New non-peptidic Inhibitors of Papain Derived from Etacrynic Acid

U. Kaeppler and T. Schirmeister*

University of Wuerzburg, Institute of Pharmacy and Food Chemistry, D-97074 Wuerzburg, Germany

Abstract: Cysteine proteases are connected to various viral and parasitic infections, as well as to other severe diseases like arthritis, stroke and cancer. Due to its α,β-unsaturated carbonyl moiety etacrynic acid, a well known diuretic, can inhibit cysteine proteases in a Michael-type reaction by reaction with the nucleophilic cysteine residue of the active site. For first structure-activity-relationship studies modifications at various positions of the etacrynic acid structure have been investigated concerning inhibition potency against the CAC1 protease papain: length of the side chain, substitution pattern of the aromatic ring as well as influence and necessity of acidic groups, esters or amides. Additionally, the effect of the aromatic ring was evaluated by replacement with a cyclohexyl moiety.

Key Words: Cysteine protease inhibitor, Michael addition, papain, etacrynic acid.

INTRODUCTION

Cysteine proteases are targets of a variety of new potential drugs (for reviews see ref. [1,2]). By now, many effective peptidic or peptidomimetic compounds containing electrophilic building blocks like three-membered heterocycles (for reviews see ref. [3,4]) or Michael-systems [5] have been synthesized and tested, whereas the number of non-peptidic inhibitors is relatively small (for a review see ref. [6]).

Among the compounds, which inhibit cysteine proteases *via* reaction of an activated double bond with the cysteine residue of the active site are two which currently undergo clinical trials against infectious diseases: Ruprintrivir (AG-7088 [5,7], Fig. **1**) against picorna viruses for treatment of rhinitis (phase III) [8] and CRA-3316 (phase I) against parasitic cysteine proteases for the treatment of trypanosomiasis (Chagas disease) [9,10] (Fig. **1**).

The diuretic etacrynic acid **7a** (4-(2-methylene)-2,3-dichloro-phenoxy acetic acid, Fig. **2**) is a weak papain inhibitor [11]. Like Ruprintrivir, etacrynic acid, which contains an α,β-unsaturated ketone moiety, can block the cysteine residue of the active site in a Michael-type addition. Therefore, this compound serves as a lead structure for new non-peptidic cysteine protease inhibitors. In the present study we investigate several structural modifications of etacrynic acid to discover essential structural elements necessary for inhibition of cysteine proteases (Fig. **2**). As a model enzyme we used papain, the prototype cysteine protease of the CAC1 family, which includes mammalian enzymes (e.g. cathepsins B, L, S) as well as protozoan ones (e.g. falcipains, rhodesain, leishmanial cathepsins) (for reviews see ref. [1,12-14]).

*Address correspondence to this author at the Institut für Pharmazie und Lebensmittelchemie, Am Hubland, D-97074 Wuerzburg, Germany; Tel: +49-931-8885440; Fax: +49-931-8885494;
E-mail: schirmei@pzlc.uni-wuerzburg.de

RESULTS AND DISCUSSION

The substituents of the aromatic ring were changed by replacing both chlorine atoms with hydrogens or methyl groups (Fig. **2, A**) (**cpds. 6b, 7c**). Moreover, the effect of truncation of the side chain from ethyl to methyl (Fig. **2, B**) (**cpd. 6b**) was investigated as well as the role of the acidic group, which was replaced by an ethyl ester or various amides (Fig. **2, C**) (**cpds. 6a, 11a-f**). To find out whether an aromatic or an aliphatic ring system favours inhibition potency (Fig. **2, D**), we synthesized the two basic scaffolds, 2-methylene-1-phenyl butan-1-one **10a** and 1-cyclohexyl-2-methylene butan-1-one **10b**.

Etacrynic acid **(7a)** was synthesized starting from the 2,3-substituted phenol **(1a)**, which was converted into the corresponding anisole **(2a)** by treatment with 1 eq. dimethyl sulfate and 1 eq. KOH (Fig. **3**). The anisoles **(2a, 2b)** were acylated in dichloromethane by Friedel-Crafts acylation using 1.5 eq. acid chlorides of the desired chain length and 1.5 eq. aluminium chloride [15]. In this step we used dichloromethane as solvent instead of the more toxic CS_2 which has been used in earlier syntheses of etacrynic acid derivatives [15,16]. In a consecutive step another 1.5 eq. of aluminium chloride were added and heated under reflux to cleave the methyl ethers to the corresponding 4-acylated phenols **(3a, 3b)**. These were treated with ethyl bromoacetate, either 1.1 eq. potassium-*tert*-butylate or 1.5 eq. K_2CO_3 and catalytic amounts of potassium iodide in THF or acetone to give the desired p-substituted ethyl phenoxy acetates **(5a, 5b)**. The use of potassium-*tert*-butylate was found to be advantageous in comparison to other bases. Compound **5c** was synthesized by alkylation of phenol **1c** with ethyl bromoacetate to yield **4c** and subsequent Friedel-Crafts acylation. *Via* a Mannich-like reaction using either 1.5 eq. hexamethylene tetramine [17] or 1.5 eq. *N,N,N',N'*-tetramethyl diamino methane [18] and 2 eq. acetic anhydride the double bond was introduced into the side chain of compounds **5** in α-position to the ketone yielding the esters **6**. These synthetic pathways are superior to the known syntheses since they proceed without isolation and subsequent elimination of

1573-4064/05 $50.00+.00

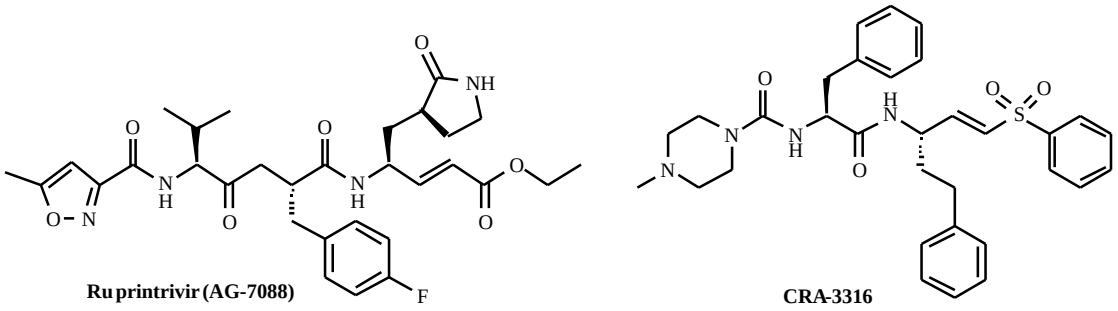


Fig. (1). Ruprintrivir (AG-7088) and CRA-3316 as peptidomimetic cysteine protease inhibitors containing an activated double bond. Both compounds are currently under clinical development.

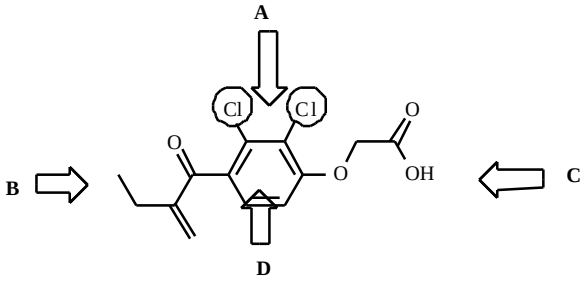


Fig. (2). Etacrynic acid **(7a)** and structural modifications addressed in this study.
A: substitution pattern of the aromatic ring; **B**: truncation of the side chain; **C**: esters, amides; **D**: variation of the ring system.

the Mannich's base obtained in the reaction with HCHO and dimethylamine [19]. The acids **7a** and **7c** were synthesized starting from **5a** and **5c**, respectively, by aldol condensation and simultaneous saponification with HCHO and K_2CO_3. An advantage of the aldol condensation over preparative methods described earlier [19] is that the acids are obtained directly and no separate ester hydrolysis and/or isolation and subsequent elimination of the Mannich's base is necessary.

To obtain 1-cyclohexyl-2-methylene butan-1-one **10b**, butanale and cyclohexyl bromide were allowed to react in a Grignard-reaction to give 1-cyclohexyl-butan-1-ol **(8b)**, which was oxidized with oxalyl chloride/DMSO[20] in dichloromethane (Fig. **4**). The obtained 1-cyclohexyl-butan-1-one **(9b)** and butyrophenone **(9a)**, respectively, were treated with 1.5 eq. hexamethylenetetramine and acetic anhydride to give the desired products **10b** and **10a**.

