

Original Research

**HOW STRUCTURAL MODIFICATIONS INFLUENCE ENVIRONMENTAL RISK:
A CASE STUDY OF STEROIDAL DERIVATIVES**

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Abstract

Steroid hormones are important endocrine-disrupting compounds due to their widespread use and continuous release into the environment via human and livestock excretion, veterinary applications, and pharmaceuticals. The environmental behavior of structurally modified steroidal hydrazones and derivatives remains largely unknown. We conducted an in silico assessment using EPI Suite to predict physicochemical properties, environmental fate, bioaccumulation, and aquatic toxicity. The analyzed derivatives exhibit higher lipophilicity (log Kow 3.27–5.60), increased molecular weight (344–530), and lower water solubility (<5 mg L⁻¹) than reference steroids, suggesting limited mobility and enhanced sorption. Predicted log Koc values (3–8) indicate strong soil and sediment adsorption, while BIOWIN3 suggests slow biodegradation. Bioaccumulation potential (log BCF/BAF) is elevated, with dietary uptake contributing for certain derivatives. Acute toxicity estimated via ECOSAR shows very high toxicity for fish (LC₅₀ 0.06–0.42 mg L⁻¹) and high to very high toxicity for daphnids (0.05–1.69 mg L⁻¹). These in silico findings indicate that the studied steroidal derivatives may pose a significant environmental risk, warranting further experimental validation and careful consideration of their fate and effects in aquatic and terrestrial ecosystems.

Keywords: *in silico* assessment, environmental fate, bioaccumulation, aquatic toxicity, lipophilicity.

INTRODUCTION

Steroid hormones represent a major class of endocrine-disrupting compounds due to their extensive medical use and continuous release from both natural and anthropogenic sources [1–2]. They are introduced into the environment through various pathways, including human and livestock excretion, veterinary applications, agricultural runoff, and pharmaceutical

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residues. As active pharmaceutical agents, they are widely employed in the treatment of reproductive disorders, growth-related conditions, and cancers.

The environmental occurrence and adverse effects of steroidal compounds, particularly estrogens, androgens, and progestogens, have been widely documented, even at trace concentrations. Their persistence and high biological activity raise significant concerns regarding ecological imbalance and potential risks to human health. At the same time, the development of novel steroid-based therapeutics, including cytotoxic derivatives, necessitates a more comprehensive evaluation of their environmental impact. Integrating environmental considerations into early-stage drug design is therefore essential, alongside improvements in wastewater treatment, analytical detection, and regulatory control.

The selected reference compounds include estrogens (estradiol and ethinyl estradiol), an androgen (testosterone), and a corticosteroid (prednisolone), representing major classes of biologically active steroids (Figure 1). Their inclusion enables a comparative evaluation of the environmental behavior and toxicity of structurally modified androstenedione- and progesterone-based derivatives. In this context, the present study provides a systematic *in silico* assessment of the environmental properties of steroidal hydrazones and their derivatives, with a comparative analysis against selected reference steroid compounds recognized as environmental contaminants.

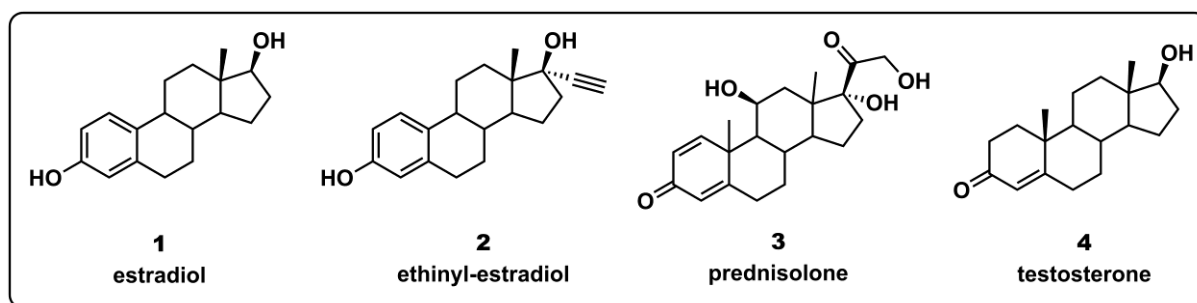


Figure 1 Chemical structures of the selected reference steroidal compounds

MATERIALS AND METHODS

Assessed compounds

The compounds analyzed in this study were previously synthesized and reported [3–5], whereas the quinoxaline derivatives were included based on data from our previous work [6].

