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Highly diastereoselective inverse electron demand (IED) Diels–Alder reaction mediated by chiral salen–AlCl complex: the first, target-oriented synthesis of pyranoquinolines as potential antibacterial agents

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Abstract—The first, target-oriented synthesis of pyranoquinolines as potential antibacterial agents by inverse electron demand (IED) Diels–Alder reaction using chiral salen–AlCl complex has been accomplished, the reactions proceed with moderate yields, and a very high degree of diastereoselectivity (>90%). The diastereoselectivity-enhanced pyranoquinolines exhibit potential bactericidal activity against seven strains of pathogenic gram-negative bacteria.

1. Introduction

The pyranoquinoline moiety is present in many alkaloids such as flindersine, oricine and verprisine[1] and derivatives of these alkaloids possess a wide range of biological activities such as psychotropic,[2] antiallergenic[3] and antiinflammatory[4] activities. The furoquinoline skeleton is present in many alkaloids like skimmianine and balfouridine.[5,6] For synthesis of quinoline products various methods are available, but reports on the synthesis of diastereoselective quinoline derivatives are limited.[7] Over the years, conventional antibiotics have been used so extensively that many of the bacteria that infect us have grown resistant to them. Moreover, no new classes of antibiotics have been developed in over 30 years and bacteria are learning how to outsmart existing drugs and there appear to be few, if any, new classes of drugs currently in clinical development. The need for research directed towards development of new lead compounds with potential antibacterial activity has never been greater.

In our quest to synthesize new lead compounds with potential antibacterial activity the products of the IED-DA reaction between 3,4-dihydro-2*H*-pyran and 2-azadiene were screened for their antibacterial activity (Scheme 1). The *exo*-diastereomer **7** exhibited potential bactericidal and bacteriolytic activity against seven strains of pathogenic gram-negative bacteria with proven clinical syndromes associated with human beings while the *endo*-diastereomer **6**, was not only inactive but also brought about the competitive inhibition of the active diastereomer **7** by binding either to the receptor sites on the bacteria or to some component of the effector mechanism, thereby preventing the active diastereomer **7** from having an effect. Hence to, synthesis the biologically active target, avoid interference from the inactive isomer, to overcome the diastereomer **6** mediated competitive inhibition of the active isomer **7** and to circumvent the time consuming and laborious separation of diastereomers, we explore the possibility of using chiral salen–AlCl complexes for a target-oriented synthesis of the active diastereomer by enhancement of diastereoselectivity for IED Diels–Alder reaction.

2. Chemistry

Salen H_2 ligands occupy five coordination sites on the central aluminium atom but presumably leave the sixth

