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Disparate SAR Data of Griseofulvin Analogues for the Dermatophytes Trichophyton mentagrophytes, T. rubrum, and MDA-MB-231 Cancer Cells

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*Supporting Information

ABSTRACT: Griseofulvin and 53 analogues of this compound have been tested against the pathogenic dermatophytes Trichophyton rubrum and Trichophyton mentagrophytes as well as against the breast cancer cell line MDA-MB-231. The modifications to griseofulvin include the 4, 5, 6, 2′, 3′, and 4′ positions. The SAR of the griseofulvin analogues toward the two fungi followed the same trend with the majority being less active than griseofulvin and none had more than twice the potency of the parent compound. A comparison of the antifungal and the anticancer SAR revealed distinct differences, as the majority of analogues showed increased activity against the cancer cell line MDA-MB-231, highlighted by 2′-benzyloxy-2′-demethoxy-griseofulvin, which showed low activity against both fungi but was among the most potent compounds against MDA-MB-231 cancer cells. Tubulin has been proposed as the target of griseofulvin in both fungal and mammalian cells, but the differences revealed by this SAR study strongly suggest that the mode-of-action of the compound class toward fungi and mammalian cancer cells is different.

INTRODUCTION

Griseofulvin (1, see Figure 1) was one of the first antifungal natural products isolated from filamentous fungi and has been known as an antifungal agent for decades.2,3 The compound was, until the approval of Terbinafine4 by the US Food and Drug Administration5 in 2007, the only drug available for treatment of tinea capitis,6 a superficial fungal skin infection caused by dermatophytes, which predominantly affects children.7 Upon administration to man, griseofulvin accumulates in the skin (stratum corneum), where it presumably binds to keratin.8 The mode of action is still not determined, but tubulin binding has been proposed.9,10

More than 400 griseofulvin analogues have been reported since its discovery, and the activity of over 300 of these have been compared with griseofulvin (1) against six dermatophytes in a study by Crosse et al.11 The curling of hyphae in Botrytis allii were also tested, but this phenotype did not correlate with the growth inhibitory effect of the analogues.11 Griseofulvin analogues with modifications at positions 4, 6, 2′, and 3′ as well as isogriseofulvin analogues with modifications at the 4, 6, 3′, and 4′ positions were tested. No 2′ analogues excelled in growth inhibition of the dermatophytes,11 but elongation did increase the curling of hyphae with the optimal analogues being 2′-propoxy (46) and 2′-butoxy analogues.12 Most griseofulvin analogues tested showed increased potency against some dermatophytes but exhibiting lower activity against others.

Position 5 has been functionalized with nitro (9) and amine (10) groups, rendering the analogues inactive at relevant concentrations against four dermatophytes.13 An ethoxycarbonyl group has also been introduced in this position and the analogue found to be inactive.14 The 4′ position has also been...
examined with the 4′ alcohol analogue (50) being inactive and the 4′ oxime (36) being 7-fold less potent compared to 1, with both analogues tested against Microsporum gypseum.15

Although the initial isolation of 1 was completed in 1939,1 both anticancer16–19 and antiviral20 properties of griseofulvin have been discovered recently. Three analogues tested for the former by Oda et al.21 against Chinese hamster V79 cells showed increased cytotoxicity, with 2′-propoxy-2′-demethoxy-griseofulvin being the most potent (46, IC50 0.7 μM; 1, 8 μM), and it was proposed that additional structural modifications at the 2′ position could enhance activity further. This was supported by ourselves in a whole-cell phenotype-based anticancer assay for spindle multipolarity induction, where increased activity was seen for analogues with modifications in the 2′ position, with the 2′-benzoyl-2′-demethoxy-griseofulvin analogue (17) being the most potent compound tested.22 Multiple papers state that griseofulvin arrests several cancer cell lines in G2/M phase of the cell cycle.17–19 Several investigators have proposed tubulin as the main target for griseofulvin, although for mammalian cells this suggestion is not undisputed.9,17,18,20,21,23,24 Recently, Panda et al.25 proposed two griseofulvin binding sites on tubulin using molecular docking studies and similar to Oda21 reported that microtubule dynamics were disrupted by 1. Using the hepatitis C virus-1b cell culture system HuH7/Rep-Feo, Jin et al. reported that G2/M phase arrest in infected cells was induced by griseofulvin (1).20 It was speculated that the effect was due to interaction with microtubule polymerization.20

Griseofulvin exhibits activity against fungi and mammalian cancer cells as well as suppressing RNA replication by the hepatitis C virus, with tubulin having been proposed to be involved in all three cases. Tubulins are very conserved within different eukaryotic cell types,26 and most of the variation among different tubulin isoforms is found in the amino acids near the C-terminus, which form a ridge on the surface of microtubules. Therefore, variations among different isoforms are expected to affect primarily the association of accessory proteins with the surface of microtubules rather than microtubule polymerization per se. In case tubulin is the sole target of griseofulvin in both fungi and mammalian cells, the activity profile of an array of analogues against these cell types should be similar. To test this hypothesis, we decided to carry out an SAR study of griseofulvin analogues. This is, to the best of our knowledge, the first study of griseofulvin (1) and analogues thereof which compares antifungal and anticancer SAR from growth inhibition assays.

Griseofulvin (1) and 54 griseofulvin analogues (11 reported for the first time here) have been tested in an antifungal assay against Trichophyton mentagrophytes and Trichophyton rubrum, two dermatophytes causing tinea capitis.7 All compounds were also tested in an anticancer assay against the human cell line MDA-MB-231 (breast adenocarcinoma), which was chosen because this cell line represents a common cancer type and is known to harbor supernumerary centrosomes which are regularly clustered into a bipolar mitotic spindle array in a high percentage of cells.26 All analogues, having alterations at the 4, 5, 6, 2′, 3′, and 4′ positions, have been synthesized from commercially available griseofulvin in one to five synthetic steps, as described here or following published procedures.

