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Benign Synthesis of Thiazolo-androstenone Derivatives as Potent Anticancer Agents

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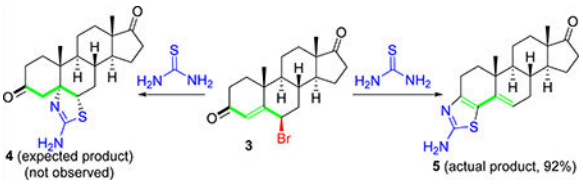
Abstract

An unprecedented reaction of thiourea derivatives with 6β-bromoandrostenedione has been discovered for the formation of aminothiazolo-androstenones via a simple, safer, cascade protocol that enables the syntheses of novel molecules by using readily available reagents. The reaction mechanism of product formation has been rationalized by density functional theory calculations. This benign methodology accentuates a domino protocol deploying a renewable solvent, ethanol, while generating novel compounds that display potent growth inhibitory effects in in vitro studies for several cancer cell lines at submicromolar concentrations.

Graphical Abstract



Steroidal hormones are involved in a number of biological signaling processes[1,2] with a large number being of natural products isolated from various plants and microorganisms. These molecules are known for their wide-ranging biological activities,[3] and therefore, not

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The authors declare no competing financial interest.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.8b02587.

^{1}H and ^{13}C NMR spectra, DFT calculation data, and X-ray diffraction data (PDF)

Accession Codes

CCDC 1858408–1858409 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

surprisingly, assorted synthetic derivatives have been reported in the pursuit of drugs, drug candidates, and other useful entities such as herbicides.[4–12] Steroidal derivatives comprise one of the broadest spectra of the therapeutic class of compounds and are being used extensively in modern medicine to treat different anomalies, including cancer.[10,13] Both natural and synthetic steroidal derivatives are known for their therapeutic properties such as agonists of cell-surface G-protein coupled bile acid receptor 1 (GP-BAR1),[8] neuroactive,[9] anticancer,[14] anti-Alzheimer,[15] and several other medicinal properties.[16] Thiazole derivatives are another class of pharmacologically important compounds with several approved drugs in this category including dasatinib and ritonavir.[17,18] Heterocyclic rings comprise several steroidal-based drugs including recently approved Emflaza (deflazacort) to treat Duchenne muscular dystrophy (DMD) and Zytiga (abiraterone acetate) to treat metastatic castration-resistant prostate cancer.[19–23] Heterocycle-attached androstanes, galeterone[22] and oleandrigenin,[23] are examples of a drug in clinical trials and a natural product, respectively (Figure 1).

In view of the importance of heterocycle-bearing steroidal derivatives, a large number of methodologies and synthetic schemes have been described,[24–30] often involving multistep synthesis.[6–9,31] Recently, Stanley et al. have reported the synthesis of heteroarylated steroidal diene by using bismuth triflate as a catalyst.[32]

In our pursuit of synthesizing bioactive heterocycles,[33–36] we envisaged the synthesis of thiazolino-androstanedione derivatives via our recently developed methodology that entails a 1,1,1,3,3,3-hexafluoroisopropanol (HFIP) mediated domino reaction of γ-bromo enones (**3**) with thioamides and thioureas to form thiazoline products (**4**) under refluxing conditions.[37] Surprisingly, hitherto unknown 3,4-thiazolo-androstenone product (**5**) was formed instead of the expected 5,6-thiazolino-androstenedione derivative (**4**) (Scheme 1). The reaction was conducted in various solvents (see Table 1, Supporting Information (SI)), and to our delight, the reaction occurred in a renewable and recyclable solvent, ethanol (EtOH), without compromising the yield and purity and precluded anhydrous reaction conditions or inert atmosphere. Product **5** was formed in 92% yield on a gram scale synthesis, and the pure material was isolated simply by filtration followed by washing with ethanol and water.

