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1,2,4-Triazolo Mercapto and Aminonitriles as Potent Antifungal Agents

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Abstract—A series of 3-mercapto-1,2,4-triazoles mono or disubstituted at 2-, 3- or 4-positions were synthesized and evaluated as antifungal agents. Many of these derivatives exhibit high activity against *Candida albicans* and *Candida tropicalis*.

Introduction

Triazoles and in particular 1,2,4-triazole nucleus have been incorporated into a wide variety of therapeutically interesting drug candidates including anti-inflammatory, CNS stimulants, sedatives, antianxiety and antimicrobial agents.[1,2]

Their antifungal activity is also documented.[3,4] If Amphotericin B is the most commonly drug used against systemic mycoses despite its toxic effect on humans, newer classes of antifungals including azole derivatives (Fluconazole, an orally active triazole agent, and Itraconazole), allylamines, thiocarbamates, fluoropyrimidines are systemic antifungal agents actually employed in patients with impaired immunity such as those who have AIDS or are neutropenic as a result of cancer therapy.

Invasive fungal infections constitute a major cause of mortality for these patients. *Candida albicans* and *Cryptococcus neoformans* are two of the most common opportunistic fungi responsible for infections. For Rabodinirina et al.[5] and Hood et al.,[6] the frequency of deeply invasive candidiasis has increased 10-fold during the past decade. Moreover, many infections due to *Candida* spp are actually refractory to antifungal therapy. While these new classes of compounds are now frequently used in treatment of fungal infections, resistance to these drugs is rising, which clearly indicates an urgent need for new antifungal agents.[7]

To overcome rapid development of drug resistance, new agents should preferably have chemical characteristics that clearly differ from those of existing agents.

An ideal heterocyclic is the 4-amino-3-mercapto-4*H*-1,2,4-triazole system which, by virtue of its vicinal nucleophiles amino and mercapto groups constitues a ready-made building block for construction of various organic heterocycles.

Prompted by the biological properties of 1,2,4-triazoles and as a part of our general search concerning chemotherapeutically important azoles heterocycles,[8] we decided to study new triazoles derivatives of 4-amino-3-mercapto-1,2,4 triazoles. This work reported here describes the synthesis and antifungal activity of some mono substituted and disubstituted amino mercapto triazoles. The influence of the thiol and cyano groups was especially evaluated.

Chemistry

The 4-amino-3-mercapto-1,2,4 triazoles **1**–**4** (Fig. 1) used in the present study for various nucleophilic substitution reactions, each bearing a different aromatic unit attached in the 5-position, were obtained by hydrazinolysis of the corresponding potassium-3-aroyldithiocarbazates with excess hydrazine hydrate following the Reid and Heindel procedure.[9]

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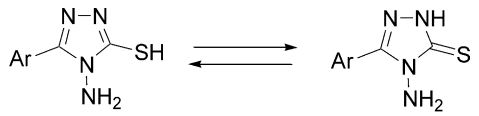


1: Ar = C_6H_5 **2**: Ar = CH_2-C_6H_5
3: Ar = p.Cl-C_6H_4 **4**: Ar = p.CH_3-C_6H_4

Figure 1. 3-Thiol/thione forms of 4-amino-3-mercapto-1,2,4-triazoles.

We therefore required a functional group that could be obtained in gram-scale amounts and could easily be attached to the 3-mercapto or the 2-amino unit of **1**. We focused our attention on a cyano group at terminal position of an aliphatic chain linked at compound **1** for several reasons:

- Firstly, relatively little is known about antifungal activity of triazolo nitriles.[10]
- Secondly, this work led to an extension of our new chemicals methods of synthesis of triazolo-nitriles discuted in a preceding publication.[8] So we are able to present here a series of amino or mercapto-1,2,4-triazolonitriles for comparison of their potent antifungal activity.
- Finally, the terminal cyano group could be easily converted into larger aliphatic group (amide). Chlorohydroxy or tetrazolo group which would mimic the methylene nitrile susbtituent were also introduced for comparisons.

