Studies on Anti-ulcer Agents. II. Synthesis and Anti-ulcer Activities of 6-Isopropylazulene-1-sodium Sulfonate Derivatives

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A series of alkylazulene-1-sodium sulfonate derivatives which has an isopropyl group at 6-position were synthesized, and their anti-ulcer activities were examined in Shay pylorus-ligated rats. The values of lipophilicity (log *P*) as a parameter of these new azulene derivatives were also examined in reference to the structure–activity relationship. The optimum value of log *P*, which showed maximal anti-ulcer activity, was about −0.46. Among the derivatives of azulene examined, 3-ethyl-6-isopropylazulene-1-sodium sulfonate (compound IXb : KT1-785) exhibited the most potent inhibitory action against Shay ulcer, and its anti-peptic activity was similar to that of 3-ethyl-7-isopropylazulene-1-sodium sulfonate (KT1-32). It also had more activity than guaiazulene sodium sulfonate (GAS). Furthermore, KT1-785 was extremely stable under heating as compared to GAS.

Keywords anti-ulcer activity; stability; lipophilicity; Shay pylorus-ligated rat; 6-isopropylazulene derivatives; structure–activity relationships; anti-peptic activity

Guaiazulene (GA) (Fig. 1) has been known to be an active component of the essential oil of *Guaiacum officinalis*, and its chemical structure was determined in 1949.[1] There are a number of reports describing anti-allergic, anti-inflammatory and anti-ulcer activities of GA.[2] In recent years, guaiazulene sodium sulfonate (GAS), a hydrophilic derivative of GA, was synthesized and it has been widely used clinically as an anti-inflammatory and anti-ulcer agent.[3] Both GA and GAS, however, are known to be unstable under heat and sunlight. In the preceding paper,[4] we reported that 3,7-dialkylazulene-1-sodium sulfonates had potent anti-ulcer activities and stability under heating. Further, we were interested in synthesizing 3,6-dialkylazulene derivatives with the hope of finding more active anti-ulcer agents. In this paper, we wish to report the synthesis and anti-ulcer activity of 6-isopropylazulene-1-sodium sulfonate derivatives and the structure–activity relationships between anti-ulcer activity and lipophilicity.

Synthesis of 4-Isopropyl-2-tosyloxytropone[5] The reaction of 4-isopropyltropolone I with tosylchloride in the presence of pyridine has been known to produce a mixture of II and III in the ratio of 4 : 6.[6] On the other hand, one of the authors (M.Y.) has developed a method for the selective synthesis of II by the use of 7-iodo-4-isopropyltropolone (IV).[7]

Treatment of I with iodine and potassium carbonate produced IV in a 100% yield. Tosylation of IV with tosylchloride in pyridine afforded V in a 96.7% yield, which was hydrogenated with 10% Pd/c in methanol in the presence of anhydrous sodium acetate to afford II in a 95% yield.

Synthesis of 6-Isopropylazulene Derivatives The reaction of 4-isopropyl-2-tosyloxytropone II[6] with 2 molar eq of dimethyl malonate (DMM) in the presence of sodium methoxide in an ice-water bath afforded methyl 6-isopropyl-2-oxo-2*H*-cyclohepta[*b*]furan-3-carboxylate VI[8] in a 98% yield. The reaction of VI with *in situ*-generated morpholino enamines of aldehydes in ethanol under reflux resulted in the formation of 3-alkyl-6-isopropylazulene-1-carboxylates VIIa—g, 3-aralkyl-6-isopropylazulene-1-car-

