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SYNTHESIS AND EVALUATION OF PYRAZOLO[3,4-b]QUINOLINE RIBOFURANOSIDES AND THEIR DERIVATIVES AS INHIBITORS OF ONCOGENIC *Ras*

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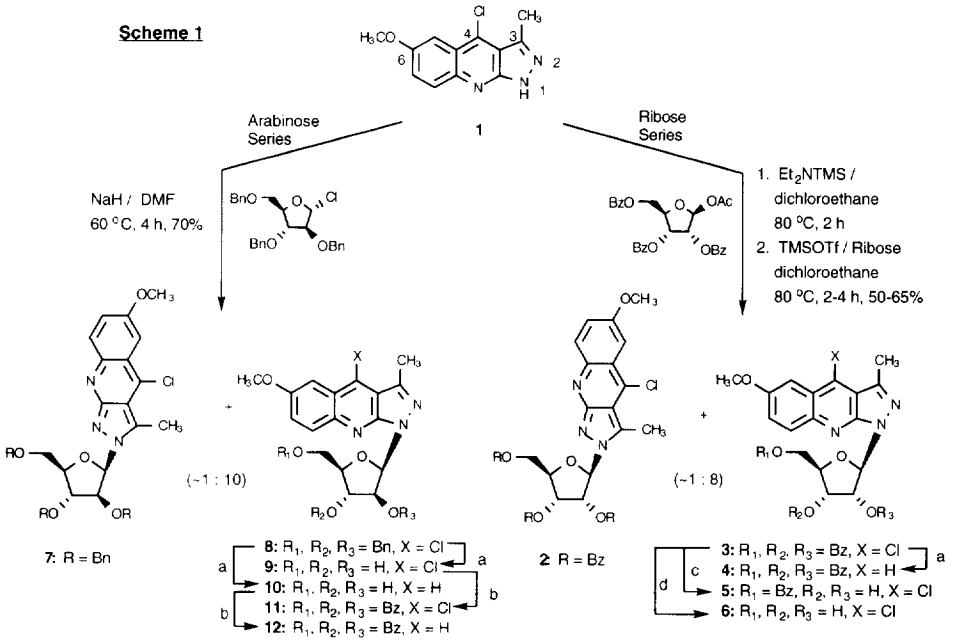
Abstract: A series of pyrazolo[3,4-b]quinoline ribofuranosides were prepared using the glycosylation methodology of Vorbruggen. Oxidative cleavage of the ribose moiety in **6** furnished the dialdehyde intermediate **36**, which cyclizes upon reductive amination providing the morpholino compound **37**. Derivatives from both the ribose and morpholino series were evaluated for their ability to inhibit the nucleotide exchange process of oncogenic *Ras* .[1a]

Current evidence suggests that *ras* oncogenes are responsible for a large percentage of human tumors, some of which include carcinomas of the pancreas, colon, and lung.[1b-e] It is believed that the protein product encoded by the *ras* gene, known as p21, plays an important role in relaying chemical signals within the cell. This 21 kilodalton protein is susceptible to single point mutations which inactivate the intrinsic GTPase activity of the protein resulting in a highly oncogenic form of *Ras*. Activation of the *Ras* pathway is also dependent upon the nature of the ligand/protein complex. When GDP is bound to *Ras* the protein resides in an inactive state; in contrast, the binding of GTP to *Ras* causes the protein to enter an active conformation favoring interaction with downstream effectors and promoting signal transduction. Because the GTP bound form of oncogenic *Ras* is stable, downstream signaling by *Ras* is constitutive resulting in abnormal cell proliferation or carcinogenesis. One mechanistic strategy we envisioned to be attractive for keeping the nucleotide exchange process in check relied on regulating or preventing GTP binding to *Ras*. We thought this objective could be accomplished by developing a non-nucleoside compound[2] capable of binding to an allosteric region of *Ras*, ultimately leading to a conformational change so as to prevent GTP ligand binding.[3]

