Experimental and Theoretical approaches to enhance Anti tubercular Activity of Chalcones

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Abstract:-Twenty three chalcones are synthesized and evaluated for antimycobacterial activity against *M. tuberculosis* $H_{37}R_v$. Ortho chloro substitutions at A and B-ring favor activity. Compound **12** with chloro and hydroxyl substitution exhibits 98% reduction in relative light units at a concentration of 100μg/ml. Methylsulfonyl chalcones also exhibit very good activity. The requirements for the anti-infective activity are explored with 2D, 3D and group based QSAR studies. The 2D technique indicates the importance of volume, refractivity and molecular weight of the compounds on the activity. The 3DQSAR indicates less bulky (at R5) and more hydrophilic substituent groups (at R3) can improve the Antitubercular activity. The GQSAR technique indicates that hydrophilic groups in R3 or R5 positions can enhance activity. The oral bioavailability of all the molecules are between 30-70%.Compound **12** is mildly acidic, soluble in water,stable at pH<2 and does not violate the Lipinski's rule of 5. Hence it appears to be a strong drug candidate.

Keywords: - QSAR, chalcones, antimycobacterial activity, anti-infective, hydrophilic groups, refractivity

1 Introduction

Infectious diseases are influencing the world with their morbidity and mortality. Tuberculosis is one among the major infectious diseases caused by *Mycobacterium tuberculosis* [1, 2]. Treatment of tuberculosis is a complex process because of various factors which include patient's inability to persist with combined treatment regimen, the spreading ability of non tubercular mycobacteria (NTM) including *M. avium* complex (MAC), the ineffectiveness of the drugs on immunosuppressed patients, and MDR (multidrug resistance) [3-5]. Antibiotics are being used to treat this infection, but this pathogenic strain is becoming resistant to antibiotics. Since the resistance increases day by day, there is a need for designing newer antibiotics [7].

Chalcones are a diverse group of compounds which could be synthesized as well as obtained from natural sources. They belong to the small molecule group and exert various biological activities which include antimycobacterial [8], antibacterial [9-10] and antifungal [11]. Small molecules play a significant role in therapeutics because of their superior pharmacokinetic (ADME/Tox) properties.

The Lipinski's rule suggests that the molecular weight of the chemical entity should not exceed 500 kDa, so small molecules can be the answer for this problem. Hence the current study focuses on synthesis and evaluation of the anti tubercular activity of chalcones. 2D, 3D and group based Quantitative structure activity relationship (QSAR) studies were also carried out to understand the structural features that are necessary to enhance their anti tubercular activity. Such a theoretical study can help in designing more active molecules than the current ones.

2 Materials and Methods

The various chalcones (Table 1) are synthesized by the method reported by Lin et al [12] and it is given in Figure 1. The 4-methylsulfonyl chalcones are synthesized from 4-methylthio chalcones (Figure 2) by the procedure reported by Davis and Zhou et al [13].

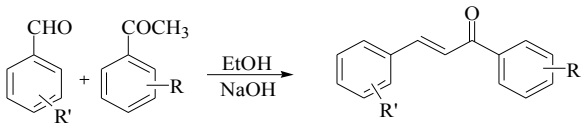


Fig. 1 Scheme for the Synthesis of chalcones

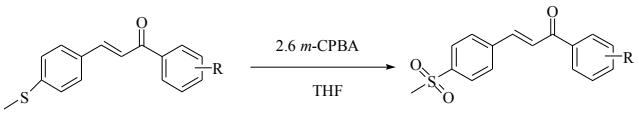


Fig. 2 Scheme for conversion of 4-methythio chalcone to 4-methylsulfonyl chalcones

The synthesized compounds are characterized by FT-IR, NMR and mass spectroscopy.

The antimycobacterial activity of these 23 chalcones were evaluated by Luciferase Reporter Phage (LRP) assay against *M. tuberculosis* $H_{37}R_v$ using the method reported by Riska et al [14].

2DQSAR, 3DQSAR and GQSAR were performed using VLifemds3.5 software (VLife Sciences Technologies Pvt. Ltd., Pune, India, http://www.vlifesciences.com). The software enables evaluation of several molecular descriptors and provides a facility to build regression equation relating the best set of descriptors with the activity which can be used later for predicting activity of new molecules. The structures of the compounds were drawn and optimized in Hyperchem software (Hypercube Inc., USA) using MM+ force field. They were then exported into the other software. For all the QSAR studies the percentage reduction in RLU at 100 µg/ml (P) were converted into activities as log [P/ (100-P)].

