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Synthesis and in vitro cytotoxicity evaluation of some fluorinated hexahydropyrimidine derivatives

Oluropo C. Agbaje [a], Olugbeminiyi O. Fadeyi [a], S. Adamson Fadeyi [b], Lewis. E. Myles [b], Cosmas O. Okoro [a,*]

[a] Department of Chemistry, Tennessee State University, 3500 John A. Merritt Blvd., Nashville, TN 37209-1561, USA
[b] Department of Biological Sciences, Tennessee State University, 3500 John A. Merritt Blvd., Nashville, TN 37209-1561, USA

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ABSTRACT

A series of trifluoromethylated hexahydropyrimidine and tetrahydropyrimidine derivatives were synthesized and their in vitro cytotoxic activities were determined in colon cancer cell line (COLO 320 HSR). Compounds **4f, 4g, 4k, 5**, and **7** proved to be the most active in this series of compounds. They represent promising new leads for the development of highly potent and selective anticancer compounds. All the compounds are lipophilic due to the trifluoromethyl group, and are thus expected to penetrate the membrane in appreciable concentration.

The unique properties of the trifluoromethyl group, such as its strong electron-withdrawing character, lipophilicity, and metabolic stability have been exploited in the design of novel targets for pharmaceutical, agrochemical and material science industries.[1,2] In particular, fluorine-building blocks are important because of their extensive use in the synthesis of drugs covering a wide variety of therapeutic areas. A fluorine atom is similar in size to a hydrogen atom. Thus, the replacement of hydrogen by fluorine is expected not to cause any significant change in molecular geometry and shape.[3] The high electronegativity of fluorine has a strong effect on the electronic properties of the basic molecules. On a molecular level this allows for the inhibition of some metabolic pathways, including the modulation of membrane permeability, as well as electrostatic interactions with the target site.[4]

From a physiological standpoint, better bioavailability and enhanced selectivity for the target site can be achieved.

In addition, a much lower dose of a fluorinated drug is often needed in some cases, compared to the unfluorinated ones. We are particularly interested in trifluoromethylated pharmaceuticals, since they are present in many commercially available drugs. Although, several trifluoromethyl building blocks are commercially available, many of them are expensive. Thus, several research laboratories, including ours are engaged in the investigation of new and easy methods for the synthesis of trifluoromethyl 'carrier reagents'.[5] The most efficient, mild and versatile nucleophilic trifluoromethylating reagent is the Ruppert–Prakash reagent (trifluoromethyl–trimethylsilane), which upon initiation with fluoride ions, delivers the trifluoromethyl group to electrophilic substrates.[6] The trifluoromethyl group has been successfully used as a probe for the hydrophobic binding site in human thymidylate synthase.[7]

The multifunctionalized dihydropyrimidine and hexahydropyrimidine scaffolds are one of the most commonly encountered heterocycles in medicinal chemistry. They exhibit diverse pharmaco logical properties, such as antiviral, antitumor, antibacterial and antiinflammatory activities.[8]

Dihydropyrimidine, monastrol and its fused bicyclic core analog dimethylenanstron are the first small molecule inhibitor of the mitotic motor Eg5 (kinesin spindle protein, KSP), and are considered as a new lead in the development of anticancer agents.[9] Hexahydropyrimidine nucleus is also present in a number of alkaloids, such as tetraponerines, verbamethine and verbametrine.[10]

In continuation of our search for biologically active fluorine-containing compounds, we report in this letter the polyphosphoric ester (PPE) promoted synthesis and preliminary in vitro cytotoxic activity of fluorinated hexahydropyrimidine and tetrahydropyrimidine derivatives.

In furtherance of our interest in biologically active fluorine-containing compounds, herein we report the synthesis and anticancer activity of a series of fluorinated hexahydropyrimidine derivatives.

* Corresponding author. Tel.: +1 615 963 5332; fax: +1 615 963 5326.
E-mail address: cokoro@tnstate.edu (C.O. Okoro).

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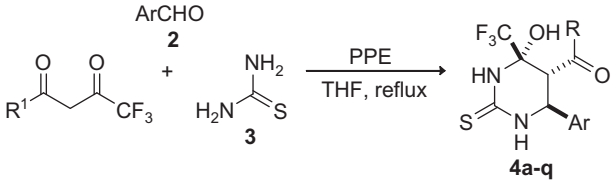
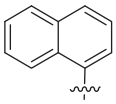
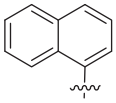
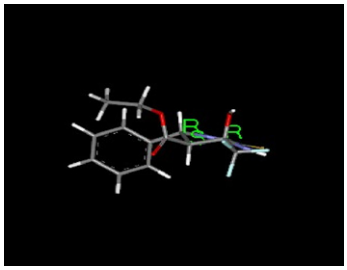
Table 1
Synthesis of fluorinated hexahydropyrimidine and in vitro anticancer activity on colon cancer cell line



