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ARTICLE TYPE

L-Proline Mediated Synthesis of Quinoxalines; Evaluation of Cytotoxic and Antimicrobial Activity

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A simple, greener and highly efficient method for the synthesis of functionalized quinoxalines has been developed employing L-proline as a catalyst in water. To the best of our knowledge this transformation is first time achieved using an organic catalyst. A small library of quinoxaline-sulphonamide conjugates have been synthesised using this protocol. The newly synthesized conjugates have been tested for their cytotoxicity and antimicrobial activity against several bacterial strains including one fungal strain. Majority of the compounds have exhibited significant cytotoxicity as well as antimicrobial activity. Compounds **5a**, **5b** and **5d** were found to be promising with respect to both cytotoxicity and antimicrobial activity.

Introduction

Quinoxalines represent as important class of biologically active compounds that are known to possess antibacterial,[1] anticancer,[2] and antiviral activities.[3] This scaffold is present in several anticancer agents such as chloroquinoxaline sulfonamide (**1**), XK469 (**2**) and NCG555879-01 (**3**)[4] and in some natural products like lzumiphenazine C (**4**).[5] It is also a part of various antibiotics such as levomycin, echinomycin and actinoleutin, which are known to inhibit the growth of gram positive bacteria.[6] In addition, quinoxaline derivatives find their application in cavitands,[7] efficient electroluminescent materials,[8] organic semiconductors,[9] dyes,[10] etc.

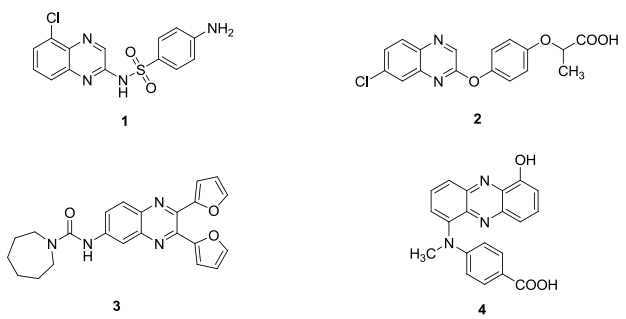


Fig. 1 Biologically important quinoxalines.

Owing to extensive applications of quinoxalines several synthetic methods for their preparation both in solution[11] as well as in solid-phase[12] have been developed. Among them the condensation of 1,2 diamines with 1,2-diketone[13] and oxidative cyclization of α-hydroxy ketones with 1,2-diamines[14] under various conditions are widely used. Recent reports indicate the use of several catalysts such as MnO_2,[15a] Ru/C in the presence of β–CD,[15b] manganese oxide octahedral molecular sieves (OMS-2),[15c] HgI_2,[15d] $RuCl_2(PPh_3)_3$-TEMPO,[15e] KF/Al $_2O_3$,[15f] and Au-NPs[15g] for onepot synthesis of quinoxaline from α-hydroxy ketones. However, they often suffer from one or more disadvantages such as long reaction time, use of hazardous organic solvents, unsatisfactory product yields and harsh reaction conditions.

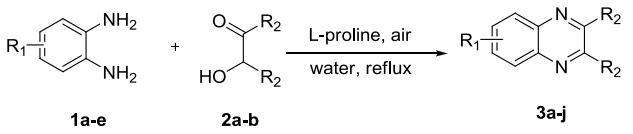
On the other hand, organic catalysis is an emerging area of organic synthesis wherein small molecules are used to catalyze organic transformations. L-proline is a versatile catalyst reported to catalyze many important organic reactions and asymmetric transformations such as aldol,[16] Mannich,[17] Michael,[18] and Diels–Alder reaction.[19] It is also excellent promoter for the copper-catalysed coupling reactions.[20] It's ease of handling, experimental simplicity, cost effectiveness and excellent solubility in water and organic solvents are some reasons for its extensive use in the development of synthetic methods. Further, use of water as a solvent is one of the greener way of organic synthesis. Therefore in order to overcome the disadvantages of previous methods and considering the advantages of L-proline as a catalyst and ecofriendly nature of water as a solvent we have developed a new method to synthesize quinoxalines. We herein report a green, simple and practical method for the synthesis of quinoxaline derivatives from hydroxy ketones and 1,2-diamines catalyzed by L-proline.

Results and discussion

Chemistry

In the beginning, a systematic study was carried out for the catalytic evaluation of L-proline towards the synthesis of quinoxalines. Initially a blank reaction was performed using benzoin and 1,2-diaminobenzene in water without any catalyst at room temperature and the completion of the reaction was

monitored by TLC. It was observed that the reaction did not proceed even untill 24 hours. Whereas the same reaction was executed in the presence of catalytic amounts of L-proline in water at room temperature and traces of the product were found (less than 5%).



Scheme 1 L-proline catalyzed synthesis of quinoxalines.

In the beginning, a systematic study was carried out for the catalytic evaluation of L-proline towards the synthesis of quinoxalines. Initially a blank reaction was performed using benzoin and 1,2-diaminobenzene in water without any catalyst at room temperature and the completion of the reaction was monitored by TLC. It was observed that the reaction did not proceed even untill 24 hours. Whereas the same reaction was executed in the presence of catalytic amounts of L-proline in water at room temperature and traces of the product were found (less than 5%). Later, this reaction was carried out under refluxing conditions and the desired transformation was observed furnishing the product in very good yield. After obtaining the desired product, the amount of catalyst and the time required for the completion of reaction were evaluated. The reaction was performed using 5, 10, 20 and 30 mole % of the catalyst and was monitored for 12-16 hours. It was observed that 20 mol% of the catalyst loading provided maximum yield (87%) in 12 hours. While 5 and 10 mole% of the catalyst afforded 64% and 72% of the product even after refluxing the reaction for 16 hours. An additional increase of the catalyst loading to 30% did not improve the yield. On the contrary, the reaction slows down on adding more than 20 mol% of the catalyst.

