Syntheses and Antigestagenic Activity of Mifepristone Derivatives

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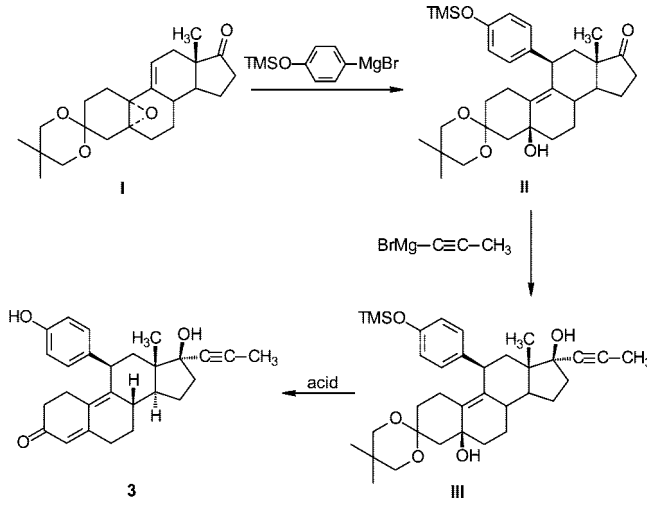
A series of mifepristone derivatives with different "linker groups" in position 4′ of the phenyl ring in the 11β-position of the steroid scaffold (**2**−**41**) have been synthesized. Their antigestagenic activites were determined in a cell-based assay (alkali phosphatase assay in T47-D breast cancer cells) and compared with that of the parent compound mifepristone. SAR and QSAR studies reveal the influence of both lipophilicity and partial charge based van der Waals surface area descriptors on biological activity. Within the series of compounds described in this study, three mifepristone derivatives are identified with considerably high antigestagenic activity. These compounds are regarded as useful starting materials for the synthesis of either physiologically stable or cleavable progesterone receptor-binding conjugates for therapeutic or diagnostic purposes.

Introduction

Worldwide, breast cancer is the most common cancer among women and the number of incidences has significantly increased since 1970, especially in Western Europe and in North America.[1] Mifepristone, or RU 486 (**1**), is a synthetic steroid compound with a *p*-(dimethylamino)phenyl group in 11β-position of the steroid scaffold and acts as a progesterone receptor (PR[a]) antagonist.[2−4] Its binding affinity is more than five times that of progesterone.[5] Therefore, it can be used as abortifacient and has been considered to be effective in treating progesterone-dependent breast cancer.[6,7]

In a preceding study, we have shown that a conjugate containing a mifepristone residue attached via an ω-thiourethano-C6-alkyl linker to a fluorescein residue is selectively accumulated in PR receptor positive T47D breast cancer cells.[8] This work has also demonstrated that such a conjugate is even translocated into the cell nuclei. Thus, the PR might serve for a site-directed delivery of pharmaceutically active agents. In a continuous effort to develop conjugates, which may be useful for a selective enrichment of anticancer agents, e.g., taxodiol or doxorubicin, into these tumor cells, we focused our attention on the nature of the linker group. In particular, the linker group used for attachment of the bioactive molecule to the mifepristone residue should impair the biological activity of **1** as low as possible. In addition, it is assumed that the linker must provide a certain distance between both molecular moieties to retain biological activity of conjugates at least partly. Therefore, linker groups of different chain length bearing additionally different groups at the ω-terminus, e.g., carboxy, ester and amide groups, were synthesized and evaluated in cell culture. Because detachment of the pharmaceutically active agent from the conjugate is required in certain cases, e.g., for the anticancer agents mentioned above, linker groups that will be cleaved in cellular environment are also considered in this study.

Scheme 1. Synthesis of *O*-Analogues of Mifepristone Starting from **I**



Derivatization of **1** at the aromatic region is necessary to retain high PR-binding affinity.[9] Therefore, the position 4′ was chosen for derivatization and attachment of the various linker groups. Mifepristone (**1**) was demethylated to **2** as reported previously,[10] which was the common starting material for compounds **6**−**41**. The different linker groups have been attached to the nitrogen atom of the secondary amino group yielding the variety of compounds summarized in Scheme 3.

The present paper describes the synthesis of a series of mostly novel mifepristone analogues as well as SAR and QSAR studies on their antigestagenic activity.

Chemistry and Structural Assignment

All compounds described in this study have been obtained by partial synthesis[9] either starting from 3,3-dimethoxy-5α,10α-

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[a] Abbreviations: PR, progesterone receptor; AP, alkaline phosphatase.

