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Anti-TB evaluation of novel 2,3-dihydroquinazolin-4(1*H*)-ones and *in silico* studies of the active compounds

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Abstract: *In vitro* anti-tubercular activity of a series of 15 novel 2,3-dihydroquinazolin-4(1*H*)-one analogues were evaluated against *Mycobacterium tuberculosis* H₃₇Ra (ATCC 25177 strain). Among the series, seven compounds showed moderate to good anti-TB activity with minimum inhibitory concentration (MIC) values ranging from 25.0-12.5 µg/mL. Further, *in silico* experiments were carried out to identify the probable ligand-protein interaction. Molecular docking of the target compounds into the active site of enzymes 1DQY Antigen 85C from *Mycobacterium Tuberculosis* and 2NSD Enoyl Acyl Carrier Protein Reductase reveals notable information on the possible binding interactions.

Keywords: Tuberculosis; Anti-tubercular activity; 2,3-Dihydroquinazolin-4(1*H*)-ones; Molecular docking

Introduction

Tuberculosis (TB) a communicable disease, spreads through air droplets containing *M. tuberculosis* bacilli. TB is treated with a combination of 1st line anti-TB agents, viz. Isoniazid, Rifampicin, Ethambutol and Pyrazinamide. The drug susceptible disease is cured after a 6 months long treatment under DOTs (Directly observed treatment) Program recommended by WHO. The present regimens of TB treatment have many drawbacks such as long treatment duration with drugs that are reported to cause organ toxicity and non-compliance to treatment [1,2]. This may result in the development of drug resistance. To improve the current regimens of TB, medicinal chemists are trying to design and synthesize new drug molecules that may overcome these shortcomings.

Quinazoline is one of the vital N-containing heterocycles bearing a benzene ring and a pyrimidine ring in its structure with chemical formula C₈H₆N₂ [3]. Quinazoline and its analogues appear in more than 100 biologically significant naturally occurring alkaloids, most commonly in the form of quinazolin-4(3*H*)-one moiety [4]. Medicinal chemists synthesized a variety of quinazoline compounds with different biological activities by introducing various active groups to the quinazoline moiety using developed as well as new synthetic protocols. The potential applications of the quinazoline derivatives in the fields of biology, pesticides and medicine have also been explored. One of the important quinazoline derivatives, deoxyvasicinone (2,3-dihydropyrrolo[2,1-*b*]quinazolin-9(1*H*)-one) is an alkaloid

isolated from the aerial parts of *Justicia adhatoda* Linn. (Sanskrit- Vasaka), an evergreen sub-herbaceous bush, used extensively as local medicine for cough, cold, bronchitis and asthma [5]. Deoxyvasicinone possesses wide spectrum of biological properties like anti-microbial, anti-inflammatory and antidepressant activities [6-9] as well as very important key intermediate for the synthesis of various natural products such as vasicinone [10], isaindigotone [11] and luotonin A [12]. Because of their diverse biological and pharmacological activities and extensive applications in pharmaceutical, research interest on the synthesis of quinazoline and its derivatives has never faded.

2,3-dihydroquinazolin-4(1*H*)-one is one of the derivatives of quinazoline that possesses broad range of biological, medicinal and pharmacological activities such as anti-tumor, antibiotic, anti-tubercular, anti-defibrillatory, anti-pyretic, analgesic, anti-hypertonic, diuretic, anti-histamine, anti-depressant, vasodilating agents and many more [13-22]. Since 2,3-dihydroquinazolin-4(1*H*)-one acquire different biological applications, in the present work, we made an attempt to synthesize some novel 2,3-dihydroquinazolin-4(1*H*)-ones with an aim to get probable novel anti-TB agent(s).

Results and Discussion

Chemistry

Our aim was to synthesize a series of novel derivatives of 2,3-dihydroquinazolin-4(1*H*)-ones and evaluation of their anti-TB potency. Using our method developed recently [23], we have synthesized ten derivatives of novel 2,3-dihydroquinazolin-4(1*H*)-ones. Afterwards, the work was extended for the construction of some novel bis-2,3-dihydroquinazolin-4(1*H*)-one analogues via Glaser coupling reaction using copper acetate in which **2a-2e** were used as starting precursors [24]. All the five reactions gave the desired novel bis-2,3-dihydroquinazolin-4(1*H*)-ones in good to excellent yields which was reported in our recent work [23]. From ¹H and ¹³C NMR spectroscopic analysis, it was confirmed that all these novel bis-2,3-dihydroquinazolin-4(1*H*)-one analogues were C₂-symmetric. Literature survey reveals that C₂-symmetric molecules have been employed in a number of catalytic reactions as ligands as they limit the different types of side reactions. Therefore, these five C₂-symmetric analogues of 2,3-dihydroquinazolin-4(1*H*)-one are also expected to have extensive application in various organic transformations in coming days. All the synthesized 15 novel 2,3-dihydroquinazolin-4(1*H*)-one compounds are depicted in **Scheme 1**.

