



Mefloquine–Oxazolidine Derivatives: A New Class of Anticancer Agents

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A series of 23 racemic mefloquine–oxazolidine derivatives, 4-[3-(aryl)hexahydro[1,3]oxazolo[3,4-a]pyridin-1-yl]-2,8-bis(trifluoromethyl)quinolines, derived from (*R**, *S**)-(±)-mefloquine and arenealdehydes, have been evaluated for their activity against four cancer cell lines (HCT-8, OVCAR-8, HL-60, and SF-295). Good cytotoxicities have been determined with IC₅₀ values ranging from 0.59 to 4.79 μg/mL. In general compounds with aryl groups having strong electron-releasing substituents, such as HO and MeO, or electron-rich heteroaryl groups, for example imidazol-2-yl, are active. However, other factors such as steric effects may play a role. As both the active and non-active conformations of the mefloquine–oxazolidine derivatives are similar, it is concluded that molecular conformations do not play a significant role either. This study is the first to evaluate mefloquine derivatives as antitumor agents. The mefloquine–oxazolidine derivatives are considered to be useful leads for the rational design of new antitumor agents.

Key words: anticancer, antitumor activity, mefloquine, oxazolidine

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Mefloquine (MQ), (*R**, *S**)-(±)- α -2-piperidinyl-2,8-bis(trifluoromethyl)-4-quinolinemethanol, also known as Lariam, Mephaquin, or Mefliam, is an important drug orally administered in the prevention and treatment of malaria. However, in the last years, this drug has been found to have promising

applications in other fields, such as against Gram-positive and Gram-negative bacteria (1), in particular against *Mycobacterium tuberculosis* (2,3) and *Mycobacterium avium* complex (MAC) (4,5) and also against schistosomiasis (6), neurodegenerative, and (neuro-) inflammatory diseases (7). A clear indication of the promise of this drug in fields other than malaria is the number of recent patent applications (5,7,8). More recently, much attention has been paid to the potential perspectives of MQ as an anticancer agent. As estimated by the National Institutes of Health (NIH), the overall costs of cancer in 2007 were \$226.8 billion.^a Cancer is a leading cause of deaths worldwide and accounted for 7.6 million deaths (13% of all deaths) in 2008.^b Louie and coworkers (9) found MQ to be the most potent anticancer drug when compared to chloroquine and the fluoroquinolone drugs cipro and levofloxacin. MQ inhibits autophagy and triggered endoplasmic reticulum stress. It also causes cell death in T47D and MDAMB-231. Cho and coworkers also investigated the effect of autophagy on mefloquine-mediated cytotoxicity in neuroblastoma cells (10).

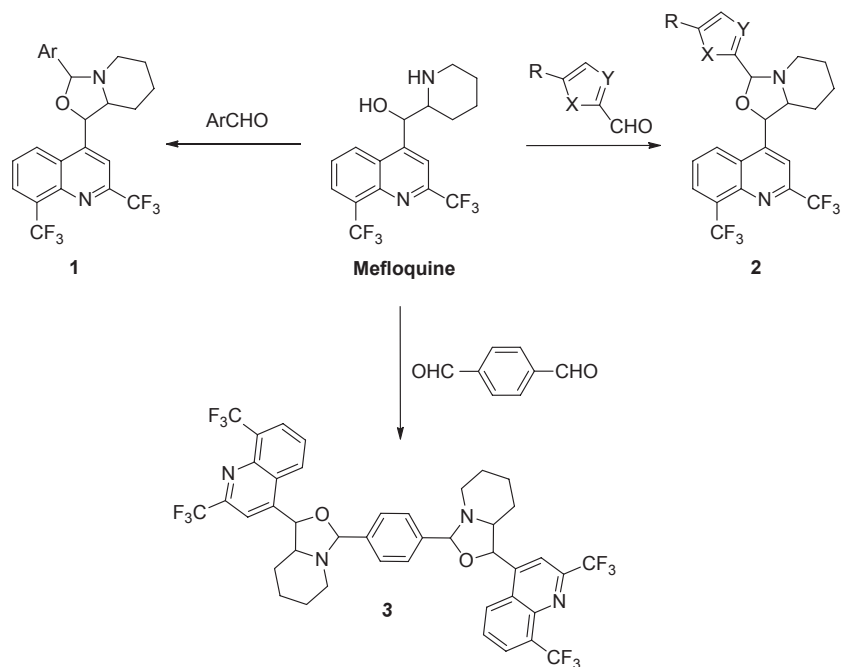
To study the potential of MQ analogues as anticancer agents, it is necessary to develop and evaluate MQ derivatives. Prime goals are to enhance the effectiveness and to minimize side-effects, as well as to provide a better understanding of the mechanism of action. In this work, we present the results of the evaluation of a series of twenty-three mefloquine–oxazolidine derivatives (Scheme 1) against several cancer cell lines, including HCT-8, OVCAR-8, HL-60 and SF-295. The results are very promising, as exemplified in Tables 1 and 2.