**RESULTS AND DISCUSSION**

**Chemistry.** The 4-phenol (4) was synthesized from 1 by treatment with freshly prepared MgI2 procured by sonication of Mg and I2 in Et2O/toluene, affording 4 in 99% yield, an improvement on prior methods (Scheme 1).22,27 Alkylation of 4 to synthesize 5 and 6 has previously been described.22,27

**Scheme 1**

```
\[(\text{a) MgI}_2, \text{toluene, Et}_2\text{O}) \quad (\text{b) RBr, Ag}_2\text{O, dioxane}) \quad (\text{c) HOOAc, 2 M H}_2\text{SO}_4 \text{M}) \quad (\text{d) 0.66 M NaOH}) \quad (\text{e) MeOH, CSA}) \quad (\text{f) MeOH, CSA (13)})\]
```

Position 4 analogues 7 and 8 were prepared from 4 with Ag2O and the appropriate alkyl bromide in dioxane as solvent.27 The syntheses of 9 and 10 have already been described.22,28

For the preparation of 11, 12, and 13, see Rønnest et al.29 and Arkley et al.27 Compound 12 was alkylated using Ag2O and EtBr followed by repeated solvolysis in MeOH with camphor sulfonic acid (CSA) to afford 14. The dichloro analogues 15 and 16 were synthesized using 3,30 POCl3, LiCl, and dioxane, a modification of a known method (Scheme 2).31

**Scheme 2**

```
\[(\text{a) LiCl, POCl}_3, \text{dioxane})\]
```

Compounds 17–33 were synthesized by 1,4-addition of the corresponding alcohol to 16 using either NaH or 1,8-diazabicyclo-[5.4.0]undec-7-ene (DBU) as base (Scheme 3).32 The iso-griseofulvin analogues 3 and 35 were synthesized in the same manner but from 15. Compound 3 has previously been prepared by treating 2 with excess diazomethane, yielding both 1 and 3.32 Compounds 26 and 38 have been reported in a patent,33 which is also the case for 19, 21, and 22.34 The dimer 34, which was conveniently synthesized together with 22 by a second 1,4-addition of 22 to 16, has previously been published in a Japanese patent.35

The analogues with an oxime functionality 36, 37, and 38 were synthesized from the corresponding ketones (1, 17, and 26) using hydroxylamine hydrochloride in ethanol and DMSO.32,22 Iso-griseofulvin (39–44) and griseofulvin analogues (45–49 and 17) were synthesized simultaneously in pairs by solvolysis.
with the respective alcohols and catalytic CSA and subsequently separated by chromatography (Scheme 4).

**Scheme 4**

![Scheme 4](image)

*Note: Substituent R at various positions in the griseofulvin framework, indicating the synthesis and activity of analogues.*

Analogues 50–55 were synthesized according to published procedures (Figure 2). The fungal secondary metabolite geodin (56) was recently isolated from Aspergillus terreus. Please see the Supporting Information for experimental details and spectral data.

**Assays.** Griseofulvin (1), geodin (56), and 53 griseofulvin analogues covering variations on six positions (see Figure 1) were tested against two dermatophytes (T. rubrum and T. mentagrophytes) and against the breast cancer cell line MDA-MB-231 in a cytotoxicity assay. For all the test results the following definitions were used: if no activity was observed at 50 μM, a given compound was deemed inactive; if activity was observed but 50% inhibition was not reached at 50 μM, a given compound is described as having low activity and no IC₅₀ value is calculated (see Supporting Information for examples). The IC₅₀ of griseofulvin (1) was determined to be 0.38 ± 0.068 μM against the T. rubrum isolate and 0.058 ± 0.0018 μM against the T. mentagrophytes isolate. All IC₅₀ values and 95% confidence intervals are given in Table 1. All dilution series were made starting with 50 μM of the tested compound in order to ensure that all compounds were soluble in the assay media, which for some compounds were not the case at higher concentrations.

**Antifungal SAR for T. rubrum and T. mentagrophytes.**

All compounds in the position 4 series of 4–8 were less active than griseofulvin (1) against the two fungi and 8 was completely inactive. For T. rubrum, 4 and 6 were inactive, 7 had low activity, and 5 was, with an IC₅₀ of 2.0 μM, approximately five times less potent than griseofulvin. For T. mentagrophytes, analogue 4 showed low activity and the rest were less potent than 1: 5 (0.29 μM), 6 (0.25 μM), and 7 (0.17 μM). The activity of 6 has previously been reported against a number of dermatophytes (Epidermophyton floccosum, Microsporum canis, Trichophyton interdigitale, Trichophyton persicolor, T. mentagrophytes, and T. rubrum) and found to be less active than 1 against all of them.

The nitro (9) and amine (10) position 5 analogues were inactive against both fungi. Compound 10 has also been reported as inactive, but 9 has been reported as weakly active against both fungi starting at around 25 μM. Other position 5 analogues (ethoxycarbonyl, methoxy, methyl, or chloro) have been reported to have lower activity than 1 against dermatophytes.

Whereas the position 6 phenols 13 and 11 were inactive against both fungi, the 6-ethyl analogue (14) had an IC₅₀ of 1.3 μM against T. rubrum and was as active as griseofulvin (1) against T. mentagrophytes with an IC₅₀ of 0.062 μM, which is in accordance with the literature.

The position 2 series is the most extensively studied due to the increased activity observed in the anticancer phenotype-based assay by these analogues. For T. rubrum, elongation to 2′-ethoxy (45, 0.69 μM) and 2′-propoxy (46, 0.62 μM) lowered the activity 2-fold, confirming the findings of Crosse et al. The bulkier 2′-isopropoxy (47, 3.1 μM) was 10 times less potent. Increasing bulkiness through 2′-cyclopentylmethoxy (48, 1.5 μM), cyclopentylxoy (49, 2.5 μM), and benzoxyl (17, 1.1 μM) led to the same trend, but all three were less active toward T. rubrum than 1. No correlation between the size of the 2′ substituent and the activity was seen for T. mentagrophytes with 46 (0.050 μM) retaining the activity of 1 and IC₅₀ values of 0.25 μM and 0.11 μM for 45 and 47, respectively. Both 45 and 46 have been reported to retain the activity of 1 against T. mentagrophytes by Crosse et al.
E. floccosum against given compound is described as having low activity and the IC50 value is not calculated. The three compounds were also tested toward T. rubrum and T. mentagrophytes, which has no 3-iodo group. The propoxy (S4) analogue showed activity against both fungi (T. rubrum 1.6 μM and T. mentagrophytes 0.23 μM) but was less potent than both 1 and the analogue 46, which does not contain iodide. Analogue 54 has been reported to be the most potent at inducing curling of hyphae but less active against all dermatophytes spare one.11

