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Design, synthesis, and structure–activity relationship studies of new phenolic DNA gyrase inhibitors

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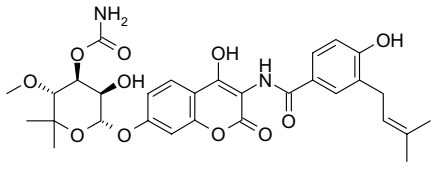
Abstract—Starting from a biased needle screening hit **3a**, we report herein the design and synthesis of a series of novel 2,3-dihydroisoindol-1-ones structurally related to cyclothialidine **2** with DNA gyrase inhibitory activity. In this series, some compounds exhibited promising antibacterial activity against Gram-positive bacterial strains.

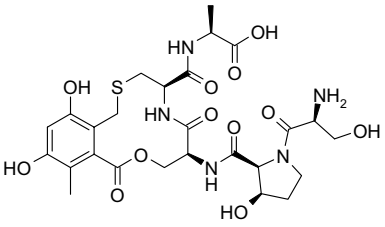
In recent years emergence and spread of resistance have caused serious problems in the effective treatment of infectious diseases.[1] Therefore there is a need for new and effective antibiotics which overcome resistance.

DNA topoisomerases are attractive targets for antibiotic research. They control the topological state of DNA in cells. They are involved in both DNA transcription and replication and are therefore essential for cell viability.[2] Like eucaryotic topoisomerases II, bacterial DNA gyrase can relax positive supercoiled DNA, but it is unique in its ability to introduce negative supercoils into DNA, processes which consume ATP. The active DNA gyrase complex consists of a tetramer A2B2 of the subunits A and B, each of about 90 kDa. Quinolones, which are successfully used as broad-spectrum antibiotics in the clinic, target DNA gyrase A by interfering with DNA cleavage and religation reactions. Subunit B binds ATP. The coumarin-containing antibiotics such as novobiocin **1**, clorobiocin, and coumermycin, known for more than 40 years, are competitive inhibitors of ATP, and demonstrated in vivo efficacy against several bacterial pathogens thus proving DNA gyrase B as a valid antibacterial target. However, the coumarins suffer from several disadvantages[3] such as toxicity and fast emergence of resistance.

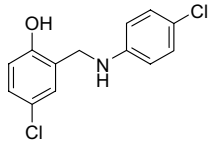
During a biased needle screening the phenol **3a**,[4] which is structurally similar to cyclothialidine **2**, a well-known inhibitor of DNA Gyrase B,[5] was identified as a weak inhibitor of DNA gyrase in the supercoiling assay (maximal non-effective concentration, MNEC = 5 μg/ml).



Novobiocin 1



Cyclothialidine 2



3a

Our hypothesis suggested the phenol group in inhibitor **3a** is probably essential for enzyme inhibition as it is the case in the natural product **2**. The phenol **3a** is an attractive starting point, since this class of compounds is readily available through reductive amination of the corresponding salicylic aldehyde with the appropriate amine (61–89% yield). It exhibits potential for further modifications and could probably be optimized based on X-ray structures of gyrase B[6] complexed with the structurally related cyclothialidine **2** or with novobiocin

Keywords: DNA Gyrase inhibitors; Antibacterial activity; Cyclothialidine; Novobiocin.
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1 and on our knowledge of the structure–activity relationship of cyclothialidines.[7]

The compounds prepared[8] were tested in the supercoiling assay.[9] The effect was measured as maximum non-effective concentration (MNEC), defined as the highest inhibitor concentration which showed no DNA gyrase inhibition.

The activity against DNA gyrase of the screening hit **3a** could not be improved substantially (Table 1). Introduction of hydroxyl-substituents (compounds **3h**, **3i**) like in cyclothialidine **2** reduced activity. Our binding model suggested these hydroxy groups are located in a lipophilic environment in the enzyme and form H-bonds to free water molecules which do not provide much additional binding energy.

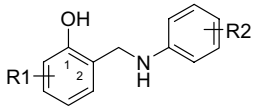
From the SAR around cyclothialidine **2** we know that the introduction of a methoxy and a carboxylic ester group in the meta positions and a lipophilic group (like methyl) in para position to the phenol improves activity. Therefore we coupled the salicylic aldehyde **4a** with various anilines to yield the acyclic benzylic amines **5** and the 2,3-dihydroisoindol-1-ones **6** (Scheme 1).

