Results in Chemistry 4 (2022) 100374

Contents lists available at ScienceDirect

Results in Chemistry

journal homepage: www.sciencedirect.com/journal/results-in-chemistry

Discovery of oxazole-dehydrozingerone based hybrid molecules as potential anti-tubercular agents and their docking for M*tb* DNA gyrase

Suraj R. Shinde [a], Shaukatali N. Inamdar [a], Vincent A. Obakachi [a], Mahadev Shinde [a], Afsana Kajee [a,b], Meenu Ghai [a], Rajshekhar Karpoormath [a,*]

[a] *Department of Pharmaceutical Chemistry, Discipline of Pharmaceutical Sciences, College of Health Sciences, University of KwaZulu-Natal, Westville Campus, Durban, South Africa*

[b] *Department of Microbiology, National Health Laboratory Services (NHLS), Inkosi Albert Luthuli Central Hospital, Durban, South Africa*

ARTICLE INFO

Keywords:
Microwave-assisted
Anti-TB activity
MTB H37Rv

ABSTRACT

The oxazole-dehydrozingerone hybrid molecules (4a-j) and oxazole-dehydrozingerone-thiophene derivatives (6a-e) were synthesized via cyclisation, coupling and aldol condensation reactions. Final compounds were characterized by FTIR, ^{1}H and ^{13}C NMR spectroscopy. Synthesized compounds were screened against Mycobacterium tuberculosis H37Rv, MDR, and XDR strains. Compound 4f showed potential activity of 6.25 μg/mL against H37Rv, while compound 4c exhibited potential activity of 12.5 μg/mL. For the XDR strain, structure 4a, 4b demonstrated moderate efficiency of 12.5 μg/mL. All of the synthesized molecules were tested in comparison with a standard drug. Computational docking studies were performed for the active compound 4f against the enzyme M*tb* DNA Gyrase. The outcomes of the presented research will broadly help to the researchers working on developing antituberculosis drugs.

1. Introduction

Tuberculosis is an air-born contagious disease caused by mycobacterium tuberculosis (Mtb) [1,2]. In 2012, World Health Organization (WHO) reported 8.6 million infections, and among them, 1.3 million people died because of infectious diseases, and in 2016, 490,000 new cases of multidrug resistance were widely estimated [3]. There is a growing resistance to existing drugs resulting from deadly diseases that become more deadly and difficult to treat. MDR and extensive drug resistance (XDR) Mtb are diseases caused by bacteria that don't respond to first-line antitubercular drugs [4]. Existing treatment consists of various drugs that need to be taken for more than a year, resulting in numerous side effects and a substantial economic burden. In developing countries, pollution is the primary reason as Mtb can increase through the air. In recent years the death rate was declined, but it is still a significant cause of death after AIDS [5]. The drugs used for the treatment are streptomycin, Isoniazid, Ethambutol, Rifampicin, Ethionamide, Cycloserine, and Kanamycin. Most of these drugs have been discovered and used for the last 70 years [1]. Hence, there is an alarming concern about the drug-resistant strains of Mtb [6-9]. A constant research is underway for understanding the reasons behind the evolution and existence of resistant strains of Mtb [10]. Synthesis and high-throughput screenings of different derivatives with a broad spectrum of novel and known scaffolds were carried out to obtain lead derivatives as anti-TB [11-13]. The drug discovery and role of heterocyclic nuclei well known since the early 18th century [14]. The heterocyclic compounds are five or six-member rings bearing heteroatoms like nitrogen (N), oxygen (O), or sulfur (S). They play an essential role in all living cells' biochemical processes and find in natural and synthetic forms [15,16].

The fused heterocycles, such as oxazole and oxazoline, were commonly disturbed in nature and attracted considerable interest because of their various medicinal activities [17]. They were initially isolated from a marine source [18]. These hetero cores contains nitrogen and oxygen atoms in an aromatic five-membered ring that can bind with different receptors and enzymes in the biological system through non -covalent interactions [19]. Several advantages of the oxazole ring in medicinal chemistry are that it has weak interactions with H-bond, ion–dipole, π-π stacking, and a weak hydrophobic character. These nuclei found their applications in medicinal and agrochemical chemistry [20]. The natural and synthetic 1,3-oxazole nuclei exerts diverse range of biological activities like anti-mycobacterial [21], anti-tubercular [22-24], anti-bacterial [25], glycomimetic inhibitors [26], antiviral [27],

* Corresponding author.
E-mail address: karpoormath@ukzn.ac.za (R. Karpoormath).

https://doi.org/10.1016/j.rechem.2022.100374
Received 3 March 2022; Accepted 17 May 2022
Available online 20 May 2022

acaricide [28], anti-microbial [29,30]. Dehydrozingerone (DZG), also known as feruloyl methane, a curcumin's half-structural analog present in Curcuma longa extract, reportedly show potent activity against H37Rv [31] According to a previous report, DZG derivative shown a wide range of activities, like antioxidant [32], anticancer [33], anti-inflammatory [34], depressive, antimalarial, antifungal [35]. Several chalcone-containing scaffolds and hybrids have been found to exhibit antitubercular activities [36]. Promising Hybridized molecule against MTb contain oxazole – Dehydrozingerone moieties depicted in Fig. 1.

Molecular hybridization is a familiar technique to synthesis active pharmacophores by combining two molecules. The oxazole containing compounds 1–3 were reported exhibiting potential antitubercular activity as 1.56 μg/mL, 0.64 μg/mL, and 0.99 μg/mL respectively against H37Rv [23,37,38]. Similarly structures 4–6 were also reported with an activity 0.22 μg/mL, 1.5 μg/mL, 0.12 μg/mL respectively [31,39,40]. (Refer Fig. 1) Hybridization outcome shows the compounds 4f and 4c depicted 6.25 μg/mL, 12.5 μg/mL against H37Rv similarly, structure 4a, 4b shows moderate efficiency 12.5 μg/mL against XDR strain. (Refer Table 1) In structure 4a, nitro thiophene increases the efficiency of the compound. Similarly in aryl-substituted halides the nitro and methoxy groups enhances the structural activity. Further, we see the hybridization of two molecule strategies, oxazole and dehydrozingerone could be an effective M*tb* drug.

Table 1
Antitubercular activity of the synthesized compounds **4a-j** and **6a-e**: (#; High potent antitubercular compounds).

No.	$H_{37}Rv$ MIC(μg/mL)	MDR MIC(μg/mL)	XDR MIC(μg/mL)
4a	50	50	12.5
4b	50	>100	12.5
4c	12.5	>100	25
4d	50	>100	>100
4e	50	50	>100
4f	**6.25**$^{\#}$	50	25
4g	>100	50	>100
4h	25	50	50
4i	100	25	50
4j	100	50	>100
6a	>100	>100	>100
6b	50	50	50
6c	25	50	50
6d	25	50	50
6e	50	50	>100
Isoniazid	<3.125	>3.125	6.25
Rifampicin	<3.125	>3.125	6.25
Moxifloxacin	<3.125	<3.125	12.5
Kanamycin	<3.125	<3.125	12.5

2. Experimental

2.1. Measurements and reagents

Analytical grade (AR) chemicals and reagents were purchased from Merck and Sigma-Aldrich and used without further purification. Thin Layer Chromatography (TLC) on pre-coated silica gel 60 F254 (mesh) (E. Merck) was used to monitor the progress of the reactions and the purity of the synthesized compounds and spots observed under UV light (long and short wavelength). For column chromatography, Merck silica gel (60e120 mesh) was utilized. Thermo Fisher Scientific (IA9000, UK) digital melting point device was used to record the melting points of all the produced compounds in open capillaries. All FT-IR spectra were recorded using a Bruker Alpha FT-IR spectrophotometer with a universal ATR sampling accessory (Billerica, MA, USA). All the synthesized compounds spectra recorded on Bruker Advance IV NMR spectrometer at 400 MHz, with the solvent $CDCl_3$ and DMSO, respectively.