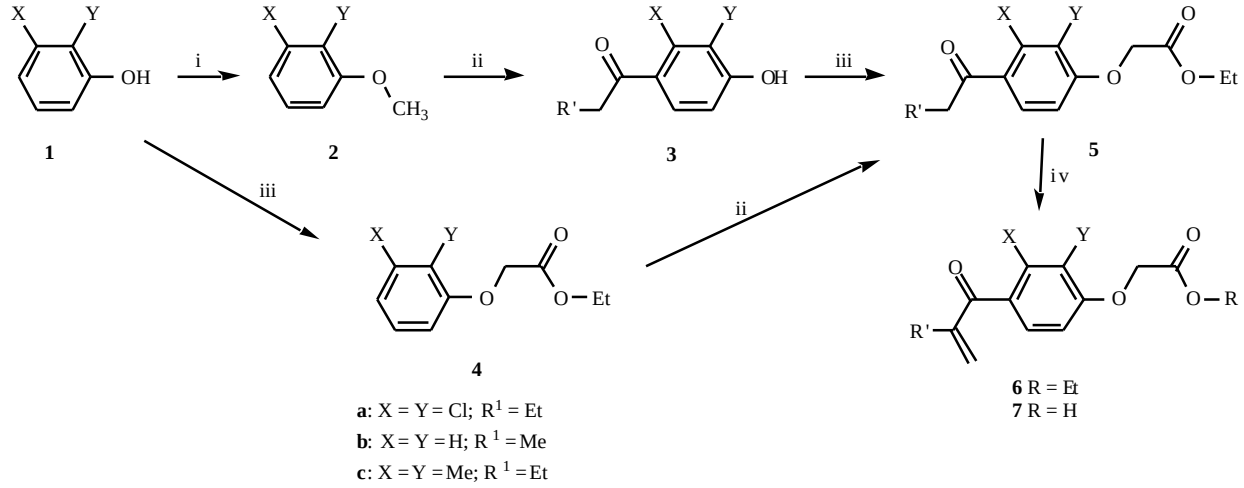


Fig. (3). Synthesis of etacrynic acid derivatives. Reaction conditions: *i*: 1 eq. dimethylsulfate, 1.25 eq. 10 % $KOH_{aq.}$; *ii*: 1.5 eq. R^1-CH_2-COCl, 1.5 eq. $AlCl_3$, CH_2Cl_2, 2 h at 0 °C; 1.5 eq. $AlCl_3$, CH_2Cl_2, 2.5 h reflux; *iii*: 1.1 eq. Br-CH_2CO_2Et, $THF_{abs.}$, 1.1 eq. K-*tert*-butylate, $KI_{cat.}$ or 2 eq. Br-CH_2CO_2Et, $acetone_{abs.}$ $KI_{cat.}$; *iv*: 1.5 eq. hexamethylenetetramine, 2 eq. Ac_2O, 4-18 h at 80 °C or 1.5 eq. *N,N,N',N'*-tetramethyldiaminomethane, 2 eq. Ac_2O, 4-18 h at 80 °C or 2 eq. HCHO, EtOH, 1 eq. K_2CO_3.

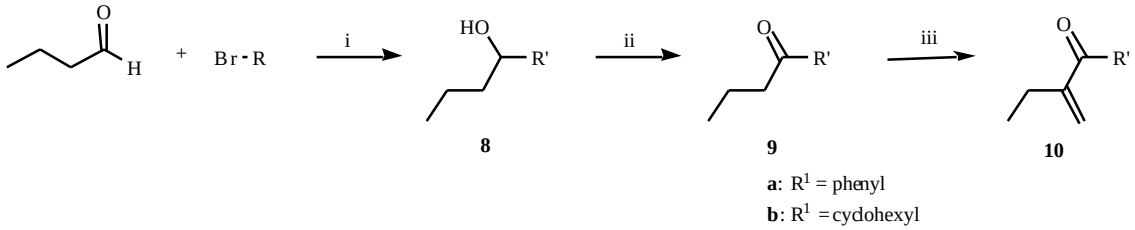
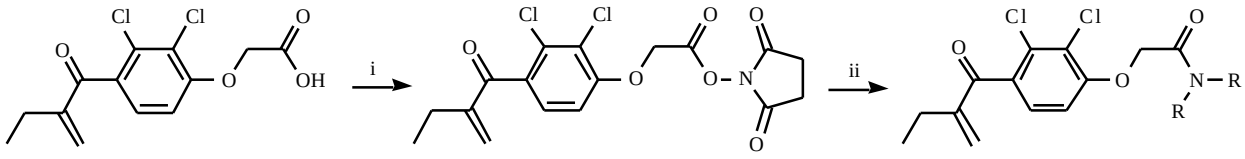


Fig. (4). Synthesis of 2-methylene-1-phenyl-butan-1-one **10a** and 1-cyclohexyl-2-methylene-butan-1-one **10b**. Reaction conditions: *i.* 1 eq. Mg, abs. diethylether; *ii.* 1.1 eq. oxalylchloride, 2.2 eq. $DMSO_{abs.}$, triethylamine, CH_2Cl_2 , -60 °C; *iii.* 1.5 eq. hexamethylenetetramine, 2 eq. Ac_2O, 80 °C, N_2-atmosphere.

The syntheses of the etacrynic acid amides **(11a-f)** were performed by transferring etacrynic acid **(7a)** into the active ester with *N*,*N*'-dicyclohexylcarbodiimide and *N*-hydroxysuccinimide. After addition of the amines the desired amides **(11a-f)** were obtained in moderate to good yields (Fig. **5**). This method is more gentle than the aminolysis of acid chlorides which can be obtained with thionyl chloride [21].

Enzyme kinetics were performed as continuous assays with the cysteine protease papain according to Tian and Tsou [22]. We used *N*-α-Benzoyl-*L*-arginine-p-nitroanilide (*L*-BAPA) as substrate in 50 mM phosphate buffer of pH 6.5. All assays were conducted at 30 °C and contained 12 % DMSO. Detailed results are shown in table **1**.

All compounds show time-dependent inhibition of papain. Since it is known that etacrynic acid reversibly reacts with



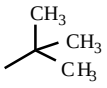
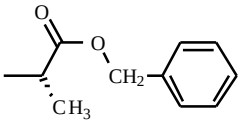
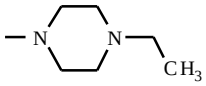
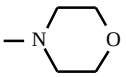
	R^1	R^2	yield
11a	H	$-CH_2-CH_2-CH_2-CH_3$	73 %
11b	H		70 %
11c	H	$-CH_2-(CH_2)_4-CH_3$	91 %
11d	H		92 %
11e	$N(R^1R^2)$ =		21 %
11f	$N(R^1R^2)$ =		74 %

Fig. (5). Synthesis of etacrynic acid amides **11a-f**. Reaction conditions: *i.* 1 eq. *N*-hydroxysuccinimide, 1 eq. *N*,*N*'-dicyclohexylcarbodiimide in dry CH_2Cl_2, room temperature (not isolated); *ii.* 1-1.5 eq. $HN-R^1R^2$, room temperature, 7-14 d.

Table 1. Time-dependent Inhibition of Papain by Etacrynic Acid Derivatives

	k_i [min^{-1}]	K_i [µM]	k_{2nd} [$M^{-1} min^{-1}$]
7a	**0.125 ± 0.020**	**375 ± 20**	**333 ± 13**[a]
6a	**0.016 ± 0.0006**	**13 ± 2.2**	**1185**[c]
6b	**0.108 ± 0.013**	**1960 ± 353**	**55**[c]
7c	**0.013 ± 0.0009**	**33 ± 9.8**	**391**[c]
10a	**0.136 ± 0.005**	**778 ± 43**	**176 ± 3**[a]
10b			**29 ± 0.24**[a,b]
11a	**0.016 ± 0.00015**	**6.15 ± 0.05**	**2572 ± 43**[a]
11b	**0.015 ± 0.00025**	**3.2 ± 0.2**	**4583 ± 386**[a]
11c			**590 ± 7**[a,b]
11d			**560 ± 8**[a,b]
11e	**0.128 ± 0.0175**	**412.5 ± 60**	**311 ± 3**[a]
11f	**0.088 ± 0.0035**	**204 ± 8**	**433 ± 33**[a]

[a] Mean value from 2 independent assays, each performed with 7 different inhibitor concentrations; [b] due to low solubility determination was restricted to the linear range, thus k_{2nd} was calculated by k_{obs}/[I]; [c] one determination performed with 7 different inhibitor concentrations.

cysteine and other SH containing compounds deactivation of the inhibitors by the buffer cysteine used for enzyme activation should be considered [23]. To evaluate the influence of the concentration of buffer cysteine on inhibition potency we performed assays with inhibitor **7a** at different cysteine concentrations (5 mM or 0.2 mM final concentration). No difference in inhibition potency could be found. This probably is due to the fact that the target active site cysteine residue in CAC1 proteases exists as a negatively charged thiolate and therefore as a considerably better nucleophile than low molecular weight thiols, even at slightly acidic conditions. Thus, the reaction between the enzyme's cysteinate and etacrynic acid may withdraw etacrynic acid from its adduct with buffer cysteine shifting the equilibrium of this side reaction into the direction of free etacrynic acid and cysteine. This is also known from assays with etacrynic acid, cysteine and DTNB, which showed that the equilibrium between cysteine and etacrynic acid on the one side and the cysteine adduct on the other side is shifted rapidly into direction of free Michael system and thiol after addition of the cysteine consuming DTNB [23].

Obviously, the introduction of a lipophilic amide residue (**11a,b**) increases k_{2nd} about tenfold compared to etacrynic acid (**7a**). This inhibition improvement is due to low dissociation constants K_i in the lower micromolar range (3-6 µM) reflecting quite good affinity to the enzyme. The same holds true for the etacrynic acid ethyl ester **(6a)** whereas longer or bulkier amide residues like hexylamine (**11c**), *N*-ethyl piperazine (**11e**), morpholine (**11f**) or alanine benzyl ester (**11d**) are only slightly more potent than or equipotent to etacrynic acid **(7a)**. Displacement of the substituents at the aromatic ring in combination with truncation of the side chain next to the double bond results in reduced second-order rate constants (cpd. **6b** *vs.* **6a**). In contrast, exchange of the chlorine atoms *vs.* methyl groups has a slightly positive effect (cpd. **7c** *vs.* **7a**). The aromatic ring system (cpd. **10a**) clearly is more advantageous than the aliphatic one (cpd. **10b**), which is shown by the very poor inactivation by compound **10b**.