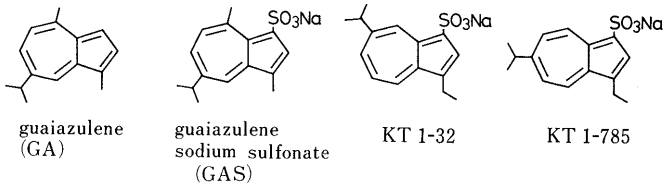


Fig. 1

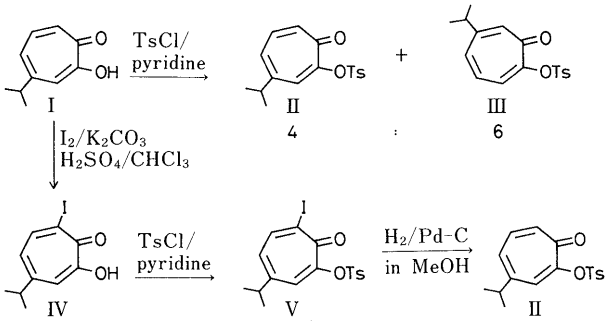
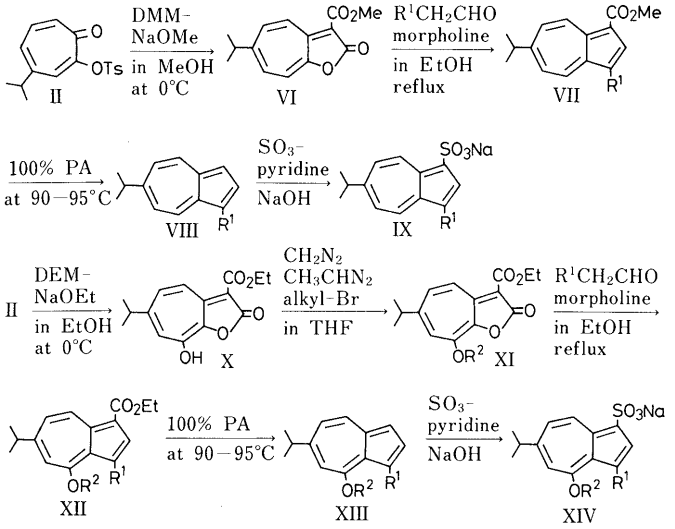


Chart 1. Synthetic Route to 4-Isopropyl-2-tosyloxytropone



R1=alkyl, aralkyl, alkenyl R2=alkyl
DMM=dimethyl malonate DEM=diethyl malonate
PA=phosphoric acid

Chart 2. Synthetic Route to Azulene Derivatives

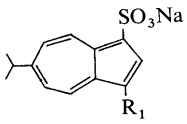
boxylates VIIh—j, and 3-alkenyl-6-isopropylazulene-1-carboxylate VIIk, respectively, in yields of more than 90%.[9] The heating of VIIa—k with anhydrous phosphoric acid (PA) at 90—95 °C for 15—30 min afforded 6-isopropylazulene derivatives VIIIa—k, respectively, in yields of over 90%. Sulfonation of VIIIa—k with a pyridine–sulfur trioxide complex in benzene, followed by treatment with sodium hydroxide, yielded the corresponding azulene sodium sulfonates IXa—k as bluish violet crystals.

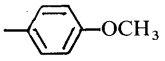
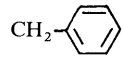
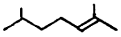
The reaction of II with diethyl malonate (DEM) in the presence of sodium ethoxide in ethanol yielded ethyl 8-hydroxy-2-oxo-2*H*-cyclohepta[*b*]furan-3-carboxylate X in a 65% yield.[10] O-Alkylation of X with diazomethane, diazoethane, or its silver salt with alkylhalide, afforded ethyl 8-alkoxy-2-oxo-2*H*-cyclohepta[*b*]furan-3-carboxylate XI. The reaction of XI with *in situ*-generated morpholino enamines of aldehydes yielded 8-alkoxy-6-isopropylazulene derivatives (XIIa—h). Decarboxylation of XII by treatment with PA yielded XIII. Sulfonation with a pyridine–sulfur trioxide complex followed by treatment with sodium hydroxide afforded the sodium 3-alkyl-4-alkoxy-6-isopropylazulene-1-sulfonates (XIVa—h).

Stability The thermal stability of KT1-785 (IXb) was compared with GAS. Samples of KT1-785 and GAS were placed in test tubes, and heated at 60 °C followed by thin layer chromatographic (TLC) analysis on silica gel plates. GAS started to decompose after heating for 20 h, whereas KT1-785 was not affected. These results indicate that KT1-785 is more stable under heating than GAS.