Scheme 1 Novel analogues of 2,3-dihydroquinazolin-4(1*H*)-ones

Biological evaluation

Proportion (agar dilution) assay [25] was used for anti-TB activity determination of the synthesized compounds in terms of Minimum Inhibitory Concentrations (MICs) against MTB H₃₇Ra (ATCC 25177). Different concentrations of the compounds ranging from 25.0 to 3.125 $\mu\text{g/mL}$ were tested to determine the MICs. From this experiment one compound (2b) was found to display appreciable anti-TB activity with MIC 12.5 $\mu\text{g/mL}$. Other six compounds (2e, 2h, 2j, 3b, 3d and 3e) showed activity at the concentration of 25.0 $\mu\text{g/mL}$. The remaining 8 compounds did not show activity up to 25.0 $\mu\text{g/mL}$, the highest concentration tested. In the present work, Ethambutol, an anti-TB drug, was used as a positive control.

Table 1 Anti-tubercular activity of newly synthesized compounds

Compounds	Structure	ClogP ^a	CMR ^a	MIC ($\mu\text{g/mL}$)
2a		3.6068	7.9272	>25
2b		2.7968	8.0678	12.5
2c		2.7968	8.0678	>25
2d		2.7968	8.0678	>25
2e		4.3198	8.4186	25
2f		5.4208	10.5199	>25
2g		5.4208	10.5199	>25
2h		3.9338	11.0775	25

2i		3.2208	10.5861	>25
2j		3.2208	10.5861	25
3a		5.6196	15.829	>25
3b		3.9996	16.1042	25
3c		5.6196	15.829	>25
3d		5.6196	15.829	25
3e		7.0456	16.8116	25
Ethambutol		0.1188	5.859	2.0

^aCLog P and CMR were calculated using Chemdraw Ultra 12.0 software by Cambridge Soft.

Molecular docking study

In silico experiments were carried out to identify the ligand-protein interaction. In this work, LibDock was used to evaluate the binding affinities between the active compounds and the enzymes 2NSD Enoyl Acyl Carrier Protein Reductase and 1DQY Antigen 85C from *M.*

tuberculosis. Among all the compounds tested against the Enoyl Acyl Carrier Protein Reductase, five compounds exhibited good docking scores (**Table 2**). Among the five compounds, compound **3b** showed the lowest CDOCKER interaction energy score of -54.5525 kcal/mol. Apart from the binding energy, it has formed one conventional H-bond with the residue Phe41. Conventional H-bonds are the major contributors for the stability in binding of protein and compounds. Apart from H-bond, it also formed some other interaction such as C-H bond, unfavorable donor-donor, pi-donor H-bond, pi-sigma, pi-pi T-shaped and pi-alkyl. The 2D and 3D structures of the docked complex is depicted in **Figure 1**.

Figure 1 2D and 3D structure of **3b** with target 2NSD

Table 2 Docking score of 2NSD with five compounds and potential amino acids of the protein interacting with ligands along with the interaction

Comp.	CDOCKER energy (Kcal/mol)	CDOCKER interaction energy (Kcal/mol)	Interacting amino acid	Interaction
2b (POSE2)	-30.8509	-36.2778	Lys165, MET147, Ala191, Gly14, Ser20, Ile21, Ala198	Conventional H-bond, C-H bond, pi-alkyl
2e (POSE2)	-23.921	-34.346	Ile21, Met161, Thr196, Ala198, Met147	Conventional H-bond, pi-alkyl
2j (POSE2)	-27.1222	-40.1077	Ile16, Ile21, Ser94, Lys165, Met161, Ala198	Conventional H-bond, C-H bond, pi-sulfur, pi-alkyl
2h (POSE2)	-22.9169	-39.1576	Phe149, Met199, Ala198, Ile21	C-H bond, pi-pi T- shaped, pi-alkyl

3b (POSE2)	-40.574	-54.5525	Phe41, Met199, Phe149, Ile21, Gly96, Ala198, Ile16	Conventional H-bond, C-H bond, unfavorable donor-donor, pi-donor H-bond, pi-sigma, pi-pi T-shaped, pi-alkyl
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Similarly an *in silico* experiment was executed for these seven compounds on 1DQY Antigen 85C from *M. tuberculosis* for exploring the anti-TB potency of the synthesized novel analogues. Only two compounds tested against the Antigen 85C showed good docking scores (**Table 3**). Compound **2b** showed the lowest CDOCKER interaction energy score of -37.0648 kcal/mol. Apart from the binding energy it has formed 2 conventional H-bonds with the residues Asp38 and Arg41 which are lying within the active pocket of the protein. Apart from H-bonds, it also formed some other interaction such as C-H bond, pi-pi T-shaped and pi-alkyl. The 3D and 2D structures of the docked complex is shown in **Figure 2**.