In summary, the most promising leads for new non-peptidic inhibitors of CAC1-proteases are the etacrynic acid *n*- and *tert*-butyl amides **11a** and **11b**, which display 50 to 100 fold better dissociation constants K_i compared to the acid. Selectivity studies as well as docking experiments to evaluate possible binding modes, and to optimize these lead structures are underway. In this connection the known structure-activity-relationship of the diuretic activity [24,25] and sulfhydryl reactivity[25] of etacrynic acid derivatives have to be taken into account. Loop diuretics like etacrynic acid reach their side of action – the luminal membrane of the cells of the thick ascending loop – by being actively secreted in the proximal convoluted tubule by the organic acid transport mechanism [26,27]. Esters act as hydrolysable prodrugs, which e. g. has also been shown for diuretic aryloxy acetic acid esters without Michael-system [28]. Thus, compounds derived from etacrynic acid usable as cysteine protease inhibitors devoid of diuretic side effects should be stable against hydrolysis to avoid generation of an acidic functional group. In this regard it is quite promising that especially the amides are more potent cysteine protease

inhibitors than acids and esters since hydrolytic stability can more easily achieved with carboxamides.

EXPERIMENTAL

General Information

Papain, DCC, and TMDM were purchased from Fluka, *L*-BAPA, *L*-Ala-OBn, phenols and anisoles from Merck, *N*-hydroxysuccinimide from Aldrich.

Melting points were determined in open capillary on a melting point apparatus 530 from Büchi, Switzerland. CHN analyses were determined with a CHNS-932, Leco, USA. HR-EI-mass spectra were recorded on a Finnigan MAT 90, Thermo Electron GmbH, Germany, at 70 eV ionisation energy. NMR spectra were recorded on an AVANCE 400 MHz spectrometer from Bruker Biospin GmbH, Germany (solvent: $CDCl_3$, ^{1}H-NMR: 400.13 MHz; ^{13}C-NMR: 100.61 MHz). IR spectra were recorded on a PharmalyzIR FT-IR spectrometer from BioRad, USA. α values were determined on a 241 polarimeter from PerkinElmer, USA. The refraction indices were determined on an apparatus from ATG GmbH, Germany. The enzyme assays were performed with a spectrophotometer from PerkinElmer, USA (Lambda EZ 210). For preparative column chromatography a MPLC from Büchi, Switzerland was used. The chromatography was performed with a LoBar column from Merck (SiO_2 Rp-18). For preparative HPLC a Symmetry RP-18 column from Waters, USA was used (19 x 150 mm, 7.5 µM, 12 mL/min). Hydrostatic column chromatography was performed with silica gel from Merck (silica gel 60, 0.063-0.2 mm or 70-230 mesh).

All solvents were purified and dried prior to use according to standard literature procedures.

Enzyme Assays

The continuous assays were performed according to ref. [22] as described previously [29]. *N*-α-Benzoyl-*L*-arginine-p-nitroanilide *L*-BAPA (final concentration 1.84 mM), dissolved in DMSO, was used as substrate. Papain was used in a final concentration of 12.5 mg ml^{-1} in 50 mM phosphate buffer of pH 6.5 containing 5 mM EDTA and 5 mM cysteine for enzyme activation. All assays were conducted at 30 °C. Inhibitors were dissolved in DMSO. Final DMSO concentration was 12 % in all assays. Assays were performed at 7 different concentrations between 10 and 2000 µM, depending on solubility. The inhibition constants were calculated by non-linear regression analysis using the software GraFit [30]. To correct K_{iapp} values K_m = 2.5 mM for papain/*L*-BAPA was used.

General Synthetic Methods

Method A, methylation of phenols: One eq. phenol is solved in KOH solution (10 %, 1.25 eq. KOH). One eq. DMS is added slowly at rt. The mixture is heated under reflux for 30 min, the organic layer is separated and the aqueous layer is extracted with EtOEt. The combined organic layers are washed with water and brine and are dried with Na_2SO_4. After filtration the solvent is removed *i. vac.*

Method B, Friedel-Crafts-Acylation of anisoles and subsequent cleavage of the methoxy group: 1 eq. anisole and 1.5 eq. acid chloride are solved under N_2 atmosphere in 50-100 mL abs. CH_2Cl_2 and are cooled to 0-5 °C. 1.5 eq. $AlCl_3$ are added within 30 min and the mixture is stirred for 2-3 h. 75-100 mL CH_2Cl_2 are removed by distillation and are substituted by the same amount of CH_2Cl_2. This procedure is repeated twice. Further 1.5 eq. of $AlCl_3$ are added and the mixture is heated under reflux for 2.5-3 h. The mixture is poured on ice and acidified with HCl conc. to pH 1. Tartaric acid is added for the complexation of aluminium until the solution clears up. The solution is extracted with EtOEt and the organic layer is washed with water and extracted with KOH solution (10 %). The aqueous layer is acidified with HCl conc. to pH 1 and the precipitated product is filtered off.

The organic layer may contain considerable amounts of bisacylated product which can be hydrolyzed with KOH 10 %.

Method C1, alkylation with ethyl bromoacetate/K_2CO_3 in acetone: 1 eq. phenol, 2 eq. ethyl bromoaceate, 1.5 eq. K_2CO_3, and a catalytic amount (0.1 eq.) KI are suspended in abs. acetone and heated under reflux for 5-6 h. After filtration the solvent is removed *i. vac.* The remaining residue is solved in EtOEt and the organic layer is washed with 5 % NaOH, water, and brine, dried with Na_2SO_4, and removed *i. vac.* The crude product is recrystallized.

Method C2, alkylation with ethyl bromoacetate/K-*tert*butylate in THF: 1 eq. phenol are solved in THF under N_2 atmosphere. 1 eq. K-*tert*butylate and a catalytic amount (0.1 eq.) of KI are added. The mixture is heated at 60°C and 1.1-2.0 eq. ethyl bromoactetate are added slowly. The mixture is stirred for 30-60 min, poured into water, acidified to pH 1 with HCl conc., and extracted with EtOEt. The organic layer is washed with KOH 10 %, water and brine, and dried with Na_2SO_4. After filtration the solvent is removed *i. vac.* and the remaining residue is recrystallized or distilled.

Method D1, Mannich reaction with hexamethylenetetramine: 1 eq. phenoxy acetic acid ester derivative, 1.5 eq. hexamethylenetetramine and 2 eq. Ac_2O are heated at 80 °C under N_2-atmosphere. The reaction is followed by ^{1}H-NMR spectroscopy. After completion of the reaction the mixture is solved in EtOAc and water. The organic layer is washed with water, $NaHCO_3$ solution 5 %, water and brine, and is dried with Na_2SO_4. After filtration the solvent is removed *i. vac.* and the remaining residue is purified by column chromatography.

Method D2, Mannich reaction with TMDM: 1 eq. phenoxy acetic acid ester derivative, 20 eq. TMDM, and 20 eq. Ac_2O are mixed. The mixture is heated under reflux at 85 °C. The reaction is followed by ^{1}H-NMR spectroscopy. After completion of the reaction the mixture is solved in 200 mL $CHCl_3$. Saturated K_2CO_3 solution is added until the gas evolvement stops. The mixture is filtered through Celite and the filtrate is dried with Na_2SO_4. After filtration the solvent is removed *i. vac.* The product is purified by column chromatography.

Method D3, aldol condensation and ester hydrolysis: 1 eq. phenoxy acetic acid derivative and 2 eq. HCHO (40 %

aqueous solution) are solved in 20 mL EtOH (= solution 1). 1 eq. K_2CO_3 is solved in 5 mL water and diluted with 6 mL EtOH (= solution 2). 10-20 mL EtOH are heated under reflux and both solutions (1 and 2) are added slowly. The mixture is heated under reflux until the reaction is completed (control by ^{1}H-NMR spectroscopy). The mixture is poured into 85 mL water containing 5 mL HCl conc. and cooled for 12 h. The precipitated product is purified by recrystallization or column chromatography.

Method E, amide syntheses with DCC/HOSuc: 500 mg (1.65 mmol) etacrynic acid **(7a)** are solved under N_2-atmosphere in 10 mL abs. CH_2Cl_2. 190 mg (1.65 mmol) *N*-hydroxy succinimide are added and the solution is stirred for 1 h. 340 mg (1.65 mmol) DCC are added and the mixture is stirred for 3 h and filtered off. The filtrate is added to a solution of 1.65 mmol amine in 5 mL abs. THF or to a mixture of 1.65 mmol amine HCl and 229 µM (1.65 mmol) TEA in 5 mL abs. THF. The mixture is stirred for several days at rt. The solvent is removed *i. vac.* and the remaining residue is stirred in EtOAc for 1 h. The mixture is filtered off and the solvent is removed *i. vac.* The crude product is purified by column chromatography.

