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Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 16 (2006) 5844–5848

Effects of α-substitutions on structure and biological activity of anticancer chalcones

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Received 28 June 2006; revised 11 August 2006; accepted 12 August 2006
Available online 1 September 2006

Abstract—Chalcones are known to exhibit antimitotic properties caused by inhibition of tubulin polymerisation. We describe here the effects of different α-substitutions, in particular α-fluorination, on the structure and biological activity of a series of chalcones.

Chalcones, 1,3-diarylprop-1-enones, are a class of compounds consisting of two aryl rings linked by an α,β-unsaturated ketone moiety. The ease of synthesis of chalcones, from substituted benzaldehydes and acetophenones, makes them an attractive drug scaffold. Some chalcones are natural products found in various plant species around the world and in the last decade or so they have been shown to display a wide range of medicinal properties including antiinflammatory,[1] anti-malarial,[2] antibacterial[3] and anticancer[4–7] effects.

The anticancer activity of certain chalcones is believed to be a result of binding to tubulin and preventing it from polymerising into microtubules. Tubulin exists as a heterodimer of two homologous α- and β-subunits. This dimer can couple together to make profilaments consisting of alternating α and β units, 12 or more profilaments can then further link together to form pipe-like structures called microtubules. These structures play an important role in a number of biochemical processes vital to cell survival and growth, one of these is the formation of the mitotic spindle, without which mitosis would not be able to take place.

Tubulin and microtubules are the targets of a number of clinically useful anticancer drugs such as the natural products paclitaxel and vincristine. Another important tubulin-binding ligand is colchicine. Many small molecules are known to bind at the colchicine site of tubulin. These include combretastatin A4,[8] its amide derivative AVE8062,[9] ZD6126.[10] The compounds cause selective damage to tumour vasculature, an effect that is related to their tubulin-binding properties.[11,12] In this way tumours are starved of oxygen and nutrients and their constituent cells die. Compounds such as these that target tumour vasculature clearly have significant clinical promise for the treatment of cancer. The tubulin molecule has three known binding sites, which are identified by the natural products known to bind to them: Taxol® and its derivatives bind to one site and prevent the depolymerisation of microtubules, vinca alkaloids such as vincristine bind to another site and colchicine binds to the third. Chalcones, which are structurally similar to colchicine, are believed to bind to the latter.

Work within our own group has led to the discovery of a number of potent chalcones, particularly **1** and **2** with cytotoxicities of 4.3 and 0.21 nM, respectively.[4] We believe that the α-methyl analogue is more active since it adopts an s-*trans* conformation (as shown by X-ray crystallography studies) as opposed to **1** which is s-*cis*, as shown in Figure 1. This structural change is thought to be a result of steric repulsion which would exist between the methyl group and the A ring in the s-*cis* conformation.

Keywords: Anticancer; Chalcone; Tubulin; Fluorine; Fluorination.
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0960-894X/$ - see front matter
doi:10.1016/j.bmcl.2006.08.065

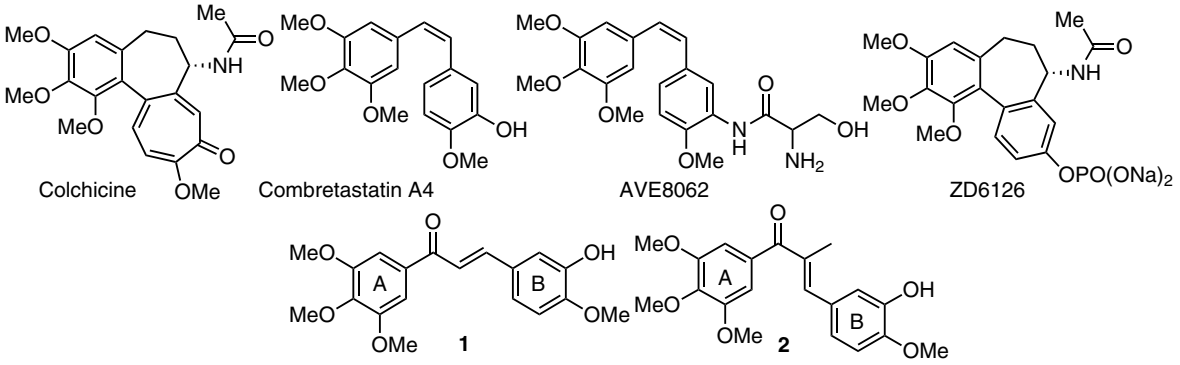


Figure 1. Representative inhibitors of tubulin polymerisation and chalcones **1** and **2**, represented as s-*cis* and s-*trans* conformers, respectively.