2.2. Synthesis

2.2.1. General synthesis of substituted 4-(chloromethyl)-2-phenyloxazole (3a-j)

The general synthesis procedure for (**3a-j**) as followed. A substituted benzamide 1 eq. (**1a-j**) and 1,3-dichloroacetone 1.2 eq. was heated at 120 °C for 1hr under microwave irradiation. After cooling the reaction mixture, the crude products were extracted with dichloromethane (3 ×

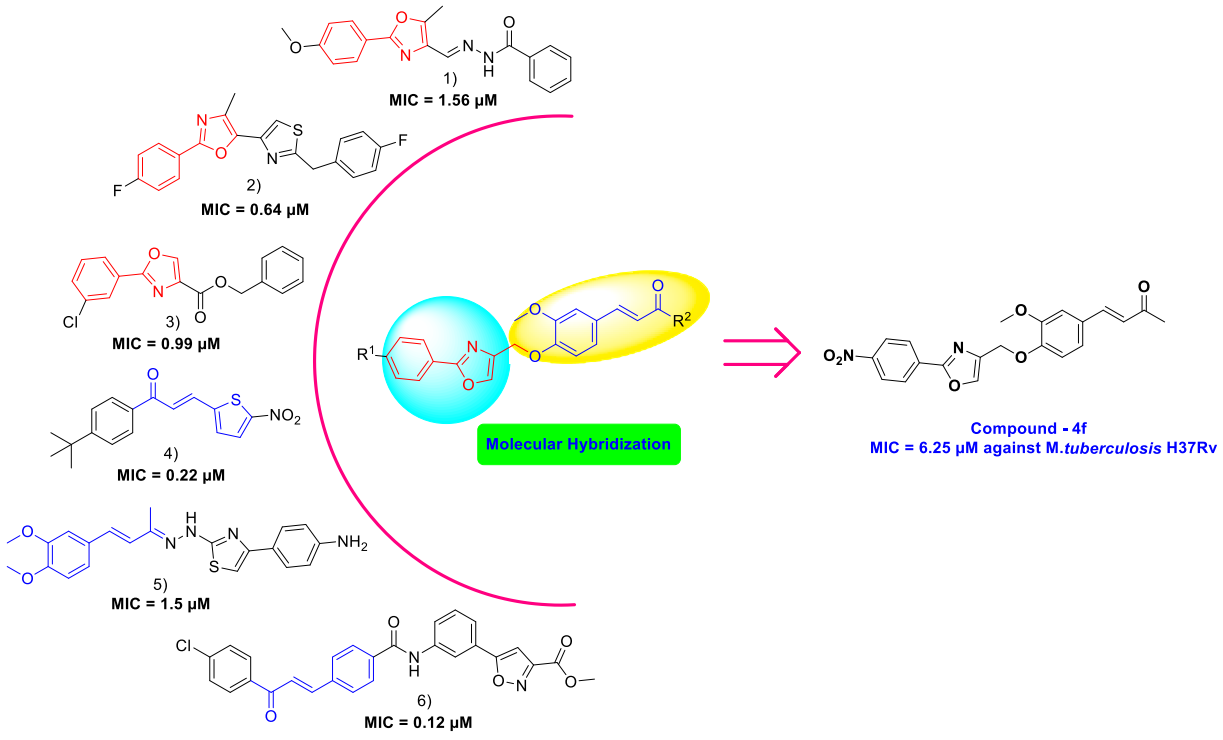


Fig. 1. Molecular hybridization of oxazole – dehydrozingerone.

15 mL), washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated in vacuum. The crude mixture on purification by flash chromatography using silica gel gave targeted compounds (**3a-j**) as shown in Scheme 1 [41].

2.2.2. General synthesis of substituted (E)-4-(3-methoxy-4-((2-phenyloxazol-4-yl)methoxy)phenyl)but-3-en-2-one (4a-j)

Procedure for general synthesis of (**4a-j**) derivatives as follows. Charge the microwave vial with intermediate (**3a-j**) 1 eq, dehydrozingerone 1.3 eq, 4 eq of dry K_2CO_3, add the dry DMF appropriately and heated 140 °C at 10 PSI pressure for 20 min under microwave irradiation. The reaction mixture was cooled to precipitate out the products. The precipitate was treated with ethyl acetate (3 × 15 mL), washed with brine, dried over anhydrous sodium sulfate, filtered, and then concentrated in vacuum. The crude mixture on purification by flash chromatography using silica gel gave desired compounds (**4a-j**) as shown in Scheme 1 [42].

*2.2.2.1. Synthesis of (E)-4-(4-((2-(4-bromophenyl)oxazol-4-yl)methoxy)-3-methoxyphenyl)but-3-en-2-one (**4a**).* Yield 82%. IR (υ/cm^{-1}) = 3744, 3650, 1657, 1513, 1261, 1138, 837. ^{1}H NMR (400 MHz, $CDCl_3$); δ (ppm) = 7.88 (d, J = 8.64 Hz, 2H), 7.74 (s, 1H), 7.57 (d, J = 8.15 Hz, 2H), 7.44 (d, J = 16.30 Hz, 2H), 7.11–7.05 (m, 2H), 7.01 (d, J = 8.33 Hz, 1H), 6.59 (d, J = 16.3 Hz, 1H), 5.14 (s, 2H), 3.89 (s, 3H), 2.35 (s, 3H). ^{13}C NMR (400 MHz, $CDCl_3$) δ (ppm) = 198.43, 161.24, 150.01, 149.82, 143.37, 138.02, 137.09, 132.10, 126.20, 127.94, 126.10, 125.59, 125.13, 122.69, 113.53, 110.26,63.52, 55.94, 27.39.

*2.2.2.2. (E)-4-(4-((2-(4-chlorophenyl)oxazol-4-yl)methoxy)-3-methoxyphenyl)but-3-en-2-one (**4b**).* Yield 85%. IR (υ/cm^{-1}) = 3744, 3650, 1657, 1513, 1425, 1261, 1138, 1095, 837. ^{1}H NMR (400 MHz, DMSO); δ (ppm) = 8.33 (s, 1H), 7.99 (d, J = 8.3 Hz, 2H), 7.58 (m, 3H), 7.34 (s, 1H), 7.27 (d, J = 8.3 Hz, 1H), 7.19 (d, J = 8.3 Hz, 1H), 6.75 (d, J = 16.3 Hz, 1H), 5.09 (s, 2H), 3.80 (s, 3H), 2.30 (s, 3H). ^{13}C NMR (400 MHz, DMSO) δ (ppm) = 198.43, 160.50, 150.07, 149.67, 143.86, 139.48, 137.96, 136.85, 135.99,129.81, 128.22, 125.93, 125.90, 123.25, 113.68, 111.23, 62.46, 56.05, 27.69.

