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In vitro antitubercular and antimicrobial activities of 1-substituted quinoxaline-2,3(1*H*,4*H*)-diones

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ABSTRACT

1-((Substituted)methyl)quinoxaline-2,3(1*H*,4*H*)-dione (**2a–e**) and 1-((substituted)acryloyl)quinoxaline-2,3(1*H*,4*H*)-dione (**4a–c**) were synthesized from quinoxaline-2,3(1*H*,4*H*)-dione **1** and evaluated for their antimicrobial activities. Results of the antitubercular screening against *Mycobacterium tuberculosis* $H_{37}Rv$ showed that the compounds **2b, 3,** and **4a** were the most effective, with minimum inhibitory concentrations of 8.012, 8.561, and 8.928 μg/ml, respectively. All the compounds exhibited significant antibacterial and considerable antifungal activities.

Tuberculosis is one of the world's most infectious diseases, killing 2 million peoples every year out of 2 billion infected individuals. Often tuberculosis is accompanied by AIDS and exists as multidrug resistant tuberculosis (MDR-TB) or as extensively/extremely resistant tuberculosis (XTR-TB), where neither standard antitubercular drug nor any of the regimens are potentially effective.[1] Owing to the ineffective remedy and risk in the treatment option, by 2020, the global burden of tuberculosis is estimated to be 2.3 million, of which 99% will be in developing countries (WHO, 1997). In 2000, the global alliance for tuberculosis drug development was established to accelerate the development of new antitubercular agents and ensure their availability and affordability in high-epidemic countries. For the first time in the last few decades, under the guidance of global alliance and pharmaceutical companies, 20 new molecules were developed with promising characteristics during in vitro and in vivo animal studies.[2] However these molecules were not brought to the realization and precluded their future development. Hence, the search for new and potent antitubercular agents is gaining interest

Nitrogen-containing heterocycles are indispensable structural units for medicinal chemists. Among the various heterocyclic compounds, quinoxalines form an attractive biologically active molecule as these are a part of various antibiotics such as hinomycin, levomycin, and actinoleutin[3,4] that are known to possess other biological potentials such as adenosine receptor antagonist, anticancer, antihelmintic, antidepressant, and anti-inflammatory.[5]

In view of the literature regarding antimicrobial potency of quinoxaline and its mode of action that prevent DNA-directed RNA synthesis by virtue of binding to CpG site on DNA, the quinoxaline nucleus is focused on synthesizing newer derivatives to explore potent antitubercular moiety for the present epidemics of tuberculosis.[6] In continuation of our earlier communications,[7–9] herein we synthesize 1-((substituted)methyl)quinoxaline-2,3(1*H*,4*H*)-dione (**2a–e**) as mannich base and 1-((substituted) acryloyl)quinoxaline-2,3(1*H*,4*H*)-dione (**4a–c**) as chalcones and subsequently evaluate their antitubercular, antibacterial, and antifungal activities.

The synthesis of the compounds was carried as outlined in Scheme 1. The starting compound, quinoxaline-2,3(1*H*,4*H*)-diones **(1)** was synthesized by Phillips condensation[10] and obtained as white silky needle crystals with good yield. 1-((Substituted)methyl) quinoxaline-2,3(1*H*,4*H*)-diones **(2a–e)** were obtained as mannich bases by acid-catalyzed mannich condensation reaction.[11] The resulted synthon structures were supported by the 1H resonance around δ 5.0 ppm and ^{13}C resonance around δ 58 ppm for the methylene linkage (–N–CH_2–) of quinoxaline-heteryl moiety. The intermediate 1-acetylquinoxaline-2,3(1*H*,4*H*)-dione **(3)** was synthesized by conventional acetylating procedure by refluxing in acetylating mixture. The yielded product was purified from byproducts by *n*-hexane precipitation followed by chromatographic techniques and was authenticated by the presence of absorption band at 2984 cm^{-1}, 1H resonance around δ 3 ppm, and ^{13}C resonance around δ 21 ppm for the methyl group (–$COCH_3$). 1-((substituted)acryloyl)quinoxaline-2,3(1*H*,4*H*)-diones **(4a–c)** were prepared as chalcones by reacting with appropriate aldehyde by conventional

