Bioorganic & Medicinal Chemistry 21 (2013) 2932–2940

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Synthesis and biological evaluation of fluoro analogues of antimitotic phenstatin

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ARTICLE INFO

Article history:
Received 17 January 2013
Revised 24 March 2013
Accepted 26 March 2013
Available online 4 April 2013

Keywords:
Phenstatin
Tubulin
Antimitotic agent
Fluorobenzophenone
Eaton's reagent

ABSTRACT

With the aim of investigating the influence of fluorine, in particular on the A-ring, a new series of fluoro analogues (**7a–l**) of phenstatin (**3**) was synthesized and tested for interactions with tubulin polymerization and evaluated for cytotoxicity on an NCI-60 human cancer cell lines panel. We have shown that the replacement of 3,4,5-trimethoxyphenyl A-ring of phenstatin with 2,4,5-trifluoro-3-methoxyphenyl unit, results in the conservation of both antitubulin and cytotoxic effect. Fluoro *iso*combretastatin **7k** was the most effective anticancer agent in the present study and demonstrated the highest antiproliferative potential on leukemia cell lines SR (GI_{50} = 15 nM) and HL-60(TB) (GI_{50} = 23 nM) and on melanoma cell line MDA-MB-435 (GI_{50} = 19 nM).

1. Introduction

The microtubule system, formed of α- and β-tubulin heterodimers, is involved in many essential cell functions[1] and is recognized as an attractive pharmacological target for anticancer drugs.[2,3] These cytoskeletal structures are involved in many cellular functions.[1] One of their key roles is in the mitotic spindle, intimately involved in cell division, which bridges chromosomes in the center and centrosomes at opposite poles of the cell, ultimately leading to, during the metaphase/anaphase transition, separation of the sister chromatids and segregation into daughter cells. The importance of microtubules in cell division makes them an important target for anticancer drugs.[3] Most of the antimitotic drugs in clinical use or in development, bind to three major tubulin sites: the vinca, taxane and colchicine sites.[4] Combretastatin A-4 **1** binds to tubulin at the colchicine binding site[5] and exhibits very strong inhibition of a variety of human cancer cell lines.[4] The trimethoxyphenyl unit and the cisoid disposition of the two aromatic rings are both essential for activity (Fig. 1).[6] Research on combretastatin A-4 to improve water solubility and in vivo activity[5] has led to the water-soluble prodrug combretastatin A-4 phosphate (CA-4P, Fosbretabulin) **2**, currently in phase II human cancer clinical trials[7] with phase III trials under way. CA-4P acts as a vascular disrupting agent (VDA).[7] CA-4P does not induce the common side effects of existing chemotherapies such as alopecia and bone marrow toxicity. However, cardiovascular toxicity and neurotoxicity are dose limiting for this compound,[8] and also it is prone to double-bond isomerization,[9] leading to the *E*-isomer which displays dramatically reduced activity.[6c] Research on combretastatin A-4 in order to improve in vivo activity has led to the discovery of phenstatin **3** (Fig. 1).[10] The water-soluble phenstatin phosphate prodrug **4** and the parent phenstatin **3** were essentially indistinguishable and very similar to the combretastatin A-4 phosphate prodrug **2** in terms of both potency and differential cytotoxicity.[10] Nevertheless this compound has not been advancing in human clinical trials, and we recently described that phenstatin **3** is subject of many metabolic transformations as well on the methoxy or hydroxy groups.[11]

The replacement of a hydrogen or a hydroxyl group by a fluorine atom is a strategy widely employed in drug development to alter the chemical properties, cellular and systemic distribution and biological activity of drugs,[12] and in CA-4 series, replacement of the ring B hydroxyl group by a fluorine atom led to compounds with activities in the nanomolar range.[13] In phenstatin series, a fluorine substituent has been placed in all positions of B-ring, sometimes to obtain synthetic intermediates,[14] but activities were modest[15] except for the OH to F replacement (compound **5**,

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http://dx.doi.org/10.1016/j.bmc.2013.03.064

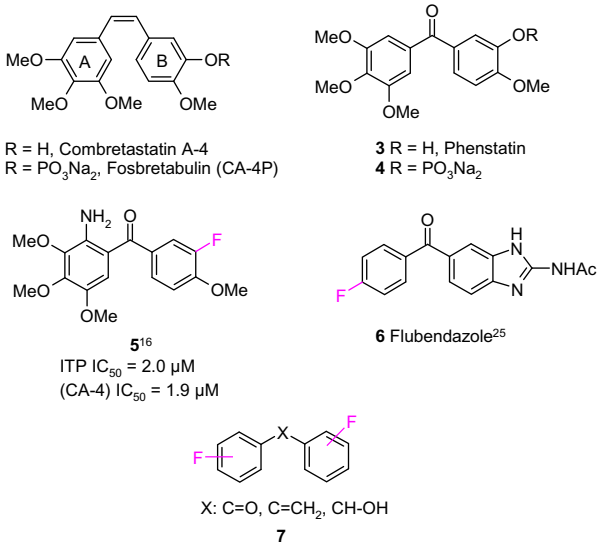


Figure 1. Structure of reference compounds CA-4 **1**, phenstatin **3** and their prodrugs **2**, **4**, structure and inhibitory activity on tubulin polymerization (ITP) of some biologically active fluorobenzophenones **5**,[16] and **6**,[25] and structure of target fluoro analogues of phenstatin **7**.

Fig. 1).[16] It is generally considered that the 3,4,5-trimethoxyphenyl ring A of phenstatin is essential for the biological activity.[6a,c,11a,17] However, it has been demonstrated that some modifications of the A ring such as 2,3,4-trimethoxyphenyl,[18] halogeno-methoxyphenyl,[19] 2,3-methylenedioxy-4-phenyl,[20] 3,4,5-trimethylphenyl moieties,[21] or even change for a heterocyclic ring[22] led to equal or better activity. In that context, we were interested by the fact that the old antiprotozoal and anthelmintic compound, flubendazole **6**,[23] is an antitubulin polymerization agent,[24,25] whose the antitumor properties were recently highlighted,[25] partly thanks to its absence of toxicity and induced neuropathy.[26] Taking these facts in consideration, we decided to study the biological properties of benzophenones and derivatives, analogues to phenstatin, and substituted in ring A or ring B by fluorine atoms. It is known that some substituents placed in the 2-position of ring A are compatible with good biological activities (OMe,[18] NH_2[16]). Thus, a fluorine atom was designed in that position as well as an isoster of these groups as for blocking a potential metabolic location, leading to the synthesis of compounds with a 2,4,5-trifluoro-3-methoxyphenyl group.

2. Chemistry

Fluorophenstatins **7a**–**f** were obtained by reacting commercially available carboxylic acids **8**–**10** with protected phenols **11**, **12**, amine **13** and commercial 3-fluoro-1,4-dimethoxybenzene **14** in Eaton's reagent (P_2O_5/$MeSO_3H$ 1:10 w/w). Protected compounds **11**–**13** were prepared as described previously.[11a] Surprisingly, the condensation of 2,4,5-trifluoro-3-methoxybenzoic acid **8** with chloroacetate **12** was accompanied by the O-demethylation of the methoxy group at C2′ position, followed by the deprotection of the chloroacetyl group to give catechol **7b** as unique product of the reaction (Scheme 1). Furthermore, a *para*-demethylation of the ketone **7e** was observed during its formation in Eaton's conditions to afford compound **7f** in 19% yield.

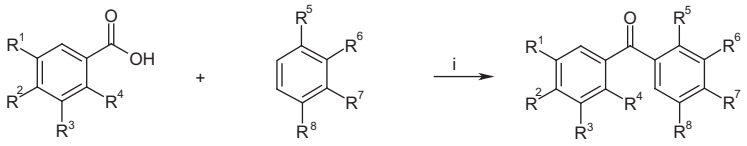
Removal of the protecting groups of benzophenones **7a**, **7c**, and **7d** was easily performed: the chloroacetyl group of ketone **7a** afforded benzophenone **7g** in 96% yield under mild basic conditions (sodium acetate, methanol) and the acetamide unit of ketones **7c** and **7d** was cleaved under acidic conditions (aqueous 10% HCl, methanol) to provide benzophenones **7h** and **7i** in 76% and 80%, respectively.