Structure–Activity Relationships The anti-ulcer activity of azulene derivatives synthesized in this study was evaluated in terms of their ability to inhibit Shay pylorus-ligated ulcers in rats. The activity was expressed as inhibition percent (%) and these data are shown in Tables I and II, where the partition coefficients (P) are expressed as $\log P = C_O/C_W$ (C_O: azulene derivatives concentration in the *n*-octanol phase; C_W: azulene derivatives concentration in the aqueous phase). Substitution at the C-3 position with a substituent

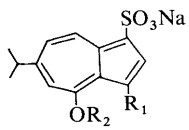
TABLE I. Anti-ulcer Activities of Azulene Derivatives



Compound	R_1	Yield (%)	mp (°C) (dec.)	Formula	Shay ulcer inhibition (%)	log P
IXa	CH_3	88.4	>280	$C_{14}H_{15}O_3SNa$	60.3	−1.20
IXb	C_2H_5[a]	90.2	180—182	$C_{15}H_{17}O_3SNa$	90.0	−1.02
IXc	*n*-C_3H_7	87.4	168—170	$C_{16}H_{19}O_3SNa$	78.7	−1.15
IXd	*n*-C_4H_9	86.5	173—175	$C_{17}H_{21}O_3SNa$	70.0	−0.98
IXe	*n*-C_5H_{11}	77.7	184—186	$C_{18}H_{23}O_3SNa$	70.1	−0.78
IXf	*n*-C_6H_{13}	72.3	181—183	$C_{19}H_{25}O_3SNa$	77.5	−0.65
IXg	iso-C_3H_7	85.3	201—203	$C_{16}H_{19}O_3SNa$	79.8	−0.79
IXh		68.2	223—225	$C_{19}H_{17}O_3SNa$	81.5	−0.39
IXi		61.2	218—220	$C_{20}H_{19}O_4SNa$	22.4	0.70
IXj		66.2	140—142	$C_{20}H_{19}O_3SNa$	26.7	0.67
IXk		70.0	150—152	$C_{21}H_{27}O_3SNa$	26.7	0.61
GAS					70.0	−1.00
KT1-32					92.7	−0.74

a) KT1-785.

TABLE II. Anti-ulcer Activities of Azulene Derivatives



Compound	R_1	R_2	Yield (%)	mp (°C) (dec.)	Formula	Shay ulcer inhibition (%)	log P
XIVa	CH_3	CH_3	54.2	198—200	$C_{15}H_{17}O_4SNa$	88.6	−0.44
XIVb	C_2H_5	CH_3	52.5	188—190	$C_{16}H_{19}O_4SNa$	88.6	−0.12
XIVc	*n*-C_3H_5	CH_3	48.7	185—187	$C_{17}H_{21}O_4SNa$	77.3	−0.15
XIVd	*n*-C_4H_9	CH_3	54.5	183—185	$C_{18}H_{23}O_4SNa$	68.0	0.20
XIVe	*n*-C_5H_{11}	CH_3	52.2	178—180	$C_{19}H_{25}O_4SNa$	35.0	0.96
XIVf	C_2H_5	C_2H_5	54.1	188—190	$C_{17}H_{21}O_4SNa$	88.9	0.11
XIVg	C_2H_5	C_3H_7	58.2	188—190	$C_{18}H_{23}O_4SNa$	73.3	0.47
XIVh	C_2H_5	C_4H_9	66.6	121—123	$C_{19}H_{25}O_4SNa$	69.7	0.58

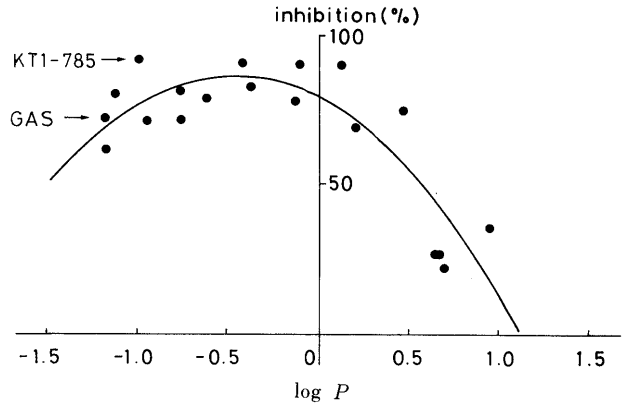


Fig. 2. Anti-ulcer Activity (Shay Ulcer) of the Compounds Plotted against log P

$$\text{Inhibition (\%)} = -32.5(\log P)^2 - 29.7(\log P) + 78.4 \quad \text{Eq. 2}$$
$$(n=19,\ r=0.834,\ S=12.34,\ F_{16}^2=18.37)$$

n, r, S and F represent the number of compounds used, correlation coefficient, standard deviation and value in the F-test, respectively.

