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ARTICLE TYPE

Regioselective synthesis, antimicrobial evaluation and theoretical studies of 2-styryl quinolines

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2-Styryl quinolines (**9a-l**) have been synthesized regioselectively from 2-methyl-quinoline by using NaOAc in water acetic acid binary solvents and evaluated for their antibacterial activity against both Gram-positive and Gram-negative strains. Among these, the compounds **12** and **8** were found to be active against both bacterial strains. Compounds **9b**, **9f**, **9g**, **9i**, **9j** and **9k** were most active among the series exhibiting MIC values ranging between 1.9-31.2 µg/ml against different bacterial strains. Compounds **9j** and **9k** were found to be as potent as standard drug ciprofloxacin against *Micrococcus luteus, Klebsiella planticola* and *Staphylococcus aureus*, respectively. In addition, the compounds showed bactericidal activity, compound **9j** was found to be better as compared to ciprofloxacin with MBC value of 0.9 µg/ml against both *M. luteus* and *K. planticola.* The compounds also inhibited biofilm formation, compound **9j** was found to be equipotent to erythromycin against *M. luteus* and *S. aureus* MLS16. Further, theoretical studies such as druggable properties and PMI plot have been carried out.

Introduction

One-third of the world's population are infected by bacterial pathogens, which results in two million fatalities per year.[1] Development of bacterial resistance to the current antibiotics and development of multidrug resistant strains like methicillin-resistant *Staphylococcus aureus* (MRSA) is a significantly increasing threat to human health.[2] Drug resistance develops as a result of gene mutation, rearrangement and genetic transfer between different bacteria.[3] In addition, bacterial resistance is induced by various cellular mechanisms involving efflux pumps, target modification, or drug inactivation by enzymes.[4] Antibiotics have been shown to be 1000- to 1500-fold less active against bacteria that are confined within a biofilm than against individual free-form planktonic bacteria.[5] These facts emphasized the urgent need to develop new affordable drugs with novel structural features, high potency, less toxicity and activity against drug resistant organisms with new mode of action.

Quinoline scaffold has gathered an immense attention among chemists as well as biologists as it is one of the key building elements for many naturally occurring compounds. The quinoline ring is endowed with various activities, such as anti-tuberculosis,[6] antimalarial,[7] anti- inflammatory,[8] anticancer,[9] antibiotic,[10] anti-hypertensive,[11] tyrokinase PDGF-RTK inhibiting agents,[12] and anti-HIV.[13,14] Compared to the successful development on new fluoroquinolones, research on chemistry of new quinoline-based antimicrobial drugs seems to be underdeveloped. However, certain synthetic small molecules based on quinine core were found to exhibit broad-spectrum of antibacterial activity. Quinine-like molecule **(1)** that acts by inhibition of bacterial gyrase and topoisomerase IV was found to be efficient against a strain of flouroquinolone-resistant *Staphylococcus pneumoniae* in a mouse lung infection model and also exhibited activity against MRSA.[15]

Since last few years, the molecular hybridization approach for quinoline-based antibacterials with structural diversification in the C-3 position of the quinoline has been used.[16-19] Recently, the new quinoline-based anti-TB drug, bedaquiline (**2**)(BQ, TMC207, R207910) was approved by FDA.[20] Chloroquine (**3**), primaquine (**4**), mefloquine (**5**) and quinine (**6**) are examples of the drugs containing quinoline scaffold (**Fig. 1**). On the basis of substitution pattern on quinoline ring in these drugs and in continuation to our research to find potent antimicrobial agents,[21-25] we herein report the design and regioselective synthesis of 2-styryl quinolines[26] and their evaluation for antimicrobial, bactericidal and anti-biofilm activities.

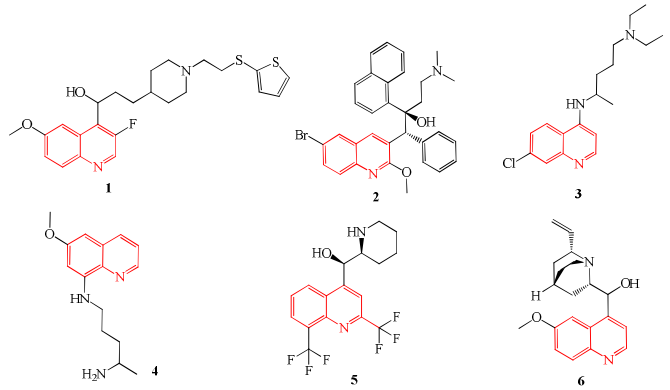

Fig. 1: Quinoline based antibacterial agents.

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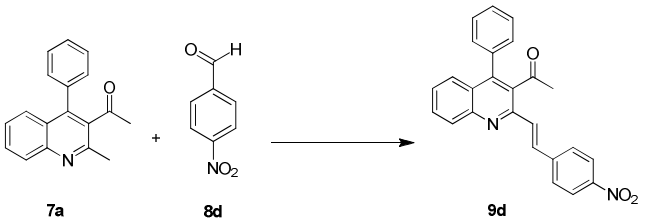
Chemistry

Intermediate **7** was synthesized by the known procedure we reported previously.[27] 2-aminobenzophenone was reacted with acetyl acetone in presence of catalytic amount of HCl in acetonitrile to afford intermediate 7. The structure of 7 was assigned on the basis of its ^{1}H NMR, two singlet at δ 1.96 and δ 2.67 ppm strongly supports the presence of –CH_3 and –$COCH_3$. Likewise two double doublets appeared at δ 7.39-7.44 and δ 7.56-7.62 with J = 8.3 Hz, 1.51 Hz confirmed the presence of two quinoline protons. To obtain final compounds 9a-l, we used a new methodology in which compound 7 was condensed with different substituted benzaldehydes (**8a-l**) in presence of sodium acetate in equal ratio of water and acetic acid as green solvents (**Table 2**). In the beginning, reaction between 2-methyl-3-acetyl-4-phenyl quinoline and 4-nitro benzaldehyde was carried out in water at room temperature and at 120 °C, the completion of the reaction was monitored by TLC (**scheme 1**). It was observed that the reaction did not proceed even until 16 hours but the use of sodium acetate affords the traces of product. Similarly with acetic solvent the reaction underwent but with low yield and when we used sodium acetate in this solvent yield was slightly increased. We did not observe any remarkable increase in the yield when we used temperature 120 °C and 80 °C.



Scheme 1: Reaction of 7a with 8d under various conditions

Table 1: Optimization of the reaction conditions

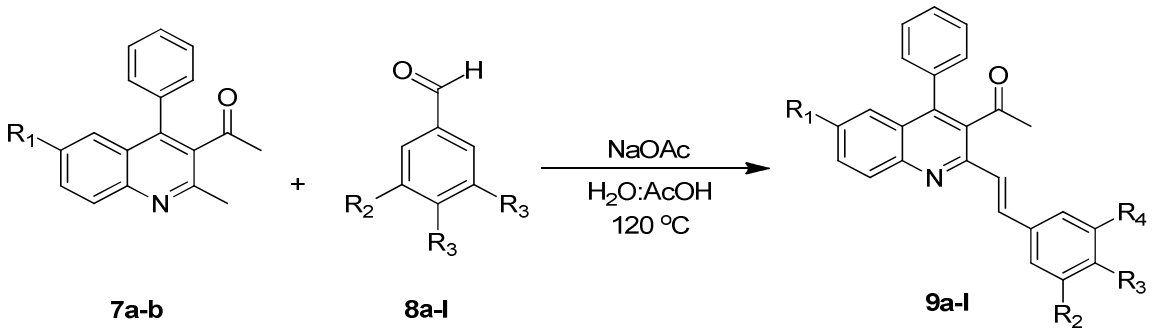
Entry	Solvent	Base	Temp (°C)	Time (h)	Yield[a](%)
1	H_2O	-[a]	120	16	Nil
2	H_2O	NaOAc	120	16	Trace
3	AcOH	-[a]	120	16	20
4	AcOH	NaOAc	120	8	38
5	AcOH	NaOAc	80	8	35
6	AcOH:H_2O[b]	-[a]	80	8	44
7	AcOH:H_2O[b]	NaOAc	80	8	69
8	AcOH:H_2O[b]	NaOAc	120	8	83

[a] No buffer used, [b] 1:1 Solvent used.

Likewise when we moved to the binary mixture of acetic acid and water we found that the use of sodium acetate and refluxing temperature at 120 °C provides us very good yield. The compound **7** was having labile methyl group at C-2 and at C-3, so we intend to condense only C-2 methyl under neutral conditions by using sodium acetate as buffer in water and in an acetic acid medium.