*2.2.2.3. (E)-4-(3-methoxy-4-((2-(3-nitrophenyl)oxazol-4-yl)methoxy)phenyl)but-3-en-2-one (**4c**).* Yield 76%. IR (υ/cm^{-1}) = 3861, 3744, 3650, 2364, 2013, 1694, 1645, 1530, 714. ^{1}H NMR (400 MHz, DMSO); δ (ppm) = 8.67 (s, 1H), 8.31 (m, 3H), 7.86 (t, J = 8.25, 1H), 7.75 (d, J = 15.62, 1H), 7.36 (s, 1H), 7.27 (d, J = 8.0 Hz, 1H), 7.21 (d, J = 8.53 Hz, 1H), 6.76 (d, J = 16.37 Hz, 1H), 5.13 (s, 2H), 3.82 (s, 3H), 2.31 (s, 3H). ^{13}C NMR (400 MHz, DMSO) δ (ppm) = 198.46, 159.51, 150.03, 149.70, 148.74, 143.84, 140.19, 138.28, 132.45, 131.62, 128.41, 128.28, 125.95, 125.67, 123.25, 120.89, 113.75, 111.25, 62.40, 56.07, 27.70.

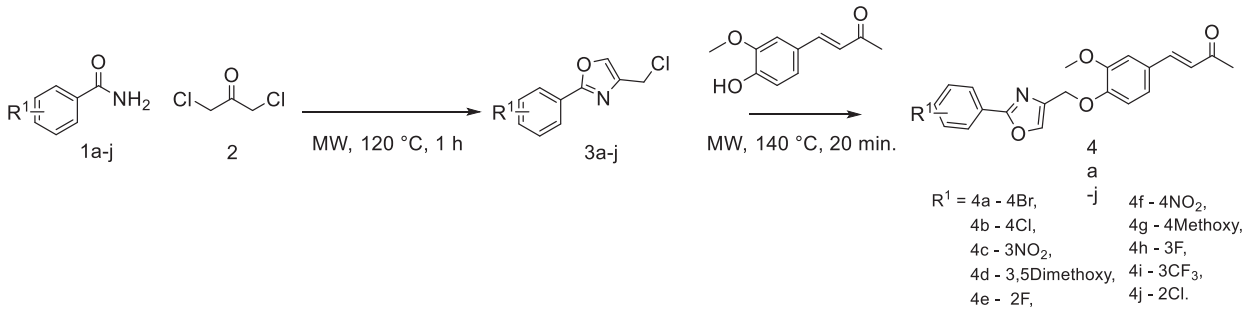
*2.2.2.4. (E)-4-(4-((2-(3,5-dimethoxyphenyl)oxazol-4-yl)methoxy)-3-methoxyphenyl)but-3-en-2-one(**4d**).* Yield 5 6%. IR (υ/cm^{-1}) = 3861, 3744, 3618, 2367, 1648, 1549, 1513, 1462, 1246, 1038, 832, 550. ^{1}H NMR (400 MHz, DMSO); δ (ppm) = 7.73 (s, 1H), 7.44 (d, J = 16.10, 1H), 7.18 (d, J = 1.84, 2H), 7.09 (d, J = 11.11, 2H), 7.01 (d, J = 8.89, 1H), 6.57 (t, J = 13.17 Hz, 2H), 5.15 (s, 2H), 3.89 (s, 3H), 3.83 (s, 6H), 2.35 (s, 3H). ^{13}C NMR (400 MHz, DMSO) δ (ppm) = 198.38, 161.97, 161.06, 150.06, 149.84, 143.39, 137.77, 136.86, 128.79, 128.17, 125.56, 122.70, 113.57, 110.28, 104.29, 103.47, 62.63, 55.94, 55.69, 27.36.

*2.2.2.5. (E)-4-(4-((2-(2-fluorophenyl)oxazol-4-yl)methoxy)-3-methoxyphenyl)but-3-en-2-one (**4e**).* Yield 86%. IR (υ/cm^{-1}) = 3843, 3743, 1657, 1511, 1464, 1245, 1135, 964, 744. ^{1}H NMR (400 MHz, DMSO); δ (ppm) = 8.39 (s, 1H), 8.03 (t, J = 7.6 Hz, 1H), 7.60–7.57 (m, 2H), 7.44–7.35 (m, 3H), 7.27–7.19 (m, 2H), 6.76 (d, J = 16.0 Hz,1H), 5.12 (s, 2H), 3.81 (s, 3H), 2.30 (s, 3H). ^{13}C NMR (400 MHz, DMSO) δ (ppm) = 198.42, 160.91, 158.38, 157.83, 150.09, 149.69, 143.87, 139.58, 137.78, 133.47, 133.39, 129.92, 128.23, 125.94, 125.59, 125.56, 123.27, 117.61, 117.40, 115.26, 115.15, 113.73, 111.25, 62.48, 56.07, 27.70.

*2.2.2.6. (E)-4-(3-methoxy-4-((2-(4-nitrophenyl)oxazol-4-yl)methoxy)phenyl)but-3-en-2-one (**4f**).* Yield 69%. IR (υ/cm^{-1}) = 1518, 1346, 1254, 1168, 981, 857, 714. ^{1}H NMR (400 MHz, DMSO); δ (ppm) = 8.29 (d, J = 8.66 Hz, 2H), 8.19 (d, J = 8.66 Hz, 2H), 7.85 (s, 1H), 7.44 (d, J = 16.36 Hz, 1H), 7.13–7.06 (m, 2H), 7.026 (d, J = 8.47 Hz, 1H), 6.59 (d, J = 16.06, 1H), 5.17 (s, 2H), 3.89 (s, 3H), 2.35 (s, 3H). ^{13}C NMR (400 MHz, DMSO) δ (ppm) = 196.32, 159.65, 149.88, 149.86, 148.80, 143.24, 138.92, 138.24, 132.54, 128.40, 127.23, 125.69, 124.18, 122.64, 113.62, 110.37, 63.43, 55.95, 27.39.

*2.2.2.7. (E)-4-(3-methoxy-4-((2-(4-methoxyphenyl)oxazol-4-yl)methoxy)phenyl)but-3-en-2-one (**4g**).* Yield 53%. IR (υ/cm^{-1}) = 1658, 1503, 1263, 1140, 1026, 839, 744, 551. ^{1}H NMR (400 MHz, DMSO) δ (ppm) = 8.22 (s, 1H), 7.92 (d, J = 8.82 Hz, 2H), 7.56 (d, J = 16.39 Hz, 1H),7.35 (s, 1H), 7.26 (d, J = 8.52), 7.18 (dd, J = 8.4 Hz, 2H), 7.08 (d, J = 8.73 Hz, 2H), 6.75 (d, J = 16.3 Hz, 1H), 5.07 (s, 2H), 3.82 (d, J = 6.47 Hz, 6H), 2.31 (s, 3H). ^{13}C NMR (400 MHz, DMSO) δ (ppm) = 198.43, 161.68, 161.53, 150.16, 149.69, 143.86, 138.47, 137.54, 128.23, 128.17, 125.89, 123.25, 119.79, 115.07, 113.70, 111.27, 62.59, 56.06, 55.85, 27.69.

*2.2.2.8. (E)-4-(4-((2-(3-fluorophenyl)oxazol-4-yl)methoxy)-3-methoxyphenyl)but-3-en-2-one (**4h**).* Yield 73%. IR (υ/cm^{-1}) = 1588, 1510, 1461, 1254, 1141, 1098, 1017, 803. ^{1}H NMR (400 MHz, DMSO) δ (ppm) = 8.34 (s, 1H), 7.83–7.72 (m, 2H), 7.57 (d, J = 17.4 Hz, 2H), 7.37 (d, J = 17.7 Hz, 2H), 7.23 (d, J = 18.8 Hz, 2H), 6.75 (d, J = 15.7 Hz, 1H), 5.10 (s, 2H), 3.81 (s, 3H), 2.31 (s, 3H). ^{13}C NMR (400 MHz, DMSO) δ (ppm) = 198.48, 164.01, 161.58, 160.28, 160.24, 150.05, 149.68, 143.86, 139.68, 138.01, 132.08, 132.00, 129.18, 129.09, 128.23, 125.92, 123.25, 122.69, 118.34, 118.13, 113.71, 113.23, 113.00, 111.23, 62.43, 56.05, 27.69.



