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Microwave prompted multigram synthesis, structural determination, and photo-antiproliferative activity of fluorinated 4-hydroxyquinolinones

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Abstract—3-Unsubstituted 4-hydroxyquinolin-2(1*H*)-one containing F and CF_3 substituent in ring is important pharmacological and synthetic target and basic synthones for a number of antibacterial fluoroquinolones and is promising potent and selective glycine site NMDA receptors. A simple facile one-step microwave enhanced multigram synthesis of such fluorinated quinolones in reasonable purity has been developed in excellent yield (85–94%) in 3–5 min, whereas conventional synthesis required the harsh conditions, long reaction period with use of environmentally unacceptable regents giving the required product in lower yield. The phototoxicity as well as the cytotoxic activities of the title compounds are evaluated against leukemia- and adenocarcinoma-derived cell lines in comparison to the normal human keratinocytes. Structure–activity relationships between the chemical structures and the antimycobacterial, antifungal activity of the evaluated compounds are also discussed.
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The synthesis of compounds belonging to 4-hydroxyquinolones constitutes an important area of research due to their use as analgesic,[1] dye-stuffs,[2] herbicides,[3] orally active antagonists,[4] anti-inflammatory,[5] antiallergenic,[6] antitubercular,[7] and cardiovascular agents.[8] They have also attracted considerable interest for various pharmacological targets including glycine NMDA receptor antagonist and glycine (5-HT_3) site antagonists related to several central nervous system disorders including stroke, epilepsy, schizophrenia, Parkinson's disease, and Alzheimer's disease.[9–12] In addition 4-hydroxyquinolones have recently been found to serve as key intermediates in the synthesis of non-peptide GnRH (gonadotropin releasing hormones) receptor antagonists. Such compounds are of interest for the treatment of sex hormone related conditions.[13]

4-Hydroxyquinolines are important synthones and they are used as synthetic precursors of many naturally occurring alkaloids,[14] polycyclic condensed heterocycles,[15] multiazahetrocycles,[16] isoxazolo quinolines,[17] pyrano quinolines,[18] piperazinyl carbomides,[19] pyrazolo quinolines, oxazino quinolines,[20] etc. These derivatives are used as potent antiemetic, migraine suppressing, antibacterial, fungicidal,[21] antipyretic, anticoagulant,[22] CNS,[23] memory enhancing agents, antibrain tumor activity in vivo,[24] etc.

Fluorinated quinolones[25] are also extensively used in medicinal chemistry, notable clinical examples are worldwide patented drugs, for example, norfloxacin, ciprofloxacin, ofloxacin, enoxacin, pefloxacin, grepafloxacin, etc., as antibacterial,[26] antimicrobial, anticonvulsant, CNS depressing,[27] hypotensive agents,[19] and some references are patented as high oral absorbents and used for the treatment of autoimmune and rheumatic diseases [28] Further literature survey that presence of F or CF_3 substituent at various positions in the quinoline nucleus plays an important role in modifying the biological activities.[19,26e]

4-Hydroxyquinolin-2(1*H*)-one derivatives have been synthesized by cyclization of *N*-acetylanthranilic acid derivatives,[29] condensation of malonates/malonic acid with anilines using $ZnCl_2$ and $POCl_3$,[7,30] and cyclization of malanodianilides using $AlCl_3$,[31] PPA,[32]

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CH_3SO_3H/P_2O_5,[33] etc. Recently, they have been synthesized by the reaction of 2-aminoacetophenone and acylating reagent such as phosgene, dimethylcarbonate or diethylcarbonate in the presence of basic catalyst sodium ethoxide, sodium hydride, 3-aminopropylamide (NAPA), potassium 3-aminopropylamide (KAPA), and refluxing in anhydrous toluene.[34]

All these methods suffer from many disadvantages like long reaction period, use of strong acids (PPA, H_2SO_4, etc.)/bases (NaOEt, sodium hydride, potassium 3-aminopropylamide (KAPA), sodium 3-aminopropylamide (NAPA), etc.), dehydrating agents ($ZnCl_2$, $AlCl_3$), and hazardous solvents/reagents like toluene, $POCl_3$, CH_3SO_4H, and P_2O_5. Further, method involving use of diethyl malonate was found unsuitable for synthesis of 3-unsubstituted 4-hydroxyquinolone.[32]

The exploitation of microwaves for assisting different organic reactions[35] has blossomed into an important tool in synthetic organic chemistry with large horizon of applications due to the timelessness, ease of workability, and ecofriendliness, so microwave-assisted organic reactions are the best alternatives to an environmentally unacceptable procedure. However, recently more interest has been focused on dry-media synthesis and particularly on solvent-free procedure using various mineral oxides and solventless reactions with neat reactants[36] in the absence of any support or solvent.

