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Structure and Cytotoxic Activity of Novel 2-(Coumarinyl-6-azo)imidazoles

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In present work, the cytotoxic activity of a series of 2-(coumarinyl-6-azo)imidazoles compounds were examined. The compounds were screened for cell viability assay in RAW 264.7 cell line and all the compounds exhibit cytotoxic activity. Single crystal X-ray diffraction study had also been carried out to confirm the structure of the compounds.

Keywords: Coumarylazoimidazole, Structure, Cytotoxicity.

INTRODUCTION

Coumarins, are a class of naturally occurring benzopyran derivatives. Depending on the type of substitution, coumarin and its different derivatives are found to have broad range of biological and pharmacological applications. Different derivatives of coumarin are found to have antibacterial [1], anticoagulant [2,3], antifungal [4], anti-HIV [5,6], antitumor [7], antiplasmodial [8], cytotoxic [9-14] and anticancer activity [15,16].

Since cytotoxic compounds have anticancer property, so the cytotoxic coumarin compounds might act as promising anticancer agents. Nowadays the common use of cytotoxic agent is as chemotherapeutic drugs. As of now, cancer is the most challenging disease which troll many lives every year and is the 2nd leading cause of death worldwide every year. Since, the anticancer drugs have many side-effects so scientists are in a search to find out drugs having minimum side effect [17, 18]. The new generation of cancer chemotherapeutic drugs are selected based on their efficacy and low toxicity profiles. Since, coumarin based anticancer drugs are found to have very less or negligible side effects, so study of coumarin based anticancer drug is a promising area of research.

Since, several coumarin derivatives are found to have potential applications in cancer chemotherapy, scientists have made huge effort to design anticancer coumarin compounds with improved activity. Example of coumarin derivatives which have remarkable anticancer activity are 6-pyrazolinylcoumarin derivatives, 3-alkyl-4-methylcoumarins, pyranocoumarins, coumarin carbox-amides, quaternary ammonium coumarins, 7-aminocoumarins and 4-aminocoumarins. Coumarin-3-(*N*-aryl) carboxamides, iodinated-4-aryloxymethylcoumarins, *etc*. [19,20] 6-bromo-coumarin-ethylidene-hydrazonyl-thiazolyl and 6-bromo-coumarin-thiazolyl- based derivative & coumarin sulfonamide derivatives have potential cytotoxic and anticancer activity [21,22].

As of now only a few reports are available where the azo compounds can exhibit cytotoxic activity [23-25] and there is rarely any report on azo-coumarin compounds till date showing cytotoxic effect except the compounds derived from 4-hydroxy coumarin [26]. Previously, the antioxidant activity of *N*-[(2-pyridyl)methyliden]-6-coumarin and its group six metal carbonyl complexes have been reported [27]. Moreover, the photophysical properties, photovoltaic activity and photochromic nature of the coumarinylazo imidazoles compounds were also explored [27]. As a part of our research interest on the chemistry and applications of coumarin azo and coumarin Schiff's base compounds and their metal complexes, herein the structure and cytotoxic activity of coumarin azo imidazoles are reported.

EXPERIMENTAL

Coumarin and methyl iodide were purchased from Sisco Research Lab, India, while 4-phenyl imidazole and 4-methyl imidazole were procured from Sigma-Aldrich (USA). 3-(4,5-Dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium (MTS), inner salt was purchased from Promega, USA. All other chemicals and solvents were of reagent grade and used without further purification. Commercially available SRL silica gel (60-120 mesh) was used for column chromatography.

X-ray crystallography: Single crystals of HCZ-4-PhH (**1c**) were grown by slow evaporation of acetonitrile-benzene solution. Crystal parameters and refined data are summarized in Table-1. The data were collected by fine focus sealed tube at 293(2) K using graphite monochromator Bruker Smart CCD Area Detector (Mo*K*α radiation, (λ = 0.71073 Å). The structure determination was performed by direct methods using SHELXS-97 and refinements with full-matrix least squares on F^2. All calculations were carried out using SHELXS 97 [28], SHELXL 97 [29], PLATON 99 [30] and ORTEP [31] programs.