After identification of the formed product, thiazolo-androstenone (**5**), under the optimized conditions, we carried out the reaction of substituted thiourea derivatives under the same reaction conditions; the expected products were formed in good to excellent yield. This reaction is very general for a wide range of substituted thiourea derivatives. Reaction of alkyl-substituted thioureas with the electrophile (**3**) afforded the products **6**–**10** in very good yields (71–79%). Morpholine, a hydrophilic substituent, attached to alkyl thiourea reacted smoothly to give the corresponding product (**11**) in 75% yield. Similarly, arylthiourea derivatives also reacted with the electrophile and delivered products without affecting the average yield and purity; *N*-phenyl thiourea provided (**12**) in 86% yield. Electron-donating groups on the aryl ring of thiourea provided the desired compounds without compromising the yield and purity; namely, toluenyl product (**13**) in 92% yield as well as methoxy-, trifluoromethoxy-, and hydroxyphenyl-substituted derivatives (**14**, **15**, and **16**) are formed efficiently. In identifying the scope of the methodology, substrates with electronwithdrawing groups on the phenyl ring were also reacted with electrophile (**3**), and the expected products

were produced such as fluoro- and chloro-substituted entities (**17**, **18**, and **19**) in 80, 83, and 74% yields, respectively. The single-crystal structure of compound **19** (CCDC 1858408) is available in the Supporting Information. Carboxylic acid substituted products (**20** and **21**) were formed in an average yield of ~73%.

This general methodology also tolerated strong electron-withdrawing substituents; the nitro group on the phenyl ring afforded **22** in 81% yield, while disubstituted products **23**–**26** were also formed efficiently (70–77%). *N,N*-Disubstituted thiourea did not hamper the reaction either, and the expected product **27** was formed in 73% yield along with pyridine and pyrimidine products **28** and **29** (Scheme 2).

To test the scope of the methodology to generate a library of new molecules as potential therapeutic agents, one of the compounds (**5**) was synthesized on multigram scale and further derivatized by simple transformations. Reaction of the amino-thiazolo derivative **5** with acetic anhydride formed acetamido product **30**, which on $NaBH_4$ reduction afforded the hydroxy product **31** (Scheme 3). The average yields of these reactions are 93%, and the products were simply obtained by filtering and washing the solid with methanol and water. In our preliminary in vitro anticancer studies, hydroxy compounds have shown several times better activity than the parent ketone compounds (data not shown).

There are four possible pathways for thiourea to undergo reaction with the electrophile, 6β-bromoandrostenedione, to create three different products (Scheme 4 and the SI).

We computed the feasibility of these four pathways by using a hybrid-density functional method (M06–2X)/6–311++G(d,p) + PCM = EtOH as implemented in the Gaussian 09 suite of programs.[34] The expected product **4**, based on our previous report,[37] is the least favorable path, and the S_N2' reaction of thiourea with β-bromoandrostenedione to generate thiazolo-androstenone is also not favorable (SI). Nucleophilic addition of thiourea to carbonyl of β-bromoandrostenedione (**3**) can form two possible intermediates, hemithioacetal (path A) or hemiaminal (path B). Gibb's free energy for the formation of hemithioacetal (**A1**, + 31.84 kcal/mol) and hemiaminal (**B1**, + 10.15 kcal/mol) is endergonic, which is achievable by refluxing the reaction mixture. We believe the formation of hemithioacetal (**A1**) and hemiaminal (**B1**) is reversible under the reaction conditions. Intramolecular S_N2' reaction of these intermediates leads to the formation of thiazoline derivatives (**A2** and **B2**). This intramolecular reaction of hemithioacetal is more favorable than that of hemiaminal (−32.70 kcal/mol vs −17.07 kcal/mol). The final step, elimination of water, is also more favorable for the hemithioacetal derivative than that of the hemiaminal (−24.82 kcal/mol vs −19.69 kcal/mol) to produce the final products **A3** and **5**, respectively (Scheme 4). Among the three steps for the formation of possible products, the first step is reversible and endergonic while the last two steps are irreversible and exergonic. Actual product **5** is formed because of the less activation energy for the first step, as a result of the nucleophilic addition to form hemiaminal **B1**. The energy profile diagram is shown in Figure 2.

Structures with absolute stereochemistry have been confirmed by single-crystal diffraction. The ORTEP diagrams (**7** and **19**) show the regiospecific reaction of this methodology in which N and S of thiazole are attached to C-3 and C-4, respectively (Figure 3 and the SI).

