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ANTI-CANCEROUS ACTIVITY OF AUTO-OXIDIZED PRODUCTS FROM 9H, 10H-INDOLIZINO [1, 2-B] INDOLE-1-ONE DERIVATIVES

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ABSTRACT

The tetracycle 9H, 10H-Indolizino [1, 2-b] indole-1-one derivatives (2a – 2d) underwent a novel auto-oxidation in the presence or absence of base [1]. The product peroxides (7a – d, 10a – d, 12a – d) were tested for their anti-cancerous activity against different cancer cell lines. Few peroxides were found out to be active against cancer cell lines with IC_{50} in low nanomolar to macromolar range. Our study proves the efficacy of the peroxide to stop propagation of cancerous cell lines *in vitro*.

Keywords: Auto-oxidation, indolizino indole, peroxides, in vitro anti-cancer activity and radicals.

INTRODUCTION

Development of new anti-cancerous drugs is the call of the hour, as cancer is becoming an almost epidemic in modern time, may be due to our lifestyle and modernization of society. In our effort to discover new anti-cancer drugs we investigate the efficacy of the product peroxides formed by the auto-oxidation of tetracycle 9H, 10H-indolizino [1, 2-b] indole-1-one derivatives (**2a–d**) by a novel mechanism of azainolate/enamine intermediates as shown by our previous publication [1] like allylic carbanion [2]. Few peroxides are known to have anticancer activity due to the formation of peroxy radical [3]. In addition, indoles are also known to have rich biological activity [4]. With this known facts we have tested the anti-cancerous activity of the peroxides (**7a – d**, **10a – d**, **12a – d**) formed by the auto-oxidation of the synthesized indolizino-indole derivatives (**2a –d**) in the presence of base/MeI [1] or with only base and without methylating agent. The peroxides were isolated by silica gel column chromatography and tested against colon HT – 29, nasopharyngeal carcinoma HONE- 1 and BM -1 & breast carcinoma MX – 1 [5-9] *in vitro*. The result is encouraging and many of the peroxides are found out to be inhibitor of cancerous cell growth *in vitro.* The IC_{50} that is the concentration of drug needed to inhibit 50% cell growth is found out in low micromolar range. We anticipate that these peroxides have a potency to be used as anti-cancerous agent. We are working on further study of these peroxides to find a clear cut picture of mode of action to use them as drug candidates.

MATERIAL AND METHODS

Compounds **2a – d** were synthesized by a known procedure using Fischer Indole [10] synthesis. Chemical required were purchased from Aldrich, Mecrk. Column chromatography was performed using silica gel G60 (70 – 230 mesh, Merck), thin layer chromatograph preparative layer chromatography were performed on silica gel GF_{254} pre-coated aluminum foil (Merck). ^{1}HNMR was observed in Bruker 400 MHz instrument.

EXPERIMENTAL

The products were subjected to auto-oxidation procedure as given below.

Procedure for auto-oxidation without methylating agent

A solution of the indolizino indole (**2a – d**, 5 mmole) in dry DMF (50 mL) and dry potassium carbonate (2.05 g, 15 mmol) was heated at 80 ^{0} C for three h. The resulting solution was filtered and the solid was filtered out. From the filtrate DMF was distilled out and the product was extracted by ethyl acetate washing with water and dried by sodium

sulphate. The product was isolated by silica gel column chromatography eluting with 1 % methanol in ethyl acetate. The product is extremely unstable and the after repeated crystallization we got a very little pure product. Procedure for auto oxidation without methylating agent : A solution of the indolizino indole (**2a – d**, 5 mmole) in dry DMF (50 mL), dry potassium carbonate (2.05 g, 15 mmol) and methy iodide (15 mL, 24 mmol) was heated at 80 ^{0} C for three h. The resulting solution was filtered and the solid was filtered out. From the filtrate DMF was distilled out and the product was extracted by ethyl acetate washing with water and dried by sodium sulphate. Different products were isolated in different polarity of the eluting solvents. Compounds 9a – d were eluted first with hexane/EA (3:1, v/v) followed by **11a –d**, **10a – d**, **7a – d** and **12a –d**.

