

## Synthesis and Biological Activity of Some New 5-Sulphonyl- 4-nitroimidazole Derivatives

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### Abstract

A series of 5-sulphonyl derivatives of 1-Benzyl-2-alkyl-4-nitro-1*H*-imidazoles (**5-9**) were prepared by the coupling method of 3-(1-Benzyl-2-ethyl-4-nitro-1*H*-imidazole-5-ylsulfanyl)propanoic acid (**4**) with appropriate amino acid ester hydrochloride, in the presence of hydroxybenzotriazole (HOBT) and DCC. Compounds (**10-12**) and (**13**) were similarly synthesized using appropriate amino alcohols, and 1-substituted piperazine, respectively. The biological activities of some of these compounds were evaluated using Ames test and were found to be nonmutagenic. They exhibited antibacterial activity with MIC values in the range 31.2 - 62.5 µg/ml using different strains of bacteria. Compounds (**5**) and (**12**) have a potent antimutagenic activity against an oxidative mutagen in TA102 strain, a result which indicates that (**5**) and (**12**) may be considered as antioxidative agents.

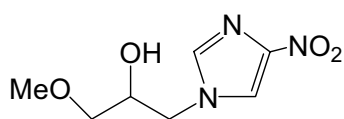
**Keywords:** 4-Nitroimidazoles, Antibacterial, Antimutagenicity, Mutagenicity

### Introduction

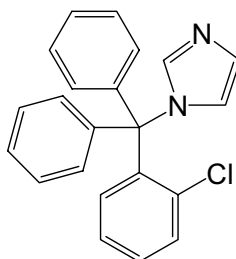
Several substituted imidazoles are of considerable pharmacological significance, particularly as potent and selective histamine H-3 receptor agonists,<sup>[1-3]</sup> mitogen-activated protein (MAP) kinases inhibitors,<sup>[4-9]</sup> nitric-oxide synthase inhibitors,<sup>[10]</sup> anti-inflammatory agents,<sup>[11]</sup> and DNA-directed alkylating agents.<sup>[12]</sup> 4-Nitro-substituted-haloimidazoles, the interesting class of such compounds, showed an important biological activity as antibacterial agents,<sup>[13,14]</sup> potential radiosensitizers<sup>[15]</sup> and cancer chemotherapeutic agents.<sup>[16]</sup> For example, misonidazole [1-methoxy-3-(2-nitro-imidazol-1-yl)-2-propanol (**1**),<sup>[17]</sup> exhibited anticancer activity, *in vivo*, with ED<sub>50</sub> = 1869 mg/kg in mouse-po. Clotrinazole [1-(2-chlorotriptyl)-1*H*-imidazole(**2**)<sup>[18,19]</sup> and metronidazole (Flagyl<sup>®</sup>) [2-(2-methyl-5-nitro-imidazol-1-yl)-ethanol(**3**)<sup>[20]</sup> are compounds of the same class with potent antifungal and antiprotozoal activities (especially for treatment of *Trichomonas vaginalis*, *Entamoeba histolytica* and *Gardia lamblia*). On the other hand, a new target for the development of anti-HIV and antitumor therapies has been reported both *in vivo* and *in vitro* using amino acid derived heterocycles. Such

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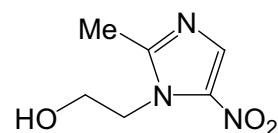
compounds are lysyl amide prodrug of 2-(4-amino-3-methylphenyl)-5-fluorobenzothiazole,<sup>[21]</sup> amino acid derivatives of Paclitaxel,<sup>[22]</sup> cysteine-modified agents<sup>[23]</sup> and isoquinoline carboxylic acid derivatives as building blocks for HIV protease inhibitors.<sup>[24]</sup> Pharmacological properties of imidazoles and amino acid derivatives prompted us to prepare new imidazoles bearing amino acid derivatives to study some of their biological activities, in particular, antibacterial, mutagenic and antimutagenic activity.



1. Misonidazole



2. Clotrinazole



3. Metronidazole  
(Flagyl<sup>®</sup>)

## Results and Discussion

### Chemistry

In our present study, 3-(1-benzyl-2-ethyl-4-nitro-1*H*-imidazol-5-ylthio)propanoic acid **4**<sup>[25]</sup> was selected as starting material for synthesis of some new potentially active substituted imidazole compounds.