Routine screening of several pyrazoloquinoline ribofuranosides identified the ribose-tribenzoate **3** as a lead candidate for inhibiting [^{3}H]GDP in a competition assay with the valine-12 mutant form of *Ras*.[4] The corresponding ribose-triols were inactive in this assay. With this result in hand, we tried to determine just how important the ester function was for activity and subsequently prepared a series of various aliphatic, substituted aromatic, and hetero-aromatic triesters (and variations thereof) as a means for establishing a structure-activity relationship (SAR). The pyrazolo[3,4-b]-quinoline nucleus **1** used in this study could be prepared in hundred gram quantities following the procedure of Radl and Zikan.[5] Heating **1** with Et_2NTMS in 1,2-dichloroethane

at 80 °C for 2 h, followed by evaporation under reduced pressure provided a silylated intermediate which was subsequently treated with TMSOTf (1.5 eq) and β-D-ribofuranose-1-acetate-2,3,5-tribenzoate (1.1 eq) in dichloroethane at 80 °C for 2-4 h.[6] The major product produced under these conditions was the β-anomeric N-1 ribofuranoside **3** (Scheme 1).

Scheme 1



Reagents: (a) Pd black, cyclohexadiene, DMF, 80 °C (b) BzCl, Pyridine (c) NH_3, MeOH (d) NaOMe, MeOH

The N-2 regioisomer **2**, which was obtained as a minor product in the glycosylation reaction could be rearranged to the more thermodynamically stable N-1 ribofuranoside **3** upon heating with excess $SnCl_4$ or TMSOTf in dichloroethane. We observed that the N-2 ribofuranoside **2** containing chlorine at the C-4 position is hydrolyzed readily to the corresponding C-4-oxo derivative under acidic conditions (1N HCl-EtOH-$CHCl_3$, 23 °C, 1 h) whereas, the N-1 ribofuranoside **3** was found to be stable even under more stringent conditions (3N HCl-EtOH-$CHCl_3$, 40 °C, 48 h). Since our synthetic plan called for retaining the C-4 chlorine atom as a handle for skeletal elaborations at a later stage, we focused our synthetic efforts in the N-1 series. Characterization of the N-1/N-2 regioisomers were determined by spectral means.[7] Additionally, a single-crystal X-ray analysis of the *p*-bromobenzoate ester **13**, which was prepared from **5**, unequivocally established the regiochemistry in the N-1 series.

Solvolysis of the N-1 ribose-tribenzoate **3** employing a saturated solution of NH_3-CH_3OH at 23 °C for 3 days afforded two compounds, the diol **5** and the triol **6**. The ratios varied slightly from experiment to

experiment, but the same two products always resulted. Proof for the C5' monobenzoate **5** was established by chemical conversion *via* **25**, to the dihydrofuran **26** utilizing the Corey-Winter protocol.[10] Hydrogenation of **26** gave the tetrahydrofuran **27**. Compounds **5** and **6** allowed us to evaluate whether a portion or all three benzoate groups in **3** were required for activity (Table 1). This question was addressed further, by evaluating a few C-5' acetophenone carbon isosteres as surrogates to the potentially labile benzoate ester. Thus, protection of **6** as the 2',3'-acetonide (dimethoxypropane, DMF, p-TsOH), followed by oxidation[11] (DMSO, DCC) of the C5' hydroxyl to the aldehyde, and subsequent Wittig homologation (Ph_3P=CHCOPh, THF) afforded **28** in 56% overall yield (Scheme 2). Deprotection of the acetonide unit using (1N HCl-THF, 50 °C, 5 h) provided ca. 3:1 mixture of **29** and **30** respectively. Treatment of **30** with (MsCl, Et_3N, CH_2Cl_2) afforded the fully unsaturated furan derivative **31** in 62% yield.[9,12] Careful reduction of the α,β-unsaturated bond in **28** (H_2, PtO_2, 1 atm, EtOAc-EtOH, 5:1, 30 min) followed by hydrolysis of the acetonide group and subsequent elimination of the diol *via* the thionocarbonate[10] provided compound **33** in an overall yield of 43%. In a similar fashion, the diol **29** was converted to the dihydrofuran **32** in 62% yield, and the triol **30** was deoxygenated to give **35** in 72% yield. Biological activities for the ribose derivatives are given in Table 1. Next, we briefly explored the stereochemical requirements within the sugar moiety by preparing the arabinofuranosides **7-12** for comparison. Condensation of 2,3,5-tri-O-benzyl-α-chloro-D-arabinofuranose[8] with the sodium salt of **1** generated from NaH in DMF provided the β-anomeric N-1 and N-2 arabinofuranosides in ca. 10:1 ratio and in 70% yield. Conversion of the tribenzyl arabinofuranoside **8** to its tribenzoate derivative **11** was accomplished in 55% yield over the two steps as outlined in Scheme 1.