Recently microwave-enhanced synthesis of some 3-substituted hydroxyquinolines has been studied.[37] Lange et al.[37a] reported the synthesis of 3-substituted 4-hydroxyquinoline under focused microwaves using substituted aniline with excess amount of substituted malonic ester under the stream of N_2 to remove the ethanol which is formed as byproduct. However, these microwave-mediated methods have disadvantages like (i) use of volatile solvents (diethyl ether, hexane, and acetone) in workup process, (ii) it was found unsuitable for the synthesis of trifluoromethyl substituted quinolones, reaction remaining at the intermediate malanodianilide stage. More interesting is that one can obtain excellent results even using inexpensive domestic microwave oven which is available elsewhere rather than to use systematically monomode oven.[35c,36a–36d,38]

Recently, 4-hydroxyquinolin-2(1*H*)-one derivatives were prepared in a new profitable way these compounds show strong photo-antiproliferative activity, higher than that of 8-methoxypsoralen (8-MOP), the most widely employed drug for photochemotherapy. Moreover, their activity is devoid of mutagenicity and skin phototoxicity.[39] For these features, fluorinated quinolinones and other analogues appeared to be very promising photochemotherapeutic agents. The great importance of this category of heterocycles and continuation to our earlier interest on environeconomic synthesis of biodynamic heterocycles under microwaves[40] oriented our attention to the synthesis of fluorinated 3-unsubstituted 4-hydroxyquinolin-2(1*H*)-one with F and CF_3 at various positions as new possible antitumoral, photochemically reactive, antimycobacterial or antifungal compounds.

Literature survey reveals that 3-unsubstituted 4-hydroxyquinolin-2-(1*H*)-one quinolines have been prepared conventionally by environmentally unacceptable procedure using hazardous reagents and volatile solvent, for example, $PPA/ZnCl_2 + POCl_3/P_2O_5$, etc.[30,32] and 3-unsubstituted fluoroquinolones are patented as pharmacological agents, the synthetic details of which are not available.[41,42]

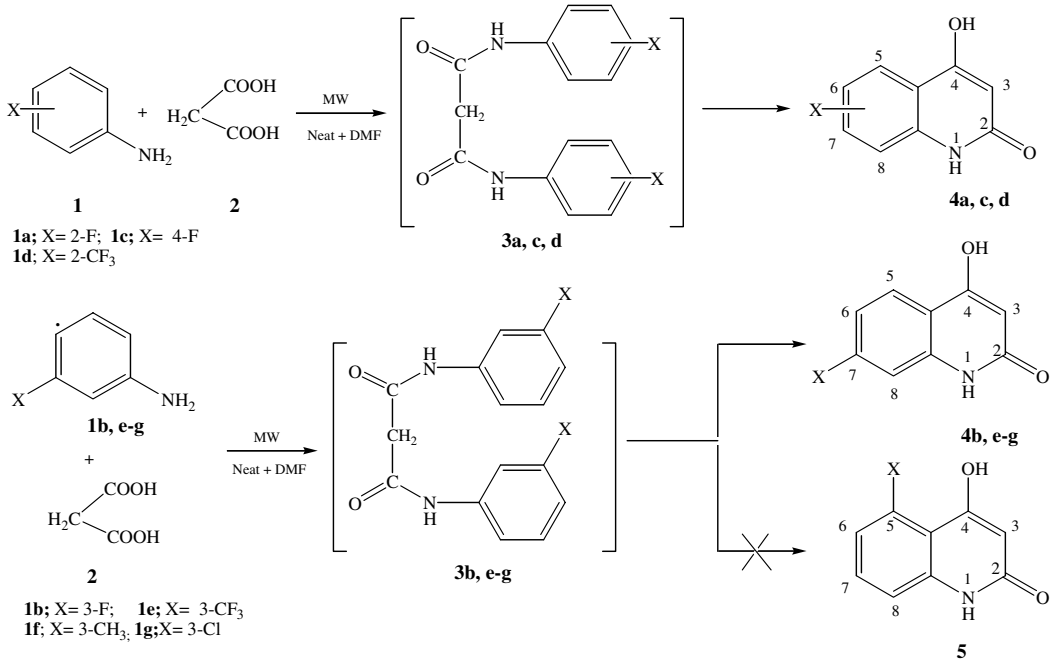
In view of these observations we have successfully synthesized fluorinated 4-hydroxyquinolin-2-(1*H*)-one (having F and CF_3 substitution at various positions) by condensation of fluorinated anilines (X = F, CF_3) with malonic acid in unexpensive multimode domestic microwave oven in reasonable purity (TLC) in 85–94% yield in 3–5 min using 4–5 drops of DMF, which act as energy transfer agent and homogenizer to increase the reaction temperature.[43] Nonreproducibility of results observed by some workers in domestic microwave oven can be overcome by previous cartography of energy distribution[38b] and performing the reactions under continuous microwave power emission without on–off cycles. The reactions carried out with similar weight of load always located in the same place as determined above in the oven are highly reproducible in present studies.