Table 1. All Available IC50 Values from the Screena

<table>
<thead>
<tr>
<th>Cmp. no.</th>
<th>T. rubrum (μM) ± STD</th>
<th>T. mentagrophytes (μM) ± STD</th>
<th>cytotox (μM) ± STD</th>
<th>Cmp. no.</th>
<th>T. rubrum (μM) ± STD</th>
<th>T. mentagrophytes (μM) ± STD</th>
<th>cytotox (μM) ± STD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.38 ± 0.05</td>
<td>0.058 ± 0.02</td>
<td>18 ± 4</td>
<td>30</td>
<td>low activity</td>
<td>low activity</td>
<td>19 ± 2</td>
</tr>
<tr>
<td>2</td>
<td>inactive</td>
<td>inactive</td>
<td>low activity</td>
<td>31</td>
<td>low activity</td>
<td>0.70 ± 0.6</td>
<td>11 ± 3</td>
</tr>
<tr>
<td>3</td>
<td>inactive</td>
<td>inactive</td>
<td>inactive</td>
<td>32</td>
<td>0.96 ± 0.4</td>
<td>0.14 ± 0.07</td>
<td>8.1 ± 2</td>
</tr>
<tr>
<td>4</td>
<td>inactive</td>
<td>inactive</td>
<td>inactive</td>
<td>33</td>
<td>inactive</td>
<td>inactive</td>
<td>low activity</td>
</tr>
<tr>
<td>5</td>
<td>2.0 ± 0.7</td>
<td>0.29 ± 0.1</td>
<td>inactive</td>
<td>34</td>
<td>inactive</td>
<td>inactive</td>
<td>8.5 ± 2</td>
</tr>
<tr>
<td>6</td>
<td>inactive</td>
<td>inactive</td>
<td>inactive</td>
<td>35</td>
<td>inactive</td>
<td>low activity</td>
<td>25 ± 5</td>
</tr>
<tr>
<td>7</td>
<td>low activity</td>
<td>0.17 ± 0.02</td>
<td>low activity</td>
<td>36</td>
<td>1.3 ± 0.5</td>
<td>0.19 ± 0.1</td>
<td>12 ± 2</td>
</tr>
<tr>
<td>8</td>
<td>inactive</td>
<td>inactive</td>
<td>inactive</td>
<td>37</td>
<td>7.9 ± 0.9</td>
<td>0.30 ± 0.2</td>
<td>1.4 ± 0.3</td>
</tr>
<tr>
<td>9</td>
<td>inactive</td>
<td>inactive</td>
<td>inactive</td>
<td>38</td>
<td>inactive</td>
<td>inactive</td>
<td>8.3 ± 0.8</td>
</tr>
<tr>
<td>10</td>
<td>inactive</td>
<td>inactive</td>
<td>inactive</td>
<td>39</td>
<td>inactive</td>
<td>inactive</td>
<td>low activity</td>
</tr>
<tr>
<td>11</td>
<td>inactive</td>
<td>inactive</td>
<td>inactive</td>
<td>40</td>
<td>inactive</td>
<td>low activity</td>
<td>43 ± 6</td>
</tr>
<tr>
<td>12</td>
<td>inactive</td>
<td>inactive</td>
<td>inactive</td>
<td>41</td>
<td>inactive</td>
<td>low activity</td>
<td>48 ± 10</td>
</tr>
<tr>
<td>13</td>
<td>inactive</td>
<td>inactive</td>
<td>inactive</td>
<td>42</td>
<td>inactive</td>
<td>low activity</td>
<td>52 ± 10</td>
</tr>
<tr>
<td>14</td>
<td>1.3 ± 0.1</td>
<td>0.062 ± 0.02</td>
<td>36 ± 3</td>
<td>43</td>
<td>inactive</td>
<td>low activity</td>
<td>27 ± 4</td>
</tr>
<tr>
<td>15</td>
<td>inactive</td>
<td>inactive</td>
<td>inactive</td>
<td>44</td>
<td>inactive</td>
<td>low activity</td>
<td>13 ± 3</td>
</tr>
<tr>
<td>16</td>
<td>low activity</td>
<td>3.2 ± 0.3</td>
<td>low activity</td>
<td>45</td>
<td>0.69 ± 0.2</td>
<td>0.25 ± 0.08</td>
<td>13 ± 3</td>
</tr>
<tr>
<td>17</td>
<td>1.1 ± 0.2</td>
<td>0.26 ± 0.1</td>
<td>2.1 ± 0.2</td>
<td>46</td>
<td>0.62 ± 0.09</td>
<td>0.050 ± 0.02</td>
<td>16 ± 5</td>
</tr>
<tr>
<td>18</td>
<td>1.3 ± 0.4</td>
<td>0.058 ± 0.02</td>
<td>4.7 ± 0.7</td>
<td>47</td>
<td>3.1 ± 1.0</td>
<td>0.11 ± 0.05</td>
<td>11 ± 3</td>
</tr>
<tr>
<td>19</td>
<td>0.83 ± very wide</td>
<td>0.041 ± 0.01</td>
<td>6.5 ± 1</td>
<td>48</td>
<td>1.5 ± 0.3</td>
<td>0.060 ± 0.02</td>
<td>9.4 ± 2</td>
</tr>
<tr>
<td>20</td>
<td>1.0 ± 0.2</td>
<td>0.24 ± 0.1</td>
<td>1.8 ± 0.3</td>
<td>49</td>
<td>2.5 ± 0.8</td>
<td>0.24 ± 0.09</td>
<td>3.2 ± 0.5</td>
</tr>
<tr>
<td>21</td>
<td>1.5 ± 0.2</td>
<td>0.15 ± 0.1</td>
<td>7.0 ± 0.9</td>
<td>50</td>
<td>low activity</td>
<td>inactive</td>
<td>inactive</td>
</tr>
<tr>
<td>22</td>
<td>low activity</td>
<td>1.7 ± 1</td>
<td>2.8 ± 0.6</td>
<td>51</td>
<td>low activity</td>
<td>3.0 ± 1</td>
<td>inactive</td>
</tr>
<tr>
<td>23</td>
<td>6.1 ± 7</td>
<td>1.6 ± 3</td>
<td>7.2 ± 1</td>
<td>52</td>
<td>inactive</td>
<td>inactive</td>
<td>inactive</td>
</tr>
<tr>
<td>24</td>
<td>low activity</td>
<td>0.28 ± 0.2</td>
<td>17 ± 2</td>
<td>53</td>
<td>inactive</td>
<td>inactive</td>
<td>inactive</td>
</tr>
<tr>
<td>25</td>
<td>inactive</td>
<td>inactive</td>
<td>inactive</td>
<td>54</td>
<td>1.6 ± 0.4</td>
<td>0.23 ± 0.08</td>
<td>8.0 ± 1</td>
</tr>
<tr>
<td>26</td>
<td>2.6 ± 0.3</td>
<td>0.25 ± 0.09</td>
<td>13 ± 2</td>
<td>55</td>
<td>inactive</td>
<td>1.1 ± 0.5</td>
<td>low activity</td>
</tr>
<tr>
<td>27</td>
<td>inactive</td>
<td>inactive</td>
<td>inactive</td>
<td>56</td>
<td>inactive</td>
<td>inactive</td>
<td>9.9 ± 1</td>
</tr>
<tr>
<td>28</td>
<td>low activity</td>
<td>1.3 ± 3</td>
<td>32 ± 6</td>
<td>29</td>
<td>low activity</td>
<td>0.35 ± 0.2</td>
<td>10 ± 2</td>
</tr>
</tbody>
</table>