To our disappointment compounds **5** did not show any improved activity against DNA gyrase (Table 2) over compound class **3**.

On the other hand, the 1,2-dihydroisoindol-1-ones **6** showed promising activity in the supercoiling assay (Table 3). We introduced residues on the aniline moiety, which could interact by H-bonds (**6c–6f**), by π–π-stacking or by charge transfer interactions (**6g–6j**) with the enzyme especially with arginine 144, which interacts with the cysteine ester residue in cyclothialidine **2**, respectively, with the coumarin residue in novobiocin **1**. This strategy proved to be successful especially when using basic aromatic residues like pyridine (**6g**) and quinolines (**6i,j**).

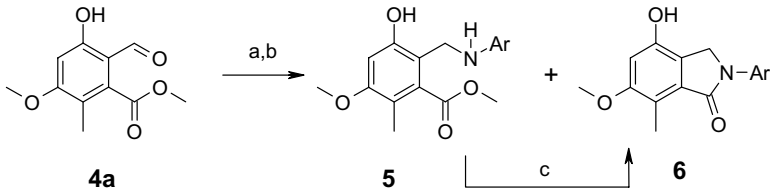
Superpositioning of lactam **6** onto cyclothialidine **2** in the X-ray structure of its complex with DNA gyrase B

Table 1. *Escherichia coli* DNA gyrase supercoiling activity for compounds **3**

Structure	Compound	R[1a]	R[2a]	MNEC[b] (μg/ml)
	**3a**	4-Cl	4-Cl	5
	3b	4-Cl	3-Cl	8
	3c	4-Cl	3,5-Cl_2	4
	3d	4-Cl	4-NC	2
	3e	4-Cl	4-NH_2SO_2	63
	3f	4,6-Cl_2	4-Cl	16
	3g	4-Br	4-Cl	16
	3h	5-OH	4-Cl	16
	3i	6-OH	4-Cl	16

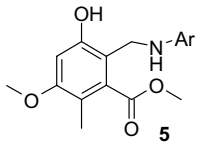
3

[a] The numbering refers to the position of the substituent on the corresponding aromatic ring.
[b] MNEC, maximal non-effective concentration.



Scheme 1. Reagents and conditions: (a) $ArNH_2$, MeOH, rt, 12 h; (b) $NaBH_4$, MeOH, rt, 1 h, 22–63% over two steps; (c) NaOMe, MeOH, reflux, 3 h, 5–81% over three steps.

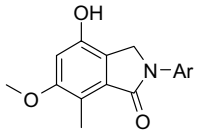
Table 2. *Escherichia coli* DNA gyrase supercoiling activity for compounds **5**

Structure	Compound	Ar	MNEC[a] (μg/ml)
	**5a**	4-Cl–Ph	16
	5b	3-Cl–Ph	4
	5c	3,4-Cl_2–Ph	2
	5d	3,5-Cl_2–Ph	4
	5e	Quinolin-6-yl	8

5

[a] MNEC, maximal non-effective concentration.

Table 3. *Escherichia coli* DNA gyrase supercoiling activity for compounds **6**

Structure	Compound	Ar	MNEC[a] (μg/ml)
 **6**	**6a**	3,4-Cl_2–Ph	4
	6b	4-MeO–Ph	8
	6c	3-HO_2C–Ph	8
	6d	3-(HO_2CCH_2)–Ph	2
	6e	3-NH_2–Ph	2
	6f	4-NH_2SO_2–Ph	1
	6g	Pyrid-3-yl	2
	6h	1-Indazol-5-yl	1
	6i	Quinolin-6-yl	1
	6j	Quinolin-3-yl	0.5

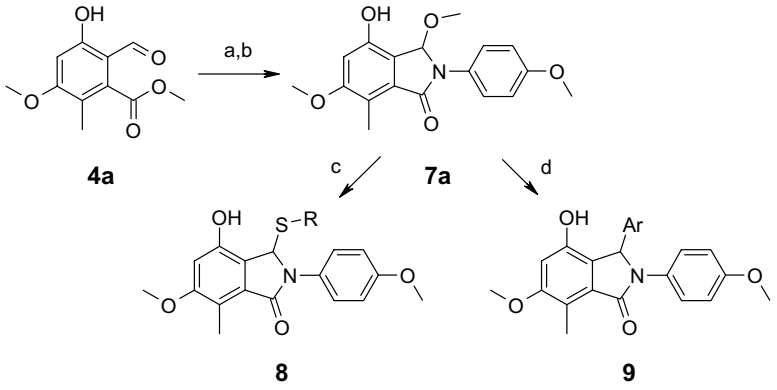
[a] MNEC, maximal non-effective concentration.

