The Synthesis, Antileukemic Activity, and Crystal Structures of Indirubin Derivatives

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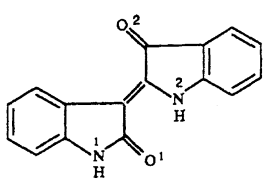
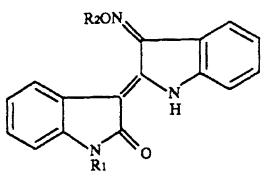
(Received November 14, 1995)

In order to search for a new kind of antileukemic drug, we synthesized four indirubin derivatives, including indirubin monooxime (**IM**), indirubin monooxime *O*-methyl ether (**IMME**), N_1-methylindirubin monooxime *O*-methyl ether (**MIMME**), and indirubin monooxime *O*-ethyl ether (**IMEE**). Their antileukemic activities *in vivo* and *in vitro* were tested; some of these compounds showed good activities. Their molecular and crystal structures were determined by an X-ray diffraction method. The results revealed that all four indirubin derivatives are planar, and have a tendency to form a big π-system. The molecular structures also showed that oximation of indirubin resulted in only a slight change in the antileukemic activity. On the other hand, the amido moiety in the compounds may play an important role in the activity of the indirubin monooxime derivatives. This conclusion was supported by the calculation results of the electrostatic-potential (esp) derived charge distribution of the indirubin derivatives, which were obtained using an *ab initio* molecular orbital of the basis set (3-21G), taking the electronic correlation into account at the MP2 level.

Danggui-luhui wan, pills of *Radix Angelicae Sinensis Aloes*, made according to the theory of traditional Chinese medicine, was found to be an active recipe against chronic granulocytic leukemia. *Indigofera tinctoria* was shown to be an active constituent after clinical studies of the recipe, from which indirubin (Chart 1) was isolated and found to have antileukemic activity.[1—4] As a new kind of cancer chemotherapeutic agent, indirubin has no inhibition to human immunity, which is the usual case, and shows a marked inhibitory action against Walker 256 and Lewis lung carcinoma in rats.[1,5] The binding between indirubin and DNA *in vitro* has been verified by means of an isotope-labeling method, a spectrophotometric method, and thermal denaturation measurements.[6—8] It is now being used clinically for treating chronic granulocytic leukemia in China. However, it was also found to have some side effects on the human digestive system, and can not be absorbed very well because of its poor dissolution ability. Therefore, a chemical modification of indirubin has been carried out since then, mainly to reduce its side effects and to improve its antileukemic activity. The roles of N_1-substitution, N_2-substitution, and the connecting position of the two indole rings in the antitumor activity were intensively investigated.[9—11] In this paper we report on our recent study of the indirubin derivatives. Four N_1- and O_2-substitution derivatives of indirubin (Chart 1) (indirubin monooxime (**IM**), indirubin monooxime *O*-methyl ether (**IMME**), N_1-methylindirubin monooxime *O*-methyl ether (**MIMME**), and indirubin monooxime *O*-ethyl ether (**IMEE**)) were synthesized, and their antileukemic activities were tested. It was found that **IMME** shows good activity, and that **IM** and **IMEE** also have some activities, in contrast to the poor activity for **MIMME** *in vitro* and *in vivo*. In order to understand their structure-activity relationship, an X-ray diffraction method was used to determine their molecular structures. Although previous paper described that substituting the H attached to N_1 by $-C_2H_5$, $-C_3H_7$, and $-COCH_3$ increased the antileukemic activity,[10] we have considered that the amido moiety may play an important role in the antileukemic activity of the derivatives of indirubin monooxime. This conclusion was supported by the calculation results of the electrostatic-potential (esp) derived charge dis-



indirubin



IM: R1= H; R2= H
IMME: R1= H; R2= CH3
MIMME: R1= CH3; R2= CH3
IMEE: R1= H; R2= C2H5

Chart 1.

tribution of indirubin derivatives obtained by using an *ab initio* molecular orbital of the basis set 3-21G, while taking the electronic correlation into account at the MP2 level.

