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DESIGN AND SYNTHESIS OF FOUR FLUTAMIDE DERIVATIVES USING SOME CHEMICAL TOOLS: THEORETICAL ANALYSES OF ITS INTERACTION WITH ANDROGEN RECEPTOR

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ABSTRACT

Several studies have shown the preparation of some flutamide derivatives as androgen receptor antagonists; however, some of these drugs require expensive and dangerous reagents. In addition, the evaluation of their interaction with the androgen receptor is very confusing. The aim of this study was synthesize 4 new flutamide derivatives (compounds **8**to **11**) by a series of reactions such as displazament of nitro group from nitro-phenylderivatives and a cycloaddition (2 + 2) of 5-alkyn-3-ol to some alkyne derivatives to form some cyclodiene analogs which were bound to flutamide. Following, the interaction between flutamide and its derivatives with androgen receptor and P-glycoprotein (P-gp) was evaluated using some models theoretical. The results showed a π- π bound (Trp741 andPhe764) for flutamide and compound **9** and cation-π bound (Phe764) for **8** and **10**. Other

data indicated that free energy of binding and lowest interactive surface for compounds **9** and **11**was higher compared with flutamide and the compounds **8** and **10**; however, the inhibition constant (Ki) for **9** was different compared with **11**. Other results showed a specific volume (Å3/atom) < 7.5 for compounds **8** to **11**. In conclusion, the theoretical results indicated that interaction of **9** with androgen receptor was higher in comparison with flutamide and the compound **11**; in addition, the compounds **8** to **11** could act as substrates to P-gp and produce a specific effect. All these data indicate that flutamide derivative could be prospect for evaluation in some cancer model.

Keywords: Flutamide, derivatives, synthesis, docking, androgen receptor

INTRODUCTION

Prostate cancer is one of the leading public health problems in men worldwide (Hammerlid *et al*., 2001; Secco *et al*., 2002; Siegel *et al*., 2016). The growth and maintenance of the prostatic cells are stimulated by some androgens such astestosterone and 5α-dihydrotestosterone which exert their biological activity via androgen receptor (Culig *et al*., 1994; Visakorpi *et al*., 1995; henlein *et al*., 2004). There are some clinical treatments for hormone dependent cancers involves androgen withdrawal using surgical or use of some drugs such as leuprolide acetate and goserelin acetate (Gonadotropin-releasing hormone agonists) which have been used inprostate cancer patients; however this drugs can produce hot flashes, loss of libido, and promotion of osteoporosis (Stege, 2000). There are other drugs such as diethylstilbestrol for treatment of prostate cancer; however, there are some reports which indicate that this drug can induce some side-effects such as gynecomastia, myocardial infarction, deep vein thrombosis, edema, and transient ischemic attacks (Emtage *et al*., 1989; Chang *et al*., 1996). Other data indicate that cyproterone acetate also had been used in prostate cancer; however, can induce severe cardiovascular complications in some patients (De-Voogt *et al*., 1986) such as gynecomastia, loss of libido, erectile dysfunction, and central nervous system symptoms, such as headache and fatigue, which limit use in treating men with prostate cancer. Other type of antiandrogen (flutamide) have used to prostate cancer patients; however, there are data which indicating that survival rates were lower for flutamide mono therapy compared to

diethylstilbestrol in men with prostate cancer metastases (Katchen and Buxbaum, 1975); in addition, can induce some side effects including gynecomastia (De Voogt *et al*., 1988). In search of pharmacotherapy to prostate cancer was evaluated the effect exerted by nitulamide which showed durable response in 91% of the previously untreated prostate cancer patients (Decensi *et al*., 1991). However, nilutamide therapy has a high incidence of side effects including visual problems, nausea, and vomiting, alcohol intolerance and respiratory disturbances compared to the other nonsteroidal anti-androgens (Dole and Holds worth, 1997; Iversen, 2002; McLeod and Iversen, 2000). These results severely limit the use of nilutamide monotherapy to treat to prostate cancer patients. Other antiandrogen used for this pathology is the bicalutamide which showed fewer side effects compared with flutamide and nilutamide (Iversen, 2002, However, although it is very effective at the beginning, resistance appears after 2-3 years of treatment; possibly this phenomenon is due to various mutations of the androgen receptor (Liu *et al*, 2016). Recently, was prepared an anti-androgen (enzalutamide), which has a higher androgen receptor binding affinity and better efficacy in prostate cancer patients (Tran *et al*., 2009). All these data show that several drugs have used for treatment of prostate cancer; however, some this drug can induce several side effects perhaps this phenomenon is due to differences in the chemical structure or to the different pharmacological approaches used. Therefore, in this study were prepared four flutamide derivatives using several strategies; moreover, a theoretical analysis of its interaction with androgen receptor and the P-glycoprotein (P-gp) was carried out.

MATERIAL AND METHODS

Chemical synthesis

The compounds evaluated in this study were purchased from Sigma-Aldrich Co., Ltd. The melting point for compounds was determined on an Electrothermal (900 model). Infrared spectra (IR) were recorded using KBr pellets on a Perkin Elmer Lambda 40 spectrometer. ^{1}H and ^{13}C NMR (nuclear magnetic resonance) spectra were recorded on a Varian VXR-300/5 FT NMR spectrometer at 300 and 75.4 MHz (megahertz) in $CDCl_3$ (deuterated chloform) using TMS (tetramethylsilane) as internal standard. EIMS (electron impact mass spectroscopy) spectra were obtained with a Finnigan

Trace Gas Chromatography Polaris QSpectrometer. Elementary analysis data were acquired from a Perkin Elmer Ser. II CHNS/02400 elemental analyzer.

1-(1-Ethyl-but-3-ynyloxy)-4-prop-2-ynyl-benzene (2)

A solution of 1-nitro-4-prop-2-ynylbenzene (100 mg, 0.62 mmol) and 5-hexyn-3-ol (100µl, 0.90 mmol) potassium carbonate anhydrous (50 mg, 0.36 mmol) in 5 ml of dimethylsulfoxide was stirring for 72 h to room temperature. The reaction mixture was evaporated to dryness under reduced pressure. The obtained solid was washed with water, yielding 68% of product, m.p. 70-72 ^{o}C; IR V_{max} = 2102 and 1058 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$)δ_H: 1.00 (s, 3H), 1.60-1.80 (m, 5H), 2.00-2.04 (m, 2H), 2.44-2.66 (m 2H), 3.50 (m, 2H),4.60 (m, 1H), 6.50-7.10 (m, 4H) ppm. ^{13}C NMR (75.4 Hz, $CDCl_3$) δ_C: 10.80 (**C**-10), 25.10(**C**-11), 25.72 (**C**-14), 29.44 (**C**-9), 67.12 (**C**-16), 71.22 (**C**-13), 81.08 (**C**-8), 81.22 (**C**-15),82.44 (**C**-12), 116.38 (**C**-2, **C**-6), 128.30 (**C**-3, **C**-5), 128.60 (**C**-4), 159.00 (**C**-1) ppm. EIMS *m/z:*212.12. Anal. Calcd. for $C_{15}H_{16}O$: C, 84.87; H, 7.60; O, 7.54. Found: C, 68.90; H,5.70.

1-[3-(4-{1-[2-(2-Hydroxy-butyl)-cyclobutadienylmethyl]-propoxy}-benzyl)cyclobuta- dienyl]-butan-2-ol (3)

Method A.

A solution of **2** (180 mg, 0.84 mmol) and 5-hexyn-3-ol (200 µl, 1.80 mmol), Copper (II) chloride anhydrous (150 mg, 1.11 mmol) in 5 ml of methanol was stirring for 72 h to room temperature. The reaction mixture was evaporated to dryness under reduced pressure. The obtained solid was washed with water, yielding 44 % of product, m.p. 72-74 ^{o}C; IR V_{max} =3400 and 1060 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 0.96 (s, 6H), 1.00 (s, 3H), 1.20-1.36(m, 4H), 1.50-1.56 (m, 2H), 2.32-2.34 (m, 2H), 2.38 (m, 1H), 2.40-2.42 (m 2H), 2.46 (m,1H), 2.50 (broad, 2H), 3.48 (m, 1H), 3.50 (m, 1H), 4.48 (m, 1H), 5.62-5.74 (m, 4H), 6.68-7.18 (m, 4H) ppm. ^{13}C NMR (75.4 Hz, $CDCl_3$) δ_C: 9.68 (**C**-10), 9.98 (**C**-28, **C**-30), 29.34(**C**-9), 33.90 (**C**-27, **C**-29), 43.48 (**C**-16), 44.00 (**C**-11), 44.68 (**C**-24), 47.00 (**C**-21),71.10 (**C**-22), 72.22 (**C**-25), 79.10 (**C**-8), 115.48 (**C**-2, **C**-6), 120.11 (**C**-20), 124.00 (**C**-19), 127.08 (**C**-4), 127.70 (**C**-13), 129.00 (**C**-3, **C**-5), 132.00 (**C**-15), 140.00 (**C**-14),140.10 (**C**-12), 149.60 (**C**-18), 152.00 (**C**-17), 158.00 (**C**-1) ppm. EI-MS *m/z:*408.26. Anal.

Calcd. for $C_{27}H_{36}O_3$: C, 79.37; H, 8.88; O, 11.75. Found: C, 79.28; H, 8.76; O, 11.68.

Method B.

A solution of **5** (200 mg, 0.64 mmol) and 5-hexyn-3-ol (100 µl, 0.9 mmol), Copper (II) chloride anhydrous (90 mg, 0.66 mmol) in 5 ml of methanol was stirring for 72 h to room temperature. The reaction mixture was evaporated to dryness under reduced pressure. The obtained solid was washed with water, yielding 38 % of product. Similar ^{1}H NMR and ^{13}C NMR data were obtained compared with method A product.

1-[3-(4-Nitro-benzyl)-cyclobutadienyl]-butan-2-ol (4)

A solution of 1-nitro-4-prop-2-ynylbenzene (100 mg, 0.62 mmol) and 5-hexyn-3-ol (100µl, 0.90 mmol), Copper (II) chloride anhydrous (90 mg, 0.67 mmol) in 5 ml of methanol was stirring for 72 h to room temperature. The reaction mixture was evaporated to dryness under reduced pressure. The obtained solid was washed with water, yielding 52 % of product, m.p. 76-78 ^{o}C; IR V_{max} = 3402 and 1570 cm^{-1}; $_{1}H$ NMR (300 MHz, $CDCl_3$) δ_H:0.96 (s, 3H), 1.20-2.40 (m, 4H), 2.50 (broad, 1H), 3.50 (m, 2H), 3.52 (m, 2H), 4.60 (m,1H), 5.62-5.64 (m, 2H), 7.66-8.16 (m, 4H) ppm. $_{13}C$ NMR (75.4 Hz, $CDCl_3$) δ_C: 9.98 (**C**-19), 33.94 (**C**-18), 44.00 (**C**-7), 47.02 (**C**-14), 71.12 (**C**-15), 125.90 (**C**-3, **C**-6), 127.70 (**C**-9), 128.90 (**C**-2, **C**-6), 132.00 (**C**-11), 140.02 (**C**-10), 140.10 (**C**-8), 142.50 (**C**-1), 145.80(**C**-4) ppm. EI-MS *m/z:*259.12. Anal. Calcd. for $C_{15}H_{17}NO_3$: C, 69.48; H, 6.61; N, 5.40; O,18.51. Found: C, 69.39; H, 6.52.

1-(3-{4-[(1-ethylbut-3-ynyl)oxy]benzyl}cyclobuta-1,3-dien-1-yl)butan-2-ol (5)

A solution of **4** (200 mg, 0.77 mmol) and 5-hexyn-3-ol (100 µl, 0.90 mmol) potassium carbonate anhydrous (50 mg, 0.36 mmol) in 5 ml of dimethyl sulfoxyde was stirring for 72h to room temperature. The reaction mixture was evaporated to dryness under reduced pressure. The obtained solid was washed with water, yielding 48 % of product, m.p. 80-82^{o}C; IR V_{max} = 2100, 1568 and 1058 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 0.96 (s, 3H),1.00 (s, 3H), 1.20-1.36 (m, 2H), 1.60-1.80 (m, 2H), 2.00 (s, 1H), 2.34-2.40 (m, 2H), 2.46(m, 1H), 2.50 (broad, 1H), 2.66 (m, 1H), 3.50 (m, 2H), 3.52 (m, 1H), 4.60 (m, 1H), 5.62-5.64 (m, 2H), 6.60-7.10 (m, 4H) ppm. $_{13}C$ NMR (75.4 Hz, $CDCl_3$) δ_C: 9.98 (**C**-23), 10.80(**C**-10), 25.70 (**C**-16), 29.40 (**C**-9), 33.94 (**C**-22), 44.00 (**C**-11), 47.02 (**C**-19), 67.12 (**C**-18),

71.12 (**C**-20), 81.10 (**C**-8), 81.20 (**C**-17), 115.32 (**C**-2, **C**-6), 127.10 (**C**-4), 127.70 (**C**-13),129.00 (**C**-3, **C**-5), 132.00 (**C**-15), 140.02 (**C**-14), 140.10 (**C**-12), 159.18 (**C**-1), ppm.EIMS*m/z:*310.19. Anal. Calcd. for $C_{21}H_{26}O_2$: C, 81.25; H, 8.44; O, 10.31. Found: C, 81.18;H, 8.40.

