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Indole- and indolizine-glyoxylamides displaying cytotoxicity against multidrug resistant cancer cell lines

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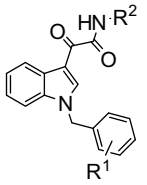
Abstract—We report herein the SAR studies of a series of indole- and indolizine-glyoxylamides that demonstrate substantial in vitro anti-proliferative activities against cancer cell lines, including multidrug resistance (MDR) phenotypes. The in vitro cytotoxic effects have been demonstrated across a wide array of tumor types of various origins (e.g., breast, colon, uterine).

One of the leading causes for the failure of chemotherapeutic agents is the development of resistance to the drug as a result of prolonged treatment. This phenomenon is known as multidrug resistance (MDR).[1] Some of the most successful cancer drugs such as paclitaxel,[2] vincristine,[3] and doxorubicin[4] suffer from MDR rendering them ineffective against certain cancers. Natural products such as the epothilones[5] discodermolide,[6] and modified taxanes[7] have been discovered that display potent activity against MDR resistant cancer cell lines. However there is a continuing search for effective small-molecule drugs that show MDR cancer cell cytotoxicity.

From our screening program, we found that indole-glyoxylamides **1** displayed in vitro cytotoxicity against a range of cancer cell lines, particularly MDR cancer cell lines. Recently, we reported that conjugated indole-imidazole derivatives **2** showed appreciable cytotoxicity against MDR cancer cell lines.[8] These compounds are structurally similar to the conjugated indole-glyoxylamide derivatives. Essentially the gloxylamide system is a biostere of the imidazole ketone system. It should be noted that others have also reported indole-glyoxylamides as anticancer agents. Bacher et al.,[9] first reported the activity of D-24851 (now known as Indibulin) and its mechanism of action has been extensively studied. The mechanism of action was shown to be through the destabilization of microtubules in cancer cells but the tubulin binding site is not the same as the well-known vincristine and colchicine binding sites. It has also been reported that Indibulin has activity against MDR cell lines, oral bioavailability, and no neurotoxicity.[9] These characteristics offer advantages over existing microtubule disrupting drugs. Indibulin is currently undergoing early stage clinical trials for the treatment of solid tumors.[10] Li et al.,[11] have synthesized and studied a series of *N*-heterocyclic indolyl glyoxylamides and found compounds possessing activity against cancer cell lines including MDR cell lines. These compounds also showed significant in vivo activity in cancer models. These findings support the continued investigation of this class of compounds as anticancer agents.

Herein we describe the synthesis, cytotoxicity against cancer cells including MDR cancer cell lines of a series indole- and indolizine-glyoxylamide derivatives. The biological activity of the indole-glyoxylamides support the potential of this class of compounds and the indolizine series represent a novel class of anticancer agents.



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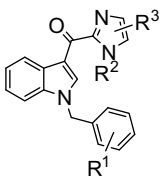

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Keywords: Indole; Indolizine; Multidrug; Resistant; Cytotoxic; Cancer; Glyoxylamide.
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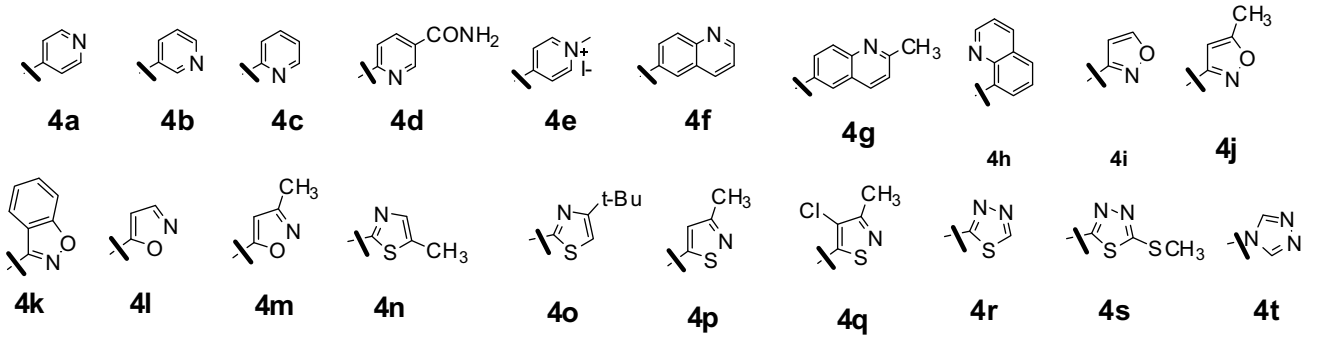