of more than two carbon atoms led to a gradual decrease in the activity as follows; ethyl(IXb) > n-propyl (IXc) = isopropyl (IXg) > n-butyl (IXd) > n-pentyl (IXe) > n-pentyl (IXf); methyl(XIVa) = ethyl (XIVb) > n-propyl (XIVc) > n-butyl (XIVd) > n-pentyl (XIVe). Therefore, two carbon atoms as alkyl substituents at the C-3 position seem to be optimal for anti-ulcer activity. While the phenyl group (IXh) showed high potency, the p-methoxy phenyl group (IXi), the benzyl group (IXj) and alkenyl group (IXk) showed low activities. These low active compounds tended to have high lipophilicity. The inhibitory effect of alkoxy groups at the C-4 position on Shay ulcer was almost parallel to that of the parent compounds. The alkyl substitution effect on C-3 of 6-isopropylazulene was the same in the case of 7-isopropylazulene, and ethyl substitution on C-3 caused high activity.[4] Among the compounds synthesized in this study, KT1-785 (IXb) showed the most potent anti-ulcer activity, and its inhibitory effect was similar to that of 3-ethyl-7-isopropylazulene-1-sodium sulfonate (KT1-32), having more activity than GAS. The lipophilicity (log P) of compounds, such as IXb, IXc, IXg, XIVa and XIVb, which have high inhibitory action against Shay ulcer, corresponded to the values of -1.1—0.1 as shown in Tables I, II and Fig. 2. Therefore, lipophilicity (log P) of the compounds may play an important role in enhancement of anti-ulcer activity as to azulene derivatives.[4]

$$\text{inhibition (\%)} = -19.6 \log P + 63.6 \quad (1)$$
$$(n=19,\ r=0.623,\ S=17.53,\ F_{16}^2=5.06)$$

$$\text{inhibition (\%)} = -32.5\ (\log P)^2 - 29.7(\log P) + 78.4 \quad (2)$$
$$(n=19,\ r=0.834,\ S=12.34,\ F_{16}^2=18.37)$$

n, r, S and F represent the number of compounds used, correlation coefficient, standard deviation and a value in F test, respectively.

As mentioned above, a quadratic equation (2) calculated on the basis of inhibition (%) and log P values of 19 compounds showed a better correlation ($r=0.834$)[11] than that ($r=0.623$) of a linear equation (1). Equation 2 had a high correlation coefficient ($r=0.834$), which was statistically significant at the 99% level with a F_{16}^2 value of ($F(=0.01)=6.23$). Expected values of inhibition percentage, as calculated from Eq. 2, were in good agreement with those observed for the compounds as shown in Table III. Consequently, the potency (inhibition percentage) of compounds can be estimated easily by Eq. 2 with log P values of these compounds as the parameter, as shown in Fig. 2. The optimum log P value is about -0.46 based on Eq. 2. The log P value of compound IXb (KT1-785), however, was about 0.5 unit smaller than optimum.

TABLE III. Comparison of Expected Values of Inhibition Percentage from Eq. 2 with Those Values Observed from the Compounds

Compound No.	log P[a]	Inhibition (%)		
		Obsd[a]	Calcd[b]	|obsd − calcd|
IXa	−1.20	60.3	67.2	6.9
IXb	−1.02	90.0	74.8	15.2
IXc	−1.15	78.7	69.5	9.2
IXd	−0.98	70.0	76.3	6.3
IXe	−0.78	70.1	81.8	11.7
IXf	−0.65	77.5	84.0	6.5
IXg	−0.79	79.8	81.6	1.8
IXh	−0.39	81.5	85.1	3.6
IXi	0.70	22.4	41.8	19.3
IXj	0.67	26.7	44.0	17.3
IXk	0.66	26.7	44.7	18.0
XIVa	−0.44	88.6	85.2	3.4
XIVb	−0.12	88.6	81.5	7.1
XIVc	−0.15	77.3	82.2	4.9
XIVd	0.20	68.0	71.2	3.2
XIVe	0.96	35.0	20.0	15.0
XIVf	0.11	88.9	74.8	14.1
XIVg	0.47	73.3	57.3	16.0
XIVh	0.58	69.7	50.3	19.4

a) From Table I and II. b) The calculated values were obtained from Eq. 2.