Various 3-mercaptonitriles (compounds **5**,[8] **6**,[8] **7**,[13] **8**,[14] **9**,[15] Scheme 1) or 2-aminonitriles (compound **10**,[8] Scheme 1) keeping a free 4-amino group (N–NH_2), were therefore synthesized using the following procedure:[8] **1** was heated under reflux in the presence of halonitriles (or chloro-tetrazole) and Et_3N (or K_2CO_3) in ethanol (or acetone) until the triazole was consumed (Scheme 1); the tautomeric 3-thiol/thione forms of **1** reacting with halonitriles to the thiol form (3-S) or the potentially tautomeric ring (2-NH), illustrating the alkylation of ambident anions derived from aminomercapto triazoles.[8]

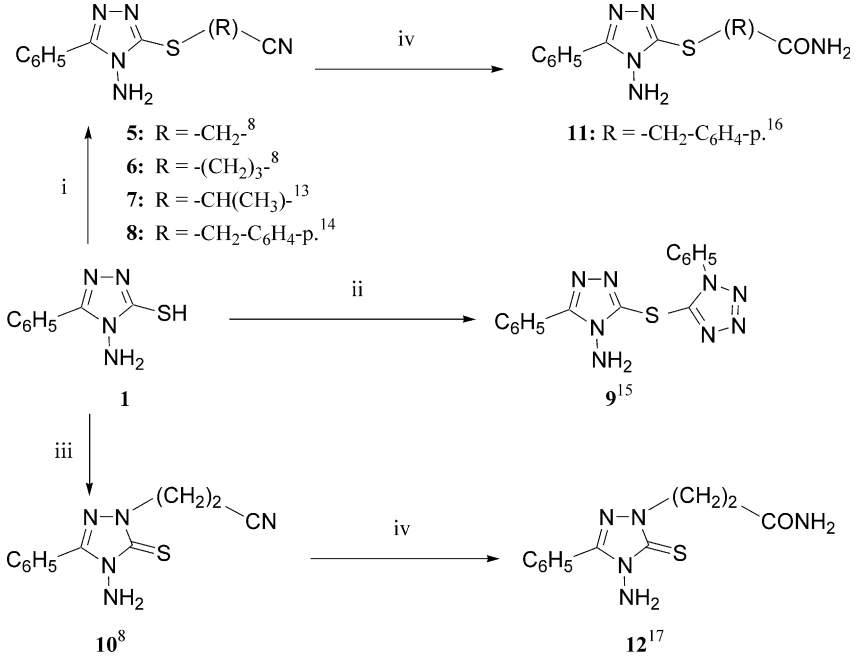
Cyano groups of compounds **8** and **10** were functionalized into the corresponding amides (compounds **11**[16] and **12**[17]) in the presence of sulfuric acid 36N.

The influence of 4-NH_2 substituting groups such as a pyrryl group (compounds **13** and **14**, Scheme 2) or an *N*-alkylnitrile group (compound **15**) on the antifungal activities was either investigated: 3-4-disubstituted 1,2,4-triazoles **13**[18] and **14**[19] were prepared respectively from products **5** and **8** using 2,5-dimethoxytetrahydrofurane in glacial acetic acid (Clauson–Kass reaction). Compound 2-4-disustituted **15** was previously synthesized using a Michael addition.[8]

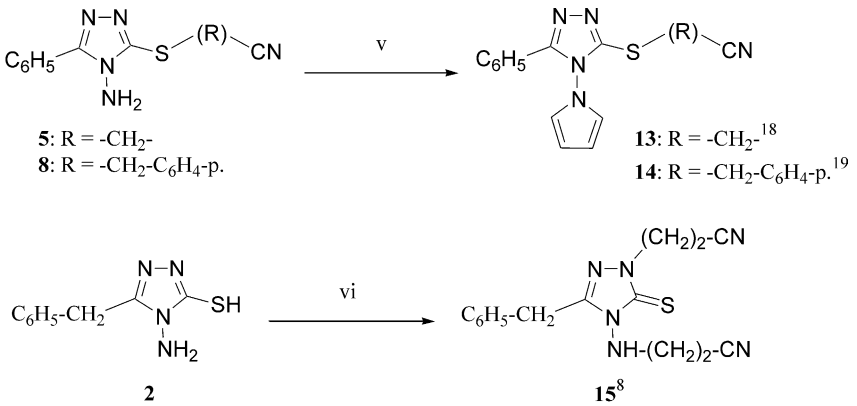
The incorporation of an alternative chlorohydroxy group in the 3-position of triazole **3** which would mimic the methylene nitrile substituent, resulted of treating **3** with epichlorhydrin/$NaHCO_3$ in absolute ethanol (compound **16**,[20] Scheme 3).

