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1,3-Azoles from *ortho*-naphthoquinones: Synthesis of aryl substituted imidazoles and oxazoles and their potent activity against *Mycobacterium tuberculosis*

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This paper is dedicated to the memory of Professor Antonio V. Pinto who passed away in 2010, a great scientist and our mentor in all aspects.

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ABSTRACT

Twenty-three naphthoimidazoles and six naphthoxazoles were synthesised and evaluated against susceptible and rifampicin- and isoniazid-resistant strains of *Mycobacterium tuberculosis*. Among all the compounds evaluated, fourteen presented MIC values in the range of 0.78 to 6.25 μg/mL against susceptible and resistant strains of *M. tuberculosis*. Five structures were solved by X-ray crystallographic analysis. These substances are promising antimycobacterial prototypes.

1. Introduction

Tuberculosis (TB) is one of the most widespread diseases in the world, responsible for approximately 1.3 million deaths annually.[1] Although the currently available clinical treatments can cure most cases of TB with drug susceptible, problems such as the long duration of treatment, the need for multiple drug therapy, antimicrobial resistance, and co-infection with HIV necessitate the discovery of new drugs to treat TB.[2] Furthermore, the complex pathobiology of *M. tuberculosis*, which enables the organism to persist in a dormant stage for years, makes treatment even more difficult.[3]

It is important to develop new drug prototypes that are both more active and more effective and that have alternative mechanisms of action against resistant and susceptible bacterial strains. Although a large number of molecules have been developed as potential new drugs, none have emerged for clinical use in the last 45 years.[4,5] However, there are a wide variety of research resources and strategies aimed at developing new drugs for TB treatment, some of which involve the screening of prototypes of synthetic molecules and natural products.[2,6] Biodiversity is an important factor in the discovery of active substances, leading to further studies of chemical structural modification to enhance their biological activity.

Recently, several imidazole and oxazole compounds (Fig. 1) were described as having antimycobacterial activity, and novel substances were designed based on this important nitrogenated class of heterocycles.[7]

Quinones represent a wide and diverse family of plant metabolites.[8] In addition to their anti-inflammatory and cytotoxic properties, the activity of quinones against viruses and pathogenic microorganisms has been reported.[9] The main deleterious action of quinones in biological systems is oxidative stress promotion, a

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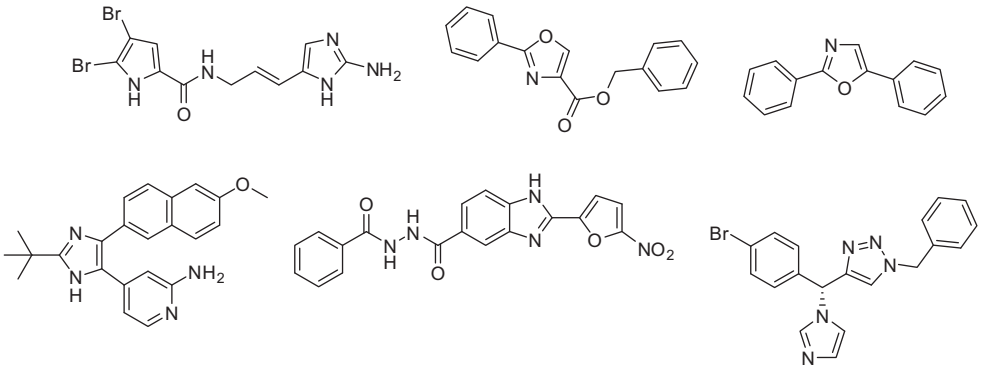


Figure 1. Synthetic drugs already tested against *Mycobacterium tuberculosis*.[7]

harmful process that causes irreversible damage by altering vital cellular components.[10] One consequence of this mechanism is the destruction of biological membranes, resulting from peroxidation of the lipid components. Moreover, quinones can interfere with a variety of biosynthetic pathways, including the destruction of proteins, damage to the structures of nucleic acids and DNA strand breaks.[11]

Our research group has described the synthesis and antimycobacterial activity of naphthoquinoidal and heterocyclic derivatives from lapachol.[12] This quinone is an important natural compound isolated from the hard core of trees of the bignoniaceous family, found mainly in the tropical areas of the Western Hemisphere.[13] Initially, phenazines from lapachones were obtained, and the compound derived from allyl-β-lapachone was identified as a potent substance (Fig. 2) with MIC values equal to 0.78 μg/mL against pan-susceptible and rifampicin-resistant strains of *M. tuberculosis*.[12] More recently, we reported a phenazine obtained from a lapachol derivative that was considered highly active (MIC ⩽3 μg/mL) against *M. tuberculosis* $H_{37}Rv$, a pan-susceptible strain. Against ATCC 35822 strains, this compound was less active, with an MIC value of 12.5 mg/mL.[14] Nor-lapachol also presented an MIC = 3.12 μg/mL against a rifampicin-resistant strain (Fig. 2).[14]