Hence, we have developed a facile, safe, and economic method for 3-unsubstituted 4-hydroxyquinolin-2-(1*H*)-one with no need of paraphernalia requiring the stream of N_2, since in present case instead of ethanol, water is formed as byproduct which is removed easily. Further workup procedure is simple involving the washing from water to give product in reasonable purity (TLC) with no need of further use of solvent for purification procedure Scheme 1).

Earlier report mentioned[32,37] that the yield of the reaction strongly depends on the relative amount of the starting material (malonates/malonic acid and anilines) used, prompting to study in detail this effect using different molar ratio of the malonic acid and anilines, and results are summarized in Table 1.

In all these cases it has been found that reactions proceed with higher yields with shorter reaction time when 1:1 molar ratio of malonic acid and anilines was used in open vessel. It is essential to work in an open vessel to enable the formed water to escape from reaction mixture, which is polar microwave active product, while the 1.5:1/2:1 molar ratio of starting materials gave lower yield with higher time which makes it unsuitable for synthesis of required fluoroquinolines. These optimal conditions were then applied for the synthesis of a range of 3-unsubstituted 4-hydroxyquinolones depicted in Table 2.

Further multigram synthesis (18–22 g) of representative quinolines (**4d**, **e**, **h**; X = 8-CF_3, 7-CF_3, H) is also carried out safely in open tall beaker (4 cm radius) in reasonable purity (TLC) in 10–15 min (Table 3) under microwaves.



Scheme 1.

Table 1. Comparative results obtained for the synthesis of **4d** and **4e** by different relative amounts of the starting material

Compound	X	Molar ratio (malonic acid:amine)	Temp[a] (°C)	Time (min)	Isolated yield (%)
4d	8-CF_3	1:1	145	5	85
4d	8-CF_3	1.5:1	142	6	80
4d	8-CF_3	2:1	141	7	78
4e	7-CF_3	1:1	142	4	88
4e	7-CF_3	1.5:1	138	7	81
4e	7-CF_3	2:1	138	8	84
4h	H	1:1	135	3	94
4h	H	1.5:1	133	6	80
4h	H	2:1	128	9	89

[a] Final temperature is measured at the end of microwave irradiation by introducing a glass thermometer in the reaction mixture in the beaker.

Table 2. Physical data of synthesized compounds **4a**–**g**

Compound	X	Reaction time				Molecular formula	Mp (°C)
		Classical method		Microwave method			
		(min)	yield[a] (%)	(min)	yield[a] (%)		
4a	8-F	600	75	3	90	$C_9H_6FNO_2$	310[33]
4b	7-F	480	67	4	89	$C_9H_6FNO_2$	318[34]
4c	6-F	960	78	3	85	$C_9H_6FNO_2$	245[34]
4d	8-CF_3	1080	62	5	85	$C_{10}H_6F_3NO_2$	295[34]
4e	7-CF_3	960	71	4	88	$C_{10}H_6F_3NO_2$	290
4f	7-CH_3	840	80	4	88	$C_{10}H_9NO_2$	305
4g	7-Cl	860	76	4	85	C_9H_6 $ClNO_2$	340
4h	H	560	81	3	94	$C_9H_7FNO_2$	360

[a] Isolated yield.