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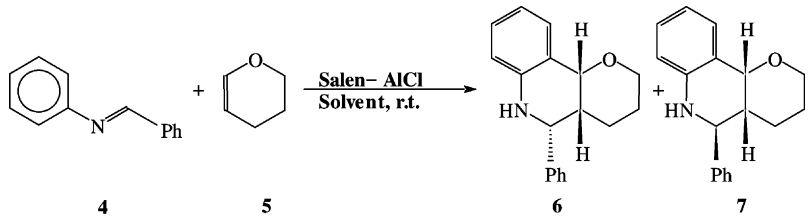
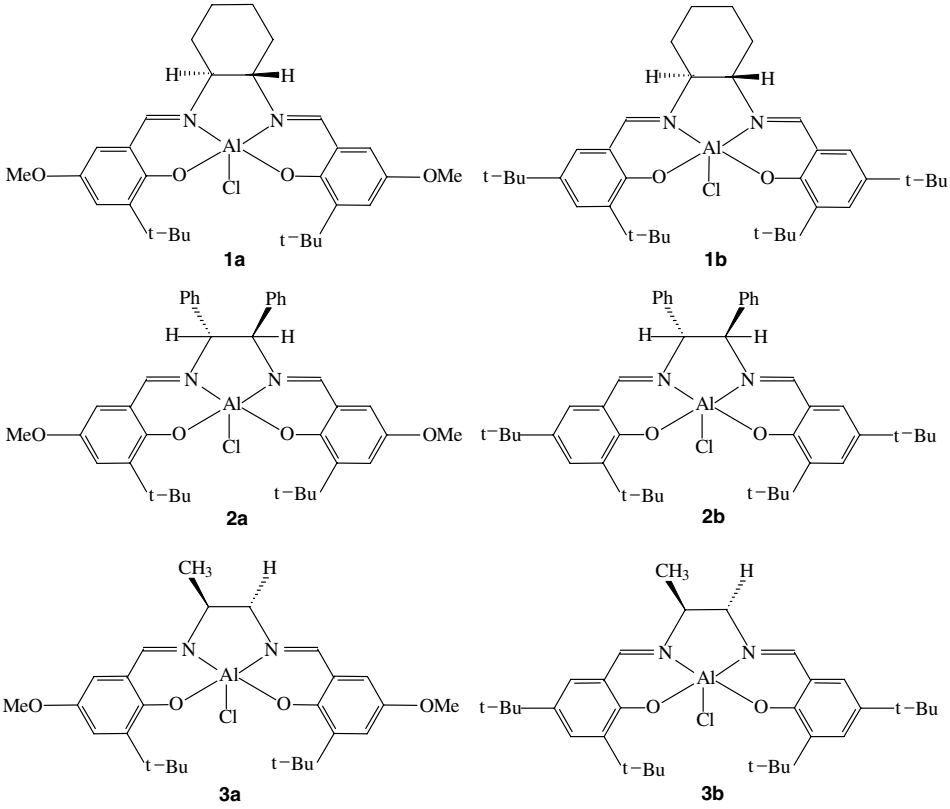


Scheme 1.

site open for substrate coordination. Thus these salen–AlCl complexes behave as coordinatively unsaturated species in the presence of a more basic substrate, such as the 2-azadiene. Here the reversible complexation of chiral salen–AlCl to the 2-azadiene would make the 2-azadiene more reactive in a concerted Diels–Alder process, because this will decrease the energy gap between the LUMO (2-azadiene) and HOMO (dienophile). The chiral salen–AlCl complexes were prepared by combining the appropriate salen H_2 ligands with R_2AlCl.[8] The electron-poor diene, benzylidene aniline was prepared and recrystallized as per literature.[9]

On examining the role of the solvent towards improving the diastereoselectivity of this reaction (Scheme 1), we found that with pure toluene there was no reaction. In dichloromethane medium alone, the products were obtained in poor yield. On switching to a more polar solvent like methanol, the amount of conversion improved considerably but the diastereomeric excess were still considerably low, possibly as a result of a high degree of solvation leading to the delinking of the ligand from the metal. However of all the solvent's examined, acetonitrile proved to be the most effective with a very high degree of diastereoselectivity (Table 1).



1a **1b** **2a** **2b** **3a** **3b**

Table 1. Effect of solvent on the IED Diels–Alder reaction

S. no.	Solvent	Salen–AlCl	Time (h)	Conversion (%)	*endo:exo* Ratio (**6**:**7**)	% De
1	Toluene	**1a**	—	—	—	—
2	Toluene:DCM (1:1)	**1a**	6	17	06:94	88
3	DCM	**1a**	6	23	05:95	90
4	Methanol	**1a**	8	90	26:74	48
5	Acetonitrile	**1a**	6	63	03:97	94

Table 2. Effect of substituents on the salen–AlCl complex

Entry	Salen–AlCl	Time (h)	Conversion (%)	*endo:exo* Ratio (**6**:**7**)	% De
1	**1b**	7	57	02:98	96
2	**2a**	12	45	01:99	98
3	**2b**	14	43	00:100	100
4	**3a**	6	55	05:95	90
5	**3b**	6	47	04:96	92

The stereochemistry of the products was assigned based on the scalar-coupling constant between H-4a and H-5. In the isomer **6**, the coupling constant $J(4a\text{-}5) = 2.2\,Hz$ is significantly smaller and typical for a *gauche* conformation. This orientation is present in both conformers of the configuration having *cis* orientation of the pyran ring and phenyl group. In the isomer **7**, the $J(4a\text{-}5) = 11.4\,Hz$ is indicative of the *anti* reciprocal orientation of protons H-5 and H-4a. This orientation is only possible when the pyran ring and phenyl ring are on opposite sides of the quinoline ring of **7**. On the basis of reactivity of the salen–AlCl complex **1a** in CH_3CN, several further derivatives of salen–AlCl complexes were examined to probe the effect of substituents on the salen backbone, in enhancing the diastereoselectivity of the reaction. Even more effective catalysis was observed with **2b** resulting in 43% conversion after 14 h at room temperature with 100% de of the biologically active diastereomer **7**. The effect of substituents on the salen–AlCl complex are summarized in Table 2.

