in this class. Thus this paper presents an assessment of the *in vitro* antiviral activity of six neutral metallocene compounds, i.e. $(\eta\text{-}C_5H_5)_2MCl_2$, when M = Ti, V, Mo, Zr and Hf, and $(\eta\text{-}C_5H_5)_2Ti$ [bis(hydrogen maleinate)]; three ferrocenium complexes, $[(\eta\text{-}C_5H_5)_2Fe^+)X^-$ where $X^- = Cl_3CO_2^-$, picrate and $FeCl_4^-$; and three titanocenium complexes, $[(\eta\text{-}C_5H_5)_2Ti(2,2'\text{-bipyridyl})^{2+}][CF_3SO_3^-]_2$, $[(\eta\text{-}C_5H_5)_2TiCl(\text{acetonitrile})^+][FeCl_4^-]$, and $[(\eta\text{-}C_5H_5)_2Ti(N\text{-methyl-}o\text{-aminothiophenolate})^+]I^-$. These complexes were evaluated against three DNA viruses, herpes simplex virus type 1, type 2 and vaccinia virus; and the RNA viruses vesicular stomatitis virus, Coxsackie virus type B4, Sindbis virus, Semliki forest virus, parainfluenza virus type 3, and the retrovirus HIV-1. Titanocene dichloride and the corresponding molybdenum analog have been the subject of a previous assessment of their antiviral potential;[9] there is virtually no overlap between the two studies.

MATERIALS AND METHODS

Metallocene and metallocenium complexes

The neutral biscyclopentadienyl metal dichlorides, $(\eta\text{-}C_5H_5)_2MCl_2$, where M = Ti, V, Mo, Zr and Hf, were synthesized and purified according to methods described in the literature.[10,12] Titanocene bis(hydrogen maleinate),[13] the three ferrocenium complexes,[14,15] and the three ionic, salt-like titanocenium complexes[16–18] were synthesized by methods described previously. For the assessment of antiviral activity the six neutral metallocenes were first dissolved in dimethylsulfoxide (DMSO) (10 mg cm^{-3}) and diluted with culture medium to give a range of final concentrations of 200–0.002 $\mu g\ cm^{-3}$. The concentration of DMSO had no effect either on viral-induced cytopathogenicity or cell growth as determined in separate experiments. The cationic ferrocenium complexes were dissolved in phosphate-buffered saline and diluted with culture medium to give final concentrations in the range 400–0.004 $\mu g\ cm^{-3}$. The three ionic titanocenium complexes were dissolved in DMSO (20 mg cm^{-3}) and diluted with medium to give concentrations in this same range.

Viruses

The origin of the viruses was as follows: herpes simplex virus type 1 (strain KOS) and herpes simplex virus type 2 (strain G), see Ref. 19; vaccinia virus, vesicular stomatitis virus, Coxsackie virus type B4 and Sindbis virus, see Ref. 20; Semliki forest virus (ATCC VR-67) and parainfluenza virus type 3 (ATCC VR-93),

American Type Culture Collection, Rockville, MD, USA. The virus stocks were grown in primary rabbit kidney (PRK) cells (herpes simplex virus types 1 and 2, vaccinia virus and vesicular stomatitis virus), Vero cells (Coxsackie virus B4 and Semliki forest virus), chicken embryo cells (Sindbis virus), or human embryonic lung cells (parainfluenza virus). HIV-1 was obtained from the culture supernatant of an H9 cell line persistently infected with HTLV-III_B,[21] which was kindly provided by Dr R C Gallo, National Cancer Institute, Bethesda, MD, USA.

