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Conjugated indole-imidazole derivatives displaying cytotoxicity against multidrug resistant cancer cell lines

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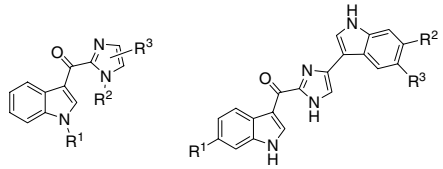
Abstract—We report herein the SAR studies of a series of indole-imidazole compounds. that demonstrate substantial in vitro anti-proliferative activities against cancer cell lines, including multidrug resistance (MDR) phenotypes. The in vitro cytotoxic effects have been demonstrated across a wide array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

The development of resistance to chemotherapy with existing anti-cancer drugs has challenged the pharmaceutical industry to rapidly identify and develop new chemical entities able to counteract this unmet medical need. Prolonged treatment of cancer cells with certain drugs can result in an acquired resistance of these cells toward multiple drugs. This phenomenon is known as multidrug resistance (MDR).[1] While the concise mechanism of MDR is not completely understood it is known that MDR is often associated with an overexpression of ATP-binding cassette (ABC) transporters.[2] The two best-known ABC transporters are P-glycoprotein (P-gp) and multidrug resistance protein 1 (MDR1) that effectively pump out the anti-cancer drug from MDR-cancer cells. Other mechanisms believed to be associated with MDR in cancer cells include: increased expression of anti-apoptotic genes and decreased expression of pro-apoptotic genes,[3] overexpression of specific tubulin isotypes,[4] decreased expression of topoisomerases,[5] and overexpression of major vault protein.[6]

Various strategies have been employed to overcome MDR, the most common being inhibition of P-gp and related proteins to effectively block the efflux of the drug.[7] Numerous MDR-reversal agents have been reported but most have undesirable side effects such as toxicity. Other complex natural products such as the epothilones,[8] discodermolide,[9] and modified taxanes[10] 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.

As part of our program to develop anti-cancer derivatives we found that conjugated indole-imidazole derivatives **1** display in vitro cytotoxicity against a range of cancer cell lines, even MDR cancer cell lines.[11] These compounds are structurally related to the bisindole alkaloids which include the topsentins **2**.[12] The topsentins display a range of biological activities including anti-tumour activity.[13] Herein we describe the synthesis, cytotoxicity against cancel cell lines, and SAR of these indole-imidazole derivatives.



1 **2**

R^1, R^2, R^3 = H or Br or OH

The synthesis of the initial series is shown in Scheme 1. Early in the program we discovered that when the indole nitrogen is protected with a benzyl-group activity was dramatically improved therefore all compounds

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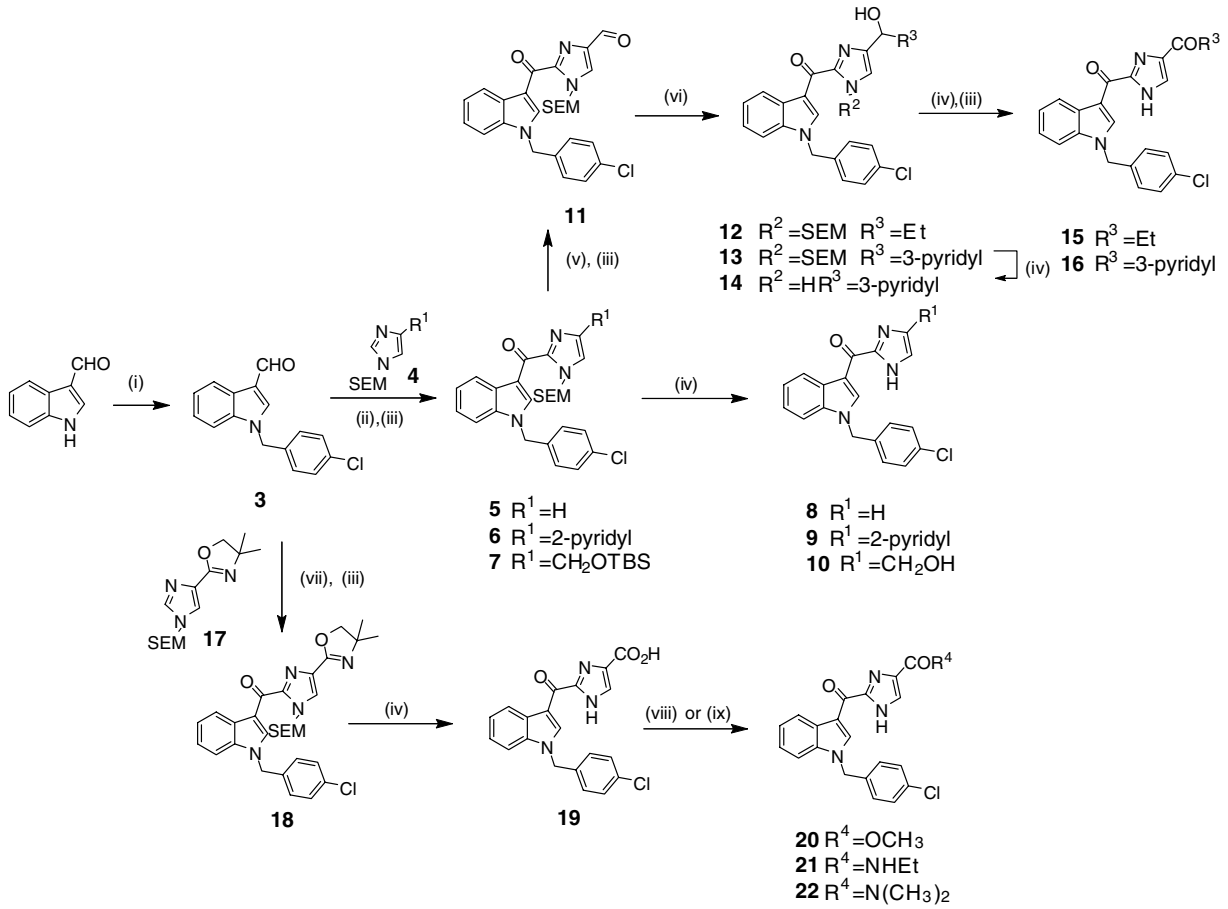