Biological assays and cytotoxic assay

The effect of the peroxides on cell growth were determined in all human tumor cells (i.e., colon HT – 29, nasopharyngeal carcinoma HONE- 1 and BM -1 &breast carcinoma MX – 1), in a 72 h. incubation, by XTT –tetrazolium assay, as prescribed in references [5-9]. After the addition of phenazine methosulphate-XTT solution at 37 ^{0} C for 6 h. absorbance at 450 and 630 nm was detected on a microplate reader. 6 to 7 different concentrations of each compound were used. The IC_{50} dose effect was calculated by a median relationship using a computer program.

^{1}H NMR and Purification Data

10-Hydroperoxy-2-cyano-3-methyl-1-methoxy-indolizino[1,2-b]-indole (7a)

Eluted by Hexane : EA = 1 : 2 (v/v), ^{1}H NMR (DMSO d_6) δ 2.45 (3H, s), 3.86 (3H, s), 6.40 (1H, s), 7.21 (1H, t, *J* = 7.6 Hz), 7.37 (1H, t, *J* = 7.2 Hz), 7.54 (1H, d, *J* = 8.4 Hz), 7.65 (1 H, d, *J* = 8 Hz), 12.37 (1H, s, exch.), 12.48 (1H, s, exch.).

10-Hydroperoxy-2-cyano-3,7,8-trimethyl-1-methoxy-indolizino[1,2-b]-indole (7b)

Eluted by Hexane : EA = 1 : 2 (v/v), ^{1}H NMR (DMSO d_6) δ 2.40 (3H, s), 2.44 (3H, s), 2.50 (3H, s), 4.02 (3H, s), 6.44 (1H, s), 7.05 (1H, brs), 7.35 (1H, brs),11.69 (1H, s, exch.), 12.38 (1H, s, exch.).

10-Hydroperoxy-2-cyano-3,6,8-trimethyl-1-methoxy-indolizino[1,2-b]-indole (7c)

Eluted by Hexane : EA = 1 : 2 (v/v), ^{1}H NMR (DMSO d_6) δ 2.40 (3H, s), 2.45 (3H, s), 2.50 (3H, s), 4.02 (3H, s), 6.43 (1H, s), 7.09 (1H, s), 7.21 (1H, s), 7.54 (1H, d, *J* = 8.4 Hz),12.19 (1H, s, exch.), 12.42 (1H, s, exch.).

10-Hydroperoxy-8-fluoro-2-cyano-3-methyl-1-methoxy-indolizino[1,2-b]-indole (7d)

Eluted by Hexane : EA = 1 : 2 (v/v), ^{1}H NMR (DMSO d_6) δ 2.41 (3H, s), 4.22 (3H, s), 6.41 (1H, s), 7.26 (2H, m), 7.44 (1H, m), 13.22 (1H, s, exch.), 13.42 (1H, s, exch.).

9-*N*-methyl-10-hydroperoxy-2-cyano-3-methyl-1-methoxy-indolizino[1,2-b]-indole (8a)

Eluted by Hexane : EA = 2 : 3 (v/v), ^{1}H NMR (DMSO d_6) δ 2.45 (3H, s), 3.86 (3H, s), 4.01 (3H, s), 6.40 (1H, s), 7.21 (1H, t, *J* = 7.6 Hz), 7.37 (1H, t, *J* = 7.2 Hz), 7.54 (1H, d, *J* = 8.4 Hz), 7.65 (1 H, d, *J* = 8 Hz), 12.47 (1H, s, exch.).

9-*N*-methyl-10-methylperoxy-2-cyano-3-methyl-1-methoxy-indolizino[1,2-b]-indole (9a)

Eluted by Hexane : EA = 3 : 1 (v/v), ^{1}H NMR (DMSO d_6) δ 2.39 (3H, s), 3.18 (3H, s), 3.84 (3H, s), 4.19 (3H, s), 6.37 (1H, s), 7.24 (1H, d, *J* = 8.4 Hz), 7.48 (2H, d, *J* = 7.6 Hz), 7.74 (1H, d, *J* = 8.4 Hz).