1-(prop-2-yn-1-yl)-4-((1-(3-(4-((1-(4-(2-(4-(prop-2-yn-1-yl)phenoxy)butyl)cyclobuta-1,3-dien-1-yl)butan-2-yl)oxy)benzyl)cyclobuta-1,3-dien-1-yl)butan-2-yl)oxy)benzene (6)

A solution of **3** (200 mg, 0.49 mmol), 1-nitro-4-prop-2-ynylbenzene (80 mg, 0.50 mmol) potassium carbonate anhydrous (50 mg, 0.36 mmol) in 5 ml of dimethyl sulfoxide was stirring for 72 h to room temperature. The reaction mixture was evaporated to dryness under reduced pressure. The obtained solid was washed with water, yielding 42 % of product, m.p. 94-96 ^oC; IR V_{max} = 2102 and 1060 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ_H:1.00 (s, 9H), 1.50-1.56 (m, 6H), 2.02 (m, 2H), 2.36-2.46 (m, 6H), 3.48 (m, 4H), 3.50 (m,2H), 4.46-4.48 (m, 3H), 5.50-5.72 (m, 4H), 6.60-7.18 (m, 10H) ppm. ^{13}C NMR (75.4 Hz,$CDCl_3$) δ_C: 9.68 (**C**-10, (**C**-40, **C**-45), 25.10 (**C**-41, **C**-46), 29.30 (**C**-9, **C**-39, **C**-44), 43.44(**C**-16, **C**-30), 44.00 (**C**-11), 45.92 (**C**-21), 71.22 (**C**-43, **C**-48), 77.94 (**C**-22), 79.10 (**C**-8,**C**-31), 82.40 (**C**-42, **C**-47), 115.52 (**C**-2, **C**-6), 116.60 (**C**-25, **C**-29, **C**-34, **C**-38), 120.10(**C**-19, **C**-20), 123.80 (**C**-13), 127.10 (**C**-4), 128.30 (**C**-26, **C**-28, **C**-35, **C**-37), 128.60 (**C**-27, **C**-36), 129.00 (**C**-3, **C**-5), 137.22 (**C**-15), 140.04 (**C**-12), 147.66 (**C**-14), 157.20 (**C**-17,**C**-18), 157.82 (**C**-24), 157.84 (**C**-33), 158.00 (**C**-1), ppm. EI-MS *m/z:*636.36. Anal. Calcd.for $C_{45}H_{48}O_3$: C, 84.87; H, 7.60; O, 7.54. Found: C, 84.80; H, 7.52

1-(3-(4-((1-(3-(4-((1-(4-(2-(4-((3-(2-hydroxybutyl)cyclobuta-1,3-dien-1-yl)methyl)phe-noxy)butyl)cyclobuta-1,3-dien-1-yl)butan-2-yl)oxy)benzyl)cyclobuta-1,3-dien-1-yl) butan-2-yl)oxy)benzyl)cyclobuta-1,3-dien-1-yl)butan-2-ol (7)

A solution of **6** (200 mg, 0.31 mmol), 5-hexyn-3-ol (50 µl, 0.45 mmol), Copper(II) chloride anhydrous (50 mg, 0.37 mmol) in 5 ml of methanol was stirring for 72 h to room temperature The reaction mixture was evaporated to dryness under reduced pressure. The obtained solid was washed with water, yielding 64 % of product, m.p. 84-86 ^oC; IR V_{max} =3400 and 1062 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ_H: 0.96 (s, 6H), 1.00 (s, 9H), 1.20-1.38(m, 4H), 1.50-

1.56 (m, 6H), 2.36-2.46 (m, 10H), 2.50 (broad, 2H), 3.50 (m, 6H), 3.52 (m,2H), 4.46-4.48 (m, 3H), 5.50-5.72 (m, 8H), 6.66-7.18 (m, 12H) ppm. ^{13}C NMR (75.4 Hz,$CDCl_3$) δ_C: 9.68 (**C**-10, (**C**-10, **C**-40, **C**-47), 9.98 (**C**-60, **C**-62), 29.30 (**C**-9, **C**-39, **C**-46),33.90 (**C**-59, **C**-61), 43.44 (**C**-16, **C**-30), 44.00 (**C**-41, **C**-48), 45.92 (**C**-21), 47.00 (**C**-53,**C**-56), 71.10 (**C**-54, **C**-57), 77.94 (**C**-22), 79.10 (**C**-8, **C**-31), 115.52 (**C**-2, **C**-6, **C**-25, **C**-29, **C**-34, **C**-38), 120.10 (**C**-19, **C**-20), 123.80 (**C**-13), 127.10 (**C**-4, **C**-27, **C**-36), 127.70(**C**-43, **C**-50), 129.00 (**C**-3, **C**-5, **C**-26, **C**-28, **C**-35, **C**-37), 132.00 (**C**-45,**C**-52), 137.22(**C**-15), 140.00 (**C**-44, **C**-51), 144.06 (**C**-12, **C**-42, **C**-49), 147.66 (**C**-14), 157.20 (**C**-17, **C**-18), 158.00 (**C**-24), 158.06 (**C**-1, **C**-33) ppm. EI-MS *m/z:*832.50. Anal. Calcd. For$C_{57}H_{68}O_5$: C, 82.17; H, 8.23; O, 9.60. Found: C, 82.09; H, 8.18.

N-[3-(1-{3-[4-(1-{2-[2-(5-Isobutytylamino-2-trifluoromethyl-phenoxy)-butyl]cyclobutadienylmethyl}-propoxy)-benzyl]cyclobutadienylmethyl}-propoxy)-4-trifluromethyl-phenyl]isobutyramide (8)

A solution of **3** (200 mg, 0.77 mmol), flutamide (215 mg, 0.78 mmol) and potassium carbonate anhydrous (50 mg, 0.36 mmol) in 5 ml of dimethyl sulfoxide was stirring for 72h to room temperature. The reaction mixture was evaporated to dryness under reduced pressure. The obtained solid was washed with water, yielding 34 % of product, m.p. 88-90^{o}C; IR V_{max} = 1632 and 1062 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: δ_H: 1.00 (s, 9H), 1.16(s, 6H), 1.18 (s, 6H), 1.50-1.56 (m, 6H), 2.36-2.46 (m, 6H), 2.54 (m, 2H), 3.50 (m, 2H),4.40-446 (m, 3H), 5.50-5.72 (m, 4H), 6.68-7.78 (m, 10H), 10.70 (broad, 1H) ppm. ^{13}CNMR (75.4 Hz, $CDCl_3$) δ_C: 9.68 (**C**-10, **C**-36, **C**-52), 19.60 (**C**-24, **C**-32), 19.62 (**C**-61, **C**-62), 29.30 (**C**-9, **C**-35, **C**-52), 33.80 (**C**-60), 38.00 (**C**-23), 43.44 (**C**-37, **C**-10), 44.00 (**C**-25), 45.92 (**C**-16), 79.04 (**C**-34), 79.50 (**C**-8), 80.60 (**C**-43), 97.30 (**C**-6, **C**-50), 113.50 (**C**-4, **C**-48), 114.22 (**C**-2, **C**-46), 115.50 (**C**-28, **C**-30), 120.10 (**C**-40, **C**-41), 123.80 (**C**-11, **C**-53), 123.83 (**C**-18), 125.86 (**C**-3, **C**-47), 127.08 (**C**-26), 129.00 (**C**-27, **C**-31), 137.22 (**C**-20), 140.10 (**C**-19), 141.12 (**C**-49), 142.66 (**C**-5), 147.66 (**C**-17), 157.20 (**C**-38, **C**-39),158.00 (**C**-29), 160.80 **C**-1, **C**-45), 174.74 (**C**-14), 175.63 (**C**-56) ppm. EI-MS *m/z:*866.40.

Anal.Calcd.for $C_{49}H_{56}F_6N_2O_5$: C, 67.88; H, 6.51; F, 13.15; N, 3.23; O, 9.23. Found: C,67.72; H, 6.44.

N-(3-{1-[3-(4-Nitro-benzyl)-cyclobutadienylmethyl]-propoxy}-4-triofluoromethylphenyl)-isobutyramide (9)

A solution of **4** (200 mg, 0.77 mmol), flutamide (215 mg, 0.78 mmol) and potassium carbonate anhydrous (50 mg, 0.36 mmol) in 5 ml of dimethyl sulfoxide was stirring for 72h to room temperature. The reaction mixture was evaporated to dryness under reduced pressure. The obtained solid was washed with water, yielding 48 % of product, m.p. 74-76^{o}C; IR V_{max} = 1630 and 1570 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 1.00 (s, 3H), 1.16(s, 6H), 1.50-2.46 (m, 4H), 2.54 (m, 1H), 3.50 (m, 2H), 4.40 (m, 1H), 5.50-5.64 (m, 2H),7.18-8.20 (m, 8H), 11.20 (broad, 1H) ppm. ^{13}C NMR (75.4 Hz, $CDCl_3$) δ_C: 9.68 (**C**-10),19.60 (**C**-24, **C**-32), 29.30 (**C**-9), 38.00 (**C**-23), 44.00 (**C**-25), 45.92 (**C**-16), 79.50 (**C**-8),97.30 (**C**-6), 113.50 (**C**-4), 114.22 (**C**-2), 123.80 (**C**-11), 123.83 (**C**-18), 125.86 (**C**-3),125.88 (**C**-28, **C**-30), 128.90 (**C**-27, **C**-31), 137.22 (**C**-20), 140.10 (**C**-19), 142.50 (**C**-26),142.66 (**C**-5), 145.74 (**C**-29), 147.66 (**C**-17), 167.74 (**C**1), 174.74 (**C**-14) ppm. EI-MS*m/z:*488.19. Anal. Calcd. for $C_{26}H_{27}F_3N_2O_4$: C, 63.93; H, 5.57; F, 11.67; N, 5.73; O, 13.10.Found: C, 63.84; H, 5.48.

N-[3-(1-{3-[4-(1-Ethyl-but-3-ynyloxy)benzyl]cyclobutadienylmethyl}-propoxy)-4-trifluoromethyl-phenyl]-isobutiramide (10)

A solution of **5** (200 mg, 0.64 mmol) and flutamide (215 mg, 0.78 mmol) potassium carbonate anhydrous (50 mg, 0.36 mmol) in 5 ml of dimethyl sulfoxide was stirring for 72h to room temperature. The reaction mixture was evaporated to dryness under reduced pressure. The obtained solid was washed with water, yielding 44 % of product, m.p. 92-94^{o}C; IR V_{max} = 2102 and 1060 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 0.98 (s, 3H), 1.00 (s,3H), 1.16 (s, 6H), 1.50-1.56 (m, 2H), 1.60-1.80 (m, 2H), 2.00 (s, 1H), 2.36 (m, 1H), 2.46(m, 1H), 2.48 (m, 1H), 2.52 (m, 1H), 2.66 (m, 1H), 3.50 (m, 2H), 4.40 (m, 1H), 4.60 (m,1H), 5.52-5.64 (m, 2H), 6.60-7.76 (m, 7H), 11.20 (broad, 1H), ppm. ^{13}C NMR (75.4 Hz,$CDCl_3$) δ_C: 9.68 (**C**-10), 10.80 (**C**-36), 19.60 (**C**-24, **C**-32), 25.70 (**C**-37), 29.30 (**C**-9),29.40 (**C**-35), 38.00 (**C**-23), 44.00 (**C**-25), 45.92 (**C**-16), 67.12 (**C**-39), 79.48 (**C**-8), 81.10
(**C**-34), 81.20 (**C**-38), 97.30 (**C**-6), 113.50 (**C**-4), 114.22 (**C**-2), 115.32 (**C**-28, **C**-

30),123.80 (**C**-11), 123.83 (**C**-18), 125.88 (**C**-3), 127.10 (**C**-26), 129.00 (**C**-27, **C**-31), 137.22(**C**-20), 140.08 (**C**-19), 142.66 (**C**-5), 147.66 (**C**-17), 159.18 (**C**-29), 160.78 (**C**-1), 174.74(**C**-14), ppm. EI-MS *m/z:*310.19. Anal. Calcd. for $C_{21}H_{26}O_2$: C, 81.25; H, 8.44; O, 10.31.Found: C, 81.18; H, 8.40.

