– die Beeinflussung des Schweregrades (0 = nicht vorhanden bis 3 = schwer) gastrointestinaler Symptome, wie Druck-, Schwere- oder Völlegefühl, Übelkeit, Aufstoßen, Erbrechen, Blähungen, Nahrungsmittelunverträglichkeit, Durchfall, Stuhlunregelmäßigkeiten, Verstopfung und Gefühl der unvollständigen Stuhlentleerung, sowie allgemeiner Symptome, wie Gewichtsabnahme und Leistungsschwäche,
– die Beurteilung des Behandlungsergebnisses durch den Prüfarzt sowie
– die Clinical Global Impressions (CGI) – Items I (Beurteilung des Schweregrades einer Krankheit), II (Gesamtbeurteilung der Zustandsänderung) und III (Nutzen/Risiko-Index).

Die CGI stellen eine validierte psychometrische Skala dar, deren Anwendung aufgrund der starken psychischen Komponente der untersuchten Indikation angezeigt erschien [23, 24].

Die Präparate wurden als therapeutisch äquivalent angesehen, wenn der Erwartungswert für die Zielgröße (intraindividuelle Differenz der Schmerzintensität zwischen Therapiebeginn und Therapieende) unter der Behandlung mit dem Prüfpräparat um maximal 10% der Länge der visuellen Analogskala kleiner war als unter der Behandlung mit dem Referenzpräparat. Die Null-Hypothese der Nicht-Äquivalenz wurde mit dem modifizierten einseitigen t-Test für verschobene Null-Hypothesen zu einer Irrtumswahrscheinlichkeit von $\alpha = 0,05$ getestet. Die statistische Auswertung erfolgte primär nach dem Intent-to-treat-Ansatz. Ergänzend wurde eine Per-protocol-Analyse durchgeführt, bei der nur die Patienten berücksichtigt wurden, für die keine relevanten Prüfplanverletzungen vorlagen. Fehlende Werte wurden nach der Last-observation-carried-forward-Methode ersetzt. Die Begleitgrößen wurden deskriptiv ausgewertet. Bei der Bestimmung des Stichprobenumfangs wurde die Patientenzahl von 200 so gewählt, daß bei einer maximalen Drop-out-Rate von 20% die Nullhypothese der Nicht-Äquivalenz bei einer Fehlerwahrscheinlichkeit 1. Art von $\alpha = 0,05$ und einer Fehlerwahrscheinlichkeit 2. Art von $\beta = 0,10$ dann abgelehnt werden konnte, wenn bezüglich der Zielgröße für Prüf- und Referenzpräparat die gleiche Wirksamkeit vorlag und die Standardabweichung in beiden Therapiegruppen 20% der Länge der visuellen Analogskala betrug.

[3] Enteroplant®; Spitzner Arzneimittel GmbH, ein Unternehmen der Firmengruppe Dr. Willmar Schwabe GmbH & Co., 76209 Karlsruhe
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In vitro cytotoxic activity of naphtho[1,2-*b*]furan, furo[2,3-*f*], furo[2,3-*g*] and furo[3,2-*g*]quinoline derivatives

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Some naphtho[1,2-*b*]furan, furo[2,3-*f*], furo[2,3-*g*] and furo[3,2-*g*]quinoline derivatives have been submitted to *in vitro* cytotoxic tests towards L 1210, MDA-MB 231 and PC_3 cell lines. Among them, the furoquinone structures exhibited the most interesting IC_{50} values.

1. Introduction

As part of our interest towards the *in vitro* cytotoxic activity of heteropolycyclic compounds [1–5], we investigated the synthetic usefulness of α, β unsaturated *N*,*N*-dimethylhydrazones [6] to afford tricyclic quinones possessing a fused furan ring. Previously, we reported the *in vitro* cytotoxic activities of 5-hydroxynaphtho[1,2-*b*]furan and 5-hydroxyfuro[2,3-*f*] quinoline derivatives [2, 5]. In the aim to complete this work with the biological assays of *ortho*- and *para*-furoquinones, we developed efficient routes to their syntheses. Since no examples of furoquinolinediones with such biological properties have been yet described so far, we report in this paper the IC_{50} values of these quinones and their derivatives towards L 1210, MDA-MB231 and PC_3 cell lines.