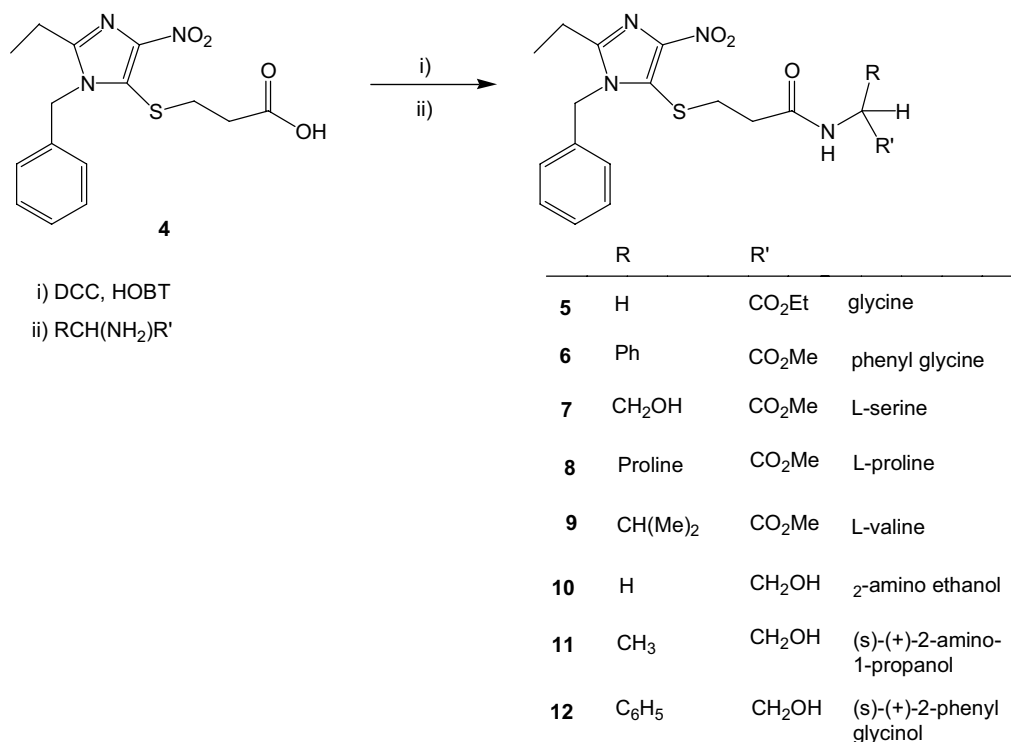
A suitable coupling method<sup>[26]</sup> was employed for the formation of peptides by reaction of the carboxylic acid group with acylated amino acid, using 1-hydroxybenzotriazole (HOBT)<sup>[27, 28]</sup> and *N,N'*-dicyclohexylcarbodiimide (DCC)<sup>[29]</sup> as coupling reagents. HOBT is currently the most frequently used activating agent for the carboxyl group of amino acid. The procedure is fast and suppresses racemization, especially in the presence of DCC.<sup>[30]</sup>

The amides (**5-9**) and (**10-12**) were performed by coupling of **4** with the appropriate acylated amino acids and amino alcohols in the presence of HOBT and DCC as coupling reagents, to give (**5-12**) in 58-83% yield (scheme 1).

The structures of the newly synthesized products (**5-12**) were confirmed by the <sup>1</sup>H, <sup>13</sup>C NMR and mass spectra. In the <sup>1</sup>H NMR spectra the phenyl and ethyl protons showed similar pattern, meanwhile the singlets in the region  $\delta_H$  5.6-5.3 were attributed to the methylene of benzyl group.

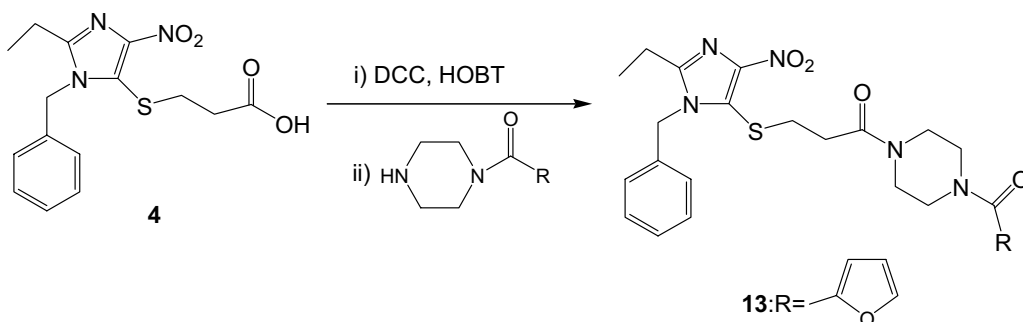
The SCH<sub>2</sub> protons appeared in the region  $\delta_H$  3.0-3.4. The signals at  $\delta_H$  2.4-2.6 represent the methylene protons adjacent to the carbonyl group of the amide bond. The other protons of the amino acid ester and the amino alcohols were fully analyzed. In the <sup>13</sup>C NMR spectra of **5-12** C-2 resonates at  $\delta_C$  150.6 and 151.0, while C-4 appeared between  $\delta_C$  148.1 and 149.2. The resonances between  $\delta_C$  136.1 and 124.7

represent C-5 and the phenyl carbon atoms. The other carbons were fully analyzed (Experimental section).