Imidazole compounds are important drug prototypes for TB.[15] Different synthetic strategies have been used to obtain imidazoles with diverse patterns of substitution, and these substances present low MIC values, proving the importance of imidazole moieties for activity against *M. tuberculosis*.[7,15]

In the last few years, a series of manuscripts showed that the functionalisation of the quinoidal moieties of lapachol and β-lapachone into naphthoimidazoles and naphthoxazoles is an important strategy in the development of new, active compounds.[16]

In this context, we synthesised naphthoimidazoles and naphthoxazoles that were evaluated against pan-susceptible *M. tuberculosis* strains that were resistant to isoniazid and rifampicin. Our strategy was to examine imidazoles and oxazoles with electron-donating and electron-withdrawing aryl substituents or that had heterocyclic substitutions, such as quinolinic, isoquinolinic or pyridinic core structures.

2. Results and discussion

2.1. Chemistry

All of the naphthoimidazoles (Fig. 3) and naphthoxazoles (Fig. 4) were obtained by the reaction of a quinonoid compound (β-lapachone) with aromatic aldehydes in the presence of ammonium acetate, as previously described.[16,17] In summary, the substances were separated by column chromatography using eluents of varying polarity: oxazoles with 94:6 hexane:ethyl acetate and imidazoles with 90:10 hexane:ethyl acetate.

Some aryl-substituted imidazoles were also obtained from nor-β-lapachone by the method described in Scheme 1. Nor-lapachol was obtained from lapachol by the Hooker oxidation method[18] and was then reacted with H_2SO_4 to obtain the cyclised product, nor-β-lapachone. The unpublished 1,3-azoles (Scheme 1) were obtained through the reaction of nor-β-lapachone, different substituted aldehydes and a nitrogenating reagent ($AcONH_4$) in acetic acid. The reaction conditions allowed us to obtain a mixture of products (imidazoles and oxazoles). In some cases both compounds were obtained, while in other cases, only the imidazole or oxazole was obtained.

Compound **30** was obtained from C-allyl lawsone (**29**), as previously described[19] and was used to prepare the respective oxazole derivative **31**, described herein for the first time (See Scheme 2).

Compounds **1**–**21** were synthesised as previously described,[16,17] and substances **22**–**28** and 31, described for the first time, were isolated and characterised by ^1H and ^{13}C NMR, infrared, and electron-impact mass spectrometry. For some of the compounds, ^{13}C NMR spectra could not be collected due to their low solubility in most of the organic solvents tested. For compounds **1**, **26**, **27**, **28** and **31**, suitable crystals were obtained, and the structures were re-confirmed by X-ray crystallographic study.

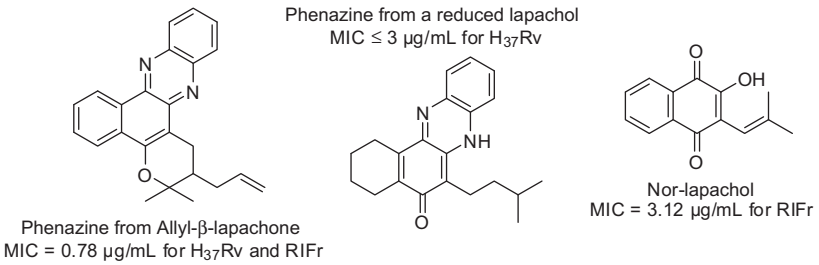


Figure 2. Lapachol derivatives with TB activity.

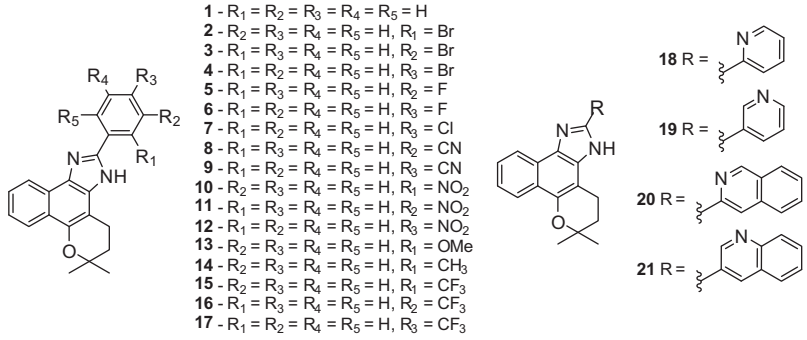


Figure 3. Naphthoimidazoles **1**–**21** obtained from β-lapachone.