2. Investigations, results and discussion

In our investigations on the reactivity of 2-ethoxybut-2-enal *N*,*N*-dimethylhydrazone **1b** towards naphthoquinone **2a** or azanaphthoquinones **2b** or **2c**, we obtained in the presence of trifluoroacetic acid 5-hydroxynaphtho[1,2-*b*]furan and 5-hydroxyfuro[2,3-*f*]quinoline derivatives **6**, through a [3 + 2] process (Scheme) [7]. In order to oxidize the phenolic group of **6** by means of bis(trifluoroacetoxy)iodo-benzene (PIFA) into the corresponding *ortho*-quinones **9**, we converted the hydrazone function of **6** into a cyano group by treatment with magnesium monoperoxyphtalate hexahydrate (MMPP) [8]. Thus, compounds **7** were isolated and then easily acetylated into the hitherto unknown products **8**.

On the other hand, structural analogoues of *ortho*-quinones **9** were synthesized using a Diels-Alder strategy between azadiene **1b** and the benzo[*b*]furan-4,5-diones **3** [8]. Thus, the furoquinolinediones **10** were obtained, while a reductive acetylation of **10b** led to the corresponding new diacetoxylated derivative **11**.

Finally, an efficient way to reach regiospecifically the *para*-furoquinones **12** and **13** was achieved through [4 + 2]

Scheme 1

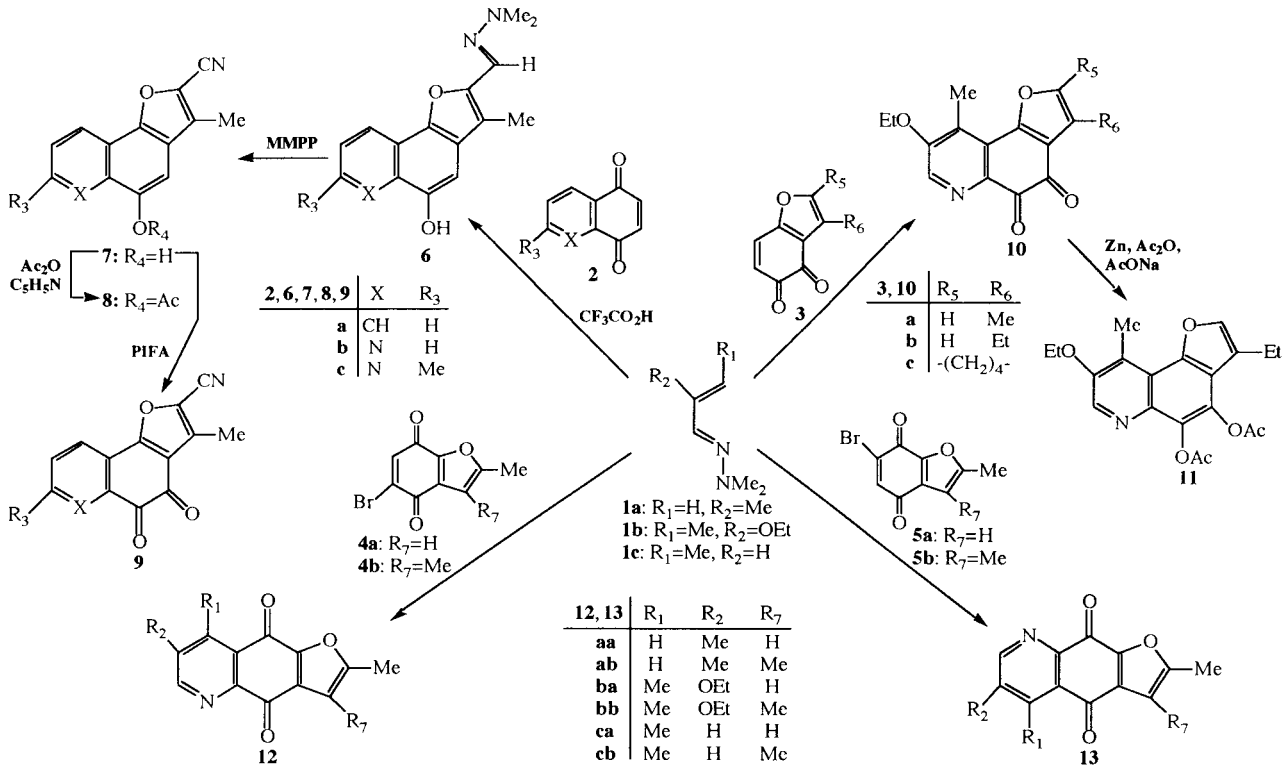

Table: Cytotoxic activity against murine lymphocytic leukemia cells L 1210, human mammary adenocarcinoma cells MDA-MB 231 and human prostate tumoral cells PC_3