Biological Results and Discussion

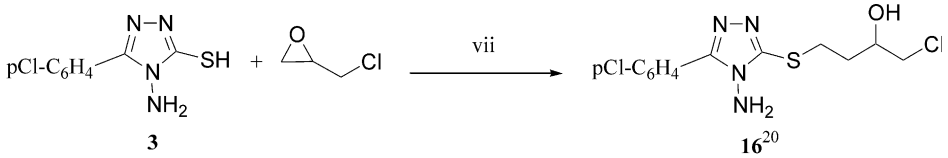
Antifungal activities of synthesized compounds were determined by the disk diffusion method[11] (Disk (1M Whatman paper) diameter: 6 mm; Sabouraud solid



Scheme 1. Synthesis of 4-amino-3-mercapto-1,2,4-triazolonitriles S- or N-substituted. Reagents and conditions: (i) **1** (1 equiv), Cl–CH_2–CN (4 equiv) or Cl–$(CH_2)_3$–CN (4 equiv) or Cl–$CH(CH_3)$–CN (4 equiv) or Cl–CH_2–C_6H_4–CN (1 equiv), Et_3N, EtOH, reflux, 6 h; (ii) **1** (1 equiv), chloro-tetrazole (1.2 equiv), K_2CO_3, acetone, reflux, 24 h; (iii) **1** (1 equiv), Cl–$(CH_2)_2$–CN (1.5 equiv), K_2CO_3, acetone, reflux, 24 h; (iv) **8** or **10**, H_2SO_4 36 N, rt, 2 h.



Scheme 2. Protection of 4-aminogroup of 4-amino-3-mercapto-1,2,4-triazolonitriles: (v) **5** or **8** (1 equiv), 2,5-dimethoxytetrahydrofuran (1 equiv), CH_3COOH, reflux, 1.5 h; (vi) **2** (1 equiv), acrylonitrile (1.5 equiv), K_2CO_3, CH_3CN, rt, 7 h.



Scheme 3. (vii) **3** (1 equiv), $NaHCO_3$ (1.17 equiv), epichlorhydrin (1.4 equiv), EtOH, rt, 3 days.

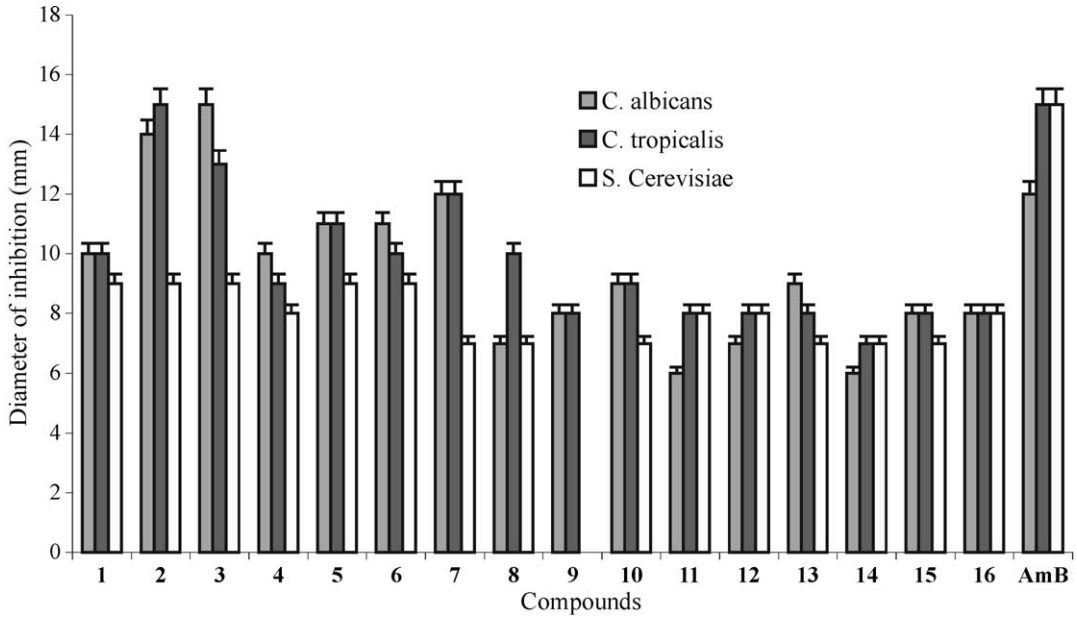
medium (2% glucose, 1% peptone, 7% agar) was used as growth medium) using following yeast strains: *C. albicans*, *C. tropicalis* and *Saccharomyces cerevisiae*. The compounds were dissolved in dimethylformamide at the concentration of 40 mg/mL (excepted Amphotericin B, 20 mg/mL). The results are summarized in Table 1.