Experimental

Syntheses. All of the chemicals used in this experiment were of chemical grade. Colunm chromatography was performed using EM Reagents silica gel 60, 230—400 mesh. The melting points were measured in open capillary tubes in an Electrothermal IA6304 apparatus, and were uncorrected. Elemental analyses for carbon, hydrogen, and nitrogen were performed using an automatic Carlo Erba elemental analyzer. 5% NaOH, methanol, acetone, tetrahydrofuran, DMF, and acetic ether were used to test the solubility of the compounds indirubin, **IM**, **IMEE**, **MIMME**, and **IMEE**, respectively. In each case, 5 ml of a solvent and 20 mg of a solute were taken at room temperature (20 °C).

Preparation of IM: $NH_2OH{\cdot}HCl$ (2 g) and KOH (8 g) were added to a suspension of indirubin (2 g) in 95% ethanol (40 ml). After refluxing for 2 h, the reaction mixture was cooled to room temperature and poured into water (350 ml). The precipitate was filtered off. Acetic acid was then added to the solution. A red product was precipitated and washed with water to give a 98% yield, and then recrystallized from acetone to give a dark-red crystal.

Preparation of IMME: CH_3I (7 ml) was added dropwise to a rapidly stirred solution of **IM** (2 g) and KOH (2.5 g) in ethanol (50 ml). After an additional 2 h of stirring, a dark-red product was precipitated. It was purified by column chromatography (1 : 2 $CHCl_3$/EtOAc) to yield 38% **IMME**, and **IMME** was recrystallized from DMF to give the dark red crystal.

Preparation of MIMME: Compound **MIMME** was prepared by the same reaction, and seperated under the same conditions as those described for **IMME** in 32% yield, and recrystallized from acetone to give a dark-red crystal.

Preparation of IMEE: $(CH_3CH_2)_2SO_4$ (10 ml) was added dropwise to a rapidly stirred solution of indirubin monooxime (2.0 g) and KOH (2.5 g) in ethanol (50 ml). After an additional 1 h of stirring, an orange-red product was precipitated to give a yield of 72%, and recrystallized from acetone to give a dark-red crystal.

Activity Test. ***In vitro* Drug Sensitivity:** 30 patients with newly diagnosed leukemia were selected to test the sensitivity of indirubin, **IM**, **IMME**, **MIMME**, and **IMEE** against human leukemic cells by a methylene-blue method *in vitro*.[12] Leukemic cells were separated from their bone marrow or peripheral blood. The cells of every patient were kept separately, and then washed twice by Roswell Park Memorial Institute Tissue Culture Medium (RPMI) 1640. They were found to have a trypen-blue viability greater than 90%. A $1—2\times10^7$ cell ml^{-1} suspension in RPMI 1640 was prepared for an activity evaluation of indirubin, **IM**, **IMME**, **MIMME**, and **IMEE**. The concentrations of 1, 5 and 10 $\mu g\,ml^{-1}$ were examined for all of these compounds, respectively, according to the following procedure. The screened compound was added, respectively, to each of the 30 tubes which contained 2 ml of the cell suspension of 30 patients; after the reaction mixture in every tube was aggitated so as to make it well-distributed, it was placed into an incubator at 37 °C. After 5 h, methylene-blue indicator was added to every tube. If the color of the sample suspension changed to blue, the tested compound was considered to have antileukemic activity. However, if the sample suspension kept its light-blue color or was colorless the compound was considered to have no antileukemic activity.

***In vivo* Study:** 20—24 g L_{7212} tumor-bearing mice and 18—26 g 6—8 week-old Kunming-strain male mice (provided by Animal Center of the Chinese Academy of Medical Sciences, Beijing) were used to test the antitumor activity and toxicity of indirubin and its derivatives, respectively, in this experiment. The animals were housed under the standard laboratory conditions (20.0±0.5 °C; relative humidity, 55—75%) and treated *per os* (po) with a daily drug treatment. The ratio of the medium survival time of the treated to the control mice, expressed as a percentage (T/C%), were used to indicate the activity.