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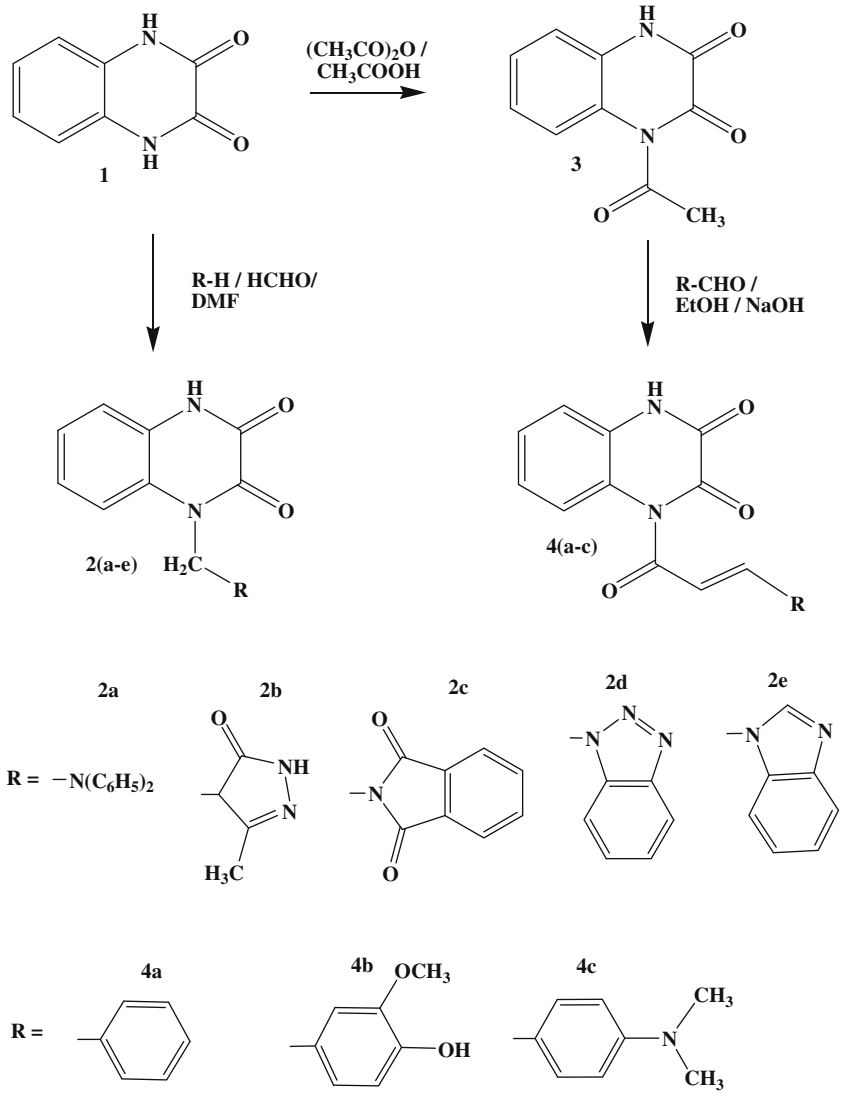

Scheme 1.

route. The structures were supported by the additional maxima in UV spectrum at 254 nm for unsaturated carbonyl chromophore (Table 1), 1H signal around δ 5–6 (dd) ppm, disappearance of ^{13}C resonance for the methyl group (–CH_3), and appearance of two ^{13}C resonances around δ 110 ppm. The compounds' molecular weight and molecular formula were supported by mass spectral data and elements analytical data.

