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Replacement of the double bond of antitubulin chalcones with triazoles and tetrazoles: Synthesis and biological evaluation

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ABSTRACT

In the chalcone scaffold, it is thought that the double bond is an important structural linker but it is likely not essential for the interaction with tubulin. Yet, it may be a potential site of metabolic degradation and interaction with biological nucleophiles. In this letter, we have replaced this olefinic portion of chalcones with two metabolically stable and chemically inert heterocyclic rings, namely triazole or tetrazole. Yet, our biologic data suggest that, unlike in other antitubulinic structures, the olephinic ring might not be merely a structural linker.

Chalcones are open-chained molecules consisting of two aromatic rings linked by a three-carbon enone fragment. Over the last few years, it has been demonstrated that some chalcones substituted on the aryl rings possess cytotoxic and antimitotic activity due to their ability to inhibit tubulin polymerization[1] (Fig. 1). These compounds exert such effect by binding to the colchi-site of tubulin in a reversible manner.[2] To date, despite the interesting pharmacological properties demonstrated by this class of compounds, there are no chalcones as antitubulinic agents in clinic or pre-clinic studies. This seems to be due to their metabolic instability in vivo. Indeed, the phenolic group can easily undergo phase II metabolism and the enone system can undergo Michael additions with biological nucleophiles such as glutathione. Other drawbacks can be: (a) chalcones are promiscuous structures with a plethora of biological activities and (b) they can have patentability problems. For these reasons, over the last decades, chalcones have been used as starting points to design and synthesize novel stable analogues with the same antimitotic effect and a better efficacy/safety window.[3] In particular, modifications on the chalcone scaffold regarding the replacement of the double bond have been fulfilled maintaining cytotoxicity and antitubulin action, suggesting that the double bond is not strictly required for this biological activity (**3**–**7**) (Fig. 2).[4] We therefore decided to take advantage of the possibility to replace the olefinic bond to set up rapid synthetic approaches that might be used to generate libraries based on the chalcone scaffold. In particular, we thought to substitute the double bond of chalcones with a triazole or a tetrazole moiety, metabolically stable and chemically inert heterocyclic rings (Fig. 3). Furthermore, these substitutions might increase the bioavailability of the new compounds. Preliminary molecular modeling studies were performed, superimposing chalcone structure **1** with scaffold **A**, **B** or **C**. Conformation of **1**, docked into the colchi-site of tubulin, was used as reference and superimpositions were made by using the VegaZZ[5] software. Visual inspection allowed us to see that, for scaffold **B** and **C** triazole or tetrazole analogues can replace the double bond of chalcone, maintaining the correct alignment of the pharmacophoric groups (Fig. 4). In this letter, we now report the synthesis, and the biological evaluation of these new chalcone-like scaffolds.

The molecules belonging to the Scaffold **A** group were easily prepared by using the Sharpless–Fokin cycloaddition[6] between the ynone **8**[7] and 18 substituted aryl azides (Fig. 5) as shown in Scheme 1. The insolubility of the ynone in polar solvents required its dissolution in dichloromethane followed by the addition of the azide, *tert*-butanol/water, copper sulfate and sodium ascorbate. This two-phase system was stirred vigorously at room temperature to give the desired click-chalcones (yields between 80% and 90%)

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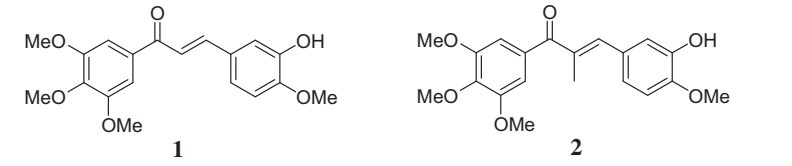


cytotoxicity	IC_{50}= 4.3 nM	IC_{50}= 0.21 nM
antitubulinic acitivity	IC_{50}= 1.0 μM	IC_{50} = 1.8 μM

Figure 1. Antitubulin chalcones.

