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Synthesis and antihepatotoxicity of some Wuweizisu analogues

WL Wu[1], SE Chen[1], WL Chang[1], CF Chen[2], AR Lee[1]

[1]*School of Pharmacy, National Defense Medical Center;*
[2]*National Research Institute of Chinese Medicine, Taipei, Taiwan, Republic of China*

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Summary — A preparation of dimethyl 4,4'-dimethoxy-5,6,5',6'-dimethylenedioxybiphenyl-2,2'-dicarboxylate (VII) was readily achieved. It provided the advantages of specificity, simplicity, and efficiency in reactions. 6-Phenyl-3,9-dimethoxy-1,2-methylenedioxy-10,11-methylenedioxy-6,7-dihydro-5H-dibenz(c, e)azepin (X) was successfully synthesized from VII (DDB) and its liver-protective property proved to be more effective than DDB and silymarin in the *in vitro* test of carbon tetrachloride-induced damage of primary cultured rat hepatocytes.

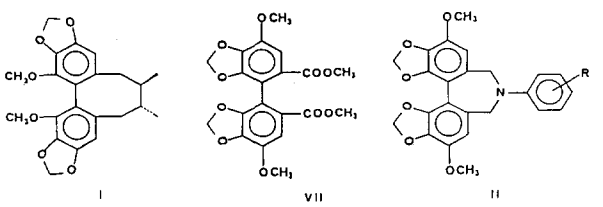
***Schizandra sinensis* / antihepatotoxic agents / dibenzoazepins / DDB**

Introduction

Fructus schisandrae (Wuweizi), is the dried fruit of *Schisandra chinensis* (Schisandraceae). It is used mainly to treat asthmatic coughs due to pulmonary asthenia, dryness in mouth due to dehydration, spontaneous or night sweating, nocturnal emission, chronic diarrhea, insomnia, amnesia and palpitations. It is therefore one of the most important herb drugs in use since ancient times in China, and is ranked as a supergrade medicine in Shen-nung-pen-tsao-ching [1]. Phytochemically, the genus of Schizandra is characterized by the fact that it is rich in lignans. More than 35 of them have been isolated and characterized by Japanese and Chinese chemists [2–10]. This group of secondary metabolites is interesting to organic chemists, pharmacologists and phytochemists not only for chemical reasons such as challenging synthetic targets but also for their remarkable biological properties. The biological activities of these metabolites span an enormous range including protection against hepatic damage [11–15], action on the cardiovascular system, effect on metabolism, antibacterial action, adaptogen-like action, stimulant action at various levels of the central nervous system, and excitatory action in the uterus etc [16].

Of all of these pharmacological activities reported, antihepatotoxic action has been investigated more than the others. Over 20 lignans have exhibited significant protective activity, both *in vitro* and *in vivo* against carbon tetrachloride-, galactosamine-induced liver damage in animals [11–15]. Wuweizisu C (I), a lignan with S configuration, is an active liver-protective component in Wuweizi reported so far [14, 15]. Those lignans possess a common skeleton of dibenzocyclooctadiene which was considered to be crucial in antihepatotoxic activity [11].

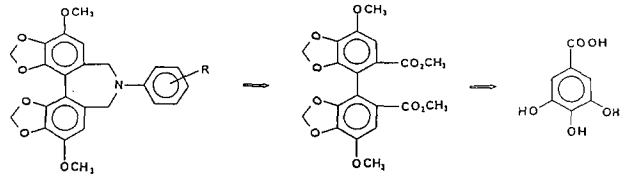
In 1982, Xie *et al* [17, 18] reported the first synthesis and liver-protective property of dimethyl-4,4'-dimethoxy-5,6,5',6'-dimethylenedioxybiphenyl-2,2'-dicarboxylate (DDB), a noncyclooctadiene analogue. Their elegant approaches involved dimerization by way of Ullmann condensation. However, the nonspecific reaction in the bromination step and the extremely low yield made the synthesis impractical. Here we report a novel synthesis of DDB (VII) and dibenzoazepins (II). The rationale to prepare II is that the seven-member ring with a basic nitrogen atom might bring about improved antihepatotoxicity by virtue of ring strain and basicity. The authors also conducted a preliminary *in vitro* antihepatotoxic activity test by the assay method using carbon tetrachloride-induced cytotoxicity in primary cultured rat hepatocytes, and found that X was superior to silymarin and DDB.