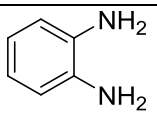
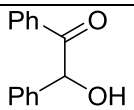
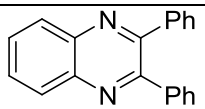
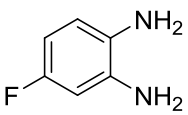
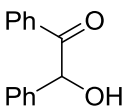
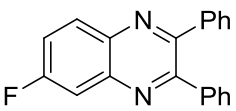
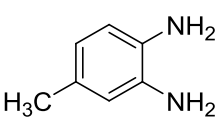
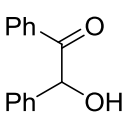
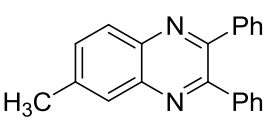
Table 1 Condensation of benzoin and 1, 2-diaminobenzene in water at different catalyst (L-proline) concentrations.

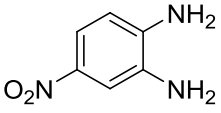
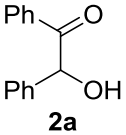
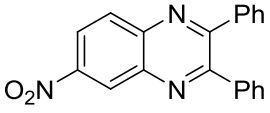
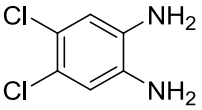
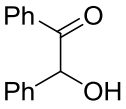
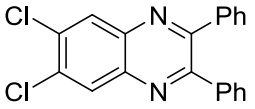
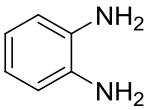
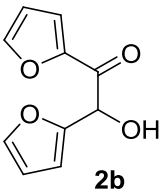
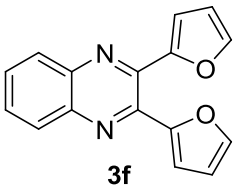
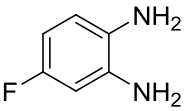
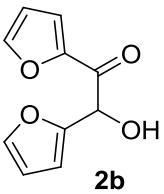
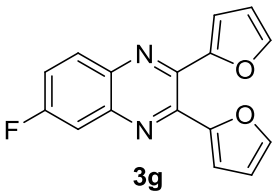
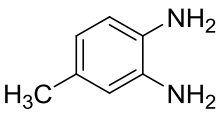
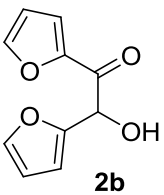
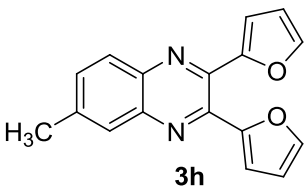
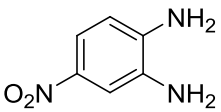
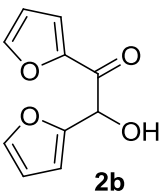
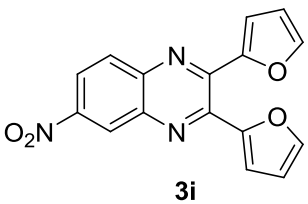
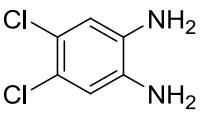
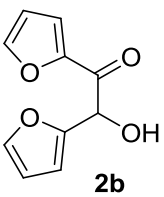
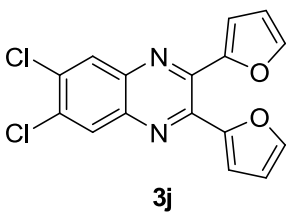
Entry	Catalyst (mole%)	Time (h)	Yield(%)[a]
1	-	24	Nil
2	5	12	62
3	5	16	64
4	10	12	71
5	10	16	72
6	20	12	87
7	20	16	87
8	30	12	82
9	30	16	86

[a]Isolated yield

With the optimized conditions in hand, the reaction was performed with differant set of substituents to explore the scope and generality of the present protocol. The quinoxaline derivatives were synthesized using two hydroxyketones namely benzoin (**2a**) and furoin (**2b**) with varying 1,2-diamines. The diamines used possessed both ring activating as well as deactivating substituents and the results of these observations are summarized in Table 2. From the results it can be concluded that the electronic factors of 1,2-diamine influences the progress of the reaction. Electron donating substituents such as methyl (entry 3 and 8) provided excellent yields of the corresponding products. In presence of weak ring deactivating groups such as *fluoro* and *dichloro* (entry 2, 5, 7 and 10) the reaction progressed smoothly and the product was obtained in good yields. This trend was also observed in the absence of substituents on the diamine moiety. However, in case of ring deactivating groups such as nitro (entry 4 and 9) the reaction was slower and the yields were also comparable very lower.

Table 2 L-Proline mediated synthesis of quinoxalines from hydroxy ketone with 1,2-diamines

Entry	1,2-Diamine (1)	Hydoxyketone (2)	Product (3a-h)	Time (h)	Yield[a] (%)
1	 **1a**	 **2a**	 **3a**	12	87
2	 **1b**	 **2a**	 **3b**	12	83
3	 **1c**	 **2a**	 **3c**	12	88

4	 1d	 2a	 3d	14	65
5	 1e	 2a	 3e	13	85
6	 1a	 2b	 3f	12	85
7	 1b	 2b	 3g	12	82
8	 1c	 2b	 3h	12	86
9	 1d	 2b	 3i	15	67
10	 1e	 2b	 3j	13	82

[a] = Isolated yield

Plausible mechanism

The plausible mechanism for the formation of quinoxaline ring from 1,2-diaminobenzene and α-hydroxy ketone is shown in Figure 2. The reaction proceeds with the formation of an iminium ion resulting from the condensation of L-proline and α-hydroxy ketone. The iminium ion gets converted to an alkene by reorganisation of proton from α-position. The alkene formed is electron rich and readily adds on oxygen from atmospheric air to form a diradical. Further rearrangement of α-hydroxyl group generates α-keto peroxide. The peroxy oxygen is then ionized with the help of lone pair of proline and leaves to form diketo

imine which is hydrolysed to form 1,2-diketobenzene. The condensation of 1,2-diketobenzene and 1,2-diaminobenzene results in the formation of functionalized quinoxalines.