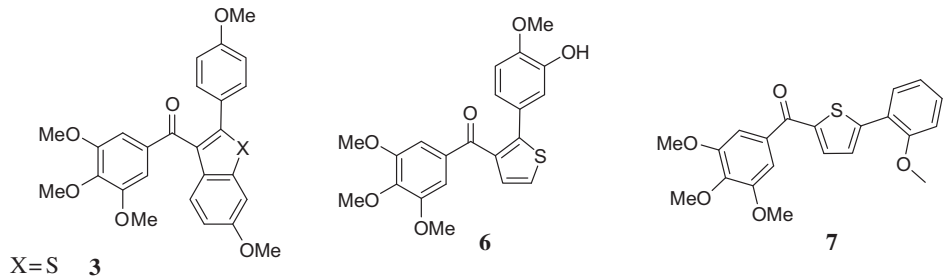


Figure 2. Antitubulin chalcone-like compounds where the double bond has been replaced with an heterocyclic ring.

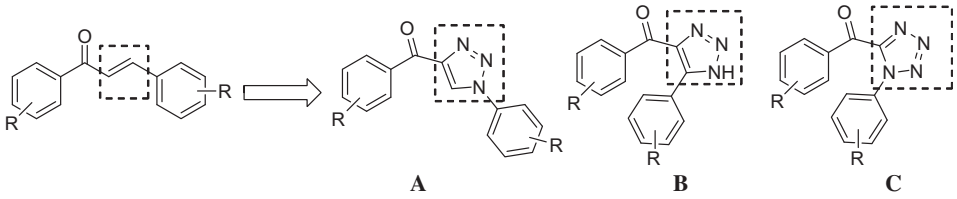


Figure 3. Substitution of the double bond with triazoles or tetrazoles.

(**8A–T**) which precipitated and were obtained by simple filtration. The two molecules belonging to scaffold **B** were prepared by reacting alkynes with acyl chlorides in a Sonogashira-type reaction[8] to give a ynonic system which can react with sodium azide in a 1,3-dipolar cycloaddition. Compound **12** was then prepared reacting trimethoxybenzoylchloride (**9**) and 4-ethynylanisole (**10**) under the Sonogashira protocol, to give the ynone derivative **11** in 72% yield. It is well known that alkynes with electron-withdrawing groups favor the cycloaddition reaction with sodium azide even a low temperature[9] and the ynone **11** was reacted at −10 °C with sodium azide to give **12** in 55% yield (Scheme 2). Compound **15** was prepared using the same protocol. In brief, the ynone **14**[10] was obtained in 65% yield by reacting 4-methoxybenzoylchloride (**13**) with 5-ethynyl-1,2,3-trimethoxybenzene, followed by the reaction with sodium azide to give the final product in 51% yield (Scheme 3). Finally, compounds belonging to scaffold **C** were prepared exploiting a novel multicomponent reaction/post transformation strategy developed by us[11] illustrated in Scheme 4. Briefly, a four component Ugi-like reaction among trimethylsilylazide, the respective isocyanide, the respective aldehyde and the respective benzylamine gives **16**, which undergoes an hydrogenolytic cleavage of the *N*-benzyl group affording the amine derivative **17**, which

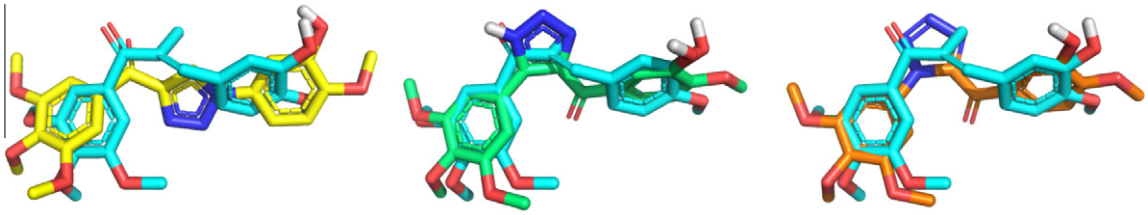


Figure 4. Superimpositions between the chalcone (cyan) and the three scaffolds (A in yellow, B in green and C in orange) show an RMSD value of 1.993, 0.930 and 1.164, respectively.