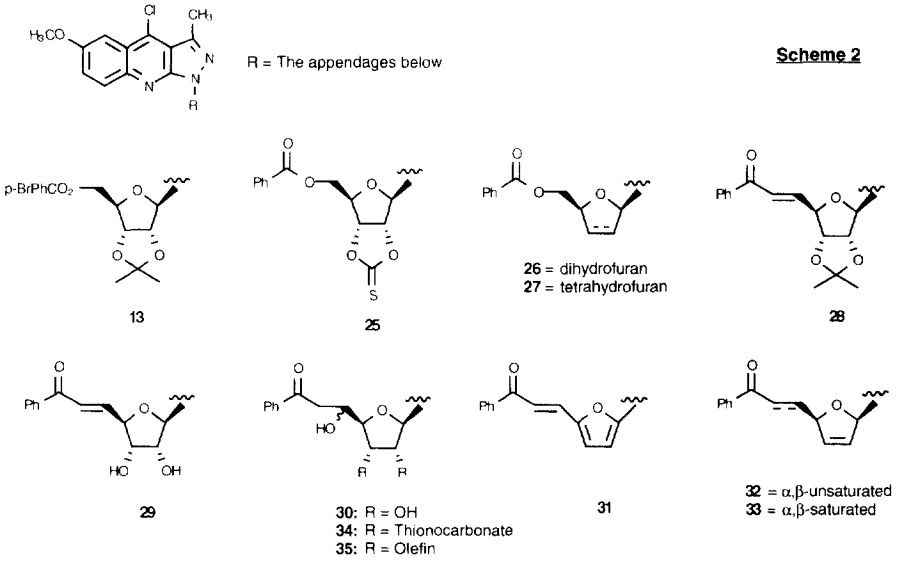
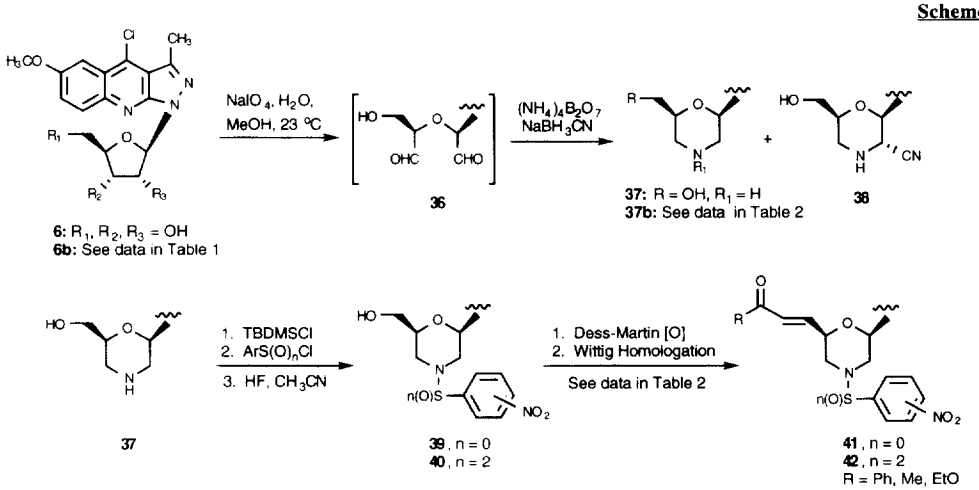


Table 1. *In Vitro* Nucleotide-Exchange Activity for the Ribose Series **6b**.
(Data Reported as % Inhibition Obtained at 50 μM, or as an IC_{50} Value for Compounds with % Inhibitions >70%)

Compd	R_1	$R_2 = R_3$	Inhib.	Compd	R_1	$R_2 = R_3$	Inhib.
3	$PhCO_2$	$PhCO_2$	10 μM	23	2-furoate	2-furoate	10.5 μM
5	$PhCO_2$	OH	16%	24	3-furoate	3-furoate	49%
14	$PhCO_2$	CH_3CO_2	44%	25	$PhCO_2$		15 μM
15	3-nicotinoate	3-nicotinoate	33%	26	$PhCO_2$	Olefin	80 μM
16	$PhCH_2CO_2$	$PhCH_2CO_2$	62%	27	$PhCO_2$	H	38%
17	$PhCH_2$	$PhCH_2$	35%	28	PhCOCH=CH	Acetonide	53%
18	$(CH_3)_2CHCO_2$	$(CH_3)_2CHCO_2$	33%	29	PhCOCH=CH	OH	10 μM
19	$PhCO_2$	$PhCH_2$	53%	32	PhCOCH=CH	Olefin	35 μM
20	CH_3CO_2	CH_3CO_2	3%	33	$PhCOCH_2CH_2$	Olefin	25%
21	p-MeO-$PhCO_2$	p-MeO-$PhCO_2$	44%	34	$PhCOCH_2(OH)CH$		1.5 μM
22	p-NO_2-$PhCO_2$	p-NO_2-$PhCO_2$	59%	35	$PhCOCH_2(OH)CH$	Olefin	48%