ANTIVIRAL ASSAYS

Confluent cell cultures in 96-well microtiter trays were inoculated with 100 $CCID_{50}$ (one $CCID_{50}$ corresponds to the virus stock dilution that is infective for 50% of the specific cell cultures under test). After adsorption of the virus to the cells for 1 h at 37°C, residual virus was removed from the wells and replaced with cell culture medium (Eagle's minimum essential medium) supplemented with 3% fetal calf serum and various concentrations of the compounds. Viral cytopathogenicity was recorded as soon as it reached completion in the untreated virus-infected cell cultures, i.e., at 1 to 2 days for vesicular stomatitis virus; at 2 days for Coxsackie virus and Semliki forest virus, at 3 days for vaccinia virus, herpes simplex virus and Sindbis virus, at 5 days for human immunodeficiency virus, and at 6 to 7 days for parainfluenza virus. The antiviral activity of the compounds was expressed as the minimum (antiviral) inhibitory concentration (MIC) ($\mu g\ cm^{-3}$) required to inhibit viral cytopathogenicity by 50%. For HIV-1-induced cytopathogenicity in MT-4 cells,[22] this activity was expressed as the ED_{50} (50% effective dose, or dose required to reduce virus-induced cytopathogenicity by 50%).

HIV-1 reverse transcriptase assay

The reverse transcriptase assay was carried out with virus preparations isolated from HIV-1-infected H9 cell cultures. Inhibition of this enzyme by the test compounds was measured during 60 min, the timeframe in which the reverse transcriptase activity was linear. The procedure was based on the method of Balzarini *et al.*,[23] with some slight modifications. Briefly, exogenous poly(rA);oligo$(dT)_{12-18}$ served as the template:primer for the enzyme. The reaction mixture (50 μL) contained 5 mmol dm^{-3} dithiothreitol, 300 μmol dm^{-3} glutathione, 50 mmol dm^{-3} Tris–HCl (pH 7.8), 5 mmol dm^{-3} $MgCl_2$, 150 mmol dm^{-3} KCl, 1.25 μg of bovine serum albumin, 1 μmol dm^{-3} of [*methyl*-^{3}H]dTTP (specific radioactivity 30 Ci $mmol^{-1}$) (5 μCi), 0.01 unit of poly(rA):oligo$(dT)_{12-18}$, 0.03% Triton X-100, 10 μL of the compound solution (containing varying concentrations of the test compounds), and 10 μL of the HIV-1 reverse transcriptase preparation [partially purified by low centrifugation of the supernatant of a H9/HTLV-III_B cell suspension, followed by filtration (0.45 μm) and ultracentrifugation (100 000 g, 2 h)]. The reaction mixture was incubated for 60 min at 37°C, at which time 200 μL of ice-cold 5% trichloroacetic acid was added to stop the reaction. After an additional 30 min at 0°C, the acid-insoluble material was filtered, washed with water, dried and analyzed for radioactivity. Suramin, a potent inhibitor of HIV reverse transcriptase,[24] was included as a standard.

Cytotoxicity

Measurement of cytotoxicity was based on the alteration of normal cell morphology. To evaluate this parameter, confluent cell cultures which had not been inoculated with virus but treated with various concentrations of the complexes were incubated in parallel with the virus-infected cell cultures. These cultures were examined microscopically at the same time as viral cytopathogenicity was recorded for the virus-infected cells. Disruption of the cell monolayer, e.g. rounding up or detachment of the cells, was considered as evidence for cytotoxicity. Cytotoxicity of the compounds against PRK and Vero cell monolayers was expressed as the minimum cytotoxic concentration (MTC) ($\mu g\ cm^{-3}$) required to cause a microscopically detectable alteration of normal cell morphology. Cytotoxicity of the compounds against MT-4 cells was expressed as the dose required to reduce the viability of the cells by 50% (CD_{50}).