In conclusion, reaction optimization showed that in basic medium, the C-3 methyl was condensed to give chalcone as the product, but in acidic medium the desired compound formed with low yield. So we tried the binary mixture of water and acetic acid in presence of sodium acetate which furnished the desired results (**Table 1**). Synthetic route for desired compounds **9a-l** is outlined in **Scheme 2**.

The structure of the target compounds were assigned by their ^{1}H and ^{13}C NMR, IR and HRMS data.[28] The coupling constants values obtained from ^{1}H NMR data indicate that the geometry of the double bond in the product is predominantly *trans*. Further, X-ray crystallographic study was performed on **9k** to precisely determine the position of double bond that is whether it was formed on the carbon of methyl group adjacent to carbonyl at C-3 position of compound **7b** or at C-2 position. The X-ray crystal structure of the compound **9k** has confirmed the regioselective formation of double bond at C-2 position through the application of sodium acetate in water acetic acid solvents (**Fig. 2**). To the best of our knowledge, the synthesized compounds and method used were not reported previously in the literature.



Scheme.2: Synthesis of 2-styrylquinoline

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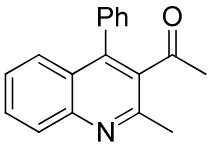
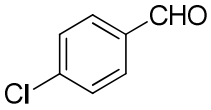
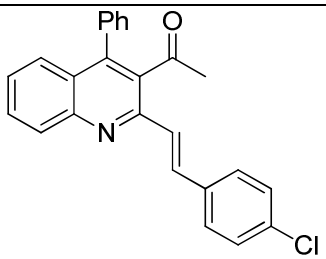
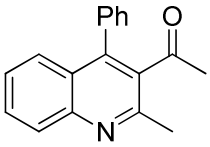
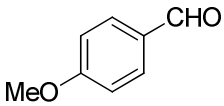
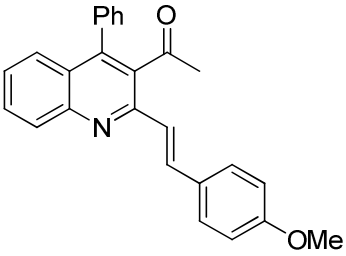
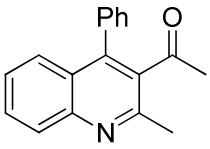
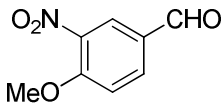
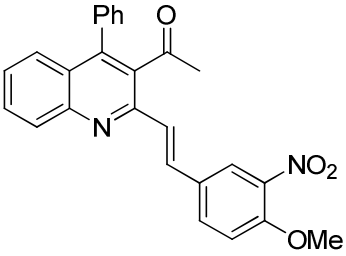
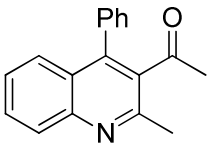
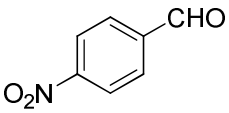
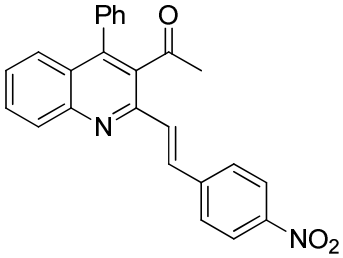
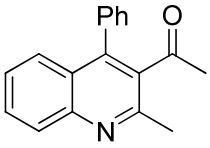
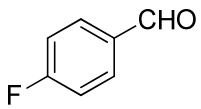
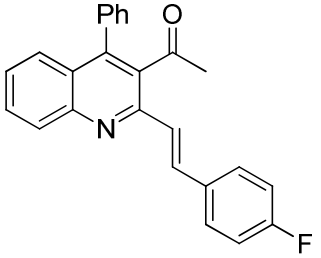
Table 2: Synthesis of 2-styrylquinoline from quinoline and aldehyde

Entry	Quinolines (7a-b)	Aldehydes (8a-l)	Products (9a-l)	Time (h)	Yield[c] (%)	M.P (°C)
1	 **7a**	 **8a**	 **9a**	10	80	201-203
2	 **7a**	 **8b**	 **9b**	12	75	213-215
3	 **7a**	 **8c**	 **9c**	8	85	209-211
4	 **7a**	 **8d**	 **9d**	8.5	86	246-248
5	 **7a**	 **8e**	 **9e**	9	85	180-182

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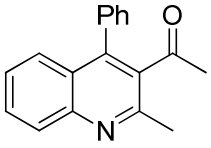
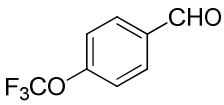
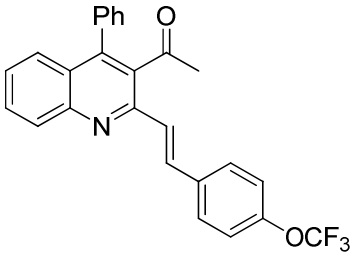
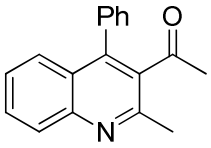
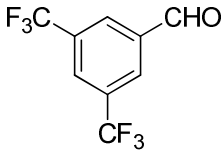
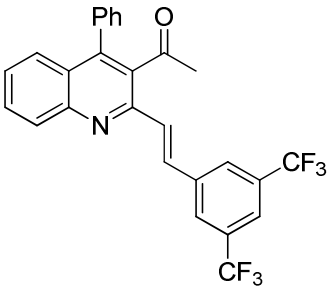
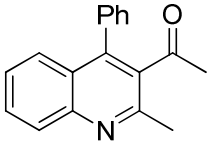
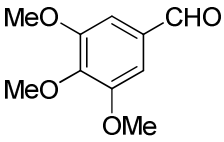
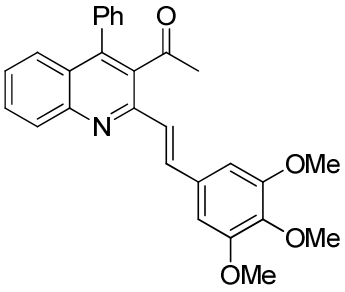
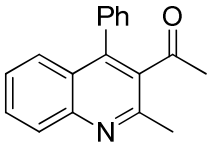
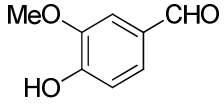
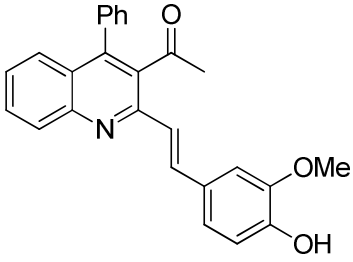
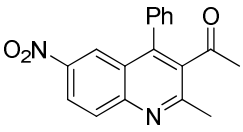
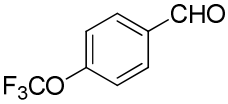
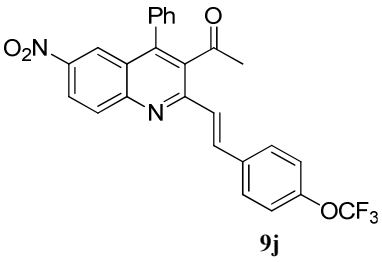
6	 **7a**	 **8f**	 **9f**	12	83	163-165
7	 **7a**	 **8g**	 **9g**	14	81	159-161
8	 **7a**	 **8h**	 **9h**	13	82	172-174
9	 **7a**	 **8i**	 **9i**	15	82	230-232
10	 **7b**	 **8j**	 **9j**	8	90	208-210

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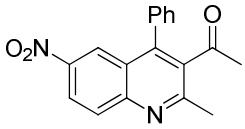
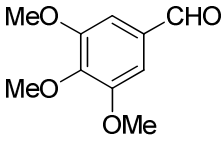
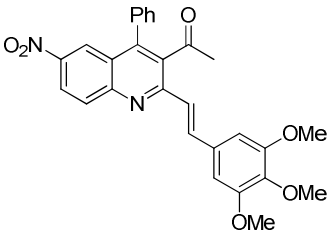
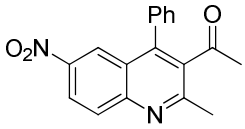
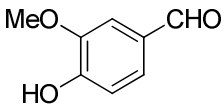
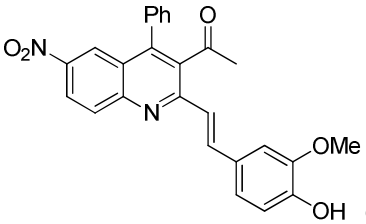
11	 **7b**	 **8k**	**9k**		9	88	217-219
12	 **7b**	 **8l**	**9l**		14	85	243-245

[c]Isolated yield.