To study the range of this electrophilic attack of the amide toward aromatic ring, some meta-substituted anilines (**1b**, **e**–**g**) with fluorine, trifluoromethyl, methyl, and chlorine as the substituent were treated with malonic acid under microwaves to yield **4b**, **e**–**g** in one step. Although in these cases two isomers (**4** and **5**) could be expected, only one isomer was obtained (**4b**, **e**–**g**), the structure of which could be unequivocally assigned as 7-substituted 4-hydroxyquinolin-2-ones on the basis of PMR spectra.

Table 3. Multigram synthesis (50 g) of compounds **4d**, **4e**, and **4h**

Compound	X	Temp[a] (°C)	Time (min)	Isolated yield	
				(%)	(g)
4d	8-CF_3	138	10	81	18
4e	7-CF_3	135	13	86	20
4h	H	137	12	89	22

The condensation of fluorinated anilines with malonic acid afforded the title compound instead of the intermediate malanodianilides, which is confirmed by spectral studies.

IR spectra of the products (**4a**–**h**) displayed characteristic absorption in the region 3390–2980 (br, OH, NH, and CH stretching), 1670–1640 (s, NH–C=O), 1610–1600 (s, C=C), and 760–740 (C–F). The ^{1}H NMR spectra of **4a** (X = 8-F) showed singlet at δ 6.38 (s, 1H, H-3), 7.32–7.65 (m, 2H, H-6 and H-7), 7.81 (dd, J = 7.2 Hz, H-5), 10.21 (br, 1H, N*H*), and 11.94 (br, 1H, OH). Disappearance of CO (acidic) at 1740 cm^{-1} and primary amino group absorption at 3450–3330 cm^{-1} further confirmed the formation of **4**.

In case of meta-substituted anilines (**1b**, **e**–**g**) (X = F, CF_3, Cl, and CH_3) two isomers (**4** and **5**) could be expected, only one isomer was obtained showing ^{1}H NMR signals at δ 6.21–6.38 (s, 1H, H-3), 7.26–7.40 (d, J = 2 Hz, H-8), 7.35–7.60 (dd, J = 2 + 7 Hz, H-6), 7.65–7.92 (d, J = 7 Hz, H-5), 10.22–10.32 (br, 1H, N*H*), and 11.76–11.94 (br, 1H, OH). On the basis of ^{1}H NMR spectra it is unequivocally identified as 7-substituted quinoline derivatives (**4b**, **e**–**g**). ^{13}C NMR of **4** showed a sharp signal at δ 85.6–86.5 (*C*H), 119.2–138.5 (aromatic carbons), 164.6–165.3 (*C*=O), and 172.3–173.4 (*C*–OH). The presence of fluorine was confirmed by ^{19}F NMR spectra, where the C–F signal was observed at δ −118.05–120.42 (compounds **4a**–**c**) and CF_3 signal at δ −63.25/64.07 ppm in case of compounds **4d**–**e**. In the mass spectra of **4a** and **4b** molecular ion peaks were observed at 179 corresponding to their molecular weight along with base peak at 109 ($C_3H_2O_2$) and **4d**, **e** molecular ion peaks were observed at 229 corresponding to their molecular weight along with base peak at 43($C_9H_5OF_3$), other characteristic peaks observed are described in Table 4.

The phototoxicity of title compounds was investigated first on a cell line of human tumor HL-60 (human promyelocytic leukemia). Table 5 shows the extent of cell survival expressed as GI50, which is the concentration, expressed in mM, which induces 50% of inhibition of cell growth, after irradiation at different UVA doses.

Control experiments with UVA light or compounds alone were carried out without significant cytotoxic effects (data not shown). The results, shown in Table 5, indicate that compound **4a** is not active, instead **4e** we observe the highest activity. Interestingly, the isomeric compound **4c** is only slightly active and substitution for a methyl group leads to an inactive derivative **4b**. From this preliminary screening the most active compounds were also evaluated on a human intestinal adenocarcinoma cell line (LoVo) and one line of immortalized, not tumorigenic, human keratinocytes (NCTC 2544). From Table 6 it appears that the phototoxicity of the most active compounds, in particular **4e**, is higher in the tumor cell lines in comparison to the normal ones (NCTC 2544). In preliminary experiments devoted to the search for a possible molecular target, compound **4e** was evaluated for its potential capability to induce single strand breaks in a plasmid DNA, as a model.

