Indolo[2,3-b]quinoxaline-Linked Pyrimidine-2-ol Derivatives: Synthesis, Characterization and Antimicrobial Studies

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Abstract:

A new set of pyrimidine-2-ol derivatives (6a–6j) bearing the indolo[2,3-b]quinoxaline scaffold was synthesized through cyclization of the corresponding chalcone precursors. The synthesized derivatives were characterized by FT-IR, 1H NMR, 13C NMR and HRMS analyses, with the obtained data supporting the proposed structures. The antimicrobial properties of the synthesized derivatives were subsequently investigated against selected bacterial and fungal strains. Within this series, 6b showed notable activity against Gram-positive bacteria, whereas 6e and 6f displayed comparatively better activity against Gram-negative bacteria. In the antifungal assay, 6e exhibited excellent activity against Saccharomyces cerevisiae. The biological findings indicate that incorporation of a pyrimidine-2-ol moiety into the indolo[2,3-b]quinoxaline framework can generate derivatives with appreciable antimicrobial potential. The identified active compounds provide a basis for further investigation of this structural class toward the development of biologically relevant heterocyclic candidates.

Introduction:

Heterocyclic chemistry represents a fundamental and continually developing area of organic chemistry, owing to the remarkable structural diversity and functional versatility of heterocyclic molecules 1,2. Incorporation of heteroatoms such as nitrogen, oxygen, and sulfur into aromatic and partially saturated frameworks can substantially influence molecular geometry, electron distribution, polarity, hydrogen-bonding characteristics and physicochemical behavior 3,4. These features have contributed to the extensive use of heterocyclic scaffolds in the design of molecules with pharmaceutical, agrochemical, and material-related applications. In particular, the rational assembly of different heterocyclic units within a single molecular framework provides an effective approach for expanding chemical diversity and developing compounds with desirable biological properties 5. Consequently, the synthesis of structurally diverse heterocyclic architectures remains an important objective in contemporary organic and medicinal chemistry 6.

The significance of heterocyclic compounds extends beyond their synthetic diversity, as numerous heterocyclic frameworks are integral components of biologically active natural products and therapeutic molecules 7,8. Modulation of heterocyclic structures through changes in ring systems, functional groups, and substitution patterns can alter their interaction with biological macromolecules and consequently influence their biological responses. A wide variety of heterocyclic compounds have therefore been investigated for anti-bacterial, anti-fungal, anti-oxidant, anti-cancer, anti-inflammatory and other pharmacological activities 9,10. In the medicinal chemistry, the integration of two or more pharmacophoric features into a

single molecular architecture is frequently explored to generate new structural entities with distinct biological profiles. Such molecular hybridization also provides a useful platform for examining the relationship between structural modifications and biological activity 11,12.

Chalcones are particularly useful synthetic intermediates for the construction of structurally diversified heterocyclic molecules. Their characteristic α,β-unsaturated carbonyl framework provides a conjugated reactive system that readily participates in several cyclization, condensation, and annulation reactions 13,14. Chalcones can generally be obtained by straightforward condensation of appropriately substituted aromatic aldehydes with ketones, allowing extensive variation of their aromatic substitution patterns. In addition to their synthetic importance, numerous chalcone derivatives have been investigated for antibacterial, antifungal, antioxidant, anti-inflammatory, and anticancer properties 15,16. The combination of facile synthesis, structural flexibility, and chemical reactivity makes chalcones valuable precursors for the preparation of new heterocyclic systems and biologically relevant molecular hybrids 17.

The indolo[2,3-b]quinoxaline framework is an attractive fused nitrogen-containing heteroaromatic system characterized by an extended π-conjugated structure and multiple nitrogen atoms that can contribute to molecular recognition and intermolecular interactions 18. Incorporation of this scaffold with another biologically relevant heterocycle may therefore provide an effective strategy for generating new molecular architectures with potentially useful biological characteristics. Among such heterocycles, pyrimidine is an important nitrogen-containing pharmacophore, while its 2-hydroxy (pyrimidine-2-ol) functionality introduces additional hydrogen-bonding and electronic features that may influence molecular interactions 19,20. On this basis, combining the indolo[2,3-b]quinoxaline and pyrimidine-2-ol motifs represents a rational approach toward developing structurally diverse heterocyclic hybrids 21 . Accordingly, the present study describes the synthesis of indolo[2,3-b]quinoxaline-based pyrimidine-2-ol derivatives (6a–6j). The synthesized derivatives were subsequently characterized by advanced techniques and their anti-bacterial and anti-fungal activities were subsequently investigated.