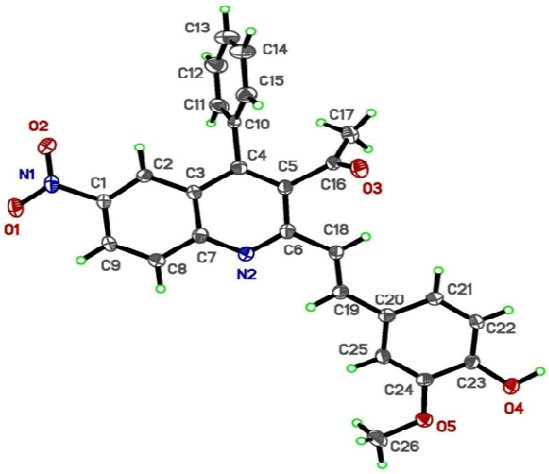


Fig. 2 : ORTEP of compound **9k**. A view of AW80, showing the atom-labeling scheme. Displacement ellipsoids are drawn at the 30% probability level and H atoms are represented by circles of arbitrary radii. (CCDC No: 1023840)

Biological evaluation

Antimicrobial activity

Compounds **9a-l** were tested for antimicrobial activity against both Gram-positive and Gram-negative bacterial strains. The results illustrate that out of **12**, compounds, **8** compounds exhibited promising antimicrobial activity (**Table 3**) and the bactericidal effects with minimum bactericidal concentration (MBC) values ranged between 0.9 to 31.2 µg/ml. Compound **9a** was found to be active against different Gram-positive bacterial strains with MIC values ranging between 15.6 to 31.2 µg/ml; however, it was more promising against Gram-negative bacteria with MIC value as low as 3.9 µg/ml against *P. aeruginosa* MTCC 2453. Compound **9b** showed lowest MIC value of 1.9 µg/ml against *M. luteus* MTCC 2470 and 3.9 µg/ml against *S. aureus* MTCC 96, *B. subtilis* MTCC 121 and *K. planticola* MTCC 530. Compound **9f** was found to be active against *S. aureus* MTCC 96 with MIC value of 1.9 µg/ml and showed good activity against *M. luteus* MTCC 2470, *B. subtilis* MTCC 121, *E. coli* MTCC 739, *P. aeruginosa* MTCC 2453 and *K. planticola* MTCC 530 with MIC value of 3.9 µg/ml. Compound **9g** showed MIC value of 1.9 µg/ml against *B. subtilis* MTCC 121 and MIC value of 3.9 µg/ml against *S. aureus* MTCC 96. Compound **9i** was found to be more promising against *M. luteus* MTCC 2470, *S. aureus* MTCC 96 and *K. planticola* MTCC 530 exhibiting MIC value of 1.9 µg/ml and was good against *B. subtilis* MTCC 121, *S. aureus* MLS16 MTCC 2940 and *B. subtilis* MTCC 121.

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Table 3: Antimicrobial activity of the compounds against several standard strains

		Minimum inhibitory concentration (µg/ml)							
Entry	**Compound**	**M.l[a]**	**S.a[b]**	**S.a[c]**	**B.s[d]**	**E.c[e]**	**P.a[f]**	**K.p[g]**	**C.a[h]**
1	**9a**	15.6±0.12	31.2±0.36	31.2±0.38	15.6±0.26	7.8±0.18	3.9±0.24	15.6±0.32	-[i]
2	**9b**	1.9±0.08	3.9±0.16	7.8±0.36	3.9±0.22	7.8±0.32	7.8±0.28	3.9±0.21	-[i]
3	**9c**	>125	>125	>125	>125	>125	>125	>125	-[i]
4	**9d**	>125	>125	>125	>125	>125	>125	>125	-[i]
5	**9e**	>125	>125	>125	>125	>125	>125	>125	-[i]
6	**9f**	3.9±0.13	1.9±0.12	7.8±0.32	3.9±0.18	3.9±0.18	3.9±0.16	3.9±0.28	-[i]
7	**9g**	7.8±0.16	3.9±0.22	15.6±0.24	1.9±0.16	15.6±0.25	7.8±0.32	15.6±0.24	-[i]
8	**9h**	>125	>125	>125	>125	>125	>125	>125	-[i]
9	**9i**	1.9±0.14	1.9±0.11	3.9±0.12	3.9±0.28	7.8±0.18	7.8±0.21	1.9±0.18	-[i]
10	**9j**	0.9±0.12	1.9±0.13	1.9±0.16	1.9±0.22	3.9±0.26	1.9±0.22	0.9±0.07	-[i]
11	**9k**	1.9±0.11	0.9±0.08	31.2±0.28	1.9±0.24	1.9±0.12	1.9±0.18	31.2±0.32	-[i]
12	**9l**	>125	>125	>125	>125	31.2±0.38	31.2±0.42	>125	-[i]
Ciprofloxacin (standard)		0.9±0.12	0.9±0.09	0.9±0.10	0.9±0.11	0.9±0.07	0.9±0.08	0.9±0.11	-[i]
Miconazole (standard)		-[i]	-[i]	-[i]	-[i]	-[i]	-[i]	-[i]	7.8±0.14

[a]Micrococcus luteus MTCC 2470, [b]Staphylococcus aureus MTCC 96, [c]Staphylococcus aureus MLS16 MTCC 2940, [d]Bacillus subtilis MTCC 121, [e]Escherichia coli MTCC 739, [f]Kseudomonas aeruginosa MTCC 2453, [g]Klebsiella planticola MTCC 530, [h]*Candida albicans* MTCC 3017,[i] No activity.

Compound **9j** was found to be most active among the series and equipotent to the standard drug ciprofloxacin with MIC value of 0.9 µg/ml against *M. luteus* MTCC 2470 and *K. planticola* MTCC 530. Compound **9j** was found to be more promising against all the remaining bacterial strains used withMIC value of 1.9 µg/ml. For *E. coli* MTCC 739, the MIC value observed was 3.9 µg/ml. Compound **9k** was found to be equipotent to ciprofloxacin against *S. aureus* MTCC 96 and

showed excellent MIC value of 1.9 µg/ml against *M. luteus* MTCC 2470, *B. subtilis* MTCC 121 *E.coli* MTCC 739 and *P. aeruginosa* MTCC 2453.

Bactericidal activity

Compounds were evaluated for bactericidal activity (**Table 4**) and they exhibited good to promising activity against all the tested bacterial strains. All the compounds showed bactericidal effect on different strains with MBC value ranging from 3.9 to 62.5 µg/ml. Compound **9j** was found to be better in this regard as compared to ciprofloxacin with MBC value of 0.9 µg/ml against *M. luteus* MTCC 2470 and promising against *S. aureus* MLS16 MTCC 2940, *P. aeruginosa* MTCC 2453 and *K. planticola* MTCC 530. Compound **9k** was also found promising against *Bacillus subtilis* MTCC 121 with MBC value of 1.9 µg/ml.

Table 4: MBC values of compounds against several bacterial strains

		Minimum bactericidal concentration (µg/ml)						
Entry	**Compound**	**M.l[a]**	**S.a[b]**	**S.a[c]**	**B.s[d]**	**E.c[e]**	**P.a[f]**	**K.p[g]**
1	**9a**	31.2±0.46	31.2±0.24	62.5±0.42	31.2±0.38	15.6±0.26	7.8±0.22	31.2±0.22
2	**9b**	3.9±0.28	3.9±0.18	15.6±0.36	7.8±0.12	7.8±0.35	15.6±0.26	3.9±0.28
3	**9f**	7.8±0.34	3.9±0.26	15.6±0.32	3.9±0.36	7.8±0.34	3.9±0.22	7.8±0.26
4	**9g**	15.6±0.42	7.8±0.32	31.2±0.28	3.9±0.28	15.6±0.26	15.6±0.38	31.2±0.18
5	**9i**	3.9±0.18	3.9±0.16	7.8±0.24	7.8±0.26	15.6±0.28	7.8±0.14	3.8±0.24
6	**9j**	0.9±0.08	3.9±0.22	1.9±0.11	3.9±0.14	7.8±0.28	1.9±0.16	0.9±0.08
7	**9k**	3.9±0.22	3.9±0.14	31.2±0.28	1.9±0.22	3.9±0.16	3.9±0.24	62.5±0.35
8	**9l**	>125	>125	>125	>125	62.5±0.52	62.5±0.48	>125
Ciprofloxacin (standard)		1.17±0.11	0.58±0.09	1.17±0.12	0.58±0.06	0.58±0.08	1.17±0.08	0.58±0.09

Anti-biofilm activity

Compounds exhibiting antibacterial and bactericidal properties were also checked for bacterial biofilm inhibition (**Table 5**). They were found to inhibit the biofilm formation with IC_{50} values ranging between 0.3 and 30 µg/ml. Compound **9j** was found to be potent as compared to that of erythromycin against *M. luteus* MTCC 2470, S. aureus MLS16 MTCC 2940 and *P. aeruginosa* MTCC 2453. However, it was observed that not even a single compound was active against the fungal strain *Candida albicans* MTCC 3017 even at the maximum tested concentration (125µg/ml).