Previously, we had found with the indole-imidazole series that substitution of the indole nitrogen was crucial for activity and a *para*-chloro group (**1** $R^1 = p$-Cl) in the phenyl ring was well tolerated.[8] Therefore, all the compounds described herein possess this substitution. A number of compounds were synthesized for initial screening using the procedure shown in Scheme 1. Indole was benzylated with 4-chlorobenzyl chloride to generate **3**. The benzylated indole **3** was then reacted with oxalyl chloride, and with various amines to generate the glyoxylamides **4**.[12]

The potency of a selection of the initial series of compounds is summarized in Table 1.[13] Compound **4a** has the same structure as D-24851 and is included as a reference. In particular we were interested in compounds that showed significant activity against multidrug resistant cell lines such as HL60/TX1000 that is resistant to Taxol.[14] The cell line HL-60/TX1000 was isolated in vitro by subculturing HL-60 in progressively higher concentration of Taxol. HL-60/TX1000 cells over-express MDR1 mRNA and p-glycoprotein, as determined by Western blot and immunofluorescence labeling with anti-Pgp antibodies.

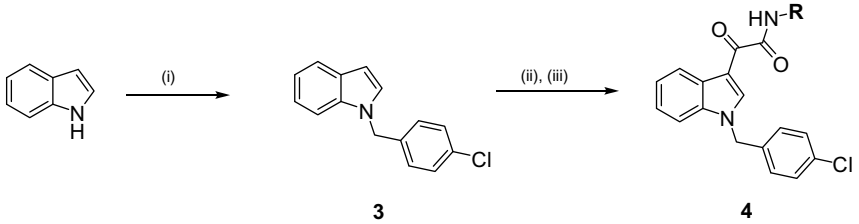
From these results, it is noted that certain heterocyclic amides show very strong activity against this cell line. Clear and interesting, SAR is shown for the pyridine series **4a**–**e**. The 4-pyridyl compound **4a** is very active whereas the 2- and 3-pyridyl isomers **4b** and **4c** have very weak or almost no potency. Interestingly, when a carboxamide group is introduced into the 4-position of the 2-pyridyl derivative as represented by **4d** strong cytotoxicity was observed.

The importance of the electronic nature of the pyridine nitrogen atom (or a group to replace the nitrogen atom) is suggested by the complete absence of activity for the pyridinium salt **4e**. A similar suggestion of the importance of the position and accessibility of a nitrogen atom was observed for the series of quinoline compounds **4f**–**h**. The quinoline amide **4f** was very active but the introduction of a methyl group adjacent to the quinoline ring nitrogen atom as shown in **4g** resulted in a 100-fold decrease of activity. An isomer of **4f** with nitrogen atom in a different position **4h** showed almost no potency against this cell line.

Numerous amides comprising of five-membered ring heterocycles were also synthesized and screened. A selec-

Table 1. Comparison of the in vitro cytotoxicity of indole-glyoxylamides against the Taxol resistant HL60/TX1000[14] cell line

Compound	Cytotoxicity IC_{50} (μM)[13]
Taxol	5
4a (D-24851)	0.05
4b	6
4c	>10
4d	0.05
4e	>10
4f	0.05
4g	0.5
4h	>10
4i	0.05
4j	0.05
4k	>10
4l	0.05
4m	0.05
4n	0.05
4o	5
4p	0.01
4q	5
4r	0.1
4s	1
4t	>10



Scheme 1. Reagents and condition: (i) NaH, 4-Chlorobenzyl chloride, rt, 77%; (ii) $(COCl)_2$, ether, reflux; (iii) R–NH_2, THF, TEA.

tion of interesting compounds is also shown in Scheme 1. Isoxazoles **4i–j** and **4l–m** were active and for both pairs of isomers simple methyl substitution did not affect cytotoxicity. However, when a larger more bulky group is introduced such as a fused phenyl ring (**4k**) then virtually all the biological effect is lost. Thiazole amides are tolerated (**4n**) but bulky substitution within the thiazole ring is detrimental to activity (**4o**). Isothiazole **4p** was shown to be the most potent derivative within this series and one of the most potent compounds we had discovered within this program (up to five times more active than the next most potent compounds). The exact reasons for its outstanding activity are uncertain but it appears to have the correct ring size and orientation of heteroatoms to produce the strong cytotoxic effect. It is quite striking to compare compound **4q** with **4p** and to note that the introduction of a chloro-substituent causes almost complete loss of potency, illustrating the importance of the positioning of the group in the correct orientations or the delicate electronic nature of the active system.