Scheme 1

Furthermore, the work was extended to include the use of 1-substituted piperazines for the synthesis of new derivatives to examine their antibacterial activity in comparison with the amino acids and amino alcohols **5-12**. Compound **13** was prepared in 86 % yield by applying the coupling method used previously in the presence of HOBT and DCC as coupling reagents (Scheme 2). The structure of **13** was determined from its <sup>1</sup>H, <sup>13</sup>C NMR spectral data and mass spectrum.



### Biological Activities

Some of the synthesized compounds were investigated for their antibacterial, mutagenic and antimutagenic activities.

### Mutagenicity assay / Ames Spot Test

Spot test was applied as a primary quick investigation for mutagenicity of

nitroimidazol compounds **4**, **5**, **12** and **13** at a concentration of 33 µg/plate. The results are presented in (table 1) ,which show that the number of revertant colonies was very close to the revertants number of negative control (DMSO) in both bacterial strains, TA102 and TA1530. Moreover, the obtained results indicated that using S9 mix did not lead to metabolism of any of the tested compounds to a mutagenic one. This indicates that such compounds are not mutagenic in the indicated strains.

**Table 1:** Spot test for nitroimidazole compounds in bacterial strains TA1530 with and without S9.

Strain Compound	TA102		TA1530	
	-S9	+S9	-S9	+S9
4	119	108	83	85
5	117	94	87	106
12	107	120	98	96
13	92	98	92	114
DMSO	116	128	73	81
S. A*	_____	_____	>1000	>1000
NQNO**	>1000	>1000	_____	_____

\* S.A.: Sodium azide

\*\* NQNO: nitroquinoline-*N*-oxide

#### *Antimutagenicity assay:*

The importance of antimutagenicity assay is to investigate the potential activity of the synthesized compounds in preventing or inhibiting the activity of mutagenic compounds. The results showed that two of the tested compounds **5** and **12** have a potent antimutagenic activity against an oxidative mutagen in TA102 strain (table 2). This indicates that these compounds may be considered as antioxidants and a promising anticarcinogenic agents. However, using TA1530 *Salmonella* strain, none of the tested compounds showed antimutagenic activity in this base pair substitution detector strain (table 3).

The percentage mutagenic repression was calculated according to the following formula. <sup>[31]</sup>

$$\% \text{Mutagenic repression} = \left( 1 - \frac{\text{experimental} - \text{negative control}}{\text{positive control} - \text{negative control}} \right) \times 100\%$$

Using data in table 2, the % of mutagenic repression as a result of using compounds **5** and **12** was calculated to be 96.4% and 97.5% for these compounds, respectively at a concentration of 33µg/plate. Given that metronidazole was shown to be mutagenic<sup>[32]</sup> and tumorigenic<sup>[33]</sup>, our results should be considered of a special importance if any practical application of such compounds is under discussion.

**Table 2:** Antimutagenicity test for nitroimidazole compounds in bacterial strain TA102.

Compound	Strain TA102	
	-S9	+S9
5 + NQNO	93	141
12 + NQNO	115	130
DMSO + NQNO	>1000	>1000
DMSO	148	105

**Table 3:** Antimutagenicity test for nitroimidazole compounds in bacterial strain TA1530.

Compound	Strain TA1530	
	-S9	+S9
4+ S A*	>1000	>1000
5 + S A	>1000	>1000
12 + S A	>1000	>1000
13 + S A	>1000	>1000
DMSO+ S A	>1000	>1000
DMSO	194	145

\* S A: sodium azide.

#### Antibacterial assay

Compounds **5** and **12** showed antibacterial activity at a minimal inhibitory concentration (MIC) 62.5 µg/ml against *Staphylococcus epidermidis*, *E. coli*, and *Pseudomonas sp.* The other derivatives **4** and **13** have the same effect except that they showed activity at MIC of 31.2 µg/ml against *Pseudomonas* strain. Metronidazole as a reference gave MIC values of > 500 µg/ml for *Staphylococcus epidermidis* and *E. coli* strains and an MIC 250 - 500 µg/ml using *pseudomonas sp.* However, although metronidazole is used mainly as antifungal and antiprotozoal agent<sup>[20]</sup>, our results indicate that, at least under aerobic conditions, the newly synthesized compounds are much more active as antibacterial. This may encourage us to say that such compounds are suspected to be very promising antibiotics.