Compd.	L 1210 IC_{50}		MDA-MB 231 IC_{50}		PC_3 IC_{50}	
	(μg/ml)	(μmol/l)	(μg/ml)	(μmol/l)	(μg/ml)	(μmol/l)
8a	8.82	33.28	>10		>10	
8b	0.30	1.14	0.74	2.79	0.58	2.17
8c	0.35	1.25	1.77	6.33	2.96	10.58
9a	0.18	0.75	0.36	1.54	0.57	2.39
9b [8]	0.05	0.21	0.49	2.08	0.24	1.00
9c [8]	0.12	0.48	0.43	1.72	0.34	1.35
10a [8]	0.05	0.19	0.10	0.37	0.09	0.33
10b [8]	0.10	0.34	0.14	0.50	0.85	2.98
10c [8]	0.13	0.40	0.46	1.47	1.10	3.54
11	0.04	0.14	0.35	0.93	0.93	2.24
12aa [9]	0.13	0.57	0.22	0.96	0.07	0.31
12ab [9]	0.14	0.58	0.22	0.91	0.14	0.60
12ba [9]	1.20	4.42	2.22	8.18	0.85	3.12
12bb [9]	>10		>10		>10	
12ca [9]	0.15	0.65	0.33	1.46	0.07	0.31
12cb [9]	0.60	2.49	0.88	3.67	0.34	1.43
13ab [9]	0.07	0.30	0.29	1.21	0.17	0.71
13bb [9]	>10		>10		>10	
13cb [9]	0.45	1.86	1.10	4.58	0.29	1.20
Doxorubicin	0.019	0.035	0.045	0.082	0.28	0.515
Taxol	0.025	0.029	0.003	0.003	0.002	0.002

cycloadditions between azadienes **1** and 5- or 6-bromoquinones **4** or **5** [9].

Compounds **8** to **13** were tested *in vitro* for cytotoxic activity against a murine lymphocytic leukemia cell line (L 1210), a human tumoral cell line (MDA-MB 231) established from a mammary adenocarcinoma [10] and a human prostate tumoral cell line (PC_3) [11, 12]. The results of this study are given in the Table.

Concerning the naphtho[1,2-*b*]furan and furo[2,3-*f*]quinoline series, compounds **8** exhibited higher IC_{50} values than the corresponding *ortho*-quinones **9**. The results obtained from the latter are, on the other hand, comparable to those of products **10**. This would indicate that neither the intracyclic nitrogen (**9a** versus **9b** or **9c**) nor the nature of the substituent on the pyridine ring (**9b** versus **9c** or versus compounds **10**), significantly influence the *in vitro* cytotoxic activity of these furoquinones. Moreover, it is important to note that similar IC_{50} values were also obtained from the diacetoxylated derivative **11**. Thus, if the *ortho*-quinone function seems to be not required for the cytotoxic activity, the presence of one oxygen atom at C-4 and another one at C-5 appears, on the contrary, essential (compound **9**, **10** and **11** versus products **8**). Lastly, the results observed with the [4 + 2] cycloadducts **10** vary with the substitution on the furan ring. Indeed, the best IC_{50} values were obtained from the 3-methyl derivative **10a** and the worst from the tetracyclic structure **10c**.

In the *para*-quinones series, the nature of the substituent on the piridine ring is important. Thus, the compounds synthesized from azadiene **1b** (**12ba, 12bb, 13bb**) exhibited no significant IC_{50} values, while better results were observed with products obtained from **1c** (**12ca, 12cb, 13cb**) and especially from **1a** (**12aa, 12ab, 13ab**). Moreover, when the furan ring was disubstituted (**12ab, 12bb, 12cb**) the cytotoxic activity decreased except for **12ab**, compared to the monosubstituted derivatives (**12aa, 12ba, 12ca**). On the other hand, if we compare the IC_{50} values of the dimethyl compounds **12** with those of their corresponding regioisomers **13ab, 13bb** and **13cb**, the regioisomeric structure seems to have no significant influence.