In 2DQSAR, Two hundred and eleven descriptors (physico-chemical, alignment Independent and atom type descriptors) were estimated. The data was divided into training and test sets (14 and 4 data points respectively) randomly. Five compounds which did not show any reduction in RLU were not included in the development of the model. Multiple forward linear regression method was used to identify the best model. Statistical parameters such as r^2, r^2adj, q^2 and F were estimated for the regression equation to determine the quality of the model fit and the predictive capability of the model.

In 3DQSAR, the position of each atom drawn is important as the descriptors were calculated on 3D space grid. The alignment of the molecules was done based on the common fragment of chalcones using template based method. To derive the descriptor fields, a 3D cubic lattice grid in *x, y* and *z* directions, was created to encompass the aligned molecules. The descriptors were calculated using an sp^3 carbon probe atom with a van der Waals radius of 1.52 Å and a charge of +1.0 to generate steric, electrostatic and hydrophobic fields with the distance dependant dielectric constant of 1.0 at each lattice point . The electrostatic energy values were truncated at a default value of 10 kcal/mol and 30 kcal/mol respectively [15].

Two thousand seven hundred and one descriptors which include all the electrostatic, steric and hydrophobic descriptor values were evaluated for eighteen compounds and was used as independent variable. Several literature reports give a description about descriptors [16-20]. The data was once again divided into training and test sets (14 and 4 data points respectively) using manual selection method [21]. The Multiple Regression Stepwise forward variable selection method with the cross-correlation limit of 1.0, q^2 as the selection criteria, cut off value as zero and auto-scaling method were applied regression. The set of descriptors that would give the statistically best model was selected.

In GQSAR, the basic chalcone template is common for all the structures and the fragments or groups at R1, R2, R3, R4, R5, and R6 positions are different which contribute to the difference in the observed anti tubercular activity. Two thousand two hundred and eighty seven descriptors (physico-chemical, alignment independent and atom type descriptors) for the various groups in these six positions were calculated. The data was once again divided into training and test sets (14 an 4 respectively). Multiple forward linear regression method was used to develop the best GQSAR equation relating the descriptors with the activity.

ADME/Tox is an *insilico* online tool service (http://pharma-algorithms.com/webboxes/) that allows users to find Absorption, Distribution, Metabolism, Excretion, and Toxicity of the molecules[22].The molecular properties should be considered to enhance further drug discovery [23,

24]. ADMET properties like Oral Bioavailability, Solubility, Permeability, LD50 etc were calculated by uploading the structures in *.skc format. Tox Boxes is a tool to predict three basic toxicity endpoints - acute toxicity, genotoxicity and organ specific health effects. It allows calculation of toxic effects of molecules solely from the chemical structure.

Molinspiration offers free on-line cheminformatics services (http://www.molinspiration.com/cgi-bin/properties). The structures of the chalcone molecule were sketched in Molinspiration, and the physicochemical properties such as logP, polar surface area, number of hydrogen bond donors and acceptors and bioactivity scores such as GPCR ligands, kinase inhibitors, ion channel modulators, nuclear receptors for the most important drug targets were predicted[25].The compounds were then tested for Lipinski's Rule of Five using the Molinspiration online property calculation toolkit [26-28].

3 Results and discussions

Twenty three of the synthesized chalcones were characterized and evaluated for antimycobacterial activity at 50 and 100 µg/ml concentrations (Table 2). It is evaluated in terms of percentage reduction in Relative Light Units (RLU). At 50 µg/ml concentration, compounds **5, 6, 7, 12** and **22** are found to be active and other compounds are inactive. Compounds with *ortho* chloro and *para* sulfonylmethyl substitutions at A-ring increase the antimycobacterial activity. At 100 µg/ml concentration, compounds **4, 5, 6, 7, 8, 9, 12, 19, 20** and **22** are active. Among these, compound **12** exhibits more than 98% reduction in RLU. *Ortho* chloro substitutions at A and B-ring (compounds **4, 5, 9, 12** and **20**) favour antimycobacterial activity. Except for compound **9,** others have Cl substitution in A-ring. Interestingly methylsulfonyl chalcones exhibit very good activity (**6, 7, 8** and **9**). So the A-ring can accommodate both hydrophobic and hydrophilic substitutions. B-ring accommodates the polarizable substitutions like NO_2, OH, F and methylenedioxy groups.