It is well known that the addition of molecular sieves to the reaction mixture sometimes increases the enantiomeric excesses and yield of the products obtained.[10] Therefore to observe the effect of molecular sieves on the diastereoselectivity of the IED-DA reaction catalyzed by salen–AlCl complex, we added various mol% of the molecular sieves to the reaction mixture of 2,3-dihydrofuran and 2-azadiene, and observed a marginal increase in the diastereomeric excess. The results are summarized in Table 3 (Scheme 2).

Table 3. Effect of molecular sieves on the IED Diels–Alder reaction

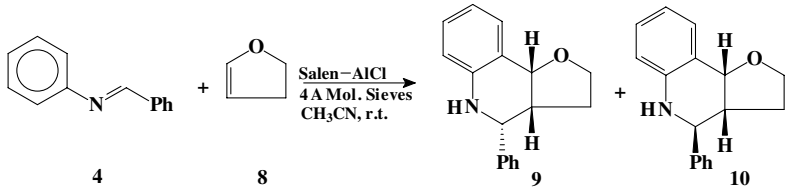
Entry	Salen–AlCl	4 Å mol sieves (mol%)	Time (h)	Conversion (%)	*endo:exo* Ratio (**9**:**10**)	% De
1	**1a**	0	7	43	05:95	90
2	**1a**	10	7	50	02:98	96
3	**1a**	20	7	47	03:97	94

Generally imines derived from phenylglyoxal are highly hygroscopic, unstable at high temperatures, difficult to purify by distillation or column chromatography and lack efficiency.[11] Henceforth we attempted a three component coupling reaction by adding phenylglyoxal monohydrate, aniline and 2,3-dihydro-2*H*-furan in the presence of salen–AlCl complex **2b** (Scheme 3). The reaction proceeded with 40% conversion after 7 h in the *endo:exo* ratio of 10:90.

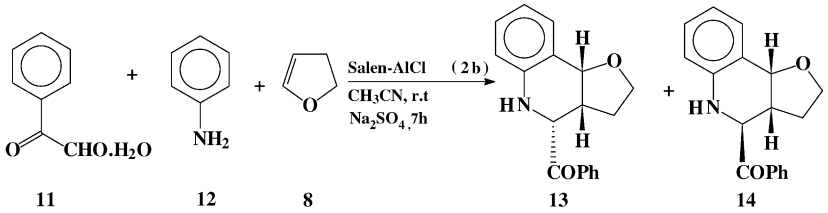
Although the mechanistic details of the present reaction are still under investigation, a hypothetical mechanism for the salen–AlCl catalyzed IED-DA reaction is proposed in Scheme 4. The chloride atom on salen–AlCl complex can easily be displaced by a more basic substrate, such as the 2-azadiene leading to the formation of an intermediate hexacoordinate octahedral Schiff base aluminium complex. Thus the catalytically active species in Schiff base–AlCl complex may be an aluminium cation.[12] Thus, a four centred transition state, with two open coordination sites occupied by 2-azadiene and cationic aluminium centre is proposed for the IED-DA reaction.