In conclusion, in the case of L 1210 and MDA-MB 231 cell lines, we can observe that the naphtho[1,2-*b*]furan and furo[2,3-*f*]quinoline derivatives lead, on average (calculated from the six best IC_{50}), to better results than those observed for furo[2,3-*g*] and furo[3,2-*g*]quinoline skeletons. In contrast, it is the opposite with the PC_3 cell line.

3. Experimental

All the results of elemental analyses (C, H, N) were in an acceptable error range.

3.1. Synthesis of acetoxylated compounds

3.1.1. 5-Acetoxy-2-cyano-3-methylnaphtho[1,2-b]furan (**8a**)

Compound **7a** (0.223 g, 1 mmol) [5] was dissolved in CH_2Cl_2 (30 ml). At room temperature, pyridine (0.716 g, 10 mmol) and $(CH_3CO)_2O$ (0.458 g, 5 mmol) were added. The mixture was stirred 24 h. Then, CH_3OH (15 ml) was added. After a continuing stirring for 1 h, H_2O (40 ml) and Et_2O (80 ml) were added. The organic layer was washed with H_2O (3 × 30 ml) and dried over $MgSO_4$. The residue obtained after evaporation under vacuum was purified by crystallization from EtOH to give **8a** as a yellow powder. Yield: 0.254 g. 96%. M.p. 155–158 °C; IR (KBr): 2220 (CN), 1775 (CO) cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz, δ, ppm) 8.33 (dd, 1H, J = 7.8 and 1.4 Hz, 6-H or 9-H); 7.94 (dd, 1H, J = 7.8 and 1.4 Hz, 6-H or 9-H); 7.67 (m, 2H, 7-H and 8-H); 7.38 (s, 1H, 4-H); 2.51 (s, 3H, 3-CH_3 or $OCOCH_3$); 2.50 (s, 3H, 3-CH_3 or $OCOCH_3$).
$C_{16}H_{11}NO_3$

3.1.2. 5-Acetoxy-2-cyano-3-methylfuro[2,3-f]quinoline (**8b**)

Following the procedure used to prepare **8a**, compound **8b** was obtained from **7b** [8] as a yellow powder. Yield: 0.261 g. 98%. M.p. 144–146 °C; IR (KBr); 2220 (CN), 1770 (CO) cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz, δ, ppm) 9.01 (dd, 1H, J = 4.3 and 1.4 Hz, 7-H); 8.65 (dd, 1H, J = 8.4 and 1.4 Hz, 9-H); 7.63 (dd, 1H, J = 8.4 and 4.3 Hz, 8-H); 7.60 (s, 1H, 4-H); 2.54 (s, 3H, 3-CH_3 or $OCOCH_3$); 2.53 (s, 3H, 3-CH_3 or $OCOCH_3$).
$C_{15}H_{10}N_2O_3$

3.1.3. 5-Acetoxy-2-cyano-3,7-dimethylfuro[2,3-f]quinoline (**8c**)

Following the procedure used to prepare **8a**, compound **8c** was obtained from **7c** [8] as a yellow powder. Yield: 0.196 g. 70%. M.p. 168–172 °C; IR (KBr); 2220 (CN), 1760 (CO) cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz, δ, ppm) 8.50 (d, 1H, J = 8.5 Hz, 9-H); 7.55 (s, 1H, 4-H); 7.48 (d, 1H, J = 8.5 Hz, 8-H); 2.78 (s, 3H, 7-CH_3); 2.53 (s, 3H, 3-CH_3 or $OCOCH_3$); 2.51 (s, 3H, 3-CH_3 or $OCOCH_3$).
$C_{16}H_{12}N_2O_3$