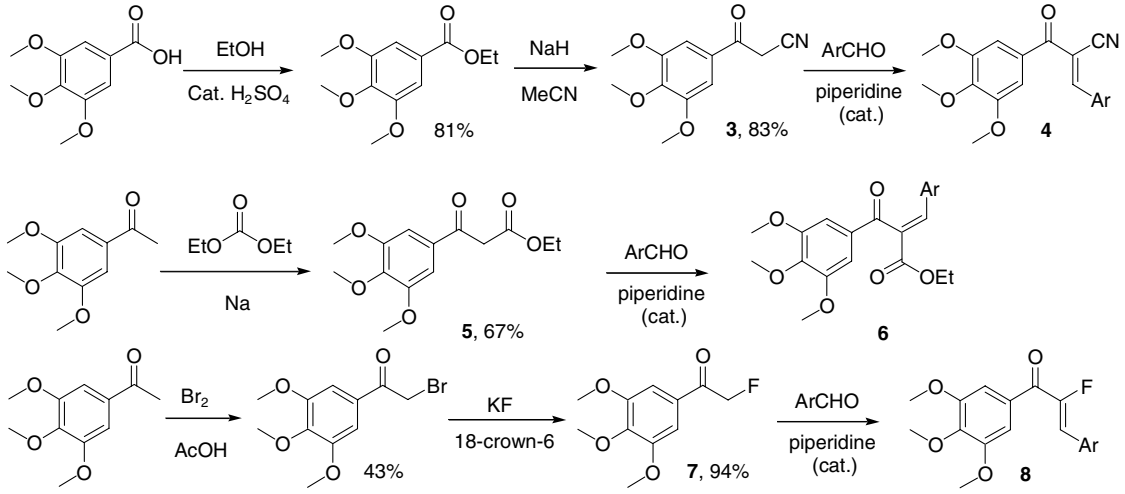
We were intrigued as to whether groups other than a methyl moiety would impart high biological activity to the chalcones. We herein report the synthesis and activity of a series of chalcones with cyano, ester or fluoro groups in the α-position along with some preliminary biological data. In particular the effects of fluorine at this position were of interest,[13] as fluorine is well known to improve activity and metabolic properties[14,15] in the many fluorinated drugs which are already in development.[16] The fluorinated chalcones would also be useful as probes to investigate their ability to induce tumour vasculature damage via ^{18}F positron emission tomography (PET) and their interaction with tubulin by ^{19}F NMR spectroscopy.

Initially a series of chalcones of the type **4** with a nitrile group at the α position was synthesized by Knoevenagel condensation of a range of benzaldehydes with the β-ketonitrile **3**. A similar strategy was also used in the preparation of α-ethyl ester chalcones of the type **6** and α-fluorochalcones **8** (Scheme 1). In both cases we have opted to retain the 3,4,5-trimethoxy substitution of the A-ring, since this is usually optimal and is a feature of many tubulin-binding agents.

The cell growth inhibitory properties of the substituted chalcones (summarized in Table 1) were determined in the K562 human chronic myelogenous leukaemia cell line using the MTT assay from a previously reported procedure.[17] The IC_{50} value is reported as the concentration which results in a 50% inhibition in cell growth after 5-day incubation. In the case of the most active compounds, the tubulin inhibition IC_{50} values are also shown in Table 1.

In the case of chalcones **6c** and **8c** crystals were grown of suitable quality for their structures to be resolved by X-ray analysis.[18,19] Chalcone **6c** showed an unusual structure in which the α,β double bond was configured *E* and the A and B rings were twisted out of plane (Fig. 2). However, chalcones bearing an α-carboalkoxy group are known to adopt this type of structure.[24] This lack of significant biological activity agrees with our previous findings that those chalcones adopting an s-trans conformation in the solid state usually possess high cytotoxicity.[4]

The structure of **8c** showed the molecule in the s-*trans* conformation, typical of α-methyl chalcones. Again this is surprising because this conformation is normally a result of steric interaction between the α group and the A ring and therefore associated with large α groups. Fluorine, with a van der Waals radius of 1.47 Å, must therefore be sufficiently large (cf. hydrogen 1.20 Å)[16] to make



Scheme 1. Synthesis of the α-chalcone classes **4**, **6** and **8**.