Results And Discussion

Chemistry

The synthetic approach utilized for the preparation of the chalcone derivatives **(5a-5j)** in **scheme 1** and target pyrimidine-2-ol derivatives **(6a-6j)** is illustrated in **Scheme 2**. The

synthesis was developed through a sequential construction of the indolo[2,3-*b*]quinoxaline framework followed by introduction of the chalcone and pyrimidine-2-ol pharmacophores.

The synthesis commenced with isatin **(1)** and 4-chlorobenzene-1,2-diamine **(2)**. Condensation of these two starting materials under acidic conditions afforded 2-chloro-6H-indolo[2,3-*b*]quinoxaline **(3)**. The chloro-substituted intermediate **(3)** was subsequently reacted with 4-aminoacetophenone to introduce the acetylphenylamino substituent, furnishing the corresponding ketone derivative **(4)**, namely 1-{4-[(6*H*-indolo[2,3-*b*]quinoxalin-2-yl)amino]phenyl}ethan-1-one.

The ketone intermediate **4** served as the key precursor for the synthesis of the chalcone series **5a–5j**. Claisen–Schmidt condensation of compound **(4)** with a range of appropriately substituted aromatic aldehydes afforded the corresponding chalcone derivatives **(5a–5j)**. The variation of substituents on the aromatic ring generated a series of structurally related α,β-unsaturated carbonyl compounds and provided a convenient platform for subsequent heterocyclization.

The synthesized chalcones (**5a–5j)** were then employed as intermediates for the construction of the target pyrimidine-2-ol derivatives. Cyclization of the respective chalcone derivatives under the selected reaction conditions resulted in transformation of the α,β-unsaturated carbonyl system into the corresponding six-membered pyrimidine ring, giving the target compounds (**6a–6j)**. The substituent pattern introduced through the individual chalcone precursor was retained in the corresponding pyrimidine-2-ol derivative, thereby generating a systematic series of indolo[2,3-*b*]quinoxaline-based pyrimidine-2-ol compounds.

The structures of the synthesized derivatives were confirmed using FT-IR, ^{1}H NMR, ^{13}C NMR and HRMS analyses. The spectroscopic data were consistent with the proposed structures, while the HRMS measurements provided further confirmation of their molecular compositions. The overall synthetic sequence, therefore, enabled the construction of a structurally diverse series of indolo[2,3-*b*]quinoxaline-based pyrimidine-2-ol derivatives (**6a–6j)** from readily accessible starting materials through the corresponding chalcone intermediates.

In vitro antimicrobial assay

The antimicrobial activity was assessed against two Gram-positive strains, *S. aureus* (ATCC-6538) and *B.subtilis* (ATCC-6633), and two Gram-negative strains, *E.coli* (ATCC-25922) and *P.aeruginosa* (ATCC-9027), In addition, the compounds were also tested against yeast strains such as Candida albicans (ATCC10,231) and Saccharomyces cerevisiae (ATCC

9763), along with the fungal strain Aspergillus brasiliensis (ATCC 16,404). The results are presented in **(Table 1)** and **(Table 2)** respectively.

Table-01 : Results of in vitro antibacterial activity of indolo[2,3-*b*]quinoxaline based pyrimidine hydroxy derivatives **(6a-6j)**

Minimum Inhibitory Concentration (µg/ml)					
Compd.	R	Antibacterial activity			
		Gram-positive		Gram-negative	
		S. Aureus	B. subtilis	E. Coli	P. Aeruginosa
6a	(H)	500	250	125	500
6b	(3-Cl)	125	62.5	500	250
6c	(2-F)	500	125	500	500
6d	(2-OCH3)	125	500	125	500
6e	(4-CN)	500	125	62.5	125
6f	(2,3-Cl)	500	125	62.5	500
6g	(2-Cl,6-F)	125	500	125	500
6h	(2-NO2)	500	125	125	500
6i	(4-Br)	125	500	125	500
6j	(4-OH)	500	125	500	500
Gentamicin		50	50	50	50
Ciprofloxacin		50	50	25	25

As shown in **Table 01**, compound **6b** displayed notable activity against Gram-positive bacterial strains, while **6e** and **6f** showed comparatively higher efficacy against Gram-negative germs. The moderately synthesised derivatives, antibacterial activity, antibacterial activity, antibacterial activity.