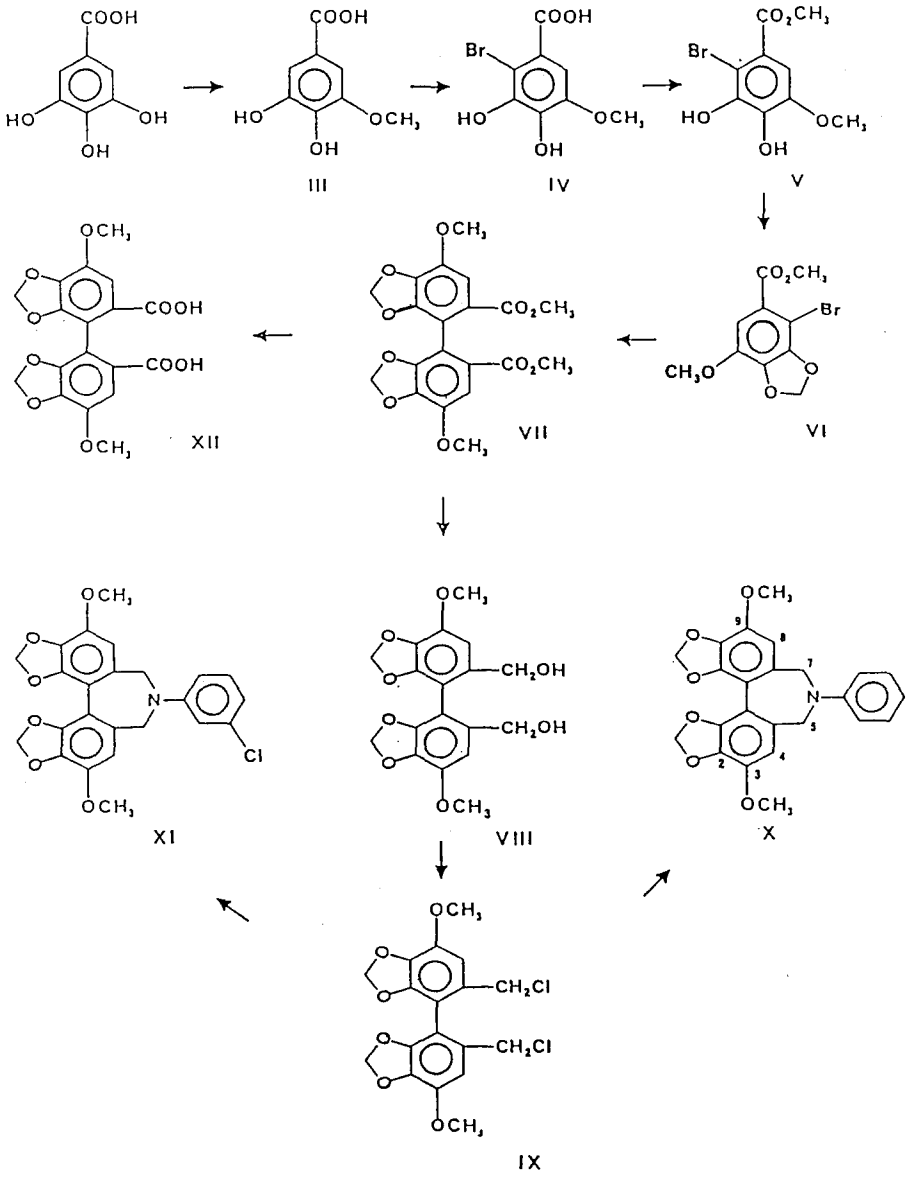
I VII II

Chemistry, results and discussion

The envisaged approach to Wuweizisu analogues is outlined retrosynthetically in scheme 1. In this approach, the overall synthesis was described and discussed. A flow-chart of the total synthesis is shown in scheme 2. Gallic acid was used as a starting material for synthesizing DDB by methylation, bromination, esterification, methylenation, and Ullmann coupling reaction. Further manipulations provided the dibenzo azepin products, which might become a new antihepatotoxic agent in the future.



Scheme 1.



Scheme 2.