In order to explore the influence of the modification of the connector between the two phenyl rings of phenstatin, we were interested in the synthesis of fluorinated analogs with an ethylene or a carbonyl-reduced linker. The direct methylenation of benzophenones **7e** and **7g** with the Wittig reagent methylenephosphorane, prepared from methyltriphenylphosphonium bromide and potassium *tert*-butoxide, afforded *iso*combretastatins **7j** and **7k** in moderate 44% and 34% yield, respectively. The reduced form of phenstatin was previously obtained from Grignard reaction of the corresponding aldehyde.[27] However, the sodium borohydride reduction of the carbonyl unit of fluorophenstatin **7g**, provided a simpler route to benzhydrol **7l** (81%) (Scheme 3).

3. Biological evaluation

The synthesized compounds **7a**–**l** were tested for their ability to interact with tubulin using an assembly assay and compounds **7c**, **7e**–**l** were selected and evaluated for cytotoxicity against the 60-human cancer cell lines panel from the National Cancer Institute (NCI). The results are summarized in Tables 1–3.

The replacement of the trimethoxyphenyl unit of parent phenstatin by a 2,4,5-trifluoro-3-methoxyphenyl moiety (compound **7g**, Scheme 2) led to the best antitubulin activity in this series (**7g**: IC_{50} = 28.23 ± 2.79 μM, Table 1). Unexpectedly, the transformation of the carbonyl bridge of compound **7g** in an olefinic one (compound **7k**) decreased the biological activity (generally speaking, the *iso*combretastatins present higher activities than the corresponding ketones),[28] while the reduction of the carbonyl linker in compound **7l** abolished the antitubulin efficacy in the same way as for dihydrophenstatin. The modification of the classical B-ring of compound **7g** by a 2′,3′-dihydroxy-4′-methoxyphenyl in compound **7b** or 3′-amino-2′,4′-dimethoxyphenyl in compound **7h** conserved the antitubulin potential, validating the structure–activity relationships concerning the C2′ position of



8: R^1=R^2=R^4=F, R^3=OMe
9: R^1=F, R^2=OMe, R^3=R^4=H
10: R^1=R^2=R^3=OMe, R^4=H

11: R^5=R^8=H, R^6=$OCOCH_2Cl$, R^7=OMe
12: R^5=R^7=OMe, R^6=$OCOCH_2Cl$, R^8=H
13: R^5=R^7=OMe, R^6=$NHCOCH_3$, R^8=H
14: R^5=R^8=OMe, R^6=H, R^7=F

7a: R^1=R^2=R^4=F, R^3=R^7=OMe, R^5=R^8=H, R^6=$OCOCH_2Cl$ 90%
7b: R^1=R^2=R^4=F, R^3=R^7=OMe, R^5=R^6=OH, R^8=H 71%
7c: R^1=R^2=R^4=F, R^3=R^5=R^7=OMe, R^6=$NHCOCH_3$, R^8=H 76%
7d: R^1=R^4=R^8=H, R^2=F, R^3=R^5=R^7=OMe, R^6=$NHCOCH_3$ 85%
7e: R^1=R^2=R^3=R^5=R^8=OMe, R^4=R^6=H, R^7=F 51%
7f: R^1=R^3=R^5=R^8=OMe, R^2=OH, R^4=R^6=H, R^7=F 19%

Scheme 1. Reagents and conditions: Eaton's reagent 4 equiv, 50 °C, 3–14 h.[11a]

phenstatin[11a] and CA-4 analogs.[6] In our previous investigations,[11a] the acetylation of 3′-amino derivatives and the protection of the phenol groups by a chloroacetyl function in the benzophenone series abolished the potency on microtubule assembly. This behavior was also observed in the current fluorobenzophenone series, protected compounds **7a**, **7c**, and **7d** being inactive (Table 1).

In the trimethoxyphenyl series, the modification of ring-B substitution by a 4′-fluoro-2′,5′-dimethoxyphenyl unit in compound **7e**[29] resulted in a sevenfold decrease of antitubulin potency compared to parent phenstatin **3**, its *iso*combretastatin analog **7j**[29] deleting all the activity. Compound **7f** (the *para*-demethylated derivative of **7e**) was also inactive on microtubule assembly.

Table 1
Inhibitory activities on tubulin polymerization

Compd No.	%TPI[a,b]	IC_{50}[b] (μM ± SD)	R^2
7a	46	N.D.[c]	—
7b	93	29.16 ± 2.04	0.97112
7c	39	N.D.	—
7d	3	N.D.	—
7e	77	24.04 ± 5.74	0.69119
7f	48	N.D.	—
7g	88	28.23 ± 2.79	0.96761
7h	89	29.20 ± 1.96	0.97561
7i	45	N.D.	—
7j	44	N.D.	—
7k	77	42.18 ± 3.49	0.94922
7l	49	N.D.	—
Phenstatin **3**	99	3.43 ± 0.70	0.93782
Desoxypodophyllotoxin	100	1.76 ± 0.44	0.97402

[a] Inhibition of tubulin polymerization at a 100 μM concentration.
[b] Values represent mean of two experiments.
[c] Not determined.

In vitro cytotoxicity of nine compounds (**7c**, **7e**–**l**) was assessed by NCI. They were tested initially at a high single dose (10 μM) in the full 60-cell panel. Only compounds which satisfied predetermined threshold inhibition criteria have progressed to the 5-dose screen in order to evaluate their GI_{50} values (Table 2).

*Iso*combretastatin derivative **7k** exhibited the most important in vitro cytotoxicity among studied compounds: inhibition of CCRF-CEM, HL-60(TB), K-562, SR, HCT-15, KM12, SW-620, M14, MDA-MB-435, MALME-3M, NCI-ADR/RES, and SF-539 cell lines with GI_{50} values ranging from 15 to 47 nM (Table 2). The closely related benzophenone **7g** showed decreased cellular activity. The ketone precursor **7e** was more active than the *iso*combretastatin **7j**. Both showed good cytotoxicity against colon cancer and melanoma cell lines. **7e** also exhibited very potent anti-proliferative activity against leukemia cell lines K-562 and SR. Amino derivative **7h** was twofold less active than analogue **7g**, showing best cytotoxic activity on MDA-MB-435 melanoma cell line (**7g**: GI_{50} = 285 nM, **7h**: IC_{50} = 407 nM, Table 2).

A mean graph midpoints (MG_MID) was calculated for each derivative selected for GI_{50} calculation, giving an average activity parameter over all cell lines for compounds **7e**, **7g**, **7h**, **7j**, and **7k**. These data indicated that compounds **7e** and **7k** possessed the best antiproliferative activity among the tested compounds (Table 2).