In the next part of this investigation, we focused our attention on transforming the ribose moiety into a morpholino subunit as a way of broadening our SAR profile (Scheme 3). The extension into this class of sugar-modified nucleotides was based upon approaches that have been successful in other therapeutic areas.[13,15] Thus, oxidative cleavage of triol **6** with $NaIO_4$ afforded the 2',3'-secodialdehyde **36**, which was condensed with ammonium biborate[14] in the presence of $NaBH_3CN$ giving rise to the morpholino compound **37** in ca. 50% yield. The cyano-morpholino adduct **38** was isolated in ca. 10% yield.[15]

Scheme 3



Derivatization of **37** was best accomplished by first protecting the C-5' hydroxyl as a TBDMS ether followed by coupling with the appropriate sulfenylchloride or sulfonylchloride. Removal of the TBDMS group with HF in CH_3CN provided the corresponding sulfenamides **39** and sulfonamides **40** in good yield. Oxidation of the C-5' hydroxyls of **39** and **40** using the Dess-Martin periodinane reagent[16] afforded the requisite aldehydes in >70% yield; Moffatt conditions were not successful in this case. Wittig homologation of the aldehydes provided the α,β-unsaturated compounds of types **41** and **42** for evaluation as illustrated in Table 2.

Table 2. *In Vitro* Nucleotide-Exchange Activity in the Morpholine Series **37b**.
(Data Reported as % Inhibition Obtained at 50 μM, or as an IC_{50} Value for Compounds with % Inhbitions > 70%)

Compd	R	R_1	Inhib.	Compd	R	R_1	Inhib.
37	HO	H	0%	51	TBDMSO	p-NO_2PhS	50 μM
43	HO	$PhSO_2$	12 μM	52	HO	p-NO_2PhS	6.7 μM
44	TBDMSO	$PhSO_2$	14 μM	53	PhCOCH=CH	p-NO_2PhS	7.4 μM
45	PHCO2	$PhSO_2$	47%	54	PhCOCH=CH	o-NO_2PhS	4.7 μM
46	HO	p-NO_2PhSO_2	8.0 μM	55	PhCOCH=CH	p-NO_2PhSO_2	7.0 μM
47	TBDMSO	p-NO_2PhSO_2	5.0 μM	56	EtO_2CCH=CH	o-NO_2PhS	16 μM
48	TBDMSO	p-AcNPhSO_2	27 μM	57	EtO_2CCH=CH	p-NO_2PhS	64%
49	TBDMSO	o-NO_2PhS	1.0 μM	58	MeCOCH=CH	o-NO_2PhS	88%
50	HO	o-NO_2PhS	14 μM	59	MeCOCH=CH	p-NO_2PhS	3.0 μM

Biological Results: A variety of functionalized ribose and morpholino pyrazoloquinolines were found to be moderate *in vitro* inhibitors of the nucleotide exchange process of oncogenic *Ras*. Of the ribose triesters evaluated, only the unsubstituted benzoate **3**, and the 2-furoate ester **23** exhibited low μM activity. Two of the acetophenone isosteres **29** and **34** wherein the C-5' oxygen atom was replaced with a carbon atom, were the only non-triester compounds which displayed equal or better activity than **3**. The greatest improvement in activity in the ribose series was seen in going from the 2'3'-diol to the 2',3'-thionocarbonate moiety; **5** ---> **25** and **30** ---> **34**.[9] In the arabinose series, only the C4-hydro derivative **12** displayed comparable activity to compound **3**; the corresponding compound **4** in the arabinose series was inactive.[9] A slight improvement in activity was observed in the morpholino series, with the *o*-nitrobenzene sulfenamide **49** exhibiting the best inhibition in this group. The observation that benzoate esters are critical for activity in the SAR of the ribose series did not translate to the morpholino series. Other functional groups appended to the morpholino nitrogen such as amides, ureas, and carbamates did not lead to an improvement in biological activity.