suggested to place a substituent on the unsubstituted position next to the nitrogen in the 1,2-dihydroisoindol-1-one moiety in order to improve lipophilic interaction with the enzyme. Condensation of aldehyde **4a** with anilines under basic condition and subsequent electrophilic additions of the formed aminal **7a** to thiols or electron-rich arenes yielded the derivatives **8** and **9** (Scheme 2).

We were hoping that the sulfur atom in compounds **8** would improve the inhibitory potency against DNA gyrase, since the sulfur atom is essential for activity in cyclothialidine **2**. This failed for the derivatives **8a** to **8d** probably because the introduced substituents are too large (Table 4). Nevertheless we could demonstrate with derivative **8e** that a small residue with an appropriate acceptor (e.g., ester group), which can putatively interact with the enzyme in the region of arginine 144, is beneficial for activity.

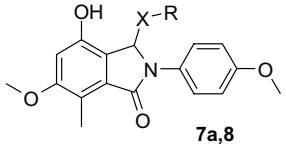
Next we introduced aromatic residues at the 3-position of the 2,3-dihydroisoindol-1-ones **6**. Unfortunately only electron-rich aromatic derivatives **9** (furans and indols) could be prepared by electrophilic addition of **7a** to arenes. Compounds **9** were almost inactive against DNA gyrase in the supercoiling assay (Table 5). The aromatic substituent is probably too large and interferes with enzyme-binding.

Therefore we introduced smaller substituents. Reacting aminal **7a** with trimethyl allyl silane yielded the allyl derivative **10** and with acetone under acidic conditions gave the ketone **11a**. Interestingly with chloroacetone



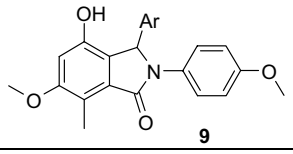
Scheme 2. Reagents and conditions: (a) 4-MeO–$PhNH_2$, MeOH, rt, 4 h, 83%; (b) NaOMe, MeOH, reflux, 3 h, 94%; (c) RSH, TFA, CH_2Cl_2, rt, 0.5 h, 24–100%; (d) ArH, TFA, CH_2Cl_2, rt, 1 h, 32–92%.

Table 4. *Escherichia coli* DNA gyrase supercoiling activity for compounds **7** and **8**

Structure	Compound	X	R	MNEC[a] (μg/ml)
 **7a,8**	**7a**	O	Me	16
	8a	S	Bu	16
	8b	S	4-Tol	31
	8c	S	Pyrimid-2-yl	4
	8d	S	Imidazo-2-yl	4
	8e	S	$MeO_2C(CH_2)_2$	0.5

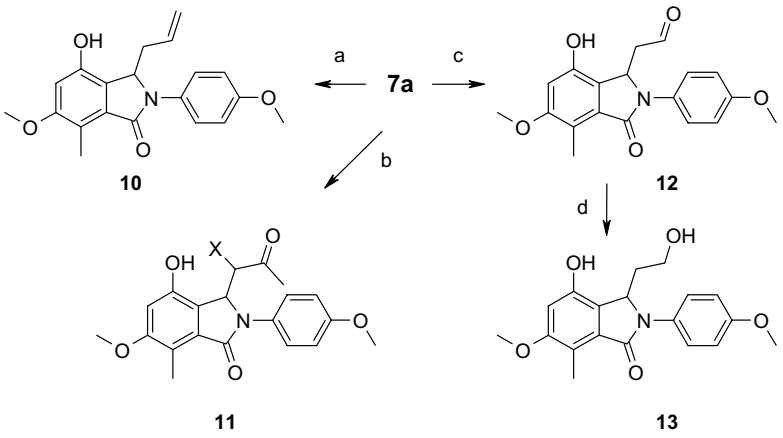
[a] MNEC, maximal non-effective concentration.