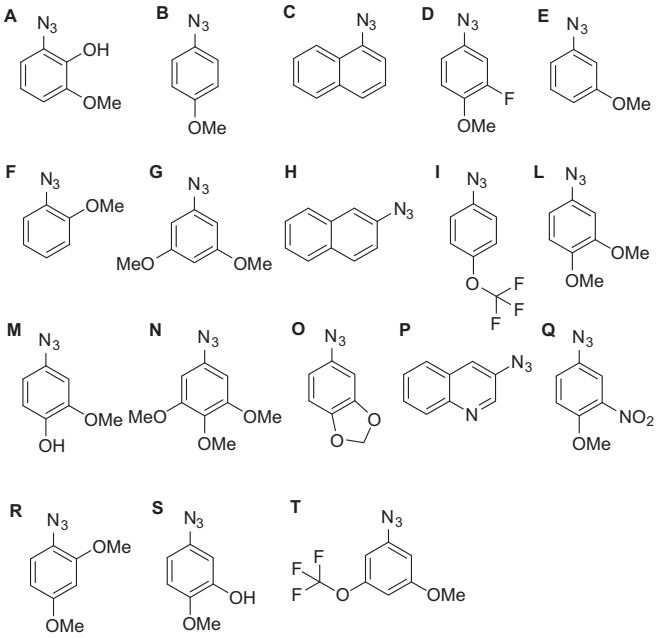
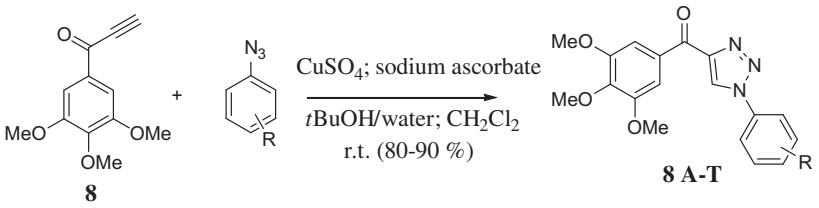


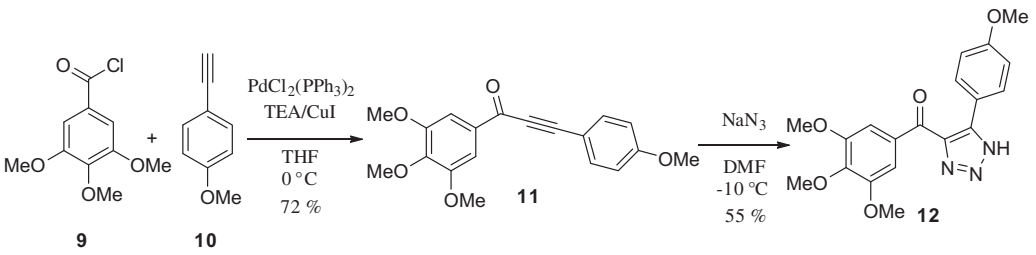
Figure 5. Azide building blocks.

is then converted in the 1-aryl-5-aroyl tetrazole (**18**) through a transamination reaction.[12] This reaction was used to prepare compounds **18a–h** and the respective intermediates (**16a–h** and **17a–h**) were also tested. Alongside, we also generated compounds in which the benzylamine of **16** was substituted to probe whether this ring could participate in the interaction with tubulin (**16i–k**) (Scheme 4).