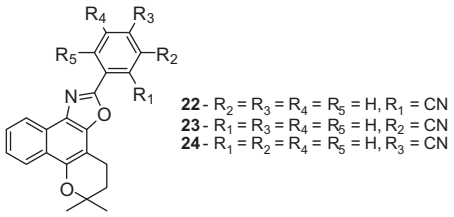


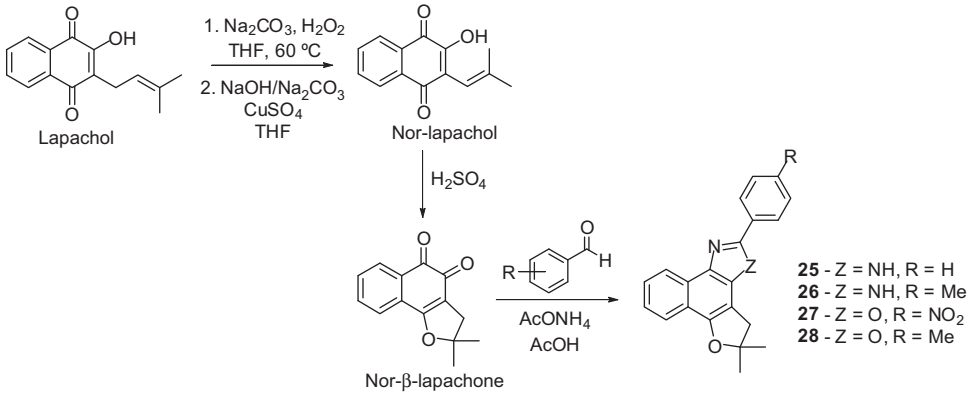
Figure 4. Naphthoxazoles **22**–**24** obtained from β-lapachone.

2.2. X-ray analysis

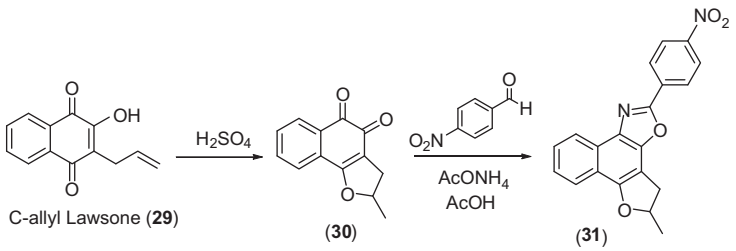
Hydrogen atoms bound to carbon and nitrogen were located at their idealised positions using appropriate *HFIX* instructions in SHELXL (*43* for the aromatic and *33* for the terminal $-CH_3$ methyl groups) and were included in subsequent refinement cycles in the riding-motion approximation with isotropic thermal displacements parameters (U_{iso}) fixed, respectively, at 1.2 or 1.5 times the U_{eq} of the carbon or nitrogen atom to which they are attached. The final difference Fourier map synthesis showed the highest peak and deepest hole between (0.480 eÅ^{-3}) and ($-$0.427 eÅ^{-3}), respectively, for all compounds. The ORTEP-3 program for Windows[20] was used for graphic presentations, and the material for publication was prepared using WinGX-Routine.[21] Figure 5 shows the ORTEP-3 diagrams for five of the structures.

2.3. Biological assays

The minimum inhibitory concentrations (MIC) for all of the compounds were evaluated against *M. tuberculosis* $H_{37}Rv$ (pan-susceptible), rifampicin-resistant (RIFr, ATCC 35338) and isoniazid-resistant (INHr, ATCC 35822) strains (Table 1). Eighteen compounds, **1**–**3**, **5**–**7**, **10**, **12**–**16**, **18**, **20** and **22**–**24,** displayed MIC values between 0.78 and 100 μg/mL against all strains. The substances **25**–**31** were considered mildly active or inactive with MIC $\geqslant$100 μg/mL for all three strains.



Scheme 1. Naphthoimidazoles and naphthoxazoles **25**–**28** obtained from nor-β-lapachone.



Scheme 2. Naphthoxazole **31** obtained from substance **30**.

