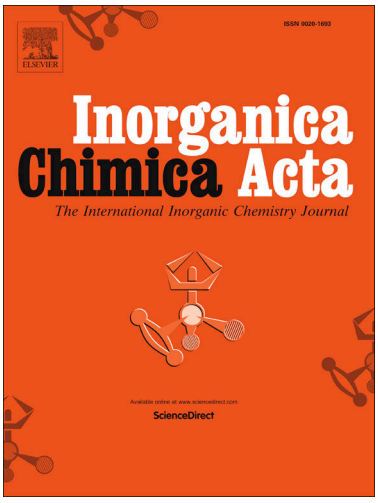
Journal Pre-proofs



Research paper

Synthesis of Theophylline-Based Iridium(I) N-Heterocyclic Carbene Complexes Including Fluorinated-Thiophenolate ligands. Preliminary Evaluation of Their *In Vitro* Anticancer Activity

Itzel Eslava-Gonzalez, Hugo Valdés, María Teresa Ramírez-Apan, Simón Hernandez-Ortega, Miriam Rosario Zermeño-Ortega, Alcives Avila-Sorrosa, David Morales-Morales

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Synthesis of Theophylline-Based Iridium(I) N-Heterocyclic Carbene Complexes Including Fluorinated-Thiophenolate ligands. Preliminary Evaluation of Their *In Vitro* Anticancer Activity.

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Abstract

A series of theophylline-based Ir(I) N-heterocyclic carbene complexes including fluorinated-thiolate ligands have been prepared and fully characterized. The molecular structures of the complexes [(NHC)Ir(SC_6F_5)(COD)] (**3a**) and [(NHC)Ir(SC_6F_4H-4)(COD)] (**3b**) were unambiguously determined by single crystal X-ray diffraction analysis. Both compounds were isostructural, having the theophylline-imidazolylidene ligand coordinated to the metal center and completing the coordination sphere with a 1,5-COD and thiophenolate ligands. Interestingly, both complexes exhibit both inter- and intra-molecular π–stacking interactions between the fluorinated ring of the thiolate and the theophylline-based NHC ligand. Furthermore, for the series of Ir(I) NHC complexes preliminary *in vitro* anticancer activity experiments were performed on six human cancer cell-lines, *i.e.* glia cells of nervous central system (U-251), prostate (PC-3), leukemia (K-562), colon (HCT-15), breast (MCF-7) and lung (SKLU-1). Being complex (**3**) the one showing the best performance (compared to cisplatin) against PC-3 and SKLU-1 with IC_{50} values of 7.8 ± 0.4 μM and 10.7 ± 0.7 μM, respectively.

Keywords: N-heterocyclic carbene; Iridium complexes; Theophylline complex; Cancer; Cytotoxic activity; Thiolate complexes; Xanthines.

1. Introduction

In the past two decades, the use of natural products as scaffolds for the preparation of complexes has attracted much attention [1]. Several natural products can be easily isolated, and their pharmaceutical applications have been well established.[1] Thus, the combination of biological active compounds with a metal center can produce species with interesting pharmaceutical applications. In this sense, xanthines are natural products that can be extracted principally from cacao, coffee and tea. Among the best-known xanthines, we have caffeine, theophylline and theobromine, whose structures are excellent scaffolds for the preparation of N-heterocyclic carbene (NHC) ligands. In general, NHC ligands may form very strong C-M bonds with almost any transition metal, avoiding NHC-metal bond dissociation, a highly desirable feature for the design of catalysts and pharmaceutical agents.

One of the first examples of xanthine-based NHC complexes was described in 1975 by Taube [2], since then few reports have been published [3]. These reports have focused in their biological [4-10] and catalytic applications [11-13], as well as their preparation [14-18] that often is not trivial. In 2014, Picquet and Casini described the antitumor activity of a series of Au(I) xanthine-NHC complexes against human ovarian cancer cell line A2780/R, which is resistant to cisplatin [6]. These compounds showed IC_{50} values in the micromolar scale, being lower than that of cisplatin. Other xanthine-NHC complexes based on transition metals such as Ag(I) [7], Pt(II) [19] and Pd(II) [10] have also been tested against some cancer cell lines (Figure 1) showing promising results.

Recently, Iridium has been used for the design of antiproliferative agents, especially when its oxidation state is +III [20-30]. However, there are some promising examples of Ir(I) complexes for this purpose [31-33]. Metzler-Nolte and co-workers described the

cytotoxic activity of a series of complexes of the type [(NHC)IrCl(COD)], where NHC corresponds to imidazolylidene and triazolylidene ligands [34-36]. These compounds showed IC_{50} values in the micromolar scale, ranging from 10.3 ± 2.9 to 46.920 ± 0.085 μM for MCF-7. Based on the aforementioned reasons, herein we reported the synthesis and preliminary cytotoxic activity of a series of Ir(I) theophylline-NHC complexes including fluorinated-thiophenolate ligands (Figure 1).