Scheme 1. Synthesis of oxazole-dehydrozingerone hybrid molecules (4a-j).

*2.2.2.9. (E)-4-(3-methoxy-4-((2-(3-(trifluoromethyl)phenyl)oxazol-4-yl) methoxy)phenyl)but-3-en-2-one(**4i**).* Yield 67%. IR (υ/cm^{-1}) = 1583, 1509, 1257, 1138, 1098, 1018, 839. ^{1}H NMR (400 MHz, DMSO); δ (ppm) = 8.39 (s, 1H), 8.33 (m, 2H), 7.85 (m, 2H), 7.57 (d, *J* = 16.59, 1H), 7.35 (s, 1H), 7.32–7.09 (m, 2H) 6.75 (d, *J* = 16.26 Hz, 1H), 5.11 (s, 2H), 3.81 (s, 3H), 2.31 (s, 3H). ^{13}C NMR (400 MHz, $CDCl_3$) δ (ppm) = 198.39, 160.70, 149.97, 149.86, 143.35, 138.23, 137.42, 137.26, 131.64, 131.31, 129.52, 129.46, 128.29, 127.95, 127.09, 127.05, 125.64, 125.07, 123.43, 123.39, 122.68, 122.37, 113.59, 110.30, 63.51, 55.95, 27.37.

*2.2.2.10. (E)-4-(4-((2-(2-chlorophenyl)oxazol-4-yl)methoxy)-3-methoxyphenyl)but-3-en-2-one (**4j**).* Yield 87%. IR (υ/cm^{-1}) = 1583, 1508, 1256, 1138, 1097, 1018, 839. ^{1}H NMR (400 MHz, DMSO); δ (ppm) = 8.38 (s, 1H), 7.83 (d, *J* = 7.8 Hz, 1H), 7.57 (d, *J* = 16.3 Hz, 1H), 7.43 (t, *J* = 7.1 Hz, 1H), 7.36 (s, 1H), 7.27 (d, *J* = 8.1 Hz, 1H), 7.22 (d, *J* = 8.3 Hz, 1H), 7.07 (d, *J* = 8.2 Hz, 1H), 7.01 (t, *J* = 7.5 Hz, 1H), 6.76 (d, *J* = 16.3 Hz, 1H), 5.16 (s, 2H), 3.82 (s, 3H), 2.31 (s, 3H). ^{13}C NMR (400 MHz, DMSO) δ (ppm) = 198.40, 161.17, 156.77, 149.96, 149.82, 143.79, 138.24, 136.22, 133.23, 128.42, 126.99, 126.01, 123.17, 120.24, 117.45, 114.13, 111.44, 111.35, 62.08, 56.14, 27.70.

*2.2.3. Synthesis of substituted 3-methoxy-4-((2-phenyloxazol-4-yl) methoxy)benzaldehyde(**5a, b, f, g**)*

Procedure for general synthesis of (**5a, b, f, g**) derivatives as follows. Charge the microwave vial with intermediate (**3a, b, f, g**) 1 eq, 4-hydroxy-3-methoxybenzaldehyde 1.3 eq, dry K_2CO_3 4 eq, add the dry DMF appropriately and heated 140 °C at 10 PSI pressure for 20 min under microwave irradiation. After cooling the reaction mixture using ice water, the compounds were precipitated out. The precipitate was treated with ethyl acetate (3 × 15 mL), washed with brine, dried over anhydrous sodium sulfate, filtered, and then concentrated in vacuum. The mixture on purification by flash chromatography using silica gel gave desired compounds (**5a, b, f, g**) as shown in Scheme 2 [42].

*2.2.4. General synthesis of (E)-3-(3-methoxy-4-((2-phenyloxazol-4-yl) methoxy)phenyl)-1-(thiophen-3-yl)prop-2-en-1-one derivatives (**6a-e**)*

KOH (1.1 eq), at 0 °C and substituted acetyl thiophene (1.0 eq) in MeOH/H_2O (5:1) solvent were taken in a round bottom flask and were stirred for 30 min. The intermediate (**5a, b, f, g**) were added to the reaction mixture and the stirring continued for next 12 hrs at room temperature. The progress of reaction was monitored using TLC (ethyl acetate-hexane 5:5). The reaction mixture was extracted with ethyl acetate (3 × 15 mL), washed with brine, dried over anhydrous sodium sulfate, filtered, and concentrated in vacuum. The crude mixture on purification by flash chromatography using silica gel gave targeted compounds (**6a-j**) as shown in (Scheme 2) [43].

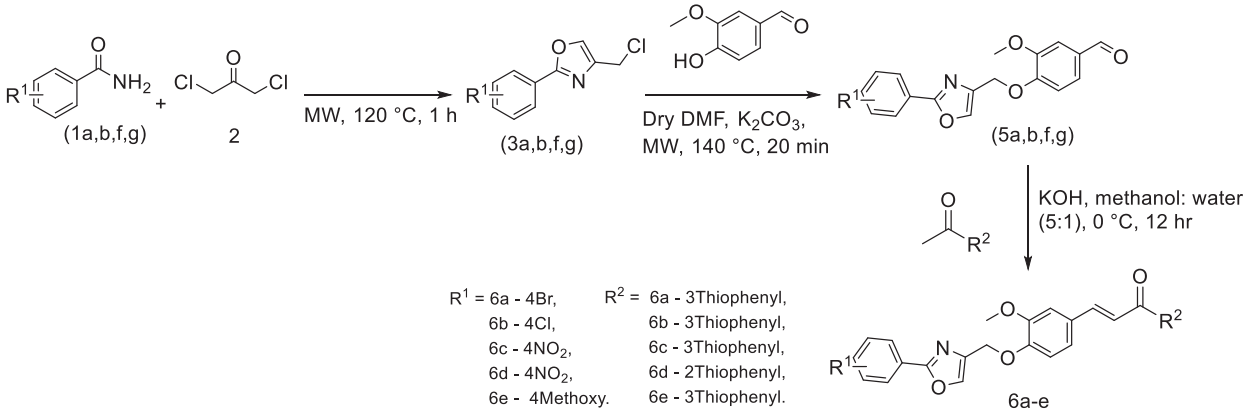
*2.2.4.1. (E)-3-(4-((2-(4-bromophenyl)oxazol-4-yl)methoxy)-3-methoxyphenyl)-1-(thiophen-3-yl)prop-2-en-1-one (**6a**).* Yield 76%. IR (υ/cm^{-1}) = 1590, 1510, 1250, 1134, 1070, 1029, 839, 705. ^{1}H NMR (400 MHz, $CDCl_3$); δ (ppm) = 7.98 (s, 1H), 7.73 (d, *J* = 7.81 Hz, 2H), 7.63–7.54 (m, 2H), 7.49 (d, *J* = 3.35 Hz, 1H), 7.41 (d, *J* = 8.02, 2H), 7.18 (s, 1H),7.15–6.94 (m, 3H), 6.88 (d, *J* = 7.27 Hz, 1H), 5.00 (s, 2H), 3.76 (s, 3H). ^{13}C NMR (400 MHz, $CDCl_3$) δ (ppm) = 183.99, 161.25, 150.07, 149.83, 144.08, 143.23, 138.11, 137.10, 132.10, 131.82, 128.71, 127.96, 127.51, 126.45, 123.15, 125.13, 122.57, 121.13, 113.70, 110.95, 77.37, 77.06, 76.74, 63.65, 56.09.