Compounds

Ethyl 2,3-dichloro-4-(2-methylene-butyryl)-phenoxy] Acetate (Etacrynic Acid Ethyl Ester) (6a) and 2,3-Dichloro-4-(2-methylene-butyryl)-phenoxy] Acetic Acid (Etacrynic Acid) (7a)

1-(2,3-Dichloro-4-hydroxy-phenyl)-butan-1-one (3a)

Method B; 35.4 g (200 mmol) 2,3-dichloro anisole **(2a)** (prepared according to method A), 31.96 g (300 mmol) butyric acid chloride, 2x 40 g (300 mmol) Al_Cl3, 1_50 mL CH2Cl2; yield: 45.46 g (195 mmol, 69 %), colourless solid; mp. 107-108 °C (methanol/water), (ref. [31]: 105-107 °C); 1H-NMR (400.13 MHz, CDCl3, 300 K, TMS): δ 0.98 (3H, t, J=7.33 Hz; H_3C-CH_2-CH_2); 7.13 (2H, sext, J=7.33 Hz; H_3C-C*H*$_2$-CH_2); 2.89 (2H, t, J=7.33 Hz; H_3C-CH_2-C*H*$_2$); 5.93 (1H, s, O*H*); 7.00 (1H, d, J=8.59 Hz, arom. H_6); 7.39 (1H, d, J=8.59 Hz, arom. H_5);

Ethyl (4-butyryl-2,3-dichlorophenoxy) Acetate (5a)

Method C1; 2.18 g (9.36 mmol) **3a**, 2.0 g (14.48 mmol) K_2CO_3, 3.0 g (17.96 mmol) ethyl bromoacetate, 20 mL abs. acetone; yield: 2.38 g (7.46 mmol, 80 %), colourless solid; mp. 55-56 °C (ethanol) (ref. [32]: 55-56 °C); ^{1}H-NMR (400.13 MHz, $CDCl_3$, 300 K, TMS): δ 0.98 (3H, t, J=7.33 Hz, *H*$_3$C-CH_2-CH_2); 1.30 (3H, t, J=7.07 Hz, *H*$_3$C-CH_2-O); 1.73 (2H, sext, J=7.33 Hz, H_3C-C*H*$_2$-CH_2); 2.89 (2H, t, J=7.33 Hz, H_3C-CH_2-C*H*$_2$); 4.28 (2H, q, J=7.07 Hz, H_3C-C*H*$_2$-O); 4.75 (2H, s, O-C*H*$_2$-CO); 6.77 (1H, d, J=8.59 Hz, arom. *H*$_6$); 7.34 (1H, d, J=8.59 Hz, arom. *H*$_5$);

Ethyl [2,3-dichloro-4-(2-methylene-butyryl)-phenoxy] acetate (etacrynic acid ethyl ester) (6a)

Method D2; 3.20 g (10 mmol) **5a**, 6.3 g (61.7 mmol) *N,N,N',N'*-Tetramethyldiaminomethan, 5.4 g (52.9 mmol) acetic anhydride, reaction time 7 d; purification by MPLC (SiO_2 RP-18, gradient H_2O/acetonitrile (95 % → 65 % water in 60 min) and recrystallization from ethanol; yield: 788 mg (2.38 mmol, 24 %), colourless solid; mp. 39-40.5 °C (ethanol) (ref. [19]: 43-45 °C); HR-EI-MS (70 eV, m/z, $[M]^+$): calc.: 166.13522; found.: 166.13515; ^{1}H-NMR (400.13 MHz, $CDCl_3$, 300 K, TMS): δ 1.45 (t, 3H, J=7.33 Hz, *H*$_3$C-CH_2-C); 1.30 (t, 3H, J=7.20 Hz, *H*$_3$C-CH_2-O); 2.47 (q, 2H, J=7.33 Hz, H_3C-C*H*$_2$-C); 4.28 (q, 2H, J=7.20 Hz, H_3C-C*H*$_2$-O); 4.75 (s, 2H, O-C*H*$_2$-CO); 5.60 (s, 1H, C=C*H*$_2$); 5.94 (s, 1H, C=C*H*$_2$); 6.79 (d, 1H, J=8.34 Hz, arom. H_6); 7.14 (d, 1H, J=8.34 Hz, arom. H_5); ^{13}C-NMR (100.61 MHz, $CDCl_3$, 300 K): δ 12.32 (*H*$_3$*C*-CH_2-C); 14.05 (*H*$_3$*C*-CH_2-O); 23.35 (H_3C-*C*H_2-C); 61.67 (H_3C-*C*H_2-O); 66.26 (O-*C*H_2-CO); 110.77 (arom. *C*$_6$, CH); 123.29 (arom. *qC*$_4$); 126.70 (arom. *C*$_5$, CH); 128.54 (=*C*H_2); 131.38 (arom. *qC*$_3$, C-Cl); 133.78 (arom. *qC*$_2$, C-Cl); 150.10 (*C*=CH_2); 155.44 (arom. *qC*$_1$); 167.65 (O-CH_2-*C*O); 195,74 (Ar-*C*O); IR (cm^{-1}): $\tilde{\nu}$ = 1074 (s, arom. C−Cl); 1206, 1293 (s, CO_2R, C−O); 1466 (m, arom. C=C); 1586 (m, C=C−C=O); 1661 (m-s, C=O); 1746 (m, CO_2R, C=O); 2940, 2972 (w, C−H);

Etacrynic Acid (7a)

Method D3; 3.19 g (10 mmol) **5a**, 1000 µl 40 % HCHO solution (13.33 mmol HCHO), 1.38 g (10 mmol) K_2CO_3, reaction time 6 h; the crude product was recrystallized from benzene; yield: 600 mg (1.98 mmol, 20 %), colourless solid; mp. 112-114°C (benzene) (ref. [16]: 118.5-120.5 °C); ^{1}H-NMR (400.13 MHz, $CDCl_3$, 300 K, TMS): δ 1.15 (3H, t, J=7.45 Hz, *H*$_3$C-CH_2); 2.47 (2H, q, J=7.45 Hz, H_3C-C*H*$_2$); 4.81 (2H, s, O-C*H*$_2$-CO); 5.60 (1H, s, =C*H*$_2$); 5.95 (1H, s, =C*H*$_2$); 6.82 (1H, d, J=8.47 Hz, arom. *H*$_6$); 7.16 (1H, d, J=8.47 Hz, arom. *H*$_5$).

[2,3-Dimethyl-4-(2-methylacryloyl)-phenoxy] Acetic Acid (7c)

Ethyl 2,3-dimethylphenoxy Acetate (4c)

Method C2; 24.44 g (200 mmol) 2,3-dimethyl phenol **(1c)**, 22.44 g (200 mmol) K-*tert*butylat, 66.8 g (400 mmol) ethyl bromoacetate, 100 mL THF; the crude product was purified by distillation at 12 mbar and 200°C oil bath temperature; yield: 27.91 g (134 mmol, 67 %), colourless solid; mp. 32-34 °C; CHN analysis for $C_{12}H_{16}O_3$: calc.: C 69.21, H 7.74; found: C 69.27, H 8.14; ^{1}H-NMR (400.13 MHz, $CDCl_3$, 300 K): δ 1.28 (3H, t, J=7.07 Hz, *H*$_3$C-CH_2-O); 2.21 (3H, s, Ar-C*H*$_3$); 2.26 (3H, s, Ar-C*H*$_3$); 4.25 (2H, q, J=7.07 Hz, H_3C-C*H*$_2$-O); 4.60 (2H, s, O-C*H*$_2$-CO); 6.58 (1H, d, J=7.90 Hz, arom. *H*); 6.80 (1H, d, J=7.90 Hz, arom. *H*); 7.01 (1H, t, J=7.90 Hz, arom. *H*$_5$);

Ethyl (4-butyryl-2,3-dimethylphenoxy) Acetate (5c)

Method B; 5.0 g (24 mmol) **4c**, 3.84 g (36 mmol) butyric acid chloride, 4.8 g (36 mmol) $AlCl_3$, 25 mL CH_2Cl_2; the crude product was purified by column chormatography (SiO_2 60, cyclohexane/EtOAc 9+1 V/V); yield: 3.84 g (13.8 mmol, 57 %), colourless solid; mp. 55-56 °C (cyclohexane/EtOAc); CHN analysis for $C_{16}H_{22}O_4$; calc.: C 69.04, H 7.97; found: C 68.93, H 7.64; ^{1}H-NMR (400.13 MHz, $CDCl_3$, 300 K, TMS): δ 0.97 (3H, t, J=7.33 Hz, *H*$_3$C-CH_2-CH_2); 1.30 (3H, t,

J=7.07 Hz, H_3C-CH_2-O); 1.71 (2H, sext, J=7.33 Hz, H_3C-CH_2-CH_2); 2.25 (3H, s, Ar-CH_3); 2.36 (3H, s, Ar-CH_3); 2.80 (2H, t, J=7.33 Hz, H_3C-CH_2-CH_2); 4.27 (2H, q, J=7.07 Hz, H_3C-CH_2-O); 4.66 (2H, s, O-CH_2-CO); 6.58 (1H, d, J=8.59 Hz, arom. H_6); 7.37 (1H, d, J=8.59 Hz, arom. H_5); ^{13}C-NMR (100.61 MHz, $CDCl_3$, 300 K): δ 11.90 (arom. C_2-CH_3); 13.82 (H_3C-CH_2-CH_2); 14.14 (H_3C-CH_2-O); 16.96 (arom. C_3-CH_3); 18.14 (H_3C-CH_2-CH_2); 44.06 (H_3C-CH_2-CH_2); 61.36 (H_3C-CH_2-O); 65.62 (O-CH_2-CO); 107.69 (arom. C_6, CH); 126.58 (arom. C_5, CH); 127.29 (arom. qC_2, C-CH_3); 133.78 (arom. qC_4); 138.09 (arom. qC_3, C-CH_3); 157.44 (arom. qC_1); 168.70 (O-CH_2-C=O); 205.19 (Ar-C=O); IR (cm^{-1}): $\tilde{\nu}$ = 812 (two vicinal Ar-H); 1121 (s, =C−O−C + COOH, C−O-R); 1205 (COOH, C−O-R); 1571 (m, arom. C=C); 1670 (m, Ar-C=O); 1754 (s, CO_2R, C=O); 2853, 2922, 2959 (m-w, C−H);

[2,3-Dimethyl-4-(2-methylacryloyl)-phenoxy] Acetic Acid (7c)