Methods and Materials

Cytotoxicity assays

Cytotoxicity against cancer cell lines

Compounds **1a–s**, **2a–c**, **3** and **MQ** (0.009–5 μg/mL) were tested for their cytotoxic activity against 3–5 cancer cell lines: SF-295 (nervous system), HCT-116 (colon), OVCAR-8 (ovarium), HCT-8 (colon), and HL60 (leukemia) (National Cancer Institute, Bethesda, MD, USA). All cell lines were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin at 37 °C with 5% CO₂. Each compound was dissolved in DMSO to give a concentration



Scheme 1: Synthesis of mefloquine-oxazolidine derivatives.

Table 1: Growth inhibition percentage (GI%) for three tumor cell lines^a, measured by MTT assay

Entry		SF-295		HCT-116		OVCAR-8	
No	Ar or heteroaryl	GI%	SD%	GI%	SD%	GI%	SD%
1a	C ₆ H ₅	33.74	0.46	56.74	3.85	43.52	4.33
1b	2-HOC ₆ H ₄	100.88	0.06	100.82	0.27	101.44	0.00
1c	2-MeOC ₆ H ₄	53.80	1.90	94.81	3.04	100.78	0.31
1d	4-MeOC ₆ H ₄	31.84	8.89	71.09	2.06	21.68	14.34
1e	3,4-(MeO) ₂ C ₆ H ₃	46.55	9.45	100.30	0.85	100.29	0.58
1f	3,4,5-(MeO) ₃ C ₆ H ₂	36.80	5.71	52.78	5.94	21.55	1.23
1g	3-EtOC ₆ H ₄	34.69	8.06	24.40	59.49	8.99	13.64
1h	4-FC ₆ H ₄	32.89	0.77	39.78	0.18	31.33	1.65
1i	2-ClC ₆ H ₄	22.28	0.59	40.09	38.92	12.26	4.78
1j	3-ClC ₆ H ₄	33.50	6.73	27.84	13.76	26.20	3.56
1k	4-ClC ₆ H ₄	80.38	28.93	101.58	0.45	101.16	0.08
1l	2,3-Cl ₂ C ₆ H ₃	24.94	3.10	40.28	3.41	8.50	0.41
1m	2,4-Cl ₂ C ₆ H ₃	17.41	8.42	19.70	1.39	-17.58	7.63
1n	3,4-Cl ₂ C ₆ H ₃	20.39	14.64	23.58	1.43	12.04	2.74
1o	3-BrC ₆ H ₄	34.50	4.56	42.02	3.95	36.17	12.52
1p	4-BrC ₆ H ₄	38.13	9.01	53.95	0.18	20.85	7.68
1q	2-O ₂ NC ₆ H ₄	22.56	22.32	23.77	48.30	23.56	3.48
1r	3-O ₂ NC ₆ H ₄	30.89	1.74	32.29	0.43	11.96	17.24
1s	4-O ₂ NC ₆ H ₄	29.12	21.72	61.47	2.24	18.70	2.74
2a	5-O ₂ N-furan-2-yl	37.80	4.45	14.42	0.32	10.38	23.13
2b	5-O ₂ N-thien-2-yl	14.14	3.86	18.99	6.64	26.41	4.99
2c	Imidazol-2-yl	29.91	3.68	84.82	12.70	97.51	0.08
3	-	37.11	5.00	-0.66	4.37	-6.62	0.25
-	Mefloquine	98.44	2.42	101.93	1.36	99.93	0.46

^aCell lines: nervous system (SF-295), colon (HCT-116), and ovarium (OVCAR-8).

of 1 mg/mL. The final concentration of DMSO in the culture medium was kept below 0.1% (v/v). Compounds **1a-s**, **2a-c**, **3**, and MQ were incubated with the cells for 72 h. The negative control received the same amount of DMSO

(0.001% in the highest concentration). The cell viability was determined by reduction in the yellow dye 3-(4,5-dimethyl-2-thiazol)-2,5-diphenyl-2H-tetrazolium bromide (MTT) to a blue formazan product as described by Mosmann (11).

