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Synthesis and Phosphatase Inhibitory Activity of 3-Alkynylestrones and their Derivatives

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Abstract

A range of 3-alkynylated 3-deoxy-estrones were prepared by Sonogashira reactions and transformed into estrone derived diones and quinoxalines. The alkynylated estrones and their derivatives exhibit significant biological activity as alkaline phosphatases inhibitors. The mode of action was illustrated based on docking studies.

Introduction

Steroidal hormones represent an important class of compounds that regulate a number of processes in a human body. Their synthetic derivatives often exhibit biological and pharmacological activity against various receptors and, thus, represent lead structures in the development of new pharmaceuticals. Estrone (**1**) is an estrogenic hormone which plays an important role in menstrual and estrous reproductive cycles of human females.[1] Related compounds such as ethynyl estradiol (**2**), are commonly used as a component of oral contraceptive pills.[2] Moreover, Schang *et al.* patented 3-alkynylestrones which show antiviral properties and can be used as prophylactic agents against various virus infections (**3**).[3] 2-Phenylethynylestrone was applied in the synthesis of 2-phenylethyl-D-homo-estrone (**4**), which is a highly active inhibitor of steroidogenic human 17β-hydroxysteroid dehydrogenase type 1 (17β-HSD 1). The IC_{50} values of this compound are in the nanomolar range and are seven-fold higher than that of estrone (Figure 1).[4]

A recent study showed that estrone sulfamate (**5**, EMATE) inhibits steroid sulfatase (STS) and, therefore, can be successfully used for the treatment of hormone-dependent breast cancer.[5] EMATE was further functionalized at position 2- and 4- in order to search for STS inhibitors lacking the undesired estrogenic effect, displayed by EMATE.[5c] Furthermore, it was shown that 3-functionalized 2-methoxyestradiols (**6**, 2ME2) show high antiproliferative and antiangiogenic activity. Investigations on their structure-activity relationships indicate high antitumor activity for functional groups in position 3, containing hydrogen donors with π-electrons, while relatively bulky substituents suppress the undesired estrogenic activity.[6]

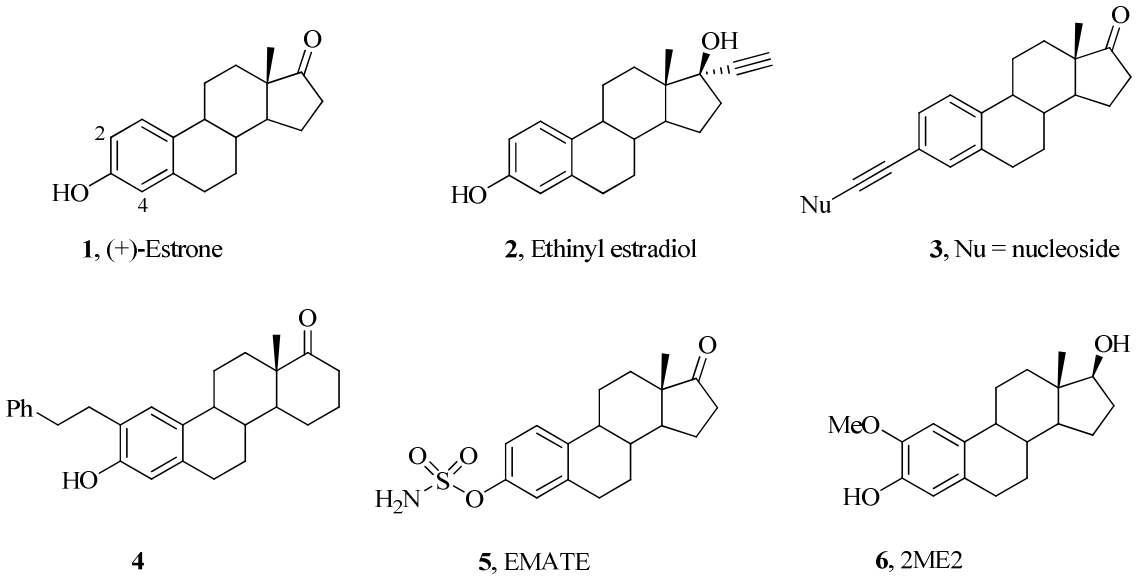


Figure 1. Estrone and its derivatives used in pharmacy

Despite of the potentially high biological and pharmacological activity of 3-alkynylated estrone derivatives or of other derivatives functionalized in position 3, there are only few reports on their synthesis, mostly lacking a wide preparative scope.[7,3] Therefore, we decided to study the synthesis of 3-alkynylated estrone derivatives and to investigate the scope and limitations of the new products.

Alkaline phosphatases (APs; EC 3.1.\3.1) are metalloenzymes expressed in a variety of tissues and exist as four different isoenzymes, each coded by a different gene. With few exceptions, APs are homodimeric enzymes and each catalytic site contains three metal ions, i.e., two Zn and one Mg, necessary for enzymatic activity.[8] The enzymes catalyze the hydrolysis of monoesters of phosphoric acid and also catalyze a transphosphorylation reaction in the presence of large concentrations of phosphate acceptors. Catalytic roles of AP involve breakdown of various nucleotides to liberate inorganic phosphate (Pi).[9] Mammalian APs have optimum activities at alkaline pH and exhibit a wide range of substrate specificity ranging from phosphomonoesters to an assortment of phosphate containing compounds, such as inorganic polyphosphates, glucose-phosphates, phosphatidates (containing fatty acid side chains), and bis(*p*-nitrophenyl) phosphate.[10] They are categorized into two groups, the tissue nonspecific alkaline phosphatase (TNAP) and tissue-specific APs that include placental AP (PLAP), intestinal AP (IAP), and

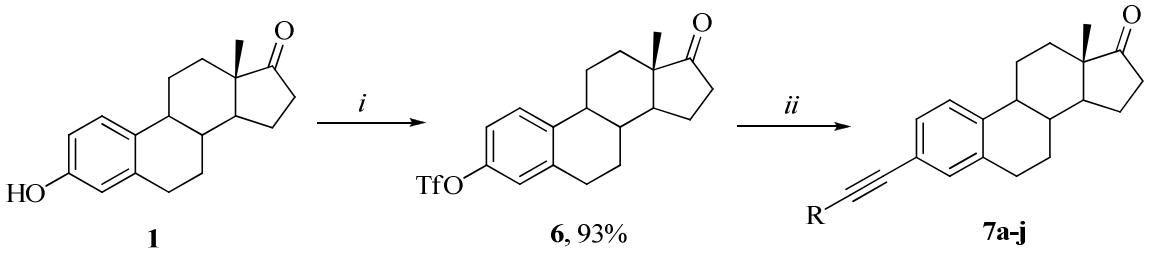
germ cell AP (GCAP). The tissue specific APs (PLAP, GCAP and IAP) share 90–98% sequence identity, whereas TNAP shares only 50% sequence identity with these tissue-specific APs.[11]

PLAP is important in the diagnosis of a variety of germ-cell (e.g seminoma) and non germ-cell tumors including lung, ovarian, gastrointestinal and uterine carcinomas.[12] The human tissue non-specific alkaline phosphatase (TNAP) is found in liver, kidney, and bone. TNAP hydrolyzes PPi (a potent inhibitor of mineralization) and is responsible for maintaining sufficient levels of extracellular PPi. TNAP acts as a potentially useful therapeutic target for the treatment of soft tissue ossification abnormalities including ankylosis, osteoarthritis and arterial calcification. IAP has also been suggested to be involved in lipid absorption as a parallel increase has been observed in triacylglycerol concentration and IAP activity, during fat absorption in thoracic duct lymph.[10]

Inhibitors of APs can help to map out the exact mechanisms and origins of pathological conditions, thus, defining footsteps that can lead to novel therapies based on inhibition of APs. The most well known and commonly used inhibitors of APs are levamisole (Ki =16 μM) and theophilline (Ki = 82 μM).[13]

Results and Discussions

At first we identified estrone triflate **6** as an easily accessible substrate for the Sonogashira reaction by conversion of the phenolic OH-group to its triflate. The latter was obtained with an excellent yield by standard conditions (Scheme 1). With estrone triflate **6** in hand, we studied suitable conditions for the subsequent Sonogashira reaction. During our optimization of the synthesis of product **7a**, using phenylacetylene, it was found that best results were obtained applying DMF as solvent in the presence of diisopropylamine as base at 100 °C for 8 h. The use of 10 mol% of $Pd(PPh_3)_4$ and 10 mol% of copper iodide are required to achieve high yields (Scheme 1, Table 1). The yield decreased when trimethylamine was used as the base. No product at all was formed when the reaction was carried out at 25 or 60 °C.



1 **6**, 93% **7a-j**

Scheme 1. Synthesis of **7a-j**. *Conditions*: *i*, **1** (1.0 equiv.), TfCl (1.2 equiv.), NEt_3 (1.2 equiv.), DCM, 0 to 25°C; *ii*, **6** (1.0 equiv.), alkyne (1.2 equiv.), catalyst, co-catalyst, base (3 equiv.), DMF, T°C.

Table 1. Optimization of the Sonogashira reaction

Entry	Catalyst	Co-catalyst	Base	T	Time	Yield[a]

	[mol%]	[mol%]	(3.0 equiv.)	[°C]	[h]	[%]
1	$Pd(PPh_3)_4$ (10)	CuI (20)	NEt_3	25	8	0
2	$Pd(PPh_3)_4$ (10)	CuI, (20)	NEt_3	60	8	0
3	$Pd(PPh_3)_4$ (10)	CuI (20)	NEt_3	100	8	64
4	$Pd(PPh_3)_4$ (10)	CuI (20)	$HN(iPr)_2$	100	8	82
5	$Pd(PPh_3)_4$ (10)	CuI (10)	$HN(iPr)_2$	100	8	87
6	$Pd(PPh_3)_4$ (5)	CuI (10)	$HN(iPr)_2$	100	16	55

[a] Isolated yields

Chen *et al.* earlier reported conditions for the direct alkynylation of estrone[7a] in a one-pot reaction. However, yields were lower as compared to the overall yields obtained by our two-step procedure. Thus, phenyl derivative **7a** was obtained in 63%. We used the optimized conditions to obtain alkynylestrones **7a-i** in good to excellent yields (Table 2). Electron-donating substituents on the phenylacetylene resulted in higher yields. We were unable to obtain the product derived from *p*-cyanophenylacetylene. The structure of **7c** was independently confirmed by an X-ray crystal structure analysis (Figure 3).

Table 2. Synthesis of 3-alkynyl-3-deoxyestrones **7a-j**.