*aIf no activity was observed at 50 μM, a given compound is deemed inactive. If activity was observed, but 50% inhibition was not reached at 50 μM, a given compound is described as having low activity and the IC50 value is not calculated.

Analogue 48 (0.060 μM) had the same activity as 1, but 17 (0.26 μM) and 49 (0.24 μM) were about four times less potent. The activities of 17, 45, and 46 have been reported against T. mentagrophytes and T. rubrum, and all showed lower activity when tested against the latter.11 Lower activity than 1 toward T. mentagrophytes was also reported for 17, but 45 and 46 had similar potency.11 The three compounds were also tested against E. floccosum, M. canis, T. interdigitale, and T. persicolor displaying the same trends, with 17 having lower activity and 45 and 46 retaining the activity of 1.11

The rest of the 2′ series includes bulkier analogues (25–27), para-substituted variations of 17 (19–22), two sets of ortho-, meta-, and para-analogues (28–30 and 31–33), modification of the linker part (23 and 24), and a vinyl sulfide analogue 18. Of the bulkier 2′ analogues, the naphthalen-1-ylmethoxy 26 was the only active analogue and 25 and 27 were inactive against both fungi. With IC50 values of 2.6 μM (T. rubrum) and 0.25 μM (T. mentagrophytes), compound 26 is three and four times less potent than 1, respectively.

The three 2′ pyridine analogues (28–30) had low activity toward T. rubrum, and 30 also showed low activity toward T. mentagrophytes. Compounds 28 and 29 had IC50 values of 1.3 and 0.35 μM, meaning they were approximately 22 and 6 times less potent toward T. rubrum compared to 1, respectively. The series with ortho-, meta-, and para-methoxy groups revealed 33 as inactive against both fungi, 31 with low activity toward T. rubrum and an IC50 of 0.70 μM against T. mentagrophytes. The meta-substituted analogue 32 (0.96 μM) was 3-fold less active against T. rubrum compared to 1 and displayed a 2-fold decrease in activity against T. mentagrophytes with an IC50 of 0.14 μM.

The 2′-phenoxy analogue (23) had IC50 values of 6.1 μM against T. rubrum and 1.6 μM against T. mentagrophytes, while the phenylethoxy analogue (24) had low activity against T. rubrum and an IC50 of 0.28 μM against T. mentagrophytes. Lower activity for 23 has previously been reported.11 The vinyl sulfide analogue 18 (1.3 μM) was as active as 17 against T. rubrum, but against T. mentagrophytes the IC50 (0.088 μM) was equal to that of griseofulvin (1).

The 3′-dimethyl analogue 52 was inactive against both fungi. The series of 3′ analogues include three 3′-iodo analogues with 2′ modifications, 2′-methoxy (53), 2′-propoxy (54), and 2′-benzoxyl (55). The 3′-iodo griseofulvin (53) was inactive against T. rubrum and had low activity toward T. mentagrophytes in accordance with Crousse et al.11 The 2′-benzoxyl (55) analogue was inactive against T. rubrum and close to 20 times less potent (1.1 μM) than 1 against T. mentagrophytes. Analogue 55 was also four times less potent than 17, which has no 3′ iodo group. The propoxy (54) analogue showed activity against both fungi (T. rubrum 1.6 μM and T. mentagrophytes 0.23 μM) but was less potent than both 1 and the analogue 46, which does not contain iodide. Analogue 54 has been reported to be the most potent at inducing curling of hyphae but less active against all dermatophytes spare one.11
The introduction of an oxime functionality on the parent compounds 1, 17, and 26 afforded the analogues 36, 37, and 38 and lowered the activity toward both fungi compared to the parent ketones (38 was inactive against both fungi altogether). The IC_{50} values for 36 were 1.3 \mu M against T. rubrum and 0.19 \mu M against T. mentagrophytes, which is in accordance with previously published data.\textsuperscript{13} Analogue 36 has also been reported active against M. gypseum.\textsuperscript{15} Compound 37 had IC_{50} values of 7.9 \mu M against T. rubrum and of 0.30 \mu M against T. mentagrophytes, 20 and 3 times less potent than 1, respectively. The 4’ alcohol 50 exhibited low activity toward both fungi, which was not in agreement with prior work, as it has been reported that 50 causes 100% growth inhibition of T. rubrum and visible inhibition against T. mentagrophytes at 28 \mu M.\textsuperscript{13} Compound 50 has however been tested inactive against M. gypseum.\textsuperscript{15}

The series including isogriseofulvin (3) and isogriseofulvin analogues 35 and 39–44 was inactive against T. rubrum and showed low activity against T. mentagrophytes. This is in accordance with the literature, where 3 and 39 have been reported to be less active than griseofulvin against a number of dermatophytes (E. floccosum, M. canis, T. interdigitale, T. persicolor, T. mentagrophytes, and T. rubrum).\textsuperscript{11}

Grisofulvic acid (2) was inactive against T. rubrum and had low activity against T. mentagrophytes contrary to prior work, which reported 2 to be inactive toward T. mentagrophytes but having visible growth inhibition at 30 \mu M against T. rubrum.\textsuperscript{13} The griseofulvin dimer 34 was inactive against both fungi. The dichloro analogue 16 showed low activity toward both fungi, while 15 was inactive against both. The reduced analogue 51 had low activity against T. rubrum and an IC_{50} of 3.0 \mu M against T. mentagrophytes, which is 50 times less potent compared to 1. Geodin (56) was inactive against both fungi.