Furthermore, the results of the present investigation suggest that it is reasonable to investigate all the synthesized compounds for their antibacterial, antimutagenic, antifungal, antiprotozoal and anticarcinogenic activity; also they may promote us to look for other types of substitutions, hoping that they will lead to more potent compounds.

### Experimental section

#### Chemistry

*General procedure for the Synthesis of imidazole-bearing amino acid esters, amino alcohols and piperazine (5-13).*

To a cold (– 5 °C) solution of the amino acid ester hydrochlorides, amino alcohols or piperazine derivatives (10 mmol) in MeCN (20 mL), the imidazole **4**<sup>[25]</sup> (3.4 g, 10 mmol), hydroxybenzotriazole (HOBT) (1.4 g, 10 mmol) and *N,N'*-Dicyclohexyl carbodiimide (DCC) (2.1 g, 10 mmol) were added, successively. The reaction mixture

was stirred at 0 °C for 1 h, 5 °C for 1 h, and at 23 °C for 16 h. The dicyclohexylurea (DCU) was filtered, the filtrate was evaporated to dryness and the residue was dissolved in ethyl acetate, filtered, washed successively with saturated NaCl solution, 5% NaHCO<sub>3</sub> solution, 1.0 mol L<sup>-1</sup> HCl, followed by washing with saturated NaCl solution and finally with water. The residue was dried (Na<sub>2</sub>SO<sub>4</sub>), filtered, evaporated to dryness and purified by chromatography using cyclohexane/ethyl acetate (1:1) as eluent.

*Preparation of [3-(1-Benzyl-2-ethyl-4-nitro-1H-imidazol-5-ylsulphanyl)-propionylamino]-acetic acid ethyl ester (5).* Prepared from **4** (3.4 g) and glycine ethyl ester hydrochloride (1.4 g); (3.5 g, 83% Yield); m.p. 72 –74°C ; <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 1.2 (t, J=7.5, 6H, 2 CH<sub>3</sub>); 2.5 (t, J=7.5, 2H, SCH<sub>2</sub>CH<sub>2</sub>); 2.7 (q, J=7.5, 2H, CH<sub>2</sub>CH<sub>3</sub>); 3.2 (t, J=6.1, 2H, SCH<sub>2</sub>CH<sub>2</sub>); 3.9 (s, 2H, NHCH<sub>2</sub>); 4.2 (q, J=7.5, 2H, OCH<sub>2</sub>); 5.3 (s, 2H, PhCH<sub>2</sub>); 6.1-6.30 (br, 1H, NH); 7.0 (d, J=6.0, 2H, Ar); 7.2-7.4 (m, 3H, Ar). <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ 11.3 (CH<sub>2</sub>CH<sub>3</sub>); 14.1 (OCH<sub>2</sub>CH<sub>3</sub>); 21.3 (CH<sub>2</sub>CH<sub>3</sub>); 32.0 (SCH<sub>2</sub>CH<sub>2</sub>); 35.7 (SCH<sub>2</sub>CH<sub>2</sub>); 41.4 (NHCH<sub>2</sub>); 47.8 (CH<sub>2</sub>Ph); 61.6 (OCH<sub>2</sub>); 124.7, 126.1, 128.3, 129.2, 135.0 (Ph-C, C-5); 148.6 (C-4); 151.0 (C-2); 169.7, 170.2 (2 C=O). Anal Calcd for C<sub>19</sub>H<sub>24</sub>N<sub>4</sub>O<sub>5</sub>S (420.48): (%),C, 54.27; H, 5.75; N, 13.32. Found (%),C, 54.11; H, 5.61; N, 13.05. MS: m/z (%) (EI) 420 (90).

*Ethy-2-(3-(benzyl-2-ethyl-4-nitro-1H-imidazol-5-ylthio)propanamido-2-phenylacetate (6).*

Prepared from **4** (3.4 g) and phenyl glycine methyl ester hydrochloride (2.0 g); (3.8 g, 79% Yield); m.p. 78 –80°C ; <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 1.2 (t, J=7.5, 3H, CH<sub>3</sub>); 2.4-2.6 (m, 2H, SCH<sub>2</sub>CH<sub>2</sub>); 2.7 (q, J=7.5, 2H, CH<sub>2</sub>CH<sub>3</sub>); 3.2 (t, J=6.1, 2H, SCH<sub>2</sub>CH<sub>2</sub>); 3.7 (s, 3H, OCH<sub>3</sub>); 5.3 (s, 2H, PhCH<sub>2</sub>); 5.5 (d, J=7.0, 1H, NHCH); 6.9 (br, 1H, NH); 7.2-7.4 (br, 10H, Ar). <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ 11.1 (CH<sub>2</sub>CH<sub>3</sub>); 21.0 (CH<sub>2</sub>CH<sub>3</sub>); 31.8 (SCH<sub>2</sub>CH<sub>2</sub>); 35.5 (SCH<sub>2</sub>CH<sub>2</sub>); 47.6 (CH<sub>2</sub>Ph); 52.6 (OCH<sub>3</sub>); 56.4 (NHCH); 124.4, 125.0, 126.3, 127.3, 128.1, 128.3, 128.5, 128.9, 129.0, 136.1 (Ph-C, C-5); 148.1 (C-4); 150.4 (C-2); 169.5, 171.0 (2 C=O). Anal Calcd for C<sub>24</sub>H<sub>26</sub>N<sub>4</sub>O<sub>5</sub>S (482.16): (%),C, 59.74; H, 5.43; N, 11.61. Found (%),C, 59.44; H, 5.61; N, 11.45. MS: m/z (%) (EI) 482 (90).