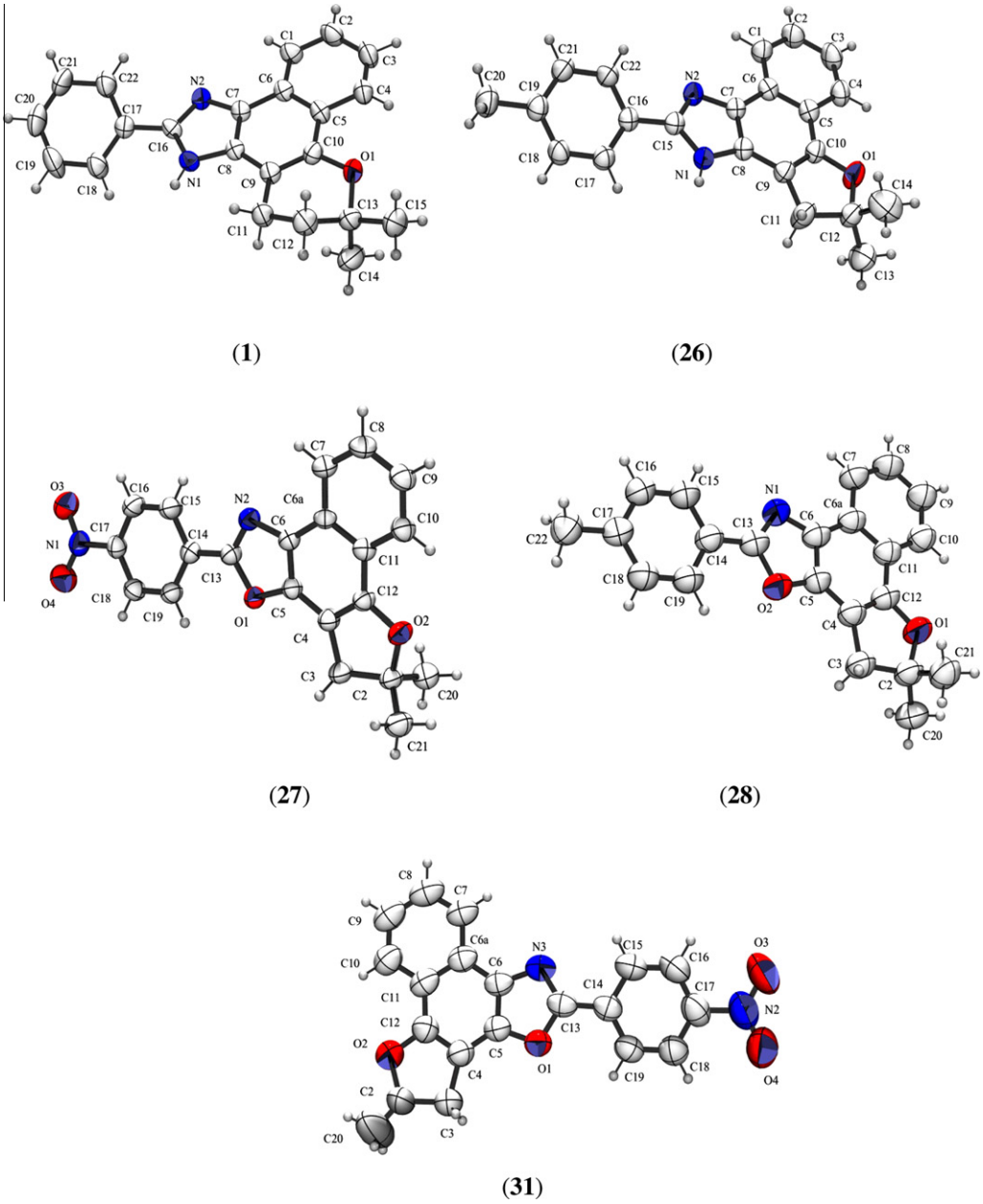


Figure 5. An ORTEP-3 projection of molecules **1**, **26**, **27**, **28** and **31**, showing the atom-numbering and displacement ellipsoids at the 50% probability level.

The insertion of an aryl-substituted ring into the imidazole group enhanced its microbicidal activity and is an important prototype for novel compounds against TB. For example, *ortho*-substituted compounds **2** (*o*-Br, MIC values of 7.6 μM for $H_{37}R_V$ and 15.3 μM for ATCC 35338 and ATCC 35822 strains) and **10** (*o*-NO_2, MIC values of 267.8, 33.5 and 16.7 μM against the $H_{37}Rv$, RIFr and INHr strains, respectively) were more potent than the naphthoimidazole **1** (no substituent on the phenyl ring, MIC >304.5 μM for all strains). Compound **15** (*o*-CF_3, MIC values = 4.0, 2.0 and 4.0 μM against $H_{37}Rv$, RIFr and INHr strains, respectively) was more active than **1** and its precursor, β-lapachone (MIC = 6.43 μM for $H_{37}RV$ and ATCC 35338) indicating that the insertion of –CF_3 group at the *ortho*-position in the phenyl ring enhances the activity. Substances **13** (*o*-OMe) and **14** (*o*-CH_3), which were substituted with electron donating groups, were highly active with MIC values in the range of 4.5–17.4 μM.

Among the *meta*-substituted phenyl derivatives, those more active against the *M. tuberculosis* were *m*-Br (**3**), *m*-F (**5**) and *m*-CF_3 (**16**), with MICs below 18.0 μM. The *m*-CN (**8**) and *m*-NO_2 (**11**) compounds were inactive. Substances **3** (*m*-Br, MIC = 15.3 μM) and **16** (*m*-CF_3, MIC = 4.0 μM) were the most active imidazoles in this series for all of the *M. tuberculosis* strains. Regarding the presence of substituents at the *para* position, the naphthoimidazole **12** (*p*-NO_2, MIC = 4.2 μM) was the most active against INHr but was inactive against the RIFr strain. The *p*-Br (**4**), *p*-CN (**9**) and *p*-CF_3 (**17**) compounds were inactive, demonstrating that the insertion of substituents in this position decreases the activity. Although compounds **1**–**17** possess substituents with different sizes, sterics and electronic properties, no consistent structure–activity relationships could be established.