TABLE-1
SELECTED CRYSTALLOGRAPHIC DATA FOR COMPOUND **1c**

Empirical formula	$C_{36}H_{24}N_8O_4$
Formula weight (g M^{-1})	632.63
Crystal system	Orthorhombic
Space group	C212121
a (Å)	11.560
b (Å)	13.714
c (Å)	18.587
α (°)	90.00
β (°)	90.00
γ (°)	90.00
V (Å^3)	2946.7
Z	4
T (K)	293(2)
Density (calculated) (Mg/m^3)	1.426
λ (Å) (Mo-K_α)	0.71073
Absorption coefficient (mm^{-1})	0.097
Total reflection collected	5693
Unique reflections	1171
Refined parameters	217
hkl range	$-8 \le h \le 10$; $-12 \le k \le 12$; $-16 \le l \le 17$
θ range (°)	2.19- 18.95
Largest diff. peak and hole (e Å^{-3})	0.127 and -0.143
R[a] (I > 2σ(I))	0.0485
wR[b]	0.0970
GOF[c]	1.037

[a]$R = \Sigma|F_0 - F_c|/\Sigma F_0$.
[b]$wR = [\Sigma w(F_0^2 - F_c^2)/\Sigma wF_0^4]^{1/2}$ where $w = 1/[\sigma^2(F_0^2) + (0.0392P)^2]$, where $P = (F_0^2 + 2F_c^2)/3$.
[c]GOF (Goodness of fit) is defined as $[w(F_0^2 - F_c^2)/(n_0 - n_p)]^{1/2}$ where n_0 and n_p denote the number of data and variables, respectively.

Cell line analyses and cell viability tests

Cell culture: A non-adherent human monocyte macrophage cell line, RAW264.7, obtained from National Centre for Cell Science (Pune, India), was maintained in RPMI 1640 supplemented with 10% fetal calf serum, penicillin (50 U/mL) and streptomycin (50 µg/mL) at 37 ºC, in a humidified 95% air, 5% CO_2 atmosphere. The cells were subcultured every 72 h using an inoculum of 1×10^6 cells/mL.

Cytotoxicity study: The cytotoxic activity of the drugs was measured by a cell viability assay using a modified MTS assay [32]. Exponentially growing RAW264.7 cells (2×10^5 cells/200 µL/well) were incubated for 48 h with different concentrations (0-100 µg/mL) of coumarylazoimidazoles along with DMSO, which represented the highest concentration of DMSO. The MTS was prepared (2 mg/mL in PBS) along with phenazine methosulphate (PMS, 0.92 mg/mL in PBS) and then stored in the dark at -20 ºC. Just before use, MTS and PMS were mixed in the ratio of 20:1 and 20 µL of solution was added to each well. The plates were then incubated at 37 ºC for 3 h and absorbance measured at 490 nm using a Multiscan ELISA reader (BioRad, USA). The percent viability was calculated as follows:

$$\text{Viability (\%)} = \frac{\text{Mean specific absorbance of treated parasites}}{\text{Mean specific absorbance of untreated parasites}} \times 100$$

Mean specific absorbance of untreated parasites: The inhibitory concentration or IC_{50} was measured by graphical extrapolation by plotting % viability *vs.* drug concentration using GRAPHPAD PRISM software (version 4).

RESULTS AND DISCUSSION

The synthesis of new coumarin derivatives is a challenging task to the chemists. In present case, we have started from the coumarin itself. After nitrating it with mixed acid and subsequent reduction with Fe powder/NH_4Cl, 6-amino coumarin was synthesized. It was diazotized and then coupled with different imidazole compounds to produce 2-(coumarinyl-6-azo)-imidazoles [27]. Two set of compounds were synthesized: 2-(coumaryl-6-azo)-4-substituted imidazole (HCZ-R) and its methylated products 1-methyl-2-(coumaryl-6-azo)-4-substituted imidazole (R′CZ-R) (**Scheme-I**).

Molecular structure: The molecular structure of 2-(coumaryl-6-azo)-4-phenyl-imidazole (**1c**) is shown in Fig. 1. The molecule is in *trans*-configuration about –N=N- bond. The azo distance, N(6)-N(7) is 1.257(7) Å, (Table-2) is comparable to the previously reported phenylazo-imidazole [33]. The C(4)-C(17) & C(2)-N(1) bond distances were 1.462(9) Å and 1.325(8) Å, respectively.