Crystallography. All crystals of **IM**, **IMME**, **MIMME**, and **IMEE** were generally of good quality. Crystals having dimensions of about $0.3\times0.3\times0.4$ mm were selected for data collection on an Enraf Nonius CAD4 diffractometer with graphite-monochromated Mo $K\alpha$ radiation ($\lambda = 0.7107$ Å) using the ω–2θ scan technique over the range $2° < 2\theta < 50°$. On a PDP 11/44 computer with an SDP program package, the structures of **IM**, **IMME**, **MIMME**, and **IMEE** were solved by a direct method and Fourier-synthesis techniques, and refined by full-matrix least-squares with anisotropic thermal parameters for all non-hydrogen atoms. All of the hydrogen atoms were located on a difference-Fourier-map, and included in a least-squares refinement with isotropic thermal parameters.

Theoretical Calculation. As a measure of the antileukemic activity of indirubin derivatives, the electrostatic-potential (esp) derived charges were calculated by the CHelpG scheme of Breneman.[13] The total possible points were about 80000, of which a quarter fit the esp. The esp itself was found by an *ab initio* molecular-orbital calculation using a basis set of 3-21G, and taking the electronic correlation into account at the MP2 level. Both calculations were done by the program Gaussian 92/DFT.[14] The molecular geometries and conformations of compounds **IM**, **IMME**, **MIMME**, **IMEE**, and indirubin determined by an X-ray analysis were used as input for the calculations. The molecular geometries and conformations of N_1-ethylindirubin (**EI**) were built from that of indirubin by setting distances of C–N 1.427 Å and C–C 1.523 Å respectively.

Results and Discussion

Syntheses. The results of elemental analyses and the melting points of **IM**, **IMME**, **MIMME**, and **IMEE** are listed in Table 1. Their solubility test showed that **IM**, **IMME**, **MIMME**, and **IMEE** have better solubilities than does indirubin in 5% NaOH, methanol, acetone, tetrahydrofuran, DMF, and acetic ether.

Activity Test. ***In vitro* Test:** 7 acute lymphatic leukemia (ALL), 3 acute monocytic leukemia (AML), and

Table 1. Analytical and Physicochemistry Data of Indirubin Derivatives

S.	Formular	Mp	Found (Calcd) / %		
		°C	C	H	N
2	$C_{16}H_{11}N_3O_2$	245	69.21 (69.30)	4.03 (4.00)	15.09 (15.16)
3	$C_{17}H_{13}N_3O_2$	267	69.95 (70.09)	4.44 (4.50)	14.36 (14.42)
4	$C_{18}H_{15}N_3O_2$	241	70.88 (70.80)	5.04 (4.95)	13.76 (13.76)
5	$C_{18}H_{15}N_3O_2$	241	70.88 (70.80)	5.07 (4.95)	13.77 (13.76)

20 chronic granulocytic leukemia (CGL) were chosen for *in vitro* studies. The results of an antileukemic activity evaluation of indirubin and its derivatives are given in Table 2. It shows the order of the sensitivity of indirubin and its derivatives against human leukemic cells, as follows: **IMME** > **IMEE** > **IM**≈indirubin. **MIMME** was almost ineffective.

***In vivo* Test:** The results are listed in Table 3. The antitumor activities of indirubin and its derivatives (evaluated *in vivo*) are in accord with that of *in vitro* test. **IMME** and **IMEE** showed a greater improvement to increase the lifespan of the tumor-bearing mice compared with indirubin. The results also showed that the indirubin derivatives had low toxicity. No mice died because of the toxicity of the indirubin derivatives during our experiment.

Crystallography. The crystal data and information concerning the collection of the intensity-data sets are summarized in Table 4. The final positional parameters are listed in Table 5. Their molecular structures with atom labeling are shown in Fig. 1. From the results of a crystal analysis we can see that all four of these indirubin derivatives are planar molecules, and have the tendency to form a big π-system. The atom mean deviations from the least-squares plane, which comprise the main skeleton of the molecule, is no more than 0.042 Å for all four indirubin derivatives. The crystal structure of indirubin was reported in 1961.[15] It was found that indirubin molecules form an infinite chain by an

Table 2. Antileukemic Activity Evaluation of Indirubin and Its Derivatives *in vitro*