N-(4((1-(3-(4-(((1-(3-(4-((1-(4-(2-(4-((3-(2-(4-isobutyramido-2-trifluoromethyl)phenoxy) butyl)cycnobuta-1,3-dien-1-yl)methyl)phenoxy)butyl)cyclobuta-1,3-dien-1-yl)butan-2-yl)oxy)benzyl)cyclobuta-1,3-dien-1-yl)butan-2-yl)oxy)benzyl)cyclobuta-1,3-dien-1-yl)butan-2-yl)oxy)-3--(trifluoromethyl)phenyl)isobutiramide (11)

A solution of **6** (200 mg, 0.49 mmol), flutamide (150 mg, 0.54 mmol) and potassium carbonate anhydrous (50 mg, 0.36 mmol) in 5 ml of dimethyl sulfoxide was stirring for 72h to room temperature. The reaction mixture was evaporated to dryness under reduced pressure. The obtained solid was washed with water, yielding 55 % of product, m.p. 102-104 ^{o}C; IR V_{max} = 1634 and 1062 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 1.00 (s, 15H),1.16 (s, 6H), 1.18 (s, 6H), 1.50 (m, 5H), 1.56 (m, 5H), 2.36 (m, 2H), 2.38-2.46 (m, 10H),2.54 (m, 2H), 3.50 (m, 6H), 4.40-4.48 (m, 5H), 5.50-5.72 (m, 8H), 6.66-7.18 (m, 18H),9.40 (broad, 2H) ppm. ^{13}C NMR (75.4 Hz, $CDCl_3$) δ_C: 9.68 (**C**-10, (**C**-10, **C**-36, **C**-52, **C**-68, **C**-84), 19.60 (**C**-93, **C**-94), 19.66 (**C**-24, **C**-32), 29.30 (**C**-9, **C**-35, **C**-51, **C**-67, **C**-83),33.78 (**C**-23), 38.00 (**C**-92), 43.44 (**C**-37, **C**-42), 44.00 (**C**-25, **C**-53, **C**-69), 45.92 (**C**-16,**C**-58, **C**-74), 77.94 (**C**-59), 79.10 (**C**-34, **C**-43), 79.52 (**C**-8, **C**-75), 115.30 (**C**-6, **C**-82),115.52 (**C**-28, **C**-46, **C**-50, **C**-62, **C**-66), 115.72 (**C**-2, **C**-78), 116.82 (**C**-3, **C**-79), 120.10(**C**-40, **C**-41), 122.14 (**C**-11, **C**-85), 122.20 (**C**-81), 123.34 (**C**-5), 123.80 (**C**-18, **C**-55, **C**-71), 127.10 (**C**-26, **C**-48, **C**-64), 127.70 (**C**-43, **C**-50), 129.00 (**C**-27, **C**-31, **C**-47, **C**-49, **C**-63, **C**-65), 131.00 (80), 134.16 (**C**-4), 137.22 (**C**-29, **C**-57, **C**-73), 140.10 (**C**-19, **C**-54, **C**-70), 147.66 (**C**-17, **C**-56, **C**-72), 150.00 (**C**-1, **C**-77), 157.20 (**C**-38, **C**-39), 158.00 (**C**-61),158.04 (**C**-29, **C**-45), 174.68 (**C**-88), 175.64 (**C**-14) ppm. EI-MS *m/z:*1290.64. Anal.Calcd.for $C_{79}H_{88}F_6N_2O_7$: C, 73.47; H, 6.87; F, 8.83; N, 2.17; O, 8.67. Found: C, 73.32; H, 6.74.

2.9.2 Evaluation of interaction of flutamide and compound 8 to 11 with androgen receptor (2ylo) using Docking Server

Docking calculations were carried out using Docking Server (Bikadi and Hazai,

2009). The MMFF94 force field (Halgren, 1999) was used for energy minimization of ligand molecule using the Docking Server. Gasteiger partial charges were added to the ligand atoms. Non-polar hydrogen atoms were merged, and rotatable bonds were defined. Docking calculations were carried out on the2ylo-transcription protein model. Essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added with the aid of AutoDock tools (Morris *et al*., 1999). Affinity (grid) maps of 20 × 20 × 20-Å grid points and 0.375-Å spacing were generated using the Autogrid program. AutoDock parameter set and distance dependent dielectric functions were used in the calculation of the Vander Waals and the electrostatic terms, respectively. Docking simulations were performed using the Lamarckian genetic algorithm (LGA) and the Solis and Wets local search method (Solis, 1981). Initial position, orientation, and torsions of the ligand molecules were set randomly. Each docking experiment was derived from two different runs that were set to terminate after a maximum of 250,000 energy evaluations. The population size was set to 150. During the search, a translational step of 0.2Å and quaternion and torsion steps of 5 were applied.

Evaluation of interaction of flutamide and compound 8 to 11 with P-glycoprotein using Biozyne P-gp software

The ClogP and total polar surface area of compounds were calculated using the BioZyneP-gp server (Pan *et al*., 2016) to show whether a compound is likely to be a substrate of the P-gp drug efflux pump.

RESULTS AND DISCUSSION

There are reports which indicate the preparation of diverse flutamide derivatives; nevertheless, expensive reagents and special conditions are required (Jacobsona *et al*., 2006; He *et al*., 2009; Paven *et al*., 2011). Therefore, inthis study a new flutamide derivative was synthetized using several strategies.

Preparation of an ether derivative

The first step was achieved by the synthesis of 1-(1-Ethyl-but-3-ynyloxy)-4-prop-2-ynylbenzene (compound **2**, Figure 1) via displacement of nitro group from 1-nitro-4-prop-2-ynylbenzene (**1**). It is noteworthy that there are several methods for displacement of nitrogroups by methoxy groups (Kornblum *et al*., 1976), fluoride ion (Attiná *et al*., 1983), nitropropane or nitrocyclohexanone (Kornblum *et al*, 1970),sodium phenoxide

(Crossley *et al*., 1997), nitrobenzamide in DMSO (Beck, 1978) and others. In this study, the compound **1** was reacted with and 5-hexyn-3-ol using a previously report for synthesis of ethers (Figueroa-Valverde *et al*., 2014). The ^{1}H NMR spectrum of **2** showed several signals at 1.00 ppm for methyl group of the arm bound to both ether and alkyne groups; at 1.60-1.80, 2.44-2.66 and 4.60ppm for methylene groups bound to both ether and alkyne groups; at 2.00-2.04 ppm foralkyne groups; at 3.50 ppm for methylene group bound to both phenyl and alkyne group; at6.50-7.10 ppm for phenyl group. The ^{13}C NMR spectra displays chemical shifts at 10.80ppm for methyl group; at 25.10 ppm for methylene group bound to both phenyl and alkynegroup; at 25.72-29.44 and 81.08 ppm for methylene groups bound to both ether and alkynegroups; at 67.12-71.22, 81.22-82.44 ppm for carbons of alkyne groups; at 116.42-159.00ppm for phenyl group. Finally, the presence of compound **2** was confirmed with mass spectrum which showed a molecular ion at 212.12.

Synthesis of a new cyclobutadiene derivative (3)

There are several reports to preparation of cyclobutadiene rings using some reagents suchas cyclopenta dienylcobalt dicarbonyl (Roidi*et al*., 1999), cyclopenta dienylcobalt dicarbonyl diphosphines (Chang *et al*., 2009), diazomethane (Maier *et al*., 2002), ferrocene (Wolf et al., 2009) and others. In this study, two methods were used; in first (Method A) the compound **2** was reacted with 5-hexyn-1-ol using CooperII chlorideas catalyst to form **3** (Figure 1). The ^{1}H NMR spectrum of **3** shows signals at 0.96 ppm form ethyl of arm bound to both to hydroxyl group and cyclobutadiene ring; at 1.00 ppm form ethyl of arm bound to ether and phenyl group; at 1.20-1.30, 2.32-2.34, 2.40-2.42 and 3.50ppm for methylene groups involved in the arm bound to both to hydroxyl group and cyclobutadiene ring; at 1.50-1.56, 2.38, 2.46 and 4.48 ppm for methylene groups involved in the arm bound to ether and phenyl groups; at 2.50 ppm for hydroxyl group; a3.48 ppmfor methylene group bound to both phenyl group and cyclobutadiene ring; at 5.62-5.34 ppm for cyclobutadiene ring; at 6.68-7.18 ppm for phenyl group. The ^{13}C NMR spectra displays chemical shifts at 9.68 ppm for methyl of arm bound to ether and phenyl group; at 9.98ppm for methyl of arm bound to both to hydroxyl group and cyclobutadiene ring; at 29.34,43.48 and 79.10 ppm for methylene groups involved

in the arm bound to ether and phenylgroups; at 33.90, 44.68-72.22 ppm for methylene groups involved in the arm bound to both to hydroxyl group and cyclobutadiene ring; at 44.00 ppm for carbon bound to both phenyl and cyclobutadiene ring; at 115.48, 127.08, 129.00 and 158.00 ppm for phenyl group; at120.11-124.00, 127.70 and 132.00-152.00 ppm for cyclobutadiene ring. In addition, the presence of compound **3** was confirmed with mass spectrum which showed a molecular ion at 408.26.

In the second method (Method B) the compound **5** (Figure 1) was reacted with 5-hexyn-1-ol using Cooper II chloride as catalyst to form **3**. It was found that yielding of **3** was lower compared with method A.

Preparation of 1-[3-(4-Nitro-benzyl)cyclobutadienyl]butan-2-ol (4).

The compound **4** (Figure 1) was prepared by the reaction of **1** with 5-hexyn-1-ol in presence of CooperII chloride. The ^{1}H NMR spectrum of **4** shows signals at 0.96 ppm form ethyl group; at 1.20-2.40 and 3.52 ppm for methylene groups bound to both hydroxylgroup and cyclobutadiene ring; at 2.50 ppm for hydroxyl group; at 5.62-5.64 for cyclobutadiene ring; at 7.66-8.16 ppm for phenyl group. The ^{13}C NMR spectra displays chemical shifts at 9.98 ppm for methyl group; at 33.94 and 47.02-71.12 ppm for methylene groups bound to both hydroxyl group and cyclobutadiene ring; at 44.00 ppm for carbon bound to both phenyl group and cyclo butadiene ring; at 125.990, 128.90 and 142.50-145.80ppm for phenyl group, at 127.70 and 132.00-140.10 ppm for cyclobutadiene ring. Finally, the presence of compound **4** was confirmed with mass spectrum which showed a molecularion at 259.12.

***Synthesis of* 1-(3-{4-[(1-ethylbut-3-ynyl)oxy]benzyl}cyclobuta-1,3-dien-1-yl)butan-2-ol(5).**

The compound **5** was prepared via displazament of nitro group involved in the chemical structure of **4** using hexyn-3-ol/DMSO (Figure 1). The ^{1}H NMR spectrum of **5** showssignals at 0.96 ppm for methyl involved in the arm bound to both hydroxyl group and cylobutadiene ring; at 1.00 ppm for methyl involved in the arm bound to both ether group and alkyne group; at 1.20-1.36, 2.34-2.40 and 3.52 ppm for methylene involved in the armbound to both hydroxyl group and cyclobutadiene ring; at 1.60-1.8'0, 2.46, 2.66 and 4.60ppm for methylene groups

involved in the arm bound to both ether group and alkyne group;
at 2.00 ppm for alkyne group; at 2.50 ppm for hydroxyl group; at 3.50 ppm for carbonbound to both phenyl group and cyclobutadiene ring; at 5.62-5.64 for cyclobutadiene ring;at 6.60-7.10 ppm for phenyl group. The ^{13}C NMR spectra displays chemical shifts at 9.68ppm for methyl involved in the arm bound to both hydroxyl group and cyclobutadiene ring;at 10.80 ppm for methyl involved in the arm bound to both ether group and alkyne group; at 25.70-29.40 and 81.10 ppm for methylene groups involved in the arm bound to both ether group and alkyne group; at 39.94 47.02 and 71.12 ppm for methylene involved in the arm bound to both hydroxyl group and cyclobutadiene ring; at 44.00 ppm for carbon bound
to both phenyl group and cyclobutadiene ring; at 67.12 and 81.20 ppm for alkyne group; at115.32-127.10, 129.00 and 159.18 ppm for phenyl group, at 127.70 and 132.00-140.10 ppm for cyclobutadiene ring. In addition, the presence of compound **5** was confirmed with mass spectrum which showed a molecular ion at 310.19.

Synthesis of a di-cyclobutadiene derivative (6)

The compound **6** (Figure 2) was prepared via displazament of nitro group of **4** by 5-hexyn-3-ol. The ^{1}H NMR spectrum of **6** shows signals at 1.00 ppm for methyl groups; at 1.50-1.56, 2.36-2.46 and 4.46-4.48 ppm for methylene groups involved in the arm bound to both ether groups and cyclobutadiene rings; at 2.02 ppm for alkyne groups; at 3.48 ppm for carbon bound to both phenyl groups and cyclobutadiene rings; at 5.50-5.72 ppm for cyclobutadiene rings; at 6.60-7.18 ppm for phenyl groups. The ^{13}C NMR spectra displays chemical shifts at 9.68 ppm for methyl groups; at 25.10 ppm for methylene groups bound to
both phenyl and alkyne groups; at 29.30-43.44, 45.92 and 77.94-79.10 ppm for methylene groups involved in the arm bound to both ether groups and cyclobutadiene rings; at 44.00ppm for carbons bound to both phenyl groups and cyclobutadiene rings; at 71.22 and 82.40ppm for alkyne groups, at 115.52-116.60, 127.10-129.00 and 157.82-158.00 ppm for phenyl groups, at 120.10-123.80 and 137.22-157.20 ppm for cyclobutadiene rings. Finally, the presence of compound **6** was confirmed with mass spectrum which showed a molecularion at 636.36.