Compound	R	**7 [%]**[a]
a	C_6H_5	87
b	4-MeC_6H_4	77
c	4-*n*-PrC_6H_4	94
d	4-*tert*-BuC_6H_4	91
e	4-$(MeO)C_6H_4$	99
f	Me_3Si	94
g	3-Pyridyl	70
h	Thien-3-yl	90
i	4-$(CF_3)C_6H_4$	55
j	4-$(CN)C_6H_4$	-

[a] Isolated yields

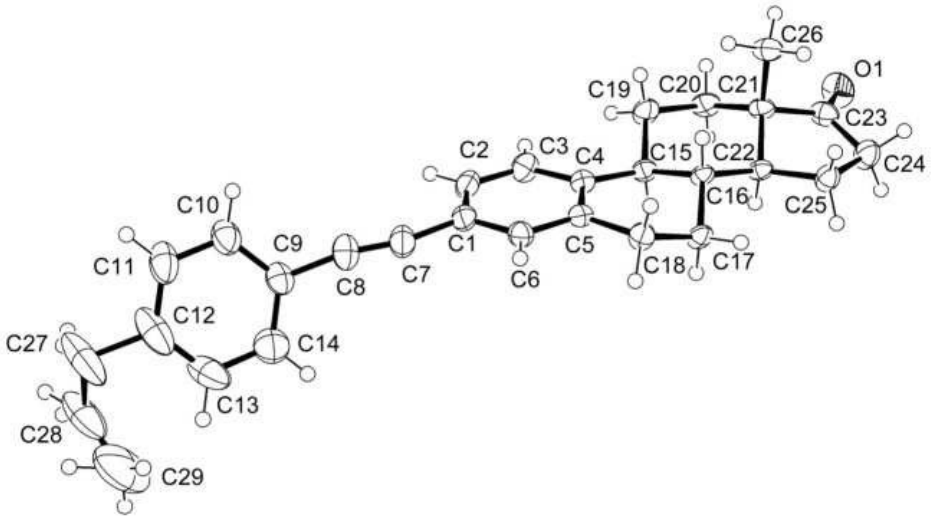
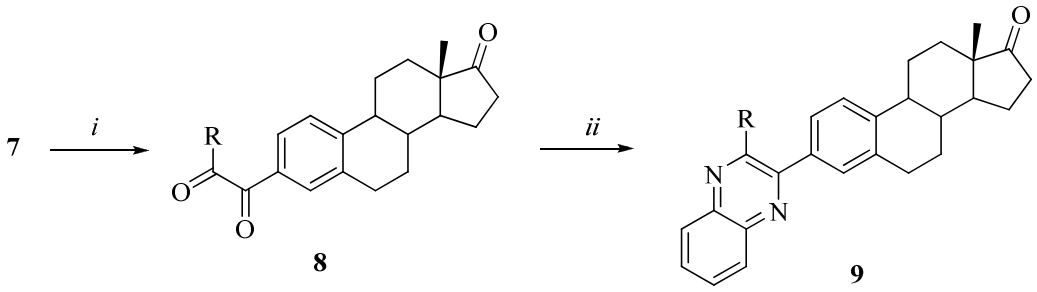


Figure 2. Molecular structure of **7c**.

Next, we studied the transformation of the triple bond to a pyrazine ring. While a one-pot reaction of estrone **7a**, in the presence of phenylenediamine and phenyliodine diacetate gave no pyrazine ring,[14] we succeeded by the establishment of a two-step procedure, consisting of a $Pd(OAc)_2/CuBr_2$ oxidation step followed by a ring closing step using phenylenediamine.[15] Ethanediones **8** were obtained in good to excellent yields. In the second step, the desired quinoxalines were obtained in high yields under mild conditions (Scheme 2, Table 3).



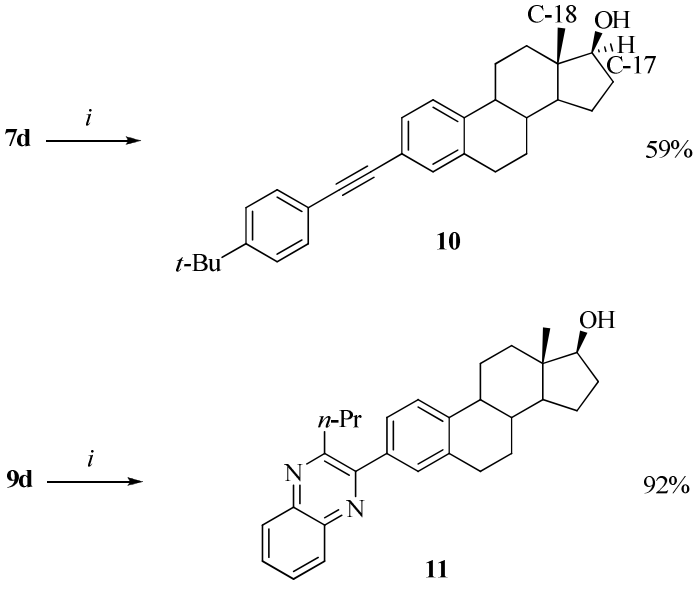
Scheme 2. Synthesis of ethanediones **8** and corresponding quinoxalines **9**. *Conditions*: *i*, $Pd(OAc)_2$ (0.1 equiv.), $CuBr_2$ (0.1 equiv.), DMSO, air, 140°C; *ii*, *o*-phenylenediamine (1.3 equiv.), ethanol, 50°C, 2h.

Table 3. Synthesis of ethanediones **8** and corresponding quinoxalines **9**.

Compound	R	**8 [%][a]**	**9 [%][a]**
a	C_6H_5	58	92
b	4-*tert*-BuC_6H_4	65	88
c	thiophen-3-yl	59	82
d	4-*n*-PrC_6H_4	79	96

[a] Isolated yields.

Finally, 3-alkyne-3-deoxyestrone **7d** and quinoxaline **9d** were transformed to the corresponding estradiol derivatives (**10** and **11**) by reduction of the carbonyl group (Scheme 3).[16] Products **10** and **11** were obtained diastereomerically pure as proved by the NMR spectra which showed only one set of signals. We can assume that this diastereomer has the structure depicted in Scheme 2 with β-position of the hydroxyl group. Although ^{1}H and ^{13}C NMR spectra of α- and β-estradiol are very similar and does not help in revealing the right structure, their NOESY-spectra are different.[17] Thus, the proton at C-17 shows strong interaction with protons of the methyl group C-18 in case of α-estradiol, whereas for β-estradiol there is no such interaction. For products **10** and **11** only insignificant interaction between protons of the methyl-group C-18 and proton at position C-17 is observed, what speaks for the β-position of the hydroxyl group in both cases.



Scheme 3. Synthesis of **10** and **11**. *Conditions*: *i*, $NaBH_4$ (2 equiv.), MeOH/DCM 1:1, 20°C, 1 h.

Alkaline Phosphatase Inhibition Studies and SAR. The estrone and quinoxaline derivatives were investigated for their inhibitory potential on both alkaline phosphatase isozymes, TNAP and IAP. The derivatives of both classes exhibited an interesting inhibitory strength towards both isozymes, comparable to their respective reference standards. Among the estrone derivatives, only one derivative **7e** showed dual inhibition on both isozymes, but more selectively towards IAP. Compounds **7b**, **7f**, **7g**, **7h** and **7i** were found to be selective inhibitors of TNAP having inhibitory value ranges from IC_{50}±SEM = 0.32 ±.001 to 2.83±0.20 µM. Compound **7i** was found to be the most potent inhibitor of TNAP and it exhibited 60 folds better improvement in the inhibition, comparable to the reference standard, i.e. Levamisole, with a value of IC_{50}±SEM = 19.21±0.001 µM. The detailed and comparitive study of this structure with the other derivatives suggested that the activity of this compound might be due to the presence of the trifluoromethyl group attached to the benzene ring which might change the electronic situation and thus biological behaviour of the ring.

Compounds **7a**, **7c** and **7d** were identified as selective inhibitors of IAP having inhibitory values of IC_{50}±SEM = 7.35±0.71, 16.2±0.64 and 19.8±0.96 µM, respectively. Among estrone derivatives, compound **7e** was found to be the most potent inhibitor with IC_{50} values of IC_{50}±SEM = 0.71±.002 µM. This compound exhibits a 113 fold enhancement in inhibition as compared to the reference standard, i.e. phenylalanine, with an IC_{50} value of IC_{50}±SEM = 80.21±0.001 µM. The comprehensive study of **7a** suggests that the activity of this compound could be attributed to the unsubstituted phenyl ring. As the substitution on the phenyl ring increased, the response of the compound towards IAP decreased, as in case of **7c** and 7**d**.

All four quinoxaline derivatives showed dual inhibition on both isozymes. These derivatives exhibited much interesting behaviour towards IAP and the inhibitory response was improved many folds in these derivatives. Among these, compound **9a** was found to be the most potent inhibitor of IAP and the activity was improved to 251 fold when compared to the reference standard. This compound exhibited more than 140 fold inhibition of IAP, when compared to estrone derivative **7i**. When the phenyl ring was unsubstituted, the compound showed higher inhibitory activity on IAP as compared to TNAP. This effect can be seen in the inhibitory response of compound **9b** and **9d**. When the phenyl ring was substituted with propyl chain, as in case of **9d**, the compound was a more potent inhibitor of TNAP. This compound exhibited inhibitory potential of IC_{50}±SEM = 0.25 ±0.001µM and it was 77 fold more potent than levamisole.

Table 4. Tissue non-specific alkaline phosphatase (TNAP) and intestinal alkaline phosphatase (IAP) inhibition data for the synthesized compounds

	TNAP	IAP

Compound	IC_{50} [a] (μM) ± SEM or (% inhibition)[b]	IC_{50} [a] (μM) ± SEM or (% inhibition)[b]
7a	34.90% [b]	7.35±0.71 [a]
7b	2.83±0.20 [a]	30.15% [b]
7c	29.39% [b]	16.2±0.64 [a]
7d	37.82% [b]	19.8±0.96 [a]
7e	0.91±.005[a]	0.71±.002 [a]
7f	1.08±.052 [a]	37.10% [b]
7g	0.90±0.01 [a]	30.42% [b]
7h	0.38±.002 [a]	48.85% [b]
7i	0.32±.001[a]	30.35% [b]
9a	0.52±0.004 [a]	0.32±0.005 [a]
9b	0.48±.003 [a]	1.95 ±0.08 [a]
9c	2.44±0.10 [a]	0.92 ±0.01 [a]
9d	0.25 ±0.001 [a]	0.44 ±0.003[a]
Levamisole	19.21±0.001[a]	
L-Phenyl alanine	-------	80.21±0.001[a]

[a] The IC_{50} is the concentration at which 50% of the enzyme activity is inhibited. [b] The % inhibition of the enzyme activity caused by 0.5 mM of the tested compound.