RESULTS AND DISCUSSION

Six neutral biscyclopentadienyl metal complexes, i.e. five metallocene dichlorides, $(\eta\text{-}C_5H_5)_2MCl_2$ (where M = Ti, V, Mo, Zr and Hf), and titanocenebis

(hydrogen maleinate) $[(\eta\text{-}C_5H_5)_2Ti(OCOCH{=}CHCOOH)_2]$; three ferrocenium complexes, $[(\eta\text{-}C_5H_5)_2Fe^+X^-$ (where $X = Cl_3CO_2^-$, $FeCl_4^-$ and picrate)]; and three cationic titanocenium complexes, i.e. $[(\eta\text{-}C_5H_5)_2Ti(2,2'\text{-bipyridyl})^{2+}][CF_3SO_3^-]_2$, $[(\eta\text{-}C_5H_5)_2Ti(acetonitrile)Cl^+][FeCl_4^-]$, and $[(\eta\text{-}C_5H_5)_2Ti(N\text{-methyl-}o\text{-aminothiophenolate})^+][I^-]$, were assessed for their antiviral potential in a number of virus assay systems.

Neutral metallocenes

The six neutral metallocenes were evaluated against herpes simplex virus types 1 and 2, vaccinia virus, and vesicular stomatitis virus in primary rabbit kidney (PRK) cells (Table 1), and against Coxsackie, Sindbis, Semliki forest and parainfluenza virus in Vero cell cultures (Table 2). The antiviral effects of these complexes were compared with those of four reference antiviral agents: (*S*)-DHPA [(*S*)-9-(2,3-dihydroxypropyl)adenine],[25] C-c^{3}Ado[3-deazaaristeromycin],[26] tubercidin[27] and ribavirin.[28] The antiviral and cytotoxic effects of the standard compounds were determined in experiments run in parallel with the title complexes. A selectivity index[29] (S.I.) was calculated for each compound. This parameter, as defined in the current study, is the ratio of the minimum cytotoxic concentration (MTC) to the minimum antiviral concentration (MIC) required to inhibit virus-induced cytopathogenicity by 50%, and thus it represents a measure of the antiviral effectiveness of a given agent. The results are listed in the final table. As a whole, the compounds were active against virus replication at concentrations that approached or coincided with those that were toxic to the host cells. Thus the selectivity index was generally close to 1. In some cases it reached 2.0–2.5. The results for $(\eta\text{-}C_5H_5)_2TiCl_2$ are consistent with those found previously.[9] The only other metallocene common to the two studies, $(\eta\text{-}C_5H_5)_2MoCl_2$, was reported to be inactive towards vesicular stomatitis virus.

Ferrocenium complexes

The three ferrocenium complexes, $(\eta\text{-}C_5H_5)_2Fe^+X^-$ where X^- = trichloroacetate, tetrachloroferrate(III) or picrate, did not exhibit antiviral activity at 200 $\mu g\,cm^{-3}$. They were also less cytotoxic than the neutral metallocenes (Tables 1 and 2).

Table 1 Antiviral and cytotoxic effects of metallocenes and metallocenium complexes in PRK cell cultures.

Compound	Minimum (antiviral) inhibitory concentration[a] ($\mu g\,cm^{-3}$)				Minimum cytotoxic concentration[b] ($\mu g\,cm^{-3}$)
	HSV-1(KOS)	HSV-2(G)	Vaccinia virus	Vesicular stomatitis virus	
$(\eta\text{-}C_5H_5)_2TiCl_2$	>40	>40	>40	>40	>100
$(\eta\text{-}C_5H_5)_2$Tibis(hydrogen maleinate)	>100	>100	>100	>100	>100
$(\eta\text{-}C_5H_5)_2VCl_2$	>10	>10	>10	>10	>10
$(\eta\text{-}C_5H_5)_2MoCl_2$	>60	>60	>60	>30	>60
$(\eta\text{-}C_5H_5)_2ZrCl_2$	>50	>50	>50	>50	50
$(\eta\text{-}C_5H_5)_2HfCl_2$	>50	>50	>30	>30	50
$[(\eta\text{-}C_5H_5)_2Fe^+][Cl_3CO_2^-]$	>200	≥200	>200	>200	≥200
$[(\eta\text{-}C_5H_5)_2Fe^+][FeCl_4^-]$	>400	>400	>200	>200	>400
$[(\eta\text{-}C_5H_5)_2Fe^+]$[picrate$^-$]	>400	>400	>200	>200	>400
$[(\eta\text{-}C_5H_5)_2Ti(bipy)^{2+}][CF_3SO_3^-]_2$	20	20	20	≥200	≥200
$[(\eta\text{-}C_5H_5)_2Ti(NCCH_3)Cl^+][FeCl_4^-]$	>40	>40	>40	>40	≥40
$[(\eta\text{-}C_5H_5)_2Ti(o\text{-}S(NHCH_3)C_6H_4^-]I^-$	>40	>40	>40	>40	≥40
Tubercidin	>0.1	>0.1	>0.1	0.2	0.4
(*S*)-DHPA	>400	300	40	70	≥400
Ribaririn	>400	>400	10	>400	≥400
C-c^{3}Ado	>400	200	0.7	7	>400