*2.2.4.2. (E)-3-(4-((2-(4-chlorophenyl)oxazol-4-yl)methoxy)-3-methoxyphenyl)-1-(thiophen-3-yl)prop-2-en-1-one (**6b**).* Yield 69%. IR (υ/cm^{-1}) = 1654, 1589, 1507, 1254, 1138, 999, 839,786. ^{1}H NMR (400 MHz, $CDCl_3$); δ (ppm) = 8.07 (s, 1H), 7.87 (d, *J* = 7.72 Hz, 2H), 7.66 (t, *J* = 6.98 Hz, 2H), 7.57 (d, *J* = 4.95 Hz, 1H), 7.33 (d, *J* = 8.28, 2H), 7.28 (m, 1H), 7.22 (m, 2H), 7.07 (s, 1H), 6.96 (d, *J* = 8.28 Hz, 1H), 5.07 (s, 2H), 3.84 (s, 3H). ^{13}C NMR (400 MHz, $CDCl_3$) δ (ppm) = 183.96, 161.16, 150.07, 149.90, 144.06, 143.21, 138.04, 137.05, 136.72, 131.82, 129.13, 128.67, 127.76, 127.48, 126.43, 125.70, 122.67, 121.07, 113.63, 110.92, 63.56, 56.02.

*2.2.4.3. (E)-3-(3-methoxy-4-((2-(4-nitrophenyl)oxazol-4-yl)methoxy) phenyl)-1-(thiophen-3-yl)prop-2-en-1-one (**6c**).* Yield 63%. IR (υ/cm^{-1}) = 1653, 1589, 1507, 1414, 1305, 1252, 1136, 999, 838,786.^{1}H NMR (400 MHz, $CDCl_3$); δ (ppm) = 8.25 (d, *J* = 8.56 Hz, 2H), 8.14 (d, *J* = 8.77 Hz, 2H), 8.08 (s, 1H), 7.79 (s, 1H), 7.69 (d, *J* = 15.28, 1H), 7.59 (s, 1H), 7.34 (m, 1H), 7.20–7.05 (m, 3H), 6.99 (d, *J* = 8.20 Hz, 1H), 5.13 (s, 2H), 3.87 (s, 3H). ^{13}C NMR (400 MHz, $CDCl_3$) δ (ppm) = 190.89, 183.98, 159.95, 149.91, 149.83, 148.83, 143.99, 143.18, 138.95, 138.23, 132.55, 131.85, 128.87, 127.48, 127.25, 126.47, 124.21, 122.63, 121.20, 113.63, 110.94, 63.46, 56.05.

*2.2.4.4. (E)-3-(3-methoxy-4-((2-(4-nitrophenyl)oxazol-4-yl)methoxy) phenyl)-1-(thiophen-2-yl)prop-2-en-1-one (**6d**).* Yield 61%. IR (υ/cm^{-1}) = 3406, 1650, 1585, 1414, 1339, 1254, 1136, 1027, 839, 708. ^{1}H NMR (400 MHz, $CDCl_3$); δ (ppm) = 8.27 (d, *J* = 8.29 Hz, 2H), 8.16 (d, *J* = 8.78 Hz, 2H), 7.81 (s, 1H), 7.74 (d, *J* = 15.61 Hz, 1H), 7.61 (d, *J* = 4.28, 1H), 7.43–7.36 (m, 1H), 7.20 (t, 7.87 Hz, 2H), 7.14–7.09 (m, 2H), 7.01 (d, *J* = 8.63 Hz, 1H), 5.15 (s, 2H), 3.89 (s, 3H). ^{13}C NMR (400 MHz, $CDCl_3$) δ (ppm) = 190.89, 183.00, 145.63, 143.96, 138.35, 138.23, 133.71, 132.56, 131.62, 130.86, 128.78, 128.20, 127.26, 126.43, 124.22, 122.68, 120.06, 113.71, 112.47, 111.06, 109.58, 63.48, 56.07.

*2.2.4.4. (E)-3-(3-methoxy-4-((2-(4-methoxyphenyl)oxazol-4-yl)methoxy) phenyl)-1-(thiophen-3-yl)prop-2-en-1-one (**6e**).* Yield 52%. IR (υ/cm^{-1}) = 1651, 1585, 1506, 1252, 1136, 1024, 839, 794. ^{1}H NMR (400 MHz, $CDCl_3$); δ (ppm) = 8.12 (s, 1H), 7.94 (d, *J* = 8.864 Hz, 2H), 7.76–7.61



Scheme 2. Synthesis of oxazole-dehydrozingerone-thiophene hybrid molecules (6a-e).

(m, 3H), 7.36–7.30 (m, 1H), 7.22 (d, $J = 5.67$, 1H), 7.18 (d, $J = 8.17$, 1H), 7.12 (s, 1H), 7.03 (d, $J = 7.72$, 1H), 6.93 (d, $J = 8.88$ Hz, 2H), 5.14 (s, 2H), 3.90 (s, 3H), 3.82 (s, 3H). ^{13}C NMR (400 MHz, $CDCl_3$) δ (ppm) = 184.03, 162.21, 161.53, 150.20, 149.80, 144.16, 143.23, 137.55, 136.28, 131.81, 128.56, 128.18, 127.50, 126.42, 122.71, 121.02, 120.02, 114.23, 113.66, 110.92, 63.72, 56.06, 55.40.

2.3. Antitubercular studies

2.3.1. In vitro evaluation of antimycobacterial activity

The in vitro antimycobacterial activity for the newly synthesized test compounds (**4a-j**) and (**6a-e**) was evaluated using the colorimetric reassuring microplate assay plate method [44-46]. Briefly, *M. tuberculosis* (ATCC H37Rv), multi-drug resistant (MDR-TB UKQC strain), extensively drug resistant tuberculosis (XDR-TB UKQC strain) were maintained on 7H11 agar plates at 37 °C O_2. The inoculum of each strain was prepared by scraping and re-suspending a loopful of colonies into Middle brook 7H9 broth, supplemented with 10% ADC and 0.04% tween 80 to avoid clump formation and incubated at 37 °C in O_2. The inoculum turbidity was adjusted to a McFarland number 1 standard and further diluted 1:10 in Middle brook 7H9 [47] broth prior to addition (100 μL) to each of the test samples and drug-free wells. A growth control and a sterile control were also included for each strain. Each of the synthesized test compounds (**4a-j**) and (**6a-e**) and reference drugs were weighed accordingly, dissolved in the appropriate solvent and filter and sterilized using a 0.2-μm polycarbonate filter (400 μg/mL). A serially diluted drug free control (DMSO) was used to check the activity on each strain. The outer-perimeter wells of a 96-well microtiter plates was filled with 200 μL of sterile double distilled water to minimize evaporation of the medium in the test wells during incubation. In the remaining test wells, an amount of 100 μL of Middlebrook 7H9 broth with ADC 10%, 100 μL of the test compounds and 100 μL of the mycobacterial strain was added to get at final concentration of 200, 100, 50, 25, 12.5, 6.25, 3.125, 1.56, 0.78, and 0.39 μg/mL. The plates were incubated at 37 °C for 7 days. After incubation, 30 μL of resazurin solution prepared at 0.02% (wt/vol) in distilled water was added to each well [45,46]. The plates were then incubated overnight at 37 °C, and assessed for color development. A positive reaction results in a color change from blue to pink ((reduction of resazurin to resorufin) indicates bacterial growth, which confirms drug resistance [45,46].