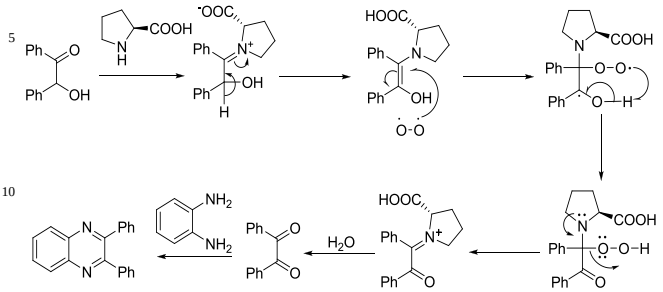


Fig. 2 Plausble mechanism of quinoxaline formation

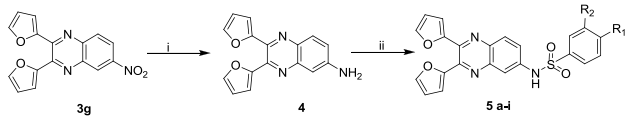
Controlled experiments

In order to determine whether the reaction is proceeding through the mechanism anticipated, a series of experiments have been performed. Firstly, the reaction was performed using other secondary amines such as piperidine, pyrrolidine and morpholine as a catalyst. The reaction was found to proceed under the similar reaction conditions. However, the reaction times were longer and the yields are lower compared to the proline catalyzed reaction. Similarly, another reaction involving heating of benzoin with proline in water without the addition of 1,2-diamine has been carried out in presence of air. Formation of benzil, the oxidized product of benzoin was observed under these conditions. Since the mechanism depicts the formation of H_2O_2, it was of interest to test its effect on the reaction. In this context, a reaction was carried out using benzoin, 1,2-diamine, proline and H_2O_2 under inert conditions (in presence of nitrogen). Surprisingly, the reaction did not proceed how ever on removal of nitrogen gas the reaction proceeded to completion. This indicates the necessity of air which serves as a source of oxygen.

Table 3: Controlled experiments to determine mechanism of L-proline catalyzed synthesis of quinoxalines

S.No.	Catalyst (mole%)	In presence of	Time (h)	Yield(%)[a]
1	Piperidine (20 mole%)	Air	16	82
2	Pyrrolidine (20 mole%)	Air	16	80
3	Morpholine (20 mole%)	Air	16	72
4	L-proline (20 mole%)	Air	12	87
5	L-proline (20 mole%) + H_2O_2	Nitrogen	12	No reaction
6	L-proline (20 mole%) + H_2O_2	Air	12	87

Since quinoxaline structure finds many applications in pharmaceutical research, it was of interest to develop a library of quinoxaline conjugates. In continuation to our research to develop newer anticancer [21] and antimicrobial agents,[22] the protocol developed was applied to synthesize a set of quinoxaline derivatives (**5a-i**). The synthetic route to access these derivatives is outlined in Scheme 2. As shown, the quinoxaline derivative **3i** obtained from the reaction of furoin with nitro-1,2-diamine was hydrogenated in presence of Pd/C to yield amino derivative(**4**).[23] This amino derivative was then reacted with substituted sulphonyl chlorides in pyridine to yield final conjugates **5a-i**. These derivatives were later evaluated for their cytotoxic as well as antimicrobial activities.



Scheme 2 *Reagents and condition*: i) H_2, Pd/C, MeOH, rt, 6h ii) Aryl sulfonyl chloride, pyridine, rt, 2h

Biology

Antiproliferative activity

Table 4 Anticancer activity of the synthesized compounds against various cancer cell lines expressed in μM

Compound	R_1	R_2	IC_{50} (μM)[a] HeLa[b]	DU145[c]	A549[d]
5a	OMe	H	**6.7 ± 1.0**	12.3 ± 1.1	**8.4 ± 0.6**
5b	OMe	OMe	**5.0 ± 1.1**	11.1 ± 0.1	**7.4 ± 1.0**
5c	NO_2	H	26.8 ± 1.4	38.9 ± 1.6	25.7 ± 1.2
5d	F	H	14.1 ± 1.7	26.5 ± 1.6	33.6 ± 1.5
5e	CF_3	H	13.2 ± 0.4	16.7 ± 0.3	16.0 ± 0.6
5f	Cl	Cl	15.3 ± 0.3	32.6 ± 0.4	20.7 ± 0.9
5g	Br	H	27.9 ± 0.7	23.6 ± 0.6	15.8 ± 0.9
5h	H	Cl	19.2 ± 1.2	21.4 ± 0.8	16.0 ± 0.4
5i	H	NO_2	18.1 ± 1.0	15.8 ± 1.1	32.1 ± 1.6
Noc	-	-	0.9±0.9	1.2±0.7	1.3±0.7

[a]IC_{50}: The amount of the compounds concentration required to reduce the cells to half of its original number; [b]HeLa: human cervical cancer cell line; [c] DU145: human prostate cancer cell line; [d]A549: Non small cell lung cancer cell line; Noc: Nocodazole.

These quinoxalin-sulphonamide conjugates **5a-i** were investigated for their antiproliferative activity against three human cancer cell lines namely Hela (cervical cancer), DU145 (prostate cancer) and A549 (non small cell lung cancer). Nocodazole was employed as reference standard and the IC_{50} values are summarized in Table 3. All the compounds (**5a-i**) have

exhibited moderate to good cytotoxicity with the IC_{50} values ranging between 5.0 and 38.9 μM. Notably, the compounds **5a** and **5b** have shown significant activity on all the cell lines examined.