Table 2: Cytotoxic activity of mefloquine–oxazolidine derivatives 1b–e and 2 on Tumor Cell Lines^a

Entry			IC ₅₀ (μg/mL)			
	No	Ar or heteroaryl	HCT-8	OVCAR-8	HL-60	SF-295
1b		2-HOC ₆ H ₄	2.699	2.824	3.338	1.606
			2.320–3.140	2.346–3.400	2.186–5.098	0.9841–2.621
1c		2-MeOC ₆ H ₄	2.957	4.074	3.635	4.785
			2.657–3.291	3.304–5.023	2.606–5.070	2.833–8.082
1d		4-MeOC ₆ H ₄	1.514	0.9450	1.913	3.598
			1.251–1.831	0.5600–1.595	1.498–2.444	3.299–3.925
1e		3,4-(MeO) ₂ C ₆ H ₃	3.077	2.839	2.343	3.250
			2.359–4.012	1.041–7.738	2.101–2.613	2.519–4.194
1k		4-Cl C ₆ H ₄	2.957	4.074	0.8873	3.524
			2.658–3.291	3.304–5.023	0.4072–1.934	3.049–4.072
2c		Imidazol-2-yl	1.732	3.297	0.5916	3.524
			1.404–2.137	2.318–4.688	0.3341–1.047	3.049–4.072
			Mefloquine	2.834	0.8176	2.831
			1.233–1.556	2.156–3.726	0.3970–1.684	1.781–4.500
			Doxorubicin	0.01	0.265	0.02
		0.01–0.02	(0.17–0.305)	0.01–0.02	0.19–0.25	

^aData are presented as IC₅₀ values, with 95% confidence intervals obtained by nonlinear regression for all cell lines leukemia (HL-60), ovary (OVCAR-8), nervous system (SF-295), colon (HCT-8) from three independent experiments. Mefloquine (MQ) was used as positive control. The trials were performed in triplicate. nd, not determined; IC₅₀, concentrations that induce 50% inhibition of cell growth in μg/mL.

Cell membrane disruption

These tests were performed in 96-well plates using a 2% mouse erythrocyte suspension in 0.85% NaCl solution containing 10 mM CaCl₂ (12). The compounds **1b–e**, **1k**, **2c**, and MQ were diluted as mentioned above and tested at a concentration of 250 μg/mL. After incubation at room temperature for 30 min, centrifugation, and removal of the supernatant, the released hemoglobin was measured by spectrophotometry at 540 nm. DMSO was used as a negative control and Triton-X-100 (1%) as a positive control. After incubation at room temperature for 1 h, centrifugation, and removal of the supernatant, the released hemoglobin was measured by visible spectrophotometry at λ = 540 nm. EC₅₀ is the calculated effective concentration that induced lysis on 50% that of the Triton-X-100.

Results and Discussion

Chemistry

In previous studies, we reported the synthesis of compounds, **1–3**, from racemic *erythro* mefloquine and appropriate arenealdehydes (Scheme 1), and their antimycobacterial evaluation against *Mycobacterium tuberculosis* H₃₇R_v and resistant strains (2,3).

The reactions between the racemic *erythro* mefloquine [stereochemistry: (R*)-C9, (S*)-C10] and arenealdehydes do not lead to changes of the stereochemistries at C-9 and C-10, but do generate new chiral center at the carbonyl center and at the piperidinyl nitrogen. The NMR data for the compounds studied here indicated the presence of a very major if not a sole stereoisomer, after initial

work-up. Crystalline solids, generated for crystallographic studies by slow recrystallization from EtOH, were found to be racemic mixtures having (R*)-C9, (S*)-C10, (S*)-C (carbonyl), and (R*)-N(piperidinyl) stereochemistries.

Cytotoxicity against cancer cell lines

Compounds **1a–s**, **2a–c**, **3**, and MQ were initially tested *in vitro* against three cancer cells at a concentration of 25 μg/mL, using the MTT assay (Table 1) (11). According to their growth inhibition (GI) in at least one of the cell line, the compounds were classified as active (95 < GI < 100), moderately active (70 < GI < 90), or inactive (GI < 50%). Compounds **1b–c**, **1e**, **1k**, **2c**, and MQ were thus classified as being active and derivative **1d** as moderately active, while all the other compounds were inactive against all cancer cell lines at 25 μg/mL.