All activities were referred to 5-phenyl-4-amino-3-mercapto-1,2,4-triazole **1**. Taking into account that pharmacomodulations effected did not involve any increase of antifungal activity against *S. cerevisiae* compared to that of **1**, we focused our comments on results obtained on *C. albicans* and *C. tropicalis* yeasts.

Modulation of the phenyl cycle

Introduction of a chlorine atom in the *para* position (**3**) and incorporation of a methylene chain between the phenyl and the 1,2,4-triazole cycle (**2**) increased the antifungal activity from 30 to 50% on the yeasts species, affording the most efficient compounds of the series.

Table 1. Antifungal activities of synthesized 1,2,4-triazoles



The presence of a *p*-methyl group (compound **4**) did not exert any significant modification on the antifungal activity of original compound **1**.

Substitution on the sulphur atome

Keeping the aromatic group constant on the tetrazolyl carbon 5 (phenyl group), alkylation of the thiol function by a methylcyano group (**5**) led to an increase (1 mm) of the diameter of inhibition on each yeast compared to that of **1**.

Enhancement of the alkyl chain (**6**) and introduction of a phenyl group (**8**) between cyano group and sulfur atom did not increase the antifungal activity. On the other hand, introduction of a methyl ramification leading to the presence of an asymmetrical carbone atome on this aliphatic chain (**7**) induced the most significant diameters of inhibition in this serie (12 mm on each yeast).

Substitution of the thiol function by a tetrazolo cycle (**9**) significantly decreased the activity.

Transformation of the nitrile group of compound **8** into amidic function (**11**) was either out of interest for antifungal activity.

At last, introduction of a 4-chloro-3-hydroxybutyl group on the sulfur atom of **3** to form compound **16** was not favorable.

Protection of the NH_2 group

Protection of the NH_2 function of compounds **5** and **8** by a pyrole group (**13** and **14**) did not favour the antifungal activity. It is in harmony with the studies of Beuchet and coll. who suggested that presence of a primary amine was necessary for the expression of the antifungal activity of amino sterols.[12]

Substitution on the intracyclic 2-nitrogen atom

We examined the interest of the tautomeric thiol/thione form illustrated by the alkylation of ambident anions derived from **1**. Substitution of the hydrogen of the intracyclic 2-nitrogen atom by an ethylcyano group (**10**), leading to the chemical stabilization of the tautomeric thione form, decreased the inhibitory activity against yeasts studied, compared to these of compound **1** and S-substituted **5** and **6** (thiol form) analogues. Supplementary substitution of the amino group in nitrogen 4 by the same ethylcyano group (**15**) did not improve antifungal activity.

Conclusion

In conclusion, the structure–antifungal activity relationships may be interpreted as follows: the balance of overhall electron density present on both the aromatic and heterocyclic rings seems to be responsible for the activity. It would be confirmed by the fact that with an electron-releasing group, the activity would tend to be maximum (compounds **2** and **3**). However, this conclusion calls for a deeper study of electron density calculations.

The second observation that is worth noting was that keeping the aromatic group constant, attachment of an alkylnitrile group to the thiol function enhanced the antifungal activity (**5**, **6** and **7**), perhaps due to the significant interaction of CN function (electron-withdrawing group) with free amino group at N-4 (electron-releasing groups). It would be confirmed by the diminution of antifungal activity observed in the following cases, cramping or suppressing such a dipole–dipole interaction:

- important steric overcrowding due to a benzyl group (**8**);
- protection of the amino function (**13** and **14**);
- replacement of the nitrile group by an amidic function (**11** and **12**), a tetrazole cycle (**9**) or a 4-chloro-3-hydroxybutyl group (**16**);
- attachment of the nitrile group at nitrogen-2 leading to steric removing of this group from the amino function at C-4 (**10** and **15** where protection of amino group is also effective).