The obtained results (data not shown) indicate that **4e**, after irradiation in the presence of DNA, is not able to induce any significant damage to DNA thus suggesting that another target at cellular level may be involved in its phototoxic effect. In parallel to the cytotoxic evaluation, flow cytometry was employed to study cell cycle variations upon irradiation. The effects of the most active compound **4e** were evaluated after 24, 48, and 72 h from irradiation in the leukemic cell line. The percentage of the cells in the different phases of the cell cycle is shown in Table 7.

It can be observed that treatment with **4e** in combination with UVA induces a reduction of the S phase at 48 and 72 h after irradiation especially for the highest dose utilized. This is accompanied by a concomitant block in G1 phase. This event is followed at 48 and 72 h after the irradiation by massive induction of apoptosis, as observed by the appearance of a sub G1 peak (apoptotic cells) that refers to cells with DNA content lower than G1.[44,45] In fact, apoptosis induces the activation of endogenous nucleases, which are responsible for nucleic acid degradation.

All compounds prepared were evaluated for their in vitro antimycobacterial activity. The highest activity (72% inhibition) against *Mycobacterium tuberculosis* was found for 7-trifluoromethyl4-hydroxyquinolin-2-(1*H*)-one (**4e**). Two other compounds, (**4d** and **4f**) with the identical substitution on the aromatic part of the molecule, exert a comparable activity. The majority of compounds exhibited only modest antimycobacterial activity (see Table 8).

The evaluation of in vitro antifungal activity of the synthesized compounds was performed against eight fungal strains. The results revealed no interesting activity against the majority of strains tested.