Molecular Docking. Molecular docking study of compound **7i** was performed to identify the putative binding mode of the newly synthesized estrones class of compounds. The in-vitro enzyme assay revealed high potency of compound **7i** against bovine tissue non-specific alkaline phosphatase as compared to that of intestinal alkaline phosphatase. Thus, a molecular docking study was performed to identify the molecular basis of the in-vitro observation as no crystallographic or NMR structure of the bovine origin alkaline phosphatase is present in protein data bank. The homology models of bovine tissue non-specific and alkaline phosphatase previously modelled[18] was used. For compound **7i** inside bovine tissue non-specific alkaline phosphatase, it was observed that the carbonyl group of compound **7i** forms hydrogen bonding interaction with amino acid His454 and one of the fluorine atom of the 4-(tri-fluoromethyl)phenyl- substitution forms hydrogen bonding contact with amino acid Arg335 and forms conventional fluorine interaction with amino acid Asp294. Additionally, the amino acid His341 was also found in pi-pi stacking interaction with our compound **7i** that is similar to interaction reported previously.[18] In contrast to the observations found above, inside intestinal alkaline phosphatase no hydrogen bonding interaction was observed for compound **7i**. However, the compound pose is stabilized by extensive pi-pi stacking interactions with amino acid residues such as His336, His339 and His451. Additionally, one fluorine atom of 4-tri-fluorophenyl substitution forms interaction with amino acid Arg169.

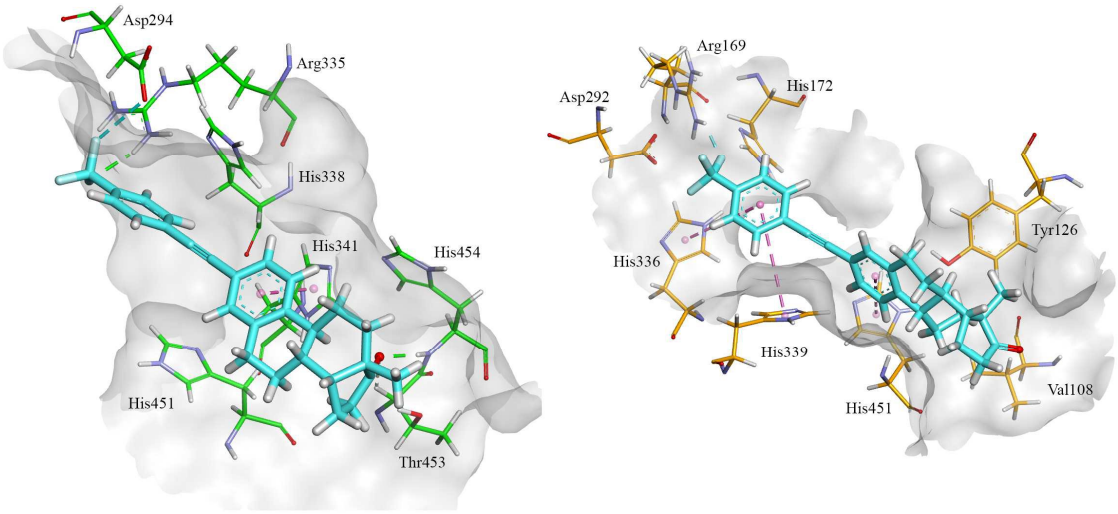


Figure 3. Binding mode of compound **7i** (colored cyan) inside bovine TNAP active site (colored green) and inside bovine IAP active site (colored brown). Hydrogen bonding interaction and Pi-Pi staking interactions are shown as green and pink dashed lines while interaction of fluorine as cyan dashed line.

Upon the HYDE assessment, compound **7i** revealed higher binding affinity of -21 $KJmol^{-1}$ inside bovine tissue non-specific alkaline phosphatase as compared to that of -13 $KJmol^{-1}$ inside intestinal alkaline phosphatase. The difference in binding affinity and the type of interaction it forms with different amino acid residues is attributable for higher potency against bovine tissue non-specific alkaline phosphatase as compared to that of bovine intestinal alkaline phosphatase. The putative binding mode of compound **7i** can be found in Figure 3.

Conclusion

In this study we successfully optimized and applied the Sonogoshira reaction in order to synthesize new alkynylated derivatives of 3-deoxyestrone. This reaction presents a convenient path towards compounds containing electron-donating and neutral alkynyl substituents. Further transformations afford access to ethanediones and 2,3-disubstituted quinaxolines, as well as to their estradiol derivatives. Biological activity studies showed that synthesized compounds inhibit alkaline phosphatase with significant lower concentrations as compared to the reference compounds and molecular docking of the most selective inhibitor was performed.

Experimental section

Chemistry

Estrone triflyl (6): Estrone (**1**) (1g, 3.7 mmol), NEt_3 (0.62 ml, 1.2 equiv.) and DCM (20 ml) were added in a round-bottom 50 ml flask and cooled at an ice bath to 0°C. Then TfCl (0.47 ml, 1.2 equiv.) was slowly added with a syringe while stirring. The reaction mixture was left to warm to 20 °C and to stay overnight. At the next day the mixture was quenched with a solution of $NaHCO_3$ (20 ml) and extracted with DCM. The product was purified by column chromatography (EA : Heptane = 1:5). **6** was isolated as a white solid (1.35 g, 91%), Mp: 97 - 98 °C (lit.[19] 87 – 89 °C), $[\alpha]_D$ = +116.0° (c 1.00, $CHCl_3$) (lit.[19] $[\alpha]_D$ = +105.8° (c 1.56, $CHCl_3$)). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.92 (s, 3H, CH_3), 1.47 – 1.68 (m, 6H, aliphatic), 1.96 – 2.21 (m, 4H, aliphatic), 2.25 – 2.33 (m, 1H, aliphatic), 2.37 – 2.44 (m, 1H, aliphatic), 2.47 – 2.56 (m, 1H, aliphatic), 2.92 – 2.96 (m, 2H, aliphatic), 6.99 – 7.05 (m, 2H, Ar), 7.34 (d, $^3J_{H\text{-}H}$ = 8.5 Hz, 1H, Ar). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 13.8 (CH_3), 21.6 (CH_2), 25.7 (CH_2), 26.1 (CH_2), 29.4 (CH_2), 31.5 (CH_2), 35.8 (CH_2), 37.8 (CH), 44.1 (CH), 47.8 (C), 50.4 (CH), 188.3 (CH), 121.2 (CH), 127.2 (CH), 139.3 (C), 140.3 (C), 147.6 (C), 220.4 (C=O). ^{19}F NMR (282 MHz, $CDCl_3$) δ = -73.0 (CF_3). IR (ATR, cm^{-1}): $\tilde{\nu}$ = 2932 (w), 2859 (w), 1734 (s), 1488 (w), 1454 (w), 1418 (s), 1372 (w), 1339 (w), 1249 (m), 1206 (s), 1137 (s), 1128 (s), 1054 (m), 1007 (m), 916 (s), 879 (m), 848 (m), 836 (s), 820 (m), 785 (m), 766 (m), 702 (m), 640 (w), 620 (s), 605 (s). MS (EI, 70 eV): m/z(%) = 403 (21), 402 (99) [M^+], 358 (62), 345 (49), 251 (64), 225 (44), 213 (100), 157 (43), 129 (45), 128 (44), 115 (67), 69 (73). HRMS (ESI): calcd. for $C_{19}H_{21}F_3O_4S$ [$M+H^+$] 403.11854; found 403.11859.

3-Phenylethynyl-estra-1,3,5(10)-trien-17-one (7a): Estrone triflyl **(6)** (100 mg, 0.25 mmol), $Pd(PPh_3)_4$ (29 mg, 0.1 equiv.), CuI (4.8 mg, 0.1 equiv.), diisopropylamine (76 mg, 3 equiv.) and DMF (3 ml) were added in a Schlenk flask under argon atmosphere. The mixture was degassed by changing vacuum and argon and phenylacetylene (31 mg, 1.2 equiv.) was added with a syringe. The mixture was degassed three times and stirred at 100°C overnight. The solvent was evaporated in vacuo. The residue was purified by column chromatography (silica gel, heptane/EtOAc = 10:1). **7a** was isolated as a yellow solid (77 mg, 87%), Mp: 216 - 217 °C (lit.[7a] 224 - 225 °C), $[\alpha]_D$ = +78.5° (c 2.18, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.85 (s, 3H, CH_3), 1.34 – 1.60 (m, 6H, aliphatic), 1.89 – 2.14 (m, 4H, aliphatic), 2.20 – 2.29 (m, 1H, aliphatic), 2.33 – 2.49 (m, 2H, aliphatic), 2.82 – 2.86 (m, 2H aliphatic), 7.19 – 7.27 (m, 6H, Ar + $CDCl_3$), 7.43 – 7.46 (m, 2H, Ar). ^{13}C NMR (63 MHz, $CDCl_3$): δ = 13.8 (CH_3), 21.6 (CH_2), 25.6 (CH_2), 26.3 (CH_2), 29.1 (CH_2), 31.5 (CH_2), 35.8 (CH_2), 38.0 (CH), 44.5 (CH), 48.0 (C), 50.5 (CH), 88.7 (C_{alkyne}), 89.5 (C_{alkyne}), 120.6 (C), 123.4 (C), 125.4 (CH), 128.1 (CH), 128.3 (2CH), 128.9 (CH), 131.5 (2CH), 132.0 (CH), 136.6 (C), 140.3 (C), 220.7 (C=O). IR (ATR, cm^{-1}): $\tilde{\nu}$ = 3056 (w), 2926 (m), 2867 (m), 1731 (s), 1595 (w), 1500 (m), 1442 (m), 1371 (w), 1261 (w), 1216

(w), 1083 (m), 1053 (m), 1008 (m), 964 (w), 912 (m), 823 (m), 794 (w), 756 (s), 691 (s), 620 (w), 577 (m), 529 (m). MS (EI, 70 eV): m/z(%) = 354 (100) [M^+], 355 (30), 241 (12), 230 (12), 229 (14), 228 (17), 215 (17), 202 (10). HRMS (EI): calcd. for $C_{26}H_{26}O$ [M^+] 354.19782; found 354.19799.