9-*N*-methyl-10-methylperoxy-2-cyano-3,7-dimethyl-1-methoxy-indolizino[1,2-b]-indole (9b)

Eluted by Hexane : EA = 3 : 1 (v/v), ^{1}H NMR (DMSO d_6) δ 2.35 (3H, s), 2.40 (3H, s), 2.44 (3H, s), 3.20 (3H, s), 3.39 (3H, s), 3.83 (3H, s), 6.43 (1H, s), 7.10 (1H, brs), 7.35 (1H, brs).

9-*N*-methyl-10-methylperoxy-2-cyano-3,7-dimethyl-1-methoxy-indolizino[1,2-b]-indole (9c)

Eluted by Hexane : EA = 2 : 1 (v/v), ^{1}H NMR (DMSO d_6) δ 2.35 (3H, s), 2.45 (3H, s), 2.56 (3H, s), 2.83 (3H, s), 3.22 (3H, s), 4.34 (3H, s), 6.40 (1H, s), 7.05 (1H, s), 7.21 (1H, s).

9-*N*-methyl-10-methylperoxy-8-fluoro-2-cyano-3-methyl-1-methoxy-indolizino[1,2-b]-indole (9d)

Eluted by Hexane : EA = 2 : 1(v/v), ^{1}H NMR (DMSO d_6) δ 2.41 (3H, s), 2.83 (3H, s), 3.68 (3H, s), 4.22 (3H, s), 6.41 (1H, s), 7.26 (2H, m), 7.44 (1H, m).

9-*N*-methyl-10-hydroperoxy-2-cyano-3-methyl-1-methoxy-indolizino[1,2-b]-indole (10a)

Eluted by Hexane : EA = 2 : 1 (v/v), ^{1}H NMR (DMSO d_6) δ 2.45 (3H, s), 4.07 (3H, s), 4.14 (3H, s), 6.42 (1H, s), 7.27 (1H, d, *J* = 8.4Hz), 7.65 (2H, m), 7.72 (1H, d, *J* = 8.2 Hz), 12.42 (1H, s, exch.).

9-*N*-methyl-10-hydroperoxy-2-cyano-3,7-dimethyl-1-methoxy-indolizino[1,2-b]-indole (10b)
Eluted by Hexane : EA = 1 : 1 (v/v), ^{1}H NMR (DMSO d_6) δ 2.35 (3H, s), 2.50 (3H, s), 3.82 (3H, s), 4.00 (3H, s), 6.94 (1H, s), 7.33 (1H, d, *J* = 8 Hz), 7.58 (2H, d, *J* = 8 Hz), 11.72 (1H, s, exch.).

9-*N*-methyl-10-hydroperoxy-2-cyano-3,6-dimethyl-1-methoxy-indolizino[1,2-b]-indole (10c)
Eluted by Hexane : EA = 2 : 1 (v/v), ^{1}H NMR (DMSO d_6) δ 2.37 (3H, s), 2.41 (3h, s), 2.56 (3H, s), 3.79 (3H, s), 3.96 (3H, s), 6.46 (1H, s), 7.07 (1H, s), 7.21 (1H, s), 12.26 (1H, s, exch.).

9-*N*-methyl-10-hydroperoxy-8-fluoro-2-cyano-3-methyl-1-methoxy-indolizino[1,2-b]-indole (10d)
Eluted by Hexane : EA = 2 : 1 (v/v), ^{1}H NMR (DMSO d_6) δ 2.43 (3H, s), 3.78 (3H, s), 4.22 (3H, s), 6.43 (1H, s), 7.27 (2H, m), 7.46 (1H, m), 13.29 (1H, s, exch.).

10-Hydroperoxy-2-cyano-1-hydroxy-indolizino[1,2-b]-indole (11a)
Eluted by Hexane : EA = 1 : 1 (v/v), ^{1}H NMR (DMSO d_6) δ 2.50 (3H, s), 3.82 (3H, s), 3.93 (3H, s), 3.96 (3H, s), 7.27 (1H, m), 7.36 (1H, s), 7.41 (1H, m), 7.69 (1H, d, *J* = 8.4 Hz), 7.98 (1H, d, *J* = 8.8 Hz).