***Preparation of a tetra-cyclobutadiene derivative* (7)**

The compound **7** (Figure 2) was prepared via cycloaddition 2+2 by the reaction of **6** with 5-

hexyn-1-ol using Cooper II chloride as catalyst. The ^{1}H NMR spectrum of **7** shows signals at 0.96 ppm for methyl groups involved in the arm bound to both hydroxyl groups and cyclobutadiene rings; at 1.00 ppm for methyl groups involved in the arm bound to both ether groups and cyclobutadiene rings; at 1.20-1.38, 2.36-2.46 and 3.52 ppm for methylene groups bound to both involved in the arm bound to both hydroxyl groups and cyclobutadiene rings, at 1.50-1.56 and 4.46-4.48 ppm for methylene involved in the arm bound to both ether groups and cyclobutadiene rings; at 2.50 ppm for hydroxyl groups; at3.50 ppm for methylene bound to both phenyl and cyclobutadiene rings; at 5.50-5.72 ppm for cyclobutadiene rings; at 6.66-7.18 ppm for phenyl groups. The ^{13}C NMR spectra displays chemical shifts at 9.68 ppm for methyl groups involved in the arm bound to both ether groups and cyclobutadiene rings; at 9.98 ppm for methyl groups involved in the armbound to both hydroxyl groups and cyclobutadiene rings; at 29.30, 43.44, 45.92, 77.94-79.10 ppm for methylene groups involved in the arm bound to both ether groups and cyclobutadiene rings; at 33.90 and 47.00-71.10 ppm for methylene involved in the armbound to both hydroxyl groups and cyclobutadiene rings; at 44.00 ppm for carbon bound toboth phenyl groups and cyclobutadiene rings; at 115.52, 127.10, 129.00 and 158.00-158.06ppm for phenyl groups, at 120.10-123.80, 127.70 and 132.00-157.20 ppm for cyclobutadiene rings. In addition, the presence of compound **7** was confirmed with mass spectrum which showed a molecular ion at 832.50.

Synthesis of cyclobutadiene-flutamide derivatives (8 to 11)

In the first stage the compound **8** (Figure 3) was prepared via displazament of nitro group involved in the chemical structure of flutamide with the compound **3** in DMSO. The ^{1}HNMR spectrum of **8** shows signals at 1.00 ppm for methyl groups involved in the arm bound to both ether group and cyclobutadiene ring; at 1.16 ppm for methyl groups bound to amide groups; at 1.50-2.46 ppm for methylene groups bound to ether group and cyclobutadiene ring; at 2.54 ppm for

methylene bound to amide and methyl groups; at 4.40-

4.46 ppm for methylene bound to both phenyl group and cyclobutadiene ring; at 5.50-5.72ppm for cyclobutadiene ring; at 6.68-7.78 ppm for phenyl groups; at 10.70 ppm for amide groups. The ^{13}C NMR spectra displays chemical shifts at 9.68 ppm for methyl groups involved in the arm bound to both ether group and cyclobutadiene ring; at 19.60-19.62 ppm for methyl groups bound to amide groups; at 29.30, 43.44 and 45.92-80.60 ppm form ethylene groups bound to ether group and cyclobutadiene ring; at 33.80-38.00 ppm form ethylene group bound to amide group; at 44.00 ppm for carbon bound to both phenyl and cyclobutadiene ring; at 97.30-11.50, 123.86, 127.08-129.00, 141.12-142.66 and 158.00-160.80 ppm for phenyl groups; at 120.10, 123.83, 137,22-140.10, 147.66 and 157.20 ppm for cyclobutadiene ring; at 123.80 ppm for methylene groups bound to fluoride atom; at174.74-175.63 ppm for amide groups. Additionally, the presence of compound **8** was confirmed with mass spectrum which showed a molecular ion at 866.40.

Also, **9** was prepared via displazament of nitro group involved in the chemical structure of flutamide with the compound **4** in DMSO (Figure 3). The ^{1}H NMR spectrum of **9** shows signals at 1.00 ppm for methyl group bound to both ether group and cyclobutadiene ring; at1.16 ppm for methyl groups bound to amide group; at 1.50-2.46 and 4.40 ppm form ethylene groups bound to both ether group and cyclobutadiene ring; at 350 ppm form ethylene bound to both phenyl group and cyclobutadiene ring; at 5.50-6.64 ppm for cyclobutadiene ring; at 7.18-8.20 ppm for phenyl group: at 11.20 for amide group. The $^{13}CNMR$ spectra displays chemical shifts at 9.68 ppm for methyl group bound to both ether group and cyclobutadiene ring; at 19.60 for methyl groups bound to amide group; at 29.30and 45.92-79.50 ppm for methylene groups bound to both ether group and cyclobutadiene ring; at 38.00 ppm for carbon bound to amide and methyl groups;; at 44.00 ppm form ethylene bound to both phenyl group and cyclobutadiene ring; at 97.30-114.22, 125.86-128.90, 142.50-145.74 and 160.77 ppm for phenyl groups; at 123.80 ppm for carbon bound

To trifluoride atoms; at 123.83, 137.22-140.10 and 147.66 ppm for cyclobutadiene ring; at174.74 for amide group. Finally, the presence of compound **9** was

confirmed with mass spectrum which showed a molecular ion at 488.19.

In the third stage the compound **10** was prepared via displazament of nitro group involvedin the chemical structure of flutamide with the compound **5** in DMSO (Figure 4). The ^{1}HNMR spectrum of **10** shows signals at 0.98 ppm for methyl group bound to both ethergroup and cyclobutadiene ring; at 1.00 ppm for methyl group bound to both ether andalkyne groups; at 1.16 ppm for methyl groups bound to amide group; at 1.50-1.56, 2.36,and 4.40 ppm for methylene groups bound to both ether group and cyclobutadiene ring; at1.60-1.80, 2.46, 2.66 and 4.60 ppm for methylene groups involved in the arm bound to bothether and alkyne groups; at 2.00 for alkyne group; at 2.52 ppm for methylene group boundto both amide and methyl groups; at 3.50 ppm for methylene bound to both phenyl groupand cyclobutadiene ring; at 5.52-5.64 ppm for cyclobutadiene ring; at 6.60-7.76 ppm forphenyl groups; at 11.20 ppm for amide group. The ^{13}C NMR spectra displays chemical lshifts at 9.68 ppm for methyl group bound to both ether group and cyclobutadiene ring; at10.80 ppm for methyl group bound to both ether and alkyne groups; at 19.60 ppm methylgroups bound to amide group; at 25.70, 29.40 and 81.10 ppm for methylene groups involved in the arm bound to both ether and alkyne groups; at 29.30, 45.92 and 79.48 form ethylene groups involved in the arm bound to both ether group and cyclo butadiene ring; at 38.00 ppm for carbon bound to amide and methyl groups; at 44.00 ppm for carbon boundto both phenyl group and cyclobutadiene ring; at 67.12 and 81.20 for alkyne group; at97.32-115.32, 125.88-129.00, 142.68-147.66 and 159.18-160.78 ppm for phenyl groups; at123.82 for carbon bound to trifluoride atoms; at 123.84, 137.22-140.08 and 147.66 ppm for cyclobutadiene ring; at 174.74 ppm for amide group. Additionally, the presence of compound **10** was confirmed with mass spectrum which showed a molecular ion at 310.19.

The following stage was achieved by reaction of **7** with flutamide to form **11** (Figure 4); the ^{1}H NMR spectrum of **11** shows signals at 1.00 ppm for methyl group involved in thearm bound to both ether group and cyclobutadiene ring; at 1.16-1.18 ppm for methylgroups bound to amide group; at 1.50-2.46 and 4.40-4.48 ppm for methylene groups bound to ether group and cyclobutadiene ring; at 2.54 ppm for methylene groups bound to both

amide and methyl groups; at 3.50 ppm for methylene group bound to both phenyl group and cyclobutadiene ring; at 5.50-5.72 ppm for cyclobutadiene ring; at 6.66-7.18 ppm for phenyl groups; at 9.40 ppm for amide group. The ^{13}C NMR spectra displays chemical shiftsat 9.68 ppm for methyl group involved in the arm bound to both ether group and cyclobutadiene ring; at 19.60 ppm for methyl groups bound to amide group; at 29.30, 43.44and 45.92-79.52 ppm for methylene groups bound to ether group and cyclobutadiene ring; at 33.78-38.00 ppm for carbons bound to both amide and methyl groups; at 115.30-116.82,122.20-123.34,127.10-134.16 and 155.00-158.00 ppm for phenyl groups; at 120.10,123.80, 33.22-147.66 and 157.20 ppm for cyclobutadiene rings; at 122.14 ppm for carbons bound to fluoride atoms; at 174.68-175.64 ppm for amide groups. Finally, the presence of compound **11** was confirmed with mass spectrum which showed a molecular ion at1290.64.

Evaluation of interaction flutamide derivatives-androgen receptor using a dockingmodel.

In order, to evaluate the possibility that flutamide derivatives could interact with androgen receptor [PDB ID:2ylo] (Lack et al., 2011) in this study, a molecular docking model (server doking) was used (Perez and Saven, 2012; Rosales and Correa, 2015). The results showed a π-π bound (Trp^{741} and Phe^{764}) for flutamide and compound **9** and cation-π bound (Phe^{764}) for **8** and **10** (Figure 5-9 and tables 1-11). Other results theoretical intramolecular parameters involved between interaction of flutamide and the compound **8** to **11** are showed in the Table 12; the results indicate that free energy of binding and lowest interactive surface for compounds **9** and **11** was higher compared with flutamide and the compounds **8** and **10**. These data suggest that **9** and **11** possess highest probability of interaction with binding site of androgen receptor (2ylo). However, the inhibition constant (Ki) is low in the compound **9** in comparison with **11**; these theoretical results indicated that interaction of **9** with androgen receptor is could be higher that compound **11**. These hypotheses are availed by other studies which indicate the influence of free energy of binding and Ki on the interaction protein ligand (Tripathi *et al*., 2012; Verapur, 2012). In addition, docking analysis, the total breakdown energy in terms of van der Waals and electrostatic energy (Table 1) indicated the strong influence for stabilizing binding;

the total inter molecular energy, the cumulative sum of van der Waals, H-bond, dissolve and electrostatic energy (vdW + Hbond + desolv energy). In addition, other theoretical results showed different the decomposed interaction energies (kcal/mol) between flutamide and the compounds **8** to **11** and the amino acid residues from androgen receptor (table 2). All these data suggest that the interaction of these compounds with the androgen receptor is conditioned by their physicochemical properties.

Interaction of flutamide and its derivatives on the glycoprotein (P-gp)

Analyzing the data above mentioned and other reports, which indicate that there is other substance such as the glycoprotein P-gp which is strongly, related to resistance development prostate cancer (Gimenez *et al*., 2004) and the efflux of the androgens (Fedoruk *et al*., 2004). It is important to mention that P-gp is a well-characterized transporter (which transports a wide variety of substrates including small molecules such as carbohydrates and organic cations, and macromolecules such as proteins and polysaccharides (Rapposelli *et al*., 2009). Additionally, several drugs used for treatment of cancer such as vinca alkaloids, anthracyclines, epipodophyllotoxins, and taxanes, are known to be P-gp substrates, resulting in some cases the loss of its therapeutic effects against cancer (Ford and Hait, 1990 Fereidoonnezhad *et al*., 2016). There are some physicochemical descriptors involved in the chemical structure of several drugs such as the size of molecule (expressed as the number of atoms), and the specific atomic volume (reciprocal density, in Å 3/atom). Analyzing these data, in this study, the Biozyne P-gp software (Levatić *et al*., 2013) was used to evaluate the possibility that the flutamide and its derivatives may be substrates or non-substrates of the glycoprotein P-gp. The theoretical data shown a specific volume ($Å^3$/atom) > 7.5 for flutamide (Figure 10); these results suggest that flutamide is non-substrate of glycoprotein P (Figure 10). Other data showed a specific volume ($Å^3$ / atom) <7.5 for compounds 8 to11 (Figure 11-14); in addition, other data showed in the table 13 indicate that octanol-water partition coefficient (XlogP) and total polar surface area was higher for compounds 8-11compared with flutamide. This phenomenon suggests the probability of these compounds could be substrates of glycoprotein P-gp (Figure 11-14).

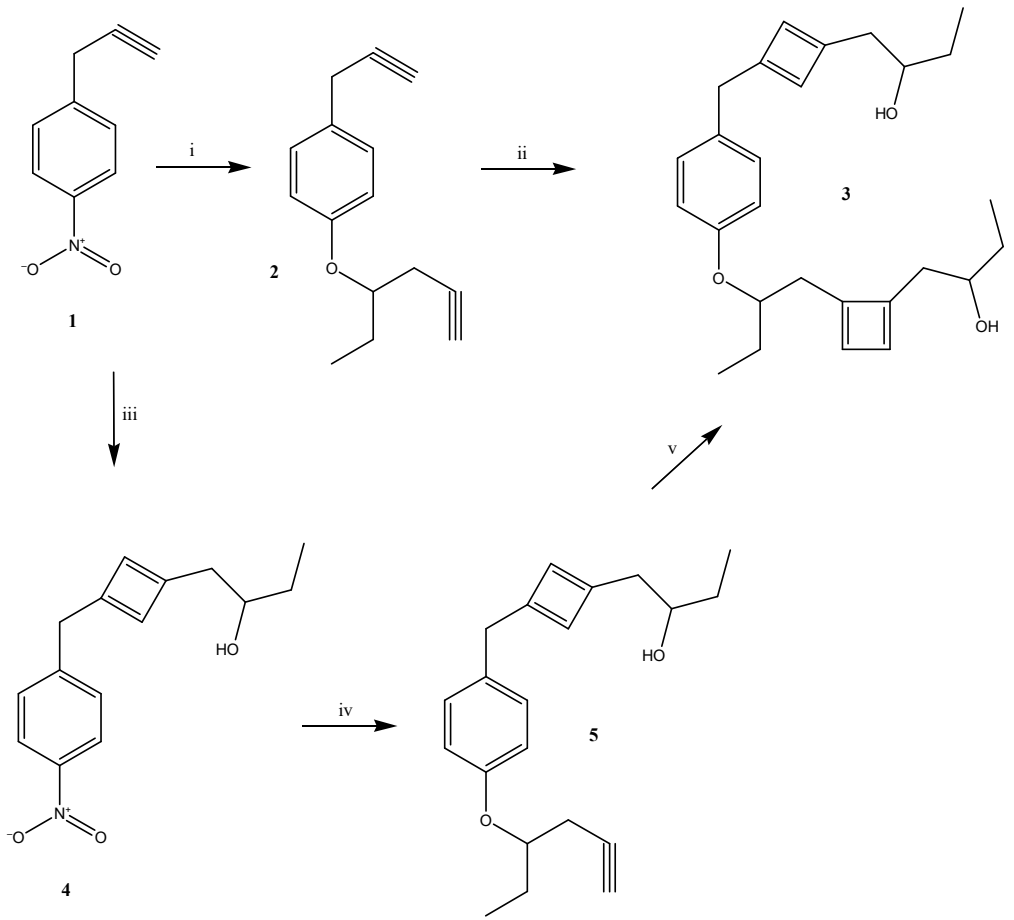

Figure 1: Preparation of acyclobutadienyl-butanol derivative (3).The first stage was achieved by synthesis of butynyloxy-propynyl-benzene (2) by the reaction of1-nitro-4-prop-2-ynylbenzene (1). After, 1 was reacted with 5-hexyn-3-ol (iii) to form a nitro-cyclobutadienyl-olderivative (4). Following, 5(oxy-cyclobutdien-ol derivative) was prepared by the reaction of 4 with 5-hexyn-3-ol (iv). Finally,3 was synthesized by the reaction of 2 or 55-hexyn-3-ol (v).