Table-02 : In-Vitro Antifungal Evaluation of Indolo[2,3-*b*]quinoxaline-Based Pyrimidin-2-ol Derivatives**(6a-6j)**

Minimum Inhibitory Concentration (µg/ml)				
Compd.	**R**	**Antifungal activity**		
		C. albicans	***S. cerevisiae***	***A. brasiliss***
6a	(H)	500	250	500
6b	(3-Cl)	125	500	125
6c	(2-F)	500	1000	1000

6d	(2-OCH_3)	500	**100**	>1000
6e	(4-CN)	125	**100**	125
6f	(2,3-Cl)	500	125	500
6g	(2-Cl,6-F)	500	125	125
6h	(2-NO_2)	125	**100**	500
6i	(4-Br)	500	500	500
6j	(4-OH)	500	500	1000
Nystatin		100	100	100

*Bold value indicates highly active compounds against microorganism

According to the antifungal results of compounds **5a–5j** presented in **Table 02**, compound **6d,6e and 6h** exhibited excellent activity against *S. cerevisiae*, whereas the remaining compounds in the series showed moderate antifungal activity against the tested fungal strains.

Experimental

Materials and methods.

All chemicals and solvents were of analytical reagent grade and employed without further purification as supplied by Sigma. The synthesized compounds were purified by recrystallization and their purity was confirmed by TLC and melting point determination. Structural characterization was carried out using advanced tecniques. The Antibacterial activity was evaluated by the broth microdilution method (MIC) against S. *aureus, B. subtilis, E. coli* and *P. aeruginosa*, using gentamicin and ciprofloxacin as standards.

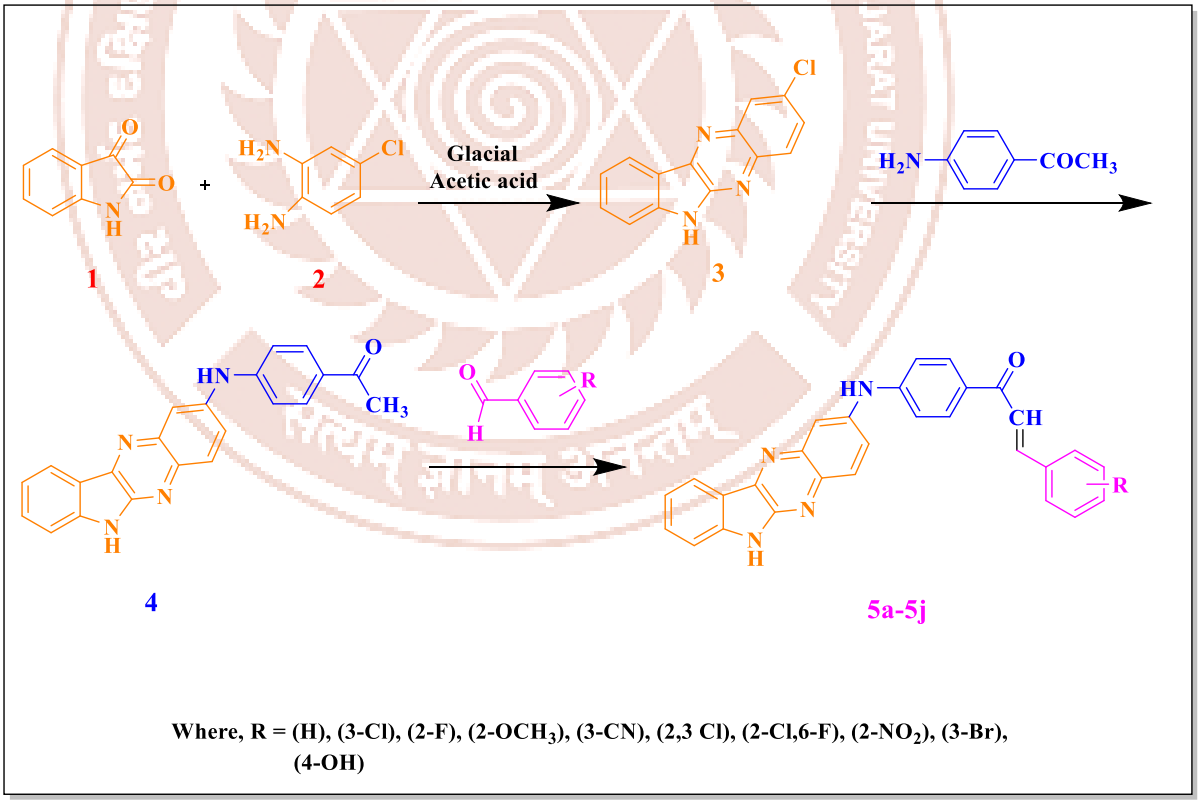
General procedure.