Benzophenones **7c**, **7f**, and **7i** and benzhydrol **7l** did not satisfy predetermined threshold inhibition criteria in the initial single-dose assay and did not progress to the 5-dose screen. Results from these studies are reported in Table 3. Fluoro derivatives **7c** and **7i**

Table 2
Results of the in vitro human cancer cell growth inhibition[a] for compounds **7e**, **7g**, **7h**, **7j** and **7k**

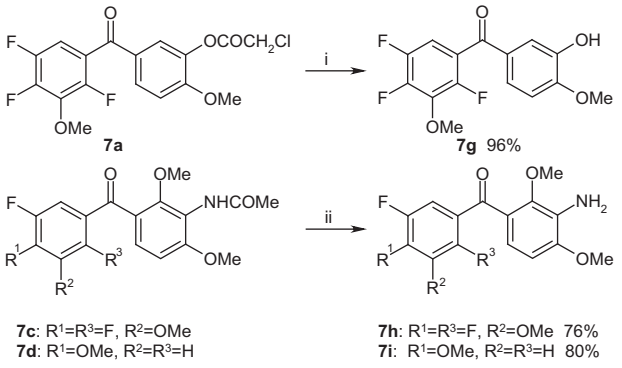
Cell type	Compound	Phenstatin **3**	**7e**	**7g**	**7h**	**7j**	**7k**
	Cell line	GI_{50}[b] (nM)					
Leukemia	CCRF-CEM	—	192	3310	3350	1100	37
	HL-60(TB)	—	203	2020	3690	273	23
	K-562	—	46	**186**	1830	340	37
	SR	—	43	969	961	455	**15**
Non-Small	NCI-H23	—	421	3750	30700	2200	385
Cell lung	NCI-H460	6.0[c]	115	2230	3000	1220	343
Cancer	NCI-H522	—	102	1340	752	601	25
Colon	HCT-15	—	55	1760	2980	666	45
Cancer	KM12	—	50	1030	890	568	47
	SW-620	—	63	703	2840	587	45
Melanoma	M14	—	72	804	N.D.[e]	547	38
	MDA-MB-435	—	**25**	285	**407**	**260**	19
	MALME-3M	—	433	9530	11500	>10000	44
Ovarian	OVCAR-3	2.0[c]	45	1500	2090	521	217
Cancer	NCI/ADR-RES	—	68	789	1670	375	38
	SK-OV-3	—	213	2440	8370	1050	94
Renal	ACHN	—	693	6840	7900	6540	67
Cancer	RXF 393	—	144	2470	4510	2160	176
	SN12C	—	602	5160	5810	498	209
Prostate	DU-145	34.0[d]	257	2080	3910	2440	139
Cancer	PC-3	—	207	3720	10200	2240	140
Central	SF-268	—	748	3850	6540	2670	75
Nervous	SF-539	—	164	2470	3590	2160	31
System	SNB-19	—	419	4390	5470	3300	67
Cancer	SNB-75	—	109	1740	1700	920	55
Breast cancer	MDA-MB-231/ATCC	—	388	2090	8550	945	94
	HS 578T	—	194	1600	3780	2190	52
	BT-549	—	365	3130	34100	4120	61
MG-MID[f] (nM)		60.1[c]	195	2880	4677	1820	295

[a] Data obtained from NCI's in vitro 60 cell 5-dose screening.[31]
[b] GI_{50} is the molar concentration of synthetic compound causing 50% growth inhibition of tumor cells.
[c] Data obtained from Ref. 10.
[d] Data obtained from Ref. 30.
[e] Not determined.
[f] Average activity parameter over all cell lines for the tested compounds.

Table 3
In vitro growth inhibition percentage (GI%) caused by the selected compounds (**7c**, **7f**, **7i**, and **7l**) against some tumor cell lines in the single-dose assay[a]

Compd No.	Panel	Most sensitive cell lines	GI%	Positive cytostatic effect[b]	Positive cytotoxic effect[c]
7c	Leukemia	SR	49	4/54	0/54
	Non-small cell lung cancer	NCI-H522	83		
		HOP-62	30		
	CNS cancer	SNB-75	44		
	Colon cancer	HCC-2998	31		
		KM12	57		
		SW-620	37		
	Melanoma	UACC-257	21		
	Renal cancer	UO-31	30		
	Prostate cancer	PC-3	27		
	Breast cancer	MDA-MB-468	81		
	Ovarian cancer	NCI/ADR-RES	55		
7f	Leukemia	SR	22	0/58	0/58
	Melanoma	MDA-MB-435	21		
7i	Leukemia	HL-60(TB)	49	9/53	1/53
		K-562	71		
		SR	61		
	Non-small cell lung cancer	NCI-H522	45		
	Colon cancer	HCC-2998	72		
		HCT-15	53		
		KM12	59		
		SW-620	77		
	Melanoma	MDA-MB-435	100		
		M14	43		
	Breast cancer	MCF-7	52		
		MDA-MB-468	81		
7l	Leukemia	K-562	41	1/59	0/59
		SR	33		
	Non-small cell lung cancer	NCI-H522	53		
	Colon cancer	KM12	26		
	Ovarian cancer	NCI/ADR-RES	35		

[a] Data obtained from NCI's in vitro disease-oriented human tumor cell screen at 10 μM concentration.[31]
[b] Ratio between number of cell lines with percentage growth from 0 to 50 and total number of tested cell lines.
[c] Ratio between number of cell lines with percentage growth of <0 and total number of cell lines.

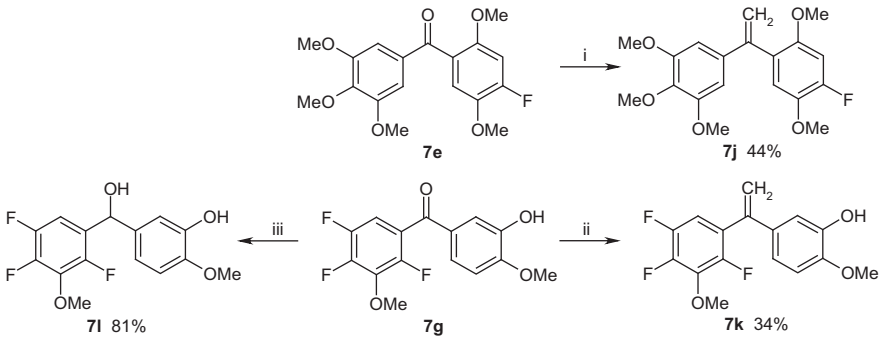


Scheme 2. Reagents and conditions: (i) $AcONa{\cdot}3H_2O$ 4.5 equiv, MeOH, reflux, 2 h; (ii) aqueous 10% HCl, MeOH, reflux, 12–16 h.

displayed a cytostatic effect on four and nine cell lines, respectively. In addition, **7i** demonstrated a minor cytotoxic effect on MDA-MB-435 cell line (growth percentage: −2.6% at 10^{-5} M concentration). Benzophenone **7f** and benzhydrol **7l** were inactive at 10 μM concentration.

4. Molecular modeling

Docking studies were realized in the colchicine binding site of tubulin, on new compounds that presented a biological potential (**7b**, **7g**, **7h** and **7k**), *iso*combretastatin A-4 and phenstatin **3** (Fig. 2) in order to gain some insights into their binding mode and therefore help the design of new pharmacomodulations. Contrary to the other compounds, phenstatin **3** occupies an upright position in the entrance of the pocket (Fig. 2e), most surely because



Scheme 3. Reagents and conditions: (i) CH_3PPh_3Br 2 equiv, *t*BuOK 5 equiv, toluene, 80 °C, 18 h; (ii) CH_3PPh_3Br 2 equiv, *t*BuOK 5 equiv, THF, rt, 24 h; (iii) $NaBH_4$ 2 equiv, EtOH/H_2O, rt, 3 h.