Moreover, it is partly in contradiction with the purpose of Ghannoum and coll. considering that both NH_2 and SH groups should be free for antimicrobial activity.[3] According to our studies, if a free amino group is necessary for optimal activity, implication of the thiol group in a thioether function leading to the introduction of a cyanoalkyl group increases the antifungal activity.

In continuation of our researches, further extensive studies for the synthesis of new 1,2,4-triazoles (dimer structures) which may provide useful antifungal agents to combat infections such as candidiasis are now in progress.

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13. Physical data of synthesized compound **7** (Scheme 1): IR (ν cm^{-1}, KBr): 3342 and 3104 (NH_2), 2240 (CN). ^1H NMR (δ, 500 MHz, DMSO-d_6): 1.70 (d, $J=7$ Hz, 3H, CH_3); 4.80 (q, $J=7$ Hz, 1H, CH); 6.25 (s, 2H, NH_2); 7.55 (m, 3H, *m*- and *p*-Ph); 8.00 (m, 2H, *o*-Ph). ^{13}C NMR (δ, 500 MHz, DMSO-d_6): 8.2 (*q*, $J=33$ Hz, CH_3); 27.8 (d, $J=54$ Hz, CH); 120.1, (m, CN); 126.4 (t, $J=7$ Hz *i*-Ph); 127.8 (dt, $J=162$ Hz, 6.5 Hz, *o*-Ph); 128.5 (dd, $J=160$ Hz, 7 Hz, *m*-Ph); 129.8 (dt, $J=161$ Hz, 7 Hz, *p*-Ph); 150.3 (d, $J=3$ Hz, C-Ph); 154.4 (s, C–S).
14. Physical data of synthesized compound **8** (Scheme 1): IR (ν cm^{-1}, KBr): 3342 and 3174 (NH_2), 2226 (CN). ^1H NMR (δ, 500 MHz, DMSO-d_6): 4.50 (s, 2H, CH_2); 6.10 (s, 2H, NH_2); 7.50 (m, 3H *m*- and *p*-Ph); 7.65 (m, 2H, *o*-Ph–CH_2); 7.80 (m, 2H, *m*-Ph–CH_2); 8.00 (m, 2H, *o*-Ph). ^{13}C NMR (δ, 500 MHz, DMSO-d_6): 34.1 (t, $J=147$ Hz, CH_2); 109.9 (t, $J=8$ Hz, C-CN); 118.7 (m, CN); 126.7 (t, $J=7$ Hz, *i*-Ph); 127.7 (dt, $J=163$ Hz and 5 Hz, *o*-Ph);128.4 (dd, $J=161$ Hz and 7 Hz, *m*-Ph); 129.6 (dt, $J=161$ Hz and 7 Hz, *p*-Ph); 129.9 (dd, $J=165$ Hz and 5 Hz, *m*-Ph–CN); 132.2 (dd, $J=168$ Hz and 6 Hz, *o*-Ph-CN); 143.8 (m, *p*-Ph–CN); 152.7 (t, C–Ph);154.2, (s, C–S).
15. Physical data of synthesized compound **9** (Scheme 1): IR (ν cm^{-1}, KBr): 3328 and 3182 (NH_2). ^1H NMR (δ, 500 MHz, DMSO-d_6): 6.30 (s, 2H, NH_2); 7.55 (m, 3H *m*- and *p*-Ph); 7.70, 7.80, 8.00 (m, 6H, Ph). ^{13}C NMR (δ, 500 MHz, DMSO-d_6): 125.0 (dd, $J=166$ Hz and 6 Hz); 126.1 (t, *i*-Ph); 127.9 (d, $J=163$ Hz, *o*-Ph); 128.5 (dd, 161 Hz and 6 Hz, *m*-Ph); 129.8 (dd, $J=165$ Hz and 7 Hz); 130.1 (dt, $J=162$ Hz and 8 Hz, *p*-Ph); 130.9 (dt, $J=162$ Hz and 8 Hz, *p*-Ph tetrazole); 132.9 (t, $J=7$ Hz, *i*-Ph tétrazole); 146.3 (s C–Ph); 149.7 (s, C–S); 154.8 (s, S–C(=N)–N).