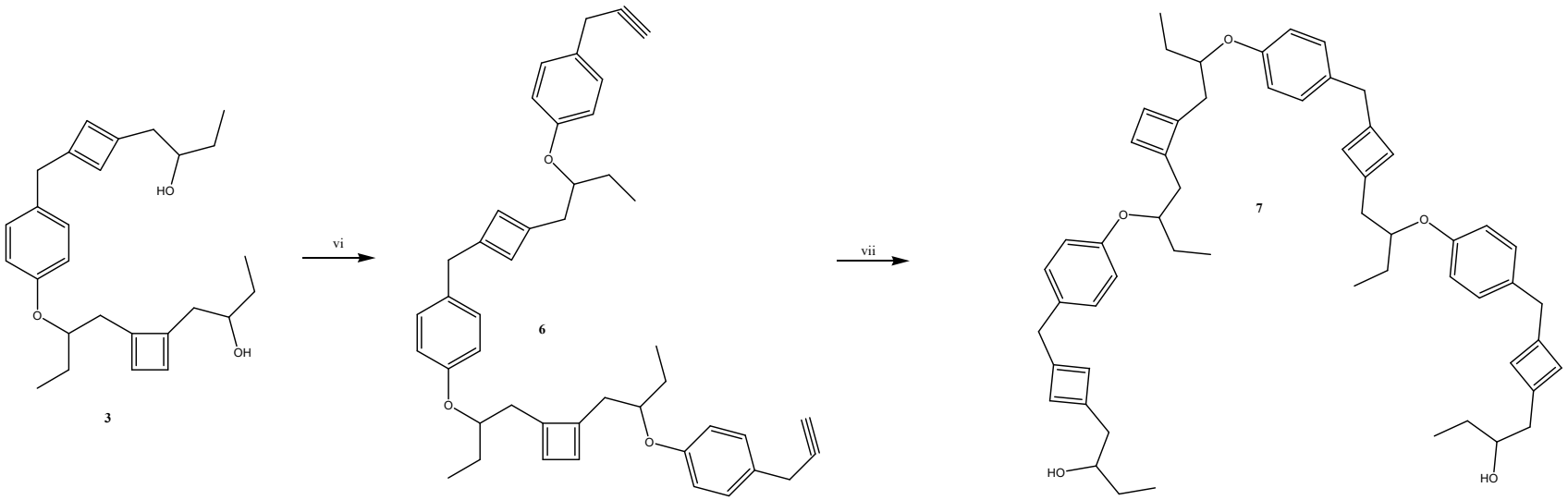

Figure 2.Synthesis of a tetra-cyclobutadiene derivative(7). Reaction of 3 with 1-nitro-4-prop-2-ynylbenzene(vi) to form a di-cyclobutadiene derivative (6). After,7synthesized by reaction of 6 with 5-hexyn-3-ol (vii).

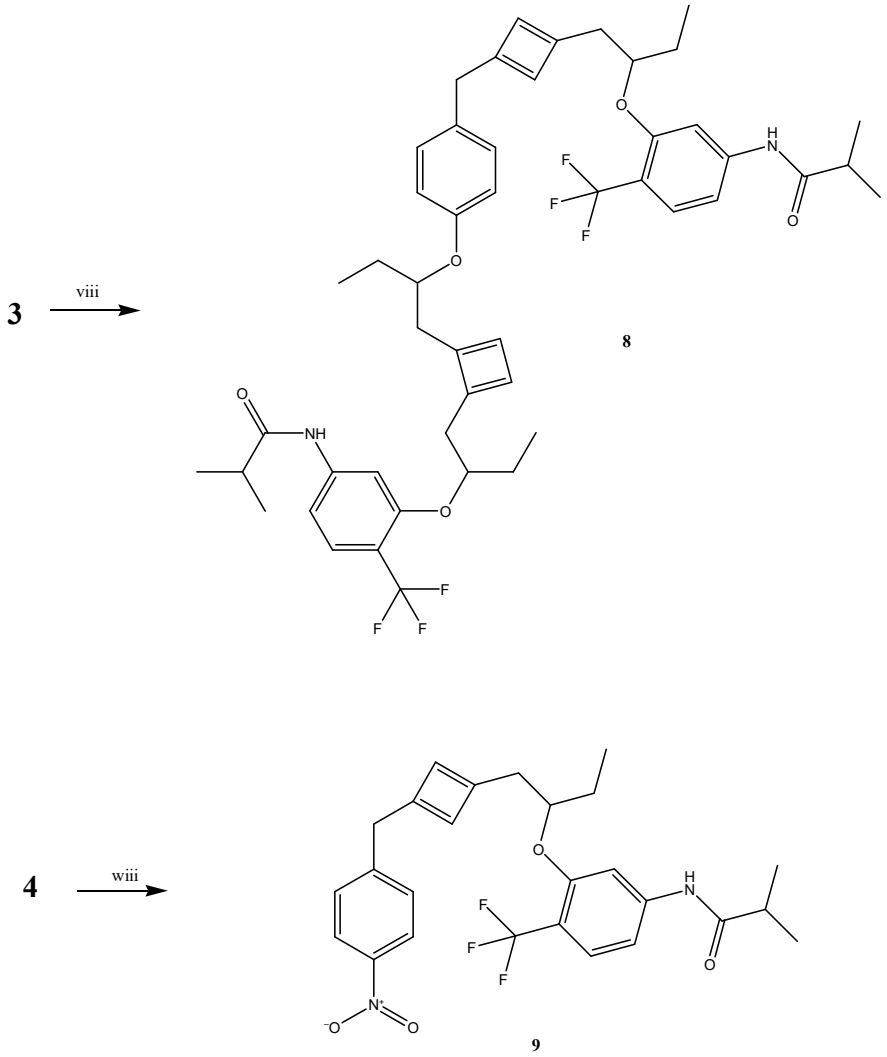

Figure 3: Synthesis of flutamide derivatives (8 and 9).Reaction of 3or4with flutamide (viii) to form 8or9.

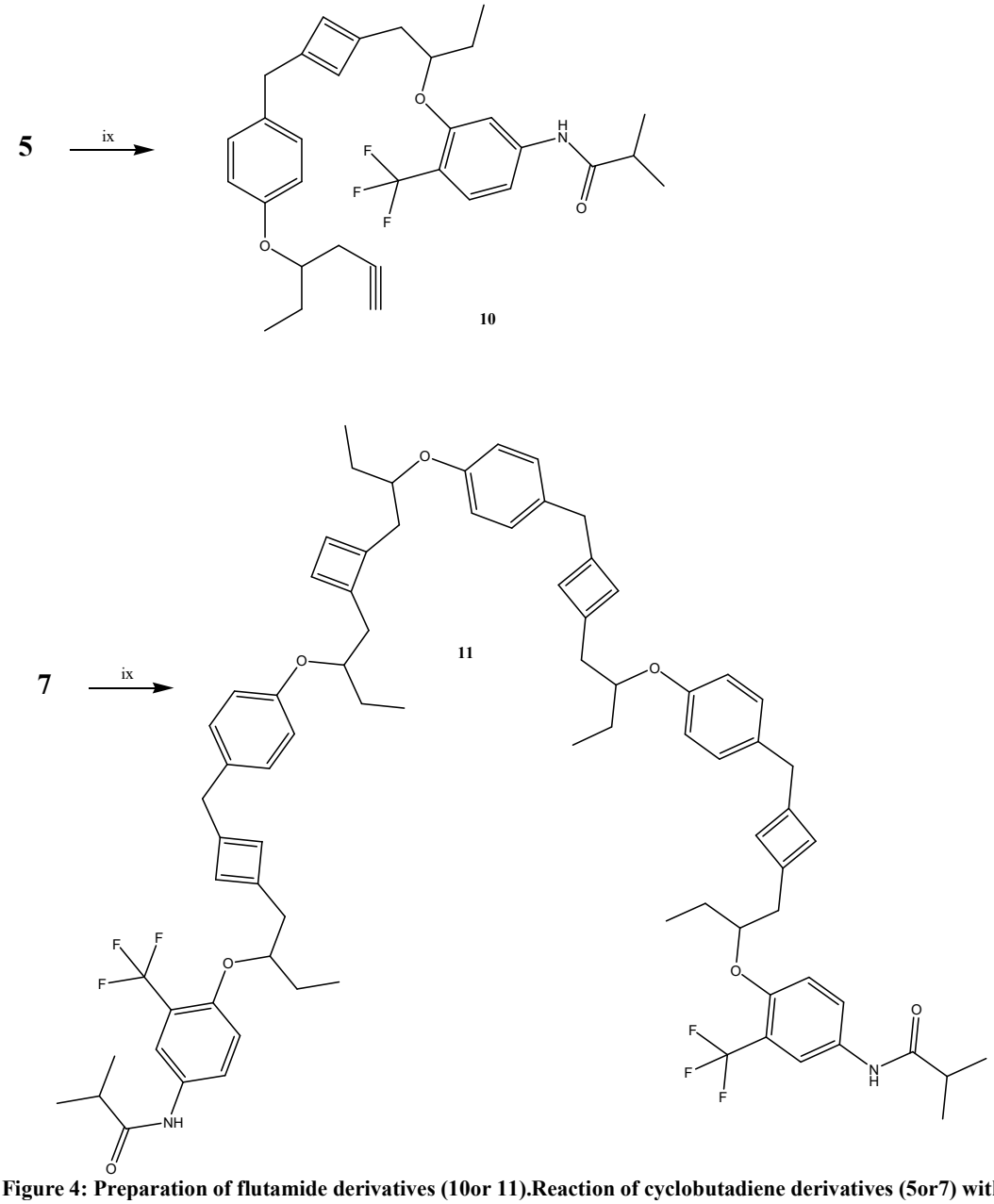

Figure 4: Preparation of flutamide derivatives (10or 11).Reaction of cyclobutadiene derivatives (5or7) with flutamide (ix) to form 10or11.

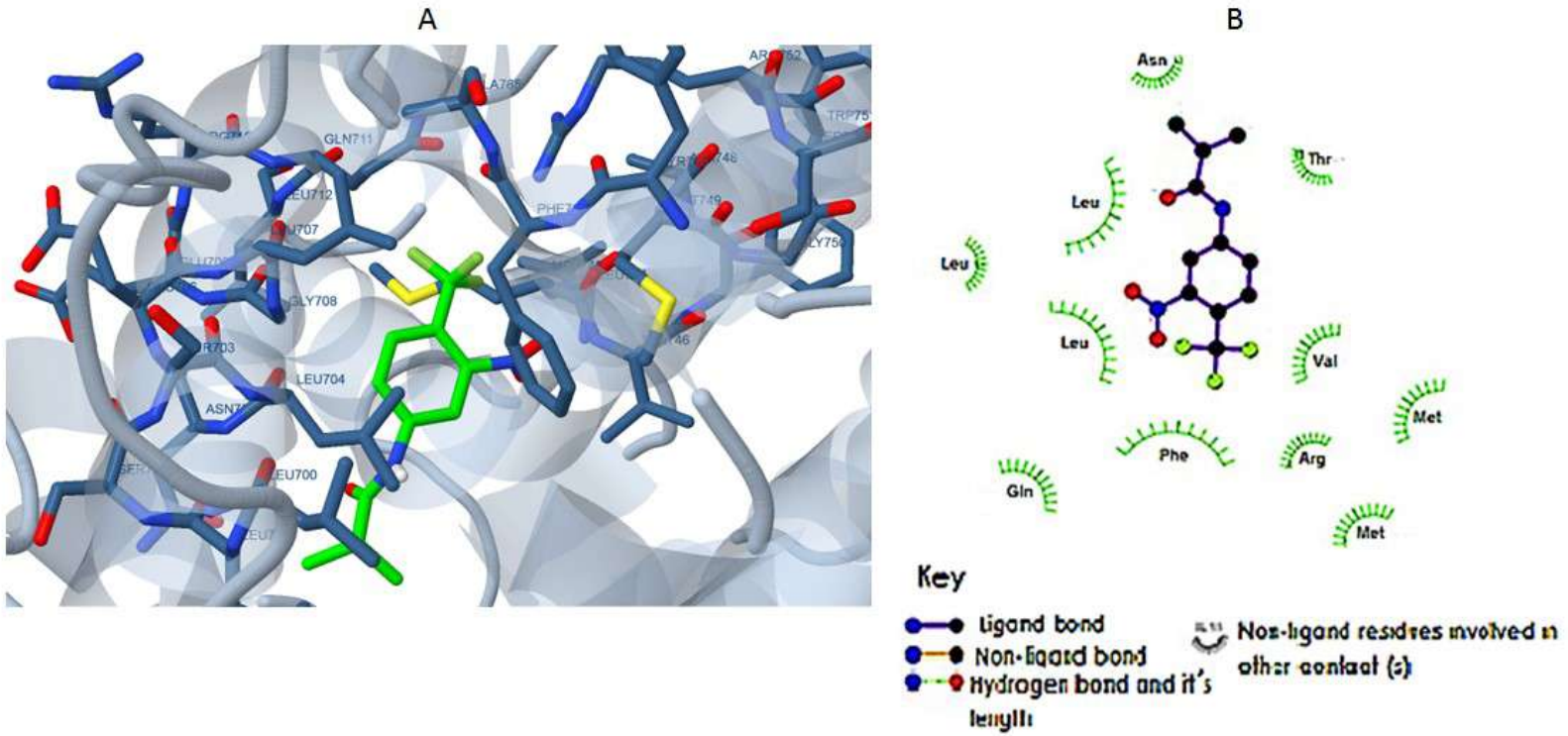

Figure 5.Site of binding for androgen receptor (2ylo protein) with flutamide (A). In addition, the scheme shown the contact site of amino acid residues involved in the 2ylo protein with flutamide(B). Visualized with GL mol Viewer after docking analysis with one-click docking.