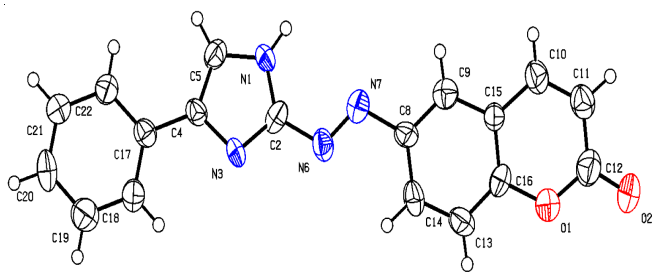
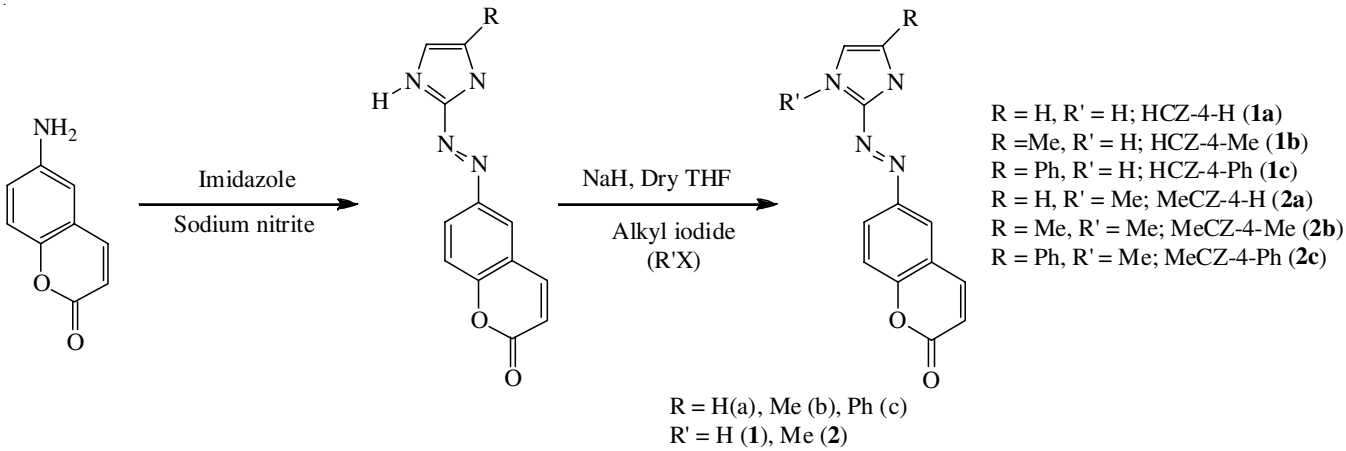


Fig. 1. X-ray crystallographic structure of HCZ-4-Ph (**1c**)

Cytotoxic activity: The cytotoxic activity of the synthesized 2-(coumarinyl-6-azo)imidazoles wes measured by a cell viability assay using a modified MTS assay wherein the conv-



Scheme-I: Coumarylazoimidazole ligand

TABLE-2
SELECTED BOND LENGTHS (Å) AND ANGLES (°) FOR HCZ-4-Ph (**1c**) WITH ESTIMATED STANDARD DEVIATIONS IN THE PARENTHESES

Bond lengths (Å)		Bond angles (°)	
N(6)-N(7)	1.257(7)	C(2)-N(6)-N(7)	113.6(6)
C(2)-N(6)	1.396(8)	N(6)-N(7)-C(8)	114.1(6)
N(7)-C(8)	1.438(8)	N(7)-C(8)-C(9)	115.7(8)
C(2)-N(3)	1.348(8)	N(7)-C(8)-C(14)	123.5(8)
C(2)-N(1)	1.325(8)	C(14)-C(8)-C(9)	120.8(7)
C(8)-C(9)	1.380(8)	N(6)-C(2)-N(1)	128.6(9)
C(8)-C(14)	1.385(9)	N(6)-C(2)-N(3)	119.2(8)
O(1)-C(12)	1.368(9)	N(3)-C(2)-N(1)	112.1(6)
O(2)-C(12)	1.229(8)	N(3)-C(4)-C(5)	107.6(6)
C(12)-C(11)	1.438(10)	C(4)-C(17)-C(22)	120.2(8)
C(4)-C(17)	1.462(9)	C(4)-C(17)-C(18)	121.2(8)
C(17)-C(18)	1.378(9)	C(22)-C(17)-C(18)	118.6(7)
C(17)-C(22)	1.406(8)	–	–

ersion of MTS to formazan by mitochondrial enzymes in the presence of the electron coupler phenazine methosulfate served as an indicator of cell viability thus, a decrease in formazan production indicated decrease in cell viability and *vice versa* [32]. Treatment of cells with the azo compounds (0-100 µg/mL) caused a dose-dependent inhibition of growth and IC_{50} values obtained were 7.91 µg/mL (HCZ-4-Ph), 22.9 µg/mL (MeCZ-4-Ph), 14.5 µg/mL (HCZ-4-H), 32.0 µg/mL (MeCZ-4-H), 7.5 µg/mL (HCZ-4-Me) and 23.3 µg/mL (MeCZ-4-Me) (Fig. 2). The highest concentration of DMSO caused no change in cell viability.

