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$AlCl_3$ induced (hetero)arylation of 2,3-dichloroquinoxaline: A one-pot synthesis of mono/disubstituted quinoxalines as potential antitubercular agents

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ABSTRACT

A direct and single-step method has been developed for the synthesis of mono and 2,3-disubstituted quinoxalines by using a $AlCl_3$ induced (hetero)arylation of 2,3-dichloroquinoxaline. Both symmetrical and unsymmetrical 2,3-disubstituted quinoxalines can be prepared conveniently by using this method under appropriate reaction conditions. The reaction proceeds via C–C bond formation and can be utilized for the preparation of a variety of quinoxaline derivatives from readily available starting materials and reagents. The molecular structure of a representative compound was confirmed by single crystal X-ray diffraction study. Some of the compounds synthesized were tested for chorismate mutase inhibitory properties in vitro and one compound showed promising activity representing one of the few examples of chorismate mutase inhibition by a heteroarene based small molecule.

1. Introduction

The quinoxaline ring has been found to be integral part of a variety of biologically active compounds and many pharmaceutical agents[1] including several antibiotics such as Echinomycin, Levomycin and Actinoleutin. Both 2-substituted and 2,3-disubstituted aryl or heteroaryl quinoxaline derivatives have shown remarkable pharmacological activities. For example, compound **A** was found to be an inhibitor of p38 alpha mitogen-activated protein kinase[2] whereas compound **B** showed low nanomolar activity against Janus kinase 2 (JAK2) and potently suppressed proliferation of SET-2 cells in vitro (Fig. 1).[3] Compounds **C** and **D** were identified as selective antagonists at human A(1) and A(3) adenosine receptors.[4] A number of quinoxaline derivatives have exhibited antibacterial activities.[5,6] 3-Aryl and heteroaryl indoles on the other hand have shown promising antibacterial properties.[7] Thus combining the structural features of quinoxaline and indole moiety in a single molecule represented by **E** (Fig. 2) was thought to be an attractive template for the identification and development of promising antibacterial agents. Due to our continuing interest in the identification of novel anti-tuberculosis agents[8,9] we now wish to report the pharmacological evaluation of a series of 2-substituted and 2,3-disubstituted quinoxaline derivatives synthesis of which was carried out by an inexpensive and scalable C–C bond forming reaction.

A number of methods are known to introduce aryl or heteroaryl group at C-2 and/or C-3 position of a quinoxaline ring.[10,11] While all these approaches have their own merit and synthetic value

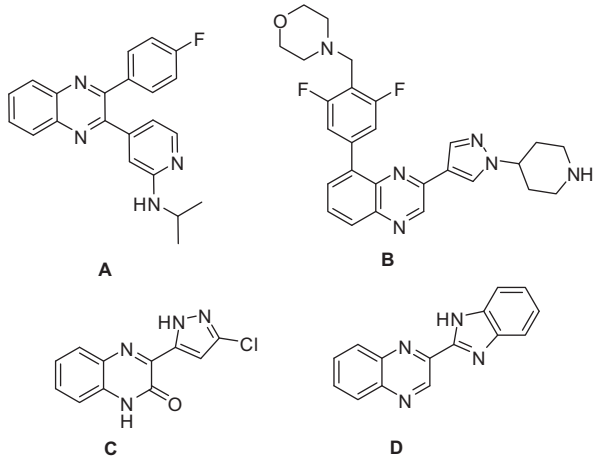

Figure 1. Quinoxaline-containing biologically active compounds.

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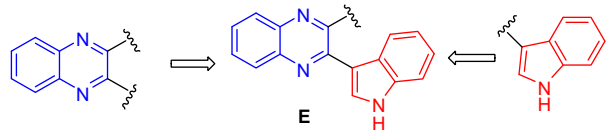
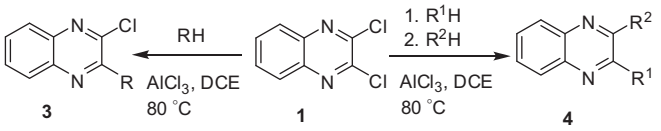


Figure 2. Design of new quinoxaline derivatives as potential antibacterial agents.



Scheme 1. $AlCl_3$ mediated mono and bis (hetero)arylation of 2,3-dichloro quinoxaline.

their use for the preparation of mono aryl or heteroaryl substituted quinoxalines however, remained inconvenient due to the lack of control or selectivity.[11] Only one method has been reported for the selective mono arylation of a quinoxaline ring using Suzuki coupling involving the use of palladium catalysts and boronic acids at 75–80 °C for 24 h.[12] In several cases the required boronic acids are either not available or often involved cumbersome preparation. We therefore were in need of a more convenient, practical and straightforward method for the preparation of 2-substituted and 2,3-disubstituted quinoxalines. In recent years we have been working on $AlCl_3$-induced direct heteroarylation of various aromatic and heterocyclic compounds via C–C bond forming reaction[13] and the methodology has found applications in the preparation of compounds of potential pharmacological interest.[14] In view of cost effectiveness and easy availability of starting materials we chose to adopt this $AlCl_3$ induced C–C bond forming reaction for our purpose (Scheme 1). To the best of our knowledge no successful mono or di (hetero)arylation of the quinoxaline ring system using a similar methodology has hitherto been described in the literature.

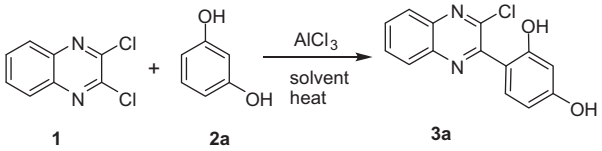
2. Results and discussion

2.1. Chemistry

For the generation of quinoxaline based small molecule library 2,3-dichloroquinoxaline[12] (**1**) was chosen as the starting compound and resorcinol (**2a**) was used as an arylating agent for our initial study. Due to the presence of two replaceable and chemically identical chloro groups in the quinoxaline **1** it was necessary to establish an optimized reaction condition initially to obtain mono substituted product **3a**. Accordingly, to avoid the second substitution the reaction of **1** and **2a** (1.0 equiv of each compound) was carried out in the presence of 1.1 equiv $AlCl_3$ in various solvents and results are summarized in Table 1. Among the solvents examined 1,2-dichloroethane (DCE), CH_2Cl_2, EtOAc, CH_3CN and $CHCl_3$ (Table 1, entries 1–4 and 6) were found to be effective whereas the use of toluene provided lower yield of the desired product **3a** (Table 1, entry 5). The best result however was obtained both in terms of reaction time and product yield using 1,2-dichloroethane (Table 1, entry 1). No formation of disubstituted product was observed in this case. It is worthy to mention that the use of 2.0 equiv of reactant, that is, (hetero)arene as well as $AlCl_3$ led to the formation of 2,3-diaryl substituted quinoxaline (see later).

With the optimized reaction condition in hand we then decided to test the generality and scope of this $AlCl_3$ mediated route to 2-substituted quinoxaline. Thus a number of activated arenes and indoles (**2**) were reacted with 2,3-dichloroquinoxaline (**1**) in the presence of $AlCl_3$ and results are shown in Table 2. The reaction

Table 1
Effect of solvents on $AlCl_3$ mediated reaction of **1** with **2a**[a]



Entry	Solvent	Temp (°C)	Time (h)	Yield[b] (%)
1	$ClCH_2CH_2Cl$	80	1.0	90
2	$ClCH_2Cl$	60	1.5	85
3	EtOAc	80	1.5	80
4	CH_3CN	80	2.0	83
5	Toluene	80	3.0	65
6	$CHCl_3$	60	2.0	80

[a] All the reactions were carried out using compound **1** (1.0 equiv), **2a** (1.0 equiv) and $AlCl_3$ (1.1 equiv) in a solvent (5 mL).
[b] Isolated yield.

proceeded well in all these cases providing the corresponding 2-aryl (Table 2, entries 1–3) or indolyl substituted quinoxalines (Table 2, entries 4–10) in good to excellent yields. As mentioned earlier the use of 2.0 equiv of $AlCl_3$ and indole afforded 2,3-diindolyl substituted quinoxaline exclusively without any mono substituted product (Table 1, entries 11 and 12). All the compounds synthesized were well characterized by spectral (NMR, MS and IR) data. Additionally, the molecular structure of a representative compound **3g** was established unambiguously by single crystal X-ray diffraction (Fig. 3).[15]

Prompted by the results of selective mono (hetero)arylation of 2,3-dichloroquinoxaline (**1**) we then decided to explore the successive substitution of both the chloro group of **1** by two different (hetero)arenes in a single pot. Accordingly, 1.0 equiv of dichloro compound **1** was reacted with two different arenes/heteroarenes (1.0 equiv each) in a sequential manner when unsymmetrically substituted quinoxaline **4** containing two different arenes/heteroarenes was isolated (Table 3).