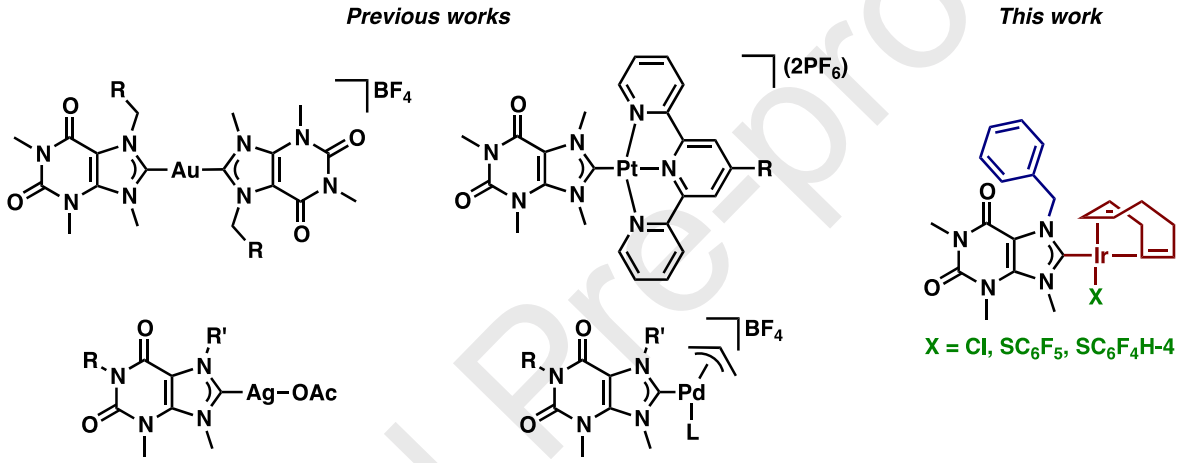
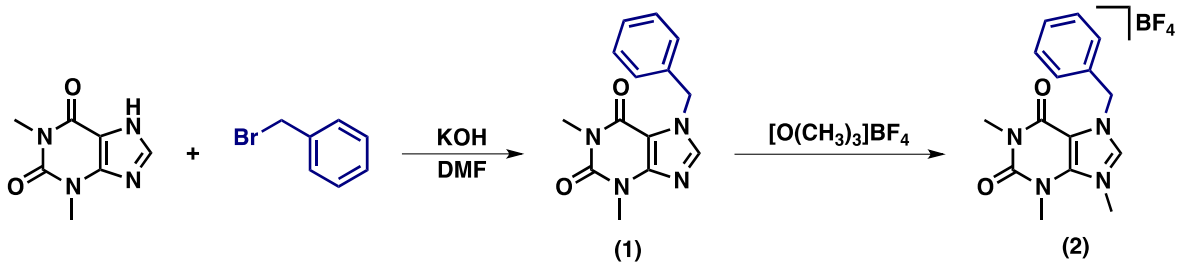


Figure 1. Cytotoxic xanthine-based complexes

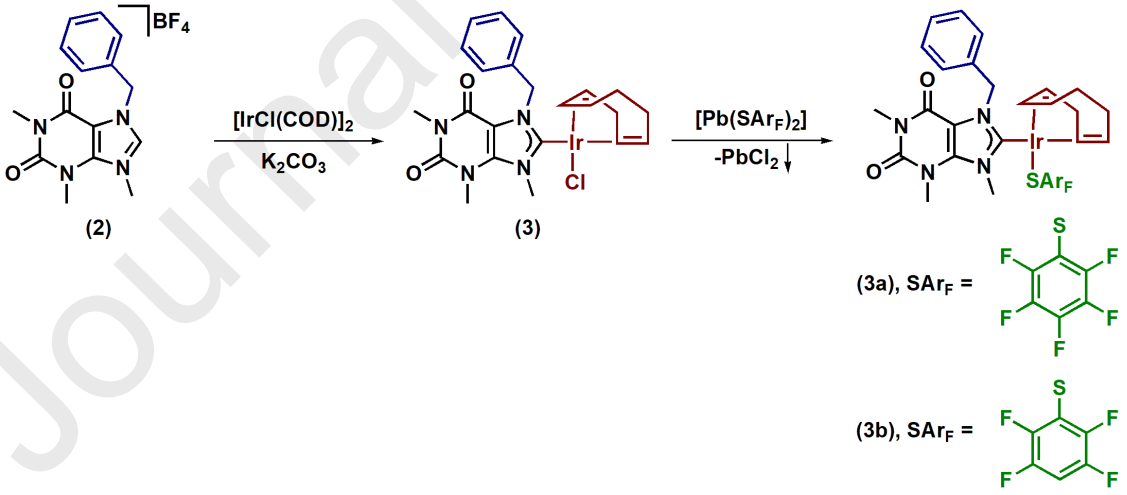
2. Results and discussion

The preparation of 7-benzyl-1,3,9-trimethylxanthinium tetrafluoroborate (**2**) was performed by a slight modification of a protocol described in the literature (Scheme 1) [6,10]. Thus, theophylline was reacted with KOH, followed by the addition of 1 eq. of benzyl bromide, affording the mono-benzylated theophylline compound (**1**) in 79 % yield. The further reaction of this compound with $[O(CH_3)_3]BF_4$ in CH_2Cl_2 afforded (**2**) in 80 % yield. Spectroscopic analyses by ^1H and $^{13}C\{^1H\}$ were in agreement with those reported [10].