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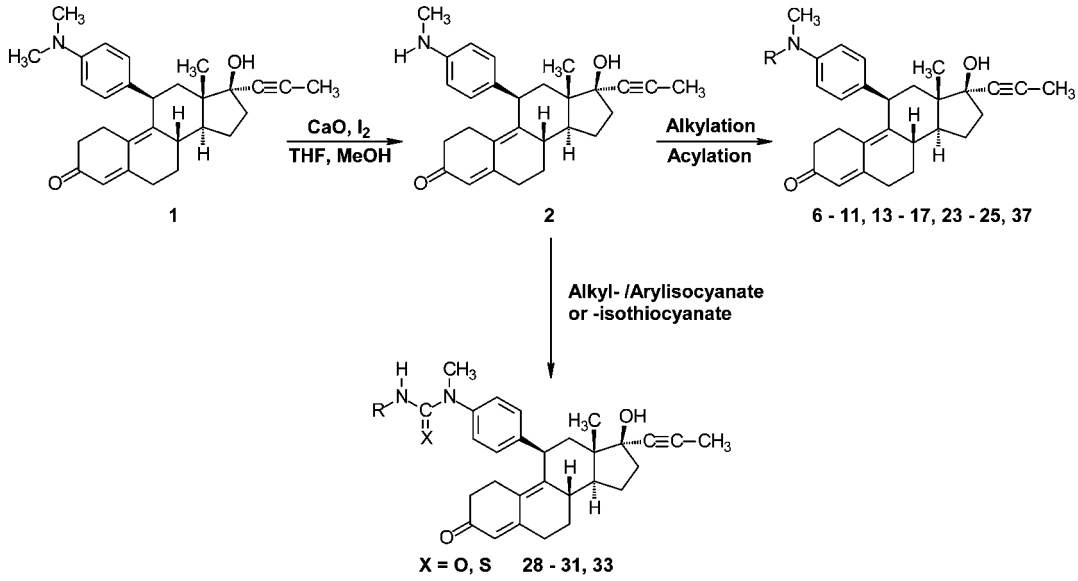
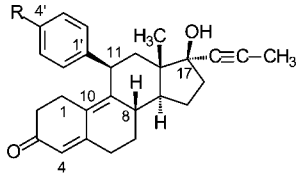
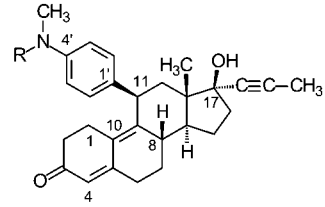
Scheme 2. Synthesis of *N*-Derivatives via Demethylation of Mifepristone



Scheme 3. Structures of compounds **1**−**42**



3: R = -OH
4: R = -OCH_3
5: R = -O-$(CH_2)_9$-Br



1: R = -CH_3
2: R = -H
6: R = -$(CH_2)_2$-CH_3
7: R = -$(CH_2)_5$-CH_3
8: R = -$(CH_2)_8$-CH_3
9: R = -$(CH_2)_2$-CH_2OH
10: R = -$(CH_2)_5$-CH_2OH
11: R = -$(CH_2)_5$-CH(O)O
12: R = -$(CH_2)_5$-CHO
13: R = -$(CH_2)_2$-COOH
14: R = -$(CH_2)_5$-COOH
15: R = -$(CH_2)_7$-COOH
16: R = -$(CH_2)_{10}$-COOH
17: R = -$(CH_2)_2$-$COOCH_3$
18: R = -$(CH_2)_5$-$COOCH_3$
19: R = -$(CH_2)_7$-$COOCH_3$
20: R = -$(CH_2)_{10}$-$COOCH_3$
21: R = -$(CH_2)_5$-$CONH_2$
22: R = -$(CH_2)_5$-$CONHC_4H_9$
23: R = -CO-$(CH_2)_4$-CH_3
24: R = -CO-$(CH_2)_3$-COOH
25: R = -CO-$(CH_2)_4$-COOH
26: R = -CO-$(CH_2)_3$-$COOCH_3$
27: R = -CO-$(CH_2)_4$-$COOCH_3$
28: R = -CO-NH-C_4H_9
29: R = -CS-NH-C_4H_9
30: R = -CO-NH-(4-Cl-C_6H_4)
31: R = -CS-NH-(4-Cl-C_6H_4)
32: R = -CO-NH-$(CH_2)_5$-COOH
33: R = -CO-NH-$(CH_2)_5$-$COOCH_3$
34: R = -CO-NH-$(CH_2)_5$-CO-L-*Leu*-O-*t*-Bu
35: R = -CO-NH-$(CH_2)_5$-CO-L-*Leu*-OH
36: R = -$(CH_2)_2$-CONH-(OH, $CONH_2$)
37: R = -$(CH_2)_3$-O-CH_2-(OH, $CONH_2$)
38: R = -$(CH_2)_2$-CH_2-OCO$(CH_2)_2$COOH
39: R = -$(CH_2)_5$-CH_2-OCO$(CH_2)_2$COOH
40: R = -$(CH_2)_2$-CH_2-OCO$(CH_2)_2$$COOCH_3$
41: R = -$(CH_2)_5$-CH_2-OCO$(CH_2)_2$$COOCH_3$
(**42**: R = -$(CH_2)_3$-OCO-O-$(CH_2)_3$-OH)

epoxyestra-9(11)-en-17-one (**I**) or mifepristone (**1**), as shown in Schemes 1 and 2, respectively.

