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Synthesis of flutamide-conjugates

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ABSTRACT

In this paper, we designed and extended modification basing on the flutamide structure. A series of flutamide-conjugates were obtained with methyl bromoacetate and ethylenediamine. Through the synthesis of two conjugates with 3,5-bis(dodecyloxy)benzoate derivatives, these flutamide conjugates were tested for anticancer activity. Among the compounds tested, the flutamide-conjugates showed good inhibition activity against cancer cell lines U-251, PC-3 and K-562. The conjugates showed a better inhibitory effect than free flutamide and did not show activity against normal COS-7 monkey kidney fibroblast cells. It was also observed that the flutamide conjugates had an inhibitory effect against human colorectal adenocarcinoma HCT-15.

Flutamide (2-methyl-N-[4-nitro-3-(trifluoromethyl)phenyl]) propanamide is used as an antineoplastic and antiandrogen drug.[1,2] It is a powerful nonsteroidal androgen antagonist which has treated prostate cancer and is believed to block androgen receptor sites, namely for testosterone and dihydrotestosterone. Hindering the binding of androgens to the androgen receptors in the prostate gland[2] impedes tumor growth.[3] In the organism, flutamide is metabolized in plasma,[4,5] or excreted in the urine.[6] Flutamide shows low solubility in water, poor membrane permeability and does not participate in acid-base equilibria.[7] These properties do not permit an optimal drug-receptor binding[8] as solubility and permeability are considered prerequisites for optimal bioactivity of the drugs. To increase the solubility and membrane permeability of the drugs and decrease the side effects,[9,10] flutamide has been modified in the aromatic ring[11], in the branched alkyl chain α to the amide carbonyl,[12] and in the amide carbonyl[13] to improve the anticancer activity. Structure-activity relationships showed that the best activity was observed with the compounds containing electron-withdrawing substituents.[14] Also, several formulation strategies have been obtained, including the use of macromolecules. In the last few years, macromolecules as dendrimers have shown good abilities in drug delivery,[15,16] and in gene delivery.[17,18] The dendrimers' amphiphillic nature *i.e.,* hydrophilic exterior and hydrophobic interior, makes them analogous to unimolecular micelles. Dendrimers are considered static unimolecular micelles and their micellar structure remains stable even at higher concentrations of solvents.[19] Micelle-like behavior of dendrimers resulted in their application to solubilize hydrophobic drugs. A developed and well-defined dendrimer structure constitutes excellent nanoscaffolding, to which one can covalently connect many different functional groups, showing specific biochemical functions.[20] In the present work, we designed and synthesized a new type of dendrimers with flutamide-derivatives and evaluated its potential for use as a vehicle for drug delivery.

The *N*-alkylation of the amide group by methyl bromoacetate was used for the synthesis of the flutamide-derivative methyl *N*-isobutyryl-*N*-(4-nitro-3-(trifluoromethyl)phenyl)glycinate **2** (Scheme 1).[21] The compound **2** was reacted with ethylenediamine at room temperature in methanol to obtain the flutamide-derivative **3**.

In the ^{1}H NMR spectra of the compound **3**, the following signals were observed: at δ_H 0.94 and δ_H 0.97 two singlets due to the CH_3 groups of the isopropyl group, at δ_H 2.27 one multiplet due to the CH group of the isopropyl proton, at δ_H 3.11 a broad signal assigned to the CH_2-CH_2-N group, at δ_H 3.86 one doublet due to the CH_2-C═O group with coupling constant J = 5.7 Hz, at δ_H 6.76–7.12 two singlets assigned to the aromatic proton with a coupling constant J = 8.7 Hz, at δ_H 7.72 one multiplet due to the aromatic proton, at δ_H 8.05 one doublet due to the other proton of the aromatic ring with a coupling constants J = 9.0 Hz. Finally, one broad signal at δ_H 8.20 due to the NH group.

On the other hand, the compounds **4**–**7** were obtained (Scheme 2) starting from the 3,5-dihidroxibenzoic acid followed by the esterification reaction with methanol in presence of sulfuric acid to obtain the compound **4**. After that, a reaction with dodecyl bromide under the Williamson reaction, yielded the compound **5**. The ester **5** was reacted with ethylenediamine in a mixture of methanol toluene to obtain the