Entry[a]	Ar	R	Product	Yield (%)[b]	IC_{50} (μM)[c](COLO 320)
1	3,4-(C–CH_2–O)-C_6H_3	OEt	**4a**	80	75
2	3,4-(C–CH_2–O)-C_6H_3		**4b**	84	62.5
3	3-(CH)-C_6H_4		**4c**	82	72
4	4-(F)-C_5H_4		**4d**	75	51
5	4-(F)-C_5H_4		**4e**	87	>100
6	4-(OMe)-C_6H_4		**4f**	88	11.5
7	4-(F)-C_5H_4		**4g**	83	9.3
8	C_6H_5		**4h**	92	63
9	4-(OMe)-C_6H_4		**4i**	89	75
10	2-(Br)-C_6H_4	OEt	**4j**	78	73
11	3,4-(O–CH_2–O)-C_6FH_3		**4k**	80	9.9
12	3-(OH)-C_6H_4		**4l**	78	>100
13	3,4-(O–CH_2–O)-C_6H_3		**4m**	67	67
14	4-(F)C_6H_4	OEt	**4n**	81	75
15	3-(OH) C_6H_4	OMe	**4o**	95	>100
16	3-(OH) C_6H_4	OEt	**4p**	93	>100
17	3-(CN)-C_6H_4	OEt	**4q**	68	>100

[a] All reactions proceeded to complete conversion.
[b] Yield after chromatography or recrystallization.
[c] IC_{50} (μM) values obtained from Alamarblue™ assays with tested cancer cell lines; means obtained from three independent experiments performed in triplicate.[15]

We initially examined the reaction of trifluoroacetoacetate **1**, appropriate aldehyde **2** and thiourea **3** in ethanol with a catalytic amount of HCl at reflux temperature.[11] Unfortunately, the reaction was found to occur only when 3-hydroxy benzaldehyde (entries 15 and 16) is used as the aldehyde, giving a low yield of the desired hexahydropyrimidine product. No reaction was observed when other aldehyde derivatives were used. This led us to Kappe's previous work, where polyphosphate ester (PPE) was proven to be an efficient reagent in the synthesis of dihydropyrimidine derivatives.[12] The PPE was prepared according to Lakshmikantham's procedure.[13] Utilizing a catalytic amount of the prepared PPE, the synthesis of the trifluoromethylated hexahydropyrimidine derivatives were achieved in a one pot condensation of 1:1:1.5 ratios of appropriate aldehyde **2**, ethyl trifluoroacetoacetate **1** and thiourea **3** in refluxing THF for 24 h.[14] The hexahydropyrimidine **4a–q** (Table 1), considered to be an intermediate in the Biginelli reaction,



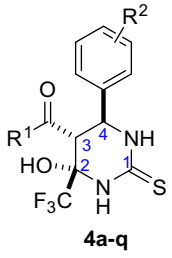
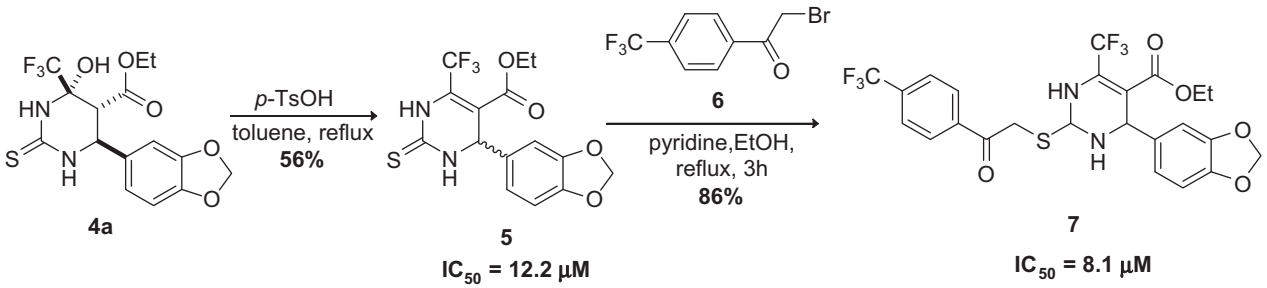


Fig. 1. RSR configuration with lowest conformational energy of −41.18 kJ mol^{-1}.