Scheme 1. Synthesis of the theophylline-azolium salt (**2**).

The coordination to iridium was achieved by reacting the imidazolium salt (**2**) with K_2CO_3 and $[IrCl(COD)]_2$ in THF (Scheme 2). The reaction was allowed to proceed for 24 h at 50 ºC. After purification *via* chromatographic column, compound (**3**) was isolated as a yellow solid in 41 % yield. With this complex on hand we were interested in exchanging the chloride ligand by aromatic fluorinated thiolates. For this purpose, we reacted the Ir(I) complex (**3**) with the corresponding thiolate lead salt $[Pb(SAr_F)_2]$ in acetone at room temperature for 24 h (Scheme 2). The high insolubility in acetone of the $PbCl_2$ produced, drives the reaction to products, affording the desired complexes in good yields (Scheme 2).



Scheme 2. Preparation of Ir(I) theophylline-NHC complexes **3**, **3a** and **3b**.

The new Ir(I) species were characterized by NMR spectroscopy and mass spectrometry. For the series of complexes, the ^1H NMR reveals the loss of symmetry of the

1,5-cyclooctadiene ligand due to coordination of the NHC to the metal. This loss of the symmetry was also noted in the $^{13}C\{^{1}H\}$ NMR spectra, producing eight signals assignable to the 1,5-cyclooctadiene moiety. The characteristic signal of the carbene carbon atom appears in a similar chemical shift (δ 188 ppm) for the three complexes. Because of the coordination of the thiolate ligand, the signals due to the olefinic carbons *trans* to the NHC are shifted upfield, while the opposite effect was observed for the signals of the carbons *trans* to the thiolate ligand. The mass spectra of the complexes provided further information, producing clean spectra showing the molecular ions at 619.926 *m/z* for (**3**), 785.326 *m/z* for (**3a**) and 767.252 *m/z* for (**3b**) respectively. All these data, as well as elemental analysis results are coherent with the proposed structures.

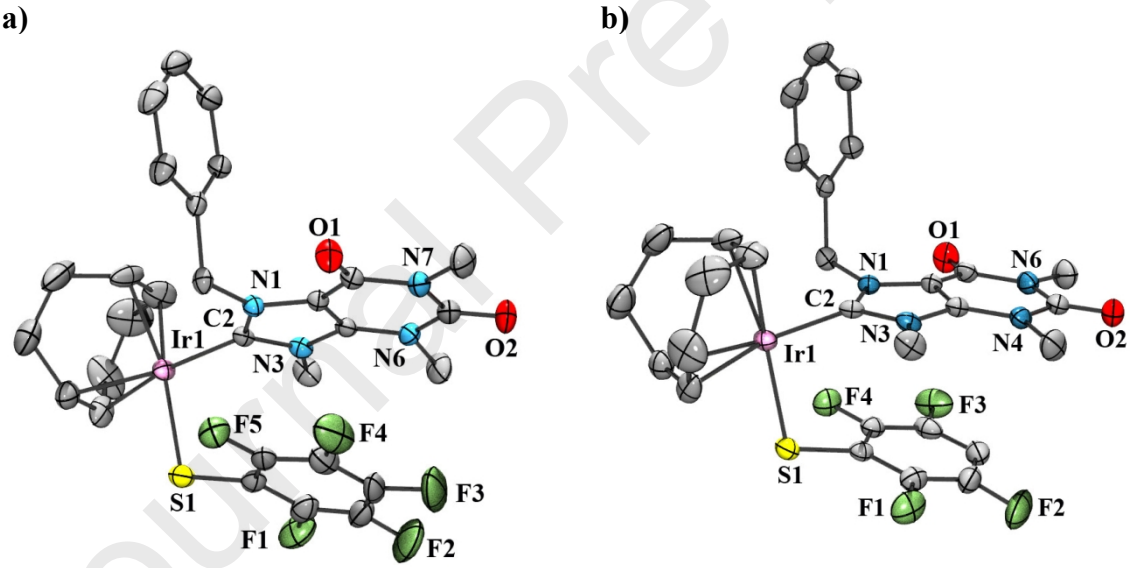


Figure 1. Molecular structure of a) (**3a**) and b) (**3b**). Hydrogen atoms are omitted for clarity. Ellipsoids are at the 50 % probability level. Selected bond lengths (Å): (**3a**): Ir(1)-C(2) 2.024(4), Ir(1)-C(20) 2.123(8), Ir(1)-C(21) 2.131(8), Ir(1)-C(24) 2.210(6), Ir(1)-C(25) 2.197(6), Ir(1)-S(1) 2.331(2). (**3b**): Ir(1)-C(2) 2.009(4), Ir(1)-C(27) 2.130(6), Ir(1)-C(26) 2.117(7), Ir(1)-C(30) 2.219(5), Ir(1)-C(31) 2.197(5), Ir(1)-S(1) 2.339(1). Selected Bond Angles (º): (**3a**): C(2)-Ir(1)-S(1) 92.0(1), C(2)-Ir(1)-C(21) 94.1(2), C(2)-Ir(1)-C(20) 93.1(2), C(2)-Ir(1)-C(24) 163.6(2), C(2)-Ir(1)-C(25) 160.6(2), C(21)-Ir(1)-S(1) 162.5(2), C(20)-Ir(1)-S(1) 157.8(2), Ir(1)-S(1)-C(28) 110.2(2). (**3b**): C(2)-Ir(1)-S(1) 91.0(1), C(2)-Ir(1)-C(27) 94.2(2), C(2)-Ir(1)-C(26) 93.2(2), C(2)-Ir(1)-C(30) 166.5(2), C(2)-Ir(1)-C(31) 157.6(2), C(27)-Ir(1)-S(1) 161.7(2), C(26)-Ir(1)-S(1) 159.3(2), Ir(1)-S(1)-C(20) 110.7(2).