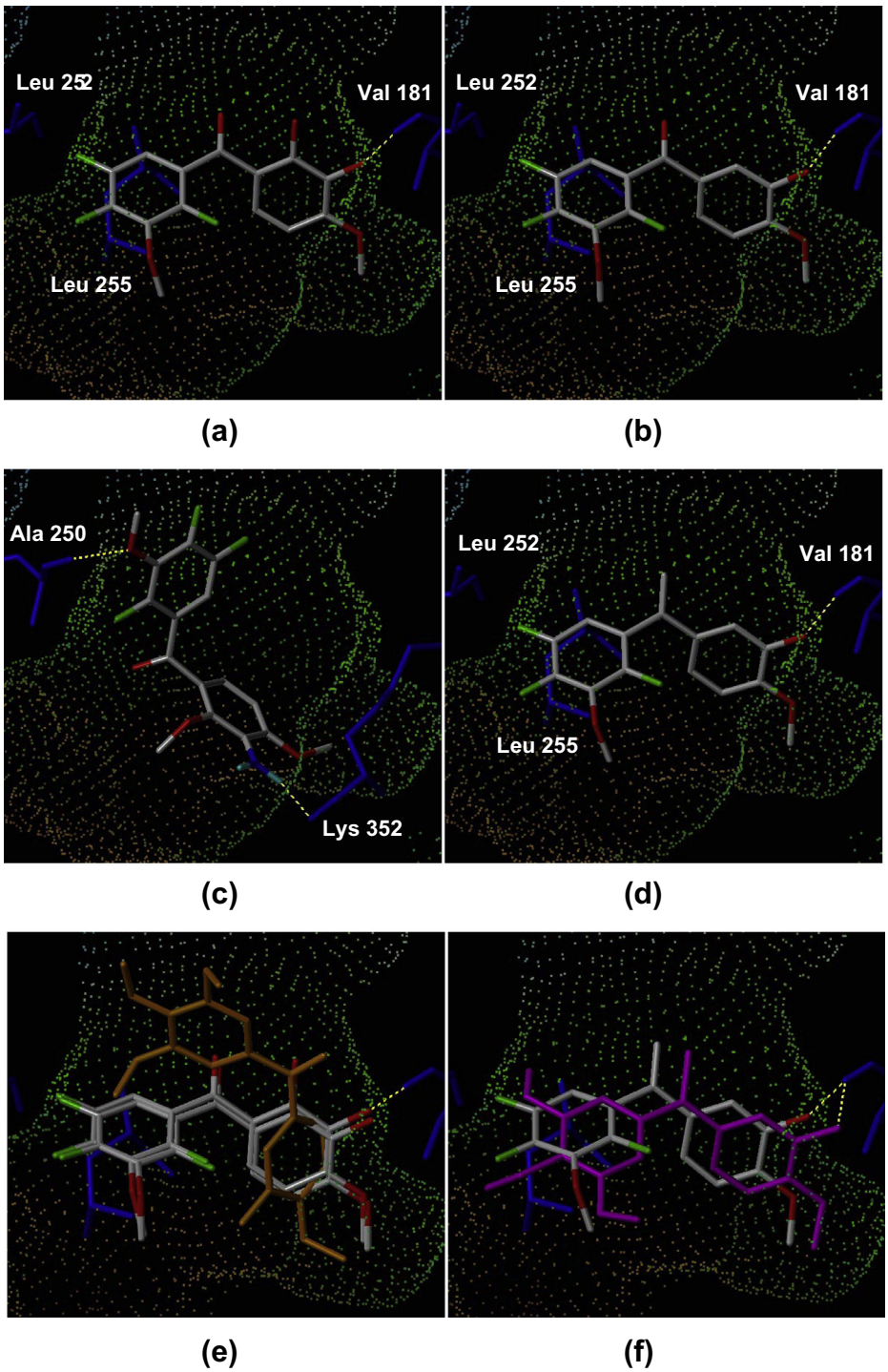


Figure 2. Structure and docking of fluorophenstatins in the tubulin binding site: (a) compound **7b**, (b) compound **7g**, (c) compound **7h**, (d) compound **7k**, (e) superimposition of compounds **7b** and **7g** with phenstatin **3** (in orange), and (f) superimposition of compound **7k** with *iso*combretastatin A-4 (in purple).

of the somewhat larger volume of its triple methoxy substituted cycle compared to the less voluminous fluorines. However, the B cycle is well superposed with the corresponding ring of its newly synthesized analogues. It nonetheless looses the hydrogen bond tying the other molecules to Val 181, which is compensated by a hydrogen bond between its *para* methoxy and Ala 250. Compound **7b** showed preferentially a conformation lying flatly in the bottom of the binding site, with its halogenated ring forming hydrophobic contacts with Leu 252 and 255. The *meta* hydroxyl on the B ring was involved in a hydrogen bond with the skeleton of Val 181. This conformation accounted for just below a half of the solutions (Fig. 2a).

Compound **7g** behaved essentially the same way, with half of the solutions occupying the binding site in the same conformation as compound **7b** and forming the same interactions (Fig. 2b).

Compound **7h**, on the contrary, displayed a rather fuzzy collection of conformations in this region of the binding site. Moreover, these conformations split equally between two general orientations, pointing the nitrogen either toward the right or the left of the pocket. A secondary set of conformations gathered six closely

related solutions that were placed upright in the binding site, with the nitrogen in the bottom. It formed two hydrogen bonds, one between the nitrogen and the backbone carbonyl of Lys 352, the other between the methoxy of the A cycle and the backbone of Ala 250. The difference in placement of this compound when compared with its congeners appears to be made up by a stronger hydrogen bond net (Fig. 2c).

Compound **7k** (Fig. 2d) displayed again a horizontal placement in the binding site. However, it showed two sets of conformations differing by their orientation. The more numerous had also a better score and was very similar to compound **7g**. It formed the same hydrogen bond with Val 181. The second cluster was rotated to point the cycles upward and had no hydrogen bond to stabilise it.

Comparing phenstatin **3** and *iso*combretastatin A-4 (Fig. 2e and f), it is interesting to note that the binding mode of *iso*combrestatin A-4 is also a flat positioning in the bottom of the pocket, where the trimethoxy barely fits and pushes the whole molecule toward Val 181. This notable difference between phenstatin and *iso*combretastatin A-4 despite their structural closeness may be related to a possible interaction with Lys 352. Although oriented away from the pocket, this residue could anchor phenstatin by both it lateral methoxy and its linker carbonyl, while *iso*combretastatin A-4 misses this last and would be therefore less stabilised by a phenstatin-like conformation displaying much less contact with the binding site.

5. Conclusions

The synthesis and the biological evaluation of a new family of fluorobenzophenones **7a**–**i** are reported. In summary, we have shown that the replacement of trimethoxyphenyl ring A of phenstatin with 2,4,5-trifluoro-3-methoxyphenyl unit results in conservation of an antitubulin and cytotoxic effect, less active than the parent compound, but which could counterbalance the metabolic degradation observed with phenstatin,[11] especially the O-demethylations of methoxy groups on ring A. The replacement of the carbonyl bridge of the fluorobenzophenone **7g** with an olefinic group in compound **7k** resulted in an increase of the antiproliferative potential, while reducing the carbonyl linker in compound **7l** abolished the biological efficacy. A docking study of the compounds also hint that the replacement of methoxy groups by fluorine in phenstatin analogs give them a putative binding mode similar to that of *iso*combretastatin A-4 due to a decreased bulk of the corresponding cycle. Further investigation on the metabolic profile of the best candidates issued from this study will be realized in due course.

6. Experimental section

6.1. Chemistry

6.1.1. Materials and methods

Starting materials are commercially available and were used without further purification. Melting points were measured on a MPA 100 OptiMelt® apparatus and are uncorrected. NMR spectra were acquired at 400 MHz for ^{1}H NMR, at 100 MHz for ^{13}C NMR, and at 376 MHz for ^{19}F NMR on a Varian 400 MHz Premium Shielded® spectrometer. Chemical shifts (δ) are given in ppm relative to $CDCl_3$ (7.26 ppm; 77.1 ppm). Splitting patterns are designed as: s, singlet; d, doublet; dd, doublet of doublets; t, triplet; m, multiplet and sym m, symmetric multiplet. Coupling constants *J* are reported in hertz (Hz). Thin layer chromatographies were realized on Macherey Nagel silica gel plates with a fluorescent indicator and were visualized with UV-lamp at 254 and 366 nm. Column chromatographies were performed using a Combi*Flash* Rf Companion (Teledyne-Isco System) and Redi*Sep* packed columns. IR spectra were recorded on a Varian 640-IR FT-IR Spectrometer. Elemental analyses (C, H, N) of new compounds were determined by 'Pôle Chimie Moléculaire', Faculté de Sciences Mirande, Université de Bourgogne, Dijon, France.