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References and Notes.

1. (a) Throughout the text, the notation "*ras*" is used to refer to the gene, whereas, the use of "*Ras*" refers to the protein product, p21. For leading references dealing with the biology of *ras* see: (b) Barbacid, M. *Annu. Rev. Biochem.* **1987**, *56*, 779. (c) Barbacid, M. *Eur. J. Clin. Invest.* **1990**, *20*, 225. (d) Hall, A. *Science*. **1990**, *249*, 635. (e) Bourne, H. R.; Sanders, D. A.; McCormick, F. *Nature*. **1990**, *348*, 125.
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7. All new compounds gave spectroscopic data consistent with their assigned structures, with particular use being made of NOE difference and Selective INEPT techniques to establish the regiochemical assignments. Additionally, the chemical shift for the C3-methyl group served as a reliable diagnostic indicator in determining whether the product was an N-1 or N-2 regioisomer. For all N-1 isomers the chemical shift for the C3-methyl group was observed upfield to the corresponding N-2 isomer. **Compd 2.** ^{1}H NMR (300 MHz, DMSO-d_6) 7.92 (d, 1H, J = 9.5 Hz), 7.51 (dd, 1H, J = 2.6, 9.5 Hz), 7.40 (d, 1H, 2.6 Hz), 6.30 (d, 1H, J = 3.2 Hz, anomeric H), 5.66 (d, 1H, 5.4 Hz, OH), 5.35 (d, 1H, J = 5.9 Hz, OH), 5.02 (t, 1H, J = 5.3 Hz, C5' OH), 4.81 (m, 1H), 4.47 (m, 1H), 4.13 (m, 1H), 3.7 (m, 1H), 3.54 (m, 1H), 3.13 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) 155.63, 154. 85, 147.84, 133.80, 133.57, 130.05, 126.40, 121.81, 111.51, 98.35, 91.18, 85.87, 74.79, 70.58, 61.82, 55.28, 11.07. MS (FAB) m/e 380 (M+1). **Compd 3.** ^{1}H NMR (300 MHz, DMSO-d_6) 7.98 (d,1H, J = 9.3 Hz), 7.58 (dd, 1H, J = 2.7, 9.3Hz), 7.49 (d, 1H, J = 2.5 Hz), 6.36 (d, 1H, J = 5.0 Hz), 5.40 (d, 1H, J = 5.7 Hz, OH), 5.20 (d, 1H, J = 5.3 Hz, OH), 4.84 (t, 1H, J = 5.4 Hz, C5'OH), 4.75 (q, 1H, J = 5.3 Hz), 4.25 (q, 1H, J = 4.7 Hz), 3.94 (m, 1H, w/ overlapping singlet, 3H), 3.6 (m, 1H), 3.5 (m, 1H), 2.79 (s, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) 155. 86, 149.59, 143.87, 140.69, 133.31, 129.25, 124.97, 121.84, 113. 57, 99.89, 87.30, 84.29, 72.09, 70.25, 61.82, 54.98, 14.37. MS (FAB) m/e 380.3 (M+1). **Compd 50.** ^{1}H NMR (400 MHz, $CDCl_3$) 8.20 (m, 2H, w/ overlapping d, J = 9.0 Hz), 7.76 (d, 1H, J = 9.7 Hz), 7.50 (m, 2H, w/ overlapping d J = 9.0 Hz), 7.35 (m, 2H), 6.51 (dd, 1H, J = 2.5, 9.9 Hz), 4.4 (bm, 1H), 4.17 (apparent t, 1H, J = 10.8 Hz), 3.99 (s, 3H), 3.80 (bs, 2H), 3.40 (dd, 1H, J = 1.5, 11.8 Hz), 3.26 (apparent t, 1H, J = 10.4 Hz), 3.18 (dd, 1H, J = 1.6, 11.7 Hz), 2.75 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) 156.55, 150.05, 149.75, 145.74, 144.30, 142.76, 134.66, 129.55, 125.38, 124.35, 123.19, 122.88, 114.28, 100.59, 79.38, 77.72, 63.57, 57.47, 56.28, 55.68, 15.22. MS, (SIMS) m/e 516.2 (M+1).
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