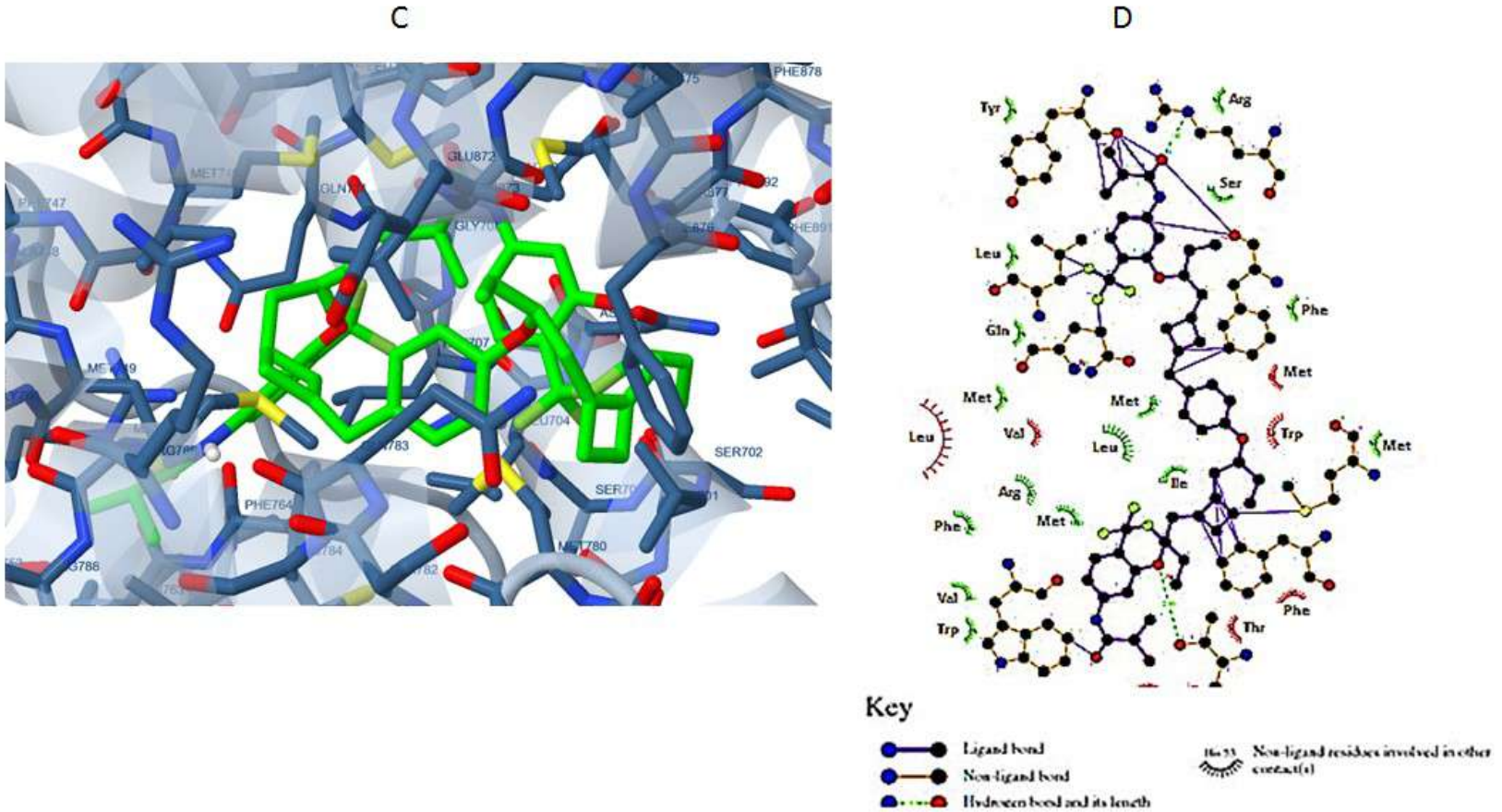

Figure 6.Site of binding for androgen receptor (2ylo protein) with the compound 8(C). In addition, the scheme shown the contact site of amino acid residues involved in the 2ylo protein with the compound 8(D). Visualized with GL mol Viewer after docking analysis with one-click docking.

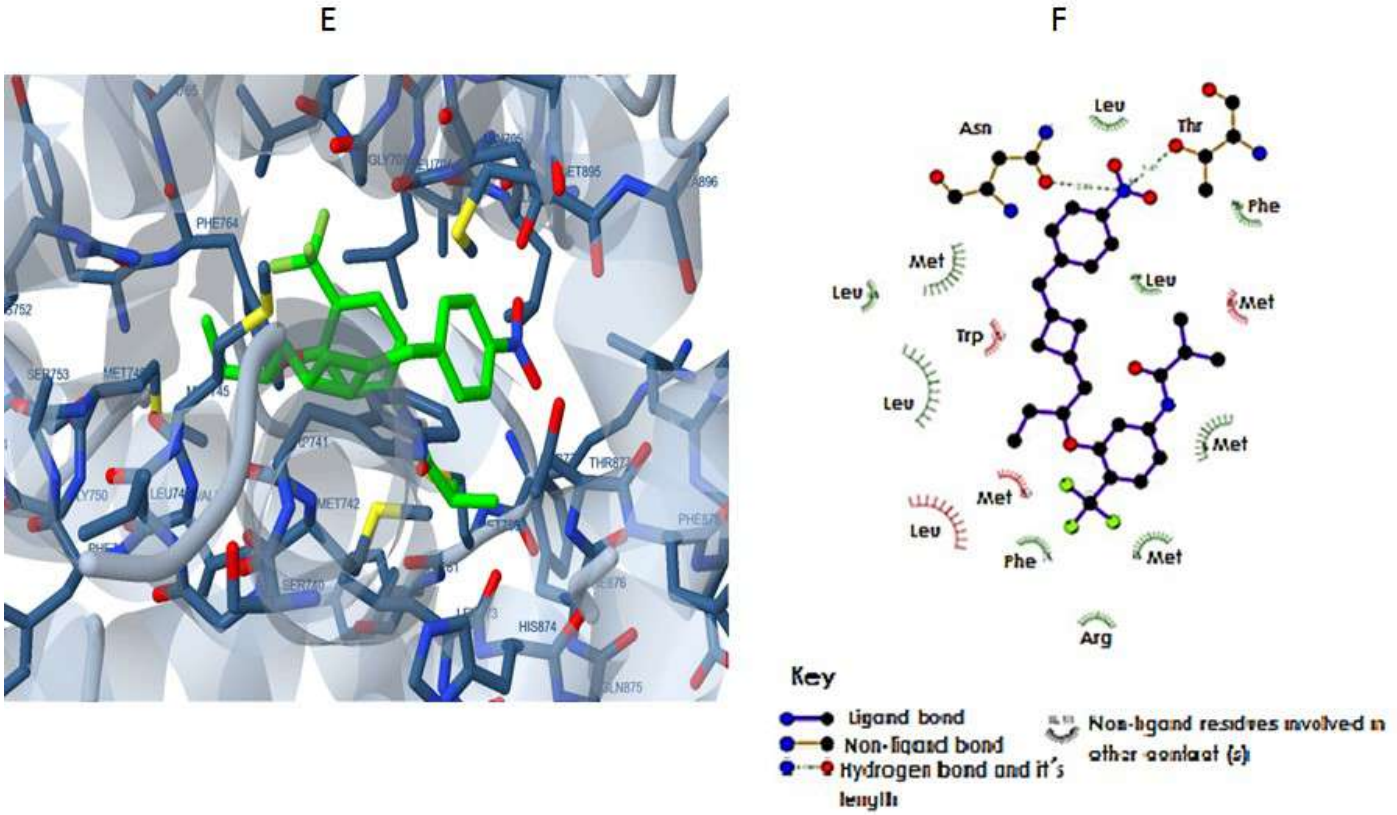

Figure 7.Site of binding for androgen receptor (2ylo protein) with the compound 9(C). In addition, the scheme shown the contact site of amino acid residues involved in the 2ylo protein with the compound 9(D). Visualized with GL mol Viewer after docking analysis with one-click docking.

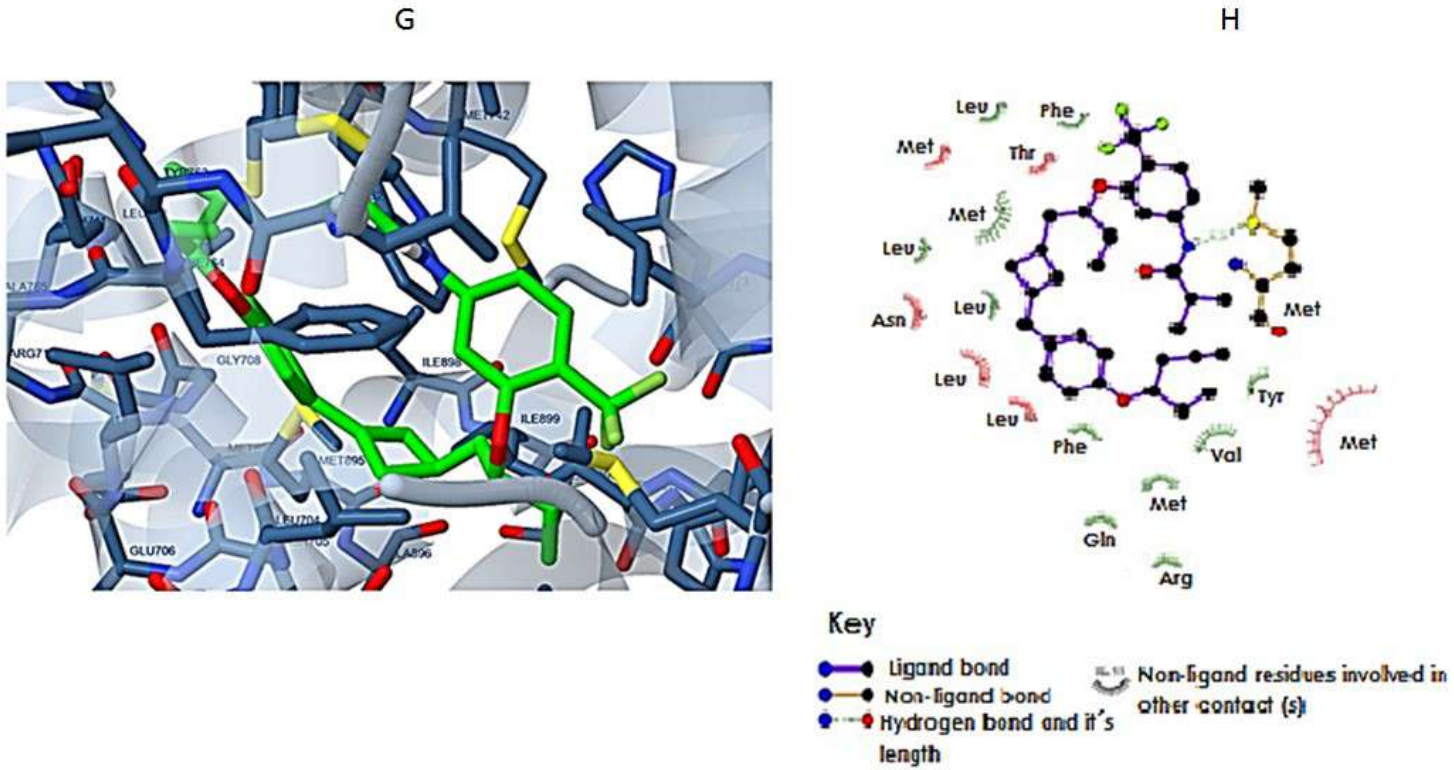

Figure 8.Site of binding for androgen receptor (2ylo protein) with the compound 10(E). In addition, the scheme shown the contact site of amino acid residues involved in the 2ylo protein with the compound 10(F). Visualized with GL mol Viewer after docking analysis with one-click docking.