Crystals of (**3a**) and (**3b**) suitable for X-ray diffraction analyses were obtained by slow diffusion of hexane into a saturated solution of the corresponding compound in CH_2Cl_2. Complexes (**3a**) and (**3b**) are isostructural, with molecular structures consisting in the theophylline-imidazolylidene ligand coordinated to iridium (C-Ir), and the 1,5-cyclooctadiene and a thiolate ligands completing the coordination sphere around the metal center (Table 1, Figure 1). The geometry of the iridium atom can be described as a pseudo-square planar as seen by the C(2)-Ir(1)-S(1) angle, being 92.0(1)º for (**3a**) and 91.0(1)º for (**3b**). While the Ir-$C_{carbene}$ distances are 2.024(4) and 2.009(4) Å, for (**3a**) and (**3b**), respectively.

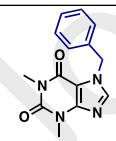
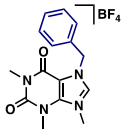
Table 1. Structural and refinement data for compounds (**3a**) and (**3b**).

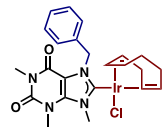
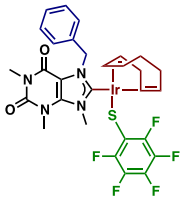
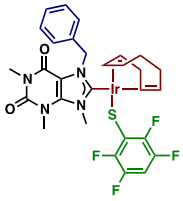
	Compound (**3a**)	Compound (**3b**)
Empirical formula	$C_{29}H_{27}F_5IrN_4O_2S$	$C_{29}H_{28}F_4IrN_4O_2S$
Formula weight	782.80	764.81
Temperature (K)	298(2)	298(2)
Wavelength (Å)	0.71073	0.71073
Crystal system	Monoclinic	Triclinic
Space group	$P2_1/n$	P-1
a (Å)	7.1495(3)	7.3026(4)
b (Å)	41.6834(16)	9.4501(5)
c (Å)	9.7013(4)	20.9232(10)
α (º)	90	78.7458(14)
β (º)	102.4180(10)	86.3229(14)
γ (º)	90	77.6856(14)
Volume ($Å^3$)	2823.5(2)	1383.19(12)
Z	4	2
ρ calc (g/cm^3)	1.842	1.836
μ (mm^{-1})	4.871	4.964
F (0 0 0)	1532	750
Crystal size (mm^3)	0.389 x 0.209 x 0.138	0.315 x 0.064 x 0.037
2Θ range for data collection (º)	2.204, 25.343	2.245, 25.364
Max. and min. transmission	0.7452 and 0.4910	0.7452 and 0.4839
Reflections collected	35638	15479
Independent reflections	5165	5059
GOF	1.258	1.172
Data/restraints/parameters	5165 / 0 / 382	5059 / 0 / 373
R1 $I \geq 2\sigma$ (I)	0.0357	0.0306
wR2 $I \geq 2\sigma$ (I)	0.0718	0.0619

With the compounds in hand, we carried out preliminary *in vitro* evaluation of their cytotoxic activity. The experiments were carried out following the sulforhodamine B protocol, using a 25 μM solution of the compound in DMSO, using six human cancer cell lines, namely: glial cells of nervous central system (U-251), prostate (PC-3), leukemia (K-562), colon (HCT-15), breast (MCF-7) and lung (SKLU-1) (Table 2). The theophylline derivatives (**1**) and (**2**) were inactive in practically all cell lines, while the Ir(I) complexes performed fine. Interestingly, the thiolate derivatives (**3a**) and (**3b**) were more active than (**3**), reaching values of inhibition above 98 % in all cases. It is well known that fluorinated aromatic rings favor non-covalent interactions (*e.g.* π-π stacking), thus, the thiolate ligand may play an important role in the activity of these species, probably *via* molecular recognition or even helping in the transport process.

Since compound (**3**) was the more selective for PC-3 and SKLU-1, we determined their IC_{50} values on these two human cancer cell lines. Having the IC_{50} value of (**3**) for PC-3 (7.8 ± 0.4 μM) being slightly better than that found for cisplatin (8.4 ± 04 μM), but less efficient for SKLU-1 (compare: (**3**) 10.7 ± 0.7 μM *vs* cisplatin: 4.3 ± 0.5 μM).