In silico environmental assessment

Physicochemical properties (log Kow, water solubility), environmental fate (log Koc), biodegradability (BIOWIN3), bioaccumulation (log BCF/BAF), and aquatic toxicity (fish and daphnid LC₅₀) of the steroidal derivatives were predicted using the EPI Suite (<https://episuitedev.org/EpiWebSuite/#/>). The calculations were performed using relevant modules, including KOWWIN, WSKOWWIN, KOCWIN, BIOWIN3, BCFBAF, and ECOSAR.

RESULTS AND DISCUSSION

Steroidal compounds chosen for the environmental assessment are presented in Figure 2.

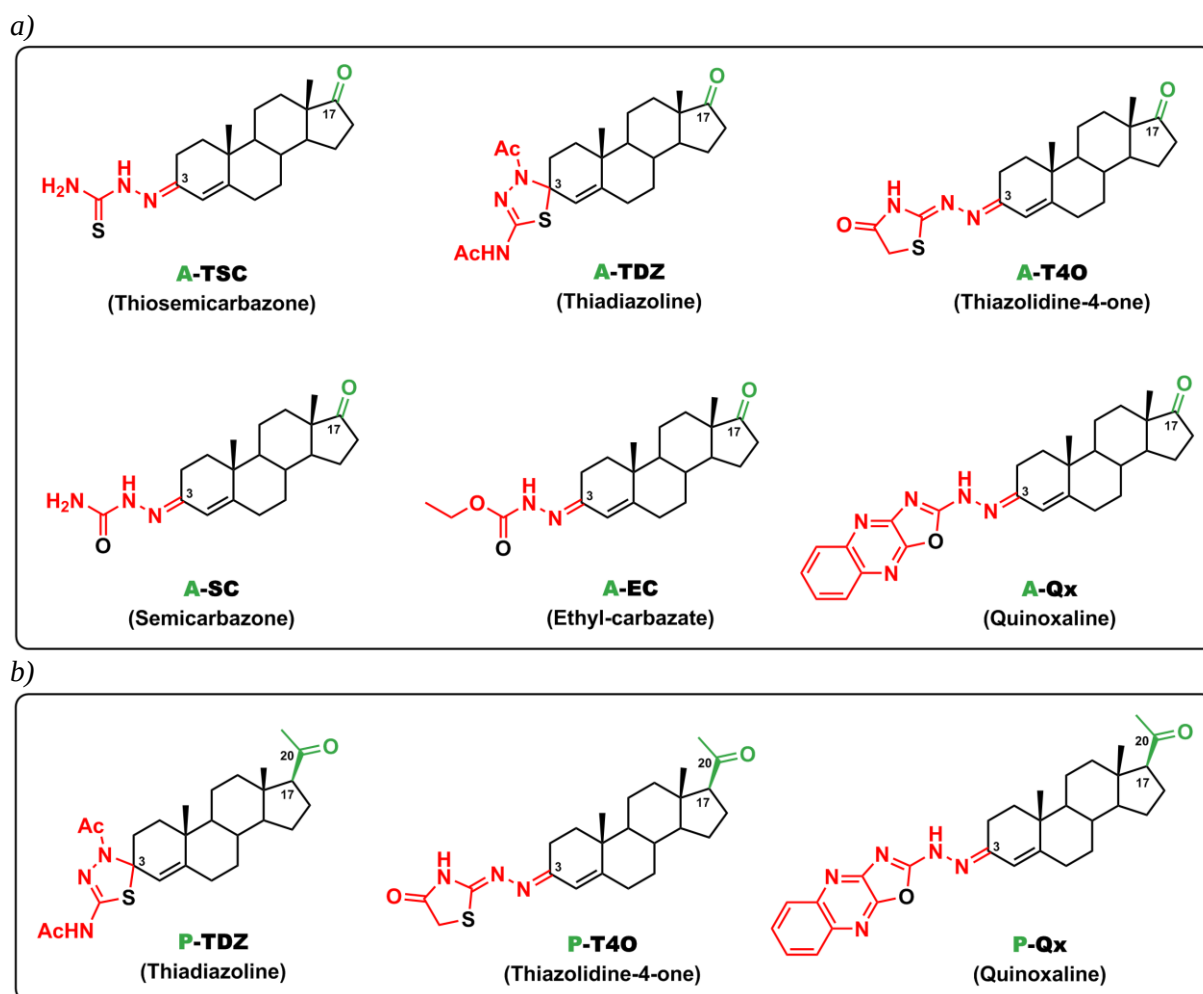


Figure 2 Chemical structures of the analyzed steroidal derivatives: a) androstenedione derivatives, and b) progesterone derivatives

To evaluate these aspects, predicted physicochemical, environmental fate, and ecotoxicity parameters of the analyzed steroidal derivatives are presented in Table 1, alongside selected reference steroid compounds for comparison (1–4).