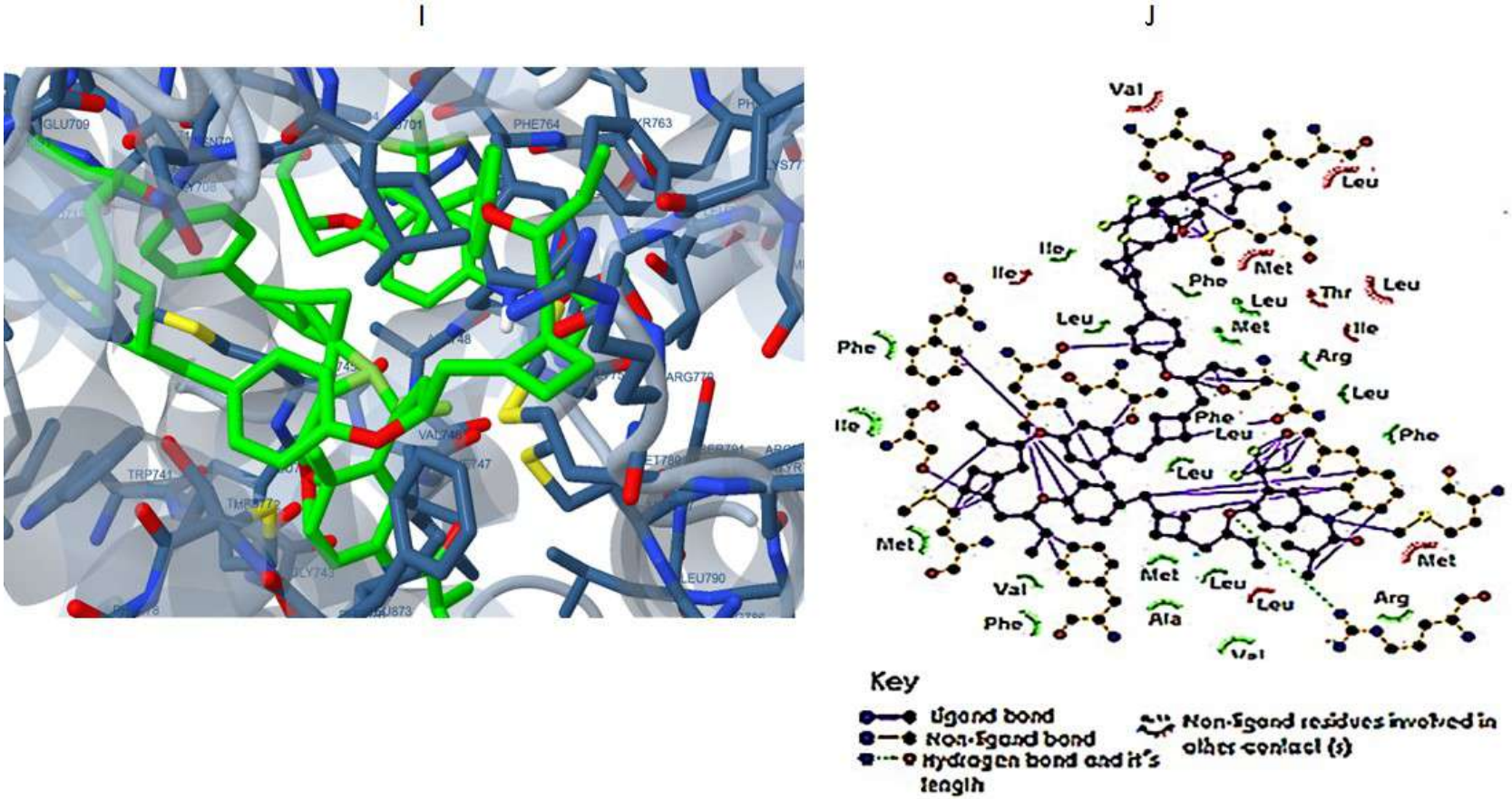

Figure 9.Site of binding for androgen receptor (2ylo protein) with the compound 11(G). In addition, the scheme shown the contact site of amino acid residues involved in the 2ylo protein with the compound 11(H). Visualized with GL mol Viewer after docking analysis with one-click docking.

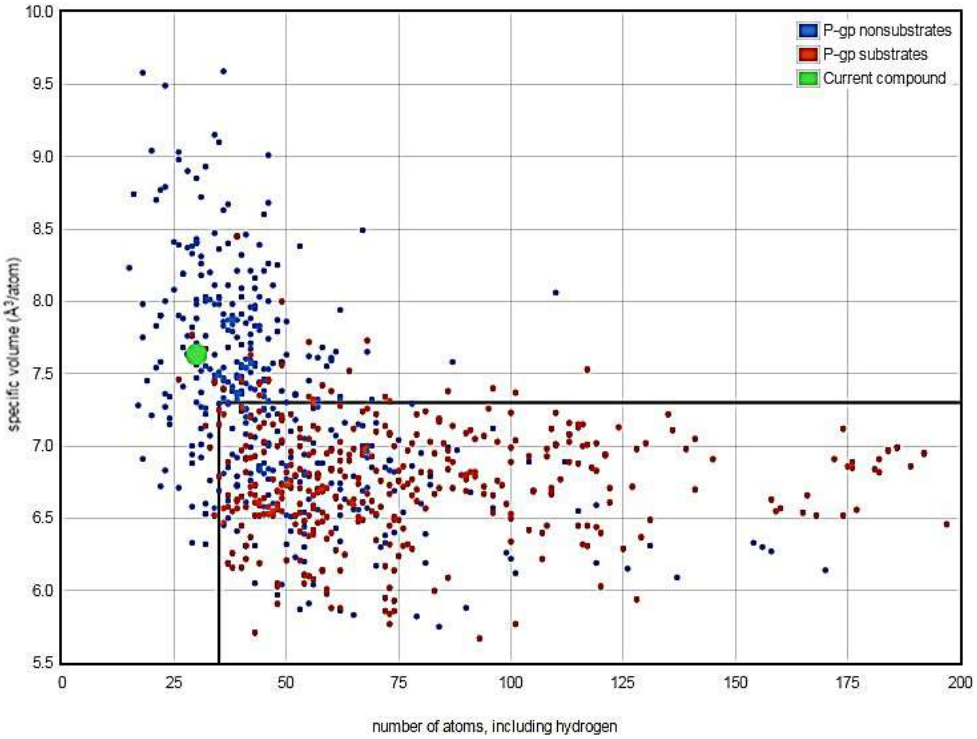

Figure 10. The scheme shows the probability of flutamide being a non-substrate for the glycoprotein P-gp. The Theoretical dat are from a Support Vector Machine (SVM) classification model, trained on a large set of 814 known P-gp substrates or non-substrates.

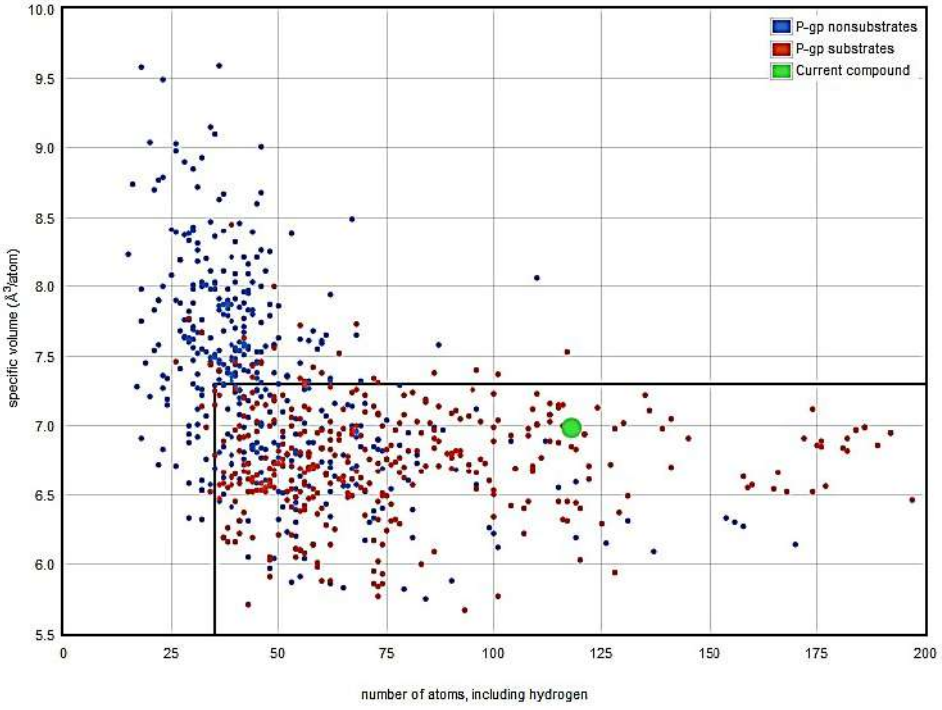

Figure 11. The graphicindicate the probability of compound 8 being a substrate for the glycoprotein P-gp. The Theoretical dat are from a Support Vector Machine (SVM) classification model, trained on a large set of 814 known P-gp substrates or non-substrates.

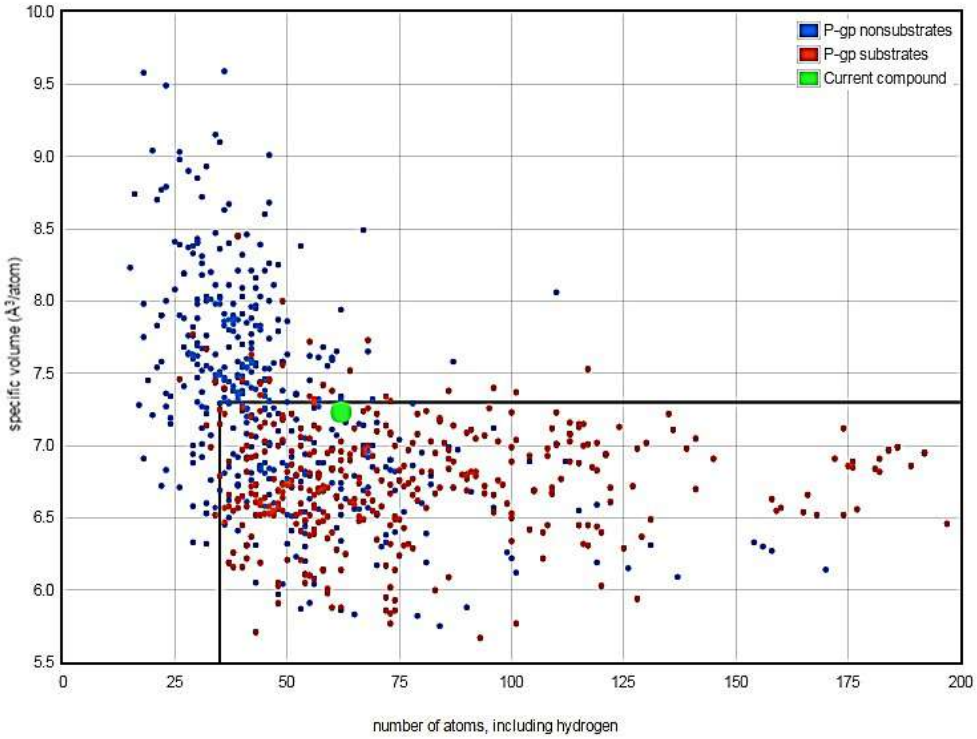

Figure 12. The schemeshown the probability of compound9 being a substrate for the glycoprotein P-gp. The Theoretical dat are from a Support Vector Machine (SVM) classification model, trained on a large set of 814 known P-gp substrates or non-substrates.

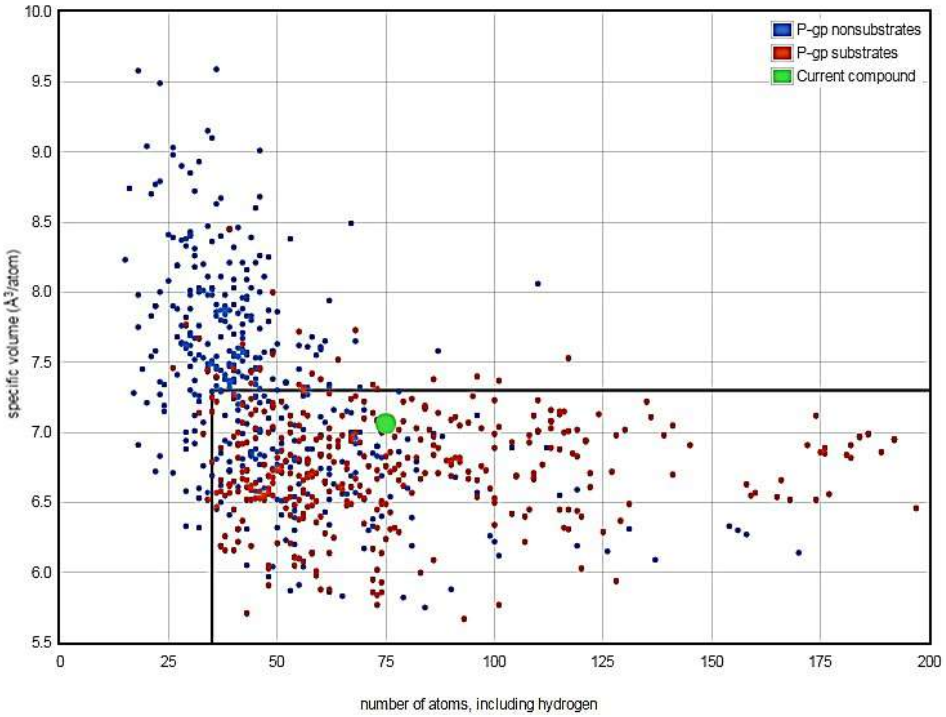

Figure 13. The schemeindicate the probability of compound10 being a substrate for the glycoprotein P-gp. The Theoretical dat are from a Support Vector Machine (SVM) classification model, trained on a large set of 814 known P-gp substrates or non-substrates.