Method D3; 0.85 g (3 mmol) **5c**, 500 µl 40 % HCHO solution (6.66 mmol HCHO), 0.5 g (3.6 mmol) K_2CO_3, reaction time 8 h; the crude product was purified by preparative HPLC (SiO_2 Rp 18, methanol-water-gradient (45 % → 75 % → 90 % methanol, 12mL/min); yield: 116 mg (0.44 mmol, 15 %), colourless solid; mp. 78-79 °C (methanol/water) (ref. [24]: 83.5-84.5°C); CHN analysis for $C_{15}H_{28}O_4$: calc.: C 68.69, H 6.92; found: C 68.50 , H 7.05; ^{1}H-NMR (400.13 MHz, $CDCl_3$, 300 K): δ 1.13 (3H, t, J=7.33 Hz, H_3C-CH_2); 2.19 (3H, s, Ar-CH_3); 2.21 (3H, s, Ar-CH_3); 2.46 (2H, q, J=7.33 Hz, H_3C-CH_2); 4.65 (2H, s, O-CH_2-CO); 5.20 (1H, s, COO*H*); 5.57 (1H, s, =CH_2); 5.85 (1H, s, =CH_2); 6.58 (1H, d, J=8.34 Hz, arom. H_6); 7.04 (1H, d, J=8.34 Hz, arom. H_5); ^{13}C-NMR (100.61 MHz, $CDCl_3$, 300 K): δ 11.78 (arom. C_2-CH_3); 12.54 (H_3C-CH_2); 17.13 (arom. C_3-CH_3); 23.77 (H_3C-CH_2); 65.49 (O-CH_2-); 107.88 (arom. C_6, CH); 126.54 (arom. C_5, CH); 126.67 (arom. qC_2, C-CH_3); 127.64 (qC=CH_2); 133.78 (arom. qC_4); 136.92 (arom. qC_3, C-CH_3); 151.42 (qC=CH_2); 156.53 (arom. qC_1); 172.90 (*C*OOH); 200.80 (Ar-*C*=O); IR (cm^{-1}): $\tilde{\nu}$ = 792 (m, two vicinal Ar-H); 1125, 1246 (s, Ar−O−C); 1579 (m, arom. C=C); 1651 (m, Ar-C=O); 1725 (s, C=O, CO_2H); 2875, 2785, 2959 (w, C−H); 2916 (w, br, O−H, CO_2H); 3038 (w, =C−H).

Ethyl [4-(2-methyl-acryloyl)-phenoxy] Acetate (6b)

1-(4-Hydroxy-phenyl)-propan-1-one (3b)

Method B; 27.0 g (250 mmol) anisole **(2b)**, 34.70 g (375 mmol) propionyl chloride, 2x 50 g $AlCl_3$, 150 mL $CHCl_2$; yield: 21.69 g (144 mmol, 58 %), colourless solid; mp. 143-146 °C (ethanol) (ref. [33]: 147 °C (ethanol)); ^{1}H-NMR (400.13 MHz, $CDCl_3$, 300 K, TMS): δ 1.22 (3H, t, *J*=7.33 Hz, H_3C-CH_2); 2.95 (2H, q, *J*=7.33 Hz, H_3C-CH_2); 5.51 (1H, s, O*H*); 6.88 (2H, m, *J*=2.02 Hz, *J*=8.84 Hz, Ar-*H*); 7.92 (2H, m, *J*=2.02 Hz, *J*=8.84 Hz, Ar-*H*);

Ethyl (4-propionyl-phenoxy) Acetate (5b)

Method C3; 12.77 g (85 mmol) **3b**, 9.54 g (85 mmol) K-*tert*butylat, 15.61 g (93.5 mmol) ethyl bromoacetate, 75 mL THF; yield: 15.6 g (66 mmol, 78 %), colourless needles [34]; mp. 31-32 °C ($CHCl_3$); ^{1}H-NMR (400.13 MHz, $CDCl_3$, 300 K, TMS): δ 1.21 (3H, t, *J*=7.33 Hz, H_3C-CH_2-CO); 1.30 (3H, t, *J*=7.07 Hz, H_3C-CH_2-O); 2.95 (2H, q, *J*=7.33 Hz, H_3C-CH_2-CO); 4.28 (2H, q, *J*=7.07 Hz, H_3C-CH_2-O-); 4.68 (2H, s, -O-CH_2-); 6.94 (2H, m, *J*=2.02 Hz, *J*=8.97 Hz, arom. H_2 + H_6); 7.95 (2H, m, *J*=2.02 Hz, *J*=8.97 Hz, arom. H_3 + H_5);

Ethyl [4-(2-methyl-acryloyl)-phenoxy] Acetate (6b)

Method D1; 2.37 g (10 mmol) **5b**, 2.10 g (15 mmol) hexamethylenetetramine, 2.04 g (20 mmol) acetic anhydride, reaction time 6 h; yield: 857 mg (3.45 mmol, 35 %); mp. 50-52 °C (methanol); CHN analysis for $C_{14}H_{16}O_4$: calc.: C 67.73, H 6.50; found: C 67.85, H 6.60; ^{1}H-NMR (400.13 MHz, $CDCl_3$, 300 K): δ 1.27 (3H, t, *J*=7.07 Hz, O-CH_2-CH_3); 2.02 (3H, s, CH_3); 4.24 (2H, q, *J*=7.07 Hz, O-CH_2-CH_3); 4.65 (2H, s, O-CH_2-CO); 5.51 (1H, s, =C*H*); 5.78 (1H, s, =C*H*); 6.90 (2H, d, *J*=9.09 Hz, arom. H_2 + H_6); 7.75 (2H, d, *J*=9.09 Hz, arom. H_3 + H_5); ^{13}C-NMR (100.61 MHz, $CDCl_3$, 300 K): δ 14.06 (O-CH_2-CH_3); 18.89 (CH_3); 61.46 (O-CH_2-CH_3); 65.15 (O-CH_2-CO); 113.99 (arom. C_2 + C_6, CH); 125.11 (C=CH_2); 130.93 (arom. qC_4); 131.72 (arom. C_3 + C_5, CH); 143.73 (*C*=CH_2); 160.98 (arom. qC_1); 168.21 (O-CH_2-*C*=O); 196.87 (Ar-*C*=O); IR (cm^{-1}): $\tilde{\nu}$ = 792 (s, 1,4-disubst. arom.); 1076 (s, =C−O−C); 1165, 1205 (s, CO_2R, C−O); 1570 (m, arom. C=C); 1599 (m, C=C−C=O); 1636 (m, C=O); 1754 (s, CO_2R, C=O); 2853, 2926, 2973 (w, C−H); 3068 (w, =C−H).

2-Methylene-1-phenyl-butan-1-one (10a)

9.88 g (66.67 mmol) butyrophenone **(9a)**, 14.02 g (100 mmol) hexamethylenetetramine, and 13.61 g (133.34 mmol) acetic anhydride are mixed under N_2-atmosphere and are heated at 80°C. After 16 h the solution is poured into a mixture of 75 mL 0.2 M NaOH and 75 mL CH_2Cl_2. The organic layer is washed with 1 M HCl and brine and is dried with Na_2SO_4. After filtration the solvent is removed *i. vac.* and the crude product is purified by vacuum distillation (70°C, 0.1 mbar); yield: 4.53 g (28.3 mmol, 42 %), yellowish liquid;; CHN analysis for $C_{11}H_{12}O$: calc.: C 82.46, H 7.55; found: C 82.21, H 7.72; ^{1}H-NMR (400.13 MHz, $CDCl_3$, 300 K, TMS): δ 1.13 (3H, t, J=7.45 Hz, H_3C-CH_2); 2.49 (2H, q, J=7.45 Hz, H_3C-CH_2); 5.57 (1H, s, =CH_2); 5.82 (1H, s, =CH_2); 7.43 (2H, m, arom. H_3 + H_5); 7.53 (1H, m, arom. H_4); 7.75 (2H, d, J=8.58 Hz, arom. H_2 + H_6); ^{13}C-NMR (100.61 MHz, $CDCl_3$, 300 K, TMS): δ 12.42 (H_3C); 25.22 (H_3C-CH_2); 124.20 (=CH_2); 128.16 (arom. C_3 + C_5); 129.48 (arom. C_2 + C_6); 132.13 (arom. C_4); 138.00 (arom. qC_1); 149.74 (qC=CH_2); 198.51 (Ar-*C*=O); IR (cm^{-1}): $\tilde{\nu}$ = 693 + 751 (s, monosubst. arom.); 1598 (m, arom. C=C); 1624 (m, C=C−CO); 1655 (s, C=O); 2877, 2934, 2968 (w, C−H); 3063 (w, =C−H).