6.1.2. General procedure A for Friedel–Crafts reactions in the presence of Eaton's reagent

Eaton's reagent was prepared from phosphorus pentoxide (P_2O_5) and methanesulfonic acid (CH_3SO_3H) (weight ratio P_2O_5/CH_3SO_3H 1:10). The mixture was heated at 40 °C under nitrogen atmosphere until complete homogeneity. Benzoic acid (1.15–1.5 equiv) and aromatic derivative (1.0 equiv) were then added to Eaton's reagent. The mixture was heated at 50 °C under inert atmosphere for 3–14 h. After cooling to room temperature, the reaction medium was diluted with dichloromethane and carefully poured into a separatory funnel containing sodium bicarbonate aqueous solution (50% $NaHCO_3$) (neutralization to pH 7). The aqueous solution was extracted with dichloromethane, and the combined organic layers were dried ($MgSO_4$). Solvent was removed under reduced pressure to produce a brownish oil. The crude product was purified by Flash chromatography on Redi*Sep* packed columns to provide pure fluorobenzophenones **7a**–**f**.

6.1.2.1. 2-Methoxy-5-(2,4,5-trifluoro-3-methoxybenzoyl)phenyl chloroacetate (7a). The general procedure A was followed using 2,4,5-trifluoro-3-methoxybenzoic acid **8** (12.8 g, 62.0 mmol), 2-methoxyphenyl chloroacetate **11** (10.4 g, 51.6 mmol) and Eaton's reagent (4.6 g P_2O_5 in 31.4 mL CH_3SO_3H). The mixture was heated at 50 °C for 14 h. The final brown oil was purified by flash chromatography with EtOAc/*n*-heptane 3:7 and recrystallized from absolute EtOH to give pure chloroacetate **7a** (18.1 g, 90%) as a white solid; mp (EtOH) 121–123 °C; TLC R_f (EtOAc/*n*-heptane 5:5) = 0.78; ^{1}H ($CDCl_3$, 400 MHz) δ (ppm) 3.97 (s, 3H, OC*H*$_3$), 4.05 (t, *J* = 1.3 Hz, 3H, OC*H*$_3$), 4.24 (s, 2H, OCOC*H*$_2$Cl), 6.89 (d, *J* = 8.2 Hz, 1H, Ar*H*), 7.00 (ddd, *J* = 9.0, 8.3, 5.6 Hz, 1H, Ar*H*), 7.38 (q, *J* = 2.2, 1.5 Hz, 1H, Ar*H*), 7.42 (t, *J* = 1.3 Hz, 1H, Ar*H*). ^{19}F NMR ($CDCl_3$, 376 MHz) δ (ppm) −147.21 (m, *J* = 20.7, 16.4, 8.3 Hz, 1F, Ar*F*), −139.02 (dddd, *J* = 23.2, 20.6, 13.5, 9.1 Hz, 1F, Ar*F*), −131.8 (m, *J* = 13.6, 6.5 Hz, 1F, Ar*F*). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 40.5 (CH_2), 56.3 (CH_3), 60.9 (t, *J* = 3.6 Hz, CH_3), 107.6 (sym m, CH), 114.6 (CH), 117.1 (CH), 124.5 (d, *J* = 1.8 Hz, CH), 130.8 (C), 145.5 (sym m, C), 146.3 (C), 146.9 (dd, *J* = 11.8, 3.5 Hz, C), 147.1 (dd, *J* = 14.8, 5.6 Hz, C), 148.6 (C), 150.8 (dd, *J* = 8.3, 2.1 Hz, C), 151.3 (C), 164.7 (C), 189.7 (C). IR ν cm^{-1}: 1755, 1655, 1605, 1430, 1099. Calcd for $C_{17}H_{12}ClF_3O_5$: C, 52.53; H, 3.11. Found: C, 52.40; H, 3.47.

6.1.2.2. (2,3-Dihydroxy-4-methoxyphenyl)-(2,4,5-trifluoro-3-methoxyphenyl)methanone (7b). The general procedure A was followed using 2,4,5-trifluoro-3-methoxybenzoic acid **8** (2.5 g, 12.1 mmol), 2,6-dimethoxyphenyl chloroacetate **12** (1.9 g, 8.1 mmol) and Eaton's reagent (0.9 g P_2O_5 in 6.1 mL CH_3SO_3H). The mixture was heated at 50 °C for 12 h. The final brown oil was purified by flash chromatography with EtOAc/*n*-heptane 3:7 to provide the deprotected and mono O-demethylated pure compound **7b** (1.9 g, 71%) as a yellow solid; mp (EtOAc/*n*-heptane) 120–121 °C; TLC R_f (EtOAc/*n*-heptane 3:7) = 0.17; ^{1}H ($CDCl_3$, 400 MHz) δ (ppm) 3.98 (s, 3H, OC*H*$_3$), 4.09 (s, 3H, OC*H*$_3$), 5.54 (s, 1H, ArO*H*), 6.51 (d, *J* = 9.2 Hz, 1H, Ar*H*), 6.97 (sym m, 1H, Ar*H*), 6.98 (d, *J* = 9.2 Hz, 1H, Ar*H*), 11.89 (s, 1H, ArO*H*). ^{19}F ($CDCl_3$, 376 MHz) δ (ppm) −146.37 (ddd, *J* = 20.5, 12.4, 9.6 Hz, 1F, Ar*F*), −138.92 (dddd, *J* = 22.4, 20.5, 13.5, 9.5 Hz, 1F, Ar*F*), −132.42 (sym m, 1F, Ar*F*). ^{13}C ($CDCl_3$, 100 MHz) δ 56.4 (CH_3), 62.2 (t, *J* = 3.1 Hz, CH_3), 103.3 (CH), 109.9 (dd, *J* = 20.2, 3.1 Hz, CH), 114.4 (C), 121.9 (sym m, C), 125.5 (d, *J* = 2.4 Hz, CH), 133.5 (C), 138.3 (m, C), 145.9 (ddd, *J* = 255.0, 15.0, 5.3 Hz, C), 147.4 (ddd,

J = 248.5, 11.7, 3.1 Hz, C), 148.5 (dt, J = 249.6, 3.3 Hz, C), 150.8 (C), 153.1 (C), 194.3 (C). IR ν cm^{-1}: 3467, 1633, 1597, 1508, 1463, 1429, 1278, 1095, 1066, 777. Calcd for $C_{15}H_{11}F_3O_5$: C, 54.89; H, 3.38. Found: C, 54.77; H, 3.17.

6.1.2.3. *N*-[2,6-Dimethoxy-3-(2,4,5-trifluoro-3-methoxybenzoyl)phenyl]acetamide (7c). The general procedure A was followed using 2,4,5-trifluoro-3-methoxybenzoic acid **8** (2.0 g, 9.7 mmol), *N*-(2,6-dimethoxyphenyl)acetamide **13** (1.3 g, 6.5 mmol) and Eaton's reagent (0.9 g P_2O_5 in 6.1 mL CH_3SO_3H). The mixture was heated at 50 °C for 14 h. The final brown oil was purified by flash chromatography with EtOAc/*n*-heptane 5:5 to give pure acetamide **7c** (1.9 g, 76%) as a white solid; mp (EtOAc/*n*-heptane) 160–162 °C; TLC R_f (EtOAc/*n*-heptane 75:25) = 0.21; ^1H ($CDCl_3$, 400 MHz) δ (ppm) 2.15 (br s, 3H, NHCOCH_3), 3.58 (s, 3H, OCH_3), 3.92 (s, 3H, OCH_3), 4.02 (s, 3H, OCH_3), 6.60 (br s, 1H, ArNH), 6.79 (d, J = 8.6 Hz, 1H, ArH), 7.18 (sym m, 1H, ArH), 7.56 (d, J = 8.7 Hz, 1H, ArH). ^{19}F ($CDCl_3$, 376 MHz) δ (ppm) −145.27 (m, 1F, ArF), −139.81 (m, 1F, ArF), −131.77 (sym m, 1F, ArF). ^{13}C ($CDCl_3$, 100 MHz) δ 23.2 (CH_3), 56.3 (CH_3), 62.1 (CH_3), 62.2 (t, J = 3.1 Hz, CH_3), 106.8 (CH), 110.6 (dd, J = 20.2, 3.1 Hz, CH), 119.3 (sym m, C), 124.0 (sym m, C), 130.6 (CH), 137.9 (sym m, C), 147.1 (sym m, C), 149.2 (sym m, C), 151.7 (sym m, C), 156.4 (d, J = 3.1 Hz, C), 156.8 (sym m, C), 159.4 (C), 166.8 (C), 188.6 (C). Due to the presence of the two methoxy groups next to the acetamide function, the signal corresponding to the methyl group at 23.2 ppm is very weak. IR ν cm^{-1}: 3210, 1660, 1595, 1510, 1461, 1349, 1089, 995. Calcd for $C_{18}H_{16}O_5NF_3$: C, 56.40; H, 4.21; N, 3.65. Found: C, 56.09; H, 4.30; N, 3.67.