3-(*p*-Tolylethynyl)-estra-1,3,5(10)-trien-17-one (7b): yellow solid, 77%. Mp: 186 - 187 °C, $[\alpha]_D$ = +120.2° (c 1.00, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 0.84 (s, 3H, CH_3), 1.37 – 1.60 (m, 6H, aliphatic), 1.88 – 2.13 (m, 4H, aliphatic), 2.19 – 2.48 (m, 6H, aliphatic + CH_3), 2.81 – 2.85 (m, 2H, aliphatic), 7.07 (d, $^3J_{H\text{-}H}$ = 7.9 Hz, 2H, Ar), 7.16 – 7.24 (m, 3H, Ar + $CDCl_3$), 7.33 (d, $^3J_{H\text{-}H}$ =8.1 Hz, 2H, Ar). ^{13}C NMR (75 MHz, $CDCl_3$) δ = 13.9 (CH_3), 21.5 (CH_3), 21.6(CH_2), 25.6 (CH_2), 26.4 (CH_2), 29.2 (CH_2), 31.6 (CH_2), 35.9 (CH_2), 38.0 (CH), 44.5 (CH), 48.0 (C), 50.6 (CH), 88.8 (C_{alkyne}), 89.0 (C_{alkyne}), 120.4 (C), 120.8 (C), 125.4 (CH), 128.9 (CH), 129.1 (2CH), 131.5 (2CH), 132.0, (CH) 136.6 (C), 138.2 (C), 140.0 (C), 220.7 (C=O). IR (ATR, cm^{-1}): $\tilde{\nu}$ = 3029 (w), 2932 (m), 2872 (w), 1731 (m), 1601 (w), 1511 (m), 1451 (w), 1425 (w), 1372 (w), 1338 (w), 1256 (w), 1178 (w), 1082 (m), 1050 (w), 1005 (m), 911 (m), 816 (s), 692 (m), 637 (w), 575 (m), 538 (m). MS (EI, 70 eV): m/z (%) = 368 (100) [M^+], 369 (30), 215 (10). HRMS (EI): calcd. for $C_{27}H_{28}O$ [M^+] 368.21347; found 368.21387.

3-(*p*-*n*-Propylphenylethynyl)-estra-1,3,5(10)-trien-17-one (7c): yellow solid, 94%. Mp: 153 - 154 °C, $[\alpha]_D$ = +106.0° (c 1.00, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 0.84 – 0.89 (m, 6H, $2CH_3$), 1.37 – 1.60 (m, 8H, aliphatic), 1.88 – 2.13 (m, 4H, aliphatic), 2.19 – 2.27 (m, 1H, aliphatic), 2.32 – 2.45 (m, 2H, aliphatic), 2.49 – 2.54 (m, 2H, aliphatic), 2.81 – 2.85 (m, 2H, aliphatic), 7.07 (d, $^3J_{H\text{-}H}$ = 8.3 Hz, 2H, Ar), 7.16 – 7.24 (m, 3H, Ar + $CDCl_3$), 7.35 (d, $^3J_{H\text{-}H}$ = 8.1 Hz, 2H, Ar). ^{13}C NMR (63 MHz, $CDCl_3$) δ = 13.7 (CH_3), 13.8 (CH_3), 21.6 (CH_2), 24.3 (CH_2), 25.6 (CH_2), 26.4 (CH_2), 29.1 (CH_2), 31.6 (CH_2), 35.8 (CH_2), 37.9 (CH_2), 38.0 (CH), 44.5 (CH), 47.9 (C), 50.5 (CH), 88.8 (C_{alkyne}), 89.0 (C_{alkyne}), 120.6 (C), 120.8 (C), 125.3 (CH), 128.5 (2CH), 128.9 (CH), 131.4 (2CH), 132.0 (CH), 136.6 (C), 140.0 (C), 143.0 (C), 220.7 (C=O). IR (cm^{-1}): $\tilde{\nu}$ = 3026 (w), 2929 (s), 2859 (m), 1736 (s), 1601 (w), 1510 (m), 1451 (m), 1404 (m), 1371 (m), 1337 (m), 1254 (m), 1214 (s), 1179 (w), 1081 (m), 1005 (m), 912 (m), 901 (m), 888 (m), 819 (s), 575 (m). MS (EI, 70 eV): m/z (%) = 396 (100) [M^+], 397 (31), 367 (18). HRMS (EI) calcd. for $C_{29}H_{32}O$ [M^+] 396.24477; found 396.24435.

3-(*p*-*tert*-Butylphenylethynyl)-estra-1,3,5(10)-trien-17-one (7d): yellow solid, 91%. Mp: 205 - 206 °C, $[\alpha]_D$ = +111.5° (c 1.00, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.84 (s, 3H, CH_3), 1.25 (s, 9H, $C(CH_3)_3$), 1.37 – 1.55 (m, 6H, aliphatic), 1.88 – 2.10 (m, 4H, aliphatic), 2.20 – 2.27 (m, 1H), 2.36 – 2.47 (m, 2H, aliphatic), 2.82 – 2.85 (m, 2H, aliphatic), 7.16 – 7.24 (m, 3H, Ar + $CDCl_3$), 7.28 (d, $^3J_{H\text{-}H}$ = 8,3 Hz, 2H, Ar), 7.37 (d, $^3J_{H\text{-}H}$ = 8,3 Hz, 2H, Ar). ^{13}C NMR (63 MHz, $CDCl_3$) δ = 13.8 (CH_3), 21.6 (CH_2), 25.6 (CH_2), 26.4 (CH_2), 29.1 (CH_2), 31.2 ($C(CH_3)_3$), 31.6 (CH_2), 34.8 ($C(CH_3)_3$), 35.8 (CH_2), 38.0 (CH), 44.4 (CH), 47.9 (C), 50.5 (CH), 88.8 (C_{alkyne}), 88.9 (C_{alkyne}), 120.4 (C), 120.8 (C), 125.3 (2CH), 125.4 (CH), 128.9 (CH), 131.3 (2CH), 132.0 (CH), 136.6 (C), 140.0 (C), 151.3 (C), 220.7 (C=O). IR (ATR, cm^{-1}): $\tilde{\nu}$ = 708 (w), 796 (m), 822 (m), 844 (m), 889 (m), 1006 (m), 1055 (m), 1082 (m), 1105 (m), 1204 (m),

1260 (m), 1294 (w), 1337 (w), 1363 (m), 1455 (m), 1464 (m), 1509 (m), 1552 (w), 1737 (s), 2861 (w), 2937 (m). MS (EI, 70 eV): m/z (%) = 410 (100) [M^+], 411 (30), 396 (29), 395 (96). HRMS (EI): calcd. for $C_{30}H_{34}O$ [M^+] 410.26042; found 410.26012.

3-(*p*-Methoxyphenylethynyl)-estra-1,3,5(10)-trien-17-one (7e): yellow solid, 99%. Mp: 206 - 207 °C, $[\alpha]_D$ = +95.9° (c 0.66, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 0.84 (s, 3H, CH_3), 1.37 – 1.59 (m, 6H, aliphatic), 1.88 – 2.12 (m, 4H, aliphatic), 2.19 – 2.27 (m, 1H, aliphatic), 2.32 – 2.47 (m, 2H, aliphatic), 2.80 – 2.85 (m, 2H, aliphatic), 3.74 (s, 3H, OCH_3), 6.79 (d, $^3J_{H\text{-}H}$ = 8.9 Hz, 2H, Ar), 7.15 – 7.23 (m, 3H, Ar + $CDCl_3$), 7.37 (d, $^3J_{H\text{-}H}$ = 8.8 Hz, 2H, Ar). ^{13}C NMR (63 MHz, $CDCl_3$) δ = 13.8 (CH_3), 21.6 (CH_2), 25.6 (CH_2), 26.4 (CH_2), 29.1 (CH_2), 31.6 (CH_2), 35.8 (CH_2), 38.0 (CH), 44.4 (CH), 47.9 (C), 50.5 (CH), 55.3 (OCH_3), 88.1 (C_{alkyne}), 88.7 (C_{alkyne}), 114.0 (2CH), 115.6 (C), 120.9 (C), 125.3 (CH), 128.8 (CH), 131.9 (CH), 133.0 (2CH), 136.5 (C), 139.9 (C), 159.5 (C), 220.7 (C=O). IR (ATR, cm^{-1}): $\tilde{\nu}$ = 2922 (m), 2871 (w), 2848 (w), 1729 (s), 1598 (w), 1568 (w), 1512 (s), 1461 (m), 1451 (m), 1443 (m), 1402 (w), 1373 (w), 1338 (w), 1286 (m), 1243 (s), 1183 (w), 1171 (m), 1106 (m), 1083 (m), 1052 (w), 1026 (s), 1007 (m), 967 (w), 912 (m), 881 (m), 836 (s), 812 (s), 783 (m), 744 (w), 709 (m), 670 (w), 638 (w), 577 (m), 535 (s). MS (EI, 70 eV): m/z (%) = 384 (100) [M^+], 385 (26), 215 (12), 202 (12). HRMS (EI): calcd. for $C_{27}H_{28}O_2$ [M^+] 384.20828; found 384.20794.

3-(Trimethylsilylethynyl)-estra-1,3,5(10)-trien-17-one (7f): brown solid, 94%. Mp: 140 - 141 °C, $[\alpha]_D$ = +95.3° (c 0.60, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 0.17 (s, 9H, TMS), 0.84 (s, 3H, CH_3), 1.36 – 1.59 (m, 6H, aliphatic), 1.87 – 2.13 (m, 4H, aliphatic), 2.17 – 2.26 (m, 1H, aliphatic), 2.30 – 2.48 (m, 2H, aliphatic), 2.78 – 2.82 (m, 2H, aliphatic), 7.15 – 7.19 (m, 3H, $C_{Ar}H$ + $CDCl_3$). ^{13}C NMR (63 MHz, $CDCl_3$) δ = 0.0 ((CH_3)$_3$Si), 13.8 (CH_3), 21.5 (CH_2), 25.5 (CH_2), 26.3 (CH_2), 29.0 (CH_2), 31.5 (CH_2), 35.8 (CH_2), 37.9 (CH), 44.4 (CH), 47.9 (C), 50.5 (CH), 93.3 (C_{alkyne}), 105.2 (C_{alkyne}), 120.4 (C), 125.2 (CH), 129.2 (CH), 132.4 (CH), 136.4 (C), 140.4 (C), 220.6 (C=O). IR (ATR, cm^{-1}): $\tilde{\nu}$ = 2938 (m), 2861 (w), 2154 (w), 1738 (s), 1493 (m), 1448 (m), 1404 (w), 1251 (m), 1086 (m), 1053 (m), 1005 (m), 912 (m), 885 (m), 832 (s), 763 (m), 700 (m), 658 (m), 579 (m), 540 (w). MS (EI, 70 eV): m/z (%) = 350 (45) [M^+], 351 (14), 336 (29), 335 (100). HRMS (EI): calcd. for $C_{23}H_{30}OSi$ [M^+] 350.20604; found 350.20587.