All the synthesized compounds were evaluated for their in vitro antibacterial activity against *S. aureus* (Gram-positive bacteria), *E. coli*, *P. vulgaris*, and *P. aeruginosa* (Gram-negative bacteria) and for their antifungal activity against *A. Niger* and *C. albicans* by agar plate disk diffusion method.[11] The antitubercular screening was performed by Alamar blue assay[12] against *Mycobacterium tuberculosis* $H_{37}Rv$ and the results are represented in Table 2. The obtained results revealed that the nature of substituents on the cyclic nitrogen of quinoxaline may have a considerable impact on the antitubercular, antibacterial, and antifungal activities of the parent pharmacophore. Introduction of heteryl-substituted methyl side

Table 1
Physical data of the compounds **2a–e, 3,** and **4a–c**

Compound	Molecular formula	Molecular weight	Melting point (°C)	% Yield	λ_{max} (nm)	R_f^* value
2a	$C_{21}H_{17}N_3O_2$	343.352	290–291	65	257	0.689
2b	$C_{13}H_{12}N_4O_3$	272.260	273–274	55	249	0.603
2c	$C_{19}H_{11}N_3O_4$	321.260	>300	47	253	0.620
2d	$C_{15}H_{11}N_5O_2$	293.280	>300	60	278	0.682
2e	$C_{16}H_{12}N_4O_2$	292.292	256–258	48	269	0.487
3	$C_{10}H_8N_2O_3$	204.182	230–231	45	250	0.714
4a	$C_{17}H_{12}N_2O_3$	292.289	281–282	63	278	0.533
4b	$C_{18}H_{14}N_2O_5$	338.314	101–102	52	284	0.757
4c	$C_{19}H_{17}N_3O_3$	335.35	98–100	64	318	0.679

* Mobile phase: benzene/methanol/water (7:2:1).

Table 2
Antitubercular and antimicrobial activities of the synthesized compounds

Compound	Antibacterial[a]				Antifungal[a]		*M. tuberculosis* H_{37} *Rv* (MIC in μg/ml)
	S. aureus	*E. coli*	*P. vulgaris*	*P. aeruginosa*	*A. Niger*	*C. albicans*	
2a	26	30	30	35	28	20	9.920
2b	33	37	32	39	23	21	8.012
2c	29	30	31	34	23	23	10.593
2d	29	39	34	39	28	20	10.775
2e	36	33	32	35	25	18	10.245
3	20	24	26	29	20	00	8.561
4a	20	26	30	29	24	18	8.928
4b	16	16	18	18	16	10	10.775
4c	18	33	33	31	00	00	16.447
Std[b]	24	25	34	22	21	13	–
Blank[c]	00	00	00	00	00	00	00

[a] The antibacterial and antifungal activities are the average results of triplicate experiments and reported as zone of inhibition in millimeter at a test concentration of 100 μg/disk.
[b] Nalidixic acid (50 μg/disk) and clotrimazole (50 μg/disk) were used, respectively, for antibacterial and antifungal activities.
[c] Dimethyl formamide for bacteria and fungi, and dimethyl sulfoxide for mycobacteria were used as blank solvents.

(**2a–e)** chain at quinoxalines-2,3(1*H*,4*H*)-dione exhibited significant antimicrobial potential against bacteria than against fungi. Among the series, 1-((3-methyl-5-oxo-4,5-dihydro-1*H*-pyrazol-4-yl)methyl)quinoxaline-2,3(1*H*,4*H*)-dione **(2b)** showed predominant antitubercular activity with the minimum inhibitory concentration of 8.012 μg/ml, while the rest of the compounds showed between 8.928 and 10.775 μg/ml, except the compound **4c** with 16.447 μg/ml among the compounds synthesized. However, the introduction of chalcones moiety at parent quinoxaline decreased the antimicrobial activity toward all the test microbes impartially, whereas heteryl substitution with ethylene linkage resulted in a significant enhancement in the antibacterial activity with moderate enhancement against antifungal activity.

Acknowledgments

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