The naphthoimidazoles **8** and **9** and the naphthoxazoles **22**–**24** with a cyano group showed diverse activity for all of the *M. tuberculosis* strains; the naphthoimidazoles were inactive, while the naphthoxazoles exhibited the opposite effect. For the heterocyclic compounds **18**–**21**, substance **20** with a quinoline group was the

Table 1
MIC (minimum inhibitory concentration) of the tested compounds against *Mycobacterium tuberculosis* $H_{37}Rv$, rifampicin-resistant *M. tuberculosis* (RIFr) and isoniazid-resistant *M. tuberculosis* (INHr)

Compd No.	CMI μg/mL (μM)		
	$H_{37}Rv$ (ATCC27294)	RIFr (ATCC 35338)	INHr (ATCC 35822)
1	>100 (>304.5)	>100 (>304.5)	12.5 (38.0)
2	3.12 (7.6)	6.25 (15.3)	6.25 (15.3)
3	6.25 (15.3)	6.25 (15.3)	6.25 (15.3)
4	Na	Na	Na
5	3.12 (9.0)	6.25 (18.0)	6.25 (18.0)
6	25 (72.2)	6.25 (18.0)	6.25 (18.0)
7	12.5 (34.4)	6.25 (17.2)	6.25 (17.2)
8	Na	Na	Na
9	Na	Na	Na
10	100 (267.8)	12.5 (33.5)	6.25 (16.7)
11	Na	Na	Na
12	100 (267.8)	Na	1.56 (4.2)
13	6.25 (17.4)	6.25 (17.4)	6.25 (17.4)
14	3.12 (9.0)	1.56 (4.5)	1.56 (4.5)
15	1.56 (4.0)	0.78 (2.0)	1.56 (4.0)
16	1.56 (4.0)	1.56 (4.0)	1.56 (4.0)
17	Na	Na	Na
18	25 (75.9)	6.25 (19.0)	6.25 (19.0)
19	Na	Na	Na
20	6.25 (16.4)	⩽0.78 (⩽2.0)	⩽0.78 (⩽2.0)
21	Na	Na	Na
22	12.5 (35.2)	6.25 (17.6)	6.25 (17.6)
23	12.5 (35.2)	6.25 (17.6)	6.25 (17.6)
24	6.25 (17.6)	1.56 (4.4)	3.12 (8.8)
25	>100 (>318.0)	100 (318.0)	>100 (>318.0)
26	>100 (>304.5)	100 (304.5)	>100 (>304.5)
27	>100 (>277.5)	Nd	>100 (>277.5)
28	>100 (>303.6)	Nd	>100 (>303.6)
29	100 (466.8)	25 (116.7)	100 (466.8)
30	3.12 (14.5)	Na	Na
31	>100 (>288.7)	100 (288.7)	>100 (>288.7)
Rifampicin	⩽0.125 (⩽0.1)	>4 (>4.8)	⩽0.125 (⩽0.1)
Isoniazid	⩽0.06 (⩽0.4)	⩽0.06 (⩽0.4)	1 (7.3)

most active, with an MIC ⩽2.0 μM for rifampicin- and isoniazid-resistant *M. tuberculosis*.

We have previously described a strategy to modify the C-ring of the β-lapachone (pyran ring versus furan ring) that was successfully used to obtain trypanocidal compounds.[22] Substances **25**–**28** and **31**, which have a dihydrofuran ring, were synthesised and evaluated against all of the *M. tuberculosis* strains to determine the effectiveness of this approach against TB. None of these substances with the dihydrofuran ring were active against *M. tuberculosis*.

For compounds **1**–**11**, **20** and **25**, the toxicity to human peripheral blood mononuclear cells (PBMC) after 72 h of drug exposure was evaluated by the Alamar Blue assay. For these substances, the IC_{50} values were higher than 25.0 μg/mL. The described azoles represent an important series of compounds with potent activity against TB with low cytotoxicity.

3. Conclusions

To discover antimicrobial compounds, we describe fourteen substances with MIC values in the range of 0.78 to 6.25 μg/mL against strains of *M. tuberculosis*. Substance **20**, with a quinoline group, was the most active, with an MIC value ⩽0.78 μg/mL for rifampicin-resistant *M. tuberculosis* (RIFr) and isoniazid-resistant *M. tuberculosis* (INHr) strains. Therefore, this substance emerges as an important derivative for the design of novel active compounds. The research described here is an important contribution in the field of biologically active heterocyclic compounds against TB, a neglected disease and an epidemic in many developing countries.

4. Experimental protocols

4.1. General procedures

Melting points were determined in a capillary Thomas Hoover apparatus (Thomas Co., Philadelphia, PA, USA) and are uncorrected. Analytical grade solvents were used. Column chromatography was performed on silica gel (Acros Organics 0.035–0.070 mm, pore diameter ca 6 nm). Infrared spectra were recorded on a Perkin–Elmer FT-IR spectrometer (Perkin–Elmer Inc., Wellesley, MA, USA). ^1H and ^{13}C NMR spectra were recorded at room temperature using a VNMR-SYS-500, Varian MR 400 instrument and Gemini 200-MHz spectrometer (Varian, Palo Alto, CA, USA) in the solvents indicated with TMS as an internal reference. Chemical shifts (δ) are given in ppm, and coupling constants (J) are reported in Hertz. The mass spectra were obtained at 70 eV in a VG Autospec apparatus. The fragments are described as a relationship between atomic mass units and the charge (m/z) and the relative abundance in percentage. All the compounds were nominated using the program ChemBioDraw Ultra 12.0.