Physicochemical parameters such as lipophilicity (log Kow), water solubility (Ws), and molecular weight (MW) are key determinants of environmental distribution and govern processes such as sorption, bioaccumulation, and biodegradation.

The predicted log Kow values of the reference compounds range from 1.40 to 4.12, with prednisolone showing the lowest and ethinyl estradiol the highest lipophilicity. In contrast, the analyzed steroidal derivatives exhibit higher log Kow values (3.27–5.60), indicating increased lipophilicity. The highest value was observed for the **P-Qx** derivative, while the lowest corresponds to the **A-TDZ** derivative. Overall, progesterone derivatives are more lipophilic than the corresponding androstane analogues, suggesting that structural modifications significantly influence hydrophobicity. Increased lipophilicity may reduce water solubility

and enhance sorption to organic matter, which can contribute to higher bioaccumulation potential and persistence in the environment. In line with these observations, the molecular weights of the analyzed steroidal derivatives (344–498) are higher than those of the reference compounds (272–360), reflecting increased structural complexity. In addition, the predicted water solubility of the modified compounds is significantly lower ($< 5 \text{ mg L}^{-1}$) compared to the reference steroids (48–341 mg L^{-1}). This reduction in water solubility is consistent with the increased lipophilicity of the analyzed compounds and suggests limited mobility in aqueous environments. Instead, such compounds are more likely to associate with organic matter and accumulate in sediments. Furthermore, the combination of higher molecular weight and low solubility may contribute to reduced biodegradation rates and enhanced bioaccumulation potential.

Table 1 Predicted physicochemical parameters, environmental fate, and ecotoxicity parameters of steroidal derivatives

Compd.	log K _{ow}	W _s (mg L^{-1})	MW	BIO3	log K _{oc}	log BCF	log BAF	F-LC ₅₀ (mg L^{-1})	D-LC ₅₀ (mg L^{-1})
A-TSC	4.63	0.60	360	1.9	5.07	2.72	2.45	0.09	0.62
A-TDZ	3.27	2.61	444	1.5	4.59	1.83	2.15	0.42	1.69
A-T4O	4.34	0.60	400	1.8	5.22	2.53	2.34	0.14	0.81
A-SC	3.68	4.85	344	1.9	4.64	2.10	1.55	0.22	1.03
A-EC	4.67	0.46	373	1.9	5.23	2.75	3.17	0.09	0.51
A-Qx	4.69	0.009	470	1.7	7.70	2.76	2.67	0.11	0.77
P-TDZ	3.50	1.12	472	1.4	5.07	1.98	2.38	0.36	1.57
P-T4O	5.25	0.07	428	1.8	5.69	3.13	2.82	0.06	0.21
P-Qx	5.60	0.001	498	1.7	8.18	3.36	3.22	0.19	0.05
1	3.94	93.53	272	2.4	4.19	2.27	1.52	4.03	2.74
2	4.12	48.42	296	2.0	4.65	2.38	1.28	3.06	2.11
3	1.40	341	360	2.0	2.05	0.59	0.43	42.85	26.51
4	3.27	74.88	288	2.3	3.34	1.82	2.17	17.17	10.96

Compd. – compound.

log K_{ow} – octanol-water partition coefficient; indicator of compound lipophilicity.

W_s – water solubility; predicted solubility of the compound in water.

MW – molecular weight.

BIO3 – aerobic biodegradability (BIOWIN3 model).

log K_{oc} – organic carbon-water partition coefficient; indicator of adsorption to soil/sediment.

log BCF – bioconcentration factor; accumulation from water into organisms.

log BAF – bioaccumulation factor; accumulation from water and food.

F-LC₅₀ – predicted lethal concentration for 50% of fish over 96 hours.

D-LC₅₀ – predicted lethal concentration for 50% of *Daphnia* spp. over 48 hours.