In comparison with Xie's method [18], which is different due to the catalytic reagents used, and skillful designation in synthesizing pathway, our synthetic procedure is more specific and time-saving. Xie *et al* synthesized DDB from gallic acid by a circuitous way of esterification, methylation, methylenation, bromination, and finally Ullmann coupling reaction.

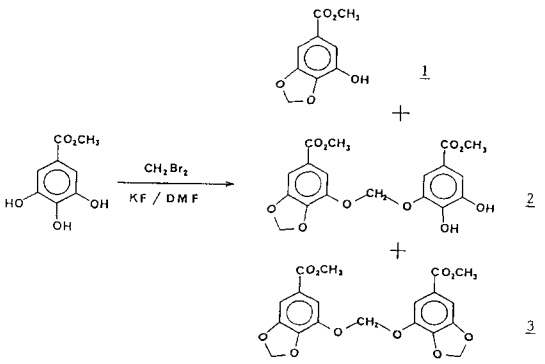
As shown in scheme 3, an attempt to selectively synthesize **1** by reaction of methyl gallate with methylene bromide failed.

The products obtained were a mixture of **1**, **2** and **3**. This was a result of intra- and intermolecular methylenation in an approximately equal ratio after chromatography on silica gel. Even using equimolar quantity of methylene bromide under meticulous conditions, the non-selective methylenation still remains.

Fortunately, we were able to overcome this obstruction to accomplish specific methylenation which is shown in scheme 2. Firstly, gallic acid was methylated with dimethyl sulfate in borax solution [19]. Two phenolic-OH's of gallic acid were chelated to the boron atom, and the third phenolic-OH was smoothly methylated to afford a mono-methylation reaction. The chelation was then broken with sodium hydroxide. The monomethylated compound, 3-0-methyl-gallic acid (III) was obtained in 77% yield. Secondly, utilizing the phenolic-OH-directed bromination procedure of Podall and Foster's [20, 21], 3-0-methyl-gallic acid readily underwent the bromination reaction in a regioselective manner to give 2-bromo-5-O-methylgallic acid (IV) in 74% yield. Thirdly, methylenation of 5-methoxy-3,4-dihydroxy-2-bromobenzoic acid methyl ester (V) at 110-120°C with dibromomethane catalized by potassium fluoride in N,N-dimethylformamide gave 5-methoxy-3,4-dimethylenedioxy-2-bromobenzoic acid methyl ester (VI) in 73% yield. It took only 40 min to complete the reaction, whereas in Xie's method the same reaction took more than 40 h. Another tedious step of synthesis in Xie's process was a non-specific bromination. They prepared compound VI from 3-methoxy-4,5-methylenedioxybenzoic acid methyl ester with bromine in acetic acid to give a mixture of three corresponding bromocompounds. Repeating chromatographic separations were necessary to obtain pure VI before the next step of synthesis. Therefore, our method provides the advantages of specificity, simplicity and efficiency in reactions.

In the preparation of DDB (VII), the bromine atom of VI now serves nicely to direct the regiochemical outcome of the Ullmann coupling reaction, resulting in DDB, a first known synthetic antihepatotoxic product obtained from the cyclooctadiene skeleton.

Further manipulations lead to the dibenzo-azepin derivatives. Direct reaction of DDB with anilines to form the imide compounds failed (scheme 4). Reduction of DDB with lithium aluminum hydride in tetrahydrofuran gave the corresponding diol (VIII, 68% yield) which after conversion with triphenyl phosphine and hexachloroacetone, the dichloride (IX, 51% yield) readily reacted with various anilines to obtain the desired dibenzo-azepins, X (80% yield) and XI (73% yield). DDB was saponified with potassium hydroxide in methanol to get XII in 83% yield.



Scheme 3.

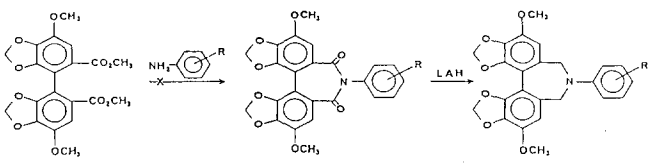
Pharmacological results and discussion

Preparation and culture of hepatocytes

Yields of 2–4 x 10^8 cells/liver with 80–90% viability in male Wistar strain rats were routinely obtained. Almost no contamination with non-parenchymal cells was observed. When placed in the medium, isolated hepatocytes attached to the surface of plastic dishes within 60 min to form a monolayer.