In order to determine the effect of substituent on the cytotoxicity the quinoxalin-sulphonamide conjugates have been synthesized with various substitutions on the phenyl ring attached to sulphonamide (Scheme 2). The compounds **5a** and **5b** possessing ring activating substituents such as methoxy group on phenyl ring have significantly inhibited the growth of HeLa and A549 cells with IC_{50} of 6.7; 5.0 μM and 8.4; 7.4 μM respectively. While the same compounds have shown moderate growth inhibition on the DU145 cell line (IC_{50}: **5a** = 12.3 and **5b** = 11.1 μM). In comparison, **5c** and **5e** functionalized with ring deactivating substituents such as nitro and trifluoromethyl on phenyl ring exhibited lower cytotoxicity with IC_{50} values of 26.8 and 13.2 μM respectively against HeLa cells. Based on these observations it is clear that substitution at C4 position on the phenyl ring plays an important role with respect to the activity. The presence of electropositive groups on the phenyl ring significantly enhances the cytotoxicity than electronegative groups such as halogen and nitro. However, there is an insignificant effect on the activity by the substitution at C_3 position of the phenyl ring.

Table 5 Antimicrobial activity of the compounds **5a-i** and values are expressed in MIC (μg/ml).

Compound	R_1	R_2	B s[a]	S c[a]	M l[a]	S a[a]	E c[b]	P a[b]	K p[b]	C a[c]
5a	OMe	H	40	10	40	40	80	80	80	40
5b	OMe	OMe	10	10	10	25	30	40	30	20
5c	NO_2	H	10	10	10	15	25	30	25	20
5d	F	H	10	16	10	25	40	40	40	20
5e	CF_3	H	40	40	40	40	>100	>100	>100	80
5f	Cl	Cl	25	50	20	30	80	80	40	30
5g	Br	H	10	50	20	40	40	40	80	25
5h	H	Cl	25	25	20	25	80	40	80	30
5i	H	NO_2	40	40	40	50	>100	>100	>100	80
chloramphenicol			20	15	10	12	14	20	25	-
nistatin			-	-	-	-	-	-	-	12

[a] = gram positive bacteria; b = gram negative bacteria; c = fungal strain; [a]B s = *Bacillus subtilis* MTCC121; [a]S c = *Staphylococcus MLS-16* MTCC2940; [a]M l =*micrococcus luteus* MTCC2470; [a]S a= *Staphylococcus aureus* MTCC 96; [b]E c= [b]*Escherichia coli* MTCC739; [b]P a= [b]*Pseudomonas ; aeruginosa* MTCC2453; [b]K p= [b]*Klebsiella planticola* MTCC530; [c]C a= [c]*Candida albicans* MTCC3017

Antimicrobial activity

The synthesized quinoxalin-sulphonamide conjugates **5a-i** were also tested for their activity against various Gram positive bacteria like *Bacillus. subtilis* MTCC121, S*taphylococcus. aureus* MTCC 96, *staphylococcus MLS-16* MTCC2940, *Micrococcus luteus* MTCC2470 and Gram negative bacteria like *Escherichia coli* MTCC739, *Pseudomonas aeruginosa* MTCC2453, *Klebsiella planticola* MTCC530 taking chloramphenicol as a positive control, and the results are summarized in Table 4. As evident from the results, most of the conjugates have shown activity against all the strains tested. Interestingly, the compounds have shown selectivity towards Gram positive bacteria. Some of the conjugates **5b**, **5c** and **5d** have exhibited excellent antibacterial activity (MIC 10 μg/ml) comparable to and sometimes even better than the standard against the pathogens *B. Subtilis*, *S. MLS* and *M. luteus*. However, other conjugates have displayed moderate activity against both Gram positive and Gram negative bacteria. Furthermore, all the conjugates were tested against a fungal strain, *Candida albicans* (MTCC 3017). All the compounds have shown moderate activity against it with MIC values ranging between 20 and 80 μg/ml. Compounds **5b**, **5c** and **5d** were found to be most effective from the series displaying MIC 20 μg/ml, however the compounds have not superseded the standard nistatin (MIC 12 μg/ml).

Conclusion

In conclusion, we have developed a simple, efficient and ecofriendly method for the synthesis of quinoxalines from 1,2-diamines and α-hydroxy ketones using cost-effective and readily available catalyst L-proline. To the best of our knowledge this transformation has not been reported with an organic catalyst. The advantages of this method over previous reports include its simplicity of operation, cleaner reactions, higher yields, shorter reaction times and use of inexpensive catalyst. The mild reaction condition makes this methodology an alternative procedure to the conventional acid or base-catalyzed processes for the synthesis of quinoxalines and has practical applicability. Further, using this protocol a short library of quinoxaline-sulphonamide conjugates have been developed. The conjugates were found to be cytotoxic and effectively inhibit the growth of many bacterial strains.

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Notes and references

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Experimental

General remarks

Melting points were determined with an electrothermal melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded on Perkin–Elmer model 683 or 1310 spectrometers with sodium chloride optics. ^{1}H NMR spectra were recorded on an Avance 300 MHz spectrometer (Bruker, Fallanden, Switzerland) and ^{13}C NMR spectra were recorded on a UNITY 300 MHz (Varian, Switzerland). Chemical shifts (d) are reported in ppm, downfield from internal TMS standard. Mass spectra were recorded using a quadruple ion trap mass spectrometer (Thermo Finnign, San Jose, CA, USA) equipped with an electro spray source.