Table 2. Growth inhibition (%) of human cancer cells by theophylline derivatives (25 μM)*

Compound	U-251	PC-3	K-562	HCT-5	MCF-7	SKLU-1
 (**1**)	1.9	4	---	---	---	22.3
 (**2**)	---	1.4	---	---	---	8.9

 (**3**)	---	99.5	55.8	55.8	69.3	100
 (**3a**)	100	99.4	100	100	100	100
 (**3a**)	100	98.4	100	100	100	100

*The results are the average of three runs.

3. Conclusions

In summary, we have synthesized a series of theophylline-based Ir(I) NHC complexes. The molecular structures of the thiolate derivatives (**3a**) and (**3b**) were unambiguously determined by different spectroscopic techniques and single crystal X-ray diffraction analyses. The structures showed the theophylline-imidazolylidene ligand coordinates to the Ir(I), and a thiolate and a 1,5-COD ligand complete their coordination spheres.

The complexes were active against six human cancer cell lines. The presence of the fluorinated-thiolate ligand improved the cytotoxic activity of the compounds, but decrease their selectivity. The IC_{50} values of (**3**) were very similar to those found for cisplatin, being in the micromolar scale.

We believe that we have a nice modular structure that deserves further studies, involving the further change of other benzyl substituents and some other thiolate ligands. These compounds in turn may allow us the determination of SAR, that combined with potential mechanistic studied might shed light to better design other similar complexes with better performance and selectivities in the fight of this global illness. Efforts aimed to achieve these goals are currently under development in our laboratories.

4. Experimental Part

All chemical compounds were commercially obtained from Aldrich Chemical Co. and used as received without further purification. The ^{1}H and $^{13}C\{^{1}H\}$ NMR spectra were recorded on a Bruker Ascend 500 spectrometer. Chemical shift are reported in ppm down field of TMS using the residual signals in the solvent ($CDCl_3$, 7.27 ppm) as internal standard. Elemental analyses were performed on a Perkin Elmer 240. CHNS analyses were performed in Thermo Scientific Flash 2000 elemental analyzed, using a Mettler Toledo XP6 Automated-S Microbalance and sulfanilamide as standard (Thermo Scientific BN 217826, attained values N = 16.40 %, C = 41.91 %, H = 4.65 % and S = 18.63 %; certified values N = 16.26 %, C = 41.81 %, H = 4.71 % and S = 18.62 %). MS-Electrospray determinations were recorded on a Bruker Daltonics-Esquire 3000 plus Electrospray Mass Spectrometer.

Synthesis of 7-benzyl-1,3-dimethylxanthine (1).

A solution of theophylline (1.082 g, 6.01 mmol) and KOH (0.374 g, 6.67 mmol) in DMF (3 mL) was stirred at 85 ºC for 15 min. Then, benzyl bromide (0.790 mL, 6.61 mmol) was added and the reaction mixture was heated at 110 ºC overnight. The reaction was

cooled to room temperature, followed by addition of 15 mL of water. The solid was filtered and washed further with water. Yield: 1.275 g (79 %). The spectroscopic data obtained was in good agreement with that reported [10].

Synthesis of 7-benzyl-1,3,9-trimethylxanthinium tetrafluoroborate (2).

To a solution of compound (**1**) (0.477 g, 1.76 mmol) in $ClCH_2CH_2Cl$ (20 mL) at 0 ºC, was added $[O(CH_3)_3]BF_4$ (0.379 g, 2.56 mmol). The resulting reaction mixture was kept at 0 ºC for 1 h. Then, was allowed to reach room temperature and let stirring for 72 h. The resulting solid was filtered and washed with CH_2Cl_2. Yield: 0.525 g (80 %). The spectroscopic data obtained were similar to those reported in the literature [10].

Synthesis of [(NHC)IrCl(COD)] (3).

A solution of compound (**2**) (0.108 g, 0.29 mmol), $[IrCl(COD)]_2$ (0.100 g, 0.15 mmol) and K_2CO_3 (0.040 g, 0.29 mmol)in THF (5 mL) was heated at 50 ºC for 24 h. Then, the solution was cooled to room temperature and all the volatiles removed under high vacuum. The crude solid was dissolved in CH_2Cl_2 and purified by column chromatography using silica gel. Elution with a CH_2Cl_2/acetone mixture (96/4) afforded the separation of a yellow band that contained the desired compound. Yield: 0.073 g (41 %). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.48 (d, $^3J_{H\text{-}H}$ = 7.1 Hz, 2H, CH_{Ar}), 7.31 (t, $^3J_{H\text{-}H}$ = 7.4 Hz, 2H, CH_{Ar}), 7.26 (d, $^3J_{H\text{-}H}$ = 6.8 Hz, 1H, CH_{Ar}), 6.13 (d, $^2J_{H\text{-}H}$ = 14.6 Hz, 1H, -CH_2-), 5.91 (d, $^2J_{H\text{-}H}$ = 14.6 Hz, 1H, -CH_2-), 4.89 – 4.79 (m, 1H, CH_{COD}), 4.75 – 4.65 (m, 1H, CH_{COD}), 4.49 (s, 3H, -CH_3), 3.79 (s, 3H, -CH_3), 3.31 (s, 3H, -CH_3), 2.97 – 2.88 (m, 1H, CH_{COD}), 2.66 – 2.51 (m, 1H, CH_{COD}), 2.39 – 2.12 (m, 3H, C$H_{2\ COD}$), 1.98 – 1.81 (m, 2H, C$H_{2\ COD}$), 1.77 – 1.65 (m, 2H, C$H_{2\ COD}$), 1.48 – 1.37 (m, 1H, C$H_{2\ COD}$). ^{13}C NMR (126 MHz, $CDCl_3$) δ 188.4 (Ir-