Compound	Effective rate (Effective No./Total No.)			
	ALL	AML	CGL	Total
Indirubin				
1 μl ml^{-1}	4/7	1/3	14/20	19/30
5 μl ml^{-1}	5/7	1/3	15/20	21/30
10 μl ml^{-1}	5/7	1/3	15/20	21/30
IM				
1 μl ml^{-1}	3/7	1/3	14/20	18/30
5 μl ml^{-1}	4/7	1/3	14/20	19/30
10 μl ml^{-1}	4/7	1/3	14/20	19/30
IMME				
1 μl ml^{-1}	5/7	2/3	17/20	24/30
5 μl ml^{-1}	6/7	2/3	19/20	27/30
10 μl ml^{-1}	6/7	2/3	20/20	28/30
IMEE				
1 μl ml^{-1}	5/7	2/3	14/20	21/30
5 μl ml^{-1}	6/7	2/3	14/20	22/30
10 μl ml^{-1}	6/7	2/3	15/20	23/30
MIMME				
1 μl ml^{-1}	0/7	0/3	1/20	1/30
5 μl ml^{-1}	1/7	0/3	1/20	2/30
10 μl ml^{-1}	1/7	0/3	1/20	2/30

Table 3. Evaluation of Indirubin and Its Derivatives Against L7212 in Mice

Compound	Dose	Toxicity[a]		Lifespan (day)[b]		T/C
	mg/kg/day	sample	control	sample	control	
Indirubin	400	0/6	0/8	9.5 ±0.29	9.0 ±0.23	109
	1000	0/7	0/11	11.5 ±0.78	9.7 ±0.50	118
IM	400	0/6	0/19	8.6 ±0.32	7.6 ±0.10	113
	1000	0/8	0/20	9.4 ±0.76	8.0 ±0.31	108
IMME	400	0/8	0/13	12.2 ±0.46	9.7 ±0.21	125
	1000	0/19	0/20	11.3 ±0.58	8.7 ±0.31	130
IMEE	400	0/6	0/18	8.3 ±0.43	7.2 ±0.13	115
	1000	0/7	0/13	9.3 ±0.71	7.5 ±0.21	124
MIMME	400	0/6	0/8	8.2 ±0.37	9.0 ±0.23	91.1
	1000	0/7	0/11	8.6 ±0.66	9.7 ±0.50	88.7

a) Sample: No. of mice dying of toxicity/No. of total mice; control: No. of mice dying during the test/No. of total mice. b) Values are averages of all surviving animals together with the average errors.

Table 4. Crystal Data and Information on Collection of the Intensity Data Sets

Compound	**IM**	**IMME**	**MIMME**	**IMEE**
Formular	$C_{16}H_{11}N_3O_2$	$C_{17}H_{13}N_3O_2$	$C_{18}H_{15}N_3O_2$	$C_{18}H_{15}N_3O_2$
Mol wt	277.28	291.31	305.34	305.34
Space group	$R3$	$P2_1/c$	$P2_1/c$	$P2_1/c$
a/Å	22.643(5)	5.061(1)	16.236(2)	9.743(1)
b/Å	22.643(5)	15.502(1)	5.504(1)	13.445(1)
c/Å	13.935(2)	17.509(1)	16.662(2)	11.807(3)
α/°	90	90	90	90
β/°	90	91.66(1)	104.17(2)	101.06(1)
γ/°	120	90	90	90
V/Å^3	6183.2(7)	1373.0(1)	1443.7(1)	1518.0(1)
Z	18	4	4	4
D_c/g cm^{-3}	1.340	1.409	1.405	1.336
No. of reflections measured	4902	2576	2941	2937
No. of reflections used in refinement $[I > 3\sigma(I)]$	1846	1941	1058	1380
R	0.047	0.0398	0.0396	0.0393
R_w	0.071	0.0403	0.0395	0.0418

intermolecular hydrogen bond in the crystal state. However, there is no such infinite chain in all four of the indirubin derivatives that we synthesized. We think that this may be the main reason that all the four indirubin derivatives have improved dissolution ability. Although indirubin and its four derivatives have different antileukemic activities, no obvious structure change in the main skeleton was found due to the N_1- or O_2-substitution of indirubin. Even the bond distances and bond angles around the amido moiety or hydroxyimino group of indirubin and its derivatives are quite similar. We can reach this conclusion based on Table 6. In this case we could not explain the activity of the indirubin derivatives based only on their structure information.

