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Influence of Sugar Residue on the Cytotoxicity and Solubility of Several Aryl-indolo[2,3-α]pyrrolo[3,4-c]carbazoles and the Corresponding Maleimides

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Indolo[2,3-α]carbazoles and the corresponding maleimides form two structurally related classes of compounds endowed with diverse biological activities. In the work we investigated the influence of sugar residue on the cytotoxicity and solubility of several aryl-indolo[2,3-a]pyrrolo[3,4-c]carbazoles and the corresponding maleimides. The results indicated that introduce a monosaccharide on a planar aromatic molecule to increase the solubility is not always effective, whereas the sugar moiety do have a profound impact on the mechanism of action for these types of compounds.

Key Words: Indolocarbazole, Maleimide, Cytotoxicity, Solubility, Glycoside.

INTRODUCTION

The indolo[2,3-α]pyrrolo[3,4-c]carbazole alkaloids and their glycosides represent an interesting class of compounds that exhibit diverse biological activities[1]. This family has raised considerable attention because of their central role in the regulation of cell cycle progression and specific enzymatic inhibitions[2-6]. There are lots of efforts to optimize the structure of this class of compounds both on the aglycone and glycosyl residue, which could provide drug candidates with high developability value. Closely related compounds such as arcyriaflavin-A and staurosporine (Fig. 1) have been described as inhibitors of different isoforms of protein kinase[2]. In addition, the rebeccamycin (Fig. 1)[3] class of indolocarbazole glycosides has shown remarkable activity in the poisoning of DNA topoisomerase I[4]. Recently, several aryl[a]pyrrolo[3,4-c]-carbazole analogues[5] have been developed as very potent CDK (cyclin-dependent kinases) inhibitors. For instance, naphtho-[2,1-a]pyrrolo[3,4-c]carbazole-5,7(6*H*,12*H*)-dione (NPCD, Fig. 1)[6], in which one indole ring is replaced by a naphthyl ring, is a very potent and selective cyclin D1-CDK4 inhibitors.

Interested in the diverse biological activities of these indolo[2,3-α]pyrrolo[3,4-c]carbazoles, in our previous work[7] we conducted the synthesis and evaluation of several NPCD glycosides on the tumor cell growth inhibitory activities. The results showed that the introduction of a sugar moiety onto NPCD didn't affect much of their cytotoxic activities, while the subtle structure of the sugar moiety affected the underlying

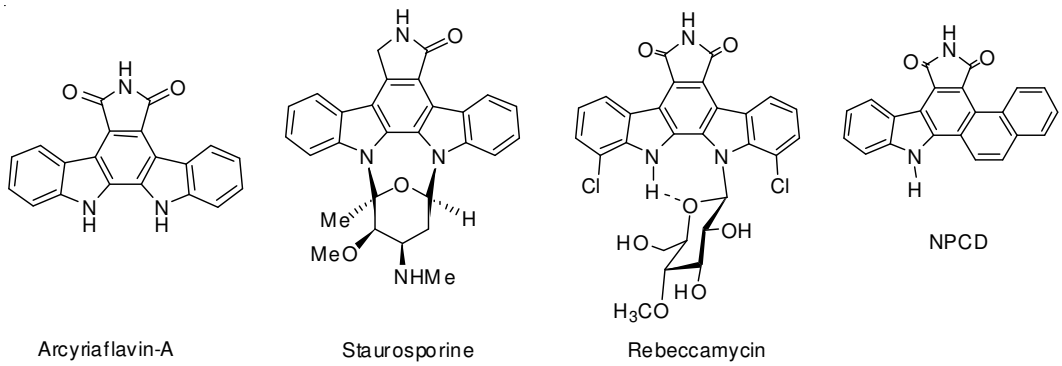


Fig. 1. Chemical structures of arcyriaflavin-A, staurosporine, rebeccamycin and naphtho[2,1-a]pyrrolo[3,4-c]carbazole-5,7(6*H*,12*H*)-dione (NPCD)

mechanism strongly. The findings make these carbazole glycosides interesting molecules for biological research.

However, all of these NPCD glycosides were found to have very low solubility in water, which embarrass further development. With our continuous interest in the effect of sugar attachments to a planar aromatic molecule on the biological activities[8] and in order to search for potential antitumor agents of NPCD class with high developability value, we decided to investigate the influence of sugar residue on the cytotoxicity and solubility of several aryl-indolo[2,3-α]pyrrolo[3,4-c]-carbazoles and the corresponding maleimides. We hope the results obtained would render new clues to the understanding of the anticancer profile for these types of compounds.