6.1.2.4. *N*-[3-(3-Fluoro-4-methoxybenzoyl)-2,6-dimethoxyphenyl]acetamide (7d). The general procedure A was followed using 3-fluoro-4-methoxybenzoic acid **9** (2.0 g, 11.8 mmol), *N*-(2,6-dimethoxyphenyl)acetamide **13** (1.5 g, 7.8 mmol) and Eaton's reagent (0.7 g P_2O_5 in 4.9 mL CH_3SO_3H). The mixture was heated at 60 °C for 5 h. The final product precipitates in dichloromethane. After filtration of the obtained precipitate, the pure acetamide **7d** (2.3 g, 85%) is obtained as a white solid; mp (CH_2Cl_2) 179–180 °C; TLC R_f (EtOAc) = 0.30; ^1H ($CDCl_3$, 400 MHz) δ (ppm) 2.18 (br s, 3H, NHCOCH_3), 3.62 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 3.96 (s, 3H, OCH_3), 6.68 (br s, 1H, N*H*COCH$_3$), 6.77 (d, J = 8.9 Hz, 1H, ArH), 6.98 (t, J = 8.0 Hz, 1H, ArH), 7.34 (d, J = 8.0 Hz, 1H, ArH), 7.60 (d, J = 8.9 Hz, 1H, ArH), 7.64 (d, J = 12.0 Hz, 1H, ArH). ^{19}F ($CDCl_3$, 376 MHz) δ (ppm) −134.58 (s, 1F, ArF). ^{13}C ($CDCl_3$, 100 MHz) δ 23.4 (CH_3), 56.2 (CH_3), 56.3 (CH_3), 62.1 (CH_3), 106.4 (CH), 112.1 (d, J = 1.6 Hz, CH), 117.1 (d, J = 19.4 Hz, CH), 125.1 (m, C), 127.8 (m, C), 129.4 (CH), 130.8 (d, J = 5.4 Hz, CH), 150.6 (C), 151.9 (d, J = 11.1 Hz, C), 153.1 (C), 155.4 (d, J = 20.0 Hz, C), 157.6 (C), 167.2 (C), 192.9 (C). Due to the presence of the two methoxy groups next to the acetamide function, the signal corresponding to the methyl group at 23.2 ppm is very weak. IR ν cm^{-1}: 3257, 1648, 1608, 1515, 1280, 1123, 1094, 1019, 833. Calcd for $C_{18}H_{18}O_5NF$: C, 62.24; H, 5.22; N, 4.03. Found: C, 62.20; H, 4.94; N, 4.17.

6.1.2.5. (4-Fluoro-2,5-dimethoxyphenyl)-(3,4,5-trimethoxyphenyl)methanone (7e)[29]. The general procedure A was followed using 2-fluoro-1,4-dimethoxybenzene **14** (1.0 g, 6.4 mmol), 3,4,5-trimethoxybenzoic acid **10** (2.0 g, 9.6 mmol) and Eaton's reagent (0.8 g of P_2O_5 in 5.4 mL of CH_3SO_3H) at 60 °C for 3 h. The crude product was purified by column chromatography on silica gel with EtOAc/*n*-heptane 3:7 to give the pure product 7e as a white solid (1.1 g, 51%); mp (EtOAc/*n*-heptane) 110–112 °C; TLC R_f (EtOAc/*n*-heptane 3:7) = 0.75; ^1H ($CDCl_3$, 400 MHz) δ (ppm) 3.70 (s, 3H, OCH_3), 3.85 (s, 6H, 2OCH_3), 3.87 (s, 3H, OCH_3), 3.94 (s, 3H, OCH_3), 6.81 (d, J = 12.0 Hz, 1H, ArH), 7.03 (d, J = 9.8 Hz, 1H, ArH), 7.07 (s, 2H, ArH). ^{19}F ($CDCl_3$, 376 MHz) δ (ppm) −127.2 (dd, J = 12.2, 9.5 Hz, 1F, ArF). ^{13}C ($CDCl_3$, 100 MHz) δ 56.3 (2CH_3), 56.5 (CH_3), 57.1 (CH_3), 60.9 (CH_3), 101.6 (CH), 107.4 (2CH), 115.3 (CH), 123.9 (C), 132.8 (C), 141.4 (C), 142.7 (C), 151.9 (C), 152.9 (2C), 155.5 (C), 193.9 (C). IR ν cm^{-1}: 2944, 1638, 1213, 1126. Calcd for $C_{18}H^{19}FO_6$: C, 61.71; H, 5.47. Found: C, 61.31; H, 5.21.

6.1.2.6. (4-Fluoro-2,5-dimethoxyphenyl)-(4-hydroxy-3,5-dimethoxyphenyl)methanone (7f)[29]. By-product from the synthesis of benzophenone **7e**; white solid (0.4 g, 19%); mp (EtOAc/*n*-heptane) 122–124 °C; TLC R_f (EtOAc/*n*-heptane 3:7) = 0.61; ^1H ($CDCl_3$, 400 MHz) δ (ppm) 3.70 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 3.89 (s, 6H, 2OCH_3), 5.98 (s, 1H, ArOH), 6.81 (d, J = 12.8 Hz, 1H, ArH), 7.02 (d, J = 9.5 Hz, 1H, ArH), 7.11 (s, 2H, ArH). ^{19}F ($CDCl_3$, 376 MHz) δ (ppm) −127.7 (dd, J = 12.3, 9.6 Hz, 1F, ArF). ^{13}C ($CDCl_3$, 100 MHz) δ 56.3 (2CH_3), 57.1 (CH_3), 60.9 (CH_3), 101.6 (CH), 107.4 (2CH), 115.3 (CH), 124.1 (C), 129.1 (C), 139.9 (C), 146.6 (2C), 151.7 (C), 152.8 (C), 155.3 (C), 193.6 (C). Calcd for $C_{17}H_{17}FO_6$: C, 60.71; H, 5.09. Found: C, 60.76; H, 5.00.

6.1.3. (3-Hydroxy-4-methoxyphenyl)-(2,4,5-trifluoro-3-methoxyphenyl)methanone (7g)