Theoretical Calculation. Some data of esp-derived charges of the indirubin derivatives, calculated by *ab initio* MO, are listed in Table 7. They revealed that the substitution of hydrogen in the amido moiety of indirubin monooxime by methyl affects the electronic state, not only the nitrogen atom of the amido moiety, but also the conjugated system from the amido moiety to the hydroxyimino group.

From the *in vivo* and *in vitro* studies of the indirubin derivatives we can conclude that the oximation of indirubin causes almost no change in the antileukemic activity; also after a hydrogen atom in the amido moiety was substituted by methyl, the compound **MIMME** showed no antileukemic activity, in contrast to no change or an increase in activity upon substituting hydrogen in hydroxyimino group by methyl or ethyl. Although **MIMME** has a better dissolution ability than does indirubin, it has almost no antileukemic activity. We therefore think that the dissolution ability is not the main factor that we should consider to improve the antileukemic activity of the indirubin derivatives. On the other hand, we think that the amido moiety may play an important role in the antileukemic activity of the derivatives of indirubin monooxime. In accord with the above conclusion, theoretical calculations of the indirubin derivatives showed that the difference in the esp-derived charge distribution between **MIMME** and the other derivatives, including indirubin, is not only at the nitrogen atom of the amido moiety, but also extends to the conjugated system. Thus, the substitution of H in the amido moiety by methyl transmits the effect from the amido group to the distant hydroxyimino group. However, the influence upon the esp-derived charge distribution caused by the H substitution in the hydroxyimino group by methyl or ethyl is not very much. As we mentioned above, a previous paper described how the substitution of an H attached to N_1 by $-C_2H_5$ (the derivative is **EI**), $-C_3H_7$, and $-COCH_3$ increases the antileukemic activity.[10] It is interesting that we found that the esp-derived charge distribution of **EI** is also quite different from that of **MIMME,** but is similar to those of the other four compounds. This means that the calculation results of the esp-derived charge distribution are coherent with the antileukemic activities of the indirubin derivatives. The results of the theoretical calculation support our conclusion that the amido moiety may play an important role in the antileukemic activity of the derivatives of indirubin monooxime, although this conclusion may be not right for all of the indirubin derivatives. Because the precise mechanisms of antileukemic action of indirubin derivatives are still unclear, and only a few indirubin derivatives have been synthesized, we can not further discuss the antileukemic action of the amido moiety in the indirubin derivatives at this moment.

We would like to thank Yoneyama Rotary Scholarship Foundation in Japan for offering a studentship for Ms. Chunmin Li to finish this work. We also would like to thank Ms. Zhengming Huang of the Reseach Institute of Chinese Medicine of Sichuan for kindly offering us indirubin.

Table 5. Fractional Atomic Coordinates and Equivalent Isotropic Thermal Parameters[a)]