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Table 5: Anti-biofilm activity of compounds against different strains.

		IC50 values (µg/ml)						
Entry	**Comp**	**M.l[a]**	**S.a[b]**	**S.a[c]**	**B.s[d]**	**E.c[e]**	**P.a[f]**	**K.p[g]**
1	**9a**	22.3 ± 0.54	25.3 ± 0.67	29.5 ± 0.66	18.4 ± 0.63	6.2 ± 0.42	3.2 ± 0.12	16.9 ± 0.58
2	**9b**	2.4 ± 0.16	3.9 ± 0.15	10.1 ± 0.38	3.2 ± 0.16	3.3 ± 0.28	7.2 ± 0.52	1.3 ± 0.12
3	**9f**	3.1 ± 0.13	2.9 ± 0.16	9.2 ± 0.28	2.1 ± 0.16	4.2 ± 0.26	2.9 ± 0.18	6.1 ± 0.32
4	**9g**	6.8 ± 0.22	6.1 ± 0.22	21.6 ± 0.56	2.6 ± 0.22	11.2 ± 0.44	8.2 ± 0.44	18.7 ± 0.44
5	**9i**	2.8 ± 0.16	3.1 ± 0.51	4.2 ± 0.21	4.6 ± 0.24	8.1 ± 0.42	2.8 ± 0.22	1.1 ± 0.18
6	**9j**	0.3 ± 0.11	2.6 ± 0.13	0.6 ± 0.12	2.6 ± 0.12	2.9 ± 0.24	0.6 ± 0.12	0.2 ± 0.08
7	**9k**	1.2 ± 0.09	3.1 ± 0.26	15.6 ± 0.34	0.8 ± 0.21	2.6 ± 0.12	2.2 ± 0.23	42.3 ± 0.51
Erythromycin		0.3 ± 0.08	0.2 ± 0.07	0.6 ± 0.08	0.3 ± 0.11	0.2 ± 0.09	0.5 ± 0.11	0.1 ± 0.05

The structure–activity relationship study revealed that NO_2 group on quinoline ring was important for antimicrobial, bactericidal as well as for anti-biofilm activity against all the tested strains. However, this NO_2 group if present on benzene ring attached to double bond (styryl benzene) was found to be undesirable for activity. Similarly, the OCF_3 and 3,4,5-trimethoxy groups on styryl benzene ring were found essential for activity against all strains. It was observed that if both NO_2 and OCF_3 groups were present then the compound exhibited activity with an increase in MIC value. The activity of compound 9f against *S. aureus* was only due to the presence of OCF_3. Likewise, the presence of 3,5-diCF_3, OMe, OH, Cl groups on the styryl benzene ring were responsible for the activity.

Comparison with FDA-approved drugs and PMI plot

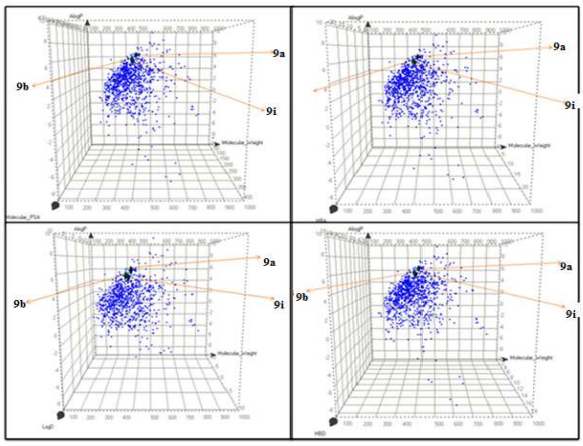

Fig.3: 1011 FDA-approved drugs (light-blue dots), as reported in the GOSTAR database (GVK Bioscience proprietary database)

Using different molecular scaffolds from various drugs, we assessed the skeletal diversity that was afforded by the 2-styryl quinolines (**9a-l**) and their related chemical descriptors by using in silico methods. We plotted them in chemical space plots that corresponded to a set of six key descriptors (i.e. polar surface area (PSA), solubility, hydrogen bond acceptor (HBA), hydrogen bond donor (HBD), logA, and logD) relative to 1011 FDA approved drugs (light-blue dots), as reported in the GOSTAR database (**Fig. 1(a)**)[29-31]. The plots showed that these quinolines (**Fig. 3**), dark-green dots) are not "outliers" with respect to these parameters when compared to marketed drugs.

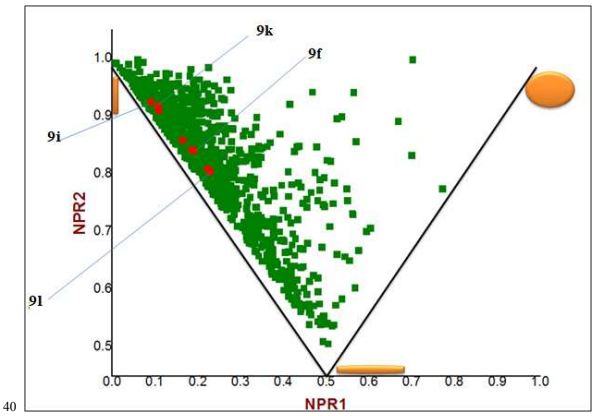

Fig.4: PMI plot of our group of molecules relative to 1011 FDA-approved drugs. The PMI calculations involved aligning each molecule to principal moment axes by using SYBYL and the normalized PMI values were calculated by using in-house software.

In addition, the synthesized compounds were plotted according to the normalized principal moment of inertia (PMI) formalism of Sauer and Schwartz.[32] Since molecular shape and biological activity are intricately related, the screening of molecules that have a higher degree of built-in molecular-shape diversity translates into a greater probability of discovering compounds with biological activity. The three corners of the isosceles triangle of the PMI plot are dominated by rods, spheres, and discs, respectively (the shapes are representations of the overall shape of the constituent molecules). From the graph, it is clear that the FDA approved drugs cover the left edge of the PMI space taking shapes intermediate between rods and discs, 2-styryl quinolines (**9a-l**) falling under the same area where the drug compounds fall in the graph. 2-styryl quinolines (**9a-l**) are falling at the top of the left edge of the PMI space (**Fig. 4**).[33] The bioactivity track record of drug compounds is well-established. It can be said that the similar shapes of our compounds to those of drug compounds will increase the likelihood of discovering biologically relevant molecules by using this method.

Conclusion

2-Styrylquinolines (**9a-l**) have been synthesized regioselectively from 2-methyl-3-acetyl-4-phenyl quinoline by using sodium acetate in binary system (water:acetic acid) as a green solvent. These compounds were further evaluated for

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their anti-microbial, bactericidal and biofilm inhibition activities. All the compounds were found to be active and few of them showed promising to excellent activities in the range between 0.9-3.9 µg/ml. The skeletal diversity and related chemical descriptors obtained from these compounds when compared with 1011 FDA approved drugs, aligns in the same region where the drugs lies in the plot. Similarly, the PMI plot showed that they are falling under the same area where the drug compounds appear in the graph. Based on the biological and theoretical data obtained, it can be concluded that the synthesized quinoline compounds have the potential to be explored for further optimization.

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Experimental section

I. Chemistry

All chemicals and reagents were obtained from Aldrich (Sigma–Aldrich, St. Louis, MO, USA) or AlfaAesar (Johnson Matthey Company, Ward Hill, MA, USA). Reactions were monitored by TLC, performed on silica gel glass plates containing 60 F-254, and visualization on TLC was achieved by UV light or iodine indicator. Column chromatography was performed with Merck 60–120 mesh silica gel. 1H and 13C NMR spectra were recorded on Gemini Varian-VXR-unity (200, 400, 500 MHz) or Bruker UXNMR/XWIN-NMR (300 MHz) instruments. Chemical shifts (δ) are expressed in ppm relative to internal standard TMS. Multiplicities of NMR signals are represented as singlet (s), doublet (d), doublet of doublet (dd), triplet (t), quartet (q) and multiplet (m). ESI spectra were recorded on Micro mass, Quattro LC using ESI+ software with capillary voltage 3.98 kV and ESI mode positive ion trap detector.

1. General procedure for the synthesis of compounds (7a-b).

2-aminobenzophenoneor5-nitro-2-aminobenzophenone(5gm, 0.025mol) and acetyl acetone (2.53gm, 0.025mol) in 100ml acetonitrile was stirred. To this solution catalytic amount of HCl was added. Completion of reaction monitored by TLC. After completion, reaction cool to room temperature then solid observed. It was filtered and washed with hexane. Yield: 83%, 5.5g, white solid (**7a**).

3-Acetyl-2-methyl-4-phenylquinoline (7a).

