Synthesis and biological evaluation of Z-isomers of Rigosertib® (ON 01910.Na) - A clinical stage (Phase III) multi kinase anti-cancer agent.

Venkat R. Pallela [1], Muralidhar R. Mallireddigari [1], Stephen C. Cosenza [2], Chen Ren [1], E. Premkumar Reddy [2] and M. V. Ramana Reddy [2].

1. Onconova Therapeutics Inc., Medicinal Chemistry, Newtown, PA , 18940.
2. Mount Sinai School of Medicine, Oncological Sciences, New York, NY, 10029.

ONCONOVA
TARGETING CANCER I PROTECTING HEALTHY CELLS

MOUNT SINAI SCHOOL OF MEDICINE

Introduction

ON 01910.Na (Rigosertib®) is a novel non-ATP competitive anticancer agent that inhibits mitotic progression, promotes G2/M arrest and induces apoptosis in a number of cancer cells while leaving nonmalignant cells virtually unaffected. ON 01910.Na has exhibited both antitumor activity and antiangiogenic activity with a low toxicity profile in various preclinical tumor xenograft models. Unlike other hypomethylating agents, ON 01910.Na exerts potent antitumor activity against Mantle Cell Lymphoma (MCL) cells by inhibition of PI-3K/Akt/mTOR pathway and down regulation of Cyclin D1 translation. Cyclin D proteins are elevated in many cancer cells including Myelodysplastic Syndrome (MDS). The fact that MDS patient's bone marrow over expresses cyclin D1 (particularly trisomy 8) and targeted deletion of this gene was selectively toxic to trisomy 8 cells. A pivotal phase III trial of ON 01910.Na in MDS patients is now underway. ON 01910.Na has also shown promising therapeutic results and drug tolerance in patients with advanced solid tumors. Additional blood cancer and solid tumor indications are being developed, both as a single agent and in combination therapy.

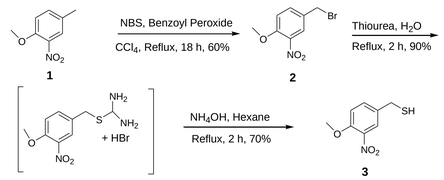
The structure activity studies of ON 01910.Na confirmed that the nature, number, and position of substituents on both aromatic rings of the molecule are critical for it's activity. As geometry of a molecule is known to play a critical role in SAR, we have undertaken the synthesis of (Z)- isomer (ON 02180.Na) of ON 01910.Na, which is an active (E)-isomer. In this presentation, we describe the total synthesis, configurational assignment and compare the biological profile of ON 02180.Na with that of ON 01910.Na.

Chemistry

Numerous synthetic methods have been explored for the stereoselective synthesis of (E)-vinyl sulfides and in contrast, it has been challenging to prepare corresponding (Z)-vinyl sulfides. Free radical initiated hydrothiolation of alkynes with aryl thiols have shown great promise in synthetic chemistry and by using this methodology we have synthesized a number of (Z) and (E)-styryl benzyl sulfides, sulfoxides and sulfones of significant biological importance.

The synthetic routes for the preparation of Z and E-isomers of styryl benzyl sulfide, sulfoxide and sulfones are described in Schemes 1- 5. Bromination of 4-methoxy-3-nitro benzene (**1**) with NBS in CCl4 in the presence of catalytic amount of benzoyl peroxide afforded 4-methoxy-3-nitrobenzyl bromide (**2**). The benzyl bromide **2** was treated with thiourea in water to get an intermediate isothiouronium salt which on reduction with ammonia yielded 4-methoxy-3-nitrobezylthiol (**3**) in low to moderate yields (**Scheme 1**).