Scheme 1. Reagents and conditions: (i) NaH, 4-chlorobenzyl chloride, rt, 76%; (ii) LDA, **4**, 32–50%; (iii) MnO_2, rt, 80–90%; (iv) HCl/EtOH, reflux, 32–85%; (v) TBAF, rt, 81; (vi) R^3MgX, rt, 41–43%; (vii) LDA, **17**, 40; (viii) concd H_2SO_4, MeOH, reflux, 89%; (ix) EDC, DMAP, NH_2R^4, rt, 60–70%.

discussed here possess this substitution. Commercially available indole-3-carboxaldehyde was readily benzylated with 4-chlorobenzyl chloride to generate **3**. The key reaction for the synthesis of this series was the coupling between the indole aldehyde **3** and various protected imidazoles. This reaction was based on a similar procedure previously described for the synthesis of topsentins.[14]

The SEM-protected imidazoles **4** were synthesized from the corresponding imidazoles by reaction with NaH/SEM-Cl. Reduction of 2-[(5-toluene-4-sulfonyl)-1*H*-imidazol-4-yl]pyridine[15] with Na/naphthalene formed 4-pyridineimidazole, protection as described provided the appropriate imidazole **4** for the synthesis of **6**. The primary hydroxyl group and the imidazole-NH of commercially available (1*H*-imidazol-4-yl)methanol were protected sequentially as a TBS-ether using TBS-Cl, imidazole followed by treatment with NaH/SEM-Cl. This produced the required doubly protected imidazole for the synthesis of the key intermediate **7**. Coupling of the aldehyde **3** with the appropriate SEM-protected imidazoles **4** produced the secondary alcohols which were readily oxidized to the conjugated ketones by manganese dioxide to produce the protected intermediates **5**–**7**. Removal of the SEM group of **5**–**7** was achieved by refluxing with HCl in ethanol to furnish the final compounds **8**–**10**.[16]

The TBS-protected intermediate **7** was a key intermediate from which numerous derivatives were synthesized as shown in Scheme 1. The TBS group was selectively removed with TBAF at low temperature followed by oxidation of the hydroxyl group to the aldehyde **11**. Reaction of **11** with ethyl magnesium bromide produced the intermediate secondary alcohol **12** which is oxidized with manganese dioxide followed by deprotection with ethanolic HCl to provide ketone **15**. Similarly, reaction of **11** with the Grignard reagent derived from 3-bromopyridine produced **13** which was oxidized and deprotected as described above to produce **16**. The secondary alcohol **14** was also isolated to provide a direct comparison with **16** which should determine the effect of the conjugated linkage between the imidazole and pyridine rings.

Carboxylic acid derivatives were synthesized via the protected imidazole derivative **17** as shown in Scheme 1. The synthesis of **17** was based on a similar procedure reported for the synthesis of 2-(4,4-dimethyl-4,5-dihydrooxazol-2-yl)-benzo[*d*]imidazo[2,1-*b*]thiazole.[17] Coupling of **17** with the indole aldeyde **3** provides the

intermediate **18**. Treatment with ethanolic HCl removes both the SEM group and liberates the dimethyloxazole producing the carboxylic acid **19**. From **19**, the methyl ester **20** and amides **21** and **22** were synthesized using standard esterification or amide coupling chemistry.