$C_{carbene}$), 152.4 (C=O), 150.6 (C=O), 140.7 ($C_{teophylline}$), 136.6 (C_{Ar}), 128.4 (CH_{Ar}), 127.8 (CH_{Ar}), 127.7 (CH_{Ar}), 109.6 ($C_{teophylline}$), 88.4 (CH_{COD}), 87.3 (CH_{COD}), 53.5 (CH_{COD}), 53.2 (-CH_2-), 52.8 (CH_{COD}), 39.0 (-CH_3), 34.4 ($CH_{2\ COD}$), 32.3 ($CH_{2\ COD}$), 31.9 (-CH_3), 30.2 ($CH_{2\ COD}$), 28.6 ($CH_{2\ COD}$), 28.4 (-CH_3). Electrospray MS (20 V, m/z): 619.926 $[M]^+$. Anal. Calcd for $C_{23}H_{28}ClIrN_4O_2$ (620.16): C, 44.54; H, 4.55; N, 9.03. Found: C, 44.60; H, 4.57; N, 9.00.

Synthesis of [(NHC)Ir(SC_6F_5)(COD)] (3a).

To a solution of compound (**3**) (0.031 g, 0.05 mmol) in acetone (15 mL) was added dropwise a solution of [$Pb(SC_6F_5)_2$] (0.019 g, 0.03 mmol) in acetone (15 mL). The resulting reaction mixture was stirred at room temperature for 24 h. After this time, the reaction was filtered over celite, and all the volatiles were removed under high vacuum, affording the desired product. Yield: 0.025 g (64 %). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.33 – 7.23 (m, 5H, CH_{Ar}), 6.02 (d, $^2J_{H\text{-}H}$ = 14.9 Hz, 1H, -CH_2-), 5.74 (d, $^2J_{H\text{-}H}$ = 14.9 Hz, 1H, -CH_2-), 4.46 (s, 3H, -CH_3), 4.45 – 4.37 (m, 1H, CH_{COD}), 4.32 – 4.24 (m, 1H, CH_{COD}), 3.80 (s, 3H, -CH_3), 3.34 (s, 3H, -CH_3), 2.93 – 2.81 (m, 1H, CH_{COD}), 2.49 – 2.39 (m, 1H, CH_{COD}), 2.35 – 2.18 (m, 2H, $CH_{2\ COD}$), 2.16 – 2.05 (m, 1H, $CH_{2\ COD}$), 1.91 – 1.70 (m, 3H, $CH_{2\ COD}$), 1.70 – 1.61 (m, 1H, $CH_{2\ COD}$), 1.40 – 1.35 (m, 1H, $CH_{2\ COD}$). ^{13}C NMR (126 MHz, $CDCl_3$): δ 188.3 (Ir-$C_{carbene}$), 152.1 (C=O), 150.4 (C=O), 148.4 – 147.6 (m, C_{ArF}), 146.1 – 145.5 (m, C_{ArF}), 140.5 ($C_{teophylline}$), 137.0 (C_{Ar}), 136.8 – 135.7 (m, C_{ArF}), 128.5 (CH_{Ar}), 127.6 (CH_{Ar}), 126.9 (CH_{Ar}), 121.6, 109.5 ($C_{teophylline}$), 84.0 (CH_{COD}), 82.9 (CH_{COD}), 57.6 (CH_{COD}), 57.6 (CH_{COD}), 53.1 (-CH_2-), 39.3 (-CH_3), 34.1 ($CH_{2\ COD}$), 31.9 ($CH_{2\ COD}$), 31.7 (-CH_3), 30.5 ($CH_{2\ COD}$), 28.7 ($CH_{2\ COD}$), 28.6 (-CH_3). Electrospray MS (20 V, m/z): 785.326 $[M+H]^+$.

Anal. Calcd for $C_{29}H_{28}F_5IrN_4O_2S$ (783.83): C, 44.44; H, 3.60; N, 7.15. Found: C, 44.54; H, 3.59; N, 7.17.

Synthesis of [(NHC)Ir(SC_6F_4H-4)(COD)] (3b).