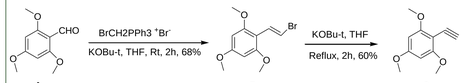
Scheme 1: Preparation of 4-Methoxy-3-nitrobenzylthiol



The intermediate 2,4, 6-trimethoxy acetylene (**6**) was made starting from 2,4,6-trmethoxybenzaldehyde (**4**) and (Bromomethyl)triphenylphosphonium bromide in the presence of potassium t-butoxide to get 2-bromovinyl-1,3,5-trimethoxy benzene (**5**) followed by dehydrobromination with potassium t-butoxide (**Scheme 2**)

Chemistry

Scheme 2: Preparation of 2,4,6-trimethoxy acetylene



The coupling reaction initiated by Et_3B-Hexane between **3** and **6** gave a mixture of (Z) and (E)-4-methoxy-3-nitrobenzyl-2,4,6-trimethoxstyryl sulfides (**7 & 8**) in 86:14 ratio (**Scheme 3**). To optimize the reaction conditions for better Z to E ratio or exclusive Z-isomer, this coupling reaction was tried with different bases, solvent, catalyst and temperature. Among the bases tested, Et_3B-Hexane afforded the best Z / E ratio with complete conversion in 2h at room temperature (**Table 1**).

Scheme 3: Synthesis of Z& E-isomers of 2, 4, 6-Trimethoxystyryl-4-methoxy-3-nitrobenzyl sulfide

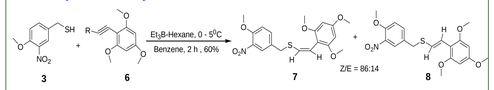


Table 1. Addition of 4-Methoxy-3-nitrobezylthiol to 2-Ethynyl-1,3,5-trimethoxybenzene

entry	R	base	solvent	catalyst	temp, ^{0}C	time, h	Yield, %	Z/E[a]
1	H	NaOH	EtOH	-	80	12	0	-
2	H	Na metal	EtOH	-	80	24	0	-
3	H	-	-	Al_2O_3/KF	60	1	10	75:25
4	H	AIBN	benzene	-	100	2	56	80:20
5	H	-	water	-	100	2	75	79:21
6	H	Et_3B-hexane	benzene	-	rt	2	82	86:14
7	H	CS_2CO_3	NMP	CUI	80	24	0	-
8	H	CS_2CO_3	DMSO	TEMPO	85	2	10	ND
9	$COOCH_3$	CS_2CO_3	DMSO	TEMPO	rt	4	0	-
10	COOH	CS_2CO_3	NMP	CUI	90	24	0	-

[a] The Z/E ratio was based on the analysis of ^{1}HNMR spectra

To further improve the efficacy of Et_3B-Hexane reaction, this reaction was tried in different solvents at various temperatures and the results showed that best Z/E ratio obtained in toluene at room temperature (**Table 2**). The sulfhydryl group added to the terminal acetylenic carbon non-stereoselctively to give a mixture of (Z)- and (E)- sulfides with an exception of the reactions where methanol was added along with the solvent in which exclusive E- sulfides were formed.

Table 2. Optimization reaction conditions of Et3B-heaxane induced radical addition of 4-Methoxy-3-nitrobezylthiol to 2-Ethynyl-1,3,5trimethoxybenzene

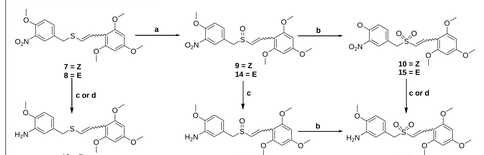
entry	solvent	temp, ^{0}C	Yield, %	Z/E[a]
1	benzene	rt	82	86:14
2	benzene	5	75	50:50
3	benzene + 4 eq. methanol	5	92	0:100
4	toluene	rt	85	88:12
5	toluene	0	80	79:21
6	Toluene +4 eq. methanol	0	95	0:100
7	acetonitrile	0	82	72:28
8	THF	0	80	62:38
9	NMP	0	85	63:37
11	DMSO	rt	60	8:92
11	DMF	0	72	28:72