Carbon tetrachloride-induced damage of cultured hepatocytes

After an initial 24-h culture of hepatocytes prepared from male Wistar strain rat, the cells were challenged with 10 mM CCl_4. Activity of glutamic pyruvic transaminase (GPT) released in the medium was determined 1 h after CCl_4 treatment.



Scheme 4.

Antihepatotoxic activity of Wuweizisu analogues

After initial incubation of hepatocytes for 24 h, Wuweizisu analogues and silymarin were added at doses of 0.01, 0.1 and 1.0 mg with 10 mM CCl_4 to the culture medium (1.0 ml). GPT activity was measured at 60 min after the treatment. The results are shown in table I.

Well-known liver-protective compounds such as DDB and silymarin exhibited significant activity [18, 22], whereas X displayed much higher antihepatotoxicity than these compounds against carbon tetrachloride-induced cytotoxicity in primary cultured rat hepatocytes. X belongs to the dibenzoazepin compound and possesses a seven-membered ring and is distinctly different from DDB, a noncyclooctadiene of biphenyl compound. It is also different from Wuweizisu C (I), an active ingredient of Wuweizi which contains a skeleton of dibenzocyclooctadiene. This may imply that the naturally occurring dibenzocyclooctadiene structure in Wuweizi can be modified from the eight-membered ring into the synthesized seven-membered ring with a basic nitrogen atom and still retains the antihepatotoxic activity as demonstrated in table I. Although the ring strain is significantly changed, there is still a common feature of the methylenedioxy group of the biphenyl skeleton among all of these compounds. This common feature is very close to Hikino's report of structure and activity relationship, that the methylenedioxy group of the dibenzocyclooctane may play an important role in antihepatotoxic activity [14].

The marked antihepatotoxic activity of X and XI with a dibenzoazepin structure, together with their specificity, simplicity and efficient in synthetic pathway may bring about a new route in the development of new liver-protective agents.

Table I. Effect of Wuseizisu analogues on carbon tetrachloride-induced cytotoxicity in primary cultured rat hepatocytes. Solvents: 10 mM CCl_4 was dissolved in 0.01 ml. Each sample was dissolved in 0.01 ml dimethylsulphoxide. Vehicle control level was 16 ± 3 IU/l, $n = 5$ (dishes).

Compd	*Dose (mg/ml)*	*GPT IU/l*	*%*
Control	–	247 ± 1	100
VII	0.01	225 ± 3	91
	0.1	186 ± 3*	75
	1.0	75 ± 1***	30
X	0.01	197 ± 2	80
	0.1	139 ± 7**	56
	1.0	47 ± 3***	19
XI	0.01	229 ± 1	93
	0.1	172 ± 2*	70
	1.0	79 ± 1***	32
XII	0.01	240 ± 1	97
	0.1	263 ± 1	106
	1.0	181 ± 6*	73
Silymarin	0.01	201 ± 8	81
	0.1	154 ± 4**	62
	1.0	82 ± 1***	33

*Significantly different from the control, $P < 0.01$, **$P < 0.001$, ***$P < 0.0001$.

Experimental protocols

Materials and methods

Melting points were determined in open capillary tubes and were uncorrected. Ultra-violet (UV) spectra were taken on a Shimadzu 210 A spectrometer. Infrared (IR) spectra were taken on a Perkin-Elmer 983 G spectrometer using polystyrene film for calibration. Mass spectra (MS) were obtained on a Jeol JMS-D 300 instrument with an ionization voltage of 70 eV and a source temperature of 250°C. ^{1}H-NMR spectra were taken on a Jeol FX 100 spectrometer and absorptions were reported as parts per million (ppm) downfield from Me_4Si. Elemental analyses were performed by the Department of Chemistry, National Taiwan University. Thin-layer chromatography (TLC) was performed on precoated plastic plates of Merck silica gel 60 F_{254} with visualization using UV or by charring with sulfuric acid. Column chromatography was performed on Merck silica gel 60 (0.040–0.063). All solvents used were of anhydrous quality and kept over 3-A or 4-A molecular sieves. CCl_4 was distilled from P_2O_5 and then kept over 4-A molecular sieves.