Figure 2 2D and 3D structures of **2b** with target 1DQY

Table 2 Docking score of 1DQY with two compounds and potential amino acids of the protein interacting with ligands along with the interaction

Comp.	CDOCKER energy (Kcal/mol)	CDOCKER interaction energy (Kcal/mol)	Interacting amino acid	Interaction
2b (POSE2)	-30.7248	-37.0648	Asp38, Trp262, Arg41, Asp216, Leu40	Conventional H-bond, C-H bond, pi-pi T shaped, pi-alkyl
2e (POSE2)	-16.9365	-31.0986	Lys165, Met147, Ala191, Gly14, Ser20, Ile21, Ala198	Conventional H-bond, C-H bond, pi-alkyl

Conclusion

In summary, we designed and successfully executed synthesis of some novel derivatives of 2,3-dihydroquinazolin-4(*1H*)-one including five C₂ symmetric bis-2,3-dihydroquinazolin-4(*1H*)-ones. The newly synthesized compounds were evaluated for their potent *in vitro* anti-tubercular activity. Among the 15 novel compounds, seven were identified as potent anti-TB agents with MIC in the range of 25.0-12.5 μ g/mL. The most potent compound, **2b** inhibited the growth of *MTB* at the concentration of 12.5 μ g/mL. Further, molecular docking of the target compounds into the active site of enzymes 1DQY Antigen 85C from Mycobacterium Tuberculosis and 2NSD Enoyl Acyl Carrier Protein Reductase reveals notable information on the possible binding interactions. These findings suggest that the newly designed compounds may be considered as potential agents for anti-TB drug discovery.

Experimental

General method for synthesis of 2,3-dihydroquinazolin-4(*1H*)-ones (2a-2j) [23]

Isatoic anhydride (1 mmol), amines (1.2 mmol), carbonyls (1.2 mmol) and [$\{\text{SiO}_2\text{-(acac)}\}_3\text{Fe}^{\text{III}}\}\text{Cl}_3$ (20 mg, 32.24 ppm Fe) in water (2 mL) were stirred in a flask equipped with a condenser at 80 °C. On completion (monitored by TLC under UV light), the resulting mixture was cooled to room temperature and then the mixture was extracted with ethyl acetate (3 x 10 mL) and washed with H₂O (3 x 10 mL). The combined extract was dried over anhydrous Na₂SO₄. The solvent of filtrate was removed with the aid of a rotary evaporator. The product was purified by column chromatography on silica gel using petroleum

ether/ethyl acetate (3:1 v/v) as eluent to obtain the pure novel compounds. The products were then characterized by ESI-MS, ^1H NMR and ^{13}C NMR analyses.

General procedure for the synthesis of compounds (3a-3e) [23]

A mixture of **2a-2e** (1 mmol), piperidine (1 mmol) and $\text{Cu}(\text{OAc})_2 \cdot \text{H}_2\text{O}$ (10 mol%) in CH_2Cl_2 (2 mL) was stirred in an open air at room temperature for 10 hours. On completion (monitored by TLC under UV light), the resulting mixture was extracted with ethyl acetate (3 x 10 mL) and washed with water (3 x 10 mL). The combined extract was dried over anhydrous Na_2SO_4 . The solvent of filtrate was removed with the aid of a rotary evaporator. The crude product was purified via column chromatography on silica gel using petroleum ether/ethyl acetate (3:1 v/v) as eluent to obtain the pure novel compounds. The compounds were then characterized by ESI-MS, ^1H NMR and ^{13}C NMR analyses.

2-(4-Fluorophenyl)-3-(prop-2-yn-1-yl)-2,3-dihydroquinazolin-4(1H)-one (2a)

Brown solid; 98% yield; Mp: 136-138 °C; IR (KBr) cm^{-1} : 3302, 2927, 2365, 2346, 1634, 1506, 1427, 1304, 1226, 1154, 860, 842, 750, 683, 651, 622, 546, 525; ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 7.67 (dd, $J = 7.7, 1.4$ Hz, 1H), 7.42 (ddd, $J = 13.0, 8.2, 2.0$ Hz, 3H), 7.23 (dddd, $J = 19.2, 8.9, 7.0, 1.8$ Hz, 2H), 6.73-6.66 (m, 2H), 5.98 (s, 1H), 3.64 (s, 2H), 3.16 (t, $J = 2.4$ Hz, 1H); ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$) δ 163.39, 162.02, 146.38, 136.16, 133.72, 128.71, 128.63, 127.60, 117.37, 115.44, 115.23, 114.34, 113.92, 78.93, 74.45, 69.63, 33.06 ; MS (ESI) calcd for $\text{C}_{17}\text{H}_{13}\text{FN}_2\text{O}$ $[\text{M} + \text{H}]^+$ 281.1090; found 281.1100.