All of the designed compounds (Table-1) were synthesized following an optimized modular synthetic approach developed by Faul *et al.*[9] (**Scheme-I**). The synthesis of compounds **1a-b**, and **2a-c** were reported in previous work[9]. Compounds **3a-d**[10] and **4a-b**[11] were prepared as shown in **Scheme-I**. The key intermediates maleimides **7a-f** were prepared in good yields by condensation of indolyl-3-glyoxylyl ester **6a** and (N-glycosyl indolyl)-3-glyoxylyl esters **6b-d** with the complementary arylacetamide **5a-b** in the presence of *t*-BuOK. Deprotection of the PMB groups on the sugar rings of **7c-f** provided compounds **8c-f**, respectively. Finally, cyclizations of **7a-b** and **8c-f** to the corresponding arylcarbazoles **3a-d** and **4a-b** were achieved by irradiation of their dry acetone solution with Osram Hg HP (HQL 125W) lamp for 48 h.

The aryl-indolo[2,3-α]pyrrolo[3,4-c] carbazoles and the corresponding maleimides as well as their glycosides were screened for their tumor cell growth inhibitory activities by 74 h drug exposure. The IC_{50} values of these compounds against two tumor cell lines along with their solubility in water are presented in Table-1.

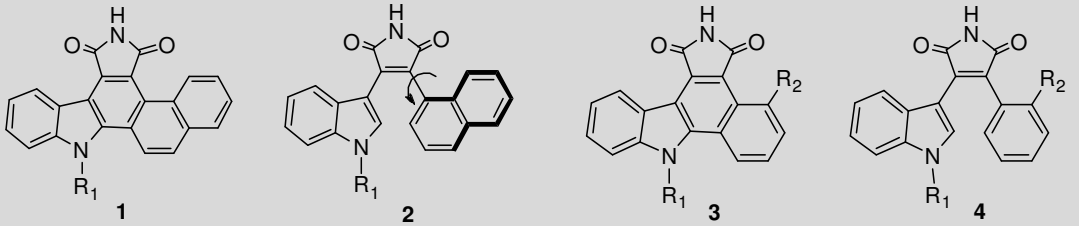
As expected, the open ring derivatives, maleimides **2a-c** and **4a-b**, have better solubility in water as compared with those ring closed carbazoles (**1a-b** and **3a-d**), because they have more flexible scaffolds. Interestingly, introduction of sugar residues into the carbazoles does not improve the solubility in water at all (**1a** *vs*. **1b**, **3a**,**b** *vs*. **3c,d**), but the solubility of maleimides were increased significantly when a sugar residue was attached on the indole N-atom (**2a** *vs*. **2b,c**). This can be explained that the flexible scaffolds of maleimides were further distorted by the presence of a sugar moiety, while the rigid carbazole scaffolds still remained planar. These results suggest that putting a monosaccharide on a planar aromatic molecule to increase the solubility is not effective.

The previous study[7] showed that introduction of a sugar moiety onto NPCD (**1a**) didn't affect much of their cytotoxic activities. For example, the IC_{50} values of compound **1b** on BxPC3 and MCF-7 cell lines are at the same level as those of **1a**. Whereas we found that introduction of a sugar moiety onto maleimide **2a** led to the decrease of cytotoxic activities (**2b-c** *vs*. **2a**). This can also be attributed to the distortion of flexible scaffolds of maleimides in the presence of a sugar moiety. The resulted conformation of the maleimides departed from their active conformation significantly.

The above results indicated that although the maleimides have better solubility in water than the carbazole derivatives, the cytotoxic activities are decreased. To obtain significant cytotoxic activity with a good solubility, based on the ring closed NPCD derivatives, we tried to cut the naphthyl ring of **1a** into one phenyl rings. As shown in Table-1, compounds **3a-d** with substituted phenyl rings were designed and synthesized.