***O*-Analogues of Mifepristone.** Compound **3** was obtained as described in literature[6−8] and converted to **4** with diazomethane. Alkylation of **3** with 1,9-dibromo nonane yielded compound **5**.

***N*-Derivatives of Mifepristone.** Starting material for all these compounds was **2**, which was prepared as described previously.[8] To introduce an alkyl residue at the nitrogen atom, **2** was alkylated with the 1-bromo alkanes, yielding compounds **6**−**8**. Alkylation with ω-bromo 1-alcohols resulted in compounds **9** and **10** (**42** was obtained as byproduct together with **9**). Reacting **2** with 6-bromohexanal ethylene glycol acetal led to compound **11**, which gave **12** after acid hydrolysis. Introduction of an ω-bromoalkyl carboxylic acid residue gave compounds **13**−**16**. Ester **17** was obtained by reaction of methyl 3-bromopropionate with **2**; conversion of compounds **14**−**16** by diazomethane resulted in the esters **18**−**20**. After activation of acid **14** by isobutyl chloroformate, amides **21** and **22** were obtained by reaction of the intermediate with ammonia or *n*-butylamine, respectively. Acid **13** was activated in the same manner and converted with 4-amino-2-hydroxybenzamide to compound **36**. Reaction of **2** with 4-(3′-bromopropoxymethyl)-2-hydroxybenzamide yielded compound **37**. Acylation of **9** and **10** with succinic acid anhydride formed acid **38** and **39**, respectively, which yielded esters **40** and **41** after treatment with diazomethane. Reaction of **2** with hexanoyl chloride or cyclic acid anhydrides led to compounds **23**−**25**; again, esterification with diazomethane yielded esters **26** and **27**. Reaction of **2** with different ω-iso(thio)cyanates gave the corresponding (thio)urea compounds **28**−**31** and **33**. Saponification of ester **33** resulted in acid **32**, which was activated by isobutyl chloroformate and further reacted with L-leucine *t*-butylester, resulting in ester **34**, which was further hydrolyzed to **35**.

Structural Assignment. Structures of all new compounds were confirmed by IR, MS, and NMR spectroscopic methods. In most cases complete and unambiguous assignments for all ^{1}H and ^{13}C resonances could be achieved on the basis of 1D and 2D experiments.

Antigestagenic Activity

To demonstrate ligand−receptor interaction of the new compounds in cell culture, antigestagenic activity was determined using two independent cell-based assays, and biological

Table 1. Calculated logP Values and Antigestagenic Activity of Compounds **1**−**41**

		alkaline phosphatase assay[a]		transactivation assay[b]	
compd	calcd logP	IC_{50} value[c]	relative potency[d]	IC_{50} value[c]	relative potency[d]
1	4.57	0.045	100	0.021	100
2	4.30	0.17	26	0.073	29
3	4.35	1.3	3		
4	4.61	0.095	47		
5	7.14	1.0	4.5		
6	5.53	0.27	17	(0.025)	(84)
7	6.85	0.66	7	0.18	12
8	8.18	1.4	3	0.38	6
9	4.32	0.12	37	0.105	20
10	5.65	0.06	75	0.071	30
11	6.02	0.17	26	0.073	29
12	5.74	0.15	30		
13	4.17	34	0.13		
14	5.49	0.44	10		
15	6.38	0.27	17		
16	7.70	0.46	10		
17	4.43	0.16	23		
18	5.76	0.40	11		
19	6.64	0.47	10		
20	7.97	0.63	7		
21	4.76	0.55	8		
22	6.51	0.9	5		
23	5.95	0.69	7		
24	4.14	∼ 100	0.04		
25	4.58	78	0.06		
26	4.41	1.55	3		
27	4.85	1.8	2.5		
28	5.19	0.79	6		
29	5.78	0.73	6		
30	6.04	0.27	17		
31	6.63	0.16	28		
32	4.71	72	0.06		
33	4.97	2.2	2		
34	7.19	1.7	2.6		
35	5.71	72	0.06		
36	4.11	0.44	10		
37	5.39	0.46	10		
38	4.46	2.1	2.1		
39	5.79	0.25	18		
40	4.73	1.2	3.8		
41	6.05	0.17	26		

[a] Inhibition of progesterone-induced (10^{-9} mol/L) alkaline phosphatase activity in T45-D cells. [b] Inhibition of progesterone-induced (10^{-9} mol/L) transcriptional activity in T47-D-C124 cells. [c] IC_{50} values were determined from dose−response curves and are given in nmol/L. [d] Relative potency is defined as the ratio of IC_{50} (**1**): IC_{50} (X) multiplied by 100 (X = **2**−**41**).

activity of each test compound was compared with that of the parent compound **1**, mifepristone, as reference antagonist. In all cases, antigestagenic activity was assessed using the alkaline phosphatase assay in T47-D breast cancer cells; in some cases also a transactivation assay in T47-D-C124, i.e., T47-D cells stably transfected with the luciferase gene linked to the steroid hormone responsive MMTV promoter, was applied. Dose-dependent inhibition of progesterone-induced increase of either alkaline phosphatase or luciferase activity was found for most test compounds. IC_{50} values determined from dose−response curves and the relative potencies (rp defined as the ratio of the IC_{50} value of the reference antagonist **1** to the IC_{50} value of the individual test compound) are summarized in Table 1. Assessment of biological activity in a cell-based assay display both receptor binding affinity as well as intracellular availability of the test compound.