[a] The Z/E ratio was based on the analysis of ^{1}HNMR spectra

Chemistry

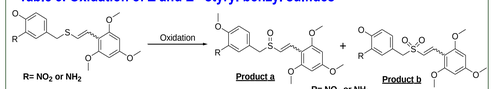
(Z) And (E)-4-methoxy-3-nitrobenzyl-2,4,6-trimethoxstyryl sulfides (**7 & 8**) were oxidized either with (a) hexafluoro-2-propanol and 30% H_2O_2 at room temperature or (b) 30% H_2O_2 in AcOH at 0 ^{0}C to the corresponding sulfoxides (**9 & 14**) which on further oxidation with MCPBA resulted in sulfones(**10& 15**). Nitro sulfones **10 & 15** were reduced with either (a) iron powder in MeOH / AcOH (2:1) or Raney Ni, NH_2-NH_2 in methanol to the corresponding anilino sulfones (**11 & 16**). These were also obtained from **9 & 14** by reduction to corresponding anilino compounds (**12 & 17**) followed by oxidation with MCPBA. Compounds **7 & 8** were reduced to corresponding amino sulfides **13 & 18** (**Scheme 4**).

Scheme 4: Synthesis of (Z) and (E)-isomers of Styryl benzyl sulfides, sulfoxides and sulfones [a]



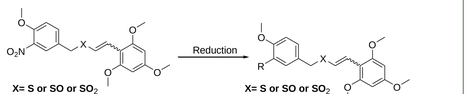
[a] Reagents and conditions: (a) 1,1,1,3,3,3-hexafluoro-2-propanol, 30%H_2O_2, 25 ^{0}C, 2 h or 30%H_2O_2, 0 ^{0}C, 5 h; (b) *m*-CPBA, CH_2Cl_2, 0 ^{0}C - rt, 5 h (c) Iron Powder, MeOH / AcOH (2:1), 80 ^{0}C, 3 h; (d) Raney Ni, NH_2NH_2, MeOH, 40 ^{0}C, 4h

Table 3. Oxidation of Z and E –styryl benzyl sulfides



entry	Oxidizing agent	Solvent	temp, ^{0}C	Product	Yield, %
1	H_2O_2	AcOH	rt	b	20
2	H_2O_2	AcOH	0	a	55
3	MCPBA	MeOH	rt	b	60
4	Oxone	2:1 THF/MeOH	rt	b	65
5	Hexafluoro-2propanol	MeOH	rt	a	45

Table 4. Reduction of Z and E–styryl benzyl sulfides, sulfoxides and sulfones

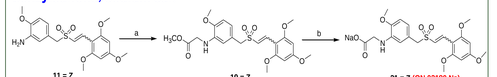


entry	catalyst	solvent	temp, ^{0}C	time, h	Yield, %
1	$Na_2S_2O_3$	Acetone/water (2:1)	50	2	20
2	Zn	AcOH	rt	3	40
3	Raney Ni/NH_2NH_2	MeOH	40	4	50
4	Indium/NH_4Cl	EtOH	80	5	50
5	$SnCl_2$	EtOH	rt	4	40
7	Fe(0)	AcOH/MeOH (1:2)	80	3	90
8	3%Pt/C (sulfided)/H_2	CH_3CN	60	3	74

Chemistry

To enhance the solubility and bioavailability of (Z) and (E)-4-methoxy-3-nitrobenzyl-2,4,6-trimethoxstyryl sulfone (**11**) and (**16**), corresponding 3-amino substituted glycine esters and acids were made. **11** And **16** were treated with methyl 2-bromoacetate in the presence of a mild base to get corresponding α-amino esters **19** and **20** which on subsequent hydrolysis afforded the corresponding sodium salt of acids (**Z**, **21**) and (**E**, **22**) -2,4,6-Trimethoxystyryl-3-[(carboxymethyl)amino]-4-methoxybenzylsulfone.