Isatin (1) (1 mmol, 0.750 g) and 4-chloro-1,2-diaminobenzene (2) (1 mmol, 0.724 g) were put in to a suitable RBF, followed by the addition of a 8 mL of glacial CH3COOH. For one hour, the reaction mixture was refluxed while being constantly stirred. Thin-layer chromatography (TLC) was used to track the reaction's development. Following completion, 2-chloro-6H-indolo[2,3-b]quinoxaline (3) was produced by allowing the reaction mixture to cool to room temperature. Recrystallisation from ethanol was used to purify the product, and TLC with n-toluene/chloroform (3:2, v/v) as the mobile phase verified its purity 22.

Compound (3) (1 mmol, 0.415 g) was dissolved in acetone in a stirred solution, and 4-aminoacetophenone (1 mmol, 0.221 g) was added gradually over the course of six hours at room temperature. A catalyst of pyridine (1.0 mL) was employed. For a further five hours at room temperature, the resultant reaction mixture was constantly agitated. Thin-layer

chromatography (TLC) was used to monitor the development of the reaction. After completion, the mixture was poured into ice-cold water with constant stirring to afford 4-(3'-acetylphenylamino)-6H-indolo[2,3-b]quinoxaline (4). It was washed thoroughly with water and dried. It was purified by recrystallization from methanol. The purity was confirmed by TLC using chloroform:ethanol (2:3) as the solvent system 23.

Synthesis of quinoxaline based chalcone derivatives (5a-5j) by Claisen–Schmidt condensation. Compound (4) was dissolved in methanol, followed by the gradual addition of a methanolic solution of the appropriate substituted benzaldehyde (0.01 mol) under heating in a water bath. Next, 2.5 mL of a 30% aqueous sodium hydroxide solution was gradually added while being continuously stirred. at order to promote Claisen-Schmidt condensation, the reaction mixture was kept at these conditions for 20–22 hours. The mixture was put into ice-cold water and acidified once the process was finished to pH 2 with concentrated hydrochloric acid. The separated product was collected by filtration, thoroughly washed with cold water, and dried. The resulting chalcone derivatives (5a–5j) were further purified by recrystallization using methanol24.

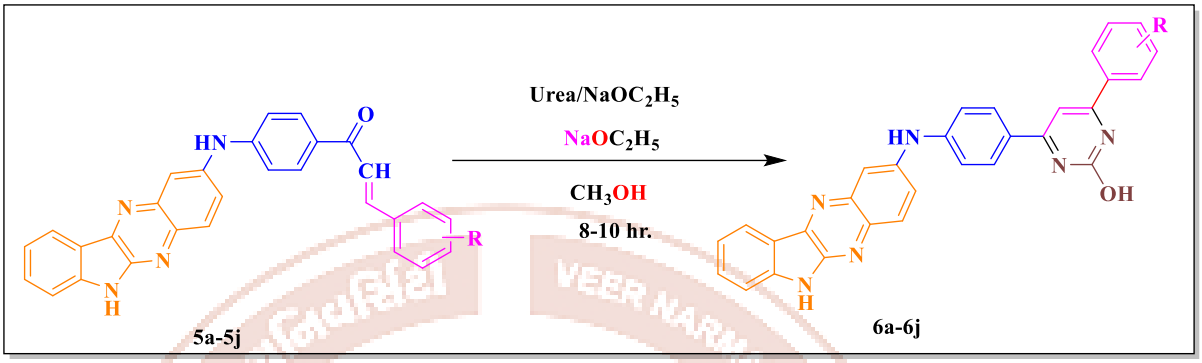


Scheme 1. Synthetic route to chalcone derivatives **(5a–5j)**.