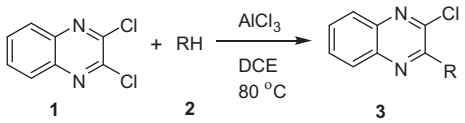
To demonstrate the utility of the present (hetero)arylation of quinoxaline ring further structure elaboration of a representative compound **3e** was carried out under Suzuki[12] and Sonogashira[16] conditions. The chloro derivative **3e** was reacted smoothly with phenylboronic acid and 3,3-dimethylbut-1-yne separately to give the corresponding aryl (**6**) and alkyne coupled product (**5**) respectively (Scheme 2).

Mechanistically the heteroarylation reaction proceeds through the complexation of $AlCl_3$ with one of the nitrogens of 2,3-dichloroquinoxaline followed by nucleophilic attack by a suitable arene or heteroarene at the adjacent carbon and finally release of $AlCl_3$ affording the desired product **3** (Scheme 3). Similarly, complexation of $AlCl_3$ with the second nitrogen facilitated the formation of product **4**. It is evident that the nucleophilicity of the reacting arenes or heteroarenes is crucial in these cases, and therefore, the reaction proceeded smoothly with electron-rich arenes or heteroarenes.

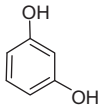
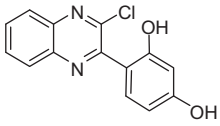
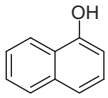
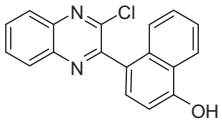
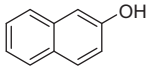
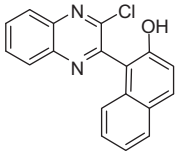
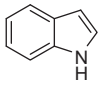
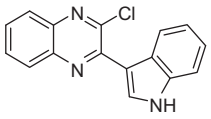
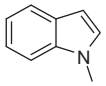
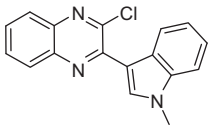
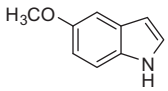
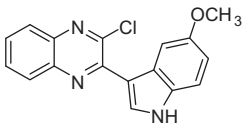
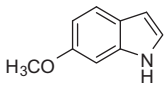
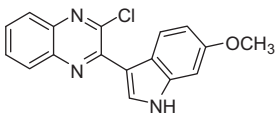
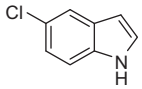
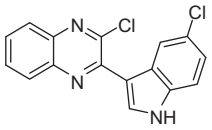
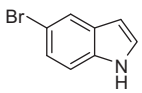
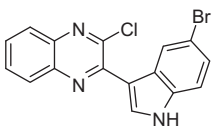
2.2. Pharmacology

Due to our interest in the identification of novel heterocycle based small molecules as inhibitors of chorismate mutase[8,9] or CM (EC 5.4.99.5) some of the compounds synthesized were tested for CM inhibiting properties in vitro. The shikimate pathway for the biosynthesis of aromatic amino acids such as phenylalanine and tyrosine involves the Claisen rearrangement of chorismate to prephenate in the presence of CM.[17] Due to the absence of this

Table 2
Synthesis of 2-(hetero)aryl substituted 3-chloroquinoxaline (**3**) via $AlCl_3$-mediated C–C bond forming reaction between **1** and (hetero)arenes (**2**)[a]

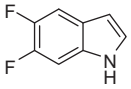
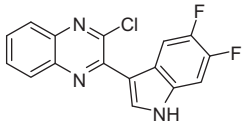
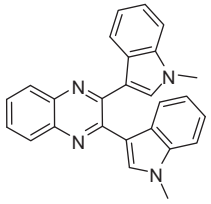
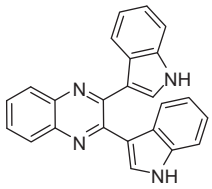


1 **2** **3**

Entry	RH; (**2**)	Products (**3**)	Time (h)	% Yield[b]
1	 **2a**	 **3a**	1.0	90
2	 **2b**	 **3b**	0.5	90
3	 **2c**	 **3c**	0.5	90
4	 **2d**	 **3d**	0.5	85
5	 **2e**	 **3e**	0.5	85
6	 **2f**	 **3f**	0.5	81
7	 **2g**	 **3g**	0.5	80
8	 **2h**	 **3h**	0.5	84
9	 **2i**	 **3i**	0.5	86

(*continued on next page*)

Table 2 (*continued*)

Entry	RH; (**2**)	Products (**3**)	Time (h)	% Yield[b]
10	 **2j**	 **3j**	0.5	90
11	**2e**	 **3h**	0.5	88[c]
12	**2d**	 **3i**	0.5	90[c]

[a] All the reactions were carried out using compound **1** (1.0 equiv), an appropriate (hetero)arene **2** (1.0 equiv) and $AlCl_3$ (1.1 equiv) in dichloroethane (5 mL) at 80 °C.
[b] Isolated yields after column chromatography.
[c] The reaction was carried out using 2.0 equiv of heteroarene (**2e** or **2d**) and $AlCl_3$.

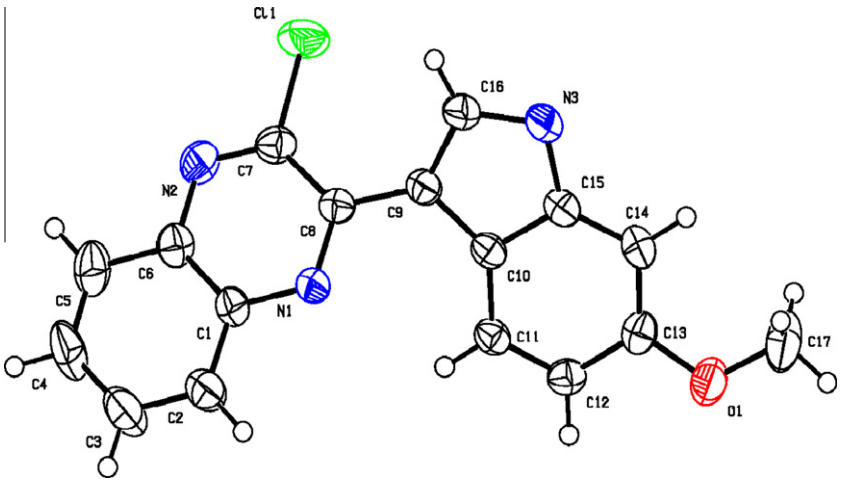
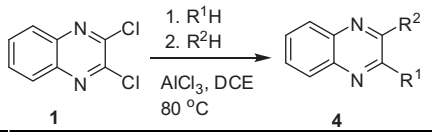
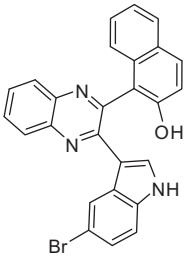
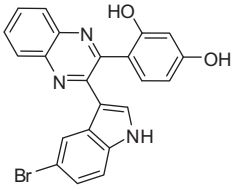
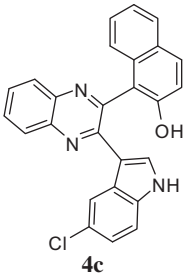
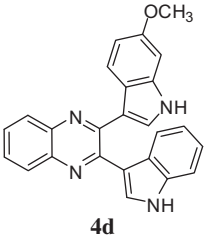
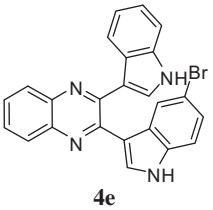
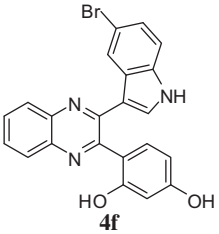
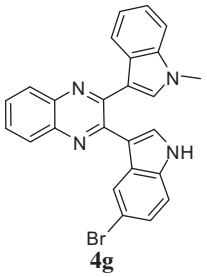
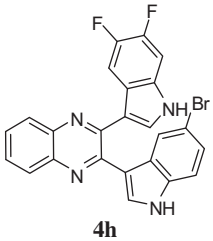