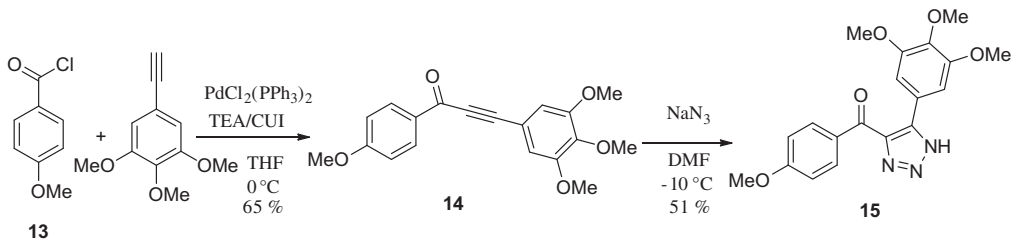
To investigate the biological activity of the synthesized compounds, we opted for SH-SY5Y cells, a neuroblastoma cell line which we have previously shown to be sensitive to antitubulin agents (e.g., combretastatin, taxol).[13] In brief, cells were treated for 48 h with the selected compounds and viability was determined by the MTT method. It should be noted that the MTT assay measures viability as well as decreased proliferation and therefore the results obtained reflect this. In this cell line, chalcone **1** displayed an IC_{50} for cell death/inhibition of proliferation of 50 ± 3 nM, confirming the choice of cellular model. All compounds were first screened at a concentration of 10 μM. None of the click-chalcones (**8A–T**) were active at this concentration (data not shown). Similarly, compounds **12** and **15**, also bearing a triazole in place of the double bond were inactive (i.e., displayed more than 70% viability at this concentration). On the other hand, their precursors (**11** and **14**), bearing a ynone group, were cytotoxic/cytostatic. Finally, the tetrazole analogues (**18a–h** and **16i–k**) were also inactive except for compound **18d**. We therefore proceeded with a concentration response curve of the active compounds under the same conditions (Fig. 6). Compound **11** displayed an IC_{50} of 1.9 ± 0.3 μM, **14** displayed an IC_{50} of 0.7 ± 0.07 μM, and **18d** displayed an IC_{50} of 4.1 ± 0.3 μM (Table 1). To confirm the mechanism of action of these compounds, we performed a cell cycle analysis. It would be expected that antitubulin agents, by disrupting the mito-



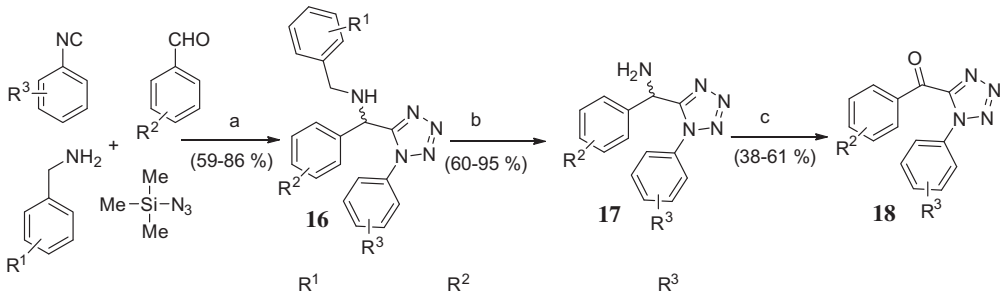
Scheme 1. General synthesis of click-chalcones.



Scheme 2. Synthesis of compound **12**.



Scheme 3. Synthesis of compound **15**.



	R^1	R^2	R^3
a	-H	3,4,5-trimethoxy	4-methoxy
b	-H	4-methoxy	3,4,5-trimethoxy
c	-H	3-hydroxy-4-methoxy	3,4,5-trimethoxy
d	-H	3,4,5-trimethoxy	3-hydroxy-4-methoxy
e	-H	3,4,5-trimethoxy	3-methoxy
f	-H	3,4,5-trimethoxy	3,4-dioxolane
g	-H	3,4,5-trimethoxy	4-phenoxy
h	-H	3-hydroxy-4-methoxy	3,4-dioxolane
i	4-methoxy	3,4,5-trimethoxy	-H
j	3,4,5-trimethoxy	4-methoxy	3-hydroxy-4-methoxy
k	3,4,5-trimethoxy	3-hydroxy-4-methoxy	3-methoxy

Scheme 4. General synthesis for tetrazolic analogues of chalcones. Reagents and conditions: (a) MeOH, rt, 3 days; (b) H_2, Pd/C 10%, MeOH, reflux, 24 h; (c) 4-formyl-1-methylpyridinium benzenesulfonate, CH_2Cl_2/DMF, rt, 8 h then TEA 20 min then satd. aq oxalic acid, rt, 16 h.