The best 2DQSAR and the statistics obtained are listed below

Activity = 15.32 - 0.433(±0.028) (smr) + 0.079(±0.002) (Volume) (1)

r^2 = 0.88, r^2adj = 0.85, q^2 = 0.83, F = 41.8 and p< 0.001

Increasing volume increases activity, while increasing smr decreases activity. The later descriptor is a function of molecular refractivity and molecular weight. The former descriptor contributes 60% and the later 40% towards activity. Fig. 3 shows the parity plot relating the experimental and predicted activities with the 99% prediction bands and Table 3 lists the predicted activity and the descriptor values.

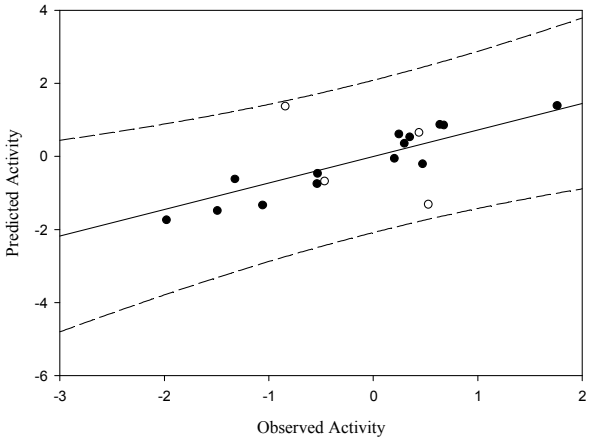


Fig. 3 Parity plot and 99% prediction interval based on 2DQSAR

The best 3DQSAR and the statistics obtained are listed below

Activity = 2.87 -0.04(±0.0004) (S_408)- 4.60(±1.014) (H_661) (2)

r^2 = 0.847, r^2adj = 0.83,q^2 = 0.79, F = 30.53, p<0.001

Fig. 4 is a parity plot showing 99% prediction interval. The Stereo view of the super imposed molecules along with the descriptors contributing to the activity are shown in Fig. 5. Table 4 lists the predicted activity and the descriptor values.

Negative contribution of S_408 in R5 indicates that negative steric potential is favorable for increasing the activity. So less bulky substituent groups are preferred in the R5 position. Negative contribution of H_661 to Hydrogen group nearer to R3 indicates that negative hydrophobic field is favorable for increasing the activity. Hence less hydrophobic or more hydrophilic substituent groups near R3 is preferred. The former descriptor contributes 56 % and the later contributes 44% towards activity.

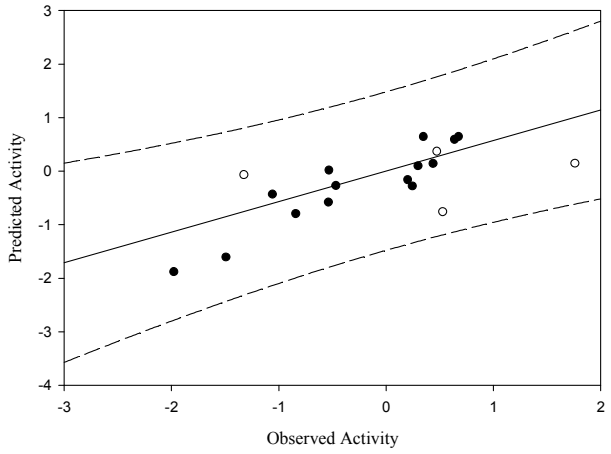


Fig. 4 Parity plot and 99% prediction interval based on 3DQSAR

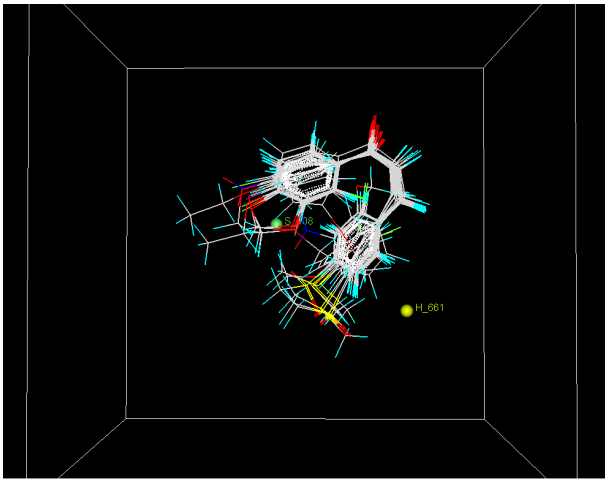


Fig.5 The Stereo view of the super imposed molecules. The descriptors which contribute to the activity are shown as dots.