The following compounds were purchased from E Merck: gallic acid, dimethyl sulfate, bromine, aniline, triphenyl-phosphine, dichloroethane, tetrahydrofuran and copper powder. Silymarin was from Aldrich Chemical Company, Inc, WI, USA.

Chemical synthesis

3-0-Methyl gallic acid (III)

Dimethyl sulfate (30 ml) and sodium hydroxide solution (13 g/50 ml H_2O) were added slowly to a solution of gallic acid (10 g) in 5% borax solution (800 ml). The mixture was stirred at room temperature for 3 h. Until the reaction was finished, the reaction mixture was acidified by diluted sulfuric acid, and then extracted with ethyl-acetate. The extract, decolored first with active charcoal was concentrated *in vacuo*, and then refluxed in 20% NaOH (100 ml) for 1 h. The aqueous solution was acidified to pH 1 with HCl, and the white precipitate was collected and recrystallized from benzene-methanol to give 7.5 g of III (77.0% yield), mp 215–216°C (lit 216). IR (KBr): 3300, 1665, 1460, 1352 cm^{-1}; MS (m/z): 184 (100%, M^+), 169 (51%, M^+-CH_3), 141 (15%, M^+-CO-CH_3). NMR (100 MHz, DMSO–d_6) 3.78 (s, 1H, OCH_3), 7.05 (t, 2H, Ar-H), 9.12 (s, 2H, -OH), 12.3 (s, 1H, COOH); analysis $C_8H_8O_5$ (C, H).

2-Bromo-5-O-methyl gallic acid (IV)

The solution of bromine (7.26 ml, 141 mmol) and 1,2-dibromomethane (30 ml) were added slowly to a solution of III (25 g, 136 mmol) in 1,2-dichloroethane (150 ml) in an ice bath. The

mixture was stirred for 5 h, and then cold water (100 ml) added. The yellow precipitate was collected, washed with cold water, and recrystallized from hot water to afford 26.5 g of IV (74%), mp 232–233°C. UV (MeOH): λ_{max} (log ε) = 271 (3.84) nm, 211 (4.39) nm. IR (KBr): 3504, 3445, 1704, 1605, 1513, 527 cm^{-1}; MS (m/z): 262 (100%, M^+), 247 (31%, M^+-CH_3), 183 (11.6%, M^+-Br); NMR (100 MHz, DMSO–d_6), 3.79 (s, 3H, OCH_3), 6.94 (s, 1H, Ar-H), 9.10 (s, 1H, OH), 12.50 (s, 1H, COOH); analysis: $C_8H_7O_5Br$ (C, H).

5-Methoxy-3,4-dihydroxy-2-bromobenzoic acid methyl ester (V)

To a solution of IV (10 g, 38 mmol) in 500 ml of anhydrous methanol a few drops of conc sulfuric acid was added and refluxed for 8 h. The methanol was then removed *in vacuo*, and the residue was decolored with active charcoal as well as recrystallized from ether/*n*-hexane to give a colorless crystal (V) (5.9 g, 56%), mp 145–145.5°C; UV (MeOH): λ_{max} (log ε) = 274 (3.80) nm, 218 (4.36) nm. IR (KBr): 3462, 1716, 1604, 1503, 600 cm^{-1}. MS (m/z): 276 (92%, M^+), 245 (100%, M^+-OCH_3), 261 (7%, M^+-CH_3). NMR (100 MHz, DMSO–d_6), 3.77 (s, 3H, $COOCH_3$), 3.76 (s, 3H, -OCH_3), 6.93 (s, 1H, Ar-H); analysis: $C_9H_9O_5Br$ (C, H).

5-Methoxy-3,4-methylenedioxy-2-bromobenzoic acid methyl ester (VI)

3.15 g of KF (54 mmol) and 2.52 ml of dibromomethane (36 mmol) were added slowly to a solution of V (5 g, 18 mmol) in anhydrous DMF (50 ml). The mixture was heated slowly and stirred at 110–120°C for 40 min. Until cooling, the mixture was filtered to remove insoluble KF and poured into 50 ml of distilled water. The precipitate was collected and recrystallized from ether/*n*-hexane to give a white powder (VI) (3.81 g, 73%), mp 103.5–104°C. UV (MeOH): λ_{max} (log ε) = 275 (3.88) nm, 218 (4.42) nm. IR (KBr): 3100, 2950, 1625, 1594, 1487, 1432 cm^{-1}. MS (m/z): 288 (97%, M^+), 257 (100%, M^+-OCH_3), 210 (6.2%, M^+-Br). NMR (100 MHz, DMSO–d_6), 3.80 (s, 3H, -$COOCH_3$), 3.84 (s, 3H, -OCH_3), 6.17 (s, 2H, -O-CH_2-O-), 7.18 (s, 1H, Ar-H); analysis: $C_{10}H_9O_5Br$ (C, H).