Figure 3. ORTEP representation of the compound **3g** (thermal ellipsoids are drawn at 50% probability level).

pathway in animals but not in bacteria CM is considered as a novel target for the identification of effective antibacterial agents.[18] Notably, only a few small molecules have been reported to possess inhibitory activity against CM. The assay[19,20] used to test our compounds involved determination of activity of enzyme CM which catalyzes the conversion of chorismate to prephenate. Thus determination of activity of CM is based on the direct observation of conversion of chorismic acid to prephenate spectrophotometrically at OD_{274}. This reaction was performed in the presence of test compounds to determine their CM inhibiting activities. A known inhibitor of CM, that is, 4-(3,5-dimethoxyphenethylamino)-3-nitro-5-sulfamoylbenzoic acid[21] was prepared and used as a reference compound the IC_{50} value of which was fund to be less than 10 μM. Our results are summarized in Table 4. In general the mono substituted compounds (**3**) were found to be superior than the 2,3-disubstituted compounds (**4**) when tested at 50 μM. Among the mono-substituted derivatives compound **3j** showed significant inhibition of CM at 50 μM (Table 4). In a dose–response study compound **3j** showed dose dependent inhibition of CM with an IC_{50} value of 19.74 μM (Fig. 4). To understand the nature of interactions of compound **3j** with the CM a docking study was performed using the energy minimization and conformational search with the MACROMODEL application in the Schrodinger package. The protein CM (PDB ID-2F6L) crystal structure was retrieved from the protein data bank and was used for our study after performing necessary steps. The docking results of compound **3j** with CM protein showed arene–cation interaction with Arg 72 and Lys 60 and Hydrogen bonding interaction with Arg134 residue of CM (Fig. 5). The binding energy −12.77 Kcal mol^{-1} suggested good interactions between **3j** and CM.

Table 3
Synthesis of unsymmetrical 2,3-disubstituted quinoxaline (**4**) via $AlCl_3$-induced (hetero)arylation of compound **1**[a]



Entry	Reactant (**2**)		Product (**4**)	Time (h)	Yield[b] (%)
	R^1H	R^2H			
1	**2i**	**2c**	 **4a**	1.0	85
2	**2i**	**2a**	 **4b**	1.0	83
3	**2h**	**2c**	 **4c**	1.0	78
4	**2d**	**2g**	 **4d**	1.0	78
5	**2i**	**2d**	 **4e**	1.0	80
6	**2a**	**2i**	 **4f**	1.0	78
7	**2i**	**2e**	 **4g**	1.0	80
8	**2i**	**2j**	 **4h**	1.0	80

[a] All the reactions were carried out using compound **1** (1.0 equiv), two different appropriate arene or heteroarene (**2**) each 1.0 equiv and $AlCl_3$ (2.2 equiv) in DCE (5 mL) at 80 °C.
[b] Isolated yields after column chromatography.

3. Conclusions

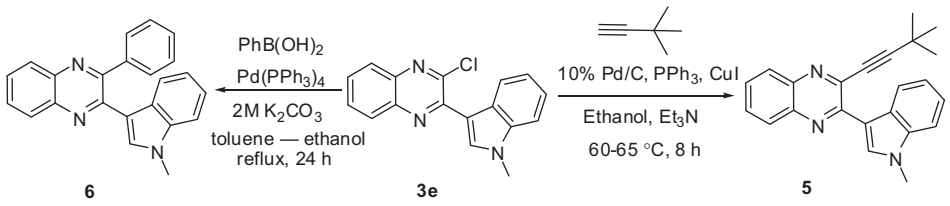
In conclusion, we have reported a new and efficient method for the synthesis of mono and 2,3-disubstituted quinoxalines from 2,3-dichloroquinoxaline in the presence of $AlCl_3$. Both symmetrical and unsymmetrical 2,3-disubstituted quinoxalines can be prepared by using this method under appropriate reaction conditions. The present operationally simple and single-step $AlCl_3$ induced (hetero)arylation of 2,3-dichloroquinoxaline proceeds via C–C bond forming reactions and can be utilized for the preparation of a variety of quinoxaline derivatives from readily available starting materials and reagents. This methodology does not require the use of expensive transition metal catalysts or organometallic reagents, and therefore has the potential to become useful alternative towards the direct one pot synthesis of library of small molecules based on quinoxaline. To the best of our knowledge this is the first example of (hetero)arylation of quinoxaline ring using $AlCl_3$ mediated method. Some of the compounds synthesized were tested for chorismate mutase inhibiting properties in vitro and one compound showed promising activity representing one of the few examples of chorismate mutase inhibition by a heteroarene based small molecule. Docking studies of this compound at the active site of chorismate mutase suggest it interacts well. The quinoxaline scaffold presented here therefore has potential for the identification and development of novel small molecule based inhibitors of chorismate mutase thereby new anti tubercular agents for further evaluation.

4. Experimental section

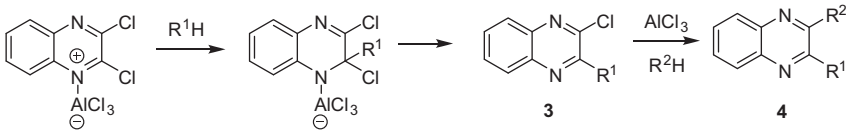
4.1. Chemistry

4.1.1. General methods

Unless stated otherwise, reactions were performed under a nitrogen atmosphere. Reactions were monitored by thin layer chromatography (TLC) on silica gel plates (60 F254), visualizing with ultraviolet light or iodine spray. Flash chromatography was performed on silica gel (100–200 and 230–400 mesh) using hexene,



Scheme 2. Structural elaboration of compound **3e**.



Scheme 3. Probable reaction mechanism leading to product **3** and subsequently **4**.

Table 4
Inhibition of chorismate mutase by compounds **3** and **4** in vitro

Entry	Compounds (**3** and **4**)	% Inhibition[a] @ 50 μM
1	**3a**	31
2	**3b**	35
3	**3f**	36
4	**3g**	42
5	**3j**	64
6	**4a**	35
7	**4d**	45
8	**4e**	31
9	**4f**	21
10	**4h**	27

[a] Average of three experiments.

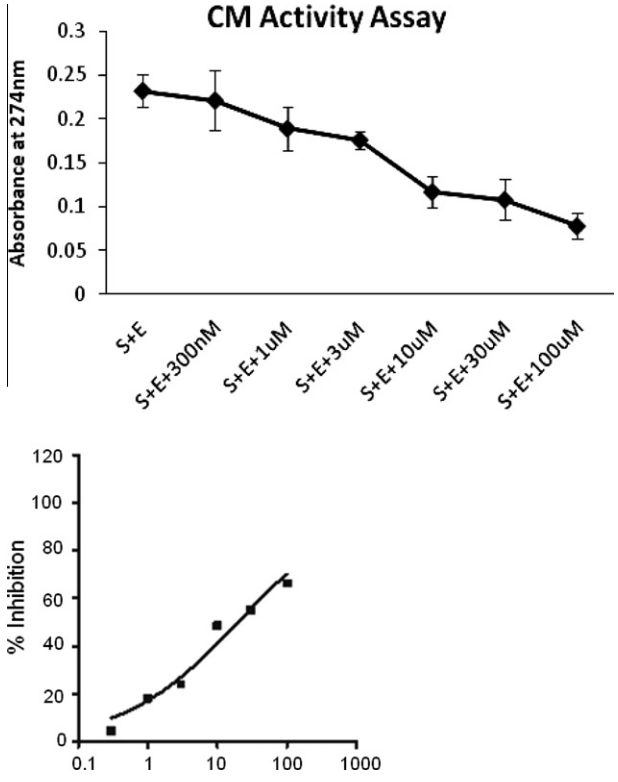


Figure 4. Dose dependent chorismate mutase inhibition (S = substrate, E = enzyme) and IC_{50} value of **3j**.