*2-[3-(1-Benzyl-2-ethyl-4-nitro-1H-imidazol-5-ylsulphanyl)-propionylamino]-3-hydroxy-propionic acid methyl ester (7).* Prepared from **4** (3.4 g) and L-serine methyl ester hydrochloride (1.6 g); (2.8 g, 64% Yield); m.p. 68 – 70°C ; <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 1.26 (t, J=7.8, 3H, CH<sub>3</sub>); 2.54 (t, J=7.1, 2H, SCH<sub>2</sub>CH<sub>2</sub>); 2.67 (q, J=7.4, 2H, CH<sub>2</sub>CH<sub>3</sub>); 3.00-3.30 (m, 2H, SCH<sub>2</sub>CH<sub>2</sub>); 3.40-3.60 (br, 1H, OH); 3.71 (s, 3H, OCH<sub>3</sub>); 3.80-3.90 (dd, 2H, CH<sub>2</sub>OH); 4.51-4.80 (m, 1H, NCH); 5.41 (s, 2H, PhCH<sub>2</sub>); 6.70-6.90 (br, 1H, NH); 7.00-7.10 (d, J=7.0, 2H, Ar); 7.30-7.40 (m, 3H, Ar). <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ 11.1 (CH<sub>2</sub>CH<sub>3</sub>); 21.3 (CH<sub>2</sub>CH<sub>3</sub>); 31.9 (SCH<sub>2</sub>CH<sub>2</sub>); 35.7 (SCH<sub>2</sub>CH<sub>2</sub>); 47.7 (CH<sub>2</sub>Ph); 52.7

(OCH<sub>3</sub>); 54.7 (NHCH); 62.8 (CH<sub>2</sub>OH); 124.7, 126.1, 128.3, 129.2, 135.0 (Ph-C, C-5); 149.0 (C-4); 150.9 (C-2); 170.6, 170.9 (2 C=O). Anal Calcd for C<sub>19</sub>H<sub>24</sub>N<sub>4</sub>O<sub>6</sub>S (436.48): (%), C, 56.28; H, 5.54; N, 12.48. Found (%), C, 56.45; H, 5.61; N, 12.62. MS: m/z (EI) 436 (80).

*1-[3-(1-Benzyl-2-ethyl-4-nitro-1H-imidazol-5-ylsulphanyl)-propionyl]-pyrrolidine-2-carboxylic acid methyl ester (8)*. Prepared from **4** (3.4 g) and L-proline methyl ester hydrochloride (1.7 g); (2.6 g, 58% Yield); oil; <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 1.3 (t, J=8.4, 3H, CH<sub>3</sub>); 1.9-2.2 (br, 4H, CH<sub>2</sub>CH<sub>2</sub>); 2.5-2.8 (br, 2H, SCH<sub>2</sub>CH<sub>2</sub>); 3.0-3.4 (br, 2H, SCH<sub>2</sub>CH<sub>2</sub>); 3.4-3.6 (br, 2H, NCH<sub>2</sub>); 3.8 (s, 3H, OCH<sub>3</sub>); 4.4-4.5 (m, 1H, NCH); 5.4 (s, 2H, PhCH<sub>2</sub>); 6.9 (d, J=7.1, 2H, Ar); 7.3-7.5 (m, 3H, Ar). <sup>13</sup>C NMR (DMSO-d<sub>6</sub>): δ 11.1 (CH<sub>2</sub>CH<sub>3</sub>); 20.8 (CH<sub>2</sub>CH<sub>3</sub>); 24.6, 29.1 (CH<sub>2</sub>CH<sub>2</sub>); 31.6 (SCH<sub>2</sub>CH<sub>2</sub>); 34.0 (SCH<sub>2</sub>CH<sub>2</sub>); 46.9 (NCH<sub>2</sub>); 47.5 (CH<sub>2</sub>Ph); 52.4 (OCH<sub>3</sub>); 58.7 (NCH); 126.4, 128.3, 129.4, 135.9 (Ph-C, C-5); 148.1 (C-4); 150.9 (C-2); 169.5, 173.2 (2 C=O). Anal Calcd for C<sub>21</sub>H<sub>26</sub>N<sub>4</sub>O<sub>5</sub>S (446.52): (%), C, 56.49; H, 5.87; N, 12.55. Found (%), C, 56.78; H, 5.59; N, 12.21. MS: m/z (%) (EI) 446 (85).