White solid, yield 83%; 1H NMR (200 MHz, CDCl3): δ 8.04 (d, J=8.3 Hz, 1H), 7.64-7.72(m, 1H), 7.56-7.62 (dd, J=8.3, 1.51 Hz,1H),7.47-7.54 (m, 3H,), 7.39-7.44 (dd, J=8.3, 1.51 Hz, 1H,), 7.32-7.38 (m, 2H,), 2.67 (s,3H,), 1.96 (s, 3H,); IR(KBr): υ 3053, 3028, 1708, 1636, 1581, 1484, 1459, 1384, 1288, 1159, 1041, 880, 709, 651; ESI-MS: 262 $(M+H)^+$.

2. General procedure for the synthesis of compounds (9a-l).

200 mg (0.76 mmol) **7a** in 14 ml H_2O:AcOH (1:1) solvent was taken with 114 mg (0.84 mmol) p-methoxy benzaldehyde stirred for 5 min and 10 equivalents of NaOAc was added to this solution. Now reaction kept under reflux 120 ^oC for 8-16 hours. Completion of reaction checked by TLC .After rotary evaporation, reaction mixture was neutralized with $NaHCO_3$ and water (5ml) and chloroform (20ml) were added and the aqueous layer was extracted with chloroform (2x25ml). The combined organic layer was dried over anhydrous Na_2SO_4 and concentrated by rotary evaporation to afford crude product which was further purified by column chromatography using ethyl acetate and hexane as solvent system.

***(E)*-1-(2-(4-Chlorostyryl)-4-phenylquinolin-3-yl) ethanone (9a).**

Yields 80%; mp: 201-203 ^oC; ^1H NMR (500 MHz, CDCl3): δ 2.07 (s, 3H), 7.16 (d, J=15.41 Hz, 1H), 7.34-7.39 (m, 3H), 7.44-7.47 (m, 2H), 7.52-7.55 (m, 5H), 7.60-7.62 (d, J=7.93,1H); 7.71-7.76 (m, 1H); 8.01-8.04 (d, J=15.41 Hz, 1H), 8.15-8.17 (d, J=8.39 Hz, 1H); ^{13}C NMR (125 MHz, CDCl3): δ 32.82, 124.57, 125.61, 126.19, 126.87, 128.72, 128.76, 128.89, 128.95, 129.52, 130.06, 130.41, 134.46, 134.99, 135.14, 135.32, 144.54, 147.92, 149.82, 205.81; IR(KBr): υ 3423, 2923, 2853, 2361, 1654, 1508, 1482, 1122, 1092, 967, 765, 669 cm-1; MS–ESIMS: m/z 384 $(M+H)^+$; HR ESIMS: m/z calcd for $C_{25}H_{19}ClNO$: 384.1149; found: 384.1152.

***(E)*-1-(2-(4-methoxystyryl)-4-phenylquinolin-3-yl) ethanone (9b).**

Yields 75%; mp: 213-215 ^oC; ^1H NMR (500 MHz, CDCl3): δ 2.08 (s, 3H), 3.84 (s, 3H) 6.91-6.93 (d, J=8.69 Hz, 2H), 7.05-7.08 (d, J=15.41 Hz, 1H), 7.36-7.39 (m, 2H), 7.40-7.44 (m, 1H), 7.53-7.55 (m, 3H), 7.56-7.59 (m, 3H); 7.71-7.74 (m, 1H); 8.02-8.05 (d, J=15.41 Hz, 1H), 8.13-8.15 (d, J=8.39 Hz, 1H); ^{13}C NMR (125 MHz, CDCl3): δ 32.84, 55.32, 114.12, 121.80 125.44, 126.15, 126.48, 128.63, 128.82, 129.05, 129.31, 129.40, 130.07, 130.22, 134.43, 135.24, 136.35, 144.23, 147.96, 150.44, 160.23, 205.95; IR(KBr): υ 3423, 2924, 2836, 2360, 1632, 1603, 1508, 1483, 1260, 1247, 1158, 1025, 971, 835, 811, 768, 755, 706 cm-1; MS–ESIMS: m/z 380 $(M+H)^+$; HR ESIMS: m/z calcd for $C_{26}H_{22}NO_2$: 380.1645; found: 380.1647.

***(E)*-1-(2-(4-methoxy-3-nitrostyryl)-4-phenylquinoline-3-yl) ethanone (9c).**

Yields 85%; mp: 209-211 ^oC; ^1H NMR (500 MHz, CDCl3): δ 2.07 (s, 3H), 4.00 (s, 3H) 7.10-7.12 (d, J=8.85 Hz, 1H), 7.13-7.16 (d, J=15.41 Hz, 1H), 7.38-7.40 (m, 2H), 7.44-7.48 (m, 1H), 7.52-7.55 (m, 3H), 7.61-7.63 (dd, J=7.62, 0.91 Hz, 1H); 7.74-7.80 (m, 2H); 8.00-8.03 (d, J=15.56 Hz, 1H), 8.07-8.08 (d, J=2.28 Hz, 1H), 8.14-8.16 (d, J=8.08 Hz, 1H); ^{13}C NMR (125 MHz, CDCl3): δ 32.79, 56.67, 113.68, 124.42, 124.65, 125.64, 126.20, 126.98, 128.75, 129.00, 129.49, 130.04, 130.50, 132.87, 133.76, 134.38, 135.09, 139.82, 144.69, 147.88, 149.55, 152.91, 205.77; IR(KBr): υ 3428, 2927, 2360, 1696, 1617, 1564, 1530, 1441, 1415, 1353, 1270, 1208, 1165, 1086, 1016, 969, 821, 764, 705, 651 cm-1; MS–ESIMS: m/z 425 $(M+H)^+$; HR ESIMS: m/z calcd for $C_{26}H_{21}N_2O_4$: 425.1495; found: 425.1494.

***(E)*-1-(2-(4-nitrostyryl)-4-phenylquinolin-3-yl) ethanone (9d).**

Yields 86%; mp: 246-248 ^oC; ^1H NMR (500 MHz, CDCl3): δ 2.06 (s, 3H), 7.34-7.37 (d, J=15.41 Hz, 1H), 7.38-7.41 (m, 2H), 7.47-7.52 (m, 1H), 7.53-7.57 (m, 3H), 7.64-7.66 (d, J=7.78, 0.76 Hz, 1H), 7.74-7.76 (d, J=8.69 Hz, 2H), 7.76-7.80 (m, 1H);

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8.12-8.15 (d, J=15.56 Hz, 1H), 8.16-8.19 (d, J=8.39 Hz, 1H), 8.24-8.26 (d, J=8.69 Hz, 2H); ^{13}C NMR (75 MHz, CDCl3): δ 32.75, 124.04, 126.25, 127.38, 128.03, 128.29, 128.81, 129.11, 129.63, 130.12, 130.68, 133.90, 134.57, 134.98, 142.84, 144.93, 147.40, 147.90, 149.06, 153.16, 205.70; IR(KBr): υ 3421, 3064, 2925, 2359, 1692, 1595, 1517, 1481, 1443, 1394, 1337, 1207, 1156, 1052, 1025, 967, 870, 839, 765, 749, 656 cm-1; MS–ESIMS: m/z 395 $(M+H)^+$; HR ESIMS: m/z calcd for $C_{25}H_{19}N_2O_3$: 395.1390; found: 395.1397.

***(E)*-1-(2-(4-fluorostyryl)-4-phenylquinoline-3-yl) ethanone (9e).**

Yields 85%; mp: 180-182 °C; ^{1}H NMR (500 MHz, CDCl3): δ 2.07 (s, 3H), 7.05-7.09(t, J=8.69 Hz, 2H), 7.10-7.14 (d, J=15.56 Hz, 1H), 7.37-7.40 (m, 2H), 7.42-7.46 (m, 1H),7.51-7.55 (m, 3H), 7.57-7.61 (m, 3H); 7.72-7.76 (m, 1H); 8.02-8.05 (d, J=15.41 Hz, 1H), 8.15-8.17 (d, J=8.24 Hz, 1H); ^{13}C NMR (125 MHz, CDCl3): δ 32.80, 115.62, 115.79, 123.76, 125.55, 126.17, 126.76, 128.69, 128.91, 129.21, 129.28, 129.48, 130.06, 130.35, 132.71, 134.43, 135.15, 135.44, 144.46, 147.92, 149.98, 205.83; IR(KBr): υ 3064, 2927, 2852, 1692, 1632, 1539, 1506, 1483, 1158, 1127, 1052, 970, 822, 766, 706 cm-1; MS–ESIMS: m/z 368 $(M+H)^+$; HR ESIMS: m/z calcd for $C_{25}H_{19}FNO$: 368.1445; found: 368.1447.