Of the 55 compounds tested, 27 analogues were inactive or exhibited low activity for both fungi (see Table 1). Nine of the compounds were active against both fungi within the same order of magnitude compared to griseofulvin (17, 20, 21, 23, 26, 32, 36, 49, and 54).

There were however some compounds that fared differently against the two fungi. The compounds 6, 7, 22, 24, 28, 29, 31, 51, and 55 were all inactive or had low activity toward T. rubrum but had IC_{50} values in the range of 0.25–3.0 \mu M against T. mentagrophytes. For other analogues (14, 18, 19, 37, 47, and 48) the activity against T. mentagrophytes was similar to that of 1 but against T. rubrum it was significantly lower. This is in accordance with earlier observations by Crosse et al., demonstrating that a given analogue was more active against some fungi but less active against others.\textsuperscript{11}

**Anticancer SAR.** Looking at the IC_{50} values for the 16 analogues (1, 17, 18, 20, 23–25, 27, 36, 37, 45–49, and 54) tested active in both the multipolarity assay\textsuperscript{19} and the cytotoxicity-based assay there was good correlation between the data with an R^2 of 0.70 (see Supporting Information).

The IC_{50} of griseofulvin (1) was determined to be 18 ± 4 \mu M (20 ± 1 \mu M in the phenotype-based spindle multipolarity assay\textsuperscript{19}), while an IC_{50} of 25 ± 4 \mu M against HeLa cells was found by Panda et al.\textsuperscript{18} In the position 4 series, 4, 5, and 7 were all inactive but 6 and 8 harboring aromatic moieties had activities similar to griseofulvin (1), with IC_{50} values of 20 (6) and 17 \mu M (8). This indicates a mode of action for cytotoxicity that does not involve induction of multipolar mitosis, as 6 was inactive in the multipolarity assay. Both the position 5 analogues 9 and 10 were inactive in the cytotoxicity assay as they were in the assay for multipolarity induction.

The 6-phenol griseofulvic acid analogue 11 was inactive, and the 6-phenol griseofulvin analogue (13) showed low activity. The 6-ethyl griseofulvin (14) analogue was approximately 50% less active than griseofulvin. The two dichloro analogues 15 and 16 were some of the most cytotoxic among the tested analogues, with IC_{50} values of 1.0 and 3.2 \mu M, respectively. 2’-Chloro analogue 16 has previously been observed by us to have a different phenotype from all other analogues in the cell-based assay for multipolarity induction.\textsuperscript{22} The highly electrophilic nature of 16 prompted an investigation of the ability of this analogue, together with 1 and 17, to act as Michael acceptors. Briefly, the three compounds were incubated in buffer with two different potential nucleophiles, 2-aminoethanol and 2-mercaptoethanol, for up to seven days (see Supporting Information for details). The experiments showed that neither 1 nor 17 underwent addition of the nucleophiles, whereas 16 did react with 2-mercaptoethanol to form the Michael addition/elimination product, which was the major constituent after 72 h of incubation. This confirms that the highly electron withdrawing properties of the chlorine atom enable 16 to react as a general alkylating agent and one could imagine the formation of a covalent bond to reactive cysteine residues in proteins. The experiment also shows that in the absence of the chlorine atom, this type of reactivity is not present for the griseofulvin analogues. Furthermore, the three representative compounds were found to be stable in the absence of nucleophiles for 1 week of incubation, showing only a slight decrease in purity.

When elongating the 2’ position from the parent methoxy (1) to ethoxy (45) and propoxy (46) and then further increasing the bulkiness with isoproxy (47), cyclopentoxy (49), and benzxyloxy (17), the activity increased through the series (see Figure 3), plateauing with 17 and 49 at 2.1 and 3.2 \mu M, respectively. The same trend was seen when these compounds were tested in the phenotype-based multipolarity assay. Moving from the benzyloxy derived analogues to even bulkier groups like the naphthalen-1-ylmethoxy was inactive in the cytotoxicity assay as they were in the assay for multipolarity induction.

The 6-phenol griseofulvic acid analogue 11 was inactive, and the 6-phenol griseofulvin analogue (13) showed low activity. The 6-ethyl griseofulvin (14) analogue was approximately 50% less active than griseofulvin. The two dichloro analogues 15 and 16 were some of the most cytotoxic among the tested analogues, with IC_{50} values of 1.0 and 3.2 \mu M, respectively. 2’-Chloro analogue 16 has previously been observed by us to have a different phenotype from all other analogues in the cell-based assay for multipolarity induction.\textsuperscript{22} The highly electrophilic nature of 16 prompted an investigation of the ability of this analogue, together with 1 and 17, to act as Michael acceptors. Briefly, the three compounds were incubated in buffer with two different potential nucleophiles, 2-aminoethanol and 2-mercaptoethanol, for up to seven days (see Supporting Information for details). The experiments showed that neither 1 nor 17 underwent addition of the nucleophiles, whereas 16 did react with 2-mercaptoethanol to form the Michael addition/elimination product, which was the major constituent after 72 h of incubation. This confirms that the highly electron withdrawing properties of the chlorine atom enable 16 to react as a general alkylating agent and one could imagine the formation of a covalent bond to reactive cysteine residues in proteins. The experiment also shows that in the absence of the chlorine atom, this type of reactivity is not present for the griseofulvin analogues. Furthermore, the three representative compounds were found to be stable in the absence of nucleophiles for 1 week of incubation, showing only a slight decrease in purity.

![Figure 3.](dx.doi.org/10.1021/jm200835c) The activity increases with the number of carbon atoms at the 2’ position from griseofulvin itself to the 2’ ethoxy (45), propoxy (46), isoproxy (47), and cyclopentoxy (48) before leveling out with the cyclopentoxy (49) and benzxyloxy (17) analogues. The bulkier analogues 25, 27, and 26 were less active than the benzxyloxy analogue (17).

does not indicate that increasing lipophilicity also leads to increasing potency, which would be the case if transport over the cell membrane was a determining parameter for activity. This is further illustrated by the analogues 17, 23, and 24, where the former is the most active (Table 1), indicating that a binding event is responsible for the activity.