Dimethyl-4,4'-dimethoxy-5,6,5',6'-dimethylenedioxybiphenyl-2,2'-dicarboxylate (VII)

3 g of VI (10.4 mmol) and pure anhydrous copper powder (2 g, 31.5 mmol) were added to 8 ml of anhydrous *N,N*-dimethyl formamide. The mixture was heated and refluxed for 3 h. After cooling down, the mixture was filtered to remove insoluble copper powder and poured into 20 ml of distilled water. The precipitate was collected, decolored, and recrystallized from ether/*n*-hexane to give a white crystal of VII (1.11 g, 51%), mp 154–155°C. UV (MeOH): λ_{max} (log ε) = 276 (4.12) nm, 225 (4.55) nm. IR (KBr): 1710, 1635, 1594, 1490, 1464, 1433, 1321 cm^{-1}. MS *(m/z)*: 418 (100%, M^+), 387 (5%, M^+-OCH_3), 373 (2%, M^+-OCH_3-$COCH_3$), 359 (16%, M^+-OCH_3-$COCH_3$). NMR (100 MHz, DMSO–d_6), 3.58 (s, 6H, 2$COOCH_3$), 3.99 (s, 6H, 2OCH_3), 5.99 (d, 4H, -O-CH_2-O-), 7.23 (s, 2H, ArH); analysis: $C_{20}H_{18}O_{10}$ (C, H).

4,4'-Dimethoxy-5,6,5',6'-dimethylenedioxy-2,2'-dibenzyl alcohol (VIII)

A solution of VII (2 g, 4.78 mmol) in anhydrous tetrahydrofuran (25 ml), was added slowly to a round flask containing lithium aluminum hydride (0.363 g, 9.56 mmol) in an ice bath and stirred for 1 h. When the reaction was complete, distilled water was added slowly along the flask wall to the mixture until no hydrogen generated. The mixture was filtered and extracted with methanol several times. The methanol and tetrahydrofuran were removed *in vacuo*, the aqueous solution was cooled down to afford a white crystal of VIII (1.18 g, 68%), mp 172–173°C. UV (MeOH): λ_{max} (log ε) = 214 (4.56) nm. IR (KBr): 3329, 1640, 1488, 1450, 1305 cm^{-1}. MS (m/z): 362 (68%, M^+), 344 (100%, M^+-H_2O), 329 (24%, M^+-H_2OCH_3), 313 (9.5%, M^+-H_2O-OCH_3), 301 (31%, M^+-H_2O-CH_3-CO). NMR (100 MHz, DMSO–d_6), 3.85 (s, 6H, -OCH_3), 4.14 (s, 4H, Ar-CH_2), 5.00 (s, 2H, -OH), 5.90 (s, 4H, -O-CH_2-O-), 6.80 (s, 2H, Ar-H); analysis: $C_{18}H_{18}O_8$ (C, H).

4,4'-Dimethoxy-5,6,5',6'-dimethylenedioxy-2,2'-dibenzyl chloride (IX)

1.42 g (3.9 mmol) of VIII was added to a solution of triphenyl phosphine (2.24 g, 8.5 mmol) in hexachloroacetone (7 ml). The mixture was stirred at 10–15°C for 20 min and filtered to remove triphenyl phosphine oxide. The mixture was separated by column chromatography (silica gel 60, mobile phase: *n*-hexane/ethyl acetate = 3:1) to give IX (0.77 g, 51%), mp 137–138°C, UV ($ClCH_2CH_2Cl$): λ_{max} (log ε) = 225 (4.27) nm. IR (KBr): 2960, 2933, 2847, 1590, 1491, 1449 cm^{-1}. MS (m/z): 398 (92.3%, M^+), 363 (9.6%, M^+-Cl), 333 (21%, M^+-Cl-OCH_2), 314 (100%, M^+-Cl-CH_2Cl), 299 (15%, M^+-Cl-CH_2Cl-CH_3). NMR (100 MHz, DMSO–d_6) 3.88 (s, 6H, -OCH_3), 4.43 (s, 4H, CH_2-Cl), 5.93 (s, 2H, -O-CH_2-O-), 6.01 (s, 2H, -O-CH_2-O-), 6.94 (s, 2H, ArH); analysis: $C_{18}H_{16}O_6Cl_2$ (C, H).