All six coumarylazoimidazoles exhibit cytotoxicity and among them HCZ-4-Me and HCZ-4-Ph shows potential cytotoxicity. The cytotoxic activity is reduced when the N-H proton is substituted by methyl group. More precise and rigorous study is needed to reach any conclusion and merits future pharma-

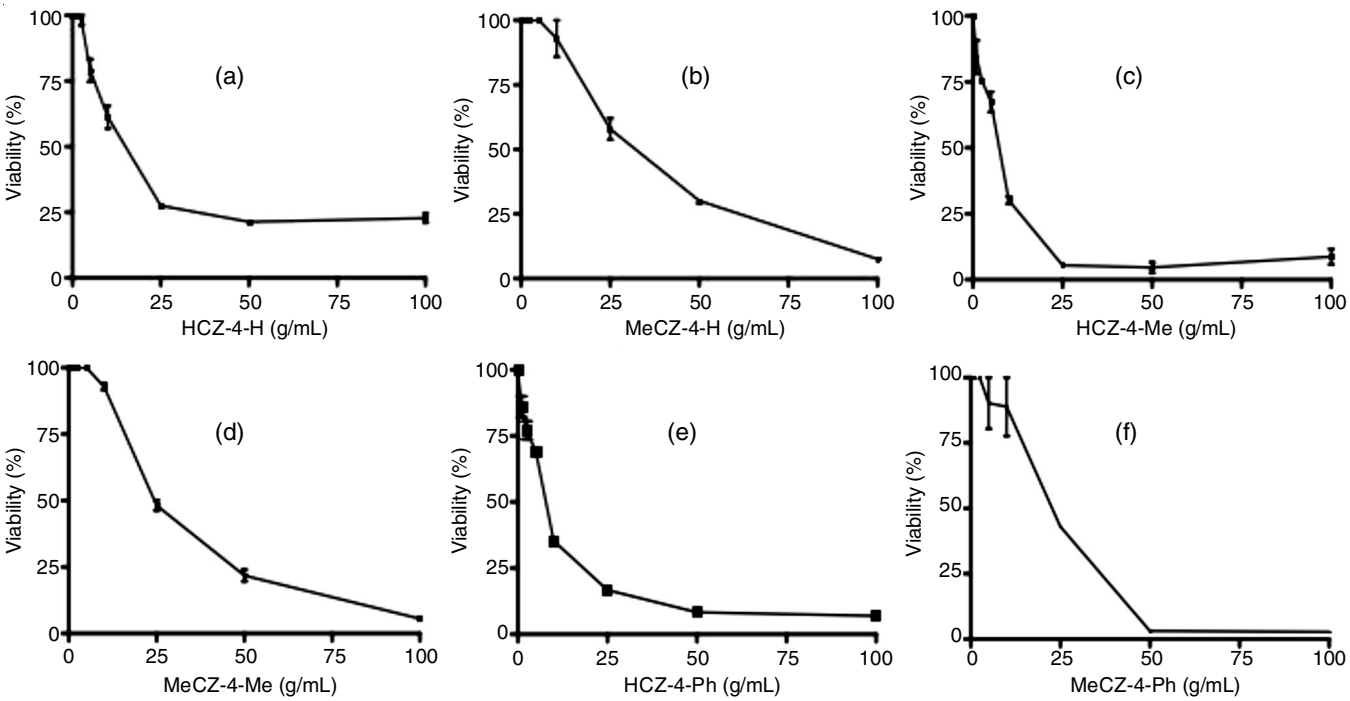


Fig. 3. Cytotoxic activity of (a) HCZ-4-H, (b) MeCZ-4-H; (c) HCZ-4-Me; (d) MeCZ-4-Me; (e) HCZ-4-Ph; (f) MeCZ-4-Ph (Each point corresponds to the mean ± SD of at least three experiments in duplicate

cological investigations to establish it as potential anticancer drug.

Conclusion

In conclusion, the cytotoxic activities of 2-(coumarinyl-6-azo)imidazoles were studied using RAW 264.7 cell line. Structure of one of the compounds has been established by single crystal X-ray diffraction study. All the synthesized compounds exhibit cytotoxic activity, however in case of HCZ-4-Me and HCZ-4-Ph the cytotoxic activity is more pronounced. Simiarly, 2-(coumaryl-6-azo)-4-substituted imidazole (HCZ-R) were found to be more cytotoxic in nature compared to 1-methyl-2-(coumaryl-6-azo)-4-substituted imidazole (R′CZ-R).

Supplementary data: Crystallographic data for the structural analysis was deposited in the Cambridge Crystallographic Data Centre and CCDC No. 751578. Copies of this information may be obtained free of charge from the Director, CCDC, 12 Union Road, Cambridge, CB2 1FZ, U.K. (email: deposit@ccdc.cam.ac.uk or http://www.ccdc.cam.ac.uk).

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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