*[3-(1-Benzyl-2-ethyl-4-nitro-1H-imidazol-5-ylsulphanyl)-propionylamino]-3-methyl-butyrac acid methyl ester (9)*. Prepared from **4** (3.4 g) and L-valine methyl ester hydrochloride (1.7 g); (3.1 g, 69% Yield); m.p. 115 - 117°C; <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 1.1 (d, J=6.4, 6H, CH(CH<sub>3</sub>)<sub>2</sub>); 1.5 (t, J=7.3, 3H, CH<sub>3</sub>); 2.3-2.5 (br, 1H, CH(CH<sub>3</sub>)<sub>2</sub>); 2.6-2.8 (br, 2H, SCH<sub>2</sub>CH<sub>2</sub>); 2.9 (q, J=7.2, 2H, CH<sub>2</sub>CH<sub>3</sub>); 3.2-3.4 (br, 2H, SCH<sub>2</sub>CH<sub>2</sub>); 4.1 (s, 3H, OCH<sub>3</sub>); 4.6-4.8 (br, 1H, NCH); 5.6 (s, 2H, PhCH<sub>2</sub>); 6.2-6.4 (br, 1H, NH); 7.2 (d, J=6.1, 2H, Ar); 7.5-7.7 (m, 3H, Ar). <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ 11.2 (CH<sub>2</sub>CH<sub>3</sub>); 17.8, 18.9 (CH(CH<sub>3</sub>)<sub>2</sub>); 21.3 (CH<sub>2</sub>CH<sub>3</sub>); 31.2 (SCH<sub>2</sub>CH<sub>2</sub>); 32.3 (CH(CH<sub>3</sub>)<sub>2</sub>); 35.9 (SCH<sub>2</sub>CH<sub>2</sub>); 47.7 (CH<sub>2</sub>Ph); 52.2 (OCH<sub>3</sub>); 57.2 (NCH); 124.5, 126.1, 128.3, 129.2, 135.0 (Ph-C, C-5); 149.2 (C-4); 150.6 (C-2); 170.0, 172.3 (2 C=O). Anal Calcd for C<sub>21</sub>H<sub>28</sub>N<sub>4</sub>O<sub>5</sub>S (448.54): (%), C, 56.23; H, 6.92; N, 12.24. Found (%), C, 56.00; H, 7.17; N, 12.48. MS: m/z (%) (EI) 448 (90).

*3-(1-Benzyl-2-ethyl-4-nitro-1H-imidazol-5-ylsulphanyl)-N-(2-hydroxy-ethyl)-propanamide (10)*. Prepared from **4** (3.4 g) and 2-aminoethanol (0.6 g); (2.3 g, 61% Yield); oil; <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 1.2 (t, J=8.0, 3H, CH<sub>3</sub>); 2.5 (t, J=8.1, 2H, SCH<sub>2</sub>CH<sub>2</sub>); 2.7 (q, J=9, 2H, CH<sub>2</sub>CH<sub>3</sub>); 2.9-3.1 (br, 1H, OH); 3.1 (t, J=8.1, 2H, SCH<sub>2</sub>CH<sub>2</sub>); 3.4 (t, J=6.1, 2H, NHCH<sub>2</sub>); 3.7 (t, J=6.0, 2H, CH<sub>2</sub>OH); 5.3 (s, 2H, PhCH<sub>2</sub>); 6.4-6.6 (br, 1H, NH); 7.0 (d, J=7.0, 2H, Ar); 7.2-7.5 (m, 3H, Ar). <sup>13</sup>C NMR (CDCl<sub>3</sub>): δ 11.2 (CH<sub>2</sub>CH<sub>3</sub>); 21.3 (CH<sub>2</sub>CH<sub>3</sub>); 32.2 (SCH<sub>2</sub>CH<sub>2</sub>); 35.9 (SCH<sub>2</sub>CH<sub>2</sub>); 47.8 (CH<sub>2</sub>Ph); 48.0 (NHCH<sub>2</sub>); 61.8 (CH<sub>2</sub>OH); 126.1, 128.4, 129.2, 134.8 (Ph-C, C-5); 148.8 (C-4); 151.0 (C-2); 171.2 (C=O). Anal Calcd for C<sub>17</sub>H<sub>22</sub>N<sub>4</sub>O<sub>4</sub>S · H<sub>2</sub>O (396.45): (%), C, 47.80; H, 5.15; N, 13.12. Found (%), C, 47.91; H, 5.23; N, 12.87. MS: m/z (%) (EI) 378 (85).

