Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography B, 818 (2005) 159–166
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Relationship between cucurbitacins reversed-phase high-performance liquid chromatography hydrophobicity index and basal cytotoxicity on HepG2 cells

Judit Bartalis, Fathi T. Halaweish*

Department of Chemistry and Biochemistry, South Dakota State University, P.O. Box 2202, Brookings, SD 57007, USA

Received 23 September 2004; accepted 22 December 2004
Available online 20 January 2005

Abstract

Drug development of cucurbitacins requires derivatives that have lower cytotoxicity. Therefore, the effect of structural modification on in vitro cytotoxicity has been investigated. Lipophilicity or chromatographic hydrophobicity index (CHI) was chosen as molecular property. CHI was determined by RP-HPLC in both aqueous acetonitrile and aqueous methanol. Compounds CHI range was wide and better defined in acetonitrile (CHI_{ACN} = 46–88 and 38–102) than in methanol (CHI_{MeOH} = 56–78). Higher resolution was achieved in acetonitrile, and higher precision on the shorter C18 column. Cucurbitacins cytotoxicity (IC_{50}) was measured on the hepatocyte-derived HepG2 cells. Strong relationship between CHI and logarithmic IC_{50} was found. As a result, cytotoxicity increased linearly with increasing hydrophobicity ($r \geq 0.90$). Other lipophilicity parameters, such as log *P* and *C* log *P* were also estimated. Cytotoxicity correlated well with log *P* ($r = 0.95$) and slightly with *C* log *P* ($r = 0.74$). The log *P* and *C* log *P* data showed good correlation with CHI ($r > 0.92$). Overall, alkylation of C1 hydroxyl, unsaturation of C1─C2 bond, and acetylation of C25 hydroxyl increased both lipophilicity and cytotoxicity. This assay should prove useful for monitoring cucurbitacin homologues or other drug candidates for their cytotoxicity.

Keywords: Cucurbitacin; Chromatographic hydrophobicity index; CHI; HepG2; IC50; Cytotoxicity; Structure-activity relationship; SAR; log *P*; *C* log *P*; RP-HPLC

1. Introduction

Plants secondary metabolites represent tremendous resources for scientific and clinical researches as well as for new drug development [1]. Cucurbitacins are particularly known in folk medicine for their strong purgative, anti-inflammatory, and hepatoprotective activities [1,2]. They are positioned on the top of the NCI list as potential anti-tumor agents in various tumor subpanels [3,5]. However, cucurbitacins strong biological activity was found to be very close to their toxic dose, which renders them unlikely biological agents [6]. On the other side, methylation of the enolic hydroxyl (a.k.a. diosphenol) of cucurbitacin E enhanced the antitumor activity and lowered the toxicity on mice [7–9].

Lipophilicity is one of the major factors that influences the transport, absorption, and distribution of chemicals in biological systems, and it is a predominant descriptor of the pharmacodynamic, pharmacokinetic and toxic aspects of drug activities in quantitative structure-activity relationship (QSAR) studies [10–13]. In the 1960s Hansch's octanol–water partition coefficient P_{oct} ($P_{oct} = C_{oct}/C_{water}$; *C*: analyte concentration) became the standard parameter to measure lipophilicity for both experimental and theoretical investigations [14]. The octanol–water partition coefficients can be obtained from other solvent systems, with certain restrictions, by applying Collander's [15] equation: $\log P_1 = a \log P_2 + b$. RP-HPLC has been long recognized as a potential method for lipophilicity determination, where

* Corresponding author. Tel.: +1 605 688 4269; fax: +1 605 688 6364.
E-mail address: fathi.halaweish@sdstate.edu (F.T. Halaweish).

1570-0232/$ – see front matter © 2004 Published by Elsevier B.V.
doi:10.1016/j.jchromb.2004.12.020

mainly hydrophobic forces dominate the retention process [16–20]. Moreover, the mobile phase/stationary phase interface models better the biological partitioning processes than the solute partitioning in the bulk octanol/water phase [21]. The chromatographic retention data is a linear free-energy related parameter and it is a more reliable descriptor in QSAR than the estimated or calculated hydrophobic, electronic and/or steric parameters [22]. Chromatographic hydrophobicity index, CHI, is deduced from the retention data and reflects not only the lipophilicity of the compound but it approximates the concentration of organic phase required achieving an equal distribution of analyte between the mobile phase and stationary phase. Thus, hydrophobicity index is a useful tool in method development [23].

Drug development would require analogues that retains or enhances the natural cucurbitacins biological activity and reduces toxicity. We choose HepG2 cell line for our in vitro study, because it is one of the best human cell lines to predict basal human cytotoxicity [24–26].