3. Results and discussion

3.1. Characterization

Novel oxazole-dehydrozingerone hybrid molecules (**4a-j**) and oxazole-dehydrozingerone-thiophene derivatives (**6a-e**) were synthesized as shown in (Scheme 1 and Scheme 2). In the absence of a solvent, dichloroacetone reacts with substituted benzamides to obtain substituted phenyl oxazoles (3a-j) via cyclization reaction. Here, the dichloroacetone reacted with benzamide to form –NH bond which on hydrolysis results in oxazole ring. Under mild conditions, K_2CO_3 reacts with dehydrozingerone to deprotonate the –OH group and attack on $–CH_2Cl$ group of substituted phenyloxazoles to forming an ether linker via SN_2 reaction to gives (**4a-j**). Same reaction pathway for the formation of (**5a, b, f, g**) in (Scheme 2), Vanillin reacts with $–CH_2Cl$ group of substituted phenyloxazoles in presence of K_2CO_3 to form (**5a, b, f, g**) via SN2 reaction. Aldehyde intermediate (**5a, b, f, g**) react with acetylthiophene in the presence of KOH to form chalcone (**6a-e**) as shown in (Scheme 2). The KOH reacted with acetylthiophene to form ketone enolate which attack on aldehyde to get chalcone derivatives (**6a-e**) via Claisen–Schmidt condensation reaction as shown in (Scheme 2).

^{1}H NMR observe (–C=C–) trans isomer of dehydrozingerone at δ7.44 ppm and δ6.59 ppm with frequency 16.24 Hz and 16.20 Hz respectively compound (4a-j), similarly oxazole peak observed as a singlet at δ7.74 ppm in the aromatic region. On another side, two methoxy (**–CH_3**) peaks appear as a singlet at δ 3.89 ppm and δ 2.35 ppm, respectively, in the aliphatic region. δ 5.14 ppm peak shows the ether linker (**–CH_2**) between dehydrozingerone and phenyl oxazole moiety. In the aromatic region, benzene ring peaks obtained at the ppm values δ 7.88, 7.57, 7.02, 7.11–7.05 respectively. ^{13}C NMR spectra analysis gave the signal at δ 137.08 ppm, indicating oxazole ring carbon (**–CH**) in (**4a-j**). Similarly, quaternary carbon of ketone (=C=O) appears at δ 198.37, further at δ 143.37, and δ 127.93 ppm represents a double bond of dehydrozingerone (–C=C–). Adjacent carbon of aromatic rings shows at δ 127.93 and δ 132.09 ppm. Another hand, in the aliphatic region, methoxy ($–CH_3$) peaks appear at δ 27.39 ppm and δ 55.94, respectively. The (–C–O) peak appeared at 63.52 ppm. In the aromatic region, peaks at δ values 126.42, 127.49, 136.7 ppm, indicates thiophenyl ring carbons in (6a-e). Oxazole moiety was also confirmed with IR bands observed at around 1512–1548 cm^{-1} in compounds (4a-j) and (5a-j). Double bond (–C=C–) of dehydrozingerone showed the stretching at around 1660–1600 cm^{-1}. IR absorption frequency for aryl halide appears at 1245.23, 731.61, 553.46 cm^{-1} for –F, -Cl, -Br respectively in structure (4a-j) and (5a-j). In compound 4c and 4f obtained frequencies were 1530.40, 1346.32 cm^{-1} that indicates asymmetrical and symmetrical stretch for the nitro functional group. The ppm values, frequency for ^{1}H, ^{13}C NMR, and IR are obtained in the specific region to conform the successfully preparation for compounds (4a-j) and (6a-e).

3.2. Biological studies

Synthesized compound (**4a-j**) and (**6a-e**) showing in vitro anti-TB activity against M*tb* H37Rv using the colorimetric reassuring microplate assay plate method. Table 1 shows the results of the evaluations and their minimum inhibitory concentration (MIC) values in μg/mL. Variation between MIC values shows compounds activity change due to their different functional groups. All the compounds show satisfactory activity compare to the standard drugs Isoniazid, Rifampicin, Moxifloxacin, Kanamycin. Compound **4f** shows potential activity **6.25 μg/mL** against $H_{37}Rv$ compare with standard drug. Similarly, both the compound **4a** and **4b** indicate mild activity **12.5 μg/mL** against XDR strain. The study reveals that methyl (**–CH_3**) group of dehydrozingerone increases the activity. The compounds (**6a-e**) showed decrease in the activity, probably due to blockage of the (**–CH_3**) group of dehydrozingerone by thiophene.

3.3. Computational study

The in vitro anti-TB activity studies against Mtb H37Rv were carried out for all the synthesized compounds. The compound **4f** was in special focus, which was exhibiting better activity with MIC = **6.25 μg/mL** against the standard drugs than other synthesized compounds. The Docking studies for the compound **4f** using the X-ray crystal structure of enzyme Mtb DNA Gyrase (PDB code – 4B6C), as displayed in Fig. 2, shows the various interactions with amino acids that may have contributed to the significant potency of compound **4f**. The presence of the methoxy group interacted with the residue VAL 77, creating a van der Waals effect. The residue ASN 52 resulted in carbon-hydrogen interaction with the methylene group. The phenyl groups were influential, resulting in Pi-Cation, Pi-Sigma, and Pi-Alkyl interactions with amino acids ARG 82, ILE 84, and PRO 85, VAL 99, ILE 171, respectively.

4. Conclusions

The current work indicates a series of hybridized oxazole-dehydrozingerone molecule derivative (**4a-j**) and (**6a-e**) synthesized via cyclization, coupling and aldol-condensation reaction. The structure of synthesized compounds characterized by ^{1}H, ^{13}C NMR and IR spectroscopy. Screened the synthesized molecules for In vitro anti-TB activity against M*tb* $H_{37}R_v$ using the colorimetric reassuring micro plate assay plate method. The compound **4f** has potential antitubercular

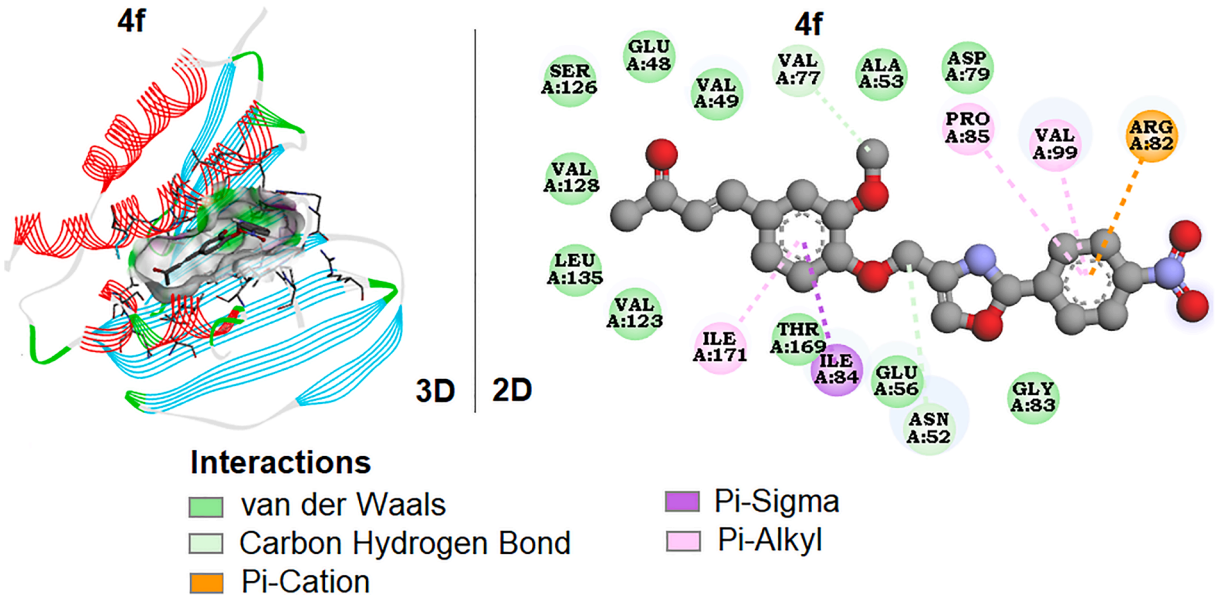


Fig. 2. 3D and 2D ligand-receptor interaction of compound **4f**.