***(E)*-1-(4-phenyl-2-(4-(trifluoromethoxy)styryl)quinolin-3-yl) ethanone (9f).**

Yields 83%; mp: 163-165 °C; ^{1}H NMR (300 MHz, CDCl3): δ 2.07 (s, 3H), 7.14-7.21 (d, J=15.86 Hz, 1H), 7.32-7.35 (m, 1H), 7.37-7.40 (m, 3H), 7.46-7.48 (d, J=7.55 Hz, 1H), 7.49-7.55 (m, 3H), 7.60-7.65 (m, 3H); 7.73-7.78 (m, 1H); 8.04-8.08 (d, J=15.86 Hz, 1H), 8.12-8.17 (d, J=8.30 Hz, 1H); ^{13}C NMR (75 MHz, CDCl3): δ 32.78, 121.05, 125.02, 125.65, 126.19, 126.91, 128.43, 128.71, 128.85, 128.95, 129.55, 129.81, 130.06, 130.41, 134.98, 144.57, 147.93, 149.29, 149.74, 205.72; IR(KBr): υ 3065, 2922, 2852, 1740, 1703, 1672, 1643, 1545, 1506, 1443, 1417, 1394, 1356, 1257, 1210, 1014, 963, 844, 767, 705, 671, 657 cm-1; MS–ESIMS: m/z 434 $(M+H)^+$; HR ESIMS: m/z calcd for $C_{26}H_{19}F_3NO_2$: 434.1362; found: 434.1360.

***(E)*-1-(2-(3,5-bis(trifluoromethyl)styryl-4-phenylquinolin-3-yl) ethanone (9g).**

Yields 81%; mp: 159-161 °C; ^{1}H NMR (300 MHz, CDCl3): δ 2.07 (s, 3H), 7.30-7.36 (d, J=15.48 Hz, 1H), 7.38-7.41 (m, 2H), 7.46-7.52 (m, 1H), 7.53-7.56 (m, 3H), 7.61-7.63 (dd, J=7.55, 0.75 Hz, 1H); 7.76-7.81 (m, 2H); 7.99-8.00 (m, 2H), 8.13-8.18 (d, J=15.67 Hz, 1H), 8.16-8.20 (d, J=8.49 Hz, 1H); ^{13}C NMR (75 MHz, CDCl3): δ 32.76, 125.88, 126.29, 127.19, 127.40, 127.67, 129.15, 129.72, 130.09, 130.25, 130.71, 131.89, 132.34, 133.43, 134.51, 135.06, 138.61, 145.00, 147.93, 148.98, 205.65; IR(KBr): υ 3428, 3069, 2925, 2854, 1739, 1704, 1672, 1610, 1560, 1542, 1451, 1382, 1249, 1183, 1126, 1023, 1009, 951, 892, 773, 764, 682, 659 cm-1; MS–ESIMS: m/z 486 $(M+H)^+$; HR ESIMS: m/z calcd for $C_{27}H_{18}F_6NO$: 486.1287; found: 486.1282.

***(E)*-1-(4-phenyl-2-(3,4,5-trimethoxystyryl)quinolin-3-yl) ethanone (9h).**

Yield 82%; mp: 172-174 °C; ^{1}H NMR (300 MHz CDCl3): δ 2.09 (s, 3H), 3.89 (s, 3H), 3.93 (s, 6H), 6.84 (s, 2H), 7.10-7.14 (d, J=15.67 Hz, 1H), 7.38-7.41 (m, 2H), 7.47 (d, J=7.55 Hz, 1H), 7.52-7.54 (m, 3H), 7.61 (d, J=7.93 Hz, 1H), 7.74 (t, J=7.55 Hz, 1H), 7.98 (J=15.48 Hz, 1H), 8.16 (d, J=8.49 Hz, 1H); ^{13}C NMR (125 MHz, CDCl3): δ 32.81, 56.19, 60.93, 104.77, 106.65, 123.40, 125.51, 126.17, 126.68, 128.65, 128.87, 129.41, 130.03, 130.32, 132.14, 134.35, 135.14, 136.87, 138.95, 144.43, 147.90, 150.03, 153.31, 205.82; IR(KBr): υ 2939, 2826, 1693, 1633, 1610, 1580, 1503, 1455, 1357, 1238, 1118, 1008, 968, 836, 768, 702, 650 cm-1; MS–ESIMS: m/z 440 $(M+H)^+$; HR ESIMS: m/z calcd for $C_{28}H_{26}NO_4$: 440.1856; found: 440.1848.

***(E)*-1-(2-(4-hydroxy-3-methoxystyryl)-4-phenylquinolin-3-yl) ethanone (9i).**

Yields 82%; mp: 230-232 °C; 1H NMR (300 MHz, CDCl3): δ 2.09 (s, 3H), 3.95 (s, 3H), 6.78-6.86 (m, 1H), 6.88-6.94 (d, J=8.30 Hz, 1H), 7.02-7.07 (d, J=15.48 Hz, 1H), 7.35-7.45 (m, 5H), 7.51-7.56 (m, 3H), 7.70-7.75 (m, 1H), 7.95-8.03 (d, J=15.48 Hz, 1H), 8.13-8.21 (m, 1H), 9.82 (s, 1H); ^{13}C NMR (75 MHz, CDCl3): δ 29.12, 54.98, 110.04, 114.74, 115.03, 120.72, 121.02, 125.66, 126.01, 128.22, 128.80, 129.52, 129.78, 134.90, 136.50, 143.84, 146.11, 147.21, 149.90, 152.56, 205.14; IR(KBr): υ 3420, 2923, 2852, 2359, 1743, 1634, 1540, 1506, 1456, 1434, 1383, 1162, 1123, 703, 615 cm-1; MS–ESIMS: m/z 396 $(M+H)^+$; HR ESIMS: m/z calcd for $C_{26}H_{22}NO_3$: 396.1594; found: 396.1604.

***(E)*-1-(6-nitro-4-phenyl-2-(4-trifluoromethoxy)styryl)quinolin-3-yl) ethanone (9j).**

Yields 90%; mp: 208-210 °C; ^{1}H NMR (500 MHz, CDCl3): δ 2.08 (s, 3H), 7.14-7.17 (d, J=15.41 Hz, 1H), 7.24-7.26 (m, 2H), 7.38-7.41 (m, 2H), 7.57-7.60 (m, 3H), 7.65-7.67 (d, J=8.69 Hz, 2H); 8.18-8.21 (d, J=15.41 Hz, 1H), 8.25-8.27 (d, J=9.15 Hz, 1H), 8.49-8.51 (dd, J=6.86, 2.28 Hz, 1H), 8.56-8.57 (d, J=2.28 Hz, 1H); ^{13}C NMR (75 MHz, CDCl3): δ 32.49, 121.19, 122.12, 123.29, 123.95, 124.74, 129.28, 129.92, 129.98, 131.23, 133.61, 134.58, 135.80, 137.51, 145.63, 146.56, 149.94, 153.25, 204.69; IR(KBr): υ 3395, 3064, 2921, 2851, 1708, 1633, 1617, 1508, 1477, 1444, 1338, 1257, 1214, 1052, 967, 844, 764, 732, 669, 642 cm-1; MS–ESIMS: m/z 479 $(M+H)^+$; HR ESIMS: m/z calcd for $C_{26}H_{18}F_3N_2O_4$: 479.1213; found: 479.1208.

***(E)*-1-(6-nitro-4-phenyl-2-(3,4,5-trimethoxystyryl)quinolin-3-yl) ethanone (9k).**

Yield 88%; mp: 217-219 °C; ^{1}H NMR (500 MHz CDCl3): δ 2.09 (s, 3H), 3.90 (s, 3H), 3.94 (s, 6H), 6.85 (s, 2H), 7.05-7.10 (d, J=15.41 Hz, 1H), 7.37-7.41 (m, 2H), 7.59-7.61 (m, 3H), 7.13-7.16 (d, J=15.41 Hz, 1H), 8.23-8.25 (d, J=7.15 Hz, 1H), 8.48-8.51 (dd, J=6.71, 2.44Hz, 1H), 8.54-8.55 (d, J=2.44 Hz, 1H); ^{13}C NMR (75 MHz, CDCl3): δ 32.55, 56.20, 60.94, 105.08, 122.21, 123.22, 123.78, 124.54, 129.16, 129.77, 129.89, 131.00, 131.46, 133.58, 139.46, 149.91, 153.37, 153.51, 204.80; IR(KBr): υ 3432, 2937, 2837, 2360, 1705, 1630, 1573, 1544, 1525, 1452, 1432, 1340, 1249, 1197, 1005, 970, 839, 790, 704, 651 cm-1; MS–ESIMS: m/z 485 $(M+H)^+$; HR ESIMS: m/z calcd for $C_{28}H_{25}N_2O_6$: 485.1707; found: 485.1700.