3-(3-Pyridinethynyl)-estra-1,3,5(10)-trien-17-one (7g): yellow solid, 70%. Mp: 197 - 198 °C, $[\alpha]_D$ = +140.1° (c 1.00, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 0.85 (s, 3H, CH_3), 1.34 – 1.60 (m, 6H, aliphatic), 1.89 – 2.14 (m, 4H, aliphatic), 2.21 – 2.29 (m, 1H, aliphatic), 2.33 – 2.49 (m, 2H, aliphatic), 2.83 – 2.87 (m, 2H, aliphatic), 7.19 – 7.28 (m, 4H, Ar + $CDCl_3$), 7.71 – 7.75 (m, 1H, Ar), 8.46 (br.s, 1H, C_{Ar}H), 8.68 (s, 1H, C_{Ar}H). ^{13}C NMR (75 MHz, $CDCl_3$) δ = 13.9 (CH_3), 21.6 (CH_2), 25.6 (CH_2), 26.3 (CH_2), 29.1 (CH_2), 31.6 (CH_2), 35.9 (CH_2), 38.0 (CH), 44.5 (CH), 48.0 (C), 50.5 (CH), 85.3 (C_{alkyne}), 93.1 (C_{alkyne}), 119.8 (C), 120.8 (C), 123.2 (CH), 125.6 (CH), 129.1 (CH), 132.2 (CH), 136.8 (C), 138.6 (CArH), 141.0 (C), 148.1 (CH), 152.0 (CH), 220.5 (C=O). IR (ATR, cm^{-1}): $\tilde{\nu}$ = 3062 (w), 2931 (m), 2873 (m), 1730 (s), 1564 (w), 1497 (m), 1452 (m), 1404 (w), 1372 (w), 1337 (w), 1258

(w), 1190 (w), 1082 (m), 1051 (w), 1022 (m), 1005 (m), 911 (m), 836 (m), 825 (m), 812 (s), 774 (m), 707 (s), 630 (m), 575 (m), 529 (m). MS (EI, 70 eV): m/z (%) = 355 (100) [M^+], 356 (29), 298 (12), 245 (10), 244 (11), 243 (11), 231 (11), 230 (16), 229 (11), 217 (12), 216 (12). HRMS (EI): calcd. for $C_{25}H_{25}ON$ [M^+] 355.19307; found 355.19293.

3-(3-Thiophenethynyl)-estra-1,3,5(10)-trien-17-one (7h): yellow solid, 90%. Mp: 192 - 193 °C, $[\alpha]_D$ = +145.3° (c 1.00, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 0.85 (s, 3H, CH_3), 1.38 – 1.60 (m, 6H, aliphatic), 1.88 – 2.13 (m, 4H, aliphatic), 2.20 – 2.28 (m, 1H, aliphatic), 2.32 – 2.48 (m, 2H, aliphatic) 2.81 – 2.85 (m, 2H, aliphatic), 7.10 – 7.12 (m, 1H, Ar)), 7.17 – 7.24 (m, 4H, Ar + $CDCl_3$), 7.41 – 7.42 (m, 1H, Ar). ^{13}C NMR (63 MHz, $CDCl_3$) δ = 13.8 (CH_3), 21.6 (CH_2), 25.6 (CH_2), 26.3 (CH_2), 29.1 (CH_2), 31.6 (CH_2), 35.8 (CH_2), 38.0 (CH), 44.5 (CH), 47.9 (C), 50.5 (CH), 83.9 (C_{alkyne}), 88.9 (C_{alkyne}), 120.5 (C), 122.5 (C), 125.3 (CH), 125.4 (CH), 128.3 (CH), 128.8 (CH), 139.9, 131.9 (CH), 136.6 (C), 140.2 (C), 220.7 (C=O). IR (ATR, cm^{-1}): $\tilde{\nu}$ = 3100 (w), 2922 (w), 2867 (w), 1731 (s), 1491 (w), 1451 (w), 1371 (w), 1353 (w), 1257 (m), 1083 (m), 1054 (m), 1005 (m), 952 (m), 891 (m), 862 (m), 819 (m), 786 (s), 701 (m), 625 (s), 577 (m). MS (EI, 70 eV): m/z (%) = 360 (100) [M^+], 361 (30), 234 (12), 221 (13). HRMS (EI): calcd. for $C_{24}H_{24}OS$ [M^+] 360.15424; found 360.15413.

3-(*p*-Trifluorophenylethynyl)-estra-1,3,5(10)-trien-17-one (7i): yellow solid, 55%. Mp: 183 - 184 °C, $[\alpha]_D$ = +85.0° (c 0.53, $CHCl_3$). ^{1}H NMR (250 MHz, $CDCl_3$) δ = 0.85 (s, 3H, CH_3), 1.42 – 1.56 (m, 6H, aliphatic), 1.88 – 2.11 (m, 4H, aliphatic), 2.21 – 2.27 (m, 1H, aliphatic), 2.32 – 2.49 (m, 2H, aliphatic), 2.82 – 2.87 (m, 2H, aliphatic), 7.22 – 7.27 (m, 3H, Ar), 7.52 (m, 4H, Ar). ^{13}C NMR (63 MHz, $CDCl_3$) δ = 13.8 (CH_3), 21.6 (CH_2), 25.6 (CH_2), 26.3 (CH_2), 29.1 (CH_2), 31.5 (CH_2), 35.8 (CH_2), 37.9 (CH), 44.5 (CH), 47.9 (C), 50.5 (CH), 87.4 (C_{alkyne}), 92.0 (C_{alkyne}), 119.8 (C), 124.0 (q, $^1J_{C\text{-}F}$ = 272.0 Hz, CF_3), 125.2 (q, $^3J_{C\text{-}F}$ = 3.8 Hz, 2*C*H-C-CF_3), 125.5 (CH), 127.3 (C), 129.1 (CH), 129.7 (q, $^2J_{C\text{-}F}$ = 32.6 Hz, C-CF_3), 131.7 (2CH), 132.2 (CH), 136.8 (C), 140.9 (C), 220.6 (C=O). ^{19}F NMR (282 MHz, $CDCl_3$) δ = -62.7 (CF_3). IR (ATR, cm^{-1}): $\tilde{\nu}$ = 2930 (m), 2858 (w), 1737 (s), 1613 (w), 1516 (w), 1492 (w), 1453 (w), 1432 (w), 1405 (m), 1380 (w), 1320 (s), 1261 (m), 1216 (w), 1165 (s), 1121 (s), 1105 (s), 1084 (m), 1064 (s), 1014 (m), 1006 (m), 914 (w), 901 (w), 889 (m), 847 (m), 838 (m), 823 (m), 800 (m), 780 (m), 750 (m), 709 (m), 694 (w), 643 (w), 627 (w), 599 (m), 576 (m), 535 (w). MS (EI, 70 eV): m/z (%) = 422 (100) [M^+], 423 (29), 365 (14), 324 (12), 312 (12), 311 (10), 310 (11), 309 (13), 298 (12), 297 (10), 296 (12), 283 (10), 215 (11). HRMS (EI, 70 eV): calcd. for $C_{27}H_{25}OF_3$ [M^+] 422.18520; found 422.18516.

(Estra-1,3,5(10)-trien-17-on-3-yl)-2-(phenylethane)-1,2-dione (8a): alkyn **(7a)** (100 mg, 0.28 mmol), palladium acetate (6.3 mg, 0.1 equiv.), $CuBr_2$ (6.2 mg, 0.1 equiv.) and DMSO (3 ml) were stirred in a flask with an access of air at 120°C overnight. The solvent was evaporated in vacuo. The residue was purified by column chromatography (silica gel, heptane/EtOAc = 5:1). **8a** was isolated as a yellow solid (63 mg, 58%). Mp: 80 - 81°C, $[\alpha]_D$ = +51.0° (c 1.38, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 0.84 (s, 3H, CH_3), 1.39 – 1.60 (m, 6H, aliphatic), 1.89 – 2.14 (m, 4H, aliphatic), 2.24 – 2.32 (m, 1H, aliphatic), 2.34 – 2.48 (m, 2H, aliphatic), 2.85 – 2.90 (m, 2H, aliphatic), 7.34 – 7.45 (m, 3H, Ar),

7.55 – 7.68 (m, 3H, Ar), 7.87 – 7.90 (m, 2H, Ar). ^{13}C NMR (75 MHz, $CDCl_3$) δ = 13.8 (CH_3), 21.6 (CH_2), 25.5 (CH_2), 26.1 (CH_2), 29.2 (CH_2), 31.5 (CH_2), 35.8 (CH_2), 37.7 (CH), 44.9 (CH), 47.8 (C), 50.5 (CH), 126.1 (CH), 127.3 (CH), 129.0 (2CH), 129.9 (2CH), 130.4 (CH), 130.7 (C), 133.1 (C), 134.8 (CH), 137.6 (C), 147.8 (C), 194.5 (C=O), 194.8 (C=O), 220.4 (C=O). IR (ATR, cm^{-1}): $\tilde{\nu}$ = 2926 (w), 2860 (w), 1734 (s), 1663 (s), 1597 (s), 1564 (w), 1450 (m), 1374 (w), 1320 (w), 1220 (s), 1160 (m), 1140 (w), 1084 (w), 1008 (w), 911 (w), 821 (w), 792 (w), 746 (m), 715 (s), 686 (m), 653 (s), 581 (w), 547 (w). MS (EI, 70 eV): m/z (%) = 386 (0.2) [M^+], 282 (20), 281 (100), 105 (11). HRMS (EI): calcd. for $C_{26}H_{26}O_3$ [M^+] 386.18765; found 386.18699.