16. Physical data of synthesized compound **11** (Scheme 1): IR (ν cm^{-1}, KBr): 3402 (NH_2 amide), 3350 and 3210 (NH_2 amine), 1658 (CO). ^1H NMR (δ, 500 MHz, DMSO-d_6): 4.50 (s, 2H, CH_2); 6.10 (s, 2H, NH_2 amine); 7.30 (s, 1H, NH amide); 7.50 (m, 5H, *m*- and *p*-Ph, *o*-Ph–CH_2); 7.80 (m, 2H, *m*-Ph–CH_2); 7.90 (s, 1H, NH amide); 8.00 (m, 2H, *o*-Ph).
17. Physical data of synthesized compound **12** (Scheme 1): IR (ν cm^{-1}, KBr): 3414 (NH_2 amide), 3292 and 3192 (NH_2 amine), 1668 (CO). ^1H NMR (δ, 500 MHz, DMSO-d_6): 2.65 (t, $J=7.5$ Hz), 2H, CH_2–CO); 4.35 (t, $J=7.5$ Hz, 2H, CH_2–N); 5.85 (s, 2H NH_2); 6.95 (s, 1H NH amide); 7.45 (s, 1H, NH amide); 7.55 (m, 3H, *m*- and *p*-Ph); 8.00 (m, 2H, *o*-Ph).
18. Physical data of synthesized compound **13** (Scheme 2): IR (ν cm^{-1}, KBr): 2250 (CN). ^1H NMR (δ, 500 MHz, DMSO-d_6): 4.30 (s, 2H, CH_2); 6.35 (t, $J=2.2$ Hz, 2H, ββ′ pyrrole); 7.30 (t, $J=2.2$ Hz), 2H, αα′ pyrrole); 7.35 (d, 8 Hz, 2H, *o*-Ph); 7.40 (t, $J=7.8$ Hz, 2H, *m*-Ph); 7.50 (t, $J=7.5$ Hz, 1H, *p*-Ph). ^{13}C NMR (δ, 500 MHz, DMSO-d_6): 17.7 (t, $J=152$ Hz, CH_2); 109.6 (dm, $J=174$ Hz and 4 Hz, ββ′ pyrrole); 117.1 (t, $J=8$ Hz, CN); 122.0 (dm, $J=194$ Hz and 4 Hz, αα′ pyrrole); 124.5 (t, $J=8.5$ Hz, *i*-Ph); 126.2 (dt, $J=162$ Hz and 7 Hz, *o*-Ph); 128.9 (dd, $J=163$ Hz and 7 Hz, *m*-Ph); 130.8 (dt, $J=163$ Hz and 7 Hz, *p*-Ph); 150.2 (t, $J=5.5$ Hz, C–Ph); 153.4 (t, $J=3.6$ Hz, C–S).
19. Physical data of synthesized compound **14** (Scheme 2): IR (ν cm^{-1}, KBr): 2224 (CN). ^1H NMR (δ, 500 MHz, DMSO-d_6): 4.50 (s, 2H, CH_2); 6.30 (t, $J=2.2$ Hz, 2H ββ′ pyrrole); 7.10 (t, $J=2.2$ Hz, 2H, αα′ pyrrole); 7.30 (d, $J=8.4$ Hz, 2H, *o*-Ph); 7.40 (t, $J=7.6$ Hz, 2H, *m*-Ph); 7.45 (t, $J=7.5$ Hz, 1H, *p*-Ph); 7.65 (d, $J=8.3$ Hz, 2H, *o*-Ph-CH_2); 7.80 (d, $J=8.3$ Hz, 2H, *m*-Ph–CH_2).
20. Physical data of synthesized compound **16** (Scheme 3): ^1H NMR (δ, 500 MHz, DMSO-d_6): 2.50 (s, 3H, CH_3; 3.30 (AB, 2H, CH_2–S); 3.69 (AB, 2H, CH_2–Cl); 4.03 (m, 1H, CH–O); 5.73 (s, 1H, OH); 6.15 (s, 2H, NH_2); 7.60 (d, 2H, *m*-Ph); 8.04 (d, 2H, *o*-Ph).