To a solution of compound (**3**) (0.032 g, 0.05 mmol) in acetone (15 mL) was added dropwise a solution of [Pb(SC_6F_4H-4)$_2$] (0.018 g, 0.03 mmol) in acetone (15 mL). The reaction mixture was stirred at room temperature for 24 h. After this time, the reaction was filtered over celite, and all the volatiles removed under high vacuum, affording the desired product. Yield: 0.021 g (53 %). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.33 – 7.24 (m, 3H, CH_{Ar}), 7.23 – 7.17 (m, 2H, CH_{Ar}), 6.63 – 6.52 (m, 1H, CH_{ArF}), 6.07 (d, $^2J_{H\text{-}H}$ = 15.2 Hz, 1H, -CH_2-), 5.67 (d, $^2J_{H\text{-}H}$ = 15.2 Hz, 1H, -CH_2-), 4.62 – 4.51 (m, 1H, CH_{COD}), 4.49 – 4.38 (m, 4H, -CH_3 and CH_{COD}), 3.77 (s, 3H, -CH_3), 3.34 (s, 3H, -CH_3), 2.91 – 2.79 (m, 1H, CH_{COD}), 2.45 – 2.36 (m, 1H, CH_{COD}), 2.34 – 2.19 (m, 2H, C$H_{2\ COD}$), 2.15 – 2.05 (m, 1H, C$H_{2\ COD}$), 1.95 – 1.79 (m, 2H, C$H_{2\ COD}$), 1.78 – 1.60 (m, 2H, C$H_{2\ COD}$), 1.44 – 1.30 (m, 1H, C$H_{2\ COD}$). ^{13}C NMR (126 MHz, $CDCl_3$): δ 188.5 (Ir-$C_{carbene}$), 152.1 (C=O), 150.4 (C=O), 144.7 – 144.0 (m, C_{ArF}), 141.3 – 140.5 (m, C_{ArF}), 140.4 ($C_{teophylline}$), 137.3 (C_{Ar}), 135.8 – 135.2 (m, C_{Ar}-F), 128.5 (CH_{Ar}), 127.5 (CH_{Ar}), 126.5 (CH_{Ar}), 109.5 ($C_{teophylline}$), 101.3 (CH_{ArF}), 84.1 (CH_{COD}), 83.1 (CH_{COD}), 57.3 (CH_{COD}), 57.2 (CH_{COD}), 53.2 (-CH_2-), 39.2 (-CH_3), 34.2 ($CH_{2\ COD}$), 31.9 ($CH_{2\ COD}$), 31.7 (-CH_3), 30.4 ($CH_{2\ COD}$), 28.7 ($CH_{2\ COD}$), 28.6 (-CH_3). Electrospray MS (20 V, m/z): 767.252 $[M+H]^+$. Anal. Calcd for $C_{29}H_{29}F_4IrN_4O_2S$ (765.84): C, 45.48; H, 3.82; N, 7.32. Found: C, 45.50; H, 3.81; N, 7.38.

4.1 Cytotoxic evaluation

4.1.1 Cell lines culture and culture medium

The compounds were screened *in vitro* against human cancer cell lines: U-251 (human glioblastoma), PC-3 (human prostatic adenocarcinoma), K-562 (chronic myelogenous leukemia), HCT-15 (human colorectal adenocarcinoma), MCF-7 (human mammary adenocarcinoma), SKLU-1 (human lung adenocarcinoma). Cell lines were supplied by National Cancer Institute (USA). The human tumor cytotoxicity was determined using the protein-binding dye sulforhodamine B (SRB) in microculture assay to measure cell growth, as described in the protocols established by the NCI1 [37]. The cell lines were cultured in RPMI-1640 medium supplemented with 10 % fetal bovine serum, 2 mM L-glutamine, 10.000 units/mL penicillin G sodium, 10.000l g/ml streptomycin sulfate and 25 µg/mL amphotericin B (Gibco) and 1% non-essential amino acids (Gibco). They were maintained at 37 ºC in humidified atmosphere with 5% CO_2. The viability of the cells used in the experiments exceeds 95% as determined with trypan blue.

4.1.2 Cytotoxic assay

Cytotoxicity after treatment of the tumors cells and normal cell with the test compounds was determined using the protein-binding dye sulforhodamine B (SRB) in microculture assay to measure cell growth [37]. The cells were removed from the tissue culture flasks by treatment with trypsin, and diluted with fresh media. Of these cell suspension, 100 µL containing 5000–10,000 cell per well, were pipetted into 96 well microtiter plates (Costar) and the material was incubated at 37 ºC for 24 h in a 5% CO_2 atmosphere. Subsequently, 100 µL of a solution of the compound obtained by diluting the stocks were added to each well. The cultures were exposed for 48 h to the compound at concentrations 25 µM. After the incubation period, cells were fixed to the plastic substratum by addition of 50 µL of cold 50% aqueous trichloroacetic acid. The plates were

incubated at 4 ºC for 1 h, washed with tap H_2O, and air-dried. The trichloroacetic-acid-fixed cells were stained by the addition of 0.4% SRB. Free SRB solution was the removed by washing with 1% aqueous acetic acid. The plates were then air-dried, and the bound dye was solubilized by the addition of 10 mM unbuffered tris base (100 μL). The plates were placed on and shaken for 10 min, and the absorption was determined at 515 nm using a ELISA plates reader (Bio-Tex Instruments).