***(E)*-1-(2-(4-hydroxy-3-methoxystyryl)-6-nitro-4-phenylquinolin-3-yl) ethanone (9l).**

Yields 85%; mp: 243-245 °C; ^{1}H NMR (300 MHz, CDCl3): δ 2.09 (s, 3H), 3.97 (s, 3H), 6.94-6.97 (d, J=8.12 Hz, 1H), 6.99-7.05 (d, J=15.29 Hz, 1H), 7.11-7.12 (d, J=1.32 Hz, 1H), 7.20-7.26 (d, J=6.79, 1.32 Hz, 1H), 7.37-7.42 (m, 2H), 7.58-7.60 (m, 3H), 8.13-8.19 (d, J=15.48 Hz, 1H), 8.21-8.24 (d, J=9.25 Hz, 1H), 8.46-8.54 (m, 2H); ^{13}C NMR (75 MHz, DMSO-

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d6+CDCl3): δ 31.48, 54.92, 110.06, 114.82, 118.83, 120.96, 121.97, 122.51, 123.24 126.55, 128.01, 128.81, 129.82, 132.60, 134.54, 138.51, 143.94, 146.86, 147.74, 152.80, 203.57; IR(KBr): υ 3290, 3004, 2963, 1666, 1615, 1594, 1543, 1517, 1445, 1422, 1375, 1313, 1263, 1148, 1087, 1035, 968, 907, 762, 709, 657, 645, 629 cm-1; MS–ESIMS: m/z 441 $(M+H)^+$; HR ESI MS: m/z calcd for $C_{26}H_{21}N_2O5$: 441.1445; found: 441.1446.

II. X-ray crystallographic study.

X-ray data for the compounds were collected at room temperature using a Bruker Smart Apex CCD diffractometer with graphite monochromated MoK□ radiation (□=0.71073Å) with ω-scan method[34]. Preliminary lattice parameters and orientation matrices were obtained from four sets of frames.Integration and scaling of intensity data were accomplished using SAINT program[34]. The structure was solved by direct methods using SHELXS97[35] and refinement was carried out by full-matrix least-squares technique using SHELXL97. Anisotropic displacement parameters were included for all non-hydrogen atoms. The O-bound H atom was located in difference Fourier maps and their positions and isotropic displacement parameters were refined. All other H atoms were positioned geometrically and treated as riding on their parent C atoms [C-H = 0.93-0.97 Å and Uiso(H) = 1.5Ueq(C) for methyl H or 1.2Ueq(c) for other H atoms]. The methyl groups were allowed to rotate but not to tip.

Crystal data for AW80: $C_{26}H_{20}N_2O_5$, M = 440.44, colorless block, 0.18 x 0.16 x 0.07 mm3, monoclinic, space group P21/c (No. 14), a = 7.1637(7), b = 26.057(3), c = 11.5507(11) Å,□ □ = 95.513(2)°, V = 2146.1(4) Å3, Z = 4, Dc = 1.363 g/cm3, F000 = 920, Bruker SMART APEX CCD area-detector, MoKλ radiation, λ = 0.71073 Å, T = 294(2)K, 2□max = 50.0º, 20605 reflections collected, 3783 unique (Rint = 0.0502). Final GooF = 1.250, R1 = 0.0826, wR2 = 0.1581, R indices based on 3081 reflections with I>2σ(I) (refinement on F2), 304 parameters, 0 restraints, μ = 0.096 mm-1. CCDC 1023840 contains supplementary Crystallographic data for the structure. These data can be obtained free of charge at www.ccdc.cam.ac.uk/conts/retrieving.html [or from the Cambridge Crystallographic Data Centre (CCDC), 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44(0) 1223 336 033; email: deposit@ccdc.cam.ac.uk].

III.PMI Plot analysis

Materials & Methods:

In silico and PMI analysis were carried out on 1100 FDA-approved drugs (randomly taken from our GOSTAR proprietary database and our compounds. Each molecule was analyzed for a set of six physiochemical properties by using online cheminformatics tools, ChemDraw, or manual inspection. The data were collected on an Excel spreadsheet. These results were used to generate the plot shown in **Fig 1(a)**.The PMI calculations involved aligning each molecule to the principal moment axes in SYBYL and the normalized PMI values were calculated by using in-house software. The 2D chemical structures of the compounds were drawn in Discovery studio 3.5 (DS 3.5), whereas those of the FDA-approved drugs were collected from the GOSTAR (GvkbioOnline Structure Activity Relationship) database.

These compounds were imported into DS 3.5 and converted into 3D structures. Energy minimization was performed for all of these 3D structures to identify global/local minima by applying the CHARMm force field. The resulting 3Dstructures were used to calculate the three principal moments of inertia; the PMI values are sorted in ascending magnitude, that is, I_1, I_2, and I_3.Normalized PMI ratios (NPR) were calculated by dividing the two smaller PMI values (I_1 and I_2) by the largest PMI value (I_3) and generated two characteristic values for each compound (I_1/I_3 and I_2/I_3). These values were plotted against each other and the resulting graph formed an isosceles triangle that was defined by its three corners, wherein the vector [I_1/I_3, I_2/I_3] was equal to [1,1], [0.5,0.5], and [0,1] (**Fig. 1(b)**).

IV. Biology

1. Antimicrobial activity:

(a) Determination of MIC

The antimicrobial activity of the quinoline based styryl conjugates were determined using well diffusion method[36] (Amsterdam, 1996) against different pathogenic reference strains which were procured from the Microbial Type Culture Collection (MTCC), CSIR-Institute of Microbial Technology, Chandigarh, India. The pathogenic reference strains were seeded on the surface of the media petri plates, containing Muller-Hinton agar with 0.1 ml of previously prepared microbial suspensions individually containing 1.5 × 108 cfu ml-1 (equal to 0.5 McFarland). Wells of 6.0 mm diameter were prepared in the media plates using a cork borer and the synthesized quinoline based styryl conjugates at a dose range of 125 - 0.9 µg well-1 were added in each well under sterile conditions in a laminar air flow chamber. Standard antibiotic solution of ciprofloxacin and miconazole at a dose range of 125 - 0.9 µg well-1 and the well containing methanol served as positive and negative controls, respectively. The plates were incubated for 24 h at 37°C for bacterial and 30°C for Candida albicans and the well containing the least concentration showing the inhibition zone was considered as the minimum inhibitory concentration. All experiments were carried out in duplicates and values are represented as mean ± S.D.

(b) Determination of MBC

Bactericidal assay (NCCLS, 2000) was performed in sterile 2.0 ml microfuge tubes against a panel of pathogenic bacterial strains,[37] including Micrococcus luteus MTCC 2470, Staphylococcus aureus MTCC 96, Staphylococcus aureus MLS-16 MTCC 2940, Bacillus subtilis MTCC 121, Escherichia coli MTCC 739, Pseudomonas aeruginosa MTCC 2453 and Klebsiella planticola MTCC 530 which were cultured overnight in Mueller Hinton broth. Serial dilutions of test compounds were prepared in Mueller Hinton broth with different concentrations ranging from 0 to125 µg ml-1. To the test compounds, 100 µl of overnight cultured bacterial suspensions were added to reach a final concentration of 1.5 × 108 cfu ml-1 (equal to 0.5 McFarland) and incubated at 37 ºC for 24 h. After 24 h of incubation, the minimum bactericidal concentration (MBC) was determined by sampling 10 µl of suspension from the tubes onto Mueller Hinton agar plates and

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were incubated for 24 h at 37 ºC to observe the growth of test organisms. MBC is the lowest concentration of compound required to kill a particular bacterium. All the experiments were carried in duplicates values are represented as mean ± S.D.

2. Biofilm inhibition crystal violet assay

The test compounds were screened in sterile 96 well polystyrene microtiter plates using the modified biofilm inhibition assay,[38] against a panel of pathogenic bacterial strains including Staphylococcus aureus MTCC 96, Staphylococcus aureus MLS-16 MTCC 2940 and Klebsiella planticola MTCC 530, which were cultured overnight in tryptone soy broth (supplemented with 0.5% glucose). The test compounds of predetermined concentrations ranging from 0 to 250 µg/ml were mixed with the bacterial suspensions having an initial inoculum concentration of 5×105 CFU/ml. Aliquots of 100 µl were distributed in each well and then incubated at 37 ºC for 24 h under static conditions. The medium was then discarded and washed with phosphate buffered saline to remove the non-adherent bacteria. Each well of the microtiter plate was stained with 100 µl of 0.1% crystal violet solution followed by 30 min incubation at room temperature. Later the crystal violet solution from the plates was discarded, thoroughly washed with distilled water for 3 to 4 times and air dried at room temperature. The crystal violet stained biofilm was solubilised in 95% ethanol (100 µl) and the absorbance was recorded at 540 nm using TRIAD multimode reader (Dynex Technologies, Inc, Chantilly, VA, USA). Blank wells were employed as background check. The inhibition data were interpreted from the dose-response curves, where IC50 value is defined as the concentration of inhibitor required to inhibit 50% of biofilm formation under the above assay conditions. All the experiments were carried out in triplicates and the values are indicated as mean ± S.D.