SAR and QSAR Studies

Molecular Modeling QSAR Studies. Structures were built within MOE (Chemical Computing Group, Montreal, Canada), and a MOE-database was generated. PEOE charges were calculated, and all compounds were subjected to energetic minimization using the MMFF94x force field (gradient 0.01) implemented in MOE.

Hansch Analyses. For multiple linear regression analyses, a set of descriptors provided by MOE was calculated. These include physicochemical parameters such as logP, polar surface area, hydrophobic van der Waals surface area, molar refractivity, number of H-bond donors and acceptors, and atomic polarizability, as well as connectivity indices and a set of fractional van der Waals surface area descriptors (PEOE_VSA, SLOG-P_VSA, SMR_VSA). For generation of a global model, the data matrix was subjected to PLS analysis using a svl-script provided by the Chemical Computing Group exchange program (auto-QSAR). For local models, regression analyses were performed.

Results and Discussion

A variety of different chemical structures have been attached to the *p*-position of the aromatic ring in 11β-position of the steroid scaffold. In total a series of 41 compounds have been synthesized and tested with respect to their antigestagenic activity in cell culture. Structural variations comprise different substitution patterns on the aniline nitrogen atom as well as exchanging the nitrogen atom by oxygen. Substituents on the nitrogen atom include alkyl and alkanoyl chains of different length bearing additionally different groups at the ω-terminus, e.g., carboxy, ester, and amide groups.

***O*-Analogues of Mifepristone.** Compound **4** exhibited considerably antigestagenic activity. The relative potency was only 2-fold lower than that of **1**. However, if the methoxy group is replaced by the more polar hydroxyl function (**3**), biological activity decreases by one decade. The same is true for compound **5**, which has an alkyl bromide side chain attached to the oxygen atom. Although **5** (calcd logP = 7.14) is more lipophilic than the *N*-derivative **10** (R = 6-hydroxyhexyl, calcd logP = 5.65), its biological activity is about 15-fold lower than that of **10** (see Table 1, Scheme 3). Therefore, we concluded that *O*-analogues of mifepristone with the phenyloxy group at position 11β are less favorable for the development of bioactive conjugates.

***N*-Derivatives of Mifepristone.** Some of these compounds have retained considerable biological activity. Most IC_{50} values are still in the subnanomolar range, and a relative potency between 10% and 25% was calculated for some of the test compounds (Table 1).

Comparison of **6**, **7**, and **8** with *n*-propyl, *n*-hexyl, and *n*-nonyl residues at the nitrogen atom reveals that the relative potency decreases from 17% over 7% to 3% as compared to **1** with increasing chain length. If, however, the alkyl residue bears an ω-hydroxyl group (**9**, **10**) the relative potency is considerable higher, exhibiting relative potencies of 37% and 75%, respectively. In this case, a longer alkyl chain seem to enhance biological activity.

In contrast, a carboxyl group at the ω-terminus of the alkyl chain resulted again in a considerable reduction of antigestagenic activity (e.g.: **13**: rp = 0.13%). In this case, length of the alkyl chain does not obviously affect biological activity as deduced from the comparison of **14**, **15**, and **16**. However, compound **13** exhibits an almost negligible antigestagenic activity for reasons unknown so far. Most likely the polar, and under physiological pH also negatively charged, carboxyl group impairs the transport of the compound through the cell membrane; consistently, esterification of the carboxyl group partly restores biological activity (**17**: rp = 23%). However,

Table 2. Antigestagenic Activity (AP-assay) of Compounds **17**−**20**

compd	side chain	IC_{50} value	relative potency
17	$-(CH_2)_2COOCH_3$	0.16	23
18	$-(CH_2)_5COOCH_3$	0.40	11
19	$-(CH_2)_7COOCH_3$	0.47	10
20	$-(CH_2)_{10}COOCH_3$	0.63	7

for the compounds with longer alkyl chains esterification does not affect antigestagenic activity (**18**−**20** as compared to **14**−**16**, Table 2).

Because the aniline derivative **7** and the anilide **23** exhibit the same biological activity, electron density at the nitrogen atom seem to be not important for ligand receptor interaction. Therefore, it is concluded that no hydrogen bonding is involved in the N-region of mifepristone analogues, which is in agreement with the present knowledge on the interaction of mifepristone with steroid hormone receptors at the structural level.[11,12] This conclusion is strengthened by comparing biological activities of the urea and thiourea derivatives (**28** vs **29** and **30** vs **31**). If, however, the anilide **23** is additionally combined with a carboxyl group at the ω-terminus of the side chain (**25**), antigestagenic activity is vanished (**25**, rp = 0.06%). Again, esterification result in a significant increase in biological activity (**27**, rp = 2.5%), which is probably due to a higher intracellular availability of the less polar compound; however, its antigestagenic activity is considerably lower than that of the corresponding N-alkyl derivative **18** (rp = 11%). Comparing mifepristone derivatives bearing a C6-alkyl chain with different oxygen functions at the ω-terminus shows that the alcohol has the highest relative potency (**10**, rp = 75%). An acetal (**11**) aldehyde (**12**), or carboxyl group (**14**) decreases the antigestagenic activity of the compounds; esterification (**18**) does not have much influence (see above). Converting the carboxyl function to an amide group (**21** and **22**) further reduces biological activity (Table 3).