We have evaluated some of the aforementioned compounds by screening them in NCI's 60 cancer cell lines,[38] and several entities have shown promising activity against several cancer cell lines at submicromolar concentrations; in vitro testing results for compounds **17** and **23** against NCI-60 cancer cell lines are shown in the SI.

These molecules have shown potent activity against several cancer cell lines including the growth inhibition of leukemia cell lines: RPMI-8226 and SR with 50% growth inhibition (GI_{50}) values at submicromolar concentration; two of the nonsmall cell lung cancer (NSCLC) cell lines were inhibited at low μM concentration. Compound **17** inhibited four of six central nervous system (CNS) cell lines with GI_{50} values of <2 μM concentration including four cell lines of the colon cancer; **17** inhibited the growth of glioblastoma (SF-295) and gliosarcoma (SF-539) cell lines with GI_{50} values of 1.19 and 1.34 μM, respectively. Five melanoma cell lines and six renal cancer cell lines were inhibited at low micromolar concentration with GI_{50} values <2 μM. These molecules (**17** and **23**) have also shown promising activity against ovarian cancer, prostate cancer, and breast cancer cell lines (Table 1).

We have discovered an efficient domino protocol to synthesize novel thiazolo-androstenone derivatives by using readily available starting materials under mild reaction conditions in benign and recyclable solvent. A large number of novel and therapeutically useful molecules are thus readily accessible via this general pathway, and interestingly, these thiazolo-androstenone derivatives could be further derivatized to generate a large library of active compounds. Further derivatization, associated anticancer studies, and mode of action of this class of compounds are currently underway and will be reported in due course.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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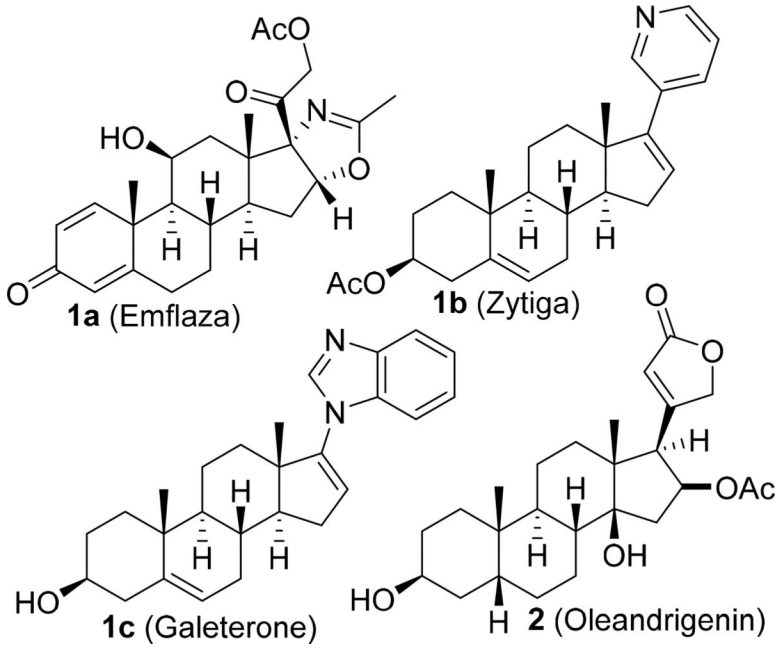


Figure 1.

Representative examples of heterocycle containing steroidal drugs (**1a**, **1b**, and **1c**) and a natural product (**2**).