Table 1. Cell growth inhibition[a] against the K562 cell line

Compound	B-ring substitution	Cytotoxicity IC_{50} (μM)	Tubulin IC_{50}
1	3-Hydroxy-4-methoxy	0.0043	10 μm
2	3-Hydroxy-4-methoxy	0.00021	1.8 μm
4a	3,4-Methylenedioxy	>10	n.d.
4b	4-Methoxy	>10	n.d.
4c	3-OTBDMS-4-methoxy	>10	n.d.
4d	3-Hydroxy-4-methoxy	4.2	n.d.
4e	3,4-Dimethoxy	>10	n.d.
4f	2,3,4-Trimethoxy	>10	n.d.
4g	4-Trifluoromethyl	>10	n.d.
4h	2-Fluoro-4-methoxy	>10	n.d.
4i	3-Fluoro-4-methoxy	>10	n.d.
4j	3-Bromo-4-methoxy	>10	n.d.
6a	4-Methoxy	>10	n.d.
6b	3-OTBDMS-4-methoxy[b]	4.29	n.d.
6c	3-Hydroxy-4-methoxy	0.87	n.d.
6d	3,4-Dimethoxy	>10	n.d.
6e	2,3,4-Trimethoxy	>10	n.d.
8a	3,4-Methylenedioxy	0.8	n.d.
8b	4-Methoxy	0.39	4.6 μM
8c	3-Hydroxy-4-methoxy	0.0137	0.6 μM
8d	3,4-Dimethoxy	1.21	n.d.
8e	2,3,4-Trimethoxy	10	n.d.
8f	2-Fluoro-4-methoxy	1.19	n.d.
8g	3-Fluoro-4-methoxy	0.77	n.d.
8h	3-Bromo-4-methoxy	6.0	n.d.

[a] As measured by the MTT assay after 5-day incubation of the drug with the cells cultured at 37 °C.
[b] TBDMS, *tert*-butyldimethylsilyl; n.d., not determined.

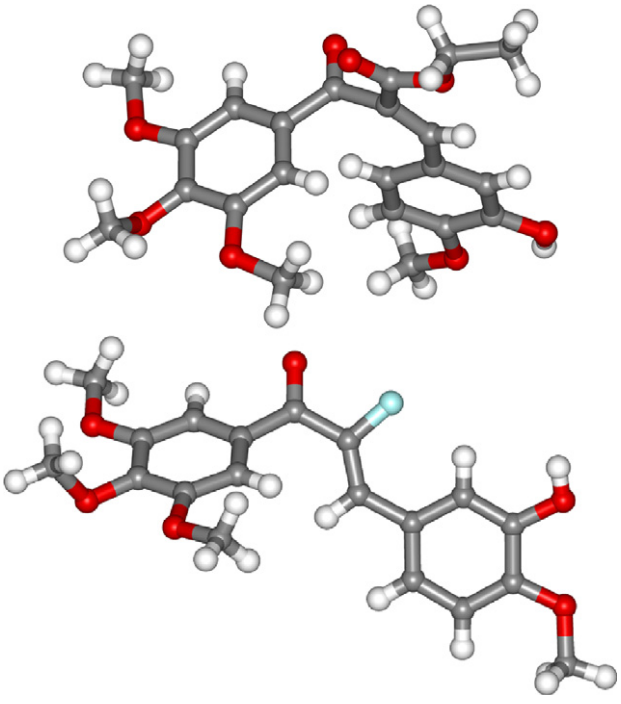


Figure 2. X-ray crystal structures of chalcones **6c** and **8c**.