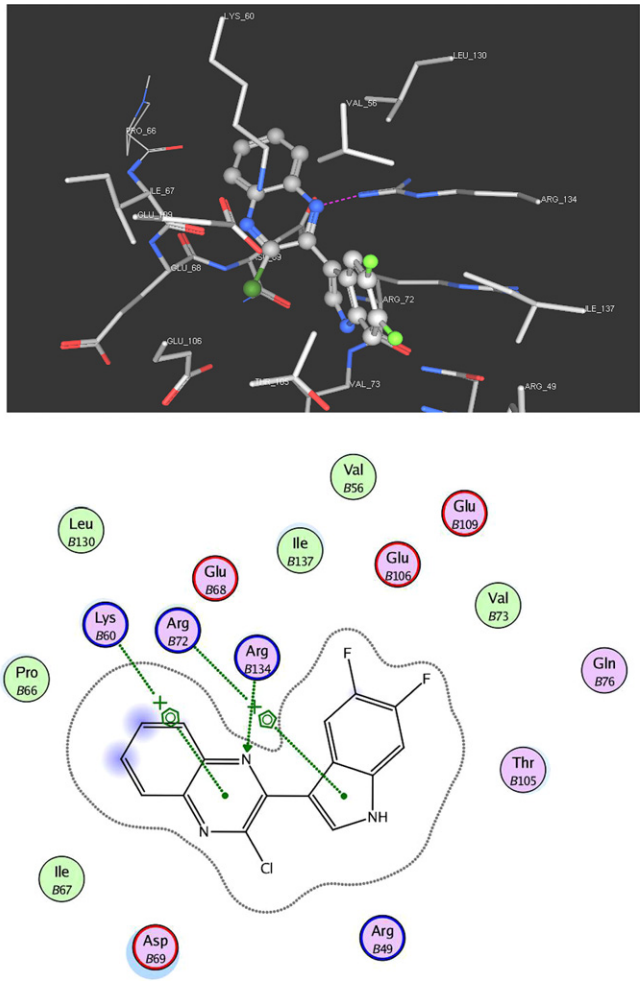


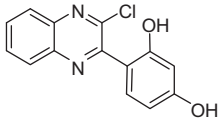
Figure 5. Docking of compound **3j** at the active site of CM.

ethyl acetate, dichloromethane. ^{1}H NMR and ^{13}C NMR spectra were determined in $CDCl_3$, DMSO-d_6, Acetone-d_6 solution by using 400 and 100 MHz spectrometers, respectively. Proton chemical shifts (δ) are relative to tetramethylsilane (TMS, δ = 0.00) as internal standard and expressed in ppm. Spin multiplicities are given as s (singlet), d (doublet), t (triplet) and m (multiplet) as well as b (broad). Coupling constants (J) are given in hertz. Infrared spectra were recorded on a FT-IR spectrometer. Melting points were determined using melting point apparatus and are uncorrected. MS spectra were obtained on a mass spectrometer.

4.1.2. General procedure for the preparation of compound 3

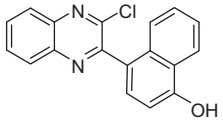
A mixture of 2,3-dichloroquinoxaline (**1**, 1.0 equiv), an appropriate (hetero)arene (**2**, 1.0 equiv) and $AlCl_3$ (1.1 equiv) in dichloroethane (5 mL) was stirred at 80 °C for time indicated in Table 2 under a nitrogen atmosphere. After completion of the reaction, the mixture was poured into ice-cold water (15 mL), stirred for 10 min and then extracted with ethylacetate (3 × 20 mL). The organic layers were collected, combined, washed with cold water (2 × 20 mL), dried over anhydrous Na_2SO_4 and concentrated under vacuum. The residue obtained was purified by column chromatography using ethylacetate/hexene to give the desired product.

4.1.3. 4-(3-Chloroquinoxalin-2-yl)benzene-1,3-diol (3a)



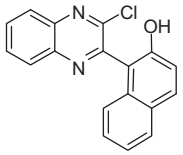
Yellow solid; mp 176–178 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.75 (bs s, 1H), 9.64 (bs s, 1H), 8.10–8.08 (m, 1H), 8.05–8.02 (m, 1H), 7.89–7.85 (m, 2H), 7.15 (d, J = 8.4 Hz, 1H), 6.40–6.34 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.3, 156.9, 153.3, 148.5, 140.7, 140.5, 131.7, 131.0, 130.9, 129.1, 128.1, 116.3, 107.0, 102.9; HPLC: 97.8%, column: Zorbax XDB C-18 150 × 4.6 mm 5 μ, mobile phase A: 0.05% Formic Acid in water mobile phase B: CH_3CN (gradient) T/%B: 0/20, 2/20, 9/95, 12/95, 15/20, 18/20; flow rate: 1.0 mL/min; UV 240 nm, retention time 7.75 min; IR (KBr) υ_{max} 3289, 3167, 1623, 1598, 1339, 1209 cm^{-1}; m/z (CI) 273 (M+1, 100%); HRMS (ESI) calcd for $C_{14}H_{10}N_2O_2Cl$ $(M+H)^+$ 273.0431, found 273.0421.

4.1.4. 2-(3-Chloroquinoxalin-2-yl)naphthalen-1-ol (3b)



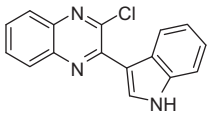
Yellow solid; mp 219–220 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18–8.13 (m, 2H), 7.91–7.82 (m, 4H), 7.41–7.39 (m, 2H), 7.39–7.38 (m, 1H), 7.23–7.22 (m, 1H), 6.75 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 153.7, 152.0, 149.0, 141.2, 141.0, 133.2, 131.9, 131.3, 131.2, 129.4, 128.6, 128.4, 128.0, 127.5, 123.6, 123.5, 118.6, 116.7; HPLC: 99.4%, column: Zorbax XDB C-18 150 × 4.6 mm 5 μ, mobile phase A: 0.05% Formic Acid in water mobile phase B: CH_3CN (gradient) T/%B: 0/70, 2/70, 9/95, 12/95, 15/70, 18/70; flow rate: 0.8 mL/min; UV 235 nm, retention time 3.77; IR (KBr) υ_{max} 3274, 3071, 1587, 1343, 1053 cm^{-1}; m/z (CI) 305 (M-1, 100%); HRMS (ESI) calcd for $C_{18}H_{12}N_2OCl$ $(M+H)^+$ 307.0638, found 307.0633.

4.1.5. 1-(3-Chloroquinoxalin-2-yl)naphthalen-2-ol (3c)



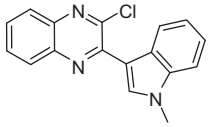
Yellow solid; mp 217–218 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.63 (bs s, 1H), 8.24 (d, J = 8.2 Hz, 1H), 8.15–8.12 (m, 2H), 7.95–7.92 (m, 2H), 7.51–7.46 (m, 2H), 7. 47–7.41 (m, 2H), 7.00–6.98 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 154.7, 153.4, 147.6, 140.7, 140.5, 132.2, 131.4, 131.0, 129.0, 128.8, 128.0, 127.1, 125.0 (2C), 124.9, 124.3, 122.5, 107.4; HPLC: 97.3%, column: Zorbax XDB C-18 150 × 4.6 mm 5 μ, mobile phase A: 0.05% Formic Acid in water mobile phase B: CH_3CN (gradient) T/%B: 0/50, 2/50, 9/95, 15/50, 12/95, 15/50, 18/50; flow rate: 1.0 mL/min; UV 225 nm, retention time 6.55 min; IR (KBr) υ_{max} 3158, 3065, 1621, 1508, 1276 cm^{-1}; m/z (CI) 307 (M+1, 100%); HRMS (ESI) calcd for $C_{18}H_{12}N_2OCl$ $(M+H)^+$ 307.0638, found 307.0635.