2-(4-Hydroxyphenyl)-3-(prop-2-yn-1-yl)-2,3-dihydroquinazolin-4(1H)-one (2b)

Brown solid; 95% yield; Mp: 58-60 °C; IR (KBr) cm^{-1} : 3358, 3253, 2926, 2854, 1634, 1611, 1500, 1456, 1428, 1405, 1266, 1232, 1167, 986, 840, 686, 593, 554, 526; ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 7.66 (d, $J = 7.9$ Hz, 1H), 7.22 (dd, $J = 13.2, 7.9$ Hz, 4H), 6.83-6.57 (m, 3H), 5.84 (s, 1H), 4.64 (d, $J = 17.3$ Hz, 2H), 3.11 (s, 1H); ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$) δ 162.91, 158.38, 147.32, 134.16, 130.30, 128.58, 128.14, 117.69, 115.71, 114.78, 114.34, 79.59, 74.63, 70.78, 33.10 ; MS (ESI) calcd for $\text{C}_{17}\text{H}_{14}\text{N}_2\text{O}_2$ $[\text{M} + \text{H}]^+$ 279.1133; found 279.1139.

2-(3-Fluorophenyl)-3-(prop-2-yn-1-yl)-2,3-dihydroquinazolin-4(1H)-one (2c)

White solid; 98% yield; Mp: 80-82 °C; IR (KBr) cm^{-1} : 3429, 3295, 1640, 1620, 1589, 1528, 1488, 1452, 1428, 1360, 1307, 1259, 1158, 1134, 1035, 919, 860, 797, 748, 656, 631, 585, 521; ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 7.66 (d, $J = 1.4$ Hz, 1H), 7.49-7.12 (m, 4H), 6.71-6.69 (m, 1H), 6.50-6.44 (m, 2H), 6.01 (s, 1H), 4.08 (s, 2H), 3.06 (t, $J = 2.5$ Hz, 1H); ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$) δ 168.51, 149.81, 146.23, 142.96, 133.76, 131.90, 128.01, 127.58,

122.38, 117.51, 116.39, 114.49, 113.67, 81.59, 74.59, 72.41, 33.41 ; MS (ESI) calcd for $C_{17}H_{13}FN_2O$ $[M + H]^+$ 281.1090; found 281.1085.

2-(2-Fluorophenyl)-3-(prop-2-yn-1-yl)-2,3-dihydroquinazolin-4(1H)-one (2d)

Light brown solid; 95% yield; Mp: 116-118 °C; IR (KBr) cm^{-1} : 3326, 3247, 2927, 2850, 2123, 1632, 1513, 1488, 1426, 1401, 1339, 1307, 1275, 1235, 1158, 1033, 988, 946, 843, 759, 693, 620, 550, 521; 1H NMR (400 MHz, DMSO- d_6) δ 7.70 (dd, $J = 7.7, 1.5$ Hz, 1H), 7.41-7.12 (m, 6H), 6.74-6.66 (m, 2H), 6.30 (s, 1H), 3.72 (s, 2H), 3.15 (t, $J = 2.4$ Hz, 1H); ^{13}C NMR (100 MHz, DMSO- d_6) δ 161.94, 160.80, 146.04, 133.72, 130.80, 127.53, 124.48, 124.45, 117.36, 116.01, 115.80, 114.35, 113.64, 78.61, 74.59, 64.59, 32.95 ; MS (ESI) calcd for $C_{17}H_{13}FN_2O$ $[M + H]^+$ 281.1090; found 281.1085.

2-(2-Chloro-6-fluorophenyl)-3-(prop-2-yn-1-yl)-2,3-dihydroquinazolin-4(1H)-one (2e)

Pale yellow solid; 92% yield; Mp: 72-74 °C; IR (KBr) cm^{-1} : 3432, 2956, 2923, 2854, 2331, 1637, 1466, 1380, 1164, 1077, 1020, 985, 916, 799; 1H NMR (400 MHz, DMSO- d_6) δ 8.59 (s, 1H), 7.66 (dd, $J = 7.7, 1.5$ Hz, 1H), 7.47-7.21 (m, 3H), 6.70-6.41 (m, 3H), 5.78 (s, 1H), 3.97 (s, 2H), 3.05 (s, 1H); ^{13}C NMR (100 MHz, DMSO- d_6) δ 163.70, 161.83, 150.25, 134.07, 132.46, 128.54, 127.82, 126.73, 115.08, 114.21, 113.89, 113.17, 82.12, 78.83, 72.92, 32.74 ; MS (ESI) calcd for $C_{17}H_{12}ClFN_2O$ $[M + H]^+$ 315.0700; found 315.0745.