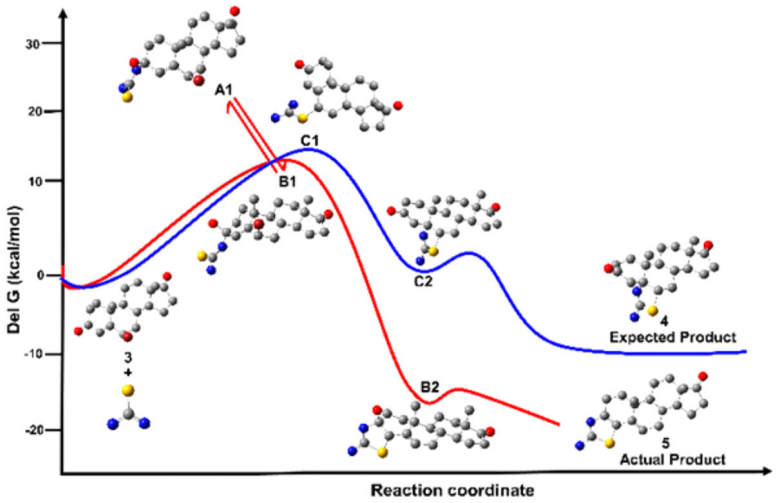


Figure 2.

Probable potential energy surface of formation of actual and expected products calculated using M06–2X/6–311++G(d,p) + PCM.

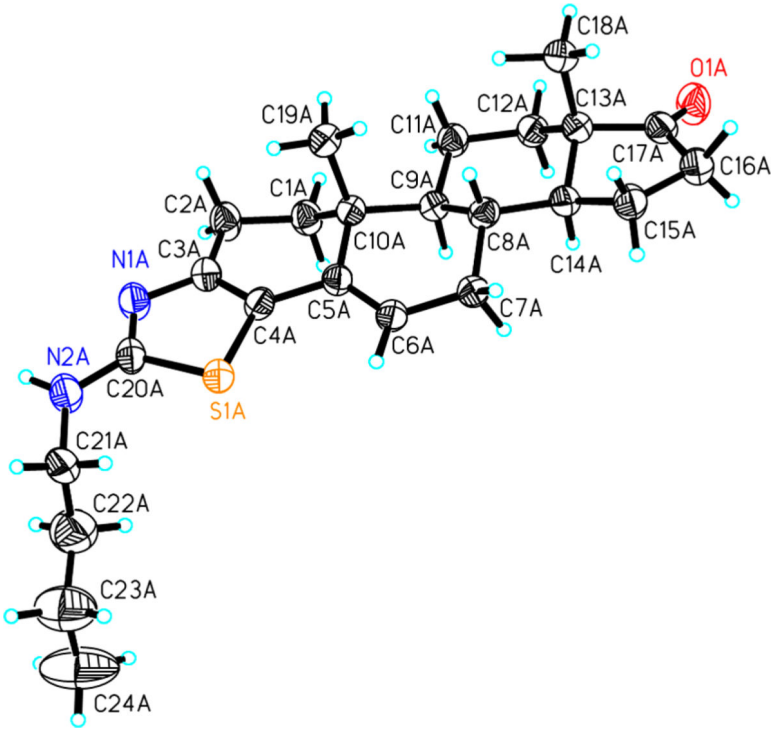
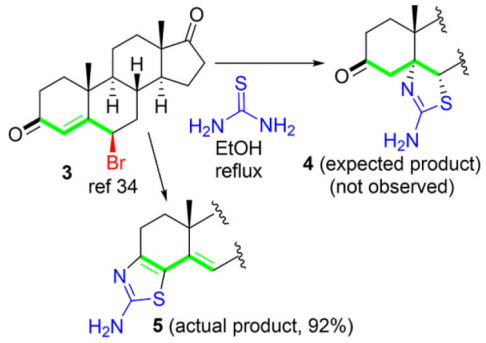
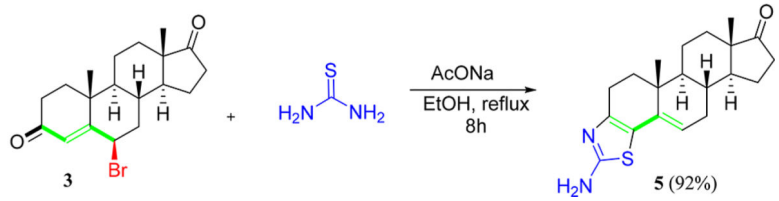


Figure 3.
ORTEP diagram of compounds **7** (CCDC 1858409).