10-Hydroperoxy-2-cyano-3-methyl-1-hydroxy-indolizino[1,2-b]-indole(12a)
Eluted by 1 % MeOH in EA (v/v), ^{1}H NMR (DMSO d_6) δ 2.45 (3H, s), 3.86 (3H, s), 6.40 (1H, s), 7.21 (1H, t, *J* = 7.6 Hz), 7.37 (1H, t, *J* = 7.2 Hz), 7.54 (1H, d, *J* = 8.4 Hz), 7.65 (1 H, d, *J* = 8 Hz), 12.37 (1H, s, exch.), 12.48 (1H, s, exch.), 13.01 (1H, s, exch.).

10-Hydroperoxy-2-cyano-3,7,8-trimethyl-1-hydrooxy-indolizino[1,2-b]-indole (12b)
Eluted by 1 % MeOH in EA (v/v), ^{1}H NMR (DMSO d_6) δ 2.40 (3H, s), 2.44 (3H, s), 2.50 (3H, s), 4.02 (3H, s), 6.44 (1H, s), 7.05 (1H, brs), 7.35 (1H, brs),11.69 (1H, s, exch.), 12.38 (1H, s, exch.), 13.72 (1H, s, exch.).

10-Hydroperoxy-2-cyano-3,6,8-trimethyl-1-hydrooxy-indolizino[1,2-b]-indole (12c)
Eluted by 1 % MeOH in EA (v/v), ^{1}H NMR (DMSO d_6) δ 2.40 (3H, s), 2.45 (3H, s), 2.50 (3H, s), 4.02 (3H, s), 6.43 (1H, s), 7.09 (1H, s), 7.21 (1H, s), 7.54 (1H, d, *J* = 8.4 Hz),12.19 (1H, s, exch.), 12.42 (1H, s, exch.), 13.23 (1H, s, exch.).

10-Hydroperoxy-8-fluoro-2-cyano-3-methyl-1-hydroxy-indolizino[1,2-b]-indole (12d)
Eluted by 1 % MeOH in EA (v/v), ^{1}H NMR (DMSO d_6) δ 2.41 (3H, s), 4.22 (3H, s), 6.41 (1H, s), 7.26 (2H, m), 7.44 (1H, m), 13.22 (1H, s, exch.), 13.42 (1H, s, exch.), 13.41 (1H, s, exch.).

RESULT AND DISCUSSION

The tetracyclic 9H, 10H-indolizino [1, 2-b] indole-1-one (**2a - d**) and its derivatives are known [1] to be auto-oxidized in the presence of base and alkylation agent to produce a series of compounds (**7a - d**, **8a - d**, **9a - d**, **10a - d**, **11a - d**, **12a – d**, Scheme 1). Among these compounds the peroxides (**7a – d**, **10a – d**, **12a - d**) are expected to be active towards propagation of anti-cancerous cells [3] due to chance of formation of peroxy radical. But the yield of peroxide is very poor may be due to the formation of methyl peroxides in the presence of methylating agent. With the aim of synthesizing enough amount of peroxide (**12a – d**) for testing it against different cancerous cell lines *in vitro*, we carried out the same reaction without methylating agent and with only base (Scheme 2). The yield of **12a – d** improved a little amount. The list of compounds synthesized was given in tabular form in Table 1 and the yields are given in Table 2 (when the reaction is carried out with methylating agent) and Table 3 (when the reaction is carried out without methylating agent and with base only).

The results clearly shows that yield of the peroxides (**12a - d**) improve a little bit when the reaction was carried out without methylating agent. In addition we have little amount of methylated peroxides (**7a–d**, **10a–d)** in our hand. The isolation and purification of the products are very complicated. After repeated purification by silica gel column chromatography and preparative thin layer chromatography we got enough purity of the compound to test the biological activity.