2-(3-Fluorophenyl)-3-(4-(methylthio)phenyl)-2,3-dihydroquinazolin-4(1H)-one (2f)

Light brown solid; 62% yield; Mp: 174-176 °C; IR (KBr) cm^{-1} : 3422, 3296, 2923, 2852, 1642, 1614, 1587, 1487, 1448, 1387, 1292, 1241, 1122, 1020, 928, 871, 828, 773, 753, 689, 669, 543, 518; 1H NMR (500 MHz, DMSO- d_6) δ 7.74-7.68 (m, 2H), 7.30-7.20 (m, 8H), 6.79-6.74 (m, 2H), 6.32 (s, 1H), 2.45 (s, 3H); ^{13}C NMR (125 MHz, DMSO- d_6) δ 162.49, 161.42, 146.77, 143.97, 137.86, 136.18, 134.25, 130.86, 128.34, 127.09, 126.46, 123.02, 123.00, 118.13, 115.69, 115.57, 115.52, 115.20, 113.95, 72.25, 15.14; MS (ESI) calcd for $C_{21}H_{17}FN_2OS$ $[M + H]^+$ 365.1124; found 365.1119.

2-(2-Fluorophenyl)-3-(4-(methylthio)phenyl)-2,3-dihydroquinazolin-4(1H)-one (2g)

Light brown solid; 85% yield; Mp: 158-160 °C; IR (KBr) cm^{-1} : 3292, 2923, 2367, 2345, 1637, 1610, 1509, 1493, 1401, 1363, 1322, 1260, 1231, 1162, 1089, 1051, 1020, 954, 820, 765, 687, 623, 529, 465, 434; 1H NMR (400 MHz, DMSO- d_6) δ 7.84-7.09 (m, 10H), 6.79-6.71 (m, 2H), 6.51 (s, 1H), 2.43 (s, 3H); ^{13}C NMR (100 MHz, DMSO- d_6) δ 162.21, 160.46, 146.38, 137.04, 136.30, 133.77, 130.70, 130.62, 128.14, 128.11, 127.84, 127.38, 125.97, 124.45, 124.42, 117.55, 115.90, 115.69, 114.59, 67.71, 14.63; MS (ESI) calcd for $C_{21}H_{17}FN_2OS$ $[M + H]^+$ 365.1124; found 365.1119.

2-(2-Chloro-6-fluorophenyl)-3-(4-(methylsulfonyl)phenyl)-2,3-dihydroquinazolin-4(1H)-one (2h)

Light brown solid; 58% yield; Mp: 90-92 °C; IR (KBr) cm^{-1} : 3473, 3375, 2923, 2852, 1670, 1620, 1588, 1568, 1518, 1489, 1453, 1420, 1301, 1247, 1160, 1113, 1050, 910, 860, 782, 749, 718, 657, 530; ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 7.84-7.62 (m, 3H), 7.20 (t, $J = 7.4$ Hz, 3H), 6.71 (d, $J = 8.3$ Hz, 2H), 6.48 (t, $J = 7.4$ Hz, 3H), 5.68 (s, 1H), 3.49 (s, 3H); ^{13}C NMR (100 MHz, $\text{DMSO-}d_6$) δ 169.97, 166.54, 151.89, 145.52, 134.81, 134.20, 131.61, 127.07, 126.12, 124.98, 123.42, 116.75, 115.06, 110.03, 77.87, 48.11 ; MS (ESI) calcd for $\text{C}_{21}\text{H}_{16}\text{ClFN}_2\text{O}_3\text{S}$ $[\text{M} + \text{H}]^+$ 431.0632; found 431.0645.

2-(3-Fluorophenyl)-3-(4-(methylsulfonyl)phenyl)-2,3-dihydroquinazolin-4(1H)-one (2i)

Pale yellow solid; 55% yield; Mp: 116-118 °C; IR (KBr) cm^{-1} : 3473, 3375, 2923, 1673, 1620, 1589, 1563, 1486, 1420, 1302, 1250, 1159, 1117, 1053, 920, 751, 657, 530; ^1H NMR (500 MHz, $\text{DMSO-}d_6$) δ 7.83-7.79 (m, 2H), 7.70 -7.67 (m, 2H), 7.22 (ddd, $J = 8.5, 7.1, 1.6$ Hz, 2H), 6.74-6.73 (m, 5H), 6.51-6.48 (m, 1H), 5.74 (s, 1H), 3.09 (s, 3H); ^{13}C NMR (125 MHz, $\text{DMSO-}d_6$) δ 163.28, 161.34, 151.85, 134.66, 134.05, 131.49, 131.18, 131.12, 125.78, 125.76, 123.28, 120.30, 116.66, 116.17, 115.99, 114.88, 109.92, 73.03, 45.80 ; MS (ESI) calcd for $\text{C}_{21}\text{H}_{17}\text{FN}_2\text{O}_3\text{S}$ $[\text{M} + \text{H}]^+$ 397.1022; found 397.1045.