1-Cyclohexyl-2-methylene-butan-1-one (10b)

1-Cyclohexyl-butan-1-ol (8b)

12.16 g (500 mmol) Mg are suspended in 50 mL abs. EtOEt. 4 g of totally 81.53 g (500 mmol) cyclohexyl bromide are added slowly and carefully. After starting of the reaction the residual cyclohexyl bromide, solved in 125 mL EtOEt is added slowly. The mixture is heated until the Mg is

fully solved. 28.84 g (400 mmol) butanale are added. The mixture is heated under reflux for 2 h. 50 g crushed ice and 6 M HCl are added until solvation of the precipitate. The organic layer is separated and the aqueous layer is extracted with EtOEt. The combined organic layers are washed with 38-40% bisulfite solution, with saturated $NaHCO_3$, and with water. The organic layer is dried with Na_2SO_4, filtered off and removed *i. vac.* The crude product is purified by vacuum distillation (200 mbar, 215°C); yield: 43.9 g (281 mmol, 56 %), colourless liquid; $n_D^{23.5} = 1.4630$ (ref. [35]: $n_D^{22} = 1.4652$); ^{1}H-NMR (400.13 MHz, $CDCl_3$, 300 K, TMS): δ 0.93 (3H, t, J=6.83 Hz, C*H*$_3$); 0.96-1.81 (16 H, m, CH_2, CH, OH); 3.36 (1H, m, C*H*-OH); ^{13}C-NMR (100.61 MHz, $CDCl_3$, 300 K): δ 14.18 (*C*H$_3$); 19.15 (*C*H$_2$); 26.26 (*C*H$_2$); 26.42 (*C*H$_2$); 26.61 (*C*H$_2$); 27.75 (*C*H$_2$); 29.31 (*C*H$_2$); 36.38 (*C*H$_2$); 43.67 (*C*H); 75.95 (*C*H-OH);

1-Cyclohexyl-butan-1-one (9b)

20.66 g (162.8 mmol) oxalylchloride are solved in 75 mL abs. CH_2Cl_2 and cooled at -60 °C. 25.44 g (325.6 mmol) DMSO in 25 mL CH_2Cl_2 are added slowly the temperature not being higher than -50°C. The mixture is stirred until no further gas evolvement can be observed. 25.33 g (148 mmol) **8b** in 25 mL CH_2Cl_2 are added within 5 min, and the mixture is stirred for 15 min. 74.88 g (740 mmol) TEA are added and the mixture is warmed to rt, washed with water, 1% sulfuric acid, 5% $NaHCO_3$ and brine. The organic layer is dried with Na_2SO_4, filtered off and removed *i. vac.* The crude product is purified by vacuum distillation (125 °C, 20 mbar); yield: 9.41 g (61 mmol, 40 %), yellowish liquid; ^{1}H-NMR (400.13 MHz, $CDCl_3$, 300 K, TMS): δ 0.90 (3H, t, J=7.33 Hz; *H*$_3$C-CH$_2$-CH$_2$); 1.18.-1.37 (5H, m, C*H*$_2$); 1.58 (2H, sext, J=7.33 Hz, H$_3$C-C*H*$_2$-CH$_2$); 1.65-1.68 (1H, m, C*H*$_2$); 1.73-1.83 (4H, m, C*H*$_2$); 2.29-2.36 (1H, m, C*H*); 2.41 (2H, t, J=7.33 Hz, H$_3$C-CH$_2$-C*H*$_2$); ^{13}C-NMR (100.61 MHz, $CDCl_3$, 300 K): δ 13.66 (H$_3$*C*); 17.00 (CH$_2$); 25.57 (CH$_2$); 25.77 (CH$_2$); 28.34 (CH$_2$); 42.39 (CH$_2$); 50.66 (*C*H); 213.95 (*C*=O);

1-Cyclohexyl-2-methylene-butan-1-one (10b)

5.87 g (34.88 mmol) **9b** are mixed with 14.02 g (100 mmol) hexamethylenetetramine and 13.61 g (133.34 mmol) acetic anhydride and heated under N_2-atmosphere at 80°C. After 15 h a mixture of 75 mL 0.2 M NaOH and 75 mL CH_2Cl_2 are added. The organic layer is washed with 1 M HCl, 15% $NaHCO_3$, and brine, dried with Na_2SO_4 and removed *i. vac.* The crude product is purified by vacuum distillation (80 °C, 10 mbar) and column chromatography (SiO_2 60, cyclohexane/EtOAc 99+1 V/V); yield: 150 mg (0.9 mmol, 2.6 %), yellowish liquid; $n_D^{22} = 1.4719$ HR-EI-MS (70 eV, m/z, $[M]^+$): calc.: 166.1352; found: 166.1351; ^{1}H-NMR (400.13 MHz, $CDCl_3$, 300 K, TMS): δ 1.01 (3H, t, J=7.46 Hz, *H*$_3$C-CH$_2$); 1.20-1.40 (5H, m, C*H*$_2$); 1.68-1.71 (1H, m, C*H*$_2$); 1.75-1.81 (4H, m, C*H*$_2$); 2.28 (2H, q, J=7.46 Hz, H$_3$C-C*H*$_2$); 2.95-3.01 (1H, m, C*H*); 5.67 (1H, s, C=C*H*$_2$); 5.92 (1H, s, C=C*H*$_2$); ^{13}C-NMR (100.61 MHz, $CDCl_3$, 300 K): δ 12.70 (H$_3$*C*-CH$_2$); 24.17 (H$_3$C-*C*H$_2$); 25.83 (*C*H$_2$); 25.92 (*C*H$_2$); 29.52 (*C*H$_2$); 45.09 (*C*H); 121.54 (C=*C*H$_2$); 149.65 (*qC*=CH$_2$); 205.78 (*C*=O); IR (cm^{-1}): $\tilde{\nu}$ = 993 (s, C=C−CO); 1627 (w, C=C−CO); 1671 (s, C=O); 2855 (m, C−H); 2930 (s, C−H); 3094 (w, =C−H).

N-Butyl-2-[2,3-dichloro-4-(2-methylene-butyryl)-phenoxy] Acetic Amide (Etacrynic acid-n-butylamide) (11a)

Method E; 479 mg (1.58 mmol), 120.6 mg (1.65 mmol) *n*-butyl amine, reaction time 20 d; the product was purified by column chromatography (SiO_2 60, cyclohexane/EtOAc 1+1 V/V); yield: 429 mg (1.20 mmol, 73 %), colourless solid; mp. 95-96 °C (EtOAc); CHN analysis for $C_{17}H_{21}Cl_2NO_3$; calc.: C 56.99, H 5.91, N 3.91; found: C 57.15, H 5.97, N 4.00; ^{1}H-NMR (400.13 MHz, $CDCl_3$, 300 K, TMS): δ 0.96 (3H, t, J=7.33 Hz, CH$_2$-CH$_2$-C*H*$_3$); 1.15 (3H, t, J=7.46 Hz, *H*$_3$C-CH$_2$); 1.40 (2H, sext., J=7.33 Hz, CH$_2$-C*H*$_2$-CH$_3$); 1.57 (2H, quin., J=7.08 Hz, J=7.33 Hz, C*H*$_2$-CH$_2$-CH$_3$); 2.48 (2H, q, J=7.46 Hz, H$_2$C-C*H*$_2$); 3.39 (2H, q, J=7.07 Hz, NH-C*H*$_2$); 4.57 (2H, s, O-C*H*$_2$-CO); 5.59 (1H, s, C=C*H*$_2$); 5.96 (1H, s, C=C*H*$_2$); 6.76 (1H, s, N*H*); 6.87 (1H, d, J=8.59 Hz, arom. *H*$_6$); 7.19 (1H, d, J=8.59 Hz, arom. *H*$_5$); ^{13}C-NMR (100.61 MHz, $CDCl_3$, 300 K, TMS): δ 12.41 (H$_3$*C*-CH$_2$); 13.70 (CH$_2$-CH$_2$-*C*H$_3$); 20.01 (CH$_2$-*C*H$_2$-CH$_3$); 23.43 (H$_3$C-*C*H$_2$); 31.49 (*C*H$_2$-CH$_2$-CH$_3$); 38.91 (NH-*C*H$_2$); 68.27 (O-*C*H$_2$-CO); 110.93 (arom. *C*$_6$, CH); 122.94 (arom. *qC*$_4$); 127.26 (arom. *C*$_5$, CH); 128.68 (qC=*C*H$_2$); 131.51 (arom. *qC*$_3$, C-Cl); 134.19 (arom. *qC*$_2$, C-Cl); 150.24 (*qC*=CH$_2$); 154.57 (arom. *qC*$_1$); 166.54 (O-CH$_2$-*C*O); 195.52 (Ar-*C*O); IR (cm^{-1}): $\tilde{\nu}$ = 1091 (m, Ar−Cl); 1244 (m-s, Ar−O−CH$_2$); 1468 (m, arom. C=C); 1555 (m-s, CO−N−H); 1586 (m, C=C−C=O); 1660 (s, C=C−C=O); 2872, 2936, 2958 (m, C−H); 3078 (w, =C−H); 3292 (m, br, N−H).

N-tertButyl-2-[2,3-dichloro-4-(2-methylene-butyryl)-phenoxy] Acetic Amide (Etacrynic acid-tert-butylamide (11b)

Method E; 116 mg (1.58 mmol) *tert*-butyl amine, reaction time 22 d; the product was purified by column chromatography (SiO_2 60, cyclohexane/EtOAc 1+1 V/V); yield: 397 mg (1.1 mmol, 70 %), colourless solid; mp. 94-96 °C (cyclohexane/EtOAc); CHN analysis for $C_{17}H_{21}Cl_2NO_3$: calc.: C 56.99, H 5.91, N 3.91; found: C 57.11, H 5.99, N 3.98; ^{1}H-NMR (400.13 MHz, $CDCl_3$, 300 K, TMS): δ 1.15 (3H, t, J=7.45 Hz, *H*$_3$C-CH$_2$); 1.43 (9H, s, C-(*CH*$_3$)$_3$); 2.48 (2H, q, J=7.45 Hz, H$_3$C-C*H*$_2$); 4.45 (2H, s, O-C*H*$_2$-CO-); 5.58 (1H, s, *H*$_2$C=C-); 5.95 (1H, s, *H*$_2$C=C); 6.66 (1H, s, N*H*); 6.85 (1H, d, J=8.47 Hz, arom. *H*$_6$); 7.19 (1H, d, J=8.47 Hz, arom. *H*$_5$); ^{13}C-NMR (100.61 MHz, $CDCl_3$, 300 K, TMS): δ 12.41 (H$_3$*C*-CH$_2$); 23.44 (H$_3$C-*C*H$_2$); 28.74 (*q*C(*C*H$_3$)$_3$); 51.47 (*qC*(CH$_3$)$_3$); 68.46 (O-*C*H$_2$-CO); 110.95 (arom. *C*$_6$, CH); 122.89 (arom. *qC*$_4$ or arom. *qC*$_2$, C-Cl); 127.26 (arom. *C*$_5$, CH); 128.64 (qC=*C*H$_2$); 131.47 (arom. *qC*$_3$, C-Cl); 134.10 (arom. *qC*$_4$ or arom. *qC*$_2$, C-Cl); 150.25 (*qC*=CH$_2$); 154.59 (arom. *qC*$_1$); 165.63 (O-CH$_2$-*C*O); 195.55 (Ar-*C*O); IR (cm^{-1}): $\tilde{\nu}$ = 1082 (m, Ar−Cl); 1264 (m Ar−O−CH$_2$); 1466 (m, arom. C=C); 1567 (m, C=C−C=O); 1658 (s, C=C−C=O); 2792, 2929, 2965 (w, C−H); 3088 (w, =C−H); 3265 (w, br, N−H).