6-Phenyl-3,9-dimethoxy-1,2-methylenedioxy-10,11-methylenedioxy-6,7-dihydro-5H-dibenz(c, e)azepin (X)

Aniline (0.14 g, 1.5 mmol) was added to a solution of IX (0.5 g, 1.25 mmol) in 5 ml of benzene. The mixture was stirred at room temperature for 2 h, and then the solvent was removed *in vacuo*. The residue was collected and recrystallized from ether to give X (80%), mp 242–243°C. UV (CH_3CN): λ_{max} (log ε) = 256 (3.66) nm. IR (KBr): 3886, 1594, 1345 cm^{-1}. MS *(m/z)*: 419 (M^+), 327 (M^+-C_6H_5-CH_3). NMR (100 MHz, DMSO–d_6) 3.81 (s, 6H, OCH_3), 4.40 (d, 4H, -CH_2-N_2), 6.00 (d, 4H, -O-CH_2-O-), 6.45–7.14 (m, 6H, Ar-H); analysis: $C_{24}H_{21}O_6N$ (C, H, N).

6-(m-Chlorophenyl)-3,9-dimethoxy-1,2-methylenedioxy-10,11-methylenedioxy-6,7-dihydro-5H-dibenz(c,e)azepin (XI)

The synthetic method is the same as that of X to give XI (73%), mp 219–220.5°C. UV (CH_3CN): λ_{max} (log ε) = 261 (4.44) nm, 237 (4.58) nm, 211 (4.54) nm. IR (KBr): 1931, 1307, 1166 cm^{-1}. MS *(m/z)*: 453 (M^+), 438 (M^+-CH_3), 422 (m^+-OCH_2), 394 (M^+-OCH_2-CO). NMR (100 MHz, DMSO–d_6) 3.86 (s, 6H, OCH_3), 4.41 (t, 4H, -CH_2-N), 6.00 (s, 2H, -O-CH_2-O-), 6.05 (s, 2H, -O-CH_3-O-), 6.60–7.20 (m, 6H, Ar-H); analysis: $C_{24}H_{20}O_6NCl$ (C, H, N).

4,4'-Dimethoxy-5,6,5',6'-dimethylenedioxy-2,2'-dibenzoic acid (XII)

A solution of NaOH (5 N, 5 ml) was added to a solution of VII (2.88 g, 5.45 mmol), and refluxed for 4 h. Upon completion of the reaction, mixture was acidified to pH 1 by diluted HCl and an orange precipitate was collected and purified to give XII (1.75 g, 83%), mp 271–272.5°C. UV (MeOH): λ_{max} (log ε) = 264 (3.97) nm, 222 (2.59) nm. IR (KBr): 2967, 2911, 2840, 1725, 1259 cm^{-1}. MS *(m/z)*: 390 (M^+), 372 (M^+-H_2O), 346 (M^+-CO_2), 328 (M^+-H_2O-CO_2), 300 (M^+-H_2O-CO_2-CO). NMR (100 MHz, DMSO–d_6) 3.88 (s, 6H, OCH_3), 5.94 (s, 2H, -O-CH_2-O-), 5.98 (s, 2H, -O-CH_2-O-), 7.24 (s, 2H, ArH), 12.25 (d, 2H, COOH); analysis: $C_{18}H_{14}O_{10}$ (C, H).

Determination of antihepatotoxicity

Assays were conducted by using CCl_4-induced cytotoxicity in primary cultured male Wistar strain rat hepatocytes as reported by Kiso *et al* [23]. All data obtained are expressed as mean ± SE, and statistical significance was evaluated by one-way analysis of variance.

Acknowledgments

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