Atom	x	y	z	B_{eq}[a)]/Å^2	Atom	x	y	z	B_{eq}[a)]/Å^2
IM					O2	0.0888(2)	1.2640(7)	0.5610(2)	5.25(8)
O1	0.8640(1)	0.0833(1)	0.9764(2)	3.80(8)	N1	0.3538(2)	0.5167(6)	0.8120(2)	3.31(8)
O2	0.6703(1)	0.1880(1)	1.1735(2)	4.00(7)	N2	0.3635(2)	1.1178(7)	0.6623(2)	3.24(8)
N1	0.8157(2)	0.0009(2)	1.0939(3)	3.82(9)	N3	0.1420(2)	1.1097(7)	0.6169(2)	4.06(9)
N2	0.8212(2)	0.1741(2)	0.9846(3)	3.46(9)	C1	0.2658(3)	0.4943(8)	0.8011(2)	3.32(9)
N3	0.7014(2)	0.1489(2)	1.1601(3)	3.36(8)	C2	0.2235(3)	0.3267(8)	0.8371(3)	4.2(1)
C1	0.7693(2)	−0.0157(2)	1.1693(3)	3.2(1)	C3	0.1357(3)	0.3450(1)	0.8196(3)	5.2(1)
C2	0.7483(2)	−0.0686(2)	1.2331(4)	4.3(1)	C4	0.0936(3)	0.5240(1)	0.7697(3)	5.1(1)
C3	0.7083(2)	−0.0740(2)	1.3045(4)	4.3(1)	C5	0.1363(3)	0.6924(9)	0.7341(3)	4.4(1)
C4	0.6803(2)	−0.0283(2)	1.3097(4)	3.9(1)	C6	0.2250(2)	0.6803(8)	0.7494(2)	3.26(9)
C5	0.7020(2)	0.0250(2)	1.2452(3)	3.4(1)	C7	0.3718(3)	0.7102(8)	0.7678(2)	3.08(9)
C6	0.7485(2)	0.0328(2)	1.1733(3)	2.9(1)	C8	0.2901(2)	0.8224(7)	0.7249(2)	2.86(9)
C7	0.8259(2)	0.0580(2)	1.0481(3)	3.2(1)	C9	0.2905(2)	1.0149(8)	0.6739(2)	2.87(9)
C8	0.7842(2)	0.0814(2)	1.0962(3)	2.8(1)	C10	0.2203(2)	1.1573(8)	0.6197(2)	3.21(9)
C9	0.7831(2)	0.1381(2)	1.0610(3)	2.9(1)	C11	0.3469(2)	1.3072(8)	0.6056(2)	3.05(9)
C10	0.7442(2)	0.1721(2)	1.0895(3)	2.80(9)	C12	0.2599(3)	1.3403(8)	0.5776(2)	3.19(9)
C11	0.8101(2)	0.2277(2)	0.9600(3)	3.3(1)	C13	0.2286(3)	1.5230(9)	0.5218(3)	4.0(1)
C12	0.7627(2)	0.2284(2)	1.0232(3)	3.0(1)	C14	0.2857(3)	1.6699(9)	0.4948(3)	4.6(1)
C13	0.7439(2)	0.2776(2)	1.0125(3)	3.7(1)	C15	0.3716(3)	1.6314(9)	0.5226(3)	4.4(1)
C14	0.7724(2)	0.3242(2)	0.9385(4)	4.5(1)	C16	0.4043(3)	1.4488(9)	0.5782(3)	4.1(1)
C15	0.8191(2)	0.3221(2)	0.8766(4)	4.5(1)	C17	0.4168(3)	0.3615(9)	0.8615(3)	4.9(1)
C16	0.8392(2)	0.2742(2)	0.8862(2)	4.1(1)	C18	0.0033(3)	1.2330(1)	0.5695(4)	6.7(2)
IMME					**IMEE**				