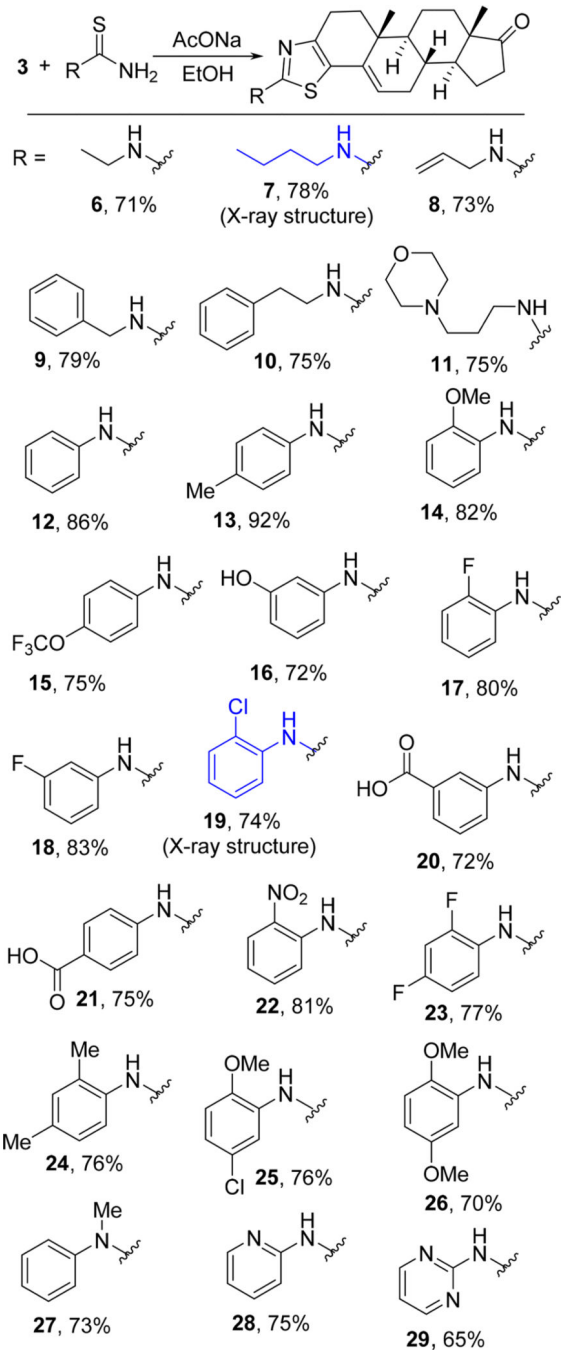


General procedure scheme in the SI



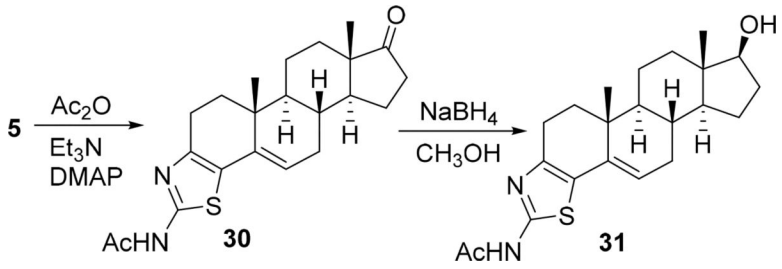
Scheme 1.

Synthesis of Thiazolo-androstenone Derivatives (5)