Alkyl amides show only moderate antigestagenic activity (**21**, rp = 8% and **22**, rp = 5%), whereas the aromatic amide **36** exhibit a somewhat higher relative potency of 10%. Comparable results are found in the case of the derivatives with an urea or thiourea bond at the nitrogen atom of the mifepristone residue (**28**, R = *n*-butyl, rp = 6% vs **30**, R = *p*-chlorophenyl, rp = 17% and **29** (rp = 6%) vs **31** (rp = 28%). However, although compounds **30** and **31** have considerable high biological activity, they offer only limited possibilities to attach linker groups. If an urea bond at the aromatic nitrogen atom is additionally combined with a carboxyl group at the ω-terminus of the side chain (**32**), antigestagenic activity is vanished (rp = 0.06%), which is again partly restored after esterification (**33**, rp = 2%).

Table 3. Relative Potencies of Compounds **10**, **11**, **12**, **14**, **18**, **21**, and **21**

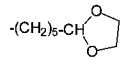
compound	side chain	IC_{50} value	relative potency
10	$-(CH_2)_5CH_2OH$	0.06	75
12	$-(CH_2)_5COH$	0.15	30
11		0.17	26
14	$-(CH_2)_5COOH$	0.44	10
18	$-(CH_2)_5COOCH_3$	0.40	11
21	$-(CH_2)_5CONH_2$	0.55	8
22	$-(CH_2)_5CONHC_4H_9$	0.90	5

Table 4. Relative Potencies of Compounds **38**, **39**, **40**, and **41**

compd	side chain	IC_{50} value	relative potency
38	$-(CH_2)_3OCO(CH_2)_2COOH$	2.10	2.1
39	$-(CH_2)_6OCO(CH_2)_2COOH$	0.25	18
40	$-(CH_2)_3OCO(CH_2)_2COOCH_3$	1.20	3.8
41	$-(CH_2)_6OCO(CH_2)_2COOCH_3$	0.17	26

Compound **35** bearing an enzymatic cleavable linker group exhibit almost negligible antigestagenic activity (rp = 0.06%). Even after esterification (**34**), biological activity remains quite moderate (rp = 2.6%). However, comparison of **34** vs **33** and **35** vs **32** reveal that attachment of the amino acid L-leucine at the ω-terminus does not significantly affect biological activity. Compound **39** with a hydrolytic cleavable linker group show considerable antigestagenic activity (rp = 18%), which is again increased after esterification (**41**, rp = 26%). In contrast, biological activities of **38** and **40** are 6−8-fold lower as compared to **39** and **41**, respectively. This indicates that in the case of a hydrolytic cleavable linker group chain length might be an important determinant for biological activity (Table 4).

QSAR Studies and Regression Analysis. The activity pattern of alkyl derivatives **6**−**8** indicate that a given logP value is needed to achieve sufficient intracellular concentrations. The general influence of lipophilicity within this series of compounds is further strengthened by a regression analysis using alkyl derivatives **6**−**8**, alcohols **9** and **10**, the acetal **11**, the aldehyde **12**, and esters **17**−**20** (r^2 = 0.64; Figure 1).

Interestingly, a negative correlation is observed. This indicates that mifepristone itself might exhibit already an optimal logP value. However, it should be stressed that increasing lipophilicity is likely to result in (nonspecific) binding of test compound to cellular membranes competing with the specific binding to PR. Compound **10** (R = 6-hydroxyhexyl) is a positive outlier showing higher biological activity than expected from its logP value. This might be due to an additional interaction mediated by the OH group. This is further strengthened by including either the topological polar surface area (TPSA) or the atom polarizability (apol) further enhancing the regression coefficient (r^2 = 0.68, Q^2 = 0.56), whereby logP shows a negative contribution and apol/TPSA exhibit positive coefficients in the equation. However, adding amides **22** and **23** to the data set dramatically decreases the predictive power of the model (r^2 = 0.45). Also within the series of anilides **23**, **26**, **27**, and urea/thiourea derivatives **28**, **29**, **30**, **31**, and **33**, a good correlation between logP(o/w) and the log(1/IC_{50}) value is observed (r^2 = 0.81). In