Representative experimental procedure for the synthesis quinoxalines (3a-j)

In a 50 mL round bottom flask 1,2-Diamine (1mmol) and α-hydroxyketone (1 mmol) were taken in water (5 mL). Catalytic amount (20 mol%) of L-proline was added and the reaction mixture was refluxed for 12 hours. The progress of the reaction was monitored by TLC. After completion of the reaction, the mixture was cooled to room temperature. The precipitated solid was collected by filtration, washed with water and recrystallized using methanol.

2,3-diphenylquinoxaline (3a)

White solid; Mp: 126–127 ˚C; ^{1}H NMR (300 MHz $CDCl_3$): δ 7.31-7.4 (m, 6H), 7.52-7.57 (m, 4H), 7.76-7.82 (m, 2H), 8.17-8.22(m, 2H); ^{13}C NMR (75 MHz $CDCl_3$): δ 127.50, 128.13, 128.40, 129.14, 129.35, 138.33, 140.38, 152.57; ESI-MS: *m/z* = 283 $(M+H)^+$.

6-fluoro-2,3-diphenylquinoxaline (3b)

Brown solid; Mp: 100–102 ˚C; ^{1}H NMR (500 MHz $CDCl_3$): δ 7.32-7.40 (m, 6H), 7.49-7.52 (m, 4H), 7.53-7.57 (m,1H), 7.81 (dd, *J* = 2.74 & 9.30 Hz, 1H), 8.16-8.19 (m, 1H); ^{13}C NMR (75 MHz $CDCl_3$): δ 112.52, 112.78, 120.20, 120.51, 128.32, 128.90, 129.08, 129.80, 129.86, 131.20, 131.34, 138.44, 138.70, 138.82, 141.88, 142.05, 152.84, 154.24, 161.19, 164.54; ESI-MS *m/z* = 301 $(M+H)^+$.

6-methyl-2,3-diphenylquinoxaline (3c)

Brown solid; Mp: 120–122 ˚C; ^{1}H NMR (300MHz $CDCl_3$): δ 2.59 (s, 3H), 7.32 (d, *J* = 6.79 Hz, 6H), 7.49 (d, *J* = 6.79 Hz, 4H), 7.59 (dd, *J* = 1.51, 8.68 Hz, 1H), 7.90 (s, 1H), 8.03 (d, *J* = 8.49 Hz, 1H); ^{13}C NMR (75 MHz $CDCl_3$): δ 20.78, 126.79, 127.04, 127.52, 127.58, 128.76, 131.18, 138.07, 138.45, 139.27, 140.02, 151.20, 151.95; ESI-MS: *m/z* = 297 $(M+H)^+$.

6-nitro-2,3-diphenylquinoxaline (3d)

Brown solid; Mp: 140–142 ˚C; ^{1}H NMR (300 MHz $CDCl_3$): δ 7.34-7.46 (m, 6H), 7.54-7.58 (m, 4H), 8.28 (d, *J* = 9.25 Hz, 1H), 8.54 (dd, *J* = 2.45 & 9.25 Hz, 1H), 9.09 (d, *J* = 2.45Hz, 1H); ^{13}C NMR (75 MHz $CDCl_3$): δ 123.28, 125.60, 128.43, 129.60, 129.74, 129.78, 129.86, 130.72, 137.97, 138.04, 139.94, 143.55, 147.84, 155.66, 156.29; ESI-MS: *m/z* = 328 $(M+H)^+$.

6,7-dichloro-2,3-diphenylquinoxaline (3e)

Brown solid; Mp: 141–143 ˚C; ^{1}H NMR (300 MHz $CDCl_3$): δ 7.31-7.39 (m, 6H), 7.48-7.52 (m, 4H), 8.29 (s, 2H); ^{13}C NMR (75 MHz $CDCl_3$): δ 127.63, 128.62, 129.05, 133.44, 137.61, 139.17, 153.77; ESI-MS: *m/z* = 351 $(M+H)^+$.

2,3-di(furan-2-yl)quinoxaline (3f)

Brown solid; Mp: 134–136 ˚C; ^{1}H NMR (300MHz $CDCl_3$): δ 6.56-6.58 (m, 2H), 6.65 (dd, *J* = 0.56 & 3.58 Hz, 2H), 7.63 (dd, *J* = 0.56 & 1.70Hz, 2H), 7.74-7.78 (m, 2H), 8.11-8.17 (m, 2H); ^{13}C NMR (75 MHz $CDCl_3$): δ 111.85, 112.93, 129.02, 130.33, 138.46, 140.51, 142.55, 144.15; *m/z* = 263 $(M+H)^+$.

6-fluoro-2,3-di(furan-2-yl)quinoxaline (3g)

Brown solid; Mp: 106–108 ˚C; ^{1}H NMR (300MHz $CDCl_3$): δ 6.56-6.58 (m, 2H), 6.66 (dd, *J* = 3.02, 9.80 Hz, 2H), 7.49-7.56 (m, 1H), 7.62 – 7.65 (m, 2H), 7.75 (dd, *J* = 3.02 & 9.06 Hz, 1H), 8.10-8.15 (m, 1H); ^{13}C NMR (75 MHz $CDCl_3$): δ 111.23, 111.61, 112.01, 112.27, 112.76, 119.92, 123.64, 130.33, 136.74, 140.46, 140.95, 142.25, 143.66, 148.74, 149.52; ESI-MS: *m/z* = 281 $(M+H)^+$.

2,3-di(furan-2-yl)-6-methylquinoxaline (3h)

Brown solid; Mp: 122–124 ˚C; ^{1}H NMR (300MHz $CDCl_3$): δ 6.61 (d, *J* = 16.42 Hz, 4H), 7.59 (t, *J* = 8.87 Hz, 3H), 7.89 (s, 1H), 7.99 (d, *J* = 8.49 Hz, 1H); ^{13}C NMR (75 MHz $CDCl_3$): δ 21.26, 111.31, 111.95, 112.20, 127.20, 127.87, 132.14, 138.33, 139.96, 140.45, 141.07, 141.82, 143.32, 143.45, 150.20; ESI-MS: *m/z* = 277 $(M+H)^+$.