3. Antibacterial activity

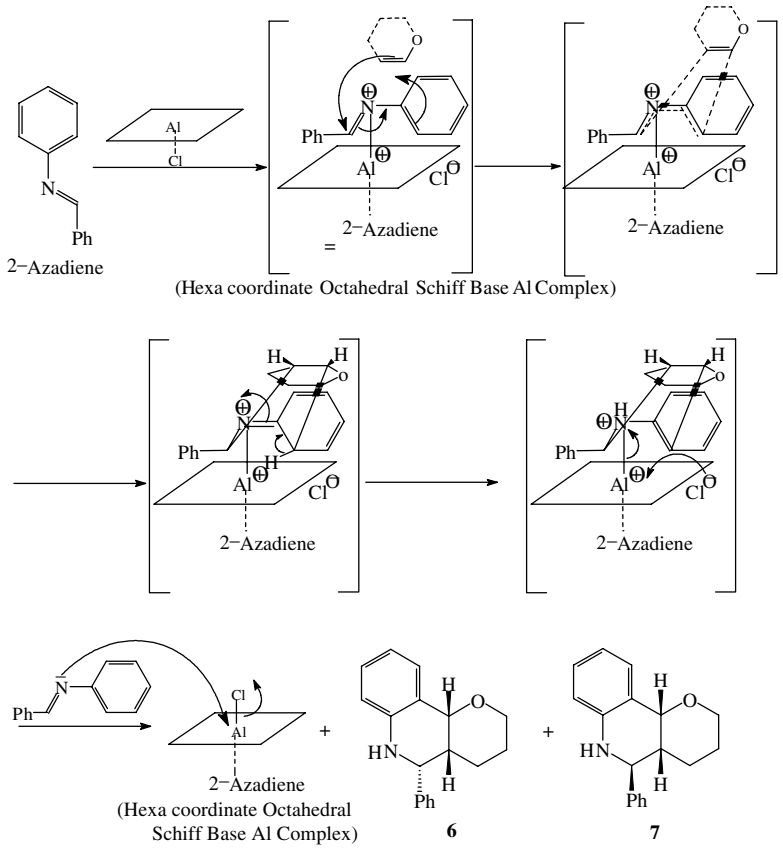
The cell wall of gram-negative bacteria is a phospholipid bilayer made up of negatively charged phosphate groups. Negative charges on the phospholipids are essential for lysosomal phospholipases to act on



Scheme 2.



Scheme 3.



Scheme 4.

phospholipid bilayers. We anticipated that the –NH-group and the –O-group on the pyranoquinolie moiety may bind with the negatively charged phosphate group on phospholipids. This in turn will cause the inhibition of the activities of lysosomal phospholipases because of the neutralization of the negative charges of phospholipid bilayer, leading to potential antibacterial activity. Moreover the outer cell wall and the plasma membrane of the gram-negative bacteria are permeable to small molecules. Henceforth the diffusion of the pyranoquinoline molecule across cell membrane and thereby binding with intracellular targets may bring about potential antibacterial activity. However when a 50:50 mixture of pyranoqinoline diastereomers **6** and **7** were screened for their bactericidal activity, no activity was found. Out of the two-pyranoqinoline diastereomers **6** and **7**, only the *exo*-diastereomer **7** has the desired bactericidal and bacteriolytic activity while the *endo*-diastereomer **6** is inactive even at very high concentrations. The contamination of the active diastereomer **7** even with low concentration of **6** significantly lowers its activity. The biologically inactive isomer **6**, binds either to the

Table 4. In vitro screening of pyranoquinoline diastereomers **6** and **7** for bactericidal activity

S. no.	Pathogens		Bactericidal activity				
	Bacteria	Clinical syndrome associated with human beings	Concentration (mg/mL)	**6** (100%)	**6** + **7** (50:50%)	**6** + **7** (25:75%)	**7** (100%)
1	*Vibrio parahaemolyticus*	Gastroenteritis, rare wound infections	2	—	—	—	+
2	*Vibrio alginolyticus*	Wound infections	2	—	—	—	+
3	*Vibrio anguillarum*	—	2	—	—	—	+
4	*Vibrio vulnificus*	Primary septicemia	2	—	—	—	+
5	*Vibrio fluvialis*	Gastroenteritis	2	—	—	—	+
6	*Vibrio mimicus*	Gastroenteritis	2	—	—	—	+
7	*Pseudomonas* species	Urinary tract infections, chronic lung infections, endocarditis and dermatitis	2	—	—	—	+
8	*E. coli*	Urinary tract infections, neonatal meningitis	2	—	—	—	+

(+): Strong bactericidal activity was observed.
(—): Bactericidal activity was not observed.