Scheme 1. Synthesis of fluorinated tetrahydropyrimidine derivatives **5** and **7**.[14]

was isolated in moderate to excellent yields (67–95%).[14] Hexahydropyrimidine **4** contains three stereogenic carbon centers, which may exist as four possible diastereomers. However spectral examination (^{1}H NMR) of products **4a–q**, confirmed the formation of one isomer only.[14]

The above observation was confirmed by characteristic signals for two doublets, which correspond to the two trans-axial methane protons (H^3–H^4) in **4** (Fig. 1). The observed coupling constants (J = 11.1–11.8 Hz) are in agreement with the values previously reported.[11,14] On a theoretical point of view, we determined the conformational energies of all possible diastereomers using *Maestro Software version 8.0 Schrodinger, Inc*. The large conformational energies of the phenyl and alkoxycarbonyl (acyl) groups favor fixed conformations where these groups take an equatorial orientation, with the RSR configuration having the lowest conformational energy (Fig. 1).

The IR spectra of products **4a–q**, showed sharp and intense signals for the hydroxyl functionality in all cases.

We examined the antiproliferative activity of the fluorinated hexahydropyrimidine (**4a–q**) derivatives on human colon (COLO 320 HSR) cancer cell line in the 72 h drug exposure Alamarblue™ assays (Table 1).[15]

The dose of the compound that inhibited 50% cell proliferation (IC_{50}) was calculated using the data generated from the assay. It was found that among the series of fluorinated hexahydropyrimidine derivatives **4e**, **4o**, **4p** and **4q** (entries 5, 15, 16 and 17) exhibited no activity at IC_{50} greater than 100 μM. Compounds **4a**, **4b**, **4c**, **4d**, **4h**, **4i**, **4j**, **4m** and **4n** showed only moderate activity with IC_{50} value ranging from 51–75 μM (Table 1, entries 1–4, 8–10 and 13–14).

Among the 17 fluorinated Hexahydropyrimidine derivatives **4a–q**, only two derivatives **4g** and **4k** containing naphthyl-substituted analogs (R = naphthyl, Table 1, entries 7 and 11) showed the highest activity with IC_{50} values of 9.3 and 9.9 μM, respectively (Table 1), also compound **4f** showed good activity of IC_{50} value of 11. 5 μM (Table 1, entry 6).

Upon refluxing compound **4a** in toluene in the presence of *p*-toluenesulfonic acid (PTSA), fluorinated tetrahydropyrimidine **5** was formed via azeotropic removal of water **5**.[14] To further optimize the synthesis of tetrahydropyrimidine, equal ratios of **5** with bromo trifluoromethyl phenyl ethanone **6** was refluxed in EtOH/pyridine to give fluorinated sulfanyl tetrahydropyrimidine **7** in good yield (Scheme 1).[14] Both compounds showed good antiproliferative activity against colon cancer cell line COLO 320 with IC_{50} values of 12.2 and 8.1 μM, respectively (Scheme 1).

In conclusion, the preliminary anticancer studies showed that **4f**, **4g**, **4k**, **5** and **7** represent novel leads for further development. Compound **7** was the most active among the series and will be subjected to in-depth structure–activity relationship (SAR). Also further biological evaluation of the fluorinated hexahydropyrimidine, tetrahydropyrimidine and other new derivatives against mitotic motor Eg5 (kinesin spindle protein, KSP) is underway, and results will be reported in due course.

Acknowledgment

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14. Experimental and spectral data for title compounds.
General procedure for the PPE-mediated preparation of Hexahydropyrimidine **4**: A mixture of the appropriate aldehyde **2** (2.0 mmol), ethyl trifluoroacetoacetate **1** (2.0 mmol), thiourea **3** (3.0 mmol), and THF (10 ml) containing 300 mg PPE was heated under reflux for the time indicated in Table 1. After cooling, the reaction mixture was poured onto 10 g of crushed ice. Stirring was continued for several hours; the solid products were filtered, washed with ice water and subsequently dried to give **4**.
Furan-2-yl(4-hydroxy-6-(4-methoxyphenyl)-2-thioxo-4-(trifluoromethyl) hexahydropyrimidin-5-yl)methanone **4f**: white solid, mp: 207 °C; IR (*nujol*) 3398, 3180, 2217, 1675, 1209 cm^{-1}. ^{1}H NMR (CD_3OD, 400 MHz) δ 3.70 (s, 3H), 4.67 (d, J = 11.6 Hz, 1H), 5.03 (d, J = 11.6 Hz, 1H), 6.52 (m, 1H), 6.80 (m, 2H), 7.26 (m, 3H), 7.69 (s, 1H); EIMS *m/z*: 137 (20%), 166 (100%), 167 (15%), 205 (15%), 207 (30%) (M + 1).
(6-(4-Fluorophenyl)-4-hydroxy-2-thioxo-4-(trifluoromethyl) hexahydropyrimidin-5-yl)(naphthalen-2-yl)methanone **4g**: brown solid, mp: 190–192 °C; 3397, 3210, 2219, 1675, 1210 cm^{-1}. ^{1}H NMR (CD_3OD, 400 MHz) δ 4.61 (d, J = 11.6 Hz, 1H), 5.00 (d, J = 11.6 Hz, 1H), 7.29 (m, 4H), 7.57 (m, 2H), 7.84 (m, 3H), 8.01 (m, 1H), 8.33 (s, 1H); EIMS m/z: 137 (20%), 166 (100%), 167 (15%), 205 (15%), 207 (30%) (M + 1).
(6-(Benzo[d][1,3]dioxol-5-yl)-4-hydroxy-2-thioxo-4-(trifluoromethyl) hexahydropyrimidin-5-yl)(naphthalen-2-yl)methanone **4k**: yellow solid, mp: 207–209 °C; 3398,