2,3-di(furan-2-yl)-6-nitroquinoxaline (3i)

Brown solid; Mp: 152–154 ˚C; ^{1}H NMR (300 MHz $CDCl_3$): δ 6.60-6.63 (m, 2H), 6.87 (dd, *J* = 3.58 & 16.80 Hz, 2H), 7.67 (dd, *J* = 0.94 & 5.09 Hz, 2H), 8.23 (d, *J* = 9.253 Hz, 1H), 8.50 (dd, *J* = 2.45 & 9.253 Hz, 1H), 9.01(d, *J* = 2.45 Hz, 1H); ^{13}C NMR (75 MHz $CDCl_3$): δ 111.28, 111.40, 113.44, 114.28, 122.39, 123.93, 129.37, 137.92, 141.78, 142.93, 143.45, 143.95, 144.41, 146.64, 148.89, 148.98; ESI-MS: *m/z* = 308 $(M+H)^+$.

6,7-dichloro-2,3-di(furan-2-yl)quinoxaline (3j)

Brown solid; Mp: 135–137 ˚C; ^{1}H NMR (300MHz $CDCl_3$): δ 6.58-6.59 (m, 2H), 6.73 (d, *J* = 3.50Hz, 2H), 7.63 (d, *J* = 1.06 Hz, 2H), 8.24 (s, 2H); ^{13}C NMR (75 MHz $CDCl_3$): δ 111.46, 113.26, 128.73, 133.72, 138.42, 142.49, 143.96, 149.50; ESI-MS: *m/z* = 330 $(M+H)^+$.

2,3-di(furan-2-yl)quinoxalin-6-amine (4)

6-Nitro-2,3-di(furan-2-yl) quinoxaline in methanol (20ml) was hydrogenated in the presence of 5% Pd/C at room temperature for 3h. After completion of the reaction, the mixture was filtered over celite. The filtrate was collected and concentrated.

General procedure for the synthesis of Quinoxalin-6-amine sulphonamides.

To a round bottom flask containing pyridine (5 ml) 2,3-di(furan-2-yl)quinoxalin-6-amine (4, 1 mmol) and corresponding benzene sulphonyl chlorides (1.5mmol) were added. The reaction mixture was stirred for 3 hours and the progress of the reaction was monitored by TLC. After completion of the reaction, aqueous $CuSO_4.5H_2O$ solution was added and the product was extracted using ethyl acetate (3x20 ml). The combined organic layer was dried over anh. Na_2SO_4 and concentrated on rotavapor. The crude product was purified by column chromatography using hexane and ethyl acetate (7:3) as eluent.

***N*-(2,3-di(furan-2-yl)quinoxalin-6-yl)-4-methoxybenzenesulfonamide (5a)**

Yellow solid; Mp: 230-232 ˚C; ^{1}H NMR (300 MHz DMSO d_6): δ 3.78 (s,3H), 6.56 (s, 2H), 6.61 (d, *J* = 2.64, 1H), 6.88 (dd, *J* = 2.07&8.87 Hz, 2H), 7.43 (s, 1H), 7.59-7.65 (m, 3H), 7.83-7.87 (m, 3H), 7.93 (dd, *J* = 2.64&8.87 Hz, 1H), 10.43 (br s, 1H); ^{13}C

NMR (75 MHz DMSO d_6): δ 54.11, 110.31, 110.48, 111.28, 111.52, 112.53, 112.80,113.57, 122.62, 127.42,127.69, 128.26, 129.43, 129.69,135.74, 138.53, 139.47, 142.19, 142.45, 148.94, 149.20; ESI-MS: *m/z* 448 $(M+H)^+$: HRMS (ESI) *m/z* for $C_{23}H_{18}O_5N_3S$ calculated *m/z*: 448.09617, found *m/z*: 448.09330.

***N*-(2,3-di(furan-2-yl)quinoxalin-6-yl)-3,4-dimethoxybenzenesulfonamide (5b)**

Yellow solid; Mp: 245-247 ˚C; ^{1}H NMR (300 MHz DMSO d_6): δ 3.84 (s, 6H), 6.57-6.61 (m, 4H), 6.85-6.88 (m, 1H), 7.40 (s, 1H), 7.49-7.51 (m, 1H), 7.60-7.65 (m, 3H), 7.84 (s, 1H), 7.89-7.92 (m, 1H), 10.57 (br s, 1H); ^{13}C NMR (75 MHz DMSO d_6): δ 55.06, 108.60, 109.56, 110.95, 111.47, 112.03, 114.41, 120.06, 123.46, 128.70, 130.09, 136.41, 139.26, 140.10, 142.92, 143.19, 147.90, 151.56; IR (KBr) (ν_{max}/cm^{-1}): ν = 3446, 2934, 1620, 1508, 1156, 1138, 1018, 913 cm^{-1}; ESI-MS: *m/z* 478 $(M+H)^+$: HRMS (ESI) *m/z* for $C_{24}H_{20}O_6N_3S$ calculated *m/z*: 478.10673, found *m/z*: 478.10325.