Table 4. Spectral data of synthesized compounds **4a-e**

Compound	IR (cm^{-1})	1H NMR (δ, ppm)			^{13}C NMR (δ, ppm)	^{19}F NMR (δ, PPM)	Mass *m/z* (%)
		CH	Ar-H	NH/OH[a]			
4a	3380–3020 (br), 1665 (s, C=O), 1605 (s), 740 (s, C–F)	6.25 (s, 1H)	7.32–7.65 (m, 2H, H-6 and H-7), 7.81 (dd, J = 7.28 Hz H-5)	10.21 (br, s, N*H*), 11.85 (br, s, O*H*)	85.6 (*C*H), 120.8–138.2 (aromatic carbons), 164.8 (*C*=O), 172.3 (*C*–OH)	−120.42 (s, 8-F)	179 ([M^+] 8.8), 160 (40.2), 151 (56), 123 (25), 109 (100), 43 (15)
4b	3390–2980 (br), 1640 (s, C=O), 1610 (s), 750 (s, C–F)	6.38 (s, 1H)	7.43 (d, J = 2 Hz, H-8), 7.56 (dd, J = 2 + 7 Hz, H-6), 8.19 (d, J = 7 Hz, H-5)	10.25 (br s, N*H*), 12.90 (br s, O*H*)	85.9 (*C*H), 119.2–135.2 (aromatic carbons), 164.9 (C=O), 172.8 (*C*–OH)	−119.20 (s, 7-F)	—
4c	3360–3090 (br), 1665 (s, C=O), 1605 (s), 760 (s, C–F)	6.35 (s, 1H)	7.34 (d, J = 8 Hz, H-8), 7.50 (dd, J = 2 + 8 Hz, H-7), 8.26 (d, J = 2 Hz, H-5)	10.21 (br s, N*H*), 11.74 (br s, O*H*)	86.2(*C*H), 121.2–137.2 (aromatic carbons), 165.3 (C=O), 173.4 (*C*–OH)	−118.05 (s, 6-F)	—
4d	3380–3090 (br), 1665 (s, C=O), 1602 (s), 750 (s, C–F)	6.50 (s, 1H)	7.38 (t, J = 7.5 Hz, H-6), 7.65–7.43 (m, 2H, H-5 and H-7)	10.28 (br s, N*H*), 10.94 (br s, O*H*)	86.4 (*C*H), 121.8–138.2 (aromatic carbons), 130.5 (*C*–CF_3), 165.8 (C=O), 173.4 (C–OH)	−64.02 (s, 8-CF_3)	229 ([M^+]12.5), 221 (4.5), 160(32.2), 120 (28.3), 105(27), 92 (54.3), 64 (23.1), 51 (48.5), 43 (100)
4e	3390–3010 (br), 1660 (s, C=O), 1605 (s), 760 (s, C–F)	6.21 (s, 1H)	7.26 (d, J = 2 Hz, H-8), 7.35 (dd, J = 2 + 7 Hz H-6), 7.65 (d, J = 7 Hz, H-5)	10.32(br s, N*H*), 11.76 (br s, O*H*)	86.3(*C*H), 120.3–135.7 (aromatic carbons), 131.2 (*C*–CF_3), 165.4 (C=O), 173.1 (C–OH)	−63.25 (s,7-CF_3)	229 ([M^+]11.8), 221 (5.6), 190 (13.8), 170 (5.6), 160 (30.5), 120 (28.3), 105 (23.6), 64 (25.3), 43 (100)
4f	320–2930 (br), 1645 (s, C=O), 1605 (w),	2.54 (s, 1H, CH_3), 6.2 (s, 1H)	7.19 (d, J = 2 Hz, H-8), 7.29 (dd, J = 2 + 7 Hz, H-6), 7.82 (d, J = 7 Hz, H-5)	10.32 (br s, N*H*), 11.76 (br s, O*H*)	28.3 (*C*H_3), 85.3 (*C*H), 121.3–134.8 (aromatic carbons), 164.8 (C=O), 172.6 (C–OH)	—	—
4g	3380–3020 (br), 1670 (s, C=O), 1605 (s), 780 (s, C–Cl)	6.32 (s, 1H)	7.12 (d, J = 2 Hz, H-8), 7.25 (dd, J = 2 + 7 Hz, H-6), 7.65 (d, J = 7 Hz, H-5)	11.32(br s, N*H*), 11.76 (br s, O*H*)	87.6 (*C*H), 119.6–132.6 (aromatic carbons), 164.9 (C=O), 172.9 (C–OH)	—	—
4h	3370–3030 (br), 1650 (s, C=O), 1600 (s)	6.33 (s, 1H)	7.32–7.76 (m, 3H), 8.26 (d, 1H, J = 8 Hz)	10.47 (br s, N*H*), 11.42 (br s, O*H*)	85.3 (*C*H), 118.6–138.4 (aromatic carbons), 165.4(C=O), 178.6 (C–OH)	—	161 (M^+), 133 (200), 105 (45), 91 (56), 43 (100)

[a] Exchangeable with deuterium.

Table 5. Photocytotoxicity of test compounds against HL-60 cell line compounds GI50 (mM)[a]

Compound	GI_{50} (μm)[a]	
	1.25[b] J cm^{-2}	2.5 J cm^{-2}
4a	>10	10
4b	>10	10
4c	>10	7.8 ± 2.2
4d	>10	7.3 ± 1.9
4e	6.2 ± 0.7	0.9 ± 0.1
4f	>10	10

[a] Concentration of compound required to inhibit the cell growth by 50% after 72 h of exposure as determined by MTT assay.
[b] UVA dose expressed in J cm^{-2} as measured at 365 nm with a Cole–Parmer radiometer.

Table 6. Photocytotoxicity of test compounds against NCTC 2544 and LoVo cell lines[a]

Compound	Cell line GI_{50} (μm)[b]			
	NCTC 2544		LoVo	
	1.25[c] J cm^{-2}	2.5 J cm^{-2}	1.25[b] J cm^{-2}	2.5 J cm^{-2}
4e	7.0 ± 1.3	2.5 ± 0.7	3.7 ± 0.8	1.8 ± 0.2
4c	>20	>20	>20	12.1 ± 1.9
4d	>20	7.8 ± 2.2	18.2 ± 2.1	10.1 ± 1.3

[a] Human cell lines: NCTC 2544 Human keratinocytes; LoVo intestinal adenocarcinoma.
[b] Concentration of compound required to inhibit the cell growth by 50% after 72 h of exposure as determined by MTT assay.
[c] UVA dose expressed in J cm^{-1} K^{-2} as measured at 365 nm with a Cole–Parmer radiometer.