The active compounds were selected for *in vitro* anticancer activity evaluations against four human cancer cell lines (HCT-8, OVCAR-8, HL-60, and SF-295) using the MTT assay (Table 2) (11). The concentrations that induced 50% inhibition of cell growth (IC₅₀) in μg/mL are presented in Table 2. None of the compounds was able to disrupt the cell membrane integrity of erythrocytes in the mouse model (data not shown) (12).

The results in Table 2 clearly indicate that compounds **1b–e**, **1k**, **2c**, and MQ exhibited good cytotoxic activity against the four cancer cell lines. All four compounds, **1b–e**, possess strong electron-releasing groups, namely 2-HO, 2-MeO, 4-MeO, and 3,4-(MeO)₂, respectively. As compound **1a** and compounds with electron-withdrawing substituents,

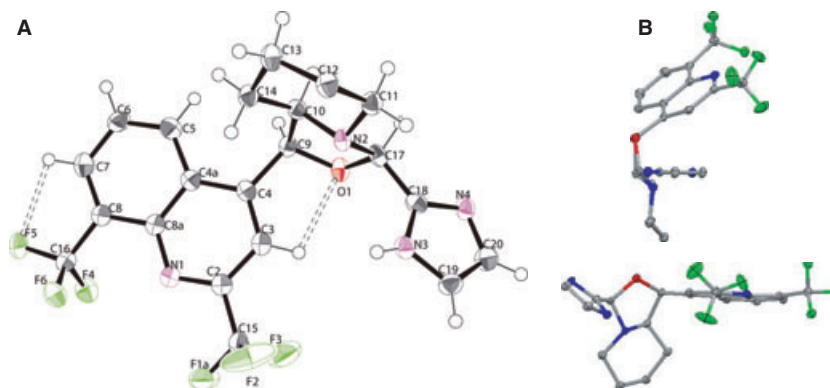


Figure 1: (A) Atom arrangements and numbering scheme for compound **2c**. Intramolecular hydrogen bonds are drawn as dashed lines. (B) Two views of the conformation of **2c**: colors: carbon gray; oxygen red; nitrogen blue; fluorine green. Hydrogen atoms have been omitted.

such as nitro and halo, were generally inactive, this strongly indicates the importance of electron-releasing substituents in the phenyl ring for activity. However, it is of interest that neither **1f** (with 3,4,5-(MeO)₃ substitution) nor **1g** (with a 3-EtO substituent) were active in the initial testing, see Table 1. This suggests that steric effects are playing some role in the activity too. Of all the compounds containing halogen substituents, compound **1k** was the only one to show activity, with IC₅₀ (μg/mL) values of 2.957, 4.074, 0.8873, and 3.524, against the cancer cell lines: HCT-8, OVCAR-8, HL-60, and SF-295, respectively.

Among the three compounds derived from heteroarenealdehydes, **2a–2c**, only the imidazole derivative, **2c**, was active. The imidazole ring is an electron-rich ring and again supports the importance of electron release for activity. The non-activity of the nitrofuranyl (**2a**) and nitrothienyl (**2b**) derivatives follows on from the non-activities of the nitrophenyl derivatives, **1q–1s**.

Currently, MQ combined with other drugs is in phase I factorial trial for the treatment of patients with glioblastoma. In this way, it is interesting to highlight that an improved cytotoxicity was observed for compound **1b**, against the glioblastoma cell line SF-295. This result displays the relevance of the proposed molecular modification for the development of new anticancer agents.