Scheme 5: 2,4,6-Trimethoxystyryl-3-[(carboxymethyl)amino]-4-methoxy-benzylsulfone, sodium salt



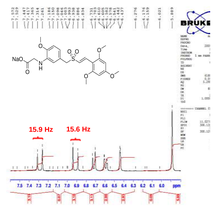
[a] Reagents and conditions: (a) $BrCH_2COOCH_3$, MeOH,CH_3COONa, 4-6 h (b) NaOH, EtOH, H_2O · CH_2Cl_2, MEK, rt, 3-4 h

Table 5: *Invitro* Cytotoxicity of Z / E-isomers of Styryl Benzyl Sulfones

Compd. #	Structure	Isomer	K562 IC_{50} (μ M)	DU145 IC_{50} (μ M)
7		Z	10-25	25-50
8		E	1-10	1-10
9		Z	50-100	>100
14		E	10-25	10-25
10		Z	N/D	N/D
15		E	2.5	2.5
13		Z	0.25	0.75
18		E	0.03	0.05
12		Z	N/D	N/D
17		E	0.02	0.04
11		Z	0.15	0.15
16		E	0.003	0.003
19		Z	0.5-1.0	1-10
20		E	0.1	0.1
21 (ON02180.Na)		Z	25-50	25-50
22 (ON01910.Na)		E	0.0075	0.08

^{1}HNMR Spectra of Z & E isomers, 21 & 22

A. NMR Spectra for 22



B. NMR Spectra for 21

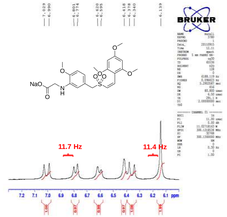


Figure 1. NMR spectra for (A) **22 (ON 01910.Na)** and (B) **21 (ON 02180.Na)**; The E-isomer is confirmed by the coupling constants (J) at 15.9 Hz and 15.6 Hz; The coupling constants (J) at 11.7 Hz and 11.4 Hz are clear indications for Z-isomer.

Summary and Conclusions

- A series of novel (E) and (Z)-styryl benzyl sulfides, sulfoxides and sulfones have been prepared by the free radical addition of 4-methoxy-3-nitrobenzylthiol with 2, 4, 6-trimethoxyphenyl acetylene.
- An optimized reaction condition for this hydrothiolation was established and the preferred method for better Z/E ratio was Et3B-Hexane in toluene.
- (Z) & (E)-Styryl benzyl sulfides were oxidized to sulfoxides and sulfones and the Nitro compounds were reduced to amino analogs. Optimum reaction conditions for oxidation and reduction were established.
- To enhance the solubility and bioavailability of these compounds, corresponding 3-amino substituted glycine esters and acids were made and tested for their cytotoxicity
- The (E)-styryl benzyl sulfides, sulfoxides and sulfones are more active than the corresponding (Z)-isomers.
- The compound **22** Rigosertib®(ON 01910.Na) exhibits broad spectrum anticancer activity and active against many drug resistant tumors. Rigosertib® is a substrate competitive inhibitor of Cyclin-D1, PI3-K / Akt / m-TOR pathway.
- Rigosertib® is being evaluated in Phase III clinical trials for the treatment of Myelodysplastic Syndrome (MDS) and other blood cancer and solid tumor indications.

References

1. Reddy, M. V. R.; Venkatapuram, P.; Mallireddigari, M. R.; Pallela, V.R.; Cosenza, S. C.; Robell, K. A.; Akula, B.; Hoffman, B. S.; Reddy, E. P. *J. Med.Chem.* 54(18), 6254 – 6276 (**2011**).
2. Reddy, M. V. R.; Mallireddigari, M. R.; Cosenza, S. C.; Pallela, V.R.; Iqbal, N. M.; Robell, K. A.; Kang, A. D.; Reddy, E. P. *J. Med.Chem.* 51(1), 86 - 100 (**2008**).
3. Reddy, E. P.; Reddy, M. V. R. *U.S. Patent* No. 6599932 B1 (**2003**).
4. Reddy, E. P.; Reddy, M. V. R. *U.S. Patent* No. 6541475 (**2002**).

Acknowledgements

Authors are thankful to Onconova Therapeutics Inc., Newtown, PA and Mount Sinai School of Medicine, New York for the financial assistance and interest in this project.