A number of variations of 17 have been tested (18–24, 28–30, and 31–33), and although all except 33 (low activity), 28 (32 μM), and (24, 17 μM; 30, 19 μM) were more active than 1, only 20 was as active as 17 with an IC₅₀ of 1.8 μM. The difference in activity between the phenoxy (23, 7.2 μM) and phenylethoxy (24, 17 μM) analogues was less pronounced in the cytotoxicity assay than in the multipolarity assay with two and a half orders of magnitude compared to the approximately 7-fold difference in the phenotype-based assay. The dimer (34) was about twice as active as 1 with an IC₅₀ value of 8.5 μM.

The introduction of an oxime functionality at the 4’ position (36) increased potency 2-fold to 12 μM, an increase in activity that was also seen in the phenotype-based assay. Introducing the oxime to analogues 17 and 26 affording 37 and 38 improved the activity further for both compounds. Apart from 15, 37 is the most active analogue in the cytotoxicity assay with an IC₅₀ of 1.4 μM. The stability of the oximes in PBS buffer (pH 7.4) was tested, and less than 5% hydrolysis to the parent ketones could be detected after 48 h (data not shown).

The isogriseofulvin analogues tested in the multipolarity assay (3 and 39–44) were all inactive. In the cytotoxicity assay, 35 retained the activity of 1, while the rest were either inactive (3), showed low activity (39, 44), or were less potent (40, 43 μM; 41, 48 μM; 42, 52 μM; 43, 27 μM).

Geodin (56) did not induce multipolar mitoses and was in that respect deemed inactive. Geodin (56) is however twice as cytotoxic as griseofulvin when tested against the MDA-MB-231 cell line, with an IC₅₀ of 9.9 μM opposed to 18 μM for 1.

The reduced analogues 50 and 51 as well as the 3’-dimethyl analogue 52 were inactive in both anticancer assays. In the 3’ iodo series, 53 (22 μM) retained activity, the 2’ propoxy analogue (54, 8.0 μM) had increased activity, and 55 exhibited low activity. 53 and 55 were inactive in the phenotype-based assay, but 54 was more potent than 1.

**Anticancer SAR versus Antifungal SAR.** The difference between the antifungal and anticancer data is illustrated in Figure 4, demonstrating that most of the analogues had increased potency against the cancer cell line but against the two fungal strains the activity was lower than for griseofulvin (1). Looking at the 4 position, there were inconsistencies throughout all three cell types. The ethyl analogue 5 was active against both fungi and inactive toward the cancer cells, while the bulkier naphthyl analogue 8 was inactive against both fungi but more potent than griseofulvin against the MDA-MB-231 cells. Analogues 6 and 7 were more potent than 5 against *T. mentagrophytes*, but both compounds were inactive or showed low activity toward *T. rubrum*. The most potent analogue toward the cancer cells (15) was inactive against both fungi and the isomer (16), which was among the most active compounds, only had low activity toward the two fungi. The two bulky analogues 25 and 27 as well as the dimer (34) were inactive in the antifungal assays but were all more potent than griseofulvin against the cancer cell line. It is also worth noticing that geodin (56) was inactive against both fungi but twice as potent as 1 against the cancer cell line.

The introduction of an oxime at the 4’ position (36, 37, and 38) decreased the potency compared to the parent compounds (1, 17, and 26) against both fungi. The opposite effect was seen for the cancer cell line, where the introduction of this moiety increased the activity for all three compounds.

There were however some similar trends for all three cell types. A number of analogues were inactive or had low activity toward both fungi and cancer cells. Among these were the position five analogues (9, 10), isogriseofulvin (3), and the series of isogriseofulvin analogues (39–44), with 35 as the sole exception. The two 6 phenols (11, 13) were virtually inactive against all cell types, which was the same for 50–52.

**CONCLUSION**

The first comparison of antifungal and anticancer SAR for griseofulvin analogues is presented in this work covering 53 analogues of griseofulvin as well as the natural product geodin (56). All compounds have been tested against *T. rubrum*, *T. mentagrophytes*, and in a cytotoxicity assay against MDA-MB-231 breast cancer cells.

Even though there were similarities between the SAR of the two fungi, with some compounds showing no activity against both fungi and some active against both, there were also some differences. Analogues 46 and 48 retain the activity of griseofulvin against *T. mentagrophytes* but were 2- and 4-fold less active against *T. rubrum* than 1, respectively. This is in accordance with reported observations by Crosse et al. that analogues differ in activity against different dermatophytes. 11

We show that there is a good correlation between the IC₅₀ values from analogues that were active in both the phenotype-based assay for spindle multipolarity and the cytotoxicity assay used in this work with an R² of 0.70. Comparing the two fungal SARs with the anticancer cytotoxicity SAR afforded distinct differences. The analogues 8, 25, 27, and 38 were inactive or had low potency against both fungi but either retained the activity (8) or were at least 2-fold more active against the cancer cells compared to 1. The 2’-benzyloxy analogue (17) was less active against both fungi in this study and against six dermatophytes in the study by Crosse et al. 11 This analogue is however nine times more potent than 1 against the cancer cell line MDA-MB-231. It is noteworthy that the two dichloro compounds 15 and 16 were inactive against both fungi but featured as some of the most cytotoxic agents against the cancer cell line, possibly due to their electrophilic nature, which is unique among the tested analogues.

Figure 4. The IC₅₀ values for each cell line have been normalized defining the activity of griseofulvin as having a value of 1. It is seen that of the 53 analogues the majority was less active against the two fungi opposed to the anticancer activity where most analogues were more active than griseofulvin.
The differences in activity observed for the two fungi and the MDA-MB-231 cell line could rise from a number of factors, such as transport over the cell membrane, efflux pumps, or different modes of action. We find it unlikely that transport or efflux pumps could explain that the majority of analogues were more active against MDA-MB-231 cells and less active against both fungi. Our conclusion therefore is that the mode-of-action of griseofulvin(s) toward fungal and mammalian cells is different, making it unlikely that tubulin itself constitutes the main cellular target in both fungi and mammalian cells. Because it has been shown that griseofulvin leads to mitotic arrest in both fungal and mammalian cells, an alternative explanation is that griseofulvin disrupts microtubule dynamics without directly interacting with tubulin, e.g., through interaction with microtubule-associated proteins (MAPs).

**EXPERIMENTAL SECTION**

**General.** Starting materials, reagents, and solvents were purchased from Sigma-Aldrich and used without further purification. 