4.2. General procedure for the preparation of the imidazoles and oxazoles

To a solution of β-lapachone (1.1 mmol) in acetic acid (6 mL), the desired aldehyde (2.5 mmol) was added, and the mixture was heated to 70 °C; at this point, ammonium acetate (16.5 mmol) was slowly added, and reflux was maintained for a determined time. All the reactions were monitored by thin layer chromatography. At the end of the reaction, after addition into water, the precipitate was purified by column chromatography using as eluent a mixture of hexane/ethyl acetate, with a gradient of increasing polarity, as previously described.[16]

4.3. 2-(6,6-Dimethyl-5,6-dihydro-4*H*-benzo[7,8]chromeno[6,5-*d*]oxazol-2-yl)benzonitrile (22)

Using 2-formylbenzonitrile (327.5 mg, 2.5 mmol), **22** was obtained as a yellow solid (53.1 mg, 0.15 mmol, 15% yield, mp 181–183 °C).

IR ν_{max}(cm^{-1}, KBr): 3061 (Ar-CH), 2225 (C≡N), 1599 (C=N), 1237 (C–O–C), 1119 (C–O–C). ^1H NMR (200 MHz, DMSO-d_6) δ: 8.46–8.28 (2H, m), 8.20 (1H, d, J = 8.2 Hz), 8.02 (1H, d, J = 7.4 Hz), 7.88 (1H, t, J = 7.4 Hz), 7.76–7.62 (2H, m), 7.56 (1H, d, J = 8.2 Hz), 3.05 (2H, t, J = 6.4 Hz), 1.98 (2H, t, J = 6.4 Hz), 1.44 (s, 6H). ^{13}C NMR (50 MHz, DMSO-d_6) δ: 157.9, 148.0, 135.0, 134.0, 133.3, 130.7, 128.7, 128.5, 127.0, 124.8, 124.5, 123.4, 122.6, 122.2, 121.1, 117.4, 108.7, 101.8, 75.6, 30.6, 26.2, 16.6. MS (70 eV, m/z) (%): [M+1] 355 (8), 354 (38), 299 (24), 298 (100), 162 (5), 130 (43), 115 (6), 114 (7), 102 (15), 41 (4).

4.4. 3-(6,6-Dimethyl-5,6-dihydro-4*H*-benzo[7,8]chromeno[6,5-*d*]oxazol-2-yl)benzonitrile (23)

Using 3-formylbenzonitrile (327.5 mg, 2.5 mmol), **23** was obtained as a yellow solid (35.0 mg, 0.1 mmol, 10% yield, mp 217–220 °C).

IR ν_{max}(cm^{-1}, KBr): 3076 (Ar-CH), 2225 (C≡N), 1601 (C=N), 1237 (C–O–C), 1118 (C–O–C). ^1H NMR (400 MHz, $CDCl_3$) δ: 8.52–8.43 (2H, m), 8.32–8.30 (1H, m), 7.85 (1H, dd, J = 0.7, 7.8 Hz), 7.73 (1H, td, J = 1.1, 7.8 Hz), 7.64 (1H, td, J = 1.1, 7.8 Hz), 7.53 (2H, ddd, J = 1.1, 7.8, 15.2 Hz), 3.16 (2H, t, J = 6.6 Hz), 2.00 (2H, t, J = 6.6 Hz), 1.50 (6H, s). ^{13}C NMR (50 MHz, DMSO-d_6) δ: 157.9, 148.4, 147.0, 132.9, 130.3, 129.8, 129.5, 129.0, 127.0, 125.2, 124.7, 124.0, 122.6, 121.5, 118.1, 113.1, 101.4, 75.3, 31.5, 26.6, 17.20. MS (70 eV, m/z) (%): [M+1] 355 (10), 354 (38), 299 (26), 298 (100), 162 (8), 130 (50), 115 (7), 114 (7), 102 (18), 41 (4).

4.5. 4-(6,6-Dimethyl-5,6-dihydro-4*H*-benzo[7,8]chromeno[6,5-*d*]oxazol-2-yl)benzonitrile (24)

Using 4-formylbenzonitrile (327.5 mg, 2.5 mmol), **24** was obtained as a yellow solid (71.0 mg, 0.2 mmol, 20% yield, mp 230–234 °C).

IR ν_{max}(cm^{-1}, KBr): 3046 (Ar-CH), 2226 (C≡N), 1606 (C=N), 1203 (C–O–C), 1120 (C–O–C). ^{1}H NMR (200 MHz, DMSO-d_6) δ: 8.40–7.80 (8H, m), 3.12 (2H, t, J = 6.7 Hz), 2.02 (2H, t, J = 6.7 Hz), 1.52 (6H, s). ^{13}C NMR (50 MHz, DMSO-d_6) δ: 158.0, 148.5, 147.0, 138.5, 132.7, 132.4, 131.5, 129.7, 126.9, 126.7, 118.3, 117.5, 117.3, 113.0, 101.4, 75.3, 31.4, 26.5, 17.1. MS (70 eV, m/z) (%): [M+1] 355 (10), 354 (39), 299 (26), 298 (100), 162 (6), 130 (56), 115 (7), 114 (7), 102 (22), 41 (5).