2-[2,3-Dichloro-4-(2-methylene-butyryl)-phenoxyl-N-hexyl-acetic Amide (Etacrynic acid-N-hexylamide (11c)

Method E; 167 mg (1.65 mmol) *N*-hexyl amine; reaction time 25 d; the product was purified by column chromatography (SiO_2 60, cyclohexane/EtOAc 1+1 V/V); yield: 577 mg (1.49 mmol, 91 %), colourless solid; mp. 63-65 °C (EtOAc); CHN analysis for $C_{19}H_{25}Cl_2NO_3$: calc.: C

59.07, H 6.52, N 3.63; found: C 59.38, H 6.62, N 3.79; HR-EI-MS (70 eV, m/z, $[M]^+$): calc.: 385.1206; found: 385.1206; ^{1}H-NMR (400.13 MHz, $CDCl_3$, 300 K, TMS): δ 0.90 (3H, t, J=6.82 Hz, CH_2-CH_2-CH_3); 1.15 (3H, t, J=7.46 Hz H_3C-CH_2); 1.29-1.40 (6H, m, NH-CH_2-CH_2-CH_2-CH_2-CH_2-CH_3); 1.58 (2H, quin., J=6.74 Hz, J=7.33 Hz, NH-CH_2-CH_2); 2.47 (2H; q, J=7.46, H_3C-CH_2); 3.38 (2H, q, J=6.74 Hz, NH-CH_2-CH_2); 4.57 (2H, s, O-CH_2-CO); 5.59 (1H, s, qC=CH_2); 5.96 (1H, s, qC=CH_2); 6.78 (1H, s, N*H*); 6.87 (1H, d, J=8.47 Hz, arom. H_6); 7.19 (1H, d, J=8.47 Hz, arom H_5); ^{13}C-NMR (100.61 MHz, $CDCl_3$, 300 K, TMS): δ 12.42 (*C*H_3-CH_2-C=CH_2); 13.99 (*C*H_3-CH_2-CH_2); 22.56 (CH_3-*C*H_2-CH_2-); 23.44 (CH_3-*C*H_2-C=CH_2); 26.50 (NH-CH_2-CH_2-*C*H_2); 29.39 (NH-CH_2-*C*H_2); 31.41 (CH_3-CH_2-*C*H_2-); 39.19 (NH-*C*H_2-CH_2); 68.27 (O-*C*H_2-CO); 110.92 (arom. C_6, CH); 122.93 (arom. qC_4 or arom. qC_2; C-Cl); 127.27 (arom. C_5, CH); 128.66 (qC=*C*H_2); 131.50 (arom. qC_3, C-Cl); 134.18 (arom. qC_4 or arom. qC_2; C-Cl); 150.24 (*qC*=CH_2); 154.58 (arom qC_1); 166.52 (O-CH_2-*C*O-); 195.50 (Ar-*C*O-); IR (cm^{-1}): $\tilde{\nu}$ = 1092 (m, Ar−Cl); 1252 (m-s, Ar−O−CH_2); 1468 (m, arom. C=C); 1555 (m-s, CO−N−H); 1586 (m, C=C−C=O); 1661 (s, C=C−C=O); 2857, 2929 (m, C−H); 3090 (w, =C−H); 3301 (m, br, N−H).

Benzyl (S)-2-{2-[2,3-dichloro-4-(2-methylene-butyryl)-phenoxy]-acetylamino}-propionate ((S)-Etacrynic acid-(benzyloxy-alanyl) Amide (11d)

Method E; 356 mg (1.65 mmol) L-Ala-*O*-benzyl ester, hydrochloride, reaction time 3 d; the product was purified by column chromatography (SiO_2 60, cyclohexane/EtOAc 1+1 V/V); yield: 707 mg (1.52 mmol, 92 %), colourless solid; mp. 59-61 °C (EtOAc); α_D^{25} = - 11.89° (methanol, c = 0.90 g/100 mL); CHN analysis for $C_{23}H_{23}Cl_2NO_5$: calc.: C 59.49, H 4.99, N 3.02; found: C 59.61, H 5.04, N 2.98; ^{1}H-NMR (400.13 MHz, $CDCl_3$, 300 K, TMS): δ 1.15 (3H, t, J=7.32 Hz, H_3C-CH_2); 1.51 (3H, d, J=7.32 Hz, Ala-CH_3); 2.48 (2H, q, J=7.33 Hz, H_3C-CH_2-); 4.58 (2H, dd, J=2.02 Hz, J=14.40 Hz, -O-CH_2-); 4.73 (1H, q, J=7.32 Hz, Ala-C*H*); 5.21 (2H, dd, J=2.28 Hz, J=12.25 Hz, benzyl-CH_2); 5.58 (1H, s, =CH_2); 5.95 (1H, s, =CH_2); 6.85 (1H, d, J=8.47 Hz, arom. H_6); 7.17 (1H, d, J=8.47 Hz, arom. H_5); 7.32-7.40 (6H, m, benzyl-C*H*, N*H*); ^{13}C-NMR (100.61 MHz, $CDCl_3$, 300 K, TMS): δ 12.41 (*C*H_3-CH_2-); 18.39 (Ala-*C*H_3); 23.43 (CH_3-*C*H_2-); 48.04 (Ala-*C*H); 67.37 (benzyl-*C*H_2); 68.19 (-O-*C*H_2-CO-); 110.98 (arom. C_6, CH); 123.22 (arom. $C_{2/3}$, C-Cl); 127.15 (arom. C_5, CH); 128.23 (arom. benzyl-*C*H); 128.56 (arom. benzyl-*C*H); 128.69 (C=*C*H_2); 131.55 (arom. $C_{2/3}$, C-Cl); 134.30 (arom. qC_4); 135.19 (arom. benzyl-$qC_{1'}$); 150.23 (*C*=CH_2); 154.56 (arom. qC_1); 166.34 (-*C*O-NH); 172.04 (-*C*O-O-benzyl); 195.56 (-*C*O-Ar); IR (cm^{-1}): $\tilde{\nu}$ = 696, 731 (m, monosubst. arom.); 1079 (w, Ar−Cl); 1203, 1250 (m-s, CO_2R, C−O); 1466 (m, arom. C=C); 1550 (m, CO−NH); 1584 (m, C=C−C=O); 1663 (s, C=C−C=O); 1739 (m-s, CO_2R, C=O); 2826, 2938, 2961 (w, C−H); 3032, 3098 (w, =C−H); 3304 (w, br, N−H).

1-{2,3-Dichloro-4-[2-(4-ethyl-piperazin-1-yl)-2-oxo-ethoxy]-phenyl}-2-methylene-butan-1-one (etacrynic acid-(N-ethyl-piperazin)-amide) (11e)

Method E; 377 mg (3.30 mmol) *N*-ethyl piperazine, reaction time 21 d; the product was purified by column chromatography (SiO_2 60, chloroform/methanol 19+1 V/V); yield: 138 mg (0.35 mmol, 21 %), colourless solid; mp. 71-73 °C (chloroform/methanol); HR-EI-MS (70 eV, m/z, $[M]^+$): calc.: 398.1164; found: 398.1165; ^{1}H-NMR (400.13 MHz, $CDCl_3$, 300 K, TMS): δ 1.09 (3H, t, J=7.20 Hz, N-CH_2-CH_3); 1.15 (3H, t, J=7.33 Hz, H_3C-CH_2); 2.40-2.50 (8H, m, H_3C-CH_2-N-CH_2; H_3C-CH_2, H_3C-CH_2-N-CH_2); 3.65 (4H, t, J=5.05 Hz, CO-N-CH_2); 4.83 (2H, s, O-CH_2-CO); 5.60 (1H, s, qC=CH_2); 5.94 (1H, s, qC=CH_2); 6.97 (1H, d, J=8.59 Hz, arom. H_6); 7.15 (1H, d, J=8.59 Hz, arom. H_5); ^{13}C-NMR (100.61 MHz, $CDCl_3$, 300 K, TMS): δ 11.89 (N-CH_2-*C*H_3); 12.41 (H_3*C*-CH_2); 23.44 (H_3C-*C*H_2); 42.24 (CO-N-*C*H_2); 45.47 (CO-N-*C*H_2); 52.18 (H_3C-*C*H_2-N); 52.30 (CH_2-*C*H_2-N-CH_2-CH_3); 52.94 (CH_2-*C*H_2-N-CH_2-CH_3); 68.74 (O-*C*H_2-CO); 110.80 (arom. C_6, CH); 122.85 (arom. qC_4 or qC_2, C-Cl); 127.07 (arom. C_5, CH); 128.59 (qC=*C*H_2); 131.39 (arom. qC_3, C-Cl); 133.70 (arom. qC_4 or qC_2, C-Cl); 150.24 (*qC*=CH_2); 155.32 (arom. qC_1); 165.06 (O-CH_2-*C*O); 195.82 (Ar-*C*O); IR (cm^{-1}): $\tilde{\nu}$ = 1016 (m, Ar−Cl); 1239 (m-s, Ar−O−CH_2); 1474 (m, arom. C=C); 1583 (m, C=C−C=O); 1649 (s, C=C−C=O); 2809, 2853, 2932, 2967 (m, C−H); 3087 (w, =C−H).