4.1.6. 2-Chloro-3-(1*H*-indol-3-yl)quinoxaline (3d)



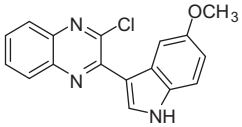
Yellow solid; mp 261–263 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.90 (bs s, 1H), 8.61 (d, J = 2.4 Hz, 1H), 8.55 (d, J = 3.2 Hz, 1H), 7.98 (d, J = 1.2 Hz, 1H), 7.96 (d, J = 1.2 Hz, 1H), 7.87–7.82 (m, 1H), 7.79–7.75 (m, 1H), 7.53–7.51 (m, 1H), 7.26–7.19 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 149.0, 144.9, 140.8, 138.6, 136.7, 131.1 (2C), 130.0, 128.5, 128.0, 127.0, 123.1, 122.6, 121.4, 112.5, 111.0; HPLC: 99.6%, column: Zorbax XDB C-18 150 × 4.6 mm 5 μ, mobile phase A: 0.05% Formic Acid in water mobile phase B: CH_3CN (gradient) T/%B: 0/70, 2/70, 9/95, 12/95, 15/70, 18/70; flow rate: 1.0 mL/min; UV 219 nm, retention time 4.59 min; IR (KBr) υ_{max} 3174, 1542, 1437, 1130 cm^{-1}; m/z (CI) 280 (M+1, 100%); HRMS (ESI) calcd for $C_{16}H_{11}N_3Cl$ $(M+H)^+$ 280.0642, found 280.0642.

4.1.7. 2-Chloro-3-(1-methyl-1*H*-indol-3-yl)quinoxaline (3e)



Yellow solid; mp 150–152 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.65 (d, J = 8.0 Hz, 1H), 8.58 (s, 1H), 8.12 (d, J = 8.0 Hz, 1H), 7.95 (d, J = 8.0 Hz, 1H), 7.85–7.81 (m, 1H), 7.79–7.74 (m, 1H), 7.56 (d, J = 8.0 Hz, 1H), 7.32–7.28 (m, 2H), 3.93 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 148.2, 144.3, 140.4, 138.2, 136.9, 134.5, 130.7, 129.6, 128.1, 127.6, 127.1, 122.8, 122.5, 121.3, 110.4, 109.6, 33.1; HPLC: 99.8%, column: Zorbax XDB C-18 150 × 4.6 mm 5 μ, mobile phase A: 0.05% Formic Acid in water mobile phase B: CH_3CN (gradient) T/%B: 0/70, 2/70, 9/95, 12/95, 15/70, 18/70; flow rate: 1.0 mL/min; UV 219 nm, retention time 7.15 min; IR (KBr) υ_{max} 3051, 2925, 1607, 1533, 1373 cm^{-1}; m/z (CI) 294 (M+1, 100%); HRMS (ESI) calcd for $C_{17}H_{13}N_3Cl$ $(M+H)^+$ 294.0798, found 294.0703.

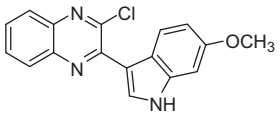
4.1.8. 2-Chloro-3-(5-methoxy-1*H*-indol-3-yl)quinoxaline (3f)



Brown solid; mp 252–253 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.79 (bs s, 1H), 8.53 (d, J = 2.8 Hz, 1H), 8.20 (d, J = 2.4 Hz, 1H), 8.13 (d, J = 8.4 Hz, 1H), 7.96 (d, J = 8.4 Hz, 1H), 7.86–7.82 (m, 1H), 7.78–7.74 (m, 1H), 7.42 (d, J = 9.2 Hz, 1H), 6.90–6.87 (m, 1H),

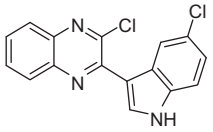
3.83 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 155.3, 149.0, 144.7, 140.8, 138.5, 131.7, 131.5, 131.2, 129.8, 128.4, 128.0, 127.7, 113.1, 113.0, 110.7, 104.6, 55.7; HPLC: 97.6%, column: X DB C-18 150 × 4.6 mm 5 μ, mobile phase A: 0.05% Formic Acid in water, mobile phase B: CH_3CN (gradient) T/%B: 0/70, 2/70, 9/95, 12/95, 15/70, 18/70; flow rate: 1.0 mL/min; UV 225 nm, retention time 4.04 min; IR (KBr) υ_{max} 3225, 2945, 1533, 1429, 1225 cm^{-1}; *m*/*z* (CI) 310 (M+1, 100%); HRMS (ESI) calcd for $C_{17}H_{13}N_3OCl$ $(M+H)^+$ 310.0747, found 310.0760.

4.1.9. 2-Chloro-3-(6-methoxy-1*H*-indol-3-yl)quinoxaline (3g)



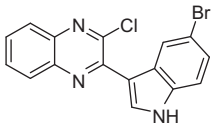
Brown solid; mp 236–238 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.70 (bs s, 1H), 8.52 (d, *J* = 8.8 Hz, 1H), 8.44 (d, *J* = 3.2 Hz, 1H), 8.12 (d, *J* = 8.0 Hz, 1H), 7.95 (d, *J* = 9.6 Hz, 1H), 7.85–7.81 (m, 1H), 7.77–7.73 (m, 1H), 7.01 (d, *J* = 2.4 Hz, 1H), 6.87–6.84 (m, 1H), 3.80 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 156.8, 148.9, 144.7, 140.8, 138.5, 137.7, 131.1, 130.2, 129.8, 128.4, 128.0, 123.6, 121.1, 111.4, 111.1, 95.3, 55.6; HPLC: 98.2%, column: Zorbax XDB C-18 150 × 4.6 mm 5 μ, mobile phase A: 0.05% Formic Acid in water mobile phase B: CH_3CN (gradient) T/%B: 0/70, 2/70, 9/95, 12/95, 15/70, 18/70; flow rate: 1.0 mL/min; UV 220 nm, retention time 4.40 min; IR (KBr) υ_{max} 3460, 3260, 2925, 1622, 1447 cm^{-1}; *m*/*z* (CI) 310 (M+1, 100%); HRMS (ESI) calcd for $C_{17}H_{13}N_3OCl$ $(M+H)^+$ 310.0747, found 310.0749.

4.1.10. 2-Chloro-3-(5-chloro-1*H*-indol-3-yl)quinoxaline (3h)



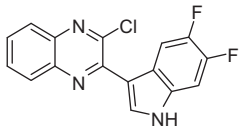
Brown solid; mp 280–282 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.08 (bs s, 1H), 8.61–8.59 (m, 2H), 8.14 (d, *J* = 8.8 Hz, 1H), 7.99–7.96 (m, 1H), 7.87–7.84 (m, 1H), 7.80–7.77 (m, 1H), 7.54 (d, *J* = 8.8 Hz, 1H), 7.27–7.24 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 148.4, 144.7, 140.6, 138.8, 135.3, 132.5, 131.3, 130.2, 128.5, 128.1, 128.0, 126.1, 123.1, 121.7, 114.1, 110.7; HPLC: 97.3%, column: Zorbax XDB C-18 150 × 4.6 mm 5 μ, mobile phase A: 0.05% Formic Acid in water mobile phase B: CH_3CN (gradient) T/%B: 0/80, 2/80, 9/98, 12/98, 15/80, 18/80; flow rate: 1.0 mL/min; UV 225 nm, retention time 97.28 min; *m*/*z* (CI) 314 (M+1, 100%); IR (KBr) υ_{max} 3211, 2599, 1536, 1440 cm^{-1}; HRMS (ESI) calcd for $C_{16}H_{10}N_3Cl_2$ $(M+H)^+$ 314.0252, found 314.0260.

4.1.11. 2-(5-Bromo-1*H*-indol-3-yl)-3-chloroquinoxaline (3i)



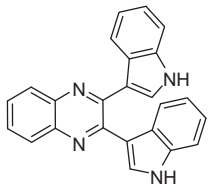
Yellow solid; mp 285–287 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.08 (bs s, 1H), 8.73 (d, *J* = 2.0 Hz, 1H), 8.59–8.57 (m, 1H), 8.12–8.10 (m, 1H), 7.98–7.96 (m, 1H), 7.87–7.76 (m, 2H), 7.51–7.48 (m, 1H), 7.38–7.34 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 148.4, 144.8, 140.6, 138.8, 135.5, 132.4, 131.3, 130.3, 128.7, 128.5, 128.0, 125.7, 124.7, 114.5, 114.2, 110.6; HPLC: 98.9%, column: Zorbax XDB C-18 150 × 4.6 mm 5 μ, mobile phase A: 0.05% Formic Acid in water mobile phase B: CH_3CN (gradient) T/%B: 0/70, 2/70, 9/95, 12/95, 15/70, 18/70; flow rate: 1.0 mL/min; UV 220 nm, retention time 6.91 min; IR (KBr) υ_{max} 3211, 1536, 1436, 1130 cm^{-1}; *m*/*z* (CI) 357 (M+1, 100%); HRMS (ESI) calcd for $C_{16}H_{10}N_3ClBr$ $(M+H)^+$ 357.9747, found 357.9763.