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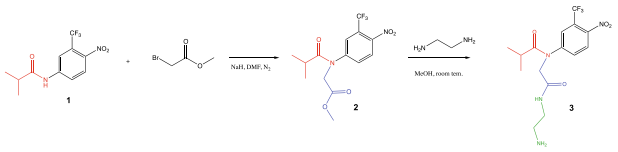
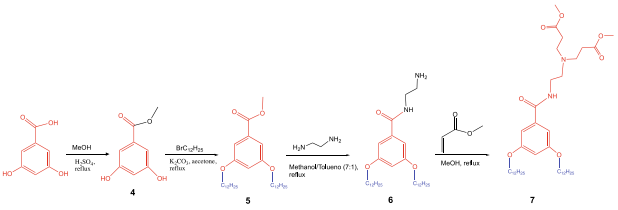
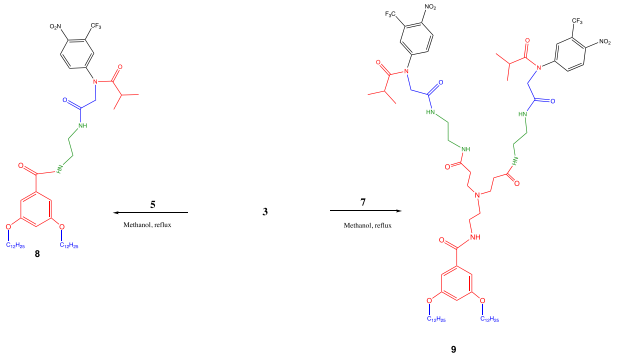


Scheme 1. Synthesis of the flutamide-derivative 3.



Scheme 2. Synthesis of the compounds **4**–**7**.



Scheme 3. Synthesis of the flutamide-derivative conjugates compounds **8** and **9**.

compound **6**. Finally, the compound **7** was treated with methyl acrylate to obtain the diester terminal groups compound **7** (See Scheme 3).

In the case of the compound **5**, crystals were grown with the quality to perform X-ray studies. Fig. 1 shows the structure of compound **5**.

The compound **7** was characterized by ^{1}H NMR spectroscopy and in the spectrum the following signals were observed; one triplet at δ_H 0.85 assigned to the –CH_3 groups with coupling constant J = 6.7, at δ_H 1.24, 1.41 and 1.74 three signals due to the –CH_2– groups from the aliphatic chain, at δ_H 2.42 one triplet assigned to the CH_2-C=O group with coupling constant J = 6.3 Hz, at δ_H 2.59, 2.72 and at δ_H 3.52 three doublets due to the CH_2-NH, CH_2-N with coupling constant J = 6.3 and J = 5.7 Hz respectively, at δ_H 3.56 one singlet assigned to the O-CH_3 group, at δ_H 3.95 one triplet due to the CH_2-O groups at the aliphatic chain with a coupling constant J = 6.4 Hz, at 6.51 and at δ_H 6.97 one triplet and one doublet due to the aromatic protons with coupling constant J = 2.1 Hz. Finally, one triplet was observed at δ_H 7.07 assigned to the NH groups with a coupling constant J = 4.8 Hz (see Supplementary Material).

Finally, the flutamide-derivative **3** was coupled to the compounds **5** and **7** to obtain the conjugates of first **8** and second generation **9**, the

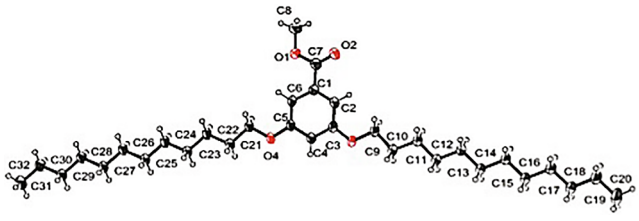


Fig. 1. X-ray structure of compound **5**.

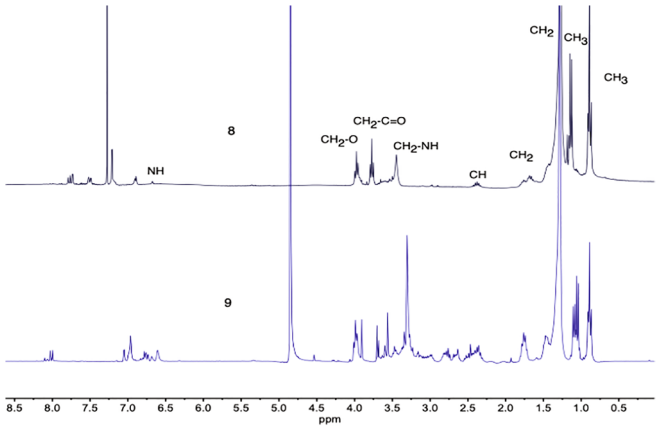


Fig 2. ^{1}H NMR spectra of the flutamide-derivative conjugates **8** and **9**.

reaction was carried out in methanol at reflux.