In a RBF, urea (0.006 mol) and methanol (20 mL) were added to the chalcone derivatives (5a–5j) (0.005 mol). The reaction mixture was continuously stirred while a 30% sodium methoxide solution was gradually added. After that, the mixture was refluxed for ten

to twelve hours. Once finished, it was poured into ice-cold water after being allowed to cool to room temperature (6a–6j). The products were recrystallized from ethanol 25.

The reaction progress and purity of the products were monitored by TLC using silica gel 60 F254 aluminium plates (E. Merck). Ethyl acetate (2:8, v/v) was used as the mobile phase and the spots were detected under short-wavelength ultraviolet (UV) light.



Scheme 2 Preparation of pyrimidin-2-ol derivatives (6a–6j)

4-(4-((6*H*-indolo[2,3-*b*]quinoxalin-2-yl)amino)phenyl)-6-phenylpyrimidin-2-ol (6a):

Yield = 70%; Mp= 232–234 °C; ^{1}H NMR (400 MHz, DMSO-d6) δ ppm: 7.85–8.20 (m, 16H, Ar–H and pyrimidine-H), 8.60 (s, 1H, –NH–), 12.10 (s, 1H, –NH of pyrrole ring), 12.50–13.00 (br s, 1H, –OH); ^{13}C NMR (100 MHz, DMSO-d6) δ 188.82, 146.01, 145.92, 145.62, 142.71, 141.30, 139.57, 138.73, 138.72, 134.87, 132.71, 130.70, 130.55, 129.75, 128.94, 128.59, 127.71, 122.42, 121.61, 121.52, 120.40, 119.94, 115.31, 110.71, 101.91 ; HRMS: m/z (M + H)$^{+}$cacld. for $C_{30}H_{20}N_6O$: 481.1620 found 481.1624.

4-(4-((6H-indolo[2,3-b]quinoxalin-2-yl)amino)phenyl)-6-(3-chlorophenyl)pyrimidin-2-ol (6b):

Yield =70% ; Mp=252–254 °C ; ^{1}H NMR (400 MHz, DMSO-*d6*) δ ppm: 6.84–8.20 (m, 17H, Ar–H and pyrimidine-H), 12.08 (s, 1H, –NH of pyrrole ring), 12.50–13.00 (s, 1H, –OH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 190.82, 146.01, 145.92, 143.12, 142.71, 141.30, 139.57, 138.73, 138.72, 136.03, 135.91, 133.67, 130.70, 130.55, 129.86, 128.67, 127.86, 126.97, 126.72, 122.48, 122.42, 121.61, 120.40, 118.91, 116.27, 112.91, 112.71 ; HRMS: m/z (M + H)$^{+}$cacld. for $C_{30}H_{19}ClN_6O$: 515.1309 found 515.1312

4-(4-((6H-indolo[2,3-b]quinoxalin-2-yl)amino)phenyl)-6-(2-methoxyphenyl)pyrimidin-2-ol (6d):

Yield =72% ; Mp=252-254°C ; 1H NMR (400 MHz, DMSO-d6) δ ppm: 1H NMR (400 MHz, DMSO-d6) δ ppm: 3.81 (s, 3H, –OCH_3), 6.85–8.20 (m, 17H, Ar–H and pyrimidine-H), 12.08 (s, 1H, –NH of pyrrole ring), 12.50–13.00 (s, 1H, –OH).;13C NMR (100 MHz, DMSO-d6) δ 187.82, 159.52, 146.01, 144.89, 142.71, 140.31, 139.95, 139.57, 138.73, 138.72, 133.70,

130.73, 130.70, 130.55, 129.35, 127.31, 126.72, 122.42, 122.20, 121.76, 121.61, 120.40, 119.94, 116.27, 114.81, 112.91, 112.71, 58.50 ; HRMS: m/z (M + H)+cacld. for C31H22N6O2: 511.1804 found 511.1808

4-(6-(4-((6H-indolo[2,3-b]quinoxalin-2-yl)amino)phenyl)-2-hydroxypyrimidin-4-yl)benzonitrile (6e):