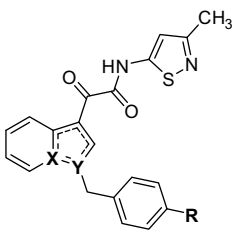
Next, we investigated different center cores in an attempt to replace the indole moiety. After screening numerous cores (the majority of which were completely inactive or possessed minimal activity) we found that the indolizine compound **5a**[15] (see Table 2) which is analogous to the indole derivative **4p** had comparable activity. This discovery shows that for this series of compounds that indolizine appears to be a suitable biostere for indole.

We also found that substituents in the *para*-position of the benzyl-substituent were well tolerated and a cyano-group (see **5b** within Table 2) provided a more potent compound. Compound **5b** was screened against a range of cancer cell lines originating from different tissues and the results are summarized in Table 3.[16]

As shown in Table 3, **5b** was effective against all cell lines including the multidrug resistant cell lines MES-SA/DX5 and HL60/TX1000 which were resistant to treatment with Taxol. Both these cell lines possess high levels of MDR1 mRNA and Pgp and show cross resistance to a wide range of common chemotherapeutic agents. This suggests that **5b** is not a substrate for the efflux pump transporters.

In summary, this SAR study has shown that the indole-glyoxylamides possess strong cytotoxicity against a range of cancer cell lines including cell lines that show resistance to Taxol. The specific orientation of substituents around the indole center was shown to be vital for potency suggesting specific interactions of these groups with a biological target. We have also shown that the indole center core can be replaced with an indolizine center core without loss of potency. These indolizine compounds represent a novel class of anticancer agents.

Table 2. Comparison of the in vitro cytotoxicity of compounds against the Taxol resistant HL60/TX1000[14] cell line



Compound	X	Y	R	Cytotoxicity IC_{50} (μM)[13]
4p	C	N	Cl	0.01
5a	N	C	Cl	0.06
5b	N	C	CN	0.02

Table 3. Comparison of the cytotoxicity of **5b** and Taxol against a range of cancer cell lines

Cell line	Cytotoxicity, IC_{50} (μM)[13,16]	
	5b	Taxol
MDA435	0.05	0.005
HL60	0.05	0.005
P388	0.01	0.01
DU145	0.05	0.005
MES-SA	0.01	0.005
MES-SA/DX5	0.05	5
HL60/TX1000	0.02	5

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12. The synthesis of **4p** described below is representative of the chemistry for the synthesis of the compounds described in this paper. A solution of oxalyl chloride (0.44 mL, 5.1 mmol) in ethyl ether (25 mL) was cooled in an ice bath. To it was added 1-(4′-chlorobenzyl)indole (1.01 g, 4.14 mmol) in ethyl ether. The resulting yellow slurry was refluxed for 2 h. After removal of the solvent in vacuo, the residue was dissolved in THF (20 mL) and cooled to 0 °C. 5-Amino-3-methylisothiazole (1.32 g, 9.73 mmol) in THF (20 mL) was added dropwise. The mixture was then warmed to room temperature, and stirred overnight. Solvent was removed in vacuo. Silica gel chromatographic purification gave the product (1.49 g) in 88% yield. ^{1}H NMR (DMSO-d_6) δ 2.37 (s, 3H), 5.67 (s, 2H), 7.07 (s, 1H), 7.36 (m, 6H), 7.61 (d, J = 9 Hz, 1H), 8.32 (d, J = 8.1 Hz, 1 Hz), 9.18 (s, 1H); ESMS Calcd ($C_{21}H_{16}ClN_3O_2S$): 409.1, found: 408.1 $(M-H)^+$.
13. *Cell culture.* Cell lines were maintained in RPMI1640(GIBCO) supplemented with 10% FC, 100 U/mL penicillin, 100 μg/mL streptomycin, and 2 nM L-glutamine. The cells were split every third day and diluted to a concentration of 2×10^5 cells/mL one day before the experiment. All experiments were performed on exponentially growing cell culture. *Drug treatment and MTS assay.* A stock solution of the drug was prepared by dissolving the compound at a concentration of 1 mM in DMSO. Final concentrations were obtained by diluting the stock solution directly into the tissue medium. Cells were incubated with various concentrations of compounds for 72 h and the IC_{50} was determined by MTS (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay.
14. The cell line HL60/TX1000 was a gift from Dr. Bhalla of Emory University School of Medicine.
15. For the synthesis of indolizine compounds such as **5a** and **5b**, see: Li, H.; Xia, Z.-Q.; Chen, S.; Koya, K.; Ono, M.; Sun, L. *Org. Proc. Res. Dev.* **2007**, *11*, 246.
16. Cell lines (tissue origin in parentheses) were all purchased from ATCC: MDA435 (breast), HL60 (leukemia), DU145 (prostate), MES-SA (uterine), and MES-SA/DX5 (MDR uterine). See Ref. 13 for cell culture conditions and assay and see Ref. 14 for origins of HL60/TX1000.