The best GQSAR and the statistics obtained are listed below

Activity = 0.609 -4.97 (±0.807) (R3-Most Hydrophobic) -7.816 (±1.579) (R5-SAAverage) (3)

r^2 = 0.85, r^2adj = 0.83, q^2 = 0.80, F = 32.6 and p < 0.001

Hydrophobicity (descriptor – R3-MostHydrophobic) in the R3 position has a negative contribution on the activity, indicating that hydrophilic groups in that position can enhance activity. Similarly descriptor – R5-SAAverage has a negative contribution on the activity, indicating that hydrophilic groups in that position can enhance activity. The former descriptor contributes 55% and the later 45% towards activity. Fig. 6 shows the parity plot relating the experimental and predicted activities with 99% prediction bands. Table 5 lists the predicted activity values and the descriptors contributing to the activity.

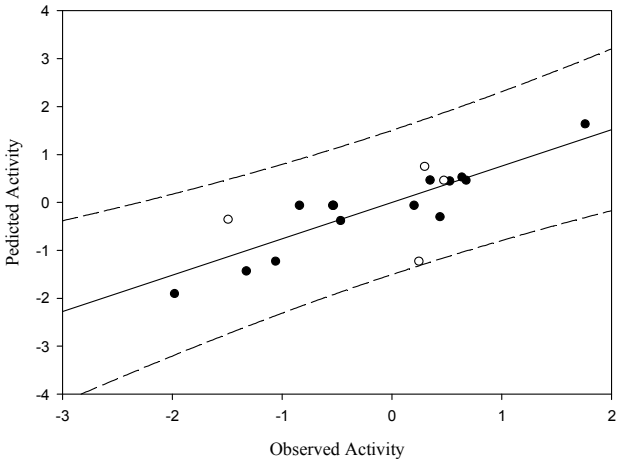


Fig. 6 Parity plot and 99% prediction interval based on GQSAR results

Lipinski's rule, in general, says that an orally active drug has no more than one violation. All chalcones have not more than 5 Hydrogen bond donors and 10 hydrogen bond acceptors [29]. Their molecular weight also less than 500 Daltons. The octanol-water partition coefficient (log P) is less than 5 except for compound **10**, which is marginally exceeding the limit study reveals that chalcones follows Lipinski's rule of five.

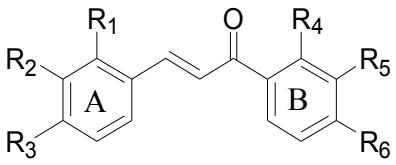
In-silico predictive models for ADME/TOX are used at the present time in the drug development [30]. From these results it is observed that Oral Bioavailability is between 30-70% (moderate) for all the molecules except for **14** and **23** which have more than 70% bioavailability. All the compounds are stable at pH<2. Most of the molecules are insoluble in buffer except compounds **6** and **14** (slightly soluble) and **12** and **23** (completely soluble). All the chalcone molecules are insoluble in pure water [31].All the 23 chalcone molecules

pass trans-cellularly (occur through the cytoplasm) by passive diffusion since the transcellular route is 100% and paracellular route is 0%. This indicates the majority of drug absorption occurs in the small intestine. The chalcones are lipophilic and they are well absorbed in intestine. There was no indication of active transport across the intestinal barrier by the carrier proteins PepT1 or ASBT [32]. Drugs, when reaching the blood stream, can bind to plasma proteins. All the compounds are neutral and they bind mostly to lipoproteins and lesser to albumin except compounds **12**, **14** and **23**. Albumin is considered the main transport protein for drugs, especially if they present an acidic behaviour. Compounds **12** and **23** are acidic which means, in plasma, these drugs predominantly bind to human serum albumin. Bases are usually bound to α1-acid glycoprotein, but can bind to albumin as well. Compound **14** (weak base) may bind predominantly to alpha1-acid glycoprotein and albumin [33] . One of the most important physicochemical properties of macromolecules is the dissociation constants for any weakly acidic or basic groups. All the chalcone molecules do not have pKa and pKb values (since there are no ionizable groups), except **12** and **23** which show acidic property. Compound **14** shows basic character [34]. All the compounds are weak H-acceptor. Probability for positive Ames test is lower for **6**, **8**, **9**,**15**,**19**,**23** and hence the molecules are non mutagenic and the other chalcones shows higher probability of positive Ames test which means they may be mutagenic[32].