The molecular arrangement of the active imidazole derivative, **2c**, was determined in this study by X-ray crystallography, see Figure 1A. The non-chiral space group, P21/c, indicates that both enantiomers of **2c** are present in the single crystal examined. The chiral centers at C9, C10, C17, and N2 (crystallographic numbering system) are (R*), (S*), (S*), and (R*), respectively. Figure 1A shows the atom arrangements in the (R), (S), (S), (R)-enantiomer. The bond angles and bond lengths are all in the expected regions. The piperidine ring has a chair shape, while the oxazolidine ring has an envelope shape with a flap at the

nitrogen atom. Two views of the conformation of the molecule can be seen in Figure 1B. As previously found with active **1b** (19), as well as inactive **1a** (20), **1f** (2), and **1q–1s** (20), both the quinoline and aryl rings are on the same side of the best plane through the combined oxazolidine and piperidine rings, thus giving a crude ‘F’ shape arrangement. Furthermore, the quinoline and aryl rings are spread like opened butterfly wings. While there are

Table 3: Interplanar angles

Compound [Reference]	Angles between planes ^a			Active or not as antitumor agents
	Heterocycles/ phenyl	Phenyl/ quinoline	Heterocycles/ quinoline	
1b (13–18)	59.7°	17.4°	78.5°	Active
2c ^{b,c}	76.2°	40.3°	71.1°	Active
3 (2)	61.9°	52.8°	77.1°	Non- active
1a	75.9°	38.5°	82.1°	Non- active
1q	72.0°	38.4°	84.0°	Non- active
1r	80.2°	24.3°	81.2°	Non- active
1s	87.2°	14.1°	78.9°	Non- active

^aHeterocycles = fused oxazolidine and piperidine rings.

^bThis study.

^cCrystal data collected at 120(2)K, colourless crystal: 0.3 × 0.06 × 0.04 mm. Formula: C₂₁H₁₆F₆N₄O, M = 454.38; monoclinic, P21/c; a = 5.1701(4)Å, b = 18.011(2)Å, β = 93.047(7)°, c = 21.008(3)Å, Z = 4, V = 1953.5(4) Å³, 4454 independent reflections [R(int) = 0.1055], 2526 observed reflections [I > 2σ(I)]; parameters refined 4454; number of restraints 0; R(F) 0.090(obs data), largest diff.peak 0.66 eÅ⁻³. Atomic coordinates, bond lengths, angles and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre, deposition number 927346.

differences in the interplanar angles in each case as shown in Table 3, there is no apparent correlation between molecular conformations and activity. The major intermolecular interactions in **2c** are classical $N_3-HN_3-N_4$ hydrogen bonds, which are augmented by weak $C-F-\pi$ interactions to form chains of molecules.

Contrasting with the good anticancer activity of MQ, 4-bromo-2,8-di(trifluoromethyl)quinolone was found to exhibit no anticancer activity (data not shown). This apparently indicates that the 2-piperidinylmethanol moiety is an important privileged structure for the anticancer activity.

Conclusion

In this work, the first to evaluate MQ derivatives as antitumor agents, a series of 23 MQ-oxazolidine derivatives were evaluated for their activity against 3–5 cancer cell lines with good cytotoxicities (IC_{50} ranging from 0.59 to 4.79 $\mu\text{g}/\text{mL}$). Compounds with aryl groups having strong electron-releasing substituents, such as HO and MeO, or electron-rich heteroaryl groups, for example imidazol-2-yl, can be considered as useful leads for the rational design of new antitumor compounds, as these are more active than MQ against ovarium (OVCAR-8) (**1d**), leukemia (HL-60), and glioblastoma (SF-295) cells. The MQ-oxazolidine derivatives are considered to be useful leads for the rational design of new antitumor agents. Further studies to obtain structure–activity relationships and the elucidation of the mechanisms of cytotoxicity presented by these compounds are in progress.