This work presents a precise and reliable technique to study the effect of structural modification on cucurbitacins cytotoxicity. The basal cytotoxicity of seventeen cucurbitacin analogues was monitored on HepG2 cells, and their hydrophobicity calculated in different ways. The lipophilic parameters are the CHI, measured by RP-HPLC, and log *P* and *C* log *P* estimated with ALOGPS software. In order to have a larger number of compounds, some cucurbitacins were isolated from plants and others generated by alkylation and acetylation of enolic analogues. Cucurbitacins drug development requires derivatives with low cytotoxicity, and correlation of lipophilicity with in vitro toxicity may lead to important conclusions regarding this issue.

2. Experimental

2.1. Extraction, isolation, and identification

Ripe fruits of *Cucurbita texana* (Cucurbitaceae) were received from Dr. D.W. Tallamy (University of Delaware, Newark, Delaware). The fruits were cut and homogenized with methanol (MeOH), filtered, and the solvent removed under reduced pressure. The residue was subjected to flash column chromatography (silica gel G60) with gradient elution (hexane/ethyl acetate and then ethyl acetate/MeOH of increasing polarity) [27] and the fractions were screened using NP-TLC (silica gel, UV_{254}, 250 μm layer). TLC plates were developed with toluene:ethyl acetate 40:60 solvent mixture, and visualized for the $\Delta^{23,24}$ cucurbitacins with vanillin/orthophosphoric acid or for the diosphenols with $FeCl_3$ solution [28]. Fractions were further separated using preparative NP-TLC (silica gel, UV_{254}, 2 mm layer) under similar developing conditions to the analytical TLC and bands were visualized with UV light. Cucurbitacins ^{13}C and ^{1}H NMR spectra (Bruker 400 MHz) were recorded in $CDCl_3$ and compared to published data [29–34]. Additional amount of cucurbitacin glycosides were isolated by preparative HPLC from the concentrate of *Citrullus lanatus* (Cucurbitaceae) (Florida Food Products, Eustis, FL).

2.2. HPLC separation

We used Dynamax liquid chromatograph (Varian Chromatography Systems) with PDA-2 photodiode array UV detector, controlled by the Dynamax PC Chromatography Data System (v. 1.9) software. Dynamax dual pump solvent delivery system, model SD-200. Cucurbitacins final purification and separation was conducted on Econosil C18 (Alltech; 250 mm × 22 mm, 10 μm) preparative column at flow rate of 13.00 ml/min, and at gradient elution in acetonitrile (Pharmco, Brookfield, CT; 20–55% in 50 min), or MeOH (Pharmco; 60–75% in 50 min). Cucurbitacins analytical separation was optimized on Alltima C18 (Alltech; 250 mm × 4.6 mm, 5 μm) HPLC column [4,35,36], at gradient elution in acetonitrile (30–70% ACN in 57 min), and in MeOH (60–75% MeOH in 50 min). Cucurbitacins stock concentration of 10^{-2} M in DMSO:ethanol (1:1) was standardized against pure cucurbitacin I (Indofine Chemical Company, Hillsborough, NJ) by analytical HPLC means. Compounds CHI was measured in both ACN, by using Alltima C18 column, and in MeOH, by using Econosil C18 column (Alltech; 150 mm × 4.6 mm, 5 μm). Analytical separations were conducted at a flow rate of 1 ml/min. The aqueous phase was buffered for the CHI measurement. For this purpose, solid ammonium acetate (Fisher Sci. Co., Fair Lawn, NJ) was dissolved in deionized distilled water at 50 mM final concentration and its pH adjusted to 7.0.

2.3. Chromatographic hydrophobicity index

2.3.1. CHI measurement in ACN

All standard compounds were purchased from Acros (Acros Organics, NJ). The chromatographic lipophilicity or hydrophobicity was determined applying Valkó's technique [37]. A standard mixture of seven compounds was prepared in solution: theophylline (**19**), benzimidazole (**20**), acetophenone (**21**), indole (**22**), propiophenone (**23**), butyrophenone (**24**), and valerophenone (**25**). In the first approach, the mixture of compounds **19**–**25**, dissolved in water:ACN (1:1), was injected at isocratic elution of 40, 45, 50, 55, and 60% ACN. The retention factor, $\log k = \log((t_R - t_0)/t_0)$, was calculated for each analyte from five good injections of 10 μl sample. The dead time (t_0) was measured by injecting $NaNO_3$ together with the sample. Then, the $\log k$ values were plot against isocratic ACN concentrations to establish the linear regression equations for each analyte. From each straight line the isocratic hydrophobicity index was computed, $\varphi_0 = (-\text{intercept/slope})$. Further, the calibration mixture was injected at fast gradient elution, 0–22 min 0–100% ACN, and three additional minutes at 100% ACN. The φ_0 values for the test compounds were plot against gradient retention