(Estra-1,3,5(10)-trien-17-on-3-yl)-2-(*p-tert*-butylphenylethane)-1,2-dione (8b): yellow solid, 65%. Mp: 80 - 81 °C, $[\alpha]_D$ = +46.1° (c 8.45, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 0.84 (s, 3H, CH_3), 1.27 (s, 9H, $C(CH_3)_3$), 1.40 – 1.56 (m, 6H, aliphatic), 1.90 – 2.14 (m, 4H, aliphatic), 2.24 – 2.49 (m, 3H, aliphatic), 2.85 – 2.90 (m, 2H, aliphatic), 7.35 (d, $^3J_{H\text{-}H}$ = 8.1 Hz, 1H, Ar), 7.43 – 7.46 (m, 2H, Ar), 7.63 – 7.68 (m, 2H, Ar), 7.81 – 7.84 (m, 2H, Ar). ^{13}C NMR (63 MHz, $CDCl_3$) δ = 13.8 (CH_3), 21.6 (CH_2), 25.5 (CH_2), 26.1 (CH_2), 29.2 (CH_2), 31.0 ($C(CH_3)_3$), 31.5 (CH_2), 35.4 (C), 35.8 (CH_2), 37.7 (CH), 44.9 (CH), 47.8 (C), 50.5 (CH), 125.98 (2CH), 126.04 (CH), 127.3 (CH), 129.9 (2CH), 130.4 (CH), 130.6 (C), 130.8 (C), 137.5 (C), 147.6 (C), 158.9 (C), 194.4 (C=O), 194.7 (C=O), 220.3 (C=O). IR (ATR, cm^{-1}): $\tilde{\nu}$ = 2925 (m), 1734 (s), 1667(s), 1601 (s), 1565 (m), 1408 (m), 1225 (s), 1184 (s), 1108 (m), 1008 (s), 949 (m), 920 (m), 854 (m), 820 (m), 774 (m), 701 (s), 672 (m), 584 (m), 545 (s). MS (EI, 70 eV): m/z (%) = 442 (1) [M^+], 282 (23), 281 (100), 161 (46). HRMS (EI): calcd. for $C_{30}H_{34}O_3$ [M^+] 442.25025; found 442.25021.

(Estra-1,3,5(10)-trien-17-on-3-yl)-2-(3-thiophenylethane)-1,2-dione (8c): yellow solid, 59%. Mp: 89 - 90°C, $[\alpha]_D$ = +117.6° (c 1.00, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 0.84 (s, 3H, CH_3), 1.46 – 1.60 (m, 6H, aliphatic), 1.90 – 2.14 (m, 4H, aliphatic), 2.24 – 2.49 (m, 3H, aliphatic), 2.86 – 2.91 (m, 2H, aliphatic), 7.31 – 7.37 (m, 2H, Ar), 7.58 – 7.60 (m, 3H, Ar), 8.11 – 8.13 (m, 1H, Ar). ^{13}C NMR (63 MHz, $CDCl_3$) δ = 13.8 (CH_3), 21.6 (CH_2), 25.5 (CH_2), 26.1 (CH_2), 29.2 (CH_2), 31.5 (CH_2), 35.8 (CH_2), 37.7 (CH), 44.9 (CH), 47.8 (C), 50.5 (CH), 126.0 (CH), 127.1 (2CH), 127.5 (CH), 130.4 (C), 130.7 (CH), 136.9 (CH), 137.5 (C), 138.2 (C), 147.7 (C), 187.5 (C=O), 193.2 (C=O), 220.4 (C=O). IR (ATR, cm^{-1}): $\tilde{\nu}$ = 3102 (w), 2926 (m), 2858 (w), 1733 (s), 1653 (s), 1600 (m), 1562 (m), 1505 (m), 1452 (m), 1408 (m), 1374 (w), 1337 (w), 1295 (w), 1256 (w), 1227 (s), 1156 (m), 1138 (m), 1080 (m), 1007 (m), 944 (w), 870 (m), 818 (m), 773 (w), 728 (s), 712 (s), 670 (m), 621 (m), 582 (m), 547 (w). MS (EI, 70 eV): m/z (%) = 392 (0.1) [M^+], 282 (20), 281 (100), 111 (11). HRMS: calcd. for $C_{24}H_{24}O_3S$ [M^+] 393.15189; found 393.15188.

(Estra-1,3,5(10)-trien-17-on-3-yl)-2-(*p-n*-propylphenylethane)-1,2-dione (8d): yellow solid, 79%. Mp: 81-82 °C, $[\alpha]_D$ = +19.1° (c 3.41, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 0.84 – 0.90 (m, 6H, aliphatic), 1.39 – 1.63 (m, 8H, aliphatic), 1.90 – 2.14 (m, 4H, aliphatic), 2.24 – 2.49 (m, 3H, aliphatic), 2.56 – 2.61 (m, 2H, aliphatic), 2.87 – 2.90 (m, 2H, aliphatic), 7.22 (d, $^3J_{H\text{-}H}$ = 8.1 Hz,

2H), 7.36 ($^3J_{H-H}$ = 8.3 Hz, 1H), 7.63 – 7.68 (m, 2H, Ar), 7.80 (d, $^3J_{H-H}$ = 8.3 Hz, 2H, Ar). ^{13}C NMR (75 MHz, $CDCl_3$) δ = 13.75 (CH_3), 13.81 (CH_3), 21.6 (CH_2), 24.1 (CH_2), 25.5 (CH_2), 26.2 (CH_2), 29.2 (CH_2), 31.5 (CH_2), 35.8 (CH_2), 37.7 (CH), 38.3 (CH_2), 44.9 (CH), 47.9 (C), 50.6 (CH), 126.1, 127.3 (2CH), 129.2 (2CH), 130.1 (2CH), 130.5 (CH), 130.9 (C), 131.0 (C), 137.6 (C), 147.6 (C), 150.7 (C), 194.5 (C=O), 194.8 (C=O), 220.3 (C=O). IR (ATR, cm^{-1}): $\tilde{\nu}$ = 2930 (m), 2870 (w), 2252 (w), 1733 (m), 1665 (s), 1602 (s), 1566 (m), 1454 (m), 1415 (m), 1221 (s), 1165 (m), 1007(m), 910 (m), 843 (m), 728 (s), 646 (m), 581 (m), 547 (m). MS (EI, 70 eV): m/z (%) = 428 (3) [M^+], 283 (14), 282 (79.93), 281 (100), 148 (12), 147 (86), 91 (12). HRMS (EI): calcd. for $C_{29}H_{32}O_3$ [M^+] 428.23460; found 428.23465.

2-(Estra-1,3,5(10)-trien-17-on-3-yl)-3-phenyl-quinoxaline (9a): dione **(8a)** (100 mg, 0.26 mmol) and *o*-phenylenediamine (36.4 mg, 1.3 equiv.) were dissolved in ethanol (4 ml) and stirred at 50°C 1 h. The solvent was evaporated in vacuo. The residue was purified by column chromatography (silica gel, heptane/EtOAc = 4:1). **9a** was isolated as a yellow solid (109 mg, 92%). Mp: 102 - 103 °C, $[\alpha]_D$ = +85.4° (c 0.85, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 0.84 (s, 3H, CH_3), 1.39 – 1.55 (m, 6H, aliphatic), 1.86 – 2.12 (m, 4H, aliphatic), 2.24 – 2.47 (m, 3H, aliphatic), 2.78 – 2.80 (m, 2H, aliphatic), 7.10 (br.s, 2H, Ar), 7.27 – 7.29 (m, 4H, Ar), 7.46 – 7.49 (m, 2H, Ar), 7.65 – 7.68 (m, 2H, Ar), 8.07 – 8.10 (m, 2H, Ar). ^{13}C NMR (63 MHz, $CDCl_3$) δ = 13.9 (CH_3), 21.6 (CH_2), 25.6 (CH_2), 26.4 (CH_2), 29.3 (CH_2), 31.6 (CH_2), 35.8 (CH_2), 38.0 (CH), 44.4 (CH), 47.9 (C), 50.6 (CH), 125.0 (CH), 127.3 (CH), 128.2 (2CH), 128.7 (CH), 129.1 (CH), 129.2 (CH), 129.7 (CH), 129.80 (2CH), 129.84 (CH), 130.3 (CH), 136.4 (C), 136.6 (C), 139.3 (C), 140.6 (C), 141.1 (C), 141.3 (C), 153.3 (C), 153.4 (C), 220.7 (C=O). IR (ATR, cm^{-1}): $\tilde{\nu}$ = 2921 (m), 2854 (w), 1734 (s), 1608 (w), 1556 (w), 1538 (w), 1497 (w), 1476 (w), 1452 (w), 1404 (w), 1373 (w), 1342 (m), 1256 (m), 1220 (w), 1172 (w), 1136 (w), 1083 (m), 1067 (w), 1051 (m), 1003 (m), 988 (w), 906 (w), 844 (w), 826 (w), 811 (w), 794 (w), 760 (s), 696 (s), 641 (w), 609 (m), 589 (m), 566 (m). MS (EI, 70 eV): m/z (%) = 458 (100) [M^+], 459 (38), 443 (12), 414 (16), 402 (12), 401 (10), 387 (17), 361 (10), 348 (12), 347 (18), 346 (11), 319 (10), 206 (13), 205 (11), 178 (23), 173 (14), 172 (17), 166 (16), 155 (10), 152 (10), 150 (11), 115 (11), 76 (13), 67 (18), 66 (14), 52 (10), 41 (13), 40 (14). HRMS (EI, 70 eV): calcd. for $C_{32}H_{30}ON_2$ [M^+] 458.23527; found 458.23613.

2-(Estra-1,3,5(10)-trien-17-on-3-yl)-3-(*p-tert*-butylphenyl)-quinoxaline (9b): yellow solid, 88%. Mp: 121 - 122°C, $[\alpha]_D$ = +104.5° (c 1.00, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 0.85 (s, 3H, CH_3), 1.26 (s, 9H, $C(CH_3)_3$), 1.45 – 1.56 (m, 6H, aliphatic), 1.88 – 2.13 (m, 4H, aliphatic), 2.23 – 2.48 (m, 3H, aliphatic), 2.77 – 2.80 (m, 2H, aliphatic), 7.10 – 7.15 (m, 2H, Ar), 7.30 (d, $^3J_{H-H}$ = 8.5 Hz, 3H, Ar), 7.42 (d, $^3J_{H-H}$ = 8.4 Hz, 2H, Ar), 7.66 (dd, $^3J_{H-H}$ = 6.4 Hz, $^4J_{H-H}$ = 3.4 Hz, 2H, Ar), 8.08 (dd, $^3J_{H-H}$ = 6.4 Hz, $^4J_{H-H}$ = 3.5 Hz, 2H, Ar). ^{13}C NMR (75 MHz, $CDCl_3$) δ = 13.9 (CH_3), 21.6 (CH_2), 25.6 (CH_2), 26.4 (CH_2), 29.3 (CH_2), 31.3 ($C(CH_3)_3$), 31.6 (CH_2), 34.7 ($C(CH_3)_3$), 35.9 (CH_2), 38.0, 44.5 (2CH), 48.0 (C), 50.6 (CH), 125.0 (CH), 125.2 (2CH), 127.3 (CH), 129.1 (CH), 129.1 (CH), 129.5 (2CH), 129.7 (2CH), 130.3 (CH), 136.3 (C), 136.5 (C), 136.5 (C), 140.6 (C), 141.10 (C), 141.13 (C), 152.0 (C),

153.3 (C), 153.5 (C), 220.8 (C=O). IR (ATR, cm^{-1}): $\tilde{\nu}$ = 2926 (w), 2865 (w), 1738 (s), 1609 (w), 1556 (w), 1540 (w), 1500 (w), 1475 (w), 1454 (w), 1404 (w), 1393 (w), 1362 (w), 1341 (m), 1255 (m), 1221 (w), 1172 (2), 1110 (m), 1084 (w), 1051 (m), 907 (w), 891 (w), 839 (m), 824 (m), 761 (s), 737 (w), 710 (w), 605 (s), 558 (m). MS (EI, 70 eV): m/z (%) = 514 (100) [M^+], 515 (39), 499 (22), 458 (10), 457 (24), 57 (10). HRMS (EI, 70 eV): calcd. for $C_{36}H_{38}N_2O$ [M^+] 514.29787; found 514.29693.