[a]Required to inhibit virus-induced cytopathogenicity by 50%; average values for two or three experiments. [b]Required to cause a microscopically detectable alteration of normal cell morphology; average of 5 or 6 experiments.

Table 2 Antiviral and cytotoxic effects of metallocenes and metallocenium complexes in Vero cell cultures.

Compound	Minimum (antiviral) inhibitory concentration[a] ($\mu g\ cm^{-3}$)				Minimum cytotoxic concentration[b] ($\mu g\ cm^{-3}$)
	Coxsackie virus B4	Sindbis virus	Semliki forest virus	Parainfluenza virus type 3	
$(\eta\text{-}C_5H_5)_2TiCl_2$	50	>100	>100	>100	>100
$(\eta\text{-}C_5H_5)_2$Tibis(hydrogen maleinate)	>200	>200	>200	>200	>200
$(\eta\text{-}C_5H_5)_2VCl_2$	>10	>10	>10	>10	>10
$(\eta\text{-}C_5H_5)_2MoCl_2$	20	>40	>40	>40	>40
$(\eta\text{-}C_5H_5)_2ZrCl_2$	40	>100	>100	>100	≥100
$(\eta\text{-}C_5H_5)_2HfCl_2$	≥60	>60	>60	>60	≥60
$[(\eta\text{-}C_5H_5)_2Fe^+][Cl_3CO_2^-]$	>200	≥200	>200	>200	≥200
$[(\eta\text{-}C_5H_5)_2Fe^+][FeCl_4^-]$	>200	>200	>200	>200	≥200
$[(\eta\text{-}C_5H_5)_2Fe^+]$[picrate$^-$]	>200	>200	>200	>200	≥200
$[(\eta\text{-}C_5H_5)_2Ti(bipy)^{2+}][CF_3SO_3^-]_2$	>40	>40	>40	>10	≥40
$[(\eta\text{-}C_5H_5)_2Ti(NCCH_3)Cl^+][FeCl_4^-]$	>100	>100	>100	>40	≥200
$[(\eta\text{-}C_5H_5)_2Ti(o\text{-}S(NHCH_3)C_6H_4^+]I^-$	>100	>100	>100	>40	≥200
Tubercidin	0.07	>0.1	>0.4	>0.1	0.4
(*S*)-DHPA	150	>400	>400	70	≥400
Ribavirin	150	100	150	70	≥400
C-c^{3}Ado	70	>400	>400	2	>200

[a]Required to inhibit virus-induced cytopathogenicity by 50%; average values for two or three experiments. [b]Required to cause a microscopically detectable alteration of normal cell morphology; average of 3 experiments.