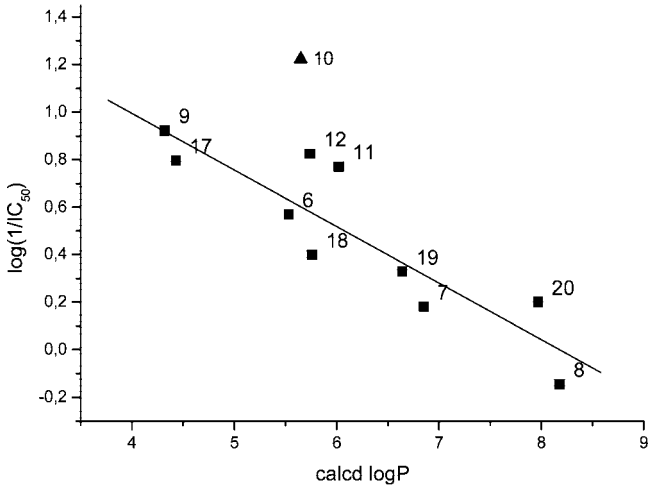


Figure 1. Plot of log potency vs calculated logP values for alkyl derivatives **6**−**8**, alcohols **9** and **10**, the acetal **11**, the aldehyde **12**, and esters **17**−**20** ($r^2 = 0.64$); excluding compound **10** increases the r^2 value to 0.79.

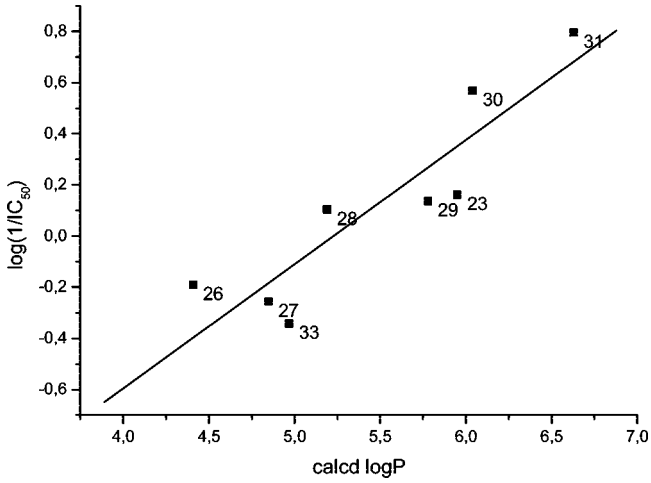


Figure 2. Plot of log potency vs calculated logP values for anilides **23**, **26**, and **27**, and ureas/thioureas **28**−**31**, and **33** ($r^2 = 0.81$).

this case, the coefficient of logP shows a positive value, indicating that increasing the value enhances biological activity (Figure 2).

These local differences in the influence of lipophilicity might be the reason why attempts to create a global model with reasonable predictivity just on basis of a set of simple ADME-type descriptors (logP, TPSA, number of H-bond donors and acceptors, molar refractivity, number of rotable bonds) failed. The best model obtained showed an r^2 value of 0.50 and a cross-validated r^2 value of 0.34 (leave one out). Therefore we used an svl-script (autoQSAR), which automatically selects out of a large set of descriptors the most important ones. This led to a final equation that shows reasonable predictivity ($r^2 = 0.73$, $r^2_{cv} = 0.62$) comprising a final set of 10 descriptors (8 components). The plot of observed vs predicted (leave one out) log(1/IC_{50}) values showed only the propanoic acid derivative **13** as a clear outlier (Figure 3). Important descriptors present in the final equation include, among others, PEOE-VSA descriptors, SlogP_VSA descriptors, and van der Waals area and volume. A full list with a short comment on the nature of the descriptors is given in Table 5. As this model covers already a relatively broad chemical space, it might serve as a useful tool for in silico

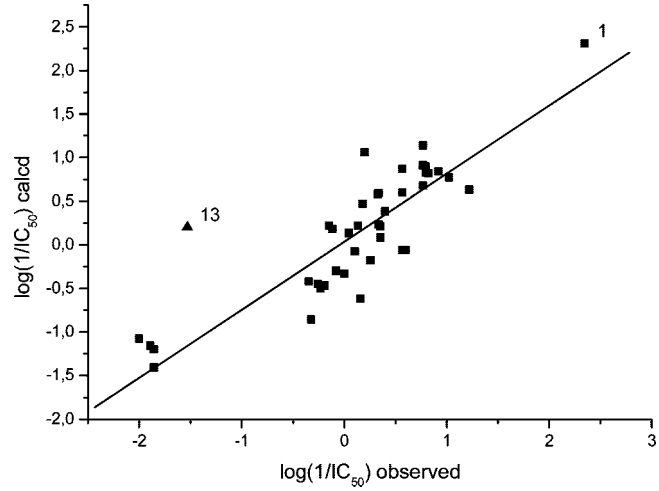


Figure 3. Plot of observed vs calculated log(1/IC_{50}) values for compounds **1**−**41** ($r^2 = 0.73$, $r^2_{cv} = 0.62$); excluding compound **13** increases the r^2 value to 0.78.