3.2. Synthesis of 2-cyano-4,5-dihydro-3-methylnaphtho[1,2-b]furan-4,5-dione (**9a**)

Following a described procedure [8], compound **9a** was obtained from **7a** [5] as a yellow powder. Yield: 78%. M.p. 222–226 °C; IR (KBr): 2220 (CN), 1675 and 1660 (CO) cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz, δ, ppm) 8.02 (d, 1H, J = 7.5 Hz, 6-H or 9-H); 7.88 (d, 1H, J = 7.5 Hz, 6-H or 9-H); 7.81 (m, 1H, 7-H or 8-H); 7.68 (t, 1H, J = 7.5 Hz, 7-H or 8-H); 2.41 (s, 3H, 3-CH_3); ^{13}C NMR (DMSO-d_6, 75 MHz, δ, ppm) 177.98 (5-CO); 174.10 (4-CO); 161.16 (C); 134.84 (CH); 134.67 (C); 131.64 (CH); 130.20 (C); 129.26 (CH); 125.95 (C); 124.10 (C); 122.81 (CH); 120.08 (CN); 110.66 (C); 9.30 (3-CH_3).
$C_{14}H_7NO_3$

3.3. Synthesis of 4,5-diacetoxy-8-ethoxy-3-ethyl-9-methylfuro[2,3-f]quinoline (**11**)

A mixture of compound **10b** [8] (0.090 g, 0.32 mmol), zinc powder (0.300 g, 4.60 mmol), CH_3CO_2Na (0.100 g, 1.20 mmol) and $(CH_3CO)_2O$ (15 ml) was heated to reflux for 10 min. After cooling to room temperature, CH_2Cl_2 (50 ml) was added and the mixture filtered. The residue obtained after evaporation under vacuum was purified by preparative circular TLC (C_6H_{14}/AcOEt 4:1), to give **11** as a white powder. Yield: 0.810 g. 68%. M.p. 132 °C; IR (KBr): 1780 (CO) cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz, δ, ppm) 8.73 (s, 1H, 7-H); 7.65 (t, 1H, J = 1.2 Hz, 2-H); 4.29 (q, 2H, J = 7.0 Hz, $OC\underline{H}_2CH_3$); 2.95 (s, 3H, 9-CH_3); 2.75 (dq, 2H, J = 7.5 and 1.2 Hz, 3-$C\underline{H}_2CH_3$); 2.59 (s, 3H, 4-$OCOCH_3$ or 5-$OCOCH_3$); 2.43 (s, 3H, 4-$OCOCH_3$ or 5-$OCOCH_3$); 1.35 (t, 3H, J = 7.0 Hz, $OC\underline{H}_2CH_3$).
$C_{20}H_{21}NO_6$

3.4. In vitro cytotoxic assays

The compounds dissolved in DMSO (Carlo-Erba, final concentration of DMSO = 0.2%) were tested at various concentrations in 3 tumor cell systems. Assays included solvent controls and reference controls (doxorubicin and taxol).

3.4.1. In vitro cytotoxic activity towards L 1210 leukemia cells

The L 1210 murine leukemia cells were cultured in suspension in a RPMI 1640 medium (Eurobio) with 10% heat-inactivated fetal calf serum (Boehringer, Mannheim), 2-mercaptoethanol (Sigma, 10 μM), L-glutamine (Boehringer, Mannheim, 2 mM), and antibiotics at 37 °C, in a 5% CO_2 humidified air atmosphere.

For the screening, the cell suspension (cells in an exponential growth phase) was adjusted to 10^4 viable cells per ml (cell viability was estimated by the Trypan blue exclusion test) and was distributed in 96 tissue culture plates (Falcon, 225 μl by well) before introducing the test compounds or the solvent (25 μl). Four days later, cells were counted with a Coulter Counter (Coultronics, France).

IC_{50} is defined as the concentration inhibiting by 50% the cell growth compared to the control after 96 h of culture and is determined from the regression line of percentage cell growth inhibition as a function of the logarithm of the dose.

3.4.2. In vitro cytotoxic activity towards human cancer cell lines MDA-MB 231 and PC_3

The MDA-MB 231 cells were maintained in DMEM (Gibco) supplemented with 10% heat-inactivated fetal calf serum (Eurobio), insulin 1 UI/ml, L-glutamine (2 mM) and antibiotics. PC_3 cells were cultured in a solution composed of: 65% F_{12} Nutriet Mixture Ham (Gibco), 25% DMEM and 10% heat-inactivated fetal calf serum, L-glutamine (2 mM) and antibiotics. All the cell suspensions were maintained at 37 °C in a 5% CO_2 humidified air atmosphere.