References and Notes

1. R. L. Monaghan, J. F. Barrett, *Biochem. Pharmacol*., 2006, **71**, 901.
2. (*a*) A. Dalhoff, *Infection.,*1994, **22**, S111.; (*b*) D. Abbanat, M. Macielag, K. Bush, *Expert. Opin. Investig. Drugs.,* 2003, **12**, 379.; (*c*) K. Poole, *Curr. Opin. Microbiol.,* 2001, 4, 500. (*d*) C. T. Walsh, G.Wright, *Chem. Rev.* 2005, **105**, 391.
3. M. E. Falagas, A. C. Kastoris, D. E. Karageorgopoulos, P. I. Rafailidis, *Int. J .Antimicrob. Agents*., 2009, 34, 111.
4. (*a*) M. C. McManus, *Am. J. Health-Syst. Pharm*., 1997, 54, 1420.; (*b*) F. C. Tenover, *Am. J. Infect. Control*., 2006, **34**, S3.
5. (*a*) H. Ceri, M. E. Olson, C. Stremick, R. R. Read, D. Morck, A. Buret, Clin. J. Microbiol. 1999, 37, 1771.; (*b*) M. Simoes, M. O. Pereira, M. J. Vieira, *Water. Sci. Technol.,* 2003, **47**, 217.; (*c*) R. Slinger, F. Chan, W. Ferris, S. W. Yeung, M. St Denis, I. Gaboury, S. D. Aaron, *Diagn. Microbiol. Infect. Dis*., 2006, **56**, 247.
6. A. Lilienkampf, J. Mao, B. Wan, Y. Wang, S. G. Franzblau, A. P. Kozikowski, *J. Med.Chem.,* 2009, **52**, 2109.
7. P. Nasveld, S. Kitchener, *Trans. R. Soc. Trop. Med. Hyg.,* 2005, **99**, 2.
8. P. A. Leatham, H. A. Bird, V. Wright, D. Seymour, A. Gordon, Eur. J. Rheumatol. Inflamm., 1983, **6**, 209.
9. W. A. Denny, W. R. Wilson, D. C. Ware, G. J. Atwell, J. B. Milbank, R. J. Stevenson, *USPat.,* 2006, 7064117.
10. A. Mahamoud, J. Chevalier, A. Davin-Regli, J. Barbe, J. M. Pages, *Curr. Drug Targ.,* 2006, **7**, 843.
11. N. Muruganantham, R. Sivakumar, N. Anbalagan, V. Gunasekaran, J. T. Leonard, *Biol. Pharm. Bull.,* 2004, **27**, 1683.
12. M. P. Maguire, K. R. Sheets, K. McVety, A. P. Spada, A. A. Zilberstein, *J. Med. Chem.,* 1994, **37**, 2129.
13. W. D. Wilson, M. Zhao, S. E. Patterson, R. L. Wydra, L. Janda, L. Strekowski, R. F. Schinazi, *Med. Chem. Res.,* 1992, **2**, 102.
14. L. Strekowski, J. L. Mokrosz, V. A. Honkan, A. Czarny, M. T. Cegla, S. E. Patterson, R. L. Wydra, R. F. Schinazi, *J. Med. Chem.,* 1991, **34**, 1738.
15. G. S. Bisacchi, J. Dumas, In: Macor JE, ed. *Annual Reports* in Medicinal Chemistry., Amsterdam Academic Press. 2009, **44**, 379.
16. (*a*) C. Ryu, J. Lee, S. Jeong, J. Nho, Bioorg. *Med. Chem. Lett.,* 2009, **19**, 146.; (*b*) R. Bhavana, S. Weng, E. Tiekink, J. Organomet. Chem., 2013,**726**, 62.
17. S. Gogoi, K. Shekarrao, A. Duarah, T. Bora, S. Gogoi, R. Boruah, *Steroids.,* 2012, **77**, 1438.
18. S. Nagarajan, P. Arjun, N. Raaman, T. Mohan Das, *Carbohydr. Res.,* 2010, **345**, 1988.
19. N. Parmar, H. Barad, B. Pansuriya, S. Teraiya, V. Gupta, R. Kant, Bioorg. *Med. Chem. Lett.,* 2012, **22**, 3816.
20. (*a*) K. Andries, P. Verhasselt, J. Guillemont, H. W. Gohlmann, J. M. Neefs, H. Winkler, J. Van Gestel, P. Timmerman, M. Zhu, E. Lee, *Science.,* 2005, **307**, 223.; (*b*) Walker, Joseph, Tadena, Nathalie, *Wall Street Journal.,* 2012, Retrieved.2013-01-01.; (c) Edney, Anna, Bloomberg. 2012, Retrieved. 2013-01-01.
21. A. Kamal, P. Swapna, R. V. C. R. N. C. Shetti, A. B. Shaik, M. P. N. Rao, S. Gupta, *Eur. J. Med. Chem.,* 2013, **62**, 661.
22. A. Kamal, S. K. Ahmed, K. S. Reddy, M. N. A. Khan, R. V. C. R. N. C. Shetty, B. Siddhardha, U. S. N. Murthy, I. A. Khan, M. Kumar, S. Sharma, A. B. Ram, Bioorg. Med. Chem. Lett., 2007, **17**, 5419.
23. A. Kamal, R. V. C. R. N. C. Shetti, S. Azeeza, P. Swapna, M. N. A. Khan, I. A. Khan, S. Sharma, S. T. Abdullah, *Eur. J. Med. Chem.,* 2011, **46**, 893.
24. A. Kamal, J. N. S. R. C. Murty, A. Viswanath, P. Sujitha, C. G. Kumar, *Bioorg. Med. Chem. Lett.,* 2012, **22**, 4891.
25. A. Kamal, S. M. A. Hussaini, S. Faazil, Y. Poornachandra, G. N. Reddy, C. G. Kumar, *Bioorg. Med. Chem. Lett.,* 2013, **23**, 6842.
26. *(a)* S. K. Chervenkov, A. I. Pavlov, S. T. Slavova, *Analytical* letters., 1995, **28**, 57.; *(b)* C. Rahime, N. Jan, D. Elena, Thomas J. J. Müller, *Org. Biomol. chem* ., 2013, **11**, 2597.
27. A. Kamal, A. Mallareddy, P. Suresh, V. L. Nayak, R.V.C.R.N.C. Shetti, N. S. Rao, J. R. Tamboli, T. B. Shaik, M.V.P.S. Vishnuwardhan, S. Ramakrishna, *Eur. J. Med. Chem.,* 2012, **47**, 530.
28. Details are available in experimetal section.
29. (*a*) GVK Biosciences Pvt. Ltd. proprietary database.; (*b*) Stable 3D structures of all of the compounds were used to calculate their physicochemical properties, such as molecular weight, number of hydrogen-bond donors (HBD), number of hydrogen-bond acceptors(HBA), A logP, log D, and polar surface area (PSA); these values were calculated by using Discovery studio 3.1, *Accelrys. Inc.*
30. (*a*) P. Ertl, B. Rohde, P. Selzer, *J. Med. Chem.,* 2000, **43**, 3714.; (*b*) P. Ertl, Molecular. Drug. Properties. R. Mannhold (Ed). *Wiley-VCH.,* 2007, 111.
31. SYBYL 8.0. The Tripos Associates., St. Louis. 2008.
32. W. H. Sauer, M. K. Schwartz, *J. Chem. Inf. Comput. Sci.,* 2003, **43**, 987.
33. A. W. Hung, A. Ramek, Y. Wang, T. Kaya, J. A. Wilson, P. A. Clemonsa, D. W. Younga, *Proc. Natl. Acad. Sci. U.S.A.,* 2011, **108**, 6799.
34. SMART & SAINT. Software reference manuals. Versions 6.28a & 5.625, Bruker Analytical X-ray Systems Inc.

View Article Online
DOI: 10.1039/C4OB02277G

Madison. Wisconsin. U.S.A., 2001.
35. G. M. Sheldrick, SHELXS97 and SHELXL97, Programs for crystal structure solution and refinement; University of Gottingen: Germany, 1997.
36. D. M.Baltimore, In: Loman, V.(Ed.) D. Amsterdam. Antibiotics in LaboratoryMedicine., 4th Edition, Williams and Wilkins, 1996, 52.
37. P. A. Wayne, National Committee for Clinical Laboratory Standards, Approved standard 5 edi, NCCLS 2000. .
38. R. E. Furlani, A. A. Yeagley, C. Melander, *Eur. J. Med. Chem.,* 2013, **62**, 59.

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