With all the required peroxides in our hand we have tested *in vitro* anti-cancerous activity assay against cancerous cell lines of colon HT – 29, nasopharyngeal carcinoma HONE- 1 and BM -1 & breast carcinoma MX – 1 by published procedure [5-9]. The result is shown in Table 4.

From the result (Table 4) it is apparent that among these peroxides compounds **7a – d** and compounds **12a – d** are most potent against almost all the cancerous cell lines in the range of nanomolar IC_{50} values. It is obvious that these two series of peroxides are having unprotected indole NH group, as shown from the structure of these products in Scheme 1 and Table 1 and Table 2. This may

be attributed due to better binding of these compounds with the cancerous cell lines. This is our speculation and to prove it we need to carry out further study. The peroxides **10a–d** are showing potent anti-cancerous activity against HT - 29 and MX - 1 cell lines, but almost inactive against HONE - 1 and BM – 1 though.

We also tried to find the *in vivo* activity of these compounds against nude mice grafted with cancerous cell. However, due to the formation of very small amount of compound and the extreme instability and also insolubility of these peroxides we could not do it. We also attempted to test *in vivo* biological activity on nude mice grafted with cancerous cell with the indolizino-indole derivatves **2a – d**, but these compounds are also extremely insoluble in solvent like DMSO. So a conclusive data we could not find.

The cause of anti-cancer activity of the peroxide *in vitro* may be due to cleaving of DNA in cancerous cell by the eroxyradical [3] as radicals are known DNA cleaving agents [12]. The peroxides may be damaging DNA of different cell lines by binding with it and thus blocking cell propagation in cell cycle. However, this conclusion we arrived by literature survey of published research of previous researchers [12]. To get a conclusive report, a more detailed study with more stable peroxides is needed.

With all these studies it is confirmed though, that the peroxides formed by the auto-oxidation of indolizino-indole derivatives (**2a – d**) have a potential to be treated as an anti-cancerous agent as shown from the *in vitro* study of these compounds against different cancerous cell lines. A detailed study to find a complete SAR is needed. Also *in vivo* anti cancerous activity of these candidates are also needed. Still we can conclude that most of the peroxides are potent against different cancerous cell line *in vitro*.

CONCLUSION

From the above data we may conclude that many of the peroxides formed by the auto-oxidation of tetracyclic 9H, 10H-indolizino [1, 2-b] indole-1-one (2a - d) have a potential to be used as an anti-cancerous agents as shown by their *in vitro* anti-cancerous activity assay against different cancerous cell lines. The *in vitro* activity assay was done against different cancerous cell lines. Among those peroxides few derivatives of 10-Hydroperoxy-2-cyano-3-methyl-1-hydroxy-indolizino[1,2-b]-indole (**12a – d**) are found out to be most active with IC_{50} in nanomolar range. It is also observed that presence of indole NH and peroxo OH is useful to increase the potency of these derivatives.

It is also found out that the yield of peroxide (**12a – d**) of the auto-oxidation of tetracyclic 9H, 10H-indolizino [1, 2-b] indole-1-one (**2a - d**) can be improved if the reaction is carried out without methylating agent, though isolation of pure product is really tough and needs a rigorous purification.