Titanocenium complexes

Because of the broad-spectrum antiviral activity, albeit rather weak, of titanocene dichloride, it was of interest to evaluate the relatively new, antitumor, ionic, biscyclopentadienyl titanium complexes, i.e. $[(\eta\text{-}C_5H_5)_2Ti(2,2'\text{-bipyridyl})^{2+}]\ [CF_3SO_3^-]_2$, $[(\eta\text{-}C_5H_5)_2Ti(\text{acetonitrile})Cl^+]\ [FeCl_4^-]$ and $[(\eta\text{-}C_5H_5)_2Ti(o\text{-}S(NHCH_3)C_6H_4^+]I^-]$. Interestingly, the former species was active towards the three DNA viruses included in this study. It inhibited these viruses at a concentration that was 10-fold lower than the minimum cytotoxic concentration (Table 1). The two other titanocenium complexes were inactive towards the DNA viruses. None of the titanoceniums showed significant activity against RNA viruses. In general, all complexes included in this study were far less efficacious as antiviral agents when compared with the most active of the four standard compounds. Although $[(\eta\text{-}C_5H_5)_2Ti(bipy)^{2+}][CF_3SO_3^-]_2$ was more inhibitory towards herpes simplex virus type 1 and 2 than the four standards, its S.I. is still relatively low when compared with that of the current clinically approved drug for herpes simplex virus infections, i.e. acyclovir.[30]

Finally, the 12 metallocenes and metallocenium complexes were assessed for their activity towards the human retrovirus, HIV-1, the etiologic agents of AIDS.[31,32] One of the strategies to combat this disease that has been adopted with some degree of success is to identify agents which specifically inhibit virus-encoded RNA-directed DNA polymerase (reverse

Table 3 Anti-HIV-1 and cytotoxic effects of metallocenes and metallocenium complexes in MT-4 cells

Compound	ED_{50}[a] ($\mu g\ cm^{-3}$)	CD_{50}[b] ($\mu g\ cm^{-3}$)
$(\eta\text{-}C_5H_5)_2TiCl_2$	>200	>200
$(\eta\text{-}C_5H_5)_2$Tibis(hydrogen maleinate)	>200	>200
$(\eta\text{-}C_5H_5)_2VCl_2$	>8	8–40
$(\eta\text{-}C_5H_5)_2MoCl_2$	>100	>100
$(\eta\text{-}C_5H_5)_2ZrCl_2$	>200	>200
$(\eta\text{-}C_5H_5)_2HfCl_2$	>200	>200
$[(\eta\text{-}C_5H_5)_2Fe^+][Cl_3CO_2^-]$	>40	52
$[(\eta\text{-}C_5H_5)_2Fe^+][FeCl_4^-]$	>8	16
$[(\eta\text{-}C_5H_5)_2Fe^+]$[picrate]	>8	16
$[(\eta\text{-}C_5H_5)_2Ti(bipy)^{2+}][CF_3SO_3^-]_2$	–[c]	–[c]
$[(\eta\text{-}C_5H_5)_2Ti(CH_3CN)Cl^+][FeCl_4^-]$	>40	155
$[(\eta\text{-}C_5H_5)_2Ti(o\text{-}S(NHCH_3)C_6H_4^+]I^-$	>200	>200

[a]50% effective dose required to inhibit HIV-1-induced cytopathogenicity in MT-4 cells by 50%. [b]50% cytotoxic dose required to reduce the viability of MT-4 cells by 50%. [c]–, Not tested. (Text reference to Table 3, p. 497.)

Table 4 Antiviral selectivity indexes (S.I.) for the metallocenes and metallocenium complexes