*3-(1-Benzyl-2-ethyl-4-nitro-1H-imidazol-5-ylsulphanyl)-N-(2-hydroxy-1-methyl-ethyl)-propanamide (11)*. Prepared from **4** (3.4) g and (s)-(+)-2-amino-1-propanol (0.8 g); (2.9 g, 74% Yield); m.p.127-128°C; <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 1.15 (d, J=7.0, 3H, CHCH<sub>3</sub>); 1.35 (t, J=7.0, 3H, CH<sub>2</sub>CH<sub>3</sub>); 2.4-2.6 (br, 2H, SCH<sub>2</sub>CH<sub>2</sub>); 2.7 (q, J=7.2, 2H, CH<sub>2</sub>CH<sub>3</sub>); 3.0-3.2 (m, 4H, CH<sub>2</sub>OH + SCH<sub>2</sub>CH<sub>2</sub>); 3.4-3.7 (m, 1H, CHCH<sub>3</sub>); 3.9-4.0 (br, 1H, OH); 5.4 (s, 2H, PhCH<sub>2</sub>); 6.2-6.4 (br, 1H, NH); 7.0 (d, J=7.1, 2H, Ar); 7.3-7.50 (m, 3H, Ar). <sup>13</sup>C NMR (CDCl<sub>3</sub>), δ 11.2 (CH<sub>2</sub>CH<sub>3</sub>); 16.8 (CHCH<sub>3</sub>); 21.3 (CH<sub>2</sub>CH<sub>3</sub>); 32.2 (SCH<sub>2</sub>CH<sub>2</sub>); 36.1 (SCH<sub>2</sub>CH<sub>2</sub>); 47.9 (CH<sub>2</sub>Ph); 48.0(NHCH); 66.6 (CH<sub>2</sub>OH); 124.7, 126.1, 128.4, 129.2, 134.8 (Ph-C, C-5); 148.9 (C-4); 150.9 (C-2); 170.7 (C=O). Anal Calcd for C<sub>18</sub>H<sub>24</sub>N<sub>4</sub>O<sub>4</sub>S (392.47): (%), C, 55.08; H, 6.16; N,14.28. Found (%), C, 55.43; H, 6.28; N, 14.28. MS: m/z (%) (EI) 392 (90).

*3-(1-Benzyl-2-ethyl-4-nitro-1H-imidazol-5-ylsulphanyl)-N-(2-hydroxy-1-phenyl-ethyl)-propanamide (12)*. Prepared from **4** (3.4 g) and (s)-(+)-2-phenyl glycinol (1.4 g); (3.5 g, 77% Yield); m.p. 63 – 65°C ;<sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 1.2 (t, J=7.2, 3H, CH<sub>3</sub>); 2.4-2.6 (br, 2H, SCH<sub>2</sub>CH<sub>2</sub>); 2.65 (q, J=7.2, 2H, CH<sub>2</sub> CH<sub>3</sub>); 3.0-3.2 (br, 3H, SCH<sub>2</sub>CH<sub>2</sub> + OH); 3.7-3.9 (br, 2H, CH<sub>2</sub>OH); 4.7-4.9 (br, 1H, CHPh); 5.3 (s, 2H, CH<sub>2</sub>Ph); 6.6-6.80 (br, 1H, NH); 7.2-7.4 (br, 10H, Ar). <sup>13</sup>C NMR (CDCl<sub>3</sub>), δ 11.2 (CH<sub>2</sub>CH<sub>3</sub>); 21.3 (CH<sub>2</sub>CH<sub>3</sub>); 32.2 (SCH<sub>2</sub>CH<sub>2</sub>); 35.9 (SCH<sub>2</sub>CH<sub>2</sub>); 47.8 (CH<sub>2</sub>Ph); 56.1 (NHCH); 66.1 (CH<sub>2</sub>OH); 125.0, 126.1, 126.8, 127.8, 128.4, 128.5, 128.8, 129.0, 129.2, 134.8, (Ph-C, C-5), 148.5 (C-4); 150.1 (C-2); 170.7 (C=O). Anal Calcd for C<sub>23</sub>H<sub>26</sub>N<sub>4</sub>O<sub>4</sub>S (454.54): (%), C, 60.77; H: 5.77; N: 12.33. Found (%), C: 60.58; H, 5.90; N, 12.25. MS: m/z (%) (EI) 455 (90).