Table 5. *Escherichia coli* DNA gyrase supercoiling activity for compounds **9**

Structure	Compound	Ar	MNEC[a] (μg/ml)
	**9a**	Furan-2-yl	>63
	9b	5-Me-furan-2-yl	>63
	9c	5-($HSCH_2$)-furan-2-yl	>63
	9d	5-($NCCH_2$)-thien-2-yl	36
	9e	3-Me-indol-2-yl	>63

9

[a] MNEC, maximal non-effective concentration.

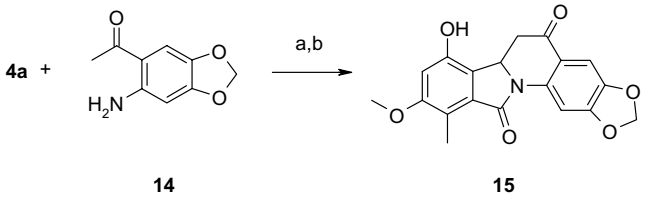


Scheme 3. Reagents and conditions: (a) TMSallyl, TFA, CH_2Cl_2, rt, 1 h, 30%; (b) 6 equiv XCH_2COMe (X = Cl, H), TFA, CH_2Cl_2, rt, 1 h, 51–99%; (c) MeCHO, TFA, THF, rt, 12 h, 91%; (d) $NaBH_4$, MeOH, THF, rt, 0.5 h, 97%.

the reaction proceeded regioselectively to the sterically more hindered product **11b** (Scheme 3).

The small aliphatic allyl residue did not improve the inhibitory activity (compound **10**, Table 6). A keto group even diminishes the activity (derivatives **11a**,**b**) and a small hydroxyl-ethyl substituent (**13**) did not result in an improved inhibition of DNA gyrase.

All previous results suggests that a substituent at the 3-position of the 2,3-dihydroisoindol-1-ones **6** is beneficial provided it is small. Therefore, we cyclized this position onto the ortho-position of the aniline. Condensation of the aldehyde **4a** with amino ketone **14** yielded the tetracyclic ketone **15** (Scheme 4). The MNEC value of 1 μg/ml is promising (Table 7) since the acyclic keto-derivatives **11a**,**b** were almost inactive (Table 6).



14 **15**

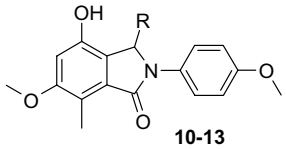
Scheme 4. Reagents and conditions: (a) MeOH, rt, 12 h; (b) 2.7 equiv NaOMe, MeOH, reflux, 12 h, 44% over two steps.

Encouraged by this finding we synthesized the unsubstituted C2-linked tetracyclic compounds. Electrophilic addition of the aminal **7** to acetaldehyde in the non-complexing solvent methylene chloride under acidic conditions (TFA) yielded the tetracyclic compound **16** as mainly one diastereomer (Scheme 5), whereas with THF as the cosolvent no cyclization to the tetracyclic compound takes place (compare Scheme 3). Subsequent reduction yielded the desired derivative **18**.

Indeed, compounds **17** and **18** showed slightly improved inhibition of DNA gyrase in comparison to the ketone **15** (Table 7). This finding encouraged us to introduce pyridine or quinoline rings into the tetracyclic structure, since these aromatic residues proved to be beneficial for the inhibitory activity against gyrase in the 2,3-dihydroisoindol-2-ones **6** (Table 3). A straightforwarded way to build such tetracycles is to condense an ortho-amino-hydroxymethyl-arene **21** with aminal **4** under basic conditions to yield the tetracyclic aminales **22** (Scheme 6). The required quinoline derivative **21a** was conveniently prepared in 3 steps from the pyridinium salt **19** and the ortho-amino-benzaldehyde **20**.[10]

Introduction of an oxymethyl-bridge did not reduce the inhibitory activity. Derivatives **22a**,**b** are as active as the carbon-linked derivatives **18**. A methyl-substituent on the aniline residue improved the activity further. Compounds **22c**,**d**,**e** are equally as potent against

Table 6. *Escherichia coli* DNA gyrase supercoiling activity for compounds **10–13**