Yield =62% ; Mp=250-252°C ; ^{1}H NMR (400 MHz, DMSO-*d6*) δ ppm: 6.88–8.22 (m, 17H, Ar–H and pyrimidine-H), 12.10 (s, 1H, –NH of pyrrole ring), 12.55–13.00 (s, 1H, –OH).;^{13}C NMR (100 MHz, DMSO-d_6) δ 191.82, 145.02, 143.92, 143.42, 142.71, 141.30, 139.57, 138.73, 138.72, 136.94, 133.67, 132.79, 130.70, 130.55, 128.51, 126.72, 122.42, 121.61, 120.86, 120.40, 119.94, 118.39, 118.31, 110.91, 110.71, 109.81 ; HRMS: m/z (M + H)$^{+}$cacld. for $C_{31}H_{19}N_7O$: 506.1651 found 506.1655.

4-(4-((6H-indolo[2,3-b]quinoxalin-2-yl)amino)phenyl)-6-(2,3-dichlorophenyl)pyrimidin-2-ol (6f):

Yield =72% ; Mp= 278-280°C; 1H NMR (400 MHz, DMSO-d6) δ ppm: 6.88–8.22 (m, 15H, Ar–H and pyrimidine-H), 8.63 (s, 1H, –NH–), 12.10 (s, 1H, –NH of pyrrole ring), 12.55–13.00 (s, 1H, –OH); 13C NMR (100 MHz, DMSO-d6) δ 190.42, 147.01, 145.92, 143.72, 142.31, 139.57, 138.73, 138.72, 138.54, 134.70, 133.68, 132.43, 132.03, 131.20, 130.70, 130.55, 127.84, 126.72, 126.71, 123.16, 122.42, 120.61, 119.41, 118.91, 116.27, 112.91, 112.71 ; HRMS: m/z (M + H)+cacld. for C30H18Cl2N6O: 549.0919 found 549.0923

4-(4-((6H-indolo[2,3-b]quinoxalin-2-yl)amino)phenyl)-6-(2-chloro-6-fluorophenyl)pyrimidin-2-ol (6g):

Yield = 70%; Mp=272-276°C ; ^{1}H NMR (400 MHz, DMSO-*d6*) δ ppm: 6.88–8.22 (m, 15H, Ar–H and pyrimidine-H), 8.63 (s, 1H, –NH–), 12.10 (s, 1H, –NH of pyrrole ring), 12.55–13.00 (s, 1H, –OH);^{13}C NMR (100 MHz, DMSO-d_6) δ 189.17, 160.94, 160.31, 146.01, 144.89, 142.71, 141.30, 139.57, 138.73, 138.72, 135.82, 135.80, 134.52, 134.49, 133.73, 131.53, 131.51, 130.70, 130.55, 126.72, 126.33, 126.32, 126.03, 126.02, 122.42, 122.17, 122.12, 121.61, 120.40, 119.94, 116.27, 115.46, 115.41, 111.96, 111.70 ; HRMS: m/z (M + H)$^{+}$cacld. for $C_{30}H_{18}ClFN_6O$: 533.1215 found 533.1219.

4-(4-((6H-indolo[2,3-b]quinoxalin-2-yl)amino)phenyl)-6-(2-nitrophenyl)pyrimidin-2-ol (6h):

Yield =66% ; Mp=230-232°C ; ^{1}H NMR (400 MHz, DMSO-*d6*) δ ppm: 6.90–8.35 (m, 17H, Ar–H and pyrimidine-H), 12.12 (s, 1H, –NH of pyrrole ring), 12.60–13.10 (s, 1H, –OH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 190.42, 149.62, 148.02, 144.89, 142.71, 140.31, 139.57, 138.73, 138.72, 133.82, 132.72, 131.46, 130.94, 130.72, 130.55, 130.49, 126.72, 125.09, 124.31,

122.42, 121.61, 120.40, 119.94, 116.27, 111.96, 111.70 ; HRMS: m/z (M + H)$^+$cacld. for $C_{30}H_{19}N_7O_3$: 526.1549 found 526.1553.