*3-(1-Benzyl-2-ethyl-4-nitro-1H-imidazol-5-ylsulphanyl)-1-[4-(furan2-carbonyl)-piperazin-1-yl]-propan-1-one (13)*. Prepared from (3.4 g) and 1-(2-furoyl)-piperazine (1.8 g); (4.8 g, 86% Yield); m.p.70 – 72°C ; <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 1.3 (t, J=7.2, 3H, CH<sub>2</sub>CH<sub>3</sub>); 2.6-2.8 (m, 4H, SCH<sub>2</sub>CH<sub>2</sub> + CH<sub>2</sub>CH<sub>3</sub>); 3.1 (t, J=6.6, 2H, SCH<sub>2</sub>CH<sub>2</sub>); 3.4-3.70 (br s, 4H, piperazine); 3.8-3.9 (br s, 4H, piperazine); 5.4 (s, 2H, CH<sub>2</sub>Ph); 6.50-6.6 (br s, 1H, furan); 7.0-7.4 (m, 6H, Ar + furan); 7.5 (br s, 1H, furan). <sup>13</sup>C NMR (CDCl<sub>3</sub>), δ 11.3 (CH<sub>2</sub>CH<sub>3</sub>); 21.3 (CH<sub>2</sub>CH<sub>3</sub>); 32.4 (SCH<sub>2</sub>CH<sub>2</sub>); 33.3 (SCH<sub>2</sub>CH<sub>2</sub>); 41.7, 45.2 (piperazine); 47.7 (CH<sub>2</sub>Ph); 111.5, 117.3 (2C, furan-C); 125.2; 126.0; 128.3; 129.2; 135.1(Ph-C, C-5); 144.0, 147.6 (2C, furan-C); 149.1 (C-4); 150.6 (C-2); 159.2, 169.0 (2C=O). Anal Calcd for C<sub>24</sub>H<sub>27</sub>N<sub>5</sub>O<sub>5</sub>S (497.57): (%), C, 57.03; H, 5.47; N, 14.08. Found (%), C, 57.72; H, 5.44; N, 14.18. MS: m/z (%) (EI) 497 (85).

## Biological Activity

### Bacterial strains:

*Salmonella typhimurium* strains TA102 and TA1530 were used to detect oxidative and base pair substitution mutagenicity, respectively. The strains were kindly supplied by Prof. Bruce Ames (Department of Biochemistry, University of California).



### *Mutagenicity assay /Ames Spot test*

The spot test was performed according to the procedure described by Maron and Ames.<sup>[34]</sup> The molten top agar was poured directly to the surface of the minimal agar plate after it had been inoculated with (0.1ml) of fresh overnight culture of one of the bacterial tester strains (TA102 and TA1530) with and without (0.5ml) S9 mix. After hardening, the tested compounds at 33 µg/plate was directly spotted to the center of the plate. After one hour of diffusion, the plates were incubated for three days at 37°C and the colonies were counted using a colony counter.

Sodium azide (1.0 µg/plate) and nitroquinoline-*N*-oxide (10 µg/plate) were used as positive controls for (TA1530) and (TA102), respectively. The negative control was dimethyl sulfoxide (DMSO).

### *Antimutagenicity assay*

The antimutagenicity assay was performed according to literature procedures.<sup>[35,36]</sup> In the antimutagenicity test, the inhibitions of mutagenic activity of the nitroquinoline-*N*-oxide (10 µg/plate) for TA102 and sodium azide (1.5 µg/plate) for TA1530 by the tested compounds at concentrations of 33 µg/plate were investigated.

The mutagen (0.1ml) was added to the mixture of test chemical (0.1ml) and (0.1 ml) of the bacteria with or without (0.5ml) of S9 mix, carefully mixed with molten top agar (2ml) and dispersed onto minimal agar plates. After hardening at room temperature, the plates were incubated at 37°C for three days and the colonies were counted using a colony counter.

Control plates containing DMSO with and without sodium azide or nitroquinoline-*N*-oxide were used.

### *4. Antibacterial assay*

The tested compounds were prepared in Dextrose broth to give the following final concentrations (0.95, 1.9, 3.9, 7.8, 15.7, 31.2, 62.5, 125, and 250 µg/ml) by serial 2 fold dilutions in dextrose broth. After dilution, each tested chemical was distributed in test tubes. The final volume was 2 ml (50 µl) of to 10<sup>-3</sup> diluted overnight culture of one of the bacteria (*Staphylococcus epidermidis*, *E. coli*, and *Pseudomonas sp.*) was added to each test tube and mixed. The mixture was incubated for 20 hours at 35°C. After the incubation period, turbidity and absorbance were recorded. The following controls were used in this test : Control 1: dextrose broth;  
Control 2: dextrose broth+ bacteria; Control 3: dextrose broth + chemical (250 µg/ml)  
Control 4:dex.+ chemical only (~1 µg/ml). Metronidazole was used as a reference compound.

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