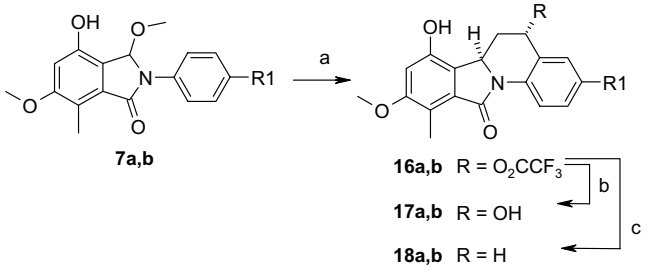
Structure	Compound	R	MNEC[a] (μg/ml)
 **10-13**	**10**	Allyl	2
	11a	2-Oxo-propanyl	>63
	11b	1-Chloro-2-oxopropanyl	63
	12	$OHCCH_2$	31
	13	$HOCH_2CH_2$	4

[a] MNEC, maximal non-effective concentration.

Table 7. In vitro *Escherichia coli* DNA Gyrase supercoiling assay activity (MNEC) and antibacterial activity (MIC)

Compound	R	X	R^1	MNEC[a] (μg/ml)	MIC[b] (μg/ml)							
					S. aureus ATCC 25923	*S. aureus NovoR*	*S. aureus Smith*	*S. aureus Smith QR-54*	*S. aureus Smith 744*	*S. epidermidis ATCC 14990*	*S. pyogenes β15*	*E. faecium vanA E23-8*
1	Novobiocin			0.25	0.25	8	0.25	0.12	0.25	0.12	2	8
3a				5	16	16	16	16	16	16	16	16
15				1	>32	>32	>32	>32	>32	32	>32	>32
17a			MeO	0.5	>32	16	32	16	>32	32	>32	>32
17b			Cl	1	8	4	4	8	16	8	32	>32
18a			MeO	0.5	32	8	16	16	>32	<2	>32	>32
18b			Cl	0.25	>32	16	>32	32	>32	32	>32	>32
22a	Me	CH	H	0.5	>32	>32	>32	>32	>32	>32	>32	>32
22b	Br	CH	H	0.5	2	2	2	2	4	>2	8	>32
22c	Me	CH	4-Me	0.5	32	16	32	16	>32	2	>32	>32
22d	Me	CH	6-Me	0.25	32	16	16	16	32	2	>32	>32
22e	Br	CH	6-Me	0.25	8	4	8	8	8	2	16	>32
22f	Me	N	H	0.13	1	0.5	1	1	2	0.5	32	>32
22g	Br	N	5,6-$(CH)_4$	0.13	0.5	16	0.5	0.5	n.d.[c]	>0.5	n.d.[c]	>32

[a] MNEC, maximal non-effective concentration.
[b] Resistance is mentioned as follows: NovoR (against novobiocin R144-I in ATC 25923), QR54 (against methicillin and quinolones), 744 (against erythromycin, methicillin, and trimethoprim), vanA E23-8 (against ampicillin, trimethoprim, and vancomycin).
[c] n.d., not determined.



Scheme 5. Reagents and conditions: (a) MeCHO, TFA, CH_2Cl_2, rt, 1 h, 79%; (b) 2 equiv K_2CO_3, MeOH, THF, rt, 12 h, 92–100%; (c) 1.1 equiv Et_3SiH, TFA, CH_2Cl_2, rt, 3 h, 43–73%.

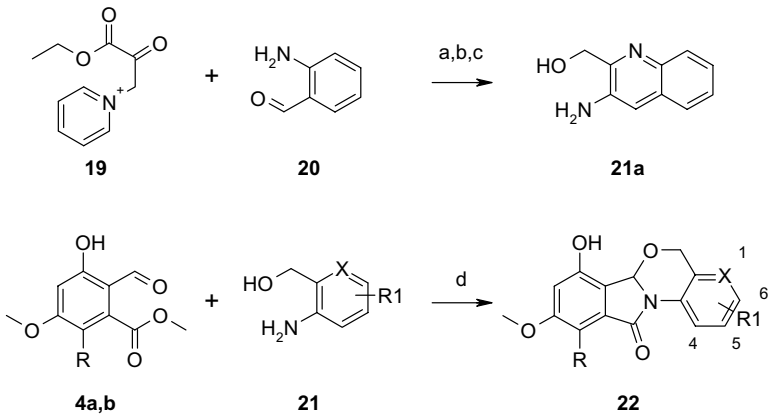
DNA gyrase as novobiocin **1**, our standard reference compound (Table 7). Replacement of the aniline moiety by an 3-amino-pyridine or by an 3-amino-quinoline residue led to the most active compounds **22f**,**g** being more effective against DNA gyrase in the supercoiling assay than novobiocin **1** (Table 7).[11]

The in vitro antibiotic activity was measured as minimal inhibitory concentration (MIC) in twofold agar dilutions (Müller–Hinton agar, inoculum 10^4 CFU/spot).