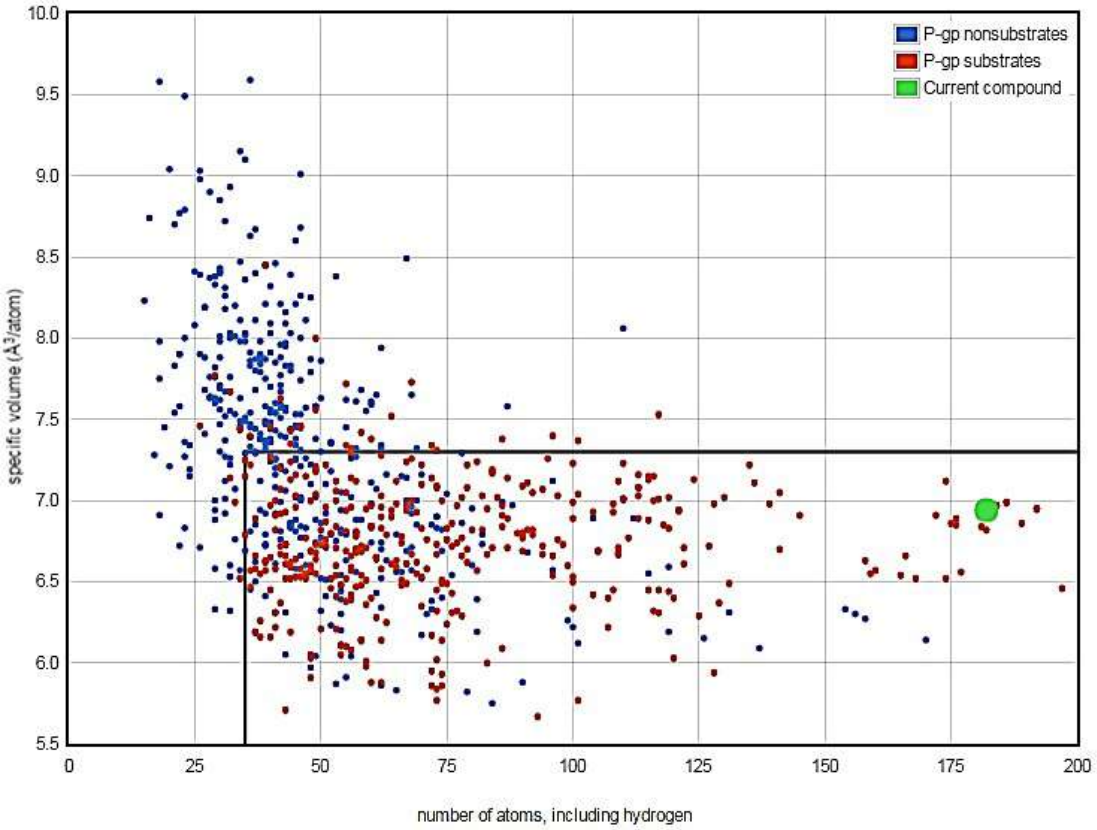

Figure 14. The graphicshown the probability of compound11 being a substrate for the glycoprotein P-gp. The Theoretical dat are from a Support Vector Machine (SVM) classification model, trained on a large set of 814 known P-gp substrates or non-substrates.

Table 1: Molecular bioactivity of flutamide and its derivatives (compounds 7 to 11).

	DRUGS				
	Flutamide	8	9	10	11
GPCR ligand	**-0.51**	**-2.79**	**-0.06**	**0.07**	**-3.93**
Ion channel modulator	**-0.38**	**-3.56**	**-0.23**	**-0.25**	**-3.97**
Kinase inhibitor	**-0.53**	**-3.39**	**-0.19**	**-0.09**	**-3.97**
Nuclear receptor ligand	**-0.36**	**-3.31**	**0.11**	**0.23**	**-3.96**
Protease inhibitor	**-0.47**	**-2.10**	**0.01**	**0.18**	**-3.90**
Enzymeinhibitor	**-0.39**	**-3.13**	**-0.02**	**0.14**	**-3.94**

Table 2: Aminoacid residues involved between the interaction of flutamide with the 2ylo protein surface

Interactions* Flutamide-2ylo			
hidrogen bonds	pi-pi	Halogen bond	others
Leu_{701} Leu_{704} Leu_{707}	Phe_{764}	Gln_{711} Met_{745} Phe_{764}	Leu_{704} Leu_{707} Gln_{711} Met_{745} Val_{746} Met_{749} Arg_{752} Phe_{764} Thr_{877}

*Aminoacids residues

Table 3: Descompesed interaction energies (Kcal/mol) involved between the flutamide and androgen receptor (2ylo) surface.

halogen bond	hydrofobic	others
Phe_{764} (-1.3493) Met_{745} (-0.6359) Gln_{711} (-0.5892)	Leu_{704} (-1.1785) Leu_{707} (-1.0819) Leu_{701} (-0.2701)	Val_{746} (-0.5454) Met_{749} (-04751) Arg_{752} (-0.2990) Asn_{705} (-0.2609) Thr_{877} (-0.2344)

Table 4: Aminoacid residues involved between the compound 8with the 2ylo protein surface.

Interactions* compound 8-2ylo						
hidrogen bonds	polar	hidrofobic bond	pi-pi	cation-pi	halogen bound	others
Arg_{752} Tyr_{763} Thr_{877}	Asn_{705} Arg_{752}	Leu_{701} Leu_{704} Leu_{707} Trp_{741} Met_{742} Met_{745} Met_{749} Tyr_{763} Phe_{764} Met_{780} Met_{787} Leu_{873} Phe_{876} Leu_{880} Val_{899} Phe_{891} Met_{895}	Phe_{764} Phe_{876}	Trp_{741} Phe_{764}	Leu_{701} Leu_{704} Leu_{707} Gln_{711}	Leu_{701} Leu_{704} Asn_{705} Leu_{707} Gln_{711} Trp_{741} Met_{749} Arg_{752} Ser_{753} Phe_{764} Met_{780} Phe_{876} Thr_{877} Met_{895} Ile_{899}

Table 5: Descompesed interaction energies (Kcal/mol) involved between the compound 8 and androgen receptor (2ylo) surface.

Hidrogen bond	halogen bond	Cation-pi	hydrofobic bond	others
Thr_{877} (-1.7282) Arg_{752} (1.1954) Gln_{711} (1.9664)	Leu_{704} (-4.3077) Asp_{705} (-1.9963) Gln_{711} (-1.8080) Leu_{707} (8.0512) Leu_{704} 13.8811)	$Thrp_{741}$ 0.0438) Phe_{764} 6.9064)	Phe_{891} (-1.1435) Met_{742} (-1.1055) Met_{787} (-0.7011) Met_{745} (-0.6372) $Valt_{869}$ (-0.5118) $Phet_{876}$ (0.1437) Met_{749}(0.6006) $Leut_{880}$(0.7105) Met_{895} (1.0148) $Leut_{873}$ (2.3580) Met_{780} (4.3330)	Ile_{899} (-0.2739) Ser_{753} (-0.2335)

Table 6.Aminoacid residues involved between the interactions of compound 9with the 2ylo protein surface.

Interactions* compound 9-2ylo					
Hidrogen- bond	polar	hydrofobic bond	pi-pi	halogen-others	
Asn_{705} Thr_{877}	Asn_{705}	Leu_{704} Trp_{741} Met_{742} Met_{745} Met_{749} Met_{780} Leu_{873} Phe_{876} Met_{895}	Trp_{741} Phe_{764}	Leu_{704}	Leu_{701} Leu_{704} Asn_{705} Leu_{707} Met_{742} Arg_{752} phe_{764} Met_{780} Leu_{873} Thr_{877} Leu_{880}

***Aminoacids residues**

Table 7: Descompesed interaction energies (Kcal/mol) involved between the compound 9 and androgen receptor (2ylo) surface.

Hidrogen bond	halogen bond	hydrofobic	others
Trp_{877} (1.7341) Asn_{705} (4.0346)	Leu_{704} (-2.8035)	Met_{895} (-1.5411) Met_{742} (-1.1856) Met_{749} (-04871) Phe_{876} (-0.2896) Phe_{764} (-0.2402) Leu_{873} (-0.1592) Trp_{741} (0.31930) Met_{780} (0.32860) Met_{745} (0.41200) Leu_{876} (2.32780)	Leu_{880} (-0.3646) Arg_{752} (-0.3429) Leu_{701} (-0.1188)

Table 8.Aminoacid residues involved between the interactions of compound 10with the 2ylo protein surface.

Interactions* compound 10-2ylo			
Hidrogen bond	hydrofobic	halogen bond	others
Met_{742}	Leu_{701} Leu_{704} Leu_{707} Met_{742} Met_{745} Val_{746} Met_{749} Phe_{764} Met_{780} Leu_{873} Phe_{876} Leu_{880} Met_{845}	Leu_{873}	Asn_{705} Leu_{707} Gln_{711} Trp_{741} Met_{742} Val_{746} Arg_{752} Asn_{764} Met_{780} Leu_{873} Phe_{876} Thr_{877}

***Aminoacids residues**

Table 9: Descompesed interaction energies (Kcal/mol) involved between the compound 10 and androgen receptor (2ylo) surface.

Hidrogen bond	halogen bond	hydrofobic	others
Met_{742} (-10.823)	Leu_{873} (-1.4808)	Met_{895} (-17.158) Val_{746} (-10.869) Met_{749} (-2.3073) Phe_{764} (-0.6638) Leu_{701} (-0.1564) Phe_{876} (-0.1252) Leu_{880} (-0.0496) Leu_{707} (0.4297) Met_{780} (2.3219) Leu_{704} (9.6646) Met_{745} (54.2601)	Trp_{741} (-3.9198) Gln_{711} (-0.3638) Arg_{752} (-0.2381) Thr_{877} (0.9041) Asn_{705} (4.3576)

Table 10: Aminoacids residues involved between the interactions of compound 11with the 2ylo protein surface.

Interactions* compound 11-2ylo			
hydrofobic	cation-pi	halogen bond	others
Leu_{707} Met_{742} Met_{745} Phe_{764} Met_{780} Met_{787} Leu_{873}	Phe_{764}	Asn_{705} Thr_{877}	Leu_{701} Trp_{741} Met_{742} Val_{746} Met_{749} Arg_{752} Phe_{764} Met_{787} Leu_{873} Phe_{876} Thr_{877} Met_{895}

*Aminoacids residues

Table 11: Descompesed interaction energies (Kcal/mol) involved between the compound 11 and androgen receptor (2ylo) surface.

.Interactions* compound 11-2ylo			
halogen bond	cation-pi	hydrofobic	others
Thr_{877} (-0.5619) Asn_{705} (-0.3441)	Phe_{760} (-1.0968)	Leu_{873} (-1.0070) Met_{742} (-0.7284) Met_{745} (-0.4449) Leu_{707} (-0.3491) Met_{787} (-3.2020) Met_{780} (-0.2537)	Val_{746} (-0.5874) Leu_{749} (-0.5241) Trp_{741} (-0.4934) Phe_{876} (-0.3817) Leu_{701} (-0.2317) Arg_{752} (-0.2099) Met_{895} (-0.1349)

*Aminoacids residues

Table 12: Intramolecular Parameters involved in the interaction of flutamide and compounds 8 to 11 with androgen receptor (2ylo)

Intramolecular Parameters	Compounds				
	Flutamide	8	9	10	11
Est. Free Energy of binding (kcal/mol)	1.25e+05	3.54e+03	-8.81	93.29	-6.72
Est. inhibition constant (Ki)	-	-	10.24	-	11.84
vdW + Hbond + desolv energy (kcal/mol)	1.04e+05	2.96e+03	-7.77	73.45	-8.07
Electrostayic energy (kcal/mol)	-0.14	-0.16	-0.06	0.08	0.16
Total intramolecularenergy (kcal/mol)	1.04e+05	2.96e+03	-7.77	7352	-7.91
Interacty surface ()	1223.711	1033.935	445.240	755.709	458.852

Table 13: Octanol-water partition coefficient (XlogP) and toxicity risk factors of flutamide and its derivatives (compounds 7 to 11)

	DRUGS				
	Flutamide	8	9	10	11
XlogP (Octanol-water parti- tion coefficient)	3.59	12.68	7.56	8.56	20,90
Toxicity risk factors (Å) (Total polar surface area)	72.24	85.89	81.47	47.56	104.35

4. CONCLUSIONS

In conclusion, the theoretical results indicated that interaction of **9** with androgen receptor was higher in comparison with the compound **11**; in addition, the compounds **8** to **11** could act as substrates to glycoprotein P and produce a specific effect.

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