4.1.12. 2-Chloro-3-(5,6-difluoro-1*H*-indol-3-yl)quinoxaline (3j)



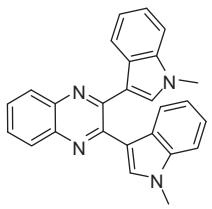
Yellow solid; mp 237–238 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.04 (bs s, 1H), 8.63 (s, 1H), 8.56–8.51 (m, 1H), 8.19 (d, *J* = 8.4 Hz, 1H), 7.97 (d, *J* = 8.4 Hz, 1H), 7.87–7.76 (m, 2H), 7.56–7.52 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 148.2, 146.5, 144.5, 140.6, 138.7, 132.7, 132.0, 131.9, 131.1, 130.2, 128.6, 127.9, 122.6, 111.5, 109.6, 109.4; HPLC: 98.5%, column: Zorbax XDB C-18 150 × 4.6 mm 5 μ, mobile phase A: 0.05% Formic Acid in water mobile phase B: CH_3CN (gradient) T/%B: 0/80, 2/80, 9/98, 12/98, 15/80, 18/80; flow rate: 1.0 mL/min; UV 225 nm, retention time 98.50 min; IR (KBr) υ_{max} 3224, 1537, 1479, 1158 cm^{-1}; *m*/*z* (CI) 316 (M+1, 100%); HRMS (ESI) calcd for $C_{16}H_9N_3ClF_2$ $(M+H)^+$ 316.0448, found 316.0453.

4.1.13. 2,3-Di(1*H*-indol-3-yl)quinoxaline (3h)



Yellow solid; mp 236–238 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.38 (bs s, 2H), 8.02–8.00 (m, 2H), 7.99–7.93 (m, 2H), 7.71–7.69 (m, 2H), 7.42–7.37 (m, 4H), 7.14–7.11 (m, 2H), 7.03–7.00 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 150.1 (2C), 140.0 (2C), 136.5 (2C), 129.3 (2C), 128.5 (2C), 128.2 (2C), 126.4 (2C), 122.4 (2C), 121.5 (2C), 120.6 (2C), 114.6 (2C), 112.3 (2C); IR (KBr) υ_{max} 3396, 3244, 2927, 1535, 1431, 1123 cm^{-1}; *m*/*z* (CI) 361 (M+1, 100%); HRMS (ESI) calcd for $C_{24}H_{17}N_4$ $(M+H)^+$ 361.1453, found 361.1446.

4.1.14. 2,3-Bis(1-methyl-1*H*-indol-3-yl)quinoxaline (3i)



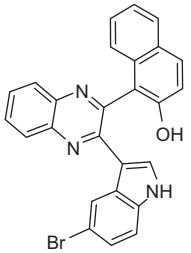
Yellow solid; mp 208–210 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.18–8.16 (m, 2H), 8.04–8.02 (m, 2H), 7.66–7.64 (m, 2H), 7.56–7.45 (m, 4H), 7.25–7.22 (m, 2H), 7.17–7.14 (m, 2H), 3.78 (s, 6H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 149.2 (2C), 139.5 (2C), 136.8 (2C), 132.1 (2C), 128.7 (2C), 128.0 (2C), 126.5 (2C), 121.9 (2C), 121.4 (2C), 120.2 (2C), 113.0 (2C), 110.2 (2C), 32.7 (2C); HPLC: 99.7%, column: Zorbax XDB C-18 150 × 4.6 mm 5 μ, mobile phase A: 0.05% Formic Acid in water mobile phase B: CH_3CN (gradient)

T/%B: 0/70, 2/70, 9/95, 12/95, 15/70, 18/70; flow rate: 1.0 mL/min; UV 225 nm, retention time 20.0 min; IR (KBr) υ_{max} 3470, 1534, 1373, 1091 cm^{-1}; *m*/*z* (CI) 389 (M+1, 100%).

4.1.15. General procedure for the preparation of compound 4

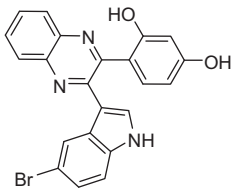
A mixture of 2,3-dichloroquinoxaline (**1**, 1.0 equiv), an appropriate (hetero)arene (**2**, 1.0 equiv) and $AlCl_3$ (1.0 equiv) in dichloroethane (5 mL) was stirred at 85 °C for 0.5 h under a nitrogen atmosphere. The reaction mixture was cooled to room temperature to which was add the second (hetero)arene (**2**, 1.0 equiv) and an additional amount of $AlCl_3$ (1.0 equiv). The reaction mixture was stirred for 0.5 h at 85 °C under a nitrogen atmosphere. After completion of the reaction (indicated by TLC), the mixture was poured into ice-cold water (15 mL), stirred for 10 min and then extracted with ethylacetate (3 × 20 mL). The organic layers were collected, combined, washed with cold water (2 × 20 mL), dried over anhydrous Na_2SO_4 and concentrated under vacuum. The residue obtained was purified by column chromatography using ethylacetate/hexene to give the desired product.

4.1.16. 1-(3-(5-Bromo-1*H*-indol-3-yl)quinoxalin-2-yl)naphthalen-2-ol (4a)



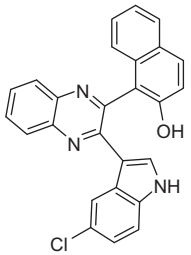
Yellow solid; mp 171–173 °C; ^{1}H NMR (400 MHz, Acetone-d_6) δ 10.42 (bs s, 1H), 9.19 (bs s, 1H), 8.70 (bs s, 1H), 8.25 (d, *J* = 8.0 Hz, 1H), 8.04–8.00 (m, 2H), 7.93–7.87 (m, 2H), 7.79–7.75 (m, 1H), 7.35–7.28 (m, 6H), 6.61 (s, 1H); ^{13}C NMR (100 MHz, Acetone-d_6) δ 152.7, 151.3, 150.9, 141.3, 139.8, 135.3, 135.1, 133.5, 128.9 (2C), 128.8, 128.7, 128.6, 128.4, 128.4, 128.1, 126.9, 125.5, 125.0, 123.8, 123.3, 120.2, 118.5, 113.7, 113.3, 113.2; HPLC: 98.9%, column: Symmetry (R) C-18 74 × 4.6 mm 3.5 μ, mobile phase A: 0.05% Formic Acid in water, mobile phase B: CH_3CN (gradient) T/%B: 0/50, 2/50, 9/95, 12/95, 15/50, 18/50; flow rate: 1.0 mL/min; UV 225 nm, retention time 7.12 min; IR (KBr) υ_{max} 3511, 3412, 3060, 2582, 1534, 1428 cm^{-1}; *m*/*z* (CI) 467 (M+2, 100%).

4.1.17. 4-(3-(5-Bromo-1*H*-indol-3-yl)quinoxalin-2-yl)benzene-1,3-diol (4b)



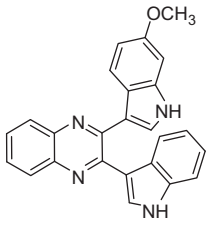
Yellow solid; mp 169–171 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.52 (bs s, 1H), 9.61 (bs s, 1H), 9.35 (bs s, 1H), 8.86 (s, 1H), 8.07 (d, *J* = 7.6 Hz, 1H), 7.96 (d, *J* = 7.6 Hz, 1H), 7.77–7.70 (m, 2H), 7.37–7.30 (m, 2H), 7.16 (d, *J* = 7.6 Hz, 1H), 6.78 (s, 1H), 6.43–6.33 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.7, 156.3, 152.9, 151.0, 140.8, 139.2, 135.2, 131.2, 130.1 (2C), 129.3 (2C), 128.8, 128.5, 125.1, 125.1, 119.3, 114.1, 113.6 (2C), 107.5, 103.2; HPLC: 98.8%, column: X Bridge C-18 150 × 4.6 mm 5 μ, mobile phase A: 0.05% Formic Acid in water, mobile phase B: CH_3CN (gradient) T/%B: 0/50, 2/50, 9/95, 12/95, 15/50, 18/50; flow rate: 1.0 mL/min; UV 225 nm, retention time 6.52 min; IR (KBr) υ_{max} 3416, 2958, 2923, 1733, 1249 cm^{-1}; *m*/*z* (CI) 432 (M+1, 100%).