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References

- Kunin C.M., Ellis W.Y. (2000) Antimicrobial activities of mefloquine and a series of related compounds. *Antimicrob Agents Chemother*;44:848–852.
- Gonçalves R.S.B., Kaiser C.R., Lourenço M.C.S., Bezerra F.A.F.M., de Souza M.V.N., Wardell J.L., Wardell S.M.S.V., Henriques M.G.M.O., Costa T. (2012) Mefloquine oxazolidine derivatives, derived from mefloquine and arenecarbaldehydes: in vitro activity including against the multidrug-resistant tuberculosis strain T113. *Bioorg Med Chem*;20:243–248.
- Gonçalves R.S.B., Kaiser C.R., Lourenço M.C.S., de Souza M.V.N., Wardell J.L., Wardell S.M.S.V., da Silva A.D. (2010) Synthesis and antitubercular activity of new mefloquine-oxazolidine derivatives. *Eur J Med Chem*;45:6095–6100.
- Bermudez L.E., Kolonoski P., Wu M., Aralar P.A., Inderlied C.B., Young L.S. (1999) Mefloquine is active in vitro and in vivo against *Mycobacterium avium* complex. *Antimicrob Agents Chemother*;43:1870–1874.
- Carson D.A., Leoni L.M., Cottam H.B., inventors (2003) Regents of the University of California, assignee. Treatment of cancer with mefloquine, its purified enantiomers, and mefloquine analogs. United States patent US 20030216426.
- Ingram K., Ellis W., Keiser J. (2012) Antischistosomal activities of mefloquine-related arylmethanols. *Antimicrob Agents Chemother*;56:3207–3215.
- Schrattenholz A., inventor (2005) Proteosys A.-G., Germany, assignee. Mefloquine, nelfinavir and saquinavir as novel agents for neurodegenerative and (neuro-) inflammatory diseases. United States patent US 2005670648.
- Seo J.B., Sun H.M., Choi S.S., Park D.Y., Park J.Y., Choi E.U., Jung J.Y., Shin D.S., Lee T.G., inventors (2012) BRNScience Co., Ltd., S. Korea, assignee. Mefloquine for preventing and treating bone disease. Republic Korean patent KR 2012045123.
- Sharma N., Thomas S., Golden E.B., Hofman F.M., Chen T.C., Petasis N.A., Schonthal A.H., Louie S.G. (2012) Inhibition of autophagy and induction of breast cancer cell death by mefloquine, an antimalarial agent. *Cancer Lett*;326:143–154.
- Shin J.H., Park S.J., Jo Y.K., Kim E.S., Kang H., Park J.H., Lee E.H., Cho D.H. (2012) Suppression of autophagy exacerbates mefloquine-mediated cell death. *Neurosci Lett*;515:162–167.
- Ahmed S.A., Gogal R.M. Jr, Walsh J.E. (1994) A new rapid and simple non-radioactive assay to monitor and determine the proliferation of lymphocytes: an alternative to [^3H]thymidine incorporation assay. *J Immunol Methods*;170:211–224.
- Sharma P., Sharma J.D. (2001) In vitro hemolysis of human erythrocytes - By plant extracts with antiplasmodial activity. *J Ethnopharmacol*;74:239–243.
- Hooft R.W.W. (1998) COLLECT, Data Collection Software. Delft, The Netherlands: Nonius BV.
- Sheldrick G.M. (2007) SADABS Version 2007/2. Madison, Wisconsin, USA: Bruker AXS Inc.
- Otwinowski Z., Minor W. Jr. (1997) Processing of X-ray diffraction data collected in oscillation mode. In: Carter C.W., Sweet R.M., editors. *Methods in Enzymology*, Vol. 276: Macromolecular Crystallography, Part A. New York: Academic Press; p. 307–326.
- Farrugia L.J. (1997) ORTEP-3 for Windows - a version of ORTEP-III with a Graphical User Interface (GUI). *J Appl Crystallogr*;30:565.
- Sheldrick G.M. (2008) A short history of SHELX. *Acta Crystallogr A*;64:112–122.
- Spek A.L. (2003) Single-crystal structure validation with the program PLATON. *J Appl Crystallogr*;36:7–13.



19. Gonçalves R.S.B., Kaiser C.R., de Souza M.V.N., Wardell J.L., Wardell S.M.S.V., Tiekink E.R.T. (2011) 2-{1-[2,8-Bis(trifluoromethyl)quinolin-4-yl]-3,5,6,7,8,8a-hexahydro-1H-1,3-oxazolo[3,4-a]pyridin-3-yl}phenol. *Acta Cryst;E67*:o1656–o1657.
20. Gonçalves R.S.B., Kaiser C.R., de Souza M.V.N., Wardell J.L., Wardell S.M.S.V., Howie R.A. (2011) Structures of 4-{3-(X-phenyl)perhydro-1,3-oxazolo[3,4-a]pyridin-1-yl}-2,8-bis(trifluoromethyl)quinolines (X = H, 2-O₂N, 3-O₂N and 4-O₂N), derived from mefloquine. *Z Krist;226*:793–803.

Notes

^aCancer.org [homepage on the Internet]. American Cancer Society. Available from: <http://www.cancer.org/>. Accessed: February 01, 2013.

^bGlobal Health Observatory [homepage on the Internet]. World Health Organization; c2013. Available from: http://www.who.int/gho/ncd/mortality_morbidity/cancer/en/index.html. Accessed: February 13, 2013.