2-(2-Fluorophenyl)-3-(4-(methylsulfonyl)phenyl)-2,3-dihydroquinazolin-4(1H)-one (2j)

Pale yellow solid; 56% yield; Mp: 112-114 °C; IR (KBr) cm^{-1} : 3474, 3375, 2922, 2854, 1674, 1615, 1588, 1564, 1487, 1421, 1304, 1248, 1160, 1116, 1053, 920, 859, 750, 708, 658, 530; ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 7.92-7.76 (m, 2H), 7.64 (dd, $J = 19.1, 7.1$ Hz, 1H), 7.24 (dt, $J = 15.1, 7.8$ Hz, 3H), 6.71 (d, $J = 8.3$ Hz, 3H), 6.48 (t, $J = 7.5$ Hz, 3H), 5.83 (s, 1H), 3.55 (s, 3H); ^{13}C NMR (100 MHz, $\text{DMSO-}d_6$) δ 162.86, 160.30, 151.87, 135.24, 135.15, 134.80, 134.21, 133.08, 132.36, 131.61, 124.94, 124.91, 123.41, 117.49, 117.27, 116.76, 115.07, 110.01, 76.02, 47.39; MS (ESI) calcd for $\text{C}_{21}\text{H}_{17}\text{FN}_2\text{O}_3\text{S}$ $[\text{M} + \text{H}]^+$ 397.1022; found 397.1056.

3,3'-(Hexa-2,4-diyne-1,6-diyl)bis(2-(4-fluorophenyl)-2,3-dihydroquinazolin-4(1H)-one)

(3a)

Light brown solid; 70% yield; Mp: 144-146 °C; IR (KBr) cm^{-1} : 3305, 2922, 1641, 1621, 1509, 1418, 1395, 1333, 1297, 1226, 1157, 1014, 978, 838, 784, 754, 695, 660, 601, 529; ^1H NMR (400 MHz, $\text{DMSO-}d_6$) δ 7.65 (d, $J = 7.5$ Hz, 2H), 7.39 (d, $J = 5.7$ Hz, 2H), 7.21 (dt, $J = 16.9, 7.9$ Hz, 8H), 6.68 (dd, $J = 13.3, 7.2$ Hz, 4H), 5.94 (s, 2H), 4.08 (s, 4H); ^{13}C NMR (100 MHz, $\text{DMSO-}d_6$) δ 162.72, 161.55, 147.02, 136.53, 134.41, 129.31, 129.23, 128.15, 118.01,

116.00, 115.79, 114.94, 114.28, 75.06, 70.68, 67.28, 34.46 ; MS (ESI) calcd for $C_{34}H_{24}F_2N_4O_2$ $[M + H]^+$ 559.1945; found 559.1951.

3,3'-(Hexa-2,4-diyne-1,6-diyl)bis(2-(4-hydroxyphenyl)-2,3-dihydroquinazolin-4(1H)-one) (3b)

Brown solid; 80% yield; Mp: 152-154 °C; IR (KBr) cm^{-1} : 3419, 2959, 2927, 2858, 2340, 1633, 1612, 1512, 1488, 1336, 1235, 1168, 923, 797, 752, 694, 659; 1H NMR (400 MHz, DMSO- d_6) δ 9.60 (s, 1H), 7.72-6.84 (m, 12H), 6.71 (dd, J = 31.0, 7.3 Hz, 4H), 5.80 (s, 2H), 4.73 (s, 4H); ^{13}C NMR (100 MHz, DMSO- d_6) δ 160.79, 156.26, 145.20, 132.04, 128.18, 126.46, 126.01, 115.56, 113.59, 112.66, 112.62, 112.22, 77.47, 72.51, 68.65, 30.98 ; MS (ESI) calcd for $C_{34}H_{26}N_4O_4$ $[M + H]^+$ 555.2032; found 555.2039.

3,3'-(Hexa-2,4-diyne-1,6-diyl)bis(2-(3-fluorophenyl)-2,3-dihydroquinazolin-4(1H)-one) (3c)

Brown solid; 85% yield; Mp: 96-98 °C; IR (KBr) cm^{-1} : 3331, 3064, 2927, 2853, 2340, 1633, 1615, 1590, 1515, 1485, 1255, 1159, 1032, 872, 752, 695, 658, 522; 1H NMR (400 MHz, DMSO- d_6) δ 7.66-7.35 (m, 4H), 7.26-7.11 (m, 4H), 6.68 (d, J = 7.9 Hz, 6H), 6.47 (dd, J = 19.7, 12.4 Hz, 2H), 5.95 (s, 2H), 4.08 (s, 4H); ^{13}C NMR (100 MHz, DMSO- d_6) δ 162.57, 161.24, 150.36, 134.43, 132.61, 131.17, 128.54, 128.12, 122.89, 118.13, 116.98, 115.12, 113.91, 76.98, 70.64, 66.13, 29.30 ; MS (ESI) calcd for $C_{34}H_{24}F_2N_4O_2$ $[M + H]^+$ 559.1945; found 559.1951.