Table 7. Percentage of HL-60 in the different phases of the cell cycle[a]

Treatment	G1	G2	S	Apoptotic cells[b]
Nonirradiated cells	39.0	9.0	51.7	0.8
UVA irradiated cells without drug (2.5 J cm^{-2})				
24 h	36.7	13.0	50.3	8.6
48 h	48.2	10.9	40.9	11.7
72 h	35.0	10.5	54.4	9.4
4c 2.5 mM CUVA (2.5 J cm^{-2})				
24 h	30.8	10.8	58.4	13.2
48 h	44.7	10.3	45.0	27.0
72 h	44.2	12.0	43.8	40.5
4c 5.0 mM CUVA (2.5 J cm^{-2})				
24 h	34.6	11.1	54.3	21.0
48 h	49.6	11.7	38.6	26.0
72 h	56.0	6.9	37.1	46.0

[a] The percentage of each phase of the cell cycle (G1, S, and G2/M) were calculated on living cells.
[b] The percentage of apoptotic cells is referred to cells population characterized by the appearance of a sub-G1 peak.

Only the 8-fluoromethyl4-hydroxyquinolin-2-(1*H*)-one (**4a**) and especially 7-fluoromethyl4-hydroxyquinolin-2-(1*H*)-one (**4b**) showed some promising in vitro antifungal activity against *Trichophyton mentagrophytes*, the most susceptible fungal strain evaluated (MIC = 31.25–62.5 μmol mL^{-1}), although this activity is only modest in comparison with fluconazole, the standard (MIC = 3.91 μmol mL^{-1} after 120 h, see Table 8). The negative antifungal screening results do not allow us to draw detailed conclusions on potential structure–activity relationships.

Table 8. Calculated antimycobaterial evaluation (% of inhibition) and antifungal susceptibility (MIC) comparison with standards: pyrazinamide (PZA) and fluconazole

Compound	X	% inhibition at 6.25 μg mL^{-1}	MIC (μmol mL^{-1})
4a	8-F	50	31.25/62.5
4b	7-F	52	31.25/31.25
4c	6-F	35	125/250
4d	8-CF_3	69	>500/>500
4e	7-CF_3	72	125/125
4f	7-CH_3	54	>500/>500
4g	7-Cl	2	>500/>500
4h	H	0	>500/>500
PZA	—	100[a]	—
Fluconazole	—	—	1.95/3.91

[a] MIC = 12.5 μg mL^{-1}, data from Ref. 46.

In summary, the synthesis and biological evaluation of eight new substituted 4-hydroxyquinolin-2-(1 H)-one are described. In the first series, among the compounds with substituted fluoro, the highest antifungal effect was found for 7-fluoromethyl4-hydroxyquinolin-2-(1*H*)-one (**4b**). In the second series, among the compounds bearing trifluoromethyl (**4d–f**) have shown the highest biological activity, that is, against *M. tuberculosis* H37Rv.

In conclusion, we have presented a new economical, safe, environmentally benign solvent free synthesis of fluorinated 4-hydroxyquinolin-2(1*H*)-one under microwave irradiation. The operational simplicity, avoiding the use of solvent and solid support, and high yield in significantly very short reaction time, can impose this procedure as a useful and attractive alternative to the currently available methods.

On the basis of the biological evaluation, compound **4e** seems to be very attractive as a potential drug for photochemotherapy and antimicrobial agents, while, among the compounds bearing trifluoromethyl (**4d–f**) have shown the highest antituberculosis activity, that is, against *M. tuberculosis* H37Rv. Hence, experiments aimed at defining the target(s) at cellular level and the phototoxicity mechanism are in progress.

Human promyelocytic leukemia cells (HL-60) were grown in RPMI-1640 medium (Sigma Co., MO, USA), human keratinocytes (NCTC 2544) were grown in DMEM (Sigma Co., MO, USA), and intestinal adenocarcinoma cells (LoVo) were grown in Ham's F12 medium (Sigma Co., MO, USA) all supplemented with 115 U mL^{-1} of penicillin G (Invitrogen, Milano, Italy), 115 μg mL^{-1} streptomycin (Invitrogen, Milano Italy) and 10% fetal bovine serum (Invitrogen, Milano, Italy). Individual wells of a 96-well tissue culture microtiter plate (Falcon BD) were inoculated with 100 mL of complete medium containing 8×10^3 HL-60 cells or 5×10^3 NCTC 2544 and LoVo cells. The

plates were incubated at 37 °C in a humidified 5% incubator for 18 h prior to the experiments. After medium removal, 100 mL of the drug solution, dissolved in DMSO and diluted with Hanks' balanced salt solution (HBSS, pH 7.2), was added to each well and incubated at 37 °C for 30 min and then irradiated.