The initial HTS-hit **3a** showed weak antibacterial activity (Table 7). We tested several other compounds from different classes (**5**,**6**,**8**,**10**,**13**) but they were all inactive against the tested strains except sometimes little activity was observed against *S. epidermidis ATCC 14990*, a bacterial strain, which has an easily permeable cell membrane.

Table 7 shows that improved MNEC values lead to higher antibacterial activity. The tetracyclic ketone **15** is devoid of any antibacterial activity except for *S. epidermidis ATCC 14990* (because of the reasons discussed above). The carbon tetracyclic compounds **17**,**18** have MNEC values, which are in the range of novobiocin **1**. Therefore these compounds should show some activity against bacteria, which is indeed the case.

The oxo-methyl-linked tetracycle **22a** with a methyl group in para-position to the phenol did not show any antibacterial activity. In contrast the corresponding bromo-derivative **22b** had consistently MIC values of around 2 μg/ml against Gram-positive bacteria. These different antibacterial activities of the para-methyl and the para-bromo derivatives were also observed within the class of cyclothialidines although not as pronounced as it is seen for this pair of compounds. This observation



Scheme 6. Reagents and conditions: (a) pyridine, EtOH, reflux, 6 h, 60%; (b) pyrrolidine, EtOH, reflux, 1 h, 83%; (c) DIBAH, THF, 0 °C, 75%; (d) NaOMe, MeOH, reflux, 3–5 h, 17–84%.

holds also true for the pair **22d**,**e**, now at a more moderate ratio of a roughly 2- to 4-fold lower MIC values when moving from methyl to bromine as it was generally seen for cyclothialidine derivatives. The reason for this observation might be a better permeability of the bromine derivative, which is due either to a higher lipophilicity or to an increased acidity of the phenol moiety. Compounds **22f**,**g** exhibited good activities against Gram-positive bacteria being often as active as novobiocin **1** although permeability of these compounds seems not to be optimal. Since the pyridine and quinoline derivatives **22f**,**g** are probably interacting similarly with gyrase B as novobiocin **1** we observed as well lower antibacterial activity of **22g** against the novobiocin resistant *S. aureus* strain. Interestingly the pyridine derivative **22g** did not show this resistance. This might be due to its smaller size.

In conclusion, we explored the chemical space around the biased needle screening hit **3a**. By applying our knowledge about the SAR of the cyclothialidines and by using the information from the X-ray structures from novobiocin **1** and cyclothialidine **2** complexed to gyrase B we could optimize this compound class. The formation of a tetracyclic core structure and the introduction of a pyridine or quinoline moiety led to derivatives **22f**,**g**, which are potent inhibitors of DNA gyrase and which exhibit promising antibacterial activity against Gram-positive bacterial strains.