**General Procedure for the Synthesis of Position 4 Ethers (7 and 8).** The appropriate alkyl bromide (1.2 mmol, 3 equiv) was added to a solution of 4 (0.65 mmol, 1 equiv), Ag2O (1.2 mmol, 3 equiv), and 1,4-dioxane (3 mL). The mixture was stirred at 50 °C for 18 h and then cooled to 20 °C. EtOAc (10 mL) was added to the solution, and the mixture was washed with brine (15 mL). The aqueous phase was dried (MgSO4) and concentrated. The following process was repeated three times: The residue was dissolved in MeOH (10 mL), CSA (0.1 mmol) was added, and the mixture was heated to 65 °C and stirred for 12 h. The mixture was then cooled to 20 °C, and EtOAc (10 mL) was added to the solution, and the mixture was washed with satd aq NaHCO3 (15 mL). The aqueous phase was extracted with EtOAc (2 × 10 mL), and the combined organic phases were dried (MgSO4) and concentrated. The residue was purified by column chromatography (EtOAc:heptane 1:3) to afford the desired product.

**Antifungal Assay.** The fungal micro broth dilution assay was performed in sterile flat bottom microplates (cat. no. 655101) and lids (cat. no. 656161) from Greiner Bio-One GmbH (Frickenhausen, Germany). Each microplate accumulated five dilution series starting at 50 μM of a given analogue. Each plate was also fitted with four negative and four positive control wells. The fungi T. rubrum (IBT29284) and T. mentagrophytes (IBT27274) were kept in the IBT fungal collection at the Department of Systems Biology at the Technical University of Denmark. The identity of the fungal cultures was verified by microscopy after the last test round in the antifungal assay. For testing against T. rubrum, a 4-fold dilution series was used, resulting in 10 concentrations with the lowest at 0.2 μM. For T. mentagrophytes, an 8-fold dilution series was used, resulting in 10 concentrations with the last one at 0.4 μM.

**Inoculum Preparation.** Inoculum preparation was performed in accordance with the document M38-A25 from the "Committee for Clinical Laboratory Standards." Inoculum was adjusted to 0.90–1.5 OD at 530 nm on a Genesys 10uv from Thermo Electron Corporation (Waltham, MA, US). The inoculums were diluted 50-fold with RPMI media (with 1-glutamine, without NaHCO3, cat. no. R6504 in MOPS buffer (cat. no.69947)), both from Sigma-Aldrich (St Louis, MO, USA). The pH was adjusted to 7.00 with 1 M NaOH, and the volume with Milli-Q water from a Milli-Q gradient filled with a Millipore 0.22 μm filter (cat. no. MPGL04001), both from Millipore (Billerica, MA, USA).

Each well contained 99 μL of RPMI media with inoculum, 99 μL of Milli-Q water, and 2 μL of DMSO. Microplates were incubated for 7 days at 37 °C in a WTB binder incubator from VWR (Radnor, PA, US), at which point the OD of the wells were measured at 530 μm on a BioTek Synergy 2 (Brendby, Denmark) using GenSTm 1.1.0.8 software. The half-maximal inhibitory concentration (IC50) values were obtained with GraphPad Prism5 (ver. 5.04) from GraphPad Software (La Jolla, CA, USA) using the "nonlinear regression, sigmoidal dose–response (variable slope)" function. The fitting method used was "automatic outlier elimination,"39 which eliminated less than 1.1% of data points.

**Cytotoxicity Assay.** MDA-MB-231 human adenocarcinoma cells were cultivated in Leibovitz’s L-15 medium in the presence of 10% fetal calf serum at 37 °C. Cell viability was examined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT). Sensa Chemical, St. Louis, MO) colorimetric assay, as previously described.40 Briefly, cells were plated in 96-well microtiter plates at a density of (2–3) × 104 cells per well, and each plate was incubated for 24 and/or 48 h, with MTT added to each well for at least 4 h. The absorbance of each well was measured at 570/630 nm using a spectrophotometer (Molecular Devices, Sunnyvale, CA). Each condition was analyzed in at least three replicates, and the results are presented as the mean ± standard deviation of replicates of a representative experiment that was repeated at least three times.

**General Procedure for the Synthesis of 2’-Ethenyl-2’-methoxyl-Benzofuran (25).** Each well contained 99 μL of RPMI media with inoculum, 99 μL of Milli-Q water, and 2 μL of DMSO. Microplates were incubated for 7 days at 37 °C in a WTB binder incubator from VWR (Radnor, PA, US), at which point the OD of the wells were measured at 530 μm on a BioTek Synergy 2 (Brendby, Denmark) using GenSTm 1.1.0.8 software. The half-maximal inhibitory concentration (IC50) values were obtained with GraphPad Prism5 (ver. 5.04) from GraphPad Software (La Jolla, CA, USA) using the "nonlinear regression, sigmoidal dose–response (variable slope)" function. The fitting method used was "automatic outlier elimination,"39 which eliminated less than 1.1% of data points.
residue was purified by column chromatography (EtOAc:heptane: 1:3) affording the product. When possible, the product was recrystallized from EtOAc:heptane.

(25.6 R)-[7-Chloro-4,6-dimethoxy-benzofuran-3-one]-2-spiro-1'-(2-(3-methoxybenzyl)oxy-6-methyl-cyclohex-2'-ene)-4'-one-4'-oxime 38. To a solution of 26 (0.08 mmol, 1.0 equiv) in EtOH (2 mL) and DMSO (1 mL) was added hydroxylamine hydrochloride (0.30 mmol, 3.5 equiv) and sodium acetate (0.37 mmol, 4.3 equiv). The mixture was stirred at 75 °C for 18 h, allowed to reach 20 °C, and diluted with EtOAc (5 mL). The mixture was washed with brine (3 × 4 mL). The organic phase was dried (MgSO4) and concentrated.

Yield: 83 mg (32%) (white crystals); Rf values (EtOAc/heptane, 5:1), 0.65; mp 173 °C.

C27H25ClNO6, found 494.1371.