The cytotoxic effect of compounds **8** to **13** against these two human cancer cell lines was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma) colorimetric method derived from Mosmann [13]. 10^4 cells/ml were seeded in 96-well tissue culture

plates (135 µl per well). After 24 h of culture, wells received either 15 µl of medium with DMSO as controls, or 15 µl of medium containing a compound solubilized in this solvent. After 5 d of culture, each well received 15 ml of a sterile solution of MTT in phosphate buffered saline (PBS) solution at 5 mg/ml before incubating 2 h. Then, the culture medium was removed and 100 µl of DMSO was added in each well for quantitation of blue formazan by reading the absorbance at 540 nm on a Titertek Multiskan II (Flow Laboratories).
The respective IC_{50} values were calculated from the regression lines plotted as the percentage of decrease in absorbance *versus* controls as a function of the logarithm of the concentration.

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Neue polare Inhaltsstoffe aus *Asiasarum sieboldii*

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Aus *Asiasarum sieboldii* wurden drei neue Verbindungen, das Monoterpen Asiasarinol (**3**), das Iridoidglycosid 6-O-(3,4-Dimethoxy-)cinnamoyl-ajugol (**14**) und das Isobutylamid Asaramid (**15**) neben 12 weiteren, für die Gattung *Asarum* zum Teil neuen Substanzen isoliert und deren Struktur mit spektroskopischen Methoden aufgeklärt. Vier dieser Verbindungen traten als *E/Z*-Isomerengemische mit unterschiedlichen Isomerenverhältnissen auf.

New polar ingredients from *Asiasarum sieboldii*

From *Asiasarum sieboldii* 3 new compounds, the monoterpene derivative asiasarinol (**3**), the iridoid glycoside 6-O-(3,4-dimethoxy-)cinnamoyl-ajugol (**14**) and the isobutylamide asaramid (**15**) were isolated besides twelve other, for the genus *Asarum* partly unknown compounds. Their structures were elucidated by spectroscopic methods. Four of these ingredients were detected as mixtures of *E/Z* isomeres.

1. Einleitung

Asiasarum sieboldii gehört aufgrund seines sehr breiten Einsatzgebietes in der chinesischen Volksmedizin zu einer der am besten auf ihre Inhaltsstoffe untersuchten Pflanze der Gattung *Asarum*. Seine unpolaren Inhaltsstoffe sind schon seit Jahrzehnten Objekt intensiver Forschung asiatischer Phytochemiker. *Asiasarum sieboldii* zeichnet sich durch das Vorkommen vieler verschiedener Naturstoffklassen aus. Darunter finden sich vor allem Monoterpene, Phenylpropanderivate, Phenolderivate, Orcinderivate, Isobutylamidderivate, Benzylisochinolinderivate, Flavonoide und Lignanoide. Übersichten hierzu finden sich bei Hegnauer [1] und Hager [2].
Das getrocknete ganze Kraut (Asari herba) soll sich in der traditionellen chinesischen Volksmedizin als Decoct bei Erkältungen, Kopfschmerzen und Husten [3], äußerlich als Fluidextrakt bei Zahnschmerzen und Gingivitis [4] und als Pflaster bei Gingivitis [4] und als Pflaster bei Gingivo-Stomatitis herpetica bewährt haben [5].
Pharmakologische Testungen auf antiallergische und antiphlogistische Wirkung [6] des *n*-Hexan-, *n*-Butanol-, Methanol- und des Wasserextraktes aus der Wurzel von *Asiasarum sieboldii* zeigten, daß insbesondere Inhaltsstoffe des polaren Butanolextraktes starke pharmakologische Aktivität besitzen. Die Studie wurde mit Hilfe eines 5-LOX Enzym Tests [7] und einer Antagonismusstudie von LTD_4 am Meerschweinchen-Ileum [8] durchgeführt. Somit sollte die Isolierung möglicher wirksamer Bestandteile aus der Butanolfraktion im Hinblick auf eine genauere pharmakologische Untersuchung weitere Informationen zur Wirksamkeit dieser häufig angewandten Arzneipflanze liefern.

2. Untersuchungen und Ergebnisse

Aus der n-Butanol- und der Dichlormethanfraktion der Ganzdroge von *Asiasarum sieboldii* konnten mit Hilfe von säulenchromatographischen Methoden insgesamt fünfzehn Verbindungen isoliert und deren Strukturen spektroskopisch eindeutig identifiziert werden. Von diesen fünfzehn