Table 5. Descriptors Present in the Final Equation

PEOE_VSA+0	sum of accessible van der Waals surface area (in Å^2) for all atoms with a partial charge q_i in the range of [0.00, 0.05)
PEOE_VSA-5	sum of accessible van der Waals surface area (in Å^2) for all atoms with a partial charge q_i in the range of [−0.30, −0.25)
Q_VSA_POS	total positive van der Waals surface area; this is the sum of the accessible van der Waals surface area of all atoms with positive partial charge.
pmiX	x component of the principal moment of inertia (external coordinates).
SlogP_VSA7	sum of accessible van der Waals surface area (in Å^2) for all atoms with an logP(o/w) contribution between (0.25, 0.30]
SlogP_VSA9	sum of accessible van der Waals surface area (in Å^2) for all atoms with an logP(o/w) contribution >0.40
SMR_VSA7	sum of accessible van der Waals surface area (in Å^2) for all atoms with an SMR value >0.56
ASA	water accessible surface area calculated using a radius of 1.4 Å for the water molecule; a polyhedral representation is used for each atom in calculating the surface area
vdw_area	van der Waals volume (Å^3) calculated using a connection table approximation
vdw_vol	area of van der Waals surface (Å^2) calculated using a connection table approximation

screening of small virtual libraries for further improvement of the linker group.

Conclusions

Within this paper we describe the synthesis, biological activity, and structure−activity relationships of a series of mifepriston analogues bearing different linker groups at the nitrogen atom of the phenyl ring in position 11β of the steroid scaffold. SAR and QSAR studies reveal the influence of both lipophilicity and partial charge based van der Waals surface area descriptors on biological activity. Some of the new compounds retain considerable antigestagenic activity (with IC_{50} values in the subnanomolar range and relative potencies between 26% and 75% as compared to the parent compound mifepristone), indicating high receptor binding affinity as well as sufficient intracellular availability. Within the series of compounds described in this study, **9** (R = 3-hydroxypropyl) and **10** (R = 6-hydroxyhexyl) are regarded as good starting materials for the synthesis of stable conjugates (without degradation within cellular environment), which will accumulate in PR-positive

tumor cells. A promising starting material for the synthesis of cleavable conjugates seem to be compound **39**, which is obtained by reacting **10** with succinic anhydride. Besides intracellular accumulation, cleavable conjugates enables additionally the detachment of the pharmacologically active agent, which seem to be essential in some cases, e.g., for anticancer agents like doxorubicin[13] or taxol.[14] Thus, it is concluded that compounds **9**, **10**, and **39** are promising and versatile starting materials for the development of stable or cleavable conjugates for diagnostic or therapeutic purposes in PR expressing tumors, i.e., breast, ovarian, and endometrial cancer. Specific targeting of PR-positive cancer cells may contribute to further improve classification, diagnosis, stratification, and treatment of cancer patients.

Experimental Section

General. All reagents and solvents for syntheses were purchased from Sigma-Aldrich, Fluka, or Merck and used without further purification. Reagent-grade solvents were purified and dried using standard methods. Solvents of analytical and spectroscopic grade as well as deuterated NMR solvents were purchased from Merck and Chemische Fabrik Uetikon, respectively. NMR spectra were recorded on a Varian Unity Inova 400/600 NMR spectrometer equipped with a tuneable broadband probe. Purity was determined by elemental analyses (Mikroanalytisches Laboratorium of the Institute of Physical Chemistry, University of Vienna, Austria) and/or HPLC; purity of key target compounds was ≥95% except otherwise noted.

***N*-Alkylation of 2 with 1-Bromo Alkanes and *ω*-Bromo-1-alkanols: General Procedure I.** Compound **2** (100 mg, 0.24 mmol) and 3-fold excess of freshly distilled 1-bromo alkane (0.72 mmol) or *ω*-bromo-1-alkanol (0.72 mmol) were dissolved in anhydrous DMF (2.0 mL). The mixture was stirred in the presence of dry K_2CO_3 (33.3 mg, 0.24 mmol) and in the absence of moisture for 23 h at 70 °C. The solvent was removed in vacuo and the residue purified by flash-chromatography (EtOAc:cyclohexane, (3:2, vol/vol)) and by preparative HPLC. After lyophilization, colorless or light-yellow solid compounds **6**−**10** were obtained in 20−55% yield.

17*β*-Hydroxy-11*β*-[4-(6-hydroxy-*N*-methylhexylamino)-phenyl]-17α-(1-propinyl)-estra-4,9-dien-3-one (10). Compound **10** was synthesized according to general procedure I reacting **2** (100 mg, 0.24 mmol) with 6-bromo-1-hexanol (0.72 mmol); the product was purified by flash-chromatography (EtOAc: cyclohexane, 3:2, vol/vol)) and preparative HPLC (YMC-Pack ODS-A (C18); acetonitrile: H_2O, 2:3, vol/vol). Yield: 54%. Anal. ($C_{34}H_{45}NO_3$) calcd C 79.18, H 8.79, N 2.72; found C 78.51, H 8.80, N 3.25.