***N*-(2,3-di(furan-2-yl)quinoxalin-6-yl)-4-nitrobenzenesulfonamide (5c)**

Yellow solid; Mp: 260-262 ˚C; ^{1}H NMR (300 MHz DMSO d_6): δ 6.56-6.63 (m, 3H), 7.61-7.65 (m, 3H), 7.70-7.78 (m, 1H), 7.81-7.86 (m, 2H), 7.89-7.94 (m, 1H), 8.19-8.23 (m, 1H), 8.34-8.39 (m, 1H), 8.70-8.72 (m, 1H), 11.10 (br s, 1H); ^{13}C NMR (75 MHz DMSO d_6): δ 110.77, 111.36, 111.92, 114.91, 120.71, 123.19, 126.14, 128.72, 129.63, 131.40, 137.85, 139.60, 140.20, 140.56, 142.77, 143.05, 143.30, 146.79, 149.20; IR (KBr) (ν_{max}/cm^{-1}): ν = 3246, 3095, 1619, 1571, 1527, 1353, 1165, 1123, 912 cm^{-1}; ESI-MS: *m/z* 463 $(M+H)^+$: HRMS (ESI) *m/z* for $C_{22}H_{15}O_6N_4S$ calculated *m/z*: 463.07068, found *m/z*: 463.06729.

***N*-(2,3-di(furan-2-yl)quinoxalin-6-yl)-4-fluorobenzenesulfonamide (5d)**

Yellow solid; Mp: 235-237 ˚C; ^{1}H NMR (300 MHz DMSO d_6): δ 6.58-6.61 (m, 4H), 7.10-7.16 (m, 2H), 7.58-7.63 (m, 3H), 7.86-7.94 (m, 4H), 10.73 (s, 1H); ^{13}C NMR (75 MHz DMSO d_6): δ 110.43, 110.52, 110.90, 111.52, 113.57, 114.74, 115.03, 122.69, 128.24, 128.34, 134.13, 135.74, 138.26, 139.35, 139.45, 141.15, 142.50, 144.81, 149.05; IR (KBr) (ν_{max}/cm^{-1}): ν = 3251, 2924, 1620, 1491, 1325, 1229, 1088, 1018, 912 cm^{-1}; ESI-MS: *m/z* 436 $(M+H)^+$: HRMS (ESI) *m/z* for $C_{22}H_{15}O_4N_3FS$ calculated *m/z*: 436.07618, found *m/z*: 436.07273.

3,4-dichloro-*N*-(2,3-di(furan-2-yl)quinoxalin-6-yl)benzenesulfonamide (5e)

Yellow solid; Mp: 236-238 ˚C; ^{1}H NMR (300 MHz DMSO d_6): δ 6.63 (d, *J* = 9.90 Hz, 4H),7.65 (t, *J* = 7.33 Hz, 4H), 7.78 (t, *J* = 7.33 Hz, 2H), 7.96 (d, *J* = 8.80 Hz, 1H), 8.01 (s, 1H), 11.01 (br s, 1H);^{13}C NMR (75 MHz DMSO d_6): δ 110.42, 110.50, 110.98, 111.58, 114.24, 122.78, 124.97, 127.13, 128.43, 129.89, 131.46, 135.51, 135.96, 137.79, 138.07, 139.33, 139.67, 141.24, 142.53, 142.84, 149.00, 149.09; IR (KBr) (ν_{max}/cm^{-1}): ν = 3247, 2924, 1619, 1491, 1327, 1157, 1068, 1022, 919 cm^{-1}; ESI-MS: *m/z* 486 $(M+H)^+$: HRMS (ESI) *m/z* for $C_{22}H_{14}O_4N_3Cl_2S$ calculated *m/z*: 486.00766, found *m/z*: 486.00459.

***N*-(2,3-di(furan-2-yl)quinoxalin-6-yl)-4-(trifluoromethyl)benzenesulfonamide (5f)**

Yellow solid; Mp: 255-257 ˚C; ^{1}H NMR (300 MHz DMSO d_6): δ 6.56-6.75 (m, 4H), 7.60-7.81 (m, 5H), 7.85-8.01 (m, 2H), 8.05-8.25 (m, 2H), 10.86 (br s, 1H); ^{13}C NMR (75 MHz DMSO d_6): δ 28.52, 111.01, 111.09, 111.70, 112.24, 115.12, 122.91, 122.95, 123.51, 128.51, 128.55, 128.98, 129.16, 129.41, 136.64, 138.43, 139.84, 140.02, 140.36, 141.93, 143.05, 143.31, 149.56; IR (KBr) (ν_{max}/cm^{-1}): ν = 3247, 2924, 1619, 1535, 1488, 1347, 1250, 1160, 919 cm^{-1}; ESI-MS: *m/z* 486 $(M+H)^+$: HRMS (ESI) *m/z* for $C_{23}H_{15}O_4N_3F_3S$ calculated *m/z*: 486.07299, found *m/z*: 486.06939.

4-bromo-*N*-(2,3-di(furan-2-yl)quinoxalin-6-yl)benzenesulfonamide (5g)

Yellow solid; Mp: 220-222 ˚C; ^{1}H NMR (300 MHz DMSO d_6): δ 6.56-6.62 (m, 4H), 7.57-7.66 (m, 5H), 7.76-7.84 (m, 3H), 7.90-7.94 (m, 1H), 10.81 (br s, 1H); ^{13}C NMR (75 MHz DMSO d_6): δ 111.68, 110.75, 111.24, 111.82, 114.20, 123.04, 126.29, 127.36, 128.57, 131.02, 136.15, 137.41, 138.40, 139.68, 139.85, 141.50, 142.70, 142.98, 149.27, 149.34; IR (KBr) (ν_{max}/cm^{-1}): ν = 3086, 2853, 1618, 1571, 1527, 1489, 1343, 1218, 1087, 914 cm^{-1}; ESI-MS: *m/z* 495 $(M+H)^+$: HRMS (ESI) *m/z* for $C_{22}H_{15}O_4N_3BrS$ calculated *m/z*: 495.99612, found *m/z*: 495.99265.