Table 5. In vitro screening of the pyranoquinoline diastereomer **7** for bacteriolytic activity (de = 100%)

S. no.	Bacteria	Bacterial suspension in optical density (OD)	Bacteriolytic activity							
			Test 1				Test 2			
			Initial OD	Final OD	Activity OD	BA	Initial OD	Final OD	Activity OD	BA
1	*Vibrio parahaemolyticus*	0.8	0.833	0.718	0.115	+++	0.833	0.712	0.121	+++
2	*Vibrio alginolyticus*	0.8	0.833	0.761	0.072	++	0.833	0.756	0.077	++
3	*Vibrio anguillarum*	0.8	0.833	0.753	0.080	++	0.833	0.767	0.066	++
4	*Vibrio vulnificus*	0.8	0.833	0.720	0.113	+++	0.833	0.731	0.102	+++
5	*Vibrio fluvialis*	0.8	0.833	0.814	0.019	—	0.833	0.812	0.021	—
6	*Vibrio mimicus*	0.8	0.833	0.757	0.076	++	0.833	0.748	0.085	++
7	*Pseudomonas* species	0.8	0.833	0.769	0.064	++	0.833	0.764	0.069	++
8	*E. coli*	0.8	0.833	0.788	0.045	+	0.833	0.791	0.042	+

(+): Mild bacteriolytic activity, (++): moderate bacteriolytic activity, (+++): strong bacteriolytic activity.
(—): bacteriolytic activity was not observed, BA = bacteriolytic activity.

receptor sites on the bacteria or to some component of the effector mechanism, in order to prevent the active isomer **7** from having an effect. Henceforth the target-oriented synthesis[13] of pyranoquinoline **7** with a diastereomeric excess of 100% offers the advantages of enhanced activity, smaller doses, increased specificity and completely rules out the competitive inhibition of the active diastereomer **7**, ultimately reaching the maximal bactericidal[14] and bacteriolytic effect.[15] The active pyranoquinoline diastereomer **7** exhibited potential bactericidal activity against seven strains of pathogenic, gram-negative bacteria as is evident from Table 4.

Encouraged by the above results, the active diastereomer was further screened for its in vitro bacteriolytic activity to ascertain its potentially active nature. The results of the bacteriolytic study are summarized in Table 5. The active diastereomer **7** exhibited potential bacteriolytic activity against *Vibrio parahaemolyticus* and *Vibrio vulnificus*.

4. Conclusion

In conclusion the paper describes: (1) the discovery of pyranoquinolines[16] as potential antibacterial agents; (2) The bactericidal and bacteriolytic studies revealed that one diastereomer was potentially active while the other was not only inactive but also brought about the competitive inhibition of the active diastereomer **7** by binding either to the receptor sites on the bacteria or to some component of the effector mechanism, thereby preventing the active diastereomer **7** from having an effect; (3) The first, target-oriented synthesis of pyranoquinolines as potential antibacterial agents by inverse electron demand (IED) Diels–Alder reaction using chiral salen–AlCl complex has been accomplished; (4) The effect of solvent, the effect of substituents on the salen–AlCl backbone and the effect of molecular sieves were probed to synthesis the target pyarnoquinoline as highly pure diastereomer; (5) The target-oriented synthesis of the active diastereomer offers the advantages of enhanced activity, smaller doses, increased specificity, circumvents the problem of effective separation of diastereomers, and completely rules out the risk of contamination from the inactive diastereomer, ultimately reaching the maximal bactericidal and bacteriolytic effect. However studies are still in progress, to test the toxicity as well as the mechanism of antibacterial action. The discovery, modification and annotation of quinoline diastereomers in terms of their ability to perturb biological targets will have an important role in the post genomic era.