Chloroacetate **7a** (18.0 g, 46.3 mmol) and sodium acetate (AcONa·3H_2O) (28.4 g, 208.7 mmol) were dissolved in MeOH (50 mL). The solution was refluxed for 2 h. After cooling at rt, the mixture was concentrated under reduced pressure. The residue was taken into distilled water. The resulting precipitate was filtered, washed with water several times to remove remaining sodium acetate and recrystallized from absolute EtOH to obtain benzophenone **7g** (13.9 g, 96%) as a white solid; mp (EtOH) 112–115 °C; R_f (EtOAc/*n*-heptane 5:5) = 0.32; ^1H ($CDCl_3$, 400 MHz) δ (ppm) 3.99 (s, 3H, OCH_3), 4.07 (t, J = 1.3 Hz, 3H, OCH_3), 5.74 (br s, 1H, ArOH), 6.91 (d, J = 8.2 Hz, 1H, ArH), 7.01 (ddd, J = 9.3, 8.1, 5.6 Hz, 1H, ArH), 7.36 (q, J = 2.2, 1.5 Hz, 1H, ArH), 7.41 (t, J = 1.3 Hz, 1H, ArH). ^{19}F NMR ($CDCl_3$, 376 MHz) δ (ppm) −146.67 (m, J = 20.4, 16.4, 8.2 Hz, 1F, ArF), −139.43 (dddd, J = 23.2, 20.4, 13.5, 9.5 Hz, 1F, ArF), −131.8 (m, J = 13.6, 6.7 Hz, 1F, ArF). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 56.2 (CH_3), 62.1 (t, J = 3.9 Hz, CH_3), 109.9 (CH), 110.4 (dd, J = 20.2, 3.9 Hz, CH), 115.5 (CH), 123.9 (d, J = 1.6 Hz, CH), 130.2 (C), 144.7 (dd, J = 14.7, 4.7 Hz, C), 145.6 (C), 146.0 (dd, J = 11.8, 3.2 Hz, C), 147.2 (dd, J = 14.8, 5.5 Hz, C), 148.0 (C), 150.5 (dd, J = 7.7, 2.4 Hz, C), 151.4 (C), 189.5 (C). IR ν cm^{-1}: 3421, 1647, 1579, 1436, 1352, 1099, 1020, 788. Calcd. for $C_{15}H_{11}F_3O_4$: C, 57.70; H, 3.55. Found: C, 57.40; H, 3.47.

6.1.4. General procedure B for deprotection of acetamide function

A mixture of acetamide **7c** or **7d** (1 equiv) and a 10% hydrochloric acid solution (5–50 mL) in methanol (10–50 mL) was stirred at reflux for 12–16 h. The final suspension was neutralized to pH 7 by slow addition of an ammoniacal solution. The aqueous solution was extracted with dichloromethane. The organic phase was dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was recrystallized from Et_2O to afford pure aniline **7h** or **7i**.

6.1.4.1. (3-Amino-2,4-dimethoxyphenyl)(2,4,5-trifluoro-3-methoxyphenyl)methanone (7h). The general procedure B was followed using acetamide **7c** (0.1 g, 0.3 mmol), 10% HCl (5 mL) in methanol (10 mL). The mixture was stirred at reflux for 16 h. The crude product was purified by column chromatography on silica gel with EtOAc/*n*-heptane 1:9 and recrystallized from absolute EtOH to give the pure product 7h as a yellow solid (0.09 g, 76%); mp (EtOH) 161–162 °C; TLC R_f (EtOAc/*n*-heptane 75:25) = 0.18; ^1H ($CDCl_3$, 400 MHz) δ (ppm) 3.63 (s, 3H, OCH_3), 3.93 (s, 3H, OCH_3), 3.97 (br s, 2H, ArNH$_2$), 4.03 (s, 3H, OCH_3), 6.65 (d, J = 8.6 Hz, 1H, ArH), 6.97 (d, J = 8.6 Hz, 1H, ArH), 7.13 (ddd, J = 12.0, 5.9, 1.6 Hz, 1H, ArH). ^{19}F ($CDCl_3$, 376 MHz) δ (ppm) −145.93 (ddd, J = 21.1, 17.1, 9.0 Hz, 1F, ArF), −140.17 (dddd,

J = 23.2, 20.5, 13.8, 9.6 Hz, 1F, Ar*F*), −132.10 (sym m, 1F, Ar*F*). ^{13}C ($CDCl_3$, 100 MHz) δ 55.9 (CH_3), 61.1 (CH_3), 62.1 (t, J = 4.0 Hz, CH_3), 105.7 (CH), 110.7 (dd, J = 20.4, 4.0 Hz, CH), 120.5 (CH), 124.3 (sym m, C), 125.0 (C), 130.1 (C), 137.82 (sym m, C), 146.4 (ddd, J = 256.3, 15.6, 5.5 Hz, C), 146.6 (C), 147.0 (ddd, J = 247.3, 11.7, 4.0 Hz, C), 150.4 (dt, J = 256.3, 4.0 Hz, C), 151.8 (C), 189.3 (t, J = 1.9 Hz, C). IR ν cm^{-1}: 3459, 3366, 1642, 1580, 1499, 1355, 1079, 950, 781. Calcd for $C_{16}H_{14}F_3O_4N$: C, 56.31; H, 4.13; N, 4.10. Found: C, 56.79; H, 3.88; N, 4.17.

6.1.4.2. (3-Amino-2,4-dimethoxyphenyl)(3-fluoro-4-methoxyphenyl)methanone (7i). The general procedure B was followed using acetamide **7d** (2.0 g, 5.8 mmol), 10% HCl (50 mL) in methanol (50 mL). The mixture was stirred at reflux for 12 h. The crude product was purified by column chromatography on silica gel with EtOAc/*n*-heptane 4:6 to give the pure product **7i** as a yellow solid (1.4 g, 80%); mp (EtOAc/*n*-heptane) 80–82 °C; TLC R_f (EtOAc/*n*-heptane 4:6) = 0.21; ^{1}H ($CDCl_3$, 400 MHz) δ (ppm) 3.65 (s, 3H, O*CH*$_3$), 3.92 (s, 3H, O*CH*$_3$), 3.96 (s, 3H, O*CH*$_3$), 3.99 (br s, 2H, ArN*H*$_2$), 6.65 (d, J = 8.5 Hz, 1H, Ar*H*), 6.79 (d, J = 8.5 Hz, 1H, Ar*H*), 6.97 (t, J = 8.5 Hz, 1H, Ar*H*), 7.61 (ddd, J = 8.5, 2.2, 1.0 Hz, 1H, Ar*H*), 7.64 (dd, J = 12.7, 2.2 Hz, 1H, Ar*H*). ^{19}F ($CDCl_3$, 376 MHz) δ (ppm) −134.97 (q, J = 10.9, 8.2 Hz, 1F, Ar*F*). ^{13}C ($CDCl_3$, 100 MHz) δ 55.9 (CH_3), 56.3 (CH_3), 61.3 (CH_3), 105.5 (CH), 111.9 (d, J = 2.4 Hz, CH), 117.4 (d, J = 19.3 Hz, CH), 118.9 (CH), 125.1 (C), 127.6 (d, J = 3.1 Hz, CH), 129.7 (C), 131.2 (d, J = 4.7 Hz, C), 145.5 (C), 150.2 (C), 151.7 (d, J = 10.9 Hz, C), 153.0 (C), 193.7 (d, J = 2.4 Hz, C). IR ν cm^{-1}: 3457, 3352, 1647, 1606, 1432, 1279, 1256, 1127, 1068, 1023, 817, 762. Calcd for $C_{16}H_{16}FO_4N$: C, 62.95; H, 5.28; N, 4.59. Found: C, 62.79; H, 5.67; N, 4.57.

6.1.5. General procedure C for Wittig reaction

A mixture of potassium *tert*-butoxide (5 equiv) and methyltriphenylphosphonium bromide (2 equiv) in THF or toluene was stirred at rt or 80 °C for 1 h. The benzophenone **7e** or **7g** (1 equiv) dissolved in THF or toluene was added and the mixture was stirred at rt or 80 °C for 18–24 h. The solution was poured into water, then extracted in dichloromethane and dried ($MgSO_4$). The solvent was removed in vacuo. The crude product was purified by column chromatography on silica gel to obtain the Wittig compound **7j** or **7k**.