The compounds **8** and **9** were characterized by ^{1}H NMR spectroscopy and in the NMR spectra (Fig. 2) the following signals were observed: at δ_H 0.89, 1.27 and at δ_H 1.43 one triplet and two broad signals due to the aliphatic chain, at δ_H 1.13 one signal assigned to the CH_3 groups at the isopropyl group. At δ_H 2.37 one multiplet due to the CH group at the isopropyl group. At δ_H 3.44 one broad signal assigned to the NH-CH_2-CH_2-NH groups. At 3.77 and 3.97 two triplets assigned to the N-CH_2-C=O and Ar-CH_2-O groups with a coupling constant J = 6.6 Hz and J = 7.2 Hz respectively. At δ_H 6.67 one triplet signal due to the NH groups with a coupling constant J = 2.4 Hz. Finally, from δ_H 6.91 to 7.76 the signals for the aromatic protons were observed (see Supplementary Material).

The synthesized compounds **2, 3**, **5**–**9** and the starting drug flutamide were screened for their cytotoxic activity against: U-251 (human glioblastoma), PC-3 (human prostatic adenocarcinoma), K-562 (human chronic myelogenous leukemia cells), HCT-15 (human colorectal adenocarcinoma) cancer cell lines and monkey kidney fibroblast cells (COS-7) according to the MTT method.[22]

The data is shown in Table 1, revealing that the free flutamide and the flutamide-derivatives **2** and **3** have very similar antiproliferative activity against the cancer cell lines tested. Cytotoxic activity tests revealed that free flutamide showed strong activity against the U-251, PC-3 and K-562 cell lines, as well the flutamide-derivatives **2** and **3**. The flutamide-derivatives **2** and **3** showed higher activity against normal COS-7 cells in comparison with free flutamide. The compounds **5**–**7** were not toxic to the listed above cancer cell lines. The flutamide conjugates **8** and **9** showed better anticancer activity against all cancer cell lines than the free flutamide and the flutamide-derivatives **2** and **3**. The flutamide conjugate **8** showed high anticancer activity IC_{50} against U-251 (human glioblastoma) 14.29 μM and PC-3 (human prostatic adenocarcinoma) 13.3 μM. The anticancer activity was better when the poly(amidoamine) chain was extended as observed for the flutamide conjugate **9** with IC_{50} value of 8.59 μM for U-251. In the case of the line PC-3, the IC_{50} was 7.16 μM. The concentration of the conjugate **9** was diluted 2 times to have the anticancer activity for one molecule of the drug to compare to the free drug and to the conjugate **8**. The flutamide conjugates **8** and **9** showed increased anticancer activity compared to the free flutamide and the flutamide conjugates **2** and **3**. It was also observed that the conjugates **8** and **9** showed good anticancer activity against HCT-15 (human colorectal adenocarcinoma). Finally, the most important is that the conjugates **5**–**9** did not show any activity against the normal monkey kidney fibroblast cells (COS-7).

In this paper, we processed structural design and extended modification based on the flutamide structure. A series of flutamide-derivatives were obtained with methyl bromoacetate and ethylenediamine and through the synthesis of two conjugates with 3,5-bis(dodecyloxy)

Table 1
In vitro growth inhibitory effect of compounds **2**, **3**, **5–9** in human cell lines U-251, PC-3, K-562, HCT-15 and monkey kidney fibroblast COS-7.

Compound	IC_{50}(μM)				
Cell line	U-251	PC-3	K-562	HCT-15	COS-7
Flutamide	22.11 ± 5.14	20.3 ± 2.14	29.2 ± 1.16	> 100	51.51 ± 1.19
2	20.11 ± 3.14	19.12 ± 1.04	14.10 ± 5.14	> 100	12.10 ± 2.25
3	24.10 ± 1.34	20.10 ± 1.54	18 ± 1.16	> 100	15.17 ± 3.14
5	> 1000	> 1000	> 1000	> 1000	> 1000
6	> 1000	> 1000	> 1000	> 1000	> 1000
7	> 1000	> 1000	> 1000	> 1000	> 1000
8	14.29 ± 4.01	13.3 ± 2.10	15.39 ± 1.52	19.15 ± 4.09	> 1000
9	8.59 ± 3.14	7.16 ± 2.11	6.79 ± 8.23	22.35 ± 2.29	> 1000

[a]IC50 (μM) values obtained from MTT assays. Cells were incubated with compounds for 48 h. The results represent the mean ± SD of two independent trials, performed in triplicate.

benzoate derivatives. These flutamide conjugates were tested for anticancer activity. Among the compounds tested, flutamide-derivatives showed good inhibition activity against U-251, PC-3 and K-562. The conjugates did not show activity against normal monkey kidney fibroblast COS-7 cells. It was also observed that the flutamide conjugates **8** and **9** had an inhibitory effect against human colorectal adenocarcinoma HCT-15. The presence of a longer poly(amidoamine) chain in the conjugates increases the anticancer activity *in vitro* against human glioblastoma, human prostatic adenocarcinoma and human chronic myelogenous leukemia cell lines. In future works, we will extend the effect of the poly(amidoamide) chain to increase the inhibitory properties.

Declaration of Competing Interest

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.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2020.127507.

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