efficacy as **6.25 µg/mL** against $H_{37}Rv$ based on screening results as well compound **4a**, **4b** showing mild activity against XDR strain. Computational studies were carried out for the active compound **4f** against enzyme M*tb* DNA Gyrase. The results of the present studies will be helpful to the researchers working on developing Antitubercular drugs.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

The authors would like to thank the College of Health Science (CHS), University of KwaZulu-Natal, Durban for the financial support. R. Karpoormath is also thankful to National Research Foundation-South Africa (NRF-SA) for funding (Grant No. 103728, 112079 and 129247). Thank you, Dr. Vuyisa Mzozoyana, for the NMR spectroscopy.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.rechem.2022.100374.

References

[1] R.G. Hall, R.D. Leff, T. Gumbo, Treatment of active pulmonary tuberculosis in adults: current standards and recent advances, Insights Soc. Infect. Dis. Pharm. Pharmacother. 29 (2009) 1468–1481.
[2] S.I. Cadmus, V.O. Akinseye, B.O. Taiwo, E.O. Pinelli, D. van Soolingen, S.G. Rhodes, Interactions between helminths and tuberculosis infections: Implications for tuberculosis diagnosis and vaccination in Africa, PLoS neglected tropical diseases, 14 (2020) e0008069-e0008069.
[3] W.H. Organization, Tuberculosis: WHO Global Tuberculosis Report, (2015).
[4] H. Herzog, History of tuberculosis, Respiration 65 (1998) 5–15.
[5] D. Marangu, H.J. Zar, Childhood pneumonia in low-and-middle-income countries: An update, Paediatr. Respir. Rev. 32 (2019) 3–9.
[6] C. Lienhardt, S.V. Cook, M. Burgos, V. Yorke-Edwards, L. Rigouts, G. Anyo, S. J. Kim, A. Jindani, D.A. Enarson, A.J. Nunn, Efficacy and safety of a 4-drug fixed-dose combination regimen compared with separate drugs for treatment of pulmonary tuberculosis: the Study C randomized controlled trial, JAMA 305 (2011) 1415–1423.
[7] M.R. Ward, Notes on the, to 2009 United States Food and Drug Administration approval of new molecular entities (NMEs), KEI Res. Note 2010 (2004) 1–14.
[8] A. Zumla, P. Nahid, S.T. Cole, Advances in the development of new tuberculosis drugs and treatment regimens, Nat. Rev. Drug Discov. 12 (2013) 388–404.
[9] N.R. Gandhi, A. Moll, A.W. Sturm, R. Pawinski, T. Govender, U. Lalloo, K. Zeller, J. Andrews, G. Friedland, Extensively drug-resistant tuberculosis as a cause of death in patients co-infected with tuberculosis and HIV in a rural area of South Africa, Lancet 368 (2006) 1575–1580.
[10] B. Müller, S. Borrell, G. Rose, S. Gagneux, The heterogeneous evolution of multidrug-resistant Mycobacterium tuberculosis, Trends Genet. 29 (2013) 160–169.
[11] H. Zhang, D. Li, L. Zhao, J. Fleming, N. Lin, T. Wang, Z. Liu, C. Li, N. Galwey, J. Deng, Y. Zhou, Y. Zhu, Y. Gao, T. Wang, S. Wang, Y. Huang, M. Wang, Q. Zhong, L. Zhou, T. Chen, J. Zhou, R. Yang, G. Zhu, H. Hang, J. Zhang, F. Li, K. Wan, J. Wang, X.-E. Zhang, L. Bi, Genome sequencing of 161 Mycobacterium tuberculosis isolates from China identifies genes and intergenic regions associated with drug resistance, Nat. Genet. 45 (10) (2013) 1255–1260.
[12] R.C. Reynolds, S. Ananthan, E. Faaleolea, J.V. Hobrath, C.D. Kwong, C. Maddox, L. Rasmussen, M.I. Sosa, E. Thammasuvimol, E.L. White, W. Zhang, J.A. Secrist, High throughput screening of a library based on kinase inhibitor scaffolds against Mycobacterium tuberculosis H37Rv, Tuberculosis (Edinb.) 92 (1) (2012) 72–83.
[13] L.G. Dover, G.D. Coxon, Current status and research strategies in tuberculosis drug development, J. Med. Chem. 54 (18) (2011) 6157–6165.
[14] R. Purchase, J.R. Hanson, Sir John Cornforth Ac Cbe Frs: His synthetic work, Sci. Prog. 98 (3) (2015) 219–229.
[15] D. Prajapat, Utility of drug discovery in medicinal and organic chemistry, Mod. Chem. Appl. 5 (2017) 1–2.
[16] J.T. Mhlongo, E. Brasil, B.G. de la Torre, F. Albericio, Naturally occurring oxazole-containing peptides, Mar. Drugs 18 (2020) 203.
[17] S. Bajaj, V. Asati, J. Singh, P.P. Roy, 1,3,4-Oxadiazoles: An emerging scaffold to target growth factors, enzymes and kinases as anticancer agents, Eur. J. Med. Chem. 97 (2015) 124–141.
[18] L. O. Haustedt, I. V. Hartung, H.M.R. Hoffmann, Graphical Abstract: Angew. Chem. Int. Ed. 24/2003, Angew. Chem. Int. Ed., 42 (2003) 2696-2702.
[19] H.-Z. Zhang, Z.-L. Zhao, C.-H. Zhou, Recent advance in oxazole-based medicinal chemistry, Eur. J. Med. Chem. 144 (2018) 444–492.
[20] X. Zheng, W. Liu, D. Zhang, Recent advances in the synthesis of oxazole-based molecules via van Leusen oxazole synthesis, Molecules 25 (2020) 1594.
[21] F.G. Avalos-Alanís, E. Hernández-Fernández, P. Carranza-Rosales, S. López-Cortina, J. Hernández-Fernández, M. Ordóñez, N.E. Guzmán-Delgado, A. Morales-Vargas, V.M. Velázquez-Moreno, M.G. Santiago-Mauricio, Synthesis, antimycobacterial and cytotoxic activity of α, β-unsaturated amides and 2,4-disubstituted oxazoline derivatives, Bioorg. Med. Chem. Lett. 27 (2017) 821–825.
[22] P. De, G. Koumba Yoya, P. Constant, F. Bedos-Belval, H. Duran, N. Saffon, M. Daffé, M. Baltas, Design, synthesis, and biological evaluation of new cinnamic derivatives as antituberculosis agents, J. Med. Chem. 54 (2011) 1449–1461.
[23] G.C. Moraski, M. Chang, A. Villegas-Estrada, S.G. Franzblau, U. Möllmann, M. J. Miller, Structure-activity relationship of new anti-tuberculosis agents derived from oxazoline and oxazole benzyl esters, Eur. J. Med. Chem. 45 (2010) 1703–1716.
[24] O.K. Onajole, M. Pieroni, S.K. Tipparaju, S. Lun, J. Stec, G. Chen, H. Gunosewoyo, H. Guo, N.C. Ammerman, W.R. Bishai, A.P. Kozikowski, Preliminary structure-activity relationships and biological evaluation of novel antitubercular indolecarboxamide derivatives against drug-susceptible and drug-resistant Mycobacterium tuberculosis strains, J. Med. Chem. 56 (2013) 4093–4103.