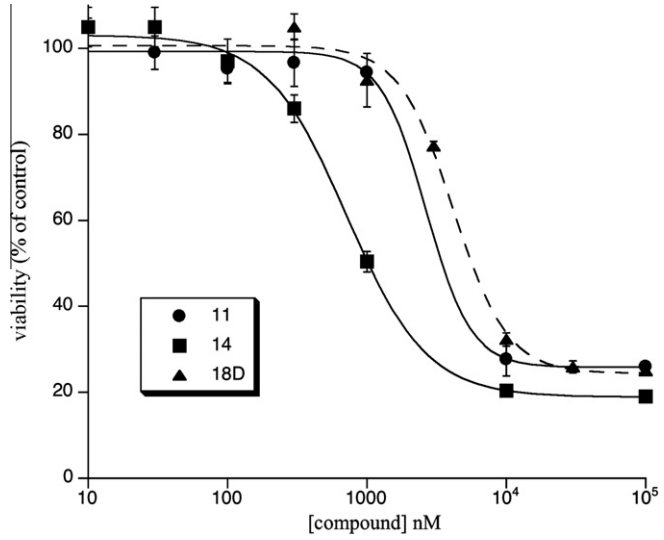


Figure 6. Dose–response curves of cell viability/proliferation as measured by the MTT assay. Values represent mean ± SEM of at least eight determinations from two separate experiments.

tic spindle, induce a G2/M block. For these experiments, cells were treated for 16 h at a concentration twice the determined IC_{50}. As expected, chalcone **1** induced a G2/M block, and this effect was reproduced when using compound **18d**. On the contrary, compounds **11** and **14** displayed a G1 block, strongly suggesting that their cytotoxic/cytostatic nature is not due to an effect on tubulin (Fig. 7 and Table 1). Given the nature of ynone, it could be speculated that the cytotoxic/cytostatic nature of **11** and **14** is aspecific and given by their strong electrophilicity.

1,2,3-Triazoles have already been used successfully to replace the double bond of combretastatin A-4.[14] This, together with the preliminary modeling of chalcones led us to speculate that this strategy could have led to a new series of potent chalcone analogues (click-chalcones) that might serve as scaffolds to generate novel antitubulin agents. The data presented here, though, shows that click-chalcones are inactive. Similarly, replacement of the double bond with tetrazole has been considered for combretastatin A-4.[15] In our hands, one compound (**18d**) retained activity, albeit displaying a very low potency compared to chalcone **1**. Our new scaffold has the advantage of being amenable to rapid synthesis via a multicomponent reaction and could therefore be envisaged to generate a large array of analogues. Yet, it should be noticed that the potency reported here for **18d** is in the same order of magnitude as other analogues previously reported which attempted to replace the olefinic bond, raising the question on whether, unlike combretastatin, the olefinic bridge on chalcones is not merely a structural linker.

Table 1
Synopsis of results obtained with the active compounds. Results of the first column were obtained with a fixed concentration of drug (10 μM) are values are mean ± SEM of three replicates. IC_{50} values were obtained from data presented in Figure 6. Cell cycle data is mean ± SEM of six separate experiments in two experimental days.

	MTT assay 10 μM (% of control)	MTT assay IC_{50} (μM)	Cell cycle		
			G_0/G_1	S	G_2/M
CTRL	100.0 ± 1.4		49 ± 5.5	36 ± 4.0	15 ± 4.8
1	17.0 ± 2.2	0.05 ± 0.003	23 ± 7.1	48 ± 6.4	30 ± 0.7
11	27.7 ± 4.0	1.9 ± 0.2	79 ± 9.2	16 ± 6.4	6 ± 2.8
14	20.4 ± 0.2	0.7 ± 0.07	76 ± 2.1	18 ± 3.5	8 ± 0.7
18d	51.6 ± 3.4	4.1 ± 0.3	41 ± 2.2	30 ± 5.3	29 ± 6.0