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13. General procedure for the preparation of pyranoquinolines: To a mixture of imine (1.1 mmol) or a mixture of phenylglyoxal monohydrate (1.1 mmol) and aromatic amine (1.1 mmol), dienophiles [cyclopentadiene (2.2 mmol) or 3,4-dihydropyran (2.2 mmol) or dihydrofuran (2.2 mmol)] in dry acetonitrile (5 mL) salen–AlCl complex (0.22 mmol) was added and stirred at room temperature for appropriate times. To the reaction mixture water was added (25 mL) and extracted with $CHCl_3$ (4×10 mL), washed with brine and dried over anhydrous Na_2SO_4, filtered and the solvent evaporated. The residue was purified by column chromatography with petroleum ether/ethyl acetate to afford the cycloadducts.
14. Procedure for bactericidal activity: The test compound (20 mg) was dissolved in 500 μL of DMSO. Five microlitres (0.2 mg approx) of the stock solution was taken and 95 μL bacterial suspension in Tris buffer saline (0.8 OD at 580 nm) was added to it. The mixture was incubated at 14 °C for 14 h. After incubation, it was subjected to plating in TCBS agar (Thiosulphate, Citric, Bile salt, sucrose agar). After 12 h, the culture plate was observed for bacterial growth.
15. Procedure for bacteriolytic activity: Bacterial suspension in TBS was prepared with an optical density of 0.8 OD at 580 nm (double beam UV spectrometer). TBS (Tris buffer saline) served as the blank. The test compound (10 mg) was dissolved in 150 L of DMSO and 2850 μL of bacterial suspension in TBS was added to it. The initial OD of the sample was recorded. The mixture was incubated for 90 min at 23 °C. Final OD of the mixture was recorded. The initial OD−final OD, gives the bacteriolytic activity.
16. All the compounds were characterized by FT-IR, NMR (500 MHz), MS and CHN analysis. The spectroscopic data were fully consistent with the assigned structures. Selected spectroscopic data: *Pyran adduct* (**6**) FT-IR (KBr): 3380, 3325, 2940, 1603, 1484, 1074 cm^{-1}; ^1H NMR δ (500 MHz, $CDCl_3$): 7.47–7.39 (m, 5H), 7.34 (t, 1H, $J = 6.8$ Hz), 7.1 (t, 1H, $J = 8.0$ Hz), 6.82 (t, 1H, $J = 7.4$ Hz), 6.6 (d, 1H, $J = 6.8$ Hz), 5.35 (d, 1H, $J = 5.75$ Hz), 4.7 (d, 1H, $J = 2.2$ Hz), 3.9 (br s, 1H, NH), 3.62 (d, 1H, $J = 11.4$ Hz), 3.4 (m, 1H), 2.21–2.16 (m, 1H), 1.62–1.30 (m, 4H); ^{13} C NMR δ (75 MHz, $CDCl_3$): 145.3, 141.26, 128.5, 128.2, 127.7, 127.6, 126.9, 120.0, 118.4, 114.5, 72.8, 60.7, 59.4, 39.0, 25.5, 18.1; MS m/z 265 (M^+); Anal. Calcd for $C_{18}H_{19}NO$: C, 81.48; H, 7.22; N, 5.28. Found: C, 81.36; H, 7.21; N, 5.26. *Pyran adduct* (**7**) FT-IR (KBr): 3368, 3026, 2936, 1610, 1489, 1073 cm^{-1}; ^1H NMR δ (500 MHz, $CDCl_3$): 7.45–7.33 (m, 5H), 7.26 (d, 1H, $J = 6.8$ Hz), 7.11 (t, 1H, $J = 8.0$ Hz), 6.73 (t, 1H, $J = 6.9$ Hz), 6.54 (d, 1H, $J = 8.0$ Hz), 4.74 (d, 1H, $J = 10.85$ Hz), 4.42 (d, 1H, $J = 2.8$ Hz), 4.10 (m, 2H), 3.75 (t, 1H, $J = 11.45$ Hz), 2.11 (m, 1H), 1.88 (m, 1H), 1.71–1.30 (m, 3H); ^{13}C NMR δ (75 MHz, $CDCl_3$): 144.8, 142.4, 131.0, 129.4, 128.7, 128.0, 127.9, 120.7, 117.5, 114.2, 74.6, 68.7, 54.9, 39.0, 24.2, 22.1; MS m/z 265 (M^+); Anal. Calcd for $C_{18}H_{19}NO$: C, 81.48; H, 7.22; N, 5.28. Found: C, 81.45; H, 7.23; N, 5.25.