4-(4-((6H-indolo[2,3-b]quinoxalin-2-yl)amino)phenyl)-6-(3-bromophenyl)pyrimidin-2-ol (6i):

Yield =68% ; Mp=252-256°C ; 1H NMR (400 MHz, DMSO-d6) δ ppm: 6.88–8.25 (m, 17H, Ar–H and pyrimidine-H), 12.11 (s, 1H, –NH of pyrrole ring) 12.60–13.05 (s, 1H, –OH); 13C NMR (100 MHz, DMSO-d6) δ 190.82, 147.01, 143.92, 142.71, 142.64, 141.30, 139.57, 138.73, 138.72, 136.08, 133.67, 132.08, 131.78, 130.70, 130.55, 130.26, 128.41, 126.72, 124.04, 122.77, 122.42, 121.61, 120.40, 119.94, 116.27, 110.91, 110.71 ; HRMS: m/z (M + H)+cacld. for C30H19BrN6O: 559.0804 found 559.0809

4-(4-((6H-indolo[2,3-b]quinoxalin-2-yl)amino)phenyl)-6-(4-hydroxyphenyl)pyrimidin-2-ol (5j):

Yield = 72%; Mp=268-272°C ; 1H NMR (400 MHz, DMSO-d6) δ ppm: 6.72–8.20 (m, 17H, Ar–H and pyrimidine-H), 9.85 (s, 1H, Ar–OH), 12.10 (s, 1H, –NH of pyrrole ring), 12.60–13.05 (s, 1H, pyrimidin-2-ol); 13C NMR (100 MHz, DMSO-d6) δ 190.89, 168.42, 150.12, 145.03, 144.89, 142.71, 141.30, 139.57, 138.73, 138.72, 133.67, 130.77, 130.70, 130.55, 128.11, 126.72, 122.42, 121.61, 120.82, 120.40, 119.94, 116.63, 116.27, 112.91, 112.71 ; HRMS: m/z (M + H)+cacld. for C30H20N6O2: 497.1649 found 497.1654

Antibacterial activity. The antibacterial activity of the synthesized quinoxaline-chalcone derivatives **(5a–5j)** was evaluated using the broth microdilution method.

Briefly, each well of a sterile 96-well microtiter plate was filled with 100 µL of Mueller–Hinton broth. Stock solutions of the synthesized compounds were prepared in dimethyl sulfoxide (DMSO), and 100 µL of each compound solution was added to the first well, followed by serial two-fold dilutions to obtain the desired concentration range. Subsequently, an appropriate volume of the bacterial suspension was added to each well to achieve the required inoculum density.

Ciprofloxacin (10 µg/mL) and Gentamicinwere used as the reference antibacterial drug, while wells containing only culture medium and bacterial inoculum served as growth controls. The microplates were incubated at 37°C for 24 hour under aerobic conditions. Following incubation, bacterial growth was assessed visually, and the The minimum inhibitory concentration (MIC) was taken as the lowest tested concentration that completely prevented visible bacterial growth. Each experiment was conducted in triplicate, and the reported MIC values represent the mean obtained from three independent measurements 26.

Conclusion

In the present study, a series of pyrimidine-2-ol derivatives (6a–6j) was successfully synthesized. The synthesized derivatives were confirmed by various methods and the obtained spectroscopic data supported the proposed molecular structures. The biological evaluation demonstrated that the synthesized derivatives possessed varying degrees of antibacterial and antifungal activity against the tested microorganisms. Among the synthesized compounds, 6b showed notable activity against Gram-positive bacteria, while 6e and 6f exhibited comparatively enhanced activity against Gram-negative bacteria. In the antifungal evaluation, 6e demonstrated excellent activity against Saccharomyces cerevisiae, whereas the other derivatives displayed moderate activity. Overall, the findings demonstrate that incorporation of the pyrimidine-2-ol moiety into the indolo[2,3-b]quinoxaline framework provides a promising structural platform for the development of heterocyclic compounds with antimicrobial potential. The identified active derivatives may serve as useful lead structures for further structural modification and detailed biological investigation.

Credit Author Statement

Bhargav B. Dave: Validation, Formal analysis, Data curation, Writing - original draft, Investigation, Biological activity; **Tarun P. Patel**: Conceptualization, review & editing,Biological activity; **Megha A. Patel**: Data curation; review & editing; Axay B. Dodiya: Formal analysis, Biological activity; **Dr.Paresh S. Patel**: Methodology, Conceptualization, Resources, review, & editing, Supervision.

Conflict Of Interest

The authors declare no conflict of interest.

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