the α-fluoro chalcone adopt an s-*trans* conformation. It is also interesting that the CO and CF dipoles are aligned in the s-*trans* conformation. This would be expected to disfavour the s-*trans* conformation but clearly this is not a feature that by itself determines the conformation in this case. However, this structure is consistent with the potent cytotoxicities and tubulin inhibition properties shown by this type of compounds. As far as we are aware this is only the second report of the X-ray crystal structure of an α-fluorochalcone. The crystal structure of the α,β-difluorochalcone bearing a *p*-tolyl A-ring and phenyl B-ring also adopts an s-*trans* conformation.[25]

In the case of the α-nitrile chalcones it was not possible to obtain suitable crystals for X-ray analysis. However, in an effort to determine their structure NOE experiments were carried out; compound **4b** showed a 29% enhancement at Hβ when irradiated at the H2/H6 position on the A ring, which suggests it is more stable in the s-*trans* conformation which is consistent with other reports.[24]

Both the α-nitrile and α-ethyl ester chalcones show very poor biological activity compared not only to the fluorinated compounds but also to **1** and **2**.

The inactivity of the α-ethyl ester chalcones can be explained by their distorted structure, as discussed above, but that of the α-nitrile is less clear. The nitrile group is more electron withdrawing than either methyl or hydrogen and also much more polar, and is able to act as a hydrogen bond acceptor. The electron-withdrawing nature of the ester and nitrile groups makes the enone system particularly electrophilic and certainly more so than **1** and **2**. It is possible that **4** and **6** are just too electrophilic and react non-specifically, perhaps via hydrolysis, and are therefore unable to target tubulin.

The fluorinated chalcones, however, showed very high cytotoxicity and tubulin inhibition and were nearly as potent as **1** and **2**.

In all cases the chalcones with the 3-hydroxy-4-methoxy B ring show the highest cytotoxicities, as expected. Where there is a fluorine atom in place of the hydroxyl group, however (as in the case of **4i** and **8g**), the activity was significantly lower. Replacement of a hydrogen or hydroxyl group by a fluorine atom is a strategy widely used in drug development to alter biological function. The substitution of the hydroxyl group with a fluorine atom would also block any potential metabolic clearance via glucuronidation. Fluorine substitution often significantly alters the chemical properties, cellular and systemic distribution and biological activity of drugs.[14] However, as measured by the MTT assay, the hydroxyl replacement is not beneficial in these cases. The most inactive compounds in all cases are those with bulky B rings such as 3-OTBDMS-4-methoxy, 2,3,4-trimethoxy and 3-bromo-4-methoxy. Clearly steric hindrance prevents these molecules from binding effectively to tubulin.

In summary, chalcones with a fluorine atom in the α position have been shown by X-ray analysis to adopt the s-*trans* conformation and show potent cytotoxic and tubulin inhibitory properties. The α-ethyl ester chalcones have an unusual, twisted structure and are poor tubulin inhibitors. The 3-hydroxy-4-methoxy B ring is

key to high biological activity and no other substitution pattern (including 3-fluoro-4-methoxy) is able to match its effect.

Acknowledgments

The support of our work by the EPSRC (Research Grant GR/S25456/01: 500 MHz NMR spectrometer; the EPSRC Chemical Database Service at Daresbury,[26] the EPSRC National Mass Spectrometry Centre at Swansea University for High-Resolution Mass Spectrometry), BBSRC (Research Grants B18009 and B18017), Cardiff University (Senior Research Fellowship to N.J.L., and studentship to R.P.P.) and the Moffitt Cancer Center is gratefully acknowledged.