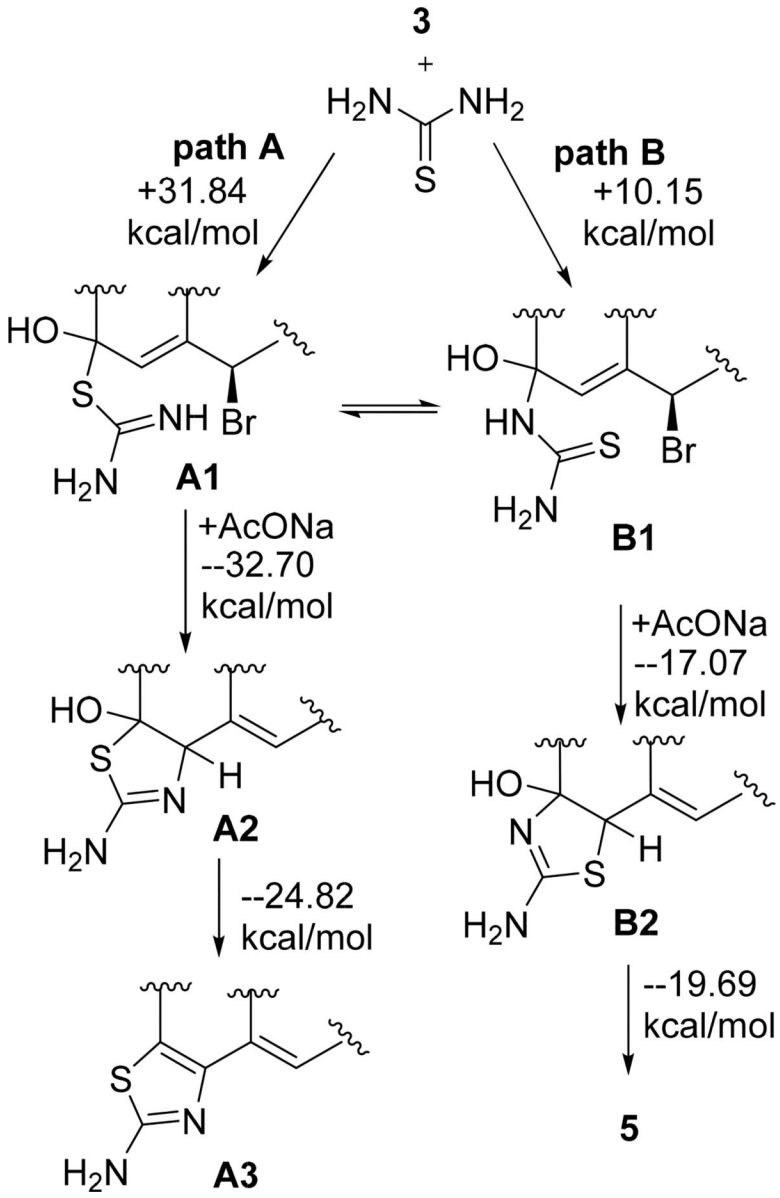


Scheme 2.

Reaction of Various Thiourea Derivatives with 6β-Bromoandrostenedione (3)



Scheme 3.
Derivatization of Heterocycle-Fused Steroidal Molecules



Scheme 4.

Plausible Mechanism for the Formation of Product (5) Using M06-2X/6-311++G(d,p) + PCM (Solvent = EtOH)

Table 1.

NCI Data for Selected Cell Lines for Two Compounds[a]

		GI_{50}	
cancer panel	**cell line**	**17**	**23**
leukemia	CCRF-CEM	1.90	2.45
	K-562	1.86	2.86
	MOLT-4	1.68	2.49
	RPMI-8226	0.86	2.43
	SR	0.85	2.32
NSCLC	HOP-62	2.07	1.42
	NCI-H522	1.63	1.65
colon cancer	HCC-2998	3.55	1.90
	HCT-116	1.59	1.49
	HCT-15	1.58	2.68
	HT29	1.49	2.55
CNS cancer	SF-295	1.19	1.81
	SF-539	1.34	1.62
	SNB-75	1.52	2.19
	U251	1.59	1.69
melanoma	LOX IMVI	1.67	1.64
	MALME-3M	1.94	1.99
	M14	2.36	1.85
	SK-MEL-2	1.86	2.19
	SK-MEL-28	1.52	2.31
	SK-MEL-5	2.02	1.86
	UACC-62	1.54	1.72
ovarian cancer	IGROV1	1.89	2.11
	OVCAR-3	1.72	2.18
renal cancer	786–0	1.43	1.62
	ACHN	1.78	2.08
	CAKI-1	1.99	2.83
	RXF 393	1.45	1.74
	TK-10	1.97	2.46
	UO-31	1.67	1.34
prostate cancer	PC-3	1.88	1.86
	DU-145	1.93	2.04
breast cancer	MCF7	1.37	2.31
	BT-549	1.54	1.49
	MDA-MB-468	2.01	1.93

[a] GI_{50} = concentration of a compound that causes 50% growth inhibition.[39]