4.1.18. 1-(3-(5-Chloro-1*H*-indol-3-yl)quinoxalin-2-yl) naphthalen-2-ol (4c)



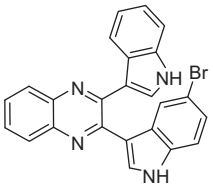
Yellow solid; mp 192–194 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.32 (bs s, 1H), 9.89 (bs s, 1H), 8.82 (s, 1H), 8.19 (d, *J* = 8.4 Hz, 1H), 8.01–7.98 (m, 2H), 7.92–7.84 (m, 2H), 7.77–7.73 (m, 1H), 7.34–7.23 (m, 4H), 7.17–7.15 (m, 1H), 7.07–7.05 (m, 1H), 6.38 (d, *J* = 2.0 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 153.1, 151.3, 151.2, 141.1 (2C), 139.6, 134.9, 133.4, 130.9, 130.7, 129.1, 129.0, 128.7 (2C), 128.3, 128.1, 127.6, 125.8, 123.7, 123.6, 122.9, 122.2, 119.9, 118.9, 113.7, 112.8; HPLC: 98.0%, column: Zorbax XDB C-18 150 × 4.6 mm 5 μ, mobile phase A: 0.05% Formic Acid in water mobile phase B: CH_3CN (gradient) T/%B: 0/70, 2/70, 9/98, 12/98, 15/70, 18/70; flow rate: 0.8 mL/min; UV 225 nm, retention time 5.84 min; IR (KBr) υ_{max} 3745, 3213, 3181, 1533 cm^{-1}; *m*/*z* (CI) 422 (M+1, 100%).

4.1.19. 2-(1*H*-indol-3-yl)-3-(6-methoxy-1*H*-indol-3-yl)quinoxaline (4d)



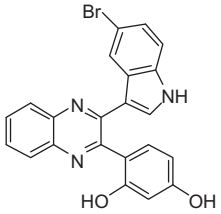
Brown solid; mp 160–162 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.27 (s, 2H), 8.12–8.10 (m, 2H), 8.05 (d, *J* = 7.6 Hz, 1H), 7.94 (d, *J* = 8.8 Hz, 1H), 7.69 (d, *J* = 6.8 Hz, 2H), 7.38–7.33 (m, 2H), 7.23–7.13 (m, 3H), 6.83–6.80 (m, 2H), 3.84 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.7, 149.9, 149.7, 140.4, 140.4, 136.7, 135.9, 128.8 (2C), 128.6 (2C), 126.6, 126.4, 125.7, 122.7, 122.5, 121.6, 120.9, 120.6, 115.5, 115.4, 111.2, 110.7, 94.5, 55.5; HPLC: 98.4%, column: X Bridge C-18 150 × 4.6 mm 5 μ, mobile phase A: 0.05% Formic Acid in water mobile phase B: CH_3CN (gradient) T/%B: 0/50, 9/95, 12/95, 15/50, 18/50; flow rate: 0.8 mL/min; UV 225 nm, retention time 6.79 min; IR (KBr) υ_{max} 3372, 2960, 2924, 1735, 1246 cm^{-1}; *m*/*z* (CI) 389 (M-1, 100%).

4.1.20. 2-(5-Bromo-1*H*-indol-3-yl)-3-(1*H*-indol-3-yl)quinoxaline (4e)



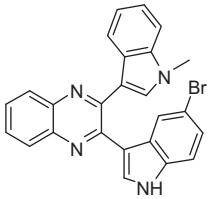
Brown solid; mp 213–215 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.44 (bs s, 1H), 8.33 (bs s, 1H), 8.24 (s, 1H), 8.15 (d, J = 6.4 Hz, 1H), 8.11 (d, J = 8.0 Hz, 1H), 7.80 (d, J = 7.2 Hz, 1H), 7.71 -7.70, (d, J = 6.8 Hz, 2H), 7.38–7.32 (m, 3H), 7.23–7.16 (m, 3H), 7.10–7.07 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 149.9, 149.6, 140.0 (2C), 136.4, 135.0, 129.6 (2C), 129.5, 128.5 (2C), 128.2, 127.8, 126.1, 125.0, 123.7, 122.4, 121.1, 120.6, 114.5, 114.3, 113.9, 113.4, 112.3; IR (KBr) υ_{max} 3411, 2962, 2923, 2875, 1732, 1248 cm^{-1}; m/z (CI) 439 (M+1, 100%).

4.1.21. 4-(3-(5-Bromo-1*H*-indol-3-yl)quinoxalin-2-yl)benzene-1,3-diol (4f)



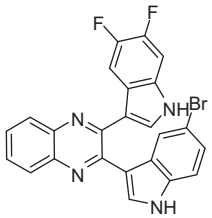
Brown solid; mp 242–244 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.20 (bs s, 1H), 8.39 (bs s, 1H), 8.29 (s, 1H), 8.12–8.10 (m, 1H), 7.96 (d, J = 8.4 Hz, 1H), 7.73–7.68 (m, 2H), 7.37–7.7.28 (m, 4H), 6.57 (d, J = 2.0 Hz, 1H), 6.07–6.05 (m, 1H), 5.18 (bs s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.7, 156.3, 152.9, 151.0, 140.8, 139.2, 135.2, 131.2, 130.2, 129.2 (2C), 128.9, 128.8, 128.4, 125.2, 125.0, 119.3, 114.1, 113.7, 113.6, 107.5, 103.3; IR (KBr) υ_{max} 3854, 3295, 1620, 1532 cm^{-1}; m/z (CI) 432 (M+1, 100%).

4.1.22. 2-(5-Bromo-1*H*-indol-3-yl)-3-(1-methyl-1*H*-indol-3-yl)quinoxaline (4g)



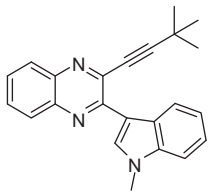
Yellow solid; mp 164–166 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.52 (s, 1H), 8.21–8.16 (m, 3H), 7.69–7.63 (m, 3H), 7.36–7.33 (m, 3H), 7.25–7.20 (m, 3H), 7.05–7.01 (m, 1H), 3.78 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 149.4, 149.2, 140.5, 140.4, 137.1, 134.5, 130.8, 129.0, 129.0, 128.7, 128.6, 128.3, 127.9, 126.6, 125.7, 124.4, 122.3, 121.5, 120.6, 114.7, 114.6, 114.6, 112.6, 109.5, 33.1; HPLC: 98.0%, column: Zorbax XDB C-18 150 × 4.6 mm 5 μ, mobile phase A: 0.05% Formic Acid in water mobile phase B: CH_3CN (gradient) T/%B: 0/80, 2/80, 9/95, 12/98, 15/80, 18/80; flow rate: 1.0 mL/min; UV 248 nm, retention time 5.82 min; IR (KBr) υ_{max} 3423, 2951, 2933, 2855, 1632 cm^{-1}; m/z (CI) 454 (M+2, 100%).

4.1.23. 2-(5-Bromo-1*H*-indol-3-yl)-3-(5, 6-difluoro-1*H*-indol-3-yl)quinoxaline (4h)



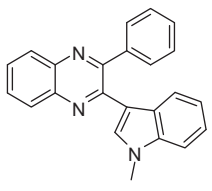
Brown solid; mp 172–174 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.38 (bs s, 1H), 8.31 (bs s, 1H), 8.13–8.10 (m, 3H), 7.90–7.85 (m, 1H), 7.73–7.71 (m, 2H), 7.33–7.31 (m, 2H), 7.27–7.25 (m, 2H), 7.17–7.13 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 148.9, 148.7, 140.5, 140.3, 134.5, 130.9, 130.8, 129.5, 129.4, 128.6, 127.9, 127.7, 127.2, 125.9, 123.9, 122.7, 121.7, 121.7, 115.5, 115.0, 114.5, 112.7, 108.5, 108.3; IR (KBr) υ_{max} 3453, 3411, 3239, 2927, 2358, 1713, 1474 cm^{-1}; m/z (CI) 475 (M+1, 100%).