4.6. 5,5-Dimethyl-2-phenyl-4,5-dihydro-3*H*-furo[3′,2′:3,4]naphtho[1,2-*d*]imidazole (25)

Using nor-β-lapachone (228 mg, 1 mmol) and benzaldehyde (265 mg, 2.5 mmol), **25** was obtained as a yellow solid (157 mg, 0.5 mmol, 50% yield, mp 265–266 °C).

IR ν_{max}(cm^{-1}, KBr): 3100 (Ar-CH), 1606 (C=N), 1110 (C–O–C). ^{1}H NMR (200 MHz, DMSO-d_6) δ: 8.47 (1H, d, J = 8 Hz), 8.2 (2H, m), 7.88 (1H, d, J = 8 Hz), 7.66–7.13 (5H, m), 2.5 (s, 2H); 1.5 (s, 6H). MS (70 eV, m/z) (%): 314 (100); 299 (75); 272 (8); 245 (3); 210 (4); 196 (11); 169 (8); 149 (11); 115 (8); 77 (6); 57 (8).

4.7. 5,5-Dimethyl-2-(*p*-tolyl)-4,5-dihydro-3*H*-furo[3′,2′:3,4]naphtho[1,2-*d*]imidazole (26)

Using nor-β-lapachone (456 mg, 2 mmol) and 4-metyl benzaldehyde (540 mg, 4.5 mmol), **26** was obtained as a white solid (98 mg, 0.30 mmol, 15% yield, mp 275–278 °C).

IR ν_{max}(cm^{-1}, KBr): 3140 (Ar-CH), 1606 (C=N), 1123 (C–O–C). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 8.48 (1H, d, J = 8.0 Hz), 8.14–8.06 (2H, m), 7.89 (1H, d, J = 8.0 Hz), 7.57 (1H, t, J = 7.5 Hz), 7.44 (1H, t, J = 7.5 Hz), 7.38–7.34 (2H, m), 3.37 (2H, s), 2.39 (3H, s), 1.58 (6H, s). MS (70 eV, m/z) (%): 328 (64), 313 (55), 299 (5), 285 (7), 196 (6), 167 (8), 140 (14), 130 (9), 115 (22), 102 (12), 91 (27), 77 (14), 65 (15), 41 (100).

4.8. 5,5-Dimethyl-2-(4-nitrophenyl)-4,5-dihydrofuro[3′,2′:3,4]naphtho[1,2-*d*]oxazole (27)

Using nor-β-lapachone (456 mg, 2 mmol) and *p*-NO_2 benzaldehyde (679.5 mg, 4.5 mmol), **27** was obtained as a red solid (410 mg, 1.14 mmol, 57% yield, mp 224–226 °C).

IR ν_{max}(cm^{-1}, KBr): 1600 (C=N),1553, 1316 (NO_2), 1256 (C–O–C), 1105 (C–O–C). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.52 (1H, d, J = 8.2 Hz), 8.45–8.34 (4H, m), 8.08 (1H, d, J = 8.2 Hz), 7.69–7.62 (1H, m), 7.57–7.50 (1H, m), 3.43 (2H, s), 1.67 (6H, s). MS (70 eV, m/z) (%): 360 (75), 330 (8), 318 (11), 197 (11), 169 (20), 47 (100).

4.9. 5,5-Dimethyl-2-(*p*-tolyl)-4,5-dihydrofuro[3′,2′:3,4]naphtho[1,2-*d*]oxazole (28)

Using β-nor-lapachone (456 mg, 2 mmol) and *p*-methyl benzaldehyde (540 mg, 4.5 mmol), **28** was obtained as a white solid (86 mg, 0.26 mmol, 13% yield, mp 198–200 °C).

IR ν_{max}(cm^{-1}, KBr): 1612 (C=N), 1258 (C–O–C), 1065 (C–O–C). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.44 (1H, d, J = 8.2 Hz), 8.08 (2H, d, J = 7.9 Hz), 7.97 (1H, d, J = 8.2 Hz), 7.56–7.48 (1H, m), 7.44–7.36 (1H, m), 7.24 (2H, d, J = 7.9 Hz), 3.33 (2H, s), 2.35 (3H, s), 1.56 (6H, s). MS (70 eV, m/z) (%): 329 (100), 314 (8), 300 (2), 287 (16), 197 (15), 169 (16), 141 (10), 139 (7), 119 (33), 91 (23).