3210, 2217, 1670, 1210 cm^{-1}. ^1H NMR (CD_3OD, 400 MHz) δ 4.60 (d, *J* = 11.6 Hz, 1H), 5.01 (d, *J* = 11.6 Hz, 1H), 6.0 (s, 2H), 6.57 (d, *J* = 8.0 Hz, 1H), 6.87 (m, 1H), 7.03 (s, 1H), 7.58 (m, 2H), 7.84 (m, 3H), 8.0 (d, *J* = 8.0 Hz, 1H), 8.35 (s, 1H); EIMS *m*/*z*: 137 (20%), 166 (100%), 167 (15%), 205 (15%), 207 (30%) (M + 1).

Ethyl-4-(benzo[d][1,3]dioxol-5-yl)-2-thioxo-6-(trifluoromethyl)-1,2,3,4-tetrahydropyrimidine-5-carboxylate **5**: (2.0 mmol) in 15 ml of toluene, 0.1 g of *p*-toluenesulfonic acid was added. The mixture was refluxed for 6 h with azeotropic removal of water. The reaction mixture was cooled to room temperature and filtered from a small amount of solid material. Recrystallization of this residue from ethanol/water gave **5** in 56% yield as white solid. Mp: 147 °C; IR (*nujol*) 3295, 3180, 2217, 1705, 1675, 1560, 1209, 1130 cm^{-1}.^1H NMR (CD_3OD, 400 MHz) δ 1.09 (t, 3H), 4.06 (q, 2H), 5.4 (s, 1H), 6.0 (s, 2H), 6.77 (d, 2H), 6.9 (s, 1H); EIMS *m*/*z*; 326 (5%), 375 (100%) (M + 1).

Ethyl-4-(benzo[d][1,3]dioxol-5-yl)-2-(2-oxo-2-(4-(trifluoromethyl)phenyl) ethylthio)-6-(trifluoromethyl)-1,2,3,4-tetrahydropyrimidine-5-carboxylate **7**: A mixture of 2-bromo-1-[4-(trifluoromethyl)phenyl]ethanone **6** (1 mmol) and ethyl-4-(1,3-benzodioxol-5-yl)-2-thioxo-6-(trifluoromethyl)-1,2,3,4-tetrahydropyrimidine-5-carboxylate **5** (1 mmol) was refluxed in ethanol (5 ml) containing pyridine(1 mmol) for 3 h. The reaction mixture was cooled to room temperature. The solid obtained was filtered, dried. Further purification by flash column chromatography on silica using hexane/ethyl acetate (1:1) gave **7** in 86% yield as yellow solid. Mp: 215 °C; IR (*nujol*) 3295, 3180, 1710, 1670 cm^{-1}. ^1H NMR (CD_3OD, 400MHz) δ 1.09 (t, 3H), 3.58(s, 2H), 4.06 (q, 2H), 5.6 (s, 1H), 6.0 (s, 2H) 6.4–6.88 (m, 3H), 7.6 (d, 2H), 7.87(d, 2H). LC/MS: 563 (M + 1).

15. *Materials and methods*: Human colon (COLO 320 HSR) cancer cell line was obtained from the American Type Culture Collection (Manassas, VA). Cell line was maintained in RPMI 1640 supplemented with 10% fetal bovine serum (FBS), 0.15% sodium bicarbonate, 0.011% sodium pyruvate, 0.24% Hepes and 10 ml/L of antibiotic solution. The cells were grown in 150 cm^2 culture plates in an air/CO_2 (95:5) atmosphere at 37 °C and passaged approximately every 3 days. Cancer cell line (1×10^4 cells per well) was plated using RPMI 1640 medium containing FBS in 96-well plates and left to attach for 24 h. Cells were then treated with either vehicle (DMSO) or the indicated concentrations of different compounds. Cells were exposed for a total of 72 h to test compounds and vehicle controls. Alamarblue™ reagent dye was added to wells in a 1:10 following exposure period and incubated at 37 °C overnight. The plates were then analyzed using a microtiter plate reader at dual wavelengths (560 nm λ excitation, 590 nm λ emission). Each experiment was done in triplicate and results are expressed as means for each determination. The IC_{50} values were calculated using linear regression method and are expressed in micromolar (μM).