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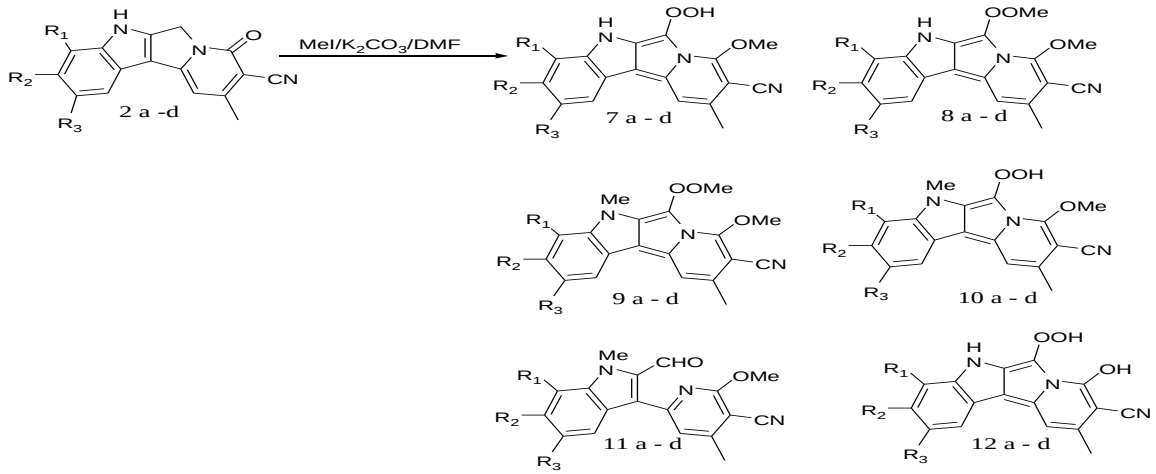
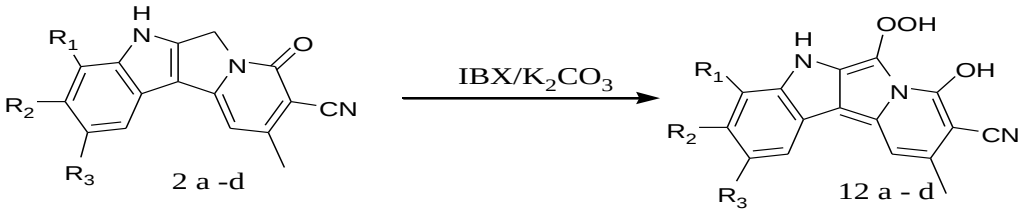


Scheme. 1: Auto-oxidation using methylating agents



Scheme. 2: Auto-oxidation without methylating agent

Table 1: List of compound synthesized

Number of Compound	R_1	R_2	R_3
2a, **7a**, **8a**, **9a**, **10a**, **11a**, **12a**	H	H	H
2b, **7b**, **8b**, **9b**, **10b**, **11b**, **12b**	Me	Me	H
2c, **7c**, **8c**, **9c**, **10c**, **11c**, **12c**	Me	H	Me
2d, **7d**, **8d**, **9d**, **10d**, **11d**, **12d**	F	H	H

Table 2: Yield of different compound produced by auto-oxidation with MeI/K_2CO_3

Compound	Product	Yield %	Yield of 7a after rigorous purification %
2a	**7a**	52	12
	8a	1	
	9a	5	
	10a	8	
	11a	11	
	12a	1	
2b	**7b**	60	10
	8b	0 (ND)	
	9b	12	
	10b	8	
	11b	0 (ND)	
	12b	0 (ND)	
2c	**7c**	55	8
	8c	0 (ND)	
	9c	15	
	10c	11	
	11c	0 (ND)	
	12c	0 (ND)	
2d	**7d**	58	5
	8d	0 (ND)	
	9d	14	
	10d	12	
	11d	0 (ND)	
	12d	0 (ND)	

Table 3: Yield of different compound produced by auto-oxidation with base K_2CO_3

Starting material	Product	Isolated Yield (%)
2a	12a	16
2b	12b	20
2c	12c	17
2d	12d	15

Table 4: Inhibition of different cell lines (IC_{50} μM)

Compound Number	Inhibition of cancerous cell growth (IC_{50} μM)*			
	HT - 29	HONE - 1	BM - 1	MX -1
7a	0.098	0.36	0.45	0.067
7b	0.085	0.86	1.1	0.092
7c	0.067	0.097	0.88	0.12
7d	0.081	1.2	0.96	0.079
10a	1.2	>100	45	1.8
10b	2.8	>100	34	0.11
10c	1.7	87	68	0.32
10d	0.97	>100	>100	0.17
12a*	0.079	0.21	0.23	0.051
12b*	0.087	0.34	0.11	0.068
12c*	0.073	0.55	0.31	0.081
12d*	0.056	0.78	0.56	0.091

* Purity of these compounds is a matter of concern and result may vary due to this.

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