4.10. 5-Methyl-2-(4-nitrophenyl)-4,5-dihydrofuro[3′,2′:3,4]naphtho[1,2-*d*]oxazole (31)

Using compound **30** (428 mg, 2 mmol) and *p*-NO_2 benzaldehyde (679.5 mg, 4.5 mmol), **31** was obtained as a red solid (138 mg, 0.40 mmol, 20% yield, mp 234–237 °C).

IR ν_{max}(cm^{-1}, KBr): 1600 (C=N), 1258 (C–O–C), 1130 (C–O–C). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.52 (1H, d, J = 8.2 Hz), 8.44–8.32 (4H, m), 8.07 (1H, d, J = 8.2 Hz), 7.68–7.64 (1H, m), 7.56–7.52 (1H, m), 5.41–5.26 (1H, m), 3.75 (1H, dd, J = 15.2, 9.2 Hz), 3.23 (1H, dd, J = 15.2, 7.4 Hz), 1.65 (3H, s). MS (70 eV, m/z) (%): 346 (92), 316 (19), 300 (20), 228 (7), 155 (24), 141 (34), 127 (23), 115 (61), 102 (31), 88 (29), 76 (100), 63 (31), 43 (77).

4.11. Minimum inhibitory concentration determination

The experiment was performed using *Mycobacterium tuberculosis* H_{37}Rv (ATCC27294), a pan-susceptible strain, RIFr with a His-526→Tir mutation in the *rpo*B gene and INH^R with a Ser-315→Tir mutation in the *kat*G gene. The strains were maintained in Ogawa-Kudoh media for 14 days at 37 °C.

The determination of antimycobacterial activity was performed by REMA (Resazurin Microtitre Assay), as previously described.[23] In brief, the bacterial suspensions were homogenized by vortex agitation, and the turbidity was adjusted in agreement with tube one of the scale 1.0 of McFarland (3.2×10^6 cfu/mL). The inoculum was prepared by diluting the bacterial suspension 1:20 in Middlebrook 7H9 OADC media (4.7 g Middlebrook 7H9 base; Difco, Becton Dickinson). The 7H9 medium consisted of 0.5 g of ammonium sulfate, 0.5 g of L-glutamic acid, 0.1 g of sodium citrate, 0.001 g of pyridoxine, 0.0005 g of biotin, 2.5 g of disodium phosphate, 1.0 g of monopotassium phosphate; 0.04 g ferric ammonium citrate, 0.05 g of magnesium sulfate, 0.0005 g of calcium chloride, 0.001 g of Zinc sulfate, 0.001 g of copper sulfate, 2 mL of glycerol, 900 mL of H_2O, and 10% OADC (oleic acid, albumin, dextrose, catalase). The assay was performed in 96-well microplates, with a concentration of 100 μg/mL in the first well and up to 0.19 μg/mL in the last well. The microplate was incubated at 37 °C for 7 days. After this, 30 μL of resazurin was added to each well, and the plate was incubated for 2 days at 37 °C. The reading was recorded from the oxi-reduction of the resazurin, noting the change of color when cellular growth was taking place.

4.12. Inhibition of PBMC proliferation

To investigate the selectivity of compounds toward a normal proliferating cell, the Alamar blue assay was performed with peripheral blood mononuclear cells (PBMC) after 72 h of drug exposure. After 24 h, compounds (0.048 to 25 μg/mL) dissolved in DMSO (0.1%) were added to each well and incubated for 72 h. Twenty-four h before the end of the incubation, 10 μL of stock solution (0.312 mg/mL) of the Alamar Blue (Resazurin, Sigma–Aldrich Co) was added to each well. The absorbance was measured using a multiplate reader (DTX 880 Multimode Detector, Beckman Coulter®) and the drug effect was quantified as the percentage of control absorbance at 570 nm and 595 nm. The absorbance of Alamar Blue in culture medium is measured at a higher wavelength and a lower wavelength. The absorbance of the medium is also measured at the higher and lower wavelengths. The absorbance of the medium alone is subtracted from the absorbance of medium plus Alamar Blue at the higher wavelength. This value is called AOHW. The absorbance of the medium alone is subtracted from the absorbance of medium plus Alamar Blue at the lower wavelength. This value is called AOLW. A correction factor, R0, can be calculated from AOHW and AOLW, where R0 = AOLW/AOHW. The

percent Alamar Blue reduced is then expressed as follows:% reduced = ALW—(AHW × R0) × 100.

4.13. X-ray crystallographic analysis

Data for each compound were collected at room temperature on an Enraf Nonius FR590 charge-coupled device (CCD) area-detector diffractometer (Mo K_α graphite-monochromated radiation, $\lambda = 0.71073$ Å) controlled by the COLLECT software package.[24] Images were processed using the Denzo and Scalepack software packages.[25] The structures were solved using direct methods with SHELXS-97[26] and refined by full-matrix least squares on F^2 using SHELXL-97.[26] All non-hydrogen atoms were successfully refined using anisotropic displacement parameters.

Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication No. CCDC 814214, 814215, 882547, 882549 and 882552. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 2EZ, UK (Fax: (+44) 1223 336033; e-mail: deposit@ccdc.cam.ac.uk).

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