4.2 Data collection and refinement for compound (3a) and (3b)

Crystals of (**3a,** CCDC 1977715) and (**3b,** CCDC 1977716) were grown from Hexane/iPrOH and mounted on glass fibers, then placed on a Bruker Smart Apex II diffractometer with a Mo-target X-ray source (λ = 0.71073Å). The detector was placed at a distance of 5.0 cm from the crystals and frames were collected with a scan width of 0.5 in ω and an exposure time of 10 s/frame. Frames were integrated with the Bruker SAINT software package[38] using a narrow-frame integration algorithm. Non-systematic absences and intensity statistics were used in monoclinic $P2_1/n$ and triclinic P1 space groups respectively. The structures were solved using Patterson methods using SHELXS-2014/7 program [39]. The remaining atoms were located via a few cycles of least squares refinements and difference Fourier maps. Hydrogen atoms were input at calculated positions and allowed to ride on the atoms to which they are attached. Thermal parameters were refined for hydrogen atoms on the phenyl groups using a Ueq = 1.2 Å to precedent atom. The final cycles of refinement were carried out on all non-zero data using SHELXL-2014/7.[39] Absorption corrections were applied using SADABS program.[40]

Supplementary information

Supplementary data for compound (**3a**) and (**3b**) have been deposited at the Cambridge Crystallographic Data Centre. Copies of this information are available free of charge on request from The Director, CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (Fax: +44- 1223-336033; e-mail deposit@ccdc.cam.ac.uk or www: http://www.ccdc.cam.ac.uk) quoting the deposition numbers CCDC 1977715-1977716.

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Author Statement

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Graphical Abstract-Pictogram

Synthesis of Theophylline-Based Iridium(I) N-Heterocyclic Carbene Complexes Including Fluorinated-Thiophenolate ligands. Preliminary Evaluation of Their *In Vitro* Anticancer Activity.

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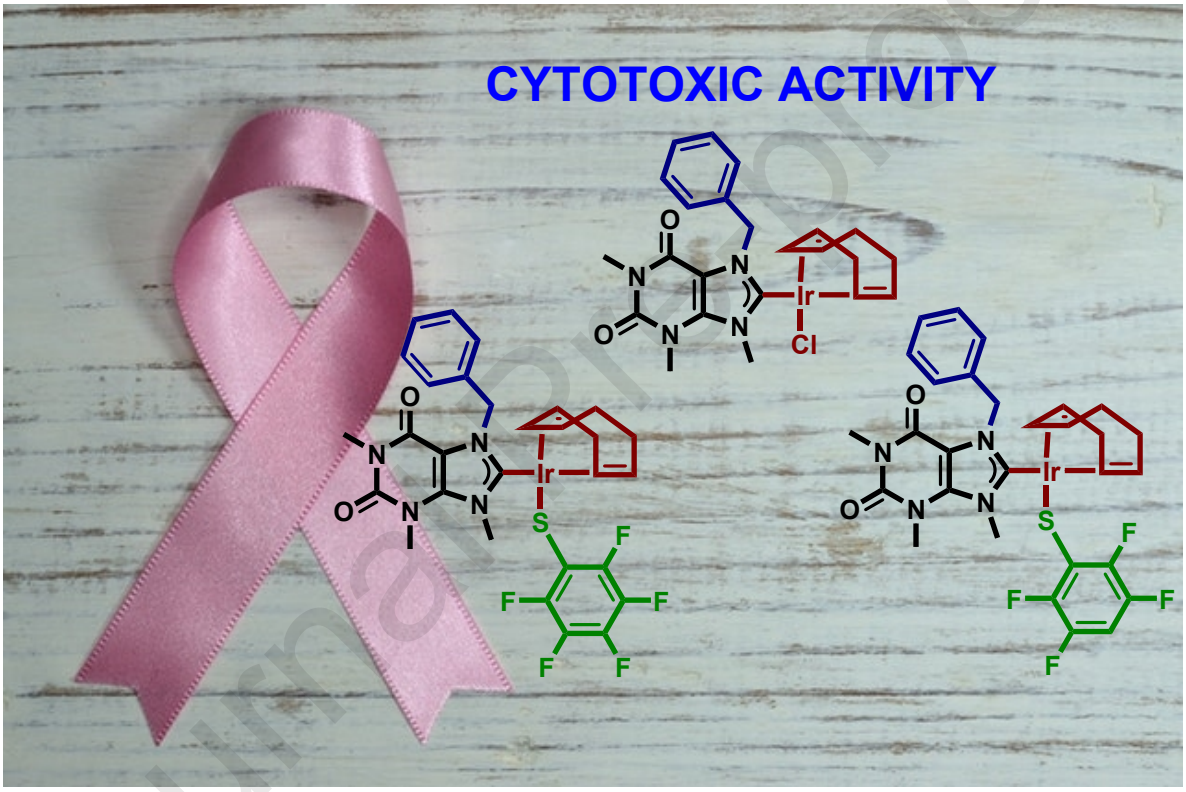

Highlights

- Three theophylline-based Ir(I) N-heterocyclic carbene complexes were synthesized.
- The complexes have excellent cytotoxic activities against different cancer cell lines.
- The cytotoxic activity of the parent complex was enhanced by substitution of the chloride ligand by a fluorinated thiophenol.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

N/A