Derivatives **23**–**25** (see Table 2 for structures) all possess an imidazopyridine linked to the indole ring via a carbonyl group. These compounds were synthesized by the coupling reaction between the indole aldehyde **3** and the appropriate protected imidazopyridine, oxidation with manganese oxide, and deprotection of the SEM group as previously described.

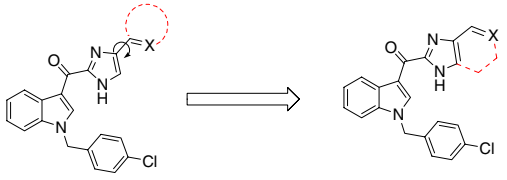
The cytotoxicity of the initial series of conjugated indole-imidazole compounds is summarized in Table 1.[18]

In particular we were interested in compounds that showed significant activity against multidrug resistant cell lines such as HL60/TX1000 that are resistant to Taxol as shown in Table 1.[19]

The first observation to note is that the unsubstituted imidazole derivative **8** shows minimal activity but substitution with certain groups produces significant activity. Susbtitution with a 2-pyridyl group **9** produces potent activity but a hydroxy(methyl) group **10** causes significant loss of activity. When a conjugated ketone group is introduced activity is maintained as shown by compound **15**. The importance of a conjugated group is clearly demonstrated by a direct comparison of compounds **14** and **16**. Compound **16** possesses a pyridyl group directly conjugated via a carbonyl to the imidazole ring and shows potent activity. When the conjugation is disrupted between the imidazole ring and the pyridine, as in the secondary alcohol **14**, a serious decrease in activity is observed.

The carboxylic acid derivatives provided further SAR insight. While the parent carboxylic acid **19** showed minimal activity the methyl ester **20** displayed strong cytotoxicity. However, both the secondary amide **21** and the tertiary amide **22** showed weak potency. The differences in activity between these derivatives could possibly be explained by poor cell permeation from the carboxylic acid and amides rather than a substituent effect.

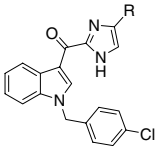
From this SAR study it appeared that compounds with a general structure **26** were active against all cell lines including the MDR cell lines. In general, compounds that possessed a hydrogen bond acceptor conjugated to the imidazole ring showed submicromolar activity. Multiple conformations resulting from rotation around the bond connecting the imidazole ring to the hydrogen bond acceptor exist as represented by **26**. We hypothesized that cyclization producing fused imidazole derivatives **27** could stabilize a conformation where the hydrogen bond acceptor is fixed in a particular conformation.



26 **27**

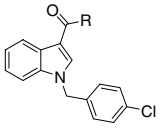
The cytotoxic activities of the imidazopyridine series of compounds against the Taxol-resistant cell line HL60/TX1000[18] are shown in Table 2.[18] Our restricted conformation hypothesis gained support when it was found that the indole-pyridoimidazole compound **23** showed a 10-fold increase in potency compared to any of the indole-imidazole derivatives shown in Table 1. Also of note is serious loss of activity for the isomer **24** where the nitrogen atom is moved adjacent to the imidazole ring. Additionally, if the NH of the fused imidazole ring is methylated as in **25** significant loss of activity was observed. This series of compounds illustrates that the

Table 1. Comparison of the in vitro cytotoxicity of indole-imidazole compounds against the Taxol-resistant HL60/TX1000[19] cell line



Compound	R	Cytotoxicity IC_{50}[18] (μM)
Taxol	—	5
8	H	10
9	2-Pyridyl	0.5
10	CH_2OH	5
14	CH(OH)-3-pyridyl	10
15	COEt	0.5
16	CO-3-pyridyl	0.5
19	COOH	10
20	$COOCH_3$	0.5
21	CONHEt	5
22	$CON(CH_3)_2$	5

Table 2. Comparison of the in vitro cytotoxicity of indole-pyridoimidazole compounds against the Taxol resistant HL60/TX1000[19] cell line



Compound	R	Cytotoxicity IC_{50}[18] (μM)
Taxol	—	5
23		0.05
24		10
25		10

Table 3. Comparison of the cytotoxicity of **23** and Taxol against a range of cancer cell lines

Compound	Cytotoxicity IC_{50}[18,20] (μM)						
	MDA435	HL60	P388	DU145	MES-SA	MES-SA/DX5	HL60/TX1000
23	0.05	0.05	0.05	0.05	0.01	0.05	0.02
Taxol	0.005	0.005	0.01	0.005	0.005	5	5

position of the nitrogen atom in the fused pyridine ring (hydrogen bond acceptor) and the presence of the imidazole NH are important for activity.