The reduced water solubility and increased lipophilicity of the analyzed steroidal derivatives are expected to influence their adsorption to soils and sediments. This behavior is further reflected in the predicted log K_{oc} values, with structural modifications leading to a wider range of soil adsorption compared to reference steroid contaminants (4.64–8.18 vs. 2.05–4.65), indicating increased variability in environmental mobility and potential accumulation in sediments. Among the reference compounds, prednisolone remains relatively mobile in the environment (log K_{oc} < 3), whereas estradiol, ethinyl estradiol, and testosterone exhibit moderate adsorption to soil organic matter (log K_{oc} = 3.34–4.65). In contrast, **A-TDZ** and **A-SC** derivatives show moderate adsorption (log K_{oc} = 3–5), while the remaining

modified compounds display very high adsorption potential ($\log K_{oc} > 5$), suggesting strong binding to soil and sediment organic matter and a tendency for accumulation in sediments. This behavior is consistent with the increased lipophilicity of the modified steroidal derivatives.

Consistent with these properties, steroid-based derivatives exhibit relatively slow predicted biodegradation rates according to the BIOWIN3 model, reflecting the known environmental persistence of steroidal contaminants. The reference compounds, testosterone and estradiol, are predicted to degrade within weeks to months (BIOWIN3 = 2.3–2.4). In contrast, heterocyclic derivatives with higher molecular weights, including both TDZ and Qx classes, show slower degradation kinetics (BIOWIN3 = 1.3–1.7), suggesting extended persistence in the environment. The remaining compounds are predicted to degrade on a timescale of months (BIOWIN3 = 1.8–2.0), indicating limited biodegradation potential. These predictions should be interpreted with caution, as BIOWIN3 provides an estimate of primary biodegradation timeframe rather than complete mineralization.

As expected from their increased lipophilicity and sorption potential, the predicted log BCF values (1.83–3.36) indicate moderate to high bioaccumulation potential for the studied compounds, exceeding that of reference steroids (0.58–2.38). Similar trends were observed for log BAF values. Notably, **A-EC** and both TDZ derivatives exhibited higher BAF than BCF values, suggesting that dietary uptake contributes substantially to overall bioaccumulation for these compounds.

The environmental relevance of these compounds is further highlighted by their predicted acute aquatic toxicity. Toxicity was estimated using ECOSAR as LC_{50} (96 h) for fish and LC_{50} (48 h) for *Daphnia spp.*, with the most conservative (lowest) predicted value among applicable models selected for each endpoint.

The analyzed steroidal derivatives exhibit pronounced aquatic toxicity, with generally higher sensitivity observed in fish than in daphnids. The higher toxicity of the analyzed compounds relative to the reference steroids may be attributed to the presence of hydrazone functionality, suggesting its role in enhancing aquatic toxicity. The predicted LC_{50} values for fish (0.06–0.42 mg L⁻¹) indicate very high toxicity of the investigated compounds, whereas the reference compounds show considerably lower toxicity (3.06–42.85 mg L⁻¹). A similar trend is observed for daphnids, with the analyzed compounds classified as highly to very highly toxic (0.05–1.69 mg L⁻¹), in contrast to the reference compounds, which range from moderately to highly toxic (2.11–26.51 mg L⁻¹). Although direct comparison between fish and daphnid toxicity should be made with caution due to differences in exposure duration and test conditions, the results suggest that fish may represent the more sensitive trophic level for the majority of the investigated compounds. This may be associated with the higher lipophilicity of the compounds, which facilitates membrane permeation and potential bioaccumulation in aquatic organisms.

These findings highlight the potential environmental risk and underscore the need for further experimental validation and environmentally conscious design of steroid-based compounds.

CONCLUSION

In summary, the analyzed steroidal derivatives exhibit increased lipophilicity, higher molecular weight, and lower water solubility compared to reference steroids, resulting in stronger sorption to organic matter, slower predicted biodegradation, and higher bioaccumulation potential. Structural modifications, particularly the introduction of heterocyclic and hydrazone functionalities, contribute to these trends and are associated with pronounced aquatic toxicity, with fish generally representing the most sensitive trophic level. Taken together, these *in silico* predictions suggest that the studied compounds may pose a significant environmental risk, highlighting the need for further experimental validation and careful consideration of their fate and effects in aquatic and terrestrial ecosystems.

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