After irradiation, the solution was replaced with the medium, and the plates were incubated for 72 h. Cell viability was assayed by the MTT [(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)] test, as described previously.[47,48]

For flow-cytometric analysis of DNA content, 5×105 HL-60 cells in exponential growth were treated at different concentrations of the test compounds for 24, 48, and 72 h. After the incubation period, the cells were centrifuged and fixed with ice-cold ethanol (70%), treated with lysis buffer containing RNAseA, and then stained with propidium iodide. Samples were analyzed on a Becton Coulter Epics XL-MCL flow cytometer. For cell cycle analysis, DNA histograms were analyzed using Multi Cycle for Windows (Phoenix Flow Systems, San Diego, CA).

The broth microdilution test[49] was used for the assessment of in vitro antifungal activity of the synthesized compounds against *Candida albicans* ATCC 44859 (CA), *Candida tropicalis* 156 (CT), *Candida krusei* E28 (CK), *Candida glabrata* 20/I (CG), *Trichosporon beigelii* 1188 (TB), *Aspergillus fumigatus* 231 (AF), *Absidia corymbifera* 272 (AC), and *Trichophyton mentagrophytes* 445 (TM). Fluconazole was used as a reference drug. The procedure was performed with twofold dilution of the compounds in RPMI 1640 medium (Sevapharma) buffered to pH 7.0 with 0.165 mol of 3-morpholinopropane-1-sulfonic acid. The final concentrations of the compounds ranged from 500 to 0.975 μmol L^{-1}. Drug-free controls were included. The minimal inhibitory concentrations (MICs) were determined after 24 and 48 h of static incubation at 35 °C. With *T. mentagrophytes*, the final MICs were determined after 72 and 120 h of incubation. The results of all compounds in vitro tested against *T. mentagrophytes*, the most susceptible fungal strain, are summarized in Table 8.

Antimycobacterial evaluation was carried out at the Tuberculosis Antimicrobial Acquisition and Coordinating Facility (TAACF), Southern Research Institute, Birmingham, AL, USA, which is a part of the National Institutes of Health (NIH). Primary screening of all compounds was conducted at 6.25 μg mL^{-1} against *M. tuberculosis* strain H37Rv in BACTEC 12B medium using the BACTEC 460 radiometric system.[50,51] The results are presented in Table 8. The synthesis,[52] structural determination[53] and photo-antiproliferative[54] activity described detail in reference and notes section.

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52. Fluorinated 4-hydroxyquinolin-2-ones were synthesized by reaction of substituted anilines (0.01 mol) of (**1**) and malonic acid (**2**) with 4–5 drops of DMF, in an open vessel loosely covered with funnel inside microwave oven for an appropriate time (monitored by TLC), to give an oily product, which was solidified on standing, washed with water, and found to be pure by (TLC), with no need of further purification process.
53. IR spectra (KBr) were recorded on a Magna FT IR–550 spectrophotometer, ^{1}H and ^{19}F and ^{13}C NMR spectra [$CDCl_3$ + $(CD_3)_2SO$] were taken on a Bruker −300DX spectrometer at 300, 84.25, and 200 MHz, respectively, using TMS as an internal standard for PMR and hexafluorobenzene as external standard for ^{19}F NMR and mass spectra were recorded on Jeol D-300 spectrometer at an ionization potential of 70 eV. Microwave-assisted reactions were carried out on a BPL BMO model, operating at 700 W, generating 2450 MHz frequency. All anilines were purchased from Aldrich Chemical Co., and were used as received.
54. For photoirradiation two HPW 125 Philips lamps, mainly emitting at 365 nm, were used. The spectral irradiance of the source was 4.0 mW cm K2 as measured,at the sample level, by a Cole–Parmer Instrument Company radiometer (Niles, IL), equipped with a 365-CX sensor.