4.1.24. Preparation of 2-(3,3-Dimethylbut-1-ynyl)-3-(1-methyl-1*H*-indol-3-yl)quinoxaline (5)



A mixture of the 2-chloro-3-(1-methyl-1*H*-indol-3-yl)quinoxaline (**3e**) (0.68 mmol), 10% Pd/C (0.013 mmol), PPh_3 (0.12 mmol), CuI (0.03 mmol), and triethylamine (2.04 mmol) in ethanol (5 mL) was stirred at 25–30 °C for 30 min under a nitrogen atmosphere. To this was added 3,3-dimethylbut-1-yne compound (**2**) (1.06 mmol). The mixture was initially stirred at room temperature for 1 h and then at 60–65 °C for 8 h. After completion of the reaction, the mixture was cooled to room temperature, diluted with EtOAc (50 mL), and filtered through celite. The filtrated was collected, washed with water (3 × 30 mL), dried over anhydrous Na_2SO_4, and concentrated. The crude residue was purified by column chromatography on silica gel using hexane/ethyl acetate to give the desired product as a yellow solid; mp 184–185 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.68–8.67 (m, 1H), 8.08 (d, J = 8.0 Hz, 1H), 7.98 (d, J = 8.0 Hz, 1H), 7.82–7.79 (m, 1H), 7.75–7.66 (m, 2H), 7.59 (d, J = 8.4 Hz, 1H), 7.34–7.25 (m, 2H), 3.94 (s, 3H), 1.43 (s, 9H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 150.3, 149.5, 140.5, 140.3, 136.9, 130.5, 129.4, 129.3, 129.0, 128.9 (2C), 122.4, 121.5, 121.1, 111.2, 110.6, 103.9, 80.1, 33.3, 30.3 (3C), 28.5; IR (KBr) υ_{max} 3464, 2951, 2220, 1534, 1372 cm^{-1}; m/z (CI) 340 (M+1, 100%); HRMS (ESI) calcd for $C_{23}H_{22}N_3$ (M+H)$^+$ 340.1820, found 340.1814.

4.1.25. Preparation of 2-(1-methyl-1*H*-indol-3-yl)-3-phenylquinoxaline (6)



A mixture of the 2-chloro-3-(1-methyl-1*H*-indol-3-yl)quinoxaline (**3e**) (0.68 mmol), phenyl boronic acid (1.49 mmol), and $Pd(PPh_3)_4$ (6 mol%) under a nitrogen atmosphere. To this was added toluene (5 mL), EtOH (0.7 mL), and K_2CO_3 (2.0 M in water, 2.2 mL) and the mixture was stirred and heated under reflux for 24 h. after completion of the reaction (indicated by TLC) the mixture was cooled to room temperature and diluted with CH_2Cl_2 (15 mL). The organic layer was separated and collected and the aqueous phase was extracted with CH_2Cl_2 (2 × 15 mL). The combined organic extracts were washed with water (20 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated. The crude residue was purified by column chromatography on silica gel using hexane/ethyl acetate to afford the desired product as a yellow solid; mp 158–160 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.37 (d, J = 8.4 Hz, 1H), 8.12 (d, J = 7.6 Hz, 1H), 8.04 (d, J = 8.0 Hz, 1H), 7.84–7.81 (m, 1H), 7.77–7.74 (m, 1H), 7.62–7.60 (m, 2H) 7.52–7.46 (m, 4H), 7.24 (t, J = 7.4 Hz, 1H), 7.16 (t, J = 7.6 Hz, 1H), 6.77 (s, 1H), 3.65 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 153.7, 149.5, 141.2, 140.3, 137.3, 137.1, 129.4 (4C), 128.9, 128.6, 127.3, 127.0, 123.1, 122.9, 122.8, 122.4, 121.5, 121.1, 110.8, 110.6, 33.3; IR (KBr) υ_{max} 2924, 2859, 1533, 1371 cm^{-1}; m/z (CI) 336 (M+1, 100%); HRMS (ESI) calcd for $C_{23}H_{18}N_3$ $(M+H)^+$ 336.1500, found 336.1501.

4.2. Single crystal X-ray data for compound 3g[15]

Single crystals suitable for X-ray diffraction of **3g** were grown from methanol. The crystals were carefully chosen using a stereo zoom microscope supported by a rotatable polarizing stage. The data was collected at room temperature on Bruker's KAPPA APEX II CCD Duo with graphite monochromated Mo-Kα radiation (0.71073 Å). The crystals were glued to a thin glass fiber using FOMBLIN immersion oil and mounted on the diffractometer. The intensity data were processed using Bruker's suite of data processing programs (SAINT), and absorption corrections were applied using SADABS.[22] The crystal structure was solved by direct methods using SHELXS-97 and the data was refined by full matrix least-squares refinement on F^2 with anisotropic displacement parameters for non-H atoms, using SHELXL-97.[23]

Crystal data of ***3g****:* Molecular formula = $C_{17}H_{11}ClN_3O$, Formula weight = 308.74, Crystal system = Monoclinic, space group = $P2(1)/n$, a = 9.0594 (12) Å, b = 9.1332(11) Å, c = 17.852(2) Å, V= 1445.3(3) Å^3, T = 298 K, Z = 4, D_c = 1.419 Mg m^{-3}, μ(Mo Kα) = 0.71073 mm^{-1}, 19170 reflections measured, 2480 independent reflections, 2156 observed reflections [$I > 2.0\sigma(I)$], R_1_obs = *0.035, Goo*dness of fit = 1.040.

4.3. Pharmacology[20,21]

4.3.1. Chorismate mutase activity assay

Mycobacterium tuberculosis chorismate mutase (MtCM) gene was PCR amplified and cloned into expression vector pET22b. MtCM was purified from over expressed culture of BL21 (DE3) harboring pET22b/MtCM by Ni-NTA affinity chromatography.

Activity of chorismate mutase enzyme is based on the direct observation of conversion of Chorismate to prephenate Spectrophotometrically at OD_{274}. The reaction volume of 100 µl contained 50 mM Tris–HCl (pH 7.5), 0.5 mM EDTA, 0.1 mg/mL bovine serum albumin, and 10 mM β-Mercaptoethanol, and chorismic acid 4 mM. The reaction was started by adding 180 pmol of purified protein to the pre-warmed chorismic acid solution. Inhibitory screening of the test compounds against chorismate mutase activity was measured at 50 µM concentration of the effectors. The reaction was allowed to proceed at 37 °C and was terminated after 5 min with 100 µl of 1 N HCl. A blank with no enzyme for every reaction was kept as a control to account for the non enzymatic conversion of chorismate to prephenate.

The percentage of enzyme inhibition caused by the test compound is calculated by the following formula

% Inhibition = 100−residual activity of CM

$$\left[\text{Residual activity of CM} = \frac{(S+E+C)-(S+C)}{(S+E)-(S)} \times 100\right]$$

S = Substrate absorbance at 274 nm; E = Enzyme absorbance at 274 nm; C = Compound absorbance at 274 nm.

Dose–response study of the compound **3j** against chorismate mutase activity was carried out using the concentration from 1 µM to 100 µM.

4.4. Docking study

The molecular docking was carried out with XP Glide application of schrodinger software with MASTERO interface 9.2 The molecule **3j** was docked in chorismate mutase protein of *Mycobacterium tuberculosis*.

4.5. Procedure

In the present study we have performed the energy minimization and conformational search with the MACROMODEL application in the Schrodinger package. The study molecule was energy minimized for flexibility and then the conformational search was performed. We used OPLS_2005 force field and water as implicit solvent. We have followed the PRCG (Polak-Ribier conjugate gradient) method of minimization with 500 iterations with a threshold gradient on 0.05 kJ/mol. The conformational search was based on Montecarlo multiple minimum torsional sampling. The ligands were then finally prepared with LIGPREP application.

The protein chorismate mutase (PDB ID-2F6L) crystal structure was retrieved from the protein data bank and it was then refined with the PROTEIN PREPERATION WIZARD application in which the hydrogen's were added and missing side chains and loops were filled with PRIME application. Waters were observed within the 5Å distance and waters were deleted beyond 5Å from het (heteroatom) groups. Finally the protein is then optimized and minimized with impref using OPLS_2005 force filed. GRID based Flexible Docking was done in the present study.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2012.01.012.

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