1H NMR (CDCl3, 300 MHz): 0.98 (0.5 C, s); 1.26 (1.0 C, s), 56.7 (1 C, s), 56.1 (0.5 C, s), 56.1 (0.5 C, s), 36.4 (0.5 C, s), 126.2 (0.5 C, s), 126.0 (0.5 C, s), 125.7 (0.5 C, s), 125.6 (0.5 C, s), 128.2 (0.5 C, s), 128.1 (0.5 C, s), 126.3 (0.5 C, s), 126.3 (0.5 C, s), 128.9 (0.5 C, s), 128.8 (0.5 C, s), 128.3 (0.5 C, s), 131.2 (0.5 C, s), 131.1 (0.5 C, s), 130.8 (0.5 C, s), 130.6 (0.5 C, s), 128.9 (0.5 C, s), 128.9 (0.5 C, s), 128.8 (0.5 C, s), 128.3 (0.5 C, s), 128.2 (0.5 C, s), 128.1 (0.5 C, s), 126.3 (0.5 C, s), 126.3 (0.5 C, s), 126.2 (0.5 C, s), 126.0 (0.5 C, s), 125.7 (0.5 C, s), 125.6 (0.5 C, s), 125.2 (0.5 C, s), 125.0 (0.5 C, s), 124.9 (0.5 C, s), 123.6 (0.5 C, s), 123.5 (0.5 C, s), 105.6 (0.5 C, s), 105.5 (0.5 C, s), 100.5 (0.5 C, s), 99.5 (0.5 C, s), 99.5 (0.5 C, s), 93.9 (0.5 C, s), 91.5 (0.5 C, s), 91.4 (0.5 C, s), 89.0 (0.5 C, s), 89.0 (0.5 C, s), 69.4 (0.5 C, s), 69.0 (0.5 C, s), 56.7 (1 C, s), 56.1 (0.5 C, s), 56.1 (0.5 C, s), 36.4 (0.5 C, s), 35.2 (0.5 C, s), 35.2 (0.5 C, s), 35.2 (0.5 C, s), 14.4 (0.5 C, s), 14.3 (0.5 C, s); [α]D 20 = +112° (c = 1.0 in CDCl3). HRMS (ESI+) calcd for M + H [C27H25ClNO6]0.149.1370, found 494.1371.

The analogue 35 was synthesized according to the general procedure for 2′ enol ethers with the starting material being 16.

(25.6 R)-[7-Chloro-4,6-dimethoxy-benzofuran-3-on]-2-spiro-1'-(4-3-(methoxybenzyl)oxy-6-methyl-cyclohex-2'-ene)-4'-one 35. Yield: 83 mg (32%) (white crystals); Rf value (EtOAc: heptane, 5:1), 0.65; mp 173–175 °C. IR (neat, cm−1): 3425, 2928, 1740, 1605, 1584. 1H NMR (CDCl3, 300 MHz) δ: 7.37–7.37 (1H, m), 7.02–6.84 (3H, m), 6.09 (1H, s), 5.44 (1H, d, J = 1.0 Hz), 5.01–4.85 (2H, m), 4.01 (3H, s), 3.93 (3H, s), 3.84 (3H, s), 3.85 (3H, s), 2.91 (1H, ddd, J = 12.0 Hz), 5.26 (0.4 H, d, J = 11.9 Hz), 5.23 (0.6 H, d, J = 11.9 Hz), 3.95 (1.8H, s), 3.93 (1.2H, s), 3.91 (1.8H, s), 3.90 (1.2H, s), 3.15 (0.6 H, dd, J = 16.8, 4.9 Hz), 3.06 (0.4 H, dd, J = 15.0, 13.3 Hz), 2.75 (0.6 H, dd, J = 16.8, 13.1 Hz), 2.70–2.75 (1 H, m), 2.44 (0.4 H, dd, J = 15.0, 4.1 Hz), 0.98 (1.8H, d, J = 6.7 Hz), 0.98 (1.2H, d, J = 6.8 Hz). 13C NMR (75 MHz, CDCl3): 130.5 (0.5 C, s), 130.3 (0.5 C, s), 129.7 (0.5 C, s), 129.5 (0.5 C, s), 128.9 (0.5 C, s), 128.9 (0.5 C, s), 128.8 (0.5 C, s), 128.3 (0.5 C, s), 128.2 (0.5 C, s), 128.1 (0.5 C, s), 126.3 (0.5 C, s), 126.3 (0.5 C, s), 126.2 (0.5 C, s), 126.0 (0.5 C, s), 125.7 (0.5 C, s), 125.6 (0.5 C, s), 125.2 (0.5 C, s), 125.0 (0.5 C, s), 124.9 (0.5 C, s), 123.6 (0.5 C, s), 123.5 (0.5 C, s), 105.6 (0.5 C, s), 105.5 (0.5 C, s), 100.5 (0.5 C, s), 99.6 (0.5 C, s), 96.8 (0.5 C, s), 93.9 (0.5 C, s), 91.5 (0.5 C, s), 91.4 (0.5 C, s), 89.0 (0.5 C, s), 89.0 (0.5 C, s), 69.4 (0.5 C, s), 69.0 (0.5 C, s), 56.7 (1 C, s), 56.1 (0.5 C, s), 56.1 (0.5 C, s), 36.4 (0.5 C, s), 35.2 (0.5 C, s), 35.2 (0.5 C, s), 35.2 (0.5 C, s), 14.4 (0.5 C, s), 14.3 (0.5 C, s); [α]D 20 = +112° (c = 1.0 in CDCl3). HRMS (ESI+) calcd for M + H [C24H24ClO7]0.149.1370, found 459.1216.

1605, 1584. 1H NMR (300 MHz, CDCl3): δ 7.82–7.73 (3H, m), 7.50–7.42 (2H, m), 7.37–7.32 (2H, m), 6.54 (0.4H, s), 5.99 (0.6H, s), 5.96 (0.4H, s), 5.84 (0.6H, s), 5.31 (0.4H, d, J = 12.0 Hz), 5.29 (0.6H, d, J = 12.0 Hz), 5.26 (0.4H, d, J = 11.9 Hz), 5.23 (0.6H, d, J = 11.9 Hz), 3.95 (1.8H, s), 3.93 (1.2H, s), 3.91 (1.8H, s), 3.90 (1.2H, s), 3.15 (0.6 H, dd, J = 16.8, 4.9 Hz), 3.06 (0.4 H, dd, J = 15.0, 13.3 Hz), 2.75 (0.6 H, dd, J = 16.8, 13.1 Hz), 2.70–2.75 (1 H, m), 2.44 (0.4 H, dd, J = 15.0, 4.1 Hz), 0.98 (1.8H, d, J = 6.7 Hz), 0.98 (1.2H, d, J = 6.8 Hz).
Analogues. Part I. Modification of the Aromatic Ring.