4 Conclusion

Twenty three chalcones (including 4-methylthio chalcones) are synthesized and are evaluated for antimycobacterial activity. The requirement for the anti-infective activity in terms of substitutions in both A and B-ring are explored. Ortho chloro substitutions at A and B-ring favour activity (in one case achieving 98% reduction in relative light units). Methylsulfonyl chalcones exhibit very good activity. The requirements for the anti-infective activity are explored with 2D, 3D and group based QSAR studies. The 2D technique indicates the importance of volume, refractivity and molecular weight of the compounds on the activity. The 3DQSAR indicates less bulky and more hydrophilic substituent groups can improve the antitubercular activity. The GQSAR technique indicates that hydrophilic groups in R3 or R5 positions can enhance activity. ADME and Tox Predictions indicate that these chalcones do not violate the Lipinski's rule of Five. None of them have any ionizable groups but compounds **12** and **23** are mildly acidic. All of them are stable below a pH of 2.

Table 1: Structures and antimycobacterial activities of chalcones



Comp No	Substitution at A-ring			Substitution at B-ring		
	R_1	R_2	R_3	R_4	R_5	R_6
1		NO_2			-O-CH_2-O-	
2			Cl		NO_2	
3		NO_2			Br	
4	Cl				OMe	
5	Cl					OEt
6			SO_2CH_3			
7			SO_2CH_3			NO_2
8			SO_2CH_3			Cl
9			SO_2CH_3	Cl		Cl
10	Cl			Cl		Cl
11	Cl				NO_2	
12	Cl				OH	
13			SMe		-O-CH_2-O-	
14			SMe		NH_2	
15			SMe		OCH_3	OCH_3
16			SMe		OCH_3	
17			SMe			OEt
18			SMe		Br	
19			SMe			F
20	Cl				-O-CH_2-O-	
21	OMe			Cl		
22			OMe		-O-CH_2-O-	
23			OH		-O-CH_2-O-	

Table 2: Antimycobacterial activity of these chalcones measured at two concentrations

Compd No	Percentage reduction in RLU	
	50µg/ml	100µg/ml
1	0	12.69
2	0	0
3	0	0
4	0	66.73
5	50.87	61.66
6	70.27	82.72
7	51.07	69.22
8	18.53	75.01
9	34.10	77.29
10	0	22.66
11	0	0
12	73.69	98.31
13	0	4.56
14	0	0
15	0	3.15
16	0	25.64
17	0	8.11
18	0	1.05
19	37.91	64.03
20	36.97	73.46
21	5.07	22.86
22	62.03	81.42
23	0	0

Table 3: The descriptor values for 2DQSAR and predicted antimycobacterial activity

Compd No	Actual activity	smr	Volume	Predicted activity
Training set				
4	0.302	77.81	238.40	0.341
5	0.206	82.42	258.64	-0.069
6	0.680	79.17	252.30	0.841
7	0.35	85.22	281.52	0.514
8	0.477	84.18	266.42	-0.219
10	-0.533	81.28	243.52	-0.758
12	1.765	72.92	224.66	1.379
13	-1.321	84.15	260.98	-0.632
15	-1.488	91.13	288.45	-1.498
17	-1.054	89.19	279.74	-1.344
18	-1.974	85.66	254.99	-1.754
19	0.250	77.98	242.64	0.598
21	-0.528	82.82	255.58	-0.479
22	0.641	78.92	251.11	0.857
Test set				
1	-0.838	78.42	254.72	1.358
9	0.532	89.19	279.98	-1.324
16	-0.462	84.58	262.62	-0.689
20	0.442	77.38	239.86	0.641

Table 4: The descriptor values for 3DQSAR and predicted antimycobacterial activity