Acknowledgments

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8. All compounds were characterized by MS and ^{1}H NMR analysis. Purities are above 95%.
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11. The preparation of **18b** and **22f** is described as representative examples. Preparation of **18b**: Step 1. 2-(4-Chloro-phenyl)-4-hydroxy-3,6-dimethoxy-7-methyl-2,3-dihydro- isoindol-1-one (**7b**): 2-Formyl-3-hydroxy-5-methoxy-6-methyl-benzoic acid methyl ester (897 mg, 4 mmol) and 4-chloroaniline (510 mg, 4 mmol) were stirred in methanol (10 ml) for 3 h at room temperature. The precipitated yellow imine was filtered off and washed with little methanol. It was suspended in methanol (10 ml) and a solution of 5.4 N NaOMe in methanol (1 ml) was added. The yellow solution was refluxed for 3 h and cooled to room temperature. Water was added and the reaction mixture was neutralized with acetic acid. The precipitate was filtered off, washed with water, dissolved in tetrahydrofurane, dried over sodium sulfate, filtered, and evaporated to yield the product as a light yellow solid (1060 mg, 79%). ^{1}H NMR (250 MHz, DMSO-d_6) δ 10.1 (s, 1H, OH), 7.78 (d, 2H), 7.50 (d, 2H), 6.72 (s, 1H), 6.59 (s, 1H), 3.81 (s, 3H), 2.81 (s, 3H), 2.40 (s, 3H). MS (EI) *m*/*z*: 333 (5) $(M)^+$, 318 (5) $(M-Me)^+$, 301 (100) $(M-MeOH)^+$.
Step 2. Trifluoro-acetic acid 3-chloro-7-hydroxy-9-methoxy-10-methyl-11-oxo-5,6,6a,11-tetrahydro-isoindolo[2,1-*a*]quinolin-5-yl ester (**16b**): To the aminal **7b** (1061 mg, 3.2 mmol) was added acetaldehyde (2 ml) and trifluoroacetic acid (10 ml) at room temperature. The resulting pink suspension was stirred at room temperature overnight. The suspension was evaporated several times with hexane. The residue was purified by column chromatography (diethyl ether then ethyl acetate) to yield the product as a white solid (1110 mg, 79%). ^{1}H NMR (250 MHz, DMSO-d_6) δ 10.1 (s, 1H, OH), 8.48 (d, 1H), 7.79 (d, 1H), 7.57 (dd, 1H), 6.69 (s, 1H), 6.24 (s, 1H), 4.80 (d, 1H), 3.80 (s, 3H), 3.01 (d, 1H), 2.42 (s, 3H), 1.88 (t, 1H). MS (ISN) *m*/*z*: 344.2/346.3 (100/37) $(M-H)^-$.
Step 3. 3-Chloro-7-hydroxy-9-methoxy-10-methyl-6,6a-dihydro-5*H*-isoindolo[2,1-*a*]quinolin-11-one (**18b**): The trifluoroacetate derivative **16b** (221 mg, 0.5 mmol) was suspended in methylene chloride (4 ml). Triethyl silane (116 mg, 1 mmol) and trifluoroacetic acid (2 ml) were added. The suspension turned blue and the reaction mixture becomes almost a solution. After stirring at room temperature for 3 h the reaction was evaporated, suspended in methylene chloride, and the product was precipitated with hexane. The product was filtered off, washed with hexane, and dried at the evaporator to yield a gray solid (120 mg, 73%). ^{1}H NMR (250 MHz, DMSO-d_6) δ 10.0 (s, 1H), 8.38 (d, 1H), 7.32 (s, 1H), 7.28 (d, 1H), 6.67 (s, 1H), 4.6 (dd, 1H), 3.79 (s, 3H), 2.74–3.12 (m, 3H), 2.41 (3H), 1.50 (m, 1H). MS (ISN) *m*/*z*: 328.1/330.2 (100/36) $(M-H)^-$.
Preparation of 7-hydroxy-9-methoxy-10-methyl-5*H*,6a*H*-6-oxa-4,11a-diaza-benzo[*a*]fluoren-11-one (**22f**): 2-Formyl-3-hydroxy-5-methoxy-6-methyl-benzoic acid methyl ester (448 mg, 2 mmol) and 3-amino-2-hydroxymethylpyridine (248 mg, 2 mmol) were stirred in methanol (5 ml) at room temperature overnight. The yellow precipitate was filtered off and washed with methanol. The imine was suspended in methanol (10 ml) and a solution of 5.4 N NaOMe in methanol (1 ml) was added. The resulted solution was refluxed for 3 h, cooled to room temperature. Water was added and the reaction was neutralized with acetic acid. A gel precipitated, which was filtered off. It was dissolved in acetone, dried over sodium sulfate, filtered, and the solvent was evaporated to yield the product as a white solid (240 mg, 40%). ^{1}H NMR (250 MHz, DMSO-d_6) δ 10.2 (s, 1H, OH), 8.43 (dd, 1H), 8.35 (dd, 1H), 7.39 (dd, 1H), 6.72 (s, 1H), 6.14 (s, 1H), 5.09 (d, 1H), 4.92 (d, 1H), 3.82 (s, 3H), 2,39 (s, 3H). MS (ISN) *m*/*z*: 297.2 (100) $(M-H)^-$.