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18. The crystals for X-ray analysis were prepared by the diffusion method between methanol and a solution of the substrate in chloroform. CCDC 617654 and 617653 contain the supplementary crystallographic data for **6c** and **8c** respectively. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via http://www.ccdc.cam.ac.uk/data_request/cif.
19. *General procedure for the synthesis of chalcones*. The appropriate ketone **3**, **5** or **7** (0.5 mmol) and the appropriate aryl aldehyde (0.5 mmol) were warmed in ethanol (50 ml) until they dissolved. The solution was then cooled to room temperature and piperidine (3–5 drops) was added. After 48 h at room temperature, the product chalcone was isolated by filtration. If after 48 h the chalcone product had not precipitated, the ethanol was removed under vacuum and the residue partitioned between DCM and water. The DCM fraction was dried and evaporated and the chalcone isolated by column chromatography (7:3 hexane/ethyl acetate, v/v).
*Ethyl 3-oxo-3-(3′,4′,5′-trimethoxyphenyl)propionate (**5**)*.[20] Diethyl carbonate (50 ml) and dibutyl ether (50 ml) were heated to reflux and sodium (2 g, 87 mmol) was added cautiously with stirring over 30 min. The resulting purple solution was stirred for 30 min and then 3,4,5-trimethoxyacetophenone (4 g, 19 mmol) was added over 30 min. The reaction mixture was refluxed for a further 4 h, cooled to room temperature and poured into crushed ice and acetic acid (200 ml) and extracted with ether. The extracts were dried and evaporated and recrystallisation from methanol yielded **5** as white needles (3.5 g, 67%). Mp 90–91 °C (lit. mp[21] 93–94 °C); ^{1}H NMR δ (400 MHz; $CDCl_3$), 1.28 (3H, t, J = 7.1 Hz, CH_3), 3.93 (6H, s, OMe), 3.94 (3H, s, OMe), 3.98 (2H, s, $COCH_2CO$), 4.23 (2H, q, J = 7.1 Hz, CH_2), 7.27 (2H, s, H2′ and H6′).
*(Z)-2-Ethoxycarbonyl-3-(3″-hydroxy-4″-methoxy-phenyl)-1- (3′,4′,5′-trimethoxyphenyl)propen-1-one (**6c**)*. Pale yellow, cubic crystals (70%); mp 123–125 °C (found: C, 63.46; H, 5.84. $C_{22}H_{24}O_8$ requires C, 63.46; H, 5.77%); ν_{max} ($CHCl_3$) 3412s,br, 2938s, 1664s, 1581s, 1510s, 1459m, 1324m, 1251s, 1128s, 754s cm^{-1}; ^{1}H NMR δ (400 MHz; $CDCl_3$), 1.14 (3H, t, J = 7.1 Hz, CH_3), 3.76 (6H, s, OMe), 3.77 (3H, s, OMe), 3.84 (3H, s, OMe), 4.16 (2H, q, J = 7.1 Hz, CH_2), 5.67 (1H, s, OH), 6.65 (1H, d, J = 8.1 Hz, H5″), 6.84 (1H, dd, J = 2.2, 8.1 Hz, H6″), overlapping 6.85 (1H, d, J = 2.2 Hz, H2″), 7.14 (2H, s, H2′ and H6′), 7.78 (1H, s, H3). ^{13}C NMR δ (100 MHz; $CDCl_3$), 14.2 (s, CH_3), 55.9 (s, OMe), 56.2 (s, OMe), 60.9 (s, OMe), 61.5 (s, CH_2), 106.4 (s, C2′ and C6′), 110.5 (s, C2″), 116.0 (s, C5″), 123.7 (s, C6″), 126.3 (s, C2),128.9 (s, C1″), 131.4 (s, C1′), 142.5 (s, C3), 143.1 (s, C3″), 145.5 (s, C4′), 148.5 (s, C4″), 153.2 (s, C3′ and C5′), 165.3 (s, COOEt), 194.7 (s, C1) (found $[M+H]^+$, 417.1547, $C_{22}H_{25}O_8$ requires $[M+H]^+$, 417.1544).
2′-Bromo-3,4,5-trimethoxyacetophenone.[22] Bromine (2.5 ml, 48.8 mmol) was added dropwise to a stirred solution of 3,4,5-trimethoxyacetophenone (10 g, 47.6 mmol) in acetic acid (50 ml) at room temperature. The mixture was then left for a further 2 h. Water (200 ml) was added and the product was extracted into DCM, washed with satd NaCl, dried over $MgSO_4$ and evaporated to leave a black oil. Purification by silica chromatography using a 3:7 mixture of EtOAc/hexane (R_f = 0.23) yielded white crystals (5.71 g, 43%). Mp 67–68 °C (lit.[23] mp 68–70 °C); ^{1}H NMR δ (400 MHz; $CDCl_3$), 3.86 (6H, s, OMe), 3.87 (3H, s, OMe), 4.35 (2H, s, H2′), 7.19 (2H, s, H2 and H6) (found $[M+H]^+$, 289, $C_{11}H_{13}O_4{}^{79}Br$ requires $[M+H]^+$, 289).
*2′-Fluoro-3,4,5-trimethoxyacetophenone (**7**)*. Potassium fluoride (4.0 g, 71 mmol) was dried by heating in a two-neck, round-bottomed flask with a Bunsen flame under high vacuum and then cooled to room temperature under N_2. The freshly prepared 2′-bromo-3,4,5-trimethoxyacetophenone (1.1 g, 4.0 mmol) and 18-crown-6 (0.2 g, 0.76 mmol) in dry MeCN (20 ml) were then added and the mixture refluxed for 24 h. The solvent was then removed under vacuum and the residue partitioned between DCM and water. The DCM fraction was dried and evaporated to leave