O1	1.2570(4)	0.5908(1)	−0.0186(1)	3.98(4)	O1	0.1820(3)	1.0285(3)	0.0876(2)	3.56(7)
O2	0.3388(4)	0.7627(1)	0.1898(1)	4.02(4)	O2	0.7738(3)	0.7921(2)	0.1116(3)	4.63(8)
N1	1.3204(4)	0.5029(2)	0.0865(1)	3.84(5)	N1	0.0996(5)	0.9021(4)	−0.0390(3)	3.18(8)
N2	0.8670(4)	0.7035(2)	−0.0015(1)	3.67(5)	N2	0.4579(4)	1.0160(3)	0.1702(3)	2.91(8)
N3	0.5374(4)	0.7038(1)	0.1716(1)	3.38(5)	N3	0.6325(5)	0.8159(5)	0.0747(4)	3.7(3)
C1	1.2107(5)	0.4911(2)	0.1574(2)	3.51(6)	C1	0.1549(3)	0.8208(5)	−0.0881(6)	2.9(6)
C2	1.2873(6)	0.4347(2)	0.2141(2)	4.41(7)	C2	0.0841(4)	0.7526(3)	−0.1655(4)	3.7(1)
C3	1.1512(6)	0.4355(2)	0.2817(2)	4.60(7)	C3	0.1607(3)	0.6764(4)	−0.2023(4)	4.1(1)
C4	0.9420(6)	0.4922(2)	0.2908(2)	4.43(7)	C4	0.3042(3)	0.6707(4)	−0.1629(2)	3.9(2)
C5	0.8632(5)	0.5484(2)	0.2334(2)	3.72(6)	C5	0.3748(4)	0.7395(2)	−0.0855(3)	3.4(2)
C6	0.9987(5)	0.5503(2)	0.1651(2)	3.16(5)	C6	0.3007(2)	0.8165(3)	−0.0460(6)	2.7(5)
C7	1.1971(5)	0.5662(2)	0.0463(2)	3.38(5)	C7	0.2018(5)	0.9526(6)	0.0321(4)	2.9(3)
C8	0.9841(5)	0.6003(2)	0.0950(2)	3.08(5)	C8	0.3349(6)	0.9000(5)	0.0330(3)	2.65(8)
C9	0.8337(5)	0.6682(2)	0.0689(2)	3.01(5)	C9	0.4542(5)	0.9330(3)	0.1025(4)	2.5(1)
C10	0.6174(5)	0.7097(2)	0.1037(2)	2.99(5)	C10	0.6000(3)	0.8936(4)	0.1280(3)	2.8(1)
C11	0.6971(5)	0.7728(2)	−0.0159(2)	3.39(5)	C11	0.5888(4)	1.0313(5)	0.2389(6)	2.8(6)
C12	0.5377(5)	0.7844(2)	0.0474(2)	3.23(5)	C12	0.6798(4)	0.9576(3)	0.2151(4)	2.9(1)
C13	0.3458(6)	0.8494(2)	0.0454(2)	3.89(6)	C13	0.8193(4)	0.9591(4)	0.2709(5)	3.7(1)
C14	0.3229(6)	0.9004(2)	−0.0189(2)	4.45(7)	C14	0.8628(4)	1.0339(5)	0.3502(3)	4.1(2)
C15	0.4853(6)	0.8885(2)	−0.0809(2)	4.58(7)	C15	0.7705(5)	1.1060(3)	0.3741(4)	4.0(2)
C16	0.6777(6)	0.8242(2)	−0.0798(2)	4.30(7)	C16	0.6309(3)	1.1068(3)	0.3180(6)	3.5(5)
C17	0.2397(6)	0.7427(2)	0.2637(2)	4.57(7)	C17	0.8050(3)	0.7052(4)	0.0489(3)	5.2(1)
MIMME					C18	0.7466(6)	0.6140(3)	0.0916(4)	6.4(1)
O1	0.4450(2)	0.7735(6)	0.7680(2)	3.87(7)					