3,3'-(Hexa-2,4-diyne-1,6-diyl)bis(2-(2-fluorophenyl)-2,3-dihydroquinazolin-4(1H)-one) (3d)

Brown solid; 90% yield; Mp: 86-88 °C; IR (KBr) cm^{-1} : 3300, 2926, 2856, 1642, 1614, 1488, 1449, 1420, 1335, 1156, 1097, 1028, 928, 753, 695, 661, 523; 1H NMR (400 MHz, DMSO- d_6) δ 7.73-7.57 (m, 6H), 7.47-7.07 (m, 6H), 6.68 (d, J = 7.3 Hz, 4H), 6.22 (s, 2H), 3.98 (s, 4H); ^{13}C NMR (100 MHz, DMSO- d_6) δ 162.61, 160.77, 146.69, 135.66, 134.39, 131.43, 131.35, 128.44, 128.24, 128.07, 127.91, 126.87, 117.96, 116.55, 116.33, 114.92, 114.03, 79.61, 74.78, 65.82, 34.49 ; MS (ESI) calcd for $C_{34}H_{24}F_2N_4O_2$ $[M + H]^+$ 559.1945; found 559.1951.

3,3'-(Hexa-2,4-diyne-1,6-diyl)bis(2-(2-chloro-6-fluorophenyl)-2,3-dihydroquinazolin-4(1H)-one) (3e)

Brown solid; 50% yield; Mp: 66-68 °C; IR (KBr) cm^{-1} : 3421, 2959, 2923, 2853, 2332, 1636, 1541, 1467, 1381, 1162, 1077, 985, 918, 803, 749; 1H NMR (400 MHz, DMSO- d_6) δ 8.65 (s, 2H), 7.45 (d, J = 7.7 Hz, 2H), 7.13 (t, J = 7.6 Hz, 6H), 6.69 (d, J = 8.2 Hz, 2H), 6.47 (dd, J = 18.6, 11.1 Hz, 4H), 5.84 (s, 2H), 4.09 (s, 4H); ^{13}C NMR (100 MHz, DMSO- d_6) δ 169.16,

169.08, 150.31, 132.57, 128.55, 127.79, 116.94, 115.10, 113.92, 76.97, 66.17, 60.22, 29.32 ; MS (ESI) calcd for C₃₄H₂₂Cl₂F₂N₄O₂ [M + H]⁺ 627.1166; found 627.1172.

Evaluation of *in vitro* anti-tubercular activity of compounds

Initially all the synthesized compounds were screened against *M. tuberculosis* H₃₇Ra (ATCC 25177 strain) at the single concentration of 25 µg/mL. The active compounds from the above screening were further tested to determine MIC using Agar Proportion assay. To make stocks (5 mg/mL), the compounds were dissolved in DMSO. Serial two fold dilutions were also made in DMSO from the stocks. 0.1 mL of compound or DMSO (negative control) or anti-TB drugs (positive controls) was added to 1.9 mL MB7H10 agar medium (in tubes, temperature 45-50 °C, with OADC supplement, final concentration 10%). Then the contents were mixed and allowed to solidify as slants. Three week old culture of *M. tuberculosis* H₃₇Ra was harvested from L-J medium and its suspension (1 mg/mL, equivalent to approximately 10⁸ bacilli) was prepared in normal saline containing 0.05% Tween-80. 10 µL of 1:10 dilution of this suspension (~10⁵ bacilli) was inoculated into each tube for 4 weeks at the temperature of 37 °C. The lowest concentration of a compound up to which there was no visible growth of bacilli was its Minimal Inhibitory Concentration (MIC). Ethambutol was used as reference drug.

CDocker algorithm in Discovery studio

The Dock Ligands (CDOCKER) protocol is an implementation of the CDOCKER algorithm. It allows running a refinement docking of any number of ligands with a single protein receptor. CDOCKER is a grid based molecular docking method that employs CHARMm. The receptor is held rigid while the ligands are allowed to flex during the refinement. For predocked ligands, prior knowledge of binding site is not required. It is possible, however, to specify the ligand placement in the active site using a binding site sphere. Random ligand conformations are generated from the initial ligand structure through high temperature molecular dynamics, followed by random rotations. The random conformations are refined by grid based (GRID 1) simulated annealing and a final grid based or full force field minimization. CDOCKER uses a detailed atomic force field that is comprised of accuracy of automated MD docking with a soft-core potential over Monte Carlo (MC) Simulations and Genetic Algorithm (GA) in searching a large configuration space.

Force-field and grid

The grid origin is situated at the centre of the active sites of the protein. A +1 point charge probe is used to map electrostatic interactions to the grid. The grid's vdW interactions are generated in one of two ways. The energies and forces are generated for the soft-core potentials in the heating-cooling stages and with normal non bond potentials in the final minimization step. The soft-core is generally approximated by the below function:

$$E_{ij}(r_{ij}) = E_{\max} - a * r_{ij}^b \text{ if } |E^*_{ij}| > \frac{|E_{\max}|}{2}$$

where E^*_{ij} is the energy of regular non bond (vdW or electrostatic) potential. The coefficients a and b were extracted from two equations that express equality of regular and soft potential and forces at the switching distance.

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Conflict of interest

The authors declare no competing interests.