a brown oil. Purification by silica chromatography using a 4:6 mixture of EtOAc/hexane (R_f = 0.22) yielded **7** as yellow crystals (0.75 g, 94%). Mp 120–122 °C (found: C, 58.46; H, 5.83. $C_{11}H_{13}O_4F$ requires C, 57.89; H, 5.74%); ν_{max} ($CHCl_3$) 2944m, 2359m, 1702s, 1587s, 1463s, 1416s, 1326m, 1128s, 990m, 820w cm^{-1}; ^1H NMR δ (400 MHz; $CDCl_3$), 3.84 (6H, s, OMe), 3.85 (3H, s, OMe), 5.49 (2H, d,J = 47.0 Hz, H2′), 7.07 (2H, s, H2 and H6); ^{19}F NMR δ (283 MHz; $CDCl_3$), −173.4 (1F, t, J = 47 Hz); ^{13}C NMR δ (100 MHz; $CDCl_3$), 56.4 (s, OMe), 61.0 (s, OMe), 83.6 (d, J = 226 Hz, C2′), 105.3 (s, C2 and C3), 128.8 (s, C1), 143.4 (s, C4), 153.3 (s, C3 and C5), 192.3 (d,J = 15 Hz, C1′) (found $[M+H]^+$, 229.0874, $C_{11}H_{14}O_4F$ requires $[M+H]^+$, 229.0876).

*(Z)-2-Fluoro-3-(3″-hydroxy-4″-methoxyphenyl)-1-(3′,4′, 5′-trimethoxyphenyl)prop-2-en-1-one (**8c**)*. Yellow crystals (75%); mp 132–135 °C (found: C, 62.39; H, 5.16. $C_{19}H_{19}O_6F$ requires C, 62.98; H, 5.25%); ν_{max} ($CHCl_3$) 3423w,br, 2940m, 1579s, 1506s, 1415m, 1334m, 1253m, 1127s, 1001w, 755w cm^{-1}; ^1H NMR δ (400 MHz; $CDCl_3$), 3.86 (6H, s, OMe), 3.88 (3H, s, OMe), 3.89 (3H, s, OMe), 5.60 (1H, s, ArOH), 6.77 (1H, d, J = 37 Hz, H3), 6.83 (1H, d, J = 8.4 Hz, H5″), 7.11 (2H, s, H2′ and H6′), 7.15 (1H, dd, J 2.0, 8.4 Hz, H6″), 7.33 (1H, d, J = 2.0 Hz, H2″); ^{19}F NMR δ (283 MHz; $CDCl_3$), −121.6 (1F, d, J = 37 Hz); ^{13}C NMR δ (100 MHz; $CDCl_3$), 56.0 (s, OMe), 56.3 (s, OMe), 61.0 (s, OMe), 106.9 (d, J = 5 Hz, C3), 110.6 (s, C2′ and C6′), 116.3 (d, J = 10 Hz, C2″), 120.1 (d, J = 5 Hz, C5″), 124.1 (d, J = 8 Hz, C6″), 124.9 (d, J = 4 Hz, C1″), 131.3 (s, C1′), 142.3 (s, C4′), 145.7 (s, C3″), 148.2 (d, J = 3 Hz, C4″), 152.9 (s, C3′ and C5′), 153.8 (d, J = 269 Hz, C2), 186.5 (d, J = 28 Hz, C1) (found $[M+H]^+$, 363.1243, $C_{19}H_{20}O_6F$ requires $[M+H]^+$, 363.1244).

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