3-chloro-*N*-(2,3-di(furan-2-yl)quinoxalin-6-yl)benzenesulfonamide (5h)

Yellow solid; Mp 216-218 ˚C; ^{1}H NMR (300 MHz DMSO d_6): δ 6.57-6.63 (m, 4H), 7.42-7.54 (m, 2H), 7.63 (d, *J* = 7.93, 3H), 7.78-7.83 (m, 2H), 7.89-7.95 (m, 2H), 10.91 (s, 1H); ^{13}C NMR (75 MHz DMSO d_6): δ 110.53, 110.61, 111,09, 111.69, 114.13, 122.86, 123.82, 125.25, 128.44, 129.36, 131.58, 133.24, 135.99, 138.12, 139.47, 139.71, 139.95, 141.34, 142.59, 142.90, 149.09, 149.17; IR (KBr) (ν_{max}/cm^{-1}): ν = 3241, 2977, 1735,1618, 1420, 1324, 1250, 1019, 1162, 911 cm^{-1}; ESI-MS: *m/z* 452 $(M+H)^+$: HRMS (ESI) *m/z* for $C_{22}H_{15}O_4N_3ClS$ calculated *m/z*: 452.04663, found *m/z*: 452.04381.

***N*-(2,3-di(furan-2-yl)quinoxalin-6-yl)-3-nitrobenzenesulfonamide (5i)**

Yellow solid; Mp 260-262 ˚C; ^{1}H NMR (300 MHz DMSO d_6): δ 6.57-6.61 (m, 4H), 7.61-7.72 (m, 4H), 7.89 (s, 1H), 7.97 (d, *J* = 9.06 Hz, 1H), 8.23 (d, *J* = 7.36 Hz, 1H), 8.37 (d, *J* = 8.12 Hz, 1H), 8.78 (s, 1H), 11.01 (brs, 1H); ^{13}C NMR (75 MHz DMSO d_6): δ 110.16, 110.24, 110.68, 111.29, 113.92, 119.84, 122.47, 125.83, 128.22, 129.50, 130.84, 135.54, 137.30, 138.83, 139.30, 140.83, 142.48, 142.82, 146.20, 148.58, 148.67; IR (KBr) (ν_{max}/cm^{-1}): ν = 3249, 1618, 1569, 1526, 1468, 1352, 1228, 1164, 1081, 911 cm^{-1}; ESI-MS: 463 $[M+H]^+$: HR-MS (ESI) *m/z* for $C_{22}H_{15}O_6N_4S$ calculated *m/z*: 463.07068, found *m/z*: 463.06717.

Materials and Methods

Cell culture and reagents

All the cell lines used in this study were obtained from the American Type Culture Collection (ATCC). DU145 (human prostate carcinoma epithelial) cells have cultured in Eagle's minimal essential medium (MEM) containing nonessential amino acids, 1mM sodium pyruvate, and 10% FBS. HeLa (human epithelial cervical cancer) and A549 (human lung carcinoma epithelial) were grown in Dulbecco's modified Eagle's medium (DMEM) containing non essential amino acids and 10% FBS. All the cells maintained under humidified atmosphere of 5% CO_2 at 37˚C. Cells were trypsinized when sub confluent from T75 flasks/90mm dishes and seeded on to 96 well test plates at a concentration of 1×10^4 cells/mL in complete medium, treated with compounds at desired concentrations and harvested as required[24].

MTT assay

Cell proliferation and viability was determined by 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay. The pale yellow colored tetrazolium salt (MTT) reduces to

a dark blue water-insoluble formazan by metabolically active cells and which is measured quantitatively after soluble in DMSO. The absorbance of the soluble formazan is directly proportional to the number of viable cells. Cells were seeded at a density of 1×10^4 cells in 200 µL of medium per well of 96-well plate. The 96-well microtiter plates were incubated for 24 h prior to addition of the experimental compounds. Cells were treated with vehicle alone (0.4% DMSO) or compounds (drugs were dissolved in DMSO previously) at different concentrations (1, 10 and 25µM) of test compounds for 48 hours. The assay was completed with the addition of MTT (5 %, 10µL) and incubated for 60 min at 37 ^{0}C. The supernatant was aspirated and plates were air dried and the MTT-formazon crystals dissolved in 100 µL of DMSO. The optical density (O.D.) was measured at 560 nm using TECAN multimode reader. The growth percentage of each treated well of 96 well plate have been calculated based on test wells relative to control wells. The cell growth inhibition was calculated by generating dose response curves as a plot of the percentage of surviving cells versus drug concentration. Antiproliferative activity of the cancer cells to the test compounds was expressed in terms of IC_{50} value, which defines as a concentration of compound that produced 50% absorbance reduction relative to control.(25)

Antimicrobial Susceptibility Testing

All the synthesized compounds were dissolved in pure DMSO of 2 mg/mL. Empty sterilized disks of 6mm were impregnated with test compounds in the range from 1 to 80 mg/disk and placed in triplicate in the medium inoculated with fresh bacterial strain (1e2 _10^7 cfu mL_1) on the freshly prepared sterile Mueller Hilton agar plates.(26) Subsequently these plates were incubated at 35 °C for 24 h for zone of inhibition, if any, around the disks. The lowest concentration (higher dilution) of the test compound required to arrest the growth of tested strains was defined as minimum inhibitory concentrations (MICs). 20 µL of DMSO was loaded on sterile disc and placed on the culture. After incubation for 24 h, observed no zone of inhibition around the disc, indicated that DMSO has no inhibitory effect on the tested strains and which was used as a negative control. chloramphenicol was employed as the reference standard. The method followed for antifungal bioassay was similar to that followed for antibacterial assay where the medium was potato dextrose agar 39 g/L. The equivalent amount of solvent (DMSO) did not exhibit any activity in the assay. The treated compounds and the controls were kept in an incubator at 28±2 °C for 48 h. All the compounds were tested for minimum inhibitory concentration (MIC). Nistatin was employed as positive control.

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