Compound	HSV-1(KOS)	HSV-2(G)	Vaccinia virus	Vesicular stomatitis virus	Coxsackie virus type B4	Sindbis virus	Semliki forest virus	Parainfluenza virus type 3	HIV-1
$(\eta\text{-}C_5H_5)_2TiCl_2$	≤ 2.5	≤ 2.5	≤ 2.5	≤ 2.5	>2	$><1$[a]	$><1$	$><1$	$><1$
$(\eta\text{-}C_5H_5)_2$Tibis(hydrogen maleinate)	$><1$	$><1$	$><1$	$><1$	$><1$	$><1$	$><1$	$><1$	$><1$
$(\eta\text{-}C_5H_5)_2VCl_2$	$><1$	$><1$	$><1$	$><1$	$><1$	$><1$	$><1$	$><1$	<5
$(\eta\text{-}C_5H_5)_2MoCl_2$	$><1$	$><1$	$><1$	≤ 2	≥ 2	$><1$	$><1$	$><1$	$><1$
$(\eta\text{-}C_5H_5)_2ZrCl_2$	<1	<1	<1	<1	≥ 2.5	$><1$	$><1$	$><1$	$><1$
$(\eta\text{-}C_5H_5)_2HfCl_2$	<1	<1	≤ 1.6	≤ 1.6	$><1$	<1	<1	<1	$><1$
$[(\eta\text{-}C_5H_5)_2Fe^+][Cl_3CO_2^-]$	≤ 1	1	≤ 1	≤ 1	<1	<1	<1	<1	<1
$[(\eta\text{-}C_5H_5)_2Fe^+][FeCl_4^-]$	$><1$	$><1$	≤ 2	≤ 2	<1	<1	<1	<1	<2
$[(\eta\text{-}C_5H_5)_2Fe^+]$[picrate]	$><1$	$><1$	≤ 2	≤ 2	<1	<1	<1	<1	<2
$[(\eta\text{-}C_5H_5)_2Ti(bipy)^{2+}][CF_3SO_3^-]_2$	≥ 10	≥ 10	≤ 10	1	<1	<1	<1	<4	–
$[(\eta\text{-}C_5H_5)_2Ti(CH_3CN)Cl^+][FeCl_4^-]$	<1	<1	<1	<1	<2	≤ 2	≤ 2	≤ 5	<4
$[(\eta\text{-}C_5H_5)_2Ti(o\text{-}S(NHCH_3)C_6H_4^-]I^-$	<1	<1	<1	<1	<2	≤ 2	≤ 2	≤ 5	$><1$
Tubercidin	<2	<4	<4	5.7	≥ 5.7	≥ 5.7	1	≥ 4	
(*S*)-DHPA	1	>1	≥ 40	≥ 20	1	≥ 5.7	1	≥ 20	
Ribavirin	1	1	≥ 100	≥ 2	≥ 2.7	>2.7	1	>5.7	
C-c^{3}Ado	1	>1	≥ 800	≥ 800	≥ 20	≥ 5.7	1	≥ 200	

[a] $><$, approx. unity.

transcriptase). For example, the 5′-triphosphate of 3′-azido-2′,3′-dideoxythymidine (AZT) is a potent and selective inhibitor of HIV-1 reverse transcriptase.[33] Accordingly, the metallocenes and metallocenium complexes were investigated for their activity towards HIV-1 reverse transcriptase activity and for their ability to inhibit the cytopathic effect of HIV in human T-lymphocyte MT4 cells. In the former experiments, only $(\eta\text{-}C_5H_5)_2VCl_2$ was found to inhibit HIV-1 reverse transcriptase activity at a concentration $<200\ \mu g\ cm^{-3}$, the highest concentration tested. Its 50% inhibitory concentration was found to be $141\ \mu g\ cm^{-3}$ ($560\ \mu mol\ dm^{-3}$), approximately 25000-fold higher than the IC_{50} of AZT 5′-triphosphate. None of the complexes was found to be effective in inhibiting HIV-induced cytopathogenicity in human T-lymphocyte MT4 cells at subtoxic concentrations (Table 3).

In conclusion, a number of antitumor neutral metallocenes and ferrocenium complexes demonstrated only marginal antiviral activity when evaluated against a select number of DNA and RNA viruses. Of the three titanocenium complexes, $[(\eta\text{-}C_5H_5)_2\ Ti(bipy)^{2+}]\ [CF_3SO_3^-]_2$ had distinct activity against DNA viruses, especially the two selected strains of herpes simplex virus and vaccinia virus. Selectivity Indexes are given in Table 4.

Acknowledgements The authors thank Professor Dr V Thewalt, Ulm, Germany, for providing a sample of the titanocenium bipyridyl complex and gratefully acknowledge the excellent technical assistance of Anita Van Lierde, Frieda De Meyer and Ann Absillis. R C T would like to thank Oakland University for a research retraining leave during the academic year 1987–88. S G W and R C T would like to express their gratitude to E D C for providing laboratory space and supplies to carry out this research at the Rega Institute.

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