1-[2,3-Dichloro-4-(2-morpholin-4-yl-2-oxo-ethoxy)-phenyl]-2-methylene-butan-1-one (etacrynic acid-morpholine amide) (11f)

Method E; 144 mg (1.65 mmol) morpholine, reaction time 23 d; the product was purified by column chromatography (SiO_2 60, EtOAc); yield: 468 mg (1.26 mmol, 74 %), colourless solid; mp. 113-115 °C (EtOAc); CHN analysis for $C_{17}H_{19}Cl_2NO_4$: calc.: C 54.85, H 5.14, N 3.76; found: C 55.25, H 5.30, N 3.99; ^{1}H-NMR (400.13 MHz, $CDCl_3$, 300 K, TMS): δ 1.15 (3H, t, J=7.46 Hz, H_3C-CH_2); 2.47 (2H, q, J=7.46 Hz, H_3C-CH_2); 3.66 (8H, m, morpholine-CH_2-); 4.83 (2H, s, O-CH_2-CO-); 5.60 (1H, s, C=CH_2); 5.94 (1H, s, C=CH_2); 6.98 (1H, d, J=8.47 Hz, arom. H_6); 7.15 (1H, d, J=8.47 Hz, arom. H_5); ^{13}C-NMR (100.61 MHz, $CDCl_3$, 300 K, TMS): δ 12.40 (H_3*C*-CH_2); 23.43 (H_3C-*C*H_2); 42.60 (morpholine-*C*H_2); 46.10 (morpholine-*C*H_2); 66.79 (morpholine-*C*H_2); 68.68 (O-*C*H_2-CO); 110.71 (arom. C_6, CH); 122.81 (arom. qC_2, or arom. C-Cl qC_4); 127.09 (arom. C_5, CH); 128.64 (C=*C*H_2); 131.46 (arom. qC_3, C-Cl); 133.86 (arom. qC_2, or arom. C-Cl qC_4); 150.21 (*qC*=CH_2); 155.10 (arom. qC_1); 165.29 (O-CH_2-*C*O); 195.73 (Ar-*C*O); IR (cm^{-1}): $\tilde{\nu}$ = 1035, 1082 (m, Ar−Cl); 1240 (s, Ar−O−CH_2); 1476 (m, arom. C=C); 1587 (m, C=C−C=O); 1654 (s, C=C−C=O + C=O−NR_2); 2867, 2928, 2957 (w, C−H).

ACKNOWLEDGEMENTS

This work was supported by the Deutsche Forschungsgemeinschaft (SCHI 441/3-1, Sonderforschungsbereich 630/TP A4) and the Fonds der Chemischen Industrie.

ABBREVIATIONS

abs = Absolute

DCC = *N*,*N*`-Dicyclohexylcarbodiimide

DMS = Dimethylsulfate

DMSO = Dimethylsulfoxide

DTNB = 5,5'-Dithiobis(2-nitrobenzoate)

eq. = Equivalent

HOSuc = *N*-Hydroxysuccinimide

i. vac. = *In vacuo*

L-BAPA = *N*-α-Benzoyl-*L*-arginine-p-nitroanilide

rt = Room temperature

TEA = Triethyl amine

THF = Tetrahydrofurane

TMDM = *N,N,N',N'*-Tetramethyldiaminomethane

TMS = Tetramethyl silane

REFERENCES

[1] Lecaille, F.; Kaleta, J.; Broemme, D.; *Chem. Rev.* **2002**, *102*, 4459-4488.

[2] Leung-Toung, R.; Li, W.; Tam, T.F.; Karimian, K.; *Curr. Med. Chem.* **2002**, *9*, 979-1002.

[3] Powers, J.C.; Asgian, J.L.; Ekici, O.D.; James, K.E.; *Chem. Rev.* **2002**, *102*, 4639-4750.

[4] Schirmeister, T.; Klockow, A.; *Mini Rev. Med. Chem.* **2003**, *3*, 585-596.

[5] Matthews, D. A.; Dragovich, P. S.; Webber, S. E.; Fuhrman, S. A.; Patick, A. K.; Zalman, L. S.; Hendrickson, T. F.; Love, R. A.; Prins, T. J.; Marakovits, J. T.; Zhou, R.; Tikhe, J.; Ford, C. E.; Meador, J. W.; Ferre, R. A.; Brown, E. L.; Binford, S. L.; Brothers, M. A.; DeLisle, D. M.; Worland, S. T.; *Proc. Natl. Acad. Sci. U S A* **1999**, *96*, 11000-11007.

[6] Schirmeister, T.; Kaeppler, U.; *Mini Rev. Med. Chem.* **2003**, *3*, 361-373.

[7] Roush, W. R.; Gwaltney, S. L.; Cheng, J.; Scheidt, K. A.; McKerrow, J. H.; Hansell, E.; *J. Am. Chem. Soc.* **1998**, *120*, 10994 - 10995.

[8] Graul, A.; Castaner, J.; *Drugs Fut.* **2001**, *26*, 62.

[9] Engel, J. C.; Doyle, P. S.; Hsieh, I.; McKerrow, J. H.; *J. Exp. Med.* **1998**, *188*, 725-734.

[10] CRA-3316, *Drug Data Report* **2002** *24*, 356.

[11] Kaeppler, U.; Schirmeister, T.; *Arch. Pharm. Pharm. Med. Chem.* **2002**, *335*, Suppl. 1, 86.

[12] Broemme, D.; Kaleta, J.; *Curr. Pharm. Des.* **2002**, *8*, 1639-1658.

[13] Sajid, M.; McKerrow, J. H.; *Mol. Biochem. Parasitol.* **2002**, *120*, 1-21.

[14] Rosenthal, P. J.; Sijwali, P. S.; Singh, A.; Shenai, B. R.; *Curr. Pharm. Des.* **2002**, *8*, 1659-1672.

[15] Woltersdorf, O. W., Jr.; Robb, C. M.; Bicking, J. B.; Watson, L. S.; Cragoe, E. J., Jr.; *J. Med. Chem.* **1976**, *19*, 972-975.

[16] Schultz, E. M.; Sprague, J. M.; *Patent US 3255241*; **1966**; 48 p.

[17] Bhattacharya, A.; Segmuller, B.; Ybarra, A.; *Synth. Commun.* **1996**, *26*, 1775-1784.

[18] Cragoe, E. J., Jr.; Woltersdorf, O. W., Jr.; Gould, N. P.; Pietruszkiewicz, A. M.; Ziegler, C.; Sakurai, Y.; Stokker, G. E.; Anderson, P. S.; Bourke, R. S.; Kimelberg, H. K.; *J. Med. Chem.* **1986**, *29*, 825-841.

[19] Cragoe, E. J., Jr.; *Patent US 3478085*; **1969**; 7 p.

[20] Tidwell, T. T.; Organic Reactions (New York) **1990**, *39*, 297-572.

[21] Patent De 1810053; **1970**; 30 p.

[22] Tian, W.-X.; Tsou, C.-L.; *Biochemistry* **1982**, *21*, 1028-1032.

[23] Koechel, D. A.; Cafrung, E. J.; *J. Med. Chem.* **1973**, *16*, 1147-1152.

[24] Schultz, E. M.; Cragoe, E. J., Jr.; Bicking, J. B.; Bolhofer, W. A.; Sprague, J. M.; *J. Med. Pharmaceut. Ch.* **1962**, *5*, 660-662.

[25] Sprague, J. M.; *Top. Med. Chem.* **1986**, *2*, 1-63.

[26] Rang, H. P.; Dale, M. M.; Ritter, J. M.; Moore, P. K., Pharmacology, 5th edition, Chirchill Livingstone, Ed.: Edinburh **2003**, p. 362.

[27] Hasannejad, H.; Takeda, M.; Taki, K.; Shin, H. J.; Babu, E.; Jutabha, P.; Khamdang, S.; Aleboyeh, M.; Onozato, M. L.; Tojo, A.; Enomoto, A.; Anzai, N.; Narikawa, S.; Huang, X.-L.; Niwa, T.; Endou, H.; *J. Pharm. Exp. Ther.* **2004**, *308*, 1021-1029.

[28] Plattner, J. J.; Fung, K. L.; Smital, J. R.; Lee, C.-M.; Crowley, S. R.; Pernet, A. G.; Bunnell, P. R.; Buckner, S. A.; Sennello, L. T., *J. Med. Chem.* **1984**, *27*, 1587-1596.

[29] Schirmeister, T.; *Arch. Pharm. Pharm. Med. Chem.* **1996**, *329*, 239-241.

[30] GraFit® vers. 3.03, **1994**, Erithacus Software Ltd. London.

[31] Patent De 1929732; **1970**; 6 p.

[32] Patent NL 6604297; **1966**; 44 p.

[33] Nakazawa, K.; Matsuura, S.; Kusuda, K.; *Yakugaku Zasshi* **1954**, *74*, 495-497.

[34] De Cointet, P.; Loppinet, V.; Sornay, R.; Morinere, J. L.; Boucherle, A.; Renson, F. J.; Voegelin, H. and Dumont, C.; *Chim. Ther.* **1973**, *8*, 574-587.

[35] Pineau *Bull. Soc. Chim. Fr.* **1936**, *5*, 2195.

Received: 24 November, 2004 Accepted: 28 January, 2005