2-(Estra-1,3,5(10)-trien-17-on-3-yl)-3-(thiophen-3-yl)-quinoxaline (9c): yellow solid, 82%. Mp: 111 - 112 °C, $[\alpha]_D$ = +90.2° (c 1.08, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 0.85 (s, 3H, CH_3), 1.44 – 1.55 (m, 6H, aliphatic), 1.89 – 2.10 (m, 4H, aliphatic), 2.28 – 2.48 (m, 3H, aliphatic), 2.83 – 2.85 (m, 2H, aliphatic), 7.19 – 7.28 (m, 5H, Ar + $CDCl_3$), 7.40 (s, 1H, Ar), 7.64 – 7.66 (m, 2H, Ar), 8.03 – 8.05 (m, 2H, Ar). ^{13}C NMR (63 MHz, $CDCl_3$) δ = 13.9 (CH_3), 21.6 (CH_2), 25.7 (CH_2), 26.4 (CH_2), 29.3 (CH_2), 31.6 (CH_2), 35.8 (CH_2), 38.0 (CH), 44.4 (CH), 47.9 (C), 50.6 (CH), 125.1 (CH), 125.3 (CH), 126.8 (CH), 127.5 (CH), 128.85 (CH), 128.98 (CH), 129.1 (CH), 129.7 (CH), 129.77 (CH), 129.82 (CH), 136.75 (C), 136.79 (C), 140.3 (C), 140.8 (C), 140.9 (C), 141.1 (C), 148.5 (C), 153.4 (C), 220.7 (C=O). IR (ATR, cm^{-1}): $\tilde{\nu}$ = 2920 (m), 2854 (w), 1734 (s), 1608 (w), 1559 (w), 1524 (w), 1500 (w), 1476 (w), 1452 (w), 1423 (m), 1374 (w), 1330 (m), 1254 (m), 1222 (w), 1182 (w), 1136 (w), 1084 (m), 1052 (m), 1006 (m), 907 (w), 867 (m), 843 (m), 817 (m), 787 (s), 760 (s), 712 (m), 655 (m), 622 (w). MS (EI, 70 eV): m/z (%) = 464 (100) [M^+], 465 (27), 463 (29), 431 (14), 353 (12), 339 (11), 327 (16), 325 (10), 314 (13), 313 (50), 301 (25), 300 (54), 299 (13), 287 (10), 185 (11), 170 (43), 161 (13), 153 (13), 149 (15), 140 (15). HRMS (EI, 70 eV): calcd. for $C_{30}H_{28}ON_2S$ [M^+] 464.19169; found 464.19139.

2-(Estra-1,3,5(10)-trien-17-on-3-yl)-3-(*p*-*n*-propylphenyl)-quinoxaline (9d): yellow solid, 96%. Mp: 114 - 115 °C, $[\alpha]_D$ = +66.0° (c 2.55, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$) δ = 0.90 – 0.95 (m, 6H, $2CH_3$), 1.44 – 1.55 (m, 4H, aliphatic), 1.59 – 1.66 (m, 4H, aliphatic), 1.94 – 2.17 (m, 4H, aliphatic), 2.31 – 2.55 (m, 3H, aliphatic), 2.58 – 2.63 (m, 2H, $CH_2CH_2CH_3$), 2.84 – 2.89 (m, 2H, aliphatic), 7.14 – 7.18 (m, 4H, Ar), 7.37 (s, 1H, Ar), 7.46 (d, $^3J_{H\text{-}H}$ = 8.1 Hz, 2H, Ar), 7.72 – 7.75 (m, 2H, Ar), 8.15 – 8.18 (m, 2H, Ar). ^{13}C NMR (75 MHz, $CDCl_3$) δ = 13.7 (CH_3), 13.9 (CH_3), 21.6 (CH_2), 24.4 (CH_2), 25.6 (CH_2), 26.4 (CH_2), 29.3 (CH_2), 31.6 (CH_2) 35.8 (CH_2), 37.8 (CH_2), 38.0 (CH), 44.4 (CH), 48.0 (C), 50.6 (CH), 125.0 (CH), 127.3 (CH), 128.4 (2CH), 129.0 (CH), 129.1 (CH), 129.7 (2CH), 129.8 (2CH), 130.3 (CH), 136.4 (C), 136.5 (C), 136.6 (C), 140.7 (C), 141.0 (2C), 143.6 (C), 153.3 (C), 153.5 (C), 220.7 (C=O). IR (ATR, cm^{-1}): $\tilde{\nu}$ = 2954 (m), 2926 (s), 2857 (s), 1736 (s), 1609 (w), 1556 (w), 1537 (w), 1500 (w), 1475 (m), 1453 (m), 1407 (m), 1392 (m), 1374 (w), 1340 (s), 1276 (m), 1255 (m), 1220 (m), 1184 (w), 1172 (w), 1136 (m), 1117 (m), 1084 (m), 1067 (m), 1051 (m), 1010 (m), 989 (m), 962 (w), 926 (w), 906 (w), 891 (w), 841 (m), 824 (m), 801 (m), 761 (s), 731 (m), 711 (m), 638 (w), 608 (m), 551 (m). MS (EI, 70 eV): m/z (%) = 500 (100) [M^+], 501 (37), 457 (17). HRMS (EI, 70 eV): calcd. for $C_{35}H_{36}ON_2$ [M^+] 500.28222; found 500.28273.

3-(*p*-*t*-butylphenylethynyl)-estra-1,3,5(10)-trien-17β-ol (10): alkyn **(7d)** (100 mg, 0.24 mmol) was dissolved in a mixture DCM-MeOH 1:1 (4 ml) and $NaBH_4$ (24.5 mg, 2.7 equiv.) was added in one portion. After stirring 1 h at 25°C 1 ml of water was added and the mixture was evaporated in

vacuo to dryness. The residue was purified by column chromatography (silica gel, heptane/EtOAc = 3:1). **10** was isolated as a white solid (60 mg, 59%). Mp: 200 - 201 °C, $[\alpha]_D$ = +41.2° (c 1.30, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) δ = 0.80 (s, 3H, CH_3), 1.33 – 1.40 (m, 12H, aliphatic + $C(CH_3)_3$), 1.46 – 1.55 (m, 4H, aliphatic), 1.69 – 1.75 (m, 1H, aliphatic), 1.88 – 1.93 (m, 1H, aliphatic), 1.96 – 2.00 (m, 1H, aliphatic), 2.10 – 2.17 (m, 1H, aliphatic), 2.23 – 2.28 (m, 1H, aliphatic), 2.33 – 2.27 (m, 1H, aliphatic), 2.85 – 2.88 (m, 2H, aliphatic), 3.73 – 3.76 (m, 1H, C*H*OH), 7.25 – 7.27 (m, 2H, Ar + $CDCl_3$), 7.29 – 7.31 (m, 1H, Ar), 7.35 – 7.37 (m, 2H, Ar), 7.45 – 7.46 (m, 2H, Ar). ^{13}C NMR (126 MHz, $CDCl_3$) δ = 11.1 (CH_3), 23.1 (CH_2), 26.0 (CH_2), 27.1 (CH_2), 29.3 (CH_2), 30.6 (CH_2), 31.2 (C(*C*H_3)$_3$), 34.8 (*C*(CH_3)$_3$), 36.7 (CH_2), 38.5 (CH), 43.2 (C), 44.5 (CH), 50.2 (CH), 81.9 (CHOH), 88.8 (C_{alkyne}), 89.0 (C_{alkyne}), 120.5 (C), 120.6 (C), 125.3 (2CH), 125.4 (CH), 128.8 (CH), 131.3 (2CH), 132.0 (CH), 136.8 (C), 140.7 (C), 151.3 (C). IR (ATR, cm^{-1}): $\tilde{\nu}$ = 3625 (w), 3605 (w), 2922 (s), 2861 (m), 1506 (m), 1453 (m), 1435 (m), 1393 (m), 1376 (m), 1362 (m), 1336 (w), 1265 (m), 1246 (m), 1203 (m), 1175 (w), 1136 (m), 1114 (w), 1105 (w), 1069 (m), 1045 (s), 1014 (m), 963 (w), 892 (m), 831 (s), 796 (m), 774 (m), 737 (w), 712 (w), 639 (w), 577 (m), 565 (s). MS (EI, 70 eV): m/z (%) = 412 (100) [M^+], 413 (32), 398 (20), 397 (66). HRMS (EI, 70 eV): calcd. for $C_{30}H_{36}O$ [M^+] 412.27607; found 412.27613.