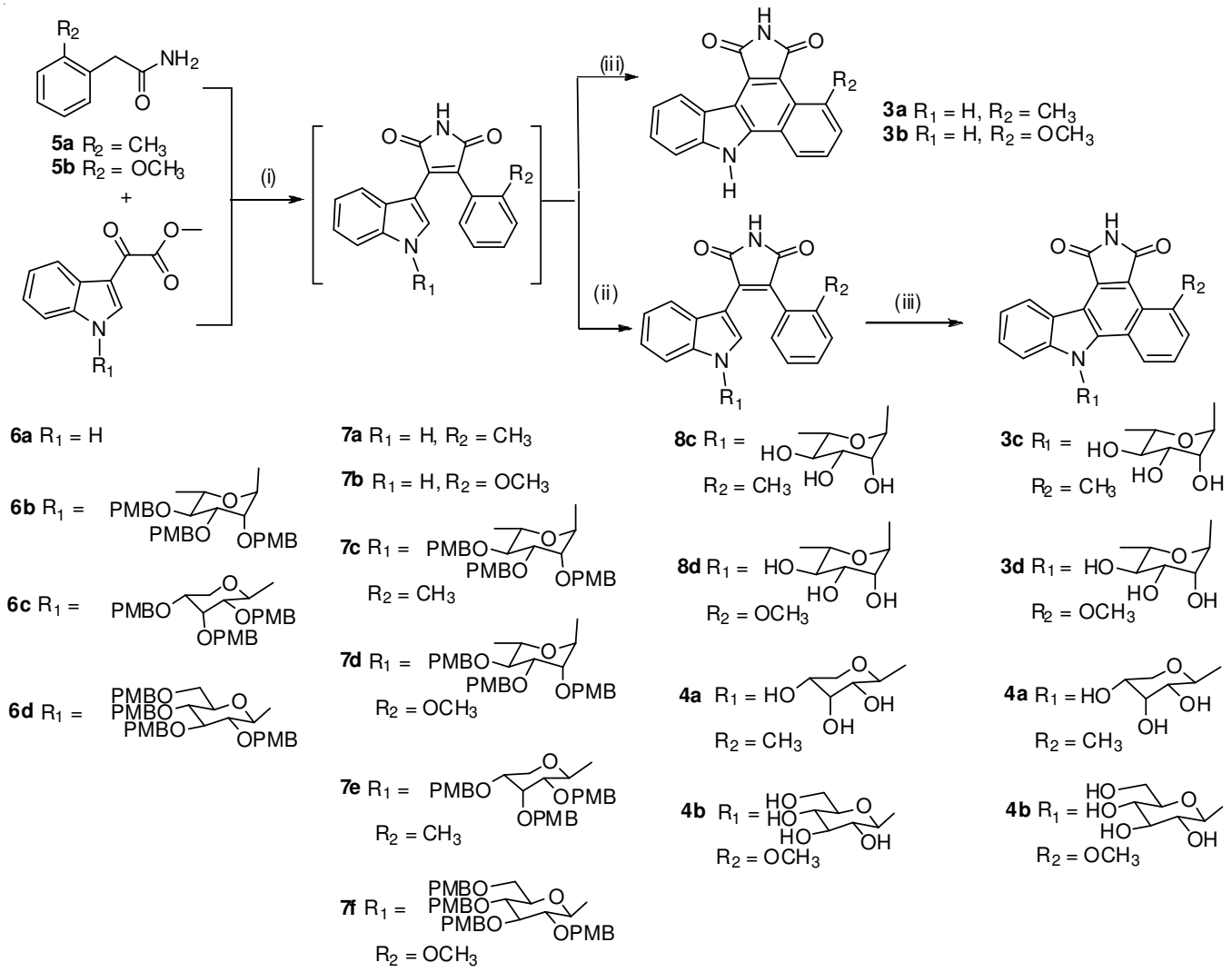
To our surprised, compounds **3a-b** did not show cytotoxic activities against the tested BxPC3 prostate cells and MCF-7 breast cells, suggesting the naphthyl ring is critical for the activity. And more interestingly, their corresponding glycosides **3c** showed significant cytotoxicity towards the two tested cell lines and **3d** was very effective to inhibit the growth of MCF-

TABLE-1
STRUCTURES, SOLUBILITY AND CYTOTOXICITY OF INDOLO[2,3-α]PYRROLO[3,4-c] CARBAZOLES, ARYL-INDOLYL MALEIMIDES AND THEIR GLYCOSIDES