References

1. Sotgiu, G.; Centis, R.; D'ambrosio, L.; Migliori, G. B. *Cold Spring Harb. Perspect. Med.*, **2015**, 5, a017822.
2. Singh, P.; Mishra, A. K.; Malonia, S. K.; Chauhan, D. S.; Sharma, V. D.; Venkatesan, K.; Katoch, V. M. *J. Commun. Dis.*, **2006**, 38, 288.
3. Srivastava, S.; Srivastava, S. *Int. J. Pharm. Sci.*, **2015**, 6, 1206.
4. Mhaske, S. B.; Argade, N. P. *Tetrahedron*, **2006**, 62, 9787.
5. Mehta, D. R.; Naravane, J. S.; Desai, R. M. *J. Org. Chem.*, **1963**, 28, 445.
6. Amin, A. H.; Mehta, D. R. *Nature*, **1959**, 183, 1317.
7. Joshi, B. S.; Newton, M. G.; Lee, D. W.; Barber, A. D.; Pelletier, S. W. *Tetrahedron Asymmetry*, **1996**, 7, 25.
8. Atal, C. K. In *Chemistry and Pharmacology of Vasicine*. Raj Bandu Industrial Co, Delhi, **1980**.
9. Ghosal, S.; Chauhan, P. S. R. B.; Mehta, R. *Phytochemistry*, **1975**, 14, 830.

10. Al-Shamma, A.; Drake, S.; Flynn, D. L.; Mitscher, L. A.; Park, Y. H.; Rao, G. S. R.; Simpson, A.; Swayze, J. K.; Veysoglu, T.; Wu, S. S. *J. Nat. Prod.*, **1981**, *44*, 745.
11. Kamal, A.; Ramana, K. V.; Rao, M. V. *J. Org. Chem.*, **2001**, *66*, 997.
12. Molina, P.; Tarraga, A.; Gonzalez-Tejero, A. *Synthesis*, **2000**, *11*, 1523.
13. Zhang, H.; Liu, H.; Luo, X.; Wang, Y.; Liu, Y.; Jin, H.; Liu, Z.; Yang, W.; Yu, P.; Zhang, L.; Zhang, L. *Eur. J. Med. Chem.*, **2018**, *152*, 235.
14. Nagaladinne, N.; Hindustan, A.; Nayakanti, D. *Indian J. Pharm. Sci.*, **2020**, *82*, 984.
15. Marvania, B.; Lee, P. C.; Chaniyara, R.; Dong, H.; Suman, S.; Kakadiya, R.; Su, T. L. *Bioorg. Med. Chem.*, **2011**, *19*, 1987.
16. El-Azab, A. S.; ElTahir, K. E. *Bioorg. Med. Chem. Lett.*, **2012**, *22*, 1879.
17. Maurya, H. K.; Verma, R.; Alam, S.; Pandey, S.; Pathak, V.; Sharma, S.; Gupta, A. *Bioorg. Med. Chem. Lett.*, **2013**, *23*, 5844.
18. Dukat, M.; Alix, K.; Worsham, J.; Khatri, S.; Schulte, M. K. *Bioorg. Med. Chem. Lett.*, **2013**, *23*, 5945.
19. Akester, J. N.; Njaria, P.; Nchinda, A.; Le Manach, C.; Myrick, A.; Singh, V.; Lawrence, N.; Njoroge, M.; Taylor, D.; Moosa, A.; Smith, A. J. *ACS Infect. Dis.*, **2020**, *6*, 1951.
20. Jafari, E.; Khajouei, M. R.; Hassanzadeh, F.; Hakimelahi, G. H.; Khodarahmi, G. A. *Res. Pharm. Sci.*, **2016**, *11*, 1.
21. Couturier, C.; Lai, C.; Pellet, A.; Upton, A.; Kaneko, T.; Perron, C.; Cogo, E.; Menegotto, J.; Bauer, A.; Scheiper, B.; Lagrange, S.; Bacque, E. *Bioorg. Med. Chem. Lett.*, **2016**, *26*, 5290.
22. Maddali, N. K.; Viswanath, I. K.; Murthy, Y. L. N.; Bera, R.; Takhi, M.; Rao, N. S.; Gudla, V. *Med. Chem. Res.*, **2019**, *28*, 559-570.
23. Dutta, A.; Trivedi, P.; Kulashetra, A.; Kumar, A.; Chaturvedi, V.; Sarma, D. *Applied Organometallic Chem.*, **2021**, *35*, e6116.
24. Balaraman, K.; Kesavan, V. *Synthesis*, **2010**, *20*, 3461.
25. McClachy, J. K. *Lab Med.*, **1978**, *9*, 47.

Figures

Figure 1

2D and 3D structure of 3b with target 2NSD

Figure 2

Novel analogues of 2,3-dihydroquinazolin-4(1H)-ones

Figure 3

2D and 3D structures of 2b with target 1DQY

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