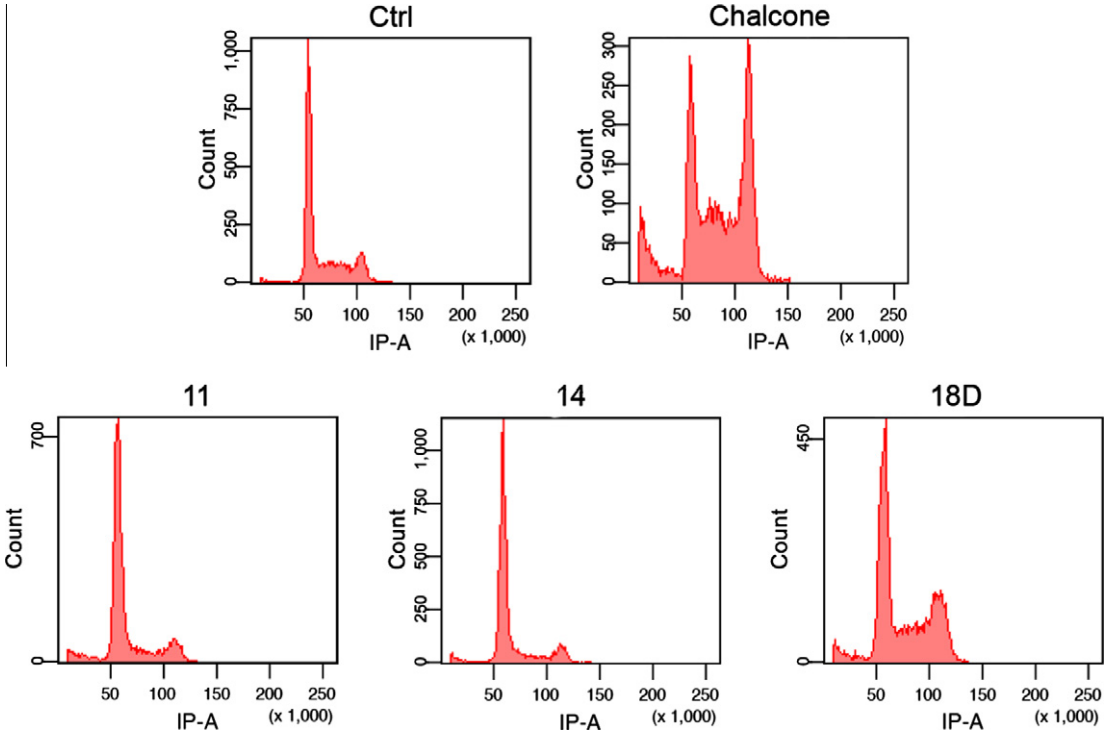


Figure 7. FACS analysis of the selected compounds. Cells were stained propidium iodide to investigate DNA content. Data are representative of at least 6 cytofluorimeter analysis in two experimental days.

Acknowledgments

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12. Synthetic procedure for compound **18d**: equimolar amounts of 5-isocyano-2-methoxyphenol, 3,4,5-trimethoxybenzaldehyde, benzylamine and trimethylsilylazide were dissolved in dry methanol (2 M) under a nitrogen atmosphere. The resulting solution was stirred at room temperature for three days. The solid formed was filtered off and washed with cold methanol to give **16d** as a yellow solid (75%). The obtained tetrazole intermediate was then stirred in methanol under a hydrogen atmosphere in the presence of Pd/C 10% heating at 60 °C for 24 h. The crude reaction was filtered through a Celite pad and evaporated to give the free amine **17d** as a yellow oil (65%) which was enough pure for the following step. The amine was dissolved in CH_2Cl_2/DMF 3:1 and 4-formyl-1-methylpyridinium benzenesulfonate (1.2 equiv) was added. The reaction mixture was stirred at room temperature for 8 h, then treated with triethylamine (1.0 equiv) and stirred for 15–20 min. The reaction was finally quenched with a cold saturated aqueous solution of oxalic acid and stirred overnight. The reaction was then diluted with water and CH_2Cl_2. After extraction with CH_2Cl_2 (3×) the combined organic phases were washed with brine (1×), dried over sodium sulfate, filtered, and evaporated under reduced pressure. The carbonyl compound was purified by column chromatography using PE/EtOAc as eluent to give **18d** as a yellow solid (46%): IR (KBr) 1643, 1514, 1255, 1124, 1021, 865 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.55 (s, 2-H), 7.01 (d, J = 3 Hz, 1-H), 7.97 (d, J = 3 Hz, 1-H), 6.94 (s, 1-H), 3.97 (s, 3-H), 3.94 (s, 3-H), 3.89 (s, 6-H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 181.0, 154.2, 151.0, 149.4, 147.4, 145.9, 130.6, 128.3, 117.8, 112.7, 111.6, 109.4, 62.1, 57.4, 57.2; MS (ESI) m/z 385 $(M-H)^-$; mp = 166–169 °C. $C_{18}H_{18}N_4O_6$: Anal. Calcd for C, 55.96; H, 4.70; N, 14.50. Found: C, 56.22; H, 4.83; N, 14.30.
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