2-(Estra-1,3,5(10)-trien-17β-ol-3-yl)-3-(*p*-*n*-propylphenyl)-quinoxaline (11): quinoxaline **(9d)** (100 mg, 0.2 mmol) was dissolved in a mixture DCM-MeOH 1:1 (4 ml) and $NaBH_4$ (20.4 mg, 2.7 equiv.) was added in one portion. After stirring 1 h at 25°C 1 ml of water was added and the mixture was evaporated in vacuo to dryness. The residue was purified by column chromatography (silica gel, heptane/EtOAc = 3:1). **11** was isolated as a white solid (92.5 mg, 92%). Mp: 121 - 122 °C, $[\alpha]_D$ = +14.7° (c 1.42, $CHCl_3$). ^{1}H NMR (250 MHz, $CDCl_3$) δ = 0.79 (s, 3H, CH_3), 0.93 (t, $^3J_{H\text{-}H}$ = 7.3 Hz, 3H, $CH_2CH_2CH_3$), 1.43 – 1.54 (m, 6H, aliphatic), 1.61 – 1.70 (m, 4H, aliphatic), 1.86 – 1.98 (m, 2H, aliphatic), 2.09 – 2.35 (m, 3H, aliphatic), 2.59 – 2.65 (m, 2H, $CH_2CH_2CH_3$), 2.80 – 2.83 (m, 2H, aliphatic), 3.70 – 3.76 (m, 1H, C*H*OH), 7.14 – 7.19 (m, 4H, Ar), 7.34 (s, 1H, Ar), 7.45 – 7.48 (m, 2H, Ar), 7.74 (dd, $^3J_{H\text{-}H}$ = 6.4 Hz, $^4J_{H\text{-}H}$ = 3.4 Hz, 2H, Ar), 8.16 (dd, $^3J_{H\text{-}H}$ = 6.4 Hz, $^4J_{H\text{-}H}$ = 3.4 Hz, 2H, Ar). ^{13}C NMR (63 MHz, $CDCl_3$) δ = 11.1 (CH_3), 13.7 (CH_3), 23.1 (CH_2), 24.4 (CH_2), 26.0 (CH_2), 27.1 (CH_2), 29.4 (CH_2), 30.6 (CH_2), 36.7 (CH_2), 37.8 (CH_2), 38.5 (CH), 43.2 (C), 44.4 (CH), 50.2 (CH), 81.8 (CHOH), 125.0 (CH), 127.1 (CH), 128.4 (2CH), 129.0 (2CH), 129.7 (4CH), 130.2 (CH), 136.1 (C), 136.5 (C), 136.8 (C), 140.97 (C), 141.01 (C), 141.3 (C), 143.5 (C), 153.4 (C), 153.5 (C). IR (ATR, cm^{-1}): $\tilde{\nu}$ = 3372 (w), 2925 (m), 2866 (m), 1708 (w), 1630 (w), 1610 (w), 1589 (w), 1556 (w), 1537 (w), 1511 (m), 1454 (m), 1411 (w), 1391 (m), 1341 (s), 1249 (m), 1221 (m), 1184 (w), 1168 (w), 1137 (m), 1076 (m), 1054 (s), 1021 (m), 1009 (m), 988 (m), 960 (w), 907 (w), 893 (w), 843 (m), 825 (m), 801 (m), 760 (s), 731 (m), 645 (m), 608 (s), 552 (m). MS (EI, 70 eV): m/z (%) = 502 (100) [M^+], 503 (36), 459 (12). HRMS (EI, 70 eV): calcd. for $C_{35}H_{38}ON_2$ [M^+] 502.29787; found 502.29819.

Alkaline Phosphatase Inhibition Assay

A luminescence assay using CDP-Star (disodium 2-chloro-5-(4-methoxyspiro[1,2-dioxetane-3,2′-(5-chlorotricyclo[3.3.1.13.7]decan])-4-yl]-1-phenyl phosphate) as a substrate was used for the determination of enzyme inhibition of compounds on bovine kidney alkaline phosphatase (TNAP) enzyme and calf intestine alkaline phosphatase (IAP). The luminescent assay is 1000 times more sensitive than colorimetric assay for the evaluation of TNAP and IAP inhibitors.[20]

The assay buffer containing 8 M diethanolamine (DEA), 2.5 mM $MgCl_2$ and 0.05 mM $ZnCl_2$, pH 9.8, was used. Initial screening was performed at a concentration of 0.2 mM of the tested compounds. Total assay volume was 50 µL, containing 10 µL of tested compound (0.2 mM) followed by the addition of 20 µL of enzyme TNAP (1:800 times diluted (0.8 units/mL) enzyme in assay buffer) or 20 µL of IAP (1:800 times diluted (1 unit/mL) enzyme in assay buffer). The mixture was pre-incubated for 3-5 minutes at 37 ^{o}C and luminescence was measured as a pre-read using microplate reader (BioTek FLx800, Instruments, Inc. USA). Then, 20 µL of CDP-star (final concentration of 110 µM) was added to initiate the reaction and the assay mixture was incubated again for 15 min at 37 ^{o}C. The change in the luminescence was measured as after-read. The inhibitory activity of each compound was compared with total activity control (without any inhibitor). Levamisole (2 mM per well) and L-phenylalanine (4 mM per well) were used as a positive controls against tissue-nonspecific alkaline phosphatase (TNAP) and calf intestinal alkaline phosphatase (IAP), respectively. The compounds which exhibited more than 50% inhibition of either the tissue-nonspecific alkaline phosphatase (TNAP) activity or calf intestinal alkaline phosphatase (IAP) activity were further evaluated for determination of inhibition constants (IC_{50} values). For this purpose 8 serial dilutions of each compound (200 µM to 20 nM) were prepared in assay buffer and their dose response curves were obtained by assaying each inhibitor concentration on both APs using the above mentioned reaction conditions. All experiments were repeated three times in triplicate. The IC_{50} values were determined by the non-linear curve fitting program PRISM 5.0 (GraphPad, San Diego, California, USA).

Molecular Docking

Molecular docking study of the compound **7i** was carried out using FlexX utility of LeadIT v2.1.8 software from BioSolveIT GmbH, Germany.[21] Chemical structure of compound **7i** was drawn using ACD/ChemSketch[22] and was 3D optimized. Homology models of bovine tissue non-specific and intestinal alkaline phosphatase previously modelled[18] was used to identify the putative binding mode of our compound. The enzyme was loaded and prepared for docking using the automated 'Load or Prepare receptor' utility of the software. Two zinc ions and a magnesium ion was selected as part of the receptor and to carry out docking, active site was defined by selecting the amino acid residues in 7.5 Å radius around the metal ions. Tautomer and protonation state of the receptor's amino acids were determined by Protoss[23] utility of LeadIT v2.1.8 software and recommended parameters of tautomer and protonation state, solvent handling and metal coordinates was used. Chemical structure of compound **7i** previously sketched and optimized was loaded into compound library for docking. Using default parameters of the software molecular docking of the compound was carried out. Top ranking 30 poses were generated and

retained. The putative binding mode of our compound was selected after careful visual and HYDE assessment.[24]

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References

1 Bruice, P. Y. Organic Chemistry (5th Edition), Prentice Hall, **2006**.

2 Inhoffen, H. H.; Hohlweg, W. *Naturwissenschaften* **1938** , *26*, 96.

3 Schang, L. M.; Vincent, A. V. Patent: US20120135954 A1, 2012.

4 Möller, G.; Duluca, D.; Gege, C.; Rosinus, A.; Kowalik, D.; Peters, O.; Droescher, P.; Elger, W.; Adamski, J.; Hillisch, A. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6740-6744.

5 a) Nussbaumer, P.; Billich, A. *Med. Res. Rev*. **2004**, *24*, 529-576; b) Geisler, J.; Lonning, P. E. *Cancer Res*. **2005**, *11*, 2809-2821; c) Lawrence Woo, L. W.; Leblond, B.; Purohit, A.; Potter, B. V. L. *Bioorg. Med. Chem*. **2012**, *20*, 2506-2519.

6 Suwandi, L. S.; Agoston, G. E.; Shah, J. H.; Hanson, A. D.; Zhan, X. H.; LaVallee, T. M.; Treston, A. M. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6459-6462.

7 a) Chen, Q.-Y.; He, Y.-B. *Synthesis* **1988**, 896-897; b) Shirbin, S. J.; Boughton, B. A.; Zammit, S. C.; Zanatta, S. D.; Marcuccio, S. M.; Hutton, C. A.; Williams, S. J. *Tetrahedron Lett*. **2010**, *51*, 2971–2974.

8 Sidique, S.; Ardecky, R.; Su, Y.; Narisawa, S.; Brown, B.; Millán, J. L.; Sergienko, E.; Cosford, N. D. 2009. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 222-225.

9 Zimmermann, H. *Drug Dev. Res*. **2001**, *52*, 44-56.

10 Al-Rashida, M.; Raza, R.; Abbas, G.; Shah, M. S.; Kostakis, G. E.; Lecka, J.; Sevigny, J.; Muddassar, M.; Papatriantafyllopoulou, C.; Iqbal, J. *Eur. J. Med. Chem.* **2013**, *66*, 438-449.

11 Hessle, L.; Johnson, K. A.; Anderson, H. C.; Narisawa, S.; Sali, A.; Goding, J. W.; Terkeltaub, R.; Millán, J. L. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 9445-9449.

12 Frieling, G. W.; Al-Zaid, T.; Prieto, V. G. *Am. J. Dermatopathol.* **2014**, *36*, 189-190.

13 Narisawa, S.; Harmey, D.; Yadav, M. C.; O'neill, W. C.; Hoylaerts, M. F.; Millán, J. L. *J. Bone Miner. Res.* **2007**, *22*, 1700-1710.

14 Chen, C.-Y.; Hu, W.-P.; Liu, M.-C.; Yan, P.-C., Wang, J.-J.; Chung, M.-I. *Tetrahedron* **2013**, *69*, 9735-9741.

[15] a) Chandrasekhar, S.; Reddy, N.K.; Kumar, V.P. *Tetrahedron Lett*. **2010**, *51*, 3623-3625; b) Gao, A.; Yang, F.; Li, J.; Wu, Y. *Tetrahedron* **2012**, *68*, 4950-4954 c) Claremon, D. A.; Ponticello, G. S. Patent: US6350744 B1, 2002.

[16] Saxena, H. O.; Faridi, U.; Kumar, J. K.; Luqman, S.; Darokar, M. P.; Shanker, K.; Chanotiya, C. S.; Gupta, M. M.; Negi, A. S. *Steroids* **2007**, *72*, 892-900.

[17] Guo, J.; Duclos Jr., R. I.; Vemuri, V. K.; Makriyannis, A. *Tetrahedron Lett*. **2010**, *51*, 3465-3469.

[18] al-Rashida, M.; Ejaz, S. A.; Ali, S.; Shaukat, A.; Hamayoun, M.; Ahmed, M.; Iqbal, *J. Bioorg. Med. Chem*. **2015**, 23, 2435-44.

[19] Lipshutz, B. H.; Petersen, T. B.; Abela, A. R. *Org. Lett*. **2008** , *10*, 1333-1336.

[20] Sergienko, E. A.; Millán, J. L. *Nat. Protoc*. **2010**, *5*, 1431-1439.

[21] LeadIT, version 2.1.8, BioSolveIT GmbH, Germany. **2004**

[22] ACD/ChemSketch, version 14.0, Advanced Chemistry Development, Inc., Toronto, On, Canada. **2015**

[23] Bietz, S.; Urbaczek, S.; Schulz, B.; Rarey, M. *J. Chem. informatics*. **2014**, 6, 6-12.

[24] Schneider, N.; Lange, G.; Hindle, S.; Klein, R.; Rarey, M. *J. Comput. Aided Mol. Des*. **2013**, 27,15-29.