Compound **23** was screened against a range of cancer cell lines originating from different tissues and the results are summarized in Table 3.[20] As shown in Table 3, **23** 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 posses high levels of MDR1 mRNA and P-gps and show cross resistance to a wide range of common chemotherapeutic agents. The success of **23** suggests that it is not a substrate for the efflux pump transporters and its mechanism of action is quite effective against these cell lines. Additionally **23** is approximately 10 times more active than structurally related topsentin derivatives against the P388 cell line.[21].

In summary, our SAR study of the indole-imidazole derivatives provided us with a lead towards developing a pharmacophore for in vitro activity against a range of cancer lines including MDR cell lines. Using this preliminary design we improved the activity 100-fold through a possible stabilization of a conformation required for activity. Further studies are being conducted to determine the mode of action of these compounds to provide an insight into the design of future drugs active against MDR-carcinoma cells.

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16. (a) The synthesis of **23** described below is representative of the chemistry for the synthesis of the compounds described in this paper: [1-(4-chlorobenzyl)-1*H*-indol-3-yl]-[1-(2-trimethyl-silanylethoxymethyl)-1*H*-imidazo[4,5-*c*]pyridin-2-yl]-methanone. A solution of 1-(2-trimethyl-silanylethoxymethyl)-1*H*-imidazo[4,5-*c*]pyridine (250 mg, 1.0 mmol) in dry THF (50 mL) was cooled to −78 °C in dry-ice acetone bath. To this solution was added lithium diisopropylamide (0.6 mL, 2 M solution in heptane/ethylbenzene/THF, 1.2 mmol) and the reaction mixture was stirred at −78 °C for 30 min. To this solution, 1-(4-chlorobenzyl)-1*H*-indole-3-carboxaldehyde (390 mg, 1.21 mmol) dissolved in THF (20 mL) was added dropwise. The reaction mixture was stirred at −78 °C for 60 min then quenched with saturated $NaHCO_3$ and allowed to warm to room temperature. The resultant solution was extracted with ethyl acetate (3× 50 mL), the combined extracts were washed with water, dried over $MgSO_4$, and the solution was filtered. Solvent was removed under reduced pressure to produce a brown oil. This product was dissolved in CH_2Cl_2 (50 mL) and MnO_2 (500 mg) was added. The resultant suspension was stirred at room temperature overnight then filtered through a plug of Celite. Solvent was removed under reduced pressure and the crude product was purified by silica gel column chromatography eluting with a gradient of ethyl acetate/hexane (1:1, v/v) to ethyl acetate. The desired product was isolated as a yellow oil which was a mixture of isomers (yield 408 mg, 74%); (b) [1-(4-Chlorobenzyl)-1*H*-indol-3-yl]-(1*H*-imidazo[4,5-*c*]pyridin-2-yl)-methanone **23**: a solution of [1-(4-chlorobenzyl)-1*H*-indol-3-yl]-[1-(2-trimethyl-silanylethoxymethyl)-1*H*-imidazo[4,5-*c*]pyridin-2-yl]-methanone (300 mg, 0.54 mmol) in ethanol (50 mL) and 2 N HCl (20 mL) was heated to reflux for 2 h. After allowing to cool to room temperature, the solution was neutralized with 2 N NaOH and ethanol was removed under reduced pressure. The resultant suspension was extracted with ethyl acetate (3× 50 mL), the combined extracts were washed with water, dried over $MgSO_4$, and the solution was filtered. Solvent was removed under reduced pressure to produce the pure desired product as a

white solid (yield 257 mg, 92%). ^{1}H NMR (DMSO-d_6) δ 5.83 (s, 2H), 7.36 (m, 2H), 7.62 (m, 4H), 7.81 (s, 1H), 8.43 (m, 2H), 9.14 (s, 1H), 9.53 (s, 1H); ESMS calcd ($C_{22}H_{15}ClN_4O$): 420.12, found: 421.1 $(M+H)^+$.

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18. *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 varying 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.
19. The cell line HL60/TX1000 was a gift from Dr. Bhalla of Emory University School of Medicine. HL-60/TX1000 was isolated in vitro by subculturing HL-60 in progressively higher concentration of Taxol. HL-60/TX1000 cells overexpress MDR1 mRNA and P-glycoprotein, as determined by Western blot and immunoflourescence labeling with anti-P-gp antibodies.
20. Cell lines (tissue origin in parentheses) were all purchased from ATCC: MDA435 (breast), HL60 (leukemia), P388 (murine leukemia), DU145 (prostate), MES-SA (uterine) and MES-SA/DX5 (MDR uterine). See Ref. 15 for cell culture conditions and assay, and see Ref. 16 for origins of HL60/TX1000.
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