1 2 3 4

Compounds	R_1	R_2	Solubility in water (μg/mL)	IC_{50} (μM)	
				BxPC3	MCF-7
1a	H	–	8	8.3 ± 2.5	8.3 ± 4.2
1b	α-L-Rhamnopyranosyl	–	8	7.3 ± 2.9	4.5 ± 1.0
2a	H	–	70	33.5 ± 2.1	29.4 ± 1.6
2b	β-D-Glucopyranosyl	–	171	>100	>100
2c	α-D-Ribopyranosyl		105	63.9 ± 2.5	80.4 ± 1.6
3a	H	$-CH_3$	3	>100	>100
3b	H	$-OCH_3$	5	>100	>100
3c	α-L-Rhamnopyranosyl	$-CH_3$	8	5.9 ± 0.8	6.6 ± 0.6
3d	α-L-Rhamnopyranosyl	$-OCH_3$	5	>100	7.9 ± 0.9
4a	α-D-Ribopyranosyl	$-CH_3$	879	36.5 ± 1.2	92.5 ± 1.5
4b	β-D-Glucopyranosyl	$-OCH_3$	1735	>100	>100



Scheme-I: Synthesis of indolo[2,3-α]pyrrolo[3,4-c]carbazoles, aryl-indolyl maleimides and their glycosides. Conditions: (i) *t*-BuOK, THF, 12 % for **7a**, 17 % for **7b**, 44 % for **7c**, 36 % for **7d**; (ii) DDQ, CH_2Cl_2/H_2O (v:v = 10:1), 77 % for **8c**, 81 % for **8d**, 31 % for **4a** over 2 steps, 16 % for **4b** over 2 steps, (iii) 57 % for **3a**, 40 % for **3b**, 65 % for **3c**, 35 % for **3d**.

7 cell lines. These results revealed that not only the carbazole framework as well known before but the sugar moiety played an important role on the cytotoxic activities for these types of compounds.

In summary, in the work we investigated the influence of sugar residue on the cytotoxicity and solubility of several aryl-indolo[2,3-α]pyrrolo[3,4-c]carbazoles and the corresponding maleimides. The results indicated that introduce a monosaccharide on a planar aromatic molecule like NPCD to increase the solubility is not always effective, whereas the sugar moiety do have a profound impact on the mechanism of action for these types of compounds.

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10. **3a**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.84 (s, 1H), 11.13 (s, 1H), 9.02 (d, *J* = 7.9 Hz, 1H), 8.55 (d, *J* = 8.1 Hz, 1H), 7.75-7.68 (m, 2H),

7.60 (d, *J* = 7.1 Hz, 1H), 7.55 (s, 1H), 7.37 (d, *J* = 7.5 Hz, 1H), 3.04 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.3, 170.2, 141.6, 140.2, 136.2, 131.1, 130.7, 127.5, 126.5, 126.4, 124.7, 124.2, 121.3, 120.8, 120.7, 120.2, 111.7, 111.5, 25.7; API-ESMS: calcd. (%) for $[M + H]^+$: m/z 301.1, found (%) 301.1. **3b**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.81 (s, 1H), 10.97 (s, 1H), 9.03 (d, *J* = 7.8 Hz, 1H), 8.22 (d, *J* = 8.1 Hz, 1H), 7.76 (t, *J* = 8.1 Hz, 1H), 7.71 (d, *J* = 8.1 Hz, 1H), 7.54 (t, *J* = 7.6 Hz, 1H), 7.35 (t, *J* = 7.6 Hz, 1H), 7.25 (d, *J* = 7.9 Hz, 1H), 3.99 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.1, 168.0, 156.8, 140.6, 140.2, 130.1, 129.0, 126.6, 124.8, 121.4, 120.7, 120.0, 118.3, 114.7, 112.1, 111.8, 108.4, 55.8; API-ESMS: calcd. (%) for $[M + H]^+$: m/z 317.1, found (%) 317.1. **3c**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.19 (s, 1H), 9.18 (d, *J* = 7.6 Hz, 1H), 8.47 (d, *J* = 8.5 Hz, 1H), 8.29 (d, *J* = 8.5 Hz, 1H), 7.74-7.68 (m, 1H), 7.62 (d, *J* = 7.0 Hz, 1H), 7.45 (t, *J* = 7.2 Hz, 1H), 7.34 (t, *J* = 7.2 Hz, 1H), 6.41 (s, 1H), 5.66 (d, *J* = 4.9 Hz, 1H), 5.06 (d, *J* = 5.4 Hz, 1H), 5.01 (d, *J* = 6.0 Hz, 1H), 4.64 (s, 1H), 3.82 (s, 1H), 3.56-3.48 (m, 1H), 3.47-3.38 (m, 1H), 3.01 (s, 3H), 1.16 (d, *J* = 5.9 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 175.1, 175.0, 146.8, 146.7, 141.3, 135.7, 134.9, 132.5, 132.3, 130.7, 130.0, 129.1, 127.4, 127.2, 126.5, 126.4, 123.8, 119.6, 92.8, 80.6, 78.1, 77.3, 76.6, 30.7, 23.1; API-ESMS: calcd. (%) for $[M + H]^+$: m/z 447.1, found (%) 447.1. **3d**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.05 (s, 1H), 9.21 (d, *J* = 7.8 Hz, 1H), 8.47 (d, *J* = 8.6 Hz, 1H), 7.95 (d, *J* = 8.6 Hz, 1H), 7.77 (t, *J* = 8.2 Hz, 1H), 7.44 (t, *J* = 7.7 Hz, 1H), 7.38-7.27 (m, 2H), 6.49 (s, 1H), 5.65 (s, 1H), 5.13 (m, 2H), 4.61 (s, 1H), 4.01 (s, 3H), 3.82 (s, 1H), 3.53 (s, 1H), 3.46 (d, *J* = 6.0 Hz, 1H), 1.19 (d, *J* = 5.9 Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 175.0, 172.7, 162.0, 146.7, 145.2, 134.3, 130.6, 130.5, 126.5, 126.2, 123.8, 121.0, 120.0, 113.1, 92.5, 80.6, 78.1, 77.3, 76.6, 61.1, 23.1; API-ESMS: calcd. (%) for $[M + H]^+$: m/z 463.2, found. (%) 463.1.