6.1.5.1. 5-[1-(4-Fluoro-2,5-dimethoxyphenyl)ethenyl]-1,2,3-trimethoxybenzene (7j)[29]. The general procedure C was followed using benzophenone **7e** (0.5 g, 1.4 mmol), potassium *tert*-butoxide (0.8 g, 7.1 mmol) and methyltriphenylphosphonium bromide (1.0 g, 2.8 mmol) in toluene (20 mL) at 80 °C. The crude product was purified by column chromatography on silica gel with EtOAc/*n*-heptane 5:5 to give the pure Wittig product **7j** as a white solid (0.2 g, 44%); mp (EtOAc/*n*-heptane) 77–79 °C; R_f (EtOAc/*n*-heptane 4:6) = 0.54; ^{1}H ($CDCl_3$, 400 MHz) δ (ppm) 3.64 (s, 3H, O*CH*$_3$), 3.80 (s, 6H, 2O*CH*$_3$), 3.85 (s, 3H, O*CH*$_3$), 3.93 (s, 3H, O*CH*$_3$), 5.32 (d, J = 1.8 Hz, 1H, C*H*$_2$), 5.59 (d, J = 1.8 Hz, 1H, C*H*$_2$), 6.54 (s, 2H, Ar*H*), 6.66 (d, J = 8.6 Hz, 1H, Ar*H*), 6.74 (d, J = 8.6 Hz, 1H, Ar*H*). ^{19}F NMR ($CDCl_3$, 376 MHz) δ (ppm) −132.71 (dd, J = 11.2, 9.5 Hz, 1F, Ar*F*). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 55.9 (2CH_3), 56.2 (CH_3), 60.5 (CH_3), 60.9 (CH_3), 104.2 (2CH), 106.2 (CH), 115.1 (CH_2), 121.1 (CH), 128.1 (C), 137.8 (C), 138.4 (C), 145.0 (C), 146.5 (C), 147.5 (C), 152.8 (C), 154.8 (C). IR ν cm^{-1}: 2927, 1574, 1124. Calcd. for $C_{19}H_{21}FO_5$: C, 65.51; H, 6.08. Found: C, 65.09; H, 6.51.

6.1.5.2. 2-Methoxy-5-[1-(2,4,5-trifluoro-3-methoxyphenyl) vinyl]phenol (7k). The general procedure C was followed using benzophenone **7g** (0.3 g, 1.0 mmol), methyltriphenylphosphonium bromide (0.7 g, 2.0 mmol) and potassium *tert*-butoxide (0.5 g, 4.8 mmol) in THF (20 mL) at rt for 24 h. The crude product was purified by column chromatography on silica gel with EtOAc/*n*-heptane 1:9. The pure Wittig product **7k** was obtained as a beige oil (0.1 g, 34%); TLC R_f (EtOAc/*n*-heptane 5:5) = 0.65; ^{1}H ($CDCl_3$, 400 MHz) δ (ppm) 3.90 (s, 3H, O*CH*$_3$), 4.02 (s, 3H, O*CH*$_3$), 5.30 (s, 1H, C*H*$_2$), 5.58 (s, 1H, ArO*H*), 5.68 (s, 1H, C*H*$_2$), 6.73–6.81 (m, 3H, Ar*H*), 6.90 (d, J = 2.3 Hz, 1H, Ar*H*). ^{19}F ($CDCl_3$, 376 MHz) δ (ppm) −152.26 (m, 1F, Ar*F*), −141.67 (ddd, J = 23.2, 11.6, 2.7 Hz, 1F, Ar*F*), −133.63 (q, 1F, Ar*F*). ^{13}C ($CDCl_3$, 100 MHz) δ 56.0 (CH_3), 62.1 (CH_3), 110.3 (2CH), 113.0 (CH), 116.6 (CH_2), 118.8 (CH), 133.3 (C), 134.4 (C), 138.7 (C), 141.9 (C), 142.1 (C), 143.0 (C), 145.5 (C), 146.6 (C), 150.1 (C). IR ν cm^{-1}: 3444, 2921, 2851, 1738, 1605, 1505, 1464, 1431, 1356, 1260, 1091, 1023, 947, 763. Calcd for ¼C_7H_{16}·$C_{16}H_{13}F_3O_3$: C, 63.58; H, 5.11. Found: C, 63.31; H, 5.30.

6.1.6. 5-[Hydroxy(2,4,5-trifluoro-3-methoxyphenyl)methyl]-2-methoxyphenol (7l)

An aqueous solution of sodium borohydride (3.7 g, 98.1 mmol in 140 mL distilled water) was added dropwise to a solution of benzophenone **7g** (13.3 g, 44.6 mmol) in absolute ethanol (190 mL). After stirring for 3 h at room temperature, the reaction media is neutralized (pH 7) with a hydrochloric acid solution (1.5 M) and extracted with ethyl acetate (3 × 100 mL). Combined organic layers were dried over magnesium sulfate and concentrated under reduced pressure. The final precipitate was washed with water, collected by filtration to give pure benzhydrol **7l** (10.8 g, 81%) as a white solid; mp (H_2O) 128–130 °C; R_f (EtOAc/*n*-heptane 5:5) = 0.69; ^{1}H ($CDCl_3$, 400 MHz) δ (ppm) 3.88 (s, 3H, O*CH*$_3$), 4.00 (t, J = 1.2 Hz, 3H, O*CH*$_3$), 5.63 (br s, 2H, ArO*H* and CHO*H*), 5.98 (br s, 1H, C*H*OH), 6.81 (d, J = 8.4 Hz, 1H, Ar*H*), 6.88 (dd, J = 8.4, 2.5 Hz, 1H, Ar*H*), 6.92 (d, J = 2.6 Hz, 1H, Ar*H*), 7.09 (sym m, 1H, Ar*H*). ^{19}F NMR ($CDCl_3$, 376 MHz) δ (ppm) −152.85 (ddd, J = 20.4, 13.6, 5.5 Hz, 1F, Ar*F*), -140.47 (sym m, J = 24.4, 21.7, 13.6, 10.9 Hz, 1F, Ar*F*), −138.75 (sym m, J = 13.6, 5.5 Hz, 1F, Ar*F*). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 56.0 (CH_3), 62.0 (CH_3), 69.0 (CH), 107.6 (dd, J = 20.3, 4.7 Hz, CH), 110.6 (CH), 112.6 (CH), 118.0 (CH), 135.4 (C), 145.8 (C), 146.4 (C). IR ν cm^{-1}: 3396, 3264, 1510, 1475, 1087, 1039. Calcd. for $C_{15}H_{13}F_3O_4$: C, 57.33; H, 4.17. Found: C, 56.94; H, 4.48.

6.2. Tubulin assembly studies

Sheep brain tubulin was purified according to the method of Shelanski[31] by two cycles assembly–dissambly and then dissolved in the assembly buffer containing 0.1 M MES, 0.5 mM $MgCl_2$, 1 mM EGTA, and 1 mM of GTP (pH 6.6) to give a tubulin concentration of about 2–3 mg/mL. Tubulin assembly was monitored by fluorescence according to reported procedure[32] using DAPI as fluorescent molecule. Assays were realized on 96-well plates prepared with Biomek NKMC and Biomek 3000 from Beckman coulter and read at 37 °C on Wallac Victor fluorimeter from Perkin–Elmer. The IC_{50} value of each compound was determined as tubulin by 50% compared to the rate in the absence of compound. The IC_{50} values for all compounds were compared to the IC_{50} of phenstatin and desoxypodophyllotoxine and measured the same day under the same conditions.

6.3. Cell proliferation assay

The compounds were tested against a panel of 60 human cancer cell lines at the National Cancer Institute, Bethesda, MD.[33a] The cytotoxicity studies were conducted using a 48 h exposure protocol using the sulforhodamine B assay.[33b]

6.4. Molecular modeling

The crystallographic structure of a heterodimer of α and β-tubuline was taken from the 1sa0[34] entry of the RCSB Protein Data Bank (http://www.pdb.org)[35] and the binding site was

thought to be that of the co-crystallised DAMA colchicine. Flexible docking of the compounds into their putative binding site was performed using GOLD 5.1 software.[36] The most stable docking models were selected according to the best scored conformation predicted by the GoldScore[36] and X-Score scoring functions[37] and a visual assessment of the consistency of the docking solutions, expressed as the closeness of the thirty generated conformations.

Acknowledgments

The authors gratefully acknowledge the 'Conseil Régional Nord-Pas-de-Calais' for the A.G., A.T. and V.S.'s PhD scholarships and the NCI (National Cancer Institute) for the biological evaluation of compounds on their 60-cell panel. The authors would like to thank Dr D. Perry for reviewing this manuscript.

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