[25] S. Eswaran, A.V. Adhikari, R. Ajay Kumar, New 1,3-oxazolo[4,5-c]quinoline derivatives: synthesis and evaluation of antibacterial and antituberculosis properties, Eur. J. Med. Chem. 45 (2010) 957–966.
[26] D. Hauck, I. Joachim, B. Frommeyer, A. Varrot, B. Philipp, H.M. Möller, A. Imberty, T.E. Exner, A. Titz, Discovery of two classes of potent glycomimetic inhibitors of Pseudomonas aeruginosa LecB with distinct binding modes, ACS Chem. Biol. 8 (8) (2013) 1775–1784.
[27] V.N. Madia, A. Messore, L. Pescatori, F. Saccoliti, V. Tudino, A. De Leo, L. Scipione, L. Fiore, E. Rhoden, F. Manetti, M.S. Oberste, R. Di Santo, R. Costi, In vitro antiviral activity of new oxazoline derivatives as potent poliovirus inhibitors, J. Med. Chem. 62 (2) (2019) 798–810.
[28] J. Suzuki, T. Ishida, I. Shibuya, K. Toda, Development of a new acaricide, etoxazole, J. Pest. Sci. 26 (2) (2001) 215–223.
[29] K. Lal, P. Yadav, A. Kumar, Synthesis, characterization and antimicrobial activity of 4-((1-benzyl/phenyl-1H-1,2,3-triazol-4-yl)methoxy)benzaldehyde analogues, Med. Chem. Res. 25 (4) (2016) 644–652.
[30] B. Aneja, M. Azam, S. Alam, A. Perwez, R. Maguire, U. Yadava, K. Kavanagh, C. G. Daniliuc, M.M.A. Rizvi, Q.M.R. Haq, M. Abid, Natural product-based 1,2,3-triazole/sulfonate analogues as potential chemotherapeutic agents for bacterial infections, ACS Omega 3 (6) (2018) 6912–6930.
[31] G.A. Hampannavar, R. Karpoormath, M.B. Palkar, M.S. Shaikh, B. Chandrasekaran, Dehydrozingerone inspired styryl hydrazine thiazole hybrids as promising class of antimycobacterial agents, ACS Med. Chem. Lett. 7 (2016) 686–691.
[32] I.R. Kubra, B.K. Bettadaiah, P.S. Murthy, L.J.M. Rao, Structure-function activity of dehydrozingerone and its derivatives as antioxidant and antimicrobial compounds, J. Food Sci. Technol. 51 (2014) 245–255.
[33] J. Tatsuzaki, K.F. Bastow, K. Nakagawa-Goto, S. Nakamura, H. Itokawa, K.-H. Lee, Dehydrozingerone, chalcone, and isoeugenol analogues as in vitro anticancer agents, J. Nat. Prod. 69 (2006) 1445–1449.
[34] P. Chibber, C. Kumar, A. Singh, S. Assim Haq, I. Ahmed, A. Kumar, S. Singh, R. Vishwakarma, G. Singh, Anti-inflammatory and analgesic potential of OA-DHZ; a novel semisynthetic derivative of dehydrozingerone, Int. Immunopharmacol. 83 (2020) 106469.
[35] G. Hampannavar, R. Karpoormath, M. Palkar, M. Shaikh, An appraisal on recent medicinal perspective of curcumin degradant: dehydrozingerone (DZG), Bioorg. Med. Chem. 24 (2016) 501–520.
[36] M. Sharma, V. Chaturvedi, M. Krishnan, S. Bhatnagar, K. Srivastava, S.K. Puri, P.M. S. Chauhan, Substituted quinolinyl chalcones and quinolinyl pyrimidines as a new class of antiinfective agents, Eur. J. Med. Chem. 44 (2009) 2081–2091.
[37] S.R. Shah, K.D. Katariya, 1,3-Oxazole-isoniazid hybrids: Synthesis, antitubercular activity, and their docking studies, J. Heterocycl. Chem. 57 (2020) 1682–1691.
[38] Y. Abhale, A. Sasane, A. Chavan, S. Saddam husen, K. Deshmukh, S. Bhansali, L. Nawale, D. Sarkar, P. Mhaske, Synthesis and antimycobacterial screening of new thiazolyl-oxazole derivatives, Eur. J. Med. Chem. 132 (2017) 333–340.
[39] M. Gomes, R. Braga, E. Grzelak, B. Neves, E. Muratov, R. Ma, L. Klein, C. Sanghyun, G. Oliveira, S. Franzblau, C. Andrade, QSAR-driven design, synthesis and discovery of potent chalcone derivatives with antitubercular activity, Eur. J. Med. Chem. 137 (2017) 126–138.
[40] S.K. Sahoo, B. Rani, N.B. Gaikwad, M.N. Ahmad, G. Kaul, M. Shukla, S. Nanduri, A. Dasgupta, S. Chopra, V.M. Yaddanapudi, Synthesis and structure-activity relationship of new chalcone linked 5-phenyl-3-isoxazolecarboxylic acid methyl esters potentially active against drug resistant Mycobacterium tuberculosis, Eur. J. Med. Chem. 222 (2021), 113580.
[41] D. Huang, A. Liu, W. Liu, X. Liu, Y. Ren, X. Zheng, H. Pei, J. Xiang, M. Huang, X. Wang, Synthesis and insecticidal activities of novel 1H-pyrazole-5-carboxylic acid derivatives, Heterocycl. Commun. 23 (2017) 455–460.
[42] M.S. Malamas, J. Sredy, M. McCaleb, I. Gunawan, B. Mihan, D. Sullivan, Antihyperglycemic activity of new 1,2,4-oxadiazolidine-3,5-diones, Eur. J. Med. Chem. 36 (2001) 31–42.
[43] A. Basnet, P. Thapa, R. Karki, Y. Na, Y. Jahng, B.-S. Jeong, T. Jeong, C.-S. Lee, E.-S. Lee, 2,4,6-Trisubstituted pyridines: synthesis, topoisomerase I and II inhibitory activity, cytotoxicity, and structure—activity relationship, Bioorg. Med. Chem. 15 (2007) 4351–4359.
[44] C.M. Mann, J.L. Markham, A new method for determining the minimum inhibitory concentration of essential oils, J. Appl. Microbiol. 84 (1998) 538–544.
[45] J.-C. Palomino, A. Martin, M. Camacho, H. Guerra, J. Swings, F. Portaels, Resazurin microtiter assay plate: simple and inexpensive method for detection of drug resistance in Mycobacterium tuberculosis, Antimicrob. Agents Chemother. 46 (2002) 2720–2722.
[46] A. Martin, N. Morcillo, D. Lemus, E. Montoro, M.A.d.S. Telles, N. Simboli, M. Pontino, T. Porras, C. León, M. Velasco, L. Chacon, L. Barrera, V. Ritacco, F. Portaels, J.C. Palomino, Multicenter study of MTT and resazurin assays for testing susceptibility to first-line anti-tuberculosis drugs, Int. J. Tubercul. Lung Dis., 9 (2005) 901-906.
[47] J. McFarland, The nephelometer: an instrument for estimating the number of bacteria in suspensions used for calculating the opsonic index and for vaccines, J. Am. Med. Assoc. XLIX (1907) 1176–1178.