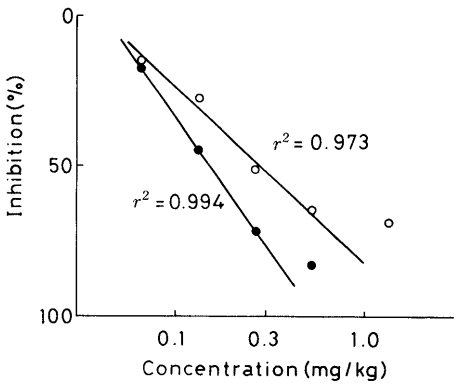


Fig. 3. Antipeptic Activity of KT1-785 and GAS

●—●, KT1-785; ○—○, GAS. The IC_{50} values of KT1-785 and GAS were 0.152 and 0.284 mg/ml, respectively.

Antipeptic Activity Dose–response curves for anti-peptic activity of azulene derivatives and GAS are shown in Fig. 3. KT1-785 (0.07—0.4 mg/ml) showed inhibitory activity against pepsin in a dose-dependent manner. KT1-785 (0.4 mg/ml) completely inhibited the peptic activity. The inhibitory effect of KT1-785 was more potent than that of GAS. The IC_{50} (concentrations which inhibit peptic activity by 50%) values of KT1-785 and GAS were 0.152 and 0.284 mg/ml, respectively.

Experimental

Melting points were determined on a Yanagimoto melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded on a Hitachi 270-30. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were measured at 90 MHz on a Hitachi R-90H Fourier Transform NMR spectrometer. Chemical shifts are quoted in parts per million (ppm) with tetramethylsilane as an internal standard. Coupling constants (J) are given in Hz. The following abbreviations are used: s = singlet, d = doublet, q = quartet, m = multiplet and br s = broad singlet. Mass spectra (MS) were recorded on a Hitachi M80B for electron impact-mass spectra (EI-MS). Ultraviolet (UV) spectra were recorded on a Hitachi 150-20 spectrometer. For column chromatography, silica gel (Merck, Kieselgel 60, 70—230 mesh) was used. TLC was performed on Silica gel 60 F_{254} plates (Merck).

3-Ethyl-6-isopropylazulene-1-carboxylate (VIIb) A mixture of *n*-butylaldehyde (17.6 g, 2.4×10^{-1} mol), morpholine (21.2 g, 2.4×10^{-1} mol) and methyl 6-isopropyl-2-oxo-2*H*-cyloheptа[*b*]furan-3-carboxylate (20.0 g, 8.1×10^{-2} mol) in EtOH (350 ml) was heated under reflux for 4 h. After the solvent was removed the residue was extracted with benzene. The organic layer was washed with H_2O and dried over Na_2SO_4. The solvent was removed and the residue was purified by column chromatography with benzene to produce VIIb (20.1 g, 96.8%) as violet crystals. mp 60—61 °C. MS *m/z*: 256 (M^+). IR (KBr) cm^{-1}: 3000, 1700, 1460, 1220. ^{1}H-NMR ($CDCl_3$) δ: 1.37 (3H, t, J=7.5 Hz, Et-Me), 1.38 (6H, d, J=7.0 Hz, iso-Pr-Me), 3.05 (2H, q, J=7.5 Hz, Et-CH_2), 3.12 (1H, m, J=7.5 Hz, iso-Pr-CH), 3.90 (3H, s, Me), 7.28 (1H, dd, J=10.0, 2.0 Hz, C_5-H), 7.30 (1H, dd, J=10.0, 2.0 Hz, C_7-H), 8.10 (1H, s, C_2-H), 8.30 (1H, d, J=10 Hz, C_4-H), 9.45 (1H, d, J=10 Hz, C_8-H). Compounds VIIa, VIIc—k and XIa—h were obtained by the same procedure as described for VIIb.

1-Ethyl-6-isopropylazulene (VIIIb) A mixture of VIIb (20 g, 7.8×10^{-2} mol), and anhydrous PA (200 ml) was heated and stirred at 90—95 °C for 15 min. The reaction mixture was then poured into H_2O (300 ml), extracted with benzene, washed with H_2O, and dried over Na_2SO_4. The solvent was removed, and the residue was purified by chromatography with benzene to produce VIIIb (14.6 g, 94.3%) as a blue colored oil. MS *m/z*: 198 (M^+). IR (neat) cm^{-1}: 2950, 1590, 1400. ^{1}H-NMR ($CDCl_3$) δ: 1.31, (3H, d, J=7.5 Hz, Et-Me), 1.32 (6H, d, J=7.0 Hz, iso-Pr-Me), 3.00 (2H, q, J=7.5 Hz, Et-CH_2), 3.05 (1H, m, J=7.5 Hz, iso-Pr-CH), 6.95 (2H, dm, J=10 Hz, C_5-H, C_7-H), 7.21 (1H, d, J=5.0 Hz, C_1-H), 7.65 (1H, d, J=5.0 Hz, C_2-H), 8.12 (2H, dm, J=10 Hz, C_4-H, C_8-H). Compounds VIIIa, VIIIc—k and XIIIa—h were obtained by the same procedure as described for VIIIb.