a) $B=4/3\sum\beta_{ij}\boldsymbol{a}_i\cdot\boldsymbol{b}_j$.

Table 6. Selected Bond Lengths (Å) and Angles (°)

Compound	N1–C7	C7–O1	C10–N3	N3–O2	C1–N1–C7	N1–C7–O1	C9–C10–N3	C10–N3–O2
IM	1.354(7)	1.256(5)	1.294(6)	1.392(6)	110.9(5)	124.1(5)	120.7(5)	111.0(4)
IMME	1.350(4)	1.244(4)	1.270(4)	1.401(3)	111.8(2)	125.3(3)	129.7(3)	114.6(2)
MIMME	1.367(3)	1.238(3)	1.288(5)	1.393(5)	110.2(3)	123.4(2)	121.1(4)	110.2(3)
IMEE	1.355(7)	1.248(8)	1.290(8)	1.399(6)	110.7(5)	124.5(5)	120.2(4)	110.6(4)
Indirubin[18)]	1.38	1.25	1.21 (C10–O2)		111	126	126 (C9–C10–O2)	

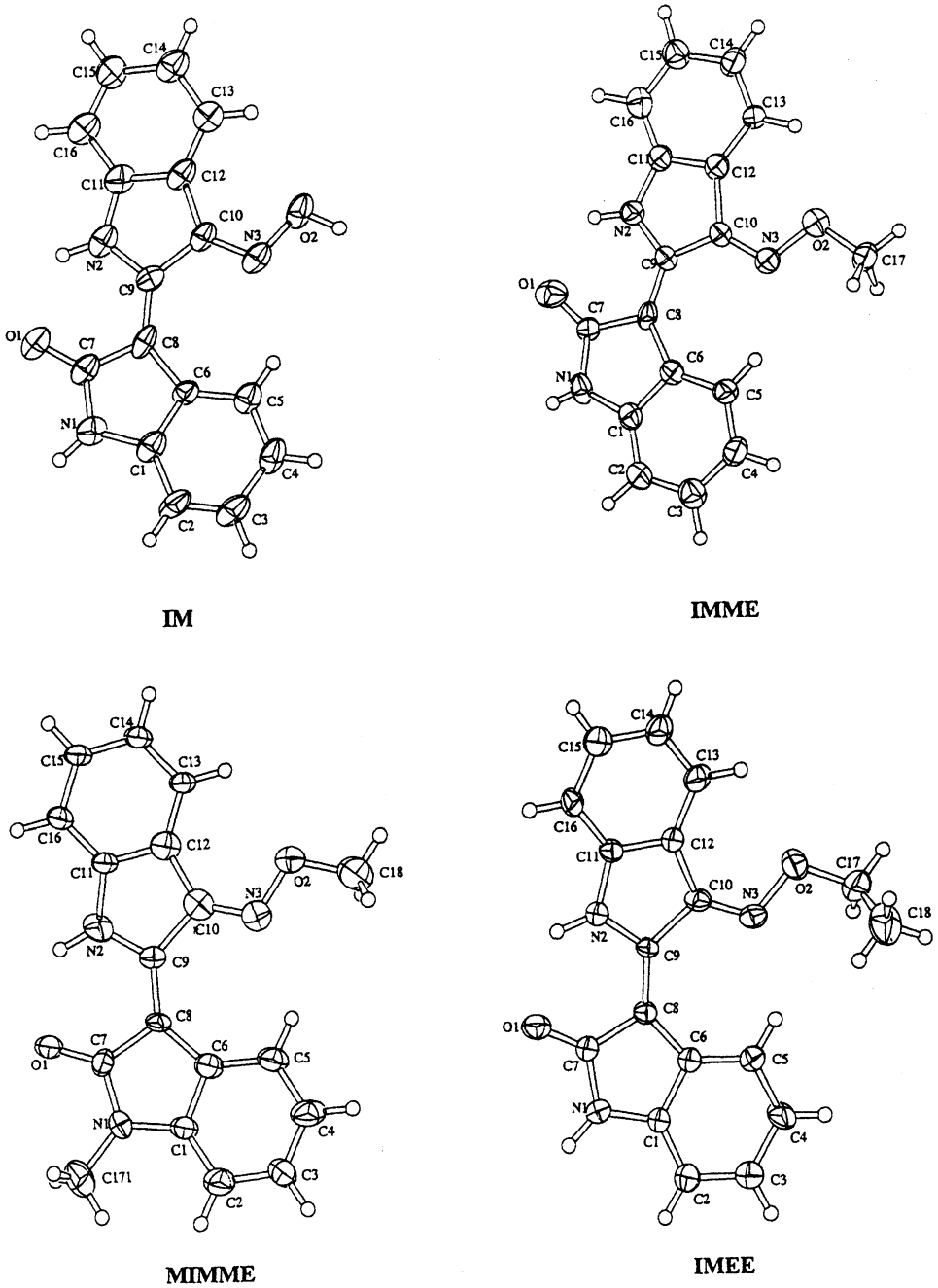


Fig. 1. Molecular structures of IM, IMME, MIMME, and IMEE.

Table 7. Effect of CH_3 Substitution of Amide on the Charge Distribution of Conjugated System from Amide to Hydroxyimino Group

Compound	N1	C1	C6	C8	C9	C10	N3	O2
MIMME	−0.878	0.689	−0.820	−0.741	1.023	−0.432	0.251	−0.253
IMME	−0.657	0.233	0.076	−0.387	0.320	0.176	−0.120	−0.159
IMEE	−0.702	0.288	0.036	−0.353	0.228	0.198	−0.102	−0.257
IM	−0.683	0.238	0.066	−0.351	0.216	0.228	−0.104	−0.439
EI	−0.446	0.201	0.063	−0.326	0.215	0.499		
Indirubin	−0.689	0.262	0.038	−0.313	0.181	0.511		

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