11. **4a**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.08 (s, 1H), 8.11 (s, 1H), 7.47 (d, *J* = 8.3 Hz, 1H), 7.29 (dd, *J* = 10.7, 5.6 Hz, 1H), 7.24-7.16 (m, 3H), 7.03 (t, *J* = 7.7 Hz, 1H), 6.60 (t, *J* = 7.6 Hz, 1H), 6.18 (d, *J* = 8.2 Hz, 1H), 5.56 (d, *J* = 9.2 Hz, 1H), 5.12 (d, *J* = 3.7 Hz, 1H), 5.07 (d, *J* = 7.6 Hz, 1H), 4.89 (d, *J* = 6.1 Hz, 1H), 4.02 (s, 1H), 3.96 (t, *J* = 8.5 Hz, 1H), 3.82- 3.69 (m, 2H), 3.60 (dd, *J* = 9.9, 4.5 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.3, 172.2, 137.3, 136.5, 133.6, 131.5, 131.0, 130.9, 130.5, 130.1, 128.9, 125.5, 122.4, 120.6, 120.5, 111.5, 111.4, 105.5, 105.4, 82.5, 82.2, 71.4, 68.7, 66.6, 65.2, 20.0; API-ESMS: calcd. (%) for $[M + H]^+$: m/z 435.1, found. (%) 435.1. **4b**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.02 (s, 1H), 8.01 (s, 1H), 7.56 (d, *J* = 8.21 Hz, 1H), 7.37 (t like, *J* = 7.44 Hz, 1H), 7.15 (m, 1H), 7.05 (t, *J* = 7.43 Hz, 1H), 6.94 (m, 2H), 6.64 (t, *J* = 7.24 Hz, 1H), 6.31(d, *J* = 8.22 Hz, 1H), 5.50 (d, *J* = 9.0 Hz, 1H), 5.33 (d, *J* = 5.08 Hz, 1H), 5.21 (d, *J* = 4.69 Hz, 1H), 5.15 (d, *J* = 5.09 Hz, 1H), 4.59 (s, 1H), 3.68 (m, 3H), 3.48 (s, 3H), 3.27 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.4, 172.0, 157.6, 137.4, 136.4, 133.3, 131.1, 130.6, 128.9, 125.4, 125.3, 122.1, 120.4, 120.3, 120.1, 111.8, 111.6, 105.9, 85.0, 79.6, 77.4, 71.9, 69.7, 60.8, 55.1; API-ESMS: calcd for $[M + H]^+$: m/z 481.2, found. (%) 481.1; $[M + Na]^+$: m/z 503.1, found. (%) 503.2.