Comp	Actual activity	S_408	H_661	Predicted activity
Training set				
1	-0.84	30.00	0.51	-0.80
4	0.30	-0.58	0.61	0.09
5	0.21	-0.10	0.66	-0.17
6	0.68	-0.48	0.49	0.64
7	0.35	-0.67	0.49	0.64
10	-0.53	-0.55	0.76	-0.59
15	-1.49	30.00	0.69	-1.62
16	-0.46	0.10	0.68	-0.28
17	-1.05	-0.49	0.73	-0.44
18	-1.97	30.00	0.75	-1.89
19	0.25	-0.18	0.69	-0.29
20	0.44	0.28	0.59	0.13
21	-0.53	0.90	0.62	-0.01
22	0.64	-0.19	0.50	0.58
Test set				
8	0.48	-0.50	0.55	0.36
9	0.53	16.38	0.63	-0.77
12	1.77	0.20	0.59	0.14
13	-1.32	-0.02	0.64	-0.08

Table 5: The descriptor values for GQSAR and predicted antimycobacterial activity

Comp	Actual activity	R3-XKMost Hydrophobic	R5-SA Average	Predicted activity
Training set				
1	-0.837	0.042	0.061	-0.072
5	0.206	0.0418	0.061	-0.071
6	0.680	-0.063	0.061	0.451
7	0.352	-0.064	0.061	0.456
9	0.531	-0.060	0.061	0.434
10	-0.533	0.042	0.061	-0.071
12	1.765	0.042	-0.157	1.626
13	-1.321	0.275	0.087	-1.440
16	-0.462	0.275	-0.048	-0.389
17	-1.054	0.276	0.061	-1.237
18	-1.974	0.275	0.148	-1.914
20	0.442	0.042	0.091	-0.310
21	-0.528	0.042	0.061	-0.071
22	0.641	-0.093	0.071	0.517
Test set				
4	0.302	0.042	-0.043	0.739
8	0.477	-0.063	0.061	0.451
15	-1.487	0.276	-0.051	-0.363
19	0.250	0.276	0.061	-1.23

Table 6: Lipinski's Rule of Five and the number of violations (using Molinspiration online tool)

Cmpd No	MW	No. of bond acceptors	No. of bond donors	Log P	No. of violations
1	297.26	6	0	3.74	0
2	287.70	4	0	4.3	0
3	332.15	4	0	4.29	0
4	272.73	2	0	4.35	0
5	286.75	2	0	4.77	0
6	286.35	3	0	2.35	0
7	331.34	6	0	2.15	0
8	320.79	3	0	2.77	0
9	355.24	3	0	3.40	0
10	311.59	1	0	5.33	1
11	287.70	4	0	4.30	0
12	258.70	2	1	3.95	0
13	298.36	3	0	4.30	0
14	269.36	2	2	3.44	0
15	314.40	3	0	3.92	0
16	284.38	2	0	4.13	0
17	298.40	2	0	4.55	0
18	333.25	1	0	4.85	0
19	272.34	1	0	4.17	0
20	286.71	3	0	4.52	0
21	272.73	2	0	4.30	0
22	282.29	4	0	3.91	0
23	268.26	4	1	3.33	0

Table 7: Absorption, Digestion, Metabolism, Excretion and Toxicity properties of the chalcone molecules

No	Solubility at pH<2	Permeability Caco-2 scale (pH=7.4) $x10^{-6}$ cm/s	LD50 Oral mg/kg	PositiveAmes Test
1	I	300.11	1700	0.966
2	I	311.62	1400	0.959
3	I	311.48	1400	0.937
4	I	312.25	1300	0.594
5	I	316.01	1400	0.437
6	Ss	197.61	1300	0.118
7	I	172.64	1600	0.831
8	I	243.25	1400	0.120
9	I	287.11	1200	0.178
10	I	318.37	960	0.620
11	I	311.62	1400	0.979
12	S	295.58	1500	0.415
13	I	311.62	1500	0.346
14	Ss	243.29	1100	0.780
15	I	304.88	1500	0.194
16	I	309.07	1300	0.400
17	I	314.35	1400	0.268
18	I	316.49	1300	0.287
19	I	309.73	930	0.186
20	I	314.07	1600	0.505
21	I	311.62	930	0.663
22	I	304.64	1700	0.461
23	S	261.99	1400	0.154

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