***N*-Alkylation of 2 with *ω*-Bromo Carboxylic Acids: General Procedure II.**[15] Compound **2** (150 mg, 0.361 mmol) was dissolved in 3.6 mL of ethanol and added to a solution of 1.80 mmol *ω*-bromo carboxylic acid (dissolved in 3.6 mL water and adjusted to pH 9.25 with 4.3 mmol $NaHCO_3$). The mixture was stirred for 20 h at 70 °C. Thereafter, the pH of the reaction mixture was adjusted to 6.1 by adding 0.7 mL of HCl (2 mol/L) in 10 mL ethanol. The solvent was removed in vacuo, the residue suspended in a small amount of water, extracted with EtOAc, and washed with brine. The organic layer was dried over Na_2SO_4. The solvent was removed in vacuo, and the residue purified by flash chromatography with EtOAc and MeOH and by preparative HPLC. After lyophilization, yellow solid compounds **13**−**16** were obtained in 30−55% yield.

17*β*-Hydroxy-11*β*-[4-(5-carboxy-*N*-methylpentylamino)-phenyl]-17α-(1-propinyl)-estra-4,9-dien-3-one (14). Compound **14** was synthesized according to general procedure II reacting **2** (150 mg, 0.361 mmol) with 6-bromohexanoic acid (1.80 mmol); the product was purified by flash-chromatography with EtOAc and MeOH and preparative HPLC (Prontosil 120−5-C 18H; acetonitrile: H_2O (56:44, vol/vol). Yield: 54%. Anal. ($C_{34}H_{43}NO_4$) calcd C 77.09, H 8.18, N 2.64; found (+ 1/4 H_2O) C 76.40, H 8.22, N 2.63.

General Procedure III. After dissolving 20 mg of **14**−**16**, **24**, or **25** (0.038−0.034 mmol) in 2 mL of THF, a 10-fold excess of diazomethane in 2 mL of diethyl ether was added and the mixture stirred for 1 h at room temperature. Solvent and excess of diazomethane solution were removed in vacuo and the residue dissolved in acetonitrile/water and lyophilized. Compounds **18**−**20**, **26**, and **27** were obtained in about 80% yield.

General Procedure IV. Compound **2** (116 mg, 0.26 mmol) and an excess of the iso(thio)cyanate were dissolved in 3 mL of dry DMF and stirred for 3 h at 70 °C for. The mixture was poured into 3 mL of ice water; pH of the mixture was adjusted to 2 with 2 mol/L HCl. After extraction with diethyl ether, the organic layer was washed with water and dried over Na_2SO_4. The solvent was removed in vacuo and the residue purified by preparative HPLC. After lyophilization, colorless solid compounds **28**−**31** and **33** were obtained in typically 33% yield.

17*β*-Hydroxy-11*β*-{4-[1,14,14-trimethyl-11-(2-methylpropyl)-2,9,12-trioxo-1,3,10-triaza-13-oxa-pentadecyl]-phenyl}-17α-(1-propinyl)-estra-4,9-dien-3-one (34). Compound **32** (115 mg, 0.2 mmol) and an equimolar amount of triethylamine (20.2 mg, 0.2 mmol) were dissolved in 3 mL of THF under argon at 0 °C. Isobutyl chloroformate (27.3 mg, 0.2 mmol) was added, and the mixture was stirred for 40 min at 0 °C. Subsequently, a solution of leucine-*t*-butyrate in 0.2 mL THF [the free base was extracted from leucine-*t*-butyrate·HCl (37 mg, 0.2 mmol) with NaOH (2 mol/L)] was added and the mixture stirred for additional 1.5 h. The product was purified by flash chromatography with EtOAc and by preparative HPLC (Prontosil 120−5-C 18H; acetonitrile: H_2O (67:33, vol/vol). Yield: 56%. Purity HPLC 100%, t_R = 4.105 min.

17*β*-Hydroxy-11*β*-[4-(12-hydroxy-1-methyl-9,12-dioxo-1-aza-8-oxa-dodecyl)-phenyl]-17α-(1-propinyl)-estra-4,9-dien-3-one (39). Compound **10** (51.6 mg, 0.1 mmol), succinic anhydride (10 mg, 0.1 mmol), 4-(dimethylamino)pyridine16 (7.3 mg, 0.06 mmol), and an excess of triethylamine (20.2 mg, 0.2 mmol) were dissolved in 4 mL of CH_2Cl_2 and refluxed for 5 h. The solvent was removed in vacuo, the residue suspended in EtOAc, and the reaction mixture adjusted to pH = 4.0 by adding HCl (2 mol/L) in MeOH; the residue was purified by flash chromatography with EtOAc as eluent. Yield: 45%. Purity HPLC 95.4%, t_R = 5.087 min.

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Supporting Information Available: Preparation, purification, spectroscopic (^{1}H NMR, ^{13}C NMR, IR, and UV/vis), and chromatographic data (TLC, HPLC) of compounds **3**−**42** as well as a description of the biological assays. This material is available free of charge via the Internet at http://pubs.acs.org.

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