3-Ethyl-6-isopropylazulene Sodium Sulfonate IXb (KT1-785) A mixture VIIIb (15 g, 7.6×10^{-2} mol) and pyridine–sulfur trioxide complex (24 g, 1.5×10^{-1} mol) in benzene (100 ml) was heated under reflux for 6 hr. Precipitates thereby formed were collected by filtration, and dissolved in H_2O (80 ml). Next, 20 ml of sodium hydroxide solution (6 g, 1.5×10^{-1} mol) was added at 10—15 °C, and the reaction mixture was stirred at 30—35 °C for 1 h. The reaction mixture was extracted with *n*-butanol and washed with a saturated NaCl solution. The crude product obtained by removal of the solvent was recrystallized in ethanol to produce IXb (KT1-785, 20 g, 90%), as bluish violet crystals. mp 180—182 °C (dec.). IR (KBr) cm^{-1}: 3600, 2980, 1420, 1200. ^{1}H-NMR (CD_3OD) δ: 1.37 (3H, t, J=7.5 Hz, Et-Me), 1.38 (6H, d, J=7.0 Hz, iso-Pr-Me), 3.05 (2H, q, J=7.5 Hz, Et-CH_2), 3.12 (1H, m, J=7.5 Hz, iso-Pr-CH), 7.28 (1H, dd, J=10.0, 2.0 Hz, C_5-H), 7.30 (1H, dd, J=10.0, 2.0 Hz, C_7-H), 7.98 (1H, s, C_2-H), 8.37 (1H, d, J=10.0 Hz, C_4-H), 9.05 (1H, d, J=10.0 Hz, C_8-H).

Compounds IXa, IXc—k and XIVa—h were obtained by the same procedure as described for IXb.

Pharmacological Method 1) Shay Ulcer: Male Donryu rats weighing 180 to 220 g were deprived of food with free access to water for 48 h prior to the experiments. Under ether anesthesia, the pylorus was ligated according to the method described by Shay *et al.*[12] Each drug (100 mg/kg) or the control vehicle was given orally immediately after ligation of the pylorus. The volume of each drug or vehicle was 0.2 ml/100 g body weight. 16 h after the pylorus ligation, each animal was sacrificed and the stomach was removed. Each stomach was fixed in 1% formalin solution for 10 min. Gastric mucosa was then exposed by opening the stomach along the greater curvature, and gastric ulcers which had developed in the forestomach were observed. The degree of ulceration was estimated using an ulcer index according to Okabe *et al.*[3] Each group was comprised of 8 rats. The inhibition ratio was calculated as follows:

$$\text{inhibition ratio (\%)} = \frac{\text{ulcer index (control)} - \text{ulcer index (sample)}}{\text{ulcer index (control)}} \times 100$$

2) Anti-peptic Activity: Bovine serum albumin (BSA) and pepsin were purchased from Wako Co. Anti-peptic activity was measured as described previously by Thiemer *et al.*[13] A mixture of 0.2 ml of BSA (25 mg/ml), 0.2 ml of 0.1 N HCl and 2 ml of water, with or without a test drug, was preincubated at 37 °C for 5 min. 0.5 ml of an enzyme solution (100 μg pepsin/ml of 0.5 N HCl) was added to this mixture, and then incubated at 37 °C for 30 min. 10% trichloro acetic acid (2.0 ml) was then added to this mixture. The pellet obtained after centrifugation at 3000 rpm for 5 min was used to determine the amount of residual BSA. The amount of BSA was measured according to the method of Lowry *et al.*[14] The percentage inhibition was calculated as follows:

$$\% \text{ inhibition} = 100 \times (A - B)/A$$

where A is the amount of digested BSA in the absence of the test drug and B is the amount of BSA digested in the presence of the test drug.

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